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## Serial polygyny and colony genetic structure in the monogynous queenless ant *Diacamma cyaneiventre*

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**Abstract** Serial polygyny, defined as the temporal succession of several reproductive females in a colony, occurs in some monogynous social insects and has so far attracted little attention. *Diacamma cyaneiventre* is a queenless ponerine ant found in the south of India. Colonies are headed by one singly mated worker, the gamergate. After the death of the gamergate or her absence following colony fission, the gamergate is replaced by a newly eclosed nestmate worker. After a replacement, colonies go through short-lived periods in which two matrilineal sisters co-occur. This is a situation which can be described as serial polygyny. To measure the consequences of serial polygyny, a genetic analysis was performed on 449 workers from 46 colonies of *D. cyaneiventre* using five microsatellite loci. The presence of more than one matriline among workers of the same nest was detected in 19% of colonies, indicating a recent change of gamergate. The average genetic relatedness among nestmate workers was 0.751 and did not significantly differ from the theoretical expectation under strict monogyny and monandry (0.75). A simple analytical model of the temporal dynamics of serial polygyny was developed in order to interpret these results. We show that the rate of gamergate turnover relative to the rate of worker turnover is the crucial parameter determining the level of serial polygyny and its effect on the genetic structure of colonies. This parameter, estimated from our data, confirms that serial polygyny occurs in *D. cyaneiventre* but is not strong enough to influence significantly the average genetic relatedness among workers.

**Keywords** Genetic relatedness · Microsatellites · Gamergate turnover · Queenless ants · Ponerinae

### Introduction

The genetic relatedness between members of animal societies is a key parameter for understanding the evolution of altruistic behaviour and reproductive division of labour (Hamilton 1964). This parameter is based on the probability that interacting individuals carry genes that are identical by descent. The sociogenetic organisation of colonies, i.e. the genealogical relationships between nestmates, is therefore a crucial factor involved in the outcome of social conflicts over sex allocation, production of males and other colony characteristics (Bourke and Franks 1995; Crozier and Pamilo 1996). In the Hymenoptera, the haplodiploid sex determination system leads to high genetic relatedness (0.75) among nestmate workers in monogynous and monandrous species. However, empirical studies have confirmed that polyandry or polygyny can drastically reduce the genetic relatedness among workers in a colony (Queller 1993; Ross 1993; Bourke and Franks 1995).

In a number of monogynous social insects, different reproductive females succeed each other in the same colony, and this may also be expected to reduce relatedness. Species exhibiting a temporal succession of reproductives have potentially immortal colonies, and these multiply by fission. This contrasts with other monogynous species in which the death of the founding queen leads to colony extinction (Hölldobler and Wilson 1990). Replacement of reproductives is also commonly observed in polygynous ants, in which new queens are regularly recruited in colonies (Evans 1996). Very fast queen turnover has been detected in some species, and this affects the genetic relatedness between workers and brood (Seppa 1994; Evans 1996; Bourke et al. 1997). However, in polygynous species, the sociogenetic consequences of queen turnover are conflated with the specific effects of polygyny, and are thus difficult to quantify. Monogynous species exhibiting queen replacement include various primitively eusocial wasps (Jeanne 1972; West-Eberhard 1978; Gadagkar et al. 1993a), queenless ponerine ants (Peeters 1993), most army ants (Gotwald

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1995), a few formicine ants (Lenoir et al. 1988) as well as honey-bees and stingless bees. In these species, referred to as “serially polygynous” (Yamane 1986; Gadagkar et al. 1993a), colonies undergo phases during which two or more matriline of sisters, produced by a succession of reproductive females, co-exist in the colony. Replacement queens may be foreign or related, depending on the taxon. There have been no genetic studies of serial polygyny in monogynous species (but see Gadagkar et al. 1993a who used pedigree analyses). As with polygyny and polyandry, queen turnover could have major implications for social evolution, because it leads to temporal variation in genetic relatedness between workers and brood (Gadagkar et al. 1993a).

In this study, we investigated the importance of serial polygyny in *Diacamma cyaneiventris*, a queenless ant. In all *Diacamma* species (subfamily Ponerinae), the queen caste does not exist. The reproductive role is fulfilled by workers, called gamergates, than can mate and lay fertilised eggs (Peeters 1991). Reproduction in *Diacamma* is regulated by a very peculiar behaviour prohibiting polygyny. The gamergate mutilates every recently emerged worker by biting off a pair of bladder-like innervated appendices present on the thorax, called gemmae (Fukumoto et al. 1989; Peeters and Higashi 1989; Gronenberg and Peeters 1993). Mutilation prevents workers from mating and thus producing diploid offspring. Thus only one adult worker in each colony retains her gemmae, mates and monopolises diploid egg production. In the absence of the gamergate, i.e. after colony fission (leading to two autonomous groups, one of which initially lacks a gamergate) or after the gamergate dies, the first worker to emerge will retain her gemmae and mutilate all subsequent callows. Mating occurs inside the nest with a male from another colony, when the future gamergate performs a behaviour called “sexual calling” which is restricted to virgin unmutilated workers (Peeters et al. 1992). Males are winged and fly away from their natal colony, searching for a nest with a worker exhibiting “sexual calling”. Following copulation, the male stays attached to the female abdomen for several hours (Wheeler and Chapman 1922; Fukumoto et al. 1989; Nakata et al. 1998), and this prevents her from re-mating immediately afterwards; in addition, a worker never exhibits “sexual calling” again after mating (C.

