Reproductive system, social organization, human disturbance and ecological dominance in native populations of the little fire ant, *Wasmannia auropunctata*

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Abstract

The invasive ant species *Wasmannia auropunctata* displays both ecologically dominant and non-dominant populations within its native range. Three factors could theoretically explain the ecological dominance of some native populations of *W. auropunctata*: (i) its clonal reproductive system, through demographic and/or adaptive advantages; (ii) its unicolonial social organization, through lower intraspecific and efficient interspecific competition; (iii) the human disturbance of its native range, through the modification of biotic and abiotic environmental conditions. We used microsatellite markers and behavioural tests to uncover the reproductive modes and social organization of dominant and non-dominant native populations in natural and human-modified habitats. Microsatellite and mtDNA data indicated that dominant and non-dominant native populations (supercolonies as determined by aggression tests) of *W. auropunctata* did not belong to different evolutionary units. We found that the reproductive system and the social organization are neither necessary nor sufficient to explain *W. auropunctata* ecological dominance. Dominance rather seems to be set off by unknown ecological factors altered by human activities, as all dominant populations were recorded in human-modified habitats. The clonal reproductive system found in some populations of *W. auropunctata* may however indirectly contribute to its ecological dominance by allowing the species to expand its environmental niche, through the fixation over time of specific combinations of divergent male and female genotypes. Unicoloniality may rather promote the range expansion of already dominant populations than actually trigger ecological dominance. The *W. auropunctata* model illustrates the strong impact of human disturbance on species' ecological features and the adaptive potential of clonal reproductive systems.

Keywords: biological invasion, clonality, human disturbance, parthenogenesis, social organization, *Wasmannia auropunctata*

Received 3 June 2009; revision received 12 October 2009; accepted 19 October 2009

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Introduction

Biological invasions are a major component of the current global change, and can have huge detrimental effects on public health, agriculture and biodiversity (Sax *et al.* 2005). There has thus been an increasing interest among biologists in understanding why some species become successful invaders (Sax *et al.* 2005; Cadotte *et al.* 2006), with a recent and important focus on the biology of invasive species in their native range (Bossdorf *et al.* 2005; Pedersen *et al.* 2006; Vogel *et al.* 2009). Paradoxically, there is still debate on the definition of an 'invasive' species in the literature (Valéry *et al.* 2008). Current conceptual frameworks basically differ in the relative importance given to three main components of biological invasions: 'range extension' (Facon *et al.* 2006), 'high local abundance' (Suarez *et al.* 1999) and 'disruption of ecosystem function' (Mooney & Hobbs 2000). High local abundance and pre-eminence in ecosystem function are distinctive of dominant species or populations. Understanding the transition from non-dominant to dominant populations hence sheds light on one key component of biological invasions. Our study of dominant and non-dominant populations of an invasive ant within its native range is designed to explore this transition.

The little fire ant, *Wasmannia auropunctata*, is considered to be one of the most successful invasive species (Lowe *et al.* 2000), now present in nearly all tropical regions (Wetterer & Porter 2003). Within its native range (i.e. tropical Central and South America), *Wasmannia auropunctata* displays both non-dominant populations (Levings & Franks 1982; Tennant 1994), and high-density populations that severely affect the functioning of some ecosystems (Delabie *et al.* 1994; Majer *et al.* 1994; Orivel *et al.* 2009).

Three characteristics seem potentially relevant in explaining the ecological dominance of some *W. auropunctata* populations within its native range. First, native populations have been shown to display either a haplodiploid reproductive system that is traditional in Hymenopterans, or an unusual clonal reproductive system, where female queens are parthenogens, males are produced clonally (through queen eggs), and workers are produced sexually (see Supplementary Fig. S1; Fournier *et al.* 2005; Foucaud *et al.* 2007). It has been commonly argued that a clonal reproductive system could help species to become numerically dominant, by providing a clear demographic advantage over sexual species (i.e. the 'two-fold cost of sex'; Maynard Smith 1978). However, this demographic advantage may not hold for *W. auropunctata*, because in ants only one inseminated queen is needed to establish a new population, and workers are still sexually produced in clonal populations (Fournier *et al.* 2005). Nevertheless, the clonal reproductive system of *W. auropunctata* has the potential to fix some favourable combinations of genes over time, while sexual reproduction cannot. A striking example of clonal advantage in an ecological dominance context via the conservation of an adaptive gene combination is the recent takeover of African lakes by a single water flea clonal genotype (Mergeay *et al.* 2006). In the case of *W. auropunctata*, mating pairs of clonal males and clonal females can be maintained over time, and some could thrive, if their sexually produced worker offspring perform particularly well in their environment. This clonal reproductive system may therefore, at least indirectly, explain the ecological dominance of some *W. auropunctata* populations through adaptive advantages, if not through direct demographic advantages.

Second, it has been shown that an invasive population of the introduced range of *W. auropunctata* is organized in a large cooperative network of nests showing no intraspecific aggression, in which workers, reproductives and brood can mix freely (Le Breton *et al.* 2004). This social organization, termed unicoloniality (Hölldobler & Wilson 1977), is the opposite of multi-coloniality, displayed by most ant species, in which colonies are genetically differentiated and mutually aggressive. A large proportion of invasive ant species have been shown to be unicolonial (Holway *et al.* 2002a), and seem to benefit from unicoloniality to attain high worker densities (Abbott 2005). Currently, the social organization of both dominant and non-dominant native populations of *W. auropunctata* is unknown.

Third, due to the increase of human activities in the native range of the species, *W. auropunctata* populations are increasingly found in contact with human-modified habitats. Human disturbance, through the use of the land for agricultural or urban purposes, has recurrently been shown to enhance ecological dominance of various taxa (McKinney & Lockwood 1999; Mooney & Hobbs 2000). Interestingly, human-modified habitats seem to promote the successful establishment of other invasive ant species such as *Linepithema humile* (Holway *et al.* 2002b; Carpintero *et al.* 2004), *Solenopsis invicta* (Tschinkel 1988; Forys *et al.* 2002; King & Tschinkel 2008), *Pheidole megacephala* (Hoffmann *et al.* 1999), *Anoplolepis gracilipes* (O'Dowd *et al.* 1999) and *Lasius neglectus* (Ugelvig *et al.* 2008). Four of these five invasive ant species, together with *W. auropunctata*, share the characteristic of belonging to the top 100 most threatening invasive species in the world (Lowe *et al.* 2000). Human disturbance of ecosystems may thus constitute a key factor triggering the ecological dominance of some populations of *W. auropunctata* within its native range.

In this study, we first verified that dominant and non-dominant native populations of *W. auropunctata* did not form two distinct evolutionary units. We then examined whether the ant's reproductive system, social organization, and/or the level of human disturbance could explain variation in the ant's dominance status across part of its native range. To address this latter question, we used a combination of genotypic and behavioural data sets of individuals collected in nests from both dominant and non-dominant populations within the native range, together with historical infor-mation and direct observations regarding human land use at sampled sites.

