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Research article

Estimating the rate of gamergate turnover in the queenless ant *Diacamma cyaneiventre* using a maximum likelihood model

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Abstract. In monogynous ants, the death of the queen generally implies the death of the colony. However, queen replacement by either unrelated or related queen has been described in few species. In queenless ants from the genus *Diacamma*, the single reproductive worker (gamergate) is replaced by one of her daughters (or occasionally by a sister). From a long-term genetic survey of nests of *D. cyaneiventre,* we estimated the rate of gamergate turnover as well as the lifespan of workers and gamergate tenure using a maximum likelihood model developed for this purpose. We specifically compared the genotypes of two cohorts of workers sampled at 2 and 16 months interval from the same nests, using five microsatellite markers. To improve the accuracy of the estimates, we also used in the model the nests from the same population sampled only once and analysed by André et al. (2001). The model indicates that the possibility of the same nest not sheltering the same colony at two different sampling dates (colony turnover) was not significantly different from zero in our sample. The likelihood of the model was maximal for a probability of gamergate change $p_\gamma = 0.005$ per day (i.e. a gamergate tenure of 200 days) and a worker lifespan $w = 60$ days, indicating that the gamergate 's tenure is about 3 times longer than workers' expected lifespan in the population studied. Moreover, the genetic analysis of the gamergate and brood in three colonies excavated completely, reveals that colony fission can occur just after a gamergate replacement with the sister of the new gamergate reproducing in the new propagule.

Keywords: Gamergate turnover, colony fission, longevity, microsatellites, *Diacamma cyaneiventre.*

Introduction

Insect societies are usually considered as fairly stable social entities with one or more reproductives. As a consequence, most theoretical and empirical studies on kin conflict have assumed that genetic structure varies between colonies but is stable within a colony (Heinze and Keller, 2000). However, many studies indicate that queen turnover is a common process in polygynous ants (e.g. Seppä, 1994; Heinze et al., 1995; Evans, 1996; Bourke et al., 1997; Pedersen and Boomsma, 1999; Hannonen et al., 2004) as well as in social wasps (e.g. Gadagkar et al., 1991, 1993; Landi et al., 2003). As a consequence of the replacement of reproductive(s), temporal variation in the genetic composition of colonies can lead to temporal variation in social conflict or induce conflicts between workers from different age cohorts (Heinze and Keller, 2000). Characterizing the temporal change in the genetic structure of insect colonies is of major importance for our understanding of kin conflict and life history strategies of queens and workers. However, few studies have directly estimated the rate of queen turnover through the genetic analysis of cohorts from different ages in a single colony (Evans, 1996; Pedersen and Boomsma, 1999; Fournier et al., 2004; Chapuisat et al., 2004).

In monogynous ants, the death of the queen generally implies the death of the colony. However, the occurrence of workers from different matrilines has been detected in several species suggesting that queen replacement can occur (DeHeer and Tschinkel, 1998; Heinze and Keller, 2000; Foitzik and Herbers, 2001; Sanetra and Crozier, 2002). From a queen perspective, seeking adoption into an existing colony can be advantageous when the cost of independent colony foundation is too high (DeHeer and Tschinkel, 1998). For instance, in the fire ant *Solenopsis invicta*, queens producing diploid males are quite common due to a population bottleneck that occurred during their introduction to the United States. These queens have a very low success in founding new colonies alone, but are as successful as other queens in

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gaining acceptance in orphaned nests (DeHeer and Tschinkel, 1998). From a worker's perspective, the adoption of queens, even if unrelated, can enhance posthumous fitness of the dead queen by allowing the colony to survive until all the brood of the dead queen has been reared (Evans, 1996). Alternatively, the adoption of new unrelated queens may have no benefit for workers but results from a process of nest usurpation and temporary intraspecific parasitism under intense competition for nest sites as in *Leptothorax nylanderi* (Foitzik and Herbers, 2001).

