Université Pierre et Marie Curie - Paris VI

Habilitation à diriger les recherches

Stratégies reproductrices chez les insectes sociaux - De la colonie à l'individu

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Sommaire

I Curriculum Vitae

Née le 07/01/1970 à Narbonne
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Situation actuelle : Maître de Conférences, classe normale, Ecole Pratique des Hautes Etudes depuis le 1/01/1998, actuellement en délégation CNRS jusqu'au 01/02/2008.

I.1 Diplômes

1995 : **Doctorat** Biologie des Populations et Ecologie**,** Université Montpellier II (UM II). "Evolution des systèmes de reproduction chez les gastéropodes hermaphrodites des eaux douces : le cas de l'aphallie chez *Bulinus truncatus*", sous la direction de B. Delay et P. Jarne, laboratoire Génétique et Environnement, ISEM, UMR 5554. Mention très honorable avec les félicitations du jury.
Financement : Ministère de la Recherche (allocation de 3 ans).

1992 : **DEA** Evolution et Ecologie*,* UM II. Mention assez bien.

1991 : **Maîtrise** Biologie des Organismes et des Populations (option Génétique des Populations), UM II. Mention bien.

1990 : **Licence** Biologie des Organismes et des Populations (option Zoologie), UM II.

1989 : **DEUG** Biologie, UM II.

1987 : **Baccalauréat C**, Narbonne.

I.2 Stages post-doctoraux

1997 : "Ecologie évolutive des interactions hôte-parasite chez les bourdons (*Bombus sp.*)", en collaboration avec P. Schmid-Hempel, Ecole polytechnique, Zürich, financé par l'Université d'accueil.

1996 : "Variabilité génétique et évolution des systèmes de reproduction chez les oiseaux" en collaboration avec M. Petrie & T. Burke, Université de Leicester, Grande Bretagne, financé par l'Université d'accueil.

I.3 Publications

(* = papiers présentés en annexe)

1. Hora RR, Poteaux C, **Doums C**, Fresneau D, Fénéron R. 2007. Egg cannibalism in a facultative polygynous ant: conflict for reproduction or strategy to survive? **Ethology,** sous presse.
2. Clémencet J, **Doums C**. 2007. Habitat-related microgeographic variation of worker size and colony size in the ant *Cataglyphis cursor.* **Oecologia,** sous presse. *
3. Zinck L, Jaisson P, Hora RR, Denis D, Poteaux C, **Doums C**. 2007. The role of breeding system on ant ecological dominance: genetic analysis of *Ectatomma tuberculatum*. **Behavioral Ecology**, sous presse.
4. André JB, Peeters C, Huet M, **Doums C.** 2006. Estimating the rate of gamergate turnover in the queenless ant *Diacamma cyaneiventre* using a maximum likelihood model. **Insectes Sociaux**, 53, 233-244.
5. Hora RR, **Doums C**, Poteaux C, Fénéron R, Valenzuela J, Heinze J, Fresneau F. 2005. Small queens in the ant *Ectatomma tuberculatum*: a new case of social parasitism. **Behavioral Ecology and Sociobiology**, 59, 285-292.
6. Clémencet J, Viginier B, Doums C. 2005. Hierarchical analysis of population genetic structure in the monogynous ant *Cataglyphis cursor* using microsatellite and mitochondrial DNA markers. **Molecular Ecology**, 14, 3735-3744.*
7. Pearcy M, Aron S, **Doums C**, Keller L. 2004. Conditional use of sex and parthenogenesis for worker and queen production in ants. **Science**, 306, 1780-1783.*
8. Pearcy M, Clémencet J, Chameron S, Aron S, **Doums C**. 2004. Characterization of nuclear DNA microsatellite markers in the ant *Cataglyphis cursor*. **Molecular Ecology Notes**, 4, 642-644.
9. Viginier B, Peeters C, Brazier L, **Doums C.** 2004. Very low genetic variability in the Indian queenless ant *Diacamma indicum*. **Molecular Ecology**, 13, 2095-2100.*
10. Baudry E, Peeters C, Brazier L, Veuille M, **Doums C**. 2003. Shift in the behaviours regulating monogyny is associated with high genetic differentiation in the queenless ant *Diacamma ceylonense*. **Insectes Sociaux***,* 50, 390-397.*
11. **Doums C**, Cabrera H, Peeters C. 2002. Population genetic structure and male-biased dispersal in the queenless ant *Diacamma cyaneiventre*. **Molecular Ecology***,* 11, 2251-2264.*

12. **Doums C**, Moret Y, Benelli E, Schmid-Hempel P. 2002. Senescence of immune defence in *Bombus* workers. **Ecological Entomology***,* 27, 138-144.
13. Myskowiak JB, **Doums C**. 2002. Effects of refrigeration on the biometry and development of *Protophormia terraenovae* (Robineau-Desvoidy) (Diptera: Calliphoridae) and its consequences in estimating post-mortem interval in forensic investigations. **Forensic Science International***,* 125, 254-261.
14. André JB, Peeters C, **Doums C.** 2001. Serial polygyny and colony genetic structure in the monogynous queenless ant *Diacamma cyaneiventre*. **Behavioral Ecology and Sociobiology***,* 50, 72-80.*
15. **Doums C**, Schmid-Hempel P. 2000. Immunocompetence in workers of a social insect, *Bombus terrestris* L., in relation to foraging activity and parasitic infection. **Canadian Journal of Zoology***,* 78, 1060-1066.
16. **Doums C.** 1999. Characterization of microsatellite loci in the queenless Ponerine ant *Diacamma cyaneiventre*. **Molecular Ecology***,* 8, 1957-1959.
17. Olsson M, Pagel M, Shine R, Madsen T, **Doums C**, Gullberg A, Tegelström H. 1999. Sperm choice and sperm competition: Suggestions for field and laboratory studies. **Oikos***,* 84, 172-175.
18. **Doums C**, Perdieu MA, Jarne P. 1998. Resource allocation and stressful conditions in the aphallic snail *Bulinus truncatus*. **Ecology***,* 79, 720-733.
19. **Doums C**, Viard F, Jarne P. 1998. The evolution of phally polymorphism. **Biological Journal of the Linnean Society***,* 64, 273-296.
20. Petrie M, **Doums C**, Moller AP. 1998. The degree of extra-pair paternity increases with genetic variability. **Proceedings of the National Academy of Sciences, USA***,* 95, 9390-9395.
21. **Doums C**, Viard F, David P, Jarne P. 1997. Phally status and size in Niger populations of *Bulinus truncatus* (Gastropoda: Planorbidae). **Journal of Molluscan Studies***,* 63, 111-115.
22. Viard F, **Doums C**, Jarne P. 1997. Selfing, sexual polymorphism and microsatellites in the hermaphroditic freshwater snail *Bulinus truncatus*. **Proceedings of the Royal Society of London, B***,* 264, 39-44.
23. **Doums C**, Bremond P, Delay B, Jarne P. 1996. The genetical and environmental determination of phally polymorphism in the freshwater snail *Bulinus truncatus*. **Genetics***,* 142, 217-225.
24. **Doums C**, Jarne P. 1996. The evolution of phally polymorphism in *Bulinus truncatus* (Gastropoda, Planorbidae): The cost of male function analysed through life-history traits and sex allocation. **Oecologia***,* 106, 464-469.
25. Doums C, Labbo R, Jarne P. 1996. Stability and genetic basis of variability of phally polymorphism in natural populations of the self-fertile freshwater snail *Bulinus truncatus*. **Genetical Research***,* 68, 23-33.
26. **Doums C**, Viard F, Pernot AF, Delay B, Jarne P. 1996. Inbreeding depression, neutral polymorphism, and copulatory behavior in freshwater snails: A self-fertilization syndrome. **Evolution***,* 50, 1908-1918.

27. **Doums C**, Delay B, Jarne P. 1994. A problem with the estimate of self-fertilization depression in the hermaphrodite freshwater snail *Bulinus truncatus*: The effect of grouping. **Evolution***,* 48, 498-504.

Soumises :

Bocher A, Tirard C, Doums C. Phenotypic plasticity of immune defence linked with foraging activity in the ant *Cataglyphis velox*. Soumise à **Journal of Evolutionary Biology**.

Clémencet J, Cournault L, Odent A**, Doums C**. Worker size, thermal tolerance and foraging activity in the thermophilic ant *Cataglyphis cursor* (Hymenoptera ; Formicidae). Soumise à **Insectes Sociaux**.

Clémencet J, **Doums C**. Genetic diversity and worker size in the highly polyandrous and partially parthenogenetic ant *Cataglyphis cursor*. 2ème soumission à **Behavioral Ecology and Sociobiology**.

Clémencet J, Rome Q, Fédérici P, **Doums C.** Larger workers have a higher reproductive potential in an orphaned colony of the ant *Cataglyphis cursor*. 2ème soumission à **Naturwissenschaften**.

I.4 Communications internationales

(* = sur invitation ; pour les communications orales, j'ai souligné la personne qui a présenté la communication)

2006 : Clémencet J, **Doums C.** Level of polyandry and worker body size in the ant *Cataglyphis cursor*. International Society Behavioural Ecology Congress (ISBE), Tours, France, 2006.

Clémencet J, **Doums C**. Worker size and colony fitness in the polyandrous ant *Cataglyphis cursor*. International Union for the Study of Social Insect Congress (IUSSI), Washington D.C., USA, 2006.

Bocher A, **Doums C**, Millot L, Tirard C. Reproductive conflicts affect labour and immune defenses in the queenless ant *Diacamma sp.* (Nilgiri, south India) International Union for the Study of Social Insects, Washington DC, USA, 2006

Zinck L, Hora RR, **Doums C**, Jaisson P. Functional organization and genetic structuration of nests in patch in the ant Ectatomma tuberculatum.XV Congress IUSSI, 30-4 August Washington, USA (Poster).

2005 : Clémencet J, Cournault L, Odent A, **Doums C.** Genetic diversity, worker polymorphism and colony fitness in the polyandrous ant *Cataglyphis cursor*. European Society for Evolutionary Biology Congress (ESEB), Cracow, Poland, 2005 (Poster).

2004 : Bocher A, **Doums C**, Millot L, Tirard C. Impact of social conflicts on immunocompetence in a queenless ant, *Diacamma sp*., from Nilgiri. The closing symposium of the EU research training network INSECTS. Helsingor Septembre 2004 (Poster).

Clemencet J, **Doums C**. Polyandry and colony size in the ant *Cataglyphis cursor*. The closing symposium of the EU research training network INSECTS. Helsingor Septembre 2004 (Poster).

Zinck L, Hora R, **Doums C**, Jaisson P. Complex genetic structure of monogynous colonies in the ant *Ectatomma tuberculatum*. The closing symposium of the EU research training network INSECTS. Helsingor Septembre 2004 (Poster).

2002 : Doums C, Viginier B, Brazier L, Peeters C. Very low genetic variation in the queenless ant *Diacamma indicum* suggests recent introduction to India. International Union for the study of Social Insect. Sapporo (Japan), Juillet 2002. (Poster).

2001 : *Doums C, Cabrera H**.** Sex-biased dispersal and population genetic structure of the queenless ant *Diacamma cyaneiventre*. European meeting of the IUSSI (International Union for the study of social insect), Berlin Septembre 2001.

2000 : Doums C, André JB. Effects of gamergate turn-over on the population and colony genetic structure of the queenless ant *Diacamma cyaneiventre*. TMR workshop on social evolution, Zurich (Swizerland), Janvier 2000.

1998 : Doums C, Schmid-Hempel P. Foraging activity reduces immune defenses in the bumble bee *bombus terrestris*. International Union for the study of Social Insect. Adelaide (Australia), Decembre 1998.

***Doums C**. Immune defence in the social insect *Bombus terrestris* in relation to foraging activity and parasitic infection. European Science Foundation workshop on Parasite Defences and Trade-offs in Evolutionary Ecology. Upssala (Sweden), octobre 1998.

Doums C, P. Schmid-Hempel P. Immune defence, foraging activity and parasites in the bumble bee *Bombus terrestris*. TMR workshop on social evolution, Upssala (Sweden), octobre 1998 (Poster).

Doums C, Shmid-Hempel P. Variation and costs of immune defenses in the bumble bee *Bombus terrestris*. TMR workshop on social evolution, Keele (U.K.), mars 1998.

1997 : Doums C. The evolution of life-history traits and mating systems. TMR workshop on social evolution, Arrhus (Danmark), juin 1997.

Doums C, Schmid-Hempel P. Immune defenses and life-history traits in the bumble bee *Bombus terrestris*. TMR workshop on social evolution, wurzburg (Germany), octobre 1997.

1996 : Doums C, Jarne P. The evolution of phally polymorphism in the hermaphrodite freshwater snail *Bulinus truncatus* : a modelisation approach. Ecological Society of America congress, Providence (USA), août 1996.

1995 : Doums C, Delay B, Jarne P. The evolution of phally polymorphism in the hermaphrodite freshwater snail *Bulinus truncatus* : genetic variation of the aphally frequency within and between populations. 5th congress of European Society for Evolutionary Biology, Edimburgh, septembre 1995.

1994 : *Doums C, Jarne P. On the evolution of aphally in the hermaphrodite freshwater snail *Bulinus truncatus*. European Science Foundation workshop on Genetic Conflicts and Parasitism, Paris, septembre 1994.

1993 : **Doums C**, Delay B, Jarne P. On the factors influencing the evolution of phally polymorphism in the hermaphrodite snail *Bulinus truncatus*. 27th meeting of Population Genetics Group, Reading (Grande-Bretagne), décembre 1993.

Doums C, Delay B, Jarne P. Life-history traits in aphallic versus euphallic *Bulinus truncatus* (hermaphrodite freshwater snail). 4th congress of European Society for Evolutionary Biology, Montpellier, août 1993.

Doums C, Jarne P. Comparative fitness of isolated aphallic and euphallic individuals. Society for the Study of Evolution meeting, Snowbird (USA), juin 1993.

I.5 Encadrement d'étudiants

I.5.1 Thèses

Encadrement complet :

2002 - 2006 : J. Clemencet - Evolution des stratégie de reproduction chez les insectes sociaux : étude de la fourmis polyandre *Cataglyphis cursor*. Thèse Paris VI. Encadrement complet.

Encadrement en co-direction :

2006 - ---- : B. Chéron – La reproduction par fission chez les fourmis : étude des conflits intra-coloniaux. Thèse Paris VI en co-direction avec T. Monnin.

2004 - ---- : A. Bocher – Défenses immunitaires et socialité. Thèse Paris VI en co-direction avec C. Tirard.

Participation à un encadrement :

2003 - ---- : L. Zink - Stratégies de reproduction et mutualisme social chez la fourmi ponérine *Ectatomma tuberculatum*. Dir. Thèse : P. Jaisson, LEEC Paris XI. Encadrement de la partie génétique.

2001 - 2006 : M. Pearcy – Stratégies reproductrices chez la fourmi *Cataglyphis cursor* Dir. Thèse : S. Aron, Université libre de Bruxelle. Encadrement de la partie génétique effectuée à l'UMR 7625.

I.5.2 Master et DEA

2007 : A.L. Devès – Réponse immunitaire et reproduction chez la fourmi *Cataglyphis cursor*. Master EBE (2ème année) de Paris VI en co-direction avec C. Tirard.

2004 : A. Bocher - Réponse immunitaire et agressivité chez la fourmi sans reine *Diacamma nilgiri*. Stage du DEA d'Ecologie de Paris VI en co-direction avec C. Tirard.

2002 : J. Clemencet - Stratégie de dispersion et reproduction chez la fourmi *Cataglyphis cursor*: approche génétique. Stage du DEA d'Ecologie de Paris VI.

2000 : H. Cabrera - Asymétrie de dispersion entre les sexes chez la fourmi sans reine *Diacamma cyaneiventre*. Stage du DEA d'Ecologie de Paris VI.

1999 : J.B. André - Organisation sociogénétique des colonies de la fourmis sans reine *Diacamma cyaneiventre*. Stage du DEA d'Ecologie de Paris VI.

I.5.3 Diplôme E.P.H.E.

2001 - 2005 : M. Deville - Phylogéographie moléculaire des espèces de *Pneumocyctis carinii* chez des populations de rongeurs insulaires. Sous la direction extérieure de J. Guillot. Service de Parasitologie-Mycologie, UMR BIPAR, Ecole Nationale Vétérinaire d'Alfort.

2001 - 2003 : A. Maros - Biologie de population des courtilières (Orthoptère, Gryllotalpidae), insectes prédateurs des œufs de tortues Luth (*Dermochelys coriacea*) en Guyane française. UPRESA 8079 Orsay.

1998 - 2000 : J.B. Myskowiak - Effets de la réfrigération sur le développement d'un insecte nécrophage, *Protophormia terraenovae* (Robineau-Desvoidy). Influence sur l'estimation du délai post-mortem.

I.6 Enseignements

I.6.1 Enseignements à l'Université Paris VI

Dans le cadre d'un échange de service avec Alexandre Hassanin (Maître de Conférences Paris VI) depuis Septembre 2004, j'ai assuré les enseignements suivants :

2006 - 2007 : Organisation des Métazoaires L2 (70 h) ; Cours M2 (3h) ; Cours ENS-Paris VI de L3 (2h)

2005 - 2006 : Organisation des Métazoaires L2 (120 h) ; Cours M2 (3h)

2004 - 2005 : Organisation des Métazoaires L2 (100 h) ; Biostatistique M1 (20 h) ; Cours M2 (3h).

I.6.2 Enseignements à l'E.P.H.E.

Responsable des modules d'enseignements qui avaient lieu tous les ans pour les années suivantes :

2000 - 2004: 'Eléments de statistique' (env. 20 h cours / an).

2002 - 2004: 'Génétique des populations' (env. 20h cours / an).

I.7 Animation de la recherche

Membres de commissions de spécialistes :

2001 - 2004 : membre titulaire de la commission de spécialiste 67/68 de l'Université de Paris VI.

2001 - 2005 : membre suppléant de la commission de spécialiste 67/68 de l'Université de Bourgogne.

Contrats de recherche :

2007 - 2010 : ANR (N°-06-BLAN-0268-01) (200 000€), chef de projet: T. Monnin, participation à 60 %.

2001 - 2004 : Action concertée incitative jeunes chercheurs (ACI) (N° 5183) (88 500 €), chef de projet.

2001 - 2003 : Programme international de coopération scientifique (PICS) avec le Prof. Gadagkar en Inde (environ 60 000F/an), chef de projet : C. Peeters.

1998 - 2000 : Programme national "Dynamique de la Biodiversité et Environnement" (N° 2T00S9) (70 000F), chef de projet.

Séminaires nationaux et internationaux : j'ai présenté mes travaux de recherche lors de séminaires effectués en France dans les laboratoires d'Orsay (UMR 8079), de Dijon (UMR 5561), de Villetaneuse (FRE 2413), de Montpellier (UMR 5175), de Perpignan (URA 698). J'ai également été amenée à présenter mes travaux à l'étranger à l'Université de Regensburg (Allemagne), à l'Université des Sciences de Bangalore (Inde), à l'Université de Jyvaskyla (Finlande), à l'université de Zürich (Suisse) et de Leicester (UK).

Arbitre international : pour les revues Behavioral Ecology and Sociobiology, Behavioral Ecology, Biological Journal of the Linean Society, Entomological Research, Heredity, Insectes Sociaux, Journal of Evolutionary Biology, Journal of Evolutionary Ecology, Molecular Ecology, Proceedings of The Royal Society London B.

Affiliations scientifiques : Membre de l'Union Internationale pour l'Etude des Insectes Sociaux ; Membre de European Society of Evolutionary Biology.

II Activités de recherche

(A chaque citation d'un article présenté en annexe, le numéro de celle-ci est indiqué)

II.1 Introduction générale

Comprendre la mise en place et la grande diversité des modes de reproduction et des traits d'histoire de vie est un objectif majeur de la Biologie Evolutive. En effet, ces traits biologiques sont fondamentaux car ils déterminent les modalités de transmission des gènes d'une génération à l'autre (modes de reproduction) et dans l'espace et le temps (traits d'histoire de vie). Ils sont donc à la base de la définition de la valeur sélective des individus, paramètre incontournable pour comprendre l'évolution de tout caractère. Dans ce contexte, l'observation d'individus dans certaines sociétés animales qui sacrifient leur propre reproduction pour celle des autres représente un paradoxe évolutif. Les insectes sociaux en sont un exemple frappant et ont toujours suscité une grande curiosité de la part des biologistes. La théorie de la sélection de parentèle (Hamilton, 1964) apporta une solution élégante à ce problème, en spécifiant qu'un individu peut augmenter la fréquence de ses propres gènes dans les générations suivantes non seulement par sa propre reproduction, mais également en favorisant celle d'individus apparentés (qui ont donc des gènes en commun).

Un très grand nombre d'études sur les insectes sociaux se sont spécifiquement attachées à résoudre des problèmes liés au fait de vivre en société, tels que la communication, la régulation de la reproduction, la répartition des tâches de travail, les conflits génétiques au sein des colonies (Bourke & Franks, 1995 ; Crozier & Pamilo, 1996). En revanche, la mise en place de la très grande diversité des sociétés d'insectes reste relativement mal comprise (Bourke & Franks, 1995). Cette diversité peut se décrire selon trois axes principaux : (i) le niveau de complexité sociale en terme de dimorphisme reine/ouvrière, de polymorphisme des ouvrières et de système de communication, (ii) l'organisation socio-génétique qui va définir les liens génétiques entre les différents membres de la colonie et qui se caractérise par de nombreux paramètres dont le nombre de reproductrices, le mode de reproduction (nombre de partenaires sexuels par reproductrice, reproduction sexuée *vs.* parthénogenèse) et le partage de la reproduction et enfin (iii) les traits d'histoire de vie (croissance, investissement reproductif, mortalité, dispersion) qui peuvent se définir aussi bien à l'échelle de l'individu que de la colonie. Ce sont ces deux derniers axes (ii et iii) qui ont principalement retenu mon attention.

De façon assez surprenante, peu d'études ont abordé l'évolution des traits d'histoire de vie chez les insectes sociaux (Bourke & Franks, 1995) bien que ce domaine soit largement développé chez les organismes solitaires (Stearns, 1992 ; Roff, 1992). En revanche, avec l'avènement des marqueurs microsatellites permettant une résolution très fine des relations génétiques entre individus, un très grand nombre de travaux ont étudié l'organisation socio-génétique de nombreuses espèces. Ces travaux ont révélé que

beaucoup de sociétés s'éloignaient du schéma classique d'une seule reine accouplée avec un seul mâle (Bourke & Franks, 1995 ; Heinze & Keller, 2000 ; Strassmann, 2001 ; Keller, 2007). La polyandrie et polygynie en entraînant une diminution de la corrélation génétique entre les membres de la colonie, peut donc représenter un challenge potentiel pour la théorie de sélection de parentèle. Ceci a favorisé l'émergence de nombreuses hypothèses sur les potentielles pressions de sélection favorisant l'évolution de ces deux paramètres et de travaux visant à les tester (Bourke & Franks, 1995 ; Keller, 1995 ; Strassmann, 2001).

La thématique générale de mes travaux de recherche porte sur l'évolution des stratégies reproductrices en terme de modes de reproduction et de fondation de nouvelles colonies, chez deux taxons de fourmis, les fourmis sans reine du genre *Diacamma* et une fourmi méditerranéenne *Cataglyphis cursor*. Ces deux taxons présentent des caractéristiques originales (pour de plus amples informations sur la biologie de ces espèces, se référer aux Encadrés 1 et 2). Tout d'abord, à l'échelle individuelle, les ouvrières sont totipotentes, à savoir qu'elles ont la possibilité, dans certaines circonstances, de produire des œufs haploides mâles mais aussi des œufs diploides femelles. Cette totipotence des ouvrières offre des possibilités originales de reproduction ainsi que de conflits sociaux. A l'échelle de la colonie, les nouvelles colonies sont fondées avec l'aide des ouvrières (fondation dépendante des colonies). Contrairement à la majorité des fourmis qui s'accouplent lors d'un vol nuptial, les femelles s'accouplent à l'entrée du nid. Cette fondation dépendante des colonies avec accouplement sans vols nuptiaux est très peu étudiée bien qu'elle représente une stratégie de production des nouvelles colonies assez commune (Peeters & Ito, 2001) et a des conséquences particulières en terme de dispersion et d'allocation des ressources.

Mes travaux de recherche s'appuient sur une approche d'écologie moléculaire, d'écologie évolutive et récemment d'immunologie évolutive avec pour objectifs principaux (i) la compréhension des stratégies de reproduction des colonies par fission et (ii) l'évolution des systèmes de reproduction chez *C. cursor*. Ces deux points seront développés dans les deux prochains chapitres. Même si ces deux aspects sont traités séparément pour une meilleure clarté, ils ne sont pas complètement indépendants. Par exemple, le système de reproduction va affecter les conflits liés à la fission.

Encadré 1 : les fourmis Ponérines sans reine du genre *Diacamma*

Distribution et types d'habitat :

Le genre *Diacamma* contient 42 espèces et sous-espèces connues que l'on peut trouver de l'Inde jusqu'au Sud-Est de l'Asie et plus au sud de l'Indonésie à l'Australie (voir ci-dessous). Les espèces étudiées se trouvent en Inde et colonisent des milieux ouverts, pauvres en végétation tels que des pâturages (voir ci-dessous).

Distribution du genre *Diacamma* (en rose)

Pâturage à vache

Entrée d'un nid de *Diacamma ceylonense*

Structure des nids et cycle de vie :

Une colonie est constituée d'un seul nid (monodomie). Chez *D. ceylonense* ou *D. cyaneiventre*, les colonies sont relativement grandes (moyenne (± SD) = 214 ± 80, n = 6). Les nids s'enfoncent profondément dans la terre (< 1,5 m) et l'entrée peut être aisément trouvée par les amas de terre et végétaux qui l'entourent (voir ci-dessus). En revanche, chez *D. indicum*, les colonies sont plus petites (88 ± 62 ouvrières, n = 11) et plus opportunistes quant à leur type de nids. Les nids peuvent se trouver aussi bien dans la terre que sous des pierres d'un muret.

Les colonies pérennes produisent du couvain femelle (ouvrières) et mâle tout le long de l'année. Il n'y a qu'une seule ouvrière fécondée et reproductrice dans la colonie (la gamergate). Lorsque la gamergate disparaît ou lors d'une fission de la colonie, elle est remplacée par la première ouvrière qui émerge. Après un accouplement au sol, le mâle laisse une partie de son abdomen dans les genitalia femelles. La monoandrie est donc de règle chez ces espèces.

Encadré 2 : la Formicine *Cataglyphis cursor*

Distribution et types d'habitat :

C. cursor est une espèce de région méditerranéenne que l'on peut trouver du littoral jusqu'à l'intérieur des terres (< 50 km). Sa distribution peut être assez large, allant de l'Espagne jusqu'à la chine (Cagniant, 1976), mais recouvre plusieurs formes qui pourraient être différentes espèces. Nous nous sommes uniquement intéressés à la forme *tibialis* qui se trouve du Nord de l'Espagne jusqu'en Camargue. Les nids se trouvent toujours dans des milieux plats, arides et pauvres en végétation, bien exposés au soleil. Au sein de son aire de répartition française, elle peut coloniser des habitats allant des chemins de terre ou terrains vagues à l'intérieur des terres à des milieux beaucoup plus sableux tels que l'arrière plage en bordure de mer (voir ci-dessous).

Bord de mer

Chemin de vigne

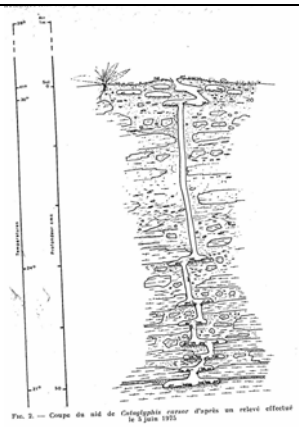


Schéma d'une coupe de nid d'après Cagniant (1976)

Structure des nids et cycle de vie :

Les colonies de taille variable (de 100 à 3000 ouvrières) n'occupent qu'un seul nid. Les nids de *C. cursor* ne possèdent qu'une seule entrée, sont profonds (< 1,2 m) et étroits. L'architecture typique est schématisée ci-dessus (d'après la Fig. 2. de Cagniant 1976).
Les colonies pérennes ont un cycle de vie annuel avec une hibernation d'octobre à mars sans couvain. Les premiers œufs pondus à la sortie d'hibernation par la reine produisent les sexués (mâles et femelles), suivis des ouvrières jusqu'à la fin de l'été. La reproduction a lieu en juin avec un accouplement au sol. Les jeunes reines fécondées par plusieurs mâles (polyandrie) peuvent fonder une nouvelle colonie avec l'aide d'ouvrières. Si la fission n'a pas lieu, les reines surnuméraires sont éliminées et la monogynie est rétablie.

II.2 Reproduction des colonies par fission

Chez les insectes sociaux, il existe deux modes principaux de fondation des nouvelles colonies. Les reines peuvent fonder seules une nouvelle colonie en s'appuyant sur leur réserve énergétique ou en partant elle-même chercher de la nourriture. On parle alors de fondation indépendante des colonies (ICF) car la fondation se fait par la reine sans l'aide des ouvrières. Par opposition, lors de la fondation dépendante des colonies (ou fission), la reine a besoin de l'aide des ouvrières pour fonder une nouvelle colonie. Même si la majorité des insectes sociaux classiquement étudiés possèdent un mode de fondation indépendante des colonies, le mode de fondation par fission reste assez commun (Peeters & Ito, 2001). Cependant les quelques travaux menés sur la fission concernent très peu d'espèces. La fission a été assez bien décrite chez les fourmis légionnaires, chez lesquelles la reproduction est assurée par des reines ergatoïdes (Franks & Hölldobler, 1987 ; Gotwald, 1995 ; Kronauer *et al.*, 2004). Cependant, il s'agit de fourmis monogynes atypiques, en raison de leur style de vie nomade et de leurs colonies énormes (plusieurs centaines de milliers à des millions d'adultes). Quelques études ont également été menées chez l'abeille (Kryger & Moritz, 1997; Fefferman & Starks, 2006).

Chez les espèces à fondation indépendante, lorsque la reine meurt accidentellement, la colonie généralement périclite. A l'inverse, chez les espèces à fondation dépendante, si les ouvrières se retrouvent orphelines en présence de couvain royal jeune, elles peuvent produire des gynes qui peuvent alors remplacer la reine. Chez les espèces à fondation dépendante, les colonies sont donc potentiellement immortelles avec une polygynie séquentielle, c'est-à-dire une succession de reines cours du temps.

L'objectif d'une partie de mes travaux vise à comprendre qu'elles sont les modalités de la fission et des changements de reines et comment elles ont pu évoluer. Pour ce faire, mes recherches, terminés, en cours ou en projet, analysent (i) les conséquences de la fission sur les capacités de dispersion, (ii) la fréquence du changement de la reproductrice ainsi que les coûts potentiels de ce changement, (iii) les stratégies d'allocation des ressources et (iv) les conflits sociaux liés à la fission. Ces deux derniers points seront principalement abordés dans le projet car nos travaux sur ces questions commencent tout juste.

II.2.1 Fission et dispersion limitée

Chez les fourmis, dans la mesure où les ouvrières ne possèdent pas d'ailes, la fondation par fission implique une dispersion femelle limitée à la distance de marche des ouvrières. Seuls les mâles, lorsqu'ils sont ailés, peuvent potentiellement disperser sur de longues distances. A l'inverse, les colonies à ICF présentent généralement des vols nuptiaux donnant la possibilité d'une dispersion sur de longues distances pour les deux sexes. Ainsi le mode de fondation des colonies peut affecter la distribution de la variabilité génétique à plusieurs échelles spatiales. A l'échelle locale, cela peut entraîner une importante viscosité

des populations (les colonies voisines sont génétiquement plus proches que les colonies éloignées) et avoir des conséquences sur l'évolution de traits liés à la socialité ainsi que sur la compétition inter-coloniale et la reconnaissance des colonies (Kelly, 1992 ; Kelly, 1994). La connaissance de la répartition de la variabilité génétique à l'échelle locale est par ailleurs indispensable à l'estimation de la corrélation génétique entre membres de la colonie (Ross, 2001). A l'échelle inter-populationnelle, une dispersion limitée devrait induire une forte structuration génétique des populations. L'étude de la structure génétique des populations est primordiale car elle permet de définir l'échelle de la population et donc des processus micro-évolutifs ainsi que des potentialités d'adaptation locales (Mopper & Strauss, 1998). Notons toutefois que les mâles, ailés, peuvent rapidement homogénéiser la structure génétique induite par la faible dispersion par voie femelle, en particulier à une échelle locale.

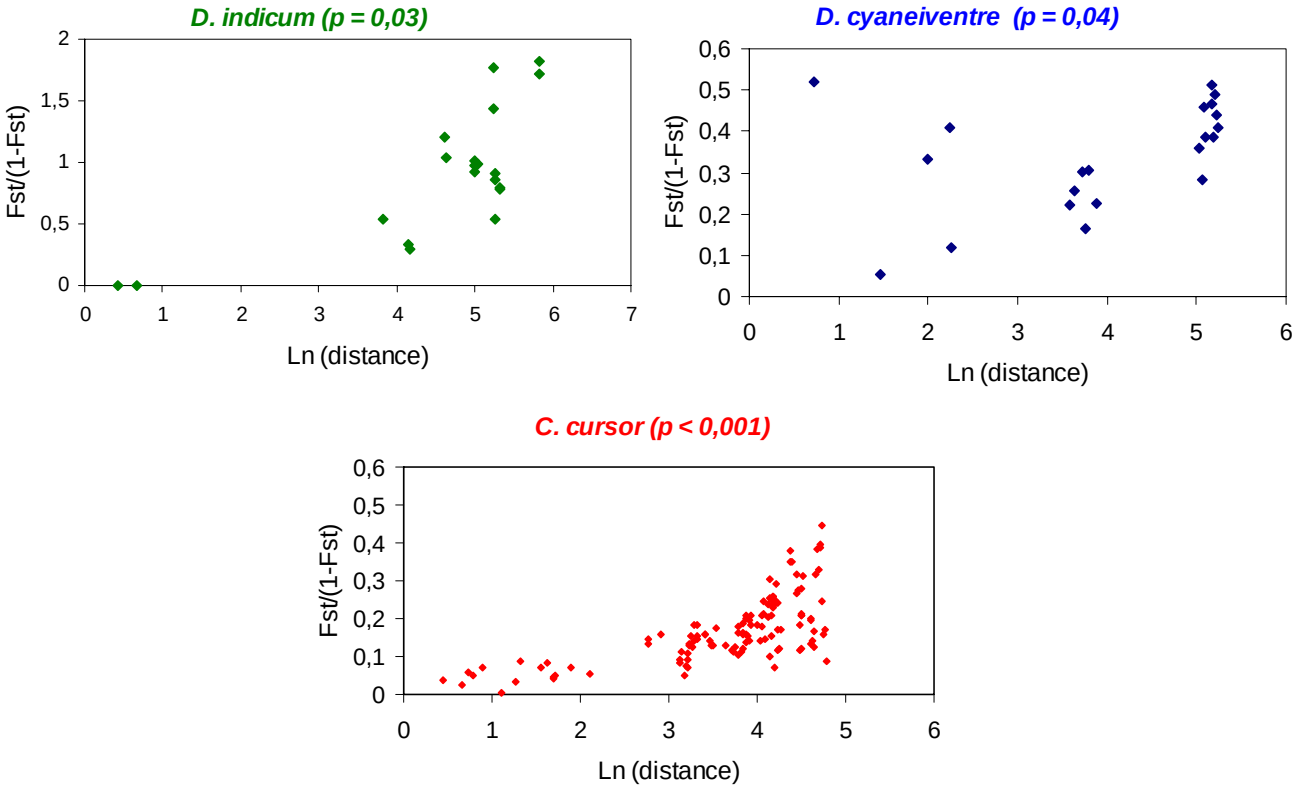
En collaboration avec C. Peeters (UMR 7625) et R. Gadagkar (Université de Bangalore, Inde), nous avons travaillé sur quatre espèces de fourmis sans reine dont deux diffèrent par des caractéristiques particulières. *Diacamma indicum* possède des traits d'histoire de vie suggérant une plus grande aptitude à fissionner (nids instables, petites colonies) par rapport aux trois autres *Diacamma*. *D. sp* "from Nilgiri" diffère par son système de régulation de la reproduction. La conclusion principale de cette étude de génétique des populations est une forte structuration génétique entre les populations, aussi bien pour l'ADN mitochondrial (qui reflète les flux de gènes femelles) que pour les marqueurs microsatellites, quelles que soient les caractéristiques des espèces (Tableau 1). Par ailleurs, la structuration génétique augmente significativement avec la distance géographique (Fig. 1). A l'échelle locale, une faible viscosité génétique a été observée chez *D. cyaneiventre,* suggérant que le flux de gène par les mâles compense localement le faible flux de gènes femelles (Doums *et al.*, 2002a[1]).

***Tableau 1** : Différentiation génétique nucléaire à différentes échelles spatiales pour différentes espèces de fourmis présentant un mode de fondation des colonies par fission. Les valeurs des Fst ainsi que la probabilité (P) d'un test exact de différentiation génétique (l'hypothèse nulle étant une absence de différentiation) sont donnés pour différentes échelles spatiales. Hs correspond à la diversité génétique (taux d'hétérozygotie attendue).*

Espèces	Caractéristiques	Echelle spatiale	*Hs*	*Fst*	*P*	Ref.
D. cyaneiventre	Monogyne/ monoandre	35 – 200 km	0,63	0,26	$< 10^{-5}$	Doums *et al.*, 2002a[1]
		2-10 km		0,15	$< 10^{-5}$	
D. ceylonense	Monogyne/ monoandre	50-100 km	0,56	0,23	$< 10^{-5}$	Baudry *et al.*, 2003[2]
D. nilgiri	Monogyne/ monoandre	1-30 km	0,44	0,21	$< 10^{-5}$	Baudry *et al.*, 2003[2]
D. indicum	Monogyne/ monoandre	15-350km	0,25	0,40	$< 10^{-5}$	Viginier *et al.*, 2004[3]
C. cursor	Monogyne/ polyandre	30-120 km	0,76	0,16	$< 10^{-5}$	Clémencet *et al.*, 2005[4]
		3-30 km		0,12	$< 10^{-5}$	

Similairement, chez *C. cursor*, une très forte différentiation génétique entre les populations a pu été mise en évidence au niveau nucléaire (Tableau 1), cette différentiation augmentant avec la distance géographique (Fig. 1, Clémencet *et al.*, 2005[4]). A l'échelle locale, le type d'habitat affecte le patron de distribution de la variabilité génétique. Au bord de mer où l'habitat est continu, la différentiation génétique entre les colonies diminue progressivement avec la distance que ce soit pour les marqueurs nucléaires ou mitochondriaux. En revanche, dans les vignobles où l'habitat est plus fragmenté, cette viscosité génétique n'a pas été observée. Seules les colonies voisines, trouvées sur un même site, sont génétiquement très proches (Clémencet *et al.*, 2005[4]).

Figure 1 *: Isolement par la distance basé sur les marqueurs microsatellites chez les différentes espèces de fourmis étudiées. La distance génétique est estimé par Fst/(1-Fst). La valeur de P correspond au test de Mantel. Notez que les échelles des différents axes ne sont pas similaires, la différentiation génétique étant beaucoup plus élevée chez D. indicum, sans doute due à la faible diversité génétique observée chez cette espèce (Viginier et al., 2004[3]).*



Il ressort de ce travail que les très faibles capacités de dispersion des mâles et des femelles des différentes espèces étudiées devrait offrir de fortes potentialités d'adaptation locale. A une échelle plus fine (< 1 km), la reproduction par fission n'induit pas une très forte viscosité génétique au niveau nucléaire, même si elle reste très forte pour l'ADN mitochondrial. La dispersion des mâles permet probablement d'effacer en partie la structuration due à la dispersion femelle limitée. Il est toutefois important de noter que nos études sont toutes basées sur un échantillonnage d'un seul individu par colonie. Ce type

d'échantillonnage pourrait potentiellement limiter la mise en évidence d'une viscosité génétique à très petite échelle par comparaison avec les études menées sur les espèces polygynes où, en général, plusieurs individus par colonie sont analysés. Notons que Pearcy & Aron (2006) ont mis en évidence une viscosité faible dans une population de *C. cursor* mais significative en ayant analysé plusieurs individus par colonie.

Quoiqu'il en soit, la viscosité génétique à une échelle locale semble plus faible que celle généralement détectée chez les espèces polygynes (i.e. Seppä & Pamilo, 1995 ; Chapuisat *et al.*, 1997 ; Giraud *et al.*, 2000). Ceci n'est pas surprenant car la polygynie est souvent associée à la polydomie (plusieurs nids par colonie) qui, à elle seule, peut générer une forte viscosité puisque les échanges d'ouvrières entre les nids vont diminuer très probablement avec la distance les séparant. La monodomie des espèces étudiée et donc l'absence d'échange d'ouvrières entre les nids font que les colonies filles vont rapidement se différentier des colonies mères. Lorsque les ouvrières de la colonie mère qui ont participées à la fission sont mortes dans les colonies filles, la corrélation génétique entre la colonie mère et la colonie fille va chuter de 0,75 à 0,375 chez des espèces monogynes monoandres comme *Diacamma*. Un seul remplacement de gamergate dans l'une ou l'autre des colonies refait chuter la corrélation génétique à 0,187. Il est donc clair que de fortes corrélations génétiques entre colonies voisines ne peuvent se percevoir que durant un assez court laps de temps. Dans le cas de *C. cursor*, c'est légèrement plus compliqué vu les modalités de reproduction originales qui ont été détectées chez cette espèce (nous les verrons plus loin), mais le principe reste le même.

II.2.2 Changement de la reproductrice

L'immortalité potentielle des colonies chez les espèces qui fissionnent est associée à une succession des reproductrices dans le temps : une nouvelle reproductrice est produite lorsque la reine meurt ou que sa fertilité décroît (Cuvillier-Hot *et al.*, 2004). Chez les fourmis sans reine du genre *Diacamma*, nous avons estimé l'importance de la polygynie séquentielle, c'est-à-dire de la fréquence du changement de la reproductrice au sein d'une colonie en utilisant une approche moléculaire (André *et al.*, 2001[5]). Ce taux de polygynie séquentielle, exprimé comme le rapport entre la longévité de la reproductrice et celle des ouvrières, s'est révélé similaire chez les différents taxons étudiés. En particulier, il était intéressant de noter que les différences majeures de comportements de régulation observées chez *D*. from 'nilgiri' n'avaient pas de conséquences drastiques sur le taux de polygynie séquentielle (Tableau 2).

***Tableau 2** : Taux de polygynie séquentielle chez différentes espèces de Diacamma. Le taux de polygynie séquentielle représente le rapport entre la longévité de la gamergate et celle des ouvrières (g/w). Il peut être estimé à partir de la proportion de colonies contenant plus d'une lignée maternelle (André et al., 2001[5]). La valeur de la corrélation génétique moyenne entre ouvrières d'une même colonie est également donnée ainsi que la valeur de probabilité que cette corrélation corresponde à une société monogyne et monoandre (t-test contre r = 0.75). N col. et N. ouv. = nombre de colonies et d'ouvrières analysées génétiquement.*

Espèces	N col.	N ouv.	*R* (± SE)	*P* value	g/w	Ref.
D. cyaneiventre	46	451	0,751 ± 0,03	0,96	5,26	André *et al.*, 2001[5]
D. ceylonense	27	283	0,744 ± 0,02	0,81	7,14	Barrate, 2005
D.' Nilgiri'	29	296	0,663 ± 0,03	< 0,01	4,76	Barrate, 2005

Le taux de polygynie séquentielle reflète le taux de mortalité de la reproductrice, mais également la fréquence des fissions. En effet, à chaque événement de fission, un changement de reproductrice va être obligatoirement induit dans une des colonies (fille ou mère). Ces deux aspects pouvant se compenser, une absence de différence de taux de polygynie séquentielle n'implique donc pas obligatoirement une différence de stratégie. En effet, un taux de mortalité élevé associé à un taux de fission faible peut induire un taux de polygynie séquentielle similaire à celui qui pourrait être observé avec un taux de mortalité faible, mais un taux de fission élevé. Connaître les taux de survie des reproductrices est donc indispensable si l'on veut pouvoir faire des inférences sur le taux de fission à partir de l'estimation de polygynie séquentielle.

Du point de vue des ouvrières, le déclenchement d'une fission, en entraînant un changement de reproductrice, change les relations d'apparentement entre individus pour les ouvrières qui vont avec la nouvelle reproductrice (au lieu d'aider des sœurs, les ouvrières aident des nièces). Si la fission a lieu après un changement de reproductrice dans la colonie mère, elle n'induit alors plus de changement d'apparentement. Il pourrait donc être préférable pour les ouvrières de fissionner à la mort de la reproductrice. De façon anecdotique, la seule observation de fission probable sur le terrain chez *D. ceylonense*, suggère effectivement qu'elle aurait eu lieu juste après un changement de gamergate (André *et al.*, 2006). Cette hypothèse pourrait être vérifiée expérimentalement.

Un changement de la reproductrice n'implique par uniquement des coûts génétiques, mais également plus écologiques. Ces coûts peuvent directement être liés à une diminution de croissance de la colonie lors d'un changement de reproductrice, puisque la colonie va subir une période sans production de couvain femelle entre la mort de la reproductrice et son

remplacement effectif. Les conflits entre ouvrières pour la reproduction, généralement associés à l'absence de reproductrice, peuvent également générer un coût. D'une façon générale, le coût des conflits a rarement été estimé dans la littérature (Gobin *et al.*, 2003) alors qu'il représente un paramètre fondamental pour comprendre comment les conflits peuvent être résolus (Hartmann *et al.*, 2003). Ce coût des conflits pourrait expliquer l'absence de conflit exprimé alors qu'un conflit serait prédit par la théorie de sélection de parentèle (Ratnieks & Reeve, 1992 ; Bourke & Franks, 1995). Par ailleurs, ces coûts peuvent influencer indirectement le taux de fission optimal des colonies.

En collaboration avec Claire Tirard du laboratoire de Parasitologie Evolutive, nous nous sommes intéressés aux coûts du changement de la reproductrice chez la fourmi sans reine *D. nilgiri*. Il est facile d'induire un conflit simplement en enlevant la gamergate présente dans une colonie. Afin d'étudier le coût potentiel de ces conflits sur la reproduction, nous avons simulé des fissions en divisant une colonie en deux parties égales dont l'une était orpheline. Les travaux de notre étudiante en thèse Aurélie Bocher ont pu montrer que les ouvrières passent moins de temps à travailler, en particulier à s'occuper du couvain, dans les groupes orphelins par rapport aux groupes contenant la gamergate (Fig. 2). Par ailleurs, les ouvrières des groupes orphelins présentent une moins bonne capacité à se défendre face à une infestation artificielle par des bactéries d' *Escherichia coli* (Fig. 3).

Figure 2 *: Pourcentage de temps passé à s'occuper du couvain dans le groupe avec gamergate (bleu) et le groupe orphelin (rouge) pour chacune des 15 colonies étudiées (effet du traitement : p = 0.001). Voir Bocher et al. (soumis)[6] pour plus de précisions sur les analyses statistiques.*

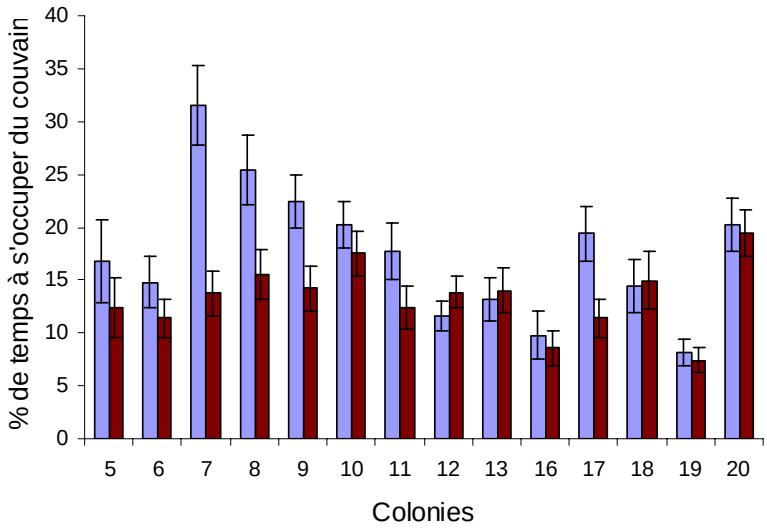
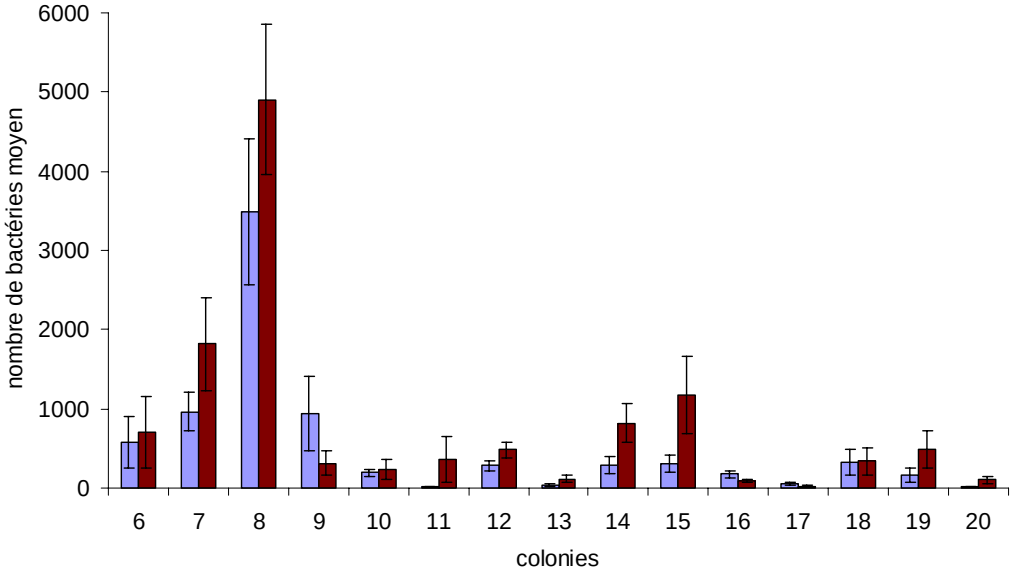


Figure 3 *: Nombre de bactéries présentes dans l'hémolymphe dans le groupe avec gamergate (bleu) et le groupe orphelin (rouge) 15 jours après la fission pour chacune des 15 colonies étudiées (effet du traitement : p = 0.008). Voir Bocher et al. (soumis)[6] pour plus de précisions sur les analyses statistiques.*



Cette diminution d'immunocompétence (augmentation du nombre de bactéries) peut s'observer chez tous les individus de la colonie, y compris ceux non impliqués directement dans les agressions (Bocher *et al.*, soumis[6]). Par ailleurs, la différence de défenses immunitaires entre le groupe avec gamergate et le groupe orphelin ne dépend pas de l'intensité du conflit dans le groupe orphelin. La diminution d'immunocompétence dans le groupe orphelin est donc plus probablement liée à une réaction au stress pouvant être induit par une situation conflictuelle que par un effet direct des agressions pendant le conflit. Un effet négatif du stress sur les défenses immunitaires a été montré chez une large gamme d'organismes allant des mammifères aux mollusques (Apanius, 1998 ; Lacoste *et al.*, 2002 ; Malham *et al.*, 2003). Bien qu'une condition de stress soit clairement moins bien définie chez les insectes que chez les vertébrés (Ottaviani & Franceschi, 1996), toute modification de l'organisation sociale pourrait induire un stress et être à l'origine d'une période de plus grande susceptibilité aux pathogènes par une diminution de l'immunocompétence. Ceci mériterait d'être plus largement testé car ce serait alors un paramètre important à prendre en compte pour comprendre l'évolution des stratégies de fission ou de remplacement de la reproductrice, qui sont associées à des modifications d'organisation sociale.

II.3 Evolution des systèmes de reproduction chez *C. cursor*

Il existe une très grande diversité de modalités de reproduction dans le règne animal. La première dichotomie oppose la reproduction sexuée à la reproduction asexuée (sans recombinaison). La prévalence de la reproduction sexuée au sein du règne animal peut être vue comme paradoxale. Pourquoi un individu décide t-il de transmettre seulement la moitié de ses gènes à sa descendance (le classique double coût du sexe (Maynard Smith, 1978) ? Pourquoi un individu qui a atteint l'âge de reproduction et qui est donc adapté à son environnement devrait-il mélanger ses gènes et par le biais de la recombinaison créer de nouvelles combinaisons de gènes dans sa descendance qui pourraient ne pas être adaptées à leur environnement ? Depuis plusieurs décennies, de nombreux travaux théoriques ont avancé diverses hypothèses sur les avantages potentiels de la reproduction sexuée (Bell, 1982 ; Michod & Levin, 1988 ; Otto & Lenormand, 2002). La majorité d'entre elles mettent en avant les avantages de la recombinaison. Mais en dépit de cet énorme investissement théorique, une explication universelle et satisfaisante n'a pas vraiment vu le jour.

Au sein de la reproduction sexuée, une large gamme de possibilité d'appariement existe. Pour des espèces hermaphrodites, l'autofécondation est une possibilité parfois sélectionnée (Jarne & Charlesworth, 1993). Pour des espèces gonochoriques (à sexe séparé), se pose la question du nombre et choix de partenaires sexuels (Arnqvist & Nilsson, 2000 ; Jennions & Petrie, 2000). L'anisogamie et l'investissement différentiel dans la descendance entre mâle et femelle confère une asymétrie entre les sexes (principe de Bateman ; Bateman, 1948). Le succès reproducteur du mâle n'est généralement pas limité par la production de sperme mais par le nombre d'accouplement qu'il peut obtenir et va ainsi dépendre du nombre de partenaires sexuels. En revanche, le succès reproducteur femelle ne dépend généralement pas du nombre de partenaires sexuels puisqu'un seul mâle confère suffisamment de sperme pour féconder tous les œufs de la femelle. Dans la mesure où l'accouplement multiple peut représenter des coûts pour la femelle, la monoandrie devrait être la règle (Thornhill & Alcock, 1983). Cependant, la polyandrie a évolué chez beaucoup d'espèces stimulant ainsi l'émergence de nombreuses hypothèses quant aux bénéfices que la femelle pourrait acquérir par la polyandrie (Arnqvist & Nilsson, 2000 ; Jennions & Petrie, 2000).