Materials and methods

Field collection

Field work was conducted in a Brazilian area and a French Guianese area that both belong to the native range of *W. auropunctata* (Fig. 1). These areas are separated by approximately 2650 km. A total of 173 nests (i.e. an
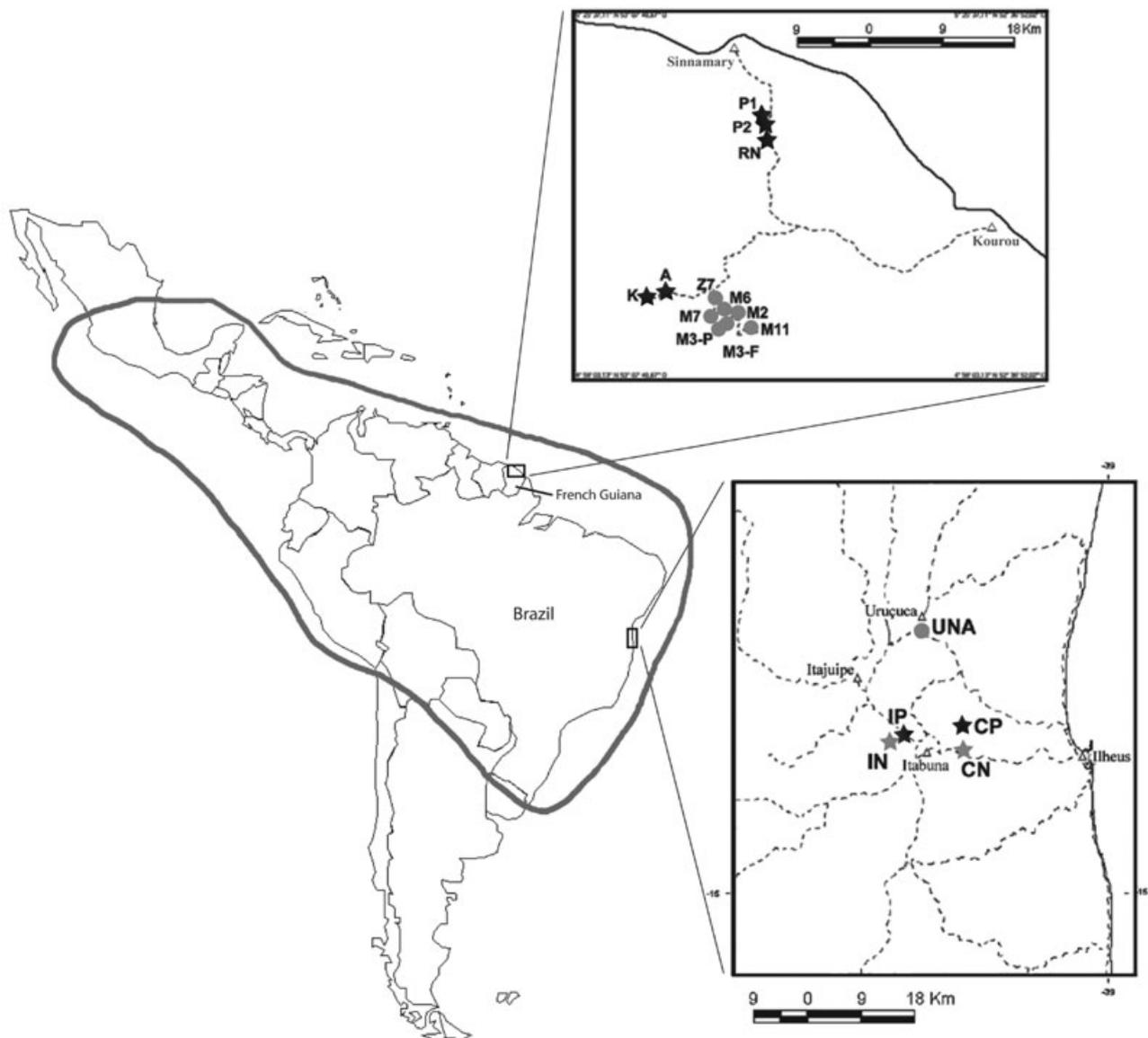
Fig. 1 Map of the 17 sampled sites. Note: Dots and stars represent natural sites and sites ecologically modified by human activities, respectively. Black and light grey colours represent sites dominated and non-dominated by *W. auropunctata*, respectively. Sampled sites are indicated with their code. See Table 1 for sampling details for each site. The large dark grey line indicates the native range of *W. auropunctata*. In each frame, plain and dashed lines indicate coast line and roads, respectively.

aggregation of workers, brood and/or queens within a fallen stick or between dead leaves) were collected in 2004, 2005 and 2006 in Brazil (5 sites) and French Guiana (12 sites). Within each country, the sampled sites were separated by at least 0.1 km and up to 30 km [mean ± standard deviation (SD): 12.6 ± 10.7 km]. The number of collected nests per site varies from four to 20 (Mean ± SD: 10 ± 5 nests). For each nest, a large number of workers (>100) and most if not all of the reproductives were collected. The distance between sampled nests was always larger than two meters. The nests collected in the field are those used for a previous genetic study focusing on the reproductive system of *W. auropunctata* (Foucaud *et al.* 2007), with the addition of five new nests collected in 2006 in the site M6.

Ecological variables

The 17 sampled sites represent various types of natural habitats or habitats ecologically disturbed by human activity (see Table 1). For each collected nest, we assessed the human disturbance level through historical data and direct visual inspection of sites (0 = sites never disturbed by human activity, corresponding to primary forests; 1 = sites disturbed by human activity, corresponding to secondary forests, plantations, and roadsides). We also recorded the dominance status of the population of the collected nest (0 = non-dominant; 1 = dominant). We consider as dominant a nest belonging to an abundant and ecologically damaging population. A recent ecological study was specifically dedicated to the description of some native populations of *W. auropunctata* in French Guiana (Orivel *et al.* 2009) and was used for classifying most of the sites studied here as dominant or non-dominant. The latter study included objective measures of population densities of *W. auropunctata* (through exhaustive sampling of 1 m^2 quadrats of leaf litter; more than 20 quadrats per site) and described its association with other native ant species (most notably species presence on baited transects). For other sampled areas (e.g., Brazil), we considered that a sampled nest belonged to a dominant population when the nest density in the area was high (>2 nests/m^2; see Orivel *et al.* 2009) as assessed by visual inspection of the site. For populations recorded as dominant (either using objective measures or by visual inspection), local abundance of *W. auropunctata* nests was so high that their discovery and sampling was immediate and straightforward. For populations recorded as non-dominant, the low nest density (typically around one per 100 m^2; Orivel *et al.* 2009) made it necessary to spend several days per site in order to col-