Queen replacement by a daughter is similar as above from a queen perspective but maximizes fitness of workers under kin selection (Crozier and Pamilo, 1996). Surprisingly, queen replacement by a daughter has rarely been reported in monogynous ants (see Sanetra and Crozier, 2002). Queenless ants of the genus *Diacamma* are an interesting study system in which colonies are strictly monogynous but the reproductive worker can be replaced by one of her daughters. The queen caste does not exist in these species, and one mated worker, called gamergate (Peeters, 1991), monopolizes the production of diploid eggs in the colony using a peculiar behavior regulating reproduction. She bites off a pair of thoracic appendages, called gemmae, for each newly emerged worker, suppressing the aggressiveness of workers and their ability to mate (Peeters and Higashi, 1989). Monogyny is then obligate since the gamergate irreversibly prevents the newly emerged workers from mating and producing female offspring. When the gamergate is absent from the colony, the first worker to emerge retains its gemmae, mates and becomes the new gamergate. As new colonies are produced by fission, gamergate replacement occurs not only after the death of a gamergate but also after colony fission since one of the daughter colonies will have no gamergate. In two other queenless ants, gamergate replacement can also be regulated by low-ranked workers if they detect a decrease in the fertility of the gamergate (Monnin and Peeters, 1999; Cuvillier-Hot et al., 2004). Such worker policing may also occur in *Diacamma*.

The occurrence of gamergate replacement was genetically detected in *Diacamma cyaneiventre* (André et al., 2001). However this previous study was conducted by genotyping nestmate workers from various nests at one given sampling date. Such an instantaneous analysis did not allow to determine the absolute turnover rate of gamergates but only the ratio of gamergate tenure relative to worker lifespan; this ratio determines the average relatedness between nestmate workers present at the same time in a given nest (André et al., 2001). In the present study, we extend this analysis in order to estimate not only the rate of gamergate turnover but also the lifespan of workers and gamergate tenure. To do so, we compared cohorts of workers sampled at 2 or 16 months interval from the same nests of *D. cyaneiventre*. Such genetic temporal survey of nests has rarely been done in social insects (but see Chapuisat et al., 2004). We then develop a maximum likelihood model in order to estimate the mean tenure of gamergates and the mean lifespan of workers from this temporal genetic data. In other ants, the estimate of queen turnover was obtained from the estimates of genetic

relatedness between workers from different cohorts (Evans, 1996; Pedersen and Boomsma, 1999; Fournier et al., 2004). However, given that a worker can not always be unambiguously assessed as being full-sister or just closely related (such as niece or aunt) to another worker, a maximum likelihood approach is the most powerful method to provide an unbiased estimate of the mean rate of reproductive turnover. Such maximum likelihood approach has been mainly used in social insects to estimate the relative contributions of queens and workers to male parentage (e.g. Toth et al., 2002; Villesen and Boomsma, 2003).

Material and methods

Samples

D. cyaneiventre is a Ponerine ant with small colonies of about 214 ± 80 ($\pm$SD) workers (André et al., 2001), that inhabits open areas in southern India. Behavioral and genetic data suggest that monoandry should be the rule in *Diacamma* species (Peeters and Higashi, 1989; Fukumoto et al., 1989; André et al., 2001). The nests are long-lived underground structures (up to 50 cm, C. Peeters, unpubl. data) composed of many chambers. Nest entrances are surrounded by a conspicuous mound of soil and pebbles, and can thus easily be found. Following foraging ants returning to their nests also allowed us to find nest entrances. Workers were collected from the nest mounds when they entered or left the entrance. For purposes of thermoregulation, the workers regularly carry the brood in the upper chambers next to the nest entrance, as already noted in another *Diacamma* by Wheeler, 1915. Therefore our sampling was not strictly restricted to foragers but could also include nurses. All the nests were marked and mapped accurately, which enabled us to locate the same nests over time.

The nests used in this study were sampled within a restricted area of 400*500 m (named deme 1 and 2 in Doums et al. (2002) near the village of Kottigehara (13°7'N, 75°31'E) in Karnataka State, India. A total of 81 samples from 60 different nests were collected over four different dates (May 1997, October 1998, October and December 1999). Twenty one nests were sampled twice, ten at 16 months intervals between May 1997 and October 1998 and eleven at 2 months intervals between October and December 1999. In three colonies that were excavated completely (in October 1999), the single worker with gemmae and some brood could also be genotyped. Before DNA extraction, the worker with gemmae was dissected to determine whether she was mated. A full spermatheca indicated that this worker was the gamergate. The genotypes of brood were not considered in the maximum likelihood model, as we aimed to estimate the lifespan of adult workers, and not the entire lifespan of a zygote from fertilization to death. A total of 585 workers were used in the model; this dataset included only the individuals for which all loci could be genotyped. Out of these, 184 workers were genotyped in this study (all nests sampled twice, except the 1997 sample, see Fig. 1), while the other worker genotypes were obtained from André et al. (2001). The number of colonies and workers analyzed per sampling date and for the nests sampled twice is given in Table 1.

