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ORIGINAL PAPER

Mechanism of facultative parthenogenesis in the ant *Platythyrea punctata*

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Abstract Thelytokous parthenogenesis, the production of diploid female offspring from unfertilized eggs, can be caused by several cytological mechanisms, which have a different impact on the genetic variation on the offspring. The ponerine ant *Platythyrea punctata* is widely distributed throughout the Caribbean Islands and Central America and exhibits facultative parthenogenesis. Workers in many field colonies from the Caribbean Islands have identical multilocus genotypes and are thus probably clonal, but the occurrence of males makes an ameiotic mechanism of thelytoky unlikely. To clarify the details of thelytoky in this species we compared the multilocus genotypes of mothers and their offspring in experimental colonies and analyzed the genotypes of haploid and diploid males. Additionally, we screened a large number of field colonies from thelytokous populations for the occurrence of recombination events. According to these data, automixis with central fusion and a reduced recombination rate is the most likely mechanism of thelytoky, as in the Cape honeybee and the ant *Cataglyphis cursor*.

Keywords Apomixis · Automixis · Diploid males · Heterozygosity · Sex determination

Introduction

A few hundred species scattered throughout the animal kingdom are characterized by thelytokous parthenogenesis, through which mothers can produce diploid, female offspring from unfertilized eggs. Thelytoky appears to be particularly common in the solitary Hymenoptera, such as parasitoid wasps and sawflies (Lamb and Willey 1987; Slobodchikoff and Daly 1971; Suomalainen et al. 1987; van Wilgenburg et al. 2006). The reason

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for this might be that some mechanisms of the haplodiploid sex determination in Hymenoptera (haploid males develop from unfertilized eggs and diploid females from fertilized eggs; Cook 1993; Cook and Crozier 1995) are rather easily transformed into thelytoky by parasitic bacteria (Bourtzis and O'Neill 1998) or mutations (Lattorff et al. 2005, 2007).

Among the social Hymenoptera (ants, bees, wasps), thelytoky is much rarer. It regularly occurs in the Cape honeybee (*Apis mellifera capensis* Baudry et al. 2004) and a few phylogenetically unrelated ant species, including *Pristomyrmex punctatus* (Tsuji 1988), *Cerapachys biroi* (Tsuji and Yamauchi 1995), *Cataglyphis cursor* (Cagniant 1983; Pearcy et al. 2004), *Wasmannia auropunctata* (Fournier et al. 2005), and *Platythyrea punctata* (Hartmann et al. 2005; Heinze and Hölldobler 1995; Schilder et al. 1999a, b).

Thelytoky can be caused by several cytological mechanisms, which have different consequences for the genetic structure of colonies and populations (Suomalainen et al. 1987). It is therefore possible, in principle, to deduce the mechanism underlying thelytoky by analyzing how commonly heterozygous mothers produce homozygous offspring (Pearcy et al. 2006). In apomictic (or ameiotic) parthenogenesis, there is no meiosis and therefore no recombination, i.e., all offspring are identical clones of their mothers and heterozygosity is maintained. In automictic parthenogenesis, meiosis takes place and diploidy is restored by the fusion of the second division sister nuclei (terminal fusion) or non-sister nuclei (central fusion). Terminal fusion usually results in complete homozygosity at all loci, but in the case of crossing-over, heterozygosity may be preserved at loci between the chiasma and the telomere. In contrast, in central fusion heterozygosity is maintained, except in the case of crossing-over at loci between the chiasma and the telomere. Thus, the effect of crossing-over on the zygosity of loci depends on the location of the chiasma on the chromosome and the position of the respective loci.

