ISSN 0962-1083

VOLUME 18
NUMBER 2
JANUARY
2009

MOLECULAR ECOLOGY

Published by
Wiley-Blackwell

Molecular Ecology (2009) **18**, 186–199 doi: 10.1111/j.1365-294X.2008.04018.x

Genetics, behaviour and chemical recognition of the invading ant *Pheidole megacephala*

DENIS FOURNIER, JEAN-CHRISTOPHE DE BISEAU and SERGE ARON
Behavioural and Evolutionary Ecology CP 160/12, Université Libre de Bruxelles, Avenue F.D. Roosevelt, 50, 1050 Brussels, Belgium

Abstract

Introduced species often become ecologically dominant, displacing native species and posing a serious threat to ecosystem function and global biodiversity. Ants are among the most widespread and damaging alien species; introductions are often accompanied by population-level behavioural and genetic changes contributing to their success. We investigated the genetic structure, chemical profile and nestmate recognition in introduced populations of the invasive big-headed ant, *Pheidole megacephala*, in Australia. Behavioural analyses show that workers are not aggressive towards conspecifics from different nests, even at large geographical scales (up to 3000 km) and between populations encompassing a wide range of environmental conditions. By contrast, interactions with workers of other species invariably result in agonistic behaviours. Genetic analyses reveal that populations have low genetic diversity. No genetic differentiation occurs among nests of the same population; differentiation between populations, though significant, remains weak. Chemical analyses indicate that cuticular lipids are similar between colonies of a population, and that differentiation between populations is low. Altogether, these results indicate that the big-headed ant *P. megacephala* forms a large unicolonial population across northern/eastern Australia.

Keywords: biological invasions, cuticular hydrocarbons, genetic structure, invasive ant, *Pheidole megacephala*, unicoloniality

Received 13 May 2008; revision received 6 October 2008; accepted 17 October 2008

Introduction

The rate of biological invasions by exotic plant and animal species (whether through accidental or deliberate introduction) has risen dramatically over the last century, with increased global trade and human mobility (e.g. Mack *et al*. 2000; Pascal *et al*. 2005). Owing to their potential for profound ecological and socio-economical disruptions, nonindigenous species are now recognized as a major threat of the 21st century (Vitousek *et al*. 1997; Wilcove *et al*. 1998; Mack *et al*. 2000; Sala *et al*. 2000; Mooney & Cleland 2001; Lockwood *et al*. 2006). The main causes of the success of invasive species stem from the combination of historical, genetic and ecological factors of both the invaders and the invaded ecosystem (Lodge 1993; Williamson 1996; Sakai *et al*. 2001; Facon *et al*. 2006; Suarez & Tsutsui 2008).

Ants are among the most abundant and influential invertebrate groups in terrestrial ecosystems. More than 12 450 ant species have been described to date (*http://antbase.org/*– September 25 2008); they occur in almost every environment on Earth (except Antarctica, Greenland and some inhospitable islands) and play a diversity of roles in terrestrial ecosystems. However, many species of ants have become established outside their native ranges (Williams 1994; McGlynn 1999), and a subset of them are among the world's worst invasive species (Lowe *et al*. 2001). In their introduced habitats, they reduce the diversity and abundance of native ants through continuous warfare, they affect other taxa through direct or indirect competition, and may alter the composition of entire ecosystems and landscapes (Passera 1994; Moller 1996; Holway *et al*. 2002a; Sanders *et al*. 2003). Invasive ants share some characteristics that facilitate their dispersal from their native ranges, their introduction into new habitats through local movements and by long distance jumps, their long-term establishment and their spread in a new context. These include a close association with humans, general nesting requirements, omnivory, polygyny (occurrence of multiple reproductive queens per colony), population genetic viscosity due to colony reproduction by

Correspondence: Denis Fournier, Fax: +32 (0)2 650 24 45;
E-mail: denis.fournier@ulb.ac.be

budding, and reduced intraspecific aggression between nests of the same unicolonial population (Passera 1994; Moller 1996; Holway *et al*. 2002a).

The ecological domination of invasive ant species is often attributed, in part, to their propensity to be unicolonial, i.e. to form expansive, polygynous populations within which individuals mix freely among physically separate nests (Holway *et al*. 2002a). By reducing costs associated with territoriality and aggression between nests, unicoloniality allows high worker densities and contributes to interspecific dominance and habitat saturation by the competitive exclusion of other ant species (Holway *et al*. 2002a). Laboratory rearings show that nonaggressive colonies experience a reduced mortality and an increased foraging activity, resulting in higher rates of resource retrieval, greater brood production and larger worker populations (Holway *et al*. 1998). Consistent with these observations, among the five ants listed on the 100 world's worst alien species (Lowe *et al*. 2001), all except one show a social organization based on unicoloniality. Furthermore, the presence of several queens accelerates colony growth (Vargo & Fletcher 1989) and increases the chance that transported propagules contain reproductive females able to initiate new colonies (Hee *et al*. 2000; Aron 2001). It was first suggested that unicoloniality is a derived trait that evolved after the introduction into new environments, due to a reduced diversity at recognition alleles through a genetic bottleneck (Tsutsui *et al*. 2000) or from a selection process against less common alleles ['genetic cleansing'; Giraud *et al*. (2002)]. However, expansive populations including polygynous supercolonies that compete for resources and potentially interbreed were documented in the native range of the Argentine ant (Suarez *et al*. 1999; Tsutsui *et al*. 2000). Therefore, Pedersen *et al*. (2006) suggested that unicoloniality is not a derived form of social organization that evolved after the introduction. This interpretation remains however, disputed by Suarez *et al*. (2008) who pointed out the strong competition between supercolonies from the same population and important differences in the genetic architecture between native and introduced populations of the species. Clearly, differences in the interpretation of the data stem from different definitions of unicoloniality. Suarez *et al*. (2008) refer to the traditional definition of unicoloniality, i.e. to situations where 'entire populations consist of nests without clear colony boundaries, whose members intermix' (Bourke & Franks 1995), a population level phenomenon. On the other hand, for Pedersen *et al*. (2006), unicolonial populations consist of one or several supercolonies, a colony level phenomenon. Here, we borrowed the population level definition of unicoloniality.

In social insects, discrimination of nestmates from non-nestmates relies on the use of nonvolatile olfactory cues, mainly cuticular lipids, that constitute a 'colony odour' (Vander Meer *et al*. 1998; Howard & Blomquist 2005). Cuticular profiles identify each individual's colony of origin and are used to form a recognition template against which to compare labels of other individuals. These recognition cues may derive from the environment (e.g. food or nesting material), they may have a genetic basis, or they may originate from both exogenous (environmental) and endogenous (genetic) sources (Vander Meer *et al*. 1998; Howard & Blomquist 2005). In unicolonial invasive species, the lack of aggressiveness between nests could stem from reduced genetic diversity at genes that encode components of their cuticular profiles due to events of range expansion and population bottlenecks directly affecting recognition capacity.

In this study, we investigated the association between genetic architecture, chemical signature and nestmate recognition in the big-headed ant *Pheidole megacephala*. This species reportedly is native from southern Africa (Wilson & Taylor 1967); it has spread to most tropical and subtropical zones of the world, including several Pacific Islands (Wetterer 2007). The most serious consequence of *P. megacephala* invading natural habitats is the elimination of native ants and its potential cascading effects on other trophic levels. Two recent surveys in semi-natural rainforest of northern Australia illustrated the impressive dynamics of the infestation. Data collected in 1996 and 2005 showed that the infested area had doubled in nine years and that *P. megacephala* abundance increased more than 20-fold while abundance of other macro-invertebrates decreased (Hoffmann *et al*. 1999; Hoffmann & Parr 2008). The species interferes with agricultural production by tending scale insects and plant lice, and by facilitating the introduction of new potentially invasive plant species by seed transportation (Hoffmann *et al*. 1999; Wetterer 2007).

Despite its ecological and economic importance, *P. megacephala* has received surprisingly little attention. In this paper, we present the first genetic data on the population structure of this species in ecologically distinct sites (wildlife and human-disturbed habitats) from Australia, using microsatellite markers. We also examined for the first time the pattern of variation of worker cuticular profiles in relation to population genetic structure and geographical location. Finally, we measure intra- and intersite tolerance on the basis of aggression tests between workers of nests at all sites, and we correlate levels of intraspecific aggression between nest pairs to the similarity of their cuticular lipid profiles to determine the potential role of these compounds as labels for nestmate recognition.

