Low relatedness among cooperatively breeding workers of the greenhead ant *Rhytidoponera metallica*

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Abstract

The greenhead ant *Rhytidoponera metallica* has long been recognized as posing a potential challenge to kin selection theory, because it has large queenless colonies where apparently many of the morphological workers are mated and reproducing. However, this species has never been studied genetically and important elements of its breeding system and kin structure remain uncertain. We used microsatellite markers to measure the relatedness among nestmates, unravel the fine-scale population genetic structure, and infer the breeding system of *R. metallica*. The genetic relatedness among worker nestmates is very low but significantly greater than zero ($r = 0.082 \pm 0.015$), which demonstrates that nests contain many distantly related breeders. The inbreeding coefficient is very close to and not significantly different from zero, indicating random mating and lack of microgeographic genetic differentiation. On average, closely located nests are not more similar genetically than distant nests, which is surprising, as new colonies form by budding and female dispersal is restricted. Lack of inbreeding and absence of population viscosity indicates high gene flow mediated by males. Overall, the genetic pattern detected in *R. metallica* suggests that a high number of moderately related workers mate with unrelated males from distant nests. This breeding system results in the lowest relatedness among nestmates reported for social insect species where breeders and helpers are not morphologically differentiated.

Introduction

The evolution and maintenance of reproductive altruism in insect societies is best accounted for by kin selection (Hamilton, 1964a,b; Bourke & Franks, 1995; Crozier & Pamilo, 1996). Colonies of social insects are usually composed of simple families, and by helping related individuals, workers indirectly transmit copies of their own genes to the next generation. However, some species have many breeders per nest. Hence, the relatedness between workers and the brood they raise may become very low, which presents a serious potential difficulty to kin selection theory (Hamilton, 1972). The maintenance of altruism in societies with low relatedness is most difficult to explain in species without morphological castes, because workers are not morphologically constrained to be helpers. In such species, workers are usually not able to breed independently. However, if the relatedness becomes too low, natural selection should favour workers that start reproducing in their colony instead of remaining helpers.

Two groups of social insects contain several species with both a high number of breeders per colony and no or very weak morphological differentiation between workers and reproductive females: the swarm-founding species of polistine wasps and the queenless species of ponerine ants (Hamilton, 1972). Those species where reproduction is divided among totipotent individuals have been referred to as cooperative breeders, which emphasizes that they are similar to many vertebrate

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societies (Crespi & Yanega, 1995; Peeters, 1997). The swarm-founding wasps have been extensively studied. Molecular analyses of seven species from phylogenetically distinct lineages revealed that the relatedness among nestmate workers is not very low, despite high queen number per nest (Queller *et al.*, 1993; Hastings *et al.*, 1998; Henshaw *et al.*, 2000; Tsuchida *et al.*, 2000). Across species, worker relatedness ranged from 0.16 to 0.49 with a mean of 0.28. This moderate relatedness among workers stems from the high relatedness among queens, which ranged from 0.34 to 0.82 with a mean of 0.59. Such elevated relatedness among nestmates is maintained by the particular colony-cycle of these wasps. The production of new queens occurs when the number of mother queens has dropped to one or a few, and the new closely related queens found colonies together (Queller *et al.*, 1993). Because the relatedness among workers is moderate, rather than extremely low, the maintenance of altruism in swarm-founding wasps may not be as problematic as it first appeared (Hamilton, 1972; Queller *et al.*, 1993; Queller & Strassmann, 1998).

In contrast to swarm-founding wasps, queenless species of ponerine ants have been relatively little studied. In the subfamily Ponerinae, workers have retained functional spermathecae (Peeters, 1991). Thus, unlike workers of most ant species, ponerine workers can actually mate and produce offspring of both sexes. Mated egg-laying workers are called gamergates to emphasize that they morphologically belong to the worker caste, rather than the queen caste (Peeters & Crewe, 1984). In approximately 80 of 1300 species of ponerine ants, morphological queens have been secondarily lost and all reproduction is by gamergates (Peeters, 1991). In some species a single gamergate becomes dominant and monopolizes reproduction, whereas in others multiple gamergates coexist in the colony (Peeters, 1993). In two species of ponerine ants, single gamergates managed to raise offspring independently under laboratory conditions (Ward, 1981; Liebig *et al.*, 1998). However, gamergates seem unable to independently found new colonies in the field (Peeters, 1993; Liebig *et al.*, 1998). Hence, colony reproduction in queenless ponerine ants occurs by budding, a process whereby one or a few gamergates with workers depart from a colony and establish a new colony in the vicinity.

Genetic studies of queenless ponerine ants are few, and all concerned species of *Rhytidoponera* with large workers. Ward (1983) studied *Rhytidoponera confusa* and *chalybea* with allozyme markers. In these two species, colonies are headed either by a single queen or by multiple gamergates. Interestingly, workers in colonies with multiple gamergates are moderately related ($r = 0.30$). On average, these colonies contain a mean of four highly related gamergates, which are often full sisters. In *Rhytidoponera* sp. 12, nestmate workers are also moderately related ($r = 0.15$–0.16, Crozier *et al.*, 1984; Tay & Crozier, 2000a). Gamergates are highly related and in some of the colonies they are full sisters (Tay & Crozier, 2000a). Moreover, colonies headed by either a single or many gamergates coexist in the same population of *R. aurata* (Komene *et al.*, 1999). These data suggest that, as in the swarm-founding wasps, periodic reductions in gamergate number and joint colony foundation by sister gamergates maintain a moderate relatedness among workers in species of *Rhytidoponera* with large workers.

