ORIGINAL PAPER

Colony fusion causes within-colony variation in a parthenogenetic ant

Katrin Kellner · Benjamin Barth · Juergen Heinze

Abstract The evolutionary stability of cooperation and altruism in colonies of social insects requires that nestmates be to some extent related. An efficient system of discrimination against non-nestmates protects the nest against unrelated conspecifics, which might exploit or parasitize the colony. The co-occurrence of unrelated individuals in mature colonies therefore is a rare event that deserves more attention. Here, we report on the relatively common incidence of colony fusion in the ant *Platythyrea punctata*. Workers of this ant can produce genetically identical female offspring from unfertilized eggs through thelytokous parthenogenesis. Consequently, the majority of colonies has a "clonal structure" and consists of individuals with identical multilocus genotypes. Nevertheless, field observations indicate that a surprisingly large percentage of colonies contain workers belonging to two or more different genetic lineages. Much of this genetic heterogeneity is incompatible with eventual recombination or mutation events, but instead appears to result from colony fusion or the adoption of unrelated individuals. Indeed, colonies of *P. punctata* from the Dominican Republic and Barbados readily merged in the laboratory and, after elimination of one of the two reproductive workers, formed stable, genetically heterogeneous colonies. We discuss the possible causes and benefits of colony fusion in natural populations.

Keywords Agonistic behavior · Colony takeover · Replacement · Thelytoky · Within colony relatedness · *Platythyrea punctata*

K. Kellner (✉) · B. Barth · J. Heinze
Biologie I, University of Regensburg,
Universitätsstrasse 31,
93053 Regensburg, Germany
e-mail: katrin.kellner@biologie.uni-regensburg.de

Present Address:
B. Barth
Institut für Biologie, Martin-Luther-Universität Halle-Wittenberg,
Hoher Weg 4,
06099 Halle/Saale, Germany

Introduction

The maintenance of cooperation and altruism in animal societies requires that they protect themselves against parasites and exploitation by unrelated individuals. The colonies of social insects are generally closed systems, which maintain their integrity through a colony odor shared by all nestmates and an efficient discrimination against non-nestmates (Wilson 1971; Crozier and Dix 1979; Hölldobler and Wilson 1990, 2009). Even in the so-called "unicolonial" species, which have been thought to completely lack colony borders (Tsutsui and Suarez 2003; Chapuisat et al. 2005), aggression between supercolonies has recently been found (Vogel et al. 2009). The adoption of alien individuals or even the complete merger of two unrelated colonies is usually a rare event (Foitzik and Heinze 1998; van Wilgenburg et al. 2006; Johns et al. 2009). The occurrence of colony fusion, its causes and consequences are therefore of considerable interest for our understanding of the stability and dynamics of insect societies.

Colony fusion may be difficult to detect when colonies have a complex genetic architecture, e.g., when queens mate multiply or when multiple queens produce offspring. It is more clearly visible when individual colonies are genetically homogeneous, as in *Platythyrea punctata* (F. Smith 1858). In

almost all studied colonies of this ponerine ant from the Caribbean islands, one or occasionally a few, unmated workers monopolize reproduction and produce offspring identical to themselves by thelytokous parthenogenesis (Heinze and Hölldobler 1995; Schilder et al. 1999a; Hartmann et al. 2005). Like in other thelytokous ants (e.g., Hasegawa et al. 2001; Fournier et al. 2005), nestmates therefore usually share the same multilocus genotype (Schilder et al. 1999a; Schilder et al. 1999b). Nevertheless, some colonies of P. punctata show considerable genetic heterogeneity, most of which cannot be explained by the occasional occurrence of sexual reproduction or recombination during parthenogenesis (Schilder et al. 1999a; Hartmann et al. 2005). Instead, such variation might result from colony fusion and/or the adoption of alien individuals into established colonies. However, a more detailed analysis of genetic variation and behavioral evidence for colony fusion has so far been missing.

The aims of this study were (a) to examine the genetic variation within natural P. punctata populations in detail, (b) to determine its origin, and (c) to substantiate fusion as its cause by laboratory experiments. We estimated the frequency of colony fusion in populations from three Caribbean islands (Hispaniola, Barbados, and Puerto Rico) by genotyping at five polymorphic microsatellite loci and subsequent exclusion of cases of heterogeneity that might have arisen through alternative mechanisms. In addition, we describe the behavior of workers during the fusion of two colonies in an experimental situation of nest site limitation.

Material and methods

Study species and sample collection

P. punctata nests in preformed cavities in decaying branches, bromeliads, or roots in hardwood hammocks, secondary rain forests, and open woody habitats throughout Central America. Colonies typically consist of a few dozen workers, of which one, rarely several dominant individuals lay eggs (five egg layers found in one colony: Heinze and Hölldobler 1995; one out of nine colonies with two egg layers: Schilder et al. 1999a). Thelytokous parthenogenesis is the standard mode of reproduction in colonies from the Caribbean islands and Florida. Despite of the sporadical occurrence of mated workers and queens (e.g., Schilder et al. 1999a; Hartmann et al. 2005), sexual reproduction has been evidenced only in one of 88 examined colonies (18 colonies in Hartmann et al. 2005, 23 in Schilder et al. 1999b, and 47 in this study). Mothers and daughters have the same multilocus genotypes (Kellner and Heinze in review) and in the majority of colonies, all nestmates are genetically identical. This suggests either an apomictic

mechanism of thelytoky (i.e., without meiosis) or automixis with central fusion (meiosis and subsequent fusion of non-sister nuclei) and a very low recombination rate.