In automictic parthenogenesis with random fusion of nuclei, all four chromatids segregate independently, and each heterozygous locus can become homozygous, independent on the position of the locus on the chromosome. In gamete duplication, the meiotically produced haploid egg undergoes an extra round of DNA replication without cell division. Gamete duplication results in complete homozygosity and appears to be the type of thelytoky induced by *Wolbachia* infection in many solitary Hymenoptera (e.g. Werren 1991; Stouthamer and Kazmer 1994). However, it is incompatible with the typical mechanism of haplodiploid sex determination in social Hymenoptera, single-locus complementary sex determination (sl-csd, Cook 1993; Whiting 1939), in which individuals homozygous at the sex locus develop into unviable or sterile males. Indeed, though *Wolbachia* infection is widespread within the social Hymenoptera (Wenseleers et al. 1998), it has never been found to lead to thelytoky (Wenseleers and Billen 2000; van Wilgenburg et al. 2006). Treatment of thelytokous ants with antibiotics (Schilder et al. 1999b) and DNA amplification with *Wolbachia*-specific primers corroborate the hypothesis that thelytoky in these species is not induced by bacteria (Wenseleers and Billen 2000), but beyond that, little is known about the cytological basis of thelytoky in social Hymenoptera other than the Cape honeybee.

The ponerine ant *Platythyrea punctata* is widely distributed throughout the Caribbean islands, Florida, and Mesoamerica (Hartmann et al. 2005; Schilder 1999; Schilder et al. 1999a). Colonies from Costa Rica contained mated workers and unmated workers were not capable of producing diploid offspring (Hartmann et al. 2005; Schilder et al. 1999b). In contrast, colonies from Florida and the Caribbean Islands almost exclusively produced offspring through thelytoky. Although males do occasionally occur (Hartmann et al. 2005; Schilder et al. 1999a; Wheeler 1905), sexual reproduction appears to be rare in the latter populations and only one reproductive worker and a few non-reproductive workers and

queens from Puerto Rico and Florida were found to be inseminated (Hartmann et al. 2005). Most colonies consist of workers with identical multilocus genotypes, which was interpreted as evidence of apomictic parthenogenesis (Schilder et al. 1999b).

To elucidate the mechanism of thelytoky in *P. punctata* in more detail, we compared mother-offspring multilocus genotypes in experimental colonies and combined this approach with the analysis of haploid and diploid males. Under apomictic parthenogenesis, diploid males are not expected to occur because the heterozygous state of the sex locus remains fixed. Diploid males are therefore only possible under automictic parthenogenesis (Oldroyd et al. 2008; Suomalainen et al. 1987; van Wilgenburg et al. 2006). Searching for diploids among the morphologically conspicuous males is therefore a simple and sensitive way of detecting rare recombination events compared to screening workers for homozygosity at microsatellite loci.

Materials and methods

Collecting, housing and set up of experimental colonies

Colonies of *Platythyrea punctata*, which were collected in Puerto Rico in 2005, were genotyped and screened for heterozygosity at five polymorphic microsatellite markers (3506, 2902, 4101, 2801, and 3302; Schilder et al. 1999b). From each of 22 colonies, which were found to contain no variation and were heterozygous at least at two loci, three callows (freshly eclosed workers, emerged in the lab) and 10–15 foragers were transferred into new nests. The callows were individually marked with dots of Edding marker paint.

Nests consisted of plastic boxes (20 × 10 × 6 cm) with plaster floors. A preformed cavity in the plaster (7 × 5 × 0.5 cm) covered with a glass plate and red foil, served as a nest chamber. Experimental colonies were kept under near natural conditions (27°C, 60% humidity, 12 L:12 D cycle) and fed ad libitum a mixed diet of honey and pieces of crickets, cockroaches and *Drosophila* flies. The plaster was regularly moistened and a tube with water and cotton served as a permanent water supply.

From each colony, freshly eclosed new callows and pupae were collected and stored at −20°C. Eggs and larvae were not used to insure that the collected offspring consisted only of females and no males. After 6–9 months, six of the 22 experimental colonies had produced 23 callows each, i.e. a number sufficient for the comparison of genotypes. These six experimental colonies were observed to determine the egg layer, which is generally the individual most often observed resting on the egg pile (Hartmann et al. 2003). At the end of the experiment, the reproductive individual was also genotyped for comparison of mother—offspring genotypes.