Table 1 Sampling design, number of sampled supercolonies, habitat type, level of human disturbance and dominance status of sampled sites

Site	Country	Number of sampled nests	Number of sampled supercolonies	Habitat	Human disturbance	Invasive status	Reproduction system
CN	Brazil	20	1	Secondary forest	D	NI	C
CP	Brazil	16	1	Cacao plantation	D	I	S
IN	Brazil	10	2	Secondary forest	D	NI	C
IP	Brazil	10	1	Cacao plantation	D	I	C
UNA	Brazil	10	NT	Primary forest	ND	NI	S/C
M2	French Guiana	8	2*	Natural backwater area	ND	NI	S
M3-F	French Guiana	10	1*	Natural backwater area	ND	NI	S
M3-P	French Guiana	13	1*	Primary forest	ND	NI	C
M6	French Guiana	8	2*	Natural backwater area	ND	NI	S/C
M7	French Guiana	15	1*	Natural backwater area	ND	NI	S
M11	French Guiana	10	2	Natural backwater area	ND	NI	S
Z7	French Guiana	6	2	Primary forest	ND	NI	S
A	French Guiana	5	1	Roadside	D	I	C
K	French Guiana	5	1	Open quarry	D	I	C
P1	French Guiana	4	1	Plantation	D	I	C
P2	French Guiana	17	4	Plantation	D	I	C
RN	French Guiana	6	1	Roadside	D	I	C
Total		173	20				

For sampled supercolonies, the site where behavioural relationships could not be tested is indicated by 'NT', and sites where one or both supercolonies are shared with other sites are indicated by '*'. Sites ecologically modified by human activities are indicated by 'HM', natural sites by 'N', sites dominated by *W. auropunctata* by 'D', non-dominated sites by 'ND', sites where reproductives reproduce sexually by 'S', sites where reproductives reproduce clonally by 'C'.

lect a sufficiently large number of nests. This clear-cut difference in local densities of *W. auropunctata* (i.e. a bimodal distribution with means differing by two orders of magnitude) allowed a safe assignment of the dominance status to the studied populations.

Intraspecific aggression tests

In order to demarcate colony boundaries and hence assess the social organization of *W. auropunctata* populations, we conducted behavioural tests using the standard aggression assays used in several studies of invasive ant species, including *W. auropunctata* (Le Breton *et al.* 2004; Pedersen *et al.* 2006). Each trial consisted of two individual workers of different nests confronted one-to-one in a neutral arena (diameter = 2 cm; height = 1 cm), whose walls were coated with fluon to prevent the ants from climbing. We scored all interactions between the workers for 5 min using the following 1–4 scale: 1 = touch (physical contact without aggressive behaviour); 2 = prolonged antennary contact (one or both ants inspect meticulously the cuticle of the other, without aggression); 3 = aggressiveness (a physical attack of one or both workers including charge, biting or leg pulling); 4 = fighting (prolonged aggressiveness of one or both workers, including the use of the sting). The highest score was averaged across trials. If this average was over 2.5, the two nests were considered aggressive to each other and therefore belonging to different colonies. On the contrary, when this average was below 2.5, the two nests were considered non-aggressive and belonging to the same colony. This cut-off value was justified by the bimodality of the distribution of values (scores either close to zero or four; see Results section). We conducted both intra and inter-site behavioural assays for nests from four of the five Brazilian sites, and all but five French Guianese nests. We first tested most intra-site nests pairs and then all pairs of behavioural groups of nests at the inter-site level within the same country (one to three replicates per pair). We could not perform behavioural tests between countries. A total of 3090 independent tests were performed to uncover the behavioural relationships between 343 nest pairs (five to ten trials per test).

Microsatellite genotyping and mtDNA sequencing

The microsatellite genotyping was carried out as described in Foucaud *et al.* (2006). Briefly, for each sampled nest, DNA was extracted from at least eight workers and all collected reproductives (including the male contributions as sampled from the queens' spermathecal contents). Our final microsatellite genotyping data set includes 2552 individuals genotyped at 12 microsatellite loci.

We also used a mitochondrial DNA sequences data set composed of 93 individuals of both dominant and non-dominant populations and three individuals of the closely related species *Wasmannia rochai* as outgroup (GenBank accession numbers EF459732-EF759824). The mitochondrial and microsatellite data sets correspond to those published in a recent genetic study focusing on the reproductive system of *W. auropunctata* (Foucaud *et al.* 2007). The microsatellite data set was supplemented with 171 individuals from five additional nests collected in the site M6.

Statistical treatment of data

To infer whether dominant and non-dominant native populations of *W. auropunctata* belong to different evolutionary units, we constructed dendrograms from individual microsatellite genotypes using the Neighbour-Joining algorithm and conducted phylogenetic analysis of the mtDNA data under the Maximum Likelihood (ML) optimality criterion (see Foucaud *et al.* 2007 for details). For the mitochondrial data set, we used the likelihood-based nonparametric Shimodaira-Hasegawa test (SH; Shimodaira & Hasegawa 1999) to formally compare our phylogeny (i.e. the best-fit ML tree) with an alternative phylogeny in which individuals from dominant and non-dominant populations were constrained to form two distinct monophyletic groups.

The reproductive system was inferred for each nest from microsatellite data both visually and using a personal computer program seeking identical multilocus genotypes, as described in Foucaud *et al.* (2007). A custom-written software package was used to compute observed heterozygosity and mean difference in allelic size within and between multilocus genotypes. Within-individual heterozygosity, Ho_w, was computed as the number of loci of an individual genotype showing different alleles, averaged over loci. Heterozygosity of a queen-male couple (i.e. a mating pair), Ho_b, was computed as the mean number of times the allele of the fathering male (determined by genotyping the queen spermatheca) was different from each queen allele at a given locus, averaged over loci. Within-individual difference in allelic size, DS_w, was computed as the difference in base pairs between the two alleles at a given locus of a single individual, averaged over loci. Difference in allelic size of a queen-male couple, DS_b, was computed as the mean difference between the male allele and the two queen alleles at a given locus, averaged over loci. Because microsatellite sequences mutate under a stepwise model, the differences in allele size between two microsatellite DNA copies measured either within or between individuals is related to the coalescence time and hence the level of divergence between

the two compared genomes. Hierarchical and non-hierarchical *F*-statistics were computed from microsatellite data using HIERFSTAT (Goudet 2005) and FSTAT v2.9.3.2 (Goudet 2001), respectively.