Entire colonies of P. punctata were collected on Puerto Rico in October 2005 (El Verde field station in the Luquillo Experimental Forest, N 18° 19.0′ W 65° 45.0′, n=9, Rio Grande, N 18° 24.5′ W 65° 49.6′, n=9; Sabana, N 18° 19.5′ W 65° 43.3′, n=6; Pico, N 18° 6.1′ W 67° 2.4′, n=12; El Tunel, N 18° 29.1′ W 66° 58.1′, n=7; WSL, near San Lorenzo, N 18° 16.4′ W 65° 54.3′, n=2; SAII, near Sabana, N 18° 19.2 W 65° 43.0′, n=2), in the Dominican Republic in November 2006 (Anton Sanchez AS, 18°49′ N 69°41′ W, n=6; El Laurel EL, 18°46′ N 69°53′ W, n=6; Miches Gallistico Mi, 18°58′ N 69° 2′ W, n = 6; Rancho Wendy/ Bonao RW, 18°53′ N 70°27′ W, n=9), and in Barbados in June 2007 (Hackleton's Cliff HaC, 13°12′ N 59° 31′ W, n=4; Harrison's Cave HC, 13°10′ N 59° 34′ W, n=1; Point 8: 13°13′ N 59° 34′ W, n=2; Turner's Hall Woods TH, 13°13′ N 59° 35′ W; n=6). Colony sizes ranged from seven to 475 (Puerto Rico, mean ± SD; 61.53±87.38), six to 50 individuals (Dominican Republic, 23.07±10.9), and five to 71 individuals (Barbados, 31.77±22.05).

Molecular methods

We sampled 12 individuals (six older foragers and six callows, i.e., young workers, larvae, or pupae) from each colony immediately after collection in Puerto Rico and the Dominican Republic. Several colonies from Barbados had insufficient numbers of callows and brood, and we therefore sampled brood later in July 2007. All individuals were stored in 100% ethanol for genetic analyses. From one colony from the Dominican Republic, 24 individuals were analyzed. In total, 1,046 individuals from 87 colonies were analyzed. DNA was extracted from complete adults and/or brood (larvae and pupae) using a modified CTAB-Method (after Sambrook and Russell 2001). Individuals were genotyped at five polymorphic microsatellite loci (2801, 2902, 3302, 3506, and 4101, Schilder et al. 1999b). PCR amplification products were denatured for 1 min at 90°C and analyzed with an ABI PRISM 310 Genetic Analyzer. Absolute allele lengths were determined using GeneScan® 3.1 Software (Applied Biosystems).

Genetic data analysis

We used exact tests as implemented in GENEPOP 4.0.7 (Raymond and Rousset 1995) to detect linkage disequilibria and deviation from Hardy-Weinberg equilibrium at each locus and in each collecting site. Colony and population genetic structure were investigated by estimating hierarchical F-statistics using the method of Weir and Cockerham (1984) as implemented in the program GDA 1.1 (Lewis and Zaykin

2001). As individuals within colonies are related and data are dependent, we considered colonies as the lowest subunit in a four-level hierarchical analysis of genetic variation. Different inbreeding coefficients are defined therefore as the following: individuals within colonies (f), individuals within the total sample (F), colonies within sample sites (Θ_{SS}), sample sites within islands (Θ_S), and the three islands within the total sample (Θ_P). The 95% confidence intervals for the statistics were determined by bootstrapping over loci with 1,000 replicates. A given result was considered significantly different from zero when its confidence interval did not overlap zero.

Within-colony relatedness (R) and between-colony relatedness (R') were calculated using the software RELATEDNESS 5.0.8 (Goodnight and Queller 1998). Standard errors were obtained by jackknifing over colonies. Genotypes of adults and callow workers/brood were compared to investigate the genetic patterns within colonies. Genotypes were recorded as 'aberrant' when they differed from the genotypes of nestmates in at least one allele at one locus. Individuals with genotypes that differed from those of their nestmates were analyzed twice in the PCR and sequencing reaction to exclude amplification and scoring errors.

Behavioral assays

Nine pairs of *P. punctata* colonies (four pairs from Barbados, collected in June 2007 and kept in the lab for 1 month, and five pairs from the Dominican Republic, collected in November 2006 and kept in the lab for 8 months, Table 1) were transferred into adjacent plastic boxes with plaster floor (20.0 cm × 20.0 cm × 9.0 cm per box) separated by a wall with a plugged tunnel and each with a nest cavity in the plaster covered with a glass plate (8.0 cm × 5.5 cm × 0.5 cm) and red foil. We confronted only colonies that differed from each other in at least one allele at one locus. Mean relatedness between colonies in natural populations was extremely low ($R' = -0.08 \pm SE\, 0.013$ in Barbados, $-0.03 \pm SE\, 0.01$ in the Dominican Republic, $-0.020 \pm SE\, 0.0009$ in Puerto Rico), and colonies used for the behavioral experiments can be considered as being unrelated to each other (the pairwise relatedness of fusion pairs ranged from -1.000 to -0.315).

Colonies were fed a mixed diet of honey and pieces of cockroaches ad libitum. Ants were marked individually with dots of Edding marker pens. After marking and transfer into the set-up boxes, we left the colonies undisturbed for 2 weeks to recover and to adapt to the new conditions. Some workers were injured during marking, and the size of the colonies used in this experiment was therefore lower than when collected (4–20 workers, Table 1).

