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Sex and Clonality in the Little Fire Ant

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Reproduction systems are controlling the creation of new genetic variants as well as how natural selection can operate on these variants. Therefore, they had historically been one of the main foci of evolutionary biology studies. The little fire ant, *Wasmannia auropunctata*, has been found to display an extraordinary reproduction system, in which both males and female queens are produced clonally. So far, native sexual populations of *W. auropunctata* have not been identified. Our goals were to identify such sexual populations and investigate the origins of female parthenogenesis and male clonality. Using mitochondrial DNA and microsatellite markers in 17 native populations, we found that traditional sexual populations occurred in *W. auropunctata* and are likely the recent source of neighboring clonal populations. Queen parthenogenesis has probably evolved several times through mutational events. Male clonality is tightly linked to queen parthenogenesis and thus appears to be female controlled. Its origin could be accounted for by 2 mutually exclusive hypotheses: either by the expected coevolution of the 2 sexes (i.e., a variant of the maternal genome elimination hypothesis) or by a shared mechanistic origin (i.e., by the production of anucleate ovules by parthenogenetic queens). Our results also show that *W. auropunctata* males and females do not form separate evolutionary units and are unlikely to be engaged in an all-out battle of sexes. This work opens up new perspectives for studies on the adaptive significance and evolutionary stability of mixed sexual and clonal reproduction systems in living organisms.

Introduction

Living organisms reproduce through a diversity of genetic systems. A significant proportion of these systems can be found in insects, in which diplodiploidy, haplodiploidy, thelytoky, mixed genetic systems, and other rare extrazygotic inheritance mechanisms have been found (Normark 2003). Studies of the origins and mechanisms of such genetic systems are of fundamental importance as they may help to resolve classical evolutionary issues, such as the paradox of sex (Maynard Smith 1978; Howard and Lively 1994; Kondrashov 2001) and the notion of species (Barraclough et al. 2003; Fontaneto et al. 2007). The little fire ant, *Wasmannia auropunctata*, is exceptional in that it displays as many as 3 different genetic systems: haplodiploidy, thelytoky, and male clonality (Fournier et al. 2005a; Foucaud et al. 2006). This myrmicine ant, ranked among the world's worse invasive species (Lowe et al. 2000), is currently widely distributed over all tropics (Wetterer and Porter 2003). In tropical Central and South America where *W. auropunctata* is native, Fournier et al. (2005a) found that female queens reproduced through thelytokous parthenogenesis and males reproduced through an unknown clonal system, whereas the sterile female workers were produced sexually.

All native populations of the little fire ant studied so far have been found to be clonal (i.e., display both parthenogenesis and male clonality). However, this situation may not apply to all populations of the native range for at least 3 reasons. First, most eukaryotic species displaying mainly clonal populations also retain sexual populations, from which new clonal lineages can arise repeatedly (Simon et al. 2003). Second, it has recently been shown that, although clonal production of reproductives (i.e., queens and males) is largely predominant, some rare sexual reproduction events also occur in populations of the introduced range of *W. auropunctata* (Foucaud et al. 2006). Finally, as previous studies of the *W. auropunctata* reproduction system in its native range were based on small sampling campaigns (5 sites in only one country), putative sexual populations may well have remained unsampled.

The conditions favoring the emergence and simultaneous maintenance of both parthenogenesis and male clonality are largely unknown. The unusual clonal reproduction system of both sexes led to the hypothesis that *W. auropunctata* males and females might be engaged in an all-out evolutionary battle of sexes as male clonality could be seen as a male strategy to counteract the reduction of male fitness by female parthenogenesis (Fournier et al. 2005a). Some authors also argued that *W. auropunctata* males and females might even belong to 2 separate species (Queller 2005). The existence (or absence) of sexual populations and their relationship to clonal populations may provide insights into the origins and conditions of coexistence of the clonal reproduction systems of the little fire ant. There is evidence for 4 main routes to parthenogenesis in animals (Simon et al. 2003). Parthenogenesis may be of spontaneous (i.e., due to the mutation of genes involved in meiosis; Turgeon and Hebert 1994; Johnson and Leefe 1999), contagious (i.e., due to recurrent crossings between parthenogenetic and sexual lineages of the same species; Hebert 1981; Pongratz et al. 1998; Schneider et al. 2002), infectious (i.e., due to vertically inherited microorganisms, such as *Wolbachia* or *Cardinium*; Werren et al. 1995; O'Neill et al. 1997; Zchori-Fein et al. 2001; Huigens and Stouthamer 2003; Groot and Breeuwer 2006), or hybrid (i.e., due to crosses between 2 distinct sexual species; Moritz 1991; Quattro et al. 1992; Spolsky et al. 1992; Delmotte et al. 2003) origin. The origin of parthenogenesis in *W. auropunctata* is currently unknown. The origin of male clonality also remains unresolved, although it has been suggested that male clonality may be an evolutionary

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Key words: reproduction system, sex, parthenogenesis, male clonality, *Wasmannia auropunctata*.