Genetic analysis

DNA was obtained using a rapid extraction procedure (see André et al., 2001). We used five microsatellites, the same than those used by André et al. (2001). PCRs were conducted following the protocol of Doums (1999), except for the co-amplification of loci D19 and D20 for which the amounts of dNTP, 33PdATP and Taq DNA polymerase were doubled.
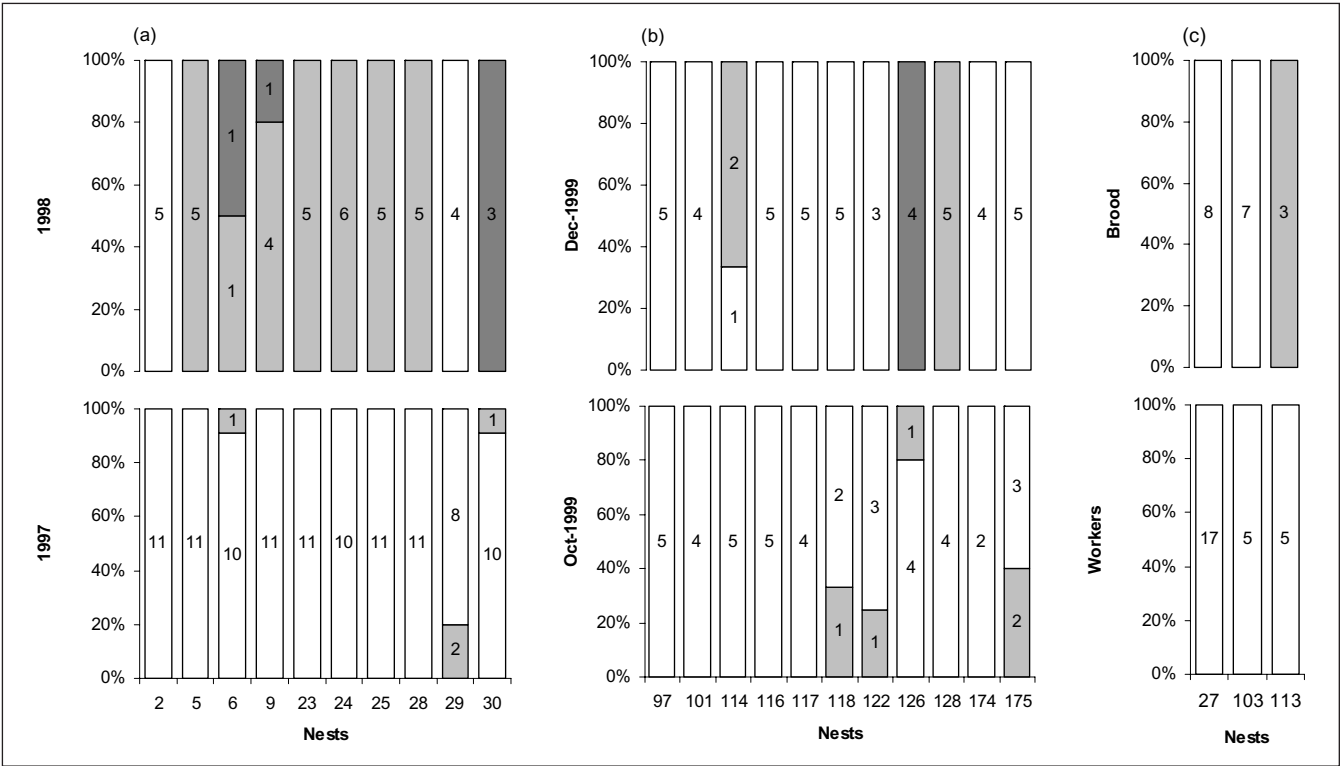
Figure 1. Pedigree analysis of the nests (a) sampled at 16 months interval, (b) sampled at 2 months interval and (c) when comparing workers and female brood for three excavated colonies. For each nest, a full-sister cohort is characterized by the same color. The number of individuals within each cohort are given within the bar. This figure is based on workers specifically analyzed in this study except for the workers sampled in 1997 for which genotypes determined by André et al. (2001) were used.