Chez les insectes sociaux, cette diversité des modes de reproduction peut également être observée. Même si la reproduction sexuée reste largement prépondérante, quelques rares espèces se reproduisent au moins en partie par parthénogenèse thélytoque (*C. cursor* Cagniant, 1979 ; *Platythyrea punctata* Schilder *et al.*, 1999 ; *Pristomyrmex pungens* (Tsuji, 1988) ; *Cerapachys biroi* (Tsuji & Yamauchi, 1995) ; *Wasmannia auropunctata* Fournier *et al.*, 2005). Il existe également une variation des taux de polyandrie, même si la plupart des espèces semblent effectivement être monoandres (Strassmann, 2001). Si les hypothèses classiquement émises pour l'évolution des modes de reproduction chez les organismes

solitaires peuvent généralement s'appliquer aux insectes sociaux, la vie coloniale génère des hypothèses spécifiques. En effet, les systèmes de reproduction agissent sur la répartition de la variabilité génétique au sein et entre les colonies et par là-même vont affecter l'intensité des deux niveaux de sélection (inter et intra colonial) ainsi que les conflits potentiels entre membres des sociétés (Bourke & Franks, 1995). La parthénogenèse augmente la variance génétique entre les colonies et la diminue à l'intérieur des colonies alors que la polyandrie a l'effet inverse. La force relative de la sélection au niveau colonial sera donc plus forte avec la parthénogenèse que sous un régime de polyandrie. A l'intérieur des colonies, des conflits génétiques peuvent être supprimés par la parthénogenèse si les individus sont génétiquement similaires (Hartmann *et al.*, 2003). La polyandrie peut également réduire certains conflits tels que ceux concernant l'allocation au sexe (Bourke & Franks, 1995). Cependant de nouveaux conflits peuvent également apparaître, même pour la reproduction par parthénogenèse lorsqu'elle offre une option reproductrice aux ouvrières.

L'objectif de cette partie de mes travaux de recherche vise à comprendre l'évolution des systèmes de reproduction chez la fourmi *C. cursor* car cette espèce combine à la fois une potentialité de reproduction par parthénogenèse et une reproduction sexuée avec polyandrie. En condition de laboratoire et en absence de la reine, les ouvrières possèdent la possibilité de produire de nouvelles ouvrières et gynes par parthénogenèse thélytoque (Cagniant, 1979 ; Lenoir & Cagniant, 1986). Par ailleurs, des observations comportementales suggèrent que la reine peut s'accoupler avec plusieurs mâles (Lenoir *et al.*, 1988). Nous avons dans un premier temps analysé le taux de polyandrie et nous verrons que cette étude de l'organisation socio-génétique a permis de révéler une modalité de reproduction par parthénogenèse conditionnelle chez la reine jusqu'alors insoupçonnée chez les insectes sociaux. Nous discuterons par la suite des coûts et bénéfices de la parthénogenèse et de la polyandrie chez cette espèce.

II.3.1 Originalité des systèmes de reproduction

En collaboration avec S. Aron et son étudiant en thèse M. Pearcy, de l'Université Libre de Bruxelle, nous avons développé des marqueurs microsatellites chez *C. cursor* (Pearcy *et al.*, 2004b). L'étude de l'organisation socio-génétique nous a permis de révéler un mode de reproduction étonnant (Pearcy *et al.*, 2004a[7]). Nous avons pu montrer que la parthénogenèse thélytoque était également utilisée par la reine pour produire les nouvelles gynes. En revanche, pour la production des ouvrières, la reine utilise la reproduction sexuée. Le nombre de mâles s'accouplant à une reine s'est révélé extrêmement élevé pour des fourmis, de l'ordre de 5 à 10 mâles (Tableau 3). La reine transmet donc tout son génome à sa descendance reproductrice (les gynes), mais maintient une diversité élevée au niveau de la colonie grâce à la reproduction sexuée utilisée pour la production des ouvrières et au fort niveau de polyandrie. Il est important de noter que les taux de polyandrie ont été généralement estimé sur du couvain et ne peuvent donc pas être affecté par un possible changement de la reine. Un tel changement de reine pourrait en effet passer inaperçu si les

deux reines sont clonales mais résulterait en une surestimation des taux de polyandrie. Le taux de production des gynes par parthénogenèse reste très élevé dans trois des quatre populations étudiées. A noter toutefois que pour la population d'Argelès 2006, le faible taux de gynes issues de parthénogenèse est dû à une colonie qui a produit une énorme quantité de gynes par reproduction sexuée (53 gynes issues de reproduction sexuée). Cette quantité de gynes produites est anormalement élevée pour une espèce fondant ses nouvelles colonies par fission (Pearcy & Aron, 2006).

Tableau 3 *: Taux de polyandrie et de production des gynes par parthénogenèse dans trois populations de C. cursor. Le nombre de mâles efficaces (M. eff.) avec le nombre de mâles minimum et maximum observés entre parenthèses sont donnés pour chaque population ainsi que le nombre de colonies étudiées (N. col.), le % de gynes produites par parthénogenèse (% G. part.) et le nombre de gynes étudiées (N).*

Population	Caractéristique	N. col.	M. eff.	% G. part. (N)	Référence
Leucate	Bord d'un étang	35	5,6 (4-8)	98% (56)	Pearcy *et al.*, 2004a[7]
Argelès 2004	Bord de mer	7	10,8 (7-12)	100 % (12)	Clémencet, 2006
Luc	Vignes en friche	7	9,7 (5-13)	63 % (11)	Clémencet, 2006
Argelès 2006	Bord de mer	8	6,3 (5-11)	24 % (86)*	Données non publiées

* A noter qu'une seule colonie possédait 53 gynes issues de reproduction sexuée. Sans considérer cette colonie étrange de par l'énorme quantité de gynes produites, 84 % des gynes étaient produites par parthénogenèse (28/33).

La parthénogenèse utilisée est une parthénogenèse automictique à fusion centrale qui induit à chaque génération une augmentation du taux d'homozygotie des individus en fonction du taux de recombinaison du locus considéré (Fig. 4 ; Pearcy *et al.*, 2006). Ce taux de recombinaison augmente avec la distance séparant ce locus du centromère. Pour un taux de recombinaison maximal (0,5), le passage de l'état hétérozygote à l'état homozygote (R) est de 0,33 (Pearcy *et al.*, 2006) et la perte d'hétérozygotes peut se faire très rapidement en quelques générations (Fig. 4).

La production des gynes sexuées peut être issue de la reine ou des ouvrières. Le couvain sexué de la reine peut se développer en gynes soit lorsque la reine décide de se reproduire par reproduction sexuée, soit car en l'absence de la reine les ouvrières peuvent modifier le déterminisme de la caste et transformer des larves, destinée à être des ouvrières, en reines. Ce dernier cas semble moins probable dans la mesure où il est rare de trouver des colonies sans reine. Les ouvrières peuvent elle-même produire par reproduction parthénogénétique des gynes. Si la parthénogenèse utilisée par les ouvrières est la même que celle de la reine (ce qui semble le plus parcimonieux), les gynes issues de la parthénogenèse des ouvrières devraient avec une certaine probabilité (dépendant du taux de recombinaison des locus) devenir homozygote pour l'allèle paternel et donc ne plus avoir

d'allèles en commun avec la reine. Il est intéressant de noter que pour la colonie ayant produit les 53 gynes par reproduction sexuée, il n'y a aucune indication que ces gynes pourraient être issues des ouvrières. Ceci devra être confirmé par des analyses plus précises et nous y reviendrons dans le projet.

Figure 4 : *Diminution de la fréquence des hétérozygotes dans une population à reproduction par parthénogenèse automictique à fusion centrale en fonction de R (taux de passage de l'état hétérozygote à l'état homozygote). La fréquence des hérérozygotes à la génération n (H_n) est calculée par l'équation suivante : $H_n=(1-R)^n H_0$. H_0 étant ici égal à 1.*

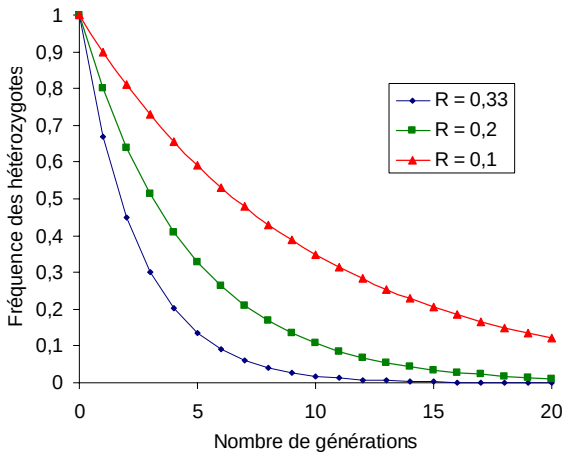


Tableau 4 *: Valeur de Fis chez les reines des colonies échantillonnées dans différentes populations. Le Fis est estimé selon Weir & Corkerham en utilisant le logiciel Genepop on the web Raymond & Rousset (1995). Les valeurs significativement différentes de 0 après une correction de Bonferroni (seuil de significativité de 0,0014) sont indiquées en gras. Le nombre de reines analysées par population est également donné (N reines). R, le taux de transition de l'état hétérozygote à l'état homozygote est donné pour chaque locus ainsi que le nombre de gynes ayant permis de l'estimer entre parenthèse.*

Population	N reines	CC-11	CC-26	CC-46	CC-63	CC-76	CC-89
Leucate 2	8	0,17	-0,29	0,11	0,22	0,18	-0,04
Canet 3	16	0,32	-0,1	0,06	0,08	-0,08	0,43
Canet 2	16	-0,05	0,12	-0,12	-0,02	0,41	0,32
Argelès 2004	11	-0,06	-0,17	0,19	0,04	0,37	0,32
Argelès 2006	16	-0,06	0,01	0,14	0,1	0,25	**0,63**
Luc	16	**-0,09**	-0,13	0,35	**0,4**	0,32	0,1
R		0 (27)	0(6)	0.19 (21)	0 (2)	0.33 (21)	0.42 (19)

Etant donné les forts taux de production des gynes par parthénogenèse, on s'attendrait à observer plusieurs reines clonales au sein d'un site. Or ceci n'a été observé que très rarement (deux reines clonales trouvées dans la population de Leucate étudiée par Pearcy *et al.* 2004a[7]). Similairement, de fort taux de parthénogenèse devraient conduire à une complète homozygotie des reines pour les locus ayant un taux de recombinaison non nul. Le taux d'hétérozygotie des reines est effectivement parfois significativement différent de 0 alors que ce n'est jamais le cas pour les ouvrières (Pearcy *et al.*, 2006 ; Clémencet *et al.,*

2005[4] ; Tableau 4). Cette diminution d'hétérozygotie va se retrouver plus facilement pour les locus les plus éloignés du centromère, présentant de plus forts taux de transition de l'état hétérozygote à l'état homozygote (Tableau 4) puisque la diminution d'hétérozygotie est plus rapide comme nous l'avons déjà noté (Fig. 4).

A partir du taux d'hétérozygotie des reines et de R, il est possible d'inférer le taux de reproduction sexuée en considérant une population à l'équilibre et un taux de reproduction sexuée fixe dans le temps (Pearcy *et al.*, 2006). En faisant l'hypothèse que les gynes sexuées seraient produites par les ouvrières, Pearcy *et al.* (2006) ont montré que plus de 60 % des reines à la tête d'une colonie devraient être issues de reproduction sexuée. En relaxant cette hypothèse et en considérant que les gynes issues de reproduction sexuée sont directement produites par la reine, nous pouvons suivre le même raisonnement que Pearcy *et al.* (2006) (Encadré 3). A partir des valeurs de *f* et *R* présentées dans le tableau 4 (en bornant *R* à 0.33 et *f* à 0), nous obtenons des taux de reproduction sexuée allant de 41 % à Luc à 68 % à Canet 2 alors que sur le terrain ce taux variait de 0 % à 76 % (Tableau 3). De par les taux élevés d'hétérozygotie des reines et le peu de reines clonales observées au sein d'un site, il semble que la proportion de reines issues de reproduction sexuée soit plus forte que ce que pouvait laisser supposer notre première analyse. Une estimation plus précise de la variation des modes de reproduction entre populations est indispensable pour comprendre comment a pu évoluer la reproduction par parthénogenèse.

Encadré 3 : estimation du taux de reproduction sexuée à partir du taux d'hétérozygotie des reines (modifié à partir de Pearcy *et al.* (2006))

A un locus donné, la probabilité d'identité par descendance des reines sera de *f*. Si l'on considère que les accouplements en reproduction sexuée se font au hasard (il y a panmixie), et que les parents ne sont pas apparentés, la consanguinité des individus issus de reproduction sexuée sera donc nulle. En revanche, la reproduction par parthénogenèse va entraîner une augmentation du coefficient de consanguinité. Sous parthénogenèse, la probabilité que deux allèles soient identiques par descendance chez une reine sera donc de 1 si la mère portait déjà des allèles identiques par descendance (avec une probabilité *f*), et de *R* si la mère ne portait pas d'allèles identiques par descendance (avec une probabilité 1-*f*). *R* correspond à la probabilité de passage de l'état hétérozygote à l'état homozygote sous la parthénogenèse automictique à fusion centrale. Si la reproduction sexuée se fait avec une probabilité *S*, le coefficient de consanguinité des reines à la génération n+1 (f^{n+1}) peut donc être exprimé en fonction du coefficient de consanguinité à la génération précédente (f^{n}) :

$$f_{n+1}=(1-S)\left[f_n+(1-f_n)R\right]$$

Sous l'hypothèse que *R* et *P* ne varient pas d'une génération à l'autre, à l'équilibre $f=f_n=f_{n+1}$ et on obtient :

$$P=\frac{R(1-f)}{f+(1-f)R}$$

II.3.2 Coûts et bénéfices de la parthénogenèse thélytoque

Quels pourraient être les avantages de la parthénogenèse chez les reines ? Le premier bénéfice réside dans l'évitement du double coût du sexe, sans avoir les inconvénients d'une réduction de variabilité à l'échelle de la colonie. Ce double coût du sexe repose sur l'hypothèse qu'une femelle se reproduisant par parthénogenèse ne produit que des filles et transmet donc tout son génome à ses filles. En compétition avec une femelle faisant de la reproduction sexuée qui produirait le même nombre de descendants, elle transmettrait donc deux fois plus de copies de ses gènes. Cependant, chez *C. cursor*, le bénéfice de la parthénogenèse n'est sans doute pas aussi élevé. Tout d'abord, les mâles continuent à être produits même dans les colonies où la reine utilise la reproduction par parthénogenèse pour produire les gynes (Pearcy & Aron, 2006). Par ailleurs, le succès reproducteur femelle va se mesurer par le nombre de propagules (colonies filles) produits plutôt que par le nombre de gynes produites. Dans des espèces à fondation indépendante de colonies, ces deux paramètres doivent être fortement corrélés. Cependant, chez les espèces où les colonies sont fondées par fission, le nombre de nouvelles propagules produites va plutôt dépendre de la taille de la colonie que du nombre de gynes produites. Celui-ci n'étant en général pas limitant.

Le deuxième bénéfice concerne la réduction de conflits potentiels sur la sélection des gynes. Dans la mesure où les colonies sont polyandres, le choix des gynes pourrait générer des conflits entre ouvrières de différentes lignées paternelles. La production des gynes par parthénogenèse pourrait éliminer ces conflits potentiellement coûteux pour la colonie. Ceci impliquerait que la polyandrie ait été sélectionnée avant la reproduction par parthénogenèse. Nous n'avons pour l'instant aucun argument pour ou contre cette hypothèse.

Qu'en est-il de la parthénogenèse chez les ouvrières ? Si la parthénogenèse a spécifiquement évolué chez les reines, celle des ouvrières pourrait alors être considérée comme un sous produit et ne pas être réellement utilisée en populations naturelles. Cependant, à partir du moment où la probabilité pour une ouvrière de se reproduire est non nulle, il est tout à fait concevable que la parthénogenèse ait été sélectionnée chez les ouvrières avec la possibilité d'évoluer vers une sorte de parasitisme comme cela a pu être observé chez les abeilles du cap (Neumann & Moritz, 2002). Estimer l'importance de la parthénogenèse des ouvrières en population naturelle est donc indispensable pour comprendre l'évolution de ce mode de reproduction chez *C. cursor*.

Une reproduction complètement issue de la parthénogenèse thélytoque de la reine souffre tout de même d'un inconvénient majeur lié au type de parthénogenèse utilisée qui est la diminution du taux d'hétérozygotie. La consanguinité de la reine peut entraîner des problèmes classiques de dépression de consanguinité que l'on trouve chez la plupart des organismes (Charlesworth & Charlesworth, 1987). La dépression de consanguinité est supposée être plus faible chez les espèces haplo-diploïdes de par la purge des allèles

délétères chez les mâles haploïdes (Werren, 1993). Cependant, seuls les allèles exprimés chez les deux sexes peuvent être contre-sélectionnés chez le mâle. Or la durée de vie d'un mâle est extrêmement faible et de nombreux gènes, notamment lié à la survie ou à la fécondité, ne pourront donc pas être purgés. Il semble donc tout à fait possible qu'il existe une dépression de consanguinité non négligeable chez les insectes haplo-diploïdes même si elle n'a que très rarement été estimée (Gerloff *et al.*, 2003 ; Henter, 2003 ; Liautard & Sundström, 2005). La consanguinité génère également des problèmes chez les espèces à détermination du sexe haplo-diploïde puisqu'elle entraîne une augmentation de la production de mâles diploïdes souvent stériles (Crozier & Pamilo, 1996).

II.3.3 Coûts et bénéfices de la polyandrie

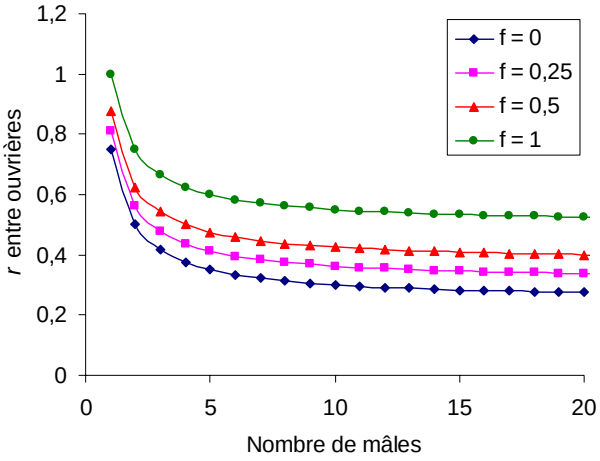
Les taux de polyandrie observés chez *C. cursor* sont tous très élevés, aucune colonie monoandre n'a à ce jour été trouvée. Il est difficile dans l'état actuel des connaissances de savoir si ces forts taux de polyandrie ont spécifiquement évolué chez *C. cursor* étant donné l'absence de connaissance sur les espèces proches. Cependant, dans la mesure où la polyandrie reste un caractère généralement flexible et sans doute coûteux (voir ci-dessous), même si la polyandrie en tant que telle n'a peut être pas évolué spécifiquement chez *C. cursor*, il est difficile de concevoir que de forts taux de polyandrie puissent se maintenir sans procurer d'avantages sélectifs.

L'estimation du coût de l'accouplement multiple reste difficile et il est d'ailleurs assez rare de trouver des estimations dans la littérature alors que de très nombreuses études s'attache à trouver un avantage à la polyandrie (Arnqvist & Nilsson, 2000 ; Jennions & Petrie, 2000). Il est intéressant de noter ici qu'une étude récente chez la fourmi champignonniste *Atta colombica,* a montré que les reines accouplées avec au moins trois mâles avaient des défenses immunitaires plus faibles que celles accouplées avec deux mâles (Baer *et al.*, 2006). Quels que soient les mécanismes sous jacents, ceci suggère un coût potentiel de l'accouplement multiple sur l'investissement pour la survie. L'accouplement multiple implique en effet non seulement une perte de temps et d'énergie mais également une augmentation des risques de prédation et d'infections parasitaires (Arnqvist & Nilsson, 2000). Chez *C. cursor*, les gynes s'accouplent au sol, hors du nid et la durée d'accouplement reste relativement brève (Lenoir *et al.*, 1988). Dans la mesure où des gynes déjà accouplées, ont été vues ressortir du nid et s'accoupler à nouveau, il est clair que l'hypothèse parfois émise d'accouplement par convenance, n'est pas très pertinente chez *C. cursor*.

Par ailleurs, dans les espèces eusociales, la polyandrie a également un coût plus indirect car elle 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, Fig. 5). Il est intéressant de noter ici que pour des reines complètement homozygotes (ce qui pourrait être le cas chez *C. cursor* après plusieurs générations de parthénogenèse), la valeur minimale de *r* n'est plus de 0,25 mais de 0,5 (Fig. 5). Notons également que l'effet

du taux de polyandrie sur *r* est plus faible lorsque le taux de polyandrie est élevé. Typiquement, pour les gammes de polyandrie trouvées chez *C. cursor* (entre 5 et 13 mâles), on attend peu de variations de *r* en fonction du taux de polyandrie, sauf bien sur si les reines diffèrent également par leur taux d'homozygotie, ce qui semble être le cas.

Figure 5 : Variation théorique de *r* (la corrélation génétique) entre ouvrières en fonction du taux de polyandrie et du degré de consanguinité (*f*) des reines.



Etant donné les coûts potentiels de la polyandrie, de nombreuses hypothèses ont été émises afin d'expliquer son évolution et son maintien chez les hyménoptères eusociaux (Boomsma & Ratnieks, 1996). Ces différentes hypothèses sont plus ou moins pertinentes chez *C. cursor* (voir Encadré 4). Dans la mesure où une majorité des gynes sont produites par parthénogenèse, il est probable que le maintien d'une diversité génétique au niveau de la colonie soit un facteur clé pour comprendre les avantages de la polyandrie. Par ailleurs, des hypothèses spécifiques liées à la parthénogenèse peuvent également être envisagées (Encadré 4) même si elles supposent que la parthénogenèse ait évolué en premier.

Notre première approche a été de s'intéresser à la taille des ouvrières et à sa variation. La taille chez les ouvrières est un trait d'histoire de vie crucial qui va affecter les performances individuelles en terme d'efficacité de recherche de nourriture ou de défense du nid, de résistance à la dessiccation ou à la privation de nourriture, et de survie en général (Calabi & Porter, 1989 ; Hölldobler & Wilson, 1990). Chez *C. cursor*, nous avons pu montrer que les ouvrières de grandes tailles avaient une meilleure résistance à la dessiccation (Clémencet, 2006). La production d'ouvrières de grande taille pourrait représenter un avantage chez cette espèce thermophile car cela permettrait aux ouvrières de rechercher la nourriture à des heures très chaudes de la journée pendant lesquelles la compétition inter-spécifique est extrêmement réduite (Cerdá *et al.*, 1989 ; Cerdá & Retana, 1997). Nos expériences de terrain n'ont cependant pas démontré que cet avantage était réellement utilisé (Clémencet, 2006). Par ailleurs, les grandes ouvrières résisteraient mieux aux faibles températures pendant l'hivernage (Cagniant, 1983).

Encadré 4 : Hypothèses pour l'évolution de la polyandrie

(D'après Boomsma & Ratnieks, 1996 ; Palmer & Oldroyd, 2000 ; Strassmann, 2001 ; Crozier & Fjerdingstad, 2001 ; Simmons, 2005)

Hypothèses générales

Accouplements forcés – Le coût de l'évitement de l'accouplement serait plus fort que d'accepter de s'accoupler. Dans la mesure où les gynes de *C. cursor* sortent volontairement du nid pour s'accoupler plusieurs fois, cette hypothèse est peu probable.

Bénéfice direct – En même temps que le sperme, le mâle peut transférer des produits annexes stimulant la fertilité. Ce genre de bénéfice n'a pas été démontré chez les insectes sociaux. Au contraire les substances transmises avec le sperme auraient plutôt un effet délétère (Baer *et al.*, 2006).

Bénéfice indirect (génétique) (Simmons, 2005)

- La polyandrie favorise la compétition spermatique ou le choix post-copulatoire des femelles pour les spermes porteurs des 'bons gènes'. A noter que cet avantage pour des espèces haplo-diploïdes est deux fois moins élevé puisque les gènes des mâles ne se retrouvent que chez leurs petits fils car ils n'ont pas de fils.
- La polyandrie permet également de diminuer les risques d'incompatibilité génétique (que ce soit en terme d'évitement de la consanguinité ou d'obtention de meilleures combinaisons génétiques). Cette hypothèse de diminution des risques d'incompatibilité génétique en terme de consanguinité pourrait être particulièrement pertinente chez *C. cursor* puisque les reines peuvent avoir une consanguinité assez élevée.
- La polyandrie permet la production de descendants génétiquement variables (nous reverrons cette hypothèse pour la production d'ouvrières dans les hypothèses spécifiques aux insectes sociaux). En restant sur l'idée émise pour des organismes solitaires, cette hypothèse suggère que la production de gynes génétiquement diverses permet d'augmenter les chances qu'elles rencontrent un environnement auquel elles seraient adaptées. Pour des espèces à fondation dépendante des colonies, la dispersion femelle limitée rend le risque de trouver un environnement différent de celui de la mère assez faible. Par ailleurs, la sélection individuelle sur les reines au moment de la fondation des colonies est moins forte puisque la reine est entourée d'ouvrières. Enfin, dans la mesure où la majorité de gynes sont produites par parthénogenèse, cette hypothèse n'est pas très pertinente pour *C. cursor*.

Hypothèses spécifiques aux insectes sociaux

Limitation spermatique – La quantité de sperme fournie par un mâle n'est pas suffisante pour féconder tous les ovocytes femelles. Chez *C. cursor*, les tailles de colonies étant relativement petites, il est peu probable que la quantité de sperme soit un facteur limitant. Des comptages de sperme réalisés par M. Pearcy vont également à l'encontre de cette hypothèse (Pearcy, 2005).

Coûts des mâles haploïdes – De part le déterminisme haplo-diploïde du sexe, les femelles doivent s'accoupler avec des mâles présentant des allèles différents au locus 'sexuel' sous peine de produire des mâles diploïdes généralement stériles et donc coûteux pour la colonie. Cette

hypothèse peut être considérée comme un cas particulier de l'hypothèse d'évitement d'incompatibilité génétique. Chez *C. cursor*, aucun mâle diploïde n'a à ce jour été trouvé.

Diminution des conflits reines-ouvrières – L'asymétrie d'apparentement liée à l'haplo-diploïdie génère des conflits potentiels entre reines et ouvrières qui peuvent diminuer avec la polyandrie. En général, l'accouplement avec quelques mâles est suffisant pour réduire ces conflits. Il est donc peu probable que cette hypothèse joue un rôle important dans le maintien d'un taux de polyandrie élevé. Par ailleurs, les conflits reines-ouvrières sur le sexe-ratio sont probablement moins importants chez les espèces à fondation dépendante des colonies car le nombre de gynes produites est généralement limité. En effet, le taux de fondation de nouvelles colonies ne dépend probablement pas du nombre de gynes produites contrairement aux espèces à fondation indépendante.

Variabilité génétique à l'intérieur de la colonie – La polyandrie augmente la diversité génétique des ouvrières à l 'intérieur d'une colonie et réduit la variance génétique entre colonies (et donc la force de sélection à l'échelle des colonies). Cette diversité génétique intra-coloniale peut générer une meilleure performance des ouvrières en terme de division du travail, une meilleure résistance de la colonie face à l'invasion par des pathogènes et une meilleure homéostasie de la colonie en particulier face à un environnement variable. A nouveau, il est clair d'après la Fig. 5 que 90 % de diversité génétique est obtenue lorsqu'une reine s'accouple avec 6 mâles. Expliquer l'évolution et le maintien de forts taux de polyandrie n'est pas toujours facile avec ces hypothèses reposant uniquement sur le bénéfice de la diversité génétique.

Il est intéressant dans ce contexte de noter que le bénéfice de la polyandrie peut résider dans une sélection à fréquence-dépendante (Fuchs & Moritz, 1998). La production d'ouvrières présentant des génotypes spécialisés dans certaines tâches pourrait être avantageuse uniquement si cette production se fait en petite quantité. Un fort taux de polyandrie permettrait alors d'avoir des ouvrières spécialisées à faible fréquence dans la colonie. Cette hypothèse reste, à ma connaissance, la seule qui puisse expliquer l'évolution vers de forts taux de polyandrie.

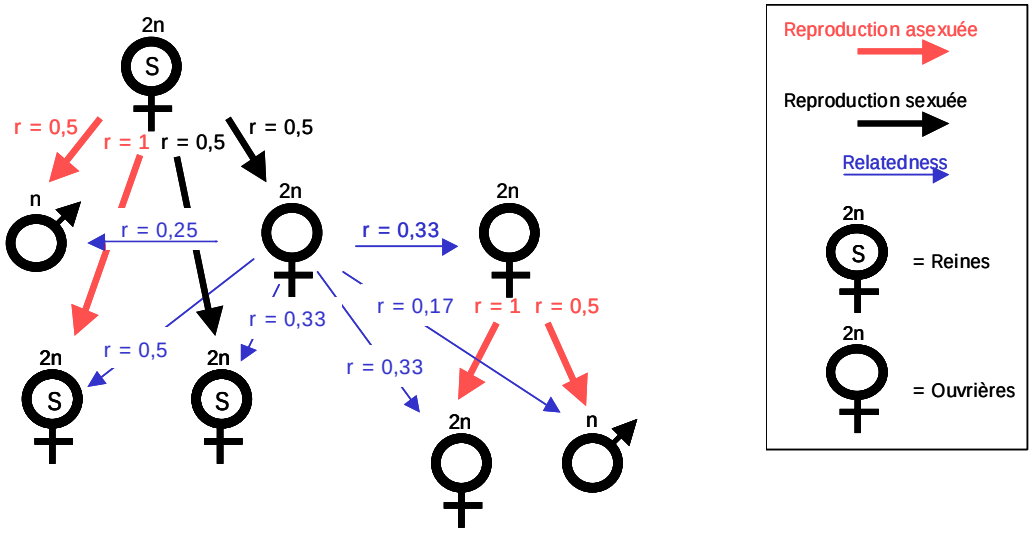
Hypothèses liées à la parthénogenèse thélytoque de *C. cursor* (Clémencet, 2006)

Si la possibilité de reproduction par parthénogenèse thélytoque est apparue avant la polyandrie, des conflits reines-ouvrières ont pu se mettre en place quant à la production de mâles (hypothèse classique) mais aussi de femelles. En effet, les ouvrières ont tout intérêt à remplacer la reine par une de leur propre fille (r = 1) plutôt que par leur sœur ou leur nièce (r = 0,75). En situation de monoandrie, les ouvrières préfèrent leur nièce (r = 0,75) à une gyne produite par parthénogenèse de la reine (r = 0,5). Cette corrélation génétique de 0,5 ne varie pas en situation de polyandrie alors que celui d'une ouvrière aux gynes produites par les ouvrières va diminuer, il passe à 0,5 pour deux mâles et à 0,25 pour trois mâles (Fig. 6). Ainsi la reine pourrait s'accoupler avec plus de deux mâles afin d'éviter un conflit quand à la production des gynes. Ce raisonnement est identique à celui proposé pour la réduction du conflit lié à la production de mâles par les ouvrières.

En suivant le même raisonnement, la reine pourrait également s'accoupler avec plusieurs mâles afin que les ouvrières préfèrent élever les gynes produites par reproduction asexuée plutôt que par reproduction sexuée. En effet, à partir du moment où la corrélation génétique entre ouvrières est supérieur à 0,5 (polyandrie >2 pour f = 0, ou le plus grand nombre de mâles possible pour f

proche de 1, voir Fig. 5), les ouvrières devraient préférer élever des gynes issues de reproduction sexuée (même apparentement) que de reproduction asexuée (0,5).

Figure 6 : schéma des corrélations génétiques attendues entre membres d'une colonie monogyne et polyandre lorsque les gynes sont produites de façon sexuée ou asexuée, d'après Clémencet (2006). 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 de corrélation génétique 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 de corrélation génétique d'une ouvrière à un neveu (r = 0,17).

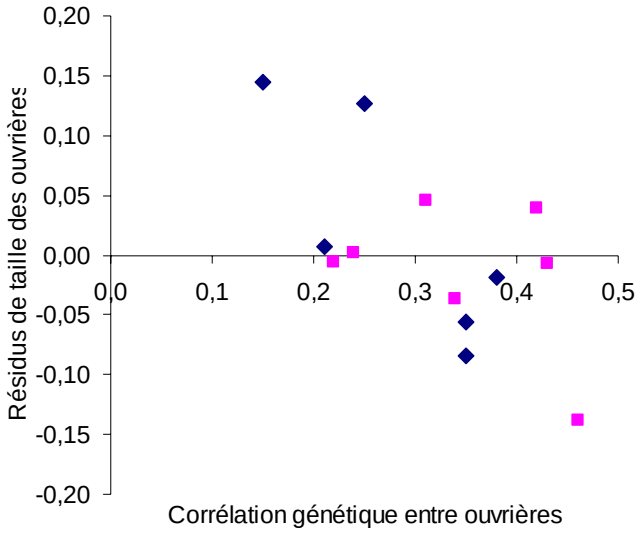


De nombreux travaux ont pu montrer qu'il peut être avantageux de produire des ouvrières de différentes tailles, voire morphologiquement différentes car cela augmente la spécialisation des tâches et procure donc une meilleure efficacité du travail à l'échelle de la colonie (Fjerdingstad & Crozier, 2006). Une hypothèse classique d'avantage à la polyandrie se base sur l'obtention d'une meilleure division du travail par la production d'une plus large gamme d'ouvrières spécialisées dans différentes tâches (Brown & Schmid-Hempel, 2003 ; voir Encadré 4). Chez *C. cursor*, nous avons spécifiquement testé si une plus grande diversité génétique permettait l'obtention d'une plus grande variabilité de taille des ouvrières.

La taille de la colonie est apparue comme un paramètre important à contrôler car elle affecte la taille moyenne des ouvrières (Clémencet & Doums, 2007[8]). En effet, des tailles plus grandes des ouvrières ne semblent possibles que dans les colonies assez grandes pour pouvoir assurer leur production plus coûteuse (Clémencet & Doums, 2007[8]). Malgré les forts taux de polyandrie chez *C. cursor*, nous avons pu mettre en évidence une corrélation

négative entre la taille moyenne des ouvrières dans la colonie et la corrélation génétique entre ouvrières, une fois la taille de colonie prise en compte (Fig. 7). Ainsi, à nombre d'ouvrières égal, les colonies à plus forte diversité génétique produiraient des ouvrières plus grandes. En revanche, nous n'avons pas observé de corrélation entre diversité génétique et variance de taille. Par ailleurs, nous n'avons pas non plus trouvé d'effet significatif des lignées paternelles sur la taille des ouvrières (Clémencet, 2006, données non publiées récemment obtenues par Hélène Magalon en stage post-doctoral de 6 mois). Ceci suggère que les colonies plus grandes produisent en moyenne des ouvrières plus grandes mais ne possèdent pas un polymorphisme plus important (Clémencet, 2006).

Figure 7 *: Relation entre la corrélation génétique entre ouvrières d'une même colonie et les valeurs résiduelles de la taille moyenne des ouvrières après avoir contrôlé pour l'effet de la taille des colonies. Chaque point représente une colonie, les colonies échantillonnées dans la population de Luc sont en bleu alors que celles en rose viennent de Argelès 2004.*



Nos résultats suggèrent que les colonies à plus forte diversité génétique pourraient obtenir plus de ressources. Ceci reste à confirmer sur le terrain. Par ailleurs, les mécanismes conférant une plus grande productivité à ces colonies à forte diversité génétique restent à analyser. Si l'hypothèse liée à la division du travail n'est pas soutenue dans le cadre de la diversité de taille des ouvrières, elle reste tout de même possible pour d'autres aspects non étudiés que l'on pourra voir dans le projet. La deuxième hypothèse classiquement émise et testée est liée à la résistance aux pathogènes (Encadré 4, Sherman *et al.*, 1988 ; Baer & Schmid-Hempel, 1999). Nous verrons dans le projet que ces deux hypothèses peuvent ne pas être complètement opposables comme elles le sont souvent dans la littérature.

Nos travaux mettent également en avant les questions liées aux stratégies d'allocation des ressources en terme de taille et nombre d'ouvrières. Un parallèle pourrait ainsi être fait avec les travaux menés chez les arbres quant au compromis entre taille et nombre de feuilles (Kleiman & Aarssen, 2007). De façon assez surprenante, l'évolution de la taille des ouvrières chez les insectes sociaux n'a reçu que peu d'attention excepté dans le

contexte de l'évolution du polymorphisme des ouvrières en relation avec la division du travail (Bourke & Franks, 1995 ; Powell & Franks, 2006 ; Fjerdingstad & Crozier, 2006). Elle ouvre pourtant des perspectives intéressantes dans la mesure où ce trait individuel des ouvrières peut non seulement être sélectionné au niveau de la colonie mais également au niveau individuel. En effet, les ouvrières de grandes tailles apparaissent plus fécondes (Clémencet, 2006) et possèdent plus d'ovarioles (Cagniant, 1983). Dans la mesure où les ouvrières peuvent non seulement produire des mâles mais potentiellement remplacer leur reine par parthénogenèse thélytoque, la sélection au niveau individuel pourrait être relativement efficace si cette opportunité était fréquente dans la nature. Se pose donc à nouveau la question de l'importance de la parthénogenèse des ouvrières en populations naturelles.

II.4 Conclusion et perspectives

En conclusion, nos travaux ont mis en évidence une dispersion très limitée chez les espèces étudiées leur conférant ainsi de fortes potentialités d'adaptation locale (Doums *et al.*, 2002a[1] ; Viginier *et al.*, 2004[3] ; Clémencet *et al.*, 2005[4]). Chez *C. cursor*, les habitats peuvent fortement varier selon les populations et pourraient donc sélectionner différentes stratégies reproductives. Nous avons effectivement montré des différences de taille de colonies et de taille d'ouvrières entre populations des différents types d'habitat (Clémencet & Doums, 2007[8]) qui pourraient résulter de différentes stratégies de fission entre populations. Similairement, la forte différentiation génétique entre populations chez les espèces du genre *Diacamma* a pu permettre l'évolution de différentes stratégies quant à la régulation de la reproduction (Baudry *et al.*, 2003[2]).

La reproduction des colonies par fission génère un changement dans le temps de la reproductrice que nous avons pu estimer chez les fourmis du genre *Diacamma* qui sont monoandres (André *et al.*, 2001[5] ; André *et al.*, 2006). Le changement de la reproductrice peut avoir des conséquences sur les stratégies de fission d'une part car les conflits qu'il induit pourraient favoriser la fission et d'autre part car ces conflits peuvent s'avérer coûteux aussi bien en terme d'efficacité de travail des ouvrières que sur leurs défenses immunitaires (Bocher *et al.*, 2007[6]). Afin de mieux comprendre les stratégies de fission, il est indispensable d'identifier les facteurs proximaux et ultimaux déterminant la fission ainsi que les conflits sociaux mis en jeux, en particulier en relation avec le changement de reproductrice.

La découverte d'un modèle unique d'utilisation conditionnelle de la reproduction sexuée pour la production d'ouvrières et asexuée pour la production des nouvelles reines chez les reines de *C. cursor* (Pearcy *et al.*, 2004a[7]) rend l'étude des systèmes de reproduction chez cette espèce particulièrement fascinante. Dans toutes les colonies étudiées, les reines étaient systématiquement accouplées avec un grand nombre de mâles, mais la variation du taux d'hétérozygotie des reines permet tout de même d'observer une variation importante de la corrélation génétique des ouvrières au sein des colonies (Pearcy *et al.*, 2004a[7] ; Clémencet, 2006). Nous avons d'ailleurs pu montrer qu'à taille de colonie égale, les colonies plus diversifiées génétiquement produisaient des ouvrières de plus grandes tailles (Clémencet, 2006). Ceci pourrait être un avantage pour cette espèce thermophile car les ouvrières de grandes tailles ont une meilleure résistance à la dessiccation mais présentent également une plus forte capacité reproductrice (Clémencet, 2006). Plusieurs aspects restent à explorer afin d'obtenir une vision plus globale de l'évolution des modes de reproduction chez *C. cursor* et d'apporter potentiellement des réponses aux questions fondamentales de l'évolution du sexe et de la polyandrie qui se pose en Biologie Evolutive. De plus larges études en populations naturelles sur le rôle de la parthénogenèse des ouvrières et la variation des taux de parthénogenèse et de polyandrie

sont nécessaires. Le rôle des mâles et de la compétition spermatique reste un domaine à explorer car ils pourraient affecter l'évolution des modes de reproduction.

Enfin, je souhaiterais poursuivre avec une approche d'écologie immunologique chez les insectes sociaux. Après une longue période d'investigations essentiellement fonctionnelles, les défenses immunitaires sont aujourd'hui de plus en plus étudiées pour leur intérêt en biologie évolutive (Schmid-Hempel & Ebert, 2003 ; Schmid-Hempel, 2005), mais la compréhension de l'évolution des défenses immunitaires dans le cadre de la socialité reste largement insuffisante. Par ailleurs, l'intégration des défenses immunitaires comme un paramètre de trait d'histoire de vie pourrait permettre une meilleure compréhension du fonctionnement des sociétés.

II.4.1 Stratégies de reproduction par fission

Cet aspect de ma recherche est mené en collaboration avec Thibaud Monnin et une étudiante en thèse que nous co-encadrons, Blandine Chéron, actuellement en première année de thèse. Deux points principaux seront considérés à savoir les stratégies de fission ainsi que les conflits associés à la fission et aux changements de reproductrices. En ce qui concerne les stratégies de fission, les questions principales portent sur le moment optimal de la fission et sur la stratégie choisie par une colonie qui fissionne face au compromis entre le nombre et la taille des propagules (colonies filles). Afin de comprendre comment le compromis entre taille et nombre de propagules est résolu, il est également nécessaire de connaître qui de la reine et des ouvrières déclenche la fission, qui de l'ancienne ou de la nouvelle reine hérite du nid, et comment les ouvrières et le couvain se répartissent (équitablement ou non, selon le degré de corrélation génétique ou aléatoirement). Nous souhaitons identifier les facteurs ultimes qui vont affecter les stratégies optimales de fission et les facteurs proximaux qui vont déclencher la fission et déterminer comment cette fission va se faire. Notons que ces facteurs ultimes et proximaux peuvent être les mêmes en agissant à la fois en tant que pression évolutive et en tant que facteur déclenchant. Des facteurs intrinsèques comme la taille de la colonie et des facteurs écologiques comme la densité de colonies aux alentours, la disponibilité en sites de nidification et en ressources alimentaires sont probablement cruciaux. Un suivi de dynamique des populations de colonies sur le terrain associé à une étude écologique permettra d'évaluer le rôle de ces facteurs. Une approche théorique a également été récemment entreprise par Mathias Gaudruchon (en stage post-doctoral de 6 mois financé par l'ANR).

Les conflits sociaux entre reines et ouvrières, classiquement étudiés chez des espèces à ICF (Bourke & Franks, 1995), ne se posent pas dans les mêmes termes pour des espèces se reproduisant par fission. Par ailleurs, des conflits particuliers peuvent se mettre en place car ces espèces à reproduction des colonies par fission possèdent la possibilité de remplacer leur reproductrice. Généralement le nombre de gynes produites est supérieur au nombre de colonies filles qui vont être crées, ce qui peut entraîner un conflit sur le choix des reines. Plusieurs questions se posent donc. Comment le choix de la reine se fait-il ? par les

ouvrières ou par une compétition directe entre reines ? Y a t-il compétition entre reines ou un choix des ouvrières selon des critères phénotypiques tels la taille, l'activité, l'âge, les signaux de fertilité et la résistance immunitaire? Choisir la reine la plus à même d'assurer la meilleure fécondité est crucial pour les ouvrières. Chez *C. cursor*, la polyandrie pourrait générer des conflits entre ouvrières sur le choix des reines. Les ouvrières de chaque lignée paternelle sont plus apparentées aux reines de la même lignée (sœur, r = 0,75) qu'à celles des autres lignées (demi-sœur, r = 0,375), de sorte qu'il pourrait y avoir un certain degré de népotisme, les ouvrières favorisant les reines de la même lignée paternelle lorsqu'elles sont issues de reproduction sexués. Le deuxième intérêt de *C. cursor* est la production de reines par parthénogenèse des ouvrières dans les colonies orphelines. Les premières données suggèrent que les reines seraient produites par des ouvrières issues de la lignée paternelle la plus représentée (Clémencet, 2006). Cela mérite d'être confirmé par l'étude d'autres colonies.

Finalement, la reine et les ouvrières pourraient avoir des intérêts divergents concernant le moment optimal de faire une fission, les ouvrières préférant fissionner plus tôt que la reine. En effet, la diminution de corrélation génétique engendrée par la fission est différente pour la reine et pour les ouvrières. La reine étant remplacée par une de ses filles dans la nouvelle colonie, elle échange la production de filles et fils (r = 0,5) pour des petites filles et petit-fils (r = 0,25), soit une diminution de moitié. Pour les ouvrières cette diminution varie selon que la nouvelle reine est une sœur, issue de la même lignée paternelle, ou une demi-sœur. L'ouvrière échange la production de sœurs, demi-sœurs et frères (0,75 ; 0,375 et 0,25) pour celle de nièces et neveux dans le premier cas (0,375), ou de «demi-nièces» et «demi-neveux» dans le second cas (0,1875). Nous développerons ce raisonnement et analyserons les conséquences sur la fission. Chez *C. cursor* la reproduction par parthénogenèse modifie ces degrés de parenté, de sorte que les intérêts des reines et des ouvrières devraient différer dans les populations produisant les nouvelles reines par parthénogenèse ou par reproduction sexuée classique.

II.4.2 Evolution des systèmes de reproduction chez *C. cursor*

Comme nous l'avons vu précédemment à plusieurs reprises, une question primordiale est l'importance de la parthénogenèse en populations naturelles. Quatre questions peuvent plus spécifiquement être abordées. (i) Quelle est la variation du taux de gynes produites par reproduction sexués ? (ii) Quels sont les facteurs écologiques qui peuvent expliquer les variations de taux de reproduction sexuée? (iii) Qui de la reine ou des ouvrières produisent ces gynes ? et (iv) Y a-t-il des différences de valeurs sélectives entre les gynes issues de reproduction sexuées et de parthénogenèse?

Dans la mesure où très peu de colonies produisent des gynes, la mesure indirecte du taux de reproduction parthénogénétique en populations naturelles par les taux d'hétérozygotie des reines (comme présentée dans l'Encadré 4 ou par Pearcy *et al.*, 2006) a l'avantage de permettre l'étude des modes de reproduction même dans des populations où il

est difficile de creuser les nids. Une approche corrélative permettrait dans un premier temps d'analyser les facteurs tels que la densité des populations, la richesse spécifique, la stabilité environnementale, qui pourrait affecter le taux de parthénogenèse suivant les hypothèses classiques émises pour l'évolution du sexe (Bell, 1982). Il serait également pertinent de regarder la prévalence de pathogènes. Malheureusement, nous n'avons toujours pas identifié d'espèces clairement pathogènes chez *C. cursor* même si des acariens sont parfois observés dans certaines colonies en populations naturelles. La présence de champignons a également été mise en évidence par une approche moléculaire (travail récemment effectué par Hélène Magalon). Une détermination plus précise de ces champignons ainsi que de leur prévalence est en cours.

Une deuxième étape est de savoir qui des reines ou des ouvrières produisent les gynes issues de reproduction sexuée. La réponse à cette question est indispensable pour l'élaboration d'un scénario évolutif de la parthénogenèse. Si les ouvrières utilisent la parthénogenèse automictique à fusion centrale comme la reine, il est alors possible, en utilisant des locus à forts taux de transition de l'état hétérozygote à l'état homozygote (fort *R*), d'identifier les gynes sexuées issues de la reproduction par parthénogenèse des ouvrières. La probabilité de non détection de gynes issues d'ouvrières est de (1-R*)* +0,5 *R* par locus. Nous n'avons pour l'instant que trois locus disponibles à fort *R*. Etant donnée les valeurs de R, la probabilité de non détection serait de $(0,835)^3 = 0,58$. Un plus grand nombre de locus est donc indispensable pour mener cette étude, les séquences obtenues lors de notre clonage des marqueurs microsatellites devraient pouvoir nous permettre de les obtenir. Cette étape est également indispensable pour une estimation plus fiable du taux de reproduction par parthénogenèse des reines à partir de leur taux d'hétérozygotie.

Le coût principal de la reproduction par parthénogenèse semble être lié à la consanguinité qu'elle entraîne. Il est cependant irréaliste d'imaginer obtenir des mesures directes de valeur sélective des gynes en population naturelle. Nous pourrions toutefois obtenir des mesures indirectes telles que le niveau de défenses immunitaires, le poids et la taille des gynes, la quantité de réserve énergétique, le niveau d'asymétrie fluctuante et le nombre d'ovarioles par ovaire. Ces paramètres devraient être plus ou moins corrélés à la fécondité ou à la survie des gynes. Des estimations plus directes du succès des gynes issues de reproduction sexuée ou parthénogénétique pourraient être obtenues en condition de laboratoire en comparant le taux de croissance et la productivité en sexués de colonies produites par des gynes issues de reproduction sexuée ou de parthénogenèse. Ces expériences nécessitent d'avoir des gynes accouplées. Ceci est possible dans la mesure où les gynes une fois accouplées peuvent rester quelques jours dans la colonie mère et donc être récupérées directement dans les populations naturelles (la majorité des gynes récoltées à Argelès en 2006 avaient une spermathèque pleine).

Le deuxième point que nous souhaitons continuer de développer est l'étude de l'évolution de la polyandrie. Dans la mesure où les taux de polyandrie de *C. cursor* se sont révélés extrêmement élevés dans les différentes populations étudiées, il semble que cela corresponde à une stratégie relativement stable au moins dans la zone géographique

étudiée. Cependant, il existe une variation non négligeable entre colonies de la corrélation génétique moyenne des ouvrières. Nous avons pu montrer que les colonies génétiquement plus diverses produisaient des ouvrières plus grosses. Deux questions sont encore sans réponses à savoir : (i) la diversité génétique procure t-elle vraiment un avantage à la colonie comme nos premiers résultats semblent l'indiquer ? et (ii) si c'est le cas, il serait pertinent de chercher quels sont les mécanismes qui procurent cet avantage. Des approches corrélatives en populations naturelles permettraient de vérifier l'hypothèse selon laquelle les colonies plus diverses sont capables d'obtenir plus de ressources. Il est difficile de modifier artificiellement la diversité génétique intra-coloniale, à moins de mettre au point des techniques d'insémination artificielles ou des manipulations relativement lourdes impliquant une détermination génétique des ouvrières avant la constitution de colonies artificielles comme cela a été fait chez les fourmis champignonnistes (Hughes & Boomsma, 2004). Nous souhaitons également rechercher le ou les mécanismes par lesquels la diversité génétique pourrait augmenter l'efficacité des colonies, en particulier en relation avec la division du travail ou la résistance aux pathogènes. Dans un premier temps, je pense qu'il est nécessaire de tester les conditions permettant à ces hypothèses d'être fonctionnelles, à savoir l'existence d'héritabilité des traits pouvant apporter un bénéfice lorsqu'ils sont exprimés avec une forte diversité dans la colonie. Ceci implique d'analyser l'architecture génétique des traits mis en avant, que ce soit en terme de résistance aux pathogènes, de défenses immunitaires ou de division du travail. Dans un deuxième temps, une diminution expérimentale de la variabilité de ces traits qui paraissent pertinents devra être menée afin de vérifier qu'une diminution de leur diversité intra-coloniale engendre bien une diminution de productivité de la colonie.

La polyandrie chez *C. cursor*, ainsi que la reproduction par parthénogenèse de la reine nous amène à nous interroger sur le rôle des mâles. En effet, les mâles ont intérêt à ce que des gynes soient produites par reproduction sexuée. Par ailleurs, dans la mesure où les gynes sont généralement produites en quantité moindre que le nombre de lignées paternelles, cela implique que tous les mâles ne transmettront pas leurs gènes. Une forte compétition spermatique peut donc être envisagée dans un tel système. Le fait que les mâles des hyménoptères sociaux soient issus de parthénogenèse arrhénotoque et donc haploïdes supprime la compétition spermatique au sein du sperme d'un seul mâle puisque tous les spermatozoïdes sont génétiquement identiques. Deuxièmement, la spermatogenèse se termine généralement au stade pupal. Les mâles ne peuvent donc pas produire de sperme au cours de leur vie adulte. Ceci implique que les mâles doivent ajuster la quantité de sperme transmise à une femelle en fonction du nombre attendu d'accouplements possibles (Boomsma *et al.*, 2005). Les questions qui peuvent se poser sont donc des questions souvent étudiées chez les animaux (Stockley, 1997 ; Birkhead & Møller, 1998), mais rarement chez les insectes sociaux. Y a-t-il un biais de parenté des différentes lignées paternelles ? Les premiers résultats semblent indiquer que c'est le cas (Clémencet, 2006 ; données non publiées obtenues par Hélène Magalon). Ce biais peut-il s'expliquer par des survies différentielles des ouvrières ? Si non, on peut alors rechercher des signes de compétition spermatique ou d'incompatibilité génétique en se posant les questions suivantes. Est-ce que ce sont les mâles les moins apparentés à la reine qui sont les plus

représentés ? Est ce que les caractères phénotypiques des mâles (taille, poids …) affectent leur taux de paternité ? Si oui, est ce que la quantité de sperme, l'ordre d'accouplement, la taille des spermatozoides affecte la compétition spermatique ? 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 labo qu'avec des mâles capturés pendant la saison de reproduction sur le terrain (A. Lenoir, Pers. Comm.). Il est important de noter qu'il existe de nombreux problèmes pour mettre en évidence la compétition spermatique. Un choix cryptique du sperme par les femelles et des effets de dépression de consanguinité peuvent être des facteurs confondants qu'il faudra donc considérer (Olsson *et al.*, 1999).

II.4.3 Défenses immunitaires et socialité

Les parasites sont ubiquistes et peuvent représenter un facteur de sélection important de par la forte promiscuité et souvent le fort degré de corrélation génétique des individus au sein des colonies (Schmid-Hempel, 1998). Chez les fourmis, l'évolution de glandes spécifiques produisant des substances antibiotiques et antifongiques (les glandes métapleurales), démontre clairement l'importance des défenses contre les pathogènes. D'une façon plus générale, les défenses immunitaires trouvées chez une grande majorité de métazoaires, représentent la dernière ligne de défense contre les pathogènes. La prise en compte des défenses immunitaires pour diverses questions d'écologie évolutive s'est largement développée (Schmid-Hempel, 2005). En collaboration avec Claire Tirard du laboratoire de Parasitologie Evolutive et Aurélie Bocher (l'étudiante en thèse que nous co-encadrons), nous avons développé une approche d'immunologie évolutive dont une partie des travaux réalisés a été présenté dans la première partie de ce manuscrit. J'ai plusieurs fois mentionné dans les perspectives que des mesures de défenses immunitaires pourraient être réalisées que ce soit comme paramètre pour comprendre le choix des gynes ou l'évolution de la polyandrie. Pour ce dernier point, je rappellerais juste ici que si l'investissement dans les différents composants des défenses immunitaires est héritable et qu'il existe des corrélations génétiques négatives entre ces composants, une plus grande variabilité des ouvrières au sein de la colonie permettrait de couvrir une plus large gamme de défenses. Par ailleurs, nous pouvons imaginer également que certains investissements dans les défenses immunitaires, extrêmement coûteux mais très efficaces pourraient être avantageux uniquement si la fréquence d'ouvrières présentant un tel système de défenses était faible. Nous retrouvons ici l'hypothèse de la fréquence dépendance mise en avant pour expliquer l'évolution de forts taux de polyandrie, hypothèse mentionnée dans l'Encadré 4.

