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Sex at the margins: parthenogenesis vs. facultative and obligate sex in a Neotropical ant

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Abstract

Geographic parthenogenesis is a distribution pattern, in which parthenogenetic populations tend to live in marginal habitats, at higher latitudes and altitudes and island-like habitats compared with the sexual forms. The facultatively parthenogenetic ant *Platythyrea punctata* is thought to exhibit this general pattern throughout its wide range in Central America and the Caribbean Islands. Workers of *P. punctata* from the Caribbean produce diploid female offspring from unfertilized eggs by thelytokous parthenogenesis, and mated females and males are rare. In contrast, workers in one colony from Costa Rica were incapable of thelytoky; instead mated workers produced all female offspring. Because sample sizes were very low in former studies, we here use microsatellite markers and explicit tests of thelytoky to examine the population genetic structure of ancestral and derived populations of *P. punctata* throughout the Caribbean and Central America. Populations from the Caribbean islands were fully capable of parthenogenesis, and population genetic signatures indicate that this is the predominant mode of reproduction, although males are occasionally produced. In contrast, the northernmost population on the mainland (Texas) showed signatures of sexual reproduction, and individuals were incapable of reproduction by thelytoky. Contrary to expectations from a geographic parthenogenesis distribution pattern, most parts of the mainland populations were found to be facultatively thelytokous, with population genetic signatures of both sexual and parthenogenetic reproduction.

Introduction

Species in which sexual and asexual or unisexual (parthenogenetic) reproduction co-occur are useful models to investigate the ecological conditions that favour either mode of reproduction and the consequences of the alternative reproductive tactics on dispersal and population structure. Geographic parthenogenesis is a distribution pattern in which parthenogenetic taxa and their sexual relatives inhabit distinct ranges. Accordingly, parthenogens tend to occur at higher altitudes and/or latitudes, island or island-like habitats instead of the mainland, in xeric rather than mesic conditions, in more continental than maritime and in disturbed rather than undisturbed habitats (Suomalainen, 1950; Glesener & Tilman, 1978).

There are a number of explanations for this pattern. Parthenogenetic reproduction is thought to be adaptive when populations are expanding into formerly uninhabited habitat, such as higher latitudes or elevations during periods of warmer climate, into deserts during periods of increased humidity, or to islands at lower sea-levels (Vandel, 1928; Maynard Smith, 1971; Williams, 1975; Glesener & Tilman, 1978; Bell, 1982; Hamilton *et al.*, 1990; Hörandl, 2009). Populations in these newly invaded, marginal habitats are often small and depleted of genetic variation (Brown *et al.*, 1996; Peck *et al.*, 1998; Pujol *et al.*, 2009; Sexton *et al.*, 2009), and individuals have difficulties in finding suitable mating partners (Baker, 1955; Cuellar, 1977; Gerritsen, 1980; Ben-Ami & Heller, 2008; Hörandl, 2009). Females capable

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of propagating without sex therefore might have an advantage in these areas. Furthermore, newly invaded areas may lack predators, pathogens and competitors, which may release the species from selection pressures commonly thought to favour sexual reproduction (Glesener & Tilman, 1978; Hamilton, 1980; Hamilton *et al.*, 1990; Lively *et al.*, 1990; Lively & Jokela, 2002). Consequently, many species of plants and animals show sexual reproduction in the core of their geographical range and asexual/unisexual reproduction in more marginal areas (Glesener & Tilman, 1978; Beaton & Hebert, 1988; Parker & Niklasson, 2000; Schön *et al.*, 2000; Stenberg *et al.*, 2003). For example, in Australian deserts, parthenogenesis appears to be an adaptive strategy of organisms such diverse as grasshoppers, geckos and *Acacia* trees (Kearney, 2003).

Although the occurrence of geographic parthenogenesis is well documented from solitary insects (Bell, 1982; Suomalainen *et al.*, 1987; Knebelsberger & Bohn, 2003; Lundmark & Saura, 2006; Schneider & Elgar, 2010) little is known about its relevance in social insects [in termites, e.g., Matsuura *et al.* (2004) and in ants, e.g., Rabeling *et al.* (2011)]. In the social Hymenoptera, thelytokous reproduction appears to be rare, despite many features of social insect biology that might make parthenogenesis particularly adaptive in some circumstances. For example, females can mate only during a very short period early in their lives (Bourke & Franks, 1995), and life-long sperm storage is costly (Tschinkel, 1987; Baer *et al.*, 2009). Additionally, inbreeding increases costs via production of inviable or sterile diploid males (Whitehorn *et al.*, 2009; Armitage *et al.*, 2010; Pearcy *et al.*, 2011).