Behavioral observations were performed in two phases: In phase 1, colonies were observed in their separate plastic boxes (24–26 observation sessions of 5 min each per colony over 24 days, total of 120–130 min per colony). The frequency of sociopositive (antennal contact and allogrooming) and aggressive behavior (antennal boxing, biting, sting smearing, immobilization of opponent, i.e., pulling on legs, and antennae; see Hartmann et al. 2003) and the location of each individual were noted (on eggs, on brood, and inside nest cavity/outside nest cavity). In addition, we determined the reproductive individual of each colony from its distinct behavioral profile, e.g., its high frequency of sitting on the egg pile (see Hartmann et al. 2003). Reproductive status was confirmed by the appearance of eggs after isolating the individual for 24 to 48 h in a separate plastic box (10.0 cm × 3.0 cm × 3.0 cm) with a nest cavity, food and water (see Hartmann et al. 2003). After separation, the reproductives were returned to the colonies and allowed to reintegrate for 3 more days. Thereafter, we opened the tunnel in the separation wall between the two boxes and forced the smaller colony to move out of its nest by removing the glass and foil cover of the nest (phase 2). We again recorded the frequency of sociopositive and aggressive behavior and the location of individuals (one observation session of 30 min each beginning with the first contact between workers from the two colonies, five sessions of 10 min each per day over 5 consecutive days after the manipulation, total 270 min per colony). Reproductive status of the presumed egg layers was investigated as above after the experiment. All statistical tests were performed with STATISTICA 6.0 (Statsoft 2003). The same colonies were also included in genetic analyses.

Results

Population structure

In total, we genotyped 145 individuals from Barbados, 336 individuals from the Dominican Republic, and 565 individuals from Puerto Rico. In samples from Barbados, the number of alleles per microsatellite locus ranged from one (locus 3506; this locus was therefore excluded from further analyses of the Barbados population) to four (locus 3302). In samples from the Dominican Republic and Puerto Rico, the number of alleles ranged from two (3506) to seven (3302). Deviations from Hardy-Weinberg equilibrium were significant ($p < 0.0001$) at all sample sites and all loci. The significant excess of heterozygotes (fixation indices f: Barbados: -0.820, 95% CI -0.920 to -0.830; Dominican Republic: -0.860, 95% CI -0.950 to -0.790; Puerto Rico: -0.940, 95% CI -0.980 to -0.911) matches expectations for predominantly clonal populations (Balloux et al. 2003; de

Table 1 Colony size and number of reproductives in colonies of the parthenogenetic ant *Platythyrea punctata* before and after experimental colony fusion

Colony fusion pair	Population	Single colonies	Original colony size (n)	Colony size before fusion (n)	Total size of fused colonies (n)	Reproductives before fusion (n)	Reproductive activity after fusion
1	Barbados	HaC-01	8	2	11	1	No
		HaC-05	12	9		1	Yes
2	Barbados	HC-01	11	11	25	2	Yes
		TH-11	16	14		2	Dead/no
3	Barbados	TH-07	12	10	20	1	Yes
		TH-13	11	10		1	No
4	Barbados	HaC-04	10	4	13	1	No
		TH-01	16	9		1	Yes
5	Dom Rep	AS-01	12	9	11	2	No/yes
		AS-14	4	2		1	Yes
6	Dom Rep	EL-01	11	4	12	1	Dead
		RW-09	20	8		1	Yes
7	Dom Rep	Mi-03	15	15	24	1	Yes
		AS-15	12	9		2	No
8	Dom Rep	EL-08	15	14	27	1	Yes
		EL-09	12	13		1	Dead
9	Dom Rep	RW-06	13	12	25	1	No
		RW-07	14	13		1	No
Mean			12.44	9.33	18.67		
SD			3.45	4.06	6.84		

Meeûs and Balloux 2005). Exact tests for linkage disequilibrium (LD) performed for each pair of loci in each population were significant ($p<0.0001$) for all but in total three locus combinations (2902×3302 on Puerto Rico, $p=0.063$, 3506×4101 and 2902×4101 in the Dominican Republic, $p=0.508$ and $p=1.000$). Significant linkage disequilibria were also found in the Barbados population, but several combinations could not be tested because of the invariability of locus 3506. The finding that different combinations of LD were found in different populations suggests that LD is caused by thelytoky and not by physical linkage of loci.

The ratio of multilocus genotypes/number of analyzed colonies was similar for the three islands (Barbados: 0.923, 12 multilocus genotypes/13 colonies; Dominican Republic: 0.815, 22/27; Puerto Rico: 0.894, 42/47). Only two clone lineages were found both in Dominican Republic and Puerto Rico, whereas Barbados contained unique clone lineages. One private allele at locus 2902 was found in Sabana/Puerto Rico. The high genetic differentiation is also reflected by the fixation indices in a four-level hierarchical analysis of variation (individuals within total population, $F=-0.148$, 95% CI: -0.305 to -0.030; colonies within sample sites, $\Theta_{SS}=0.406$, 95% CI: 0.337 to 0.461; sample sites within islands, $\Theta_S=0.231$, 95% CI 0.173 -0.301; variation among islands, $\Theta_P=0.045$, 95%CI $= 0.008 - 0.100$).

Colony structure

Mean nestmate relatedness R was $0.902\pm$SE 0.036 in colonies from Barbados, $0.918\pm$SE 0.046 in colonies from the Dominican Republic, and $0.937\pm$SE 0.018 in colonies from Puerto Rico, again as expected from a predominantly clonal colony structure. The deviation from a relatedness value of 1, expected for complete clonality, is explained by genetic variation among workers in six of 13 colonies (46%) from Barbados, ten of 27 colonies (37%) from the Dominican Republic, and 19 of 47 colonies (40%) from Puerto Rico. In these colonies, nestmate relatedness ranged from 0.53 to 0.90 in Barbados, from 0.38 to 0.97 in Dominican Republic and from 0.20 to 0.98 in Puerto Rico (see also Table 2).