Male production in the field and laboratory

During field work, males of *P. punctata* were rarely found, and also rarely eclosed in laboratory maintained colonies. For an overview of the number of males found in different *P. punctata* populations and produced in the lab, see Table 1. Males were not found in all populations, probably because of seasonal variation in male production. Some samples were brought to the laboratory only as alcohol material and could therefore not be monitored for further male production. In total, 37 males were obtained for genetic analysis.

Table 1 Male production in field and laboratory colonies

Place of collection	Date of collection	No. of colonies collected	No. of field males/ No. of male producing colonies	No. of lab males/No. of male producing colonies	Duration of male production in laboratory colonies
Barbados	June 2007	22	2/2	2/2	Jul 07–Jan 09
Belize	November 2007	10	0	7/3	Dec 07–Jan 09
Dominica	October 2008	7	0	/	
Dominican Republic	November 2006	51	4/4	6/6	Dec 06–Jan 09
Florida	July 2006	12	6/2	16/9	Aug 07–Jan 09
Grand Bahama Island	November 2008	2	0	/	
Grenada	June 2007	14	0	/	
Honduras	April 2008	5	0	6/3	May 08–Jan 09
New Providence Island	July 2007	6	0	/	
Puerto Rico	October 2006	73	0	10/7	Nov 05–Jan 09
Texas	January 2008	5	0	3/3	Feb 08–Jan 09

Colonies from Grenada, New Providence Island, Grand Bahama Island and Dominica were brought to the laboratory as alcohol material and therefore were not screened for male production in the laboratory

Screening of field colonies

Field colonies from Puerto Rico ($n = 47$), Dominican Republic ($n = 27$), Barbados ($n = 13$), Grenada ($n = 10$), Dominica ($n = 7$), Grand Bahama Island ($n = 2$), New Providence Island, Bahamas ($n = 5$) and Florida ($n = 9$) were investigated for intra-colonial variation by genotyping up to 12 individuals per colony. Thelytokous reproduction is in general monopolized by a single individual per colony (Heinze and Hölldobler 1995), and therefore we consider the genotyped individuals as offspring of one mother. In total, 1,420 female workers from 120 colonies were analyzed with the same set of five polymorphic microsatellites as the experimental colonies and the males.

Genotyping of experimental set ups, field colonies and males

From each of the six experimental colonies, the egg layer and 23 offspring, in total 37 males, and 1,420 females from field colonies were genotyped. DNA was extracted from complete individuals following modified CTAB method (Sambrook and Russell 2001). Isolated DNA was washed with 100% Ethanol and twice with 70% Ethanol, dried and resuspended in 50 µl double distilled water and stored at −20°C until use.

Microsatellite fragments were amplified for the loci the mother colony had been found heterozygous (=informative loci), in field colonies and in the males, microsatellite fragments were amplified for all 5 loci. PCR conditions were modified after Hartmann et al.

(2005). DNA was amplified in a total reaction volume of 20 µl, containing 1 µl template DNA. Each reaction contained 2 µl of 10× reaction buffer (for 3506, 2902, 4101 and 2801: Fermentas 10× Taq Buffer + KCl–$MgCl_2$; for 3302: Fermentas 10× Taq Buffer + $(NH_4)_2SO_4$–$MgCl_2$), 2 µl (5 pmol/µl) of each primer (forward primer labeled with different types of fluorescent dye, Applied Biosystems), 4 µl dNTPs (1 mM of each), 0.5 µl Taq polymerase (1U/µl Fermentas), 1.2 µl 25 mM $MgCl_2$ and 7.3 µl PCR-H_2O (Sigma). After an initial 5 min denaturation step at 94°C, the reaction mix was incubated at the following temperature cycles: 30 cycles of 1 min denaturation at 94°C, 1 min primer annealing at 50–54°C (locus 3506: 50°C; 2801: 53°C; 3302, 4101 and 2902: 54°C), and 1 min extension at 72°C. The reaction was terminated by a final 5 min extension step before cooling to 4°C. The run time for each of the steps, denaturation, annealing and extension was extended from 1 to 1.5 min for locus 2801 and 3302. The amplified microsatellite fragments were scored on an ABI Prism 310 Genetic Analyzer. Allele lengths were determined using GeneScan© software.