Platythyrea punctata is one of the few species characterized by the co-occurrence of sexual reproduction and the production of diploid, female offspring from unfertilized eggs by automictic thelytoky with central fusion (Kellner & Heinze, 2011). Thelytoky is thought to be the typical mode of reproduction on the islands of Puerto Rico, Barbados, Hispaniola (Dominican Republic) and in southern Florida (Schilder *et al.*, 1999a,b; Hartmann *et al.*, 2005; Kellner *et al.*, 2010), although a few mated workers ('gamergates') and queens and numerous males have been collected (Hartmann *et al.*, 2005; Kellner & Heinze, 2011). In contrast, a single colony collected in Costa Rica contained three fertile, mated workers, and unmated workers from this population were incapable of producing diploid offspring (Hartmann *et al.*, 2005). As is characteristic of most ants in the subfamily Ponerinae, colony size in *P. punctata* is generally small (up to a few hundred workers). In most colonies, a single, unmated worker monopolizes reproduction (Heinze & Hölldobler, 1995). This simple social organization facilitates the investigation of the genetic structure of colonies and populations (Kellner *et al.*, 2010).

Here, we document the results of an extensive population survey throughout most of the distribution range of *P. punctata*. Contrary to expectations from geographical parthenogenesis patterns, we find evidence for obligate sexual reproduction only at the northernmost population of the species' range. This matches the earlier finding of sexual reproduction in the southernmost population (Hartmann *et al.*, 2005). Controlled laboratory-rearing experiments document that workers from the northernmost population are incapable of thelytoky in contrast to workers from the other studied populations.

Materials and methods

Sample collection

Entire colonies of *P. punctata* were collected in Florida (July 2007) and on eight Caribbean islands: Grand Bahama Island (November 2008), New Providence (July 2007), Jamaica (February 2009), Hispaniola (Dominican Republic, November 2006), Puerto Rico (October 2005), Dominica (October 2008), Barbados and Grenada (both June 2007), which are henceforth referred to as the 'Caribbean population' (Florida and the islands). On the North- and Central American mainland, colonies were collected in Texas (January 2008), Belize (November 2007) and Honduras (May 2008). Colonies were located by following foragers to their nests and collected by breaking hollow twigs, branches, rotten logs and stems. After collection, complete colonies were stored in 100% ethanol (colonies from Grenada, New Providence, Dominica, Grand Bahama and Jamaica) or transferred alive to the laboratory for further investigation. Colony sizes were recorded immediately after collection when possible or after arriving in the laboratory. An overview over sample localities, number of colonies and colony sizes is given in Fig. 1 and Supplementary Table S1.

Molecular techniques and genotyping

A total of 1591 individuals from 142 colonies from 12 populations were analysed. From each colony, up to 12 individuals (six adults and six brood items (larvae or pupae) or callows, i.e., freshly eclosed workers) were genotyped. Eleven colonies contained 3–9 workers and smaller numbers of brood; in these cases, all individuals available were used. Whole ants/larvae/pupae were crushed in liquid nitrogen, and total genomic DNA was extracted following a modified CTAB extraction protocol (Sambrook & Russell, 2001). Isolated DNA was washed with 100% ethanol and twice with 70% ethanol, dried and re-suspended in double-distilled water (50 μL for individual ants and pupae, 40 μL for larvae) and stored at −20 °C. We determined the genotypes at microsatellite loci 3506, 3302, 2902, 4101 and 2801 using primers developed by Schilder *et al.* (Schilder *et al.*, 1999b). PCR conditions were as in the study by Kellner & Heinze, 2011;. The number of microsatellite