We classified the observed within-colony variation into three different categories: Type A, aberrant multilocus genotypes with two alleles at one locus, which were not present in their nestmates, resulting in a total of four different alleles at least at one locus within a colony; Type B, aberrant homozygous genotypes in colonies in which other nestmates were heterozygous at this locus; Type C, genotypes with one allele at one single locus, which was not present in other nestmates, i.e., three different alleles at one locus were present in a colony. Genotypes of categories B and C might hypothetically result from mutations or

Table 2 Within-colony relatedness R and patterns of genetic variation in heterogeneous colonies of the parthenogenetic ant *Platythyrea punctata*

	Colony	No. of individuals	Locus					Individuals causing variation	Category	R
			3506	2902	4101	2801	3302			
Barbados	TH5	6	203/203	185/185	201/201	386/386	240/242			0.68
		6			*201/211*			2c, 4a	C	
	TH7	11	203/203	185/185	201/201	386/386	238/240			0.86
		1					*240/242*	1a	C	
	HC	11	203/203	183/185	201/211	386/388	240/242			0.78
		1		*185/185*				1a	B	
	HaC2	10	203/203	185/185	201/211	386/388	238/238			0.61
		2					*242/244*	2a	A	
	P81	11	203/203	185/185	211/211	386/386	240/242			0.81
		1				*388/388*		1a	A	
	P82	2	203/203	185/185	211/211	386/386	240/242			0.53
		1			*203/211*	*386/390*	*240/240*	1a	C, B	
Dominican Republic	AS6	10	193/203	183/185	199/211	382/382	238/244			0.56
		1			*211/211*	*382/386*	*238/238*	1c	B, C, B	
		1			*201/201*	*382/386*	*236/244*	1a	A	
	AS11	9	193/193	183/185	199/211	382/382	238/244			0.56
		3	*193/203*				*238/246*	1c, 2a	C, C	
	Mi1	10	193/203	183/185	201/203	384/384	238/244			0.79
		2				*386/386*		2c	A	
	Mi2	11	193/203	183/185	201/203	386/386	238/242			0.88
		1					*240/244*	1a	A	
	Mi3	13	193/203	183/185	203/211	382/390	238/238	5c, 8a		0.34
		9			*201/201*	*382/382*	*236/244*	7c,2a	A	
		1			*211/211*	*382/390*	*238/238*	1c	B	
		1			*203/203*	*382/390*	*238/238*	1a	B	
	Mi9	11	193/203	183/185	201/201	382/386	236/244			0.80
		1			*199/211*	*382/382*	*238/244*	1a	A	
	EL1	11	203/203	183/185	199/211	382/386	238/244			0.96
		1				*386/386*		1c	B	
	EL8	11	203/203	183/185	199/211	386/386	238/244			0.97
		1			*211/211*			1c	B	
	EL11	11	203/203	185/185	199/211	386/386	238/244			0.97
		1			*211/211*			1c	B	
	RW12	11	203/203	185/185	211/211	386/386	242/244			0.93
		1				*368/386*		1c	C	
Puerto Rico	RG 6	11	193/203	183/185	211/211	368/384	238/242			0.91
		1					*236/240*	1a	A	
	RG 13	6	203/203	183/185	211/211	384/386	238/246			0.90
		6				*386/386*		6a	B	
	RG 15	11	203/203	183/185	211/211	384/384	238/244			0.97
		1				*368/384*		1c	C	
	EV 2	11	203/203	183/185	199/211	368/386	238/242			0.94
		1	*193/203*					1a	C	
	EV 6	11	203/203	183/185	199/211	368/386	238/244			0.94
		1			*211/211*			1a	B	
	EV 17	10	193/203	183/185	199/211	368/386	238/244			0.89
		2			*203/211*			2a	C	
	P 12	7	193/203	183/185	211/211	368/386	238/244			0.79
		5			*203/211*			5a	C	
	P 15	9	193/203	183/185	199/211	382/388	238/242			0.46
		1	*203/203*		199/211	*382/386*	*240/244*	1a	A	
		1	*203/203*		*211/211*	*386/386*	238/242	1a	A	

Table 2 (continued)

Colony	No. of individuals	Locus					Individuals causing variation	Category	R
		3506	2902	4101	2801	3302			
	1	193/203		199/211	*382/386*	238/242	1a	C	
P 18	6	203/203	183/185	203/203	382/382	240/242			0.84
	5					*236/242*	5c	C	
	1	*193/203*		*211/211*			1c	A	
ET 3	11	193/203	183/185	199/211	382/386	238/244			0.90
	1				*382/382*		1c	B	
ET 6	7	193/203	183/185	199/211	382/386	238/244			0.68
	4			*211/211*			4c	C	
	1			*211/211*	*386/386*		1c	C, C	
ET 10	11	193/203	183/185	199/211	382/386	238/244			0.90
	1				*382/382*		1a	C	
SA 1	10	193/203	183/185	199/203	368/368	238/244			0.90
	1			*199/199*			1c	B	
	1			*199/211*			1a	C	
SA 4	9	193/203	183/185	199/211	382/386	238/244			0.55
	3			*211/211*		*238/246*	3c	B, C	
SA 5	6	193/203	183/185	199/211	368/386	238/242			0.20
	1					*234/238*	1c	C	
	1	203/203		*199/203*		*234/238*	1a	C, C	
	1	193/203		199/211	*382/386*	238/242	1a	C	
	1	193/203		*199/203*	368/368	*234/238*	1a	C, B, C	
	1	203/203		*203/211*	368/382	*234/238*	1a	C, C, C	
	1	193/203		*203/211*	368/386	*234/238*	1a	C, C	
SA 7	11	193/203	183/185	199/211	382/386	238/244			0.86
	1			*203/211*			1a	C	
WSL 1	11	203/203	185/185	201/211	384/384	238/244			0.98
	1	*193/203*					1a	C	
SAII 1	11	193/203	183/185	203/211	386/386	238/238			0.96
	1				*368/386*		1a	C	
SAII 2	7	193/203	183/185	203/211	386/386	238/238			0.91
	5				*386/388*		5c	C	