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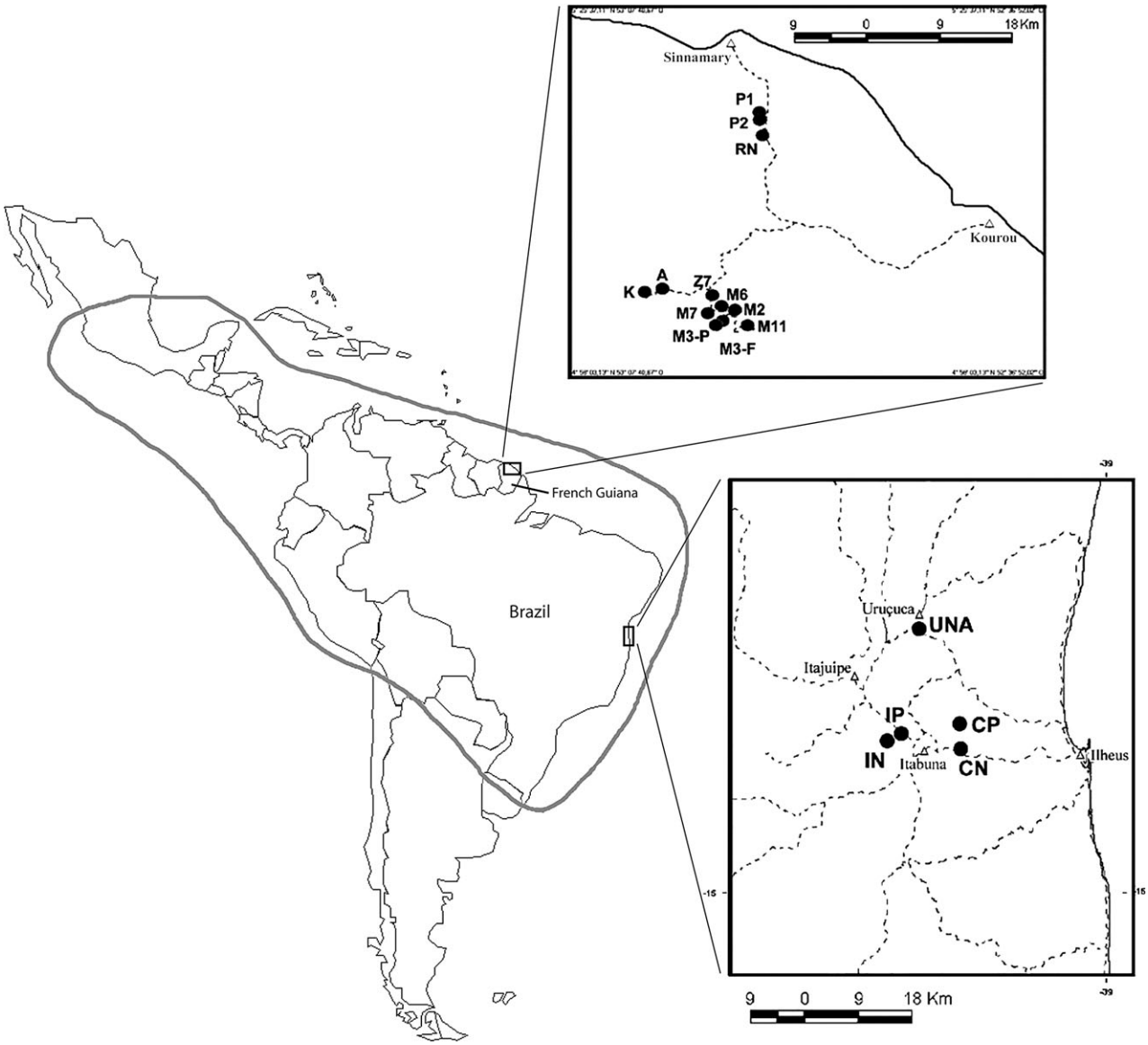

FIG. 1.—Localization of the 17 sampled sites. The large gray line indicates the native range of *Wasmannia auropunctata*. In each frame, sampled sites are indicated with black dots and their code. Plain and dashed lines indicate coastline and roads, respectively. The number of sampled nest and genotyped individuals is detailed in table 1.

response by males to the reduction of their fitness by female parthenogenesis (Fournier et al. 2005a).

The mechanisms of parthenogenesis and male clonality in *W. auropunctata* remain a matter of speculation, whereas their determination is of prime importance as it would provide insights into the origins of the 2 systems and make it possible to evaluate the evolutionary stability of male–female clonal populations. To date, male clonality is hypothesized to result from the elimination of the maternal genome by the paternal genome during fertilization (Fournier et al. 2005a), and thelytokous parthenogenesis has been successively hypothesized to be apomictic (i.e., without meiosis; Fournier et al. 2005a) and automictic with central fusion (i.e., with meiosis; Foucaud et al. 2006). However, more data are required to evaluate these hypotheses.