Materials and methods

Study species

The ant *Pheidole megacephala* was introduced into Australia about a century ago. It was first reported in north Queensland;

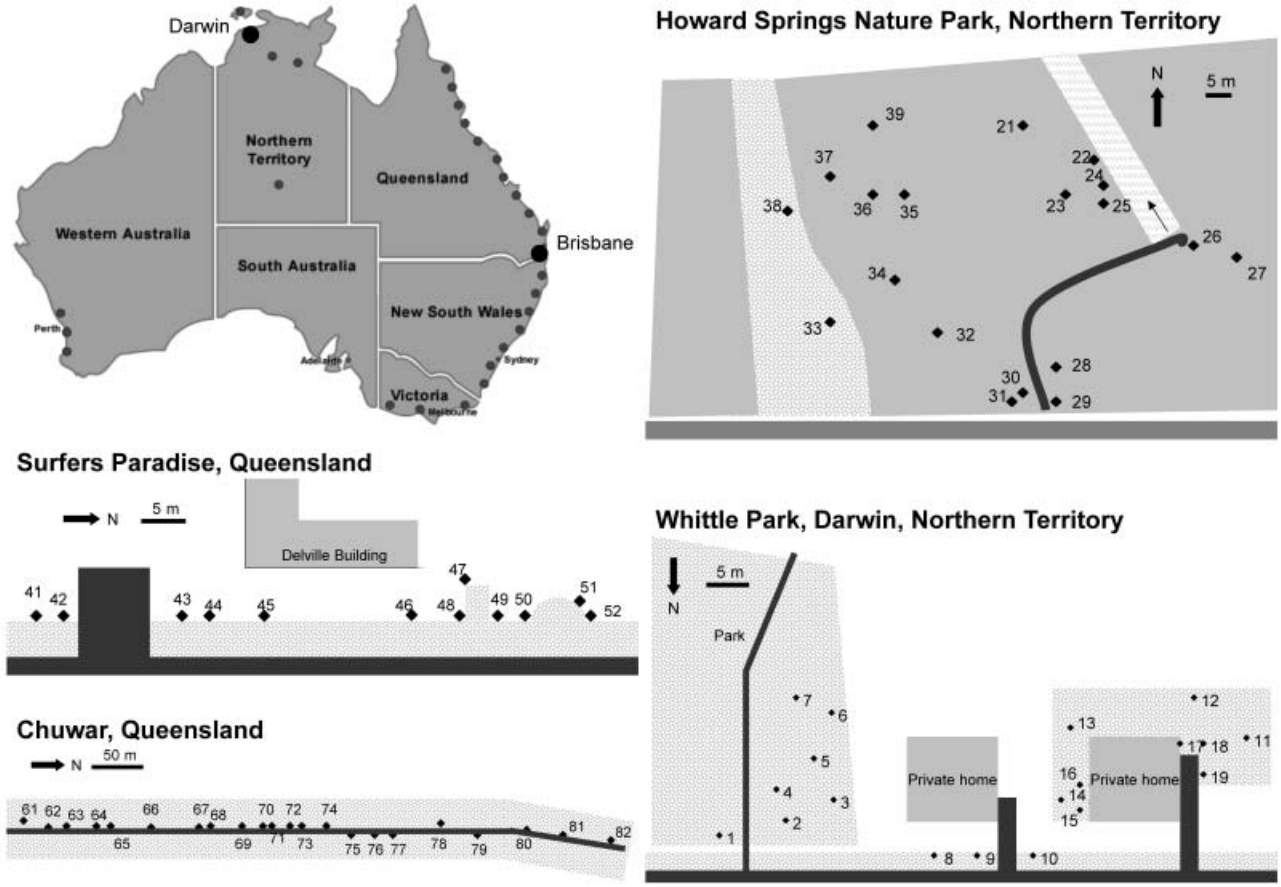

Fig. 1 Site and nest locations of the big-headed ant *Pheidole megacephala* in Australia. Places where *Pheidole megacephala* was recorded are marked by a dot (from http://www.discoverlife.org). Four sites were sampled, Surfers Paradise, Whittle Park, Howard Springs Nature Park and Chuwar; they extended over 630, 2460, 6100 and 63 000 m2, respectively. Each was inhabited by a single, continuous population. Diamonds and their number identify the nests sampled.

since then, its range expanded and now includes subtropical southern Queensland, warm-temperate coastal New South Wales, the mediterranean Perth region, the Darwin region of the top end of the Northern Territory and, over the last decade, different areas of the Tiwi Islands where it has negatively affected the ecological integrity of the native island biota (Hoffmann *et al*. 1999; Andersen *et al*. 2004) (Fig. 1).

Field collection and sampling

Fieldwork was conducted in spring 2005, in two regions about 3000 km apart, Darwin (Northern Territory) and Brisbane (Queensland) (Fig. 1). Colony material was collected at four sites belonging to distinct ecological environments: (i) in a wildlife rainforest habitat at Howard Springs Nature Reserve ('HS'; 12°27′28″ S–131°3′6″ E; $N = 18$ nests); and in 3 human-disturbed habitats; (ii) in an urban park in Whittle Park ('WP'; 12°23′48″ S–130°55′3″ E; $N = 16$); (iii) along an urban road in Surfers Paradise ('SP'; 27°59′30″ S–153°25′48″ E; $N = 11$); and (iv) in gardens of a suburb in Chuwar ('CH'; 27°33′46″ S–152°47′18″ E; $N = 16$). The distances between the two sites sampled in Darwin and Brisbane were 20 and 80 km, respectively. Samples of workers and queens were collected from each nest and kept alive for behavioural assays. *Pheidole megacephala* have dimorphic worker castes (majors and minors), but the great majority of the workers exploring the soil surface are minors. Therefore, we used only minor workers for the behavioural assays, chemical and genetic analyses. A subsample of 50 workers from each nest was immediately used for chemical extraction. From these, eight workers per nest were chosen randomly and stored in ethanol for subsequent genetic analyses. All queens were chemically and genetically analysed.

Behavioural assays

Intraspecific aggression between pairs of workers was quantified immediately after collection using standardized

fighting trials (Holway *et al*. 1998). Two individuals were placed together in a neutral arena (diameter = 2 cm, height = 1 cm) coated with Fluon™. Interactions were scored over five min according to a scale ranging from 1 to 4, with 1 = short antennation (< two s); 2 = prolonged antennation; 3 = aggression, i.e. lunging and attempts of biting; and 4 = fight, i.e. prolonged biting and pulling. Levels 1 and 2 are referred to as nonaggressive behaviours, whereas levels 3 and 4 are referred to as aggressive behaviours. Five trials were conducted for each nest pair and the highest score was averaged across trials. Pairs of nests were chosen at random and different workers were used for each trial. Interactions between individuals from the same nests were considered as control assays; interactions between individuals belonging to different nests, whether from the same or different sites, were considered as experimental assays.

Extraction and isolation of cuticular lipids

Samples of about 50 workers from each nest were washed in 1 mL of hexane for five min just after collection. Chemical profiles were obtained on a Thermo Polaris Q™ electron impact ion trap mass spectrometer interfaced to a Thermo Trace GC Ultra™ gas chromatograph equipped with a DB-5MS fused silica capillary column (30 m × 0.25 mm, diameter × 0.25 μm film thickness) (Thermo Finnigan). Qualitative and quantitative data were acquired by running the Thermo Xcalibur™ data system (Thermo Finnigan). Following splitless injection of 1 μL of the sample, oven temperature was maintained at 80 °C for two min, increased to 270 °C at 20 °C/min, and then to 310 °C at 3 °C/min, using helium as carrier gas (1.2 mL/min). The injection port and transfer line were set at 240 °C and 310 °C, respectively. We used the relative amount (i.e. area) of the peaks corresponding to cuticular lipids as a quantitative measure of each profile. Compounds with a relative peak area of less than 1% in all profiles were excluded from the data. Cuticular lipids were identified by analysis of their mass spectra produced by both electron impact and chemical ionization with methane.

Molecular procedures and genetic analyses

DNA extraction. Genetic analyses were performed on a sample of 144 workers, 16 queens and their spermatheca from Howard Springs Nature Reserve; 128 workers, 24 queens and their spermatheca from Whittle Park; 88 workers, 7 queens and their spermatheca from Surfers Paradise; and 128 workers, 36 queens and their spermatheca from Chuwar. Individual ants were ground in digestion solution (100 mM NaCl, 50 mM Tris, 1 mM EDTA, 0.5% SDS, and 200 μg/mL proteinase K). To isolate sperm DNA, queens were dissected and the spermatheca removed and placed in a drop of distilled water. The envelope was ruptured with forceps and seminal fluid was recovered using a micropipette, then resuspended in 20 μL of digestion solution. Individual ants and seminal fluid were incubated for two hours at 55 °C in 100 μL of digestion solution; DNA was purified through phenol/chloroform extractions and ethanol precipitation following standard protocols (Sambrook & Russell 2001). The DNA pellet was resuspended in 100 μL of sterile H_2O.