Aberrant genotypes are shown in italics. Individuals with aberrant genotypes are either callow workers or brood (c) or adults (a). The types of genetic variation is explained by A: two foreign alleles, B: homozygosity instead of heterozygosity, C: one foreign allele; Colonies with variation of category "A" are underlined

recombination during the parthenogenetic formation of gametes (switches from hetero- to homozygosity). Sexual reproduction, which in principle also might explain variation of types B and C, is unlikely: the observed occurrence of one or a few individuals with an aberrant genotype amongst a majority of genetically identical individuals does not match the genotype frequencies predicted from sexual reproduction. Furthermore, mated individuals appear to be exceptionally rare in populations from the Caribbean islands (Hartmann et al. 2005).

Genetic variation of type A, i.e., the presence of individuals with two aberrant alleles (and the presence of four different alleles within a colony), can be explained neither by sexual reproduction nor by thelytokous recombination. Instead, this variation suggests the mixture of workers belonging to different genetic lineages (at least two of six heterogeneous colonies from Barbados, five of ten heterogeneous colonies from the Dominican Republic, and three of 19 heterogeneous colonies from Puerto Rico). The pattern observed in colony Mi3 with at least four different multilocus genotypes (see Table 2) might result from multiple fusions / adoptions.
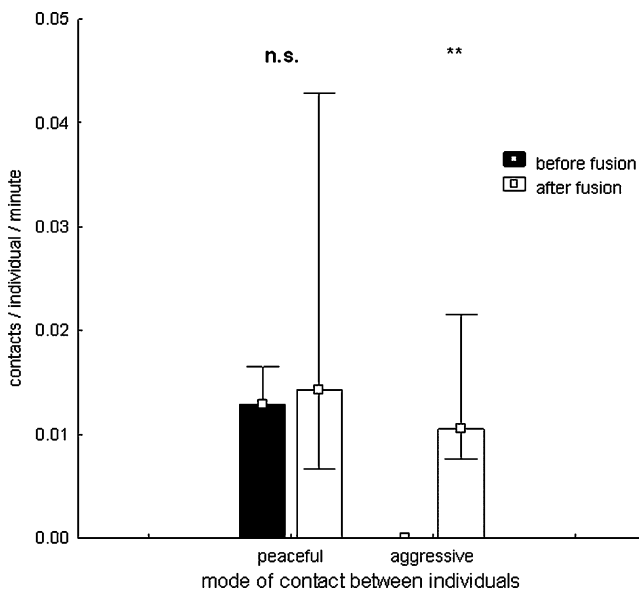
Fusion can result in the transient presence of reproductives from different colonies. Even if one of the two stops reproducing or dies (see below), other young members of the same clone lineage might later take over reproduction. This might result in the occurrence of callow workers with genotypes different from other callows (e.g., in colony P18, see Table 2).
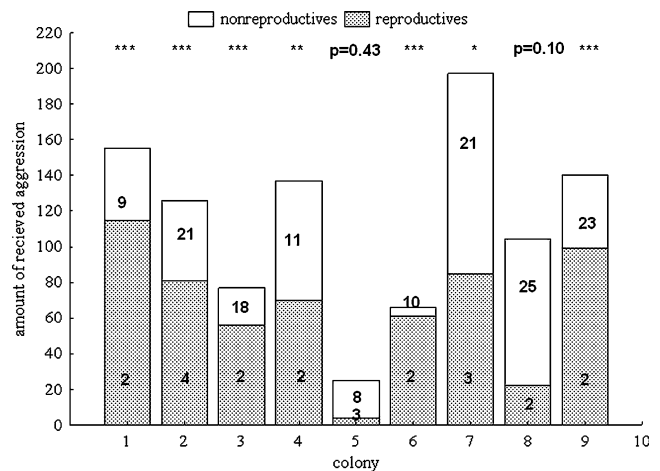
Colony fusion

All nine pairs of colonies in our experiments eventually fused and finally inhabited the one remaining nest site. Workers from the destroyed nest started moving into the intact nest within 30 min after discovering it. They did apparently not discriminate between nestmates and non-nestmates, and workers from the resident colony never attempted to prevent intruders from moving into their nest. Instead, workers from both colonies carried brood and young workers from the destroyed nest cavity into the intact nest. Nevertheless, the frequency of aggression increased significantly after the manipulation (Wilcoxon's matched-pairs test, $Z=2.67$; $p=0.007$; Fig. 1). The frequency of sociopositive interactions did not change significantly ($Z=1.24$; $p=0.21$; Fig. 1).

