

Colony kin structure and breeding system in the ant genus *Plagiolepis*

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Abstract

Relatedness is a central parameter in the evolution of sociality, because kin selection theory assumes that individuals involved in altruistic interactions are related. At least three reproductive characteristics are known to profoundly affect colony kin structure in social insects: the number of reproductive queens per colony, the relatedness among breeding queens and queen mating frequency. Both the occurrence of multiple queens (polygyny) and multiple mating (polyandry) decrease within-colony relatedness, while mating among sibs increases relatedness between the workers and the brood they rear. Using DNA microsatellites, we performed a detailed genetic analysis of the colony kin structure and breeding system in three ant species belonging to the genus *Plagiolepis*: *P. schmitzii*, *P. taurica* and *P. maura*. Our data show that queens of the three species mate multiply: queens of *P. maura* mate with 1–2 males, queens of *P. taurica* with 3–11 males and queens of *P. schmitzii* may have 1–14 different mates. Moreover, colonies are headed by multiple queens: *P. taurica* and *P. maura* are facultatively polygynous, while *P. schmitzii* is obligately polygynous. Despite polyandry and polygyny, relatedness within colonies remains high because all species are characterized by sib-mating, with a fixation index $F_{it} = 0.25$ in *P. taurica*, 0.24 in *P. schmitzii* and 0.26 in *P. maura*, and because the male mates of a queen are on average closely related.

Keywords: ant, inbreeding, *Plagiolepis*, polyandry, polygyny, population genetics

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Introduction

Kinship among group members has long been recognized as a main factor promoting the evolution of sociality and reproductive altruism (Hamilton 1964a,b). Lifetime monogamy (i.e. strict monandry) certainly was pivotal in the evolution of reproductive altruism because relatedness to siblings is equivalent to average relatedness towards own offspring ($r = 0.5$). Hence, any minute benefit/cost advantage ($b/c > 1$) may be sufficient to select for a transition towards eusociality (Boomsma 2009; Cornwallis *et al.* 2010). In social Hymenoptera (ants, bees and wasps), directional selection for the loss of reproductive totipotency of workers may have been reinforced by the haplodiploid genetics (Bourke & Franks 1995; Crozier & Pamilo 1996). Under haplodiploidy, fertilized eggs develop into diploid

females and unfertilized eggs develop into haploid males (Cook & Crozier 1995). Assuming random mating, a single reproductive queen per colony (monogyny) and single mating (monandry), females are on average more related to their sisters ($r = 0.75$) than to their own offspring ($r = 0.5$). Comparative studies across a wide range of ants, bees and wasps give clear evidences that close relatedness played a decisive role in the evolution of eusociality in Hymenoptera (Hughes *et al.* 2008a; Boomsma 2009). Both functional monogyny and monandry, two factors maximizing relatedness within colonies, are the ancestral states in all lineages investigated.

However, molecular genetic studies increasingly showed that colony kin composition may greatly differ from monogyny/monandry, because of derived breeding systems (Hughes *et al.* 2008a; Boomsma 2009). In ants, the occurrence of several reproductive queens in the same nest (polygyny) and/or of multiple mating by queens (polyandry) has been reported in several species (Bourke & Franks 1995; Crozier & Pamilo 1996). Both

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polygyny and polyandry greatly reduce the relatedness among the workers and the brood they rear and, hence, dilute the benefits from helping. Conversely, mating among relatives (i.e. inbreeding) increases relatedness within groups (Crozier & Pamilo 1996). Inbreeding is exceptional in ants, for at least two reasons. First, mating usually takes place during population-wide nuptial flights involving male and female sexuals from numerous colonies, thereby greatly limiting the probability of mating among relatives (Crozier 1980; Hölldobler & Wilson 1990). Second, when sexuals have reduced dispersal and mate close to their natal colony, as is typically the case in polygynous ants, young queens preferentially mate with non-nestmate males (Kaufmann *et al.* 1992; Keller & Passera 1993; Passera & Keller 1994). Nevertheless, moderate to high levels of inbreeding were reported in a few species suggesting a lack of inbreeding avoidance (Cole & Wiernasz 1999; Haag-Liautard *et al.* 2009; Lenoir *et al.* 2007; Schrempf *et al.* 2005; Sundström *et al.* 2003).