Peeters, unpublished data). After mating, the young gamergate does not disperse but reproduces in her natal colony. Thus for a certain period of time, the colony will have two matriline of full sisters, aunts and nieces. The frequency of such periods with multiple matriline in a colony life cycle determines the average relatedness among nestmates, especially between workers and brood, and should therefore be an important parameter for understanding the evolution of these queenless societies.

The aim of this study was (1) to determine the frequency of colonies with multiple matriline and thereby (2) to analyse the effects of serial polygyny on the socio-genetic organisation of the colonies, more specifically on the genetic relatedness among workers.

## Methods

### Species and sampling

*Diacamma cyaneiventris* is a ponerine ant that inhabits open areas in southern India. Colony size is relatively small [mean  $214 \pm 80$  ( $\pm$ SD) workers,  $n=6$ ; unpublished data]. Colonies are found in deep perennial nests (up to 50 cm underground; unpublished data). Their life cycle shows no seasonality since female and male brood can be found throughout the year. An average of 9.8 workers per colony (minimum 4, maximum 17) were collected and kept in 90% alcohol. In this species, nest entrances are surrounded by a mound of soil and pebbles; we collected workers only from this mound, when they entered or left the nest. Forty-six colonies of *D. cyaneiventris* were sampled in one site on 30 and 31 May 1997 (337 workers from 33 colonies), and in another site 300 m away on 23 October 1998 (112 workers from 13 colonies). These two sites were approximately  $300 \times 300$  m in area and were located near the village of Kotigehara ( $13^{\circ}7' N$ ,  $75^{\circ}31' E$ ), in the district of Chikmagalur, 230 km west of Bangalore, in Karnataka State, India.

### Genetic analysis

For most individuals, DNA was extracted using the following quick extraction procedure. The tissues of each ant head were homogenised in 50  $\mu$ l of a solution containing 10 mM Tris HCl, 1 mM EDTA, 25 mM NaCl, and 200  $\mu$ g ml<sup>-1</sup> of proteinase K. After 2 h at 37°C and 2 min at 92°C, the solution was centrifuged for 5 min at 6,000 rpm. The supernatant was then recovered and kept at -20°C. One microlitre of this DNA solution was used per polymerase chain reaction (PCR). Five microsatellite loci developed for *D. cyaneiventris* (Doums 1999) were used in this study (Table 1). The PCRs were conducted following the protocol of

**Table 1** Genetic variability of the five loci. The number of alleles per locus ( $n_{all}$ ), expected heterozygosity ( $H_E$ ) and Weir and Cockerham  $F_{IS}$  are given for the five loci used in this study, for the 1997 sample (337 individuals originating from 33 colonies) and the 1998 sample (112 individuals from 13 colonies).  $n_{all}$  and  $H_E$

were calculated by weighting the colonies equally using the Relatedness 5.0 programme (Queller and Goodnight 1989).  $F_{IS}$  was calculated using one random individual per colony with the Genepop programme (Raymond and Rousset 1995). More information about the loci can be found in Doums (1999)

Locus	1997 sample				1998 sample			
	$n_{all}$	$H_E$	$F_{IS}$	$P$	$n_{all}$	$H_E$	$F_{IS}$	$P$
DC 11	10	0.61	-0.099	0.93	9	0.57	0.056	0.15
DC 18	25	0.93	0.005	0.25	15	0.90	0.010	0.52
DC 19	5	0.78	-0.025	0.56	7	0.78	0.012	0.37
DC 20	5	0.77	-0.104	0.79	5	0.71	0.053	0.72
DC 52	6	0.66	0.025	0.59	5	0.64	0.005	0.46

Doums (1999), except for the co-amplification of loci D19 and D20 for which the amounts of dNTP,  $^{33}\text{PdATP}$  and Taq DNA polymerase were doubled.

Heterozygote deficiency at each locus and linkage disequilibrium between each pair of loci were examined in the two samples, by an exact test using the Genepop programme (Raymond and Rousset 1995). The tests were performed using one randomly selected individual per colony. Expected heterozygosity was calculated using the allelic frequencies of each sample. None of the ten  $F_{IS}$  values was significantly higher than zero (overall combined  $P$ -value=0.76; Table 1; Sokal and Rohlf 1995, p. 794). No significant linkage disequilibrium was observed for the ten pairs of loci (combined  $P$ -values over the two samples >0.3). Using the colonies containing a single matriline of full sisters (see Results), and headed by a heterozygous mother at a given locus, we checked for any significant deviation from the balanced Mendelian segregation of 1:1. For each locus, a  $G$ -test was performed by colony and each  $P$ -value combined over colonies to find a global significance level. None of the combined  $P$ -values was significant at the 5% level, indicating that each locus followed Mendelian segregation.

#### Data analysis

Two main parameters were estimated from the genetic data: the proportion of multiple matriline colonies and the within-colony genetic relatedness. The proportion of multiple matriline colonies was determined by checking whether the genotypes observed in each colony over the five loci were compatible with a full-sister hypothesis. A sample of workers having a common allele (from their father) and a maximum of two other alleles (from their mother) at each locus was considered as one matriline of full sisters. In any other case, the sample was considered as containing several matriline, as polyandry is very unlikely in this species. Moreover, the high polymorphism of the markers used and the absence of a high level of inbreeding make the probability of no detecting a gamergate turnover extremely low. For example, the probability of two random males sharing exactly the same genotype equals 0.005 and 0.001 for 1997 and 1998, respectively.