The present study had 2 main aims: 1) to determine whether sexual and potentially ancestral populations of *W. auropunctata* exist in its native range and 2) to gain insight into the origin and mechanisms of the *W. auropunctata* thelytokous parthenogenesis and male clonality systems. To address these questions, we extensively sampled native populations in Brazil and French Guiana (see fig. 1 and table 1), sequenced a region of a mitochondrial DNA (mtDNA) gene and genotyped individuals at 12 microsatellite loci.

Materials and Methods

Field Collection

Fieldwork was carried out in areas of Brazil and French Guiana within the native range of *W. auropunctata*

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Table 1
Sampling and Genotyping Design of *Wasmannia auropunctata* Populations

Country	Site	Sampled Nests	Number of Genotyped		
			Female Reproductives	Males	Workers
Brazil	CN	20	47	35	135
Brazil	CP	16	144	62	152
Brazil	IN	10	9	8	80
Brazil	IP	10	50	30	80
Brazil	UNA	10	0	0	80
French Guiana	M2	8	27	16	126
French Guiana	M3-F	10	26	6	120
French Guiana	M3-P	13	6	5	128
French Guiana	M6	3	10	24	48
French Guiana	M7	15	24	25	168
French Guiana	M11	10	3	3	80
French Guiana	Z7	6	3	1	48
French Guiana	A	5	17	11	40
French Guiana	K	5	29	27	40
French Guiana	P1	4	40	11	32
French Guiana	P2	17	62	57	136
French Guiana	RN	6	11	11	48
Total		168	508	332	1,541

NOTE.—Geographic locations of sampled sites are given in figure 1.

(fig. 1). These areas are separated by approximately 2,650 km. In total, 168 nests (i.e., an aggregation of workers, brood, and/or queens within a woodstick or between dead leaves) were collected in 2004 and 2005 in Brazil (66 nests from 5 sites) and French Guiana (102 nests from 12 sites). The distance between sampled nests was always larger than 2 m. 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; fig. 1). The 17 sampled sites are representatives of various types of habitat, including plantations, roadsides, primary forest, and natural backwater areas. The number of collected nests per site varies from 3 to 20 (mean ± SD: 10 ± 5 nests). In all, 34 of the 168 collected nests, from 5 sites in French Guiana, were previously analyzed by Fournier et al. (2005a). The other 135 nests were specifically sampled and analyzed for this study.

For each nest, at least 30 workers and most if not all the reproductives were collected. Voucher specimens from the investigated Brazilian nests were deposited at the UESC Genetics Laboratory Ilhéus, Brazil. Queens were present in 107 of the 168 collected nests (1–23 queens per nest), gynes (i.e., virgin female reproductives) were present in 6 nests (1–16 gynes per nests), and males were present in only 2 nests (1 and 15 males per nest).

Microsatellite Genotyping and mtDNA Sequencing

For each nest, individual DNA extractions were processed for all collected reproductives and at least 8 workers. These individuals were genotyped at 12 microsatellite loci, as described by Fournier et al. (2005b). We also analyzed the spermathecal contents of 299 queens, as described by Chapuisat (1998). We genotyped 2,381 specimens in total (queens, gynes, workers, males, spermathecal contents, and sexual larvae). The number of genotyped individuals for each caste is presented in table 1. Polymerase chain reaction (PCR) products were separated on a MegaBace DNA sequencer (GE Healthcare Bio-Sciences, Uppsala, Sweden), and gel files were analyzed using GENETIC PROFILER (GE Healthcare Bio-Sciences).

We obtained mtDNA sequences for 93 individuals from both Brazil and French Guiana and 3 individuals of the closely related species *Wasmannia rochai* as outgroup (GenBank accession numbers EF459732–EF759824). We used PCR to amplify a 520-bp region of the cytochrome oxydase I gene with the primers LCO and HCO (Folmer et al. 1994). PCR mixtures contained 1.0 μl of DNA solution, 0.1 μl $MgCl_2$ (25 mM), 0.4 μl deoxynucleoside triphosphate s (10 mM), 1.0 μl 10× Qiagen *Taq* Buffer, 0.2 μl of each primer (10 μM), 5 units of Qiagen *Taq* polymerase, and 7 μl H_2O. Thermal cycling conditions were as follows: denaturation at 95 °C for 3 min, then 37 cycles of denaturation at 94 °C for 1 min, annealing at 55 °C for 1 min, and extension at 74 °C for 1 min, followed by a final extension at 74 °C for 10 min. PCR products were purified and sequenced by Macrogen Inc. (Seoul, South Korea).