Determining the mode of thelytoky

For determining the mechanism of thelytoky in *P. punctata*, we adopted an approach similar to that of Pearcy (Pearcy et al. 2006). The probability of selecting two identical alleles (a or b) among two pairs of alleles (aa and bb), without replacement, is 1/3, which equals the probability of a heterozygous locus becoming homozygous through recombination. This means that the probability of recombination depends on the location of the locus on the chromosome and ranges from 1/3 (locus is far away from the centromere) to 1 (locus is close to the centromere). Therefore, by calculating the proportion of homozygous offspring of a heterozygous mother it is possible to infer the most likely mechanism. If thelytoky were apomictic, all offspring would be identical clones of the heterozygous mother and the proportion of homozygous offspring would be 0. However, under this mechanism the production of males is unlikely, because meiosis must take place to produce males. If thelytoky followed an automictic mechanism, we would expect the proportion of homozygous offspring to be: (a) for central fusion (fusion of second division non-sister nuclei): 0 without recombination (all offspring being heterozygous) and ranging from 0 to 1/3 in case of recombination; (b) for terminal fusion (fusion of second division sister nuclei) without recombination 1 (all offspring being homozygous) and ranging from 1/3 to 1 in case of recombination. Possibility (c) random fusion, would lead to a probability of 1/3 for each locus, independent of the location of the locus (for a more detailed explanation of these predictions see Oldroyd et al. 2008). Under gamete duplication, the proportion of homozygote offspring would be 1 in any case, but this mechanism is incompatible with sl-csd, as all offspring would be homozygous at the sex determining locus and therefore diploid males.

Determination of haploid and diploid males

Individuals (females or males) were sexed based on their morphology prior to DNA extraction. Diploid males were identified by heterozygosity at least at one locus (3506, 2902, 4101, 2802, and 3302). For males, which were found to be diploid, PCR and sequencing were repeated to exclude scoring errors. Male genotypes were compared to the available genotypes of the colony of origin.

Results

Mother-offspring comparison

In each of the six experimental colonies, all 23 analyzed offspring were identical to each other and to their mother at all scored microsatellite loci (Table 2). No changes from heterozygosity to homozygosity were observed at the informative loci. The proportion of homozygote offspring is therefore 0. Males were never produced in the experimental colonies.

Male production

Diploid males arise through recombination between the sex locus and the respective centromere. We therefore focused on males and determined how many males were diploid. Four of the 37 genotyped males were found to be heterozygous at one of five loci (locus 2801) and therefore considered to be diploid. Each of the heterozygous males carried the same heterozygous allele combination as their worker nestmates. The remaining four loci were not heterozygous, which can be explained by homozygosity of the mother colony at these loci. Three of the four males stemmed from thelytokous colonies from Florida and Puerto Rico, and one male came from a colony from Honduras. All other analyzed males appeared to carry only one allele, even though female individuals in their colonies were heterozygous. We therefore consider these males to be haploid, hemizygous males. Whereas some colonies produced more than one male (especially colonies from Central

Table 2 Genotypes of reproductives of the thelytokous ant *Platythyrea punctata* and their offspring at the informative, heterozygous loci

Colony	Analyzed individuals	Informative loci			
		3302	4101	2801	
Tm1	Reproductive	240/244	201/203	382/386	
	Offspring ($n = 23$)	240/244	201/203	382/386	
		3302	2801	4101	
Tm2	Reproductive	240/244	368/386	203/211	
	Offspring ($n = 23$)	240/244	368/386	203/211	
		3302	4101		
Tm3	Reproductive	238/244	201/211		
	Offspring ($n = 23$)	238/244	201/211		
		3302	4101	2801	
Tm4	Reproductive	240/242	201/203	382/386	
	Offspring ($n = 23$)	240/242	201/203	382/386	
		3302	3506	2801	
Tm5	Reproductive	238/244	193/203	382/386	
	Offspring ($n = 23$)	238/244	193/203	382/386	
		3302	4101	3506	2801
Tm6	Reproductive	238/244	199/211	193/203	382/386
	Offspring ($n = 23$)	238/244	199/211	193/203	382/386