The proportion of multiple matriline colonies,  $M_{\text{obs}}$ , was estimated in the 46 colonies. In 1 of these colonies, one individual was not compatible with the others for only one allele at a given locus, which differed by only 2 bp from one of the other alleles of the colony. We suspected a mutation event and decided to consider that the individual belonged to the same matriline of full sisters. Another colony sample was mainly constituted of one matriline except one individual, which could not be an aunt or a niece of the others. Our sampling of workers was restricted to the nest mound but this might not be sufficient to ensure that all the workers collected in fact came from the same colony. We therefore preferred to be conservative with respect to the number of colonies with multiple matriline and considered this colony sample as a single matriline by removing the incompatible individual from the data. The number of workers analysed per colony being limited, a better estimate could be obtained by taking into account the error of sampling only one matriline in a colony that in fact contained two. Assuming that the colonies cannot contain more than two successive matriline among their workers, the corrected proportion of colonies containing two matriline,  $M$ , is given in Eq. 1 (see derivations in Appendix 1):

$$M = M_{\text{obs}} \times \frac{1}{\frac{1}{C} \sum_{i=1}^C \frac{n_i - 1}{n_i + 1}} \quad (1)$$

with  $C$  being the number of colonies sampled, and  $n_i$  the number of workers sampled in colony  $i$ .

This estimate is subject to variations due to binomial sampling and colony sample size. A bootstrap procedure was therefore used to estimate its confidence interval. Forty-six colonies were randomly sampled from our data set (resampling of the same colony being allowed), and  $M$  was estimated for this new sample. This procedure was repeated 10,000 times to generate 10,000 values of

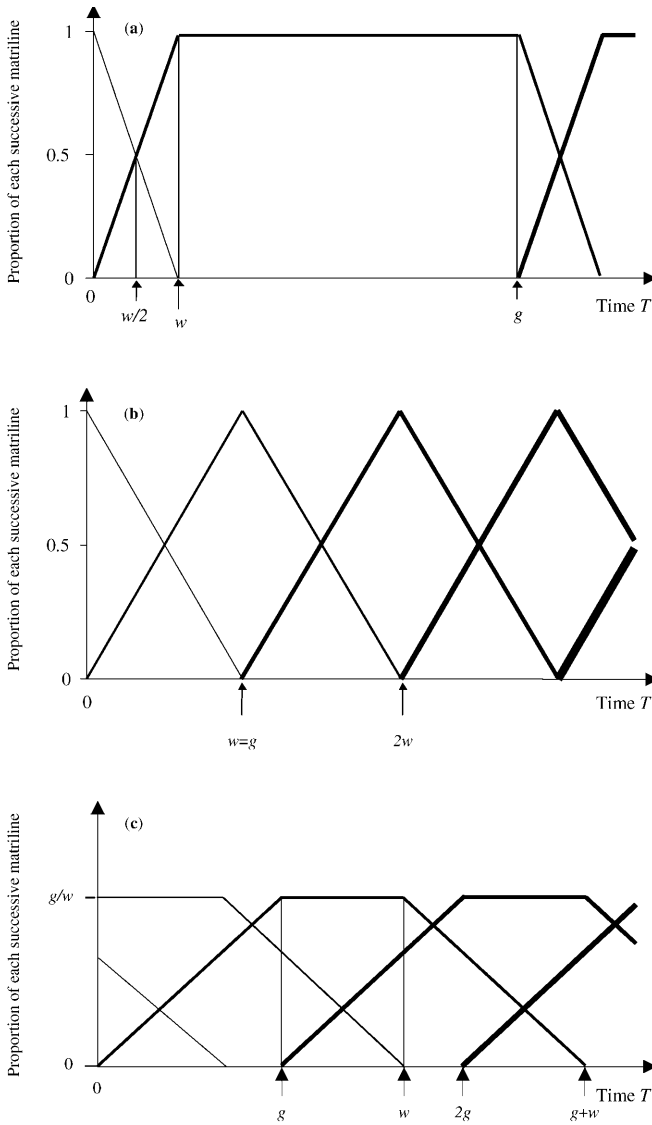
$M$ , from which the 250 lowest and the 250 highest values were excluded (5%). The remaining 9,500 values therefore constituted a confidence interval for  $M$ , at the 95% level.

Genetic relatedness among collected nestmate workers was estimated for each colony, and averaged over colonies, using the Relatedness 5.1 programme (Queller and Goodnight 1989). The standard errors of the means were obtained by jackknifing over colonies. For the allelic frequencies and the average relatedness estimates, colonies were weighted equally. The allelic frequencies were estimated separately for each colony by excluding that colony in order to avoid sample bias. These relatedness estimates could then be tested for any deviation from a given relatedness value using a  $t$ -test (Sokal and Rohlf 1995, p. 174). The relatedness estimates are affected by sub-population genetic differentiation. Two factors could induce such differentiation in our study and bias our results. First, in *Diagamma*, the colonies reproduce by fission which could generate some population viscosity over a short distance. However, in the studied population no significant isolation by distance was detected (C. Doums, unpublished results). Second, given that the sampling was not carried out at exactly the same site in the 2 different years (33 colonies in 1997 and 13 in 1998), the allelic frequencies could differ between the two samples. To test for this potential difference, the allelic frequencies of the two samplings were compared for each locus using the Struc routine in the Genepop programme (Raymond and Rousset 1995). This programme gives the exact probability that the samples come from the same gene pool. The significance over the five loci was calculated using a combined probability test (Sokal and Rohlf 1995, p. 794). However, in our samples, the individuals collected from the same colony cannot be considered as independent. To solve this problem, two artificial samples were generated for each site, using the observed average allelic frequencies of this site (calculated with Relatedness 5.1 by weighting the colonies equally) but with a number of individuals equal to three times the number of colonies sampled in the site. The underlying reasoning is that a colony represents a minimum of three independent genomes: one from the male, and two from the gamergate. In our samples, the genotypes of at least six workers per colony were determined, thus ensuring at the 5% probability level that both alleles of the gamergate have been detected. Over the five loci, the allelic frequencies of these two artificial samples differed significantly (overall combined probability test,  $P=0.016$ ; Sokal and Rohlf 1995, p. 794). The mean allelic frequencies needed for estimating the genetic relatedness were therefore computed separately for each sample (1997 and 1998 were considered as two demes in the Relatedness 5.0 programme).