The ant genus *Plagiolepis* comprises a hundred species distributed throughout Eurasia, Africa and Oceania. Yet, detailed genetic analyses of the breeding system and population structure have been documented for two species only: the pygmy ant *Plagiolepis pygmaea* (Trontti *et al.* 2005, 2007; Thurin & Aron 2008, 2009) and its workerless social parasite *P. xene* (Trontti *et al.* 2006). These studies revealed large between-colony variations in queen number and an unusually high level of sib-mating in both species. In addition, a significant rate of polyandry was reported for *P. pygmaea*, with queens mating with up to six males (Trontti *et al.* 2007). Here, we investigated whether such life-history traits occur in other ant species belonging to the genus *Plagiolepis*. We performed a detailed genetic analysis of the colony kin structure and breeding system of three species: *P. taurica*, *P. schmitzii* and *P. maura*. We estimated for each species the level of inbreeding, the frequency of sib-mating and the relatedness among worker nestmates. We also determined queen mating frequency from laboratory mother-offspring genetic combinations.

Materials and methods

Sampling

Colonies of *Plagiolepis* were sampled during the first warm sunny days in early spring, under which conditions both queens and workers accumulate near the surface of the nests. Twenty-six colonies of *P. taurica* (previously known as *P. vindobonensis*) were excavated in February 2009 at Perpignan (southern-France; 42.38°N—3.01°E). The population was located along a 130-m-long sandy embankment at the side of Mediterra-

nean sea. Sixty colonies of *P. schmitzii* were collected in March 2009 from a population at Estreito (Madeira, Portugal; 32.44°N—17.10°W). Colonies were distributed under flat stones on a south-facing short lawn on an area of 60 × 150 m. For *P. maura*, 66 colonies were collected in March 2009 from a population of Chefchaouen (Morocco; 35.11°N—5.18°W) on an area of 50 × 100 m. Colonies were established close to vegetation spots, under stones. All three species studied were unambiguously identified (X. Espadaler, personal communication). Because colonies of *Plagiolepis* ants may be polydomous and can occupy several nests (Passera *et al.* 2001), we chose a minimal distance of 1.5–2 m between sampled nests to exclude sampling the same colony twice (see Thurin & Aron 2008 for average distance between colonies). Genetic analyses confirmed that the nests sampled belonged to different colonies. In each population, the spatial location of the nests was mapped using decametre with a precision of ±0.05 m. For each colony, a sample of workers was immediately preserved in 98% ethanol for subsequent genetic analyses.

Genetic analyses

Nine microsatellite loci (P01, P06, P07, P11, P20, P22, P23, P24 and P25; Trontti *et al.* 2003) were used to score genotypes. Individual ant DNA was extracted and amplified by the same protocol as previously reported (Trontti *et al.* 2003). The DNA was extracted from finely ground samples by incubating 90–120 min in 40 µL of Chelex (Bio-Rad) at 85 °C. Samples were centrifuged for 30 s at 10 000 g, and 1.5 mL of the supernatant was amplified by polymerase chain reaction (PCR) following the fluorescent analysis protocols described in Trontti *et al.* (2003), using a T-Professional Biometra® thermal-cycler and Taq Gold polymerase (Fermentas). The amplified products were separated in an ABI® capillary sequencer and sized against Rox-GS-350HD size standard (Applied Biosystems). All populations studied showed an excess of homozygotes (see Results) that may be due to nonrandom mating and/or the presence of null alleles, which can result in overestimation of *F*. Therefore, the frequency of null alleles at each locus was estimated using the *Expectation-Maximization (EM)* algorithm implemented in INEst (http://genetyka.ukw.edu.pl/INEst10_setup.exe). This approach allows to use data from several loci at the same time, to estimate simultaneously null allele frequencies at each locus and the average level of the intrapopulation inbreeding as a multilocus parameter (Chybicki & Burczyk 2009). We found evidence for null alleles at four loci: P01 in *P. taurica*; P23 in *P. schmitzii*; P06, P07 and P23 in *P. maura*; these markers were excluded from the analysis. No other locus showed evidence of null alleles.