Genomic DNA genotyping. Genotypes of ants were determined at eight statistically independent microsatellite loci: *Pmeg-06*, *Pmeg-07*, *Pmeg-09*, *Pmeg-10*, *Pmeg-11*, *Pmeg-12*, *Pmeg-14* and *Pmeg-15* (Fournier *et al*. 2008). Polymerase chain reaction (PCR) amplifications were carried out in a 10 μL volume, containing 2 μL of genomic DNA (about 4–8 ng of DNA), 5 μL of Qiagen HotStarTaq Master Mix (providing a final concentration of 0.5 unit HotStarTaq DNA polymerase, 1 × PCR buffer with 1.5 mM $MgCl_2$ and 200 μM of each dNTP) and 0.3 μM of each primer's pair. PCR were performed with a MJ Research PTC-200 thermocycler. After an initial denaturing step of 15 min at 95 °C, the PCR consisted of 35 cycles of 30 s at 94 °C, 90 s at the annealing temperature (see detailed protocol in Fournier *et al*. 2008), and 60 s at 72 °C, followed by a final extension step of 30 min at 60 °C. Microsatellite loci were analysed using an ABI 3100 automated sequencer (Applied Biosystems); the lengths of PCR products were determined using GENEMAPPER Software (Applied Biosystems) and used to construct a multilocus genotype for each individual.

Statistical analyses

Genetic markers. Basic descriptive statistics, including allelic richness (an estimate of allelic diversity that compensates for unequal sample size), expected (H_E) and observed (H_O) heterozygosities were calculated using FSTAT 2.9.3 (Goudet 2001) and averaged across loci. Departure from the Hardy–Weinberg equilibrium for each locus across and within populations was tested using GENEPOP 3.4 (Raymond & Rousset 1995). We compared the allelic information between native [South Africa; data from Fournier *et al*. (2008)] and introduced (Australian) regions. In addition, a hierarchical analysis of molecular variance (AMOVA) was performed using GENALEX 6 software (Peakall & Smouse 2006) to assess the component of genetic diversity attributable to: (i) variance between Australian and South African regions; (ii) variance between sites within regions; and (iii) variance among individuals within sites. We computed estimates of population divergence using both variance in allelic frequency (*F*-statistics; Weir & Cockerham 1984) and allelic size (*R*-statistics; Michalakis & Excoffier 1996).

Genetic differentiation was analysed over pairs of nests in each site and over pairs of sites in Australia by testing for

genotypic differentiation, using exact tests as implemented in GENEPOP 3.4. *F*-statistics were estimated according to the method of Weir & Cockerham (1984) using GDA 1.1 software (Lewis & Zaykin 1999). The effect of spatial separation on genetic structure was tested by a Mantel test (Mantel 1967) based on 999 permutations using GENALEX 6. Hierarchical analyses were conducted across three levels represented by: (i) individuals within the nest; (ii) nests within the investigated site; and (iii) sites within Australia. f and F_{IS} give information on the amount of inbreeding due to nonrandom mating, F_{ST} gives information on gene flow. The statistical significance of fixation indices and 95% confidence intervals were evaluated by bootstrapping over loci 1000 times.

Gene flow (*Nm*) was calculated from the private alleles method of Slatkin (1985) using GENEPOP 3.4. This estimate is based on a linear relationship between the logarithm of *Nm* and the average frequency of the private alleles, corrected for differences in sample size (Barton & Slatkin 1986).

Populations that have experienced a recent reduction of their effective population size – for instance after introduction in a new environment – show reduction of both allele number (k) and gene diversity (H_E), but the former diminishes faster than the latter (Cornuet & Luikart 1996). We assessed the deviation in observed heterozygosity from that expected under mutation–drift equilibrium using the coalescent program BOTTLENECK 1.2.02 (Piry *et al*. 1999). Calculations were performed using both a two-phased mutation model (TPM) set at 90% stepwise and 10% infinite allele mutation (IAM). Significant deviations in observed heterozygosity over all loci were tested using a nonparametric Wilcoxon test. Moreover, recent bottlenecks provoke a shift away from an L-shaped distribution of allele frequencies, to one with fewer alleles in the low frequency categories. This was also assessed using BOTTLENECK 1.2.02.

Relatedness coefficients among workers were estimated using the program RELATEDNESS 5.0.8 according to the algorithm described by Queller & Goodnight (1989). The mean relatedness was estimated between nestmates workers relative to the collection site, and among workers in a site relative to the four sites sampled. Standard errors (SE) and 95% confidence intervals were obtained by jackknifing over sites and nests, respectively. Pairwise individual allele-shared distances were estimated as described in Fournier *et al*. (2005a).

The effective mean number of reproductive queens per nest, weighted by the respective contribution of each queen to the production of workers, was inferred from relatedness indices among workers according to Ross (1993) and Seppä (1994). Mating frequency per queen was assessed by comparing a queen's genotype with that of her sperm DNA. Because males of Hymenoptera are haploid, all sperm cells of an individual bear the same allele at a given locus; thus, sperm samples collected from the queen spermatheca showing multiple bands at a locus indicate that a female mated multiply. Genotyping of sperm DNA can leave multiple mating undetected when two males share the same genotype at all loci. This probability can be estimated according to Pamilo (1993), as

$$\prod_j \sum_i p_{ij}^2$$

where p_{ij} is the population frequency of allele i at locus j.

Cuticular profile. The diversity of cuticular compounds over nests, i.e. the constancy of the proportions of peaks in the cuticular profiles, was estimated using Nei's index, $I = 1 - \sum_i p_i^2$ where p_i is the relative proportion of peak i over all chemical peaks identified as cuticular lipids. Non-parametric Kruskal–Wallis tests were used for multiple independent comparisons among sites; a *posteriori* nonparametric pairwise comparisons of within-population cuticular compounds variation among sites were performed applying Dunn's procedure (Siegel & Castellan 1988).

The similarity of cuticular profiles between workers from different nests in a site (the within-site cuticular profiles variation) was estimated by nest-by-nest Euclidean distances based on the relative amounts of chemical compounds. Euclidean distances vary between 0 and 1; 0 means that the chemical profiles are identical, whereas 1 indicates that no compound is shared by the two profiles. Non-parametric Kruskal–Wallis tests were used for multiple independent comparisons among sites; Dunn's multiple comparisons tests were performed to define specific differences of within-population cuticular compounds variation among sites.

To discriminate between different patterns of chemical profile amongst sites, we performed a canonical discriminant function analysis on relative amounts (percentages) of the chemical peaks identified. Each peak had an equal statistical weight and cuticular profile was reduced to a few essential and independent components. To estimate the chemical variation attributable to the differences among sites and among nests within sampling sites, we applied the analysis of molecular variance (AMOVA) approach to a matrix of nest-by-nest Euclidean distances based on the relative amounts of chemical compounds. The significance of each variance component was tested by a random permutation test (999 permutations) using GENALEX 6.

Correlations between divergence in chemical profiles (i.e. mean pairwise Euclidean distance of relative amounts of chemical compounds between nests, each sampled in a different site) and geographical distance among sites were tested by performing a Mantel test (Mantel 1967) based on 999 random permutations, using GENALEX 6.

Association between variation of the cuticular profile and genetic and spatial data. To test for a possible association between variations of the cuticular profile and genetic or spatial

Table 1 Statistics (mean ± SE) on behavioural assays (*Ag:* level of aggressiveness), on genetic analyses (*Ar*: allelic richness; H_O: observed heterozygosity; H_E: expected heterozygosity; F_{ST}: fixation index), and chemical analyses (*I*: Nei index; *E*: Euclidean distance).[1] Comparisons of statistics between sites were performed using nonparametric Kruskal–Wallis tests followed by posthoc Dunn procedures (different letters inside the parentheses indicate significant differences).[2] Comparisons within- vs. between-sites were assessed using Mann–Whitney tests. ns: $P > 0.05$; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$

	Aggressiveness	Genetic data				Chemical data	
	Ag	*Ar*	H_O	H_E	F_{ST}	*I*	*E*
Whittle Park (WP)	1.039 ± 0.013(a)	1.334 ± 0.018(a)	0.290 ± 0.019(a)	0.296 ± 0.020 (a)	0.064 ± 0.026(b)	0.948 ± 0.002(b)	0.060 ± 0.003(a)
Howard Springs (HS)	1.103 ± 0.025(a)	1.386 ± 0.012(a)	0.361 ± 0.013(a)	0.352 ± 0.011 (a)	–0.003 ± 0.012(a)	0.935 ± 0.003(a)	0.059 ± 0.003(a)
Surfers Paradise (SP)	1.141 ± 0.038(a)	1.355 ± 0.014(a)	0.342 ± 0.018(a)	0.325 ± 0.010 (a)	0.017 ± 0.022(a)	0.930 ± 0.005(a)	0.122 ± 0.014(b)
Chuwar (CH)	1.104 ± 0.026(a)	1.300 ± 0.018(a)	0.217 ± 0.019(a)	0.226 ± 0.013 (a)	0.011 ± 0.017(a)	0.935 ± 0.007(a,b)	0.135 ± 0.015(b)
Chi-squared values[1]	10.315*	0.801 ns	4.412 ns	3.642 ns	49.366***	11.626**	35.068***
Within-sites	1.086 ± 0.012	—	—	—	0.020 ± 0.004	—	0.085 ± 0.004
Between-sites	1.067 ± 0.038	—	—	—	0.151 ± 0.003	—	0.128 ± 0.004
Mann–Whitney *U*[2]	12 626 ns	—	—	—	90 324***	—	5451***

data, we performed Mantel correlation tests using distance matrices from interpopulation chemical (as pairwise Euclidean distances), geographical and genetic (as pairwise F_{ST}) distances.