The Australian greenhead ant *R. metallica* F. Smith, which belongs to the group of *Rhytidoponera* species with small workers, might represent a more puzzling case. As recognized by Hamilton (1972), its presumed breeding system is likely to generate a very low relatedness among nestmates. In *R. metallica,* like in other queenless ants, some of the morphological workers mate and become reproductive gamergates (Haskins & Whelden, 1965; Haskins & Haskins, 1983). However, the number of gamergates per nest is likely to be higher then in the species of *Rhytidoponera* with large workers mentioned above. Haskins & Haskins (1983) mentioned that from 5 to 15% of the females become gamergates in *R. metallica*, and nests of our study population contained around 500 adult females, on average (range from 79 to 1092, mean ± SE = 464 ± 114, $n = 8$, Gartlan, 1999). These data suggest that there might be up to 150 gamergates in a single nest, but it is difficult to obtain direct estimates of gamergate number in the field, as it requires complete excavation of colonies and dissection of all individuals. If the number of gamergates is high, the level of relatedness among nestmates will critically depend on the amount of gene flow among nests. In combination, three factors are likely to result in high gene flow among nests and thus low relatedness among nestmates in *R. metallica*. First, high gene flow may occur through males that can fly between distant nests to mate with new gamergates (Hölldobler & Haskins, 1977). Secondly, if gamergates mate with unrelated males, the amount of gene flow between nests depends on the turnover of gamergates. High turnover of gamergates is expected, because gamergates have short lifespan (Peeters, 1993), and because frequent colony budding requires the production of new gamergates. Thirdly, there might be some movement of workers or gamergates among colonies, as documented in *Rhytidoponera* sp. 12 (Pamilo *et al.*, 1985; Tay *et al.*, 1997).

However, other elements of the breeding system of *R. metallica* may contribute to increase the relatedness among nestmates, in spite of high gamergate number (Hamilton, 1972). First, the relatedness among gamergates may be high if the number of gamergates per colony is periodically reduced to one or a few, as observed in swarm-founding wasps and inferred for two species of *Rhytidoponera* with large workers (Komene *et al.*, 1999; Tay & Crozier, 2000a). Secondly, one or a few gamergates may monopolize reproduction. Thirdly, there is a possibility for inbreeding, because *R. metallica* gamergates mate close to their natal nest (Hölldobler

& Haskins, 1977), and colonies have been kept as closed inbred units in the laboratory for up to 14 years (Haskins & Haskins, 1979, 1983). Fourthly, some population viscosity is expected, because new colonies are obligatorily formed by budding, and dispersal by workers and gamergates is on foot. Thus, female dispersal is restricted, and bud nests should be similar genetically to nearby mother nests, at least transiently. A molecular approach is needed to estimate how these various elements of the breeding system actually affect the relatedness among nestmates, but apart from karyotypes no genetic studies on *R. metallica* have been published so far. We used highly polymorphic microsatellite markers to measure relatedness, inbreeding and population viscosity in *R. metallica*, and make inferences about the breeding system generating the observed genetic pattern.

Methods

Study species, study site and sampling

The greenhead ant *R. metallica* is one of the commonest and most widely distributed ant species in Australia (Brown & Wilson, 1956; Haskins & Whelden, 1965). It occupies a wide variety of habitats throughout temperate and subtropical areas. *Rhytidoponera metallica* nests in the soil and forages individually over the ground. It is a general predator and scavenger, with a flexible diet that also includes seeds. The species is opportunistic and is often particularly abundant in disturbed habitat, for example after fires (Andersen, 1991). Morphological queens are rarely produced, and no functional queenright colonies have been reported in the field, although queens were able to found colonies under laboratory conditions (Ward, 1986).

Our study site was located in the You Yangs regional park, between Melbourne and Geelong in Victoria. *Rhytidoponera metallica* is abundant on the upper part of the granite hills, in areas covered with shrubs and young forest which is regenerating after a wildfire in 1985. Individuals were collected at the nest entrance. This procedure ensured that only workers were collected, as gamergates stay within nests (Peeters, 1997; Tay & Crozier, 2000b). Thirty nests were sampled within a 500 × 200 m area at the beginning of the Flinders Peak and East walks. All nests were mapped with a GPS, compass and metric rope. The distance between sampled nests ranged from 0.5 to 465 m (mean ± SD = 155 ± 117 m). Voucher specimens have been deposited in the Australian National Insect Collection, Canberra.

Genetic markers and statistical analysis

Eight individuals per nest were genotyped at six microsatellite markers, as described previously (see Chapuisat *et al.*, 2000 for markers Rmet7, Rmet10, Rmet12, Rmet15, Rh12-13525, and Tay & Crozier, 2000a for Rh12-1373). With a mean number of alleles per locus of 14.5 and a mean expected heterozygosity of 0.68, this panel of microsatellites has a high power to reveal social structures and fine-scale population genetic structures.

The fixation index F_{it} was calculated with the computer program FSTAT (Goudet, 1995; http://www.unil.ch/izea/softwares/fstat.html). Standard errors were obtained by jack-knifing over nests for the single-locus estimates, and by jack-knifing over loci for the multi-loci estimates. Significant deviation from zero was tested by permuting alleles among nests.

A principal component analysis was performed to describe the relationship between the 30 nests on the basis of their allele frequencies, using the computer program PCAGEN (developed by J. Goudet, http://www.unil.ch/izea/softwares/pcagen.html). Each nest was treated as an independent object and intranest frequencies of each allele formed the variables. To further investigate the significance of the separation in two groups, Nei's genetic distance between pairs of nests and UGPMA clustering of nests were obtained with the computer program PHYLIP (Felsenstein, 1993, http://evolution.genetics.washington.edu/phylip.html). Deviation from Hardy–Weinberg equilibrium was measured with an exact test, the 'probability test' implemented in the computer program GENEPOP (Raymond & Rousset, 1995; http://www.cefe.cnrs-mop.fr/). Linkage disequilibrium was tested with Fisher exact tests, using GENEPOP.

Relatedness among nestmates was calculated with the method of Queller & Goodnight (1989), as implemented in the computer program Relatedness 5.0.4 (http://gsoft.smu.edu/GSoft.html). Nests were weighted equally, standard errors were obtained by jack-knifing over nests, and the significance of the deviation from zero was estimated with one-tailed *t*-tests with degrees of freedom equal to the number of nests minus one. Genetical differentiation between pairs of nests was estimated by calculating pairwise F_{st} using the computer program FSTAT and the significance of the correlation with geographical distances was assessed with a Mantel test.