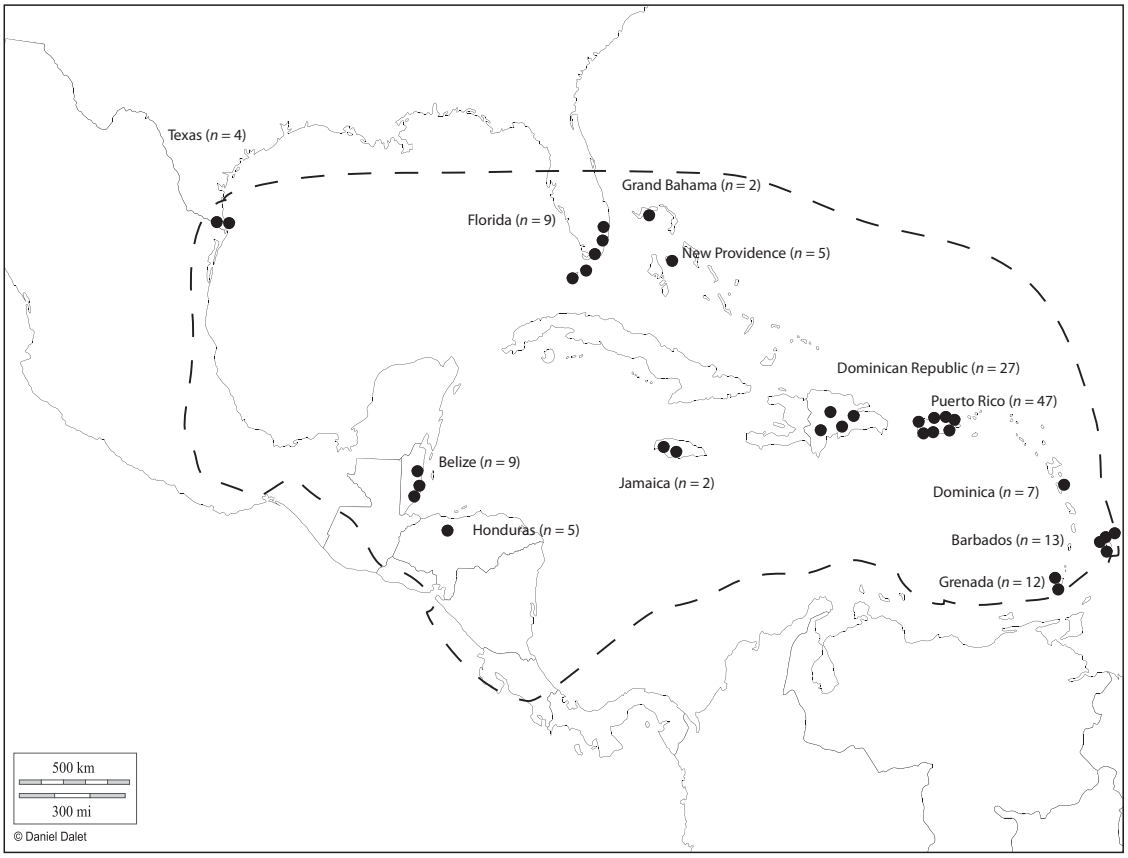

Fig. 1 Map of the Caribbean and nearby Central America, the distribution area of the ponerine ant *Platythyrea punctata*. The approximate distribution is circled in dotted line. Named areas indicate islands or countries where specimens were collected and used in this study. Number of colonies analysed in this study is given in parentheses. Dark spots indicate collecting sites. For GPS data of collection sites and colony sizes, see Table S1.

markers and the number of detectable alleles have been shown to be powerful enough to distinguish among genotypes (Kellner *et al.*, 2010; Kellner & Heinze, 2011). The amplified microsatellite fragments were scored on an ABI Prism 310 Genetic Analyzer. Allele lengths were determined using GENESCAN© software (Applied Biosystems, Foster City, California). Raw microsatellite data were deposited in the Dryad repository (doi: 10.5061/dryad.89q2c).

Comparative analysis of populations

Using the algorithms implemented in RELATEDNESS 5.0.8 (Goodnight & Queller, 1998), we calculated within-colony relatedness (standard errors obtained by jack-knifing over loci) and the average relatedness between colonies (standard errors obtained by jack-knifing over colonies). The number of multilocus genotypes (i.e. the number of different lineages) was inferred for each population, and both ratios of distinct genotypes per analysed individuals (Genotype/Individuals ratio) and per colony (Genotype/Colony ratio) were calculated. Departures from Hardy–Weinberg equilibrium were calculated using GENEPOP 4.0 (Raymond & Rousset, 1995), with a global test (Score (U) test) for each population, testing the hypothesis of heterozygote excess using the Markov chain method (100 batches, 10 000 iterations per batch). Probability of genotypic linkage disequilibrium was tested as implemented in GENEPOP (Markov chain parameters: 10 000 dememorization, 100 batches, 10 000 iterations per batch).

Observed and expected heterozygosities for each population and for each of the five loci were calculated using the program GDA 1.0 (Lewis & Zaykin, 2001). Expected and observed heterozygosities were compared statistically using the Wilcoxon test for matched samples. All statistical tests were carried out in STATISTICA 6.0 (Statsoft, 2003). For each population, a two-level analysis of molecular variance was performed, defining colonies as subpopulations. Fixation indices, describing differentiation among individuals within colonies (f), differentiation among individuals within the population (F) and differentiation among colonies within the population (θp) were calculated following the methods of Weir and Cockerham

(Weir & Cockerham, 1984; Weir, 1996) as implemented in GDA 1.0. 95% confidence intervals were obtained by bootstrapping over loci (1000 pseudoreplicates).

Tests for thelytokous vs. sexual reproduction

Absence of sperm in a worker's sperm storage organ (spermatheca) normally suggests that it has never mated (except in the rare cases of sperm depletion in very old individuals). In contrast, the presence of sperm in a worker's spermathecea does not necessarily prove that sperm is used to fertilize eggs. We therefore determined whether a population is predominantly thelytokous or sexual by the following four methods.

First, we compared the ratio between the number of distinct genotypes and the number of studied individuals (Genotype/Individuals ratio) and the number of distinct genotypes per colony (Genotype/Colony ratio). The Genotype/Individuals ratio is expected to approach 0 in a strictly clonal population and 1 in a strictly sexual population (Halkett *et al.*, 2005). The Genotype/Colony ratio describes the distribution of genotypes within colonies. A monoclonal colony will have a Genotype/Colony of 1, whereas a Genotype/Colony > 1 would mean that colonies contain more than one lineage, due to multiple parthenogenetic lineage, sexual reproduction or colony mixing.