Population structure

The structure of populations was determined from a subsample of 15 colonies for *P. taurica* and *P. maura* and 12 colonies for *P. schmitzii*. For each species, we analysed the genotype of 8–16 workers per colony (mean \pm SD = 15.6 \pm 1.3). Allele frequencies and *F*-statistics were estimated from worker genotype frequency data using the programme ESTAT 2.9.3 (Goudet 2001). Inbreeding coefficients (F_{it}) reflect the deficiency of heterozygotes owing to nonrandom mating within the populations sampled. The frequency of sib-mating α was estimated from $F = \alpha/(4-3\alpha)$ (Suzuki & Iwasa 1980; Pamilo 1985). To test for a possible difference in inbreeding between queens and workers, we also estimated heterozygote deficit separately for queens from established colonies.

Regression relatedness among colony members r was estimated using the algorithm of Queller & Goodnight (1989) implemented in the program RELATEDNESS 5.0.8. Colonies were weighted equally, and standard errors were obtained by jackknifing over colonies.

Queen mating frequency

We estimated the number of fathers contributing to the progeny of queens by reconstructing each paternal genotype from mother–offspring allele combinations under laboratory conditions. We settled 145 experimental nests, each containing a single queen and about 200 nestmate workers ($N = 10$ nests for *P. taurica*, 73 for *P. schmitzii* and 62 for *P. maura*). All brood was carefully removed from the nests. The ants were fed honey and water and kept at 26 ± 1 °C. After 40 days, worker larvae and pupae were removed from the nests; they were counted and stored in ethanol. Most nests of *P. schmitzii* and *P. maura* produced less than 10 offspring and were removed from the experiments. Overall, our sample consisted in 862 offspring from 34 experimental nests: 270 offspring for *P. taurica* (range: 20–43 offspring per queen, $N = 10$ nests), 304 for *P. schmitzii* (10–52 offspring per queen, $N = 12$) and 288 for *P. maura* (24 offspring per queen, $N = 12$). In *Plagiolepis*, eggs laid in spring usually develop into workers under natural conditions (Passera 1969), but a small proportion of larvae may include also haploid male offspring (Aron *et al.* 2004). Therefore, larvae with a homozygous genotype at all nine loci were considered as hemizygous males and were excluded from our paternity analysis.

The number of distinct male genotypes inferred from mother–offspring allele combinations provides the minimal number of mates of each queen (M_p). Because males may contribute unequally to the offspring, we

also estimated the effective mating frequency (M_{ep}) following Nielsen *et al.* (2003):

$$M_{ep} = \frac{(n-1)^2}{\sum_{i=1}^k p_i^2 (n+1)(n-2) + 3 - n}$$

where n is the total number of offspring of a queen, k is the number of males and p is the proportional contribution to the brood of the i th male. This estimator has the advantage of being unbiased by the relative contribution of each male and gives a lower variance than other estimators. The effective number of patrines equals the absolute mating frequency (M_p) when all males contribute equally. We determined the probability of nondetection of a father contributing ten per cent of the progeny because of reduced sampling of offspring, using the equation of Foster *et al.* (1999):

$$P_{\text{nonsampling}} = (1-p)^n$$

where p is the father's contribution to the progeny and n the number of sampled offspring. We also estimated the probability of nondetection of additional patrines owing to two fathers sharing the same alleles at all loci studied. Because mating usually proceeds among relatives in the nest (see Results), the probability of nondetection was calculated for each colony by using the equation:

$$P_{\text{nondetection}} = \frac{\sum_k^n \Pi_j \sum_i f_{ijk}^2}{n}$$

where f_{ijk} is the level frequency of allele i at locus j in colony k and n is the number of nests (Boomsma & Ratnieks 1996). Inbreeding further increases the probability of such nondetection errors.

Number of matrines per colony

Because we found significant inbreeding, multiple mating by queens and a positive relatedness between the mates of a single queen for all three species studied (see Results), our data did not meet the conditions to estimate the level of polygyny from worker genotype data with classical methods (e.g., MATESOFT, Moilanen *et al.* 2004; Colony 1.1, Wang 2004). Also, they did not allow correcting relatedness values for inbreeding, as models may not be applied when both polygyny and polyandry co-occur (Pamilo 1985, p. 196), and employing standard equations (Ross 1993; Seppä 1994). As a consequence, colony queen number was determined from field observation only ($N = 26$ for *P. taurica*, $N = 60$ for *P. schmitzii*, $N = 66$ for *P. maura*).

For statistical comparisons, nonparametric tests were used when assumptions for parametric standard tests (i.e. normality and homoscedasticity of the data) were not fulfilled. All statistical tests were carried out with the computer program GraphPad InStat 3.06 and XLSTAT.