Allelic frequencies

In order to get a precise estimate of the allelic frequency in the locality studied, we pooled the colonies analyzed in André et al. (2001) and Doums et al. (2002) and the new colonies from this study. As a preliminary step, the allelic frequencies were compared between the three years (we pooled the two samplings at 2 months interval in 1999) using the *Struc* routine in the *Genepop* program (Raymond and Rousset, 1995). This program gives the exact probability that the samples at each date 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). As the individuals collected from the same colony cannot be considered as independent, we first estimated the allelic frequency for each colony, by weighting the colonies equally, using the program Relatedness 5.1 (Queller and Goodnight, 1989). For each year, an artificial sample was generated, using the allelic frequency estimated by Relatedness. The size of the artificial samples was determined by the number of colonies analyzed. When only one individual per colony was analyzed (data from Doums et al., 2002), each colony was represented by two genomes. When at least five individuals were sampled per colony, each colony were represented by three genomes. The underlying reasoning is that a monogynous and monoandrous colony represents three independent genomes (two from the mother and one from the father). One of the five loci (DC18, the most variable) detected a marginally significant difference in allelic frequency between the three sampling data ($P = 0.046$). However, over the five loci, we did not detect a significant difference between the three sampling data neither between pairs of sampling data ($P > 0.05$) using a combined probability test (Sokal and Rohlf, 1995, p. 794). The allelic frequencies at each locus were therefore estimated from the whole data set and all sampling dates pooled.

Genetic relatedness

The average genetic relatedness among nestmate workers was estimated for each sampling date and between workers from the same nest at dif-

Table 1. Average within-nest genetic relatedness. The estimates are given with their standard error ($r \pm SE$) over all samples, separately for each sampling date and between workers collected at different sampling date. The estimated relatedness was tested against the expected value of a full-sister relationship (0.75) and/or an aunt-niece relationship (0.375), using a t-test. N_{nests} is the number of nests and N_{ind} is the number of workers analyzed. Note that over all samples, the nests sampled at two different times were considered as different.

	N_{nests}	N_{ind}	$r \pm SE$	p-value of the t-test (expected value)
Over all samples	81	585	0.73 ± 0.015	0.29 (0.75)
1997 samples	33	336	0.75 ± 0.019	0.90 (0.75)
1998 samples	21	129	0.76 ± 0.029	0.69 (0.75)
Oct-99 samples	13	56	0.70 ± 0.047	0.28 (0.75)
Dec-99 samples	14	64	0.75 ± 0.012	0.96 (0.75)
Between 1997–1998	10	153	0.32 ± 0.103	0.002 (0.75)
				0.59 (0.375)
Between oct99–dec99	11	94	0.56 ± 0.081	0.04 (0.75)
				0.05 (0.375)

ferent sampling dates using the Relatedness 5.1 program (Queller and Goodnight, 1989). The standard errors of the means were obtained by jackknifing over colonies and was used to test for any given deviation of the relatedness estimates from a given relatedness value, using a *t*-test (Sokal and Rohlf, 1995, p. 174). Whatever the estimate of genetic relatedness, we used the allelic frequencies from the whole data set as described above.

Three colonies were completely excavated allowing the genetic analysis of the single worker with gemmae and some of the brood. For these colonies, as well as those sampled at two different dates, a visual inspection of the genotypes was performed to determine whether or not nestmate workers could be considered as full-sisters. This information was used to get a visual impression of our basic dataset (Fig. 1).

Maximum likelihood model

Maximum likelihood analysis is performed on the full dataset, including both the colonies sampled once and colonies sampled twice. Three simplifying assumptions were made: (i) gamergates have a constant "renewal probability" (p_γ) per unit of time (including death and fission), hence their tenure as reproductive follows a geometric distribution; (ii) workers have a constant lifespan w and (iii) gamergate tenure is long relative to worker lifespan hence the probability that the gamergate changes more than once during the life of a worker is negligible (see André et al., 2001). The possibility that the same nest does not shelter the same colony during the two sampling dates (emigration and re-colonization of one nest by different colonies) is taken into account by considering a probability per unit of time (p_c) of such a colony turnover. Knowing the life history parameters p_c, p_γ and w, as well as the allelic frequencies at each locus, we calculate the likelihood of our observed genetic data using a computer program built for this purpose and available upon request from J.B. André. The model assumed no inbreeding and no genetic sub-structure. These assumptions were verified for the two demes of the population Kottigehara1 studied here (Doums et al., 2002). The aim of the model was to find the life history parameters (p_γ, w, p_c) which maximize the likelihood of our dataset. Estimating only three parameters does not require the use of a Markov chain. Here we simply calculate the likelihood for each possible triplet of parameters (p_γ, w, p_c) on a grid, and search for the maximum. The principles underlying the calculation are given in the Appendix.