The relationship between the dominance status, the reproductive system, and the human disturbance level was assessed by performing a contingency analysis on each pair of factors, and a factor-analysis. Because the social organization turned out to be similar for all nests (see Results section), it was not included in our analyses. The data sets for both analyses consists of a table containing the values of our three nominal variables, coded in binary mode (i.e. dominant/non-dominant, sexual/clonal, and disturbed/non-disturbed habitat), for our sampled nests. First, we conducted our analysis considering only nests for which the reproductive system could be unambiguously inferred (see Results section; $n = 129$). Because sexual reproduction is more difficult to demonstrate than clonal reproduction, using only the nests for which the reproductive system could be unambiguously inferred is expected to induce a bias in our analysis. We therefore conducted a second analysis, adding nests for which the reproductive system was at least suggested by our microsatellite data ($n_{added} = 33$ nests; $n_{total} = 162$ nests). Finally, because nests of the same supercolony are not fully independent observations, we conducted a third analysis with supercolonies as observations. Supercolonies represent mostly independent replicates, even if we previously uncovered that Guianese clonal queens were somewhat related (Foucaud *et al.* 2007 and see Fig. S3). In particular, spatial proximity does not preclude independence between supercolonies because (i) supercolony boundaries were confidently assessed through aggression tests (see Results section), and (ii) most genetic variance between individuals appeared to be explained by the supercolony level (i.e. see Results section). We could not use the sampled sites per se as independent replicates, because some sites turned out to be occupied by several genetically differenciated supercolonies, or a given supercolony could be present at several sites. Note that the gain in terms of statistical independence when considering supercolonies as observations is considerably reduced by a much smaller sample size (i.e. 20 supercolonies vs. 129 or 162 nests). In the factor-analysis, factors were extracted using the principal component method and the factor structure was rotated using the normalized Varimax method. All analyses were performed using STATISTICA v7.

Computer simulations

A previous genetic study of the *W. auropunctata* reproductive system showed that, within the native range, sexual populations were most probably the recent source of neighbouring clonal populations (Foucaud *et al.* 2007). The results we obtained in the present study further raised the question of whether the queen-male couples founding clonal populations (here supercolonies as identified through aggression tests) were compatible with random mating between individuals from sexual supercolonies or whether these couples were the result of non-random matings of queens and males originating from the same or different sexual supercolonies. To address this question, we used computer simulations to determine whether the Ho_b and DS_b statistic values of the observed clonal and sexual couples were compatible with males and queens randomly drawn from the same or from different sexual supercolonies. Using a predefined worker data set (see below), we randomly chose a worker genotype to be the female of the simulated couple and randomly picked half of another worker genotype to be the male of the simulated couple, either from the same supercolony or from different supercolonies. We then computed Ho_b and DS_b for each simulated couple. This process was iterated 10^6 times using a personal computer program written in the Pascal object programming language (available from the authors upon request). We then computed the joint densities of Ho_b and DS_b for a 'within supercolony' and a 'between supercolonies' treatments, using the locfit package (Loader 1996) implemented in version 2.2.1 of the R software (R Development Core Team 2005).

Because we needed several sexual supercolonies in order to compute statistics both within and between supercolonies, we only ran this simulation with the Guianese data set, using the workers of all sexual supercolonies as the baseline for the generation of simulated couples (except M11-B, because of the low number of nests collected in this supercolony). It is worth stressing here that we found different levels of genetic structure within sexual supercolonies (see Results section). Therefore, we also ran simulations for each supercolony individually ($n = 5$) and for each pair of supercolonies ($n = 10$). Finally, we plotted the $Ho_b \times DS_b$ data set of observed clonal and sexual Guianese couples within the $Ho_b \times DS_b$ plan used to draw the simulated joint densities.

Results

Our 17 sampled sites encompassed seven sites where *W. auropunctata* was dominant and ten sites where it was not dominant (hereafter referred to as 'dominated' or 'non-dominated' sites; Fig. 1 and Table 1). We found both dominated and non-dominated sites in Brazil ($n_{dominated} = 2$ and $n_{non-dominated} = 3$) and French Guiana ($n_{dominated} = 5$ and $n_{non-dominated} = 7$).

Genetic relationships between dominant and non-dominant populations

The trees constructed using the microsatellite or mtDNA data sets did not show any monophyletic clustering of dominant vs. non-dominant populations (see Supplementary Figs S2 and S3 for illustrations). This result holds when treating together or separately the Guianese and the Brazilian data sets (results not shown). For the mitochondrial data set, this finding was further confirmed by the Shimodaira-Hasegawa test, which indicated a significant lack of support for the clustering of individuals as a function of the dominance status of their population (SH test: $P < 0.001$; i.e. topologies including this clustering yielded significantly lower ML scores than topologies free of this constraint). Dominant and non-dominant native populations hence did not belong to different evolutionary units.

Social organization

Results of aggression tests were highly bimodal. Most aggression tests show either no aggression, or intense fights between nests pairs. The behavioural boundaries of colonies were thus confidently assessed. The 141 tested nests were organized in 20 supercolonies (i.e. networks of geographically separated nests showing no intraspecific aggression and cooperating as single units). Two supercolonies were present in three distinct sites (most probably connected through creeks) and six sites were hosting more than one supercolony (Mean ± SD: 2.33 ± 0.82 supercolonies; Table 1). These supercolonies encompassed a few hundred to a few thousand square meters, spatially continuous and distinct from each other. All nests tested for aggressiveness, whether dominant or non-dominant, belonged to a supercolony. The social organization was thus clearly not a sufficient factor to explain the ecological dominance of some populations of *W. auropunctata*.

Reproductive system

Over the 173 nests analyzed for the type of reproductive system, the reproductives (i.e. males and queens) reproduce exclusively clonally in 89 nests and exclusively sexually in 40 nests. Three nests display a mix of clonality and sexual reproduction. The reproductive system could not be unambiguously determined in the 41 remaining nests. These latter nests lacked reproductives at the time of collection and either parental genotypes could not be confidently inferred from individual worker genotypes, or the suggested parental genotypes did not match any known genotype (see Foucaud *et al.* 2007 for details). However, for 33 of the 41 undeter-

mined nests, our microsatellite data strongly suggested one particular type of reproductive system (usually sexual) without providing definitive proof.

Each of the 173 nests could be labelled as ''dominant'' (i.e. belonging to a dominant population; $n = 63$) or 'non-dominant' ($n = 110$), respectively. Of the 63 dominant nests, 16 are sexual (25%), 46 are clonal (73%) and one is undetermined (2%, though probably clonal). Of the 110 non-dominant nests, 24 are sexual (22%), 43 are clonal (39%), three are mixed clonal and sexual (3%), and 40 are undetermined (33%; though most of them are probably sexual, i.e. 27 sexual nests vs. five clonal nests, the rest remains undetermined).