Results

Two separate gene pools

There was an overall deficit of heterozygotes in the total sample, relative to Hardy–Weinberg equilibrium. The fixation index F_{it} over all markers was 0.053 ± 0.026, which is highly significant when tested by permuting alleles among nests (Table 1). Surprisingly, the deficit of heterozygotes was not consistent across markers. It ranged from –0.017 to 0.382, and was significant for only half of the markers. These three single-locus estimates remained significant after a sequential Bonferroni correction for multiple comparison (Rice, 1989).

Table 1 Fixation index from the total sample (F_{it}). $N = 30$ nests, $n = 240$ individuals.

Locus	F_{it} ± SE
Rmet7	0.056 ± 0.034**
Rmet10	−0.004 ± 0.023
Rmet12	0.053 ± 0.027**
Rmet15	−0.017 ± 0.039
Rh12-1373	0.382 ± 0.127***
Rh12-13525	0.040 ± 0.054
All	0.053 ± 0.026***

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

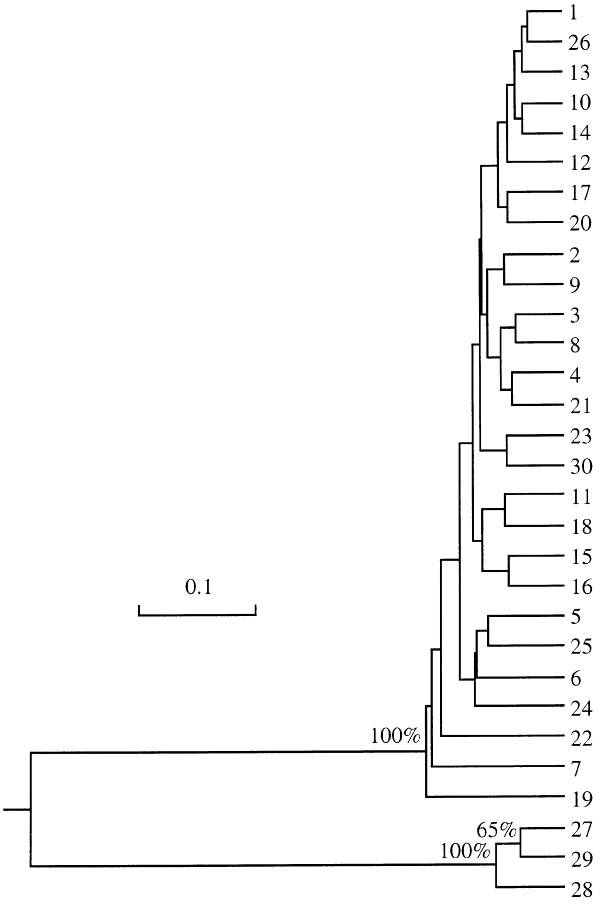

Fig. 2 UGPMA clustering of nests based on Nei's genetic distance among nests. Bootstrap values greater than 45% are indicated.

This discrepancy among markers suggested an anomaly in our data set, and prompted further investigation.

It appeared that our sample was heterogeneous, and contained nests from two separate gene pools that most likely represent sibling species. A principal component analysis on nest allele frequencies revealed that the first principal component explained 41.7% of the variance among the 30 nests and was highly significant ($P < 0.01$). The second principal component accounted for 7.5% of the variance and was not significant ($P = 1.0$). Along the first principal component, nests grouped in two well-separated clusters (Fig. 1). Hereafter, the 27 nests in the first cluster will be referred to as type I nests, and the three nests in the second cluster will be referred to as type II nests. The high genetic differentiation between type I and type II nests appeared when clustering nests by UGPMA on the basis of pairwise Nei's genetic distance (Fig. 2). The genetic distance between type I and II nests was very large (0.74), and the separation in two groups was supported by very high bootstrap values (Fig. 2). Other tree-construction methods gave similar topologies.

The distribution of alleles suggests that the two groups are reproductively isolated. Three lines of evidence indicate that gene flow from type I to type II nests is restricted. First, three alleles from two markers are found in all type I nests, but in none of type II. Secondly, one

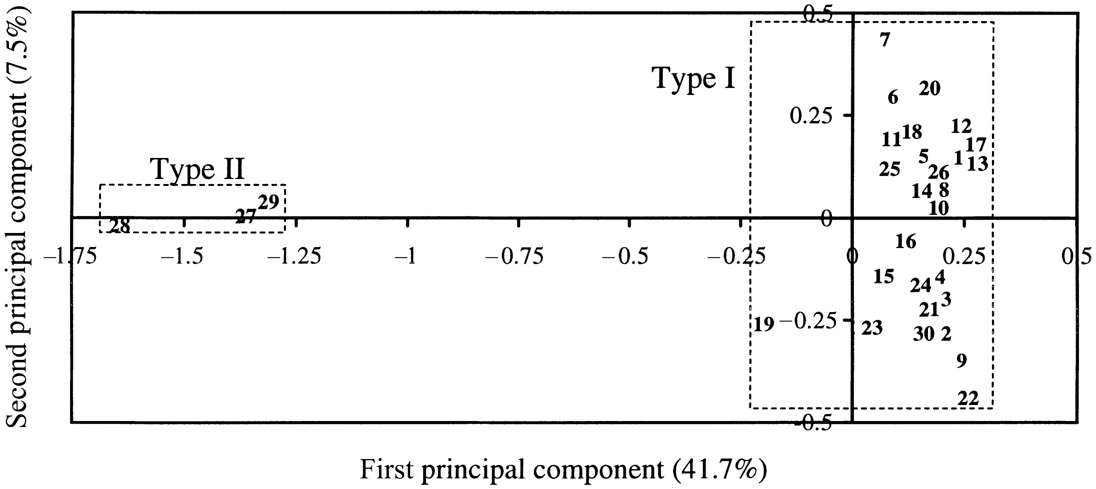

Fig. 1 Principal component analysis based on allele frequencies of the 30 nests (labelled 1–30).

marker is polymorphic in all type I nests, but is fixed in all type II nests. Thirdly, 50 of 87 alleles are found only in type I nests. This last result can partly be explained by unequal sample size. However, the five most frequent private alleles are at frequencies as high as 0.42, 0.31, 0.22, 0.20 and 0.18 in type I nests, strongly suggesting reproductive isolation. Reciprocally, gene flow from type II to type I nests seems restricted as well. Nine of 87 alleles are found only in type II nests, with two of these private alleles being at frequencies as high as 0.40 and 0.27.