Deviations of the variables from normality were tested using the Shapiro-Wilk test: normally distributed data sets were consequently analysed with parametric tests; when logarithmic or angular transformations did not suffice to normalize the data, we used nonparametric statistics (Sokal & Rohlf 1995). Unless otherwise specified, means are presented ± SE and all statistical tests are two-tailed; they were carried out with the computer program SPSS 13.0 (SPSS Inc., 1989–2005).

Results

Field observations showed that no other ant species were found when *Pheidole megacephala* was present, and that the density of nests and ant workers were invariably high.

Patterns of aggression between nests

In all 552 of the 5-min aggression tests ($n = 187, 145, 85$ and 135 confrontations for the sites WP, HS, SP and CH, respectively), workers showed no sign of aggressiveness, the maximum level of intraspecific aggression being equal to level 2 Table 1). Similarly, encounters between workers from different sites were always peaceful ($n = 45$ confrontations; maximum scored = 2) (Table 1). As a consequence, no relationship was found between the level of intraspecific aggression and the distance between nests pairs, within or between sites (Spearman's coefficient of rank correlations, all $P > 0.163$).

In contrast, interactions between *P. megacephala* workers and two local competitors (*Linepithema humile* and *Iridomyrmex purpureus; n* = 10 confrontations with each species) always resulted in very high levels of aggression. Both opponents initiated aggression; individuals started fights with prolonged biting and pulling (level four) resulting in the death of one or other of the two antagonists.

Patterns of variation of the genetic markers

Genetic diversity in Australia, measured as allelic richness and observed and expected heterozygosity, was relatively low compared to that reported for South Africa (Fournier *et al*. 2008). While the number of alleles ranged from 4 to 10 in South African populations, we found two to four alleles in our Australian sample. Similarly, levels of observed heterozygosity per locus in Africa varied between 0.350 and 0.900, whereas it ranged from 0.146 to 0.477 in Australia (Table 2). The hierarchical gene diversity analysis revealed that 59% and 86% of the total genetic variance was the result of within sites differences, when it was estimated from the variance in allelic frequency and size, respectively. In addition, a significant proportion of the allelic variance was attributed to differences among regions (34% and 12% for F_{RT} and R_{RT}, respectively), and, at a lesser extent, among populations (7% and 2% for F_{SR} and R_{SR}, respectively) (Table 3). Given a loss of 3.75 alleles on average per locus, we calculated the minimum number of queens that must have been introduced in Australia to account for the observed diversity in the study sample. We performed 200 simulations and assumed conservatively that no alleles were lost after the introduction of the first population in Australia. Our simulation estimated that at least two unrelated queens (i.e. six haploid genomes) must have been introduced in Australia.

Across the eight loci examined, no statistical difference in the genetic variability occurred between the four sites (Table 1). The genotypic proportions observed did not deviate from those expected under the Hardy–Weinberg

Table 2 Comparison of genetic diversity (mean ± SE) between Australian and South African populations, measured as number of alleles (*Na*), allelic richness (*Ar*) and observed and expected (H_O, H_E) heterozygosity. Data for the South African population are taken from Fournier *et al*. (2008). Comparisons were assessed using Mann–Whitney tests. ns: $P > 0.05$; *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$

	Na	*Ar*	H_O	H_E
Australia ($n = 488$)	5.125 ± 0.742	2.545 ± 0.226	0.314 ± 0.036	0.392 ± 0.046
	Min. 3	1.978	0.146	0.166
	Max. 9	3.673	0.477	0.507
South Africa ($n = 20$)	7.125 ± 0.833	6.129 ± 0.631	0.626 ± 0.071	0.737 ± 0.031
	Min. 4	3.549	0.350	0.583
	Max.10	8.148	0.900	0.826
Mann–Whitney *U*	16 ns	1***	6***	0***

Table 3 Hierarchical analysis of molecular variance (AMOVA) of microsatellite loci among *Pheidole megacephala* populations. The components of genetic variance were estimated (i) among Australian and South African regions; (ii) among populations within regions; and (iii) among individuals within populations. ns: $P > 0.05$; *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$

	Source of variation	Variance components	Per cent variation	Fixation indices
Molecular distance expressed as allele frequencies	Among regions	1.128	34.26%	$F_{RT} = 0.343$*
	Among populations	0.212	6.43%	$F_{SR} = 0.098$*
	Within populations	1.953	59.31%	$F_{ST} = 0.407$*
Molecular distance expressed as allele length	Among regions	2651.418	12.42%	$R_{RT} = 0.124$*
	Among populations	458.769	2.15%	$R_{SR} = 0.025$*
	Within populations	18 237.703	85.43%	$R_{ST} = 0.146$*

Table 4 Deficit in heterozygotes and relatedness between nestmates in the four sites sampled (†relative to both South African and Australian populations, ‡relative to the Australian populations only, or §relative to the collection site)

	F-statistics		Relatedness		
	Mean ± SE [95% CI]		Mean ± SE [95% CI]		
	F_{IS}	F_{IT}	*r*†	*r*‡	*r*§
Whittle Park (WP)	0.092 ± 0.084	0.151 ± 0.097	0.402 ± 0.051	0.371 ± 0.052	0.013 ± 0.040
	(–0.028–0.296)	(0.007–0.378)	(0.293–0.511)	(0.260–0.483)	(–0.027–0.111)
Howard Springs (HS)	0.052 ± 0.022	0.049 ± 0.032	0.124 ± 0.029	0.092 ± 0.029	–0.005 ± 0.022
	(0.021–0.1)	(0.001–0.114)	(0.064–0.184)	(0.031–0.154)	(–0.046–0.033)
Surfers Paradise (SP)	0.042 ± 0.054	0.058 ± 0.056	0.448 ± 0.025	0.096 ± 0.025	0.032 ± 0.042
	(–0.058–0.135)	(–0.048–0.16)	(0.093–0.204)	(0.041–0.152)	(–0.024–0.122)
Chuwar (CH)	0.157 ± 0.084	0.167 ± 0.09	0.142 ± 0.033	0.102 ± 0.034	0.020 ± 0.028
	(0.021–0.291)	(0.015–0.3)	(0.071–0.213)	(0.030–0.174)	(–0.029–0.067)

equilibrium for most single-locus comparisons within each site, except a significant heterozygote deficiency observed at *Pmeg-12* for CH ($P = 0.038$). *F*-statistics indicate a positive inbreeding coefficient in three out of the four sites studied (WP, HS, CH; Table 4); the combined analysis of the four sites gives an overall estimates of $F_{IT} = 0.199$. Consistent with this result, the average relatedness ± standard errors between the queens and their mates was 0.364 ± 0.053 (95%CI: 0.259–0.469). Within each site no genetic differentiation occurred between nests, indicating that they belonged to a single, huge colony (Table 1).

A total of one, two, and seven private alleles (i.e. occurring at only one site) at a mean frequency of 0.006, 0.005 and 0.013 were, respectively, found in CH, WP and HS; no private allele was observed in SP. Over all four Australian populations sampled, colony pairs shared on average 27.1% of their alleles (range: 0–77.3%; median: 26.1%). Accordingly, the tree-level hierarchical analysis revealed a