Results

Relatedness and brood analysis

The average within–colony genetic relatedness calculated from the whole dataset was not significantly different from 0.75. This result held for each sampling date (Table 1), indicating that on average, at a given time, there was no significant deviation from monogyny and monoandry, as already observed by André et al. (2001) for the 1997 sample. When the workers from the same nest were compared between two sampling dates, their relatedness were significantly lower than 0.75 (Table 1). More specifically, the relatedness between workers collected from the same nests at 16 months interval was not significantly different from that expected from an aunt-niece relatedness (Table 1). Such relatedness would be expected if, on average, the gamergate has changed once for each colony during this time period. A visual inspection of the genotypes indeed showed that nestmate workers from two sampling dates could not be full-sisters in

eight out of 10 nests at 16 months interval and in two out of 11 nests at 2 months interval (Fig. 1).

The pattern of full-sister relationships was different in each of the three colonies excavated completely (Fig. 1). In the two large colonies (no. 27 and 103), the workers and brood analyzed could be full-sisters or brothers. In these two colonies, the worker with gemmae was mated (the spermatheca was full) and could be considered as the gamergate. The genotype of the gamergate of colony 103 confirmed that she was actually the mother of the workers and brood, whereas the genotype of the gamergate of colony 27 suggested that she was the sister and not the mother of workers and female larvae. Therefore the gamergate had just changed in colony 27. In the third colony, 113, the female larvae could not be the full-sisters of the workers but instead nieces, suggesting a gamergate replacement. In agreement with this, the genotype of the worker with gemmae was compatible with being the mother of the male and female brood and the sister of the workers. However, this worker with gemmae had an empty spermatheca and could therefore not have laid diploid eggs and be the mother of the female brood. Because nest size and colony size (14 workers) were very small, it is very unlikely that we failed to collect the gamergate. Moreover, the unmutilated worker was really present in the field since no workers emerged after collection. We therefore interpret this to be a newly started colony. Soon after a gamergate change in the original colony, a small group of full-sister workers left the colony with some brood and a sister of the new gamergate (having exactly the same genotype) that probably emerged from a cocoon in the newly founded colony or eventually managed to retain her gemmae in the original colony. Note that given the genotypes observed in workers of this colony, the probability that two workers harboured the same multilocus genotype was 12.5% (2 locus with a single genotype in the colony and three locus with two genotypes).

Estimated parameters of the maximum likelihood model

As a first step, we tested whether the probability of colony turnover, Pc, is significantly different from zero. For this purpose, we derived the maximum likelihood of the dataset in the general model (L^1_{max}) as well as in a sub-model where the probability of colony turnover p_c is supposed null (L^0_{max}). The log-likelihood ratio test gave $2 \cdot \ln L^1_{max}/L^0_{max}$ which follows a chi-2 distribution with one degree of freedom. The test was not significant ($p = 0.2$), thus the hypothesis that every nest sampled twice contains the same colony over the two sampling dates could not be rejected, and the probability of colony turnover could not be estimated from our data. We consequently estimated the two other parameters, supposing $p_c = 0$. The likelihood was hence maximal for a probability of gamergate turnover $p_\gamma = 0.005$ per day (i.e. a gamergate expected tenure of 200 days), and a worker lifespan $w = 60$ days.