Results

The number of alleles per locus and expected and observed heterozygosity are given in Table 1. Excluding null alleles, six loci were polymorphic in *P. taurica*; they showed 2–3 alleles with a mean expected heterozygosity across all colonies and loci $H_E = 0.24$ (SE: 0.10, range: 0.07–0.60) and a mean observed heterozygosity $H_O = 0.17$ (SE: 0.07, range: 0.02–0.55). Five loci were polymorphic in *P. schmitzii*; they had 2–4 alleles with $H_E = 0.53$ (SE: 0.06, range: 0.33–0.69) and $H_O = 0.42$ (SE: 0.06, range: 0.20–0.61). In *P. maura*, the seven loci showed 3 to 20 alleles with $H_E = 0.70$ (SE: 0.09, range: 0.37–0.91) and $H_O = 0.53$ (SE: 0.07, range: 0.23–0.73).

Population structure

A significant deviation from random mating was found in the three populations sampled (Table 2). The fixation index F was significantly different from 0 in *P. taurica*, *P. schmitzii* and *P. maura* (two-tailed t -test, $t \geq 5.74$, $P < 0.001$ for all comparisons). Mating among relatives was also supported by the positive relatedness between the queens and their pedigree-estimated mates (from mother–offspring combinations genetic analyses), with $r_{q-m} = 0.25$, 0.28 and 0.31, respectively (life-for-life relatedness of males to queens; two-tailed t -test, $t \geq 4.17$,

$P < 0.001$ for all comparisons; Table 3). The frequency of sib-mating α was 0.57 in *P. taurica*, 0.56 in *P. schmitzii* and 0.58 in *P. maura*. Heterozygote deficit was not different between the queens and the workers, for the three species studied ($t \leq 0.41$, $P \geq 0.69$ for all comparisons; Table 2).

For each species, the mean genetic relatedness among nestmate queens (r_w) was not different from the within-colony genetic relatedness among nestmate workers (r_q) (two-tailed t -test, $t \geq 0.39$, $P > 0.70$ for all comparisons; Table 2), indicating that reproductive daughters are recruited as new queens in their natal colony after mating.

Queen mating frequency

The distribution of genotypes in parent–offspring combinations was consistent with queens mating multiply in the three *Plagiolepis* species (Table 3). The average absolute mating frequency M_p was 4.9 in *P. taurica*, 3.34 in *P. schmitzii* and 1.14 in *P. maura*. The average effective number of fathers M_{ep} was 2.91, 2.62 and 1.10, respectively.

The male mates of a queen were on average highly related to each other (Table 3). The inferred average relatedness among the different mates of a single queen r_m was 0.20 ± 0.03 in *P. taurica*, 0.39 ± 0.01 in *P. schmitzii* and 0.61 ± 0.07 in *P. maura*. The probability of nondetection of an additional mate because of identical paternal genotypes ($P_{\text{nondetection}}$) was 0.34 ± 0.05 , 0.07 ± 0.02 and 0.05 ± 0.01 , respectively. The probability of nondetection of a male contributing up to 10% of the progeny ($P_{\text{nonsampling}}$) was equal to 0.07 ± 0.02 , 0.11 ± 0.03 and 0.08 ± 0.00 , respectively. These

Table 1 Number of alleles (A), expected heterozygosity (H_E) and observed heterozygosity (H_O) for nine microsatellite markers typed in *Plagiolepis taurica*, *Plagiolepis schmitzii* and *Plagiolepis maura*

	<i>P. taurica</i> (15)			<i>P. schmitzii</i> (12)			<i>P. maura</i> (15)		
	A (size, bp)	H_E	H_O	A (size, bp)	H_E	H_O	A (size, bp)	H_E	H_O
P01	N	—	—	3 (128–136)	0.60	0.45	9 (127–143)	0.79	0.66
P06	2 (161–165)	0.07	0.02	2 (150–165)	0.33	0.20	N	—	—
P07	2 (108–109)	0.16	0.02	1 (108)	—	—	N	—	—
P11	3 (173–181)	0.60	0.55	4 (175–192)	0.69	0.61	11 (177–224)	0.83	0.73
P20	1 (192)	—	—	1 (193)	—	—	5 (189–197)	0.37	0.32
P22	2 (155–157)	0.22	0.25	1 (165)	—	—	3 (157–165)	0.58	0.23
P23	2 (127–131)	0.08	0.09	N	—	—	N	—	—
P24	—	—	—	4 (114–132)	0.62	0.51	20 (114–214)	0.91	0.61
P25	3 (172–178)	0.32	0.11	2 (166–168)	0.41	0.33	7 (166–196)	0.70	0.58

The number of colonies sampled for each species is indicated between brackets. Amplification of locus P24 was not successful in *P. taurica*. We found evidence for null alleles (N) at four loci: P01 in *P. taurica*; P23 in *P. schmitzii*; P06, P07 and P23 in *P. maura*; these markers were excluded from the analysis.