Second, we examined the inbreeding coefficients obtained by *F*-statistics. Mean inbreeding coefficient f across loci in a strict clonal population is expected to approach -1 and the F value is expected to be ≤ 0. Additionally, the observed heterozygosity (H_o) is expected to be approximately twice as high as the expected heterozygosity (H_e) [the Meselson effect, (Balloux *et al.*, 2003; de Meeûs & Balloux, 2005)]. The mechanism of thelytoky in *P. punctata* most likely is automixis with central fusion of daughter nuclei following meiosis, but with a low recombination rate (Kellner & Heinze, 2011). This has the consequence of heterozygosity at a given locus most likely being preserved. In contrast, in a sexual population, we expect observed and expected heterozygosity to be more balanced.

Third, we visually examined the multilocus genotypes for traces of recombination that might be due to sexual reproduction. Due to haplo-diploid sex determination, related workers have to share at least one allele at a given locus (under the assumption of a single reproductive mother, singly mated). If workers differ in more than one allele at a given locus, they are unrelated and thus the observed variation pattern cannot be due to sexual recombination. This was necessary because the interpretation of population genetic data in *P. punctata* is slightly complicated by the organization of individuals into colonies (which increases relatedness but lowers variation within colonies and leads to inbreeding coefficients f different from 0), the rare occurrence of crossing over during automictic parthenogenesis with central fusion (Kellner & Heinze, 2011), and the merging of unrelated colonies, which leads to multi-clone colonies (Kellner *et al.*, 2010).

Fourth, in a laboratory experiment, we tested for the ability of young workers to produce diploid, female offspring from unfertilized eggs. Thelytokous colonies of *P. punctata* can be kept under laboratory conditions for years without failing to rear brood from unfertilized eggs. In this study, nineteen colonies were chosen among those that were in the laboratory from the Caribbean islands, Honduras, Belize and Texas. We used all of the colonies available from the mainland (Honduras ($n = 5$), Belize ($n = 1$), and Texas ($n = 3$)) and Florida ($n = 5$). Haplotype analysis indicates that most colonies collected in Florida are Caribbean in origin, despite being on the North American mainland (Seal *et al.*, 2011). In addition, five colonies were randomly chosen among 30 colonies collected in the Dominican Republic. Colony size (number of workers) of these 19 colonies did not differ between the mainland and Caribbean populations ($F_{1,18} = 0.629$, $P = 0.438$). In May 2008, all brood items (callow adults, pupae and larvae) were removed from each colony and placed into new nest boxes with a plaster floor. Each box contained a freshly eclosed, unmated nurse worker from the original colony to take care of the otherwise orphaned brood. The newly established colonies were kept in a climate-controlled environmental chamber (12:12 L: D) and fed three times per week a mixed diet of honey, *Drosophila*, and pieces of crickets and cockroaches. The plaster floor was regularly moistened. Nest boxes were regularly checked for the presence of newly laid eggs, and presence/absence of cocoons and female offspring developing from unfertilized eggs was recorded over three months. The test for thelytoky was repeated by isolating brood and a young nurse worker from those colonies that had produced female offspring. Observations ended in December 2008. Although males are produced in laboratory and field colonies (Kellner & Heinze, 2011), none were observed in our experiment, and all workers therefore remained unmated. The inability of an unmated individual to produce eggs was seen as evidence for obligate sexual reproduction and the absence of thelytokous parthenogenesis. Growth rates were analysed using repeated measures analysis of variance with Statistica version 6. Untransformed data were used because none of the critical assumptions were violated for general linear models [normality, random distribution of error variance and sphericity, (Sokal & Rohlf, 1995; Keselman *et al.*, 2001)].

Results

Population genetic analyses

Across a total of 124 colonies from 12 different sites in Central America and the Caribbean (see Fig. 1, Table

Table 1 Number of multilocus genotypes/lineages, intracolonial variation and average within-colony relatedness (*R*, with standard errors (SE) given in parenthesis) measured with five microsatellite genotypes in the ant *P. punctata*. The five markers revealed 143 distinct genotypes (lineages).

Population	Number of genotypes found	Number of colonies analysed (Number of colonies containing variation)	%	Average *R* (SE) within colonies
Florida	11	9 (6)	66.7	0.90 (0.077)
Grand Bahama	2	2 (0)	0	1.00 (0.00)
New Providence	1	5 (0)	0	1.00 (0.00)
Jamaica	2	2 (0)	0	1.00 (0.00)
Dominican Republic	22	27 (10)	37.0	0.92 (0.046)
Puerto Rico	42	47 (20)	42.6	0.94 (0.018)
Dominica	5	7 (1)	14.3	0.79 (0.204)
Barbados	12	13 (6)	46.2	0.90 (0.036)
Grenada	4	12 (1)	8.3	0.99 (0.008)
Caribbean total	94*	124 (44)	35.5	0.93 (0.025)
Texas	17	4 (4)	100	0.60 (0.135)
Belize	21	9 (8)	88. 9	0.76 (0.056)
Honduras	11	5 (3)	60	0.85 (0.124)
Mainland total	49**	18 (15)	83.3	0.74 (0.073)

*94 is the number of unique lineages (only 5 lineages were not unique but encountered several times: Puerto Rico and Dominican Republic share two lineages, Dominica and Grenada share one lineage, Dominica shares two lineages with Barbados, and one of these is shared with New Providence and Grand Bahama Island).
**All encountered lineages are unique.

