Meiotic Recombination Dramatically Decreased in Thelytokous Queens of the Little Fire Ant and Their Sexually Produced Workers

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Abstract

The little fire ant, *Wasmannia auropunctata*, displays a peculiar breeding system polymorphism. Classical haplo-diploid sexual reproduction between reproductive individuals occurs in some populations, whereas, in others, queens and males reproduce clonally. Workers are produced sexually and are sterile in both clonal and sexual populations. The evolutionary fate of the clonal lineages depends strongly on the underlying mechanisms allowing reproductive individuals to transmit their genomes to subsequent generations. We used several queen-offspring data sets to estimate the rate of transition from heterozygosity to homozygosity associated with recombination events at 33 microsatellite loci in thelytokous parthenogenetic queen lineages and compared these rates with theoretical expectations under various parthenogenesis mechanisms. We then used sexually produced worker families to define linkage groups for these 33 loci and to compare meiotic recombination rates in sexual and parthenogenetic queens. Our results demonstrate that queens from clonal populations reproduce by automictic parthenogenesis with central fusion. These same parthenogenetic queens produce normally segregating meiotic oocytes for workers, which display much lower rates of recombination (by a factor of 45) than workers produced by sexual queens. These low recombination rates also concern the parthenogenetic production of queen offspring, as indicated by the very low rates of transition from heterozygosity to homozygosity observed (from 0% to 2.8%). We suggest that the combination of automixis with central fusion and a major decrease in recombination rates allows clonal queens to benefit from thelytoky while avoiding the potential inbreeding depression resulting from the loss of heterozygosity during automixis. In sterile workers, the strong decrease of recombination rates may also facilitate the conservation over time of some coadapted allelic interactions within chromosomes that might confer an adaptive advantage in habitats disturbed by human activity, where clonal populations of *W. auropunctata* are mostly found.

Key words: parthenogenesis, thelytoky, recombination, inbreeding, biological invasion, *Wasmannia auropunctata*.

Introduction

The ubiquitous nature of sexual reproduction in the tree of life is a key question that is addressed by evolutionary biologists but not yet resolved. Sexually produced individuals need both a mother and a father, so sexual offspring are considered to suffer from the "2-fold cost of meiosis" (Maynard Smith 1971). Several theoretical models have been proposed to highlight the potential evolutionary benefits counteracting this cost of requiring two parents. These models have generated about 20 hypotheses accounting for the long-term advantage of sexual reproduction (reviewed in Kondrashov 1993 and Barton and Charlesworth 1998). However, the short-term advantages of sexual reproduction remain a matter of debate, and asexuality appears to emerge easily and independently from sexual lineages (Simon et al. 2003). There is both theoretical and empirical evidence to suggest that, in specific conditions, asexual populations displace related sexual populations in the short term (Burger 1999; Neiman and Linksvayer 2006; Hoffmann et al. 2008). The most direct line of argument in favor of asexual lineages is demographic, as such lineages are not subject to the 2-fold cost of meiosis. In other words, two parthenogens produce two offspring at the same time as a male and a female produce just one. However,

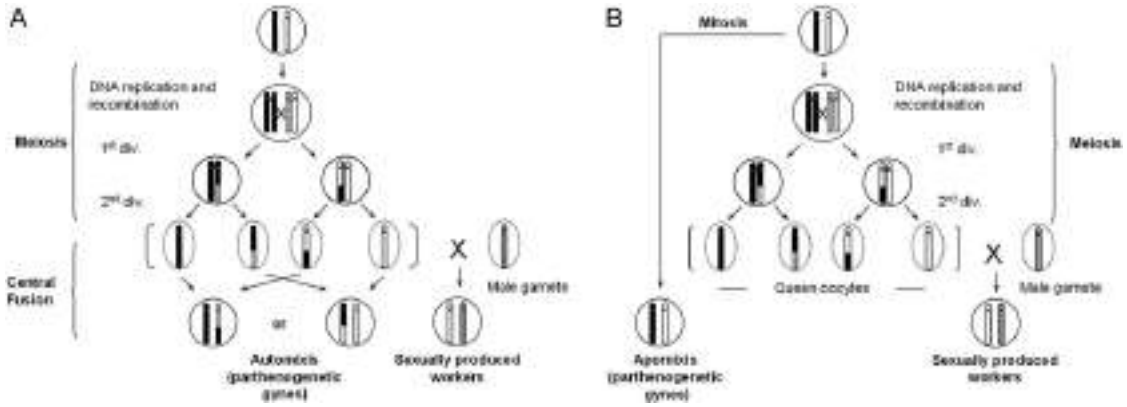

FIG. 1. Hypothetical cytological mechanism of parthenogenesis in queens displaying the ability to use parthenogenesis conditionally for the production of gynes and sexual reproduction for the production of workers. In (A), the queen lineage is automictic, and there is only one meiotic process for producing oocytes, which then either fuse together to generate parthenogenetic gynes or are fertilized by male gametes for the sexual production of workers. In (B), the queen lineage is apomictic, and the cytological mechanism of queen parthenogenesis is independent of the meiosis used to generate oocytes for fertilization by male gametes.

asexuality may also have other evolutionary advantages. In a relatively uniform environment with few biotic interactions, asexuality facilitates the conservation of favorable coadapted allelic interactions over time (Burger 1999; Neiman and Linksvayer 2006), whereas sexual reproduction breaks up both interchromosomal (i.e., via segregation) and intrachromosomal (i.e., via recombination) allelic associations. This advantage applies to strictly asexual lineages, but asexuality occurs in highly diverse forms.