Each supercolony included either clonal or sexual nests. The 83 clonal nests tested for aggressiveness were organized in 13 supercolonies. Clonal supercolonies were generally headed by a unique queen genotype. The queens of the clonal supercolonies were usually mated to a single clonal male genotype (nine supercolonies), and more rarely to two or three different clonal males genotypes (four supercolonies). Two clonal male genotypes were shared between three and four supercolonies, respectively, a situation never observed with clonal queens. This suggests that workers of a given clonal supercolony may recruit alien male genotypes, whereas alien queen genotypes might not be accepted. Consistent with this observation, controlled fertilization experiments using males and queens from different supercolonies showed that all fertilized gynes are accepted back in their supercolony of origin, but killed if placed in the supercolony of their male mate ($n = 22$; JO, unpublished results). The 39 sexual nests tested for aggressiveness are also organized in supercolonies. Three supercolonies consist of only known sexual nests, one contains both sexual nests and nests for which the reproductive system could not be confidently assessed (but that are probably sexual), and three supercolonies include only nests of the latter type (probably sexual nests).

Relationships between ecological dominance, reproductive system and human disturbance

Because both dominant and non-dominant populations turned out to be unicolonial (i.e. formed supercolonies), social organization was not included in our contingency and factor-analyses.

Our contingency analyses showed that the dominance status of a population (i.e. a supercolony) is significantly associated with human disturbance. This result was obtained using all nests for which the reproductive system could be unambiguously inferred ($n = 129$ nests, $\chi^2 = 51.728$; d.f. = 1; Cramer's $V = 0.633$; $P < 0.001$), using all nests for which the reproductive system could

be at least suggested ($n = 162$ nests, $\chi^2 = 76.487$; d.f. = 1; Cramer's $V = 0.687$; $P < 0.001$), or using populations as observations ($n = 20$ populations, $\chi^2 = 6.429$; d.f. = 1; Cramer's $V = 0.598$; $P = 0.011$). All dominant populations of *W. auropunctata* were indeed found in areas where human activities were present now or in the near past (e.g. secondary forests, quarries or roadside; Table 1). On the contrary, non-dominant population occurred in areas that were never exploited by man (e.g. primary forests; Table 1).

Our contingency analyses showed different answers regarding the relationship between the dominance status of a population and its reproductive system depending on the data set considered. We found that ecological dominance was not significantly associated with reproductive system when using all nests for which the reproductive system could be unambiguously inferred ($\chi^2 = 1.51$; d.f. = 1; Cramer's $V = 0.108$; $P = 0.219$) as well as when considering populations as observations ($\chi^2 = 1.945$; d.f. = 1; Cramer's $V = 0.329$; $P = 0.163$). The dominance status of a population becomes significantly associated with the reproductive system when considering nests for which the reproductive system was suggested but not proved ($\chi^2 = 10.829$; d.f. = 1; Cramer's $V = 0.259$; $P < 0.001$). Altogether, our analyses indicate a trend for dominant populations to be clonal, but this relationship is however far from strict. As a matter of fact, although most dominant supercolonies have a clonal reproductive system, one human-modified area is dominated by a sexual supercolony (CP, Table 1), and three clonal supercolonies are not dominant (M3-P, CN and IN).

On the other hand, the reproductive system was always significantly associated with human land use (data sets using nests as observations: both $\chi^2 > 24$, both Cramer's $V > 0.435$, both $P < 0.001$; data set using populations as observations: $\chi^2 = 8.288$; d.f. = 1; Cramer's $V = 0.679$, $P = 0.004$). This is because, in human-modified habitats, clonal production of reproductives is much more common than sexual reproduction (83% of sampled nests), and in natural habitats, the large majority of nests were found to be sexual, even if a few clonal populations were also sampled.

Our factor-analyses confirmed the three main findings of the contingency analyses. The first factor of our analysis (always explaining more than 50% of variance in our three data sets) showed a strong association between the dominance status of *W. auropunctata* populations and the human disturbance level recorded in their area (Fig. 2). The second factor, which always explained more than 30% of the total variance of our three data sets, showed an association between the type of reproductive system and the human disturbance level, as shown by the large positive factor-values of
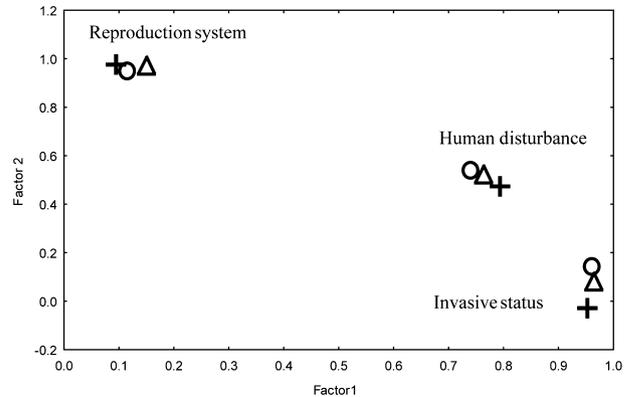
Fig. 2 Projection of the dominance status, the type of reproductive system and level of human disturbance onto a plane defined by the first and second factor axes of the factor-analyses. Note: Crosses, triangles and circles represent the results of the factor-analyses using (i) only the nests for which the reproductive system could be unambiguously inferred ($n = 129$), (ii) the same nests plus those for which the reproductive system was suggested but not proved ($n = 162$) and (iii) populations instead of nests as observations ($n = 18$), respectively. Factors 1 and 2 represent 52% and 38% of the total variance for treatment (i), 51% and 41% of the total variance for treatment (ii), and 50% and 42% of the total variance for treatment (iii).

both variables on the second axis of Fig. 2. In all datasets, the presence of human disturbance is strongly associated with a clonal reproductive system. Finally, both factors showed that the type of reproductive system was not directly linked to the ecological dominance of *W. auropunctata* populations (Fig. 2).

In brief, we found that human disturbance favours ecological dominance and, less strongly, clonal populations. Hence, human disturbance causes a mild correlation between ecological dominance and reproductive system.

Genetic structure and mating pattern

Consistent with the results based on behavioural assays, a hierarchical *F*-statistics analysis shows that the highest level of genetic structure is found between supercolonies (Table 2). This partitioning of genetic variation is more pronounced for clonal than sexual supercolonies, suggesting that clonal supercolonies accept fewer migrants from other colonies than sexual supercolonies. The level of genetic structure within supercolonies was found to considerably differ for the seven non-clonal supercolonies (i.e. including sexual nests and/or nests with undetermined type of reproduction, although probably sexuals; see Table 3). Some supercolonies showed no genetic structure, whereas some others showed substantial structure at the nest level (Table 3). The levels of genetic structure within non-clonal super-

Table 2 Hierarchical F-statistics analysis

	All nests ($n = 133$)	Clonal nests ($n = 84$)	Sexual nests ($n = 29$)	Undetermined nests ($n = 20$)
$F_{Country-Total}$	0.048	0.099	NC	NC
$F_{Supercolony-country}$	0.265	0.329	0.149	0.062
$F_{Nest-Supercolony}$	0.143	0.109	0.122	0.301

Computations were processed over all nests, over clonal nests only, and over sexual nests only. The analysis includes three hierarchical levels: country, supercolony and nest. Because the Brazilian nests that were confidently assessed as sexual were found in a single supercolony, the hierarchical analysis for sexual nests included only two levels and was based only on samples from French Guiana.
NC, not computed.