If a sample contains individuals coming from two isolated gene pools, both deviation from Hardy–Weinberg equilibrium and linkage disequilibrium are expected. However, the results might be biased if the data set includes multiple individuals from the same nest, because the genotypes of relatives are not independent. Therefore, we resampled one individual per nest one hundred times. First, we considered all 30 nests and generated 100 resampled data sets containing 30 individuals from different nests. Interestingly, both deviation from Hardy–Weinberg equilibrium and linkage disequilibrium were detected, despite the small sample size. Significant deviations from Hardy–Weinberg equilibrium were detected in 87 of the 100 resampled data set, and they were always caused by a heterozygous deficit. Linkage disequilibrium between markers Rh12-1373 and Rh12-13525 was significant in 67 of the 100 resampled data sets. For other pairs of markers, significant disequilibrium was detected in at most 10 of 100 resampled data sets.

Deviation from Hardy–Weinberg equilibrium and linkage disequilibrium totally disappeared when the analysis was restricted to the 27 nests belonging to type I, excluding type II nests. In this second analysis, deviation from Hardy–Weinberg equilibrium was significant in only three of the 100 tests. For any pair of markers, including Rh12-1373 and Rh12-13525, linkage disequilibrium was detected in at most 11 of the 100 resamples. Together, these data demonstrate that type I and type II nests belong to distinct gene pools, and should be analysed separately.

Table 2 Relatedness (r-I) and inbreeding coefficient (F_{it}-I) in *R. metallica* type I. $N = 27$ nests, $n = 216$ individuals.

Locus	r-I ± SE	F_{it}-I ± SE
Rmet7	$0.071 \pm 0.024^{**}$	0.006 ± 0.022
Rmet10	$0.097 \pm 0.030^{**}$	−0.013 ± 0.021
Rmet12	$0.126 \pm 0.032^{**}$	0.041 ± 0.026
Rmet15	0.039 ± 0.029	−0.037 ± 0.039
Rh12-1373	0.039 ± 0.038	−0.006 ± 0.043
Rh12-13525	0.053 ± 0.034	−0.039 ± 0.043
All	$0.082 \pm 0.015^{***}$	−0.004 ± 0.015

$^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

Relatedness, inbreeding and population structure

The relatedness among nestmate workers from *R. metallica* type I was 0.082 ± 0.015 when measured over all loci (Table 2). This relatedness value is very low, but significantly greater than zero. Relatedness estimates from single loci were consistent across loci, and ranged from 0.039 to 0.126. The relatedness within single nests ranged from −0.05 ± 0.08 to 0.24 ± 0.11. Within each nest, between five and 11 alleles were detected at the most polyallelic locus, in only eight individuals. Together, these results indicate that all the type I nests sampled contained multiple reproductives and multiple lineages.

No inbreeding was detected in *R. metallica* type I. The inbreeding coefficient for type I nests (F_{it}-I) was very close to and not significantly different from zero, both for single-locus estimates and over all loci (Table 2).

Surprisingly, no isolation by distance was detected in our sample of *R. metallica* type I (Fig. 3). On average, nests that are just a few metres apart are not more similar genetically than nests separated by several hundreds of metres.

Nests of *R. metallica* type II had a genetic structure very similar to that of type I nests. The relatedness among type II nestmates was also very low (r-II $= 0.037 \pm 0.056$, $N = 3$ nests, $n = 24$ individuals). Because of the small

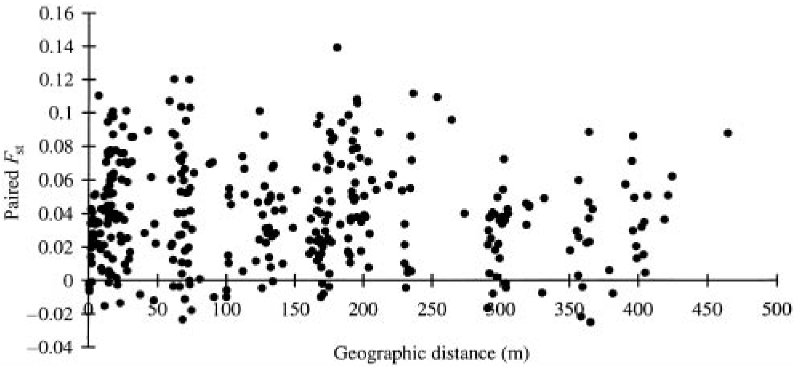

Fig. 3 Correlation between genetic differentiation (F_{st} for pairs of nests) and geographical distance for *R. metallica* type I. Matrix correlation $= -0.06$, $P = 0.68$.

sample size, this estimate has a large standard error, and is not significantly greater than zero. Within each nest, 9–10 alleles were detected at the most polyallelic locus in eight individuals, and this marker had a total of 17 alleles in the three type II nests. These results indicate that type II nests contain many lineages. The inbreeding coefficient for type II nests was very close to and not significantly different from zero (F_{it}-II = –0.003 ± 0.038).

Model

A simple model was used to examine how the number and relatedness of gamergates affect the relatedness among workers. The model assumes that gamergates mate with a single unrelated male, and that they share reproduction equally. These assumptions are reasonable for our study population. First, typing of stored sperm with four microsatellites was consistent with single mating in a sample of seven *R. metallica* gamergates (Gartlan, 1999), and 97% of the gamergates had mated with a single male in *Rhytidoponera* sp. 12 (Tay & Crozier 2001). Secondly, the absence of inbreeding in workers indicates that *R. metallica* gamergates mate with unrelated males. Thirdly, in other queenless ponerine species, gamergates have low rates of egg-laying and tend to share reproduction equally (Ward, 1983; Peeters, 1993, 1997; Tay & Crozier, 2000b).