Thelytokous parthenogenesis (i.e., thelytoky) is the development of a female individual from an unfertilized egg. Two main types of thelytoky, defined cytologically, are recognized (Suomalainen 1950 in Suomalainen 1962): ameiotic (i.e., apomictic) and meiotic (i.e., automictic). Apomictic thelytoky generates offspring that are strictly genetically identical to the parent (barring mutations and gene conversions), thus maintaining overall levels of heterozygosity. By contrast, meiosis, and hence recombination, occur in automictic thelytoky. A process for restoring diploidy in the resulting reduced oocytes is therefore required. Two such processes are known, each with different genetic consequences. First, diploid restoration through gamete duplication leads to the complete loss of heterozygosity, thus erasing genetic diversity in the resulting offspring. There are only a few known examples of this mechanism in nature, generally in insects, and in most cases, this situation has been shown to result from manipulation by endosymbiotic bacteria, such as *Wolbachia* (e.g., Stouthamer and Kazmer 1994; Gottlieb et al. 2002). Second, diploidy may also be restored by the fusion of two reduced oocytes. If both oocytes arise from the same nucleus (i.e., terminal fusion), global heterozygosity is lost throughout the genome. However, recombination events during the first meiotic division may conserve some heterozygosity, particularly for loci far from chromosomal centromeres. If oocytes from different nuclei fuse (i.e., central fusion), overall heterozygosity should be conserved, except at loci far from the centromeres, which are affected by recombination (fig. 1A). Regardless of the cytological mechanism underlying automixis described above, the offspring displays a partial, or total loss of heterozygosity, which may lead to inbreeding depression (Engelstädter 2008). There is a peculiar mode of automictic parthenogenesis, the premeiotic doubling mechanism, which preserves overall heterozygosity in the resulting offspring. In this case, a premeiotic doubling of chromosomes is achieved through endomitosis, and the number of chromosomes is subsequently reduced through meiosis. During the first division of meiosis, all chromosomes pair with their genetically identical counterpart eliminating the effect of recombination during pairing. As a result, the four resulting daughter cells are diploids and all identical to the mother cell (i.e., overall heterozygosity is preserved baring mutations and gene conversions). As for apomixy, the mechanism of premeiotic doubling only gives rise to diploid gametes and was described in species were females solely reproduce under parthenogenesis (e.g., Terhivuo and Saura 2006; Lutes et al. 2010). Because of its similarities with apomixis with respect to genomic outcomes (i.e., diploid resulting oocytes, preservation of overall heterozygosity), this peculiar mode of automictic parthenogenesis was classified as a specific case of apomixy in some studies (van Wilgenburg et al. 2006). We will hence hereafter use the term "apomixy" to refer to both standard apomictic parthenogenesis (i.e., mitotic parthenogenesis) and premeiotic doubling.

In some species, reproductive modes are a combination of both sexual and parthenogenetic reproduction. In social insects, males are classically haploid and develop from unfertilized reduced oocytes through arrhenotokous parthenogenesis, whereas diploid females are produced sexually (Crozier and Pamilo 1996). There is generally a reproductive cast (i.e., queens and males) responsible for reproduction, whereas the workers share the tasks necessary for the maintenance of populations. Automictic thelytoky with central fusion has recently been demonstrated in some individuals from three different social insect species. In the ant *Cataglyphis cursor*, queens take advantage of the caste organization to benefit from the

advantages of both sexual and asexual reproduction (Pearcy et al. 2004, 2006). Queens make use of automictic thelytoky with central fusion to produce female reproductive individuals (i.e., gynes), thus increasing the transmission of their own genes to production of the "germline" lineage, whereas they use sexual reproduction to produce workers, thereby increasing the genetic diversity of the "somatic" lineage. *Cataglyphis cursor* queen lineages would be expected to suffer a progressive loss of heterozygosity, due to the frequent recombination events associated with automictic thelytoky with central fusion. Pearcy et al. (2004, 2006) argued that the maintenance of populations over time implies the continual replacement of queens by sexually produced parthenogenetic queens or nonsterile workers (i.e., pseudoqueens). In the Cape honeybee, *Apis mellifera capensis*, pseudoqueens produce new queens by automictic thelytoky with central fusion (Verma and Ruttner 1983). Baudry et al. (2004) demonstrated a genetically controlled mechanism for reducing the frequency of recombination events during pseudoqueen meiosis. This process avoids the loss of heterozygosity resulting from thelytoky with central fusion automixis and is thought to provide pseudoqueens with a means of limiting inbreeding depression in the resulting queen offspring. A similar genetically controlled mechanism for reducing the recombination associated with thelytoky has been suggested in the ponerine ant, *Platythyrea punctata* (Kellner and Heinze 2011).

Thelytoky has also been demonstrated for queens of some native and all invasive populations of the little fire ant, *Wasmannia auropunctata* (Fournier, Estoup, et al. 2005; Foucaud et al. 2007, Foucaud, Estoup, et al. 2009). These populations (hereafter referred to as 'clonal' for the sake of simplicity; Foucaud et al. 2007) are found almost exclusively in ecological environments disturbed by human activity (e.g., plantations, open quarry). Molecular studies have indicated that these populations probably emerge recurrently from populations displaying classical sexual haplo-diploid reproduction (hereafter referred to as 'sexual'; Foucaud et al. 2007). Unlike clonal populations, sexual populations are found principally in primary forests. The specific ecological features of clonal populations, differentiating them from sexual populations, suggest that clonality may play a major role in the invasive potential of *W. auropunctata* populations (Foucaud, Orivel, et al. 2009, 2010; Orivel et al. 2009). As in *C. cursor*, the clonal queens of *W. auropunctata* use thelytoky and sexual reproduction in a conditional manner, to produce gynes and workers, respectively. *W. auropunctata* is unusual among thelytokous species, in that queen thelytoky is closely associated with androgenesis (i.e., the production of haploid sons strictly identical to their father baring mutations; Fournier, Estoup, et al. 2005; Foucaud, Estoup, et al. 2009). Moreover, contrary to other species in which queens reproduce by thelytoky, unmated queens of *W. auropunctata* (irrespectively to their reproductive system) were found to be unable to lay viable eggs (Foucaud, Estoup, et al. 2009). Hence, the production of parthenogenetic eggs by clonal queens seems strictly dependent on the fertilization process.