In seven of nine colonies, the number of attacks directed towards reproductives was higher than expected (see Fig. 2). Averaged over all colonies ($n=146$ individuals), reproductives ($n=22$) received 57.7% of all recorded aggressive acts (χ^2 test, $\chi^2=115.21$; $p<0.001$). Worker aggression towards reproductives deviated from equal distribution among the reproductives ($\chi^2=112.55$; $p<0.001$ over all colonies), but also from skewed distribution towards the foreign reproductives ($\chi^2=72.96$; $p=0.009$ over all colonies). Workers may therefore focus their attacks on a certain reproductive but did not consistently prefer their own

Fig. 2 Total number of aggressive acts (antennal boxing, biting, smearing, and immobilization) received by reproductives and non-reproductive workers in nine fused *Platythyrea punctata* colonies, summed over the total observation time. In all colonies except colonies 5 and 8 aggression was significantly skewed towards reproductives (χ^2 test; *$p<0.05$; **$p<0.01$; ***$p<0.001$; colony 5, $p=0.430$; colony 8, $p=0.100$). The numbers in the *bars* represent the number of individuals contributing to the aggression

reproductive. In three colonies, aggression started with a delay of 1 day, during which the reproductives themselves were disproportionately aggressive towards each other (χ^2 test for colonies 1: $\chi^2=7.82$, p=0.005; 4: $\chi^2=6.18$; p=0.013, and 9: $\chi^2=4.43$; p=0.035).

Aggression against reproductives resulted in the re-establishment of "monogyny" or at least the decrease of the number of reproductives. Before fusion, colonies had one, less commonly two egg layers, and directly after fusion two to four reproductives. At the end of the experiment, in each colony at least one egg layer had ceased to lay eggs or died in response to aggression (see Table 1). Colony size decreased slightly between the start of the experiment and before fusion was initiated (see Table 1). However, not a single non-reproductive worker died due to aggression during or after fusion.

Colony fusion did not lead to any bias in task allocation among the former colonies, i.e., workers that had engaged in nursing or foraging continued to do so after fusion. No significant differences were found between the ratio of indoor/outdoor workers in the original colonies and the subsequently fused colonies (Wilcoxon's matched-pairs test for indoor workers of fused colonies 2: $Z=1.11$, 3: $Z=1.36$, 4: $Z=1.83$, 6: $Z=0.37$, 7: $Z=0.28$, 9: $Z=0.12$; outdoor workers of colonies 2: $Z=0.66$, 3: $Z=1.27$, 4: $Z=1.6$, 6: $Z=0.37$, 7: $Z=0.00$, 9: $Z=0.12$; $p>0.1$ in all comparisons). Only in colony pair 8, workers of the intruder colony performed more indoor than outdoor work ($Z=2.39$ for indoor and $Z=2.63$ for outdoor; $p<0.05$). For fused colony pairs 1 and 5 no comparisons could be done due to insufficient sample size.

Fig. 1 Peaceful and aggressive behavior within *Platythyrea punctata* colony pairs before and after colony fusion ($n=9$ fusion pairs). *Spots* represent medians of interactions per individual per minute over all colonies; *bars* show percentiles, and *whiskers* show the range excluding outliers. Aggressive behavior increased significantly after fusion, whereas the frequency of peaceful interactions remained unchanged (Wilcoxon's matched-pairs test; **$p<0.01$; *n.s.* not significant)

Discussion

The majority of colonies of the thelytokous ant *P. punctata* in populations on the Caribbean islands consist of homogenous colonies with genetically identical individuals, but a considerable fraction of colonies contain workers with two or more multilocus genotypes (between 37% and 46% in Dominican Republic, Puerto Rico, and Barbados). Some genetic variation might in principle result from mutations or recombination events during parthenogenesis, e.g., changes from hetero- to homozygosity during automictic partheno-genesis. The very unequal distribution of genotypes within heterogeneous colonies and the rarity of mated individuals in the study populations (Schilder et al. 1999a; Hartmann et al. 2005) make it unlikely that sexual reproduction contributes to genetic variation.

However, recombination or mutation cannot easily explain the presence of workers with genotypes that are completely incompatible with those of their nestmates. Instead, such cases—and perhaps many simpler cases of genetic heterogeneity—may have been caused by the fusion of different colonies or the adoption of individual drifting workers or reproductives. The alternative explanation of brood raiding appears unlikely in *P. punctata*, as it is normally found in ants with a very different life history, i.e., independent founding, large colony size, and territoriality (Bartz and Hölldobler 1982; Pollock and Rissing 1989; Fowler 1992; Bourke and Franks 1995; Tschinkel 2006).

Our hypothesis that natural, genetically heterogeneous colonies are the product of colony fusion is corroborated by the observation that *P. punctata* workers readily moved in with another colony when their nests have been destroyed. After some initial aggression, workers formed a stable, mixed society. The merging of colonies closely resembles that in *Temnothorax* spp. These ants are widely distributed in temperate, deciduous forests and nest in rotting wood, hollow acorns, or hazelnuts. Because of the rapid decay of these nest sites, searching for and moving into new nest sites commonly occur in the life history of these ants. When populations are very dense and nest sites are limited, colonies may merge with another established colony and permanently form mixed colonies.

Nest site limitation might similarly explain colony fusion in *P. punctata*. In its natural habitat, *P. punctata* nests in preformed cavities in rotten wood on the ground, dead branches in trees, or preformed holes in the soil. Workers are apparently not capable of enlarging their nest sites when the colony grows, and nests in dead wood appear to be fragile and easily destroyed by strong winds or the seasonal hurricanes. Suitable nest sites may thus often become limited, forcing colonies, which have lost a nest site, to merge with another colony. Resident ants accepted the adoption of foreign workers into their colonies after some

initial aggression. Like in *Temnothorax* (Foitzik and Heinze 1998, 2000; Strätz et al. 2002), reproductives engaged in fighting, and in most cases, at least one of them ceased laying eggs or died. In contrast to expectations from kin and nestmate recognition models and previous observations in other ants (Kikuchi et al. 2007), *P. punctata* workers did not preferentially attack the alien reproductive but directed their aggression randomly towards own and alien repro-ductives. As yet, it is not known whether workers favor the more fertile over the less fertile reproductive, as shown in experiments with fire ants, *Solenopsis invicta* (Fletcher and Blum 1983). The equal survival rate of resident and invader reproductives also excludes the occurrence of parasitic lineages, which usurp other nests, as in the thelytokous ant *Pristomyrmex punctatus* (Dobata et al. 2009) and the thelytokous Cape honey bee *Apis mellifera capensis* (Neumann and Moritz 2002; Härtel et al. 2006).