#### A simple analytical model of serial polygyny

For a better understanding of serial polygyny in *D. cyaneiventris*, a simple model simulating the dynamics of the genetic structure of colonies over time was developed. The reasoning underlying the model is schematically described in Fig. 1 and can be summarised as follows when the workers' lifespan  $w$  is shorter than the gamergate's tenure  $g$ . Effective gamergate replacement takes place at time  $T=0$  when the first cocoon of the new gamergate ecloses. From  $T=0$ , the proportion of workers of the new matriline increases while the proportion of workers of the previous matriline decreases at the same rate of  $1/w$  ( $w$  being the workers' lifespan). This assumes a constant colony size and continuous and constant egg-laying activity by the gamergate. At  $T=w/2$ , if we assume an equal egg-laying rate by successive gamergates, each matriline is equally represented in the colony and the average genetic relatedness among workers is minimal and equal to 0.5625 (the average of 0.75 and 0.375). At  $T=w$ , all the workers of the previous gamergate are dead and the colony once again contains of a single matriline of full sisters. From  $T=0$  to  $T=w$ , the colony includes two matriline, whereas from  $T=w$  to  $T=g$  ( $g$  being the gamergate's tenure) the colony contains a single matriline (Fig. 1a).

The effect of varying the ratio  $w/g$  is illustrated in Fig. 1, assuming an equal egg-laying rate by all gamergates. In this figure,



**Fig. 1** Temporal variation in the proportion of workers from each successive matriline of full sisters in a single colony. Each curve represents the temporal dynamics of the proportion of workers from a given matriline in the colony under: low serial polygyny ( $g=5w$ ) – most of the time, the colony contains only one matriline and rarely two (**a**); intermediate serial polygyny ( $g=w$ ) – the colony always contains exactly two matrilines (**b**); high serial polygyny ( $g<w\leq 2g$ ) – the colony contains either two or three different matrilines (**c**).  $T=0$  is the time of effective gamergate replacement,  $T=w$  and  $T=g$  are the workers' lifespan and gamergate's tenure, respectively

$g$  varies whereas  $w$  is kept constant; however, the reverse would give the same pattern, since it is the ratio  $w/g$  which is actually important. When the gamergate's tenure is much longer than the workers' lifespan, colonies contain only one matriline for most of the time ( $M\sim 0$ ) (Fig. 1a). On the other hand, with a ratio  $\geq 1$  (workers' lifespan is identical to or longer than the gamergate's tenure), colonies always contain at least two matrilines (Fig. 1b,c). The ratio  $w/g$  thus directly measures the effect of serial polygyny on the average genetic structure of colonies.

Under this model, assuming that the workers' and gamergate's longevity  $w$  and  $g$  have no variance and that gamergate replacement occurs randomly among colonies, the expected proportion  $M$  of colonies containing more than one matriline is given by:

$$M = \frac{w}{g} \text{ For } w \leq g \quad (2)$$

$$M = 1 \text{ For } w \geq g. \quad (3)$$

The major estimate which is also derived from  $w/g$ , with the same assumptions as above combined with the additional assumption that every gamergate lays eggs at the same rate, is the average life-for-life genetic relatedness among adult workers. The detailed derivations of the formula are given in Appendix 2.

$$R_{\text{worker-worker}} = R_S + \frac{w}{g} \left[ \frac{r-RS}{3} \right] \text{ For } w \leq g \quad (4)$$

$$\begin{aligned} R_{\text{worker-worker}} = & r^m [1 + m(1-r)] \\ & - \frac{w}{g} \left[ \frac{r^m(1-r)}{3} \right] \\ & + \frac{g}{w} \left[ \frac{2(r-r^m)}{1-r} + r^m [1 - 2m - m^2(1-r)] + R_S \right] \\ \text{For } w \geq g & \\ & + \frac{g^2}{w^2} \left[ \frac{2(r^m-r)}{(1-r)^2} + \frac{2r^m(m-1)}{1-r} \right. \\ & \left. + r^m \left( \frac{m^3(1-r)}{3} + m(m-1) + \frac{1}{3} \right) - \frac{RS}{3} \right]. \end{aligned} \quad (5)$$

Where  $R_S$  is the coefficient of relatedness among sisters,  $r$  is the relatedness between workers of successive matrilines and  $m$  is the integer part of the ratio  $w/g$ .