The underlying mechanism of thelytoky in *W.auropunctata* queens has yet to be demonstrated convincingly. In a recent study, Foucaud, Estoup, et al. (2009) observed a limited number of transitions from heterozygosity to homozygosity during female thelytoky. However, it was not possible, based on the results of this study, to discriminate between apomixis with few gene conversion events and automixis with central fusion associated with a loss of recombination, as described in Cape honeybee workers. The genomic consequences of these two mechanisms may differ considerably in the sexually produced worker offspring. Under apomixis (and premeiotic doubling), the production of diploid unfertilized eggs destined to develop into gynes would be independent of the production of haploid oocytes destined to be fertilized for the production of workers (fig. 1*B*). By contrast, if automixis occurs, oocytes destined to develop into queens (fusion of two maternal oocytes) or workers (fertilization of oocytes by sperm) may follow the same process of meiotic division. In particular, oocytes with different fates might originate from the same first meiotic division, during which potential meiotic recombination events occur (fig. 1A). In this case, sexually produced workers hatching from eggs laid by clonal queens would be expected to display a very low recombining maternal genome.

In this study, we first characterized the underlying mechanism of thelytoky in *W. auropunctata* clonal queens by 1) comparing global and locus-by-locus patterns of heterozygosity in both clonal and sexual queens and 2) by directly analyzing the rate of heterozygosity to homozygosity transition at 33 microsatellite loci in female reproductive offspring of clonal queens. We then investigated the potential decrease in recombination rate in sexually produced workers hatching from eggs laid by clonal queens. We did this by comparing recombination rates between these 33 microsatellite loci in workers produced by both clonally and sexually reproducing queens. We found several lines of evidence to suggest that clonal queens use automictic thelytokous parthenogenesis with central fusion combined with low recombination rates. Consistent with this finding, we demonstrated that clonal queens produced meiotic oocytes for the generation of sexually produced workers with dramatically low levels of recombination. These findings have evolutionary consequences for both queens and workers, in the particular ecological context of invasive clonal populations of the little fire ant.

Materials and Methods

Sampling, Experimental Set-Up, and Microsatellite Genotyping

In 2008 and 2009, we sampled 4 sexual and 10 clonal populations of *W. auropunctata* in the native range (i.e., French Guiana) and from various locations within the area of introduction (i.e., New Caledonia, Israel, Florida; table 1). Previous investigations had precisely determined the reproductive system (i.e., clonal or sexual) of all the sampled populations (Foucaud, Orivel, et al. 2009, 2010). Ten clonal

Table 1. Sampling Sites and Reproductive Systems of the Collected *Wasmannia auropunctata* Populations.

				Geographical coordinates		
Reproductive system	Range	Country	Site name	*x*	*y*	Number of lineages
Sexual	Native	French Guiana	M1	−52.980300	5.067717	2
Sexual	Native	French Guiana	M3	−52.950000	5.022817	5
Sexual	Native	French Guiana	M7	−52.974967	5.049300	1
Sexual	Native	French Guiana	Pi32	−52.959967	5.048367	1
					Total	9
Clonal	Native	French Guiana	Cay	−52.213234	4.485450	1
Clonal	Native	French Guiana	RN	−52.917417	5.270533	1
Clonal	Native	French Guiana	Ker	−53.045750	5.071333	1
Clonal	Native	French Guiana	M3 C	−52.949967	5.041300	1
Clonal	Native	French Guiana	M6 C	−52.973950	5.048650	1
Clonal	Native	French Guiana	P2	−52.917800	5.287783	1
Clonal	Native	French Guiana	P3	−52.914833	5.291750	1
Clonal	Introduced	USA (Florida)	Orl	−81.352333	28.590000	1
Clonal	Introduced	Israel	Hz	32.174577	34.834464	1
Clonal	Introduced	New Caledonia	Mp	−20.587833	164.821333	1
					Total	10

NOTE.—The number of analyzed monogyne families per site is indicated in the last column. Note that our experiment initially started from 33 sexual and 23 clonal lineages; many of queens died before laying enough worker offspring for genetic analysis.

and 9 sexual fertilized queens originating from the sampled clonal and sexual populations were isolated in the laboratory, together with 50 workers each from the same populations. These ants were placed in individual monogynous artificial nests consisting of 8 × 10.5 cm boxes (height × diameter). Microsatellite genotyping showed that each monogyne lineage was composed by different queen and male genotypes and were hence genetically different. These 19 monogyne lineages (i.e., families) were housed in a walk-in climatic chamber at a constant temperature of 25°C, with 70% relative humidity and a 12:12 h (light:dark) photoperiod. They were fed ad libitum with a honey–yeast–water solution and *Ephestia* eggs throughout the experiment. For each of the 19 families, we collected 48 newly produced workers at the last pupal stage, after at least 10 weeks, to ensure that these workers were produced by the focal queens. Workers were stored in 95% ethanol, and DNA was extracted for each of the 912 newly produced workers and their parents (i.e., queens and fathers), according to a Chelex-based protocol (Estoup et al. 1996). Individual genotypes for each worker were obtained for 33 microsatellite markers (Fournier, Foucaud, et al. 2005; Almany et al. 2009). Polymerase chain reaction products were separated on an ABI 3130 DNA sequencer (Applied Biosystems). Genetics profiles were analyzed with GeneMapper software version 4.0. (Applied Biosystems).

Indirect Evidence for the Underlying Mechanism of Thelytoky

Traditionally, cytological methods have been applied to dissected mature ovaries to demonstrate directly the mode of thelytoky (i.e., apomixis vs. automixis and mechanism of diploidy restoration; Verma and Ruttner 1983). Unfortunately, such methods cannot be used in *W. auropunctata* because unmated clonal queens of this species cannot lay viable eggs. Furthermore, once they are mated, queens predominantly lay fertilized eggs generating sexually produced workers. They lay parthenogenetic eggs for queen production unpredictably and much less frequently. We therefore looked for indirect evidence about the underlying mechanisms of thelytoky, by analyzing three different microsatellite data sets.