Number of offspring is given in parenthesis; all analyzed offspring show identical genotypes to the mother

American locations, which might give a hint on more frequent sexual reproduction in these populations; Kellner, Seal and Heinze unpublished data), most colonies from thelytokous populations did not produce a single male. Only in a few colonies from thelytokous populations more than one male was produced, and in most cases these males had the same genotype, which is not surprising given the low sample size. An overview over males and colony genotypes for thelytokous colonies is given in Table 3. The occurrence of only three diploid males in thelytokous populations either suggests an extremely low recombination rate between the sex locus and the centromere or an efficient culling of diploid males in a very early developmental stage.

Recombination rates

While the comparisons of mothers and daughters at five microsatellite loci did not reveal recombination, the rare occurrence of diploid males suggests occasional recombination between the sex locus and the centromere. Among the 1,420 genotyped individuals and several hundred more collected in the field and produced in the lab, only three were diploid males, thus the presumed recombination rate at the sex locus must be lower than 1%.

Additional field data supports our hypothesis that recombination at microsatellite loci does occasionally occur: 15 out of 120 analyzed field colonies from thelytokous populations contained intra-colonial variation due to homozygosity at least at one locus, with 35 out of 1,420 investigated workers presenting homozygote genotypes although their nestmates were heterozygote (2.46%) (Table 4).We calculated recombination rates of microsatellite loci based on the number of heterozygous individuals (informative meioses) and used the ratio of heterozygous to homozygous individuals to calculate the proportion of undetected recombination events in homozygous individuals. Results for recombination rates are shown in Table 5. For each locus, recombination events, if occur at all, seem to be extremely low. This might explain why we did not observe changes from hetero- to homozygosity in the mother-offspring comparison no: the number of produced offspring was probably too low to detect the recombination events.

Discussion

Our results show that in the thelytokous ant *Platythyrea punctata*, offspring and their mothers exhibit identical genotypes, and no recombination events (changes from hetero- to homozygosity) were detected in the experimental colonies. This is in accordance with the frequent genetic uniformity of field colonies (Hartmann et al. 2005; Schilder et al. 1999b) and population genetic analyses (Kellner et al. 2010), which reveal a population structure with a heterozygote excess, as is typical for clonal diploid organisms without recombination (Balloux et al. 2003).

Thelytokous parthenogenesis may be caused by a number of cytological mechanisms, most of which, however, do not result in clonality of female offspring without an increase of homozygosity. For *P. punctata*, we thus can exclude random fusion, terminal fusion with a low frequency of crossing-over, and gamete duplication, because they all more commonly lead to a transition from heterozygosity to homozygosity. The production of males and the rare occurrence of diploid males allow distinguishing between the two remaining mechanisms, apomictic thelytoky and automixis with central fusion and a reduced recombination rate. Whereas a sexual colony from the Central American mainland commonly produced males (Hartmann et al. 2005), in colonies from the thelytokous island

Table 3 Male and colony genotypes in thelytokous colonies of the ant *Platythyrea punctata*

