

RARE SEXUAL REPRODUCTION EVENTS IN THE CLONAL REPRODUCTION SYSTEM OF INTRODUCED POPULATIONS OF THE LITTLE FIRE ANT

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Abstract.—A unique reproductive system has previously been described in *Wasmannia auropunctata*, a widespread invasive ant species, where males are produced clonally, female queens are parthenogens, and female workers are produced sexually. However, these findings were mostly based on samples originating from only a limited part of the native range of the species in South America. We used microsatellite markers to uncover the reproductive modes displayed by a large number of nests collected in various invasive *W. auropunctata* populations introduced 40 years ago into New Caledonia, where the species now forms a single 450-km-long supercolony. Although the main reproduction system in New Caledonia remained clonality for both male and female reproductives, we found evidence of rare sexual reproduction events that led to the production of both new queen and male clonal lineages. All clonal lineages observed in New Caledonia potentially derived from sexual reproduction, recombination, and mutation events from a single female and a single male genotype. Hence, the male and female gene pools are not strictly separated in New Caledonia and the two sexes do not follow independent evolutionary trajectories. Our results also suggest genetic determination for both parthenogenesis and caste. We discuss the evolutionary implications of the emergence of sex in the clonal reproduction system of introduced populations of *W. auropunctata*.

Key words.—Caste determination, invasion, parthenogenesis, reproduction system, *Wasmannia auropunctata*.

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Insects display a wide variety of genetic systems, such as diploidy, thelytoky, haplodiploidy and mixed systems, as well as other rare extrazygotic inheritance mechanisms (reviewed in Normark 2003). Ants (Formicidae) generally produce diploid queens and sterile workers by sexual reproduction (i.e., meiosis and fertilization) and haploid males by arrhenotokous parthenogenesis (i.e., unfertilized meiotic eggs developing into haploid individuals). More unusual modes of reproduction have been described in some ant species (Normark 2003), including the species studied here, *Wasmannia auropunctata* (Fournier et al. 2005a).

In this small myrmicine species, female workers are produced sexually, whereas female queens reproduce by thelytokous parthenogenesis (i.e., diploid eggs produced from female genetic material only, by mitosis or the fusion of meiotic products), a genetic system uncovered in only five other ant species: *Pristomyrmex punctatus* (Tsuji 1988), *Cataglyphis cursor* (Percy et al. 2004), *Platythyrea punctata* (Heinze and Hölldobler 1995), *Cerapachys biroi* (Tsuji and Yamauchi 1995), and *Messor capitatus* (Grasso et al. 2000). The male reproduction system of *W. auropunctata* is even more unique, in that males also reproduce clonally, a feature suspected, but not strictly demonstrated, in only one other social hymenopteran species, *Apis mellifera* (Koeniger et al. 1989). In most ant species, males achieve virtually all their reproductive success through the sexual transmission of their genome to the new generation of female reproductives. The reproductive success of *W. auropunctata* males is thus potentially

reduced to zero by the parthenogenetic production of female queens. Male clonal reproduction could therefore be considered as an evolutionary response to overcome a complete reduction of their fitness (Fournier et al. 2005a). Male clonal reproduction in *W. auropunctata* is thought to involve paternal elimination of the maternal genome from the egg (Fournier et al. 2005a). Although elimination of the paternal genome has been uncovered in a variety of taxa (Normark 2003), *W. auropunctata* represents one of the few known examples of clonal reproduction involving elimination of the maternal genome (McKone and Halpern 2003). This unique reproduction system results in the complete separation of male and female gene pools, potentially allowing the two sexes to follow different evolutionary pathways, provided that they continue to cooperate in the mutualistic production of sterile workers (Queller 2005). This system might allow male and female sexuals to reorganize their genomes independently and potentially escape the load of carrying functional genes from the other sex, thereby directly increasing their own gene transmission rate. Sexual production of workers increases their genetic diversity, potentially enhancing overall colony function through efficient division of labor (Robinson 1992; Percy et al. 2004), resistance to parasites (Hughes and Boomsma 2004), or increasing the range of environments tolerated by the colony (Crozier and Page 1985).

The *W. auropunctata* reproduction system is not the only remarkable feature of this species; it is also a so-called tramp

ant species (Passera 1994). Tramp ants are often ecologically dominant within various habitats of their introduced range, where they pose serious threats to both natural ecosystems and human activities (Sakai et al. 2001; Holway et al. 2002). These ants, which belong to various taxa, usually possess a colony structure known as unicolonality, in which reproductives, workers, and brood can mix freely between nests. This social organization ultimately leads to the formation of vast cooperative networks of nests showing no intraspecific aggression, called supercolonies (Markin 1968). Four tramp species, including *W. auropunctata*, have been classified among the most damaging invasive species on Earth (Lowe et al. 2000). *Wasmannia auropunctata* originates from the South American rainforest. Long-distance human transport has readily spread the species in nearly all the tropics, from Africa to Oceania (Jourdan 1997; Walsh et al. 2004), where it has now become a serious concern for the conservation of biodiversity and human activities (Jourdan et al. 2001; Le Breton et al. 2003, 2005). It was first recorded in the mid-1960s in New Caledonia (Jourdan 1997), a large Pacific island (19,000 km²) classified as a biodiversity hotspot (Myers et al. 2000). In the 40 years since its introduction, *W. auropunctata* has invaded the entire island and now forms a single 450-km-long supercolony (Le Breton et al. 2004).

The unusual reproduction system of *W. auropunctata* may constitute a key element in its invasive success. It is commonly argued that a clonal reproduction system can help a species to establish itself successfully early in invasion, because of the assurance of reproduction of initially small populations (Sakai et al. 2001). Clonal reproduction systems may also constitute a major demographic advantage (the two-fold cost of sex) during the range-expansion phase (Vorburger et al. 2003). Consistent with these hypotheses, clonal plants and animals have been shown to be effective colonists (Baker 1955; Samadi et al. 1999). However, such arguments may not apply to *W. auropunctata* or to ants in general. A single inseminated queen, whether clonal or sexual, alone or with a few workers, may have the reproductive potential to establish a new population, as the queen does not need to encounter a male when already inseminated (Moller 1996). Moreover, the unusual reproduction system of *W. auropunctata* does not render the male sex obsolete because workers are produced sexually.