While nest site limitation commonly leads to intra- and interspecific competition and the replacement of an established colony by another (Herbers 1986; Herbers and Banschbach 1999; Yamaguchi 1992), colony fusion in ants appears to be generally rare and on a first glance non-adaptive, in particular when one of two reproductives is eliminated after fusion.

Why, then, do colonies of *P. punctata* merge?

On the one hand, nest fusion might be a non-adaptive byproduct of weakened nestmate discrimination. For example, genetically determined odor cues might be rather invariable, as suggested for unicolonial, invasive ants (Tsutsui et al. 2000; Vásquez et al. 2008), or the odor might be dominated by environmental cues, as in *T. nylanderi* (Heinze et al. 1996; Pusch et al. 2006). Though the populations of *P. punctata* consist of different clonal lineages, mtDNA sequences indicate that the overall genetic similarity is very high (Hartmann et al. 2005), presumably due to founder effects during the colonization of the Caribbean islands. This might impair the nestmate discrimination ability in these ants and facilitate the fusion of colonies. Furthermore, field observations suggest that workers from neighboring colonies do not engage in aggressive interactions (Schilder 1999).

On the other hand, fusion may result in benefits for the genetic lineage whose reproductive survives. Recent studies have documented that increased genetic heterogeneity might be beneficial in terms of parasite resistance (Liersch and Schmid-Hempel 1998; Baer and Schmid-Hempel 1999), productivity (Jones et al. 2004; Mattila and Seeley 2007), and/or task efficiency and division of labor (reviewed in Oldroyd and Fewell 2007; Smith et al. 2008). In this way, even the lineage whose reproductive has been expelled or killed might benefit from fusion, if one

of its clone lineage members had a realistic chance of superseding the surviving queen in the future (e.g., Johns et al. 2009).

Genetic heterogeneity through nest fusion might be more common in social insects than currently thought. However, fusion might often be overlooked because the usually more complex genetic structure of colonies in sexual species makes it more difficult to separate heterogeneity due to nest fusion from other causes of intracolony variation. Thelytokous ants with clonal colonies might therefore be ideal systems for investigating the prevalence of genetic within colony heterogeneity in the field. Future work should investigate the fitness of colonies to determine if genetically diverse colonies have higher resistance to parasites and higher productivity rates than homogeneous colonies.

References

Baer B, Schmid-Hempel P (1999) Experimental variation in polyandry affects parasite loads and fitness in a bumble-bee. Nature 397:151–154

Balloux F, Lehmann L, de Meeûs T (2003) The population genetics of clonal and partially clonal diploids. Genetics 164:1635–1644

Bartz SH, Hölldobler B (1982) Colony founding in *Myrmecocystus mimicus* Wheeler (Hymenoptera: Formicidae) and the evolution of foundress associations. Behav Ecol Sociobiol 10:137–147

Bourke AFG, Franks NR (1995) Social evolution in ants. Princeton University Press, Princeton

Chapuisat M, Bernasconi C, Hoehn S, Reuter M (2005) Nestmate recognition in the unicolonial ant *Formica paralugubris*. Behav Ecol 16:15–19

Crozier RH, Dix MW (1979) Analysis of two genetic models for the innate components of colony odor in social Hymenoptera. Behav Ecol Sociobiol 4:217–224

Dobata S, Sasaki T, Mori H, Hasegawa E, Shimada M, Tsuji K (2009) Cheater genotypes in the parthenogenetic ant *Pristomyrmex punctatus*. Proc R Soc Lond B 276:567–574

Fletcher DJC, Blum MS (1983) Regulation of queen number by workers in colonies of social insects. Science 219:312–314

Foitzik S, Heinze J (1998) Nest site limitation and colony takeover in the ant *Leptothorax nylanderi*. Behav Ecol 9:367–375

Foitzik S, Heinze J (2000) Intraspecific parasitism and split sex ratios in a monogynous and monandrous ant (*Leptothorax nylanderi*). Behav Ecol Sociobiol 47:424–431

Fournier D, Estoup A, Orivel J, Foucaud J, Jourdan H, Le Breton J, Keller L (2005) Clonal reproduction by males and females in the little fire ant. Nature 435:1230–1235

Fowler HG (1992) Patterns of colonization and incipient nest survival in *Acromyrmex niger* and *Acromyrmex balzani* (Hymenoptera: Formicidae). Insect Soc 39:347–350

Goodnight KF, Queller DC (1998) Relatedness 5.0.8. Goodnight Software, available at http://gsoft.smu.edu/Gsoft.html, Houston, Texas

Härtel S, Neumann P, Raassen FS, Moritz RFA, Hepburn HR (2006) Social parasitism by Cape honeybee workers in colonies of their own subspecies (*Apis mellifera capensis*). Insect Soc 58:183–193

Hartmann A, Wantia J, Torres JA, Heinze J (2003) Worker policing without genetic conflicts in a clonal ant. Proc Natl Acad Sci USA 100:12836–12840

Hartmann A, Wantia J, Heinze J (2005) Facultative sexual reproduction in the parthenogenetic ant *Platythyrea punctata*. Insect Soc 52:155–162

Hasegawa E, Sanada S, Sato T, Obara Y (2001) Microsatellite loci and genetic polymorphism among colony members in the parthenogenetic ant *Pristomyrmex pungens* (Hymenoptera, Formicidae). Entomol Sci 4:399–402