Un aspect qui me paraît particulièrement à développer est l'intégration des défenses immunitaires dans le contexte de la division du travail des ouvrières. La défense de la colonie contre les pathogènes et donc les défenses immunitaires des individus peuvent être considéré comme une partie importante du travail des ouvrières. L'investissement dans les défenses immunitaires et donc dans la résistance aux pathogènes pourrait correspondre à une tâche comme celles plus classiquement étudiées du soin au couvain ou de la recherche

de nourriture. Intégrer les défenses immunitaires dans le contexte de la division du travail, permet de créer un lien entre les deux hypothèses classiquement mises en compétition dans la littérature pour expliquer l'évolution de la polyandrie, à savoir un avantage de la diversité génétique face aux pathogènes ou en terme d'efficacité de travail (exemple Brown & Schmid-Hempel, 2003). En effet, s'il existe des compromis entre investissement dans l'activité et défenses immunitaires, comme cela a été observé chez les bourdons (König & Schmid-Hempel, 1995 ; Doums & Schmid-Hempel, 2000), une corrélation négative entre activité et défenses immunitaires pourrait exister. Ceci reste valable pour les ouvrières restant à l'intérieur du nid et qui ont donc un environnement similaire. Par exemple, les fourmis inactives, souvent observées dans les colonies, qui ne servent pas systématiquement de réserve en cas de perte des fourrageuses, pourraient investir plus dans leur système immunitaire et constituer une sorte de barrière à une infection généralisée de la colonie par un pathogène. Maintenir une diversité génétique dans la colonie permettrait d'avoir à la fois des ouvrières efficaces dans certains aspects du travail tel que le fourragement et des ouvrières qui vont moins travailler mais investir plus dans les défenses immunitaires. L'existence de compromis génétique entre défenses immunitaires et travail pourrait donc représenter un avantage pour la colonie en augmentant à la fois son efficacité de travail et sa résistance contre les pathogènes. Le mécanisme mis en jeu est le même que celui mentionné dans le paragraphe précédent.

Intégrer les défenses immunitaires dans le contexte de la division du travail peut également nous permettre de comprendre l'évolution de l'investissement dans ces défenses. Ce projet est en partie abordé par l'étudiante en thèse A. Bocher sur la fourmi *Cataglyphis velox*. Par exemple, les fourrageuses et les ouvrières restant dans le nid sont soumises à des conditions environnementales très différentes qui peuvent nous amener à prédire des investissements différentiels dans les défenses immunitaires entre ces deux groupes de fourmis. Une première différence est liée aux taux de mortalité extrinsèque qui est beaucoup plus élevé chez les fourrageuses. Or, la théorie de la sénescence prédit que l'investissement dans les traits liés à la survie, comme les défenses immunitaires, diminue avec l'âge d'autant plus rapidement que la mortalité extrinsèque est élevée. Les pressions de sélection pour la survie vont effectivement diminuer si celle-ci est, pour des raisons extrinsèques, peu probable à plus ou moins court terme (Rose, 1991). On peut donc faire la prédiction que les ouvrières ont un investissement immunitaire d'autant plus réduit que la mortalité extrinsèque associée à leur tâche est élevée. Notons cependant que, chez de nombreuses espèces, la répartition du travail est liée à l'âge, les fourrageuses étant les plus vieilles. Les capacités immunitaires pourraient donc diminuer avec l'âge (Doums *et al.*, 2002b ; Palacios *et al.*, 2007). Une deuxième différence fondamentale entre les fourrageuses et les ouvrières internes réside dans la probabilité d'être blessées. Les fourrageuses pourraient courir un risque de blessure plus élevé et donc investir dans des paramètres du système immunitaire telle que l'enzyme phénol oxydase qui joue un rôle clé dans la réparation des blessures (Plaistow *et al.*, 2003). Dans ce cas, de plus forts niveaux de phénol oxydase devraient être observés chez les fourrageuses. Ainsi selon les compartiments des défenses immunitaires analysés, différentes prédictions peuvent être faites concernant l'investissement optimal d'une ouvrière en fonction de son activité.

II.5. Références

André, J. B., C. Peeters, C. Doums. 2001. Serial polygyny and colony genetic structure in the monogynous queenless ant *Diacamma cyaneiventre*. Behavioral Ecology and Sociobiology 50: 72-80.

André, J. B., C. Peeters, M. Huet, C. Doums. 2006. Estimating the rate of gamergate turnover in the queenless ant *Diacamma cyaneiventre* using a maximum likelihood model. Insectes Sociaux 53: 233-240.

Apanius, V. 1998. Stress and immune response. *in* A. P. Møller, M. Milinski, P. J. B. Slater, editors. Stress and behavior. Academic Press, San Diego, California.

Arnqvist, G., T. Nilsson. 2000. The evolution of polyandry: Multiple mating and female fitness in insects. Animal Behaviour 60: 145-164.

Baer, B., S. A. O. Armitage, J. J. Boomsma. 2006. Sperm storage induces an immunity cost in ants. Nature 441: 872-875.

Baer, B., P. Schmid-Hempel. 1999. Experimental variation in polyandry affects parasite load and fitness in a bumble bee. Nature 397: 151-154.

Barrate, S. 2005. Régulation irréversible de la reproduction chez la fourmi sans reine *Diacamma ceylonense* : gènes, comportement et évolution. Thèse Université Paris VI, Paris.

Bateman, A. J. 1948. Intra-sexual selection in *Drosophila*. Heredity 2: 349-368.

Baudry, E., C. Peeters, L. Brazier, M. Veuille, C. Doums. 2003. Shift in the behaviours regulating monogyny is associated with high genetic differentiation in the queenless ant *Diacamma ceylonense*. Insectes Sociaux 50: 390-397.

Bell, G. 1982. The masterpiece of nature - The evolution and genetics of sexuality. University of California Press, Berkeley.

Birkhead, T. R., A. P. Møller. 1998. Sperm competition and sexual selection. Academic Press, London.

Bocher, A., C. Doums, L. Millot, C. Tirard. Soumis. Reproductive conflicts affect labour and immune defences in the queenless ant *Diacamma 'nilgiri*'. Evolution.

Boomsma, J. J., B. Baer, J. Heinze. 2005. The evolution of male traits in social insects. Annual Review of Entomology 50: 395-420.

Boomsma, J. J., F. L. W. Ratnieks. 1996. Paternity in eusocial Hymenoptera. Philosophical Transaction of The Royal Society London B 351: 947-975.

Bourke, A. F. G., N. R. Franks. 1995. Social evolution in ants. Princeton University Press, Princeton, N.J.

Brown, M. J. F., P. Schmid-Hempel. 2003. The evolution of female multiple mating in social hymenoptera. Evolution 57: 2067-2081.

Cagniant, H. 1976. Distribution, écologie et nid de la fourmi *Cataglyphis cursor* Fonscolombe. Hyménoptères Formicidae. Vie Milieu 26: 265-276.

Cagniant, H. 1979. La parthénogenèse thélytoque et arrhénotoque chez la fourmi *Cataglyphis cursor* Fonsc. (Hym., Form.). Cycle biologique en élevage des colonies avec reine et des colonies sans reine. Insectes Sociaux 26: 51-60.

Cagniant, H. 1983. La parthénogenèse thélytoque et arrhénotoque des ouvrières de la fourmi *Cataglyphis cursor* Fonscolombe (Hyménoptères Formicidae) étude biométrique des ouvrières et de leurs potentialités reproductrices. Insectes Sociaux 30: 241-254.

Calabi, P., S. D. Porter. 1989. Worker longevity in the fire ant *Solenopsis invicta* ; ergonomic considerations of correlation between temperature, size and metabolic rates. Journal of Insect Physiology 35: 643-649.

Cerdá, X., J. Retana. 1997. Links between worker polymorphism and thermal biology in a thermophilic ant species. Oikos 78: 467-474.

Cerdá, X., J. Retana, J. Bosch, A. Alsina. 1989. Daily foraging activity and food collection of the thermophilic ant *Cataglyphis cursor* (Hymenoptera, Formicidae). Vie Milieu 39: 207-212.

Chapuisat, M., J. Goudet, L. Keller. 1997. Microsatellites reveal high population viscosity and limited dispersal in the ant *Formica paralugubris*. Evolution 51: 475-482.

Charlesworth, D., B. Charlesworth. 1987. Inbreeding depression and its evolutionary consequences. Annual Review of Ecology and Systematics 18: 237-268.

Clémencet, J. 2006. Evolution des stratégies de dispersion et de reproduction chez la fourmi *Cataglyphis cursor*. Thèse Paris VI, Paris.

Clémencet, J., C. Doums. 2007. Habitat-related microgeographic variation of worker size and colony size in the ant *Cataglyphis cursor*. Oecologia DOI: 10.1007/s00442-006-0646-2.

Clémencet, J., B. Viginier, C. Doums. 2005. Hierarchical analysis of population genetic structure in the monogynous ant *Cataglyphis cursor* using microsatellite and mitochondrial DNA markers. Molecular Ecology 14: 3735-3744.

Crozier, R. H., E. J. Fjerdingstad. 2001. Polyandry in social Hymenoptera: disunity in diversity. Annales Zoologici Fennici 38: 267-285.

Crozier, R. H., P. Pamilo. 1996. Evolution of Social Insect Colonies. Oxford University Press, Oxford.

Cuvillier-Hot, V., A. Lenoir, C. Peeters. 2004. Reproductive monopoly enforced by sterile police workers in a queenless ant. Behavioral Ecology 15: 970-975.

Doums, C., H. Cabrera, C. Peeters. 2002a. Population genetic structure and male-biased dispersal in the queenless ant *Diacamma cyaneiventre*. Molecular Ecology 11: 2251-2264.

Doums, C., Y. Moret, E. Benelli, P. Schmid Hempel. 2002b. Senescence of immune defence in *Bombus* workers. Ecological Entomology 27: 138-144.

Doums, C., H. P. Schmid. 2000. Immunocompetence in workers of a social insect, *Bombus terrestris L*., in relation to foraging activity and parasitic infection. Canadian Journal of Zoology 78: 1060-1066.

Fefferman, N., P. Starks. 2006. A modelling approach to swarming in honey bees (*Apis mellifera*). Insectes Sociaux 53: 37-45.

Fjerdingstad, E. J., R. H. Crozier. 2006. The evolution of worker caste diversity in social insects. American Naturalist 167: 390-400.

Fournier, D., A. Estoup, J. Orivel, J. Foucaud, H. Jourdan, J. L. Breton, L. Keller. 2005. Clonal reproduction by males and females in the little fire ant. Nature 435: 1230-1234.

Franks, N. R., B. Hölldobler. 1987. Sexual competition during colony reproduction in army ants. Biological Journal of the Linnean Society 30: 229-243.

Fuchs, S., R. F. A. Moritz. 1998. Evolution of extreme polyandry in the honeybee *Apis mellifera*. Behavioral Ecology and Sociobiology 9: 269-275.

Gerloff, C. U., B. K. Ottmer, P. Schmid-Hempel. 2003. Effects of inbreeding on immune response and body size in a social insect, *Bombus terrestris*. Functional Ecology 17: 582-589.

Giraud, T., R. Blatrix, C. Poteaux, M. Solignac, P. Jaisson. 2000. Population structure and mating biology of the polygynous ponerine ant *Gnamptogenys striatula* in Brazil. Molecular Ecology 9: 1835-1841.

Gobin, B., J. Heinze, M. Strätz, F. Roces. 2003. The energetic cost of reproductive conflicts in the ant *Pachycondyla obscuricornis*. Journal of Insect Physiology 49: 747-752.

Gotwald, W. H. J. 1995. Army ants: The biology of social predation. Cornell University Press, Ithaca.

Hamilton, W. D. 1964. The genetical evolution of social behaviour I,II. Journal of theoretical Biology 7: 1-52.

Hartmann, A., J. Wantia, J. A. Torres, J. Heinze. 2003. Worker policing without genetic conflicts in a clonal ant. Proceedings of the National Academy of Science, USA 100: 12836-12840.

Heinze, J., L. Keller. 2000. Alternative reproductive strategies: a queen perspective in ants. Trends in Ecology and Evolution 15: 508-512.

Henter, H. J. 2003. Inbreeding depression and haplodiploidy: experimental measures in a parasitoid and comparisons across diploid and haplodiploid insect taxa. Evolution 57: 1793-1803.

Hölldobler, B., H. B. Wilson. 1990. The ants. Springer-Verlag, Berlin.

Hughes, C. R., J. J. Boomsma. 2004. Genetic diversity and disease resistance in leaf-cutting ant societies. Evolution 58: 1251-1260.

Jarne, P., D. Charlesworth. 1993. The evolution of the selfing rate in functionally hermaphrodite plants and animals. Annual Review of Ecology and Systematics 24: 441-466.

Jennions, M. D., M. Petrie. 2000. Why do females mate multiply? A review of the genetic benefits. Biological Reviews of the Cambridge Philosophical Society, London 75: 21-64.

Keller, L. 1995. Social life: the paradox of multiple-queen colonies. Trends in Ecology and Evolution. 10: 355-360.

Keller, L. 2007. Uncovering the Biodiversity of Genetic and Reproductive Systems: Time for a More Open Approach. American Naturalist 169: 1-8.

Kelly, J. K. 1992. Restricted migration and the evolution of altruism. Evolution 37: 1492-1495.

Kelly, J. K. 1994. The effect of scale dependent processes on kin selection: Mating and density regulation. Theoretical Population Biology 46: 32-57.

Kleiman, D., L. W. Aarssen. 2007. The leaf size/number trade-off in trees. Journal of Ecology 95: 376-382.

König, C., P. Schmid-Hempel. 1995. Foraging activity and immunocompetence in workers of the bumble bee, *Bombus terrestris* L. Proceedings of the Royal Society London B 260: 225-227.

Kronauer, D. J. C., C. Schoning, J. S. Pedersen, J. J. Boomsma, J. Gagau. 2004. Extreme queen-mating frequency and colony fission in African army ants. Molecular Ecology 13: 2381-2388.

Kryger, P., R. F. A. Moritz. 1997. Lack of kin recognition in swarming honeybees (*Apis mellifera*). Behavioral Ecology and Sociobiology 40: 271-276.

Lacoste, A., S. K. Malham, F. Gelebart, A. Cueff, S. A. Poulet. 2002. Stress-induced immune changes in the oyster *Crassostrea gigas*. Developmental & Comparative Immunology 26: 1-9.

Lenoir, A., H. Cagniant. 1986. Role of worker thelytoky in colonies of the ant *Cataglyphis cursor* (Hymenoptera: Formicidae). Entomologica Generalis 11: 153-157.

Lenoir, A., L. Querard, N. Pondicq, F. Berton. 1988. Reproduction and dispersal in the ant *Cataglyphis cursor* (Hymenoptera, Formicidae). Psyche 95: 21-44.

Liautard, C., L. Sundström. 2005. Estimation of individual level of inbreeding using relatedness measures in haplodiploids. Insectes Sociaux 52: 323-326.

Malham, S. K., A. Lacoste, F. Gélébart, A. Cueff, S. A. Poulet. 2003. Evidence for a direct link between stress and immunity in the mollusc *Haliotis tuberculata*. Journal of Experimental Zoology 295A: 136-144.

Maynard Smith, J. 1978. The evolution of sex. Cambridge University Press, Cambridge.

Michod, R. E., B. R. Levin. 1988. The evolution of sex. Sinauer Associates Inc., Sunderland, Massachusetts.

Mopper, S., S. Y. Strauss. 1998. Genetic structure and local adaptation in natural insect populations. Effects of ecology, life-history and behavior. Chapman & Hall, New York.

Moret, Y., P. Schmid-Hempel. 2001. Immune defence in bumble-bee offspring. Nature 414: 506.

Neumann, P., R. F. A. Moritz. 2002. The cape honeybee phenomenon: the sympatric evolution of a social parasite in real time ? Behavioral Ecology and Sociobiology 52: 271-281.

Olsson, M., M. Pagel, R. Shine, T. Madsen, C. Doums, A. Gullberg, H. Tegelström. 1999. Sperm choice and sperm competition: Suggestions for field and laboratory studies. Oikos 84: 172-175.

Ottaviani, E., C. Franceschi. 1996. The neuroimmunology of stress from invertebrates to man. Progress in Neurobiology 48: 421-440.

Otto, S. P., T. Lenormand. 2002. Resolving the paradox of sex and recombination. Nature Genetics 3: 252-261.

Palacios, M., J. Cunnick, D. Winkler, C. Vleck. 2007. Immunosenescence in some but not all immune components in a free-living vertebrate, the tree swallow. Proceedings of the Royal Society London B 274: 951-957.

Palmer, K. A., B. P. Oldroyd. 2000. Evolution of multiple mating in the genus *Apis*. Apidologie 31: 235-248.

Pearcy, M. 2005. Stratégies reproductrices chez la fourmi *Cataglyphis cursor*. Thèse Université Libre de Bruxelles, Bruxelles.

Pearcy, M., S. Aron. 2006. Local resource competition and sex ratio in the ant *Cataglyphis cursor*. Behavioral Ecology 17: 569-574.

Pearcy, M., S. Aron, C. Doums, L. Keller. 2004a. Conditional use of sex and parthenogenesis for workers and queen production in ants. Science 306: 25-28.

Pearcy, M., J. Clémencet, S. Chameron, S. Aron, C. Doums. 2004b. Characterization of nuclear DNA microsatellite markers in the ant *Cataglyphis cursor*. Molecular Ecology Notes 4: 642-644.

Pearcy, M., O. Hardy, S. Aron. 2006. Thelytokous parthenogenesis and its consequences on inbreeding in an ant. Heredity 96: 377-382.

Peeters, C., F. Ito. 2001. Colony dispersal and the evolution of queen morphology in social hymenoptera. Annual Review of Entomology 46: 601-630.

Plaistow, S. J., Y. Outreman, Y. Moret, T. Rigaud. 2003. Variation in the risk of being wounded: an overlooked factor in studies of invertebrate immune function? Ecology Letters 6: 489-494.

Powell, S., N. R. Franks. 2006. Ecology and the evolution of worker morphological diversity: a comparative analysis with *Eciton* army ants. Functional Ecology 20: 1105-1114.

Ratnieks, F. L. W., H. Reeve. 1992. Conflicts in single-queen Hymenopteran societies: the structure of conflict and processes that reduce conflict in advanced eusocial species. Journal of Theoretical Biology 158: 33-65.

Raymond, M., F. Rousset. 1995. Genepop (version 1.2) population genetics software for exact tests and ecumenicism. Journal of Heredity 86: 248-249.

Reichardt, A. K., D. E. Wheeler. 1996. Multiple mating in the ant *Acromyrmex versicolor*: a case of female control. Behavioral Ecology and Sociobiology 38: 219-225.

Roff, D. A. 1992. The evolution of life histories. Theory and Analysis. Chapman & Hall, New York.

Rose, M. R. 1991. Evolutionary biology of aging. Oxford university Press, New York.

Ross, K. C. 2001. Molecular ecology of social behaviour: analyses of breeding systems and genetic structure. Molecular Ecology 10: 265-284.

Schilder, K., J. Heinze, B. Hoelldobler. 1999. Colony structure and reproduction in the thelytokous parthenogenetic ant *Platythyrea punctata* (F. Smith) (Hymenoptera, Formicidae). Insectes Sociaux 46: 150-158.

Schmid-Hempel, P. 1998. Parasites in social insects. Princeton University Press, Princeton, New Jersey.

Schmid-Hempel, P. 2005. Evolutionary ecology of insect immune defenses. Annual Review of Entomology 50: 529-551.

Schmid-Hempel, P., D. Ebert. 2003. On the evolutionary ecology of specific immune defence. Trends in Ecology and Evolution 28: 27-32.

Seppä, P., P. Pamilo. 1995. Gene flow and population viscosity in *Myrmica* ants. Heredity 74: 200-209.

Sherman, P. W., T. D. Seeley, H. K. Reeve. 1988. Parasites, pathogens, and polyandry in social Hymenoptera. American Naturalist 131: 602-610.

Simmons, L. W. 2005. The evolution of polyandry: Sperm Competition, Sperm Selection, and Offspring Viability. Annual Review of Ecology, Evolution, and Systematics 36: 125-146.

Stearns, S. C. 1992. The evolution of life histories. Oxford University Press.

Stockley, P. 1997. Sexual conflict resulting from adaptations to sperm competition. Trends in Ecology and Evolution 12: 154-159.

Strassmann, J. 2001. The rarity of multiple mating by females in the social Hymenoptera. Insectes Sociaux. 48: 1-13.

Thornhill, R., J. Alcock. 1983. The evolution of insect mating system. Havard University, Cambridge.

Traniello, J. F. A., R. B. Rosengaus, K. Savoie. 2002. The development of immunity in a social insect: Evidence for the group facilitation of disease resistance. Proceedings of the National Academy of Science, USA 99: 6838-6842.

Tsuji, K. 1988. Obligate parthenogenesis and reproductive division of labor in the Japanese queenless ant *Pristomyrmex pungens*. Behavioral Ecology and Sociobiology 23: 247-255.

Tsuji, K., K. Yamauchi. 1995. Production of females by parthenogenesis in the ant *Cerapachys biroi*. Insectes Sociaux 42: 333-336.

Viginier, B., C. Peeters, L. Brazier, C. Doums. 2004. Very low genetic variability in the Indian queenless ant *Diacamma indicum*. Molecular Ecology 13: 2095-2100.

Werren, J. H. 1993. The evolution of inbreeding in haplodiploid organisms. Pages 42-49 *in* N. W. Thornhill, editor. The natural history of inbreeding and outbreeding. University of Chicago Press, Chicago.

ANNEXE 1

Population genetic structure and male-biased dispersal in the queenless ant *Diacamma cyaneiventre*

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Population genetic structure and male-biased dispersal in the queenless ant *Diacamma cyaneiventre*

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Abstract

In this study we investigated the population genetic structure of the queenless ant *Diacamma cyaneiventre*. This species, lacking winged queens, is likely to have a restricted female dispersal. We used both mitochondrial and microsatellite markers to assess the consequence of such restricted female dispersal at three geographical scales: within a given locality (< 1 km), between localities within a given region (< 10 km) and between regions (> 36 km). Within a locality, a strong population structure was observed for mitochondrial DNA (mtDNA) whereas weak or nonexistent population genetic structure was observed for the microsatellites (around 5% of the value for mtDNA). Male gene flow was estimated to be about 20–30 times higher than female gene flow at this scale. At a larger spatial scale, very strong genetic differentiation for both markers was observed between localities — even within a single region. Female dispersal is nonexistent at these scales and male dispersal is very restricted, especially between regions. The phylogeographical structure of the mtDNA haplotypes as well as the very low genetic diversity of mtDNA within localities indicate that new sites are colonized by a single migration event from adjacent localities, followed by successive colony fissions. These patterns of genetic variability and differentiation agree with what is theoretically expected when colonization events are kin-structured and when, following colonization, dispersion is mainly performed by males.

Keywords: *Diacamma cyaneiventre*, gamergate, hierarchical genetic structure, microsatellites, mtDNA, population viscosity

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Introduction

Dispersal is a fundamental life history trait affecting gene flow and therefore shaping the distribution of genetic variability within and between populations (Waser & Strobeck 1998). In social insects, especially in ants where workers are wingless, patterns of dispersal are linked tightly to the mode of colony foundation. Colony foundation can be independent when the queen(s) founds the colony alone, or dependent when the queen(s) is helped by a group of workers. Female dispersal is restricted to 'walking distance' under dependent colony foundation whereas independent colony foundation is often associated with mating in flight, providing the potential for dispersal to take place over longer distances (Peeters & Ito 2001).

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In some polygynous ant species, in which dependent colony foundation predominates, restricted female gene flow within populations (at the scale of a few hundred metres) was detected using mitochondrial DNA (Stille & Stille 1993; Ross *et al*. 1997; Tay *et al*. 1997; Goodisman & Ross 1998; Liautard & Keller 2001). On the other hand, in monogynous forms of *Solenopsis invicta* in which the queen founds the colony alone, genetic differentiation for mtDNA was detected at the scale of few kilometres but not at the scale of few hundred metres within a given population (Ross *et al*. 1997).

Restricted female dispersal can induce some population viscosity for nuclear genes, i.e. an increase in genetic similarity between neighbouring colonies, if male dispersal is also restricted. A knowledge of local population genetic structure is important when analysing breeding system and within-colony genetic structure (Ross 2001). By increasing the genetic relatedness between interacting

individuals, population viscosity can also affect the evolution of social behaviours (Kelly 1992; Queller 1992). Many studies on ants have investigated the extent of population viscosity, and tend to indicate that polygyny is generally associated with population viscosity (Pamilo 1983; Crozier *et al*. 1984; Crozier & Pamilo 1986; Seppä & Pamilo 1995; Chapuisat *et al*. 1997; Beye *et al*. 1998; Giraud *et al*. 2000; Tsutsui & Case 2001), whereas no or very low population viscosity is detected in monogynous species or forms (Sundström 1993; Seppä & Pamilo 1995; Ross *et al*. 1997). Population viscosity is expected in polygynous species but not in monogynous species for two reasons. First, polygyny is often associated with dependent colony foundation and monogyny with independent colony foundation (Bourke & Franks 1995; Keller 1991). Second, movements between nests produced by budding has been found in polygynous species but is extremely rare in monogynous species (e.g. Chapuisat *et al*. 1997). Such exchange of workers can decrease in intensity with increasing geographical distance between nests and lead to some population viscosity. In this case, population viscosity does not necessarily reflect limited sexual female gene flow, as the pattern of microgeographical structure can disappear when nests from different colonies are studied (e.g. Chapuisat *et al*. 1997).

Queenless ants of the genus *Diacamma* provide an original model for investigating the consequences of restricted female dispersal on population genetic structure. In queenless species, female dispersal is limited to ant walking distance. These species have a dependent mode of colony foundation and are monogynous with a single nest per colony (Peeters *et al*. 1992). Males are winged and have the potential to disperse genes over longer distances than females. Such potential male-biased dispersal should leave some trace in the population genetic structure when biparentally and maternally inherited markers are compared (Ennos 1994). The consequence of limited female dispersal on the population genetic structure of markers with constrasting modes of inheritance has rarely been investigated in ants (but see Ross *et al*. 1997; 1999).

In this study, we used variation in mtDNA and in eight microsatellite loci to investigate: (i) the extent of microgeographical genetic structure in one locality of the queenless ant *D. cyaneiventre* found in southern India. In this species, the nests extend deep underground (up to 1 m), are the result of an elaborate construction effort and colonies do not emigrate frequently (C. Doums & C. Peeters, unpublished data). *D. cyaneiventre* therefore provides a good model for investigating population viscosity. (ii) The consequence of restricted female dispersal at larger spatial scales, more specifically at two hierarchical scales, i.e. between localities within a region (separated by few kilometres) and between regions (from 36 to 188 km apart). If male gene flow is also restricted, strong genetic differentiation is expected, especially at a larger spatial scale.

Materials and methods

Samples

Diacamma cyaneiventre is a Ponerine ant that inhabits open areas. As far as we know, the distribution area of this species is restricted to the southwest of India. Two hundred and twenty-one colonies of *D. cyaneiventre* were sampled in seven localities distributed in three regions in Karnataka state (Fig. 1). This sampling represents the species' known range. The exact position of each locality, as determined by GPS, is given in Table 1. The number of samples collected was larger in Kottigehara 1 compared to other localities, as this locality had been the subject of a previous study (André *et al*. 2001). Moreover, the topology of this site made it possible to map the colonies. The shape of the sampling area was determined mainly by the dense forest surrounding the site, in which *Diacamma* ants are not found. In *D. cyaneiventre*, the average genetic relatedness between nestmates is 0.75 (André *et al*. 2001). We therefore determined the genotype of only one individual per colony to avoid the nonindependence of genotypes attributable

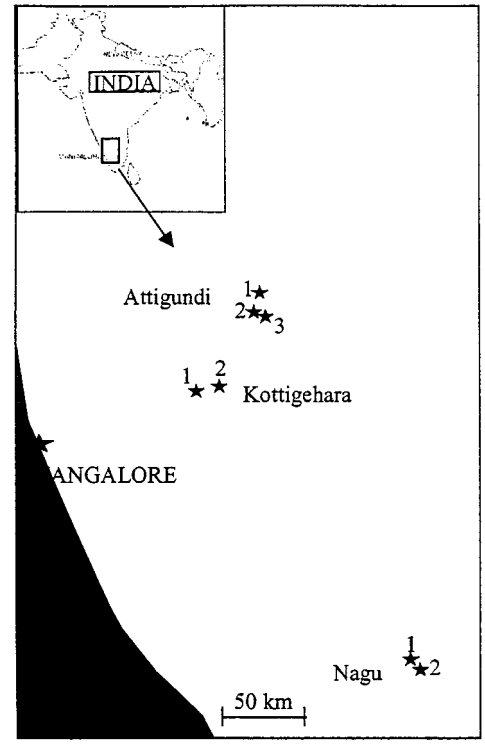

Fig. 1 Geographical position of *D. cyaneiventre* localities in the south of India. The seven localities sampled, depicted by stars, are distributed in three regions: Attigundi, Kottigehara and Nagu.

Table 1 Population position and genetic diversity of mitochondrial and microsatellite DNA. *N*, the number of colonies analysed (one individual per colony); H_a, the mitochondrial haplotype detected in each site with their accession number in gene bank in parentheses; H_E^{Nuc} and H_E^{mt}, the gene diversity for microsatellite (average expected heterozygosity over loci ± SD) and mtDNA (probability that two randomly chosen haplotypes are different ± SD), respectively (Nei 1987)

Population	Position	*N*	H_a	H_E^{mt}	H_E^{Nuc} ± SD
Attigundi 1	13°30′06,3″ N 75°44′22,7″ E	15	1 (AF467546)	0	0.63 ± 0.15
Attigundi 2	13°25′01,6″ N 75°44′27,4″ E	12	2 (AF467547)	0	0.58 ± 0.28
Attigundi 3	13°26′6,5″ N 75°44′15,0″ E	12	2 (AF467547)	0	0.54 ± 0.28
Kottigehara 1	13°6′57,3″ N 75°30′54,2″ E	101	3 (AF467548) 4 (AF467549) 5 (AF467550)	0.53 ± 0.23	0.64 ± 0.22
Kottigehara 2	13°7′16,6″ N 75°36′11,4″ E	20	6 (AF467551)	0	0.68 ± 0.21
Nagu 1	11°59′39,2″ N 76°26′21,3″ E	24	7 (AF467552)	0	0.67 ± 0.22
Nagu 2	11°57′21,5″ N 76°27′13,5″ E	28	8 (AF467553)	0	0.68 ± 0.19

to family structure. Three hierarchical levels of genetic differentiation were therefore investigated: (i) the population viscosity within the locality Kottigehara 1; (ii) the genetic differentiation between localities within a region (a few kilometres apart); and (iii) between regions (from 36 to 188 km apart).

Genetic analysis

DNA was obtained using a rapid extraction procedure (see André *et al*. 2001). Eight microsatellite loci developed for *D. cyaneiventre* (Doums 1999) were used in this study. Polymerase chain reactions (PCR) were conducted following the protocol of Doums (1999), except for the coamplification of loci D19 and D20, for which the amounts of dNTP, ^{33}PdATP and Taq DNA polymerase were doubled.

Haplotypes of the mtDNA were scored following PCR amplification of a 280-base pair (bp) segment of the COII gene and separation of the products using the single-strand conformation polymorphism analysis (SSCP) technique (Orita *et al*. 1989). This fragment was amplified using the conserved primers (300P 5′-GGTCATCAATGATACTGATC-3′ and 630 M 5′-AATCATAGATTTATACCAAT-3′). Each PCR reaction was run in a 10-μL volume containing 1 μL of DNA solution, 75 μM of each dNTP, 0.025 μci dATP, 0.4 μM of each primer, 1 × taq buffer and 0.25 units of *Taq* polymerase (Quiagen) using a PTC-100 thermal cycler (MJ Research). The thermal cycle profile was as follows: an initial hot-start of 3 min at 94 °C; 30 amplification cycles of denaturation for 30 s at 94 °C, annealing for 30 s at 40 °C and extension for 1 min at 72 °C; and a final extension for 10 min at 72 °C. The amplified products were loaded onto 6% denaturing acrylamide gel. Gels were run at 15 W for 15 h at a constant room temperature of 20 °C and autoradiographed for approximately 24 h. For each haplotype detected, the PCR product of two individuals was sequenced using the Thermo Sequenase Kit (US Biochemical Corp.) after purification using a gel band purification kit (Amersham).

Data analysis

Hardy–Weinberg equilibrium was tested for the eight loci at each locality using exact tests (GENEPOP 3.2; Raymond & Rousset 1995). Fisher's method of combining independent test results (Sokal & Rohlf 1995: 794) was used to determine the overall significance for each locality and each locus. Linkage disequilibrium between pairs of loci was tested for each locality using GENEPOP 3.2.

Population viscosity. Three different analyses were conducted to investigate the extent of population viscosity in Kottigehara 1. As the colonies were not distributed continuously throughout the locality, due mainly to environmental heterogeneity (forest, bushes, road and river, Fig. 2), the locality was divided into three demes. Demes 1 and 2 were separated by a river and demes 2 and 3 by dense bushes. However, these three demes were connected by roads (see Fig. 2). In demes 1 and 2 (Fig. 2) the sampling was nearly exhaustive. First, genetic

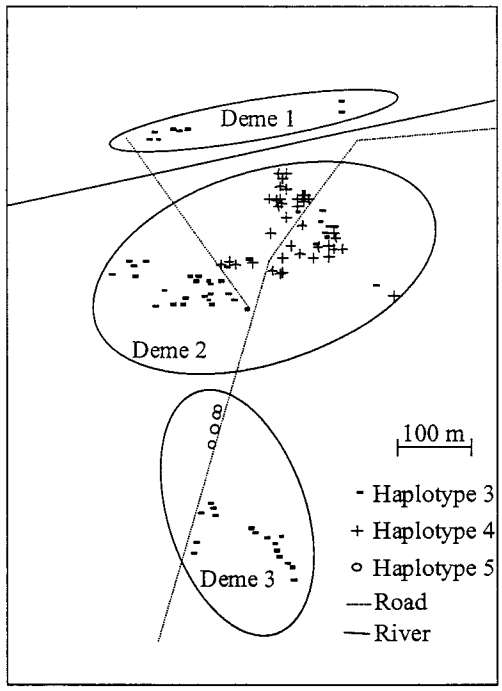

Fig. 2 Map of *D. cyaneiventre* colonies sampled from Kottigehara 1. Each colony is depicted by a symbol characterizing its mitochondrial haplotype. The three circles characterize the three demes used for estimating the genetic substructuration within this locality.

differentiation among the three demes was analysed using *F*-statistics as described below for both types of makers. Second, the population viscosity was tested by checking whether neighbouring colonies were more closely related than non-neighbouring colonies, using all colonies or only colonies within the largest, deme 2, containing 73 continuously distributed colonies. Neighbouring colonies were defined using a Gabriel graph (Gabriel & Sokal 1969). The average relatedness between neighbouring colonies was calculated using RELATEDNESS 5.0 (Queller & Goodnight 1989). The standard error of the estimate was obtained by jackknifing over individuals and was used to test whether the estimated relatedness was significantly different from 0 using a *t*-test (Sokal & Rohlf 1995). Third, within deme 2, population viscosity was also investigated by plotting the genetic differentiation between colonies against geographical distance (Rousset 2000). The estimator a_r of genetic differentiation between individuals (here colonies), analogous to $F_{ST}/(1 - F_{ST})$, was calculated using GENEPOP 3.3. The significance of the Pearson correlation coefficient between genetic differentiation and geographical distance was assessed with a Mantel test (GENEPOP 3.3, Raymond & Rousset 1995).

Genetic differentiation between localities. The significance of the genetic differentiation between localities was examined by conducting exact tests of allele frequency differentiation (GENEPOP 3.2, Raymond & Rousset 1995). The joint probabilities of differentiation over all microsatellite loci were obtained using Fisher's combined probability test (Sokal & Rohlf 1995).

For the microsatellites, we computed both *F*- and *R*-statistics assuming the infinite allele model (Wright 1978) or a stepwise mutation process (Slatkin 1995), respectively. The mutation process will influence estimations of differentiation if the coalescence times of genes between populations are sufficiently long for two or more mutation events to have occurred (Rousset 1996). This may be the case in our study, as female dispersal should be very restricted. Population genetic differentiation was partitioned at two levels; across all localities and between localities within each region (see Fig. 1). Hierarchical *F*-statistics and *R*-statistics were estimated with a two-level hierarchical AMOVA (Excoffier *et al.* 1992) using ARLEQUIN 2.00 (Schneider *et al.* 2000). The same analyses were conducted on either haplotype frequency (equidistant ϕ_{ST}) or the proportion of nucleotide differences between haplotypes (euclidean ϕ_{ST}). to estimate equidistant and euclidian ϕ_{ST} for the mitochondrial markers (ARLEQUIN 2.00, Schneider *et al.* 2000). *F*-statistics between pair of localities were estimated according to Weir & Cockerham (1984), and *R*-statistics according to Rousset (1996) using FSTAT version 2.8 (Goudet 1995) or GENEPOP 3.3 (Raymond & Rousset 1995). The significance of the Pearson correlation coefficient between genetic differentiation and geographical distance between localities was assessed with a Mantel test (GENEPOP 3.3, Raymond & Rousset 1995).

The genetic relationship between mitochondrial haplotypes was investigated by constructing a minimum spanning tree reflecting the unrooted genealogical relationships between the mtDNA haplotypes using ARLEQUIN 2.00 (Schneider *et al.* 2000) as well as a neighbour-joining tree using MEGA software (Kumar *et al.* 2001). The neighbour-joining tree was rooted at the midpoint of the longest distance between any two haplotypes. The difference between both trees is that haplotypes can be ancestral to other haplotypes in a minimum spanning tree but not in a neighbour-joining tree. For constructing both trees, we used the Jukes and Cantor distance, an appropriate measure for the level of genetic distance detected (Nei & Kumar 2000).

Male and female gene flow. Although estimates of *Nm* from F_{ST} cannot be directly translated into a number of migrants moving between populations (Bossart & Prowell 1998; Hedrick 1999; Whitlock & McCauley 1999), these estimates still provide a straightforward method of comparing the relative intensity of male and female gene flow. Under the assumptions of Wright's infinite island model (Wright 1978) at migration-drift equilibrium, genetic differentiation are given by: $F_{ST}^{mt} = 1/(2N_{ef}m_f + 1)$ for maternally inherited

genes with one copy per individual (mtDNA) and a female migration rate of m_f, $F_{ST}^{mt} = 1/(4N_e m + 1)$ for biparentally inherited genes (microsatellites) and migration rate m (Hartl & Clark 1997).

For haplodiploid species, $N_e = 9N_{ef}N_{em}/(2N_{ef} + 4N_{em})$ (Hartl & Clark 1997) and under the assumption of dispersal before mating, $m = (2m_f + m_m)/3$ with m_f and m_m being the migration rate of females and males, respectively (Berg *et al*. 1998). This model of dispersal is the most appropriate, as males of *D. cyaneiventre* disperse before mating. *D. cyaneiventre* can be considered a monoandrous species (André *et al*. 2001) and the male loses a part of his abdomen during mating and consequently can mate only once (pers. obs.). We therefore assumed an even effective sex-ratio that is $N_f = N_m = 1/2N$. From these equations and the *F*-statistics estimates of both mitochondrial and microsatellite markers, we estimated male and female gene flow between demes within Kottigehara 1 and between localities at the two spatial scales (within and between regions).

Results

Within-locality genetic diversity

Allele frequencies and gene diversities (Nei 1987) are given in Appendix I for each locality and each locus. Over all localities, the total number of microsatellite alleles per locus varied between seven (DC5) and 38 (DC18) and the gene diversity between 0.44 (DC20) and 0.90 (DC18). The within-locality genetic diversity for the different localities is given in Table 1.

Eight mitochondrial haplotypes were identified by SSCP. Within a given haplotype, the sequence of the two individuals examined was always identical. Over the entire sample, 29 variable sites were detected out of 263 bp (percentage of polymorphic sites = 11%). The overall nucleotide diversity (Nei 1987: average number of nucleotide differences per site between two sequences) was 3.8% (± 1.9 SD), while the overall gene diversity (Nei 1987: probability that two randomly chosen haplotypes are different) was 83.6% (± 10.9 SD). The within-locality diversity is given in Table 1. Basically, a single haplotype was observed in each locality except in Kottigehara 1, where three haplotypes were identified (Table 1). The sequencing of an individual in Attigundi 2 and Attigundi 3 confirmed that the SSCP haplotype observed in the two sites was identical.

Tests for linkage disequilibrium and Hardy–Weinberg equilibrium

Of 182 tests of linkage disequilibrium, only 10 were significant ($0.007 < P < 0.05$). Given that 10 tests could be significant by chance alone, we considered the eight microsatellites to be independent. Ten of the 56 probability tests for Hardy–Weinberg equilibrium were significant at $P < 0.05$ (details in Appendix I). This is higher than would be expected by chance alone on the basis of type I errors. Eight of these significant tests were associated with a deficit of heterozygotes but they did not concern specific loci or localities. Only two of these tests were highly significant ($P < 0.0001$) and remained significant after a Bonferroni correction (Sokal & Rohlf 1995). The deficit in heterozygotes in Kottigehara 1 for the loci DC 18 could be explained by a Wahlund effect (see below) and there could be a null allele for the DC 52 locus in Nagu 2. However, any null allele would have a low frequency because amplification was obtained for all individuals. In general, these data suggest a general lack of extensive inbreeding. This agrees with behavioural observations on laboratory colonies in which sister–brother mating was never seen (V. Cuvillier-Hot, pers. com.).

Population viscosity (microgeographical genetic differentiation within Kottigehara 1)

Genetic structure within Kottigehara 1 was first investigated by testing for genic differentiation between the three identified demes (see Fig. 2). For the microsatellite data, although the average value of F_{ST} over all loci was low (0.0195), the exact test of genic differentiation was highly significant ($P = 0.0002$). When comparing each pair of demes, the exact tests of genic differentiation were significant ($P < 0.005$) for the two comparisons involving deme 3. The value of F_{ST} was higher between the two demes which are the furthest apart (F_{ST} between deme 1 and 3 = 0.036) than between demes 1 and 2 ($F_{ST} = 0.015$) and demes 2 and 3 ($F_{ST} = 0.019$).

The exact test of mtDNA genic differentiation between demes was also highly significant ($P < 0.0001$). The geographical structure of the three haplotypes is shown in Fig. 2. The genetic structure observed for mtDNA was approximately 15 times higher than that for the microsatellites, whatever the distance used (equidistant $\Phi_{ST} = 0.33$; euclidean $\Phi_{ST} = 0.41$). When comparing each pair of demes, the exact tests of genic differentiation were significant ($P < 0.004$) for the two comparisons involving deme 2. The lowest value of Φ_{ST} was between demes 1 and 3 (equidistant and euclidean $\Phi_{ST} = 0.08$), while the higher values were obtained between demes 1 and 2 (equidistant and euclidean $\Phi_{ST} = 0.37$) and between demes 2 and 3 (equidistant $\Phi_{ST} = 0.31$; euclidean $\Phi_{ST} = 0.42$).

The results obtained by estimating the relatedness among neighbouring colonies also showed contrasting results between the two markers. The relatedness among neighbouring colonies was low and nonsignificant different from 0 for the entire locality ($r = 0.07$; *t*-test = 1.58, d.f. = 97, $P > 0.05$) and within deme 2 ($r = 0.035$; *t*-test = 0.72, d.f. = 70, $P > 0.05$) for microsatellites. On the other

hand, using mitochondrial data, the relatedness among neighbouring colonies was high and significantly different from 0 for both the entire locality ($r = 0.74$; t-test = 7.03, d.f. = 88, $P < 10^{-5}$) and within deme 2 ($r = 0.68$; t-test = 4.7, d.f. = 67, $P < 10^{-5}$). Note that for mtDNA, the expected relatedness between individuals from the same matriline is just one whereas the expected relatedness between individuals from different matrilines is just 0. Therefore the high mtDNA relatedness detected here indicates that neighbouring colonies share in general the same matriline and therefore that mtDNA haplotypes showed a strong spatial clustering as seen in Fig. 2.

In agreement with the results on relatedness, no pattern of isolation by distance was detected within deme 2 (which contains a sufficient number of colonies to perform the analysis) for the microsatellite data. The Spearman rank correlation coefficient between genetic differentiation between individuals and the logarithm of geographical distance was not significant (Mantel test $P > 0.05$). The slope of the regression was negative and close to 0 ($b = -0.0027$).

These results revealed a strong population viscosity for the mtDNA at a fine spatial scale while such population viscosity is low (in the entire site) or nonexistent (in deme 2) for the microsatellite markers.

Genetic differentiation between localities

Exact tests of genic differentiation computed across all localities were highly significant at each microsatellite locus, as well as over all loci ($P < 10^{-5}$). Combined probability tests were also highly significant ($P < 10^{-5}$) for all pairs of localities. In agreement with this high genetic heterogeneity, the values of F_{ST}, and R_{ST} over all localities were high and varied among loci from 0.067 (DC18) to 0.42 (DC8), and from 0.03 (DC5) to 0.72 (DC29) for R_{ST}. The value of R_{ST} tended to be larger than the value of F_{ST} (Wilcoxon's signed rank test, $Z = 1.96$, $P = 0.049$). When using hypervariable loci F_{ST} is known to underestimate levels of genetic differentiation, with its maximal possible value being constrained by the average expected within-sample homozygosity (Hedrick 1999). In our study, 63% of the variation of F_{ST} among loci was explained by variation in the average expected homozygosity (Spearman's rank correlation $r_s = 0.833$, $N = 8$, $P = 0.01$), whereas such a correlation was not significant when performed with R_{ST} ($r_s = 0.095$, $N = 8$, $P > 0.05$). The significant correlation for F_{ST} indicates that F_{ST} values are influenced strongly by the extent of within-population genetic diversity, as mentioned by Charlesworth (1998). This emphasizes the importance of taking into account the level of genetic diversity when comparing F_{ST} values either between markers or between set of populations.

The results of the hierarchical analysis of molecular variance are given in Table 2 for microsatellite and mitochondrial markers. For both markers, significantly positive estimates of the genetic structure were obtained for both hierarchical levels. The fixation indices for mtDNA were two to three times larger than those obtained for microsatellites across all localities and five to six times larger between localities within region for both types of estimates.

If we consider each region separately, using microsatellites, both F- and R-statistics varied according to the region considered. They were largest in Attigundi ($F_{ST} = 0.29$; $R_{ST} = 0.31$) followed by Kottigehara ($F_{ST} = 0.10$; $R_{ST} = 0.001$) and Nagu ($F_{ST} = 0.05$; $R_{ST} = 0.006$), with all values statistically significant. Given that three localities were included in Attigundi, we also investigated the genetic differentiation between each pair of localities in Attigundi. A high and significant level of genetic differentiation was detected for both statistics whatever the pair of localities considered (between Attigundi 1 and 2: $F_{ST} = 0.29$; $R_{ST} = 0.26$; between Attigundi 1 and 3: $F_{ST} = 0.25$; $R_{ST} = 0.39$; between Attigundi 2 and 3: $F_{ST} = 0.34$; $R_{ST} = 0.23$).

We did not perform such an analysis using mtDNA, as most localities were characterized by a single unique haplotype. Interestingly, the two localities sharing the same mitochondrial haplotype were in the Attigundi region, which was the region characterized by the greatest amount of microsatellite genetic differentiation.

Table 2 Hierarchical analysis of molecular variance for microsatellite and mitochondrial markers using the number of different alleles, F_{ST} or haplotypes (equidistant Φ_{ST}) and the sum of squared size difference of alleles, R_{ST}, or a distance measure taking into account the haplotype sequences (euclidean ϕ_{ST}). The significance of the fixation indices was tested using the nonparametric approach of Excoffier *et al.* (1992), based on more than 1000 permutations of individuals (or haplotypes) among localities and among regions or within region according to the hierarchical level tested

	Microsatellites		Mitochondrial	
	F_{ST}	R_{ST}	Equidistant Φ_{ST}	Euclidean Φ_{ST}
Across all localities	0.26	0.38	0.75	0.97
Between localities within region	0.15	0.14	0.75	0.93

All values significant at $P < 0.0001$.

Using all localities, a significant isolation by distance was detected for microsatellites using both *F*-statistics (Mantel test between the logarithm of distance and $F_{ST}/(1 - F_{ST})$; $P = 0.04$) and *R*-statistics (Mantel test between the logarithm of distance and $R_{ST}/(1 - R_{ST})$; $P = 0.04$).

The genetic relationships between mtDNA haplotypes are reflected in the minimum spanning tree and the neighbour-joining tree in Fig. 3. Both trees showed that the haplotypes from Kottigehara and Attigundi regions clustered together, as did the three haplotypes of Kottigehara 1. On the other hand, the two haplotypes from Nagu are highly divergent. Moreover, the Kottigehara haplotypes appeared to be clearly separated from those of the other regions.

Male and female gene flow

The estimates of male and female gene flow are given in Table 3 for the different spatial scales (between demes within Kottigehara 1, between localities within region and across all localities). These values should not be considered

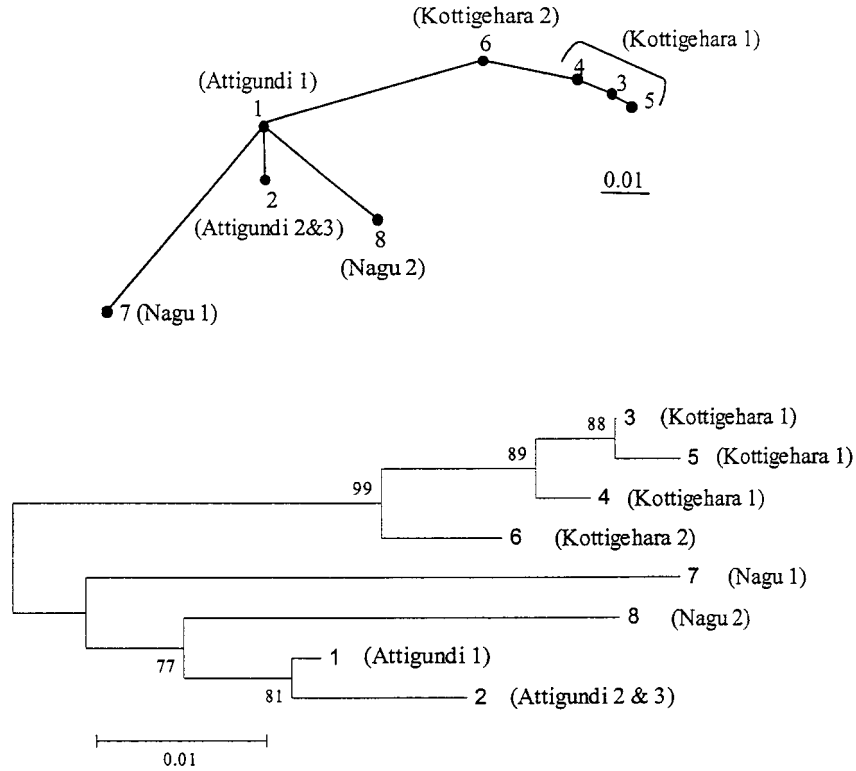

Fig. 3 Minimum spanning tree (top) and neighbour-joining tree (bottom) reflecting the mtDNA haplotypes relationships. Both trees were based on the genetic distance between mitochondrial sequences using the Jukes and Cantor correction. For the neighbour joining tree, bootstrap values are obtained from 500 iterations.

Table 3 Estimates of male (Nm_m) and female (Nm_f) gene flow, derived for the three spatial scales from the estimates of mitochondrial and microsatellite F_{ST}. For the two largest spatial scales, we also used R_{ST} estimates from microsatellites. The estimates of Nm_m and Nm_f assume that dispersal occurs before mating and take into account the haplodiploidy (see Materials and methods section for more details), allowing a direct estimation of the relative male and female gene flow

	Nm_m		Nm_f	
Spatial scale	From F_{ST}	From R_{ST}	From equidistant ϕ_{ST}	From euclidean ϕ_{ST}
Across all localities	2.2	1.6	0.3	0.03
Between localities within region	5	6	0.3	0.07
Between demes within Kottigehara 1	46.2	—	2.0	1.44

– Rst was not estimated at this lower spatial scale as the mutational process should not matter at this scale.

as 'true' estimates of the dispersal given the strong assumptions underlying the infinite island model (Bossart & Prowell 1998; Hedrick 1999; Whitlock & McCauley 1999). However, whatever the fixation index used, these estimates show clearly that female gene flow is very restricted or even nonexistent, regardless of the spatial scale considered, whereas male gene flow is efficient at small spatial scales but restricted at larger scales.

Discussion

Restricted female gene flow (mtDNA)

Because of the absence of a winged queen caste in *D. cyaneiventre*, a low level of gene flow is expected via female dispersal. The genetic structure observed for mtDNA strongly supports this hypothesis, as a highly significant genetic differentiation was observed at all spatial scales; within a deme (less than 100 m), between demes within a locality (a few hundred metres), between localities within region (few kilometres) and between all localities (up to 188 km). Basically, the estimates of female gene flow between-localities are lower than 1, regardless of the spatial scale considered. This suggests that female gene flow is not sufficient to prevent the divergence of the mtDNA due to mutational events. In agreement with this, differentiation between-localities at both spatial scales was larger when the degree of difference between the haplotypes was taken into account (euclidean distance). The genetic tree (Fig. 3) showed a phylogeographical structure of the mtDNA haplotypes. The three haplotypes of Kottigehara 1 and the haplotypes of localities from the same region clustered together (except for Nagu), suggesting that occasional migration events occur between nearby localities.

Interestingly, a very low genetic diversity was observed within localities. In fact, seven of eight localities contained only one haplotype, suggesting that new sites are colonized by a single migration event followed by a succession of colony buddings. Such very low genetic variability within localities for mtDNA is expected if colonization events are kin-structured and if migration after colonization is performed mainly by dispersing males (Wade *et al*. 1994). Even though each colonization event implies a strong bottleneck, the trace of this bottleneck will persist longer in cytoplasmic diversity than in nuclear diversity, especially when migration is mainly performed by males (Wade *et al*. 1994). This may explain why the genetic diversity of microsatellite markers in our study was not low. All these results suggest a metapopulation dynamic of mtDNA with extinction of populations and colonization of new sites by adjacent populations.

A very strong genetic differentiation of mtDNA has also been observed in the ant *Formica exsecta* between pastures separated by 1–6 km (Liautard & Keller 2001). Interestingly, *F. exsecta* queens can fly but do not seem to do so in nature. At a similar scale the value of genetic differentiation of mtDNA in the other studies on ants with dependent colony foundation is slightly lower, although highly significant (Ross & Shoemaker 1997; Ross *et al*. 1997; Tay *et al*. 1997; Goodisman & Ross 1998). All these studies show that dependent colony foundation in ants strongly restricts the dispersal ability of females.