First, the most striking contrast between apomixis and automixis is that meiotic recombination events lead to a gradual loss of heterozygosity over generations in automictic lineages, whereas heterozygosity is maintained and may even increase through the accumulation of mutations in apomictic lineages (Suomalainen 1962). We therefore compared global and locus-by-locus heterozygosities at 33 microsatellite loci in the 10 clonal and 9 sexual queens of our monogynous nests reared in the laboratory. A non-parametric Mann–Whitney *U* test was carried out to assess the significances of differences in mean individual heterozygosity between clonal and sexual queens.

Second, as meiotic recombination does occur in automictic thelytoky, transitions from heterozygosity to homozygosity would be expected to occur frequently in the gyne offspring of a given queen. By contrast, heterozygosity in newly produced gynes would be expected to be retained following rare gene conversion events in apomictic thelytoky. We extended the individual multilocus genotypes obtained for 38 F1 gynes and their respective mothers from the various clonal lineages ($n = 8$) studied by Foucaud, Estoup, et al. (2009), by genotyping 21 new microsatellite markers (Almany et al. 2009), giving a total of 33 analyzed loci. We also produced two entirely new queen-gyne data sets, each for 96 gynes produced by a clonal queen sampled in Israel (both queens originated from the same clonal lineage). Genotypes from individuals displaying homozygosity to maternal heterozygote loci were replicated twice (i.e., two independent DNA extractions and two independent genotyping procedures) to ensure that homozygosity at the concerned loci were not due to genotyping error. Mean and single-locus rates of transition from heterozygosity to homozygosity were estimated directly from these queen-gyne data sets and compared with theoretical expectations for each mechanism of thelytoky.

Third, as the observed transition rate turned out to be very low in F1 gyne offspring (see Results), we analyzed an additional data set, in which we investigated the pattern of transition event accumulation on linkage groups over several generations in a populational sample of queens. This multigenerational pattern of transition events on linkage groups has the potential to inform on the parthenogenetic mode characterizing *W. auropunctata* clonal queens. Under apomixis, transitions to homozygosity result from gene conversion or mutational alteration. In this case, it is expected that, for a given maternal linkage group, a transition event to homozygosity will affect a unique marker of the group without altering heterozygosity at the neighboring markers. By contrast, under automixis, transition events to homozygosity are more likely to result from recombination events. When such a recombination event occurs between the centromere and a given linkage group, several if not all markers of the group are likely to transit from heterozygosity to homozygosity simultaneously. Under automixis, one might therefore expect an excess of nonindependent cotransitions to homozygosity over independent single locus transitions for loci located within the same linkage group. We therefore analyzed the microsatellite genotypes of 105 queens and gynes collected at different locations in the field for an invasive clonal population present in New Caledonia since about 1970 (Foucaud et al. 2006). All these queens were known to originate from a well-known single clonal lineage (i.e., the Q0 clonal lineage; Foucaud et al. 2006), making it easy to identify transition events. All individuals were genotyped at the same 33 microsatellite loci, and we focused our analysis of transition events on groups of microsatellite loci found to belong to the same linkage groups in *W. auropunctata* (see Mapping of Microsatellite Loci).

Analysis of Recombination Rates Based on Worker Families

Cytological Analysis

Analyses of mitochondrial DNA and microsatellite markers indicated that queens (or males) did not cluster according to their reproduction system (i.e., clonal or sexual) and hence that sexual and clonal individuals did not correspond to different species or subspecies (Foucaud et al. 2007). However, we nonetheless analyzed mitotic metaphases from workers from a subset of populations (i.e., three sexual and four clonal lineages), to identify potential differences in chromosome number between populations with different reproductive systems. Such differences might account, at least in part, for differences in recombination pattern between clonal and sexual populations. Chromosome preparations were obtained from the cerebral ganglia of worker nymphs, as described by Imai et al. (1988), but with the following modifications to the protocol. As the larvae of this species are very small, we placed the entire body in a hypotonic colchicine citrate solution (0.005%). We made a small hole in the cephalic capsule, allowing the colchicines solution to penetrate into the brain. Two hours later, the brain was extracted, dissociated directly on a slide, and fixed in a series of solutions of acetic acid, ethanol, and distilled water. Slides were stained with DAPI (i.e., 4′,6-diamidino-2-phenylindole) and observed under a Zeiss A1 microscope (Zeiss S.A.S., Le Pecq, France). Images were acquired, and the number of chromosomes was counted with Genus Software (Applied Imaging, Genetixn Queesway, United Kingdom).

Mapping of Microsatellite Loci

The 33 microsatellite loci studied were mapped based on the microsatellite genotypes obtained from worker families produced by queens from sexual populations. When maternal and paternal genotypes were not obtained directly, they were deduced indirectly from the genotypes of the workers. This inference is straightforward in *W. auropunctata*, as the queens are monoandrous (i.e., each queen mates only once; Foucaud, Estoup, et al. 2009). Once all detectable mistyping errors had been ruled out and the few mutations observed in worker genotypes had been discarded from the data set, we tested for distorted segregation at each locus, in each family, by performing χ^2 tests. In the absence of distorted segregation, the two maternal alleles should be even distributed among workers. The number of significant χ^2 (at the 0.05 threshold) observed for all sexual families was compared with the distribution of the number of significant χ^2 obtained by simulating the same number of families with the same number of genotyped workers (as in the observed data set) and assuming no segregation distortion. We simulated data for 10 million families with a custom-developed computer program for calculating the probability that the observed number of significant χ^2 could be obtained by chance, without segregation distortion. We tested for differences in segregation distortion between clonal and sexual lineages for 27 of the 33 loci studied (6 loci were homozygous in all clonal lineages), using a Wilcoxon signed-rank test.