Heinze J, Hölldobler B (1995) Thelytokous parthenogenesis and dominance hierarchies in the ponerine ant, *Platythyrea punctata*. Naturwissenschaften 82:40–41

Heinze J, Foitzik S, Hippert A, Hölldobler B (1996) Apparent dear-enemy phenomenon and environment-based recognition cues in the ant *Leptothorax nylanderi*. Ethology 102:510–522

Herbers JM (1986) Nest site limitation and facultative polygyny in the ant *Leptothorax longispinosus*. Behav Ecol Sociobiol 19:115–122

Herbers JM, Banschbach VS (1999) Plasticity of social organization in a forest ant species. Behav Ecol Sociobiol 45:451–465

Hölldobler B, Wilson EO (1990) The ants. Harvard University Press, Cambridge

Hölldobler B, Wilson EO (2009) The superorganism: the beauty, elegance and strangeness of insect societies. W.W. Norton, London

Johns PM, Howard KJ, Breisch NL, Rivera A, Thorne BL (2009) Nonrelatives inherit colony resources in a primitive termite. Proc Natl Acad Sci USA 106:17452–17456

Jones JC, Myerscough MR, Graham S, Oldroyd BP (2004) Honey bee nest thermoregulation: diversity promotes stability. Science 305:402–404

Kikuchi T, Tsuji K, Ohnishi H, Le Breton J (2007) Caste-biased acceptance of non-nestmates in a polygynous ponerine ant. Anim Behav 73:559–565

Lewis PO, Zaykin D (2001) Genetic Data Analysis: computer program for the analysis of allelic data. Version 1.0 Free program distributed by the authors over the internet from http://lewis.eeb.uconn.edu/lewishome/software.html

Liersch S, Schmid-Hempel P (1998) Genetic variation within social insect colonies reduces parasite load. P Roy Soc Lond Bio B 265:221–225

Mattila HR, Seeley TD (2007) Genetic diversity in honey bee colonies enhances productivity and fitness. Science 317:362–364

de Meeûs T, Balloux F (2005) F-statistics of clonal diploids structured in numerous demes. Mol Ecol 14:2695–2702

Neumann P, Moritz RFA (2002) The Cape honeybee phenomenon: the sympatric evolution of a social parasite in real time? Behav Ecol Sociobiol 52:271–281

Oldroyd BP, Fewell JH (2007) Genetic diversity promotes homeostasis in insect colonies. Trends Ecol Evol 22:408–413

Pollock GB, Rissing SW (1989) Intraspecific brood raiding, territoriality, and slavery in ants. Am Nat 133:61–70

Pusch K, Seifert B, Foitzik S, Heinze J (2006) Distribution and genetic divergence of two parapatric sibling ant species in Central Europe. Biol J Linn Soc 88:223–234

Raymond M, Rousset F (1995) GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. J Hered 86:248–249

Sambrook J, Russell DW (2001) Preparation and analysis of eukaryotic genomic DNA. In: Sambrook J, Russell DW (eds) Molecular cloning: a laboratory manual, vol 1, 3rd edn. Harbor Laboratory Press, New York, pp 23–25

Schilder K (1999) Safer without sex? Thelytoke Parthenogenese und die Regulation der Reproduktion in der Ameise *Platythyrea punctata*. Dissertation, Universität Würzburg

Schilder K, Heinze J, Hölldobler B (1999a) Colony structure and reproduction in the thelytokous parthenogenetic ant *Platythyrea punctata* (F. Smith) (Hymenoptera, Formicidae). Insect Soc 46:150–158

Schilder K, Heinze J, Roy G, Hölldobler B (1999b) Microsatellites reveal clonal structure of populations of the thelytokous parthenogenetic ant *Platythyrea punctata* (F. Smith) (Hymenoptera: Formicidae). Mol Ecol 8:1497–1507

Smith CR, Toth AL, Suarez AV, Robinson GE (2008) Genetic and genomic analyses of the division of labour in insect societies. Nat Rev Genet 9:735–748

Statsoft (2003) STATISTICA (data analyses software system). Tulsa, Oklahoma

Strätz M, Strehl C-P, Heinze J (2002) Behavior of usurping queens in colonies of the ant species *Leptothorax nylanderi* (Hymenoptera: Formicidae). Entomol Gener 26:73–84

Tschinkel WR (2006) The fire ants. Harvard University Press, Cambridge

Tsutsui ND, Suarez AV (2003) The colony structure and population biology of invasive ants. Conserv Biol 17:48–58

Tsutsui ND, Suarez AV, Holway DA, Case TJ (2000) Reduced genetic variation and the success of an invasive species. P Natl Acad Sci USA 97:5948–5953

Van Wilgenburg E, Mulder RA, Elgar MA (2006) Intracolony relatedness and polydomy in the Australian meat ant, *Iridomyrmex purpureus*. Austr J Zool 54:117–122

Vásquez GM, Schal C, Silverman J (2008) Cuticular hydrocarbons as queen adoption cues in the invasive Argentine ant. J Exp Biol 211:1249–1256

Vogel V, Pedersen JS, D'Ettorre P, Lehmann L, Keller L (2009) Dynamics and genetic structure of argentine ant supercolonies in their native range. Evolution 63:1627–1639

Weir BS, Cockerham CC (1984) Estimating F-statistics for the analysis of population structure. Evolution 38:1358–1370

Wilson EO (1971) The insect societies. Harvard University Press, Cambridge

Yamaguchi T (1992) Interspecific interference for nest sites between *Leptothorax congruus* and *Monomorium intrudens*. Insect Soc 39:117–127