Table 2 Overview over the average relatedness between colonies and the proportion of distinct genotypes per analysed individuals (Genotype/Individuals ratio) and per colony (Genotype/Colony ratio). Means and standard deviation are given below (SD: standard deviation) for the Caribbean and the mainland population.

	Average *R* between colonies	Number of genotypes/number individuals Genotype/individual ratio	Number of genotypes/number of colonies Genotype/colony ratio
Caribbean islands			
Florida	−0.08	0.12	1.22
Grand	−1.00	0.08	1.00
Bahama			
New	1.00	0.02	0.20
Providence			
Jamaica	−1.00	0.08	1.00
Dominican	−0.03	0.07	0.82
Republic			
Puerto	−0.02	0.07	0.89
Rico			
Dominica	−0.18	0.08	0.83
Barbados	−0.08	0.08	0.92
Grenada	−0.11	0.08	0.33
Mean (SD)	0.17 (0.59)	0.08 (0.03)	0.80 (0.33)
Mainland			
Texas	−0.26	0.49	4.25
Belize	−0.12	0.30	5.25
Honduras	−0.29	0.26	2.20
Mean (SD)	−0.22 (0.09)	0.35 (0.12)	3.90 (1.55)

S1 and Table 1), the microsatellite loci used were highly polymorphic, with the total number of alleles ranging from nine at locus 3506 to 20 at locus 3302. This made it possible to detect considerable genetic diversity in terms of multiple lineages in nearly all the populations (ranging from a single lineage on New Providence, Bahamas, to 42 lineages on Puerto Rico, see Table 2). No significant linkage disequilibrium was detected in the mainland populations ($P = 0.06$–1.00) (results of island populations indicate non-independent inherence of loci, see Kellner *et al.*, 2010). There was no sign of the presence of null alleles at the microsatellite loci.

The population genetic characteristics of colonies and populations matched the prediction from former studies (Schilder *et al.*, 1999b; Hartmann *et al.*, 2005) that reproduction in the Caribbean is mainly through thelytokous parthenogenesis, whereas sexual reproduction is common on the mainland: mainland populations showed a higher genotype/individual ratio and also a higher genotype/colony ratio than populations in the Caribbean region (*t*-test, $t_{10} = -6.917$, $P < 0.0001$, $t_{10} = -6.157$, $P < 0.0001$; see also Table 2).

In the Caribbean, approximately two-thirds of the colonies (80 of 124) did not exhibit any within-colony variation and thus appeared to consist entirely of genetic identical individuals with a Genotype/Colony ratio of 1. The remaining 44 colonies (36%) showed some intra-colonial variation (Table 1). Genetic heterogeneity often (in 17 colonies) was caused by the presence of a single worker with a non-matching genotype and could not be explained by sexual recombination. Additionally, variation among individuals was hardly ever observed among analysed brood, suggesting genetic heterogeneity resulted from the adoption of stray workers or merging of colonies. Other incongruous genotypes may have arisen from sporadic automictic recombination events [see also (Kellner *et al.*, 2010; Kellner & Heinze, 2011)].

Within-colony relatedness in the Caribbean islands ranged from $R = 0.20$ (colonies which contained several unrelated lineages) to $R = 1$ (colonies which completely consisted of a single lineage). Since only a minority of colonies contained more than one lineage, the overall average of within-colony relatedness over all populations from the Caribbean was high ($R = 0.93$; SE 0.025). This

value is significantly different from 0.75, the expected relatedness among sexually produced offspring of a single, singly-mated female in a haplo-diploid Hymenoptera [(Cook, 1993), *t*-test, $t_8 = 7.2$, $P < 0.0001$; see details in Table 1].

In contrast, on the mainland, the vast majority of the colonies collected (83%; 15 out of 18) showed intracolonial variation ($\chi^2 = 14.82$, d.f. = 1, $P = 0.0001$). Variation was usually expressed in more than one individual and in more than one locus. This is also reflected in the average relatedness values (see Table 1). The overall average of within-colony over all mainland population relatedness of $R = 0.74$ (SE 0.07) was not significantly different from 0.75 (*t*-test, $t_2 = 0.137$, $P = 0.91$). Nevertheless, three colonies from the mainland (one from Guanacaste, Belize, and two from Lancetilla, Honduras) did not show any variation (see Table 1) across all individuals and alleles.