Table 2 Population-wide inbreeding coefficient among workers (F_w), inbreeding coefficient among queens (F_q), mean within-colony relatedness among workers (r_w) and among queens (r_q), estimated from field colonies in *Plagiolepis taurica*, *Plagiolepis schmitzii*, *Plagiolepis maura* and *Plagiolepis pygmaea*

	Species			
	<i>P. taurica</i>	<i>P. schmitzii</i>	<i>P. maura</i>	<i>P. pygmaea</i>
# Workers typed (# colonies)	240 (15)	192 (12)	224 (15)	1040 (65)
$F_w \pm SE$	0.25 ± 0.12	0.24 ± 0.04	0.26 ± 0.07	0.39 ± 0.04
$F_q \pm SE$	0.22 ± 0.07	0.26 ± 0.02	0.26 ± 0.03	—
$r_w \pm SE$	0.47 ± 0.07	0.34 ± 0.05	0.71 ± 0.05	0.39 ± 0.02
$r_q \pm SE$	0.46 ± 0.02	0.36 ± 0.03	0.69 ± 0.02	0.46 ± 0.03

Data for *P. pygmaea* are taken from Trontti *et al.* (2005, 2007) and Thurin & Aron (2008).

Table 3 Average relatedness values among workers sharing the same mother (r_s), among the male mates that have inseminated a queen (r_m) and between the queens and their male mates (r_{q-m}), as well as absolute (M_p) and effective (M_{ep}) mating frequency of queens in *Plagiolepis taurica*, *Plagiolepis schmitzii*, *Plagiolepis maura* and *Plagiolepis pygmaea*

	Species			
	<i>P. taurica</i>	<i>P. schmitzii</i>	<i>P. maura</i>	<i>P. pygmaea</i>
# Workers typed (# nests)	270 (10)	304 (12)	288 (12)	224 (18)
$r_s \pm SE$	0.55 ± 0.02	0.54 ± 0.03	0.76 ± 0.01	0.75 ± 0.09
$r_m \pm SE$	0.18 ± 0.04	0.38 ± 0.01	0.58 ± 0.08	0.46 ± 0.05
$r_{q-m} \pm SE$	0.25 ± 0.06	0.28 ± 0.08	0.31 ± 0.09	0.30 ± 0.16
$M_p \pm SE$ (range)	4.90 ± 0.64 (3–11)	3.34 ± 1.05 (1–14)	1.14 ± 0.13 (1–2)	2.89 ± 0.32 (1–6)
$M_{ep} \pm SE$ (range)	2.91 ± 0.80 (1.8–7.0)	2.62 ± 0.90 (1–11.2)	1.10 ± 0.08 (1–1.8)	2.37 ± 0.25 (1.0–3.9)

Mating frequencies were obtained from laboratory mother–offspring combination genetic analyses. Data for *P. pygmaea* are given in Trontti *et al.* (2007).

probabilities were reasonably low and should not significantly affect our paternity estimates.

Number of matriline per colony

Field collections showed that, for each species, the number of queens per colony may vary from 1 to 10 (Table 4). The harmonic mean number of queens per colony N_f in the populations sampled was 1.38 in *P. taurica*, 1.90 in *P. schmitzii* and 1.20 in *P. maura*.