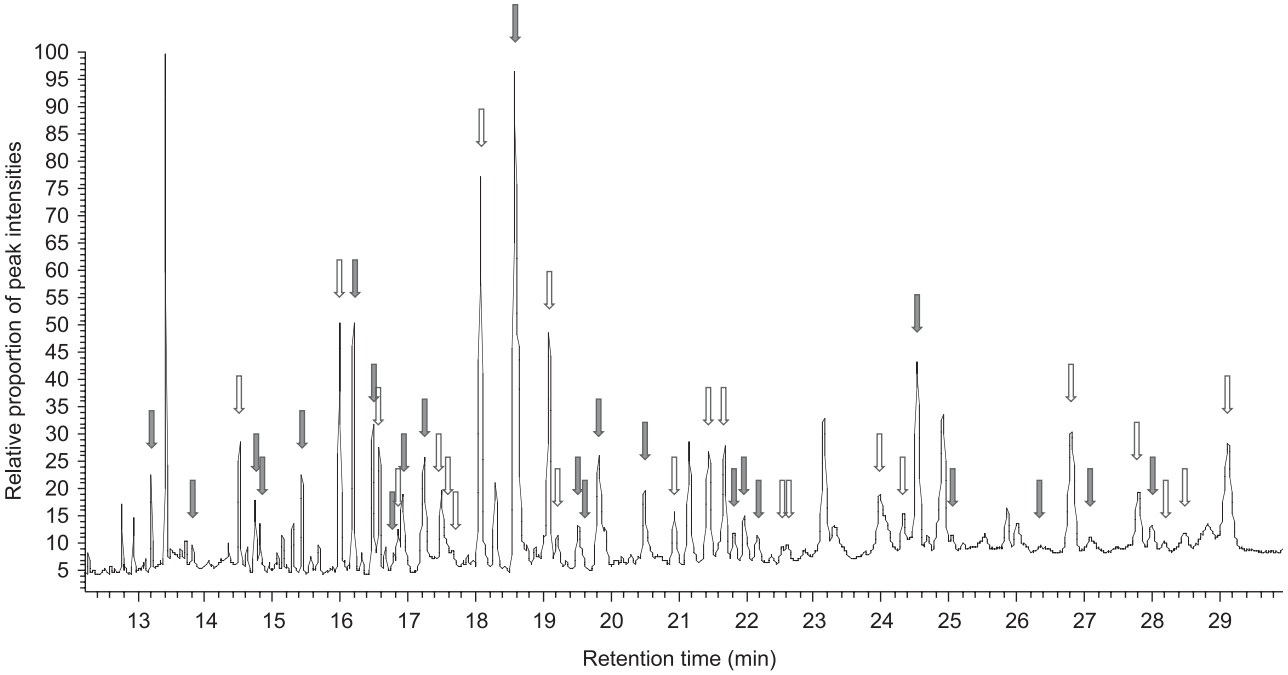

Fig. 2 Cuticular profile of *Pheidole megacephala* workers. Cuticular lipids yield 47 peaks; they are indicated by an arrow (two cuticular lipids are outside the retention time shown here). The 23 lipids likely involved in nestmate recognition are pointed by plain arrows.

moderate but significant genetic differentiation among sites ($F_{\text{sites-Australia}} = 0.140$; 95% CI = 0.047–0.212). This result was confirmed by significant genotypic differentiation between pairs of sites for all pairwise comparisons (all $P < 0.001$), and by a positive relatedness among nestmate workers (mean ± SE = 0.174 ± 0.086; one tailed *t*-test, $t = 6.681$; $P < 0.001$) when all nests of the four collection sites were used as a reference (Table 4). Correlation analysis showed that the geographical and genetic distances between the sites were not significantly associated (Mantel test, $r_{\text{geo,gen}}$: $P = 0.836$), as expected under a scenario of jump dispersal.

Gene flow, assessed using the private alleles estimates of *Nm*, was consistent with the F_{ST} estimates and genotypic differentiation observed between pairs of sites. Howard Springs Natural Park (HS) showed consistently low gene flow with other regions with *Nm* varying between 0.98 and 7.96, whereas Whittle Park (WP), Surfers Paradise (SP) and Chuwar (CH) showed higher gene flow ranging from 9.76 to 28.85.

Despite the low genetic diversity in terms of allelic richness and levels of heterozygosity, no bottleneck signature (i.e. significant heterozygosity excess than expected from the observed allele number) was detected in the four sites under study when the set of all polymorphic loci were analysed (one-tailed Wilcoxon test for *H* excess, infinite allele mutation, all $P > 0.191$; two-phased mutation model, all $P > 0.422$). Moreover, none of the allele distributions showed a departure from the standard L-shape in the mode-shift test.

The average genetic relatedness among worker nestmates did not differ from zero when nests of the same site were taken as the reference population (one tailed *t*-test, all $P > 0.232$; Table 4). As a result, the estimated number of queens contributing to reproduction (estimated from worker relatedness) was extremely high. Genotyping of seminal fluid contained in spermatheca of 83 queens indicated that 72 (87%) were singly mated and 11 (13%) were double-mated. The probability of nondetection due to two males bearing the same alleles at all loci was reasonably low and varied from 0.02 to 0.06.

Patterns of variation of the cuticular profile

Cuticular lipids yielded 47 peaks ranging in size from C_{19} to C_{40} (Fig. 2). Electronic and chemical ionizations revealed the presence of lipids including linear alkanes, alkenes and methyl-branched alkanes. Proportion of compounds in the cuticular profiles (*I*) differed significantly between the four sites (Kruskal–Wallis test, $P = 0.009$; Table 1), with a significantly higher diversity in WP than in HS and SP (Dunn's procedures, $P < 0.05$ for both comparisons). Within each site, cuticular profiles were very similar among nests (mean Euclidean distance ± SE = 0.085 ± 0.004; range: 0.059–0.135; Table 1). Within-site cuticular profiles variation differed significantly between the four sites under study (Kruskal–Wallis test, $P < 0.001$; Table 1). *A posteriori* pairwise comparisons showed that variation was identical for HS and WP (the two sites collected in the

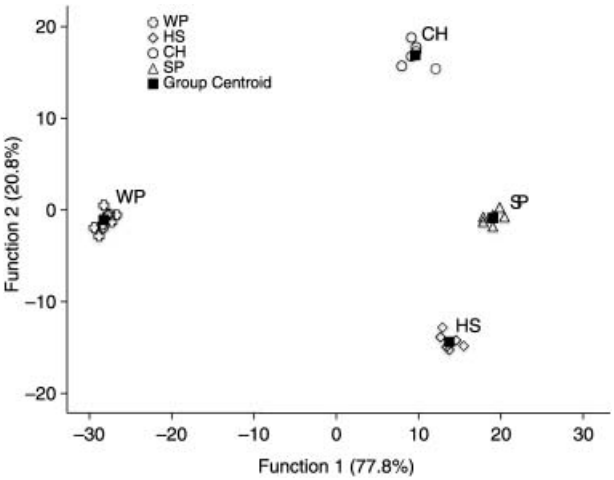

Fig. 3 Chemical differentiation among the four sites sampled (Whittle Park, WP; Howard Springs Nature Reserve, HS; Surfers Paradise, SP; and Chuwar, CH), as illustrated by a factor map of the two first axes of the canonical discriminant analysis on relative proportions of cuticular lipids. Functions one and two account for 98.7% (77.8% and 20.8%, respectively) of the total variability among sites.

Northern Territory) and for SP and CH (the two sites collected in Queensland) (Dunn's procedures, all $P > 0.05$), but lower in HS and WP compared to SP and CH (all $P < 0.01$) (Table 1).

In agreement with these results, a canonical discriminant function (CDF) analysis performed on relative amounts of the 47 chemical peaks identified discriminate the four sites significantly (Fig. 3). Among these, the relative proportion of 24 compounds varied significantly between the four study populations (Fig. 2). The two first principal components account for 98.6% of the overall variance among groups; the canonical correlation values associated to these two CDFs are, respectively, Cc1 = 0.999 and Cc2 = 0.996. 100% of original grouped cases were correctly classified; overall, 22.2% of cross-validated samples were assigned correctly to their site by the two CDFs (WP, 44.4%; HS, 0%; SP, 16.7%; CH, 16.7%). The results of AMOVA show that 30% of the total variation was attributable to differences between sites and 70% was accounted for by the differences among nests within sampling sites (variance components were significant: random permutation test, $P = 0.010$). The peaceful behaviour observed during encounters between ants from the four different sites (see above) indicates that the 24 heterogeneous chemicals can be eliminated as likely nestmate recognition cues.

Within the four sites, there was no significant correlation between divergence in chemical compounds (i.e. Euclidean distances between pairs of nests) and geographical distance among nests (Mantel tests, all $P > 0.138$).

Association between variation of the cuticular profile and genetic and spatial data

Correlation tests showed that the chemical distances between populations were not significantly correlated with geographical (Mantel test, $r_{geo,chem}$: $P = 0.180$) or genetic distances (Mantel test, $r_{gen,chem}$: $P = 0.120$).

Discussion

Our results show that the big-headed ant *Pheidole megacephala* forms large unicolonial populations with very high numbers of reproductive queens in northern/eastern Australia. Workers are not aggressive towards conspecifics from different nests. This lack of aggression occurs even at large geographical scales (up to 3000 km) and between populations encompassing a wide range of environmental conditions. By contrast, aggression is invariably extremely high between heterospecific workers, generally leading to the death of one protagonist. In agreement with these results, within the four study populations we found no genetic differentiation between nests and a high similarity in the cuticular profiles. Importantly, the peaceful behaviour of workers also occurs despite genetic and chemical differentiations between populations. Our data reveal that the weak genetic differentiation between populations primarily stems from the presence of private alleles in three of the four study sites. The total genetic diversity is low and hierarchical gene diversity analysis indicated that most genetic variance was attributed to within sites differences. Chemical analyses also show that the four populations differ in the relative proportion of the cuticular compounds identified. Among these, we found several double-bonded and methylated hydrocarbons, two classes of compounds reportedly involved in nestmate recognition (Vander Meer *et al*. 1998; Howard & Blomquist 2005). Altogether, these results suggest that all four populations studied belong to the same supercolony and arise from independent introductions from the same population established in the Australian continent.