Calculated by loci, observed heterozygosity (H_o) in the Caribbean populations was significantly higher than the expected heterozygosity (H_e) (Wilcoxon matched pairs test, $n = 9$, Z = 2.192, $P = 0.028$). The same result was found for calculation by colonies ($n = 9$, Z = 2.666, $P = 0.008$). Such an overabundance of heterozygote individuals is usually interpreted as evidence of unisexuality/parthenogenesis (Balloux *et al.*, 2003; de Meeûs & Balloux, 2005). In contrast, mainland populations did not have a significant heterozygote excess ($n = 3$; calculated by locus: $Z = 1.069$, $P = 0.285$; by colony: $Z = 1.604$, $P = 0.109$; see Table 3).

Tests for Hardy–Weinberg equilibrium revealed significant departure ($P < 0.001$) in all analysed populations. Fixation indices obtained for each population revealed *f* values (differentiation of individuals within colonies) close to −1 in most populations from the Caribbean islands, whereas populations from the mainland were less negative (closer to 0) (see Table 3 for details.). Since colonies represent families, which always exhibit a certain amount of genetic similarity, these findings are in accordance with the obtained values for within-colony relatedness. The strong negative values reflect the high genetic similarity among nestmates. *F* values (overall inbreeding coefficient) were also negative or statistically not different from 0 for the Caribbean populations and in the populations of Texas and Honduras. Only for the Belize population, *F* was significantly higher than 0. θp (differentiation among colonies within population) was significantly positive in the case of Puerto Rico, Dominican Republic and Barbados, that is, those islands where a high number of multilocus genotypes was encountered. In the Florida population, a similar pattern was found. In contrast, where a low number of different genotypes was recorded (Grenada, Dominica, the two Bahamas Islands and Jamaica), genetic differentiation among colonies was low. Comparing Caribbean and mainland population, we found that *f* values are significantly closer to −1 in the Caribbean population than in the mainland population (*t*-test; $t_{10} = -4.34126$, $P < 0.001$), whereas no significant value difference was found between *F* and θp values

Table 3 Overview of expected and observed heterozygosities (H_o, H_e) calculated over loci and populations, and the fixation indices for each population. The upper and the lower bound of the 95% CI are given in parentheses. Values significantly different from 0 are indicated in bold font. The following coefficients were calculated following the methods of Weir and Cockerham (Weir & Cockerham, 1984): *f*, differentiation among individuals within colonies; *F*, differentiation among individuals within the population; and θp, differentiation among colonies within the population.

	By locus		By population				
Population	H_e	H_o	H_e	H_o	*f*	*F*	θp
Caribbean							
Florida	0.45	0.399	0.268	0.432	**−0.668** (−0.112; −0.903)	0.369 (0.884; −0.197)	**0.622** (0.924; 0.365)
Grand Bahama	0.569	1	0.539	1	**−1** (−1.000; −1.000)	**−0.6** (−0.333; −1.000)	0.2 (0.333; 0.000)
New Providence	0.504	1	0.523	1	**−1** (−1.000; −1.000)	**−1** (−1.000; −1.000)	0 (0.000;0.000)
Jamaica	0.204	0.2	0.104	0.2	**−1** (−1.000; −1.000)	0.333 (1.000; −1.000)	0.667 (1.000; 0.000)
Dominican	0.582	0.622	0.338	0.619	**−0.864** (−0.790; −0.952)	−0.053 (0.156; −0.271)	**0.435** (0.539; 0.347)
Republic							
Puerto Rico	0.576	0.796	0.428	0.795	**−0.946** (−0.911; −0.979)	**−0.374** (−0.176; −0.657)	**0.293** (0.383; 0.161)
Dominica	0.372	0.453	0.286	0.476	−0.732 (1.000; −1.000)	−0.163 (1.000; −0.275)	0.328 (0.362; −0.013)
Barbados	0.39	0.449	0.253	0.44	**−0.867** (−0.826; −0.924)	−0.122 (0.057; −0.037)	**0.399** (0.484; 0.289)
Grenada	0.611	0.864	0.464	0.887	−0.994 (−0.972; −1.000)	−0.376 (0.155; −1.000)	0.31 (0.571; 0.000)
Mean (SD)	0.47 (0.13)	0.64 (0.29)	0.36 (0.14)	0.65 (0.28)	−0.90 (0.13)	−0.22 (0.43)	0.36 (0.20)
Mainland							
Texas	0.431	0.464	0.349	0.438	**−0.478** (−0.061; −0.699)	0.027 (0.314; −0.096)	**0.342** (0.473; 0.237)
Belize	0.687	0.462	0.395	0.511	**−0.385** (−0.242; −0.472)	**0.369** (0.432; 0.220)	**0.545** (0.611; 0.475)
Honduras	0.685	0.582	0.373	0.567	**−0.686** (−0.555; −0.847)	0.266 (0.582; −0.110)	**0.565** (0.745; 0.378)
Mean (SD)	0.60 (0.15)	0.50 (0.07)	0.37 (0.02)	0.51 (0.06)	−0.52 (0.15)	0.22 (0.18)	0.48 (0.12)

Table 4 Example of genotypic distribution of workers in a *P. punctata* colony from Texas. Two lineages were found within the colony with 6 individuals each. Three of the five analysed microsatellite loci show variation (loci 2902, 2801 and 3302; variable alleles in *italic*). The observed pattern of relatedness can be explained by sexual reproduction: the two lineages are full sisters, and share one of the two alleles from their diploid mother and one allele from the haploid father as predicted after haplo-diploid sex determination of the Hymenoptera. Putative mother and father genotypes are given below. The mean relatedness of this colony was 0.748 ± 0.26 (SE, jack-knifed over loci), which approaches the predicted value of 0.75 of social Hymenopteran colonies with a single mother singly mated. Like the other colonies from Texas, unmated workers of this colony were also unable to lay eggs.