Statistical Treatment of Data

Phylogenetic analysis of the mtDNA data was conducted under the maximum likelihood (ML) optimality criterion. We also performed phylogenetic analyses using distance, maximum parsimony, and Bayesian methods, which yielded similar topologies (not shown). The best model under the likelihood criterion (and the associated parameters) was obtained using Modeltest v3.7 (Posada and Crandall 1998). The best-fit ML tree was further reconstructed using PHYML v2.4.4 (Guindon and Gascuel 2003). Nonparametric bootstrapping (Felsenstein 1985) was performed with 1,000 replicates using the SEQBOOTand CONSENSE programs of the PHYLIP v3.6 package (Felsenstein 1989). We additionally used the likelihood-based nonparametric Shimodaira–Hasegawa (SH) test (Shimodaira and Hasegawa 1999) to compare our phylogeny

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(i.e., the best-fit ML tree) with an alternative phylogeny in which clonal and sexual individuals were constrained to form 2 distinct monophyletic groups. The constrained tree was built using TREEVIEW v1.6.6 (Page 1996). For both phylogenetic hypotheses, branch lengths were further reestimated in PAUP* v4.0b10 (Swofford 2003) using the previous substitution model parameters. The reestimated log likelihoods method (Kishino et al. 1990), as implemented in PAUP*, was used to resample the log likelihoods (1,000 replicates) in the SH test.

We characterized the reproductive systems and the relationships between genotypes by investigating individual microsatellite genotypes visually and using 2 programs we developed in the Pascal object programming language (inquiries about details of the programs should be sent to the corresponding author). The first program was used to identify clones (i.e., identical multilocus genotypes) in a given sample of genotypes and to compute basic population genetic statistics (i.e., number of alleles, observed heterozygosity, and mean difference in allele size within multilocus genotypes). Within-individual difference in allelic size, DS, was computed as the difference in base pairs between the 2 alleles at a given locus of a single individual, averaged over loci. The second program was used to construct dendrograms from individual genotypes using the Neighbor-Joining (NJ) algorithm (Saitou and Nei 1987). The genetic distance used to construct the dendrograms was a variant of allele-shared distance of Chakraborty and Jin (1993), as defined in Fournier et al. (2005a).

Results

Reproduction Systems

On the 508 genotyped female reproductives (i.e., queens, gynes, and female sexual larvae), 268 cluster in 20 groups of genotypes, identical at all 12 microsatellite loci (2–50 queens per group), and hence show direct evidence of clonality (see supplementary table S1 [Supplementary Material online] for an illustration). These clonal queens were found in 64 nests. On the 332 genotyped male reproductives (i.e., males, spermathecae contents, and male sexual larvae), 205 cluster in 24 groups of identical genotypes (2–34 males per group). These clonal males were distributed in 63 nests.

We also indirectly inferred the occurrence of clonal reproduction in 24 other nests. This was the case when genotyped reproductives differed from known clonal reproductive genotypes either 1) by only one dinucleotide repeat at one of the 12 genotyped loci (as this pattern is likely to correspond to one mutational event at a microsatellite locus) or 2) by homozygosity for one allele at a single heterozygous locus of the clonal queen genotype (as this pattern probably corresponds to a recombination event during thelytoky). We also considered a nest to be clonal if, in the absence of sampled reproductives in this nest, the male and female reproductives inferred from the genotypes of workers were identical to known clonal reproductive genotypes. In total, we identified 87 clonal nests among the 168 sampled (63 and 24 presenting direct and indirect evidence of clonality, respectively), located both in Brazil and French Guiana. In all but 2 nests, if one sex was found to be clonal, the other sex was also found to be clonal. Because ant queens keep the sperm of their mate in a vesicle (the spermatheca), we were able to determine the genotypes of the mates of 299 queens (corresponding to 110 different queen genotypes). All males mated to the 210 clonal queens of this sample were clonal.

Most importantly, we also obtained direct evidence of classical sexual production of reproductives in 39 nests from both Brazil and French Guiana. The female reproductives of these nests never have identical genotypes but clearly display sexual recombination patterns of the same allelic pool (see supplementary table S1 [Supplementary Material online] for an illustration). Some of these queens show allelic patterns consistent with full-sister relationships expected under sexual reproduction of only one mating pair (i.e., sharing 75% of their alleles). The genotypes of the male reproductives of these nests almost always bear alleles found in the female reproductives of the same nest, consistent with arrhenotokous production (i.e., unfertilized meiotic eggs developing into haploid individuals). In these populations, the occurrence of sexual production of reproductives is also clear because queens and workers are indistinguishable on the basis of their genotypes and because males and females share all their alleles (i.e., the male and female gene pools are completely mixed, as expected under sexual reproduction).

Nests are either clonal or sexual. We almost never found sexually and clonally produced reproductives in the same nest. In all but 2 nests ($n = 168$), if clonal males were sampled, clonal females were also sampled. In the 2 remarkable exceptions, we found direct evidence of sexual production of the female reproductive and some males bearing female alleles and other males displaying identical multilocus genotypes (i.e., arrhenotokous and clonal males, respectively). We never found clonal and sexual nests mixed within a single population. On the contrary, clonal and sexual nests were spatially separated into different populations. Thus, male clonality and female parthenogenesis are almost strictly associated, both at the nest and populational level.