Unicoloniality has been reported in other invasive ants such as *Linepithema humile* (Newell & Barber 1913; Markin 1970), *Anoplolepis gracilipes* (Greenslade 1972; Haines & Haines 1978), *Wasmannia auropunctata* (Clark *et al*. 1982), polygyne forms of *Solenopsis invicta* (Porter *et al*. 1988; Vander Meer *et al*. 1990) or *Lasius neglectus* (Boomsma *et al*. 1990). However, the extent of the networks of nests varies according to species and populations. A single 450-km-long supercolony of the clonal ant *W. auropunctata* inhabits New Caledonia (Fournier *et al*. 2005a; Foucaud *et al*. 2006); two supercolonies of the Argentine ant *L. humile* coexist in Europe, the main one ranging from Italy to the Spanish Atlantic coast (Giraud *et al*. 2002); and a mosaic of colonies of the yellow crazy ant *A. gracilipes*, ranging from small isolated nests to

supercolonies, splits up different regions in Borneo (Drescher *et al*. 2007). Variations in the distribution of supercolonies, as well as the relations between them, reflect the invasion history of the species. For instance, the New Caledonian supercolony of the little fire ant most likely arose from a single introduction of only one male and one female genotype, of which daughter populations spread through serial introduction events (Foucaud *et al*. 2006). In contrast, the coexistence of two mutually aggressive supercolonies of the Argentine ant in Europe is consistent with two independent introduction events (Jaquiéry *et al*. 2005). The main supercolony of the Argentine ant in Europe could have resulted from a single founding event involving as few as 6–13 unrelated queens (Giraud *et al*. 2002). In *P. megacephala*, we estimated that at least two mated queens, i.e. six haploid genomes, must have been introduced in Australia to account for the observed diversity. The number of introduced individuals influences directly the genetic diversity of the newly established population which, in turn, affects the diversity of the recognition cues encoded in the ants' cuticular profiles (Beye *et al*. 1998; Stuart & Herbers 2000; Tsutsui *et al*. 2000). Our data did not pinpoint an association between the genetic and chemical distances. The large variability in environmental conditions between the study sites may scramble the genetic part of the cuticular hydrocarbon profiles (see also Ugelvig *et al*. 2008). Under these conditions, the significant but low genetic differentiation between populations could not be sufficient to induce aggressive interactions between non-nestmate workers. Consistent with this result, workers are nonaggressive despite a weak average pairwise allele-shared distance value of 27%. This contrasts with those reported by Tsutsui *et al*. (2000) in *L. humile,* where workers from nests sharing less that 60% alleles display moderate to high intraspecific aggression, whereas those sharing more than 75% alleles were nonaggressive. However, a significantly different pattern was reported in this species: colony pairs were almost aggressive despite high allele sharing (70%) and peaceful despite relatively low allele sharing (50%) (Buczkowski *et al*. 2004). Altogether, these results confirm no clear relationship between intraspecific aggression and genetic similarity when using neutral microsatellite markers (Buczkowski *et al*. 2004). Finally, because workers are peaceful irrespective of the environmental conditions and genetic relationships, our study gives no evidence of an intraspecific recognition mechanism in *P. megacephala*. However, one may not exclude that intraspecific recognition occurs in some populations, but it is not expressed in the populations under study.

Reduction in allelic diversity and pronounced bottleneck are genetic consequences accompanying introduction of invasive species in new environments (Wares *et al*. 2005; Dlugosch & Parker 2008). Our within- and between-population genetic analyses are only partly consistent with these expectations: we found a relatively low genetic diversity, but no heterozygosity excess. The lack of heterozygosity excess indicates no sign of founder effect. However, one may not exclude that populations recovered from such an effect, what may happen when the bottleneck is brief or recent, when the invading propagule is large and/or if population growth is rapid (Wares *et al*. 2005; Dlugosch & Parker 2008). *Pheidole megacephala* fulfils at least this latter condition. Many queens head colonies of the species and reproduction takes place all the year round. In addition, queens are highly fertile and can successfully revive an experimental nest even if accompanied by only a few workers (Chang 1985). An alternative explanation is that the nondetection of a bottleneck signature stems from an artefact of the analysis, since all populations have few polymorphic loci, reducing the detection power of the heterozygosity excess method applied in this study (Piry *et al*. 1999). In addition, spatial population structure may bias inferences of demographic and evolutionary processes (Leblois *et al*. 2006). Yet, our study shows that the reduction in genetic diversity in Australian populations of *P. megacephala* is well marked. Comparison with native African colonies indeed reveals two-times higher allelic richness in Africa than Australia (i.e. a proportional loss of allelic diversity of 0.526 (Fournier *et al*. 2008). Such a reduction in genetic diversity in introduced populations, as compared to native populations, has been reported in other invasive ant species. For instance, *Solenopsis invicta, Linepithema humile* and *Wasmannia auropunctata* reportedly experience a proportional loss of allelic diversity of 0.183, 0.492, and 0.718, respectively (Ross *et al*. 1993; Tsutsui *et al*. 2000; Fournier *et al*. 2005b).

Reduced genetic diversity may have two detrimental consequences (Allendorf & Lundquist 2003). First, it usually leads to inbreeding depression and limits population growth. In Hymenoptera, a direct cost of inbreeding is the production of homozygous diploid males, which are generally sterile. Such males arise because of the complementary sex-determining system (*csd*) of Hymenoptera, whereby heterozygous individuals at the sex locus develop into females, whereas hemizygous and homozygous individuals develop into males (Crozier 1971). However, the male-haploid sex determining system allows rapid purge of deleterious alleles and, hence, forsake below-average genotypes (Crozier 1970; Schmid-Hempel *et al*. 2007; but see Zayed & Packer 2005). Second, low genetic diversity limits evolutionary opportunities of populations to evolve. But a minimum level of genetic diversity could be maintained thanks to multiple introductions and the concomitant interbreeding between individuals from introduced populations. This process creates new exotic variants and contributes to the evolutionary success of invading species (Suarez & Tsutsui 2008). It has been recently documented as providing most of the evolutionary potential of an invasive

population of the snail *Melanoides tuberculata* (Facon *et al*. 2008). Our results indicate a significant level of inbreeding in the populations studied. A positive inbreeding coefficient may result either from a Wahlund effect (i.e. a subpopulation structure) or from true inbreeding due to matings between close relatives, as pointed out by the positive relatedness between the queens and their mates. The remarkable success of *P. megacephala* in its introduced range indicates that the species is not endangered by the potential effects of low genetic diversity and associated inbreeding.

Two main hypotheses, not mutually exclusive, have been proposed to account for the evolution of unicoloniality. First, unicoloniality may have resulted from a reduction of within-colony relatedness. Polygyny tends to counter-select the set-up of kin discrimination mechanism and to favour acceptance of unrelated individuals and workers exchanges (Holzer *et al*. 2006). Our results indeed show that colonies of *P. megacephala* are headed by a high number of queens. Second, mechanisms leading to a cessation of aggressiveness could be based on a reduction of self-nonself recognition. These mechanisms may include: (i) the sharing of a collective gestalt odour (acquired when individuals mix together in winter nests; Elias *et al*. 2005) instead of a colony-specific odour; or (ii) the loss of genetic diversity at recognition loci following genetic bottlenecks (Holway *et al*. 1998; Tsutsui *et al*. 2000; but see Pedersen *et al*. 2006) or genetic cleansing (Giraud *et al*. 2002). The occurrence of differences in chemical profiles between populations does not plead in favour of the collective gestalt odour hypothesis. Rather, our data show a limited genetic diversity in Australian populations, consistent with a founder effect. The lack of aggressive behaviour in the big-headed ant, despite a low but significant chemical differentiation between the study sites, could also result from the threshold of self-nonself recognition being not reached or from the absence of discrimination behaviour (failing to respond to chemical differences). On the other hand, Steiner *et al*. (2007) recently proposed that the first stage of the formation of a supercolony may be the abandonment of aggression between members belonging to different colonies, despite the maintenance of self-nonself discrimination. Consistent with their hypothesis, the authors showed that workers of the noninvasive, monogynous ant *Lasius austriacus* behave peacefully towards conspecifics from other colonies, despite high within-colony relatedness and self-nonself discrimination. Agonistic behaviour towards conspecifics in populations of *P. megacephala* could be counter-selected to favour the conquest of new territories and the search of food, and in turn, the saturation of the environment. Whether *P. megacephala* has lost its aggressiveness and forms supercolonies in its native range awaits further genetic, chemical and behavioural analyses.