Population	Colony	Genotypes	Loci				
			3506	2902	4101	2801	3302
Florida	JP5	Male	205	185	201	386	238
		Colony	205/205	185/185	201/201	368/386	238/238
	JP4	M	205	185	201	386	238
		M	205	185	201	386	238
		M	205	185	201	**368/386**	238
		Colony	205/205	185/185	201/201	368/386	238/238
	JP1	M	205	185	201	**368/386**	238
		M	205	185	201	368	238
		Colony	205/205	185/185	201/201	368/386	238/238
			205/205	185/185	201/201	386/386	238/238
	JP2	M	205	185	201	**368/386**	238
		Colony	205/205	185/185	201/201	368/386	238/238
	SI	M	205	185	201	368	238
		M	205	185	203	386	238
		Colony	205/205	185/185	201/203	386/388	238/238
			205/205	185/185	201/203	368/386	238/238
Dominican Republic	Mi1	M	193	183	203	386	238
		Colony	193/201	183/185	201/203	386/386	238/244
			193/201	183/185	201/203	384/384	238/244
Puerto Rico	WSL1	M	203	185	211	384	238
		M	203	185	211	384	238
		Colony	203/203	185/185	201/211	384/384	238/244
	EV2	M	203	185	211	386	242
		Colony	203/203	183/185	199/211	368/386	238/242
	Sa1	M	193	183	203	368	238
		Colony	193/203	183/185	199/203	368/368	238/244
	Sa5	M	203	183	203	368	234
		Colony	193/203	183/185	199/203	368/386	234/238
	Sa9	M	203	181	211	382	244
		M	203	181	211	382	244
		Colony	193/203	181/183	211/211	382/386	238/244
Barbados	HaC4	M	203	185	211	386	242
		M	203	185	211	386	242
		Colony	203/203	185/185	201/211	386/388	242/244

Most colonies consisted only of workers with a single multilocus genotype, except colonies JP1, SI, and Mi1. For these, both multilocus genotypes are shown. Males appear to carry only one allele, even when the mother colony is heterozygous. Heterozygous diploid males are indicated in bold

populations males were only sporadically observed. Nevertheless, that males are produced at all suggests that meiosis takes place in the ovaries of thelytokous females. Furthermore, assuming that sex determination in *P. punctata* follows the typical sl-csd mechanism reported for other social Hymenoptera, the finding of a few diploid males indicates homozygosity at the sex locus, presumably due to recombination. Both results point

Table 4 Intra-colonial variation in field colonies of *P. punctata*

Colony	# IND	3506	2902	4101	2801	3302
PR1	6	203/203	183/185	211/211	384/386	238/246
	6	203/203	183/185	211/211	**386/386**	238/246
PR2	11	193/203	183/185	199/211	382/386	238/244
	1	193/203	183/185	199/211	**382/382**	238/244
PR3	11	193/203	183/185	199/211	382/386	238/244
	1	193/203	183/185	199/211	**382/382**	238/244
PR4	5	193/203	183/185	203/211	386/388	238/238
	7	193/203	183/185	203/211	**386/386**	238/238
PR5	11	193/203	183/185	199/203	368/368	238/244
	1	193/203	183/185	**199/199**	368/368	238/244
PR6	2	193/203	183/185	**211/211**	382/386	238/244
	7	193/203	183/185	199/211	382/386	238/244
	1	193/203	183/185	**211/211**	**386/386**	238/244
PR7	11	203/203	183/185	199/211	368/386	238/244
	1	203/203	183/185	**211/211**	368/386	238/244
PR8	5	193/203	183/185	203/211	368/386	238/244
	7	193/203	183/185	**211/211**	368/386	238/244
BB1	11	203/203	183/185	201/211	386/388	240/242
	1	203/203	**185/185**	201/211	386/388	240/242
FL1	5	205/205	185/185	201/201	386/388	238/238
	1	205/205	185/185	201/201	**386/386**	238/238
FL2	5	205/205	185/185	201/201	368/386	238/238
	1	205/205	185/185	201/201	**386/386**	238/238
GR1	5	203/203	185/185	201/211	384/388	240/242
	1	203/203	185/185	201/211	**384/384**	240/242
DR1	11	203/203	183/185	199/211	382/386	238/244
	1	203/203	183/185	199/211	**386/386**	238/244
DR2	11	203/203	183/185	199/211	386/386	238/244
	1	203/203	183/185	**211/211**	386/386	238/244
DR3	11	203/203	185/185	199/211	386/386	238/244
	1	203/203	185/185	**211/211**	386/386	238/244

In 15 out of 120 colonies variation was observed due to homozygous workers among heterozygous nestmates. All aberrant individuals were homozygous at one locus, except one worker from colony PR5