Population viscosity for nuclear DNA

Restricted female dispersal due to dependent colony foundation is supposed to induce some population viscosity, i.e. an increase of genetic similarity between neighbouring colonies. Population viscosity using nuclear markers has often been detected in polygynous species in which dependent colony foundation predominates (Pamilo 1983; Crozier *et al*. 1984; Crozier & Pamilo 1986; Seppä & Pamilo 1995; Chapuisat *et al*. 1997; Beye *et al*. 1998). Interestingly, in the queenless ant *Rhytidoponera metallica*, in which colonies contain many mated reproductive workers, no population viscosity was detected (Chapuisat & Crozier 2001). However, the very low genetic relatedness estimated within nests strongly restricted the possibility to detect population viscosity. In this context, our study is informative and novel because *D. cyaneiventre* has a dependent colony foundation but is monogynous with a single nest per colony. We did not detect any population viscosity using nuclear markers at a very fine spatial scale (within a deme of 300 m × 150 m). This indicates an effective dispersal by males which is in agreement with the general lack of inbreeding observed in the populations studied. However, we did observe a significant but low genetic differentiation between demes in Kottigehara 1. This emphasizes the importance of scale and habitat heterogeneity when looking for population viscosity, as noted by Boomsma *et al*. (1990).

Genetic differentiation among localities for nuclear DNA

In *D. cyaneiventre*, restricted female and male dispersal is associated clearly with very high levels of genetic differentiation between localities. Interestingly, the level of genetic differentiation was three to six times larger in Attigundi than in the two other regions. Different explanations can be put forward, most of them not exclusive. First, these differences might be due to an underestimation of genetic differentiation in Kottigehara and Nagu. However, this is unlikely given that the level of differentiation in these regions was probably too low to be constrained by the level of genetic diversity. Second, male gene flow in the Attigundi region might be more limited. This could be due to environmental factors but the habitat

did not appear to be more fragmented than in the other regions. Lower male gene flow could also result from a lower rate of gamergate replacement (see below). A third explanation could be a higher level of genetic drift due to lower effective population size. Indeed, the restricted sample size in the Attigundi localities (see Table 1) reflects the low density of colonies in these areas. Finally, different population histories between regions could also result in variations in the level of genetic differentiation.

Whatever these differences between regions, genetic differentiation is very high in *D. cyaneiventre*. The few studies that have been made of large-scale genetic differentiation in ants have also detected significant genetic differentiation using nuclear markers, but the F_{ST} values were generally lower (Ward 1980; Pamilo 1982; Ross & Shoemaker 1997; Ross *et al*. 1997). For instance, the fixation indices between isolated populations of *F. cinerea* in northern Europe were 0.11 (F_{ST}) and 0.08 (R_{ST}) (Goropashnaya *et al*. 2001), two to four times lower than in our study. The values estimated in our study are more of the order of those obtained from microsatellite data when comparing populations from different continents (e.g. Franck *et al*. 1998 for honey bees; and Paetkau *et al*. 1999; Goodman *et al*. 2001; for large mammals). With such levels of genetic differentiation, substantial homoplasy might underestimate the genetic differentiation due to the high mutation rates and finite number of unique alleles in microsatellites (Jarne & Lagoda 1996). However, the main result indicating that localities are strongly genetically differentiated cannot be questioned.

Such very restricted gene flow might favour processes of behavioural divergence. A population of *Diacamma* in the south of India, called *D.* '*nilgiri*', was discovered which differs by a social behaviour regulating reproduction (Peeters *et al*. 1992) from its closest relative *D. ceylonense* (C. Peeters, unpublished data, http://www.biologie.ens.fr/fr/ecologie/phylogenie.html). The genetic differentiation between these two taxa is currently being analysed. A large number of *Diacamma* species are found in the southeast Asia and most of them seem to show a restricted geographical distribution (C. Peeters, unpublished data). Even though we lack precise information on these distributions, this might reflect a high rate of speciation in this genus of queenless ants which could be due to their very restricted female dispersal abilities.

Male-biased gene flow

The comparison of genetic structures observed using mtDNA and microsatellites makes it possible to assess the extent of sex-biased dispersal (Ennos 1994; McCauley 1995). Even without sex-biased dispersal, higher population genetic structure is expected with mtDNA markers because of the smaller effective population size and the correspondingly larger susceptibility to genetic drift, compared to nuclear markers (Chesser & Baker 1996). In our study, the discrepancy of genetic structure observed between the two markers is so strong that it probably reflects true differences in dispersal pattern between the two sexes, and in particular a very restricted female gene flow (see above).

Male gene flow is clearly lower between localities than between demes within a locality. Such a decrease of male gene flow with increasing geographical distance was also detected in the marginally significant test of isolation by distance. This result should, however, be interpreted with caution. Localities separated by high levels of genetic differentiation might be more likely to be at a mutation/drift than a migration/drift equilibrium, as the mutation rate can be high compared to the migration rate, especially for microsatellite markers (Goldstein *et al*. 1995). This could lead to an overestimation of gene flow between regions if homoplasy is important (Gaggiotti *et al*. 1999; Balloux *et al*. 2000). In this case, the lower estimate of gene flow based on R_{ST} might be more reliable than that based on F_{ST} (Gaggiotti *et al*. 1999). From these gene flow estimates, we can say that within a locality, male gene flow is about 20–30 times higher than female gene flow (see Table 3). At larger spatial scales, estimating the relative male-biased dispersal does not make sense given that female gene flow is likely to be simply nonexistent (see above).

Direct estimates of male ant dispersal ability are very scarce, and nothing is known about the flying abilities of male *Diacamma*. In laboratory experiments on *S. invicta*, males flew at approximately 2 m/s for a maximum of 30 min (Vogt *et al*. 2000). This suggests that males may rely on passive flight for long-distance dispersal (more than a few kilometres). However, male dispersal does not necessarily imply gene flow. The availability of future gamergates willing to mate is a prerequisite of male gene flow. This situation occurs only after the replacement of the gamergate or after colony fission. The genetic trace of gamergate replacement has been detected in a genetic analysis of colonies collected at Kottigehara 1 by André *et al*. (2001), and the frequency of gamergate replacement was indirectly estimated in this locality to be around 0.8 per colony per year (C. Doums unpublished data). The temporal and spatial variability of the frequency of gamergate replacement is still unknown but might be crucial parameters affecting male gene flow.

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References

André JB, Peeters C, Doums C (2001) Serial polygyny and colony genetic structure in the monogynous queenless ant *Diacamma cyaneiventre*. *Behavioral Ecology and Sociobiology*, **50**, 72–80.

Balloux F, Brunner H, Lugon MN, Hausser J, Goudet J (2000) Microsatellites can be misleading: an empirical and simulation study. *Evolution*, **54**, 1414–1422.

Berg LM, Lascoux M, Pamilo P (1998) The infinite island model with sex-differentiated gene flow. *Heredity*, **81**, 63–68.

Beye M, Neumann P, Chapuisat M, Pamilo P, Moritz RFA (1998) Nestmate recognition and the genetic relatedness of nests in the ant *Formica pratensis*. *Behavioral Ecology and Sociobiology*, **43**, 67–72.

Boomsma JJ, Brouwer AH, Van Loon AJ (1990) A new polygynous *Lasius* species (Hymenoptera: Formicidae) from central Europe. II. Allozymatic confirmation of species status and social structure. *Insectes Sociaux*, **37**, 363–375.

Bossart JL, Prowell DP (1998) Genetic estimates of population structure and gene flow: limitations, lessons and new directions. *Trends in Ecology and Evolution*, **13**, 202–206.

Bourke AFG, Franks NR (1995) *Social Evolution in Ants*. Princeton University Press, Princeton, NJ.

Chapuisat M, Crozier R (2001) Low relatedness among cooperatively breeding workers of the greenhead ant *Rhytidoponera metallica*. *Journal of Evolutionary Biology*, **14**, 564–543.

Chapuisat M, Goudet J, Keller L (1997) Microsatellites reveal high population viscosity and limited dispersal in the ant *Formica paralugubris*. *Evolution*, **51**, 475–482.

Charlesworth B (1998) Measures of divergence between populations and the effect of forces that reduce variability. *Molecular Biology and Evolution*, **15**, 538–543.

Chesser RK, Baker RJ (1996) Effective sizes and dynamics of uniparentally and diparentally inherited genes. *Genetics*, **144**, 1225–1235.

Crozier RH, Pamilo P (1986) Relatedness within and between colonies of a queenless ant species of the genus *Rhytidoponera* (Hymenoptera: Formicidae). *Entomologia Generalis*, **11**, 113–117.

Crozier RH, Pamilo P, Crozier YC (1984) Relatedness and microgeographic genetic variation in *Rhytidoponera mayri*, an Australian arid-zone ant. *Behavioural Ecology and Sociobiology*, **15**, 143–150.

Doums C (1999) Characterisation of microsatellites in the queenless ponerine ant *Diacamma cyaneiventre*. *Molecular Ecology*, **8**, 1957–1959.

Ennos RA (1994) Estimating the relative rates of pollen and seed migration among plant populations. *Heredity*, **722**, 250–259.

Excoffier L, Smouse P, Quattri J (1992) Analysis of molecular variance inferred from metric distances among DNA haplotype: application to human mitochondrial DNA restriction data. *Genetics*, **131**, 343–359.

Franck P, Garnery L, Solignac M, Cornuet JM (1998) The origin of West European subspecies of honeybees (*Apis mellifera*): new insights from microsatellite and mitochondrial data. *Evolution*, **52**, 1119–1134.

Gabriel KR, Sokal RR (1969) A new statistical approach to geographic variation analysis. *Systematic Zoology*, **18**, 259–278.

Gaggiotti OE, Lange O, Rassmann K, Gliddon C (1999) A comparison of two indirect methods for estimating average levels of gene flow using microsatellite data. *Molecular Ecology*, **8**, 1513–1520.

Giraud T, Blatrix R, Poteaux C, Solignac M, Jaisson P (2000) Population structure and mating biology of the polygynous ponerine ant *Gnamptogenys striatula* in Brazil. *Molecular Ecology*, **9**, 1835–1841.

Goldstein DB, Ruiz Linares A, Cavalli-Sforza LL, Feldman MW (1995) An evalutation of genetic distances for use with microsatellite loci. *Genetics*, **139**, 463–471.

Goodisman MAD, Ross KG (1998) A test of queen recruitment models using nuclear and mitochondrial markers in the fire ant *Solenopsis invicta*. *Evolution*, **52**, 1416–1422.

Goodman SJ, Tamate HB, Wilson R, Nagata J, Tatsuzawa S, Swanson GM *et al.* (2001) Bottlenecks, drift and differentiation: The population structure and demographic history of sika deer (*Cervus nippon*) in the Japanese archipelago. *Molecular Ecology*, **10**, 1357–1370.

Goropashnaya AV, Seppä P, Pamilo P (2001) Social and genetic characteristics of geographically isolated populations in the ant *Formica nicerea*. *Molecular Ecology*, **10**, 2807–2818.

Goudet J (1995) FSTAT (vers. 1.2): a computer program to calculate *F*-statistics. *Journal of Heredity*, **86**, 485–486.

Hartl DL, Clark AG (1997) *Principles of Population Genetics*. Sinauer Associates, Sunderland, MA.

Hedrick PW (1999) Highly variable loci and their interpretation in evolution and conservation. *Evolution*, **53**, 313–318.

Jarne P, Lagoda PJL (1996) Microsatellites, from molecules to populations and back. *Trends in Ecology and Evolution*, **11**, 424–429.

Keller L (1991) Queen number, mode of colony founding, and queen reproductive success in ants (Hymenoptera Formicidae). *Ethology, Ecology and Evolution*, **3**, 307–316.

Kelly JK (1992) Restricted migration and the evolution of altruism. *Evolution*, **37**, 1492–1495.

Kumar S, Tamura K, Jakobsen IB, Nei M (2001) MEGA *2: Molecular Evolutionary Genetics Analysis Software*. Arizona State University, Tempe, AZ.

Liautard C, Keller L (2001) Restricted effective queen dispersal at a microgeographic scale in polygynous populations of the ant *Formica exsecta*. *Evolution*, **55**, 2484–2491.

McCauley DE (1995) The use of chloroplast DNA polymorphism in studies of gene flow in plants. *Trends in Ecology and Evolution*, **10**, 198–202.

Nei M (1987) *Molecular Evolutionary Genetics*. Columbia University Press, New York.

Nei M, Kumar S (2000) *Molecular Evolution and Phylogenetics*. Oxford University Press, New York.

Orita M, Iwahana H, Kanazawa H, Hayashi K, Sekiya T (1989) Detection of polymorphisms of human DNA by gel electrophoresis as single-strand conformation polymorphisms. *Proceedings of the National Academy of Science USA*, **86**, 2766–2770.

Paetkau D, Amstrup SC, Born EW *et al.* (1999) Genetic structure of the world's polar bear populations. *Molecular Ecology*, **8**, 1571–1584.

Pamilo P (1982) Genetic population structure in polygynous *Formica* ants. *Heredity*, **48**, 95–106.

Pamilo P (1983) Genetic differentiation within subdivided populations of *Formica* ants. *Evolution*, **37**, 1010–1022.

Peeters C, Billen J, Hölldobler B (1992) Alternative dominance mechanisms regulating monogyny in the queenless ant genus *Diacamma*. *Naturwissenschaften*, **79**, 572–573.

Peeters C, Ito F (2001) Colony dispersal and the evolution of queen morphology in social Hymenoptera. *Annual Review of Entomology*, **46**, 601–630.

Queller DC (1992) Does population viscosity promote kin selection. *Trends in Ecology and Evolution*, **7**, 322–324.

Queller DC, Goodnight KF (1989) Estimating relatedness using genetic markers. *Evolution*, **43**, 258–275.

Raymond M, Rousset F (1995) GENEPOP Version 1.2: population genetics software for exact tests and ecumenicism. *Journal of Heredity*, **86**, 248–249.

Ross KG (2001) Molecular ecology of social behaviour: analyses of breeding systems and genetic structure. *Molecular Ecology*, **10**, 265–284.

Ross KG, Krieger MJB, Shoemaker DD, Vargo EL, Keller L (1997) Hierarchical analysis of genetic structure in native fire ant populations: results from three classes of molecular markers. *Genetics*, **147**, 642–655.

Ross KG, Shoemaker DD (1997) Nuclear and mitochondrial genetic structure in two social forms of the fire ant *Solenopsis invicta*: insights into transitions to an alternate social organization. *Heredity*, **78**, 590–602.

Ross KG, Shoemaker DD, Krieger MJB, DeHeer CJ, Keller L (1999) Assessing genetic structure with multiple classes of molecular markers: a case study involving the introduced fire ant *Solenopsis invicta*. *Molecular Biology and Evolution*, **16**, 525–543.

Rousset F (1996) Equilibrium values of measures of population subdivision for stepwise mutation processes. *Genetics*, **142**, 1357–1362.

Rousset F (2000) Genetic differentiation between individuals. *Journal of Evolutionary Biology*, **13**, 58–62.

Schneider S, Roessli D, Excoffier L (2000) ARLEQUIN, Version 2.000: *a Software for Population Genetics Data Analysis*. Genetics and Biometry Laboratory, University of Geneva, Switzerland.

Seppä P, Pamilo P (1995) Gene flow and population viscosity in *Myrmica* ants. *Heredity*, **74**, 200–209.

Slatkin M (1995) A measure of population subdivision based on microsatellite allele frequencies. *Genetics*, **139**, 457–462.

Sokal RR, Rohlf FJ (1995) *Biometry*. Freeman Co., New York.

Stille M, Stille B (1993) Intrapopulation nestclusters of maternal mtDNA lineages in the polygynous ant *Leptothorax acervorum* (Hymenoptera: Formicidae). *Insect Molecular Biology*, **1**, 117–121.

Sundström L (1993) Genetic population structure and sociogenetic organisation in *Formica truncorum* (Hymenoptera; Formicidae). *Behavioral Ecology and Sociobiology*, **33**, 345–354.

Tay TW, Cook JM, Rowe DJ, Crozier RH (1997) Migration between nests in the Australian arid-zone ant *Rhytidoponera* sp. *12* revealed by DGGE analyses of mitochondrial DNA. *Molecular Ecology*, **6**, 403–411.

Tsutsui ND, Case TJ (2001) Population genetics and colony structure of the argentine ant (*Linepithema humile*) in its native and introduced ranges. *Evolution*, **55**, 976–985.

Vogt JT, Appel AG, West MS (2000) Flight energetics and dispersal capability of the fire ant, *Solenopsis invicta* Buren. *Journal of Insect Physiology*, **46**, 697–707.

Wade MJ, McKnight ML, Shaffer HB (1994) The effects of kin-structured colonization on nuclear and cytoplasmic genetic diversity. *Evolution*, **48**, 1114–1120.

Ward PS (1980) Genetic variation and population differentiation in the *Rhytidoponera impressa* group, a species complex of ponerine ants (Hymenoptera: Formicidae). *Evolution*, **34**, 1060–1076.

Waser P, Strobeck C (1998) Genetic signatures of interpopulation dispersal. *Trends in Ecology and Evolution*, **13**, 43–44.

Weir BS, Cockerham CC (1984) Estimating *F*-statistics for the analysis of population structure. *Evolution*, **38**, 1358–1370.

Whitlock MC, McCauley DE (1999) Indirect measures of gene flow and migration: $F_{ST} \neq 1/(4Nm + 1)$. *Heredity*, **82**, 117–125.

Wright S (1978) *Evolution and the Genetics of Populations*. University of Chicago Press, Chicago, IL.

This study is part of a research programme on the behaviour and genetics of queenless *Diacamma* performed in collaboration with the group of R. Gadagkar in Bangalore (India). Claudie Doums is a molecular evolutionary biologist with a particular interest in social insects. At the time of this study, Humberto Cabrera was a masters student undertaking the genetic screening of individuals using microsatellites. Christian Peeters studies the behavioural regulation of reproduction in queenless ants and conducted fieldwork in this study.

Appendix I

Observed allele frequency distributions of eight microsatellite loci in seven Indian localities of *Diacamma cyaneiventre*. Alleles are identified by the size (bp) of the amplified fragment. The number of chromosomes successfully screened per locus is given between parenthesis for each locality. Loci are further described in Doums (1999). The gene diversity was calculated using an unbiased estimator (Nei 1987; 164)

Locus DC5	Allele (bp)	Attigundi 1 (30)	Attigundi 2 (24)	Attigundi 3 (24)	Kottigehara 1 (200)	Kottigehara 2 (40)	Nagu 1 (48)	Nagu 2 (54)
	137							0.037
	141	0.200						
	143		0.042		0.165	0.025	0.021	
	145	0.021						
	147	0.500	0.250	0.708	0.255	0.125	0.625	0.407
	149	0.033	0.708	0.250	0.580	0.850	0.250	0.537
	151	0.267		0.042			0.083	0.019
	Gene diversity	0.657	0.447	0.451	0.574	0.267	0.554	0.552
	F_{IS}	−0.116*	−0.305	−0.109	0.007	−0.123	0.324†	−0.208
Locus DC8	Allele (bp)	Attigundi 1 (30)	Attigundi 2 (24)	Attigundi 3 (24)	Kottigehara 1 (192)	Kottigehara 2 (40)	Nagu 1 (46)	Nagu 2 (54)
	128	0.167						
	130						0.022	
	132						0.304	0.074
	134		0.167	0.042			0.022	
	136	0.633			0.005			0.056
	138	0.033	0.042		0.089	0.100	0.500	0.722
	140				0.089		0.130	0.111
	142	0.167	0.250	0.917	0.807	0.600	0.022	0.019
	144				0.010	0.300		0.019
	146		0.458	0.042				
	148		0.083					
	Gene diversity	0.557	0.716	0.163	0.334	0.550	0.658	0.465
	F_{IS}	−0.197	−0.164	−0.023	0.003	−0.273	0.339	−0.035
Locus DC11	Allele (bp)	Attigundi 1 (28)	Attigundi 2 (24)	Attigundi 3 (24)	Kottigehara 1 (202)	Kottigehara 2 (40)	Nagu 1 (42)	Nagu 2 (54)
	205				0.005			
	209	0.036	0.042					0.019
	211							0.019
	213				0.005	0.150		
	215				0.030	0.100		
	219	0.643	0.208	0.273	0.574	0.100	0.024	
	221	0.143	0.375	0.136	0.069	0.225	0.286	0.093
	223						0.024	0.019
	225						0.024	0.037
	227				0.010	0.200	0.024	0.056
	229	0.143			0.020	0.025	0.071	
	231			0.455	0.035	0.025	0.048	0.019
	233		0.25		0.025		0.048	0.111
	235						0.095	0.019
	237				0.040	0.025	0.048	0.093
	239				0.005	0.025	0.048	0.074
	241				0.005		0.048	0.130
	243	0.036			0.025		0.024	0.019
	245				0.005		0.024	
	247						0.071	0.074
	249				0.005	0.050	0.024	0.074
	251			0.045	0.129			0.019
	253		0.083		0.015	0.075	0.048	0.037
	255			0.045				
	257			0.045				0.037
	259		0.042					0.056
	263						0.024	
	Gene diversity	0.566	0.792	0.723	0.646	0.879	0.904	0.945
	F_{IS}	0.117	0.474†	−0.132	0.004	0.033	0.051	0.177*

Appendix I *Continued*

Locus DC18	Allele (bp)	Attigundi 1 (30)	Attigundi 2 (22)	Attigundi 3 (24)	Kottigehara 1 (146)	Kottigehara 2 (40)	Nagu 1 (22)	Nagu 2 (52)
	182							0.019
	184	0.067						
	188	0.033						
	190	0.033						0.019
	194				0.007			
	198	0.033			0.041	0.075		
	200	0.033			0.075			
	202	0.133			0.096		0.045	
	204				0.034			
	206				0.021			
	208				0.014	0.025	0.045	
	210				0.021	0.050		
	212				0.240	0.050	0.136	
	214	0.033	0.045			0.125		0.045
	216	0.133	0.136		0.014	0.125	0.096	
	218	0.033	0.045	0.167	0.021			0.019
	220		0.091		0.048	0.050		0.077
	222		0.045	0.042		0.400	0.045	0.115
	224	0.133	0.091	0.167				0.115
	226		0.091	0.083	0.007		0.182	0.058
	228	0.167	0.091		0.048			0.154
	230		0.318	0.042	0.007			0.058
	232					0.050	0.045	0.019
	234		0.045		0.062			0.019
	236	0.067			0.048	0.050	0.227	0.038
	238	0.067		0.083	0.096		0.091	0.019
	240				0.041			
	242				0.021			0.038
	244			0.083			0.045	0.019
	246				0.007			
	248				0.014			
	250			0.042				0.077
	252			0.042	0.014			0.019
	254				0.007		0.045	
	256			0.042			0.045	
	260			0.042				0.019
	262	0.033						
	266			0.167				
	Gene diversity	0.926	0.873	0.920	0.908	0.814	0.927	0.935
	F_{IS}	−0.080	−0.146	−0.086	0.125‡	0.202*	0.314	0.053
Locus DC19	Allele (bp)	Attigundi 1 (30)	Attigundi 2 (20)	Attigundi 3 (22)	Kottigehara 1 (194)	Kottigehara 2 (36)	Nagu 1 (42)	Nagu 2 (56)
	221						0.333	0.607
	223						0.357	0.054
	225				0.005			
	229		0.050		0.082	0.083	0.167	0.071
	231	0.033					0.024	0.036
	233	0.200			0.242		0.024	0.107
	235			0.182	0.165	0.250		
	237	0.633	0.100	0.091	0.196	0.139	0.095	0.107
	239	0.067		0.136	0.021	0.056		
	241			0.273	0.253	0.222		
	243		0.300		0.036	0.028		
	245	0.067		0.273		0.222		0.018
	247			0.045				
	249		0.150					
	253		0.150					
	255		0.150					
	257		0.100					
	Gene diversity	0.574	0.867	0.827	0.808	0.833	0.739	0.608
	F_{IS}	0.303	0.077	0.011	0.030†	0.067	−0.095	−0.175

Appendix I *Continued*

Locus DC20	Allele	Attigundi	Attigundi	Attigundi	Kottigehara	Kottigehara	Nagu	Nagu
	(bp)	1 (30)	2 (24)	3 (24)	1 (200)	2 (36)	1 (48)	2 (56)
	152				0.005		0.021	0.054
	154				0.005			
	156				0.115	0.167	0.062	0.018
	158					0.139	0.854	0.679
	160	0.400	0.958	0.083	0.235	0.472	0.062	0.214
	162	0.500	0.042	0.917	0.360	0.167	0.018	
	164	0.100			0.155	0.056		0.018
	166	0.125						
	Gene diversity	0.595	0.083	0.159	0.766	0.717	0.270	0.501
	F_{IS}	−0.232	0.000	−0.048	−0.018	−0.084	0.383*	0.215
Locus DC29	Allele	Attigundi	Attigundi	Attigundi	Kottigehara	Kottigehara	Nagu	Nagu
	(bp)	1 (28)	2 (22)	3 (24)	1 (200)	2 (38)	1 (46)	2 (54)
	208				0.110	0.211		
	212	0.036		0.417	0.075	0.132		
	214	0.821	0.318	0.500	0.815	0.658	0.087	0.148
	216	0.143	0.682	0.083			0.109	0.019
	218						0.022	0.019
	220						0.304	0.185
	222						0.283	0.444
	224						0.065	0.167
	226						0.043	
	228						0.022	0.019
	230						0.065	
	Gene diversity	0.313	0.464	0.595	0.320	0.519	0.815	0.729
	F_{IS}	−0.140	0.412	0.019	0.093	−0.014	0.040	−0.169
Locus DC52	Allele	Attigundi	Attigundi	Attigundi	Kottigehara	Kottigehara	Nagu	Nagu
	(bp)	1 (30)	2 (24)	3 (24)	1 (198)	2 (40)	1 (48)	2 (56)
	147							0.125
	149							0.411
	155						0.521	0.161
	157						0.042	0.304
	161		0.750			0.175	0.417	
	163	0.433		0.083	0.202			
	165					0.250	0.021	
	167		0.167	0.708		0.200		
	169	0.200	0.042	0.208	0.414	0.025		
	171	0.267	0.042		0.045			
	173	0.033			0.071	0.025		
	175	0.033						
	177	0.033			0.247	0.125		
	179					0.050		
	181					0.150		
	189							
	Gene diversity	0.717	0.428	0.477	0.723	0.846	0.563	0.714
	F_{IS}	−0.209	0.221	0.476	0.036	−0.005	−0.109*	0.300‡

*$P < 0.05$; †$P < 0.005$; ‡$P < 0.0009$ the threshold value after a Bonferonni correction.

ANNEXE 2

Shift in the behaviours regulating monogyny is associated with high genetic differentiation in the queenless ant *Diacamma ceylonense*

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Research article

Shift in the behaviours regulating monogyny is associated with high genetic differentiation in the queenless ant *Diacamma ceylonense*

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Summary. In the queenless ant genus *Diacamma*, one mated worker (called gamergate) maintains reproductive monopoly in a colony by mutilating newly emerged workers. However, in several populations from south India, referred to as *'nilgiri'*, gamergates do not mutilate their nestmates but monopolize reproduction using dominance interactions . Various lines of evidence indicate that *'nilgiri'* populations are closely related to the neighboring species *D. ceylonense*. To determine whether this important behavioural difference between *'nilgiri'* and *D. ceylonense* is associated with significant genetic differentiation, we have used microsatellite and mitochondrial markers to examine genetic variation within and between *'nilgiri'* and *D. ceylonense*. We found a very high genetic differentiation between the two forms, which suggests a lack of gene flow. There was an unexpected pattern of mitochondrial variation, because all *'nilgiri'* populations show identical or very closely related COII sequences except one population with a very different haplotype. This divergent haplotype is genetically much more distant from the other *'nilgiri'* haplotypes than are *D. ceylonense* haplotypes. This pattern is not observed at the nuclear level, which suggests that introgression of mitochondrial DNA probably occurred in some *'nilgiri'* populations.

Key words: Regulation of reproduction, genetic differentiation, microsatellites, mtDNA introgression, gamergates.

Introduction

Social insects generally exhibit extreme reproductive skew and this is achieved in various ways. In the ant subfamily Ponerinae, several species lack the queen caste altogether (Peeters, 1991). In such ants, workers have retained a functional spermatheca and are able to mate and produce diploid offspring. In colonies of queenless ants, one or more mated worker, called gamergate, monopolizes reproduction using dominance hierarchies based on aggression (Monnin and Peeters, 1999; Monnin and Ratnieks, 2001). However, in the monogynous genus *Diacamma*, the gamergate enforces her monopoly by a unique behavioural mechanism. All workers are born with a pair of innervated dorsal appendages ('gemmae') that apparently release an exocrine signal (Peeters and Billen, 1991; Gronenberg and Peeters, 1993). Only workers that keep their gemmae are able to perform sexual calling and mate with a foreign male. The gamergate mutilates the gemmae of all freshly emerged workers, thus irreversibly preventing them from sexual reproduction. This mode of regulation of monogyny appears to be general in the *Diacamma* genus (Fukumoto et al., 1989; Peeters and Higashi, 1989; Sommer et al., 1993; André et al., 2001; Cuvillier-Hot et al., 2002) with only one known exception. In several Indian populations, referred to as *'nilgiri'*, the gamergates do not mutilate their nestmates and yet retain reproductive monopoly (Peeters et al. 1992, in which *'nilgiri'* is incorrectly called *D. vagans*). These populations offer a unique opportunity to investigate evolutionary shifts in reproductive regulation.

As a first step, we assessed the level of genetic differentiation between these peculiar populations and their closest known relatives. Several lines of evidence suggest that *'nilgiri'* is closely related to *D. ceylonense*, of which the nearest population is about 80 kilometres distant (Fig. 1). (i) *'nilgiri'* workers are morphologically indistinguishable from *D. ceylonense* except that they all retain their gemmae. (ii) A molecular phylogeny of the *Diacamma* genus has shown that *D. ceylonense* is the closest known relative of *'nilgiri'* (http://www.biologie.ens.fr/fr/ecologie/thematiques/socialiteetgenetique/molecularphylogeny/molecularphylogeny.html). (iii) Crossing experiments in the laboratory have demonstrated that *'nilgiri'* and *D. ceylonense* can produce viable F1 hybrids (L. Cournault, unpublished results), though it is not currently known whether these F1 hybrids are fertile. These data indicate that *'nilgiri'* are closely related to *D. ceylonense*

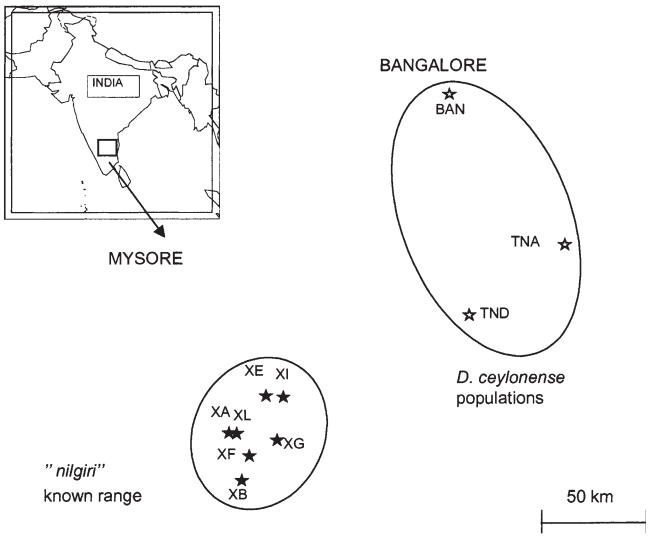

Figure 1. Geographical location of *'nilgiri'* (filled stars) and *D. ceylonense* populations (open stars) in the states of Karnataka and Tamil Nadu. The known range of '*nilgiri*' includes the town of Masinugodi

but we ignore whether they are only atypical populations or a distinct species. To account for this ambiguity, we thereafter refer to *'nilgiri'* and *D. ceylonense* as 'forms'.

To determine whether the crucial difference in behavioural regulation between *'nilgiri'* and *D. ceylonense* is associated with significant genetic differentiation, we examined nuclear and mitochondrial variation for seven populations of *'nilgiri'* and three populations of *D. ceylonense*. We then compared the differentiation observed within *'nilgiri'* populations and within *D. ceylonense* populations to that between the two forms.

Material and methods

Samples

D. ceylonense is distributed in the south east of India (Karnataka and Tamil Nadu states) and in Sri Lanka, while *'nilgiri'* inhabits a very small area (about 10 × 30 km) north of the Nilgiri Hills, on the western edge of the *D. ceylonense* range (C. Peeters, pers. obs.). *'nilgiri'* was sampled at seven localities that cover their entire known range. *D. ceylonense* was sampled at three localities located more than 60 km apart (Fig. 1). In each population, a single worker from 15 to 27 colonies was analysed with seven microsatellite loci (Table 1). Only one individual was sampled per colony to avoid the non-independence of genotypes due to intracolony relatedness. The maximum distance between two colonies of a population was about one kilometre.

Genetic analyses

DNA was extracted from whole workers with QIAgen DNAeasy kit, following the manufacturer protocol (Valencia, CA). Extracted DNA was resuspended in 100 µl elution buffer.

To study nuclear polymorphism, we used 4 microsatellites (DC8, DC11, DC20 and DC52) developed for *Diacamma cyaneiventre* that are polymorphic in *'nilgiri'* or *D. ceylonense* (Doums, 1999) and another 3 loci (DCI-56, DCI-78 and DCI-2122) developed for *D. ceylonense* (Gopinath, 2001). One primer of each pair was 5′-labeled with 6-FAM, NED or VIC derivative of fluoerescein (Applied Biosystems). Each PCR reaction was run in a 10 µl volume containing 1 µl of DNA solution, 100 µM of each dNTP, 0.15 µM of each primer and 0.1 units of Taq polymerase. Loci DC8, DC20, DC52, DCI-56, DCI-78 and DCI-2122 were coamplified. Thermocycle conditions were 94 °C for 30 s, 55 °C for 30 s and 72 °C for 30 s, for a total of 30 cycles. The PCR products were loaded on an ABI Prism 310 (Applied Biosystems). Allele sizes were estimated using the GENESCAN software. For six out of seven loci, we observed shared alleles between *'nilgiri'* and *D. ceylonense* (see Results). To determine whether these shared alleles are truly identical by descent, we sequenced one shared allele per locus in one individual from both *'nilgiri'* and *D. ceylonense*.

Mitochondrial DNA variation was assayed following the amplification of a 731 bp fragment of the Cytochrome Oxidase II gene. The fragment was amplified by PCR using the following primer pair.

CO2-P ATA AAG AAT TTC TTT TAT TA and
CO2-M AAA ATC AAT ACT AAT TAA GT

Each PCR reaction was run in a 50 µl volume containing 1 µl of DNA solution, 200 µM of each dNTP, 0.15 µM of each primer and 1.25 units of Taq polymerase. Thermocycle conditions were 94 °C for 45 s, 47 °C for 45 s, and 72 °C for 1 min, for a total of 30 cycles. Purified template DNA was sequenced with an ABI 310 automatic sequencer (Perkin-Elmer). To determine the importance of within population polymorphism, 15 individuals were first scored in the *'nilgiri'* population XE. Given the very low level of variability that we observed (see Results), only five individuals per population were subsequently scored in all other populations.

Data analysis

The analysis of the microsatellite data was carried out with GENEPOP version 3.3 (Raymond and Rousset, 1995) and Arlequin version 2.00 (Schneider et al., 2000). Hardy-Weinberg equilibrium was tested for the seven loci at each locality using exact probability tests employing a Markov chain to estimate without bias the exact probability value of this test (Guo and Thompson, 1992). To ensure independence of loci, we tested linkage disequilibrium in each population between all pairs of loci. For both Hardy-Weinberg and linkage disequilibrium tests, we use the Bonferroni correction to take multiple tests into account.

The significance of the genetic differentiation between localities was examined by conducting exact tests of allele frequency differentiation with GENEPOP 3.3. The joint probability of differentiation over all microsatellite loci was obtained using Fisher's combined probability test (Sokal and Rolf, 1995). Additionally, we have calculated both F_{ST} (Weir and Cockerham 1984) and R_{ST} statistics (Slatkin, 1995; Rousset, 1996). F_{ST} statistics are based on probabilities of identity between alleles and have been developed for loci evolving under an Infinite Allele Model (IAM). In contrast, R_{ST} statistics are based on size differences between alleles and have been developed for the estimation of genetic distances and population differentiation under a Stepwise Mutation Model (SMM). Because microsatellites are thought to evolve predominantly under SMM (Rienzo et al., 1998; Weber and Wong, 1993; Xu et al., 2000), R_{ST} statistics could be thought as better suited to the analysis of microsatellite data. However, a recent study (Balloux and Goudet, 2002) has shown that even under a strict SMM none of the two statistics is best overall, the best estimator being function of the level of gene flow. We have thus calculated both statistics. To determine whether population and form have an influence on genetic variability, we have performed a hierarchical estimation of *F* and *R* statistics with a two-level hierarchical AMOVA (Evans, 1995) using the software Arlequin (Schneider et al., 2000).

We have tested whether Slatkin's isolation-by-distance model (Slatkin, 1983) of increased genetic distance with increased geographic distance between populations is appropriate for populations of *'nilgiri'*. The significance of the Pearson correlation coefficient between genetic differentiation and geographic distance was assessed with a Mantel test using GENEPOP 3.3.

Table 1. Geographical location of populations and genetic diversity of mitochondrial and microsatellite loci, with Ncol the number of sampled colonies, mtDNA haplotype the mitochondrial haplotypes observed in five individuals, $\overline{Na}$ the mean number of microsatellite allele ± standard deviation and $\overline{He}$ the mean heterozygosity ± standard deviation

Population	Location	Ncol	mtDNA haplotype	$\overline{Na}$ ± s.d.	$\overline{He}$ ± s.d.
'*nilgiri*'					
XA	11°42′12.6″N 76°38′28.8″E	23	H1, H5	2.6 ± 1.3	0.35 ± 0.21
XB	11°34′32.7″N 76°38′48.2″E	15	H7, H8	3.0 ± 1.9	0.41 ± 0.29
XE	11°47′22.7″N 76°45′53.8″E	18	H1, H6	2.7 ± 1.4	0.46 ± 0.25
XF	11°39′03.3″N 76°39′07.9″E	27	H2	3.7 ± 2.0	0.46 ± 0.22
XG	11°39′25.4″N 76°42′41.0″E	15	H3	5.1 ± 2.7	0.65 ± 0.19
XI	11°46′58.2″N 76°47′44.3″E	21	H1	3.3 ± 1.6	0.34 ± 0.18
XL	11°42′08.0″N 76°38′59.0″E	20	H4	2.3 ± 1.0	0.44 ± 0.29
D. ceylonense					
BAN	13°00′00.0″N 77°32′00.0″E	22	H9	3.7 ± 1.9	0.48 ± 0.26
TNA	12°36′52.0″N 77°52′51.0″E	21	H10	6.3 ± 4.1	0.70 ± 0.27
TND	12°04′13.0″N 77°29′15.4″E	19	H11	3.5 ± 1.0	0.51 ± 0.12

The genetic relationships between the observed mitochondrial haplotypes were investigated by constructing a minimum spanning tree of the haplotypes with ARLEQUIN 2.00 (Schneider et al., 2000). The tree was constructed using Kimura two parameters distance (Kimura, 1980). For comparison between the mtDNA and nuclear data, relationships between *'nilgiri'* populations were also established from microsatellite genotypes with the neighbour-joining algorithm using Cavalli-Sforza and Edwards' chord distance (1967), based on allele identity and Goldstein et al. distance (1995), based on allele size.

Results

Within-population genetic diversity

For the seven *'nilgiri'* populations, the number of microsatellite alleles per locus varied between 4 (DC8 and DC11) and 15 (DCI-56). These values ranged from 5 (DCI-78) to 25 (DC52) for the three *D. ceylonense* populations. Observed heterozygosities within populations over all loci range from 0.34 to 0.65 among *'nilgiri'* populations and from 0.48 to 0.70 among *D. ceylonense* (Table 1). Among 210 linkage disequilibrium tests, only seven were significant at the 0.05 level and none were significant after a Bonferroni correction. All the loci can therefore be considered as genetically independent. Only one of the 70 Hardy-Weinberg tests was significant at the 0.05 level and none were significant after a Bonferroni correction. We have thus considered all ten populations to be at Hardy-Weinberg equilibrium.

We observed a very low level of within-population variation at the mitochondrial COII locus. In *D. ceylonense*, a single haplotype is present per population (Table 1). Only one haplotype is observed in four out of seven *'nilgiri'* populations. Three populations, XA, XB and XE exhibit two haplotypes. In all three cases, the second haplotype is found only once and it differs from the more frequent one by one base pair only.

Genetic differentiation between populations and between forms

Exact tests of genic differentiation computed for all pairs of populations, within and between forms, were highly significant at each microsatellite locus, as well as over all loci ($p < 10^{-5}$). In agreement with this important differentiation, we observed high values of F_{ST} and R_{ST} both between forms and within forms (Table 2). Within *'nilgiri',* both statistics indicate a similar level of genetic differentiation. F_{ST} values for population pairs range from 0.001 to 0.374 with a value of 0.213 over all populations. R_{ST} range from 0.008 to 0.461 with a value of 0.259 over all populations. F_{ST} and R_{ST} values for a given pair of *'nilgiri'* populations are not significantly different (Wilcoxon signed rank test, $p = 0.17$). Slatkin's isolation-by-distance model (1983) of increased genetic distance with increased geographic distance between populations was not verified among populations of *'nilgiri'*. Indeed, a Mantel test detected no significant correlation between geographic and genetic distance estimated through F ($p = 0.33$) or R ($p = 0.18$) statistics.

Between the two forms, F (allele identity) and R (allele size) statistics differ markedly (Table 2). F_{ST} values between a *'nilgiri'* and a *D. ceylonense* population have an average of 0.358 (min. 0.146, max. 0.484) while R_{ST} values have an average twice higher of 0.734 (min. 0.529, max. 0.897). A Wilcoxon signed rank test showed that *Rst* values between

Table 2. Pairwise, multilocus estimates of F_{ST} are shown below the diagonal and R_{ST} above. Comparisons between '*nilgiri*' and *D. ceylonense* are in bold

	'*nilgiri*'							D. ceylonense		
	XA	XB	XE	XF	XG	XI	XL	BAN	TNA	TND
XA		0.293	0.134	0.107	0.392	0.406	0.008	**0.737**	**0.722**	**0.914**
XB	0.140		0.250	0.133	0.227	0.217	0.336	**0.587**	**0.576**	**0.826**
XE	0.146	0.242		0.037	0.160	0.362	0.162	**0.680**	**0.665**	**0.881**
XF	0.228	0.275	0.169		0.175	0.238	0.137	**0.684**	**0.682**	**0.854**
XG	0.131	0.177	0.131	0.113		0.395	0.410	**0.592**	**0.595**	**0.813**
XI	0.253	0.344	0.266	0.374	0.253		0.461	**0.578**	**0.567**	**0.783**
XL	0.001	0.150	0.169	0.237	0.130	0.309		**0.742**	**0.723**	**0.925**
BAN	**0.528**	**0.498**	**0.529**	**0.519**	**0.381**	**0.505**	**0.540**		0.005	0.308
TNA	**0.370**	**0.347**	**0.363**	**0.392**	**0.221**	**0.374**	**0.380**	0.113		0.244
TND	**0.461**	**0.416**	**0.454**	**0.449**	**0.298**	**0.501**	**0.462**	0.360	0.217	

forms are significantly higher than the corresponding F_{ST} values ($p < 10^{-4}$). However, if the values of the two statistics differ markedly between the two forms, they are both much higher than the values observed within *'nilgiri'*. The higher values of between forms differentiation obtained with R_{ST} compared to F_{ST} can largely be explained by the very different allelic distribution in *'nilgiri'* and *D. ceylonense* at three loci. For DC11, DC52 and DCI-56, in *D. ceylonense* populations we observe many longer alleles that are absent or very rare in *'nilgiri'* populations (Fig. 2). This pattern is extreme at DC11, for which there is no shared allele between the two forms. In contrast, four loci (DC8, DC20, DCI-78 and DCI-2122) show relatively similar allelic distribution between the two forms, with many shared alleles. To determine whether these shared alleles are identical by descent or are due to size homoplasy, we sequenced one shared allele per locus in one *'nilgiri'* and one *D. ceylonense* individual. We found identical sequences between the two forms, suggesting that the shared alleles are truly identical by descent.

The hierarchical analysis of microsatellite variation (Table 3) confirms the above results by clearly showing the contrasting pattern between allele identity (*F*) versus allele size (*R*) statistics between the forms. The fixation indices calculated from allele identity variation and allele size variation are close within forms (0.22 versus 0.19) but between forms values are much higher when *R* statistics are used compared to *F* statistics (0.73 *versus* 0.30). All fixation indices are highly significant ($p < 10^{-5}$). Likewise, the percentage of variation explained between forms is much higher when allele size variation is used compared to allele identity variation (73.1 versus 29.8%).

The mitochondrial haplotypes found in *'nilgiri'* and *D. ceylonense* populations and the estimated phylogenetic relationships between these haplotypes are shown in Fig. 3a. These relationships show a surprising pattern. Three out of seven *'nilgiri'* populations (XA, XE and XI) show the same haplotype H1. The mtDNA haplotypes of three other populations (XF, XG and XL) differ respectively by only four, two and one substitutions from H1. However the XB population shows two closely related haplotypes (H7 and H8) that are extremely divergent from H1. In fact, the genetic distance between H1 and H7-H8 is much higher than the distance between H1 and the *D. ceylonense* haplotypes, H9, H10 and H11.

To compare this unexpected mtDNA pattern with the nuclear relationships between populations, we used a neighbour-joining tree of populations based on microsatellite data. Fig. 3b shows the tree constructed with the chord distance of Cavalli-Sforza and Edwards' (1967), based on allele identity. A similar topology was obtained with Goldstein's distance (1995), which is based on allele size differences (not shown). The tree reveals two clearly distinct clusters that correspond to *'nilgiri'* and *D. ceylonense* populations (Fig. 3b). Population XB of *'nilgiri'* with a very divergent mtDNA haplotype thus falls well within the *'nilgiri'* clade based on nuclear data.

To confirm the position of XB within the *'nilgiri'* clade, we assayed nuclear polymorphism using two rDNA internal transcribed spacers. A 500 bp fragment of both ITS1 and ITS2 was PCR amplified and sequenced using universal primers (Campbell et al., 1993; Sappal et al., 1995). We obtained identical sequences at the two loci for four *'nilgiri'* individuals (two from XA and two from XB) and two *D. ceylonense* individuals from TNA. This lack of differences between *'nilgiri'* and *D. ceylonense* does not allow to infer the position of XB within the *'nilgiri'* clade but it indicates that the two forms diverged very recently.

Discussion

Within-form genetic structuring

We observed a high level of nuclear genetic differentiation between the populations of each form. Similar studies made in other ant species also detected significant genetic differentiation between populations but the F_{ST} values were lower. For example, the F_{ST} values between populations were respectively 0.11, 0.17 and 0.19 in *Formica cinerea*, *Gnamptogenys striatula* and *Camponotus floridanus* (Gadau et al., 1996; Giraud et al., 2000; Goropashnaya et al., 2001). This is especially remarkable given that the geographical distances between populations in the above studies were much higher than those separating '*nilgiri'* populations. In contrast, a recent study of *Diacamma cyaneiventre* by Doums et al.

Table 3. Hierarchical analysis of molecular variance using either allele identity or the sum of squared allele size difference. The significance of the fixation indices was assayed using the non-parametric approach described in Excoffier et al. (1992), consisting in permuting 1000 times individuals or populations among populations or forms, depending on the hierarchical level tested. All fixation indices are highly significant and have a probability inferior to 10^{-5}

	Allele identity		Squared allele size difference	
	Percent of variation	F_{ST}	Percent of variation	R_{ST}
Between forms	29.8%	0.30	73.1%	0.73
Within form, between populations	15.1%	0.22	5.1%	0.19

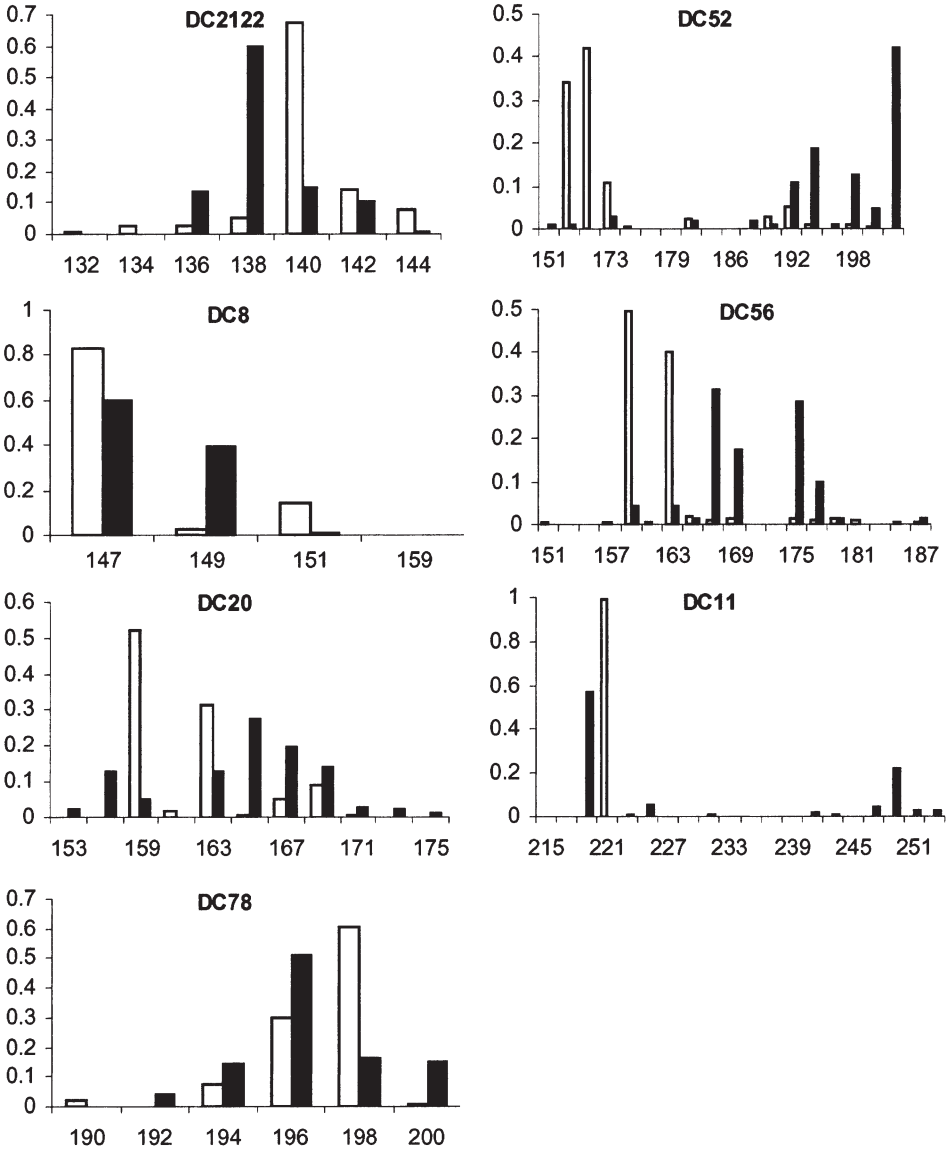

Figure 2. Allele distribution at seven microsatellite loci in *'nilgiri'* (open bars) and *D. ceylonense* (solid bars). Allele size is indicated as number of base pairs

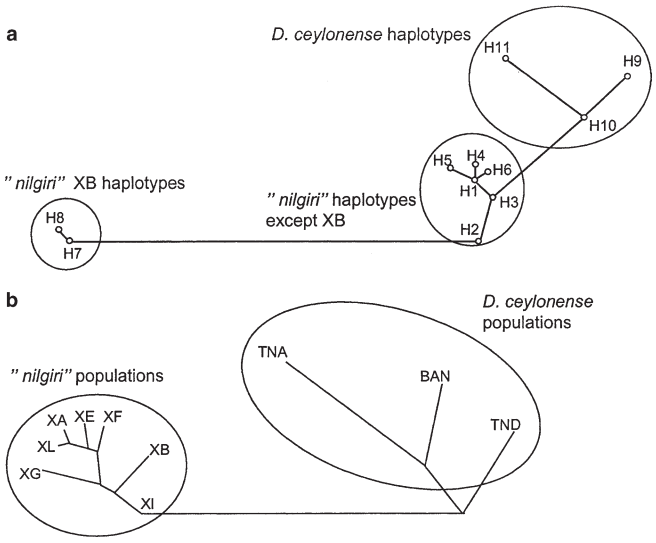

Figure 3. Comparison of mitochondrial and nuclear relationships. a: Minimum spanning tree reflecting the relationships between mtDNA haplotypes. The tree was constructed using Kimura two parameters distance (Kimura, 1980). b: Neighbour-joining tree of populations obtained from microsatellite data using Cavalli-Sforza and Edwards' chord distance (1967), based on allele identity

(2002) showed a level of structure between populations similar to that in our study. This suggests that genetic differentiation between populations is usually large in the *Diacamma* genus, although more species need to be studied to confirm this. This high differentiation is most likely the consequence of restricted dispersal. In the *Diacamma* genus, new colonies are founded by a wingless gamergate helped by a group of workers. Female dispersal is therefore restricted to ant 'walking distance'. There is currently little data on male dispersal in *Diacamma* but our results suggest that it is limited. Additionally, genetic differentiation in *'nilgiri'* and *D. ceylonense* could be enhanced by high levels of genetic drift caused by low effective population size. Indeed, all the populations that we sampled are composed of a limited number of colonies, probably a few hundreds. This is in agreement with the relatively low level of intrapopulation genetic variability observed both in *D. ceylonense* and especially in *'nilgiri'* (mean microsatellite heterozygosity of 0.44).

Between forms genetic structuring

We have used both F and R estimates to measure genetic differentiation. Interestingly, if the two types of estimators produce similar values within the forms, R_{ST} values are much higher than F_{ST} values between the forms. This is not unexpected given the high level of differentiation found between the two forms, as R_{ST} is expected to better reflect true differentiation in highly structured populations because the effect of mutation becomes more important than migration (Balloux et al., 2000). This tendency has been confirmed in several empirical studies (cursory review in Lugon-Moulin et al., 1999).

Whatever the estimator used, our results show a very high genetic differentiation between *'nilgiri'* and *D. ceylonense.* The absence of shared alleles at one microsatellite locus and their quasi-absence at two other loci suggest that gene flow is very restricted or even nonexistent between the two forms. Moreover, the very high fixation index between forms (0.73) has to our knowledge never been reported within a species. It would thus be tempting to speculate that the two forms are in reality two distinct species. However, genetic differentiation per se does not allow to conclude about species status. Reproductive isolation is only loosely correlated with genetic distance (Coyne and Orr, 1989, 1998). Therefore, especially when natural or sexual selection is involved, differentiation at neutral loci does not always reflect differentiation at loci governing reproductive isolation (Wu, 2001). Crossing experiments between the two forms and mating preference tests will be necessary to definitely ascertain the species status of *'nilgiri'*.