Discussion

Mating among relatives

A high level of inbreeding, reaching on average 0.25, characterizes populations of the three species of *Plagiolepis* sampled. Intranidal mating among relatives was also previously reported in *P. pygmaea* (Table 2; Trontti *et al.* 2005), suggesting that it could be a feature of the genus. In *P. pygmaea*, nonrandom mating was shown to stem from (i) workers actively repelling unrelated males entering their colony and (ii) queens preferentially

Table 4 Harmonic mean number of queens per colony (N_f) determined from field collection in *Plagiolepis taurica*, *Plagiolepis schmitzii*, *Plagiolepis maura* and *Plagiolepis pygmaea*

	Species			
	<i>P. taurica</i>	<i>P. schmitzii</i>	<i>P. maura</i>	<i>P. pygmaea</i>
# Colonies	26	60	66	200
N_f (range)	1.38 (1–5)	1.90 (1–28)	1.20 (1–10)	23.6 (15–200)

Data for *P. pygmaea* are taken from Passera (1969).

mating with related males than with unrelated males (Thurin & Aron 2009). Whether the proxies of inbreeding are similar in the three species of *Plagiolepis* studied here remains unknown.

The average relatedness among workers within colonies is unexpectedly high for species with colonies headed by several, multiply-mated queens. This suggests that dilution of relatedness owing to the occurrence of polygyny and/or polyandry is at least partly compensated by the high level of sib-mating found in the three species studied (Fig. 1). Comparison between

the expected relatedness among workers in single-queen colonies under random mating [$r = 0.25 + 1/(2.M_{ep})$, Boomsma & Ratnieks 1996] and the observed relatedness among workers from mother-offspring combinations shows that sib-mating increases within-colony relatedness by 0.13 (0.42 vs. 0.55, respectively) in *P. taurica*, by 0.10 (0.44 vs. 0.54) in *P. schmitzii* and by 0.06 (0.70 vs. 0.76) in *P. maura* (Fig. 1). Using the same calculation, sib-mating increases within-colony relatedness by 0.29 (0.46 vs. 0.75) in the species *P. pygmaea* (data from Trontti *et al.* 2005).

Apart from its positive influence on relatedness, inbreeding may negatively affect several fitness components by reducing birth weight and survival fecundity and/or by increasing susceptibility to pathogens, predation or environmental stress (inbreeding depression; Charlesworth & Charlesworth 1987, 1999; Keller & Waller 2002; Acevedo-Whitehouse *et al.* 2003). In ants, inbreeding was shown to induce several damaging effects. In *Cardiocondyla obscurior*, inbreeding depression results in a decrease in queen lifespan and brood survival (Schrempf *et al.* 2006). Moreover, haploid males from inbred colonies suffer a decrease in sperm production, fertilization capability or viability. In *Formica exsecta*, increased worker homozygosity lessens investment in sexual production, while queen homozygosity results in reduced queen lifespan and colony survival (Haag-Liautard *et al.* 2009). Whether inbreeding reduces colony/individual fitness in *Plagiolepis* populations remains unknown. Our data show that homozygosity is

not different between the queens and the workers in the three species studied. This suggests that inbreeding does not influence the likelihood that female larvae develop into queens vs. workers or that inbred queens are less fertile or experience higher mortality than inbred workers. It should be noted, however, that the heterozygosity-fitness correlation is typically a weak and unstable signal that is hard to detect statistically and showing up in a context-dependent fashion (e.g. very large natural populations) (Szulkin *et al.* 2010). A major inbreeding load specific to hymenoptera is the production of diploid males. Such males arise when there is homozygosity at the sex locus (Cook 1993; Beye *et al.* 2003). Usually, diploid males are non-viable (Petters & Mettus 1980), they produce infertile sperm (Cook 1993) or they father a sterile, triploid progeny (Smith & Wallace 1971; Krieger *et al.* 1999; Cournault & Aron 2009). Diploid males were never observed in any species of *Plagiolepis* (Trontti *et al.* 2005, 2006; Thurin & Aron 2008). One may not completely exclude the possibility that diploid males are produced but eliminated before reaching adulthood, as it occurs in the honeybee (Woyke 1963, 1980; Santomauro *et al.* 2004). However, their complete absence strongly suggests that they are rare or even absent. Indeed, it has been suggested that species with regular sib-mating could evade the cost of production of diploid males by evolving mechanisms preventing homozygosity at the sex locus, such as multiple-loci complementary sex determination (Cook 1993; Buschinger 1986; Schrempf