Table 3 $F_{nest-supercolony}$ statistics for each of the seven non-clonal supercolonies

Supercolony	Nests type	Sample size	$F_{nest-supercolony}$	Significance
Z7-A	S	3	0.006 ± 0.009	NS
CP	S	10	0.008 ± 0.007	NS
M7	S	15	0.038 ± 0.004	***
M11-B	U	3	0.085 ± 0.025	***
Z7-B	U	3	0.233 ± 0.052	***
M3-F	S/U	16	0.255 ± 0.014	***
M11-A	U	5	0.278 ± 0.024	***

Supercolony names were coded with the name of the site (as in Table 1), followed by a letter if the site includes several supercolonies. The presence of sexual nests and nests of undetermined type of reproduction is indicated with the letter 'S' and 'U', respectively. Estimated F-values are given ± standard deviation. The level of significance of each F-value was obtained by 10 000 permutations of genotypes among nests (NS, non significant; ***$P < 0.001$).

colonies were particularly high within supercolonies including nests undetermined with regards to their reproductive system. This is because most of these undetermined nests consisted of monogynous and monandrous worker lineages.
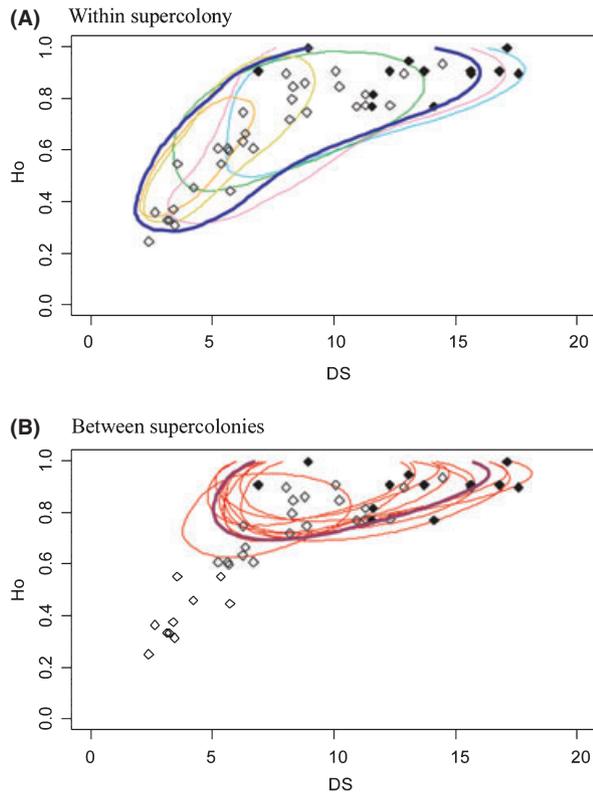
We could determine the genotype of each mate of 299 queens (corresponding to 105 individual queen genotypes) using the sperm stored in the queens' spermatheca. We found that the heterozygosity (Ho_b) and difference in allelic size (DS_b) between the male and queen genotypes of a given couple were both significantly larger in the 21 clonal couples sampled than in the 89 sexual couples sampled (Mann–Whitney U-test : $P < 0.001$; Fig. 3A). Consistent with this trend for outbreeding observed in all clonal supercolonies relative to sexual supercolonies, we found that the heterozygosity
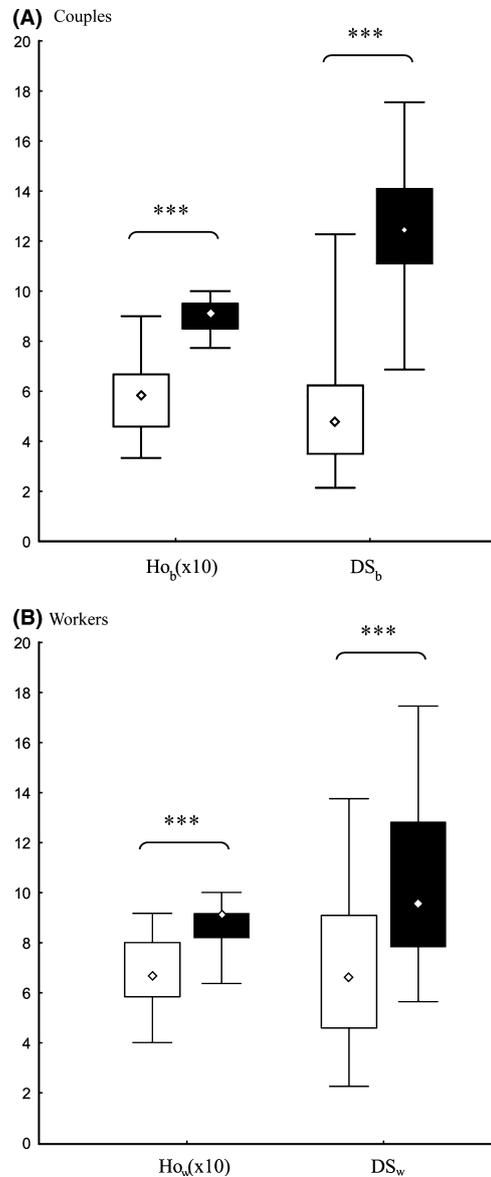
Fig. 3 Observed heterozygosity (H_O) and difference in allelic size (DS) of clonal and sexual queen-male couples (A) and of workers (B). Note: Blocks in white and in black indicate sexual and clonal nests, respectively. Diamonds indicate means, blocks and horizontal bars indicate 50% and 95% percentiles, respectively. Within-individual heterozygosity, Ho_w, was computed as the number of loci showing different alleles in a given individual, averaged over loci. Within-individual difference in allelic size, DS_w, was computed as the difference in base pairs between the two alleles at a given locus of a single individual, averaged over loci. Heterozygosity of a couple (i.e. a mating pair), Ho_b, was computed as the mean number of times the allele of the fathering male was different from each allele of the queen he has mated at a given locus, averaged over loci. Difference in allelic size of a couple, DS_b, was computed as the mean difference between the male allele and the two queen alleles at a given locus, averaged over loci. Ho_w and Ho_b values were multiplied by ten to homogenize scales with DS. Mann-Whitney U tests: *** corresponds to $P < 0.001$.