We established linkage groups in our microsatellite loci at a two-point logarithm of odds (LOD) score threshold of 3.0, using the Carthagene software "group" option (de Givry et al. 2005). As the phase of the alleles was unknown, we expected there to be some bias when mapping loci in linkage groups including more than three loci. We therefore inferred the phase in each linkage group with more than two loci, as follows. We first identified a central locus (i.e., linked to all other loci of the linkage group) by a two-point analysis. We then arbitrarily assigned a code to each worker: A or H (corresponding to usual "F2 backcross" data types in genetic mapping software), depending on the particular maternal allele it displayed in each family. In parallel, we constructed a table in which, for each pair of linked loci, the number of recombinant and parental types was calculated (the parental type being the predominant association of two alleles at two loci). We then determined the phase of all linked loci, taking into account the identified parental and recombinant worker types at the central locus. Several genetic maps were then designed for each linkage group (from 3 to10, depending on the number of loci constituting the group) using the Build option of Carthagene software. Maps displaying the

maximum likelihood were eventually selected and genomic distances between loci were estimated.

Comparison of Recombination Rates in Sexual and Clonal Queen Lineages

The rates of recombination between each pair of loci (r) were calculated as the total number of recombinant worker genotypes divided by the total number of genotyped workers, based on a LOD score approach in multiple families originating from either clonal or sexual lineages.

We compared recombination rates between sexual and clonal lineages for pairs of loci within each linkage group. Three of the 28 microsatellite loci belonging to defined linkage groups were homozygous in all clonal lineages and were therefore discarded from the comparative analysis. We found that r estimates between some loci from different linkage groups mapped in sexual lineages were significantly lower than 0.5 in clonal lineages. We included these r estimates in our comparative analysis, which was thus based on a total of 29 pairwise r estimates calculated in both sexual and clonal lineages. We compared r values for each pair of loci, using Fisher's exact tests. We corrected the 5% significance threshold by the number of tests performed using the Benjamini and Hochberg false discovery rate procedure (Benjamini and Hochberg 1995). We then calculated mean recombination rates ($\bar{r}$) in clonal and sexual lineages. We assessed the significance of differences in $\bar{r}$ between clonal and sexual lineages, with a nonparametric Mann–Whitney U test.

Results

Mechanism Underlying Queen Thelytoky

The thelytokous queens of our experimental lineages displayed a significantly lower level of heterozygosity than sexual queens (mean $H_{obs} = 0.55$ and 0.80, respectively; $P = 9 \times 10^{-4}$, Supplementary table S1, Supplementary Material online). Locus-by-locus analysis revealed that the observed lower level of heterozygosity was accounted for mostly by six loci that were homozygous in all clonal queens but heterozygous in most, if not all sexual queens (Supplementary table S1, Supplementary Material online). By contrast, none of the 33 loci was found to be homozygous in all sexual populations.

There are several possible reasons for such a large decrease in heterozygosity at the same six loci in all clonal lineages. First, clonal lineages may originate from the same common ancestor, which was homozygous at these six loci. In this case, all clonal lineages would be expected to have the same allele at a given locus. The high degree of allelic diversity between clonal lineages observed for these six loci (from three to eight alleles per locus) does not support this hypothesis. Second, a lower level of heterozygosity may result from selection or bottleneck events occurring during the transition from sexuality to clonality or during the establishment of clonal populations. Transition to homozygosity would not be expected to occur for the same 6 microsatellite loci in all 10 clonal lineages under both evolutionary forces unless those 6 loci were genetically linked. However, our mapping results demonstrated that these six loci belonged to five different linkage groups (see below). Third, in conditions of apomixis, episodic transitions to homozygosity resulting from mutations and/or gene conversion might be expected. However, it is unlikely that such events would occur recurrently, at the same six loci, in different clonal lineages. By contrast, in conditions of automixis with central fusion, recombination events would be expected to lead to transitions to homozygosity for all loci located far from the centromeres, whatever the origin of the clonal lineage. The observed pattern would thus be easy to explain by a mechanism of automictic thelytoky if the six homozygous loci were actually located at some distance from the centromere.

We detected very few single-locus transitions to homozygosity in our various thelytokous queen-gyne offspring data sets. The mean transition rate per locus ranged from 0 to 2.8%. The rate of homogenization during automixis with central fusion is expected to range from 0 in the absence of recombination events to 1/3 in the presence of recombination (Pearcy et al. 2004; Oldroyd et al. 2008). The observed pattern of transition rates is hence consistent with what would be expected under conditions of automixis with central fusion only if the number of recombination events is globally very small all over the genome.

The small number of transition events observed in the queen-gyne offspring data sets concerned unlinked loci. We were therefore unable to check for the occurrence of cotransitions for loci from the same linkage group in these data sets. Such cotransitions would be expected to occur with automixis, but not with apomixis with episodic gene conversion events. Only when we analyzed our multigenerational population data set including data for 105 reproductive females from New Caledonia did we find a clear excess of nonindependent cotransitions to homozygosity ($n = 8/10$) over independent single-locus transitions ($n = 2/10$) for loci belonging to the same linkage group (Supplementary Table S2, Supplementary Material online).

Comparison of Recombination Rates in Sexual and Clonal Queen Lineages

We found that 28 of the 33 microsatellite loci genotyped in worker families produced in sexual queen lineages were dispersed over 11 different linkage groups (fig. 2). The five remaining loci displayed no significant linkage and could not, therefore, be mapped. We compared recombination rates between the 29 pairs of loci estimated from worker families produced in either clonal or sexual queen lineages (table 2; fig. 2). No recombination events were detected in worker families produced by clonal queens, for 19 of the 29 pairs of loci considered. The mean recombination rates were 0.555% in clonal queens (ranging from 0% to 2.50%) and 25.2% in sexual queens (ranging from 0.368% to 47.7%). The mean recombination rate was thus lower in clonal queens, by a factor of 45.3 (table 2). Consistent with these strikingly different patterns of recombination, several linkage groups that were considered independent in analyses of worker families from sexual queens were found to be