The type of reproduction system could not be unambiguously determined for 42 nests. These nests lacked reproductives at the time of collection, and either the parental genotypes could not be confidently inferred from individual worker genotypes or the suggested parental genotypes did not match any known clonal or sexual genotype.

All the individual genotypes of workers in our samples are consistent with their sexual production both in clonal and sexual nests (see supplementary table S1 [Supplementary Material online] for an illustration). In clonal or sexual nests containing a single male–female couple, all workers display a pattern of allelic segregation fully consistent with their sexual production by the local male and female genotypes.

Relationships between Clonal and Sexual Genotypes

The ML tree obtained from a 520-bp region of the mtDNA COI gene shows that clonal and sexual groups

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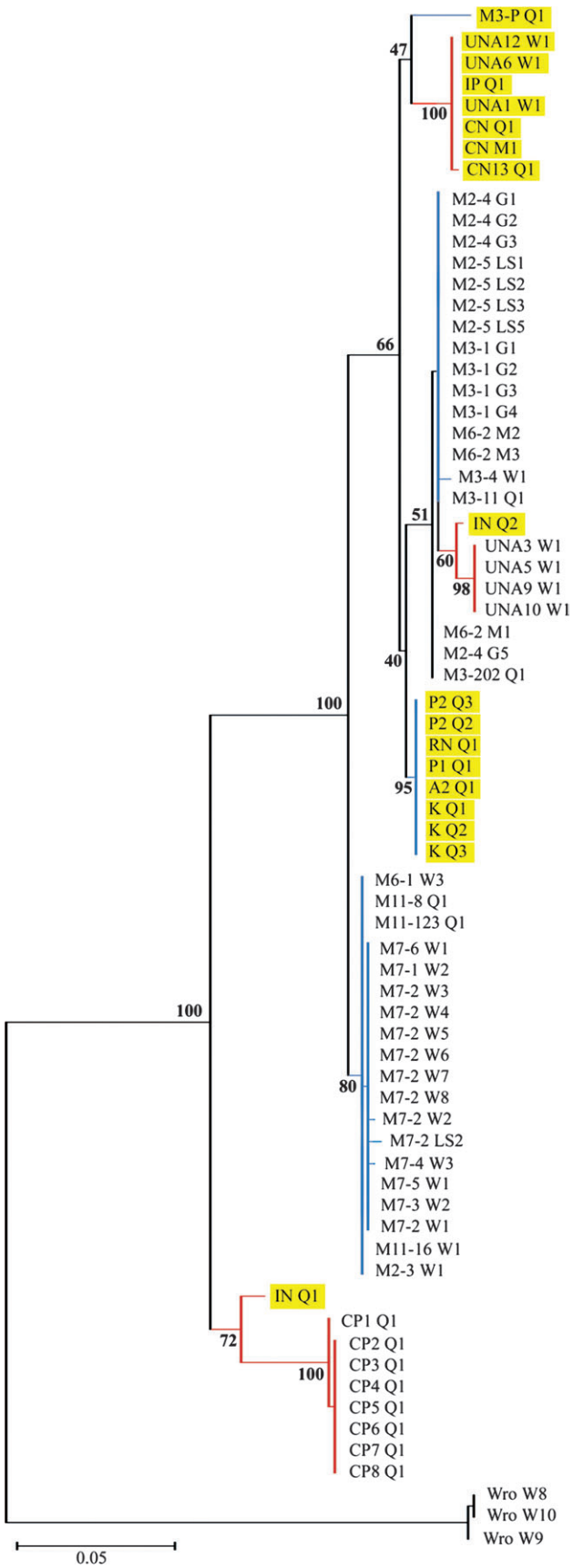

of individuals do not form 2 distinct clusters (fig. 2). This finding is confirmed by the SH test, which indicated a significant lack of support for the clustering of individuals as a function of reproduction system (SH test: $P < 10^{-3}$). Whatever the reproduction system, all groups of haplotypes are very similar to each other within the *W. auropunctata* clade (the mean pairwise distance between clonal and sexual haplotypes is 1.8% using the K2P model) although being extremely different from the closely related outgroup species, *W. rochai* (mean pairwise distance of 16.9% using the K2P model).

The NJ trees obtained from individual queen and male microsatellite genotypes also show that clonal and sexual groups of individuals do not form 2 distinct clusters (fig. 3). In agreement with this finding, almost all alleles of the clonal queens are observed in local sexual populations of *W. auropunctata*. Likewise, all Brazilian and 6 of the 8 Guianese clonal male genotypes display alleles present in local sexual populations.

Altogether, this pattern of genetic variation indicates that clonal and sexual types do not represent 2 genetically distinct evolutionary units of *W. auropunctata* and that clonal reproductives most probably arose recently from local sexual populations. The alternative scenario of a single origin of asexuality followed by multiple recent reversions to sexuality would be far less parsimonious to account for this pattern of genetic variation. A single origin of asexuality is unlikely because at least some groups of clonal queens are more distantly related than groups of sexual queens, as shown by both mtDNA and microsatellite data. Multiple reversions from asexuality to sexuality are also unlikely because the microsatellite allelic pools of sexual populations are far more diverse than those of clonal populations.