Number of individuals	Locus 3506	Locus 2902	Locus 4101	Locus 2801	Locus 3302
6	197/197	*199*/201	190/190	364/*366*	*238*/244
6	197/197	201/*211*	190/190	364/*364*	*240*/244
Putative mother genotype	197/197	199/211	190/190	364/366	238/240
Putative father genotype	197	201	190	364	244

($t_{10} = -1.67830$, $P = 0.12$ and $t_{10} = -0.96310$, $P = 0.36$, respectively).

A visual inspection of colonies which contained variation suggested recombination due to sexual reproduction only for four colonies from Texas (see Table 4): in this case, nestmate workers showed patterns suggesting them to be full sisters following the typical haplo-diploid sex determination mechanism of Hymenoptera (Cook, 1993). Additional unrelated genotypes were found in two of these four colonies, suggesting the adoption of alien workers or colony fusions as well.

Brood-rearing observations

Colonies consisting of brood and unmated nurse workers from Belize, Honduras and the Caribbean islands produced female offspring and exhibited positive growth in all studied colonies. Examination of total productivities (numbers of workers, callows (recently eclosed workers) and pupae) strongly suggest that colonies from the Central Mainland and the Caribbean doubled or tripled in size during the 7-month period (Fig. 2; $F_{1,16} = 19.3$, $P = 0.001$). However, growth did not occur among the colonies from Texas and remained significantly smaller than those from the Caribbean or the Central Mainland (Fig. 2; $F_{2,16} = 3.78$, $P = 0.04$). Moreover, the significant interaction between region and time indicates that positive growth rate was restricted to colonies collected from the Caribbean islands and Central Mainland ($F_{2,16} = 3.81$, $P = 0.04$, Fig. 2). Thus, all colonies from the Caribbean, Belize and Honduras reproduced parthenogenetically, whereas

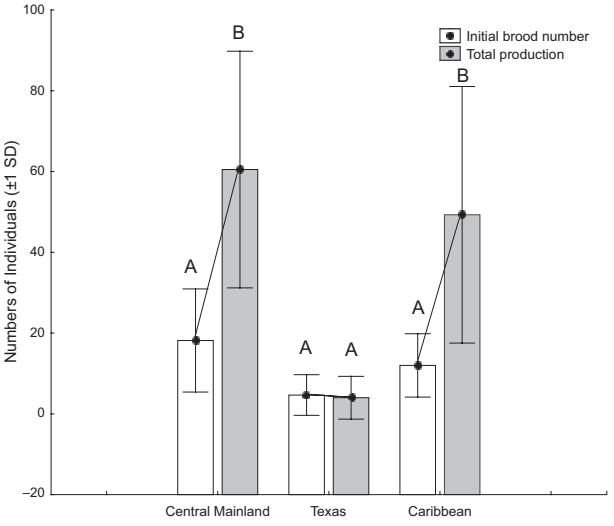

Fig. 2 Mean growth rates (± 1 SD) of Central Mainland, Texas and Caribbean populations of the ant *Platythyrea punctata*. Open bars indicate the number of brood items (larvae, pupae and callows) at the beginning of observations and solid bars indicate the total production (numbers of adults and pupae) at the end of observations (ca. 7 months later). All of these experimental colonies were started with brood and one non-reproductive nurse worker. Except for colonies from Texas ($n = 3$), all other colonies (Central Mainland $n = 6$, Caribbean $n = 10$) exhibited significant growth, suggesting that one or several unmated workers eclosing from the brood had started to produce female offspring by thelytokous parthenogenesis. Significant differences are denoted by different letters (Fisher's LSD test, $P < 0.05$).

the three colonies reared from brood from Texas did not produce new eggs; no new workers or males emerged and consequently exhibit no growth (Fig. 2). Thus, it seems unlikely that the Texas colonies could conduct thelytokous parthenogenesis. Hence, the most likely mode of reproduction of Texas colonies in the field is sexual reproduction.

Discussion

The most surprising result of our study is the discovery that thelytokous reproduction in the ant *P. punctata* does not only occur on the Caribbean islands as expected, but is also common in Central American populations. Traces of parthenogenesis are clearly evident in genotypes from colonies from Belize and Honduras, and unmated workers from these populations are capable of producing daughters. Nevertheless, the lowered relatedness and the distribution of genotypes in mainland colonies indicate at least occasional sexual recombination. This matches previous observations that males are more common in mainland than island populations (Kellner & Heinze, 2011).