Our sampling design involved only workers that were active outside the nest. Therefore our sample mainly consists of foragers. This introduces a potential bias which needs to be evaluated carefully. Given that in *Diacamma*, as in most ant species, task partitioning is age dependent (Nakata 1995), a sample composed primarily of foragers also contains a restricted age range. Such a restriction decreases the probability of sampling two distinct matrilines in the same colony and thereby increases the estimated relatedness among nestmate workers. To investigate this bias, we consider the extreme hypothesis that only foragers of a given age cohort were sampled and then estimate the relatedness among nestmate workers from such a restricted sample. In our model,  $M$  would then represent the proportion of colonies containing more than one matriline among foragers and not among all nestmate workers. Every worker is assumed to start foraging at the same age and for the same period of time ( $w_f$ ), which represents a proportion  $k$  of their entire lifespan  $w$  ( $w_f = kw$ ) (i.e. at any given time, a proportion  $k$  of workers are foragers).  $M$  is therefore equal to  $w_f/g$  ( $w_f$  replaces  $w$  in Eq. 2) and the experimentally measured ratio is not  $w/g$  but  $w_f/g$ . Nevertheless,  $\frac{w}{g}$  is equal to  $\frac{w_f}{k \times g}$  and, if the value of  $k$  is known, the predicted relatedness among nestmate workers can be estimated from a sample of foraging workers by replacing  $\frac{w}{g}$  by  $\frac{w_f}{k \times g}$  in Eqs. 4 and 5.

## Results

### Proportion of colonies with more than one matriline among workers

Seven out of the 46 samples (15%) contained more than one matriline of full sisters (six in the 1997 samples, and one in 1998). Correcting for sample bias (Eq. 1), the proportion of colonies with more than one matriline ( $M$ ) was 0.19 with a confidence interval (CI) ranging from 0.08 to 0.32. Thus at any given time, 19% of colonies contained at least two successive matrilines of full sisters.



**Table 2** Average within-colony genetic relatedness among workers. Values for the single- and multiple-matriline colonies were also computed separately. The genetic relatedness estimate ( $R$ ) is given with its SE. Each estimate was tested against the value of 0.75 expected in a matriline of full sisters, using a  $t$ -test ( $n_{\text{col}}$  is the number of colonies)

	$n_{\text{col}}$	$R \pm \text{SE}$	$t$	$P$ -value
1997 samples	33	$0.752 \pm 0.038$	0.09	0.93
1998 samples	13	$0.749 \pm 0.071$	0.04	0.97
Overall	46	$0.751 \pm 0.032$	0.05	0.96
Single matriline	39	$0.781 \pm 0.024$	2.66	0.01
Multiple matriline	7	$0.583 \pm 0.113$	4.14	0.006

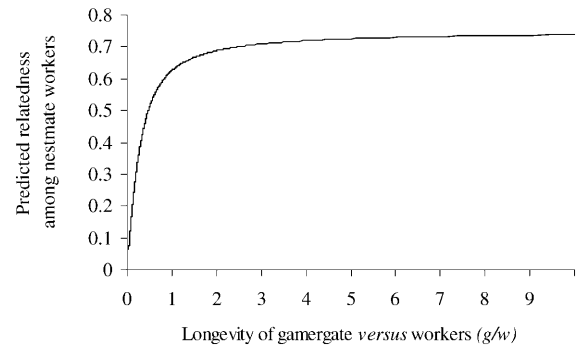
### Genetic relatedness among collected workers

The average within-colony genetic relatedness estimated among collected workers was  $0.751 \pm 0.032$  ( $\pm 95\%$  CI) which is not significantly different from 0.75 (Table 2). This estimate did not differ significantly between the two samples ( $t=0.04$ ,  $P=0.97$ ) and neither differed significantly from 0.75 (Table 2). These results indicate that, on average, there was no deviation from monogyny and monandry in *D. cyaneiventris*. However, when the multiple-matriline and the single-matriline colonies were considered separately, the relatedness among foragers was significantly lower in the former (0.583) than in the latter (0.781) ( $t=6.84$ ,  $P<0.001$ ). Average genetic relatedness in multiple-matriline colonies was significantly lower than 0.75 (Table 2), whereas average relatedness in single-matriline colonies was unexpectedly significantly higher than 0.75 (Table 2). This last result can be explained by inbreeding. Nevertheless, the amount of inbreeding was not high enough to be detected in the  $F_{\text{IS}}$  calculations. A low amount of inbreeding, undetected by the  $F_{\text{IS}}$  calculations, could have, however, been sufficient to increase the genetic relatedness. Mating between individuals from related colonies might have induced some inbreeding. Such a slight level of inbreeding should not change the detection probability of a gamergate turnover in the sampled colonies.

### Parameters estimated from the model

From our corrected estimate of the proportion of colonies containing more than one matriline ( $M$ ), the ratio of the workers' lifespan over the gamergate's tenure ( $w/g$ ) would be equal to 0.19 in our studied population (with a CI ranging from 0.08 to 0.32) (Eq. 2). Even though the confidence interval is large, it does not include 1, indicating that  $g$  was significantly higher than  $w$ .

Figure 2 shows the average coefficient of relatedness among nestmate workers as a function of the ratio  $g/w$  predicted from Eqs. 4 and 5 with  $R_{\text{FS}}=0.75$  and  $r=0.375$  (in our case, gamergates are always replaced by their daughters, and successive matriline of workers are therefore aunts and nieces). Note that Eqs. 4 and 5 and Fig. 2 give the within-colony relatedness averaged across



**Fig. 2** Average relatedness among nestmate workers predicted from the analytical model (Eqs. 4 and 5 with  $R_{\text{FS}}=0.75$  and  $r=0.375$ ) as a function of the ratio  $g/w$  of the gamergate's tenure ( $g$ ) over the workers' lifespan ( $w$ )