The microsatellite NJ tree of individual queens also indicates that clonal queen genotypes are likely to share a recent ancestor within each country. Visual inspection of those genotypes reveals that the clonal queen clusters could correspond to groups of full sisters that slightly diverged during successive generations of clonality, through a few mutation and parthenogenetic recombination events (results not shown). It is also worth noting that observed heterozygosity (H_o) and mean difference in the size of the 2 alleles observed at each locus (DS) are not significantly different between clonal and sexual queens (Mann–Whitney *U* tests:

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FIG. 2.—Best-fit ML tree of individual haplotypes of the mtDNA COI gene. Branch lengths are included. The best model of evolution (HKY + G; Hasegawa et al. 1985) was determined using Modeltest 3.7. Nonparametric bootstrap values are provided for major nodes. Each represented individual corresponds to a unique microsatellite genotype (clones are hence only represented once). Clonal genotypes (assessed by microsatellite data) are highlighted in yellow and sexual genotypes (also assessed by microsatellite data) are not highlighted. Blue and red branches correspond to individuals collected in French Guiana and Brazil, respectively. Names of the *Wasmannia auropunctata* individuals were coded as follows: name of the site, number of nest (except for clones present in several nests), type of individual (Q = queen, G = gyne, M = male, LS = larval stage, W = worker), and number of individual. Three individuals of the closely related species *Wasmannia rochai* (coded Wro) were used as outgroup.

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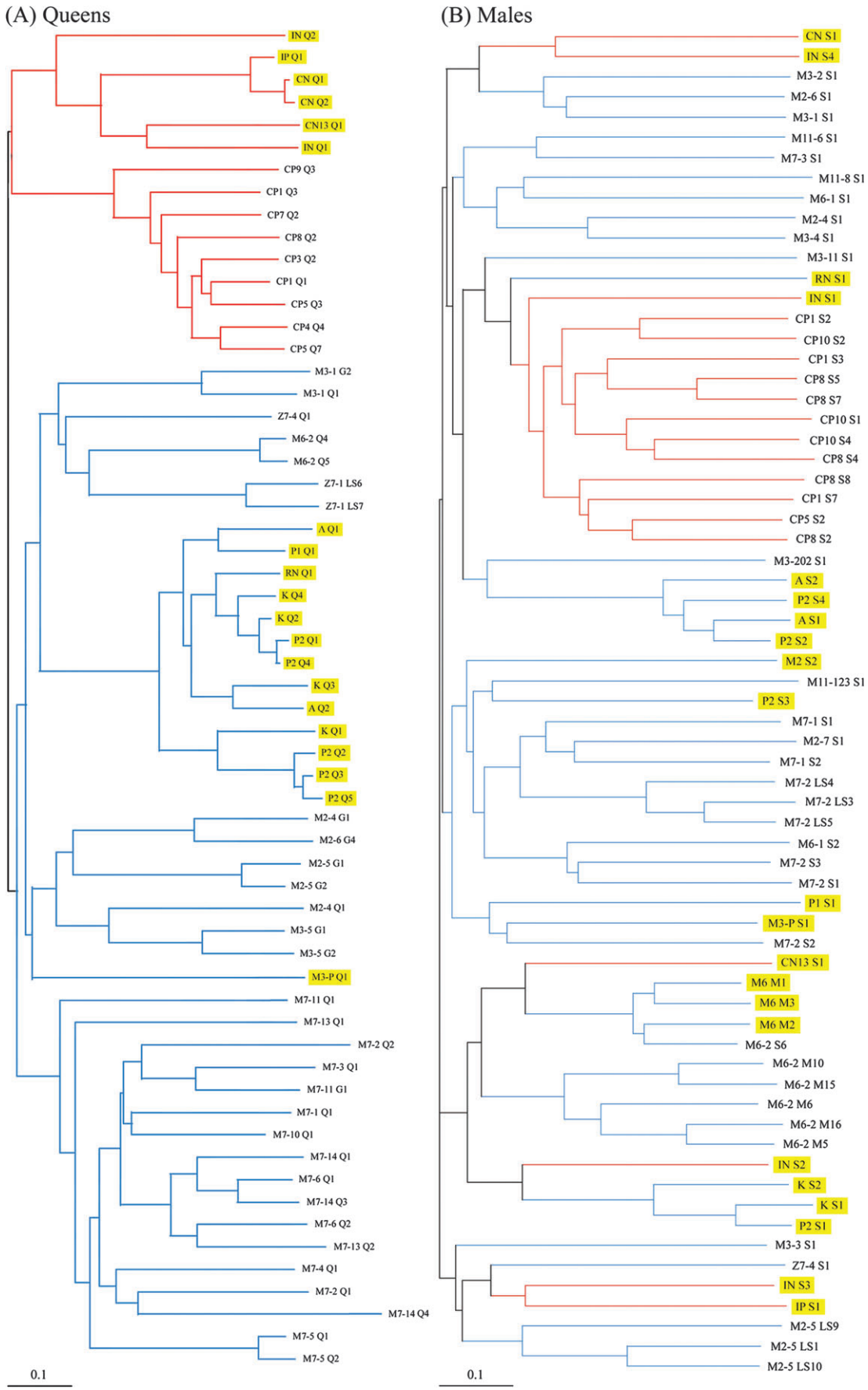