Mitochondrial variation

We observed an unexpected pattern of mtDNA: while most *'nilgiri'* populations show identical or very closely related mitochondrial haplotypes, the XB population shows a different haplotype that is genetically much more distant from the other *'nilgiri'* haplotypes than are the *D. ceylonense* haplotypes. In contrast, at the nuclear level, this XB population falls well within the *'nilgiri'* clade. Two hypotheses can be proposed to explain this surprising pattern. First, reconstructed relationships between genes can differ from the history of species (Nichols, 2001). The topology of the mtDNA trees of *'nilgiri'* and *D. ceylonense* populations is the result of coalescence events, which are highly stochastic (Hudson, 1990). We could therefore suppose that the observed mtDNA pattern does not reflect any special event but rather the randomness of coalescence events. However, given the very high divergence of the XB haplotype, this hypothesis seems relatively unlikely. Alternatively, we can suppose that a foreign mtDNA introgressed into *'nilgiri'* populations. Several examples of anomalous mtDNA pattern most probably caused by interspecific introgression are known in ants (Shoemaker et al., 2000) and *Drosophila* (Lachaise et al., 2000; Rousset and Solignac, 1995). In our case, the postulated introgression could have occurred in two ways. A first possibility is that the haplotypes from all populations except XB represent the original *'nilgiri'* mtDNA. In such a case, the mtDNA from XB would result from introgression. We have checked that the XB haplotype is not close to any currently known *Diacamma* haplotypes (Veuille, Brusadelli, Brazier and Peeters, unpublished data), so there is no obvious candidate for the origin of the introgressed DNA. Alternatively, if we suppose that the divergent XB haplotype represents the original *'nilgiri'* mtDNA, then it is the haplotypes from the other *'nilgiri'* populations that originated by introgression, most probably from *D. ceylonense* because their haplotypes are very closely related.

The shift away from mutilation in the regulation of monogyny in *'nilgiri'* appears to have resulted in a high

genetic differentiation from *D. ceylonense,* both at the mitochondrial and nuclear levels. Since regulation based on dominance is reversible, this shift could also influence gene flow within *'nilgiri',* because it is possible that matings with foreign males occur more often. In *D. ceylonense*, as in other *Diacamma* species, all workers are mutilated and therefore unable to mate. On the contrary, all *'nilgiri'* workers retain their gemmae and can potentially reproduce sexually. This could lead to a higher frequency of gamergate replacement in *'nilgiri'* and thus to a higher level of gene flow between populations. It would therefore be interesting to study whether the populations of *'nilgiri'* show a lower genetic differentiation than the populations of *D. ceylonense* at a similar spatial scale.

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References

André, J.-B., C. Peeters and C. Doums, 2001. Serial polygyny and colony genetic structure in the monogynous queenless ant *Diacamma cyaneiventre*. *Behav. Ecol. Sociobiol. 50*: 72–80.

Balloux, F., H. Brunner, N. Lugon-Moulin, J. Hausser and J. Goudet, 2000. Microsatellites can be misleading: an empirical and simulation study. *Evolution 54*: 1414–1422.

Balloux, F. and J. Goudet, 2002. Statistical properties of population differentiation estimators under stepwise mutation in a finite island model. *Mol. Ecol. 11*: 771–783.

Campbell, B.C., J.D. Steffen-Campbell and J.H. Werren, 1993. Phylogeny of the *Nasonia* species complex (Hymenoptera: Pteromalidae) inferred from an internal transcribed spacer (ITS2) and 28S rDNA sequences. *Insect. Mol. Biol. 2*: 225–237.

Cavalli-Sforza, L. and A. Edwards, 1967. Phylogenetic analysis: models and estimation procedures. *Am. J. of Hum. Genet. 19*: 233–257.

Coyne, J. A., and H.A. Orr, 1989. Patterns of speciation in *Drosophila*. *Evolution 43*: 362–381.

Coyne, J.A. and H.A. Orr, 1998. The evolutionary genetics of speciation. *Phil. Trans. R. Soc. Lond. B Biol. Sci. 353*: 287–305.

Cuvillier-Hot, V., R. Gadagkar, C. Peeters and M. Cobb, 2002. Regulation of reproduction in a queenless ant: aggression, pheromones and reduction in conflict. *Proc. R. Soc. Lond. B 269*: 1295–1300.

Doums, C., 1999. Characterization of microsatellite loci in the queenless Ponerine ant *Diacamma cyaneiventre*. *Mol Ecol 8*: 1957–1959.

Doums, C., H. Cabrera and C. Peeters, 2002. Population genetic structure and male-biased dispersal in the queenless ant *Diacamma cyaneiventre*. *Mol. Ecol. 11*: 2251–2264.

Evans, J.D., 1995. Relatedness threshold for the production of female sexuals in colonies of a polygynous ant, *Myrmica tahoensis*, as revealed by microsatellite DNA analysis. *Proc. Natl. Acad. Sci. USA 92*: 6514–6517.

Excoffier, L., P. Smouse and J. Quattro, 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: Application to human mitochondrial DNA restriction data. *Genetics 131*: 479–491.

Fukumoto, Y., T. Abe and A. Taki, 1989. A novel form of colony organization in the 'queenless' ant *Diacamma rugosum. Physiol. Ecol. Jpn 26*: 55–62.

Gadau, J., J. Heinze, B. Hölldobler and M. Schmid, 1996. Population and colony structure of the carpenter ant *Camponotus floridanus. Mol. Ecol. 5*: 785–792.

Giraud, T., R. Blatrix, C. Poteaux, M. Solignac and P. Jaisson, 2000. Population structure and mating biology of the polygynous ponerine ant *Gnamptogenys striatula* in Brazil. *Mol. Ecol. 9*: 1835–1841.

Goldstein, D., A. Ruiz Linares, L. Cavalli-Sforza and M. Feldman, 1995. An evaluation of genetic distances for use with microsatellite loci. *Genetics 139*: 463–471.

Gopinath, A., 2001. Identification of polymorphic loci in the queenless, ponerine ant *Diacamma ceylonense*. *Mol. Ecol. Notes 1*: 126–127.

Goropashnaya, A., P. Seppa and P. Pamilo, 2001. Social and genetic characteristics of geographically isolated populations in the ant *Formica cinerea*. *Mol. Ecol. 10*: 2807–2818.

Gronenberg, W. and C. Peeters, 1993. Central projections of the sensory hairs on the gemma of the ant *Diacamma*: substrate for behavioral modulation? *Cell Tissue Res. 273*: 401–415.

Guo, S. and E. Thompson, 1992. Performing the exact test of Hardy-Weinberg proportion for multiple alleles. *Biometrics 48*: 361–372.

Hudson, R.R., 1990. Gene genealogies and the coalescent process. In: *Oxford Surveys in Evolutionary Biology* (D.F.A.J. Antonovics, Ed.), pp. 1–44, Oxford University Press, Oxford.

Kimura, M., 1980. A simple method for estimating evolutionary rates of base substitution through comparative studies of nucleotide sequences. *J. Mol. Evol. 16*: 111–120.

Lachaise, D., M. Harry, M. Solignac, F. Lemeunier, V. Benassi and M.L. Cariou, 2000. Evolutionary novelties in islands: *Drosophila santomea*, a new *melanogaster* sister species from Sao Tome. *Proc. R. Soc. Lond. B. 267*: 1487–1495.

Lugon-Moulin, N., H. Brunner, A. Wyttenbach, J. Hausser and J. Goudet, 1999. Hierarchical analyses of genetic differentiation in a hybrid zone of *Sorex araneus*. *Mol. Ecol. 8*: 419–431.

Monnin, T. and C. Peeters, 1999. Dominance hierarchy and reproductive conflicts among subordinates in a monogynous queenless ant. *Behav. Ecol. 10*: 323–333.

Monnin, T. and F. Ratnieks, 2001. Policing in queenless ponerine ants. *Behav. Ecol. Sociobiol. 50,* 97–108.

Nichols, R., 2001. Gene trees and species trees are not the same. *Trends Ecol. Evol. 16*: 358–364.

Peeters, C., 1991. The occurrence of sexual reproduction among ant workers. *Biol. J. Linn. Soc. 44*: 141–152.

Peeters, C. and J. Billen, 1991. A novel exocrine gland inside the thoracic appendages ('gemmae') of the queenless ant *Diacamma australe*. *Experientia 47*: 229–231.

Peeters, C. and S. Higashi, 1989. Reproductive dominance controlled by mutilation in the queenless ant *Diacamma australe*. *Naturwissenschaften 76*: 177–180.

Peeters, C., J. Billen and B. Hölldobler, 1992. Alternative dominance mechanism regulating monogyny in the queenless ant genus *Diacamma*. *Naturwissenshaften 79*: 572–573

Raymond, M. and F. Rousset, 1995. GENEPOP (version 1.2): A population genetics software for exact test and ecumenicism. *J. Hered. 86*: 248–249.

Rienzo, A.D., P. Donnelly, C. Toomajian, B. Sisk, A. Hill, M. L. Petzl-Erler, G.K. Haines and D.H. Barch, 1998. Heterogeneity of microsatellite mutations within and between loci, and implications for human demographic histories. *Genetics 148*: 1269–1284.

Rousset, F., 1996. Equilibrium values of measures of population subdivision for stepwise mutation processes. *Genetics 142*: 1357–1362.

Rousset, F. and M. Solignac, 1995. Evolution of single and double *Wolbachia* symbioses during speciation in the *Drosophila simulans* complex. *Proc. Natl. Acad. Sci. USA 92*: 6389–6393.

Sappal, N.P., R.S. Jeng, M. Hubbes and F. Liu, 1995. Restriction fragment length polymorphisms in polymerase chain reaction amplified ribosomal DNAs of three *Trichogramma* (Hymenoptera: Trichogrammatidae) species. *Genome 38*: 419–425.

Schneider, S., D. Roessli and L. Excoffier, 2000. Arlequin: A software for population genetics data analysis, Genetics and Biometry Lab, Dept. of Anthropology, University of Geneva.

Shoemaker, D.D., K.G. Ross, L. Keller, E.L. Vargo and J.H. Werren, 2000. *Wolbachia* infections in native and introduced populations of fire ants (*Solenopsis* spp.). *Insect Mol. Biol. 9*: 661–673.

Slatkin, M., 1983. Isolation by distance in equilibrium and non-equilibrium populations. *Evolution 47*: 264–279.

Slatkin, M., 1995. A measure of population subdivision based on microsatellite allele frequencies. *Genetics 139*: 457–462.

Sokal, R.R. and F.J. Rolf, 1995. *Biometry*, Freeman & Company, New York.

Sommer, K., B. Hölldobler and H. Rembold, 1993. Behavioral and physiological aspects of reproductive control in a *Diacamma* species from Malaysia (Formicidae, Ponerinae). *Ethology 94*: 162–170.

Weber, J. and C. Wong, 1993. Mutation of human short tandem repeats. *Hum. Mol. Genet. 2*: 1123–1128.

Weir, B. and C. Cockerham, 1984. Estimating F-statistics for the analysis of population structure. *Evolution 38*: 1358–1370.

Wu, C., 2001. The genic view of the process of speciation. *J. Evol. Biol. 14*: 851–865.

Xu, X., M. Peng and Z. Fang, 2000. The direction of microsatellite mutations is dependent upon allele length. *Nat. Genet. 24*: 396–399.

ANNEXE 3

Very low genetic variability in the Indian queenless ant *Diacamma indicum*

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SHORT COMMUNICATION

Very low genetic variability in the Indian queenless ant *Diacamma indicum*

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Abstract

We developed microsatellite markers and combined them with mitochondrial markers to analyse the population genetic structure of the queenless ant *Diacamma indicum*. This species, lacking winged queens, is likely to have a restricted female dispersal but exhibits various life history traits suggesting higher dispersal abilities than the other *Diacamma* species. Only 4 of 11 microsatellites were polymorphic and only 1 had more than 4 alleles over 166 individuals originating from 7 populations from the south of India. Only one mitochondrial DNA (mtDNA) haplotype was detected throughout India (including one population in the north) and Sri Lanka. Such a level of polymorphism is particularly low compared with other *Diacamma* species having much smaller ranges in the south of India. A strong genetic differentiation was observed between populations separated by more than a few kilometres. We also analysed the genetic differentiation between the Indian populations and two populations from the Japanese island of Okinawa, which are morphologically similar and might belong to the same species. The genetic differentiation was high for both markers, suggesting an absence of ongoing gene flow between these populations.

Keywords: *Diacamma indicum*, gamergate, microsatellites, mtDNA, population genetics

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Introduction

The population genetic structure of a species is affected by its population history, habitat characteristics and life history traits. Disentangling these effects is a difficult but central task of population genetic studies. Correlative studies have shown some association between the life history traits and population genetics of a species in plants and animals (Hamrick & Godt 1996; Arndt & Smith 1998). For instance, in ants with dependent colony foundation, the queen depends on the help of workers to establish a new colony, and dispersal is limited to ant walking distance (reviewed in Peeters & Ito 2001). Species or populations with dependent colony foundation show a higher level of genetic differentiation than species or populations with independent colony foundation by flying queens (Sundström 1993; Seppä & Pamilo 1995; Ross *et al*. 1997).

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The aim of this study was to examine the level of genetic diversity and pattern of population genetic structure in the queenless ant *Diacamma indicum*. All *Diacamma* species have no queens and are peculiar among ants by the occurrence of a pair of tiny appendages on the workers' thorax, the gemmae, which play an essential role in the regulation of reproduction (Peeters *et al*. 1992; Tsuji *et al*. 1999). Colonies are monogynous, with a single mated worker (the gamergate) retaining her gemmae and producing all the diploid eggs. The absence of winged females and the obligate occurrence of colony fission strongly restrict the dispersal and colonization abilities of these species, as shown in previous population genetic studies of two *Diacamma* species from the south of India (Doums *et al*. 2002; Baudry *et al*. 2003). *D. indicum* differs from these previously studied *Diacamma* species in various ecological characteristics that are expected to result in higher dispersal rates and/or colonization abilities.

1 Even though *Diacamma* ants typically nest underground in open areas, *D. indicum* is more opportunistic with regard

to its nesting preferences. During our field sampling, we observed typical underground nests, but also nests under stones, in abandoned rice paddies, in fissures of walls in an ancient fort and even in tree branches. Related to this opportunistic nesting habit, the nests of *D. indicum* are generally shallow, with little signs of construction.

2 Colonies of *D. indicum* are small (88 ± 62 workers, N = 11; unpublished data) and are prone to emigrate. Nest relocation can be triggered by slight physical disturbance of the nests (personal observation in India; Fukumoto & Abe 1983 in Okinawa), whereas in the other *Diacamma* species from the south of India, workers retreat to the deeper chambers when the nest is disturbed.

3 *D. indicum* has a larger distribution area relative to other species. Indeed, *D. indicum* has been found in a large part of southern India and in Sri Lanka, as well as in the north (near Calcutta), whereas the geographical distribution of the other *Diacamma* species from the south of India are, as far as we know, restricted to small and nonoverlapping areas. In contrast, *D. indicum* can be sympatric with the other *Diacamma* species. Furthermore, a species referred in the literature as *Diacamma* sp. from Japan occurs throughout the Ryukyus islands (south of Japan), and specimens from Okinawa were identified as *D. indicum* on the basis of male genitalia (W.L. Brown, unpublished monograph).

Based on these life history and ecological traits, we therefore expected a lower population genetic structure in *D. indicum* than that previously observed in other *Diacamma* (Doums *et al*. 2002; Baudry *et al*. 2003). To address this issue, we used a mitochondrial marker (a fragment of the COII gene) and developed microsatellite markers. Unexpectedly, both types of markers showed a very low level of polymorphism. However, the use of four variable microsatellites was sufficient to reveal a strong genetic structure at a large spatial scale. We also analysed individuals from two populations of Okinawa Island in order to test whether these *Diacamma* sp. from Japan are genetically very close to *D. indicum* from India, as postulated by W.L. Brown (unpublished monograph), and whether they also harboured such low level of polymorphism.

Materials and methods

Field sampling

Worker ants were collected from 166 colonies distributed in seven populations from the south of India (Fig. 1) in October 1998 and from 45 colonies distributed in two populations on Okinawa Island (Japan) in August 2002. As far as we know, *Diacamma indicum* has never been found on the mainland between India and Okinawa preventing us to obtain samples between these two distant set of populations. The mean (± SD) number of colonies studied per population was 17.3 ± 4.4. The exact location of each population, as determined by GPS, is available upon request. For each colony, a single individual was analysed using microsatellite markers developed specifically for this study (see below). For the mitochondrial DNA (mtDNA), we also analysed one individual from Sri Lanka, kindly collected by S. Yamane, and one individual from one population in the north of India (Bhubaneshwar, near Calcutta), collected by C. Peeters.

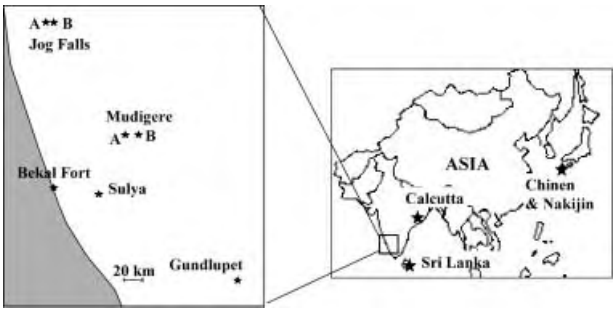

Fig. 1 Geographical position of the seven *D. indicum* populations from south India (Karnataka State) and the two populations from Japan (stars) analysed. The two Japanese populations, named Nakijin and Chinen, were distant from each other by 60 km. For the mtDNA, one individual was analysed for each of these populations as well as one individual from Sri Lanka and one near Calcutta.

Microsatellites cloning and screening

Genomic DNA was extracted from four larvae from Jog Fall A population by classic high salt procedure using NaCl. The digestion, cloning and screening of the microsatellites were performed following a standard protocol detailed in Doums (1999). Synthetic oligonucleotides $(TG)_{10}$ and $(TC)_{10}$ were used to screen ~1200 recombinant colonies. Of 88 positive clones, 40 were purified and sequenced by Genome Express (Meylan, France). Primers could be designed for 11 loci using PRIMER 3 software (Rozen & Skaletsky 2000) and all produced successful amplification. The sequences of these loci are available on GenBank with Accession nos AY258154 to AY258164. We first tested the level of polymorphism of each microsatellite marker by genotyping one individual originating from each of the seven Indian populations using a radioactive amplification. Each polymerase chain reaction (PCR) was carried out according to Doums (1999) with an annealing temperature of 53 °C. Only 4 of the 11 loci were polymorphic and used for the population genetic survey (Table 1).

Genetic analysis

Genomic DNA was extracted using a high salt procedure or an extraction kit Perfect gDNA Blood Mini (Eppendorf).

Table 1 Characteristics and number of alleles over all Indian populations for 11 microsatellite markers. The number of alleles (NaS) for all markers was assessed by genotyping one individual for each of the seven Indian populations. For the polymorphic markers, the total number of alleles (NaT) observed from the genotyping of 166 individuals originating from the seven Indian populations is also given

Loci	Motif	Primers (5′–3′)	NaS	NaT
DI5	[GA]^{21}	F: GCGAAGGTCGAGAAGTGC	1	—
		R: TCGGACTGGGAAGCAATC		
DI8	[GA]^{9}	F: GATGGTGGTAGTGGCAGTGG	1	—
		R: AAGCGCGTGCATAGCGTA		
DI11	[CA]^{8}CT[CA]^{2}	F: CGGCGAGTAAATTCGTAATAG	1	—
		R: CAGGCTTCGCAGGAGAAC		
DI13	[GA]^{11}	F: GTTTCGCAAAGGTGGTTCTC	1	—
		R: TTCACTTCGCTCCAGTCTCC		
DI16	[TC]^{10}	F: CCGATAGATAGGCGAACACA	1	—
		R: ACGTTCCGATTCCCGTTCT		
DI26	[CT]^{17}	F: TCAAGTTCGGCATCTCCTTA	1	—
		R: AACCGGACCTAAATGCGTTA		
DI28	[AG]^{11}	F: TGACGGAAGGACATCGTATC	1	—
		R: GTAATAAAGGCGGACGGAAG		
DI14	[TC]^{10}	F: TTATCGCGGCTTTCTATTCC	2	2
		R: GCATATCTTTCCGTGAGGTTG		
DI31	[CA]^{10}	F: TCTTCCTTTCACGCTCTAATTC	2	3
		R: TGGCAGTGAGCAAGTGTAAA		
DI32	[AC]^{14}	F: GTAGGATGACGGTGGTGGAA	2	2
		R: CGGCTAAGGTAGAGCTGGAA		
DI33	[GT]^{24}	F: CGCCACGTTAGAACTATACGAA	4	11
		R: GGCAATTTCGTCGTTGCT		

PCR were run in 10 μL with 40 ng of template DNA, 1 μL Buffer 10×, 0.25 U *Taq* DNA polymerase (Qiagen), 0.2 μM of each primer and 200 μM of each dNTPs using a GeneAmp 2700 thermal cycler (Applied Biosystems). The thermal cycle profile was as follows: 15 min at 94 °C; 10 amplification cycles of 12 s at 94 °C, 15 s at 53 °C and 30 s at 72 °C; 20 amplification cycles of 15 s at 89 °C, 15 s at 53 °C and 30 s at 72 °C; and a final step for 10 min at 72 °C. The loci *DI14*, *DI31*, *DI32* were co-amplified with the same PCR conditions. The genotypes were determined using a ABI prism 310 sequencer (Applied Biosystems).

A 675 bp fragment of the COII gene was amplified from the genomic DNA of one individual from each of the seven populations. The amplification was performed using the forward 5′-GTGCAATGGATCTAAAATCTA-3′ and reverse 5′-ATATATTATGTTTGATTTAA-3′ primers designed by L. Brazier. PCR were run in 50 μL with 40 ng of template DNA, 1 μL Buffer 10×, 1.25 U *Taq* DNA polymerase (Qiagen), 0.5 μM of each primer and 200 μM of each dNTPs using a GeneAmp 2700 thermal cycler (Applied Biosystems). The thermal cycle profile was as follows: 5 min at 94 °C; 30 amplification cycles of 45 s at 94 °C, 45 s at 48 °C and 1 min at 72 °C; and a final extension step for 8 min at 72 °C. PCR products were sequenced using the Bigdye Terminator V3.0 cycle sequencing kit (Applied Biosystems) and loaded in a ABI Prism 310 sequencer (Applied Biosystems).

Data analysis

Data were analysed using GENEPOP 3.3. (Raymond & Rousset 1995). Hardy–Weinberg equilibrium (HWE) was tested for the four loci at each locality using exact tests (Raymond & Rousset 1995). Fisher's method of combining independent test results (Sokal & Rohlf 1995) was used to determine the overall significance for each locality and each locus. Only 1 of the 19 Hardy–Weinberg tests was significant at the 0.05 level and showed a deficit in heterozygotes ($P = 0.001$) for locus *DI33* in Sulya. The two other loci variable in this population did not show any significant deviation from HWE. We have thus considered all populations to be at HWE. Linkage disequilibrium between pairs of loci was tested for each locality. Among 18 linkage disequilibrium tests, 2 were significant at the 0.05 level and none was significant after a Bonferroni correction. The significance of the genetic differentiation between populations was examined by conducting exact tests of allele frequency differentiation (Raymond & Rousset 1995). The level of genetic differentiation was estimated by *F*-statistics, assuming the infinite allele model (Wright 1978). To test for a pattern of isolation-by-distance, the significance of the Spearman rank correlation coefficient between genetic differentiation and geographical distance was assessed with a Mantel test.

We first compare the number of allele observed per locus (including the nonpolymorphic ones) during our first screening of seven individuals (one from each population) with the one observed in a similar sampling in *D. ceylonense*. To do so, we randomly selected one individual for each population (eight populations) studied by Doums *et al*. (2002) and estimated the number of alleles observed in this reduced data set. We perform this random selection 100 times using a modified SAS procedure of randomization kindly provided by P.L. Leberg (Leberg 2002) and estimated the mean number of allele per locus over the 100 randomly selected dataset. We then compared the number of alleles per locus (including the nonpolymorphic ones) between the two species using a Kolmogorov–Smirnov nonparametric exact test using SAS version 8. The same test was used to compare genetic diversity within populations between *D. indicum* and the others *Diacamma*. We compared both the mean number of alleles and the mean heterozygosity per population. Given that only three populations were analysed for *D. ceylonense*, we pooled these three populations with those of the closely related taxon *Diacamma* sp. from nilgiri from which the same microsatellite loci were screened (Baudry *et al*. 2003) under the species name *D. ceylonense*. In order to avoid comparing

populations with different sample size (Leberg 2002), we did not consider the population with a very large sample size in *D. cyaneiventre* (Kottigehara 1 in Doums *et al*. 2002). The different species had then similar sample size analysed per population (*D. indicum* = 17.3 ± 4.4 (± SD); *D. cyaneiventre* = 18.5 ± 6.6; *D. ceylonense* = 20.1 ± 3.65).

Results and discussion

Genetic diversity over all Indian samples

The level of polymorphism detected was very low. Indeed, only 4 of 11 microsatellite markers were polymorphic, with only one locus (*DI33*) having more than four alleles over all populations (Table 1). Note that the alleles observed in the single individual from Bhubaneshwar (near Calcuta) and Sri Lanka were not different from those already detected in the other Indian populations. Taking these individuals into account does not, therefore, change the number of polymorphic loci or number of alleles in the Indian sample. The percentage of polymorphic loci (36%) was much lower than in *Diacamma cyaneiventre* (80%) (Doums 1999), even though the screening procedure was the same. The absence of polymorphism in most microsatellites cannot be due to small numbers of repeats or impurities (see Table 1). In order to compare the diversity observed in both species, one individual per population (eight populations) was randomly selected in the data set of *D. cyaneiventre* (Doums *et al*. 2002; see Materials and methods). The number of alleles detected per locus (including the nonpolymorphic ones) was significantly lower in *D. indicum* (mean [range] = 1.5 [1–4]) than in *D. cyaneiventre* (mean of the 100 random selections [range] = 5.6 [1–10.5]; Kolmogorov–Smirnov [KS] exact test, $P = 0.004$).

No polymorphism was observed for the mtDNA in the 675 bp of the COII gene fragment over the nine individuals from India and Sri Lanka. It is difficult to explain such absence of mutations by a peculiarity of this mitochondrial gene, because again polymorphism was observed for the same gene in *D. cyaneiventre* and *D. ceylonense*, even within a single population (Doums *et al*. 2002; Baudry *et al*. 2003). Moreover, in the few mtDNA studies on ants, mtDNA polymorphism was observed within populations, suggesting that mtDNA generally harboured polymorphism in ants (Tay *et al*. 1997; Ross *et al*. 1999; Liautard & Keller 2001).

Two main explanations can be put forward to explain such low level of polymorphism for the two classes of markers observed in our species: the occurrence of recent population bottlenecks or a very low effective population size of this species. This last explanation appears less likely when comparing with the other species of *Diacamma* for two main reasons. First, the large distribution area and more opportunistic nesting habits of *D. indicum* do not really suggest a lower effective population size. Second, the level of population fragmentation, which might also affect the effective population size, appears to be similar in all the *Diacamma* species studied (see below).

The occurrence of bottlenecks could be due either to an extinction of most Indian populations followed by a recolonization, or to an introduction of this species into India. Unfortunately, we do not have any historical indication that could favour either of these scenarios. Both scenarios imply a history of rapid colonization of India and suggest that *D. indicum* has high colonization abilities. Its small colonies and unstructured nests might increase the rate of fission and also facilitate passive transport by human activities. It is interesting to note that most ants with high colonization abilities are polygynous, unlike *D. indicum*. Polygyny appears beneficial in association with fission because a reproductive is needed for the propagules to be successful (Holway *et al*. 2002). However, colony fission will succeed in monogynous *Diacamma* as long as cocoons or other brood are present; all emerging workers are capable of becoming reproductives (Peeters *et al*. 1992).

Whatever the causes of such low genetic diversity, this could have major consequences on the social organization of colonies. For instance, in the Argentine ant, internest aggression disappeared in introduced populations with a resulting switch to unicoloniality (Tsutsui *et al*. 2000 but see Giraud *et al*. 2002). In the fire ant, the decrease of allelic diversity at the sex-determining locus or loci generated a genetic load (production of sterile diploid males) in the introduced populations (Ross *et al*. 1993). Further studies are necessary to test whether such effects occur in *D. indicum*.

Within- and among-population genetic structure

When considering the level of polymorphism within populations, the number of alleles per locus varied from one to eight (Table 2). Note that more than two alleles can be observed only for locus *DI33* in three populations and in these cases, most of the alleles occur at low frequency (Table 2). The mean number [range] of alleles per population in *D. indicum* (2 [1.5–3]) is significantly lower than that detected in *D. cyaneiventre* (Doums *et al*. 2002; 6.4 [4.7–8.9]; exact KS test, $P = 0.012$) and *D. ceylonense* (Baudry *et al*. 2003; 3.6 [2.6–6.4]; exact KS test, $P = 0.002$) (Table 2). The pattern was the same when considering the mean expected heterozygosity per population which varied from 0.13 to 0.38 with a mean of 0.25 in *D. indicum*. This value is significantly lower than that detected in *D. cyaneiventre* (Doums *et al*. 2002; 0.63 [0.54–0.68]; exact KS test, $P = 0.012$), and *D. ceylonense* (Baudry *et al*. 2003; 0.44 [0.29–0.70]; exact KS test, $P = 0.002$). The level of polymorphism was also very low in the two Japanese populations (Table 2). Such low level of polymorphism within population was expected given the low level of polymorphism of the markers observed over all samples. This level of polymorphism was

Table 2 Allelic frequencies within Indian and Japanese (Chinen and Nakijin) populations of *Diacamma indicum* for four microsatellite markers. Alleles are named according to their size in base pairs. For each population, the number of individuals (one individual per colony) studied is given between parentheses

	DI14		*DI31*			*DI32*		*DI33*										
	108	110	121	123	125	183	185	209	215	217	219	229	231	233	235	237	239	241
JogFallsA (18)	1.00	—	0.80	—	0.20	—	1.00	0.14	—	—	—	0.08	0.14	0.06	0.44	0.08	0.03	0.03
JogFallsB (21)	1.00	—	0.71	—	0.29	—	1.00	0.19	—	—	—	—	0.31	0.07	0.43	—	—	—
MudigereA (20)	1.00	—	0.76	0.24	—	0.58	0.42	—	0.02	0.98	—	—	—	—	—	—	—	—
MudigereB (22)	1.00	—	0.77	0.23	—	0.64	0.36	—		0.91	0.09	—	—	—	—	—	—	—
BekalFort (10)	0.78	0.22	1.00	—	—	0.50	0.50	1.00	—	–	–	—	—	—	—	—	—	—
Sulya (17)	0.44	0.56	0.97	0.03	—	0.68	0.32	0.14	—	0.68	0.18	—	—	—	—	—	—	—
Gundlupet (13)	1.00	—	0.67	—	0.33	0.96	0.04	1.00	—	—	—	—	—	—	—	—	—	—
	108		136			181	183	217	224	228	232							
Chinen (22)	1.00		1.00			0.93	0.07	—	0.32	—	0.68							
Nakijin (23)	1.00		1.00			0.82	0.18	0.02	0.22	0.02	0.74							

of the order of that observed in the introduced populations of invasive ants such as the Argentine ant *Linepithema* humile (with an expected heterozygosity of 0.2 in introduced Californian populations; Tsutsui *et al*. 2000) and lower than that observed in introduced American populations of the fire ant *Solenopsis invicta* (with an expected heterozygosity of 0.62; Ross *et al*. 1999).

Exact tests of genic differentiation computed across all localities were highly significant at each microsatellite locus ($P < 10^{-5}$) with associated high fixation indices ($F_{ST} = 0.45$). Most of the values of fixation indices between each pair of Indian populations were high and the tests of genic differentiation were significant, except for the two pairs of neighbouring populations (Jog Falls A and B; Mudigere A and B). A Mantel test detected a significant correlation between geographical and genetic distances ($P = 0.03$). This pattern of isolation-by-distance should, however, be confirmed using more polymorphic markers.

Such high level of genetic structure has already been found in other *Diacamma* species (Doums *et al*. 2002; Baudry *et al*. 2003). Colony fission clearly restricts female dispersal in ants. Nothing is known about the ecological dynamics of male dispersal but the high population genetic structure observed at the scale of few kilometres in other *Diacamma* species also suggests restricted male dispersal (Doums *et al*. 2002; Baudry *et al*. 2003). Also, genetic differentiation between populations could be enhanced by habitat fragmentation and by high levels of genetic drift within populations, the number of nests per population being generally lower than a few hundreds. Moreover, if *D. indicum* colonized India, the founding events associated with the establishment of each population, probably involving a few individuals from a single colony, are likely to increase the divergence between populations (Wade & McCauley 1988).

Genetic differentiation between Indian and Japanese populations

The two Japanese localities were not significantly differentiated (exact test $P = 0.27$; $F_{ST} = -0.003$). We therefore pooled the two localities and considered a single Japanese population. The genetic differentiation between the Japanese population and the various Indian populations were significant with values of F_{ST} ranging from 0.72 to 0.78. These extremely high fixation indices reflect the few common alleles observed between Indian and Japanese populations (Table 2). For the mtDNA, the two Japanese populations shared the same haplotype, which differed from that observed in India by 53 variable sites, i.e. 7.8% of sites. This value is of the order of that found between the most differentiated haplotypes of *D. cyaneiventre* (7.2% of sites for the same gene, Doums *et al*. 2002).

The Indian and Japanese populations are thus highly differentiated. It is of course difficult from pure genetic data to conclude on the species status of these populations. Even though *D. indicum* is phylogenetically closest to *Diacamma* sp. from Japan (ump. data on mtDNA), the very high genetic differentiation observed between all the Indian populations studied and *Diacamma* sp. from Japan indicates a lack of gene flow. *D. indicum* belongs to the *D. vagans* complex; its most-closely related species is *D. pallidum*, which occurs in Hong Kong (differing from Japanese population by 13.6% of sites), and in Hainan island (14.2%; unpublished mtDNA data). As far as we know, *D. indicum* has never been found on the mainland between India and Okinawa. A phylogeographical study on this species complex would be necessary to clearly resolve the potential origin of *D. indicum* populations of India and Japan.

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References

Arndt A, Smith MJ (1998) Genetic diversity and population structure in two species of sea cucumber: differing patterns according to mode of development. *Molecular Ecology*, **7**, 1053–1064.

Baudry E, Peeters C, Brazier L, Veuille M, Doums C (2003) Shift in the behaviours regulating monogyny is associated with high genetic differentiation in the queenless ant *Diacamma ceylonense*. *Insectes Sociaux*, **50**, 390–397.

Doums C (1999) Characterization of microsatellite loci in the queenless Ponerine ant *Diacamma cyaneiventre*. *Molecular Ecology*, **8**, 1957–1959.

Doums C, Cabrera H, Peeters C (2002) Population genetic structure and male-biased dispersal in the queenless ant *Diacamma cyaneiventre*. *Molecular Ecology*, **11**, 2251–2264.

Fukumoto Y, Abe T (1983) Social organization of colony movement in the tropical ponerine ant, *Diacamma rugosum* (Le Guillou). *Journal of Ethology*, **1**, 101–108.

Giraud T, Pedersen JS, Keller L (2002) Evolution of supercolonies: the Argentine ants of southern Europe. *Proceedings of the National Academy of Sciences of the USA*, **99**, 6075–6079.

Hamrick JL, Godt MJW (1996) Effects of life history traits on genetic diversity in plant species. *Philosophical Transactions of the Royal Society of London B*, **351**, 1291–1298.

Holway DA, Lach L, Suarez AV, Tsutsui ND, Case TJ (2002) The causes and consequences of ant invasions. *Annual Review of Ecology and Systematics*, **33**, 181–233.

Leberg PL (2002) Estimating allelic richness: effects of sample size and bottlenecks. *Molecular Ecology*, **11**, 2445–2449.

Liautard C, Keller L (2001) Restricted effective queen dispersal at a microgeographic scale in polygynous populations of the ant *Formica exsecta*. *Evolution*, **55**, 2484–2491.

Peeters C, Billen J, Hôlldobler B (1992) Alternative dominance mechanisms regulating monogyny in the queenless ant genus *Diacamma*. *Naturwissenschaften*, **79**, 572–573.

Peeters C, Ito F (2001) Colony dispersal and the evolution of queen morphology in social hymenoptera. *Annual Review of Entomology*, **46**, 601–630.

Raymond M, Rousset F (1995) GENEPOP Version 1.2: population genetics software for exact tests and ecumenicism. *Journal of Heredity*, **86**, 248–249.

Ross KG, Krieger MJB, Shoemaker DD, Vargo EL, Keller L (1997) Hierarchical analysis of genetic structure in native fire ant populations: results from three classes of molecular markers. *Genetics*, **147**, 642–655.

Ross KG, Shoemaker DD, Krieger MJB, DeHeer CJ, Keller L (1999) Assessing genetic structure with multiple classes of molecular markers: a case study involving the introduced fire ant *Solenopsis invicta*. *Molecular Biology and Evolution*, **16**, 525–543.

Ross KG, Vargo EL, Keller L, Trager JC (1993) Effects of a founder event on variation in the genetic sex-determining system of the fire ant *Solenopsis invicta*. *Genetics*, **135**, 843–854.

Rozen S, Skaletsky HJ (2000) Primer 3 on the www for general users and for biologist programmers. In: *Bioinformatics Methods and Protocols: Methods in Molecular Biology* (eds Krawetz S, Misener S), pp. 365–386. Humana Press, Totowa, NJ, USA.

Seppä P, Pamilo P (1995) Gene flow and population viscosity in *Myrmica* ants. *Heredity*, **74**, 200–209.

Sokal RR, Rohlf FJ (1995) *Biometry*. WH Freeman, New York.

Sundström L (1993) Genetic population structure and sociogenetic organisation in *Formica truncorum* (Hymenoptera; Formicidae). *Behavioral Ecology and Sociobiology*, **33**, 345–354.

Tay TW, Cook JM, Rowe DJ, Crozier RH (1997) Migration between nests in the Australian arid-zone ant *Rhytidoponera* sp. 12 revealed by DGGE analyses of mitochondrial DNA. *Molecular Ecology*, **6**, 403–411.

Tsuji K, Egashira K, Hölldobler B (1999) Regulation of worker reproduction by direct physical contacts in the ant *Diacamma* sp. from Japan. *Animal Behaviour*, **58**, 337–343.

Tsutsui ND, Suarez AV, Holway DA, Case TJ (2000) Reduced genetic variation and the success of an invasive species. *Proceedings of the National Academy of Sciences of the USA*, **97**, 5948–5953.

Wade MJ, McCauley DE (1988) Extinction and recolonization: Their effects on the genetic differentiation of local populations. *Evolution*, **42**, 995–1005.

Wright S (1978) *Evolution and the Genetics of Populations*. University of Chicago Press, Chicago.

This study is part of a research programme on the behavior and genetics of queenless *Diacamma* performed in collaboration with the group of R. Gadagkar in Bangalore (India). Claudie Doums is a molecular evolutionary biologist with a particular interest in social insects. At the time of this study, Barbara Viginier was a master student and Lionel Brazier provided technical help with the mtDNA screening. Christian Peeters studies the behavioral regulation of reproduction in queenless ants and conducted fieldwork in this study.

ANNEXE 4

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

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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

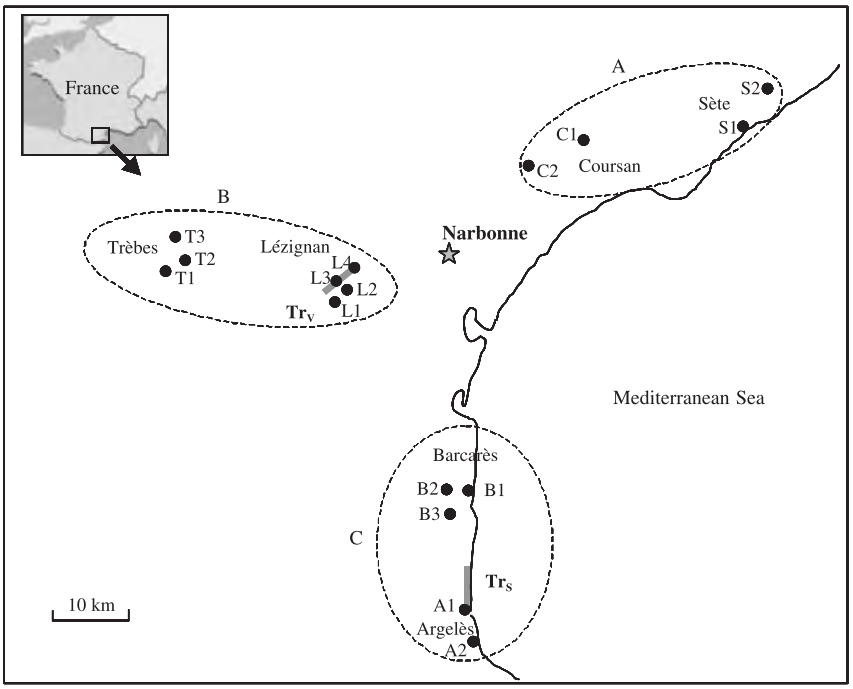

Fig. 1 Geographical location of *Cataglyphis cursor* sites (black points) and transects (grey lines) in Languedoc-Roussillon, France. Three regions (ovals A, B, C), two subregions within each region (characterized by the name of the closest village) and 16 sites (characterized by the first letter of 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 ($A_{Trèbes} = 4.1$, $H_{S\,Trèbes} = 0.64$) significantly differed from those obtained when considering all others subregions together ($A_{other} = 5.58$, two-side P value after a 10 000 permutations test: $P_A = 0.0012$ and $H_{S\,other} = 0.78$, $P_{Hs} = 0.0014$). However, no significant difference was detected when comparing between the two types of habitats ($A_{Seaside} = 5.23$, $A_{Vineyard} = 5.52$, $P_A = 0.53$ and $H_{Seaside} = 0.74$, $H_{Vineyard} = 0.78$, $P_{Hs} = 0.44$).

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

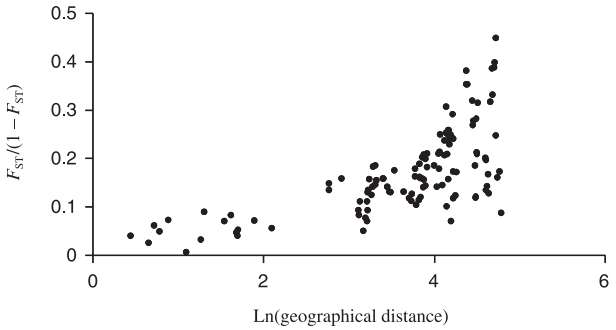

Fig. 2 Relationship between logarithm of geographical distance and nuclear genetic differentiation between sites, estimated as $F_{ST}/(1-F_{ST})$. The correlation is high and significant ($R = 0.69$, $P < 10^{-5}$).

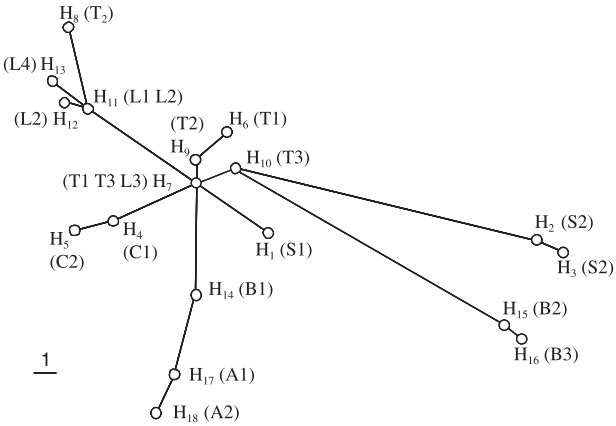

Fig. 3 Minimum-spanning network depicting relationships between the mtDNA haplotypes (408 pb) detected in the 16 sites. The tree was constructed using the pairwise distance. For each haplotype, the site in which it was detected is indicated in parentheses.

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.

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

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

	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

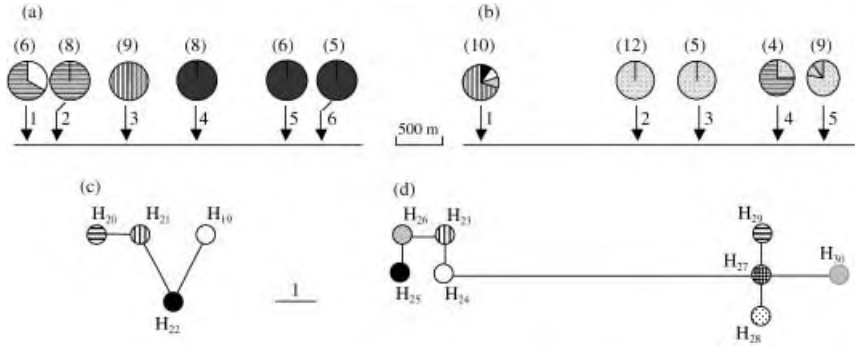

Fig. 4 Distribution of sampled patches and haplotype frequencies within patches along both seaside (a) and vineyard (b) transects. Along each transect, patches are represented by a circle labelled by a number and the number of workers successfully sequenced is given into parentheses. Within a transect, 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.

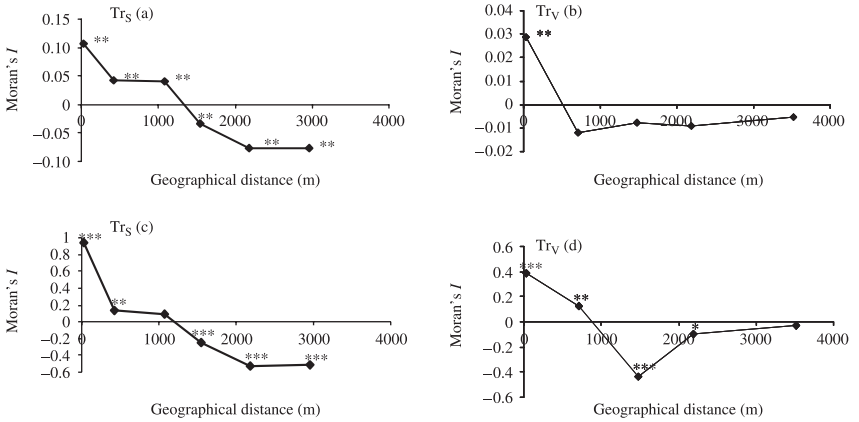

Fig. 5 Spatial autocorrelograms estimated from multilocus microsatelitte genotypes for seaside (a) and vineyard (b) transects and from mtDNA haplotypes, for seaside (c) and vineyard (d) transects. Significance levels of Moran's *I* are indicated by stars (* for $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).

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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References

Bourke AFG, Franks NR (1995) *Social Evolution in Ants*. Princeton University Press, Princeton, New Jersey.

Cagniant H (1976) Distribution, Ecologie et nid de la fourmi *Cataglyphis cursor* fonscolombe (Hyménoptères: Formicidae). *Vie et Milieu*, **26**, 265–276.

Chesser RK, Baker RJ (1996) Effective sizes and dynamics of uniparentally and biparentally inherited genes. *Genetics*, **144**, 1225–1235.

Clobert J, Danchin E, Dhondt AA, Nichols JD (2001) *Dispersal*. Oxford University Press, New York.

Doums C, Cabrera H, Peeters C (2002) Population genetic structure and male-biased dispersal in the queenless ant *Diacamma cyaneiventre*. *Molecular Ecology*, **11**, 2251–2264.

Durka W (1999) Genetic diversity in peripherical and subcentral populations of *Corrigiola litoralis* L. (Illecebraceae). *Heredity*, **83**, 476–484.

Ehrich D, Stenseth NC (2001) Genetic structure of Siberian lemmings (*Lemmus sibiricus*) in a continuous habitat: large patches rather than isolation by distance. *Heredity*, **86**, 716–730.

Filatov DA (2002) PROSEQ: a software for preparation and evolutionary analysis of DNA sequence data sets. *Molecular Ecology Notes*, **2**, 621–624.

Foitzik S, Herbers JM (2001) Colony structure of a slavemaking ant. I. Intracolony relatedness, worker reproduction, and polydomy. *Evolution*, **55**, 307–315.

Goodisman MAD, Ross KG (1998) A test of queen recruitment models using nuclear and mitochondrial markers in the fire ant *Solenopsis invicta*. *Evolution*, **52**, 1410–1422.

Goudet J (1995) FSTAT (version 1.2): a computer program to calculate *F*-statistics. *Journal of Heredity*, **86**, 485–486.

Hamilton WD (1964) The genetical evolution of social behaviour. II. *Journal of Theoretical Biology*, **7**, 17–52.

Hardy OJ, Vekemans X (2002) SPAGEDI: a versatile computer program to analyse spatial genetic structure at the individual or population levels. *Molecular Ecology Notes*, **2**, 618–620.

Hedrick PW (1999) Highly variable loci and their interpretation in evolution and conservation. *Evolution*, **53**, 313–318.

Hutchison DW, Templeton AR (1999) Correlation of pairwise genetic and geographic distance measures: inferring the relative influences of gene flow and drift on the distribution of genetic variability. *Evolution*, **53**, 1898–1914.

Jarne P, Lagoda PJL (1996) Microsatellites, from molecules to populations and back. *Trends in Ecology & Evolution*, **11**, 424–429.

Keller L (1995) Social life: the paradox of multiple-queen colonies. *Trends in Ecology & Evolution*, **10**, 355–360.

Kelly JK (1992) Restricted migration and the evolution of altruism. *Evolution*, **37**, 1492–1495.

Keller L, Passera L (1989) Size and fat content of gynes in relation to the mode of colony founding ants (Hymenoptera; Formicidae). *Oecologia*, **80**, 236–240.

Lenoir A, Quérard L, Pondicq N, Berton F (1988) Reproduction and dispersal in the ant *Cataglyphis cursor* (Hymenoptera, Formicidae). *Psyche*, **95**, 21–44.

Lewis P, Zaykin D (2001) *GENETIC DATA ANALYSIS: Computer Program for the Analysis of Allelic Data*, Version 1.0. Free program distributed over the Internet from. http://hydrodictyon.eeb.uconn.edu/people/plewis/software.php.

Liautard C, Keller L (2001) Restricted effective queen dispersal at a microgeographic scale in polygynous populations of the ant *Formica exsecta*. *Evolution*, **55**, 2484–2492.

Nei M (1987) *Molecular Evolutionary Genetics*. Columbia University Press, New York.

Passera L, Keller L (1990) Loss of mating flight and shift in the pattern of carbohydrate storage in sexuals of ants (Hymenoptera; Formicidae). *Journal of Comparative Physiology*, **160**, 207–211.

Pearcy M, Aron S, Doums C, Keller L (2004a) Conditional use of sex and parthenogenesis for worker and queen production in ants. *Science*, **306**, 1780–1783.

Pearcy M, Clémencet J, Chameron S, Aron S, Doums C (2004b) Characterization of nuclear DNA microsatellite markers in the ant *Cataglyphis cursor*. *Molecular Ecology Notes*, **4**, 642–644.

Queller DC (1992) Does population viscosity promote kin selection. *Trends in Ecology & Evolution*, **7**, 322–324.

Raymond M, Rousset F (1995) GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. *Journal of Heredity*, **86**, 248–249.

Ross KG, Shoemaker DD (1997) Nuclear and mitochondrial genetic structure in two social forms of the fire ant *Solenopsis invicta*: insights into transitions to an alternate social organization. *Heredity*, **78**, 590–602.

Ross KG, Krieger MJB, Shoemaker DD, Vargo EL, Keller L (1997) Hierarchical analysis of genetic structure in native fire ant populations: results from three classes of molecular markers. *Genetics*, **147**, 643–655.

Ross KG, Shoemaker DD, Krieger MJB, DeHeer CJ, Keller L (1999) Assessing genetic structure with multiple classes of molecular markers: a case study involving the introduced fire ant *Solenopsis invicta*. *Molecular Biology and Evolution*, **16**, 525–543.

Rousset F (1997) Genetic differentiation and estimation of gene flow from *F*-statistics under isolation by distance. *Genetics*, **145**, 1219–1228.

Rousset F (2000) Genetic differentiation between individuals. *Journal of Evolutionary Biology*, **13**, 58–62.

Ruppell O, Stratz M, Baier B, Heinze J (2003) Mitochondrial markers in the ant *Leptothorax rugatulus* reveal the population genetic consequences of female philopatry at different hierarchical levels. *Molecular Ecology*, **12**, 795–801.

Sanetra M, Crozier RH (2003) Patterns of population subdivision and gene flow in the ant *Nothomyrmecia macrops* reflected in microsatellite and mitochondrial DNA markers. *Molecular Ecology*, **12**, 2281–2295.

Schneider S, Roessli D, Excoffier L (2000) *ARLEQUIN: a software package for population genetics data analysis Version 2.000*. Genetics and Biometry Laboratory, University of Geneva, Switzerland.

Seppa P, Gyllenstrand M, Corander J, Pamilo P (2004) Coexistence of the social types: genetic population structure in the ant *Formica exsecta*. *Evolution*, **58**, 2462–2471.

Shoemaker DD, Ross KG (1996) Effects of social organization on gene flow in the fire ant *Solenopsis invicta*. *Nature*, **383**, 613–616.

Simon C, Frati F, Beckenbach AT *et al*. (1994) Evolution, weighting and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. *Annals of the Entomological Society of America*, **87**, 651–701.

Slatkin M (1993) Isolation by distance in equilibrium and non-equilibrium populations. *Evolution*, **47**, 264–279.

Sokal RR, Oden NL (1978) Spatial autocorrelation in biology. 1. Methodology. *Biological Journal of the Linnean Society*, **10**, 199–228.

Sokal RR, Rohlf FJ (1995) *Biometry*. Freeman & Company, New York.

Stow AJ, Sunnucks P, Briscoe DA, Gardner MG (2001) The impact of habitat fragmentation on dispersal of Cunningham's skink (*Egernia cunninghami*): evidence from allelic and genotypic analyses of microsatellites. *Molecular Ecology*, **10**, 867–878.

Sundström L, Keller L, Chapuisat M (2003) Inbreeding and sex-biased gene flow in the ant *Formica exsecta*. *Evolution*, **57**, 1552–1561.

Weir BS (1996) *GENETIC DATA ANALYSIS II: Methods for Discrete Population Genetic Data*. Sinaueur Associates, Sunderland, Massachusetts.

Weir BS, Cockerham CC (1984) Estimating F-statistics for the analysis of population structure. *Evolution*, **38**, 1358–1370.

Wright S (1943) Isolation by distance. *Genetics*, **28**, 139–156.

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.

ANNEXE 5

Serial polygyny and colony genetic structure in the monogynous queenless ant *Diacamma cyaneiventre*

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Serial polygyny and colony genetic structure in the monogynous queenless ant *Diacamma cyaneiventre*

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Abstract Serial polygyny, defined as the temporal succession of several reproductive females in a colony, occurs in some monogynous social insects and has so far attracted little attention. *Diacamma cyaneiventre* is a queenless ponerine ant found in the south of India. Colonies are headed by one singly mated worker, the gamergate. After the death of the gamergate or her absence following colony fission, the gamergate is replaced by a newly eclosed nestmate worker. After a replacement, colonies go through short-lived periods in which two matrilines of sisters co-occur. This is a situation which can be described as serial polygyny. To measure the consequences of serial polygyny, a genetic analysis was performed on 449 workers from 46 colonies of *D. cyaneiventre* using five microsatellite loci. The presence of more than one matriline among workers of the same nest was detected in 19% of colonies, indicating a recent change of gamergate. The average genetic relatedness among nestmate workers was 0.751 and did not significantly differ from the theoretical expectation under strict monogyny and monandry (0.75). A simple analytical model of the temporal dynamics of serial polygyny was developed in order to interpret these results. We show that the rate of gamergate turnover relative to the rate of worker turnover is the crucial parameter determining the level of serial polygyny and its effect on the genetic structure of colonies. This parameter, estimated from our data, confirms that serial polygyny occurs in *D. cyaneiventre* but is not strong enough to influence significantly the average genetic relatedness among workers.