To date, the unique reproduction system of *W. auropunctata* has been described in a limited part of the native range (i.e., French Guiana) and only in a small sample from the introduced range of the species (i.e., four nests from New Caledonia; Fournier et al. 2005a). However, new ecological conditions encountered by introduced populations of *W. auropunctata* such as those of New Caledonia are likely to represent new selective pressures that may affect their modes of reproduction. In particular, sexual reproduction has the potential to generate new genetic variants more rapidly than parthenogenesis (Colegrave 2002; but see Lushai et al. 2003), a feature that can be critical for the successful establishment and spread of a species in a new environment (Sakai et al. 2001). However, the clonal production of reproductives can maintain efficient combinations of genes over time, whereas sexual reproduction randomly breaks up favorable genetic combinations at each generation (Rouzine et al. 2003). There-

fore, introduced populations of *W. auropunctata* may, at least theoretically, display a mixed reproduction system.

Here, we used microsatellite markers to uncover the reproductive modes displayed by a large number of nests and introduced New Caledonian populations of *W. auropunctata*, with a particular interest in identifying evolutionary transitions between clonal and sexual modes of reproduction. The second aim of this study was to provide insights into the colonization process of New Caledonia by *W. auropunctata*.

MATERIALS AND METHODS

Field Collection

A total of 82 nests (i.e., an aggregation of workers, brood, and/or queens within a woodstick or between dead leaves) were collected in August 2003 and October 2004 from nine sites located across the main island of New Caledonia (Fig. 1). Sites were separated by 1–451 km (mean \pm SD = 214 \pm 132 km) and represent various types of ecologically disturbed or natural habitats: Port-Laguerre (secondary forest, first known occurrence of *W. auropunctata* in New Caledonia, 25 nests), Boulouparis-Thio (roadside, 20 nests), Bois du Sud (tropical rainforest, 13 nests), Hienghène (plantations, five nests), Paouta (plantations, five nests), Pindai (tropical dry forest, five nests), Koumac (mesophyll forest, four nests), and two sites in the locality of Mont Panié, Mont Panié 1 (tropical rainforest, two nests) and Mont Panié 2 (secondary forest, three nests). For each nest, at least 30 workers and most if not all of the reproductives present were collected. Queens were present in 68 of the 82 collected nests (one to 37 queens per nest), gynes (i.e., virgin female reproductives) were present in 14 nests (one to 23 gynes per nest), and males were present in seven nests (three to 29 males per nest). The distance between sampled nests was never less than 2 m. Four of these nests included inseminated queens, gynes, and males at the larval stage (i.e., three nests from Mont Panié and one nest from Bois du Sud). Only these latter four nests were previously analyzed by Fournier et al. (2005a) because they provided direct parent-offspring relationships and therefore allowed confirmation of more indirect evidence of clonality obtained from a large number of nests collected in French Guiana. The remaining 78 nests were specifically analyzed for the present survey.

Microsatellite Genotyping and Statistical Analysis

DNA was extracted from at least eight workers and all collected reproductives for each nest. We carried out genotyping at 12 microsatellite loci, as described by Fournier et al. (2005b). We also analyzed the spermathecal contents of 121 queens, as described by Chapuisat (1998). We genotyped 1488 specimens in total (queens, workers, males, and spermathecal contents). The number of genotyped individuals for each caste and site are presented in Table 1. The genotype dataset can be obtained from the authors upon request. Polymerase chain reaction products were separated on a MegaBace DNA sequencer (GE Healthcare, Uppsala, Sweden), and gel files were analyzed using Genetic Profiler (GE Healthcare).

To characterize the reproductive systems and the relation-

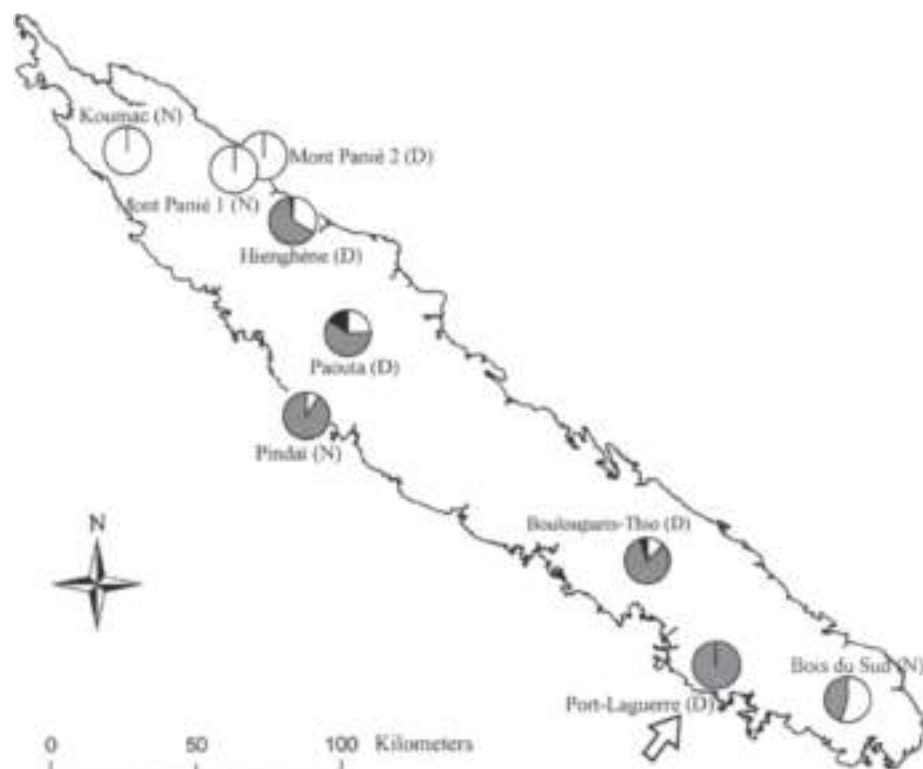


FIG. 1. Study sites and queen type distribution. White, Q0 queens; gray, Q2; black, Q3 queens (for details see results sections Main Modes of Reproduction of Reproductives and Alternative Modes of Reproduction of Reproductives). Ecological status of site in parentheses: D, disturbed; N, natural. The white arrow indicate the original introduction point (Port-Laguerre).

ships between genotypes, individual microsatellite genotypes were investigated visually and using three personal programs developed in the Pascal object programming language (inquiries about details of the programs should be sent to J. Foucaud). The first program was used to identify clones (i.e., identical multilocus genotypes) in a given sample of genotypes. The second program was used to compute the probability of observing identical individual genotypes assuming sexual reproduction given: (1) the number of heterozygous loci in the mother's genotype x ; (2) the number of genotyped diploid offspring y ; and (3) the number and size of groups of offspring with identical multilocus genotypes. More specifically, this program simulates the production through sexual reproduction of y diploid genotypes (without mutation)

TABLE 1. Number of genotyped queens, workers, males, and spermathecal contents for each sampled site.