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$P = 0.34$ and $P = 0.50$ for H_o and DS, respectively; fig. 4). Thus, the parents of the clonal queens do not differ more genetically than the parents of the sexual queens. Therefore, hybridization is unlikely to be involved in the origin of parthenogenesis in the little fire ant. Altogether, our results suggest that queen parthenogenesis in *W. auropunctata* has arisen several times, through several independent mutational or infectious events within local sexual populations.

Assuming that male clonality has emerged as a male response to their reduction of fitness by female parthenogenesis (Fournier et al. 2005a), we would expect there to be only one or a few clonal male lineages because it is unlikely that such a male response would evolve independently a large number of times. Contrary to this expectation, the microsatellite NJ tree of individual male genotypes shows that clonal males do not cluster into groups of individuals of close coancestry but are widely dispersed among sexual males. Regarding the issue of the genetic relationship between *W. auropunctata* males and queens (Queller 2005), both the ML tree of the COI gene and an NJ tree of individual queens and males microsatellite genotypes show that queens and males do not form 2 distinct evolutionary units (fig. 2 and supplementary fig. S1 [Supplementary Material online]).

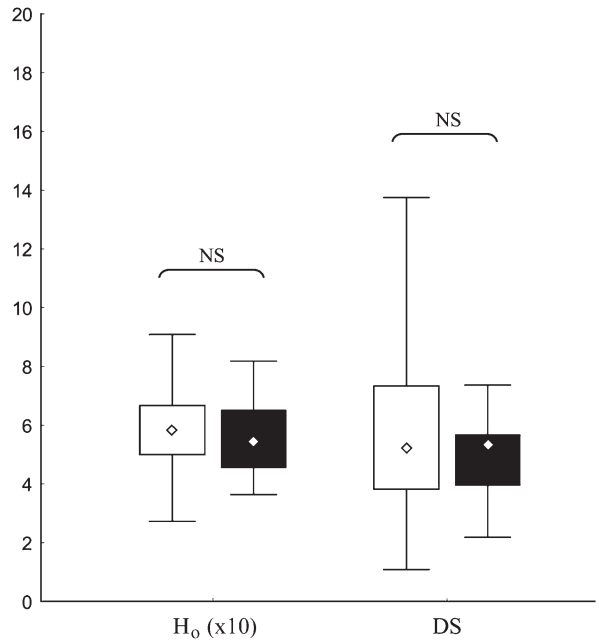

FIG. 4.—Observed heterozygosity (H_o) and difference in allele size (DS) of clonal and sexual queens. Difference in allele size, DS, was computed as the difference in base pairs between the 2 alleles at a given locus of a single individual, averaged over loci. Blocks in white and black indicate sexual and clonal nests, respectively. Diamonds indicate means, and blocks and horizontal bars indicate 50% and 95% percentiles, respectively. H_o values were multiplied by 10 to homogenize scales with DS. Mann–Whitney *U* tests: nonsignificant (NS) corresponds to $P > 0.3$.

Discussion

This study is the first to document the sexual production of *W. auropunctata* reproductives in the native range of the species. These sexual populations were not encountered previously because most occur at low density in remote areas (primary forest and natural backwater areas), whereas clonal populations often occur in accessible areas, such as plantations or roadsides. This impaired our understanding of the reproduction system of the little fire ant to date. mtDNA and microsatellite data have shown that queens, males, and workers do not cluster according to their reproduction system (i.e., clonal or sexual). Most clonal reproductive lineages (i.e., 42 of 44) are characterized by microsatellite genotypes that could have been produced by neighboring sexual populations. Finally, we found 2 nests in which queens were produced sexually and males were produced through clonal or traditional (i.e., arrhenotokous parthenogenesis) reproduction systems. These results indicate that sexual and clonal populations do not form 2 separate evolutionary units and that clonal populations most likely recently arose from local sexual populations.