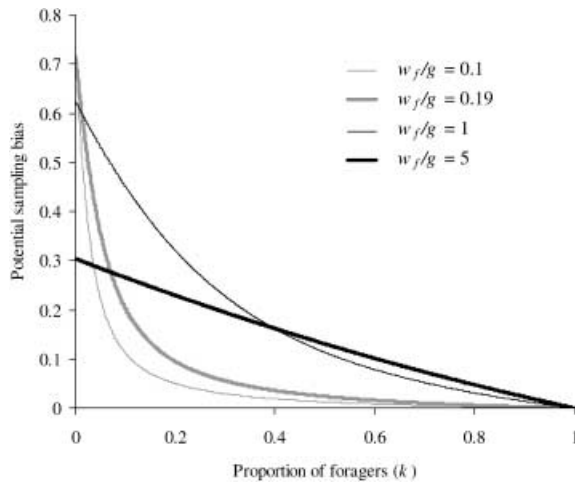
colonies. Each colony will deviate from this mean according to the time since the last gamergate replacement. This variance around the mean will be large for high  $g/w$  and low as  $g/w$  decreases (when a large number of matriline are always present in the colony).

As expected, the relatedness among nestmate workers decreases with increasing level of serial polygyny (decreasing  $g/w$ ). In this study of *D. cyaneiventris*, workers were replaced faster than reproductive females and therefore  $g/w > 1$ . In this case, the predicted within-colony relatedness only varies from 0.75 to 0.625 (Fig. 2).

Using the value 0.19 for the ratio  $w/g$  ( $g/w=5.26$ ) obtained from the proportion of multiple-matriline colonies in Eq. 4, the predicted relatedness among workers in our studied population would be 0.726 with a CI ranging from 0.71 to 0.74. Our empirical estimate, based on the microsatellite genotypes (Table 2) was not significantly different from this predicted value ( $t=1.53$ ,  $P=0.13$ ).

### Correction for the bias due to restriction of sampling to foragers

The difference between the relatedness among nestmate workers estimated either assuming a sample of foragers of a given age cohort (with correction) or a representative sample of the colony (without correction) gives the magnitude of the potential bias due to sample limitation. This potential sampling bias is given as a function of  $k$  in Fig. 3, for different levels of serial polygyny (different values of  $w_f/g$ ). This bias decreases with increasing proportion of foragers in the colony. Intuitively, a large percentage of foragers will provide a better representation of all nestmates than a small percentage of foragers. A less intuitive result of this graph is that the sampling bias is a non-linear function of  $k$  for low values of  $w_f/g$  such as the one obtained for *D. cyaneiventris* (bold curve  $w_f/g=0.19$ ). For low values of  $k$  ( $<0.2$ ), small variations of  $k$  lead to large variations in the potential sampling bias. In such a case, estimating the relatedness among nestmates from foragers is inaccurate unless  $k$  can be very precisely estimated, which is rarely the case.



**Fig. 3** The potential sampling bias due to collecting only foragers is given as a function of the proportion of foragers ( $k$ ) in a colony, for different values of the ratio  $w_f/g$ . The potential bias is expressed as the difference between the two estimates of relatedness among nestmates assuming either that only foragers were sampled (correction) or that the sample represented the age range of the entire colony (no correction) (see text for more details).  $w_f$  is the age range during which workers forage and  $g$  is the gamergate's tenure

## Discussion

Our results demonstrate the existence of serial polygyny in the queenless ponerine ant *D. cyaneiventris*. The behavioural regulation of reproduction in this species (see Introduction) prohibits polygyny. However, successive replacements of the reproductive female are likely to occur during the lifetime of a colony. Seven out of the 46 colonies analysed contained more than one matriline of full sisters among collected workers. Correcting for sampling bias, in the population studied and at a given time, 19% of colonies had more than one matriline among workers, i.e. had undergone a relatively recent change of gamergate. This gamergate turnover had no effect on the average genetic relatedness among collected workers. The value estimated over the 46 colonies was 0.751 and did not significantly differ from the relatedness expected in a monogynous and monandrous colony.

The main potential bias of these results follows from the fact that we sampled only foragers, which do not ensure a representative sample of workers with respect to age. This is an important and general problem for the study of serial polygyny, as it is often difficult to obtain a representative sample of the entire colony, especially for species whose nests are deep underground. On the other hand, this problem may be less important than it first appears, because behavioural flexibility may increase the age range represented by foragers. Indeed in *Diacamma*, the age at which a worker becomes a forager varies and depends on various colony characteristics, such as brood number (Nakata 1995). Our model shows that the potential sampling bias introduced by sampling only foragers is particularly difficult to control when the ratio of foragers is low ( $k < 0.2$ ), since in such a case, small varia-

tions of  $k$  lead to large variations in the potential sampling bias. In *D. ceylonense*, a species closely related to *D. cyaneiventris* (M. Veuille, A. Brusadelli, L. Brazier and C. Peeters, unpublished data),  $k$  was about 20% in different colonies (R. Gadagkar, unpublished data). Using this value of  $k$  and the measured value of  $w_f/g = 0.19$ , the predicted relatedness among nestmates would be approximately 0.63. The ratio  $w_f/g$  would be approximately 1, indicating that the lifespan of workers and the tenure of gamergates would be approximately equal. On the other hand, assuming that the collected workers represent a random distribution of age (no correction), the ratio  $w_f/g$  would be 0.19 which would indicate that the tenure of gamergates is about five times the lifespan of workers. The reality certainly lies between these two extreme values. The only data available come from a laboratory study on *Diacamma* sp. from Japan by Tsuji et al. (1996) indicating that gamergates live about three times longer than workers ( $w_f/g = 0.3$ ).