Site	Queens	Workers	Males	Sperm	Total
Bois du Sud	161	104	7	52	324
Pindai	45	40	0	0	85
Mont Panié 1	42	40	16	12	110
Koumac	45	40	29	0	114
Boulouparis-Thio	55	159	0	21	235
Port Laguerre	102	199	0	16	317
Mont Panié 2	40	40	35	18	133
Hienghène	45	40	0	0	85
Paouta	45	40	0	0	85
Total	580	702	87	119	1488

from a mother's genotype x and compares the simulated number and size of groups of offspring with identical genotypes with those observed in the samples. This process is iterated 10^6 times, and the probability that the observed configuration can be produced by sexual reproduction alone is computed as the proportion of times the number of groups in the simulated dataset is at least as large as that in the observed dataset. A third personal program was used to construct dendrograms from individual genotypes using the neighbor-joining algorithm. The genetic distance used to construct the dendrograms was a variant of the Chakraborty and Jin's (1993) allele-shared distance, as defined in Fournier et al. (2005a). A four-level hierarchical F -statistics analysis was performed for the entire worker dataset using HierFstat (Goudet 2005). The hierarchical levels were individual, nest, site, and across New Caledonia.

RESULTS

Main Modes of Reproduction of Reproductives

For 203 of the 206 male genotypes, at least one other male in the sample displayed an identical genotype at all 12 microsatellite loci. These males clustered into five groups, comprising two to 93 genotypically identical individuals. Of 580 genotyped queens, 512 clustered into 30 groups of two to 242 individuals with identical genotypes at all 12 loci. These results indicate that most male and female reproductives were produced by means of two different clonal reproduction sys-

tems (i.e., clonality for males and thelytokous parthenogenesis for queens).

This pattern is consistent with the findings of Fournier et al. (2005a) in *W. auropunctata* populations from French Guiana (and four nests from New Caledonia, see Materials and Methods section). However, a neighbour-joining dendrogram including reproductives from this study and from the dataset of Fournier et al. (2005a) showed different relationships among the New Caledonian genotypes and among the Guianese genotypes (Fig. 2). Whereas Guianese genotypes gather together into well-defined clusters connected by long branches, the New Caledonian genotypes form a single dense aggregation of genotypes connected by short branches. This pattern indicates a closer coancestry and hence a more recent diversification of New Caledonian genotypes than Guianese genotypes. Moreover, New Caledonian male and queen genotypes do not form strictly separated clusters, as for the Guianese populations. The New Caledonian male and queen main clusters are connected via a number of intermediate queen genotypes, and one New Caledonian male genotype is nested within the New Caledonian queen cluster (Fig. 2). This pattern indicates that male and queen gene pools are not strictly separated in New Caledonia and that sexual reproduction may occur, probably at low frequency, in this area, in contrast to what has been previously found for the Guianese populations (Fournier et al. 2005a). Finally, we note that New Caledonian and Guianese genotypes form two completely distinct clusters, indicating that the introduced New Caledonian population does not originate from the previously sampled sites of French Guiana. Similar individual tree topologies were obtained using genetic distances traditionally used for microsatellite population data (i.e., Nei et al.'s [1983] D_A distance and Cavalli-Sforza and Edwards' [1967] chord distance; Takezaki and Nei 1996).

The five New Caledonian male clonal groups, plus two unique male genotypes (at the very bottom of the dendrogram in Fig. 2), differed from each other by a single locus, with one allele shorter or larger by a single dinucleotide repeat. This allelic pattern is typical of mutational events at microsatellite loci. The observed genotypes are therefore consistent with the transmission of a single male genome via a clonal reproduction system, which slowly diversifies through rare mutational events. These 205 males will be hereafter referred to as M0 males.

Of the 30 New Caledonian queen clonal groups, 29 included a moderate number of queens (from two to 38), whereas the last group was much larger (242 queens). The genotypes shared by the queens of one small group (three individuals) differed only slightly from that of the largest group, in that these queens were homozygous for one of the two alleles of the largest group at a single locus. Because the two original alleles differed by four dinucleotide repeats, this variation is more likely to result from a recombination event during thelytokous parthenogenesis (Haccou and Schneider 2004) than from a mutational event. The queens of these last two groups (245 of the 580 genotyped queens) will be referred to hereafter as Q0 queens.

Interestingly, Q0 queens and M0 males do not share any alleles at 11 of the 12 genotyped loci. This almost complete separation of the clonal Q0 and M0 gene pools is consistent

with the situation observed by Fournier et al. (2005a). Note that the four nests from New Caledonia analyzed in Fournier et al. (2005a) included only Q0 and M0 genotypes. We hence defined two categories of allele at the 11 loci differing between males and queens: Q0 alleles and their mutational derivatives were classified as "female" alleles, and M0 alleles and their mutational derivatives were classified as "male" alleles (Table 2). This classification facilitated further investigation of the evolutionary origin of non-Q0 queens and non-M0 males.