The role of human activities is central in invasion processes, as invasions generally occur through human-trade, as well as from and to anthropogenically disturbed areas. Like other invasive ants (Tsutsui & Case 2001; Ugelvig *et al*. 2008), jump dispersal via human mediated transport is very likely in *P. megacephala* as no correlation between geographical and genetic distances among sites was detected. On the other hand, human modifications to the environment may favour the introduction of an alien species by modifying biotic and abiotic environmental conditions (Davis & Pelsor 2001; Keane & Crawley 2002; Holway & Suarez 2006) (*Solenopsis invicta*, Tschinkel 1988; *Anoplolepis gracilipes*, O'Dowd *et al*. 1999; *Linepithema humile*, Tsutsui & Case 2001; Holway *et al*. 2002b; Carpintero *et al*. 2004; *Wasmannia auropunctata*, Foucaud *et al*. unpublished data). The present work emphasizes the close relationship between the human presence and the invasion of *P. megacephala* in northern/eastern Australia. High gene flow arises between the three human-modified environments (Whittle Park, Surfers Paradise and Chuwar), whereas it is reduced with the wildlife habitat (Howard Springs Nature Reserve).

In conclusion, this study shows that encounters between two *P. megacephala* workers never yield aggression, irrespective of the chemical, genetic or geographical distances between the actors. This set of characters may have been pivotal in the ecological success of the species. If the lack of intraspecific aggressiveness is a recurrent factor present to most ant invasions, the underlying mechanisms leading to this 'entente cordiale' are nevertheless multifarious. Beside the lack of intraspecific aggressiveness, our study suggests that other factors are involved in the ecological success of this species: it can live in the vicinity of humans, it is very aggressive towards other species and does not seem to suffer from a reduction in genetic diversity. These life-history traits make *P. megacephala* a redoubtable species, able to invade human-modified environments and, once introduced, well able to acclimatize to varying conditions in the new range.

Acknowledgements

We are grateful to A. Andersen and B. Hoffmann (CSIRO, Darwin) and S. Moloney (Fire Ant Control Centre, Oxley) for assistance during field trips, L. Grumiau, O. Hardy and N.J. Vereecken for their technical advice, and R. H. Crozier, L. Keller, F. Rousset, A. Suarez, J. Foucaud and four anonymous referees for their helpful comments on the manuscript. This work was funded by the Belgian National Fund for Scientific Research through personal (postdoctoral researcher fellowship to DF and research director to SA) and various FRFC grant supports, and the Université Libre de Bruxelles (ULB).

References

Allendorf FW, Lundquist LL (2003) Introduction: population biology, evolution, and control of invasive species. *Conservation Biology*, **17**, 24–30.

Andersen AN, Woinarski JCZ, Hoffmann BD (2004) Biogeography of the ant fauna of the Tiwi Islands, in northern Australia's monsoonal tropics. *Australian Journal of Zoology*, **52**, 97–110.

Aron S (2001) Reproductive strategy: an essential component in the success of incipient colonies of the invasive Argentine ant. *Insectes Sociaux*, **48**, 25–27.

Barton NH, Slatkin M (1986) A quasi-equilibrium theory of the distribution of rare alleles in a subdivided population. *Heredity*, **56**, 409–416.

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.

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

Buczkowski G, Vargo EL, Silverman J (2004) The diminutive supercolony: the Argentine ants of the southeastern United States. *Molecular Ecology*, **13**, 2235–2242.

Carpintero S, Reyes-López J, Arias de Reyna L (2004) Impact of human dwellings on the distribution of the exotic Argentine ant: a case study in the Doñana National Park, Spain. *Biological Conservation*, **115**, 279–289.

Chang VCS (1985) Colony revival, and notes on rearing and life history of the big-headed ant. *Proceedings of the Hawaiian Entomological Society*, **25**, 53–58.

Clark DB, Guayasamin C, Pazmino O, Donoso C, Paez de Villacis Y (1982) The tramp ant *Wasmannia auropunctata*: autecology and effects on ant diversity and distribution on Santa Cruz Island, Galapagos. *Biotropica*, **14**, 196–207.

Cornuet J-M, Luikart G (1996) Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. *Genetics*, **144**, 2001–2014.

Crozier RH (1970) On the potential for genetic variability in haplo-diploidy. *Genetica*, **41**, 551–556.

Crozier RH (1971) Heterozygosity and sex determination in haplo-diploidy. *American Naturalist*, **105**, 399–412.

Davis MA, Pelsor M (2001) Experimental support for a resource-based mechanistic model of invasibility. *Ecology Letters*, **4**, 421–428.

Dlugosch KM, Parker IM (2008) Founding events in species invasions: genetic variation, adaptive evolution, and the role of multiple introductions. *Molecular Ecology*, **17**, 431–449.

Drescher J, Blüthgen N, Feldhaar H (2007) Population structure and intraspecific aggression in the invasive ant species *Anoplolepis gracilipes*. Malaysian Borneo. *Molecular Ecology*, **16**, 1453–1465.

Elias M, Rosengren R, Sundström L (2005) Seasonal polydomy and unicoloniality in a polygynous population of the red wood ant *Formica truncorum*. *Behavioral Ecology and Sociobiology*, **57**, 339–349.

Facon B, Genton BJ, Shykoff J *et al*. (2006) A general eco-evolutionary framework for understanding bioinvasions. *Trends in Ecology and Evolution*, **21**, 130–135.

Facon B, Pointier J-P, Jarne P, Sarda V, David P (2008) High genetic variance in life-history strategies within invasive populations by way of multiple introductions. *Current Biology*, **18**, 363–367.

Foucaud J, Jourdan H, Le Breton J *et al*. (2006) Rare sexual reproduction events in the clonal reproduction system of introduced populations of the little fire ant. *Evolution*, **60**, 1646–1657.

Fournier D, Dubois D, Aron S (2008) Isolation and characterization of microsatellite loci from the invasive ant *Pheidole megacephala*. *Molecular Ecology Resources*, **8**, 919–922.

Fournier D, Estoup A, Orivel J *et al*. (2005a) Clonal reproduction by males and females in the little fire ant. *Nature*, **435**, 1230–1234.

Fournier D, Foucaud J, Loiseau A *et al*. (2005b) Characterization and PCR multiplexing of polymorphic microsatellite loci for the invasive ant *Wasmannia auropunctata*. *Molecular Ecology Notes*, **5**, 239–242.

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

Goudet J (2001) *Fstat, a Program to Estimate and Test Gene Diversities and Fixation Indices*. Institute of Ecology. University of Lausanne, Switzerland.

Greenslade PJM (1972) Comparative ecology of four tropical ant species. *Journal of Experimental Biology*, **19**, 195–212.

Haines IH, Haines JB (1978) Colony structure, seasonality and food requirements of the crazy ant, *Anoplolepis longipes* (Jerd.), in the Seychelles. *Ecological Entomology*, **3**, 109–118.

Hee JJ, Holway DA, Suarez AV, Case TJ (2000) Role of propagule size in the success of incipient colonies of the invasive Argentine ant. *Conservation Biology*, **14**, 559–563.

Hoffmann BD, Parr CL (2008) An invasion revisited: the African big-headed ant (*Pheidole megacephala*) in northern Australia. *Biological Invasions*, **10**, 1171–1181.

Hoffmann BD, Andersen AN, Hill GJE (1999) Impact of an introduced ant on native rain forest invertebrates: *Pheidole megacephala* in monsoonal Australia. *Oecologia*, **120**, 595–604.

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

Holway DA, Suarez AV (2006) Homogenization of ant communities in mediterranean California: the effects of urbanization and invasion. *Biological Conservation*, **127**, 319–326.

Holway DA, Suarez AV, Case TJ (1998) Loss of intraspecific aggression in the success of a widespread invasive social insect. *Science*, **282**, 949–952.

Holway DA, Suarez AV, Case TJ (2002b) Role of abiotic factors in governing susceptibility to invasion: a test with the Argentine ant. *Ecology*, **83**, 1610–1619.

Holzer B, Chapuisat M, Kremer N, Finet C, Keller L (2006) Unicoloniality, recognition and genetic differentiation in a native *Formica* ant. *Journal of Evolutionary Biology*, **19**, 2031–2039.

Howard RW, Blomquist GJ (2005) Ecological, behavioral, and biochemical aspects of insect hydrocarbons. *Annual Review of Entomology*, **50**, 371–393.

Jaquiéry J, Vogel V, Keller L (2005) Multilevel genetic analyses of two European supercolonies of the Argentine ant, *Linepithema humile*. *Molecular Ecology*, **14**, 589–598.

Keane RM, Crawley MJ (2002) Exotic plant invasions and the enemy release hypothesis. *Trends in Ecology and Evolution*, **17**, 164–170.

Leblois R, Estoup A, Streiff R (2006) Genetics of recent habitat contraction and reduction in population size: does isolation by distance matter? *Molecular Ecology*, **15**, 3601–3615.

Lewis PO, Zaykin D (1999) *Genetic Data Analysis, a computer program for analysis of allelic data*. University of Conneticut, CT.

Lockwood J, Hoopes M, Marchetti M (2006) *Invasion Ecology*. Blackwell Scientific Press, Oxford.