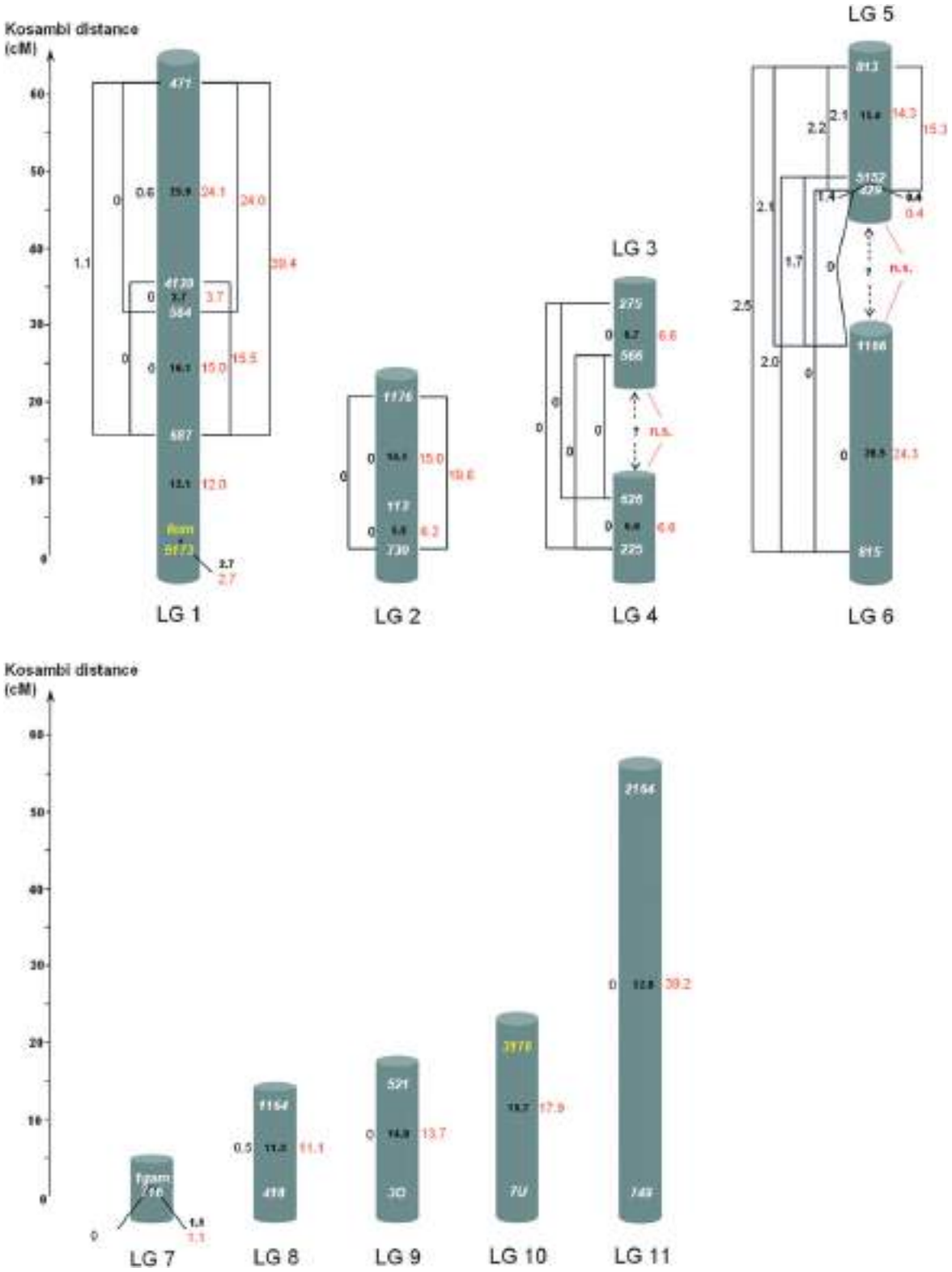

FIG. 2. Linkage groups in *Wasmannia auropunctata* deduced from families of workers from sexual lineages and estimates of recombination rates for both sexual and clonal queen lineages. Recombination rates estimated from sexual queen lineages are shown on the right of linkage group (red characters) whereas those estimated from clonal queen lineages are shown on the left (black characters). Kosambi distances between loci, inferred from sexual lineages, are shown in bold black characters within linkage groups. The names of microsatellite loci are shown in white. Microsatellite loci in yellow are homozygous in all clonal lineages analyzed, making it impossible to estimate the recombination rate for these loci in these lineages. Other microsatellite loci did not display any significant linkage.

Table 2. Mean Recombination Rates ($\bar{r}$) and Recombination Rates for Each Pair of Linked Markers (r) Estimated from Workers Produced Sexually in Clonal and Sexual Lineages.

		Clonal				Sexual					
Locus 1	Locus 2	*N* recombinant	*N* parental	*rc*	lod	*N* recombinant	*N* parental	*rs*	lod	*rc*/*rs*	*P* value
113	1176	0	172	0	51.777	45	255	15	35.235	0	7.92E-10
1166	429	0	340	0	102.350	116	158	42.336	1.404	0	2.20E-16
1166	5152	5	293	1.678	78.678	136	183	42.633	1.509	0.039	2.20E-16
1166	813	1	47	2.083	12.338	138	175	44.089	0.952	0.047	4.10E-10
1166	815	0	324	0	97.534	61	190	24.303	15.109	0	2.05E-01
1gam	716	0	360	0	108.371	1	92	1.075	25.595	0	0.21
2164	749	0	388	0	116.800	120	186	39.216	3.116	0	2.20E-16
225	566	0	85	0	25.588	66	73	47.711	0.077	0	0.02
225	626	0	82	0	24.684	12	171	6.557	35.852	0	2.20E-16
275	225	0	87	0	26.190	107	127	45.726	0.372	0	2.20E-16
275	566	0	130	0	39.134	15	211	6.637	44.069	0	0.001503
275	626	0	213	0	64.119	78	109	41.711	1.121	0	2.20E-16
418	1164	2	418	0.476	120.922	37	297	11.078	50.045	0.043	6.72E-12
429	5152	5	345	1.429	93.979	1	271	0.368	79.012	3.883	0.24
429	813	1	45	2.174	11.755	42	232	15.328	31.508	0.142	2.20E-16
429	815	0	288	0	86.697	85	127	40.094	1.819	0	2.20E-16
471	4139	1	180	0.552	51.796	74	233	24.104	18.782	0.023	1.14E-15
471	687	3	273	1.087	75.897	134	206	39.412	3.336	0.028	2.20E-16
5152	813	1	47	2.083	12.338	39	233	14.338	33.323	0.145	0.02
5152	815	5	241	2.033	63.444	106	146	42.063	1.385	0.048	2.20E-16
521	30	0	222	0	66.829	25	158	13.661	23.396	0	9.06E-10
566	626	0	81	0	24.383	59	84	41.259	0.954	0	1.23E-14
584	4139	0	136	0	40.940	10	258	3.731	62.134	0	0.02
584	471	0	118	0	35.522	63	200	23.954	16.287	0	7.84E-12
584	687	0	140	0	42.144	45	256	14.950	35.466	0	2.23E-08
687	4139	0	196	0	59.002	54	294	15.517	39.532	0	1.27E-11
730	113	0	171	0	51.476	22	332	6.215	70.769	0	2.34E-04
730	1176	0	185	0	55.691	70	287	19.608	30.734	0	1.81E-14
815	813	1	39	2.500	10.010	117	132	46.988	0.196	0.053	4.19E-09
Global			$\bar{r}_c$ = 0.555%				$\bar{r}_s$ = 25.161%			$\bar{r}_c/\bar{r}_s$ = 45.335%	