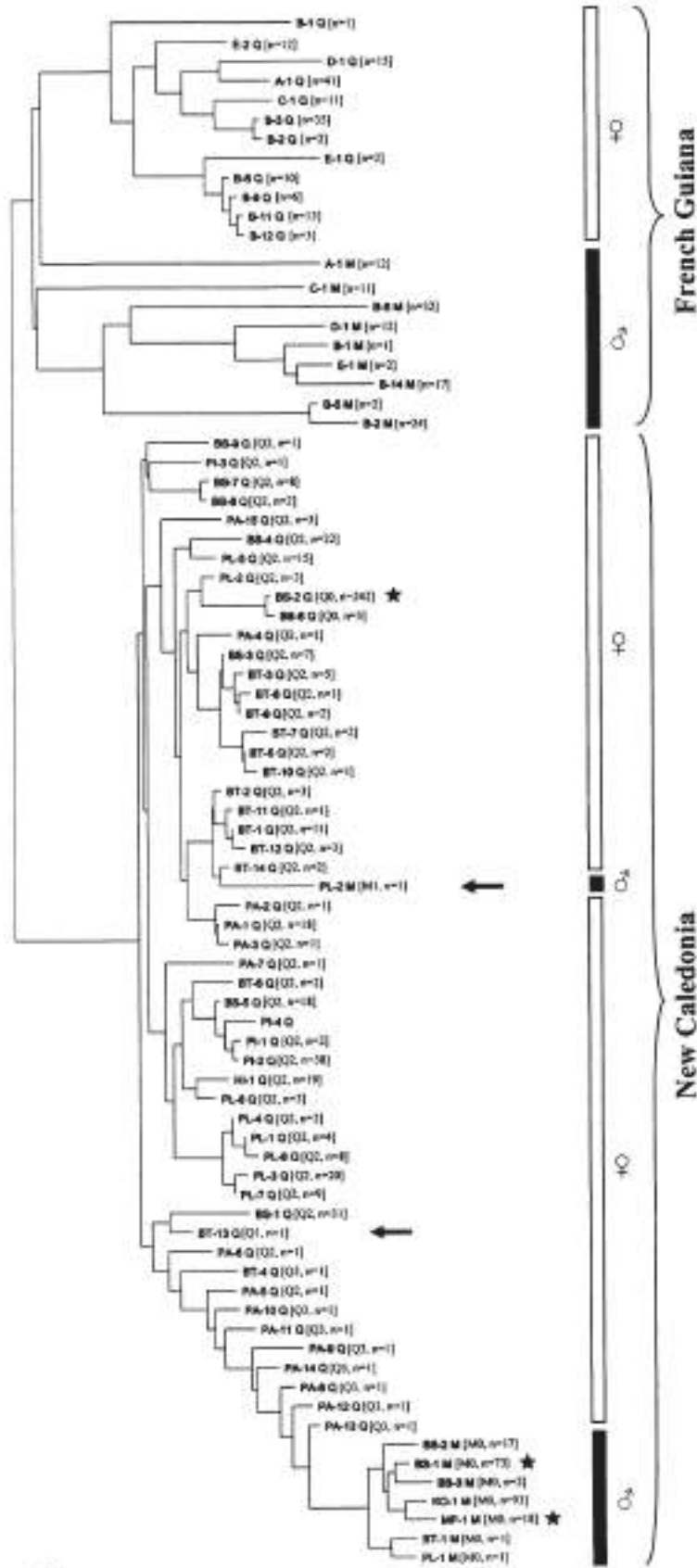
Alternative Modes of Reproduction of Reproductives

The 335 non-Q0 queens of our sample can be classified into three types according to the number of male alleles present in their genotypes (Fig. 3A).

First, only one queen in our sample, hereafter referred to as Q1, had 50% of male alleles and 50% of female alleles at the diagnostic loci and was, therefore, probably the result of a sexual reproduction event between a Q0 queen and a M0 male (which do not share any alleles, Figs. 3B and 4). This Q1 queen probably produced the only non-M0 male of our sample, hereafter named M1, by classic arrhenotokous parthenogenesis. This unique M1 male had 50% of male alleles and 50% of female alleles at the diagnostic loci. We must stress that no male bearing only female alleles was observed in our sample, indicating that Q0 queens do not produce any male through arrhenotokous parthenogenesis.

Second, we observed 323 queens (Q2 type) with three to 10 male alleles at the 11 diagnostic loci (Fig. 3A). This pattern probably reflects the occurrence of several independent sexual reproduction events between Q0 queens and different M1 male genotypes (Figs. 3B and 4). Finally, 11 queens (Q3 type) had 13 to 18 male alleles at the diagnostic loci (Fig. 3A). Such genotypes probably resulted from several Q2 \times M0 sexual events (Figs. 3B and 4).

Following their production by a Q0 \times M1 sexual reproduction event, we found evidence that most Q2 queens reproduce parthenogenetically. First, Q2 queens formed 28 groups of individuals showing identical genotypes (between two and 38 individuals per group, mean = 9.5). Secondly, all Q2 queens and their daughter gynes collected within the same nest displayed identical genotypes (11 occurrences in four different sites). All genotypes of the 56 Q2 queens that did not belong to any group of clones differed from one of the 28 Q2 clonal lineages by a single mutation step or a single recombination step, as defined above, indicating that they might also have been clonally produced. Some of the 28 clonal lineages also differed by a single mutation step or a single recombination step from each other. Altogether, these observations suggest that Q2 queens mainly reproduce parthenogenetically. Close examination of the relationships between multilocus queen genotypes indicated that the emergence of the 28 Q2 clonal lineages may be explained by 13 independent Q0 \times M1 sexual events, and 14 recombination and two mutation events consistent with parthenogenetic reproduction (Fig. 5). Moreover, as Q3 genotypes imply Q2 \times M0 reproduction events, at least 11 additional sexual reproduction events might have occurred in New Caledonian populations of *W. auropunctata*.



Unlike Q0 and Q2 queens, we found no evidence of clonal reproduction for Q1 and Q3 queens. None of the Q1 or the Q3 queens had strictly identical genotypes or genotypes that could be derived through recombination or mutation events. However, the small sample size for both Q1 (one individual) and Q3 (11 individuals) queens precludes any definitive conclusion on this point. Nonetheless, the strong underrepresentation of Q1 and Q3 queens in our sample may itself indicate that these queens could be unable to reproduce clonally.

Mode of Worker Production

Unlike reproductives, we found that the workers were produced by sexual reproduction. In the 31 nests in which the spermathecal content of queens has been genotyped, a majority of worker genotypes (63%) displayed an allelic segregation fully consistent with sexual production by the local queens and their sperm content. The rest displayed either a genotype that differed at a single locus with one allele shorter or larger by a single dinucleotide repeat, hence suggesting a mutational event at this locus (2%), or a genotype consistent with their sexual production in a neighboring nest (35%).

Although 78 groups of two to seven workers displayed identical genotypes, this pattern is still compatible with sexual reproduction being the main, if not the only means of producing workers. First, none of the workers in our sample of 702 individuals carried only female alleles, indicating that at least Q0 queens did not produce workers parthenogenetically. Second, 57 of these 78 groups included workers from geographically remote sites that could not have originated from the same mother queen. Third, the relatively small numbers of male and queen genotypes increases the probability of individuals produced by sexual means having identical genotypes. Simulation-based computations considering Q0-derived workers (Q0 queens being heterozygous at eight loci) showed that the probability of obtaining the observed pattern of groups of individuals with identical genotypes was equal to 0.995 when workers are assumed to be produced by sexual reproduction alone.

Invasion Scenarios

All non-Q0 lineages could derive from a single Q0 lineage and a single M0 lineage through sexual reproduction, recombination, and mutation events. This pattern is therefore consistent with the introduction into New Caledonia of a single Q0 genotype and a single M0 genotype.