Thelytoky could not be confirmed in the colonies from Texas, where genotypes within colonies exhibited

clear patterns consistent with sexual reproduction and unmated workers appeared to be incapable of producing diploid offspring. Thelytoky thus appears to occur in all studied populations except the most northern (Texas) and the most southern [Costa Rica, Hartmann *et al.* (2005)].

What explains this geographical pattern? Mapping the distribution of reproductive modes onto the species phylogeography (Seal *et al.*, 2011) suggests that both the sexual populations in Texas and Costa Rica and the thelytokous Caribbean populations are derived from the facultatively thelytokous mainland populations of Belize and Honduras. The caveat to this interpretation is that simple trait mapping might not reliably represent evolutionary history as the transition rates from sexual to unisexual reproduction and *vice versa* are unknown for this species and social Hymenoptera in general. Nevertheless, assuming that such transitions are infrequent, it is likely that thelytokous parthenogenesis evolved on the Central American mainland. It may have facilitated subsequent colonization of the Caribbean islands, as new colonies could have been founded by small groups of unmated workers without the presence of queens or males.

On the Caribbean islands, *P. punctata* almost exclusively reproduces through thelytoky. It has become one of the most common ants in primary or secondary tropical forests with little variation in annual rainfall and mean temperature, while it appears to be less common and rather patchily distributed in similar mainland forests (K. Kellner, unpublished data). Our analysis substantiated the co-existence of different genotype lineages in most populations. Hence, the success of thelytokous populations on the islands is not due to a single clonal lineage performing best across a wide range of environments, as proposed by the multi-purpose genotype hypothesis (Lynch, 1984; Hörandl, 2009). In contrast, the abundance of thelytokous *P. punctata* in the Caribbean islands might be explained by the release from enemies, pathogens and competitors. The islands harbour far fewer ant species than the mainland, especially predatory army ants and ponerine hunters, which compete with *P. punctata* on the mainland.

In the phylogeographic analysis, the sexual populations in Texas and Costa Rica are situated in separate clades, somewhat derived relative to the basal sister lineages in Honduras (with both thelytoky and sexual reproduction) and Chiapas, Mexico [mode of reproduction unknown, Seal *et al.* (2011)]. Thus, these populations cannot be considered to be basal sexual populations that share a common ancestor with all thelytokous populations (Seal *et al.*, 2011). One possibility is that thelytoky never evolved in these marginal populations, which implies that thelytoky evolved independently elsewhere in Central America and the Caribbean islands. Alternatively, under a simpler scenario, facultative thelytoky may have been lost independently in the Texan and Costa Rican populations. It is presently not known whether the apparent absence of thelytoky in these two populations is an adaptation to the seasonally hot and dry environments in which they occur – a seasonally dry tropical forest [Santa Rosa, Costa Rica, (Hartmann *et al.*, 2005)] and an area of high humidity but low rainfall (south Texas). Noteworthy is that the population in Texas appears extremely small: all colonies were located in just a few square kilometres, literally at the southernmost tip of Texas along the Rio Grande River (*Rio Bravo del Norte*). Searches elsewhere were unproductive. Considering the general lack of suitable intact subtropical forest along the river due to agriculture and other habitat destruction, these populations may be the only ones left in the entire region and the lack of thelytoky may be hindering its own viability.

Social insect species combining sexual and parthenogenetic reproduction might benefit from the predicted demographic advantages of thelytoky through fast colony growth (Vorburger *et al.*, 2003) and high dispersal potential (Sakai *et al.*, 2001). At the same time, occasional sexual recombination increases genetic diversity and might thus improve overall colony performance (Hughes & Boomsma, 2004; Pearcy *et al.*, 2004). Recent research has documented a special type of facultative parthenogenesis, in which queens produce workers from fertilized eggs and female sexuals from unfertilized eggs, in a few ant species (Fournier *et al.*, 2005; Foucaud *et al.*, 2006; Ohkawara *et al.*, 2006; Pearcy *et al.*, 2011). In one of them, the little red fire ant *Wasmannia auropunctata*, female sexuals, may be produced both from fertilized and unfertilized eggs in the introduced populations of New Caledonia (Foucaud *et al.*, 2006), whereas they only arise through parthenogenesis in other populations (Fournier *et al.*, 2005).

Our study demonstrated facultative parthenogenesis for *Platythyrea punctata*, with different populations being clearly characterized by alternative strategies of reproduction. *P. punctata* might therefore be a valuable system to trace the evolution and maintenance of thelytoky, and populations with sympatric occurrence of sexual and thelytokous reproducing colonies might become focus for further studies on the ecological benefits of either mode of reproduction and parameters determining the occurrence of sex and parthenogenesis.

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Supporting information

Additional Supporting Information may be found in the online version of this article:

Table S1 Overview over the populations and colonies of *Platythyrea punctata* sampled and used in this study. Colony sizes are given by median (upper and lower quartile).

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