PR Puerto Rico, *DR* Dominican republic, *FL* Florida, *GR* Grenada, *BB* Barbados; aberrant genotypes are indicated in bold

towards automixis with central fusion and a low recombination rate as most likely mechanism of thelytoky in *P. punctata* (see Fig. 1) and refute apomixis. Automixis with central fusion appears to underlie thelytoky also in the Cape Honeybee and the ant *Cataglyphis cursor* (Baudry et al. 2004; Oldroyd et al. 2008; Verma and Ruttner 1983; Pearcy et al. 2006, 2009), while apomixis has been suggested for the little fire ant, *Wasmannia auropunctata* (Fournier et al. 2005) and the fungus-growing ant *Mycocepurus smithii* (Fernández-Marín et al. 2005; Himler et al. 2009). As Rabeling et al. (2009) point out, genetic analyses with a low number of loci might not always allow distinguishing

Table 5 Recombination rates for microsatellite loci in *P. punctata*

	Locus				
	3506	2902	4101	2801	3302
Ratio Ho/He	1.925	0.823	0.445	0.585	0.194
Number of informative meioses	1,410	1,420	1,419	1,412	1,420
Lower 95% CI number of recombination events	0.000	0.011	0.000	0.002	0.000
Upper 95% CI number of recombination events	7.098	5.253	4.564	4.824	4.080
Lower 95% CI recombination rate	0.000	0.000	0.000	0.000	0.000
Upper 95% CI recombination rate	0.005	0.004	0.003	0.003	0.003

between apomixis and automixis. However, as we show here, the occurrence of males and in particular of diploid males might help clarifying whether meiosis and recombination do occur in those species in which the number of genetic markers is limited or high chromosome numbers make karyological studies difficult, as in *P. punctata* ($2n = 84$, Schilder 1999).

Evolution is expected to decrease recombination rates under automixis with central fusion to maintain genetic diversity and heterozygosity at the sex locus (Belshaw and Quicke 2003; Baudry et al. 2004). Indeed, reduced rates of recombination have previously been reported for the Cape honey bee (Baudry et al. 2004; Oldroyd et al. 2008). Our study in *P. punctata* points in the same direction. Only three of several thousand diploid individuals from thelytokous colonies were diploid males, i.e., the recombination rate at the presumed sex locus appears to be close to zero. Assuming that recombination is similarly infrequent between the investigated microsatellite loci and their respective centromers, it is not surprising that we failed to detect recombination in our mother-offspring comparisons. We point out, however, that recombination rates might be higher than our estimates because crossing-over events in homozygous individuals may have remained undetected. The scattered occurrence of isolated, homozygous workers among heterozygous nestmates in field colonies also suggests infrequent recombination, but field data in general need to be interpreted with caution. The history of field colonies is unknown and the presence of homozygous individuals amongst heterozygous nestmates might occasionally be a consequence of alternative mechanisms, such as colony fusion, the adoption of unrelated individuals workers and the replacement of reproductives by non-relatives (Kellner et al. 2010). In any case, comparative analyses show that most individuals from thelytokous populations on the Caribbean islands are heterozygous at least at one of the five studied microsatellite loci. This indicates that heterozygosity is maintained over comparatively long time spans either through the prevention of recombination or through occasional sex (e.g., Hartmann et al. 2005). A more regular combination of thelytoky and sexual reproduction has recently been documented in the ants *Cataglyphis cursor*, *Wasmannia auropunctata*, and *Vollenhovia emeryi* (Pearcy et al. 2004; Fournier et al. 2005; Ohkawara et al. 2006), where workers develop from fertilized eggs and female sexuals from unfertilized eggs through thelytokous parthenogenesis. The strikingly diverse mechanisms of sex and also caste determination in social Hymenoptera (reviewed in Heinze 2008) add hitherto hidden dimensions to the genetic diversity of insects (Normark 2003). At present, the causes and consequences of this variation are not well understood. Thelytokous parthenogenesis allows colonies to propagate without sex and might thus facilitate the fast colonization of habitat patches. Thelytokous populations of *P. punctata* are widespread