If n is the number of gamergates and r_{g} is the relatedness among gamergates, then the relatedness among workers r_{w} is given by the following relationship:

$$r_{\text{w}} = \frac{3}{4n} + \frac{n-1}{4n} r_{\text{g}}$$

(Queller, 1993; Ross, 1993, 2001; Chapuisat & Keller, 1999).

If the relatedness among gamergates is identical to the relatedness among workers, as expected at equilibrium when gamergates are adopted back in their natal nest, the above formula simplifies to:

$$r = \frac{3}{3n-1}$$

(Pamilo & Varvio-Aho, 1979; Tay & Crozier, 2000a).

The relatedness among workers for various numbers of gamergates and relatedness among gamergates is shown in Fig. 4. This model can be used to infer the average number and relatedness of gamergates that are compatible with the observed relatedness among workers.

If gamergates are unrelated, a harmonic mean of 9.4 gamergates per nest would yield the observed relatedness among workers. This is the minimum number of gamergates compatible with our data. If gamergates are as related as workers are, which is expected if all gamergates arise as nestmates, the harmonic mean raises to 11.8 gamergates. If gamergates are more related than workers are, the number of gamergates may be very high. Finally, gamergates cannot be more related than 0.32 on average.

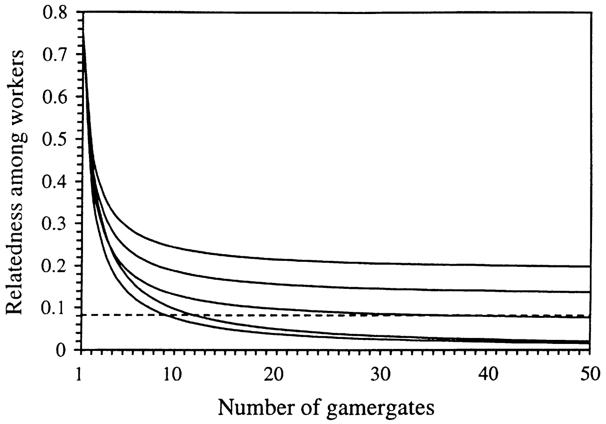

Fig. 4 Relatedness among worker nestmates as a function of gamergate number and relatedness. From top to bottom, solid lines correspond to a relatedness among gamergates of 0.75, 0.5, 0.25, equals to that of workers, and zero. The dashed line corresponds to the observed relatedness among workers in *R. metallica* type I.

Discussion

An unexpected finding of this study was that our sample of '*R. metallica*' contained two isolated gene pools, which presumably represent sibling species. The argument that the two groups are reproductively isolated is bolstered by several facts. First, there was a high genetic distance between the two groups of nests. Secondly, each group of nests contained several private alleles. Thirdly, there were significant Hardy–Weinberg and linkage disequilibria, even when considering the small data sets made by resampling one individual from each of the 30 nests. Hardy–Weinberg and linkage disequilibria disappeared when the largest group of nest was analysed separately. The finding of cryptic sibling species in this widely distributed ant 'species' is not really surprising. Early work in other areas of Victoria revealed extensive chromosomal variation, suggesting that *R. metallica* is in fact a complex of species, with two or possibly three sibling species (Crozier, 1969, 1981). Systematists consider *R. metallica* as a complex with up to nine sibling species (Hanna Reichel, personal communication; Brown, 1958; Ward, 1986). Provisionally, the two groups found in this study are referred to as *R. metallica* type I and II, but more work is needed to resolve the systematics of the *R. metallica* complex.

The main finding of this study is that the relatedness among nestmate workers is very low in *R. metallica* type I (r-I = 0.082 ± 0.015). In combination, the genetic data and the model reveal that there are many gamergates per nest. If gamergates are unrelated and divide up reproduction equally, the effective number of gamergates per nest is nine. If gamergates are related, or if some of the gamergates get a disproportionate share of reproduction, the average number of gamergates can be very high.

These results are in agreement with the few data available from field colonies, which suggest that there are many gamergates per nest (Haskins & Whelden, 1965; Haskins & Haskins, 1983).

The low relatedness among workers indicates that gamergates are not highly related, on average. Single-nest estimates of worker relatedness were below 0.1875 (the minimum value expected if nests are headed by a large number of full sisters) in 25 of 27 nests. This suggests that gamergates might be full siblings in only very few, if any, of the nests. Moreover, none of the single-nest estimates were compatible with the hypothesis that all workers are full sibs, or even half-sibs. Hence, we found no indication that gamergate number varies cyclically with periodic reduction to a single gamergate per nest. Some variation in gamergate number is likely to occur in *R. metallica*. However, it seems much less pronounced than in the larger species of *Rhytidoponera* where gamergates are often full sibling (Ward, 1983; Tay & Crozier, 2000a), or where the population consists of colonies headed by either a single or many gamergates (Komene *et al.*, 1999). With no marked variation in gamergate number, the colony-cycle of *R. metallica* also contrasts with the one of the swarm-founding wasps (Queller *et al.*, 1993).

Lack of population viscosity and absence of inbreeding suggest that workers mate with unrelated males coming from distant nests. This contrasts with many studies of queenright ants showing that polygyny (the presence of multiple queens in the same nest) is associated with restricted dispersal, local mating, and significant population viscosity (Pamilo, 1982, 1983; Sundström, 1993; Herbers & Grieco, 1994; Seppä & Pamilo, 1995; Banschbach & Herbers, 1996; Shoemaker & Ross, 1996; Chapuisat *et al.*, 1997; Ross *et al.*, 1997, 1999; Chapuisat & Keller, 1999; Pedersen & Boomsma, 1999a,b). Population viscosity was also detected in the queenless ant *Rhytidoponera* sp. 12, both with allozymes (Crozier *et al.*, 1984) and mitochondrial markers (Tay *et al.*, 1997). In contrast, gene flow among nests is high enough to homogenize allele frequencies in this local population of *R. metallica*, thus completely erasing the genetic signature of colony fission at nuclear markers. In *R. metallica*, dispersal by workers and gamergates is obligatorily on foot, whereas winged males can fly among nests. Hence, high gene flow among nests is best explained by long-distance dispersal of winged males coupled with high turnover of gamergates and frequent colony fission. High gene flow through males is consistent with ancient field observations of winged males entering foreign colonies after a dispersal flight (W. L. Brown, unpublished observations, reported in Haskins & Whelden, 1965), and with laboratory observations of the mating behaviour of workers (Haskins & Whelden, 1965; Hölldobler & Haskins, 1977). Workers gathered by the nest entrance and released a sex pheromone to attract males, and only males that had flown for about half an hour mated with these workers.