A first alternative scenario would be that the originally introduced queen type is Q2 or Q3, and that Q0 is a derived

TABLE 2. New Caledonian "male" and "female" alleles at the 11 diagnostic loci. Frequent alleles (i.e., with a frequency >0.25 in the given sex) are in bold-italic characters, other alleles are rare (i.e., with a frequency <0.05). Note that frequent female alleles represent the Q0 genotype and that frequent male alleles represent the M0 genotype (see text for details). Male alleles 326 and 328 of locus *Waur-2164* have been found at high frequency and hence both appear in bold-italic characters. Classification of alleles 118 of locus *Waur-418* and 178 of locus *Waur-730* are tentative, because of their intermediate size between clearly defined male and female alleles. We classified them as male and female, respectively, because they were found in a single male and a single queen.

Locus	Male alleles			Female alleles		
<i>Waur-1gam</i>	298	300		288	294	
<i>Waur-2164</i>	324	326	328	330	288	290
<i>Waur-225</i>		225			223	
<i>Waur-275</i>		115			113	129
<i>Waur-3176</i>	232	234			224	226
<i>Waur-418</i>	118	120	122		102	116
<i>Waur-521</i>		200			206	208
<i>Waur-566</i>		267			263	
<i>Waur-680</i>	165	167			173	
<i>Waur-716</i>	190	192			184	196
<i>Waur-730</i>	174	176			156	158
					178	180

type. This scenario seems much less parsimonious than the above Q0 introduction scenario for at least three reasons. First, a male bearing only female alleles would be required to generate a Q0 queen from a Q2 queen. We never observed such a male genotype despite extensive sampling and the male clonal reproduction. Second, we observed only two groups of Q0 queens, with only slightly different highly heterozygous genotypes. Even if the Q0 queen type was to be a successful Q2-derived type, we would expect to observe many different Q0 genotypes with various level of heterozygosity (as found for the Q2 queen type). Third, generating a Q0 queen type from a Q3 type seems difficult, as this would require many different Q3 genotypes and a number of intermediate male and queen genotypes that were not observed in our sample.

A second alternative scenario would be that the observed genotype pattern is due to multiple introductions of related queens. Again, this scenario seems unlikely for two main reasons. First, given the large distance and the scarcity of trading between New Caledonia and the *W. auropunctata* native area, introduction events are unlikely to be frequent. Second, in its native range in South America, *W. auropunctata* is organized in a patchwork of small (generally less than 1 km²) colonies that are genetically different one from another (Fournier et al. 2005a). Therefore, the multiple intro-

FIG. 2. Neighbor-joining dendrogram of the genetic (allele-shared) distance between a subset of genotypes of reproductives from New Caledonia and genotypes of reproductives from French Guiana (Fournier et al. 2005a). White bars on the right indicate queens (Q) and black bars males (M). The queen type (Q0, Q1, Q2, Q3) or male type (M0, M1) and the number of identical genotypes (*n*) are indicated in brackets. The New Caledonian subset consists of all male and female clonal lineages (*n* > 1) and 24 randomly chosen unique genotypes of reproductives (*n* = 1). The unique M1 and Q1 genotypes of our sample are indicated with a black and a gray arrow, respectively. Black stars indicate the New Caledonian genotypes in Fournier et al. (2005a). New Caledonian sampling sites are given for each genotype by a two letter code: BS, Bois du Sud; PI, Pindaf; MP, Mont Panié; KO, Koumac; BT, Boulouparis-Thio; PL, Port Laguerre; HI, Hienghène; PA, Paouta (see Fig. 1). French Guiana sites are given by a single letter code as in Fournier et al. (2005a). The rooting shown should not be taken as evidence for evolutionary transitions and cannot be used for inference of the ancestral genotypes first introduced in New Caledonia.