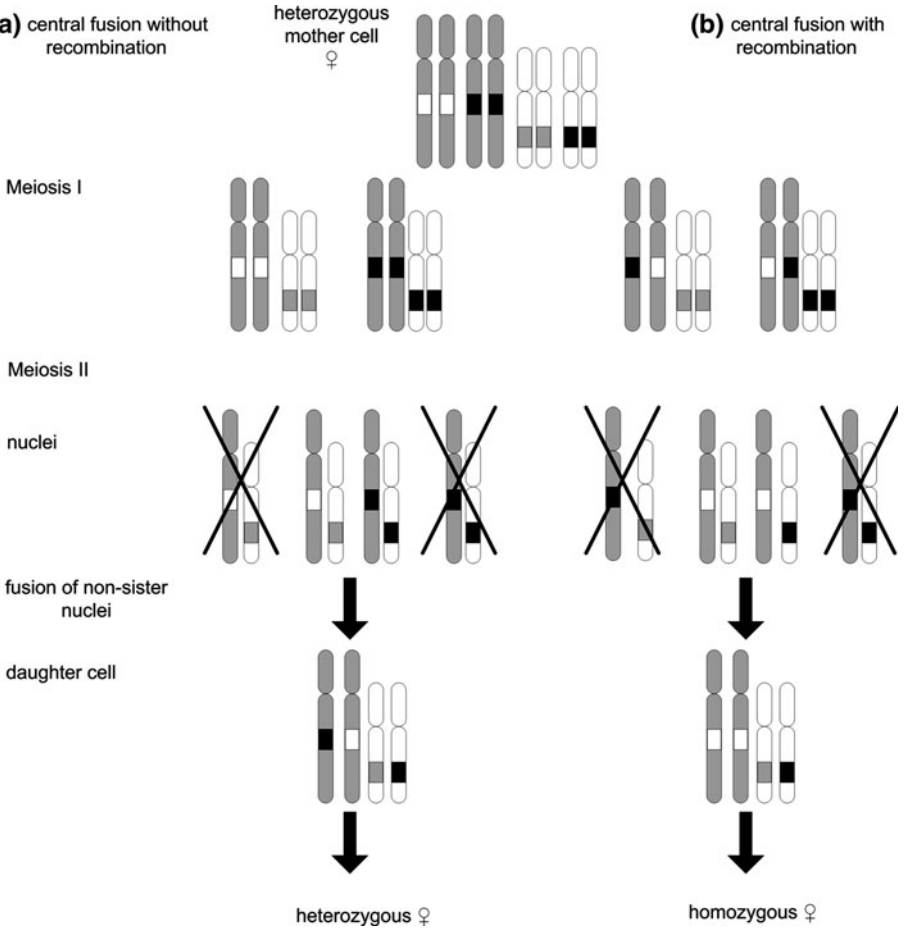

Fig. 1 Automictic parthenogenesis with central fusion, the presumed mechanism of thelytoky in the ant *Platythyrea punctata*. A heterozygous egg cell can undergo meiosis I without **(a)** or with recombination **(b)** at a microsatellite locus. After meiosis II, the two non-sister nuclei fuse to restore diploidy. Under central fusion without recombination, the daughter cell will become a heterozygous diploid, identical to the mother cell, whereas with recombination, the outcome is a homozygous diploid. The sex determination locus (CSD) is not affected under this mechanism, as long as no recombination occurs. Chromosomes are shown as bars. *Grey bars* indicate the chromosome carrying the microsatellite locus (alleles as *white* and *black* fields), and *white* chromosomes carry the CSD locus (alleles as *grey* and *black* fields)

throughout the Caribbean and can be found even on remote islands with a depauperate ant fauna, such as Barbados (Wilson 1988). Other thelytokous ants, such as *Cerapachys biroi*, *Pristomyrmex punctatus*, *Wasmannia auropunctata*, *Pyramica membranifera* and *Vollenhovia emeryi* appear to successfully invade and thrive in anthropogenically disturbed habitat, but given that population genetic and sociogenetic studies have been conducted only a minority of taxa it is likely that future research will reveal additional cases of ants with unusual genetics.

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