Overall, this breeding system results in very low relatedness among nestmates. To our knowledge, this is the lowest value reported for social insect species where workers have the option to mate and reproduce, and are not trapped in the helper role by their morphology. Lower values of relatedness have been found in some highly polygyne ant populations, for example in *Solenopsis invicta* (Ross *et al.*, 1996; Ross & Shoemaker, 1997; Goodisman & Ross, 1998) and *Linepithema humile* (Kaufmann *et al.*, 1992; Krieger & Keller, 2000). However, workers of these ant species have no spermatheca and no functional ovaries (Bourke & Franks, 1995). They have no other option than helping the queens, and this morphological constraint may account for short-term maintenance of altruism despite low relatedness. This argument does not apply to cooperative breeders with totipotent individuals. Among the cooperative breeders, the low relatedness found in *R. metallica* contrasts with the moderate relatedness detected in other ponerine species with multiple gamergates (Ward, 1983; Crozier *et al.*, 1984; Tay & Crozier, 2000a) and in several species of swarm-founding wasps (Queller *et al.*, 1993; Hastings *et al.*, 1998; Henshaw *et al.*, 2000; Tsuchida *et al.*, 2000).

The maintenance of worker altruism despite low relatedness is surprising. Hamilton (1972) suggested that the social structure of *R. metallica* should be unstable and transient, mentioning that 'the trend in genera like *Rhytidoponera* is degenerate and is probably headed for ultimate extinction'. However, *R. metallica* is one of the most successful and widespread ants in Australia (Haskins & Whelden, 1965). Its breeding system, life history and ecology make it an opportunistic species, adapted to thrive in a wide variety of habitats from desert areas of the interior to open subtropical forest, as well as in urban and disturbed areas (Brown & Wilson, 1956; Andersen, 1991). High number of breeders coupled with colony budding are efficient strategies to face perturbations and catastrophic events, as well as to monopolize patches of habitats. Dependent colony founding should also result in high intrinsic rate of increase of the population, as it decreases generation time (Tsuji & Tsuji, 1996).

Even if the life-history, dispersal and breeding system of *R. metallica* makes it a successful and productive species, explaining the maintenance of worker behaviour remains difficult. Workers gain some indirect fitness benefit by helping to raise related individuals. However, the relatedness in the colony is so low that gamergates must almost invariably get higher fitness than workers do. In our study population, a female is on average 12 times more related to her own offspring than to the offspring of nestmates. Hence, the question arises, what prevents young females from mating and becoming gamergates, rather than remaining unmated workers? One possible answer is that workers or gamergates police young females, preventing them from mating and reproducing. When the relatedness among group members is low, individuals are selected to invest resources in mutual policing, thereby collectively repressing competition and maintaining cooperation (Frank, 1995). In *R. metallica*, both workers and gamergates increase their inclusive fitness if they can limit the number of gamergates by forcing most new females to become workers. The effect is two-fold. First, it contributes to maintain an elevated relatedness among nestmates and confers a high proportion of reproduction to existing gamergates. Secondly, it probably increases colony productivity, because gamergates never forage outside the colony (Peeters, 1997; Tay & Crozier, 2000b).

A second possibility is that mating and reproduction is a lottery, with some workers remaining unmated because of mate unavailability (Peeters, 1993) or other constraints. However, such a lottery is bound to be unstable, and selection should favour more effective mating strategies. This may include increased production and dispersal of males, as well as more efficient attraction mechanisms and longer periods of receptivity by females.

A third possibility is that worker strategy encompasses hidden benefits. Workers may increase their direct fitness by producing males. Males have been produced in laboratory colonies composed of unmated workers of *R. metallica* (Haskins & Whelden, 1965), and 56% of the unmated workers had developed ovaries in one colony of our study population (Gartlan, 1999). However, developed ovaries may also reflect the production of trophic eggs, which has been documented in *Rhytidoponera* ants (Pamilo *et al*., 1985; Tay & Crozier, 2000b). More studies are needed to assess if unmated workers actually produce males under natural conditions, and if they outperform gamergates in doing so.

A final possibility is that workers are waiting for an opportunity to mate and become gamergates at some later stage. Unrelated helpers waiting for a chance of becoming replacement reproductives are common in certain vertebrate societies, and were recently found in paper wasps (Stacey & Koenig, 1990; Solomon & French, 1997; Queller *et al*., 2000). These alternative hypotheses enlighten the diversity of reproductive strategies in the social insects. Further studies are needed to gain a better understanding of the proximate and ultimate factors determining the partitioning of reproduction among morphologically similar individuals in such societies with very low relatedness.

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References

Andersen, A.N. 1991. *The Ants of Southern Australia*. CSIRO, Melbourne.

Banschbach, V.S. & Herbers, J.M. 1996. Complex colony structure in social insects: I. Ecological determinants and genetic consequences. *Evolution* **50**: 285–297.

Bourke, A.F.G. & Franks, N.R. 1995. *Social Evolution in Ants*. Princeton University Press, Princeton, NJ, USA.

Brown, W.L. 1958. Contributions toward a reclassification of the Formicidae. II. Tribe Ectatommini (Hymenoptera). *Bull. Mus. Comp. Zool.* **118**: 173–362.

Brown, W.L. & Wilson, E.O. 1956. Character displacement. *Syst. Zool.* **5**: 49–64.

Chapuisat, M. & Keller, L. 1999. Extended family structure in the ant *Formica paralugubris*: the role of the breeding system. *Behav. Ecol. Sociobiol.* **46**: 405–412.