Most of the alleles of the clonal queens are present in sexual populations and clonal queens cluster into a few groups of closely related individuals. Thelytokous parthenogenesis therefore seems to appear at low frequency within sexual populations. Hybridization has repeatedly been found to lead to asexuality in various taxa including insects (Mantovani et al. 2001; Delmotte et al. 2003; Gomez-Zurita et al. 2006). However, in *W. auropunctata*, we found similar levels of heterozygosity and difference in allele size between clonal and sexual queens and, hence, no evidence of hybridization. Therefore, queen parthenogenesis in the little fire ant is unlikely to be due to hybridization between genetically distant queen and male lineages. Whereas parthenogenesis-inducing bacteria have already been uncovered in Hymenoptera (Stouthamer 1997), they are not expected to occur in haplodiploid species due to their single-locus sex determination system and have already been ruled out as a possible origin of parthenogenesis in the 6 other thelytokous ant species uncovered so far (Wenseleers and Billen 2000). Parthenogenesis in *W. auropunctata* is therefore likely due to rare mutational events (i.e., spontaneous origin of parthenogenesis, Simon et al. 2003).

It has been suggested that male clonality may arise from the male genetic contribution of the sperm removing the maternal genetic material from the egg during fertilization (Fournier et al. 2005a). A strict "maternal genome elimination" (MGE) mechanism seems unlikely, for at least 3 reasons: 1) all workers are produced sexually without MGE, 2) given the large number of diverse clonal male

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FIG. 3.—NJ dendrograms of the microsatellite (allele shared) distances between clonal and sexual queens (*A*) and males (*B*). All clonal and, due to space limitation, a randomly chosen subset of sexual genotypes were included for both sexes. Similar results were obtained when using all individual genotypes (not shown). Dendrograms are not rooted due to the absence of PCR amplification of *Wasmannia auropunctata* microsatellite loci in *Wasmannia rochai*. Color and individual name codes are as in figure 2.

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genotypes encountered in our sample, male clonality would have arisen independently a large number of times, which seems unlikely (McKone and Halpern 2003), and 3) MGE cannot account for the finding that male clonality is almost strictly associated with queen thelytokous parthenogenesis. Queen control over male clonality therefore seems required to account for our results. It is thus unlikely that male clonality is an evolutionary response by males to the reduction of their fitness by queen parthenogenesis. It rather appears that any male mated to a clonal queen becomes clonal and that male clonality is a female rather than a male trait. Experimental studies are needed to confirm this point.

Two mutually exclusive hypotheses might account for the origin of male clonality. First, a variant of the MGE hypothesis—the "permissive MGE" hypothesis—could account for the 3 issues identified above for the strict MGE hypothesis. This modified hypothesis stems from the expected history of struggle between males and females for access to the egg. In haplodiploid species like *W. auropunctata*, it is predicted that the egg fate should be determined by a history of male moves and female countermeasures to take over the egg because males and females have distinct evolutionary stable strategies. We thus expect the sperm to be adapted for replacing the egg's nucleus whenever the egg lacks counter-adaptations to prevent it. If outbreeding is favored or if the loss of a gene essential for the production of arrhenotokous males occurred in parthenogenetic queens (as may be the case in *W. auropunctata*, see Foucaud et al. 2006), some permissive eggs (i.e., less-defended eggs in which the maternal genetic material could be destroyed by paternal genetic material) could be produced by queens and result in the production of clonal males (Normark B, personal communication).

Alternatively, male clonality may result from the production by parthenogenetic queens of "anucleate ovules" later fertilized. Such production of anucleate ovules by queens might occur simultaneously with the production of thelytokous ovules (with the whole nucleus being passed to one daughter cell and only the cytoplasm to the other), accounting for the observed strong linkage between queen parthenogenesis and male clonality. This hypothetical meiotic mechanism differs considerably from the standard mechanism of automictic central-fusion parthenogenesis, which has been put forward to account for the high level of heterozygosity and the pattern of recombination observed in parthenogenetic queens (Foucaud et al. 2006). As in the permissive MGE mechanism, the production of anucleate ovules may also be favored by an advantage of outbreeding at the worker level or the loss of a male-essential gene. However, the anucleate ovules hypothesis does not necessarily require on ongoing conflict-ridden coevolution of sexes. Cytological studies are required to discriminate between the permissive MGE versus anucleate ovules hypotheses.

In conclusion, this study demonstrates that clonal populations of *W. auropunctata* most likely recently arose from local sexual populations and that males and queens do not form separate evolutionary entities. Hence, in contrast to previous suggestions (Queller 2005), *W. auropunctata* males do not merit the title "first all-male species." As male clonality appears to be a female rather than a male trait, this unusual reproduction system cannot be seen as a male strategy to counteract the reduction of male fitness by female parthenogenesis. It therefore seems unlikely that *W. auropunctata* males and queens are currently engaged in an all-out male–female war. However, our study leaves intact the possibility of a more ordinary conflict-ridden coevolution of sexes (as expected in haplodiploid species) that could explain the strong linkage between parthenogenesis and male clonality. Alternatively, the anucleate ovules hypothesis could mechanistically explain this coexistence of sex-specific clonal systems. This work should pave the way to future studies on the adaptive significance and the evolutionary stability of mixed sexual and clonal reproduction systems found in many living organisms.

Supplementary Material

Supplementary table S1 and figure S1 are available at *Molecular Biology and Evolution* online (http://www.mbe.oxfordjournals.org/).

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