NOTE.—r values for each pair of loci were compared using Fisher's exact test.

linked in analyses of worker families from clonal queens (i.e., linkage groups 3 and 4, and linkage groups 5 and 6; fig. 2).

The observed much lower rates of recombination in clonal than in sexual queen meiosis could not be accounted for by differences in chromosome number between sexual and clonal lineages. Our cytological analysis indeed revealed that *W. auropunctata* workers had the same number of chromosomes ($2N = 32$) whether produced by clonally or sexually reproducing queens (Supplementary fig. S1, Supplementary Material online). There were therefore no major cytological differences between clonal and sexual *W. auropunctata* populations.

Finally, we tested for an excess of meiotic segregation distortion in low recombining clonal lineages due to more widespread hitchhiking effects in such lineages in comparison with sexual ones. We found only a small percentage of significant distortion segregation in the sexually produced worker offspring from either clonal or sexual queen lineages. The proportion of observed significant χ^2 values in all tests was, however, similar to the proportion expected in the absence of distortion segregation, and the observed distortion segregations were randomly distributed over loci and lineages. The proportion of significant distortion segregation at the 0.05 level did not differ significantly between workers from clonal and sexual lineages (Wilcoxon signed-rank test $P = 0.93$, $n = 27$). The segregation of our microsatellite loci during queen meiosis hence appeared similar in both clonal and sexual lineages, despite the large differences in recombination patterns.

Discussion

Thelytoky and Loss of Recombination in *Wasmannia auropunctata* Queens

Wasmannia auropunctata (Foucaud et al. 2007), like *Vollenhovia emeryi* (Ohkawara et al. 2006) and *C. cursor* (Pearcy et al. 2004, 2006), is one of the rare species of the Hymenoptera in which some queens are able to make conditional use of both sexuality and thelytoky in the production of workers and queens, respectively. In this study, we obtained indirect evidence that *W. auropunctata* queens from clonal populations give rise to gyne offspring via a mechanism of automictic thelytokous parthenogenesis with central fusion. This is not in agreement with van Wilgenburg et al. (2006) who classified *W. auropunctata* queen parthenogenesis as apomictic (premeiotic doubling) based on the first study showing parthenogenesis in some queens of this species (Fournier, Estoup et al. 2005). Automixis with central fusion, also found in *V. emeryi* and *C. cursor*, seems to be a common way for individuals to use both sexuality and thelytoky in the production of their offspring. This mechanism appears to be the simplest

mechanism for transition from sexuality to parthenogenesis, as it does not require major changes in the cytological mechanism of meiosis (Schwander and Crespi 2009). Clonal queens produce meiotic oocytes, which may either fuse together for gyne production or be fertilized by male gametes for the production of workers (fig. 1*A*).

Theoretically, automictic thelytoky with central fusion leads to partial genetic homogenization in the newly produced offspring, due to recombination events during meiosis. However, we found unexpectedly low rates of transition from heterozygosity to homozygosity in thelytokous queen lineages, confirming, for a much larger data set, the low rates previously observed by Foucaud, Estoup, et al. (2009). A genetically controlled mechanism for decreasing recombination, thereby limiting the transition to homozygosity, has been demonstrated in the Cape honeybee, whose orphaned workers (i.e., pseudoqueens) produce new queens via automictic thelytoky with central fusion (Moritz and Haberl 1994; Baudry et al. 2004). Kellner and Heinze (2011) provided indirect evidence of the existence of a similar mechanism in the ponerine ant *P. punctata*. In this study, we showed that workers produced sexually by thelytokous queens also displayed a much lower recombination rate than workers produced from sexual queens (by a factor of about 45). Our results hence demonstrate that *W. auropunctata* clonal queens produce meiotic oocytes with normal chromosome segregation but with very low rates of recombination during the first meiotic division. These cytological features apply to gyne production, as the diploid restoration system during automixis involves the fusion of reduced oocytes (i.e., central fusion) that probably originated from the same first meiotic division process (fig. 1*A*).

Baudry et al. (2004) argued that automictic thelytoky and low recombination rates were probably controlled by different genes in the Cape honeybee. We observed very low rates of recombination in all *W. auropunctata* clonal lineages (genetically different according to our microsatellite data), whereas such low rates of recombination were not observed in any of the nine sexual queens from four genetically different populations studied. This suggests that, in *W. auropunctata*, thelytoky and the reduction of recombination are two coadapted traits that have emerged together, several times, in different lineages, during the evolutionary shift from sexuality to clonality. We therefore suggest that, in *W. auropunctata*, both traits are likely to be genetically controlled by either the same gene(s) or by gene(s) close enough together to be fixed jointly in various clonal lineages. It is also possible that selection pressure against transitions to homozygosity is particularly strong in thelytokous queen lineages, resulting in rapid counter selection against clonal lineages not bearing the gene(s) for low recombination rates.