Chapuisat, M., Goudet, J. & Keller, L. 1997. Microsatellites reveal high population viscosity and limited dispersal in the ant *Formica paralugubris*. *Evolution* **51**: 475–482.

Chapuisat, M., Painter, J.N. & Crozier, R.H. 2000. Microsatellite markers for *Rhytidoponera metallica* and other ponerine ants. *Mol. Ecol.* **9**: 2219–2221.

Crespi, B.J. & Yanega, D. 1995. The definition of eusociality. *Behav. Ecol.* **6**: 109–115.

Crozier, R.H. 1969. Chromosome number polymorphism in an Australian ponerine ant. *Can. J. Genet. Cytol.* **11**: 333–339.

Crozier, R.H. 1981. Genetic aspects of ant evolution. In: *Evolution and Speciation. Essays in Honour of M. J. D. White* (W. R. Atchley & D. C. Woodruff, eds), pp. 356–370. Cambridge University Press, Cambridge.

Crozier, R.H. & Pamilo, P. 1996. *Evolution of Social Insect Colonies: Sex Allocation and Kin Selection*. Oxford University Press, Oxford.

Crozier, R.H., Pamilo, P. & Crozier, Y.C. 1984. Relatedness and microgeographic genetic variation in *Rhytidoponera mayri*, an Australian arid-zone ant. *Behav. Ecol. Sociobiol.* **15**: 143–150.

Felsenstein, J. 1993. *PHYLIP (Phylogeny Inference Package) version 3.5c*. Department of Genetics, University of Washington, Seattle.

Frank, S.A. 1995. Mutual policing and repression of competition in the evolution of cooperative groups. *Nature* **377**: 520–522.

Gartlan, K. 1999. *Genetic influences on polyethism in the ponerine ant Rhytidoponera metallica*. BSc (Honours) Thesis, La Trobe University, Bundoora.

Goodisman, M.A.D. & Ross, K.G. 1998. A test of queen recruitment models using nuclear and mitochondrial markers in the fire ant *Solenopsis invicta*. *Evolution* **52**: 1416–1422.

Goudet, J. 1995. FSTAT (Version 1.2): a computer program to calculate F-statisics. *J. Hered.* **86**: 485–486.

Hamilton, W.D. 1964a. The genetical evolution of social behaviour. I. *J. Theor. Biol.* **7**: 1–16.

Hamilton, W.D. 1964b. The genetical evolution of social behaviour. II. *J. Theor. Biol.* **7**: 17–52.

Hamilton, W.D. 1972. Altruism and related phenomena, mainly in social insects. *Annu. Rev. Ecol. Syst.* **3**: 193–232.

Haskins, C.P. & Haskins, E.F. 1979. Worker compatibilities within and between populations of *Rhytidoponera metallica*. *Psyche* **86**: 299–312.

Haskins, C.P. & Haskins, E.F. 1983. Situation and location-specific factors in the compatibility response in *Rhytidoponera metallica* (Hymenoptera: Formicidae: Ponerinae). *Psyche* **90**: 163–174.

Haskins, C.P. & Whelden, R.M. 1965. 'Queenlessness', worker sibship, and colony versus population structure in the Formicid genus *Rhytidoponera*. *Psyche* **72**: 87–112.

Hastings, M.D., Queller, D.C., Eischen, F. & Strassmann, J.E. 1998. Kin selection, relatedness, and worker control of reproduction in a large-colony epiponine wasp, *Brachygastra mellifica*. *Behav. Ecol.* **9**: 573–581.

Henshaw, M.T., Strassmann, J.E. & Queller, D.C. 2000. The independent origin of a queen number bottleneck that promotes cooperation in the African swarm-founding wasp, *Polybioides tabidus*. *Behav. Ecol. Sociobiol.* **48**: 478–483.

Herbers, J.M. & Grieco, S. 1994. Population structure of *Leptothorax ambiguus*, a facultatively polygynous and polydomous ant species. *J. Evol. Biol.* **7**: 581–598.

Hölldobler, B. & Haskins, C.P. 1977. Sexual calling behavior in primitive ants. *Science* **195**: 793–794.

Kaufmann, B., Boomsma, J.J., Passera, L. & Petersen, K.N. 1992. Relatedness and inbreeding in a French population of the unicolonial ant *Iridomyrmex humilis* (Mayr). *Ins. Soc.* **39**: 195–200.

Komene, Y., Higashi, S., Ito, F. & Miyata, H. 1999. Effect of colony size on the number of gamergates in the queenless ponerine ant *Rhytidoponera aurata*. *Ins. Soc.* **46**: 29–33.

Krieger, M.J.B. & Keller, L. 2000. Mating frequency and genetic structure of the Argentine ant *Linepithema humile*. *Mol. Ecol.* **9**: 119–126.

Liebig, J., Hölldobler, B. & Peeters, C. 1998. Are ant workers capable of colony foundation? *Naturwissenschaften* **85**: 133–135.

Pamilo, P. 1982. Genetic population structure in polygynous *Formica* ants. *Heredity* **48**: 95–106.

Pamilo, P. 1983. Genetic differentiation within subdivided populations of *Formica* ants. *Evolution* **37**: 1010–1022.

Pamilo, P. & Varvio-Aho, S.L. 1979. Genetic structure of nests in the ant *Formica sanguinea*. *Behav. Ecol. Sociobiol.* **6**: 91–98.

Pamilo, P., Crozier, R.H. & Fraser, J. 1985. Internest interactions, nest autonomy, and reproductive specialization in an Australian arid-zone ant, *Rhytidoponera* sp.12. *Psyche* **92**: 217–236.

Pedersen, J.S. & Boomsma, J.J. 1999a. Effect of habitat saturation on the number and turnover of queens in the polygynous ant, *Myrmica sulcinodis*. *J. Evol. Biol.* **12**: 903–917.

Pedersen, J.S. & Boomsma, J.J. 1999b. Positive association of queen number and queen-mating frequency in *Myrmica* ants: a challenge to the genetic-variability hypotheses. *Behav. Ecol. Sociobiol.* **45**: 185–193.