For these estimates, we obtained a confidence interval by generating 1000 artificial samples assuming (i) the estimated parameters $p_\gamma = 0.005$ and $w = 60$ for the probability of

gamergate turnover and the longevity of workers, (ii) the allelic frequencies at each locus as estimated from real data, (iii) the same dataset as in our sample: 39 colonies sampled once, 11 colonies sampled twice with an interval of 6 timesteps (the chosen time-step is 10 days), 10 colonies sampled twice with an interval of 49 time-steps, with an average of 9.75 (585/60) sampled workers in each colony. For each artificial samples, we estimated the parameters p_γ and w. From the 1000 different values of the estimated parameter, we removed the 2.5 percents lowest and highest for each parameter. We obtained a confidence interval at 95% with $0.0035 \leq p_\gamma \leq 0.0060$ (i.e. gamergates live between 165 and 285 days) and $30 \leq w \leq 90$ (i.e. workers live between 30 and 90 days).

Discussion

Life-history parameters

We estimated various parameters using a maximum likelihood model applied to a long-term genetic survey of nests of the queenless ant *D. cyaneiventre*. We first show that during the time period investigated (a maximum of 16 months), the probability of colony turnover at a single nest is not significantly different from zero. This is in agreement with different information concerning this species. (i) The nests are deep underground, suggesting that nest construction requires a high investment. (ii) When the nest is disturbed, the workers do not escape with the brood, as observed in another Indian species, *Diacamma indicum* (pers. obs.) but take shelter in the deepest chamber of the nest. Such behaviour is less likely to lead to colony emigration following a natural disturbance. (iii) The genetic analysis at a fine spatial scale of the mitochondrial DNA, performed in the population studied, revealed a very strong clustering of the mitochondrial haplotypes (Doums et al., 2002). Colony emigration is therefore not a common process as it would otherwise decrease this high genetic clustering.

The mean lifespan of workers and gamergate was estimated to be 60 days and 200 days, respectively. Concerning the gamergate, our estimate does not reflect exactly her lifespan, but the time spent as the reproductive female in a given nest. Indeed, we do not know whether, during a fission event, the gamergate stays in or leaves the original colony. If the gamergate leaves the colony, we will underestimate the gamergate lifespan. Under laboratory conditions, in *Diacamma* sp. from Japan (Tsuji et al., 1996), the average longevity of workers and gamergates was 208.8 and 579.2 days respectively (based on the study of 5 colonies), six times more than in our study. The lower worker longevity in our study could be due to the high mortality rate that ant workers are known to suffer during foraging trips under natural conditions. In *D. ceylonense*, a closely related species, the mean lifespan of foraging workers over 5 studied colonies ranged from 9 to 23 days with a mean of about 15 days (Karpakakunjaram et al., 2003). Moreover our value of gamergate longevity is underestimated since a fission event could be confounded with

senescence (as explained above). In spite of the differences between laboratory and natural estimates, it is interesting to note that the relative rate of serial polygyny (g/w) stays the same (0.30 in our study, and 0.36 from laboratory estimates of longevities). Direct estimates in natural populations of life-history parameters such as individual lifespan are very scarce in ants (Tschinkel, 1991; Keller, 1998). Our study shows that combining genetic data and a maximum likelihood approach is a promising tool for assessing life history parameters.

Rate of serial polygyny and consequences on colony conflicts

The rate of serial polygyny detected in this study is in agreement with the first estimate obtained by André et al. (2001) using a single date sampling. The replacement of reproductive females in colonies makes these potentially immortal. In polygynous species, by comparing cohorts of workers (or brood), the rate of genetic effective queen turnover can be estimated and can reflect the possible change in the group of reproducing queens (Pedersen and Boomsma, 1999). This rate was low and similar to that observed in our study (about 2% per year) in the polygynous colonies of *Pheidole pallidula* (Fournier et al., 2004) and *Formica selysi* (Chapuisat et al., 2004) but high in the polygynous ant *Myrmica sulcinodis* (from 45% to 98% per year; Pedersen and Boomsma, 1999) and *M. tahoensis* (about 40%; Evans, 1996). Such process of queen turnover was described in only few monogynous ants. In the obligate slave maker *Protomognathus americanus*, genetic evidence suggesting queen turnover was detected in 44% of colonies, most of them involving a related queen (Foitzik and Herbers, 2001). In the ant *Nothomyrmecia macrops*, pedigree analysis suggests that the queen has been replaced by a daughter queen in 10% of colonies, suggesting that queen longevity could be at most 10 years (Sanetra and Crozier, 2002). From the colony life-cycle proposed for this species, workers were supposed to live less than one year, giving a low level of serial polygyny of 0.1 (Sanetra and Crozier, 2002). The level of serial polygyny in *D. cyaneiventre* lies between these two, with no marked consequences on the relatedness among nestmate workers.