Keywords Genetic relatedness · Microsatellites · Gamergate turnover · Queenless ants · Ponerinae

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Introduction

The genetic relatedness between members of animal societies is a key parameter for understanding the evolution of altruistic behaviour and reproductive division of labour (Hamilton 1964). This parameter is based on the probability that interacting individuals carry genes that are identical by descent. The sociogenetic organisation of colonies, i.e. the genealogical relationships between nestmates, is therefore a crucial factor involved in the outcome of social conflicts over sex allocation, production of males and other colony characteristics (Bourke and Franks 1995; Crozier and Pamilo 1996). In the Hymenoptera, the haplodiploid sex determination system leads to high genetic relatedness (0.75) among nestmate workers in monogynous and monandrous species. However, empirical studies have confirmed that polyandry or polygyny can drastically reduce the genetic relatedness among workers in a colony (Queller 1993; Ross 1993; Bourke and Franks 1995).

In a number of monogynous social insects, different reproductive females succeed each other in the same colony, and this may also be expected to reduce relatedness. Species exhibiting a temporal succession of reproductives have potentially immortal colonies, and these multiply by fission. This contrasts with other monogynous species in which the death of the founding queen leads to colony extinction (Hölldobler and Wilson 1990). Replacement of reproductives is also commonly observed in polygynous ants, in which new queens are regularly recruited in colonies (Evans 1996). Very fast queen turnover has been detected in some species, and this affects the genetic relatedness between workers and brood (Seppa 1994; Evans 1996; Bourke et al. 1997). However, in polygynous species, the sociogenetic consequences of queen turnover are conflated with the specific effects of polygyny, and are thus difficult to quantify. Monogynous species exhibiting queen replacement include various primitively eusocial wasps (Jeanne 1972; West-Eberhard 1978; Gadagkar et al. 1993a), queenless ponerine ants (Peeters 1993), most army ants (Gotwald

1995), a few formicine ants (Lenoir et al. 1988) as well as honey-bees and stingless bees. In these species, referred to as "serially polygynous" (Yamane 1986; Gadagkar et al. 1993a), colonies undergo phases during which two or more matrilines of sisters, produced by a succession of reproductive females, co-exist in the colony. Replacement queens may be foreign or related, depending on the taxon. There have been no genetic studies of serial polygyny in monogynous species (but see Gadagkar et al. 1993a who used pedigree analyses). As with polygyny and polyandry, queen turnover could have major implications for social evolution, because it leads to temporal variation in genetic relatedness between workers and brood (Gadagkar et al. 1993a).

In this study, we investigated the importance of serial polygyny in *Diacamma cyaneiventre*, a queenless ant. In all *Diacamma* species (subfamily Ponerinae), the queen caste does not exist. The reproductive role is fulfilled by workers, called gamergates, than can mate and lay fertilised eggs (Peeters 1991). Reproduction in *Diacamma* is regulated by a very peculiar behaviour prohibiting polygyny. The gamergate mutilates every recently emerged worker by biting off a pair of bladder-like innervated appendices present on the thorax, called gemmae (Fukumoto et al. 1989; Peeters and Higashi 1989; Gronenberg and Peeters 1993). Mutilation prevents workers from mating and thus producing diploid offspring. Thus only one adult worker in each colony retains her gemmae, mates and monopolises diploid egg production. In the absence of the gamergate, i.e. after colony fission (leading to two autonomous groups, one of which initially lacks a gamergate) or after the gamergate dies, the first worker to emerge will retain her gemmae and mutilate all subsequent callows. Mating occurs inside the nest with a male from another colony, when the future gamergate performs a behaviour called "sexual calling" which is restricted to virgin unmutilated workers (Peeters et al. 1992). Males are winged and fly away from their natal colony, searching for a nest with a worker exhibiting "sexual calling". Following copulation, the male stays attached to the female abdomen for several hours (Wheeler and Chapman 1922; Fukumoto et al. 1989; Nakata et al. 1998), and this prevents her from remating immediately afterwards; in addition, a worker never exhibits "sexual calling" again after mating (C. Peeters, unpublished data). After mating, the young gamergate does not disperse but reproduces in her natal colony. Thus for a certain period of time, the colony will have two matrilines of full sisters, aunts and nieces. The frequency of such periods with multiple matrilines in a colony life cycle determines the average relatedness among nestmates, especially between workers and brood, and should therefore be an important parameter for understanding the evolution of these queenless societies.

The aim of this study was (1) to determine the frequency of colonies with multiple matrilines and thereby (2) to analyse the effects of serial polygyny on the sociogenetic organisation of the colonies, more specifically on the genetic relatedness among workers.

Methods

Species and sampling

Diacamma cyaneiventre is a ponerine ant that inhabits open areas in southern India. Colony size is relatively small [mean 214±80 (±SD) workers, n=6; unpublished data]. Colonies are found in deep perennial nests (up to 50 cm underground; unpublished data). Their life cycle shows no seasonality since female and male brood can be found throughout the year. An average of 9.8 workers per colony (minimum 4, maximum 17) were collected and kept in 90% alcohol. In this species, nest entrances are surrounded by a mound of soil and pebbles; we collected workers only from this mound, when they entered or left the nest. Forty-six colonies of *D. cyaneiventre* were sampled in one site on 30 and 31 May 1997 (337 workers from 33 colonies), and in another site 300 m away on 23 October 1998 (112 workers from 13 colonies). These two sites were approximately 300×300 m in area and were located near the village of Kotigehara (13°7′ N, 75°31′ E), in the district of Chikmagalur, 230 km west of Bangalore, in Karnataka State, India.

Genetic analysis

For most individuals, DNA was extracted using the following quick extraction procedure. The tissues of each ant head were homogenised in 50 µl of a solution containing 10 mM Tris HCl, 1 mM EDTA, 25 mM NaCl, and 200 µg ml^{-1} of proteinase K. After 2 h at 37°C and 2 min at 92°C, the solution was centrifuged for 5 min at 6,000 rpm. The supernatant was then recovered and kept at –20°C. One microlitre of this DNA solution was used per polymerase chain reaction (PCR). Five microsatellite loci developed for *D. cyaneiventre* (Doums 1999) were used in this study (Table 1). The PCRs were conducted following the protocol of

Table 1 Genetic variability of the five loci. The number of alleles per locus (n_{all}), expected heterozygosity (H_E) and Weir and Cockerham F_{IS} are given for the five loci used in this study, for the 1997 sample (337 individuals originating from 33 colonies) and the 1998 sample (112 individuals from 13 colonies). n_{all} and H_E were calculated by weighting the colonies equally using the Relatedness 5.0 programme (Queller and Goodnight 1989). F_{IS} was calculated using one random individual per colony with the Genepop programme (Raymond and Rousset 1995). More information about the loci can be found in Doums (1999)

Locus	1997 sample				1998 sample			
	n_{all}	H_E	F_{IS}	P	n_{all}	H_E	F_{IS}	P
DC 11	10	0.61	–0.099	0.93	9	0.57	0.056	0.15
DC 18	25	0.93	0.005	0.25	15	0.90	0.010	0.52
DC 19	5	0.78	–0.025	0.56	7	0.78	0.012	0.37
DC 20	5	0.77	–0.104	0.79	5	0.71	0.053	0.72
DC 52	6	0.66	0.025	0.59	5	0.64	0.005	0.46

Doums (1999), except for the co-amplification of loci D19 and D20 for which the amounts of dNTP, ^{33}PdATP and Taq DNA polymerase were doubled.

Heterozygote deficiency at each locus and linkage disequilibrium between each pair of loci were examined in the two samples, by an exact test using the Genepop programme (Raymond and Rousset 1995). The tests were performed using one randomly selected individual per colony. Expected heterozygosity was calculated using the allelic frequencies of each sample. None of the ten F_{IS} values was significantly higher than zero (overall combined *P*-value=0.76; Table 1; Sokal and Rohlf 1995, p. 794). No significant linkage disequilibrium was observed for the ten pairs of loci (combined *P*-values over the two samples >0.3). Using the colonies containing a single matriline of full sisters (see Results), and headed by a heterozygous mother at a given locus, we checked for any significant deviation from the balanced Mendelian segregation of 1:1. For each locus, a *G*-test was performed by colony and each *P*-value combined over colonies to find a global significance level. None of the combined *P*-values was significant at the 5% level, indicating that each locus followed Mendelian segregation.

Data analysis

Two main parameters were estimated from the genetic data: the proportion of multiple matriline colonies and the within-colony genetic relatedness. The proportion of multiple matriline colonies was determined by checking whether the genotypes observed in each colony over the five loci were compatible with a full-sister hypothesis. A sample of workers having a common allele (from their father) and a maximum of two other alleles (from their mother) at each locus was considered as one matriline of full sisters. In any other case, the sample was considered as containing several matrilines, as polyandry is very unlikely in this species. Moreover, the high polymorphism of the markers used and the absence of a high level of inbreeding make the probability of no detecting a gamergate turnover extremely low. For example, the probability of two random males sharing exactly the same genotype equals 0.005 and 0.001 for 1997 and 1998, respectively.

The proportion of multiple matriline colonies, M_{obs}, was estimated in the 46 colonies. In 1 of these colonies, one individual was not compatible with the others for only one allele at a given locus, which differed by only 2 bp from one of the other alleles of the colony. We suspected a mutation event and decided to consider that the individual belonged to the same matriline of full sisters. Another colony sample was mainly constituted of one matriline except one individual, which could not be an aunt or a niece of the others. Our sampling of workers was restricted to the nest mound but this might not be sufficient to ensure that all the workers collected in fact came from the same colony. We therefore preferred to be conservative with respect to the number of colonies with multiple matrilines and considered this colony sample as a single matriline by removing the incompatible individual from the data. The number of workers analysed per colony being limited, a better estimate could be obtained by taking into account the error of sampling only one matriline in a colony that in fact contained two. Assuming that the colonies cannot contain more than two successive matrilines among their workers, the corrected proportion of colonies containing two matrilines, *M*, is given in Eq. 1 (see derivations in Appendix 1):

$$M = M_{obs} \times \frac{1}{\frac{1}{C}\sum_{i=1}^{C}\frac{n_i - 1}{n_i + 1}} \quad (1)$$

with *C* being the number of colonies sampled, and n_i the number of workers sampled in colony *i*.

This estimate is subject to variations due to binomial sampling and colony sample size. A bootstrap procedure was therefore used to estimate its confidence interval. Forty-six colonies were randomly sampled from our data set (resampling of the same colony being allowed), and *M* was estimated for this new sample. This procedure was repeated 10,000 times to generate 10,000 values of *M*, from which the 250 lowest and the 250 highest values were excluded (5%). The remaining 9,500 values therefore constituted a confidence interval for *M*, at the 95% level.

Genetic relatedness among collected nestmate workers was estimated for each colony, and averaged over colonies, using the Relatedness 5.1 programme (Queller and Goodnight 1989). The standard errors of the means were obtained by jackknifing over colonies. For the allelic frequencies and the average relatedness estimates, colonies were weighted equally. The allelic frequencies were estimated separately for each colony by excluding that colony in order to avoid sample bias. These relatedness estimates could then be tested for any deviation from a given relatedness value using a *t*-test (Sokal and Rohlf 1995, p. 174). The relatedness estimates are affected by sub-population genetic differentiation. Two factors could induce such differentiation in our study and bias our results. First, in *Diacamma*, the colonies reproduce by fission which could generate some population viscosity over a short distance. However, in the studied population no significant isolation by distance was detected (C. Doums, unpublished results). Second, given that the sampling was not carried out at exactly the same site in the 2 different years (33 colonies in 1997 and 13 in 1998), the allelic frequencies could differ between the two samples. To test for this potential difference, the allelic frequencies of the two samplings were compared for each locus using the Struc routine in the Genepop programme (Raymond and Rousset 1995). This programme gives the exact probability that the samples come from the same gene pool. The significance over the five loci was calculated using a combined probability test (Sokal and Rohlf 1995, p. 794). However, in our samples, the individuals collected from the same colony cannot be considered as independent. To solve this problem, two artificial samples were generated for each site, using the observed average allelic frequencies of this site (calculated with Relatedness 5.1 by weighting the colonies equally) but with a number of individuals equal to three times the number of colonies sampled in the site. The underlying reasoning is that a colony represents a minimum of three independent genomes: one from the male, and two from the gamergate. In our samples, the genotypes of at least six workers per colony were determined, thus ensuring at the 5% probability level that both alleles of the gamergate have been detected. Over the five loci, the allelic frequencies of these two artificial samples differed significantly (overall combined probability test, *P*=0.016; Sokal and Rohlf 1995, p. 794). The mean allelic frequencies needed for estimating the genetic relatedness were therefore computed separately for each sample (1997 and 1998 were considered as two demes in the Relatedness 5.0 programme).

A simple analytical model of serial polygyny

For a better understanding of serial polygyny in *D. cyaneiventre*, a simple model simulating the dynamics of the genetic structure of colonies over time was developed. The reasoning underlying the model is schematically described in Fig. 1 and can be summarised as follows when the workers' lifespan *w* is shorter than the gamergate's tenure *g*. Effective gamergate replacement takes place at time *T=0* when the first cocoon of the new gamergate ecloses. From *T=0*, the proportion of workers of the new matriline increases while the proportion of workers of the previous matriline decreases at the same rate of 1/*w* (*w* being the workers' lifespan). This assumes a constant colony size and continuous and constant egg-laying activity by the gamergate. At *T=w/2*, if we assume an equal egg-laying rate by successive gamergates, each matriline is equally represented in the colony and the average genetic relatedness among workers is minimal and equal to 0.5625 (the average of 0.75 and 0.375). At *T=w*, all the workers of the previous gamergate are dead and the colony once again contains of a single matriline of full sisters. From *T=0* to *T=w*, the colony includes two matrilines, whereas from *T=w* to *T=g* (*g* being the gamergate's tenure) the colony contains a single matriline (Fig. 1a).

The effect of varying the ratio *w*/*g* is illustrated in Fig. 1, assuming an equal egg-laying rate by all gamergates. In this figure,

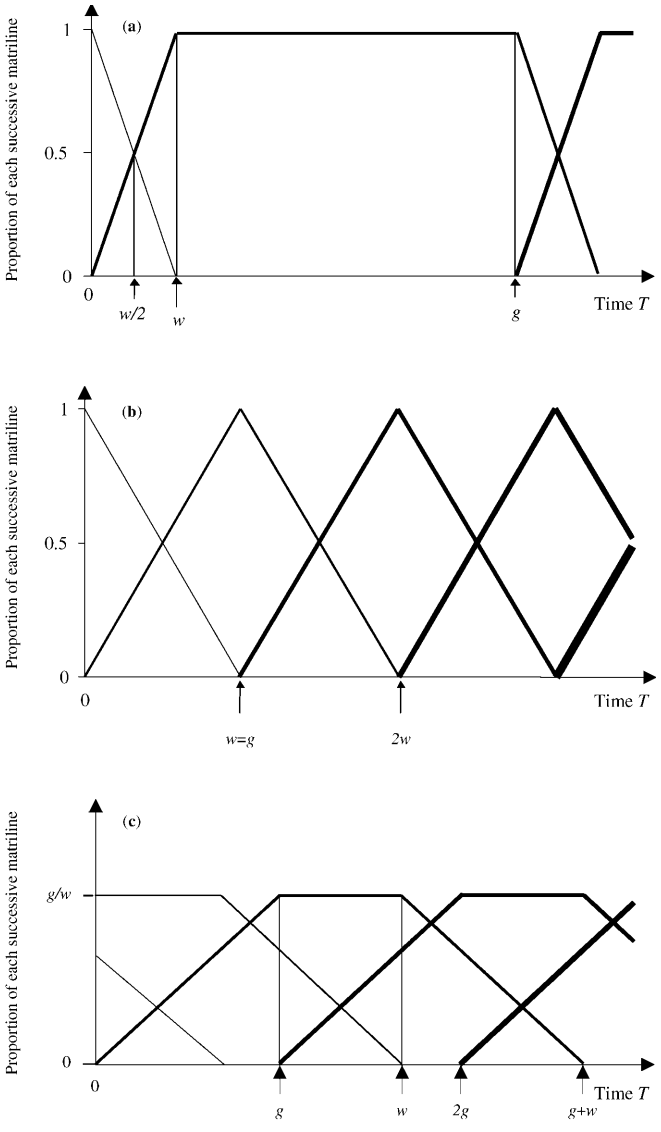

Fig. 1 Temporal variation in the proportion of workers from each successive matriline of full sisters in a single colony. Each curve represents the temporal dynamics of the proportion of workers from a given matriline in the colony under: low serial polygyny ($g=5w$) – most of the time, the colony contains only one matriline and rarely two (**a**); intermediate serial polygyny ($g=w$) – the colony always contains exactly two matrilines (**b**); high serial polygyny ($g<w\leq 2g$) – the colony contains either two or three different matrilines (**c**). T=0 is the time of effective gamergate replacement, $T=w$ and $T=g$ are the workers' lifespan and gamergate's tenure, respectively

g varies whereas w is kept constant; however, the reverse would give the same pattern, since it is the ratio w/g which is actually important. When the gamergate's tenure is much longer than the workers' lifespan, colonies contain only one matriline for most of the time (M~0) (Fig. 1a). On the other hand, with a ratio ≥1 (workers' lifespan is identical to or longer than the gamergate's tenure), colonies always contain at least two matrilines (Fig. 1b,c). The ratio w/g thus directly measures the effect of serial polygyny on the average genetic structure of colonies.

Under this model, assuming that the workers' and gamergate's longevity w and g have no variance and that gamergate replacement occurs randomly among colonies, the expected proportion M of colonies containing more than one matriline is given by:

$$M = \frac{w}{g} \text{ For } w \leq g \quad (2)$$

$$M = 1 \text{ For } w \geq g. \quad (3)$$

The major estimate which is also derived from w/g, with the same assumptions as above combined with the additional assumption that every gamergate lays eggs at the same rate, is the average life-for-life genetic relatedness among adult workers. The detailed derivations of the formula are given in Appendix 2.

$$R_{\text{worker-worker}} = R_S + \frac{w}{g}\left[\frac{r - RS}{3}\right] \text{ For } w \leq g \quad (4)$$

$$\begin{aligned} R_{\text{worker-worker}} = {} & r^m[1+m(1-r)] \\ & -\frac{w}{g}\left[\frac{r^m(1-r)}{3}\right] \\ & +\frac{g}{w}\left[\frac{2(r-r^m)}{1-r} + r^m[1-2m-m^2(1-r)] + R_S\right] \\ & \text{For } w \geq g \\ & +\frac{g^2}{w^2}\left[\frac{2(r^m-r)}{(1-r)^2} + \frac{2r^m(m-1)}{1-r}\right. \\ & \left. +r^m\left(\frac{m^3(1-r)}{3} + m(m-1) + \frac{1}{3}\right) - \frac{RS}{3}\right]. \end{aligned} \quad (5)$$

Where R_S is the coefficient of relatedness among sisters, r is the relatedness between workers of successive matrilines and m is the integer part of the ratio w/g.

Our sampling design involved only workers that were active outside the nest. Therefore our sample mainly consists of foragers. This introduces a potential bias which needs to be evaluated carefully. Given that in *Diacamma*, as in most ant species, task partitioning is age dependent (Nakata 1995), a sample composed primarily of foragers also contains a restricted age range. Such a restriction decreases the probability of sampling two distinct matrilines in the same colony and thereby increases the estimated relatedness among nestmate workers. To investigate this bias, we consider the extreme hypothesis that only foragers of a given age cohort were sampled and then estimate the relatedness among nestmate workers from such a restricted sample. In our model, M would then represent the proportion of colonies containing more than one matriline among foragers and not among all nestmate workers. Every worker is assumed to start foraging at the same age and for the same period of time (w_f), which represents a proportion k of their entire lifespan w ($w_f=kw$) (i.e. at any given time, a proportion k of workers are foragers). M is therefore equal to w_f/g (w_f replaces w in Eq. 2) and the experimentally measured ratio is not w/g but w_f/g. Nevertheless, $\frac{w}{g}$ is equal to $\frac{w_f}{k \times g}$ and, if the value of k is known, the predicted relatedness among nestmate workers can be estimated from a sample of foraging workers by replacing $\frac{w}{g}$ by $\frac{w_f}{k \times g}$ in Eqs. 4 and 5.

Results

Proportion of colonies with more than one matriline among workers

Seven out of the 46 samples (15%) contained more than one matriline of full sisters (six in the 1997 samples, and one in 1998). Correcting for sample bias (Eq. 1), the proportion of colonies with more than one matriline (M) was 0.19 with a confidence interval (CI) ranging from 0.08 to 0.32. Thus at any given time, 19% of colonies contained at least two successive matrilines of full sisters.

Table 2 Average within-colony genetic relatedness among workers. Values for the single- and multiple-matriline colonies were also computed separately. The genetic relatedness estimate (R) is given with its SE. Each estimate was tested against the value of 0.75 expected in a matriline of full sisters, using a t-test (n_{col} is the number of colonies)

	n_{col}	R±SE	t	P-value
1997 samples	33	0.752±0.038	0.09	0.93
1998 samples	13	0.749±0.071	0.04	0.97
Overall	46	0.751±0.032	0.05	0.96
Single matriline	39	0.781±0.024	2.66	0.01
Multiple matrilines	7	0.583±0.113	4.14	0.006

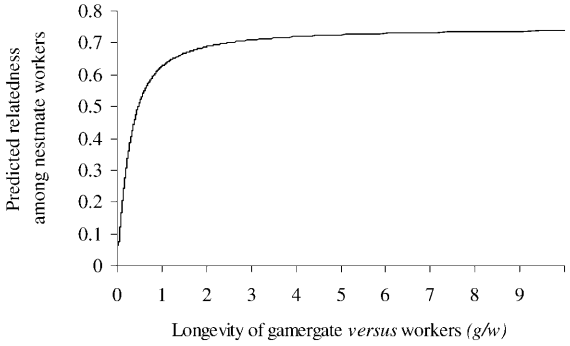

Fig. 2 Average relatedness among nestmate workers predicted from the analytical model (Eqs. 4 and 5 with R_{FS}=0.75 and r=0.375) as a function of the ratio g/w of the gamergate's tenure (g) over the workers' lifespan (w)

Genetic relatedness among collected workers

The average within-colony genetic relatedness estimated among collected workers was 0.751±0.032 (±95% CI) which is not significantly different from 0.75 (Table 2). This estimate did not differ significantly between the two samples (t=0.04, P=0.97) and neither differed significantly from 0.75 (Table 2). These results indicate that, on average, there was no deviation from monogyny and monandry in *D. cyaneiventre*. However, when the multiple-matriline and the single-matriline colonies were considered separately, the relatedness among foragers was significantly lower in the former (0.583) than in the latter (0.781) (t=6.84, P <0.001). Average genetic relatedness in multiple-matriline colonies was significantly lower than 0.75 (Table 2), whereas average relatedness in single-matriline colonies was unexpectedly significantly higher than 0.75 (Table 2). This last result can be explained by inbreeding. Nevertheless, the amount of inbreeding was not high enough to be detected in the F_{IS} calculations. A low amount of inbreeding, undetected by the F_{IS} calculations, could have, however, been sufficient to increase the genetic relatedness. Mating between individuals from related colonies might have induced some inbreeding. Such a slight level of inbreeding should not change the detection probability of a gamergate turnover in the sampled colonies.

Parameters estimated from the model

From our corrected estimate of the proportion of colonies containing more than one matriline (M), the ratio of the workers' lifespan over the gamergate's tenure (w/g) would be equal to 0.19 in our studied population (with a CI ranging from 0.08 to 0.32) (Eq. 2). Even though the confidence interval is large, it does not include 1, indicating that g was significantly higher than w.

Figure 2 shows the average coefficient of relatedness among nestmate workers as a function of the ratio g/w predicted from Eqs. 4 and 5 with R_{FS}=0.75 and r=0.375 (in our case, gamergates are always replaced by their daughters, and successive matrilines of workers are therefore aunts and nieces). Note that Eqs. 4 and 5 and Fig. 2 give the within-colony relatedness averaged across colonies. Each colony will deviate from this mean according to the time since the last gamergate replacement. This variance around the mean will be large for high g/w and low as g/w decreases (when a large number of matrilines are always present in the colony).

As expected, the relatedness among nestmate workers decreases with increasing level of serial polygyny (decreasing g/w). In this study of *D. cyaneiventre*, workers were replaced faster than reproductive females and therefore g/w>1. In this case, the predicted within-colony relatedness only varies from 0.75 to 0.625 (Fig. 2).

Using the value 0.19 for the ratio $w/g\cdot(g/w$=5.26*)* obtained from the proportion of multiple-matriline colonies in Eq. 4, the predicted relatedness among workers in our studied population would be 0.726 with a CI ranging from 0.71 to 0.74. Our empirical estimate, based on the microsatellite genotypes (Table 2) was not significantly different from this predicted value (t=1.53, P=0.13).

Correction for the bias due to restriction of sampling to foragers

The difference between the relatedness among nestmate workers estimated either assuming a sample of foragers of a given age cohort (with correction) or a representative sample of the colony (without correction) gives the magnitude of the potential bias due to sample limitation. This potential sampling bias is given as a function of k in Fig. 3, for different levels of serial polygyny (different values of w_f/g). This bias decreases with increasing proportion of foragers in the colony. Intuitively, a large percentage of foragers will provide a better representation of all nestmates than a small percentage of foragers. A less intuitive result of this graph is that the sampling bias is a non-linear function of k for low values of w_f/g such as the one obtained for *D. cyaneiventre* (bold curve w_f/g=0.19). For low values of k (<0.2), small variations of k lead to large variations in the potential sampling bias. In such a case, estimating the relatedness among nestmates from foragers is inaccurate unless k can be very precisely estimated, which is rarely the case.

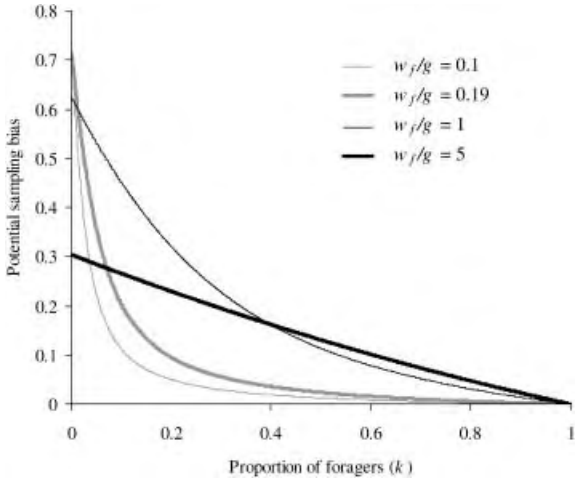

Fig. 3 The potential sampling bias due to collecting only foragers is given as a function of the proportion of foragers (*k*) in a colony, for different values of the ratio w_f/g. The potential bias is expressed as the difference between the two estimates of relatedness among nestmates assuming either that only foragers were sampled (correction) or that the sample represented the age range of the entire colony (no correction) (see text for more details). w_f is the age range during which workers forage and *g* is the gamergate's tenure

Discussion

Our results demonstrate the existence of serial polygyny in the queenless ponerine ant *D. cyaneiventre*. The behavioural regulation of reproduction in this species (see Introduction) prohibits polygyny. However, successive replacements of the reproductive female are likely to occur during the lifetime of a colony. Seven out of the 46 colonies analysed contained more than one matriline of full sisters among collected workers. Correcting for sampling bias, in the population studied and at a given time, 19% of colonies had more than one matriline among workers, i.e. had undergone a relatively recent change of gamergate. This gamergate turnover had no effect on the average genetic relatedness among collected workers. The value estimated over the 46 colonies was 0.751 and did not significantly differ from the relatedness expected in a monogynous and monandrous colony.

The main potential bias of these results follows from the fact that we sampled only foragers, which do not ensure a representative sample of workers with respect to age. This is an important and general problem for the study of serial polygyny, as it is often difficult to obtain a representative sample of the entire colony, especially for species whose nests are deep underground. On the other hand, this problem may be less important than it first appears, because behavioural flexibility may increase the age range represented by foragers. Indeed in *Diacamma*, the age at which a worker becomes a forager varies and depends on various colony characteristics, such as brood number (Nakata 1995). Our model shows that the potential sampling bias introduced by sampling only foragers is particularly difficult to control when the ratio of foragers is low ($k<0.2$), since in such a case, small variations of *k* lead to large variations in the potential sampling bias. In *D. ceylonense*, a species closely related to *D. cyaneiventre* (M. Veuille, A. Brusadelli, L. Brazier and C. Peeters, unpublished data), *k* was about 20% in different colonies (R. Gadagkar, unpublished data). Using this value of *k* and the measured value of $w_f/g=0.19$, the predicted relatedness among nestmates would be approximately 0.63. The ratio w/g would be approximately 1, indicating that the lifespan of workers and the tenure of gamergates would be approximately equal. On the other hand, assuming that the collected workers represent a random distribution of age (no correction), the ratio w/g would be 0.19 which would indicate that the tenure of gamergates is about five times the lifespan of workers. The reality certainly lies between these two extreme values. The only data available come from a laboratory study on *Diacamma* sp. from Japan by Tsuji et al. (1996) indicating that gamergates live about three times longer than workers ($w/g=0.3$).

The tenure of gamergates cannot be directly considered as the gamergate lifespan. Indeed, gamergate turnover results from fission events or death (at least reproductive death) of the gamergate. A long lifespan of gamergates and/or low rate of fission would be associated with a low level of serial polygyny. In *Diacamma*, the rate of fission in natural populations is unknown. The nests of *D. cyaneiventre* are deep underground, with many chambers constructed at different levels, and therefore require a high investment. Moreover, when disturbed, the ants do not leave the nest as in another indian species *D. indicum*, but take refuge in the deepest chamber of the nest (C. Peeters, unpublished data). Finally, no population viscosity was detected in our population (C. Doums, unpublished data) even though this would be expected if fission was a common event. From these indirect data, fission does not seem to be the primary cause of gamergate turnover in *D. cyaneiventre*. Note, though, that several ecological factors influence the rate of fission, such as the availability of empty sites, which is high in a recently colonised site. This was unlikely to be the case in the studied population, as the density of nests was high (mean distance between neighbouring nests was about 5 m; personal observation). Therefore, our results on the lifespan of workers relative to the tenure of gamergates give a good indication of the relative longevity of workers and gamergates in a natural population. We can therefore conclude that the differential longevity observed by Tsuji et al. (1996) in *Diacamma* from Japan under laboratory conditions can also occur in *D. cyaneiventre* in the wild. This differential longevity may be partly driven by physiological differentiation occurring at the adult stage, given that all the female larvae in *Diacamma* have the same developmental pathway (i.e. there are no castes).

Evidence of queen turnover has recently been detected using genetic markers in several polygynous ants (Seppa 1994; Evans 1996; Bourke et al. 1997). For all these species, the turnover rate was considered to be high. For example, in *Leptothorax acervorum*, Bourke

et al. (1997) suggested that the reproductive lifespan of queens in a given colony is just 1 year. Similarly, in *Myrmica tahoensis*, 35–50% of queens disappeared each year (Evans 1996) and in *M. sulcinodis*, the effective genetic turnover of queens was 45–98% between two age cohorts of workers separated by 1 year (Pedersen and Boomsma 1999). However, the model developed here emphasises the importance of considering the queen's reproductive tenure relative to the lifespan of workers, in order to appreciate the effects of serial polygyny (see Eqs. 4 and 5). In the species cited above, the queen's reproductive tenure was shorter than or of the same magnitude as the workers' lifespan (Bourke et al. 1997). Therefore, in both species, queen turnover had major consequences on colony genetic structure.

Our results demonstrate genetic evidence for queen turnover in a monogynous ant species. In *D. cyaneiventre*, gamergate turnover has weak consequences on genetic relatedness among workers which was higher than 0.63 (even taking into account the potential bias of sampling only foragers). The consequences of gamergate turnover should however be more pronounced on the relatedness between workers and brood since, under serial polygyny, the average relatedness between two different age classes decreases with the age distance between these classes (Pedersen and Boomsma 1999). Further studies would be required to investigate the consequences of low serial polygyny on worker-brood relatedness.

Serial polygyny differs from polygyny and polyandry by being associated with temporal variations in colony genetic structure, which are actually more pronounced than their average effect. The outcome of social conflicts could therefore vary over time. For example, Gadagkar et al. (1993b) showed that in the wasp *Ropalidia marginata*, queen reproductive success is correlated with worker-brood genetic relatedness and therefore varies as a consequence of serial polygyny. In contrast to polyandry or polygyny, under serial polygyny, workers can detect a gamergate turnover simply by detecting the absence of a gamergate in the colony. *Diacamma* workers can detect the absence of the gamergate (Cuvillier-Hot et al., 2001). If this information could be integrated with perception of the age of brood (different developmental stages), workers could obtain a simple indicator of their genealogical relationship with the brood. This would enable them to adapt their sex allocation strategies to the different brood stages. Controlled laboratory experiments would be required to investigate this possible effect of serial polygyny on optimal sex allocation strategies.

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Appendix 1

Correction for sampling errors in the observed proportion of multiple-matriline colonies

Let us assume that the correct proportion of colonies with two matrilines M is known. Then, M_{obs} (the observed proportion of colonies with two matrilines) can be derived as a function of M. The probability of detecting two distinct matrilines of full sisters among n workers sampled in the same colony is given by Eq. 6 as a function of y, the proportion of workers of the younger matriline in that colony.

$$p_n(y)=1-y^n-(1-y)^n. \quad (6)$$

Then $p_n(y)$ can be integrated over y between 0 and 1 to give the probability P_n of detecting two distinct matrilines of full sisters in a colony taken at random:

$$P_n = M \times \frac{n-1}{n+1}. \quad (7)$$

C colonies are sampled, with n_i workers sampled in the colony i, the sum of P_{ni} over C colonies gives the expected observed proportion of multiple-matriline colonies M_{obs}:

$$M_{obs} = M \times \frac{1}{c} \sum_{i=0}^{C} \frac{n_i - 1}{n_i + 1}. \quad (8)$$

Eq. 8 leads directly to:

$$M = M_{obs} \times \frac{1}{\frac{1}{C} \sum_{i=1}^{C} \frac{n_i - 1}{n_i + 1}}. \quad (9)$$

Eq. 1 in the text. The only assumption about the dynamic of worker turnover is that the proportion y of workers from the younger matriline follows a uniform distribution between 0 and 1 (i.e. colonies have an equal chance of containing either a minority or a majority of workers from the younger matriline). For example, this implies that gamergate replacement in different colonies is not synchronised.

Appendix 2

Predicted relatedness among workers under a simple analytical model of serial polygyny

The relatedness among workers can be predicted as a function of w/g under the model of serial polygyny described in the text, i.e. assuming (1) a constant, continuous and equal egg-laying activity of all the gamergates (even at the time of gamergate change), (2) no variance in the longevity of workers (w) and gamergates (g) and (3) gamergate replacements among colonies are not synchronised. In each equation, R_S will be the relatedness among sisters and r the relatedness between two matrilines produced by two successive reproductive females.

The colony will be considered as a group of workers whose ages are uniformly and continuously distributed between 0 and w (hypothesis 1 and 2). Let us first consider the case when the workers' lifespan is shorter than the gamergate's tenure ($w \le g$). Let us take a colony randomly selected with respect to the time of gamergate replacement, and state that the last gamergate turnover occurred x units of time ago ($0 \le x \le g$).

When $0 \le x \le g$, the colony contains two matrilines, respectively including workers of age 0 to x for the younger, and x to w for the older (hypothesis 2). This strict partition of matrilines by age, combined with hypothesis 1, permits us to calculate directly the proportion of workers belonging to each matriline: it is given by the ratio of the age range of the matriline divided by the total age range of the colony ($=w$). The average relatedness among adult workers is therefore:

$$R(x) = RS \times \left[\left(\frac{w-x}{w} \right)^2 + \left(\frac{x}{w} \right)^2 \right] + 2r \times \left[\frac{x}{w} \times \frac{w-x}{w} \right] \quad \text{for } x \in [0;w]. \tag{10}$$

When $w \le x \le g$, every worker of the older matriline is dead and the colony contains only one matriline. The average relatedness among adult workers is therefore:

$$R(x) = RS \quad \text{for} \quad x \in [w;g]. \tag{11}$$

$R(x)$ is then integrated over x between 0 and g and the integral divided by g. This gives the mean within-colony relatedness expected if several colonies are sampled (hypothesis 3):

$$R_{\text{worker-worker}} = R_S + \frac{w}{g}\left[\frac{r - R_S}{3} \right].$$

Eq.4 in the text.

Let us now consider the case when the workers' lifespan is longer than the gamergate's tenure ($w \ge g$). The relatedness between two matrilines separated by l generations is r^l ($l>0$). m is defined as the integer part of the ratio w/g (the highest integer inferior to the ratio w/g). Let us consider a colony randomly selected with respect to its life cycle, and state that the last gamergate turnover occurred x units of time ago ($0 \le x \le g$).

If $0 \le x \le g-mg$, the colony includes $m+2$ successive matrilines of sisters, the age ranges of which, from the youngest to the oldest, are, respectively:

$[0;x];[x;x+g];[x+g;x+2g];[x+2g;x+3g];$ .....;
$[x+(m-1)g;x+mg];[x+mg;w]$.

The proportion of workers belonging to each of them is therefore x/w for the youngest matriline, $(w-x-mg)/w$ for the oldest, and g/w for the m other matrilines.

Within-colony relatedness is then derived by pure calculus (available upon request), and defined as $R_m(x)$ with

$$\begin{aligned} R_m(x) = {} & \frac{1}{1-r}\left[2mr^{m+1}\frac{g^2}{w^2} + 2r(1-r^m)\frac{g}{w} \right] \\ & + \left(\frac{1}{1-r} \right)^2 \left[2r(r^m-1)\frac{g^2}{w^2} \right] \\ & + RS\left[m(m+1)\frac{g^2}{w^2} - 2m\frac{g}{w} + 1 \right] \\ & + 2x(RS - r^{m+1})\left[m\frac{g}{w^2} - \frac{1}{w} \right] \\ & + x^2\left[2(RS - r^{m+1})\frac{1}{w^2} \right]. \end{aligned} \tag{12}$$

If $w-mg \le x \le g$, every worker of the oldest matriline is dead and the colony includes only $m+1$ successive matrilines of sisters. The proportion of workers belonging to each of them is x/w and $[w-x-(m-1)g]/w$ for the youngest and the oldest matrilines, respectively, and g/w for the $m-1$ others. Therefore, the within colony relatedness is simply $R_{m-1}(x)$.

Relatedness is then integrated over x and the integral divided by g to obtain the expected average within-colony relatedness:

$$R_{\text{worker-worker}} = \frac{1}{g}\left[\int_0^{w-mg} R_m(x)dx + \int_{w-mg}^{g} R_{m-1}(x)dx \right]. \tag{13}$$

Eq.13 leads to

$$\begin{aligned} R_{\text{worker-worker}} = {} & r^m[1+m(1-r)] \\ & - \frac{w}{g}\left[\frac{r^m(1-r)}{3} \right] \\ & + \frac{g}{w}\left[\frac{2(r-r^m)}{1-r} + r^m[1-2m-m^2(1-r)] + R_S \right] \\ & + \frac{g^2}{w^2}\left[\frac{2(r^m-r)}{(1-r)^2} + \frac{2r^m(m-1)}{1-r} \right. \\ & \left. + r^m\left(\frac{m^3(1-r)}{3} + m(m-1) + \frac{1}{3} \right) - \frac{RS}{3} \right]. \end{aligned}$$

Eq. 5 in the text.

References

Bourke AFG, Franks NR (1995) Social evolution in ants. Princeton University Press, Princeton, NJ

Bourke AFG, Green HAA, Bruford MW (1997) Parentage, reproductive skew and queen turnover in a multiple-queen ant analysed with microsatellites. Proc R Soc Lond B 264:277–283

Crozier RH, Pamilo P (1996) Evolution of social insect colonies. Oxford University Press, Oxford

Cuvillier-Hot V, Cobb M, Malosse C, Peeters C (2001) Sex, age and ovarian activity affect cuticular hydrocarbons in *Diacamma ceylonense*, a queenless ant. J Insect Physiol 47:485–493

Doums C (1999) Characterisation of microsatellites in the queenless ponerine ant *Diacamma cyaneiventre*. Mol Ecol 8:1957–1959

Evans JD (1996) Queen longevity, queen adoption, and posthumous indirect fitness in the facultatively polygynous ant *Myrmica tahoensis*. Behav Ecol Sociobiol 39:275–284

Fukumoto Y, Abe T, Taki A (1989) A novel form of colony organization in the "queenless" ant *Diacamma rugosum*. Physiol Ecol Jpn 26:55–62

Gadagkar R, Chandrashekara K, Chandran S, Bhagavan S (1993a) Serial polygyny in the primitively eusocial wasp *Ropalidia marginata*: implications for the evolution of sociality. In:

Keller L (ed) Queen number and sociality in insects. Oxford University Press, Oxford, pp 1–15
Gadagkar R, Chandrashekara K, Chandran S, Bhagavan S (1993b) Queen success is correlated with worker-brood genetic relatedness in a primitively eusocial wasp (*Ropalidia marginata*). Experientia 49:714–717
Gotwald WH (1995) Army ants: the biology of social predation. Cornell University Press, Ithaca
Gronenberg W, Peeters C (1993) Central projections of the sensory hairs on the gemma of the ant *Diacamma*: substrate for behavioural modulation? Cell Tissue Res 273:401–415
Hamilton WD (1964) The genetical evolution of social behaviour. J Theor Biol 7:1–52
Hölldobler B, Wilson EO (1990) The ants. Springer, Berlin Heidelberg New York
Jeanne RL (1972) Social biology of the neotropical wasp *Mischocyttarus drewseni*. Bull Mus Comp Zool Harvard Univ 144:63–150
Lenoir A, Querard L, Pondicq N, Berton F (1988) Reproduction and dispersal in the ant *Cataglyphis cursor* (Hymenoptera, Formicidae). Psyche 95:21–44
Nakata K (1995) Age polyethism, idiosyncrasy and behavioural flexibility in the queenless ponerine ant, *Diacamma sp.* J Ethol 13:113–123
Nakata K, Tsuji K, Hölldobler B, Taki A (1998) Sexual calling by workers using the metatibial glands in the ant, *Diacamma* sp., from Japan (Hymenoptera: Formicidae). J Insect Behav 11: 869–877
Pedersen JS, Boomsma JJ (1999) Effect of habitat saturation on the number and turnover of queens in the polygynous ant, *Myrmica sulcinodis*. J Evol Biol 12:903–917
Peeters C (1991) The occurrence of sexual reproduction among ant workers. Biol J Linn Soc 44:141–152
Peeters C (1993) Monogyny and polygyny in ponerine ants with or without queens. In: Keller L (ed) Queen number and sociality in insects. Oxford University Press, Oxford, pp 234–261
Peeters C, Higashi S (1989) Reproductive dominance controlled by mutilation in the queenless ant *Diacamma australe*. Naturwissenschaften 76:177–180
Peeters C, Billen J, Hölldobler B (1992) Alternative dominance mechanisms regulating monogyny in the queenless ant genus *Diacamma*. Naturwissenschaften 79:572–573
Queller DC (1993) Genetic relatedness and its components in polygynous colonies of social insects. In: Keller L (ed) Queen number and sociality in insects. Oxford University Press, Oxford, pp 132–151
Queller DC, Goodnight KF (1989) Estimating relatedness using genetic markers. Evolution 43:258–275
Raymond M, Rousset F (1995) Genepop (version 1.2) population genetics software for exact tests and ecumenicism. J Hered 86:248–249
Ross KG (1993) The breeding system of the fire ant *Solenopsis invicta*, and its effects on colony genetic structure. Am Nat 141:554–576
Seppa P (1994) Sociogenetic organization of the ants *Myrmica ruginodis* and *Myrmica lobicornis*: number, relatedness and longevity of reproducing individuals. J Evol Biol 7:71–95
Sokal RR, Rohlf FJ (1995) Biometry. Freeman, New York
Tsuji K, Nakata K, Heinze J (1996) Lifespan and reproduction in a queenless ant. Naturwissenschaften 83:577–578
West-Eberhard MJ (1978) Polygyny and the evolution of social behavior in wasps. J Kans Entomol Soc 51:832–856
Wheeler WM, Chapman JW (1922) The mating of *Diacamma*. Psyche 29:203–211
Yamane S (1986) The colony cycle of the Sumatran paper wasp *Ropalidia* (*Icariola*) *variegata jacobsoni* (Buysson), with reference to the possible occurrence of serial polygyny (Hymenoptera: Vespidae). Monit Zool Ital 20:135–161

ANNEXE 6

Reproductive conflicts affect labour and immune defences in the queenless ant *Diacamma 'nilgiri'*

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En preparation pour une 2ème soumission à Evolution

REPRODUCTIVE CONFLICTS AFFECT LABOUR AND IMMUNE DEFENCE IN THE QUEENLESS ANT DIACAMMA 'NILGIRI'

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ABSTRACT

In many species of social Hymenoptera, totipotency of workers induces potential conflicts over reproduction. However, actual conflicts remain rare despite the existence of a high reproductive skew. One of the current hypotheses assumes that actual conflicts are costly and thus selected against. We studied the costs of conflict in 20 colonies of the queenless ant *Diacamma 'nilgiri'* by testing the effects of conflicts on labour and worker immunocompetence, two parameters closely linked to worker indirect fitness. In this species, the dominant female is the only mated worker (gamergate) and monopolizes reproduction. We experimentally induced conflicts by splitting each colony into two groups, a control group containing the gamergate and an orphaned group displaying a high level of aggressions until a new dominant worker arises. To estimate the level of immunocompetence, we challenged workers with the bacteria *E. coli*, and measured the clearance 10 hours after injection. Time budget analysis revealed a lower rate of labour and especially brood care in groups affected by conflicts, supporting the existence of a cost of conflicts on labour. A lower immunocompetence was also found in orphaned groups compared to control groups when the test was conducted 15 days after splitting. We propose that this decrease in immunocompetence induced by conflicts could stem from stress and not directly from aggression. Accordingly, immunocompetence correlated positively with the number of aggressions given or received by each worker within orphaned group, which could be linked to variability in quality and ovarian activation of competing workers.

Keywords :

Cost of conflicts, aggressions, immune defence, immunosuppression, labour, regulation of reproduction, queenless ants

Animal societies can be viewed as harmonious and cooperative systems, however conflicts between members of the society are widespread. These conflicts result from divergent interests between individuals of the same sex mainly over reproduction. In most vertebrate societies, dominance interactions that can include aggressive behaviours lead to a hierarchy where dominant individuals try to monopolize reproduction (Faulkes et al. 1991; Emlen 1995; Mock and Parker 1997). Such conflict can be found in primitively eusocial insects (Keller and Vargo 1993; Peeters 1997). In higher eusocial insects, this conflict is partly solved by the extreme division of reproductive labour with only the queen caste monopolizing reproduction. The worker caste, instead, performs all the other tasks of the colony which includes foraging, brood care and maintenance of the nest. Because of the haplodiploid system of sex determination of Hymenoptera (haploid males are produced by arrhenotokous parthenogenesis whereas diploid females result from fertilized eggs), relatedness asymmetries between colony members generate specific conflicts between queen and workers (Bourke and Franks 1995; Crozier and Pamilo 1996). In particular, conflicts over the production of males by workers or the optimal colony sex-ratio have largely been investigated as they provide elegant tests of the kin selection theory (Bourke and Chan 1999). However in many cases, even though conflicts are predicted, no evidence of manipulation can be detected, indicating that the conflicts are already resolved. For example, workers eggs laying rarely occurs in monogynous colonies, even though this is predicted by kin selection theory (Ratnieks 1988).

Several sets of hypotheses suggest why actual conflicts are often absent in situations where potential conflicts are predicted. One of the leading hypotheses proposes that conflicts are costly and thus pacific solutions are selected for (Ratnieks

and Reeve 1992; Bourke and Franks 1995). For example, the cost of worker reproduction (West-Eberhard 1975; Cole 1986) could favor the spread of self-restraint or worker policing alleles in populations, resulting in the increase of social harmony (Ratnieks 1988). Besides, direct aggressions between colony members appear to be very rare in insect societies, which indirectly suggests that they should be associated with high costs. Fighting behaviours are described between queens after pleometric foundation (Röseler 1991; Choe and Perlman 1997), between workers and eventually queen for male production in stingless bees (Peters et al. 1999) or between workers for dominance and reproductive status in some leptothoracine and queenless ponerine ants (Heinze et al. 1994; Monnin and Peeters 1999). In the queenless ponerine ants, the queen caste does not exist and workers have retained the ability to reproduce leading to basic conflicts over reproduction. In the monogynous queenless species, with a single mated reproductive worker (called gamergate), aggressions are just used for the establishment of the gamergate in orphaned colonies. But thereafter, workers restrain their reproduction as long as the gamergate keeps a correct level of oogenesis which can be detected by a fertility signal based on cuticular hydrocarbons (Monnin et al. 1998; Cuvillier et al. 2004a; Cuvillier et al. 2004b). This switch from aggressive to chemical regulation of reproduction strongly suggests that aggressions are costly.

Costs of aggressive conflicts can be expressed in different ways. An energetic cost of worker aggressions was observed in orphaned colonies of *Pachycondyla obscuricornis*, through a higher metabolic rate measured by the total CO_2 emission of the colony (Gobin et al. 2003). As the time budget of a worker is limited, workers involved in aggressive behaviours could also invest less time in labour, which would decrease colony efficiency. Such decrease of work load in aggressive workers has

been observed in different species (Cole 1986; Schmid-Hempel 1998; Monnin and Peeters 1999; Monnin and Ratnieks 1999). Direct costs on the quality of individuals have still remained unexplored. In this study we proposed to address this question by investigating potentials costs of conflicts on immune defence. Although this approach is new, immune defence could be an interesting trait to consider for two reasons. First, the level of immune defence is known to affect the probability of getting infected by a pathogen (Adamo 2004) and should therefore be linked to worker survival and colony productivity, all the more in social insects, for which living in groups composed of closely related individuals facilitates disease transmission. Second, it is now generally assumed by ecological immunologists that immune defence are costly in insects (Lochmiller and Deerenberg 2000; Rolff and Siva-Jothy 2003; Schmid-Hempel 2005), and integrating immunity as a life-history trait has already revealed useful in understanding the evolution of life-history strategies in insects.

The invertebrate immune system is based on both cellular and humoral components, and infection stimulates a range of diverse defensive responses (Gillespie et al. 1997). Haemocytes attach to invading organisms and then isolate them by phagocytosis or by forming around them an organized multi-cellular capsule. These responses are often associated with proteolytic activation of the phenoloxydase zymogene that is present in the haemolymph. An other component of insect immune response to pathogens is the synthesis by fat body and haemocytes of a variety of antimicrobial proteins and peptides, which are secreted in the haemolymph. Insect immunity thus relies on complex interactions between humoral and cellular components, which should be considered when trying to assess the relative strength of the immune system (Adamo 2004).

126 In the present study, we investigated costs of aggressive reproductive
127 conflicts in a queenless monogynous species *Diacamma 'nilgiri',* both on working
128 efficiency and on quality of individuals, assessed by their level of immune defence.
129 We studied an immune response that involves several components, by measuring the
130 clearance of *E. coli* bacteria that we injected in ants. In order to assess the cost of
131 conflicts, aggressive interactions were induced by splitting a colony into two groups,
132 a control group (with gamergate) and an orphaned group (without gamergate).
133 Behavioural studies allowed us to test for an effect of conflicts on work load in
134 orphaned groups. The existence of a cost of conflicts on immune defence and its
135 origin within orphaned groups were also investigated. Two kinds of costs can be
136 present. First, conflicts may induce a stress response on all individuals of the
137 orphaned groups, which could negatively affect immune defence as observed in
138 many species (Lackie 1988; Apanius 1998). A lower immune response should thus
139 be expected in most workers of orphaned groups. Second, aggression by itself could
140 represent a cost. In such case, a decrease of immune defence in orphaned groups
141 would be due especially to individuals involved in aggressive interactions.
142
143

MATERIALS AND METHODS

Studied Species

We conducted the study on an indian population of *Diacamma* which is highly related to *Diacamma ceylonense and* referred to as *'nilgiri'* (Baudry et al. 2003). All the species of the genus *Diacamma* are queenless, and have one singly mated worker called gamergate. A peculiar behaviour reduces the conflict over reproduction in all species, except in *Diacamma 'nilgiri'* (Cournault and Peeters 2006; Peeters et al. 1992). This behaviour consists in biting off a pair of thoracic appendages (called gemmae) of all emerging workers (Fukumoto et al. 1989; Peeters and Higashi 1989; Peeters and Billen 1991). By doing so, the gamergate prevents them from mating and reproducing sexually (Peeters and Higashi 1989), therefore removing any potential conflict over the reproduction of females. Interestingly, in *Diacamma 'nilgiri'*, in which mutilation does not occur, the regulation of reproduction is similar to the one found in many monogynous queenless species. In an orphaned colony, aggressive interactions are used to determine a hierarchy and the future gamergate. In *Diacamma 'nilgiri'*, these aggressions can include antennal boxing, morsures, immobilizations (several workers holding another worker for hours), thoracic attacks (repeated attacks on gemmes) and sting smearing (Peeters et al. 1992, Cournault and Peeters, personnal communication). Cuvillier et al. (2004a) demonstrated, in a different queenless species, that once the gamergate is established, aggressions are replaced by chemical fertility signalling. Aggressive interactions can therefore be observed after the death of a gamergate or after colony fission. Even though the details of this mode of colony foundation are not known, it implies that after fission one of the groups of workers will not have a gamergate, and might thus show aggressive behaviours.

168

169 *Ants collection and rearing*

170 Twenty colonies of *Diacamma 'nilgiri'* were collected in February and
171 November 2004 near Gundlupet, Tamil Nadu (mean initial colony size ± SE: 278.9 ±
172 15.0). Colonies were reared in the laboratory in plaster nests at 25°C with 12h light -
173 12h dark cycle and fed at libitum on *Tenebrio molitor* pupae and crickets. Workers
174 are monomorphic, with a body size of about 1.5 cm and very few size variation. All
175 ants were individually marked with spots of coloured paint, and each colony was
176 observed before the start of the experiment in order to identify the gamergate, the
177 foragers and the nurses.