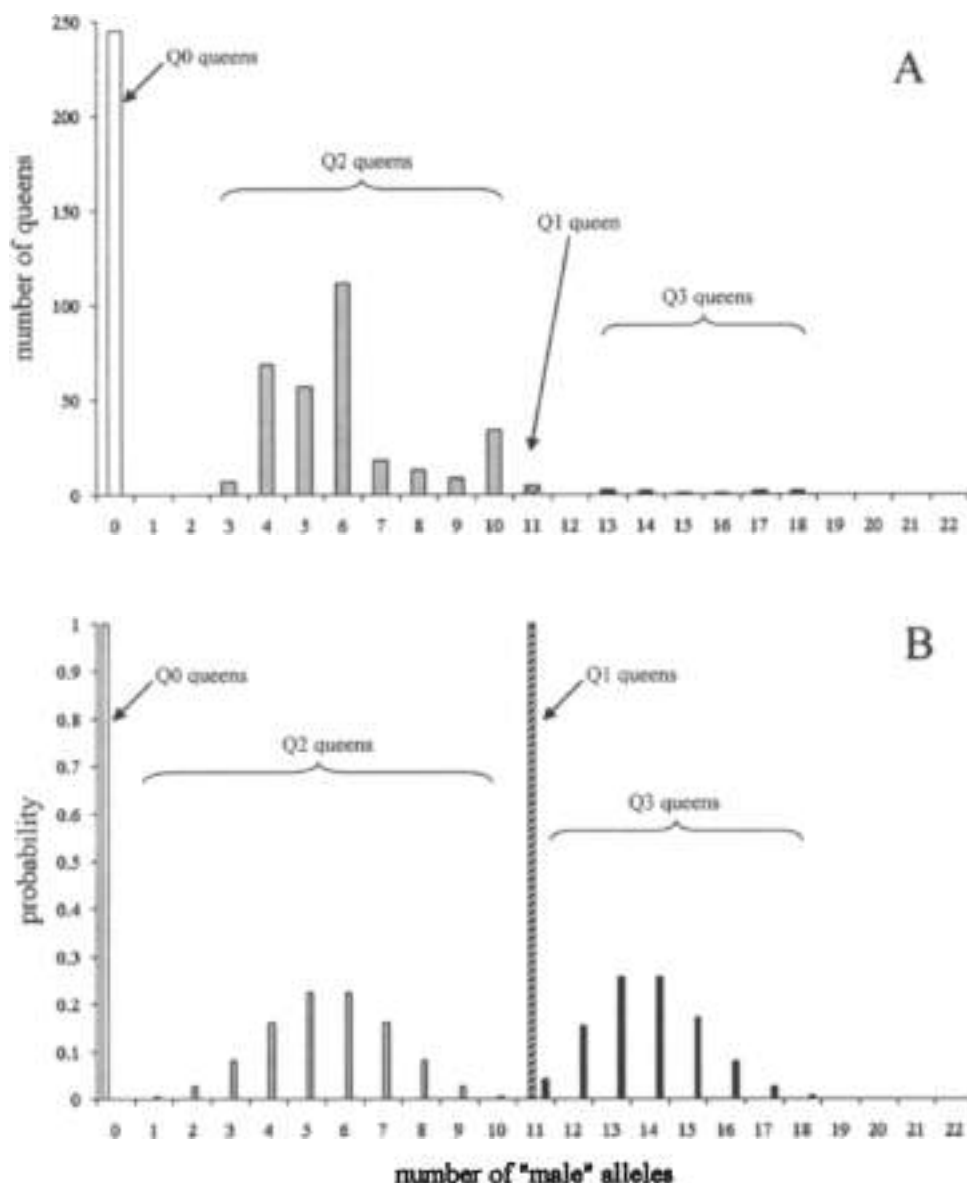


FIG. 3. Distribution of the number of "male" alleles in queens over 11 diagnostic loci: (A) observed distribution; (B) theoretical distribution. White, dashed, gray, and black bars indicate Q0, Q1, Q2, and Q3 queens, respectively. In (B), the probabilities of bearing i male alleles for Q2 queens and of bearing $i + 11$ male alleles for Q3 queens were analytically calculated as $P_{Q2}(i) = \sum_{j=0}^i C_{11}^j (1/2)^{11}$ and $P_{Q3}(i + 11) = \sum_{j=0}^i C_{11}^j (3/4)^{11-j} (1/4)^j$ respectively.

ductions postulated under the second alternative scenario are unlikely, because they should originate from a very small geographic area to account for the genotype pattern observed in New Caledonia.

Hence, an invasion scenario involving a single introduction of the Q0 genotype and the M0 genotype, which further differentiate through mutation, recombination, and sexual reproduction events represents the most parsimonious scenario to account for the observed genotype data.

Spatial Distribution and Dispersal of Reproductives

The initial introduction site, Port-Laguerre, is a no-Q0, all-Q2 site, whereas the sites the furthest (Koumac, Mont Panié

1 and 2) are all-Q0, no-Q2 sites (Fig. 1). The five remaining sites contained a mixture of Q0 and Q2 queens. The spatial distribution of queen types appears to be related to the distance from the original point of introduction. Although not statistically significant (but note that $n = 8$), a strong positive correlation could be observed between the queen type ratio $Q0/(Q0 + Q1 + Q2 + Q3)$ and the distance to the point of introduction (linear distance, $r = 0.66$, $P = 0.077$; distance by road, $r = 0.62$, $P = 0.10$). We considered both linear distance and distance by road because the large-scale migration of reproductives is thought to be mediated primarily by human transport (Passera 1994; Walsh et al. 2004).

The 28 Q2 clonal lineages were highly structured spatially,

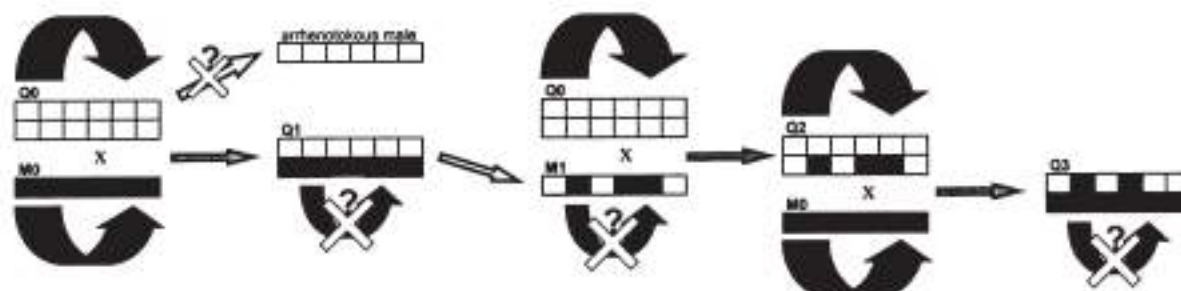


FIG. 4. Reproduction modes of reproductives in New Caledonian populations of *Wasmannia auropunctata*. Black arrow indicates clonal reproduction, gray arrow indicates sexual reproduction, white arrow indicates arrhenotokous parthenogenesis, crossed arrows with question mark indicate other potential but not observed reproductive modes, broad arrows indicate frequent events, and narrow arrows indicate rare events.

with 25 of these lineages found at a single site and most lineages differing by recombination or mutation events found at the same site (Fig. 5). This pattern indicates a low dispersal rate of queens in New Caledonian populations. Consistent with this, significant queen genetic differentiation was observed between sites ($F_{\text{site-total}} = 0.099$, $P < 10^{-4}$) and between nests within sites ($F_{\text{nest-site}} = 0.132$, $P < 10^{-4}$). A lower but significant level of worker differentiation was observed between sites ($F_{\text{site-total}} = 0.025$, $P < 10^{-4}$) and between nests within sites ($F_{\text{nest-site}} = 0.021$, $P < 10^{-4}$). The lower differentiation for workers than for queens is an expected outcome of the sexual production of workers involving almost exclusively clonal M0 males. Moreover, three of the five M0 clonal groups of males were widely distributed throughout New Caledonia, a pattern compatible with the occurrence of more frequent long-distance dispersal events for males than for queens.

DISCUSSION

A recent genetic survey of *W. auropunctata* restricted to a small geographic area of the native range of the species (French Guiana) and four nests from New Caledonia showed a unique reproduction system in which males and females reproduce separately through clonal systems, while only the workers are produced sexually (Fournier et al. 2005a). The present extensive survey (82 nests collected at various locations across New Caledonia) confirms that reproductives and workers were primarily produced by similar reproduction systems all over New Caledonia. However, we found that, unlike the populations in French Guiana (which only displayed clonal production of reproductives, leading to a strict separation of male and female gene pools), New Caledonian populations showed the hallmarks of rare sexual reproduction events in the production of queens and males. Such sexual