Lodge DM (1993) Biological invasions: lessons for ecology. *Trends in Ecology and Evolution*, **8**, 133–137.

Lowe S, Browne M, Boudjelas S (2001) *100 of the World's Worst Invasive Alien Species. A selection from the Global Invasive Species Database*, p. 11. IUCN-ISSG, Auckland.

Mack RN, Simberloff D, Lonsdale WM *et al*. (2000) Biotic invasions: causes, epidemiology, global consequences, and control. *Ecological Applications*, **10**, 689–710.

Mantel N (1967) The detection of disease clustering and a generalized regression approach. *Cancer Research*, **27**, 209–220.

Markin GP (1970) Foraging behavior of the Argentine ant in a California citrus grove. *Journal of Economic Entomology*, **63**, 740–744.

McGlynn TP (1999) The worldwide transfer of ants: geographical distribution and ecological invasions. *Journal of Biogeography*, **26**, 535–548.

Michalakis Y, Excoffier L (1996) A generic estimation of population subdivision using distances between alleles with special reference for microsatellite loci. *Genetics*, **142**, 1061–1064.

Moller H (1996) Lessons for invasion theory from social insects. *Biological Conservation*, **78**, 125–142.

Mooney HA, Cleland EE (2001) The evolutionary impact of invasive species. *Proceedings of the National Academy of Sciences, USA*, **98**, 5446–5451.

Newell W, Barber TC (1913) *The Argentine Ant*, p. 98 U S Department of Agriculture — Bureau of Entomology Bulletin No. 122, Washington, D.C.

O'Dowd DJ, Green PT, Lake PS (1999) *Status, Impact, and Recommendations for Research and Management of Exotic Invasive Ants in Christmas Island National Park*. Centre for the Analysis and Management of Biological Invasions. Monash University, Clayton, Victoria.

Pamilo P (1993) Polyandry and allele frequency differences between the sexes in the ant *Formica aquilonia*. *Heredity*, **70**, 472–480.

Pascal M, Lorvelec O, Vigne J-D (2005) Holocene turnover of the French vertebrate fauna. *Biological Invasions*, **7**, 99–106.

Passera L (1994) Characteristics of tramp species. In: *Exotic Ants: Biology, Impact, and Control of Introduced Species* (ed. Williams DF), pp. 23–43. Westview Press, Boulder, CO.

Peakall R, Smouse PE (2006) GenAlEx 6: genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes*, **6**, 288–295.

Pedersen JS, Krieger MJB, Vogel V, Giraud T, Keller L (2006) Native supercolonies of unrelated individuals in the invasive Argentine ant. *Evolution*, **60**, 782–791.

Piry S, Luikart G, Cornuet J-M (1999) Bottleneck: a computer program for detecting recent reductions in the effective population size using allele frequency data. *Journal of Heredity*, **90**, 502–503.

Porter SD, Van Eimeren B, Gilbert LE (1988) Invasion of red imported fire ants (Hymenoptera: Formicidae): microgeography of competitive replacement. *Annals of the Entomological Society of America*, **81**, 913–918.

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 (1993) The breeding system of the fire ant *Solenopsis invicta*: effects on colony genetic structure. *American Naturalist*, **141**, 554–576.

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

Sakai AK, Allendorf FW, Holt JS *et al*. (2001) The population biology of invasive species. *Annual Review of Ecology and Systematics*, **32**, 305–332.

Sala OE, Chapin FS, Armesto JJ *et al*. (2000) Global biodiversity scenarios for the year 2100. *Science*, **287**, 1770–1774.

Sambrook J, Russell D (2001) *Molecular Cloning: a Laboratory Manual*, 3rd edn. Cold Spring. Harbor Laboratory Press, NY.

Sanders NJ, Gotelli NJ, Heller NE (2003) Community disassembly by an invasive species. *Proceedings of the National Academy of Sciences, USA*, **100**, 2474–2477.

Schmid-Hempel P, Schmid-Hempel R, Brunner PC, Seeman OD, Allen GR (2007) Invasion success of the bumblebee, *Bombus terrestris*, despite a drastic genetic bottleneck. *Heredity*, **99**, 414–422.

Seppä P (1994) Sociogenetic organization of the ants *Myrmica ruginodis* and *Myrmica lobicornis*: number, relatedness and longevity of reproducing individuals. *Journal of Evolutionary Biology*, **7**, 71–95.

Siegel S, Castellan NJ Jr (1988) *Nonparametric Statistics for the Behavioral Sciences*, 2nd edn. McGraw-Hill Company, Singapore.

Slatkin M (1985) Gene flow in natural populations. *Annual Review of Ecology and Systematics*, **16**, 393–430.

Sokal RR, Rohlf FJ (1995) *Biometry: the Principles and Practice of Statistics in Biology Research*. W.H. Freeman and Company, New York.

Steiner FM, Schlick-Steiner BC, Moder K *et al*. (2007) Abandoning aggression but maintaining self-nonself discrimination as a first stage in ant supercolony formation. *Current Biology*, **17**, 1903–1907.

Stuart RJ, Herbers JM (2000) Nest mate recognition in ants with complex colonies: within- and between-population variation. *Behavioral Ecology*, **11**, 676–685.

Suarez AV, Tsutsui ND (2008) The evolutionary consequences of biological invasions. *Molecular Ecology*, **17**, 351–360.

Suarez AV, Tsutsui ND, Holway DA, Case TJ (1999) Behavioral and genetic differentiation between native and introduced populations of the Argentine ant. *Biological Invasions*, **1**, 43–53.

Suarez AV, Holway DA, Tsutsui ND (2008) Genetics and behavior of a colonizing species — The invasive Argentine ant. *American Naturalist*, **172**, S72–S84.

Tschinkel WR (1988) Distribution of the fire ants *Solenopsis invicta* and *S. geminata* (Hymenoptera: Formicidae) in northern Florida in relation to habitat and disturbance. *Annals of the Entomological Society of America*, **81**, 76–81.

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.

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, USA*, **97**, 5948–5953.

Ugelvig LV, Drijfhout FP, Kronauer DJC *et al*. (2008) The introduction history of invasive garden ants in Europe: integrating genetic, chemical and behavioural approaches. *BMC Biology*, **6**, 11.

Vander Meer RK, Breed MD, Espelie KE, Winston ML (1998) *Pheromone Communication in Social Insects: Ants, Wasps, Bees, and Termites*, p. 368. Westview Press, Boulder, CO.

Vander Meer RK, Obin MS, Morel L (1990) Nestmate recognition in fire ants: monogyne and polygyne populations. In: *Applied Myrmecology: a World Perspective* (eds Vander Meer RK, Jaffe K, Cedeno A), pp. 322–328. Westview Press, Boulder, CO.

Vargo EL, Fletcher DJC (1989) On the relationship between queen number and fecundity in polygyne colonies of the fire ant *Solenopsis invicta*. *Physiological Entomology*, **14**, 223–232.

Vitousek PM, D'antonio CM, Loope LL, Rejmánek M, Westbrooks R (1997) Introduced species: a significant component of human-caused global change. *New Zealand Journal of Ecology*, **21**, 1–16.

Wares JP, Hughes AR, Grosberg RK (2005) Mechanisms that drive evolutionary change — Insights from species introductions and invasions. In: *Species Invasions: Insights Into Ecology, Evolution, and Biogeography* (eds Sax DF, Stachowicz JJ, Gaines SD), pp. 229–257. Sinauer Associates, Sunderland, MA.

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

Wetterer JK (2007) Biology and impacts of Pacific Island invasive species. 3. The African big-headed ant, *Pheidole megacephala* (Hymenoptera: Formicidae). *Pacific Science*, **61**, 437–456.

Wilcove DS, Rothstein D, Dubow J, Phillips A, Losos E (1998) Quantifying threats to imperiled species in the United States. *BioScience*, **48**, 607–615.

Williams DF (1994) *Exotic Ants: Biology, Impact, and Control of Introduced Species*, p. 332. Westview Press, Boulder, CO.

Williamson M (1996) *Biological Invasions*. Chapman & Hall, London.

Wilson EO, Taylor RW (1967) The ants of Polynesia (Hymenoptera: Formicidae). *Pacific Insects Monograph*, **14**, 1–109.

Zayed A, Packer L (2005) Complementary sex determination substantially increases extinction proneness of haplodiploid populations. *Proceedings of the National Academy of Sciences, USA*, **102**, 10742–10746.

Denis Fournier is a Research Associate at the Belgian National Science Foundation (F.R.S.-F.N.R.S) and works on mating systems and population genetics of ants. Jean-Christophe de Biseau is an Associated Professor at the University of Brussels and works on chemical ecology of solitary and social insects. Serge Aron is a Research Director at the Belgian National Science Foundation (F.R.S.-F.N.R.S), heads the laboratory of Behavioural and Evolutionary Ecology at the University of Brussels, and works on kin conflicts and sex ratio determination in various ant species.