Peeters, C.P. 1991. The occurence of sexual reproduction among ant workers. *Biol. J. Linn. Soc.* **44**: 141–152.

Peeters, C. 1993. Monogyny and polygyny in ponerine ants with or without queens. In: *Queen Number and Sociality in Insects* (L. Keller, ed.), pp. 234–261. Oxford University Press, Oxford.

Peeters, C. 1997. Morphologically 'primitive' ants: comparative review of social characters, and the importance of queen-worker dimorphism. In: *The Evolution of Social Behavior in Insects and Arachnids* (J. C. Choe & B. J. Crespi, eds), pp. 372–391. Cambridge University Press, Cambridge.

Peeters, C.P. & Crewe, R.M. 1984. Insemination controls the reproductive division of labour in a ponerine ant. *Naturwissenschaften* **71**: 50.

Queller, D.C. 1993. Genetic relatedness and its components in polygynous colonies of social insects. In: *Queen Number and Sociality in Insects* (L. Keller, ed.), pp. 132–152. Oxford University Press, Oxford.

Queller, D.C. & Goodnight, K.F. 1989. Estimating relatedness using genetic markers. *Evolution* **242**: 258–275.

Queller, D.C. & Strassmann, J.E. 1998. Kin selection and social insects. *Bioscience* **48**: 165–175.

Queller, D.C., Strassmann, J.E., Solis, C.R., Hughes, C.R. & DeLoach, D.M. 1993. A selfish strategy of social insect workers that promotes social cohesion. *Nature* **365**: 639–641.

Queller, D.C., Zacchi, F., Cervo, R., Turillazzi, S., Henshaw, M.T., Santorelli, L.A. & Strassmann, J.E. 2000. Unrelated helpers in a social insect. *Nature* **405**: 784–787.

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

Rice, W.R. 1989. Analyzing tables of statistical tests. *Evolution* **43**: 223–225.

Ross, K.G. 1993. The breeding system of the fire ant *Solenopsis invicta*: effects on colony genetic structure. *Am. Nat.* **141**: 554–576.

Ross, K.G. 2001. Molecular ecology of social behaviour: analyses of breeding systems and genetic structure. *Mol. Ecol.* **10**: 265–284.

Ross, K.G. & Shoemaker, D.D. 1997. Nuclear and mitochondrial genetic structure in two social forms of the fire ant *Solenopsis invicta*: insights into transitions to an alternate social organization. *Heredity* **78**: 590–602.

Ross, K.G., Vargo, E.L. & Keller, L. 1996. Social evolution in a new environment: the case of introduced fire ants. *Proc. Natl. Acad. Sci. USA* **93**: 3021–3025.

Ross, K.G., Krieger, M.J.B., Shoemaker, D.D., Vargo, E.L. & Keller, L. 1997. Hierarchical analysis of genetic structure in native fire ant populations: results from three classes of molecular markers. *Genetics* **147**: 643–655.

Ross, K.G., Shoemaker, D.D., Krieger, M.J.B., DeHeer, C.J. & Keller, L. 1999. Assessing genetic structure with multiple classes of molecular markers: a case study involving the introduced fire ant *Solenopsis invicta*. *Mol. Biol. Evol.* **16**: 525–543.

Seppä, P. & Pamilo, P. 1995. Gene flow and population viscosity in *Myrmica* ants. *Heredity* **74**: 200–209.

Shoemaker, D.D. & Ross, K.G. 1996. Effects of social organization on gene flow in the fire ant *Solenopsis invicta*. *Nature* **383**: 613–616.

Solomon, N.G. & French, J.A. 1997. *Cooperative Breeding in Mammals*. Cambrige University Press, Cambridge.

Stacey, P.B. & Koenig, W.D. 1990. *Cooperative Breeding in Birds: Long-Term Studies of Ecology and Behavior*. Cambridge University Press, Cambridge.

Sundström, L. 1993. Genetical population structure and sociogenetic organisation in *Formica truncorum*. *Behav. Ecol. Sociobiol.* **33**: 345–354.

Tay, W.T. & Crozier, R.H. 2000a. Microsatellite analysis of gamergate relatedness of the queenless ponerine ant *Rhytidoponera* sp 12. *Ins. Soc.* **47**: 188–192.

Tay, W.T. & Crozier, R.H. 2000b. Nestmate interactions and egg-laying behaviour in the queenless ponerine ant *Rhytidoponera* sp 12. *Ins. Soc.* **47**: 133–140.

Tay, W.T. & Crozier, R.H. 2001. Mating behaviour of *Rhytidoponera* sp 12 ants inferred from microsatellite analysis. *Mol. Ecol.* **10**: 167–173.

Tay, W.T., Cook, J.M., Rowe, D.J. & Crozier, R.H. 1997. Migration between nests in the Australian arid-zone ant *Rhytidoponera sp. 12* revealed by DGGE analyses of mitochondrial DNA. *Mol. Ecol.* **6**: 403–411.

Tsuchida, K., Ito, Y., Katada, S. & Kojima, J. 2000. Genetical and morphological colony structure of the Australian swarm-founding polistine wasp, Ropalidia romandi (Hymenoptera, Vespidae). *Ins. Soc.* **47**: 113–116.

Tsuji, K. & Tsuji, N. 1996. Evolution of life history strategies in ants: variation in queen number and mode of colony founding. *Oikos* **76**: 83–92.

Ward, P.S. 1981. Ecology and life history of the *Rhytidiponera impressa* group (Hymenoptera: Formicidae) II. Colony origin, seasonal cycles, and reproduction. *Psyche* **88**: 109–126.

Ward, P.S. 1983. Genetic relatedness and colony organization in a species complex of ponerine ants. I. Phenotypic and genotypic composition of colonies. *Behav. Ecol. Sociobiol.* **12**: 285–299.

Ward, P.S. 1986. Functional queens in the Australian greenhead ant, *Rhytidoponera metallica* (Hymenoptera: Formicidae). *Psyche* **93**: 1–12.

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