This absence of effect on the average relatedness among nestmate workers does not prevent the occurrence of within colony conflicts linked to gamergate replacement. Because regulation is based on mutilation in *Diacamma*, there is no direct conflict over which worker will become the gamergate, in contrast to other queenless ants (Monnin and Ratnieks, 1999). However, two main conflicts, over male production and over sex-ratio can be present. Prior to the emergence of the future gamergate and during the first two weeks of her life, workers are in competition for the production of male eggs, even though at this time the workers are likely to be full-sisters. This conflict is characterized by an increase in the level of aggression following the orphaning of the colony (Peeters and Tsuji, 1993) until the future gamergate is able to produce eggs (Cuvillier-Hot et al., 2002). In

contrast, the conflict over sex-ratio begins once the gamergate has started to lay diploid eggs. On the basis of genetic relatedness, the older workers (daughters of the previous gamergate, and full-sisters of the new one) will favor an equal sex-ratio given that they are as related to the new workers (their nieces) than to their nephews (0.375). On the other hand, the new workers (daughters of the new gamergate) will favor a female-biased sex-ratio (as expected in a monogynous, monoandrous colony). Such conflict has not been investigated yet.

In the closely related *D. ceylonense*, it takes 65 days from eggs to adult emergence (Baratte et al., 2005) and about 19 days before the future gamergate starts to lay eggs (Cuvillier-Hot et al., 2002). Using these values for *D. cyaneiventre*, we can estimate that the conflict over male production lasts a minimal value of 19 days (if a cocoon ecloses just after the gamergate death) and a maximal value of 84 days (if only eggs were present just after the gamergate death). Given that gamergates are replaced every 200 days (our results), the period of conflict represents 10 to 40 % of colony life. The cost induced by these periods, either because aggression itself is costly (Gobin et al., 2003) or because colony growth is stopped (no female production), may be an important factor determining the optimal frequency of gamergate replacement. Note though that the frequency of gamergate replacement will also be affected by the rate of colony fission. Therefore, ecological factors, such as low nest density, can indirectly increase the rate of gamergate replacement by favoring colony fissions.

Gamergate replacement can also have some positive feedback on the rate of fission. Just after a gamergate change, the next callow to emerge becomes the new gamergate; the remaining brood can never become gamergate in this colony because it emerged too late. Once the new gamergate starts laying eggs, the brood will be gradually replaced by nieces of the present workers in approximately 65 days (developmental time from egg to emergence, Baratte et al., 2005). Before the replacement of all the brood, the workers have a last opportunity to produce a descendant colony headed by a full-sister, by leaving the nest with some brood among which the first worker to emerge (their full-sister) will be the gamergate of the new colony. Hence the unstable phase of gamergate replacement could be a propitious time for colony fission. Very little is known about the events triggering colony fission in ants (Peeters and Ito, 2001), but we can speculate on the basis of our genetic data from the very small excavated colony. The worker with gemmae (future gamergate) had the same genotype than the mother of the brood but could not be the mother since she was still virgin. This future gamergate was also the sister of the five workers genotyped in the colony. This suggests that this colony originated by fission of an original colony in which the gamergate had changed recently. A few workers, a future gamergate (either a cocoon or an already emerged worker that retained its gemmae), and some young brood laid by the new gamergate of the mother colony (sister of the workers and mother of the brood), left this colony and established a new one. Such scenario is possible because the de-

velopmental time from egg to emergence (65 days, Baratte et al., 2005) is longer than the time to sexual maturity and mating (19 days, Cuvillier-Hot et al., 2002). Hence, the only fission event genetically analysed so far in a queenless ant probably occurred just after a gamergate replacement. Clearly, the behavioural mechanisms underlying fission merit more attention as they may offer an interesting and original test of kin selection theory.

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Appendix (a fully detailed Appendix is provided as supplementary online material)

Parameters used in the model

p_c: probability of colony turnover (for a given nest a colony is replaced by another)

p_γ: probability of gamergate turnover

w: worker lifespan

k: number of gamergate turnover

Δt: time laps between the two sampling dates

$C_{\Delta t}^k$: the number of distinct ways to choose k dates among Δt possible ones

The likelihood of obtaining the observed whole dataset is the product of the likelihood of obtaining the observed data for each nest (i.e. the nests are assumed to be independent of each other). Data for the nests sampled at only one date are considered independently of the nests sampled at two dates.