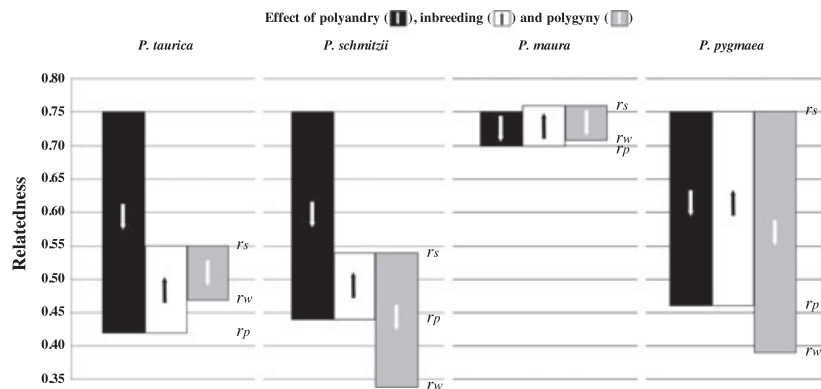


Fig. 1 Schematic representation of the effects of inbreeding, polyandry and polygyny on within-colony relatedness in *Plagiolepis taurica*, *Plagiolepis schmitzii*, *Plagiolepis maura* and *Plagiolepis pygmaea*. Data for *P. pygmaea* are taken from Trontti *et al.* (2005, 2007). r_w , relatedness among workers from field colonies; r_s , relatedness among workers from laboratory mother-offspring combinations; r_p , relatedness among workers in colonies headed by a single, multiply-mated queen under random mating [$r_p = 0.25 + 1/(2.M_{ep})$] (Boomsma & Ratnieks 1996). The effect of polyandry on relatedness is approximated by comparing the expected relatedness among workers in colonies headed by a single, multiply-mated queen (r_p), with 0.75 expected under monogyny and monandry ($M_{ep} = 1$). M_{ep} values for each species were obtained from laboratory mother-offspring combinations. The positive effect of inbreeding on relatedness is approximated by the comparison between the observed relatedness among workers from mother-offspring combinations (r_s) and the expected relatedness among workers in single-queen colonies under random mating (r_p). The effect of polygyny on relatedness is estimated from the comparison between the relatedness among workers from field colonies (r_w) and from single-queen laboratory rearing (r_s), assuming no reproductive skew among nestmate queens from natural colonies.

et al. 2005). This might also be the case in inbred species belonging to the genus *Plagiolepis*.

Polyandry

Our study also reveals that queens of the three species of *Plagiolepis* studied mate multiply. Both facultative polyandry ($\geq 50\%$ of queens being singly mated and a minority with to 2–5 males) and obligate polyandry (almost always ≥ 2 and usually ≥ 5 matings per queen) were identified in a number of ant genera (Boomsma *et al.* 2009 and references therein). Obligate multiple mating is characteristic for taxa with large colony size and with no exception to date, where monogyny is the rule (Boomsma *et al.* 2009; Hughes *et al.* 2008b; Kronauer *et al.* 2007). According to the above classification, *P. maura* falls into the class of facultatively polyandrous species, as more than 50% of the queens were found singly mated and none were mated more than twice. On the other hand, *P. taurica* and *P. schmitzii* belong to the class of obligately polyandrous species, because more than 90% of the queens were mated with at least two males; in *P. schmitzii*, queens can mate with up to 14 males. Previous studies (Trontti *et al.* 2007) showed that more than 80% of *P. pygmaea* queens mate twice, some mating with up to six males; therefore, this species must be considered as obligately polyandrous. Consistent with this classification, the effective mating frequency of queens (M_{ep}) does not differ between *P. taurica*, *P. schmitzii* and *P. pygmaea* (Kruskal–Wallis test with Dunn's multiple comparison test, $KW = 20.64$, $P > 0.05$ for all comparisons). By contrast, the queen mating frequency in the facultatively polyandrous *P. maura* is significantly lower than that estimated for the above species ($P < 0.05$ for all comparisons).

Polyandry, like polygyny, increases the genetic diversity within colonies and dilutes within-colony relatedness. However, because of sib-mating and the significant relatedness between the male mates of a queen, the impact of multiple mating by queens on relatedness values is greatly limited in *Plagiolepis*. Considering our estimates of M_{ep} values for each species (Table 3), the expected relatedness in single-queen colonies under random mating and no relatedness among the male mates of a queen is, as indicated above, 0.70 in *P. maura*, 0.44 in *P. schmitzii*, 0.42 in *P. taurica* 0.46 in *P. pygmaea* (estimated from the data of Trontti *et al.* 2005). Yet, our data show that inbreeding and the relatedness between the mates of a queen counteract the effects of polyandry on the average relatedness in *P. maura* ($r_s = 0.76$) and *P. pygmaea* ($r_s = 0.75$), and that reduction in relatedness because of multiple mating is greatly buffered in *P. schmitzii* ($r_s = 0.54$) and in *P. taurica* ($r_s = 0.55$) (Fig. 1).