178

179 *Experimental groups*

180 To induce social conflicts, we splitted each initial colony into two groups: the
181 control group containing the gamergate, and the orphaned group, without the
182 gamergate. We allocated randomly the workers to the two groups, while ensuring
183 that they were identical in size and composition with respect to the proportion of
184 foragers and nurses. The brood was also equitably shared. This process corresponds
185 to the start of the experiment (day zero). Aggressive behaviours are known to occur
186 in orphaned groups whereas they are absent in presence of the gamergate (Peeters et
187 al. 1992). The size of these groups varied among colonies from 52 to 80 ants (mean
Table 1 188 group size ± SE = 70.2 ± 2.2; see Table 1). If the size of the initial colony exceeded
189 160 individuals, a maximum of 80 ants was used in each group.

190

191 *Behavioural observations*

After splitting, we estimated the intensity of aggressions in orphaned groups by counting the number of aggressive interactions during a continuous 30-min observation period twice a day until the end of the experiment. Antennal boxing and sting smearing were rarely observed. Aggressiveness generally increased on the first or second day after colony-splitting, and lasted for about eight days. Furthermore, for 14 colonies, scans of the orphaned and control groups were performed twice a day until the end of the experiment. Each scan consisted in recording the behaviour performed by each individual. Ants were considered resting when they remained totally motionless or when they moved only antennas. We also recorded working behaviours, comprising brood care, foraging and nest maintenance. As the time spent in nest maintenance is low, we included it with foraging in "working behaviors others than brood care" which corresponds to nest provisioning and maintenance. Other behaviours were also recorded, including social interactions, grooming, moving around the nest and eating, but were not individually considered in the analysis.

Immunological measurements

We tested the effect of conflicts on immunocompetence at two different times: eight days after splitting, when the aggressions were at the highest level and only started to decrease in most colonies, and 15 days after splitting, when very few or no aggression were observed. The immunocompetence measurement was performed 8 days after splitting for 5 colonies, 15 days after splitting for 7 colonies, and both 8 days and 15 days after splitting for 8 colonies (see Table 1). The immune response was evaluated by measuring the resistance to bacterial infection (see below). In order to perform the test the same day in the orphaned and control groups

of a given colony, the immune response was measured on a maximum of 40 ants in each group. To ensure a representative distribution of ants according to the level of aggressions they received, ants were categorized by the number of aggressions received (0, 1-4, 5-9, 10-19, 20-49, 50-100, >100) and the proportion of each class was reproduced in the subsample.

The antibacterial response was measured as the persistence of a bacterial infection with *Escherichia coli* strain CIP 103470 (Pasteur Institute Collection, Paris), after injection in haemolymph, as described in Gorman and Paskewitz (Gorman and Paskewitz 2000). The resistance of an animal to a pathogen depends on different parameters, including the existence of behavioural adaptations, the level of genetic variation in factors that determine pathogen intrusion and recognition, and the efficiency of the immune response, which corresponds to immunocompetence. Indeed, a variation of resistance to a pathogen between individuals is not always related to differences in the capacity of the immune system, and caution may be required in interpretation of results (Apanius 1998). In our experiment, defence mechanisms other than immune system should not interfere: first, the behavioural and cuticular lines of defence are not involved in resistance since the pathogen is directly introduced in the haemolymph. Second, it is unlikely that *E. coli* and ants have coevolved since *E. coli* is a general and ubiquitous pathogen. Third, ants from a given colony are very close genetically, thus the problem of genetic variation of resistance based on individual differences in pathogen recognition should not be important.

Bacteria solutions were prepared by diluting an overnight culture in a LB culture medium to a concentration of 300 bacteria/µl. Bacteria were counted using a neubauer haemocytometer. Ants from orphaned and control groups from a given

242 colony were injected the same day with the same bacterial solution. 1µl of the
243 bacterial solution was injected to cold-anesthetized ants, using a 10µl syringe
244 connected with a flexible capillary to a 0.3mm–diameter dental needle, through the
245 intersegmental membrane between the 3rd and 4th abdominal segments. Injected ants
246 from each group were kept together in a plastic box with water supply, separately
247 from the rest of the colony. Ten hours later, most ants had survived (97.7%), and the
248 haemolymph was sampled using a disposable graduated capillary tube through a hole
249 performed in the abdomen. From 0.3 to 1.5µl of haemolymph was sampled from
250 each ant and diluted 100 fold in LB culture medium. From this solution, 30µl were
251 spread on LB agar plates containing tetracycline at a concentration of 10µg/ml (the
252 strain used is resistant to tetracyclin). The plates were incubated overnight and then
253 scored for the number of *E. coli* colonies. The level of the immune response of each
254 ant was then characterized by the bacterial count, a low bacterial count indicating a
255 high immune response and inversely. Ants that died during immunological
256 manipulation or for which no haemolymph could be sampled were excluded from
257 analysis (in total 3.4% of manipulated ants). The concentration of bacteria injected
258 and the time between injection and haemolymph sampling were selected to allow a
259 good survival of the ants and a good variance in the number of bacteria between
260 individuals.
261
262 *Data analysis*
263 For each colony, the level of aggressiveness in control and orphaned group
264 was compared using a Mann-Whitney test on the mean number of aggressions per
265 individual. To test for the effect of conflicts on behaviour and immune defence,
266 analyses were carried out using mixed models, with treatment (control/orphaned) as

fixed factor and colony and interaction treatment by colony as random factors. For behavioural data, the dependant variable was the ratio of the number of scans for which the individual was observed performing a given behaviour divided by the total number of scans, and the binomial distribution was used (lmer function in R 2.4 for Windows). For immune defence data, the bacterial count (number of bacteria in 30µl of 100 fold-diluted-haemolymph) was the dependant variable, following a negative binomial distribution (glmmADMB function in R 2.4 for Windows). Such models better described our data than models using a Poisson distribution. Effects are tested by comparing two models, one having the term of interest removed, using the likelihood (L) ratio test (LRT). To compare a model1 and a model2, the LRT statistic is calculated as 2 [log(L2)-log(L1)] and follows a χ^2 distribution. The degree of freedom is the difference in the numbers of parameters between the models. In all tests the interaction terms were removed when not significant.

Concerning immune defence data, given that we plated always the same volume of haemolymph the number of bacteria counted reflects the concentration of bacteria in haemolymph. For such a measure it would be relevant to control for the global level of hydration of ants. These data were not available but we included in the models the volume of haemolymph that we were able to remove from ants as it might be correlated to the total volume of haemolymph present in the body. Moreover, the colonies tested 15 days after splitting included eight colonies for which at least half of the workers were removed eight days after splitting (see Table 1). To control for a possible disturbance induced by the reduction of the colony size, the interaction treatment by size reduction was initially included in the model (see below).

291 In order to test for a direct cost of aggressions on immune defence, we
292 performed an analysis within orphaned groups using a mixed model with the
293 bacterial count as dependant variable. As above, we used a negative binomial
294 distribution for the bacterial counts. The independent variables were the volume of
295 haemolymph removed from ants and the number of aggressions ants were engaged in
296 (received or given) as fixed factors and the colony as random factor. The effect of the
297 interaction number of aggressions by colony was not considered because our data are
298 not appropriate to test such an effect since the range of aggressions experienced by
299 ants largely differ between orphaned groups. To investigate the potential effect of
300 aggressions, similar models were also built with either the number of aggressions
301 received or the number of aggressions given.
302
303 RESULTS
304 *Aggressions and time activity budgets*
305 The number and pattern of aggressions varied strongly among the orphaned
306 groups (mean total number of aggressions in orphaned groups ± SE: 654.4 ± 89.0;
307 see Table 1). However, for each colony, aggressiveness was significantly higher in
308 orphaned than in control group (Mann-Whitney analysis on the mean number of
309 aggressions per individual, all P <0.05). Splitting the colonies into two groups was
310 thus a successful way to induce aggressions in the orphaned groups as predicted.
311 In all models analysing behavioural scans the effect of the interaction
312 treatment by colony was not significant, and was therefore removed. Individuals
313 spent less time working in orphaned groups than in control groups (17.7% of
Table2 314 reduction in total labour, see Table 2 and Fig. 1C) and the difference was significant
Fig 1 315 ($\chi^2_1 = 8.30$, $P = 0.0039$). Brood care was more affected (23.7% of reduction, $\chi^2_1 =$

316 6.03, $P = 0.0145$) than nest maintenance and foraging (11.2% of reduction, $\chi^2_1 =$
317 1.50, $P = 0.221$). Besides, no difference in resting rate was found between control
318 and orphaned groups ($\chi^2_1 = 0.02$, $P = 0.88$).

319 The time spent in aggressive behaviours was quite low (5.9%), and therefore
320 not sufficient to explain the lower working activity in orphaned groups. This was
321 checked by calculating the frequency of each behaviour by individual, not based on
322 all scans, but after having removed the scans involving an aggressive act. As
323 expected, the same qualitative results were found (data not shown), indicating that
324 the difference in workers time budget between the two groups can not simply be
325 explained by the occurrence of aggressive acts in orphaned groups. The social
326 conflict in orphaned groups therefore induced disturbance that decreased the working
327 effort.

328

329 *Effects of conflicts on immune response*

330 Data on antibacterial response were collected for a total of 1926 ants from 20
331 colonies. The level of immune response of each ant was characterized by the
332 bacterial count 10 hours after injection, a low bacterial count indicating a high
333 immune response and inversely. The results suggested a trend to a lower bacterial
334 count in orphaned groups eight days after splitting. At the opposite a significantly
Fig 2 335 higher bacterial count in orphaned groups was detected 15 days after splitting (see
Table 3 336 Fig. 2; main statistical results are reported in Table 3). This indicated a lower
337 resistance to infection for groups displaying conflicts.

338 The volume of haemolymph removed from ants had no significant effect on
339 the bacteria count. The interaction treatment by group size-reduction was neither

340 significant ($\chi^2_1 = 1.68$, $P = 0.19$) which showed that the possible disturbance
341 induced by the removal of 40 individuals eight days after splitting did not influence
342 the effect of treatment at 15 days. The mean bacterial counts in control groups at
343 eight days and 15 days were significantly correlated ($r^2 = 0.88$, $P = 0.003$, n = 8),
344 indicating that colonies differed in their basic level of immune response, a level
345 which is stable over time. This is not surprising since individuals from the same
346 colony share a common environment as well as 75% of their genotype (in a
347 monogynous and monoandrous colony).

348 No effect of the interaction treatment by colony, considered as a random
349 effect, was detected. However, both 8 and 15 days after splitting, the intensity of
350 treatment seemed to vary according to the colony (see Fig. 2). We used a GLM
351 model to look for the effect of the explanatory independent factors (colony size at
352 sampling, total mortality in both groups during the experiment, and total number of
353 aggressions in orphaned group) on the difference in bacterial count between the two
354 treatments within colonies. The results did not reveal any significant effect, neither
355 for colonies tested eight days after splitting (Colony size: $F_{1,12} = 0.08$, $P = 0.79$; Total
356 mortality: $F_{1,12} = 1.44$, $P = 0.26$; Total number of aggressions: $F_{1,12} = 0.41$, $P =$
357 0.54) nor for colonies tested 15 days after splitting (Colony size : $F_{1,12} = 0.01$, $P =$
358 0.93; Total mortality: $F_{1,12} = 0.26$, $P = 0.62$; Total number of aggressions: $F_{1,12} =$
359 0.14, $P = 0.72$).

360 In order to determine whether all ants from orphaned groups are equally
361 affected by conflicts, or whether only those really involved in conflicts suffer
362 specific costs, we analyzed data collected 15 days after splitting after having
363 removed from orphaned groups the ants directly involved in conflicts. The same
364 mixed model as for complete groups was built, using negative binomial distribution

365 and including treatment and volume of haemolymph as fixed factors and colony and
366 interaction treatment by colony as random factors. We considered that workers can
367 receive 1 or 2 aggressions accidentally and thus we excluded from the analysis only
368 ants that received at least 3 aggressions. The average bacterial number was still
369 higher in orphaned group ($\chi^2_1 = 5.8$, $P = 0.016$), suggesting that ants receiving few
370 or no aggressions were also concerned by an immuno-suppression 15 days after
371 splitting and that the cost of conflicts is not only due to a direct effect of aggressive
372 interactions. Note that similar results were found whatever the number of aggressions
373 considered as a threshold. For instance, if only workers suffering at least 10
374 aggressions were removed, the treatment effect was still significant (Treatment:
375 $\chi^2_1 = 8.28$, $P = 0.004$). On the contrary, the effect observed 8 days after splitting is
376 likely to be due to individuals involved in conflicts. When individuals receiving at
377 least 3 aggressions were removed, no difference remain between groups ($\chi^2_1 = 1.16$,
378 $P = 0.281$). Besides these ants suffering at least 3 aggressions had a lower bacterial
379 count, thus a higher immunocompetence, than ants from control groups ($\chi^2_1 = 3.78$,
380 $P = 0.052$).

381 Within orphaned group, 15 days after splitting, bacterial count was found to
382 be negatively correlated with the number of aggressions received or given ($\chi^2_1 = 4.7$,
383 $P = 0.030$). Workers involved in aggressions thus show a higher
Fig 3
384 immunocompetence, which is the reverse than what would be expected if aggression
385 by itself induced a cost on immune defence. Further analyses showed a negative
386 relation between bacterial count and the number of aggressions given that is
387 marginally significant ($\chi^2_1 = 3.3$, $P = 0.069$) but the effect of aggressions received

388 was not significant (even if there is also a negative relation: $\chi^2_1 = 2.04$, $P = 0.153$).
389 No effect of the number of aggressions on bacterial count was detected 8 days after
390 splitting (aggressions received: $\chi^2_1 = 0.66$, $P = 0.417$; aggressions given: $\chi^2_1 = 0.80$,
391 $P = 0.371$).
392
393 DISCUSSION
394 *Aggressions and time activity budgets*
395 The splitting of colonies was followed by a period of conflicts in orphaned
396 groups. The number of aggressions in these groups was highly variable, which
397 confirmed previous observations made on this species (Peeters et al. 1992; Cournault
398 and Peeters 2006). The age distribution of individuals within the orphaned groups is
399 probably a major factor determining the intensity of reproductive conflicts since
400 young individuals are more prone to seek access to reproduction. Moreover, in some
401 ponerine species, a hierarchy among high-ranking individuals is observed even in the
402 presence of the gamergate (Monnin and Peeters 1999). If such underlying hierarchy
403 occurs in *Diacamma 'nilgiri'*, the conflict could be reduced in orphaned groups
404 containing a high-ranking worker, since this worker is already dominant. Given that
405 such potential underlying hierarchy as well as the age distribution of workers are not
406 known, these uncontrolled parameters could explain the large range of conflict
407 intensity observed among orphaned groups.
408 Workers in orphaned groups spent less time working, and this reduction in
409 work load was stronger concerning brood care than maintenance and foraging. Since,
410 at a given time, in a colony, a large percentage of workers are resting (around 30%),
411 they may represent a reserve that could be mobilized if necessary. We could have

412 then expected a lower percentage of inactive workers in orphaned groups. However,
413 our results did not confirm such role of reserve since the percentage of inactive
414 workers was the same in both groups, even if the time spent in working behaviors
415 was lower in orphaned groups.

416 Two non exclusive hypotheses can be put forward to explain the decrease in
417 labor, and especially brood care, in orphaned groups. First, dominant individuals
418 could realize a trade-off between working and fighting for time and metabolic
419 reserves. In colonies of *Leptothorax allardycei,* Cole (1986) analyzed the time
420 budget of the three top ranking ants and observed a negative relation between
421 dominance activity and brood care. Similarly, a recent study on hover wasps
422 demonstrated that helpers adjust their working effort according to the probability of
423 attaining breeding status themselves (Field et al. 2006). The authors experimentally
424 removed a high ranking worker and found that subordinates that consequently rose in
425 the hierarchy worked less afterwards. The evolutionary basis of this response should
426 lie in a trade-off between working effort, that increases the indirect component of
427 their fitness, and future direct fecundity and survival. A similar phenomenon could
428 occur in queenless ants, even if the hierarchy is not always well-defined. In our
429 species, removing the gamergate increases the probability for the workers to inherit
430 the nest, and could lead them to invest less in collective labour, at least temporally. A
431 second hypothesis would be that conflicts between workers competing for
432 reproduction may disturb peaceful workers and prevent them from working (Cole
433 1986). In accordance with this hypothesis, aggressions occurred most of the time
434 close to the brood area and fighting ants frequently induced movements in nearby
435 quiet ants. Working ants may also waste some time in submission behaviour to the
436 dominant individuals patrolling.

Whatever the mechanisms leading to a reduced work load in orphaned groups, the likely consequence of a disturbance of brood care is a delay in brood development or the death of part of the brood (not measured here). Moreover the decrease in foraging and nest maintenance is expected to affect provisioning and quality of the nest. This should result in a decrease of colony productivity and represent a cost of conflict. A cost of conflicts was detected in *Pachycondyla obscuricornis* at the colony level by Gobin et al. (2003). A higher metabolic rate, measured by total CO2 emission, was observed in orphaned groups displaying conflicts, thus demonstrating an energetic cost. Both ours and their results strongly suggest a cost of conflicts at the colony level that could negatively affect productivity.

Effects of conflicts on immune response

The technique used here to measure the level of immune defence is a test of resistance to a pathogen. However it is often argued that the immune response differs depending on the type of pathogen and that a variation in resistance to infection by a particular pathogen could result from a change in allocation of resources to different elements of the immune system (Adamo 2004). Similarly, a decrease in maintenance of the immune system may remain undetected if it affects a component of the immune system little involved in the resistance to the tested pathogen. As a consequence it would be relevant to associate different measurements of immunity. However, most immune defence measurements require a sample of haemolymph, and applying them on the same individuals is not possible in organisms like ants in which the volume of haemolymph is limited. To circumvent such problem, we used

here an immune response, the clearance of a generalist bacteria, that involves several components of the immune system.

The immune response was lower in workers from orphaned than control groups 15 days after splitting. The differences found between orphaned and control groups from a same colony could be partly due to the distribution of worker during splitting, as age and status of workers may influence their immune defence. However as ants were randomly distributed between groups this should not result in the pattern found, which is a lower immunocompetence in most orphaned groups. This immunosuppression was detected only 15 days after splitting, and not eight days after splitting. Moreover, the intensity of immunosuppression appeared to differ among colonies. This could be expected since the intensity of conflicts also varied. However, the parameters measured (colony size at sampling, mortality in both groups during the experiment, and total number of aggressions in orphaned group) did not significantly covaried with the intensity of immunosuppression. A lower immunocompetence was still found in some orphaned groups with a low number of aggressions (e.g. colony 12) and even when workers that received aggressions were excluded from the analysis.

All these results suggest that aggressions by themselves do not represent a cost in the orphaned group. Instead, we propose that a conflict-derived stress response mediates the immunosuppression found in most colonies. The variation in the intensity of immunosuppression could then just reflect a variability in the capacity to cope with social conflicts among colonies. In *Diacamma 'nilgiri'*, conflicts frequently induce avoidance or submission reactions of surrounding workers which could represent a stress factor in orphaned colonies for most of the workers and not only for workers involved in hierarchy. Moreover, removing the

gamergate could itself represent a stressful situation for ants even if the intensity of conflict remains low. Many studies on social insects have clearly showed that the presence of the reproductive can easily be perceived by workers, either directly by specific pheromones produced by the reproductive (Sledge et al. 2001; Hannonen et al. 2002), even in queenless species (Monnin et al. 1998; Tsuji et al. 1999; Cuvillier-Hot et al. 2004) or indirectly by the presence of the brood (Endler et al. 2004).

Much is known about stress in vertebrates, with many studies arguing that stress could lead to an immunosuppression (Apanius 1998). In invertebrates, research on stress is relatively recent and few studies have been performed until now, but some experiments performed on molluscs (Lacoste et al. 2001a; Lacoste et al. 2001b) and crustaceans (Le Moullac et al. 1998; Perazzolo et al. 2002; Pascual et al. 2003) strongly suggest that invertebrates also present a typical stress response which basic characteristics are relatively similar to those found in vertebrates (Ottaviani and Franceschi 1996). Moreover in vitro and in vivo experiments in molluscs have also revealed that a stress response could exert an inhibitory effect on immune function and increase susceptibility to pathogen infection (Stefano and Salzet 1999; Lacoste et al. 2002; Malham et al. 2003). During a stress response, immunity could be temporarily down-regulated to make nutrients available for other organismal processes that have a higher priority (Sheldon and Verhulst 1996). Actually, immune defence have been shown to be costly in many invertebrates studies (Rolff and Siva-Jothy 2003; Schmid Hempel 2005) and among them a few in social insects (Doums and Schmid-Hempel 2000; Moret and Schmid-Hempel 2000). An alternative hypothesis proposes that, during a stress response, the immune system could be down-regulated to prevent hyperactivation and ensuing autoimmune response, that

510 are more susceptible to occur in a context of high physical workload and production
511 of stress proteins (Raberg et al. 1998).

512 In orphaned groups, workers involved in aggressions were found to be more
513 immunocompetent. This relation could first result from a proximate effect of
514 physical harm caused by aggressive interactions. Actually, if the cuticule is damaged,
515 the immune system may be, at least partly, activated (Brey et al. 1993; Plaistow et al.
516 2003) which could improve the antibacterial response measured. However, the result
517 found did not remain significant when only aggressions received were considered,
518 therefore the potential effect of wounds is not likely to be involved. The relation
519 observed is the opposite of what could have been expected if aggression by itself
520 represented a cost, and is in accordance with the previous results suggesting that
521 immunosuppression was not directly related to aggression. This could also help to
522 understand why immunocompetence was lightly higher in orphaned groups 8 days
523 after splitting. If the cost of conflict is delayed and not present at this time, such an
524 effect of aggressions on immunocompetence could result in a higher
525 immunocompetence in orphaned groups compared to control groups. Although 8
526 days after splitting no relation was detected between the number of aggression and
527 immunocompetence among workers in orphaned groups, their higher
528 immunocompetence compared to workers from control groups was mostly due to
529 individuals involved in conflicts.

530 Within orphaned groups, the higher immune response of workers involved in
531 conflicts seems to be mainly related to the aggression given. Such a positive relation
532 within orphaned groups between immunocompetence and aggressiveness could be
533 explained by an intrinsically higher quality of workers competing for dominance
534 status. It is also tempting to link this effect to the classic observation that the

reproductives have a higher longevity compared to workers (Keller and Genoud 1997; Hartmann and Heinze 2003) even in queenless ants (Tsuji et al. 1996; André et al. 2001). This higher longevity could result, at least in part, from a higher immune response (Tian et al. 2004; DeVeale et al. 2004). Even if in our experiment the variation in immune response was observed within the worker caste, it could still be explained by hormonal modifications linked to the stimulation of ovarian activity and enhancing immune defence. Actually, dominant workers, involved in aggressive acts, are known to be those that stimulate their reproductive functions (Peeters et al. 1992) and produce more vitellogenine, a reproductive hormone (Cuvillier-Hot et al. 2004). This reproductive hormone has been shown to affect positively the number of functional hemocytes in honey bee (Amdam et al. 2004; Seehuus et al. 2006). Moreover, vitellogenine can increase the resistance to oxidative stress (Seehuus et al. 2006) and thus play a role in the regulation of longevity in social insects (Keller and Jemielity 2006). Further experiments are needed to test whether the positive link between reproduction and immune function is general in social insect and could really explain our results.

In conclusion, our study provides arguments in favour of the existence of a decrease of immune defence in orphaned groups probably linked to the stress response generated by the absence of the reproductive and not to a direct cost of aggressions. In addition, the behavioural study confirmed that conflicts affect worker labour and especially brood care, potentially leading to a decrease in colony productivity. Different studies have applied the principles of ecological immunology to social insects (Doums and Schmid-Hempel 2000; Traniello et al. 2002; Rolff and Siva-Jothy 2003; Vainio et al. 2004; Evans and Pettis 2005; Schmid-Hempel 2005; Baer et al. 2006). Our study is, to our knowledge, the first to have considered the

immune defence as a life-history trait for investigating the costs of conflicts at the individual level.

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LITERATURE CITED

Adamo, S. A. 2004. How should behavioural ecologists interpret measurements of immunity? Animal Behaviour 68:1443-1449.

Amdam, G. V., Z. L. P. Simões, A. Hagen, K. Norberg, K. Schrøder, Ø. Mikkelsen, T. B. L. Kirkwood, and S. W. Omholt. 2004. Hormonal control of the yolk precursor vitellogenin regulates immune function and longevity in honeybees. Experimental Gerontology 39:767-773.

André, J. B., C. Peeters, and C. Doums. 2001. Serial polygyny and colony genetic structure in the monogynous queenless ant *Diacamma cyaneiventre*. Behavioral Ecology and Sociobiology 50:72-80.

Apanius, V. 1998. Stress and immune response. Pp. 133-154 *in* A. P. Møller, M. Milinski and P. J. B. Slater, eds. Stress and behavior. Academic Press, San Diego, California.

Baer, B., S. A. O. Armitage, and J. J. Boomsma. 2006. Sperm storage induces an immunity cost in ants. Nature 441:872-875.

Baudry, E., C. Peeters, L. Brazier, M. Veuille, and C. Doums. 2003. Shift in the behaviours regulating monogyny is associated with high genetic differentiation in the queenless ant *Diacamma ceylonense*. Insectes Sociaux 50:390-397.

Bourke, A. F. G., and G. L. Chan. 1999. Queen-worker conflict over sexual production and colony maintenance in perennial social insects. The American Naturalist 154:417-426.

Bourke, A. F. G., and N. R. Franks. 1995. Social evolution in ants. Princeton University Press, Princeton, N.J.

Brey, P. T., W. Lee, M. Yamakawa, Y. Koizumi, S. Perrot, M. François, and M. Ashida. 1993. Role of the integument in insect immunity: Epicuticular abrasion and induction of cecropin synthesis in cuticular epithelial cells. Proceedings of the National Academy of Science USA 90:6275-6279.

Choe, J., and D. Perlman. 1997. Social conflict and cooperation among founding queens in ants (Hymenoptera: Formicidae). Pp. 392-406 *in* J. Choe and B. Crespi, eds. The evolution of social behavior in insects and arachnids. Cambridge University Press, Cambridge.

Cole, B. 1986. The social Behaviour of *Leptothorax allardycei* (Hymenoptera, formicidae): time budgets and the evolution of worker reproduction. Behavioural Ecology and Sociobiology 18:165-173.

Cournault, L., and C. Peeters. 2006. Aggressive regulation of monogyny in non-mutilating *Diacamma* ants. Behav Ecol *in press*

Crozier, R. H., and P. Pamilo. 1996. Evolution of Social Insect Colonies. Oxford University Press, Oxford.

Cuvillier, H. V., A. Lenoir, R. Crewe, C. Malosse, and C. Peeters. 2004a. Fertility signaling and reproductive skew in queenless ants. Anim Behav 68:1209-1219.

Cuvillier, H. V., A. Lenoir, and C. Peeters. 2004b. Reproductive monopoly enforce by sterile police workers in queenless ant. Behavioral Ecology 15:970-975.

Cuvillier-Hot, V., A. Lenoir, R. Crewe, C. Malosse, and C. Peeters. 2004. Fertility signaling and reproductive skew in queenless ants. Anim Behav 68:1209-1219.

DeVeale, B., T. Brummel, and L. Seroude. 2004. Immunity and aging: the enemy within? Aging Cell 3:195-208.

Doums, C., and P. Schmid-Hempel. 2000. Immunocompetence in workers of a social insect, *Bombus terrestris L.*, in relation to foraging activity and parasitic infection. Canadian Journal of Zoology 78:1060-1066.

Emlen, S. T. 1995. An evolutionary theory of the family. Proc Natl Acad Sci U S A 92:8092-8099.

Endler, A., J. Liebig, T. Schmitt, J. E. Parker, G. R. Jones, P. Schreier, and B. Holldobler. 2004. Surface hydrocarbons of queen eggs regulate worker reproduction in a social insect. Proceedings of the National Academy of Science USA 101:2945-2950.

Evans, J. D., and J. S. Pettis. 2005. Colony-level impacts of immune responsiveness in honey bees, *Apis mellifera*. Evolution 59:2270-2274.

Faulkes, C. G., D. H. Abbott, C. E. Liddell, L. M. Georges, and J. U. Jarvis. 1991. Hormonal and behavioral aspects of reproductive suppression in female naked mole-rats. *in* P. U. Press, ed. The Biology of the Naked Mole-Rat. P.W. Sherman, J.U.M. Jarvis et R.D. Alexander, Princeton.

Field, J., A. Cronin, and C. Bridge. 2006. Future fitness and helping in social queues. Nature 441:214-217.

Fukumoto, Y., T. Abe, and A. Taki. 1989. A novel form of colony organization in the "queenless" ant *Diacamma rugosum*. Physiol. Ecol. Japan 26:55-61.

Gillespie, J., M. R. Kanost, and T. Trenczeck. 1997. Biological mediators of insect immunity. Annual Review of Entomology 42:611-643.

Gobin, B., J. Heinze, M. Strätz, and F. Roces. 2003. The energetic cost of reproductive conflicts in the ant *Pachycondyla obscuricornis*. Journal of Insect Physiology 49:747-752.

Gorman, M. J., and S. M. Paskewitz. 2000. Persistance of infection in mosquitoes injected with bacteria. Journal of Invertebrate Pathology 75:296-297.

Hannonen, M., M. Sledge, S. Turillazzi, and L. Sundstrom. 2002. Queen reproduction, chemical signaling and worker behaviour in polygyne colonies of the ant Formica fusca. Anim Behav 64:477-485.

Hartmann, A., and J. Heinze. 2003. Lays eggs, live longer: Division of labor and life span in a clonal ant species. Evolution 57:2424-2429.

Heinze, J., B. Holldobler, and C. Peeters. 1994. Conflict and Cooperation in Ant societies. Naturwissenschaften 81:489-497.

Keller, L., and M. Genoud. 1997. Extraordinary lifespans in ants: a test of evolutionary theories of ageing. Nature 389:958-960.

Keller, L., and S. Jemielity. 2006. Social insects as a model to study the molecular basis of ageing. Experimental Gerontology 41:553-556.

Keller, L., and E. L. Vargo. 1993. Reproductive structure and reproductive roles in colonies of eusocial insects *in* L. Keller, ed. Queen Number and Sociality in Insects. Oxford University Press, Oxford.

Lackie, A. M. 1988. Haemocyte behaviour. Advances in insect physiology 21:85-178.

Lacoste, A., S. K. Malham, A. Cueff, F. Gélébart, and S. A. Poulet. 2001a. Evidence for a form of adrenergic response to stress in the oyster *Crassostrea gigas*. J Exp Biol 204:1247-1255.

Lacoste, A., S. K. Malham, A. Cueff, and S. A. Poulet. 2001b. Stress-induced Catecholamines changes in the haemolymph of the oyster *Crassostrea gigas*. Gen Comp Endocrinl 122:181-188.

Lacoste, A., S. K. Malham, F. Gélébart, A. Cueff, and S. A. Poulet. 2002. Stress-induced immune changes in the oyster *Crassostrea gigas*. Developmental and Comparative Immunology 26:1-9.

Le Moullac, G., C. Soyez, D. Saulnier, D. Ansquer, J. C. Avarre, and P. Levy. 1998. Effect of hypoxic stress on the immune response and the resistance to vibriosis of the shrimp *Penaeus stylirostris*. Fish and Shellfish Immunology 8:621-629.

Lochmiller, R. L., and C. Deerenberg. 2000. Trade-offs in evolutionary immunology: just what is the cost of immunity? Oikos 88:87-98.

Malham, S. K., A. Lacoste, F. Gélébart, A. Cueff, and S. A. Poulet. 2003. Evidence for a direct link between stress and immunity in the mollusc *Haliotis tuberculata*. Journal of Experimental Zoology Part A-Comparative Experimental Biology 295A(2):136-144.

Mock, D. W., and G. A. Parker. 1997. The evolution of sibling Rivalry. Oxford University Press, Oxford.

Monnin, T., C. Malosse, and C. Peeters. 1998. Solid-phase extraction and cuticular hydrocarbon differences related to reproductive activity in queenless ant *Dinoponera quadriceps*. Journal Of Chemical Ecology. 24:473-490.

Monnin, T., and C. Peeters. 1999. Dominance hierarchy and reproductive conflicts among subordinates in a monogynous queenless ant. Behav. Ecol. 10:323-332.

Monnin, T., and F. L. W. Ratnieks. 1999. Reproduction versus work in queenless ants: when to join a hierarchy of hopeful reproductives? Behavioral Ecology and Sociobiology 46:413-422.

Moret, Y., and P. Schmid-Hempel. 2000. Survival for immunity: The price of immune system activation for bumblebee workers. Science 290:1166-1168.

Ottaviani, E., and C. Franceschi. 1996. The neuroimmunology of stress from Invertebrates to man. Progress in Neurobiology 48:421-440.

Pascual, C., A. Sanchez, A. Sanchez, F. Vargas-Albores, G. Le Moullac, and C. Rosas. 2003. Haemolymph metabolic variables and immune response in *Litopenaeus setiferus* adult males: the effect of an extreme temperature. Aquaculture 218:637-650.

Peeters, C. 1997. Morphologically 'primitive' ants: comparative review of social characters, and the importance of queen-worker dimorphism. Pp. 372-391 *in* J. C. Choe and B. J. Crespi, eds. Social Behavior in Insects and Arachnids. Cambridge University Press, Cambridge.

Peeters, C., J. Billen, and B. Hôlldobler. 1992. Alternative dominance mechanisms regulating monogyny in the queenless ant genus *Diacamma*. Naturwissenschaften 79:572-573.

Peeters, C., and J. P. J. Billen. 1991. A novel exocrine gland inside the thoracic appendages ('gemmae') of the queenless ant *Diacamma australe*. Experientia 47:229-231.

Peeters, C., and S. Higashi. 1989. Reproductive dominance controlled by mutilation in the queenless ant *Diacamma australe*. Naturwissenschaften 76:177-180.

Perazzolo, L. M., R. Gargioni, P. Ogliari, and M. A. A. Barracco. 2002. Evaluation of some hemato-immunological parameters in the shrimp *Farfantepenaeus paulensis* submitted to environmental and physiological stress. Aquaculture 214:19-33.

Peters, J. M., D. C. Queller, F. V. L. Imperatriz, D. W. Roubik, and J. E. Strassmann. 1999. Mate number, kin selection and social conflicts in stingless bees and honeybees. Proceedings of the Royal Society of London Series B Biological Sciences 266:379-384.

Plaistow, S. J., Y. Outreman, Y. Moret, and T. Rigaud. 2003. Variation in the risk of being wounded: an overlooked factor in studies of invertebrate immune function? Ecology Letters 6:489-494.

Raberg, L., M. Grahn, D. Hasselquist, and E. Svensson. 1998. On the adaptive significance of stress-induced immunosuppression. Proceedings of the Royal Society of London Series B Biological Sciences 265:1637-1641.

Ratnieks, F., and H. Reeve. 1992. Conflicts in single-queen Hymenopteran societies: the structure of conflict and processes that reduce conflict in advanced eusocial species. Journal of Theoretical Biology 158:33-65.

Ratnieks, F. L. W. 1988. Reproductive harmony via mutual policing by workers in eusocial Hymenoptera. American Naturalist 132:217-236.

Rolff, J., and M. T. Siva-Jothy. 2003. Invertebrate ecological immunology. Science 301:472-475.

Röseler, P.-F. 1991. Reproductive competition during colony establishment. Pp. 309-335 *in* M. R. Ross KG, ed. The social biology of wasps. Cornell University Press, Ithaca.

Schmid Hempel, P. 2005. Evolutionary ecology of insect immune defense. Annual Review of Entomology 50:529-551.

Schmid-Hempel, P. 1998. Parasites in social insects. Princeton University Press, Princeton, New Jersey.

Schmid-Hempel, P. 2005. Evolutionary ecology of insect immune defense. Annual Review of Entomology 50:529-551.

Seehuus, S.-C., K. Norberg, U. Gimsa, T. Krekling, and G. V. Amdam. 2006. Reproductive protein protects functionally sterile honey bee worker from oxydative stress. Proceedings of the National Academy of Science USA 103:962-967.

Sheldon, B. C., and S. Verhulst. 1996. Ecological immunology: Costly parasite defences and trade-offs in evolutionary ecology. Trends in Ecology & Evolution 11:317-321.

Sledge, M., F. Boscaro, and S. Turillazi. 2001. Cuticular hydrocarbons and reproductive status in the social wasp *Polistes dominulus*. Behav ecol sociobiol 49:401-409.

Stefano, G., and M. Salzet. 1999. Invertebrates opioid precursors: evolutionary conservation and the significance of enzymatic processing. Int Rev Cytol 187:261-286.

Tian, H., S. B. Vinson, and C. J. Coates. 2004. Differential gene expression between alate and dealate queens in the red imported fire ant, *Solenopsis invicta* Buren(Hymenoptera: Formicidae). INSECT BIOCHEMISTRY AND MOLECULAR BIOLOGY 34:937-949.

Traniello, J. F. A., R. B. Rosengaus, and K. Savoie. 2002. The development of immunity in a social insect: Evidence for the group facilitation of disease resistance. Proceedings of the National Academy of Science, USA 99:6838-6842.

Tsuji, K., K. Egashira, and B. Hölldobler. 1999. Regulation of worker reproduction by direct physical contacts in the ant *Diacamma sp.* from Japan. Animal Behaviour 58:337-343.

Tsuji, K., K. Nakata, and J. Heinze. 1996. Lifespan and reproduction in a queenless ant. Naturwissenschaften 83:577-578.

Vainio, L., H. Hakkarainen, M. J. Rantala, and J. Sorvari. 2004. Individual variation in immune function in the ant *Formica exsecta*; effects of the nest, body size and sex. Evolutionary Ecology 18:75-84.

West-Eberhard, M. J. 1975. The evolution of social behavior by kin selection. Quarterly Review of Biology 50:1-33.

Fig1: Percentage of scans where workers from control (white bars) and orphaned groups (black bars) were recorded performing a given behaviour for each colony. A: Total Labour ; B: Brood care ; C: Resting. Bars represent standard errors.

Fig 2 : Comparison of mean bacterial count in control groups (white bars) and orphaned groups (black bars). A: 8 days after splitting. B: 15 days after splitting. Bars represent standard errors.

Fig 3: Relationship between bacterial count and number of aggressions given or received in orphaned groups 15 days after splitting.

Table 1: Characteristics of the 20 colonies and basic data on aggressions. For each colony is given the number of workers ($N_{workers}$) in each group after splitting (the number was the same for the two groups of a given colony), the total number of aggressions in the orphaned group, the number of days between the splitting event and the immune response measurement (N_{days}), and the number of workers which died during this interval in both groups. The last column indicates whether (+) or not (-) behavioural scans were performed for each colony.

colony	$N_{workers}$	Total number of aggressions	N_{days}	$N_{dead\ workers}$		Behavioural scan
				Control g.	Orphaned g.	
1	80	1104	8	2	13	-
2	80	1363	8	5	3	-
3	80	970	8	6	1	-
4	80	606	8	5	5	-
5	52	220	8	9	2	+
6	75	496	8 / 15	6 / 7	7 / 10	+
7	67	1015	8 / 15	3 / 3	8 / 12	+
8	67	767	8 / 15	8 / 8	2 / 2	+
9	72	1478	8 / 15	5 / 7	4 / 4	+
10	81	360	8 / 15	2 / 3	1 / 3	+
11	82	325	8 / 15	1 / 2	4 / 7	+
12	78	37	8 / 15	0	1 / 1	+
13	70	377	8 / 15	10 / 11	4 / 8	+
14	78	867	15	0	7	-
15	57	431	15	1	2	-
16	53	753	15	5	6	+
17	62	435	15	6	4	
18	58	58	15	7	6	+
19	68	720	15	3	3	+
20	63	705	15	3	7	+

Table 2: Time activity budgets in 14 control and orphaned groups. For each individual, the frequency of each behaviour was calculated as the pourcentage of scans in which the behaviour was observed. The values presented in the table correspond to the average of the frequency among individuals for each group. The total labour was subdivided in "Brood care" and "Others", which includes nest maintenance and foraging.

	Frequency (%, ±SE)	
	Control group	Orphaned group
Total labour	35.6 ± 0.9	29.3 ± 0.8
- Brood care	17.7 ± 0.7	13.5 ± 0.6
- Others	17.9 ± 0.9	15.9 ± 0.8
Resting	29.2 ± 0.7	28.8 ± 0.7
Aggressive interactions	0.2 ± 0.04	5.9 ± 0.4
Others	35.1 ± 0.6	35.9 ± 0.6

Table 3: Analysis of the bacterial count using mixed models (glmmADMB function in R), including treatment (T) and volume (V) as fixed factors and colony (Col) and interaction treatment by colony (T(col)) as random factors. For each model the log(likelihood) (L) and the number of parameters (npar) are given. Effects are tested by comparing models using the likelihood ratio test (LRT). Significant *P*-values ($P < 0.05$) are indicated in bold-faced.

8 days	**Models compared**			**Likelihood Ratio Test**		
Effect tested	**Fixed terms / random terms**	**Log(L)**	**npar**	χ^2	**Df**	***P***
Treatment	1. T + V / Col	- 6157.34	6	4.12	1	0.042
	2. V / col	- 6159.40	5			
Volume	1. T + V / T(Col) + Col	- 6157.08	7	1.32	1	0.25
	3. T / T(Col) + Col	- 6157.74	6			
Treatment(col)	1. T + V / T(Col) + Col	- 6157.08	7	0.52	1	0.47
	5. T + V / Col	- 6157.34	6			

15 days	**Models compared**			**Likelihood Ratio Test**		
Effect tested	**Fixed terms / random terms**	**Log(L)**	**npar**	χ^2	**Df**	***P***
Treatment	1. T + V / Col	- 5046.34	6	7.1	1	**0.008**
	2. V / col	- 5049.89	5			
Volume	1. T + V / T(Col) + Col	- 5046.35	7	0.24	1	0.62
	3. T / T(Col) + Col	-5046.47	6			
Treatment(col)	1. T + V / T(Col) + Col	- 5046.35	7	0.02	1	0.89
	5. T + V / Col	- 5046.34	6			

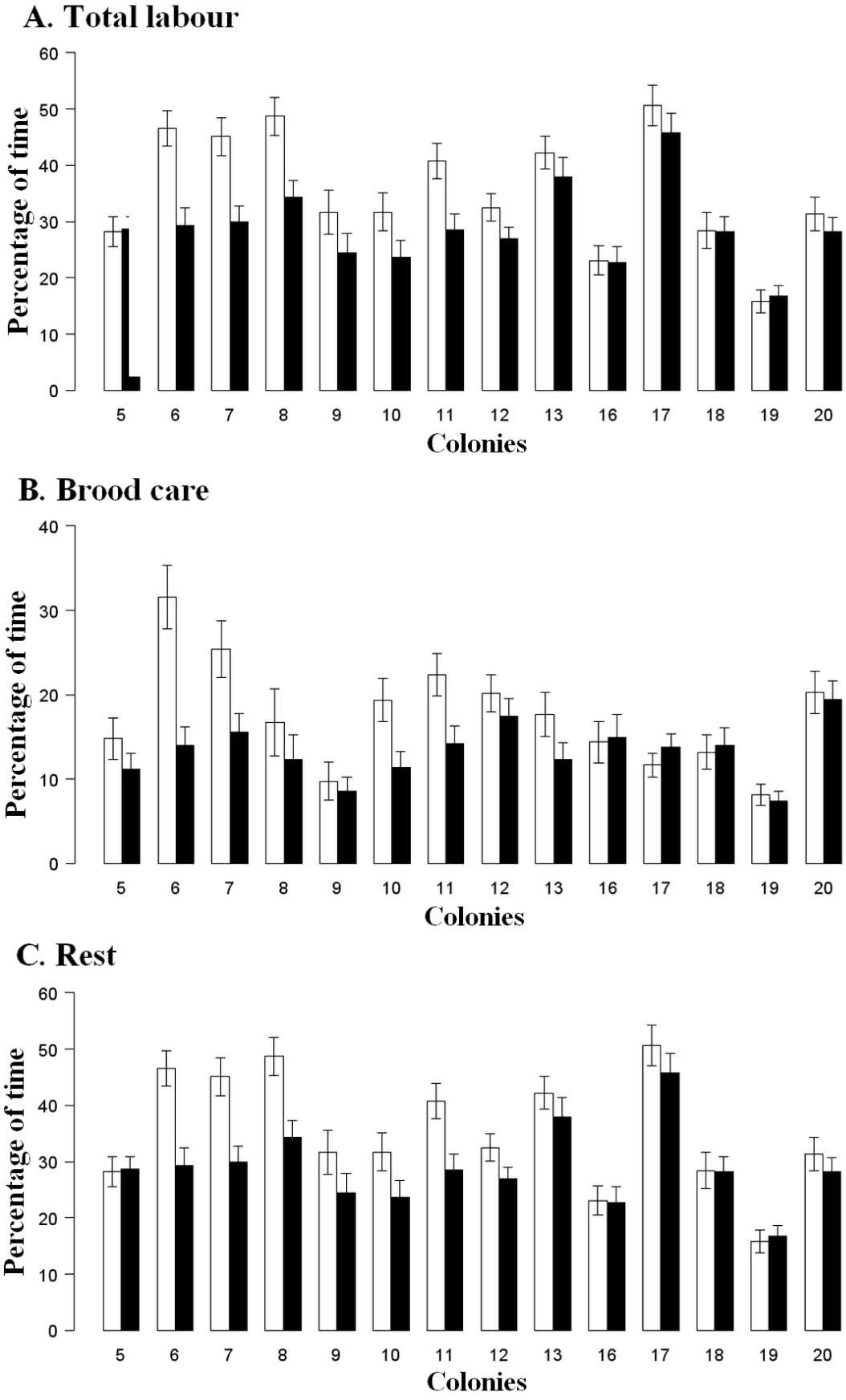
A. Total labour
Percentage of time
0
10
20
30
40
50
60
5
6
7
8
9
10
11
12
13
16
17
18
19
20
Colonies
B. Brood care
Percentage of time
0
10
20
30
40
5
6
7
8
9
10
11
12
13
16
17
18
19
20
Colonies
C. Rest
Percentage of time
0
10
20
30
40
50
60
5
6
7
8
9
10
11
12
13
16
17
18
19
20
Colonies

Fig. 1

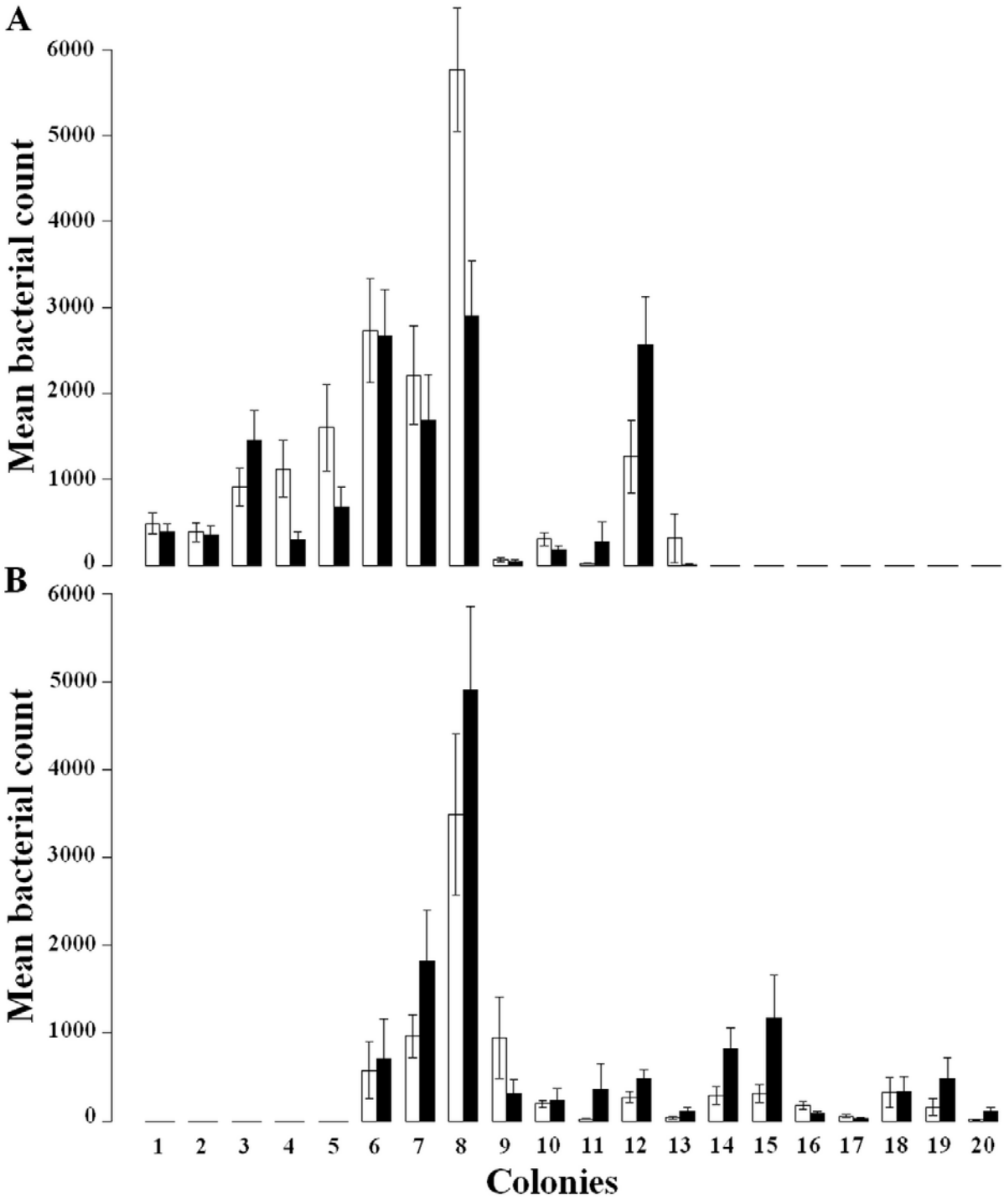
A
B
Mean bacterial count
Mean bacterial count
0
1000
2000
3000
4000
5000
6000
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
Colonies

Fig. 2

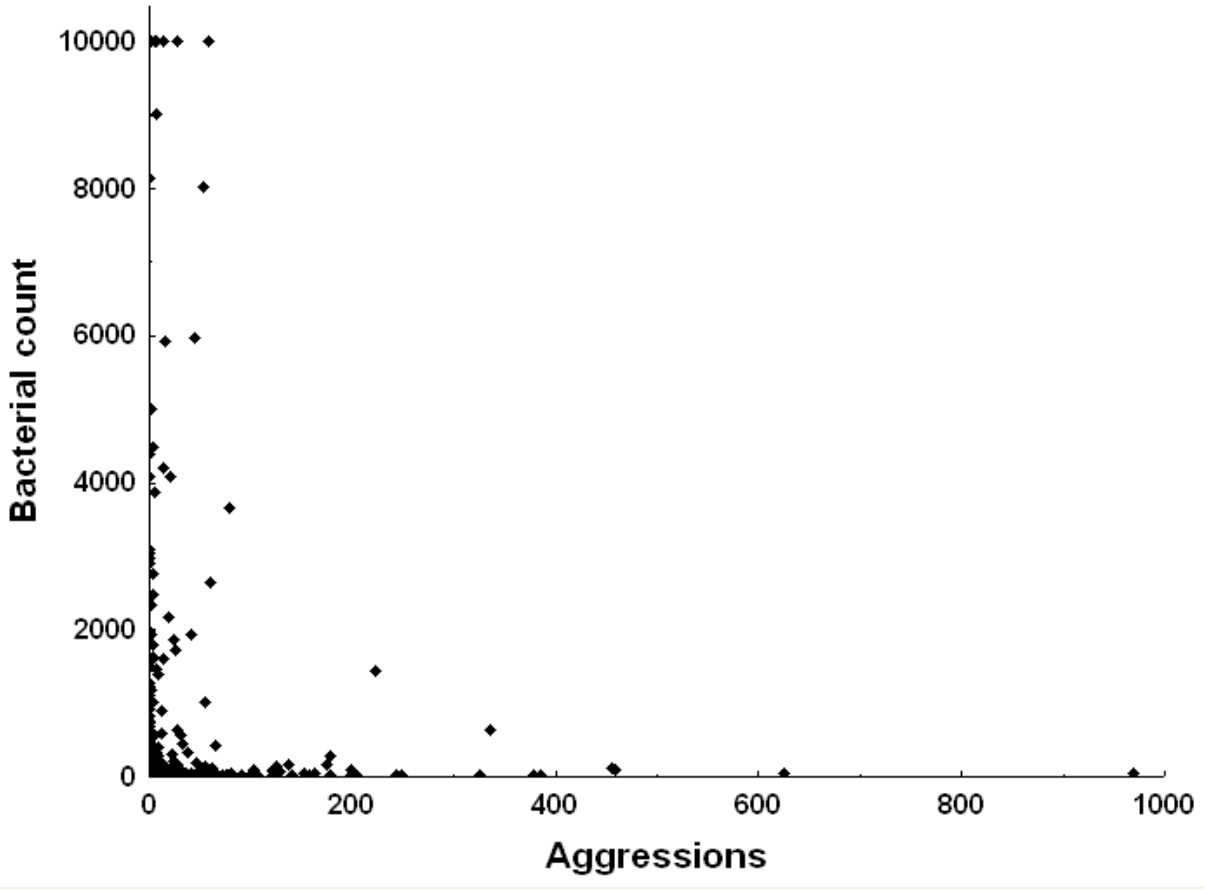
Bacterial count
10000
8000
6000
4000
2000
0
0
200
400
600
800
1000
Aggressions

Fig. 3

ANNEXE 7

Conditional use of sex and parthenogenesis for worker and queen production in ants

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Many variations of the method are possible. The activity and affect descriptors used to describe episodes should fit the particular topic of investigation. Interactive questionnaires offer further opportunities to tailor the affect terms to the respective episode; for example, when the individual identifies an episode as an interaction with customers rather than with family members, different descriptors could be presented. Other variations could make the method more practical for adoption in conventional surveys. Our preliminary work suggests that much of the benefit of the DRM in producing accurate emotional recall could be retained if respondents are asked to retrieve specific recent episodes of a designated type (e.g., "the last occasion on which you went out to dinner"). When samples are large and interviewing time is scarce, the allocation of different situations to subgroups of respondents makes it possible to achieve comprehensive coverage of situations while minimizing respondent burden. In conjunction with time-use data obtained from other sources, affect profiles of the main activities in which people engage could be integrated to produce a duration-weighted assessment of the experience of the population and of subgroups. The DRM or its variants could also contribute to the development of an accounting system for the well-being of society, a potentially important tool for social policy (*32*, *33*).