1. The likelihood of obtaining the observed data for a nest sampled at two dates

This likelihood is calculated as a weighted sum of two terms. (i) The combined likelihood of the two samples as an unique colony sampled twice (derivation described in section 3), weighted by the probability that the colony has not been replaced between the two sampling dates $(1 - p_c)^{\Delta t}$, where Δt is the time laps between the two dates. (ii) The likelihood of the two samples taken independently of each other (as the product of the independent likelihoods of each sample), weighted by the probability that the colony has been replaced between the two sampling dates $1 - (1 - p_c)^{\Delta t}$.

2. The likelihood of obtaining the observed data for a nest at a given sampling date

This likelihood is calculated as a weighted sum of all possible colony histories. (i) For the unimatriline colony, the likelihood of obtaining the observed data is conditional on the fact that the colony actually contains only one matriline of workers and is calculated as described in the section 4. This likelihood can be null if the colony sample actually contains two matrilines with different genotypes. This likelihood is then weighted by the probability that the colony is actually unimatriline, i.e. that no gamergate turnover has occurred in the w time steps preceding the sampling date $(1 - p_\gamma)^w$. (ii) For bimatriline colony, the likelihood of obtaining the observed genotypes in a given colony is conditional on the fact that the colony actually contains two matrilines of workers and is calculated as in section 4. Note that this likelihood is never null, because a collection of co-workers containing a single matriline can always be explained as a sample taken from a colony with two matrilines (either if the two matrilines have same genotype or if they have been unequally sampled, one of the matrilines being absent from the data). This is the case where one gamergate replacement occurred in the w preceding time-steps. The date of the gamergate replacement is supposed to follow a uniform distribution between -1 and $-w$ time steps before sampling (this date determines the proportion of workers of each cohort present in the colony). The likelihood of obtaining the observed sample for a colony is hence calculated as the average of all likelihood for each possible date of gamergate replacement. The average likelihood is finally weighted by the probability that one gamergate replacement has occurred in the w time steps preceding the sampling date $w \cdot p_\gamma \cdot (1 - p_\gamma)^{w-1}$. (iii) The probability that the colony contains more than two matrilines of workers (i.e. there was more than one gamergate replacement in the w time-steps preceding the sampling date) is neglected.

3. The combined likelihood of obtaining the two observed samples considering them as a single colony

The likelihood of two samples taken from the same colony at two dates is calculated as a weighted sum of all possible colony histories. A first partition is done conditional on whether the colony at each date is unimatriline or bimatriline. A partition is then done depending on the number of gamergate replacements that occurred between the two sampling dates. The coupled likelihood is calculated for each number of replacements k and weighted by the probability that k replacements actually occurred in the meantime Δt between the two sampling dates $C_{\Delta t}^k \cdot (p_\gamma)^k \cdot (1 - p_\gamma)^{\Delta t - k}$. The maximum possible number of gamergate replacements between two dates is limited in the derivations (10, in this paper). The probability that more than 10 gamergate replacements occurred in Δt is neglected.

4. The likelihood of obtaining the observed genetic data for a given colony conditional on its history

The history of a colony is telling whether the colony is uni or bimatriline and, in the case where the colony has been sampled twice, how many times the gamergate has been replaced between the two sampling dates. The likelihood of obtaining the observed genetic data for a colony, conditional on its history, is calculated as a product of the likelihood for each locus. The five loci are hence supposed independent; indeed no linkage disequilibrium has been found in a preliminary study on the same loci (Doums et al., 2002).

For each locus the likelihood of obtaining the observed genetic data for a colony, conditional on its history, is again calculated as a weighted sum. For each possible parental genotype (i.e. gamergate(s) and male(s) genotypes) the likelihood of the data is calculated as the product of the likelihood of each worker conditional on the parental genotype. The likelihood is then weighted by the probability of such a parental genotype, that is the product of the frequencies of all parental alleles. In the case where a colony is bimatriline, all the possible genotypes for the two gamergates (mother-daughter of each other) and the two independent males must be considered. In the case where the colony has been sampled twice, all the possible parental genotypes for each sampling date should be considered.

Electronic supplementary material

Appendix with detailed equations

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