(Ho_w) and difference in allelic size (DS_w) were significantly higher in workers of clonal nests than in workers of sexual nests (Mann–Whitney U-tests: $P < 0.001$; Fig. 3B).

Computer simulations confirmed that the clonal couples (i.e. mating pairs) tend to be more outbred than the sexual couples. The comparison of the joint distributions of Ho_b and DS_b for the 'within-supercolony' and 'between-supercolonies' simulations show that a large proportion of observed sexual couples could have been formed only by individuals originating from the same supercolony (Fig. 4). On the contrary, clonal couples
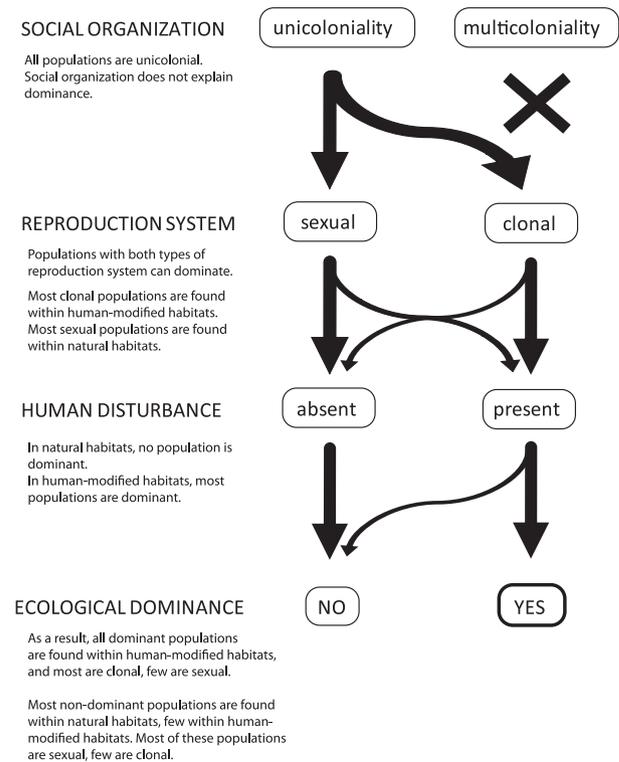
could have been formed only within the few supercolonies showing substantial genetic structure at the nest level (e.g. M3-F, M11-A in Table 3) or by individuals originating from different supercolonies (Fig. 4). In other words, established clonal supercolonies have more parents that are less related to each other than are parents of sexually reproducing supercolonies.

Discussion

A flowchart summarizing the main results and inferred relationships between ecological dominance and social organization, reproductive system and human disturbance factors is presented in Fig. 5.

Role of the reproductive system in ecological dominance

Although clonal reproduction is often argued to be a key factor enabling some species to become dominant, or even invasive (Kolar & Lodge 2001), we did not find a strict association between the clonal or sexual reproductive systems and the dominance status of *W. auro-*

Fig. 4 Plot of the joint densities for the correlated pair of statistics Ho_b and DS_b for simulated couples. Note: Queen-male couples were formed with genotypes randomly drawn within the same supercolony (A) or between different supercolonies (B) from French Guiana (see Materials & Methods section for details). In (A) and (B), white and black diamonds represent observed values for sexual ($n = 31$) and clonal ($n = 13$) couples, respectively. In (A), the large blue line represents the 95% highest density contour of the global within supercolony simulation. Other coloured lines represent the 95% highest density contours of individual within supercolony simulations as follows: turquoise = M3-F, violet = M11-A, green = M7, light green = Z7-A and yellow = Z7-B. In (B), the large violet line and the thin red lines represents the 95% highest density contours of the global between supercolonies simulation and of the between supercolonies simulations for each pair of supercolonies ($n = 10$), respectively.

Fig. 5 Flowchart summarizing the main results and inferred relationships between ecological dominance and social organization, reproductive system and human disturbance factors. Note: Large and thin arrows represent frequent and rare events, respectively.

punctata populations (supercolonies). We found both one sexual population to be dominant and several clonal populations to be non-dominant. The results of the factor-analyses consistently show that the reproductive system is not the best predictor of dominance of *W. auropunctata* populations. Interestingly, the low densities of several clonal populations suggest that there is no demographic advantage inherent to the clonal reproductive system in that species. This is congruent with the fact that even in clonal populations of *W. auropunctata* both sexes remain necessary to produce workers through sexual reproduction.

We found that the type of reproductive system occurring in a nest is significantly associated with the level of human disturbance. Although this association between reproductive system and human disturbance is not strict, sexual populations generally occurred in natural habitats and clonal populations in disturbed habitats, irrespective of the dominance status of the population. This suggests that clonal and sexual populations occupy different ecological niches, and that human disturbance commonly drives habitats toward ecological conditions favouring the establishment of clonal populations. We also found that, although clonal reproductives of both sexes likely originate from local sexual populations (Foucaud *et al.* 2007), clonal couples were composed of a male and a female significantly less related than those of sexual couples (i.e. clonal couples are more outbred than sexual couples and therefore workers of clonal nests are more heterozygous).

Contrary to sexual reproduction, the *W. auropunctata* clonal reproductive system is expected to limit recombination and promote selection on queen, male and worker genomes (through the conversion of non-additive into additive genetic variance; Neiman & Linksvayer 2006). We thus propose that the clonal reproduction system of *W. auropunctata* may be selected for in disturbed environments because it enables the species to maintain over time particular combinations of male and female genotypes that produce highly heterozygous worker genotypes. By limiting recombination at the population level, this clonal system promotes lineage selection, and by maximizing the relatedness between colony members, it also increases the efficient of selection much above what is usually possible in low relatedness supercolonies of many other unicolonial ant species (Helanterä *et al.* 2009). In addition to the break of putative beneficial combinations of alleles, sexual reproduction would also lead to a loss of alleles by genetic drift, especially if the number of founders is low, and increase diploid male load (i.e. the cost of producing sterile diploid males, homozygous for the sex determination locus; Foucaud *et al.* 2006). *Wasmannia auropunctata* may hence illustrate that consequences on adaptive potential and genetic diversity might be at least as important as demographic differences in driving the evolution of sexual vs. clonal reproductive systems (Lushai *et al.* 2003).

The question of why highly heterozygous genotypic combinations seem to be more adapted to disturbed habitats remains open to future studies. Briefly, the heterozygosity excess could enable *W. auropunctata* workers from clonal populations (i) to deal with the extreme spectrum of environmental conditions met in human-modified habitats (e.g. Kearney & Shine 2004; Ferreira & Amos 2006), and/or (ii) to better exploit resource-rich human-modified habitats (e.g. Reznick *et al.* 2000; Vorburger 2005). As a matter of fact, *W. auropunctata* has repeatedly been shown to be successful both in harsh habitats (Orivel *et al.* 2009), and in plantations where high densities of Hemiptera or plant extrafloral nectaries are present (Delabie *et al.* 1994; Wetterer & Porter 2003).