Evolutionary Consequences of Reducing Recombination Rates During Automixis

Given the high genomic recombination rates reported in others social insects (Gadau et al. 2000; Wilfert et al. 2007), the number of linkage groups found in *W. auropunctata* worker families produced in sexual queen lineages (i.e., 11 groups of 2 or more markers) might seem at first sight particularly low considering that only 33 microsatellite markers was used in this study. This might indicate that genomic recombination rates are already low in sexual queens of this species. Unfortunately, the present data set has a too limited resolution (i.e., include a too small number of markers) to allow a robust comparison of the number and configuration of linkage groups with previous mapping studies conducted in other social insects.

The decrease in recombination rates in *W. auropunctata* thelytokous queen meiosis compared with their sexually reproducing relatives seems to be much greater than that reported for Cape honeybees (factor of >45 vs. >10, respectively; Baudry et al. 2004). This extreme decrease in recombination rates is probably a response to particularly strong selective pressure against recombination in queens during the establishment and maintenance of clonal lineages.

Decrease in meiotic recombination is expected to limit the negative effects of the loss of heterozygosity associated with automictic thelytoky with central fusion (particularly for genes far from the centromere). Theoretically, this loss of heterozygosity may lead to inbreeding depression, which has been shown to have various negative effects on life history traits in many species (Keller and Waller 2002; Charlesworth and Willis 2009). In some ant species, brood quality, queen life span, male quality, and, more generally, colony survival are negatively affected by inbreeding depression (Schrempf et al. 2006; Haag-Liautard et al. 2009; but see Thurin and Aron 2009). Moreover, additional costs of inbreeding are likely in Hymenoptera due to the system of single-locus complementary sex determination in this order (van Wilgenburg et al. 2006), resulting in individuals homozygous at the sex locus developing into nonviable or sterile males (Petters and Mettus 1980; Cook 1993; but see Cowan and Stahlhut 2004).

The maintenance of heterozygosity through a large decrease in recombination rate in *W. auropunctata* thelytokous queens would therefore be expected to increase the life time of clonal lineages. In *C. cursor*, no decrease in recombination has been observed in queens reproducing by automictic thelytokous parthenogenesis with central fusion, and populations are thought to be maintained partly by a renewal of parthenogenetic lineages suffering from inbreeding depression (Pearcy et al. 2004). This renewal is achieved through both sexual events for gyne production and the production of new queens from sexually produced workers, by thelytokous parthenogenesis (Pearcy et al. 2004). By contrast, *W. auropunctata* workers are sterile in both sexual and clonal populations, and although sexual reproduction events to produce new queens do occur in clonal populations, they are much rarer than parthenogenesis events (Foucaud et al. 2006; Foucaud, Estoup, et al. 2009). Moreover, the presence within each clonal population of a single maternal genome as well as a single paternal genome reproducing through androgenesis would lead to the rapid increase of homozygosity for both gyne and

worker offspring within such sexually produced lineages (Foucaud et al. 2006, 2010). The large decrease in recombination observed in clonal populations of *W. auropunctata* thus constitutes the only means by which this species can preserve clonal lineages over time. Consistent with this hypothesis, Mikheyev et al. (2009) suggested that a particular *W. auropunctata* clonal lineage has been maintained in Gabon (Africa) since the beginning of the last century.

In a lineage reproducing by automictic thelytoky with central fusion associated with a large decrease in recombination, the most if not the entire genome is transmitted to the next generation and selection acts on the genome as a whole. In a given stable environment, an emerging thelytokous parthenogenetic lineage adapted to the environment will conserve interactions of coadapted alleles over time (Maynard Smith 1978; Lynch 1984; Burger 1999). Consistent with this hypothesis, several authors have noted that thelytoky often occurs in habitats in which biotic interactions are limited, the diversity of biotic interactions being a significant element in environmental uncertainty (Lynch 1984). *Wasmannia auropunctata* clonal populations are found mostly in habitats disturbed by human activity, which often display fewer biotic interactions than natural habitats (Wetterer and Porter 2003; Orivel et al. 2009). Well-adapted thelytokous queens may therefore have an advantage over their sexual relatives in such areas. Furthermore, in the sterile worker offspring with limited recombination, lower rates of recombination during queen meiosis may also preserve maternal allelic interactions, at least on individual chromosomes. Although sterile, workers make a major contribution to the inclusive fitness of reproductive individuals, including queens in particular (Crozier and Pamilo 1996). Furthermore, whereas queens are generally confined to favorable conditions within the nest, selection is more likely to act on workers, which are faced with biotic and abiotic environmental factors during foraging. In changing environments, restricted meiotic recombination may make it difficult for clonal lineages to adapt sufficiently rapidly (Lynch 1984; Otto and Michalakis 1998). Consistent with this, sexual *W. auropunctata* populations are maintained in native primary forests, which are characterized by major biotic interactions (Orivel et al. 2009) and in which very few clonal populations have ever been found (Foucaud, Orivel, et al. 2009; Rey O and Orivel J, personal observation).

In the present study, we provided new insights on the extraordinary reproductive system of *W. auropunctata* clonal lineages. In particular, we determined how clonal queens produce their female offspring (i.e., worker and gyne). Our findings indicate that parthenogenetic queens produce a unique kind of meiotic oocytes with a drastic reduction of recombination that may either fuse together for gyne production (automictic parthenogenesis with central fusion) or be fertilized by male gametes for the production of workers. Unfortunately, our results do not inform on how and to which extent, queens control the conditional production of workers or gynes. The mechanism underlying the capacity of parthenogenetic queens to lay eggs that develop into haploid males strictly identical to their father (i.e., the so-called male clonality reproductive system) is also still unresolved. We are currently leading some laboratory cross experiments between reproductives (i.e., queens and males) originating from lineages characterized by different reproductive systems to clarify the respective role of the male and queen genetic contribution in this uncommon reproductive system.

Supplementary Material

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

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