The tenure of gamergates cannot be directly considered as the gamergate lifespan. Indeed, gamergate turnover results from fission events or death (at least reproductive death) of the gamergate. A long lifespan of gamergates and/or low rate of fission would be associated with a low level of serial polygyny. In *Diacamma*, the rate of fission in natural populations is unknown. The nests of *D. cyaneiventris* are deep underground, with many chambers constructed at different levels, and therefore require a high investment. Moreover, when disturbed, the ants do not leave the nest as in another indian species *D. indicum*, but take refuge in the deepest chamber of the nest (C. Peeters, unpublished data). Finally, no population viscosity was detected in our population (C. Doums, unpublished data) even though this would be expected if fission was a common event. From these indirect data, fission does not seem to be the primary cause of gamergate turnover in *D. cyaneiventris*. Note, though, that several ecological factors influence the rate of fission, such as the availability of empty sites, which is high in a recently colonised site. This was unlikely to be the case in the studied population, as the density of nests was high (mean distance between neighbouring nests was about 5 m; personal observation). Therefore, our results on the lifespan of workers relative to the tenure of gamergates give a good indication of the relative longevity of workers and gamergates in a natural population. We can therefore conclude that the differential longevity observed by Tsuji et al. (1996) in *Diacamma* from Japan under laboratory conditions can also occur in *D. cyaneiventris* in the wild. This differential longevity may be partly driven by physiological differentiation occurring at the adult stage, given that all the female larvae in *Diacamma* have the same developmental pathway (i.e. there are no castes).

Evidence of queen turnover has recently been detected using genetic markers in several polygynous ants (Seppa 1994; Evans 1996; Bourke et al. 1997). For all these species, the turnover rate was considered to be high. For example, in *Leptothorax acervorum*, Bourke

et al. (1997) suggested that the reproductive lifespan of queens in a given colony is just 1 year. Similarly, in *Myrmica tahoensis*, 35–50% of queens disappeared each year (Evans 1996) and in *M. sulcinodis*, the effective genetic turnover of queens was 45–98% between two age cohorts of workers separated by 1 year (Pedersen and Boomsma 1999). However, the model developed here emphasises the importance of considering the queen's reproductive tenure relative to the lifespan of workers, in order to appreciate the effects of serial polygyny (see Eqs. 4 and 5). In the species cited above, the queen's reproductive tenure was shorter than or of the same magnitude as the workers' lifespan (Bourke et al. 1997). Therefore, in both species, queen turnover had major consequences on colony genetic structure.

Our results demonstrate genetic evidence for queen turnover in a monogynous ant species. In *D. cyaneiventris*, gamergate turnover has weak consequences on genetic relatedness among workers which was higher than 0.63 (even taking into account the potential bias of sampling only foragers). The consequences of gamergate turnover should however be more pronounced on the relatedness between workers and brood since, under serial polygyny, the average relatedness between two different age classes decreases with the age distance between these classes (Pedersen and Boomsma 1999). Further studies would be required to investigate the consequences of low serial polygyny on worker-brood relatedness.

Serial polygyny differs from polygyny and polyandry by being associated with temporal variations in colony genetic structure, which are actually more pronounced than their average effect. The outcome of social conflicts could therefore vary over time. For example, Gadagkar et al. (1993b) showed that in the wasp *Ropalidia marginata*, queen reproductive success is correlated with worker-brood genetic relatedness and therefore varies as a consequence of serial polygyny. In contrast to polyandry or polygyny, under serial polygyny, workers can detect a gamergate turnover simply by detecting the absence of a gamergate in the colony. *Diacamma* workers can detect the absence of the gamergate (Cuvillier-Hot et al., 2001). If this information could be integrated with perception of the age of brood (different developmental stages), workers could obtain a simple indicator of their genealogical relationship with the brood. This would enable them to adapt their sex allocation strategies to the different brood stages. Controlled laboratory experiments would be required to investigate this possible effect of serial polygyny on optimal sex allocation strategies.

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## Appendix 1

Correction for sampling errors in the observed proportion of multiple-matriline colonies

Let us assume that the correct proportion of colonies with two matrilines  $M$  is known. Then,  $M_{\text{obs}}$  (the observed proportion of colonies with two matrilines) can be derived as a function of  $M$ . The probability of detecting two distinct matrilines of full sisters among  $n$  workers sampled in the same colony is given by Eq. 6 as a function of  $y$ , the proportion of workers of the younger matriline in that colony.

$$p_n(y) = 1 - y^n - (1 - y)^n. \quad (6)$$

Then  $p_n(y)$  can be integrated over  $y$  between 0 and 1 to give the probability  $P_n$  of detecting two distinct matrilines of full sisters in a colony taken at random:

$$P_n = M \times \frac{n-1}{n+1}. \quad (7)$$

$C$  colonies are sampled, with  $n_i$  workers sampled in the colony  $i$ , the sum of  $P_{n_i}$  over  $C$  colonies gives the expected observed proportion of multiple-matriline colonies  $M_{\text{obs}}$ :

$$M_{\text{obs}} = M \times \frac{1}{C} \sum_{i=1}^C \frac{n_i - 1}{n_i + 1}. \quad (8)$$

Eq. 8 leads directly to:

$$M = M_{\text{obs}} \times \frac{1}{\frac{1}{C} \sum_{i=1}^C \frac{n_i - 1}{n_i + 1}}. \quad (9)$$

Eq. 1 in the text. The only assumption about the dynamic of worker turnover is that the proportion  $y$  of workers from the younger matriline follows a uniform distribution between 0 and 1 (i.e. colonies have an equal chance of containing either a minority or a majority of workers from the younger matriline). For example, this implies that gamergate replacement in different colonies is not synchronised.