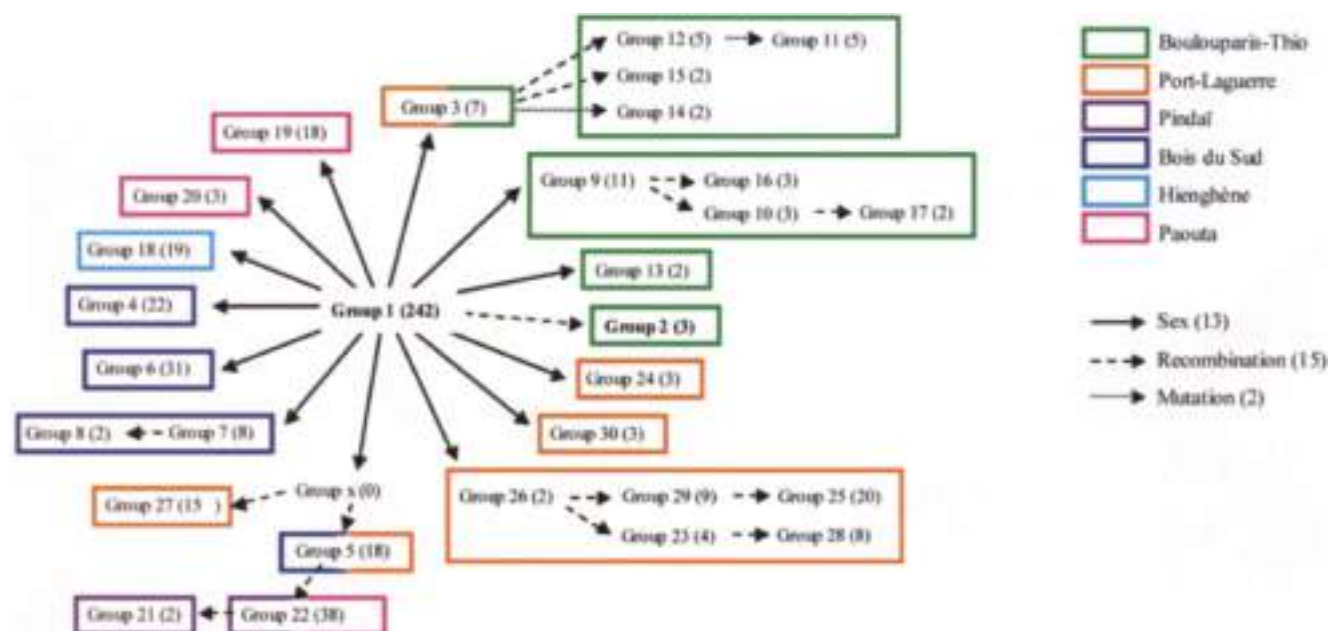


FIG. 5. Evolutionary origin and geographic distribution of the two Q0 and 28 Q2 clonal queen lineages found in New Caledonia. Q0 queens are shown in bold characters, the other queens are Q2 queens. The number of individuals in each group is shown in parentheses. A total of 13 sexual reproduction events, 15 recombination events (14 in Q2 lineages and one in the Q0 lineage), and two mutational events could be inferred. An additional 11 sexual reproduction events were inferred from Q3 queens (not shown).

reproduction events led to the production of new derived clonal lineages. As a consequence, although the separation of male and female gene pools remains strong, this separation is not complete in New Caledonian populations. *Wasmannia auropunctata* males and females therefore do not strictly form separate evolutionary units, at least in the particular historical and ecological context of New Caledonian introduced populations. Our findings also suggest the possibility of variation in the reproduction systems of *W. auropunctata*, with populations showing different rates of clonal versus sexual production of males and queens.

Only a single sampled queen and a single sampled male provided direct evidence of sexual reproduction events in introduced populations of New Caledonia (see Fig. 2). However, close examination of both the relationships between genotypes and the distribution of alleles in the different lineages observed in the 82 nests provided strong indirect evidences of additional rare sexual reproduction events, with a total of at least 24 sexual reproduction events inferred since the introduction of *W. auropunctata* in New Caledonia 40 years ago. Such a pattern could not have been inferred from the four nests from New Caledonia analyzed in Fournier et al. (2005a) because they contained only Q0 and M0 genotypes. Moreover, the relationships between the genotypes of the different clonal lineages observed in populations from French Guiana did not show the close coancestry and recent diversification through sexual reproduction events, as we have found for New Caledonian genotypes (Fig. 2). Although extensive datasets are now available for populations from both French Guiana and New Caledonia, laboratory experiments with controlled pedigrees would be particularly useful to definitively confirm the *W. auropunctata* reproduction systems and to study their proximate mechanisms. These experiments are under way in our laboratory. Given the occasional sexual reproduction events in New Caledonia, such events now seem more plausible than previously expected in both native and introduced populations of *W. auropunctata*.

Male Clonality

The male clonality pattern observed in *W. auropunctata* is consistent with a maternal genome elimination mechanism (i.e., the elimination of the maternal genome by the male genome in the egg after fertilization), as hypothesized in Fournier et al. (2005a). Although rarely observed, maternal genome elimination has been previously demonstrated in several species of *Corbicula* clams (Komaru et al. 1998) and in *Bacillus* stick insects (Scali et al. 2003). However, a mechanism of maternal genome elimination induced by the male genome after fertilization cannot easily explain the observed worker to male ratio in *W. auropunctata* nests (i.e., few if any males vs. several hundred workers per nest in most nests; data not shown). The large number of sexually produced workers implies that the maternal genome is not systematically eliminated by the male genetic contribution. Female control would therefore be required to account for the observed worker-to-male ratio in nests, either by queens at the level of fertilization or by workers at the level of egg policing (i.e., worker control on egg raising). A more parsimonious hypothesis would be that clonal males are the outcome of

normal fertilization of eggs lacking maternal genomic material, produced by a limited number of queens.

Our study demonstrated the lack of arrhenotokous male production by Q0 queens. Although this striking feature remains to be explained, we note that worker control over egg raising may account for this absence. Because workers share only 0.25 of their genes with arrhenotokous males and 0.50 of their genes with the competing M0 clonal males, kin selection theory (Hamilton 1964) predicts that workers should favor the rearing of M0 males.

Female Parthenogenesis

In Fournier et al. (2005a), only a single queen genotype among the 142 analyzed queens showed evidence of recombination, hence suggesting that queens from Guianese populations were produced by apomictic parthenogenesis (i.e., eggs produced from unreduced diploid cells, without meiosis). Our study (580 queens genotyped) reveals that 14 of the 30 clonal queen lineages sampled in New Caledonia were derived from independent heterozygote-to-homozygote locus recombination events, suggesting that queen parthenogenesis may be automictic (i.e., eggs produced from the fusion of two gametes, allowing restoration of diploidy) rather than apomictic. The rate of recombination was found to be low in *W. auropunctata*, resulting in the maintenance of high levels of heterozygosity. The low rate of recombination in *W. auropunctata* suggests that its female parthenogenesis mechanism might be automixis with central fusion, as recently found in *C. cursor* (Pearey et al. 2006) and in the automictic *A. mellifera capensis* subspecies (Baudry et al. 2004).