In ants, mating usually takes place outside the natal colony during a nuptial flight. Multiplication of mating phases increases energy expenditure, the risk of predation and/or the probability of transmission of diseases, parasites or pathogens. These costs are likely greatly reduced in *Plagiolepis*, because mating proceeds inside the colony (Trontti *et al.* 2007; this study). Conversely, multiple mating brings several fitness benefits that may have favoured its evolution (reviewed in Crozier & Fjerdingstad 2001; Boomsma *et al.* 2009). Increased genetic diversity within colonies was shown (i) to raise overall colony productivity (Robinson & Page 1995; Mattila & Seeley 2007; Wiernasz *et al.* 2008), (ii) to improve defence against pathogens (Tarpay 2003; Baer & Schmid-Hempel 1999; Hughes & Boomsma 2004, 2006) and (iii) to reduce kin conflicts within social insect colonies (Trivers & Hare 1976; Moritz 1985; Ratnieks *et al.* 2006). Females may also derive direct benefits from mating multiply by receiving a sufficient sperm reserve to maintain large and long-lived colonies (Cole 1983; Fjerdingstad & Boomsma 1998). The high level of inbreeding and the close relatedness between the male mates of a queen reported in all species of *Plagiolepis* studied so far should greatly reduce the genetic benefits associated with multiple mating. On the other hand, increasing sperm supply storage to maintain large and long-lived colonies should not favour much polyandry in polygynous species with recruitment of daughter queens. Although the benefits of multiple mating can vary between species in *Plagiolepis*, the apparent absence of cost may have been pivotal in the evolution and maintenance of polyandry in this ant genus.

Polygyny

Finally, our results also show that colony queen number greatly varies both among and within species. The number of queens ranges from 1 to 5 in *P. taurica* and from 1 to 10 in *P. maura*; it is on average < 2 indicating that both species are facultatively polygynous (*sensu* Boomsma *et al.* 2009). In *P. schmitzii*, queen number varies from 1 to 28 and is on average 5. Moreover, the vast majority of colonies contained more than one queen. Therefore, *P. schmitzii* may be considered as obligately polygynous. The occurrence of multiple reproductive queens usually increases the genetic diversity within colonies (Bourke & Franks 1995). Nevertheless, our data indicate that nestmate queens in *Plagiolepis* are closely related. Daughters are recruited as new queens in their natal colony, thereby greatly limiting the reduction in relatedness because of polygyny. Assuming no reproductive skew among nestmate queens from natural colonies, the comparison between the relatedness among workers from field colonies (r_w ,

Table 2) and from single-queen laboratory rearing (r_s , Table 3) shows that polygyny reduces only weakly within-colony relatedness in the facultatively polygynous *P. maura* and *P. taurica* (see also Fig. 1). The decrease in worker relatedness amounts 0.05 (0.76 vs. 0.71) in *P. maura* and 0.08 (0.55 vs. 0.47) in *P. taurica*. In the obligately polygynous species *P. schmitzii* and *P. pygmaea*, reduction of within-colony relatedness because of polygyny is much higher and amounts 0.20 (0.54 vs. 0.34) and 0.36 (0.75 vs. 0.39), respectively.

Collectively, our results for *P. taurica*, *P. schmitzii* and *P. maura* combined with those reported for *P. pygmaea* show that sib-mating maintains high relatedness values within colonies despite polygyny and polyandry. Mating among relatives could result from a long history, which is expected to reduce depression in species with regular inbreeding. Moreover, the co-occurrence of polyandry and polygyny is infrequent in social Hymenoptera. It shows that obligate multiple mating is not restricted to ant taxa where monogyny is the rule. The causes of their co-evolution certainly merit further studies.

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Data accessibility

Sampling locations and microsatellite genotype data for all individuals are available in Table S1 (Supporting information).

Supporting information

Additional supporting information may be found in the online version of this article.

Table S1 Sampling locations of *Plagiolepis taurica*, *P. schmitzii* and *P. maura*, accession numbers of DNA sequences and microsatellite genotype data for all individuals.

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