References and Notes

1. M. Bergner, M. L. Rothman, *Annu. Rev. Public Health* **8**, 191 (1987).
2. S. Cohen, W. Doyle, R. Turner, C. Alper, D. Skoner, *Psychol. Sci.* **14**, 389 (2003).
3. M. Marmot, *The Status Syndrome* (Bloomsbury, London, 2004).
4. F. T. Juster, in *Time, Goods, and Well-Being*, F. Juster, F. Stafford, Eds. (Institute for Social Research, Ann Arbor, MI, 1985), pp. 397–414.
5. P. van de Ven, B. Kazemier, S. Keuning, *Measuring Well-Being with an Integrated System of Economic and Social Accounts* (Department of National Accounts, Voorburg, Netherlands, 1999).
6. F. Y. Edgeworth, *Mathematical Psychics: An Essay on the Application of Mathematics to the Moral Sciences* (C. K. Paul, London, 1881).
7. D. Kahneman, P. Wakker, R. Sarin, *Q. J. Econ.* **112**, 375 (1997).
8. G. Dow, J. F. Juster, in *Time, Goods, and Well-Being*, F. Juster, F. Stafford, Eds. (Institute for Social Research, Ann Arbor, 1985), pp. 397–413.
9. L. Flood, *Household, Market, and Nonmarket Activities; Procedures and Codes for the 1993 Time-Use Survey* (Uppsala Univ. Dept. Economics, Uppsala, Sweden, 1997), vol. VI.
10. A. Campbell, *The Sense of Well-Being in America* (McGraw-Hill, New York, 1981).
11. F. M. Andrews, S. B. Whithey, *Social Indicators of Well-Being: Americans' Perceptions of Life Quality* (Plenum, New York, 1976).
12. M. Csikszentmihalyi, R. E. Larsen, *J. Nerv. Ment. Dis.* **175**, 526 (1987).
13. A. A. Stone, S. S. Shiffman, M. W. DeVries, in *Well-Being: The Foundations of Hedonic Psychology*, D. Kahneman, E. Diener, N. Schwarz, Eds. (Russell-Sage, New York, 1999), pp. 61–84.
14. M. D. Robinson, G. L. Clore, *Psychol. Bull.* **128**, 934 (2002).
15. R. Belli, *Memory* **6**, 383 (1998).
16. Materials and methods are available as supporting material on *Science* Online.
17. A. A. Stone *et al.*, *J. Pers. Soc. Psychol.* **74**, 1670 (1998). Respondents with high levels of job stress were recruited in New York (n = 88 men, 91 women; average age 40.0 years; 89% white; 58% married; 50% had some college). Situations and affect descriptors were similar to the DRM study.
18. K. N. Fortson, *Monthly Labor Rev.* **127**, 18 (2004).
19. We conducted an auxiliary random-digit-dial phone survey of 69 women, who ranked the activities from most to least enjoyable. Kendall's W coefficient of concordance among the ranks was 0.47, slightly higher than the concordance of economists' rankings of journals (*20*).
20. K. Axarloglou, V. Theoharakis, *J. Euro. Econ. Assoc.* **1**, 1402 (2003).
21. Thirty-nine job features (e.g., tenure and job level) were entered into a regression to predict duration-weighted enjoyment at work (*16*).
22. N. K. Semmer, S. Grebner, A. Elfering, in *Research in Occupational Stress and Well-being*, P. Perrewé, D. Ganster, Eds. (JAI, Amsterdam, 2004), vol. 3, pp. 207–263.
23. Twenty personal features (e.g., age and marital status) predicted duration-weighted enjoyment at work (*16*).
24. We computed enjoyment at home for respondents who reported "no problem at all" in keeping up "enough enthusiasm to get things done" during the last month and for respondents who reported "somewhat of a problem." The difference between the groups spanned 46% of the enjoyment scale.
25. R. A. Easterlin, *J. Econ. Behav. Org.* **27**, 35 (1995).
26. P. Brickman, D. T. Campbell, in *Adaptation-Level Theory*, M. Appley, Ed. (Academic Press, New York, 1971), pp. 215–231.
27. The first question in the survey was "How satisfied are you with your life as a whole these days? Are you very satisfied, satisfied, not very satisfied, or not at all satisfied?".
28. D. Kahneman, in *Choices, Values and Frames*, D. Kahneman, A. Tversky, Eds. (Cambridge Univ. Press, New York, 2000), chap. 38.
29. D. A. Schkade, D. Kahneman, *Psychol. Sci.* **9**, 340 (1998).
30. S. Lyubomirsky, K. M. Sheldon, D. Schkade, *Rev. Gen. Psychol.*, in press.
31. G. Becker, *Econ. J.* **75**, 493 (1965).
32. D. Kahneman, A. B. Krueger, D. A. Schkade, N. Schwarz, A. A. Stone, *Am. Econ. Rev.* **94**, 429 (2004).
33. E. Diener, M. Seligman, *Psychol. Sci. Pub. Int.* **5**, 1 (2004).

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Supporting Online Material

www.sciencemag.org/cgi/content/full/306/5702/1776/DC1
Materials and Methods

2 August 2004; accepted 30 September 2004

Conditional Use of Sex and Parthenogenesis for Worker and Queen Production in Ants

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The near-ubiquity of sexual reproduction in animal species has long been considered a paradox because sexually reproducing individuals transmit only half of their genome to their progeny. Here, we show that the ant *Cataglyphis cursor* circumvents this cost by using alternative modes of reproduction for the production of reproductive and nonreproductive offspring. New queens are almost exclusively produced by parthenogenesis, whereas workers are produced by normal sexual reproduction. By selectively using sex for somatic growth and parthenogenesis for germline production, *C. cursor* has taken advantage of the ant caste system to benefit from the advantages of both sexual and asexual reproduction.

The main advantage of asexual reproduction is that it confers a twofold advantage over sexuality by allowing, generation by generation, the transmission of twice the number of genes to offspring (*1*, *2*). However, asexual reproduction is also associated with both short-term and long-term disadvantages, including a lower genetic diversity of offspring and a reduced rate of adaptive evolution of species (*3*, *4*). The nature and the degree of the cost associated with asexual reproduction is expected to vary across taxa, depending on the biology of the species and the type of environment in which they live (*1*–*3*).

In ants, as in other Hymenoptera, females are usually produced by sexual reproduction and are diploid, whereas males develop from unfertilized eggs and are haploid (*3*). The diploid fertilized eggs can develop into either new queens (gynes) or workers, with the developmental switch generally under environmental control (*5*). In the Cape honey bee and five ant species, however, unmated workers may reproduce by thelytokous parthenogenesis (*6*–*11*); that is, they may produce female

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offspring from unfertilized eggs. While conducting a population genetic study of one of these species, the ant *Cataglyphis cursor*, we discovered that not only unmated workers but also mated queens can use thelytokous parthenogenesis. Pedigree analyses indicated that queens use automictic parthenogenesis with central fusion where two of the four products of meiosis merge. Unlike workers, queens use this mode of reproduction specifically to produce new queens.

Cataglyphis cursor is a common ant in the dry forests of Europe. Colonies usually contain a single queen and up to 3000 workers. Only few colonies produce gynes, and the number of gynes produced per colony is small. This is because *C. cursor* has an unusual mating system whereby gynes mate near the parental nest before leaving the colony with adult workers to initiate new colonies 3.2 to 11.3 m away (*12*). Previous studies also indicate that *C. cursor* workers can produce both gynes and workers parthenogenetically in colonies that have lost the mother queen (*7*).

We collected 38 large colonies in Southern France and genotyped 532 workers at four highly polymorphic microsatellite loci (expected heterozygosities, 0.833 to 0.944) (*13*). The genotypes indicated that 35 of these colonies contained a single reproductive queen (monogyny), whereas three colonies contained offspring from at least two queens. Analysis of lab-raised worker progeny (n = 437 freshly eclosed workers) from 12 queens showed that they had mated with an average of 5.6 ± 1.3 males (range, 4 to 8).

A detailed analysis of the 35 monogynous colonies showed that most of the workers in these colonies could only have been produced by sexual reproduction. Overall, 476 of the 489 workers (97.3%) genotyped in the 35 colonies harbored, at one or several loci, alleles that were not present in the mother queen and came from one of the queen's mates. It is impossible to determine whether the 13 workers harboring only alleles identical to those of their mother were fathered by a male that had no allele distinct from those of the queen or whether they had been parthenogenetically produced. Because the four microsatellite loci were highly polymorphic, the probability of mating with a male harboring no diagnostic allele at any of the four loci was low, ranging from 0.0001 to 0.013 across colonies according to the queen's genotype. Thus, of the 476 workers, only one was expected to have no diagnostic alleles if they were all sexually produced. Hence, it is likely that some or all of the 13 workers with no diagnostic paternal allele may indeed have been asexually produced (the estimated proportion of asexually produced workers is 2.5% when corrected for the probability of nondetection of paternal alleles).

A total of 56 gynes were produced by 10 of the 35 monogynous colonies. In contrast to workers, most of these gynes (54 of 56) had alleles at the four loci that could all be attributed to the queen (Fig. 1A), hence these gynes had been produced by parthenogenesis. The alternative explanation, that these 54 gynes had been fathered by a male having no diagnostic alleles, can be ruled out. Queens and males came from the same gene pool, as indicated by a lack of significant difference in allele frequencies for the four loci (Fisher exact test, all $P > 0.05$) and the workers' *Fis* value (an index of observed versus expected homozygosity), which was not significantly different from zero (*Fis* = 0.011 ± 0.015, n = 35 colonies; two-tailed t test, $t = 0.691$, $P = 0.494$). This, together with the high allelic diversity, resulted in a very high probability to detect a male's genetic contribution. However, none of the gynes produced in nine of the 10 colonies had any diagnostic allele, even though the likelihood of such a matched mating was lower than 0.013 in each of the nine colonies (range, 0.0001 to 0.013). Overall, the probability that all the fathers of the gynes produced in the nine colonies had no diagnostic alleles was $P < 10^{-28}$. Indeed, the genotypes of workers in these nine colonies confirmed that all or most of the males that mated with the queens had diagnostic alleles at one or more loci (Fig. 1B). The outcome of the vast majority of gynes being produced by parthenogenesis was that the relatedness between queens and gynes was very high (r = 0.864 ± 0.046, n = 56 gynes) and significantly greater ($P < 0.001$) than the theoretical value of 0.50 expected under sexual reproduction.

Most of the 54 parthenogenetic gynes were neither genetically identical to each other within a colony nor genetically identical to their mother queen. The discrepancies resulted from gynes being homozygous at some loci where the mother queen was heterozygous. In all cases, the gynes were homozygous for one of the two maternal alleles. This is the expected pattern under automictic parthenogenesis with central fusion. Because two of the four products of meiosis merge, the offspring have the same genotype as their mother for the loci that did not cross over, whereas the offspring is homozygous for one of the two maternal alleles if crossing-over did occur (*14*, *15*). The frequency of transition from heterozygosity is expected to vary across loci depending on their distance to the centromere (*15*). Consistent with this prediction, the frequency of transition from heterozygosity to homozygosity varied significantly across the four loci, presumably reflecting differences in the distance between each locus and the centromere (Table 1; $\chi^2 = 25.53$, $P < 0.0001$).

The expected outcome of automictic parthenogenesis is a gradual increase in homo-

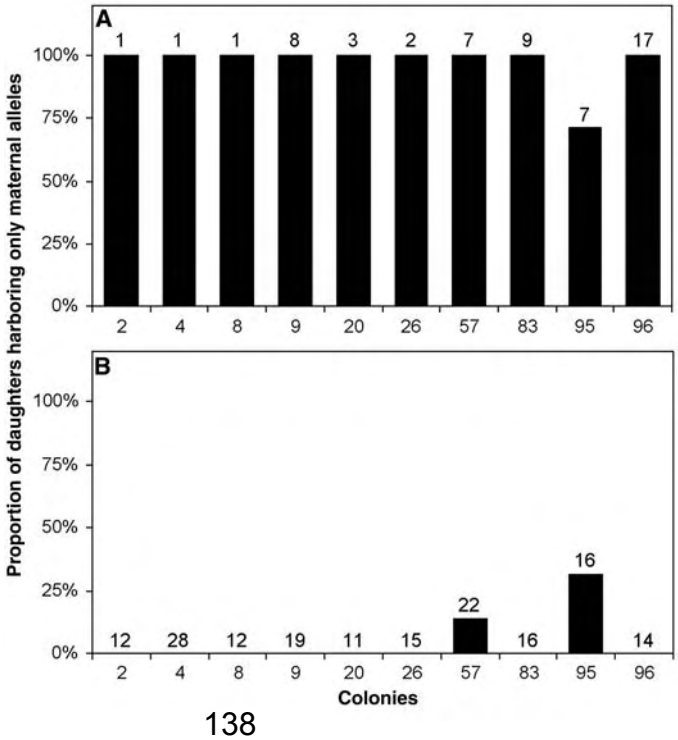

Fig. 1. Respective proportion of gynes (**A**) and workers (**B**) harboring maternal alleles only, and therefore interpreted as parthenogenetic daughters, in each of 10 colonies (colony numbers are laboratory designations). The sample size for each colony is indicated above the bars.

Table 1. Proportion of gynes homozygous for a given locus when the mother was heterozygous at that locus. The sample size for each locus is indicated.

Locus	Sample size	Percentage of gynes homozygous
Ccur11	53	5.7
Ccur46	47	46.8
Ccur58	47	34.0
Ccur63b	41	17.1

zygosity over time (*16*). Indeed, the overall level of homozygosity was significantly higher in gynes than in workers (Fig. 2; Fisher's exact test on the number of homozygous versus heterozygous loci in gynes and workers: $P < 0.0001$). Accordingly, F statistics revealed a significant excess of homozygosity in gynes ($F = 0.396 \pm 0.12$, $P < 0.001$) and queens ($F = 0.255 \pm 0.051$, $P < 0.001$) but not in workers from the same colonies ($F = 0.002 \pm 0.016$, $P = 0.45$). By increasing the levels of homozygosity, parthenogenesis should result in reduced queen survival and fitness, much like inbreeding does. However, the fitness effect might be limited for ant queens because they stay in the protected environment of the nest, except during colony founding. Even at this stage, the intensity of this cost should vary according to the mode of colony founding, with selection against more homozygous queens being higher in species where queens start a new colony on their own and lower in species, such as *C. cursor*, where queens do not go through a stage of independent colony founding (*12*).

In addition, two processes appear to counteract the process of genetic homogenization induced by automictic parthenogenesis. The first is the occasional production of gynes by sexual reproduction. The overall production of such gynes was 3.6% (2 of 56) in the 10 colonies studied. The second process is the occasional queen production by worker parthenogenesis. Because workers are usually produced by sexual reproduction, their contribution to gyne production will contribute to the maintenance of heterozygosity in gynes and queens, just as under queen sexual reproduction.

Although *C. cursor* queens do not require mating to produce diploid offspring, they have retained sexual reproduction to produce workers, which suggests that sexual reproduction has important benefits for colony function. The observed mating frequencies in this species lie on the high end of the continuum of mating frequencies reported in ants (*17*). A possible explanation is that genetic input from an increased number of mates compensates for the negative effect of high queen homozygosity on colony genetic diversity. Parthenogenetic production of workers at the level observed for gynes would lower colony genetic diversity, which could lead to reduced defense against parasites, less efficient division of labor, and a decreased range of environmental conditions that a colony can tolerate (*18–20*). These costs are akin to those thought to lead to the instability of parthenogenetic reproduction in nonsocial organisms (*2*). Multiple mating lowered the overall relatedness of nestmate workers to $r = 0.42$ ($SE_{jackknife} = 0.02$, $n = 35$), a value well within the range of values reported in other ants (*21*). Thus, the high queen mating frequency may cancel out reduced genetic diversity at the colony level stemming from the relatively high queen homozygosity.

Using alternative modes of reproduction for the queen and worker castes may also enhance cooperation within the social group by aligning the interests of queens and workers. Parthenogenetic production of gynes by queens reduces conflict with workers because, just like queens, workers are significantly more closely related ($t = 2.31$, df = 43, $P = 0.03$) to the parthenogenetic gynes ($r = 0.59$, $SE_{jackknife} = 0.07$, $n = 10$) than they would be to sexually produced gynes or to gynes produced parthenogenetically by other workers (these two values are identical to the relatedness between workers, $r = 0.42$). As a result, workers should police the reproduction of other workers (*22*). The almost complete lack of worker-produced gynes in colonies containing a queen is consistent with this idea.

Conditional use of parthenogenesis for queen production might also occur in other ants, yet it may remain unnoticed because it primarily occurs in dependent-founding species where it is most difficult to detect. In ants there is a strong association between the mode of colony founding and the number of queens, with dependent colony founding being almost exclusively restricted to species with high numbers of queens per nest (*23*, *24*). The likelihood of detecting parthenogenesis with genetic markers is low in such species because it is very difficult to determine the maternity of female offspring. As a result, only a handful of studies in highly polygynous ants are sufficiently detailed to have enabled the detection of parthenogenesis.

This study shows that by taking advantage of the social caste system, *C. cursor* colonies can benefit from the advantages of both sexual and asexual reproduction. By using alternative modes of reproduction for the queen and worker castes, queens can increase the transmission rate of their genes to their reproductive female offspring while maintaining genetic diversity and social cohesion in the worker population. These findings, together with those of other recent genetic studies (*25–29*), indicate greater flexibility of the ant reproductive and social systems, thus providing an ideal ground to test various evolutionary predictions.

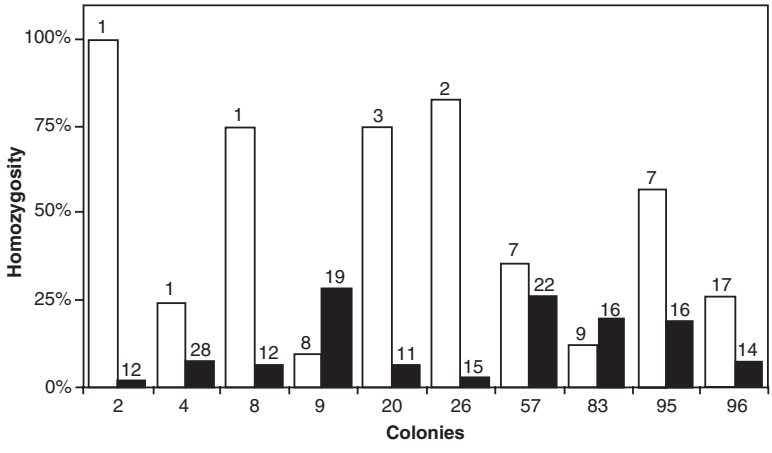

Fig. 2. Overall homozygosity detected in gynes (white) and workers (black) at all four loci, for each of 10 colonies. The sample size for each colony is indicated above the bars.

References and Notes

1. G. C. Williams, *Sex and Evolution* (Princeton Univ. Press, Princeton, NJ, 1975).
2. J. Maynard Smith, *The Evolution of Sex* (Cambridge Univ. Press, Cambridge, 1978).
3. G. Bell, *The Masterpiece of Nature: The Evolution and Genetics of Sexuality* (Univ. of California Press, Berkeley, CA, 1982).
4. S. A. West, C. M. Lively, A. F. Read, *J. Evol. Biol.* **12**, 1003 (1999).
5. E. O. Wilson, *The Insect Societies* (Harvard Univ. Press, Cambridge, MA, 1971).
6. S. Verma, F. Ruttner, *Apidologie (Celle)* **14**, 41 (1983).
7. H. Cagniant, *Insect. Soc.* **26**, 51 (1979).
8. T. Itow *et al.*, *Insect. Soc.* **31**, 87 (1984).
9. K. Tsuji, K. Yamauchi, *Insect. Soc.* **42**, 333 (1995).
10. J. B. Heinze, B. Hölldobler, *Naturwissenschaften* **82**, 40 (1995).
11. D. A. T. Grasso, T. Wenseleers, A. Mori, F. Le Moli, J. Billen, *Ethol. Ecol. Evol.* **12**, 309 (2000).
12. A. Lenoir, L. Quérard, N. Pondicq, F. Berton, *Psyche (Stuttgart)* **95**, 21 (1988).
13. See supporting data on *Science* Online.
14. E. Suomalainen, A. Saura, J. Lokki, *Cytology and Evolution in Parthenogenesis* (CRC Press, Boca Raton, FL, 1987).
15. E. Baudry *et al.*, *Genetics*, in press.
16. J.-C. Simon, F. Delmotte, C. Rispe, T. Crease, *Biol. J. Linn. Soc.* **79**, 151 (2003).
17. J. E. Strassmann, *Insect. Soc.* **48**, 1 (2001).
18. P. W. Sherman, T. D. Seeley, H. K. Reeve, *Am. Nat.* **131**, 602 (1988).
19. R. H. Crozier, R. E. Page, *Behav. Ecol. Sociobiol.* **18**, 105 (1985).
20. B. J. Cole, D. C. Wiernasz, *Science* **285**, 891 (1999).
21. R. H. Crozier, P. Pamilo, *Evolution of Social Insect Colonies: Sex Allocation and Kin Selection* (Oxford Univ. Press, Oxford, 1996).
22. F. L. W. Ratnieks, *Am. Nat.* **132**, 217 (1988).
23. L. Keller, *Ethol. Ecol. Evol.* **3**, 307 (1991).
24. A. F. G. Bourke, N. R. Franks, *Social Evolution in Ants* (Princeton Univ. Press, Princeton, NJ, 1995).
25. S. Helms Cahan *et al.*, *Proc. R. Soc. London Ser. B.* **269**, 1871 (2002).

26. G. E. Julian, J. H. Fewell, J. Gadau, R. A. Johnson, D. Larrabee, *Proc. Natl. Acad. Sci. U.S.A.* **99**, 8157 (2002).
27. V. P. Volny, D. M. Gordon, *Proc. Natl. Acad. Sci. U.S.A.* **99**, 6108 (2002).
28. S. Helms Cahan, L. Keller, *Nature* **424**, 306 (2003).
29. A. Hartmann, J. Wantia, J. A. Torres, J. Heinze, *Proc. Natl. Acad. Sci. U.S.A.* **100**, 12836 (2003).
30. We thank L. Beukeboom, M. Chapuisat, P. Christe, R. Hammond, S. H. Cahan, J. M. Pasteels, K. Parker, and I. Sanders for their comments on the manuscript. Supported by a doctoral grant from Fonds pour la Recherche dans l'Industrie et l'Agriculture (M.P.) and grants from the Belgian Fond National de la Recherche Scientifique (S.A.), the French Ministry of Research "Action concertée incitative jeunes chercheurs 2001" (C.D.), and the Swiss National Science Foundation (L.K.).

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Materials and Methods
References

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Status and Trends of Amphibian Declines and Extinctions Worldwide

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The first global assessment of amphibians provides new context for the well-publicized phenomenon of amphibian declines. Amphibians are more threatened and are declining more rapidly than either birds or mammals. Although many declines are due to habitat loss and overutilization, other, unidentified processes threaten 48% of rapidly declining species and are driving species most quickly to extinction. Declines are nonrandom in terms of species' ecological preferences, geographic ranges, and taxonomic associations and are most prevalent among Neotropical montane, stream-associated species. The lack of conservation remedies for these poorly understood declines means that hundreds of amphibian species now face extinction.

Scientists first became concerned about widespread amphibian population declines when they met in 1989 at the First World Congress of Herpetology. Historical data indicate that declines began as early as the 1970s in the western United States (*1*, *2*), Puerto Rico (*3*), and northeastern Australia (*4*). Subsequent reports revealed the severity of the declines. At one site in Costa Rica, 40% of the amphibian fauna disappeared over a short period in the late 1980s (*5*). Sudden disappearances of montane species were noted simultaneously in Costa Rica, Ecuador, and Venezuela (*5–8*). In some regions, many declines took place in seemingly pristine habitats (*1–8*). These reports were initially received with some skepticism because amphibian populations often fluctuate widely (*9*), but tests of probabilistic null models showed that the declines were far more widespread and severe than would be expected under normal conditions of demographic variation (*5*). This finding, in addition to many further reports of declines in the 1990s (*8*, *10–13*), was pivotal in convincing most herpetologists that amphibian declines are nonrandom unidirectional events.

The lack of a comprehensive picture of the extent and severity of amphibian declines prompted us to conduct the IUCN–The World Conservation Union Global Amphibian Assessment (GAA) to gather data on the distribution, abundance, population trends, habitat associations, and threats for all 5743 described species of amphibians (*14*, *15*). From this information, we used the IUCN Red List Criteria (*16*) to determine the level of threat to every species. The raw GAA data are publicly available (*14*). The results demonstrate that amphibians are far more threatened than either birds (*17*) or mammals (*18*), with 1856 species (32.5%) being globally threatened [that is, listed in the IUCN Red List Categories (*16*) of Vulnerable, Endangered, or Critically Endangered], as compared with 12% of birds (1211 species) (*17*) and 23% of mammals (1130 species) (*18*). At least 2468 amphibian species (43.2%) are experiencing some form of population decrease, whereas only 28 (0.5%) are increasing and 1552 (27.2%) are stable; 1661 (29.1%) species have an unknown trend.

Many amphibian species are on the brink of extinction, with 427 species (7.4%) listed as Critically Endangered (CR) (the IUCN category of highest threat), as compared with 179 birds (1.8%) (*17*) and 184 mammals (3.8%) (*18*). The level of threat to amphibians is undoubtedly underestimated because 1294 species (22.5%) are too poorly known to assess [Data Deficient (DD)], as compared with only 78 birds (0.8%) (*17*) and 256 mammals (5.3%) (*18*). A significant proportion of DD amphibians is likely to be globally threatened. Analysis of trends in population and habitat availability indicates

Table 1. Habitat preferences and biogeographic affinities of rapidly declining and enigmatic-decline amphibians in relation to all amphibian species (*15*). Rapidly declining species are those that now qualify for listing in a IUCN Red List Category of higher threat than they would have had in 1980. Enigmatic-decline species are rapidly declining species that have shown dramatic declines, even where suitable habitat remains, for reasons that are not fully explained.

Habitat preferences	Total number of species (%)	Number of rapidly declining species (%)	Number of enigmatic-decline species (%)
Forest	4699 (81.8)	365 (82.6)	187 (90.3)***↑
Savanna	487 (8.5)	7 (1.6)***↓	0 (0.0)***↓
Shrubland	814 (14.2)	47 (10.6)*↓	14 (6.8)***↓
Grassland	953 (16.6)	81 (18.3)	39 (18.8)
Flowing water	2650 (46.1)	277 (62.7)***↑	164 (79.2)***↑
Marshes/swamps	760 (13.2)	43 (9.7)*↓	14 (6.8)**↓
Still water bodies	2030 (35.3)	107 (24.2)***↓	28 (13.5)***↓
Artificial terrestrial habitats	1304 (22.7)	40 (9.0)***↓	22 (10.6)***↓
Tropical lowland habitats	3392 (59.1)	212 (48.0)**↓	79 (38.2)***↓
Tropical montane habitats	2714 (47.3)	251 (56.8)***↑	155 (74.9)***↑
Biogeographic realms			
Afrotropical	951 (16.6)	28 (6.3)***↓	1 (0.5)***↓
Australasian/Oceanic	561 (9.8)	36 (8.1)	23 (11.1)
Australia and New Zealand	219 (3.8)	32 (7.2)***↑	23 (11.1)***↑
Indomalayan	938 (16.3)	59 (13.3)	1 (0.5)***↓
Nearctic	331 (5.8)	24 (5.4)	9 (4.3)
Neotropical	2,825 (49.2)	279 (63.1)***↑	174 (84.1)***↑
Palearctic	451 (7.9)	34 (7.7)	2 (1.0)***↓

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ (*27*). ↑Significantly higher than average; ↓significantly lower than average.

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ANNEXE 8

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

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

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Abstract In social insects, colony size is a crucial life-history trait thought to have major implications for 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 to the type of habitats colonized (seaside vs. vineyard). The high level of the water table in the seaside habitat could constrain the depth of *C. cursor* underground nests and directly constrain its colony size. If worker size increases with colony size, as observed in other 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 were significantly larger in the vineyard populations. We further determined 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 was positively correlated with colony size and colony size with worker size. Using a type II regression approach, we further showed that the difference between the two populations in the depth of nest was sufficient to explain the difference in colony size, and similarly, 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

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Introduction

Individual size in insects, as in other organisms, is often considered an important life-history trait that 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 size has often been investigated at a macrogeographical scale, often in relation to latitude, with the aim of testing Bergmann's rule (Blanckenhorn and Demont 2004 for review). In social insects, variation in individual size has received a lot of attention as it is linked to a key social parameter, the division of labor among colony members (Oster and Wilson 1978; Hölldobler and Wilson 1990). The reproductive individual, the queen, is generally larger than workers, and in *Carebara vidua* can even exhibit thorax volume up to 8,000 times larger than the workers (M. Molet, personal communication).

Even though the workers usually do not reproduce, their body size is still an important life-history trait that can affect the ability of a colony to rear offspring and

therefore impact their indirect fitness. For various ant species, larger workers perform better than smaller workers at carrying out particular tasks linked to foraging and nest defense (Cerdá and Retana 1997; Reyes-Lopez and Fernandez-Haeger 2001; Braendle et al. 2003; Nowbahari et al. 1999, 2000). Such a pattern has also been observed at the interspecific 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 however are more energetically expensive to manufacture and maintain than small workers, and colonies have to face the traditional trade-off between worker number and size (Backus 1993; 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 similar to 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. Similarly to 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), with only large colonies 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 and Tschinkel 1981; Porter and Tschinkel 1985; Gibson 1989; Tschinkel 1988, 1993, 1998; Wetterer 1994; Kaspari and Byrne 1995) suggesting that only large colonies can 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 the evolution of life-history traits in social insects, an area still largely unexplored (Bourke and Frank 1995), but also for understanding the evolution of social complexity such as the evolution of worker castes (Hölldobler and Wilson 1990; Fjerdingstad and Crozier 2006) or the reproductive division of labor (Bourke 1999).

In ants, the size of the nesting cavity, and the nature and the availability of 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 vertical well leading to chambers located up to 1 m 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; A. Lenoir, personal observation). The depth of the water table is a simple ecological factor that clearly limits the depth of nests (Cagniant 1976; Lenoir et al. 1988). Interestingly, *C. cursor* colonizes two main types of habitat that clearly differ in the depths of their water tables: from 60 cm at the seaside (sandy soil) to 1.20 m 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 to the type of habitats. *C. cursor* is a monogynous species with dependent colony foundation, the queen founds a new colony with the help of workers (Lenoir et al. 1988). Even if 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 2,500 workers; Lenoir et al. 1988; A. Lenoir, personal observation).

If the two hypotheses about positive correlations between nest depth and colony size, and between colony size and worker size, are correct in *C. cursor*, we would expect larger colony size and larger workers in the vineyard populations. This prediction was supported by comparing the mean worker size between populations from both types of habitats. Moreover, in two populations (seaside and vineyard), the depth of nests was positively correlated with colony size, and colony size with worker size. Using a type II regression approach, we 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 in 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 (300 × 150 m 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 microsatellite 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): body length from the beginning of the clypeus in top view to the end of the abdomen (BL); tibia length of the right hind leg (TL); scape length, i.e. straight-line distance from base to apex of the scape (SL); head length from the beginning of the clypeus in top view to the end of the head capsule (HL) and head width 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, Cary, NC) 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 divergence 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 10,000 values obtained by random permutation of row and column elements in the independent matrices using XLSTAT-PRO 7.5.

Variation in 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 inland population L4 while eleven other colonies were sampled in the seaside population A2. 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, where the queen was 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 examined 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 (a type II regression approach; 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, $P > 0.05$), 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 SMATR v.1 software (Falster et al. 2003). The same SMA procedure was conducted to examine relationships between means as well as variance in worker size and colony size in both populations.

Results

Variation in worker size among populations

We confirmed that in *C. cursor*, worker size is normally distributed with unimodal distribution and that the five

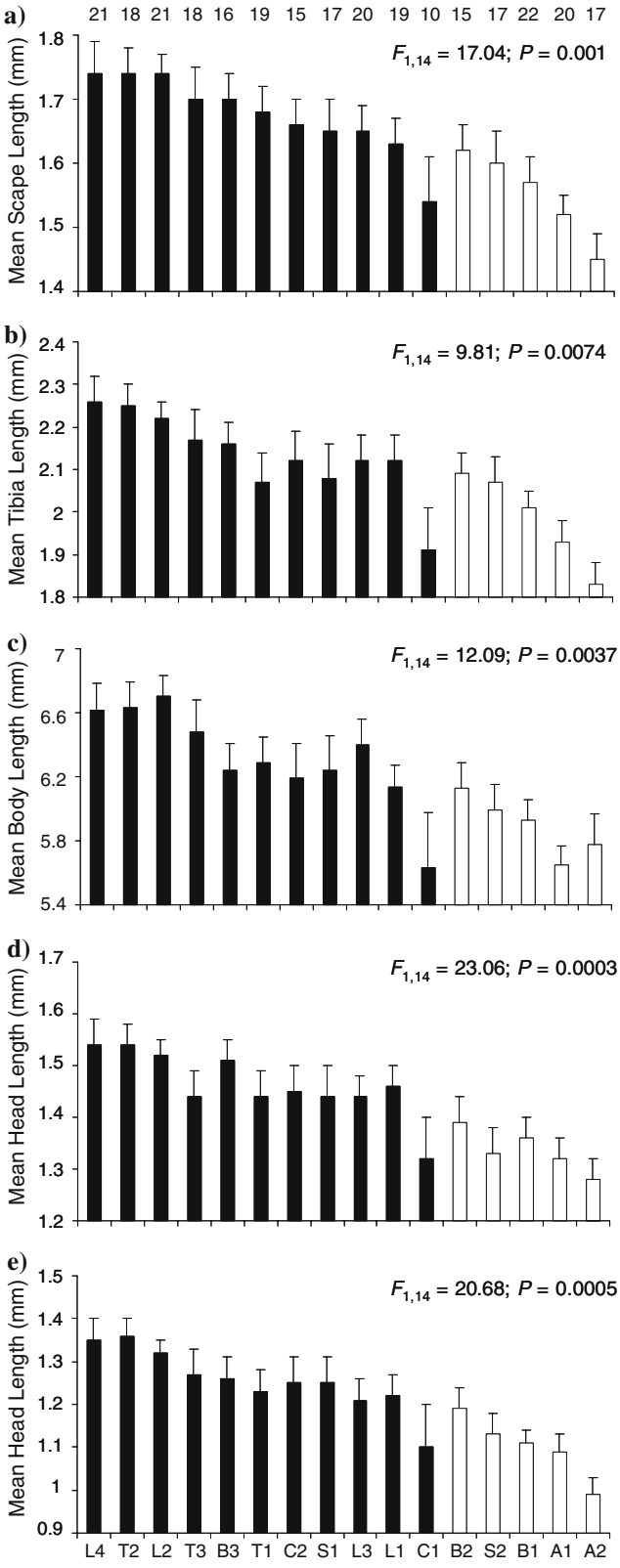

Fig. 1a–e Worker-size variation among populations. Morphological measurements [mean ± SE (mm)]: scape (**a**), tibia (**b**), body (**c**) and head length (**d**) as well as head width (**e**) are given for each of the 16 populations studied. The number of workers measured in each population is given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

measures taken were highly correlated (correlation coefficients ranging from 0.76–0.92, all significant at $P < 10^{-4}$). Overall size amplitude and coefficient of variation, ranging from 10.7% for the scape length to 16% for the head width, were very close to those reported by Cagniant (1983). Means (±SE) of the five morphological traits for the 16 populations are given in Fig. 1. The analysis of variance for each of the five morphological traits measured showed that workers from seaside populations were significantly smaller (15% for BL to 19% for HW) than workers from vineyard populations ($P < 0.01$ for the five measurements, Fig. 1). All the vineyard populations except one (C1; $n = 10$) 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 in 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 as deep 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 as high in the vineyard population (mean ± SE; $m_{L4} = 1{,}107.8 \pm 485.8$; $\text{range}_{L4} = 260$–$1{,}714$) than in the seaside population ($m_{A2} = 577.7 \pm 275.85$; $\text{range}_{A2} = 208$–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 nonnegligible 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. 2a; $P_{L4} = 0.009$; $P_{A2} = 0.049$), 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*-axis (colony size), was detected between the two populations ($F_{1,23} = 13.03$, $P = 0.002$). No significant difference in 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. 2b; $P_{L4} = 0.0066$; $P_{A2} = 0.017$) with the two slopes not significantly different between populations ($P = 0.081$). A significant separation along the common slope, i.e. along the *x*-axis (colony size), was also detected between the two populations ($F_{1,23} = 14.39$, $P = 0.001$) reflecting the difference in colony size detected between the two populations. Interestingly, no significant difference in 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 either population (see Fig. 2c, $P_{L4} = 0.94$; $P_{A2} = 0.19$). 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 in the intercept value was detected ($F_{1,23} = 2.367$, $P = 0.138$), indicating that, on average, variance values were the same in both populations.

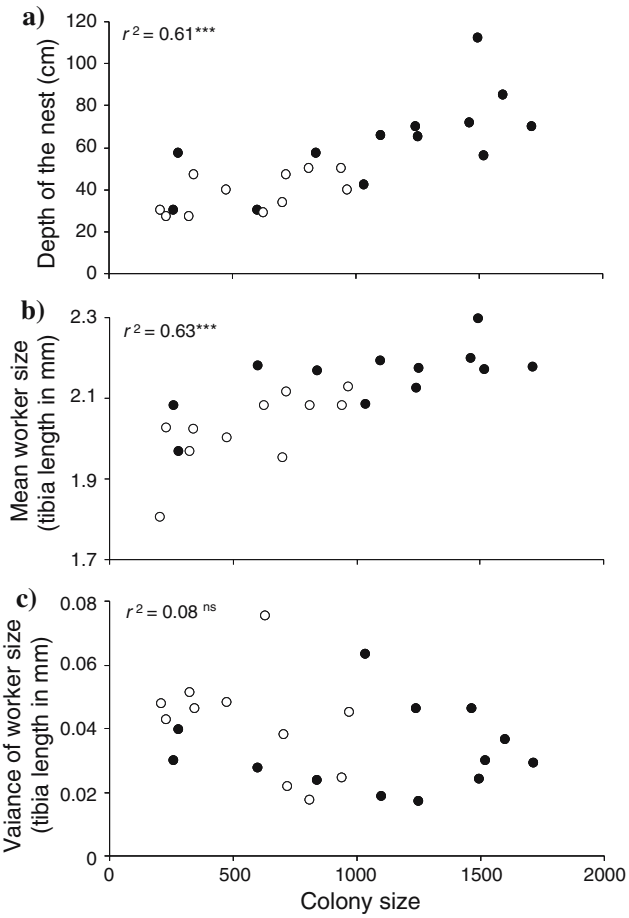

Fig. 2 Regression of depth of the nest (**a**), mean worker size (**b**) and variance of worker size (**c**) on colony size. *White circles* are colonies sampled in the population A2 at the seaside and *black circles* are colonies sampled in the population L4 in the vineyard. Significance ($^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$) and r^2 values are given for each regression. Equations for the common regression lines are **a** $y = 0.04x + 16.7$, **b** $y = 2.37.10^{-4}x + 1.9$ and **c** $y = -3.88.10^{-5}x + 0.07$

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; H. Cagniant, our observation). We found that the mean worker size was up to 27% smaller (for head width) in the seaside populations than in the vineyard. This 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) method (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 largest nests were twice as deep in the vineyard population than at the seaside, with the depth corresponding approximately to the appearance of humid soils (Cagniant 1976; H. Cagniant, personal observation). Interestingly, the maximum colony size was about twice as big in the vineyard as in the seaside population, even though the minimum colony size was

the same (see Fig. 2). It is therefore tempting to conclude that differences in a simple proximate ecological factor can 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 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 to worker size variation (Oster and Wilson 1978; Frumhoff and Ward 1992; Fjerdingstad and Crozier 2006). For instance, the number of queens per colony has been shown to affect 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 *C. cursor* populations that we studied exhibited 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 (J. Clémencet et al., personal communication)]. 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. Experimental manipulations are however needed to conclude decisively on the causal link of the relationships observed here. Within the two populations studied, nest depths explained a remarkably high part (62%) of the variation in colony size, a parameter generally supposed to be affected by various ecological and social factors (Bourke 1999). Within populations, variation in nest depths should reflect variation in colony growth and age even though it can still be constrained by micro environmental variations. As suggested by the intercept of the regression line of nest depth and colony size, freshly established propagules settle in shallow nests, which is in agreement with field observations (never deeper that 25–30 cm; personal observation May 2006; Lenoir et al. 1988).

As generally observed in ants (see Brian 1957; Elmes 1974; Wood and 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 observed in species with independent colony founding in which queens sacrifice worker size for worker number, the first workers being the smallest, the so-called nanitic workers (Wilson 1971). This pattern is also commonly observed in ants with clearly polymorphic worker castes. In such cases, 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 in the variance in worker size (Brian 1957; Gibson 1989). In *C. cursor*, none of these explanations hold since this ant founds colonies dependently and has no distinct worker caste (Cagniant 1983).

The minimum 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 minimum propagule size during a colony fission event, and agrees with the observations of Lenoir et al. (1988) estimating around 250 workers as the size of a propagule. Basically, a colony of 200 workers cannot be as energetically constrained as a queen founding a colony alone. Two hypotheses could explain our correlation between colony size and worker size. First, a small colony might not be able to afford the production of large workers because these workers could specialize in particular tasks. Such physical specialization could be costly for a small colony by decreasing its flexibility in response to environmental variation (Wheeler 1991). This explanation based on the division of labor 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. As worker size is normally distributed, it is unlikely that smaller colonies simply do not produce the largest workers. A more likely proximate explanation would be that the level of resources a colony can obtain linearly increases with its size in the colony size range observed in our study. Larger colonies could then obtain more resources leading to a shift of the distribution of worker size toward large size. This would mean that colonies never reached the point at which the curve of the resources gained as a function of colony size becomes asymptotic. Note that species that have a possibility to undergo fission when this critical point is attained should be favored to do so (Tsuji 1995).

From an evolutionary perspective, given that there is a trade-off between the number and size of workers, the increase in worker size with colony size suggests that investing in larger workers rather than in a higher number of small workers is advantageous for large colonies. In thermophilic ants, worker size is generally a major parameter affecting their thermal tolerance (Cerdá and Retana 1997). A higher resistance to temperature in larger workers has also been found in

C. cursor (Clémencet et al., personal communication). In this non-territorial ant, being the most dominated species of the Mediterranean ant community (Cerdà et al. 1997), larger worker size could therefore allow colonies to expand their daily activity period and avoid interspecific competition by foraging mainly during the hottest hours of the day.

In agreement with models that predict an advantage to producing many small workers when colonies are territorial and engage in battles with other colonies (Francks and Partridge 1993; Mc Glynn 2000), in *C. cursor*, the production of many small workers is unlikely to be advantageous in the context of inter- and intraspecific competition. At the colony level, selection should then favor 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 has been observed in different species (Tsuji 1995; Heinze et al. 1999; Heinze and Oberstadt 1999; Dietemann et al. 2002; Gobin and Ito 2003; Ravary and Jaisson 2004), individual-level selection should favor the evolution of an optimum size for worker reproduction (Oster and Wilson 1978; Fjerdingstad and Crozier 2006). This optimum might diverge from the one favored at the colony level.

Such conflicting selective pressures potentially occur in *C. cursor*. In this species, 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 optimum 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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References

Backus VL (1993) Packaging of offspring by nests of the ant *Leptothorax longispinosus*: parent–offspring conflict and queen–worker conflict. Oecologia 95:283–289

Bailey RC, Byrnes J (1990) A new method for assessing measurement error in both univariate and multivariate morphometric studies. Syst Zool 39:124–130

Blanckenhorn WU, Demont M (2004) Bergmann and converse Bergmann latitudinal clines in arthropods: two ends of a continuum? Integr Comp Biol 44:413–424

Bourke AFG, Franks NR (1995) Social evolution in ants. Princeton University Press, Princeton

Bourke AFG (1999) Colony size, social complexity and reproductive conflict in social insects. J Evol Biol 12:245–257

Braendle C, Hockley N, Brevig T, Shingleton AW, Keller L (2003) Size-correlated division of labour and spatial distribution of workers in the driver ant, *Dorylus molestus*. Naturwissenschaften 90:277–281

Brian MV (1957) The growth and development of colonies of the ant *Myrmica*. Insectes Soc 4:177–190

Cagniant H (1976) Distribution, écologie et nid de la fourmi *Cataglyphis cursor* Foscolombe Hyménoptères Formicidae. Vie Milieu 26:265–276

Cagniant H (1983) La parthénogénèse thélytoque et arrhénotoque des ouvrières de la fourmi *Cataglyphis cursor fonscolombe* (Hyménoptères Formicidae) étude biométrique des ouvrières et de leurs potentialités reproductrices. Insectes Soc 30:241–254

Calabi P, Porter SD (1989) Worker longevity in the fire ant *Solenopsis invicta*; ergonomic considerations of correlation between temperature, size and metabolic rates. J Insect Physiol 35:643–649

Campbell NA, Atchley WR (1981) The geometry of canonical variable analysis. Syst Zool 30:268–280

Cerdá X, Retana J (1997) Links between worker polymorphism and thermal biology in a thermophilic ant species. Oikos 78:467–474

Clémencet J, Viginier B, Doums C (2005) Hierarchical analysis of population genetic structure in the monogynous ant *Cataglyphis cursor* using microsatellite and mitochondrial DNA markers. Mol Ecol 14:3735–3744

Crozier R, Consul PC (1976) Conditions for genetic polymorphism in social Hymenoptera under selection at the colony level. Theor Popul Biol 10:1–9

Davidson DW, Cook SC, Snelling RR (2004) Liquid-feeding performances of ants (Formicidae): ecological and evolutionary implications. Oecologia 139:255–266

Dietemann V, Hölldobler B, Peeters C (2002) Caste specialisation and differentiation in reproductive potential in the phylogenetically primitive ant *Myrmecia gulosa*. Insectes Soc 49:289–298

Elmes GW (1974) The effect of colony population on caste size in three species of *Myrmica* (Hymenoptera: Formicidae). Insectes Soc 2:213–230

Falster DS, Warton DI, Wright IJ (2003) SMATR: standardised major axis tests and routines. Version 1.0. http://www.bio.mq.edu.au/ecology/SMATR/

Fjerdingstad EJ, Crozier R (2006) The evolution of worker caste diversity in social insects. Am Nat 167:390–400

Franks NR, Partridge LW (1993) Lanchester battles and the evolution of combat in ants. Anim Behav 54:197–199

Frumhoff PC, Ward PS (1992) Individual-level selection, colony-level selection and the association between polygyny and worker monomorphism in ants. Am Nat 139:559–590

Gibson RL (1989) Soldier production in *Camponotus novaeboracensis* during colony growth. Insectes Soc 36:28–41

Gobin B, Ito F (2003) Suma wrestling in ants: major workers fight over male production in *Acanthomyrmex ferox*. Naturwissenschaften 90:318–321

Goodisman MAD, Ross KG (1996) Relationship of queen number and worker size in polygyne colonies of the fire ant *Solenopsis invicta*. Insectes Soc 43:303–307
Hansell M (1987) Nest building as a facilitating and limiting factor in the evolution of eusociality in the Hymenoptera. In: Harvey PH, Partridge L (eds) Oxford surveys in evolutionary biology, vol. 4. Oxford University Press, Oxford, pp 155–181
Heinze J, Lipski N, Schlehmeyer K, Hölldobler B (1995) Colony structure and reproduction in the ant, *Leptothorax acervorum*. Behav Ecol 6:359–367
Heinze J, Foitzik S, Oberstadt B, Ruppell O, Hölldobler B (1999) A female caste specialized for the production of unfertilized eggs in the ant *Crematogaster smithi*. Naturwissenschaften 86:93–95
Heinze J, Oberstadt B (1999) Worker age, size and social status in queenless colonies of the ant *Leptothorax gredleri.* Anim Behav 58:751–759
Heinze J, Foitzik S, Fischer B, Wanke T, Kipyatkov VE (2003) The significance of latitudinal variation in body size in a holarctic ant, *Leptothorax acervorum*. Ecography 26:349–355
Hölldobler B, Wilson EO (1990) The ants. Springer, Berlin
Kaspari M, Byrne MM (1995) Caste allocation in litter Pheidole: lessons from plant defense theory. Behav Ecol Sociobiol 37:255–263
Kaspari M (2005) Global energy gradients and the regulation of body size: worker mass and worker number in ant colonies. Proc Natl Acad Sci USA 102:5079–5083
Lenoir A, Querard L, Pondicq N, Berton F (1988) Reproduction and dispersal in the ant *Cataglyphis cursor* (Hymenoptera, Formicidae). Psyche 95:21–44
Mantel N (1967) The detection of disease clustering and a generalized regression approach. Cancer Res 27:209–220
Mc Glynn TP (2000) Do Lanchester's laws of combat describe competition among ants? Behav Ecol 11:686–690
Ness JH, Bronstein JL, Andersen AN, Holland JN (2004) Ant body size predicts dispersal distance of ant-adapted seeds: implications of small-ant invasions. Ecology 85:1244–1250
Nijhout HF (2003) The control of body size in insects. Dev Biol 261:1–9
Nowbahari E, Feneron R, Malherbe MC (1999) Effect of body size on aggression in the ant, *Cataglyphis niger* (Hymenoptera; formicidae). Aggress Behav 25:369–379
Nowbahari E, Feneron R, Malherbe MC (2000) Polymorphism and polyethism in the formicinae ant *Cataglyphis niger* (Hymenoptera). Sociobiology 36:485–496
Nylin S, Gotthard K (1998) Plasticity in life history traits. Annu Rev Entomol 43:63–83
Oster GF, Wilson EO (1978) Caste and ecology in the social insects. Princeton University Press, Princeton
Porter SD, Tschinkel WR (1985) Fire ant polymorphism: factors affecting worker size. Ann Entomol Soc Am 78:381–386
Ravary F, Jaisson P (2004) Absence of individual sterility in thelytokous colonies of the ant *Cerapachys biroi* Forel (Formicidae, Cerapachyinae). Insectes Soc 51:67–73
Reyes-Lopez JL, Fernandez-Haeger J (2001) Some factors determining size-matching in the harvester ant *Messor barbarus*: food type, transfer activity, recruitment rate and size-range. Insectes Soc 48:118–124
Schwander T, Rosset H, Chapuisat M (2005) Division of labour and worker size polymorphism in ant colonies: the impact of social and genetic factors. Behav Ecol Sociobiol 59:215–221
Sokal RR, Rohlf FJ (1995) Biometry. WH Freeman, San Francisco
Sorvari J, Hakkaraien H (2005) Deforestation reduces nest mound size and decreases the production of sexual offspring in the wood ant *Formica aquilonia.* Ann Zool Fenn 42:259–267
Stearns SC (1992) The evolution of life histories. Oxford University Press, New York
Tschinkel WR (1988) Colony growth and the ontogeny of worker polymorphism in the fire ant, *Solenopsis invicta*. Behav Ecol Sociobiol 22:103–115
Tschinkel WR (1993) Sociometry and sociogenesis of colonies of the fire ant *Solenopsis invicta* during one annual cycle. Ecol Monogr 63:425–457
Tschinkel WR (1998) Sociometry and sociogenesis of colonies of the harvester ant, *Pogonomyrmex badius*. I. Worker characteristics in relation to colony size and season. Insectes Soc 45:385–410
Tsuji (1995) Reproductive conflicts and levels of selection in the ant *Pristomyrmex pungens*: contextual analysis and partitioning of covariance. Am Nat 146:586–607
Warton DI, Weber NC (2002) Common slope tests for bivariate errors-in-variables models. Biom J 44:161–174
Wetterer JK (1994) Ontogenetic changes in forager polymorphism and foraging ecology in the leaf-cutting ant *Atta cephalotes*. Oecologia 98:235–238
Wilson EO (1959) Some ecological characteristics of ants in New Guinea rain forests. Ecology 40:437–447
Wilson EO (1971) The insect societies. Harvard University Press, Cambridge
Wheeler DE (1991) The developmental basis of worker caste polymorphism in ants. Am Nat 138:1218–1238
Wood LA, Tschinkel WR (1981) Quantification and modification of worker size variation in the fire ant *Solenopsis invicta*. Insectes Soc 28:117–128

Résumé

Mes travaux de recherche portent sur l'étude des stratégies de reproduction chez les insectes sociaux, et, plus précisément, les fourmis que ce soit à l'échelle de la colonie que de l'individu. Les espèces étudiées présentent un mode de fondation des colonies par fission, *i.e.* la reproductrice part fonder une nouvelle colonie avec l'aide des ouvrières. J'ai pu montrer que ce mode de fondation, rarement étudié chez des espèces monogynes (une seule reproductrice par colonie), mène à des structurations génétiques significatives à l'échelle de quelques centaines de mètres, qui augmentent fortement avec la distance géographique. Une autre conséquence de ce mode de fondation est l'existence d'une succession de reproductrices dans la colonie au cours du temps qui affecte les corrélations génétiques entre ouvrières. Par ailleurs, nous avons pu montré que les conflits qui peuvent être observés lors des changements de reproductrices peuvent s'avérer coûteux en terme de diminution du temps de travail des ouvrières et de leurs défenses immunitaires. Ces coûts peuvent influencer le taux optimal de changement de reproductrices ainsi que le taux optimal de fission. A l'échelle individuelle, je me suis particulièrement intéressée à l'évolution de la polyandrie (plus d'un mâle par femelle) et parthénogenèse chez la fourmi *Cataglyphis cursor*. Nos travaux ont pu mettre à jour un système de reproduction jusqu'alors insoupçonné chez les fourmis, à savoir une utilisation par la reine de la reproduction sexuée avec un fort taux de polyandrie pour produire les ouvrières et de la reproduction parthénogénétique pour la production des nouvelles reines. Ceci permet à la fois d'augmenter le taux de transmission des gènes tout en conservant une forte diversité génétique à la colonie. Mes projets de recherche visent à analyser plus finement l'évolution de ces systèmes de reproduction originaux ainsi que les stratégies optimales de reproduction des colonies par fission. Je souhaite également renforcer une approche d'immunologie évolutive car la prise en compte des défenses immunitaires peut nous aider à comprendre des paramètres des sociétés tels que les systèmes de reproduction ou la division du travail et inversement car la vie sociale peut affecter l'investissement optimal dans les défenses immunitaires.

Abstract

My research focused on the evolution of reproductive strategies in social insects, more precisely in ants both at the colony and individual level. The species studied are characterised by a mode of colony foundation by fission, *i.e.* new colonies are founded with the help of workers. Colony fission has rarely been studied in monogynous species (species with a single reproductive in each colony). A population genetic approach has showed that limited dispersal associated to fission lead to significant genetic differentiation at the scale of few hundred meters, this differentiation strongly increasing with geographical distance. Another consequence of fission is the succession of reproductive females over time within colonies which affect the relatedness between colony members. Moreover, each change of reproductive females is generally associated with conflicts that we showed to be costly for workers both by decreasing the time spent at working and by decreasing their immune defences. These costs can affect the optimal rate of reproductive changes as well as the rate of fission. At the individual scale, I was especially interested in the evolution of mating system, especially of polyandry (more than one male per female) and parthenogenesis in the ant *Cataglyphis cursor*. Our work revealed a peculiar reproductive strategy of the ant queens, *i.e.* a conditional use of sexual reproduction with high level of polyandry for the production of workers and of parthenogenesis for the production of new queens. Such reproductive strategy increases the rate of gene transmission by the queen, keeping at the same time the advantages of a genetically diverse workers force. In my research project, I plan to investigate in more details the evolution of such reproductive strategy as well as the optimal strategies of fission. I also would like to develop an evolutionary immunology approach for a better understanding of the evolution of mating strategy and of some aspects of division of labour. On the other way around, I would also be interested to study how social life could affect optimal investments in immune defences.