## Appendix 2

Predicted relatedness among workers under a simple analytical model of serial polygyny

The relatedness among workers can be predicted as a function of  $w/g$  under the model of serial polygyny described in the text, i.e. assuming (1) a constant, continuous and equal egg-laying activity of all the gamergates (even at the time of gamergate change), (2) no variance in the longevity of workers ( $w$ ) and gamergates ( $g$ ) and (3) gamergate replacements among colonies are not synchronised. In each equation,  $R_s$  will be the relatedness among sisters and  $r$  the relatedness between two matrilines produced by two successive reproductive females.

The colony will be considered as a group of workers whose ages are uniformly and continuously distributed between 0 and  $w$  (hypothesis 1 and 2). Let us first consider the case when the workers' lifespan is shorter than the gamergate's tenure ( $w \leq g$ ). Let us take a colony randomly selected with respect to the time of gamergate replacement, and state that the last gamergate turnover occurred  $x$  units of time ago ( $0 \leq x \leq g$ ).

When  $0 \leq x \leq g$ , the colony contains two matriline, respectively including workers of age 0 to  $x$  for the younger, and  $x$  to  $w$  for the older (hypothesis 2). This strict partition of matriline by age, combined with hypothesis 1, permits us to calculate directly the proportion of workers belonging to each matriline: it is given by the ratio of the age range of the matriline divided by the total age range of the colony ( $=w$ ). The average relatedness among adult workers is therefore:

$$R(x) = RS \times \left[ \left( \frac{w-x}{w} \right)^2 + \left( \frac{x}{w} \right)^2 \right] + 2r \times \left[ \frac{x}{w} \times \frac{w-x}{w} \right] \quad (10)$$

| for  $x \in [0; w]$ .

When  $w \leq x \leq g$ , every worker of the older matriline is dead and the colony contains only one matriline. The average relatedness among adult workers is therefore:

$$R(x) = RS \quad \text{for } x \in [w; g]. \quad (11)$$

$R(x)$  is then integrated over  $x$  between 0 and  $g$  and the integral divided by  $g$ . This gives the mean within-colony relatedness expected if several colonies are sampled (hypothesis 3):

$$R_{\text{worker-worker}} = R_S + \frac{w}{g} \left[ \frac{r - R_S}{3} \right].$$

Eq.4 in the text.

Let us now consider the case when the workers' lifespan is longer than the gamergate's tenure ( $w \geq g$ ). The relatedness between two matriline separated by  $l$  generations is  $r^l$  ( $l > 0$ ).  $m$  is defined as the integer part of the ratio  $w/g$  (the highest integer inferior to the ratio  $w/g$ ). Let us consider a colony randomly selected with respect to its life cycle, and state that the last gamergate turnover occurred  $x$  units of time ago ( $0 \leq x \leq g$ ).

If  $0 \leq x \leq g - mg$ , the colony includes  $m+2$  successive matriline of sisters, the age ranges of which, from the youngest to the oldest, are, respectively:

$$[0; x]; [x; x+g]; [x+g; x+2g]; [x+2g; x+3g]; \dots; [x+(m-1)g; x+mg]; [x+mg; w].$$

The proportion of workers belonging to each of them is therefore  $x/w$  for the youngest matriline,  $(w-x-mg)/w$  for the oldest, and  $g/w$  for the  $m$  other matriline.

Within-colony relatedness is then derived by pure calculus (available upon request), and defined as  $R_m(x)$  with

$$R_m(x) = \frac{1}{1-r} \left[ 2mr^{m+1} \frac{g^2}{w^2} + 2r(1-r^m) \frac{g}{w} \right] + \left( \frac{1}{1-r} \right)^2 \left[ 2r(r^m-1) \frac{g^2}{w^2} \right] + RS \left[ m(m+1) \frac{g^2}{w^2} - 2m \frac{g}{w} + 1 \right] + 2x(RS - r^{m+1}) \left[ m \frac{g}{w^2} - \frac{1}{w} \right] + x^2 \left[ 2(RS - r^{m+1}) \frac{1}{w^2} \right]. \quad (12)$$

If  $w - mg \leq x \leq g$ , every worker of the oldest matriline is dead and the colony includes only  $m+1$  successive matriline of sisters. The proportion of workers belonging to each of them is  $x/w$  and  $[w-x-(m-1)g]/w$  for the youngest and the oldest matriline, respectively, and  $g/w$  for the  $m-1$  others. Therefore, the within colony relatedness is simply  $R_{m-1}(x)$ .

Relatedness is then integrated over  $x$  and the integral divided by  $g$  to obtain the expected average within-colony relatedness:

$$R_{\text{worker-worker}} = \frac{1}{g} \left[ \int_0^{w-mg} R_m(x) dx + \int_{w-mg}^g R_{m-1}(x) dx \right]. \quad (13)$$

Eq.13 leads to

$$R_{\text{worker-worker}} = \frac{r^m [1 + m(1-r)]}{1-r} - \frac{w}{g} \left[ \frac{r^m (1-r)}{3} \right] + \frac{g}{w} \left[ \frac{2(r-r^m)}{1-r} + r^m [1 - 2m - m^2(1-r)] + R_S \right] + \frac{g^2}{w^2} \left[ \frac{2(r^m-r)}{(1-r)^2} + \frac{2r^m(m-1)}{1-r} \right] + r^m \left[ \frac{m^3(1-r)}{3} + m(m-1) + \frac{1}{3} \right] - \frac{RS}{3}.$$

Eq. 5 in the text.

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