Roles of social organization in ecological dominance

The unicolonial social organization seems to provide a strong ecological advantage to introduced populations of invasive ant species showing this trait, through both lower intraspecific competition and efficient interference competition with other ant species (Holway & Suarez 2004; Le Breton *et al.* 2005). However, we found that all studied dominant and non-dominant populations of *W. auropunctata* were unicolonial. Therefore, contrary to previous ideas (Passera 1994; Le Breton *et al.* 2004), the unicolonial social structure does not seem to be the critical factor enabling *W. auropunctata* to become ecologically dominant, at least in its native range, and thus may not be a triggering factor of invasions in this species. In the same vein, Pedersen *et al.* (2006) and Vogel *et al.* (2009) found that the Argentine ant also displays non-dominant supercolonies of small size in its native range, and Ugelvig *et al.* (2008) predict small-scale non-dominant supercolonies of *Lasius neglectus* to occur in the native range of the species. These three examples support the idea that unicoloniality, even if undoubtedly associated with ant invasions (Holway *et al.* 2002a), might only be a promoting factor during the range-expansion phase of invasions. As a matter of fact, the benefits of a reduced intraspecific competition increase when the density of the invading species becomes high, that is in the latter stages of an invasion process (Mooney & Hobbs 2000; Sakai *et al.* 2001). Unicoloniality may hence be beneficial only at the latter stages of ant invasions, whereas ecological transitions (e.g., breaking a distribution barrier) may be the key factor during the early stages of invasions.

We found that the supercolonies encountered in the native range of *W. auropunctata* are several orders of magnitude smaller than the supercolonies of the introduced range of the species. In Brazil and French Guiana, the size of the supercolonies ranges between a few hundred to a few thousand meters squared, while the whole island of New Caledonia (approximately 19 000 km^2) is invaded by a single supercolony (Le Breton *et al.* 2004). Small-scale supercolonies occur in a variety of unicolonial species (Helanterä *et al.* 2009), such as *Linepithema humile* (Tsutsui & Case 2001; Buczkowski *et al.* 2004; Vogel *et al.* 2009) or *Formica paralugubris* (Chapuisat *et al.* 1997). This difference in size between native and introduced supercolonies could simply be due to the rarity of remote introduction events, allowing introduced supercolonies to attain greater sizes than native supercolonies. When the frequency of introduction events is low (i.e. for distant introduction events as opposed to local migration), it is likely that the first propagule that succeeds in settling will have enough time to saturate the environment prior to the arrival of following propagules, and hence efficiently prevent their settlement by aggressive behaviours.

Roles of human land use in ecological dominance

We found *W. auropunctata* ecological dominance to be strongly associated with habitats altered by human activities. This fact is not surprising given that human activities have been recognized to facilitate the worst cases of ecological dominance, i.e. biological invasions (e.g. Mooney & Hobbs 2000; Occhipinti-Ambrogi & Savini 2003; Ervin *et al.* 2006), including for other invasive ant species such as *Linepithema humile* (Holway *et al.* 2002b; Carpintero *et al.* 2004) and *Solenopsis invicta* (Tschinkel 1988; Forys *et al.* 2002). The impact of human activities on ecosystems is certainly complex, so it seems difficult to precisely identify the ecological factor(s) that promote(s) *W. auropunctata* dominance in disturbed habitats. Potential mechanisms include the modification of abiotic conditions in human-modified habitats (Salmun & Molod 2006), the reduction of biotic interactions with natural enemies or competitors (i.e. the enemy release hypothesis; Keane & Crawley 2002), or the increase of food sources (Davis & Pelsor 2001). Recent work by King & Tschinkel (2008) experimentally examined the relative contribution of disturbance and invasion by fire ants (*S. invicta*) and concluded that disturbance itself has a negative impact on native ants and the invasive ant may be secondary to disturbance in impact.

Because human disturbance of a natural habitat also favours the establishment of clonal populations of *W. auropunctata*, it is not surprising that most dominant native populations were found to be clonal. We expect most populations introduced in remote non-native areas (e.g. in Pacific islands, tropical Africa, Florida; Wetterer & Porter 2003) to be also clonal for at least three reasons. First, nests that disperse outside their native range through human means are likely to originate from habitats disturbed by human activities and hence probably from clonal nests. Second, the environment where the introduced nests have to settle is also probably highly disturbed by human activities (e.g. ports, urban areas…), and hence probably better fits the presumed environmental niche of clonal nests. Finally, clonal *W. auropunctata* populations are protected from any fitness cost due to the erosion of genetic diversity as well as inbreeding effect during bottleneck events associated to population founding, contrary to sexual populations. In agreement with this expectation, we only found remote introduced populations of *W. auropunctata* to be clonal so far (unpublished data; Foucaud *et al.* 2006; Mikheyev *et al.* 2009).

Conclusions

This study sheds light on the biological diversity of populations of an invasive ant species, within its native range. We found that *W. auropunctata* populations were non-dominant, mostly sexual and rarely clonal in ecologically undisturbed areas, and dominant, mostly clonal and rarely sexual in human-modified habitats. The *W. auropunctata* model therefore illustrates how the presence of human activities may lead to extensive changes in selective pressures over species that can result in spectacular shifts in both ecological and genetical traits.

So far, the native dominant populations of *W. auropunctata* have shown striking similarities to introduced invasive population with regards to demographical, reproductive, and behavioural features (this study, Orivel *et al.* 2009; Foucaud *et al.* 2006; Mikheyev *et al.* 2009). Further investigations are needed to assess the evolutionary and historical relationships between native dominant and introduced invasive populations, in order to gain deeper insights into the emergence of invasiveness in *W. auropunctata*.

Besides the importance of human activity in the process of ecological dominance, our results point to the adaptive potential of clonal genomes. The debate over the evolution of sex might have seriously overlooked the significance of clonality in terms of power of selection (Neiman & Linksvayer 2006), while the demographical 'two-fold cost of sex' retained much attention (West *et al.* 1999; Doncaster *et al.* 2000). The *W. auropunctata* model reminds us that the evolution of reproductive systems is only a part of a much larger question regarding the evolution of genomic recombina-

tion, and its role in creating new genotypic variants and in adjusting the power of selection over these variants.

Acknowledgements

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Supporting information

Additional supporting information may be found in the online version of this article.

Fig. S1 Schematic representation of the two types of reproductive system in *W. auropunctata*.

Fig. S2 Best-fit ML tree of mtDNA COI haplotypes obtained from *W. auropunctata* individuals sampled in dominant and non-dominant populations.

Fig. S3 Neighbour-Joining dendrograms of the microsatellite (allele-shared) distances between *W. auropunctata*

workers sampled in dominant and non-dominant populations.

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