Not all queen types seem to reproduce parthenogenetically. We found no evidence of clonal reproduction for Q1 and Q3 queens, unlike Q0 and Q2 queens. The main difference between these two groups of queen types is that at least half of the genome of Q1 and Q3 queens is of male origin. This pattern suggests that female parthenogenesis may be under the control of a single locus, with two female alleles required for parthenogenesis, as recently found in *A. mellifera capensis* (Lattorff et al. 2005).

Genetic Determination of Caste

In ants, caste is usually considered to be environmentally determined and controlled by the workers (Crozier and Pamilo 1996). However, worker control is unlikely to account for the existing distribution of queen and worker genotypes in *W. auropunctata*. If this were the case, *W. auropunctata* workers should favor the rearing of their own sexually produced sisters (with which they share 0.75 of their genes) as queens. Instead, we found that clonally produced eggs, who share only 0.50 of their genes with workers, invariably develop into the queen caste. Alternatively, queens themselves could favor their clonal eggs to become queens and eliminate sexually produced queen larvae. However, queen control could not account for the pattern uncovered in the male sex, where virtually no queen alleles are transmitted. Caste determination may therefore be under genetic control in this species. Our analyses show that 98% of sampled queens have a high proportion of female alleles (75–100% female alleles, so that most if not all loci possess two female alleles) and that all

workers have a high proportion of male alleles (50–75% male alleles, with no loci possessing two female alleles). A model as simple as a single biallelic locus with homozygotes directed to one caste and heterozygotes to the other would account for the observed distribution of castes in *W. auropunctata*. Although rare in social insects, genetic caste determination has been demonstrated in harvester ants (*Pogonomyrmex rugosus-barbatus*; Cahan et al. 2004), for which a simple model like that the one suggested here may also account for the observed pattern of genetic dimorphism between queens and workers.

Colonization of New Caledonia

We found our genotypic data to be consistent with the introduction into New Caledonia of a single male and a single female genotype (i.e., the M0 and Q0 genotypes, respectively). Genetically based cues are often assumed to enable nestmate recognition in ants (Vander Meer and Morel 1998). The genetic homogeneity at recognition loci resulting from the extreme bottleneck that occurred in New Caledonia may be insufficient to induce aggressive interactions between non-nestmates (Tsutsui et al. 2000), leading to the formation of a single 450-km-long supercolony (Le Breton et al. 2004).

The geographic distribution of clonal lineages in New Caledonia differs greatly from that observed in French Guiana (Fournier et al. 2005a). The latter populations are characterized by several small supercolonies (a few tens to hundreds of meters long), each one headed by a single male-female pair of genotypes different from that of the neighboring supercolonies. The human-mediated displacement of a small fraction of a single local supercolony from the native range would logically result in the introduction of a single male-female pair of genotypes, as inferred from our data in New Caledonia. Once an introduced propagule has successfully developed, the time to the next introduction event may be sufficiently long for the first propagule to saturate the environment, hence preventing any following propagule from establishing themselves, even if better adapted to the new environment (Tsutsui et al. 2003). Given that New Caledonian habitats are largely saturated by the Q0-M0 clone pair and its derived clonal lineages, the successful establishment of other *W. auropunctata* introduction propagules on the island seems unlikely in the near future.

Unicoloniality is repeatedly cited as one of the most important biological features of invasive ant species, including *W. auropunctata* (Passera 1994; Jourdan et al. 2001; Holway et al. 2002; Tsutsui and Suarez 2003; Le Breton et al. 2004). By definition, an unicolonial ant species is organized into networks of geographically separated nests cooperating as single units (Markin 1968). Several recent genetic studies of unicolonial ant species uncovered a low level of relatedness between workers of the same supercolony, contrary to expectations of kin-selection theory predicting that such situations can be evolutionarily unstable (Chapuisat and Keller 1999; Gizaud et al. 2002; Tsutsui et al. 2003; Elias et al. 2005). The genetic data obtained for *W. auropunctata* populations (this study; Fournier et al. 2005a) show that the unicoloniality of the species is rather unique as compared to other known unicolonial species (Holway et al. 2002; Tsutsui

and Suarez 2003). The clonal reproductive system of *W. auropunctata* provides each supercolony with a family structure genetically identical (cf. Guianese populations, Fournier et al. 2005a) or close (our New Caledonian populations) to a monogynous-monoandrous colony for which the absolute worker relatedness is theoretically expected to be high (i.e., ≈ 0.75). Kin selection theory predicts that the high-relatedness unicoloniality displayed by *W. auropunctata* might be evolutionarily more stable and hence persist over longer time than other known unicolonial ant species. The clonal reproduction system may combine the advantage of social cohesion due to high absolute relatedness with the ecological advantage of being demographically polygynous-polyandrous. This system may therefore constitute a key factor of the invasive success of *W. auropunctata*.

Emergence of Sexual Reproduction

Apart from the size of the supercolonies, the main difference between New Caledonian and Guianese populations of *W. auropunctata* is the occurrence of sexual reproduction events in New Caledonia. The initial introduction site of *W. auropunctata* in New Caledonia (Port-Laguette) now only comprises sexually derived Q2 clones, suggesting that the original Q0 clone may have been completely replaced at this site. The spatial distributions of Q0 and Q2 + Q3 queens throughout New Caledonia suggests that this putative replacement of the original Q0 clone by sexually derived queens could be a local process currently occurring all over the island. The source population of the New Caledonian supercolony is currently unknown and would be difficult to sample due to the high level of genetic structure observed over short distances in native populations of the species (Fournier et al. 2005a). Therefore, we cannot determine from our data whether rare sexual reproduction events are positively selected or developmentally constrained (as defined by Maynard Smith et al. 1985).

Experimental studies focusing on putative differences of fitness between queen types are needed to assess whether sexual reproduction is beneficial. Temporal surveys of the demography and queen type ratio in New Caledonia could also shed light on the evolution of this supercolony of large size. Together, such studies might lead to a better understanding of the cause and consequences of the emergence of sexual reproduction in the New Caledonian *W. auropunctata* supercolony.

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