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Colony kin structure, reproductive dominance and colony founding strategies in the ant *Rhytidoponera* sp. 12 (Hymenoptera: Formicidae)

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Abstract

Rhytidoponera sp. 12 A.N.I.C. is a queenless Australian arid zone ant with multiple mated workers (= gamergates) breeding in the colonies. *Rhytidoponera* sp. 12 colonies are classified as Type 1 with full sisters, or Type 2 with related females from overlapping generations as co-existing gamergates. While Type 2 colonies can arise from workers accepting mated nestmates into the natal colony, the origin of Type 1 colonies is more difficult to explain, as no monogynous colony founding stage is known in this species. We applied microsatellites to study colony kin structure and its temporal variation over three sampling years, and used the data to infer colony-founding strategies and reproductive dominance. The average worker relatedness was always significantly higher than zero, but it differed greatly between colonies within each sampling occasion, ranging from approximately zero to 0.34. Furthermore, worker relatedness fluctuated in individual colonies over the sampling period, probably reflecting different stages of colony life cycles. Although gamergates lack dominance behaviour, assumption of some degree of reproductive skew must be invoked to explain the discrepancy between worker nestmate relatedness and the theoretical expectation based on the observed number of gamergates and relatedness among them. The effective (genetic) turnover rate of gamergates was 17% per year. This estimate results probably not only from actual changes in the gamergate pools, but also from changes in the reproductive skew between the gamergates. Incorporating gamergate relatedness estimates with worker nestmate relatedness estimates over multiple years suggested that one alternative for the origin of Type 1 colonies could be colony fission that involves kin-association between gamergates along matrilines.

Key words: *Rhytidoponera* sp. 12, gamergate turn over, reproductive skew, queenless ant, colony fission, temporal variation.

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Introduction

The social biology of social insects is largely shaped by kin selection (HAMILTON 1964a, b, CROZIER 2008), and the colony kin structure and its variation among colonies or populations are often associated with differences in some characteristics of social life. For instance, the queen-worker conflict over resource allocation, sex allocation or male production critically depend on the colony kin structure, with expectations and outcomes depending on the number of queens breeding in the colonies and queen mating frequency (e.g., TRIVERS & HARE 1976, RATNIEKS 1988, BOOMSMA & GRAFEN 1991, CROZIER & PAMILO 1996). Kin structure of social insect colonies is determined by the number and performance of breeding individuals in the colonies and relatedness among them, and it is also influenced by life history characteristics such as the mode of colony founding. Knowledge on colony kin structure, including relatedness patterns and division of reproduction between co-existing breeders, is essential information when studying possibilities and restrictions of kin selection to direct evolution of social insects.

In species with a single once-mated queen (monogyny, monandry), the interpretation of sociogenetic organisation is straightforward (e.g., *Aphaenogaster rudis*, see CROZIER 1974, *Solenopsis invicta*, see ROSS & FLETCHER 1985). However, many species have multiple queens (polygyny) breeding in nests, multiple mating by queens (polyandry), spatially isolated nests remaining socially connected (polydomy) and worker migration / raiding behaviour between nests. These features lead to more complicated sociogenetic structures and low relatedness among worker nestmates. Life history and colony structure are also intimately linked with the ecology of the species concerned (BOURKE & FRANKS 1995). For example, fluctuation in queen numbers in the polygynous red ants *Myrmica rubra* and *M. limanica* have been explained by annual or even seasonal changes through effects of temperature on the size of the worker force (ELMES & PETAL 1990). Similarly, nest boundaries in the polydomous ant *Leptothorax longispinosus* fluctuate seasonally (HERBERS 1984, 1990, HERBERS & STUART 1990), and frequencies of queen death and colony

orphanage are essential in interpreting colony life history in *L. ambiguus* (see HERBERS & GRIECO 1994), *M. sulcinodis* (see PEDERSEN & BOOMSMA 1999) and *Formica selysi* (see CHAPUISAT & al. 2004).

Queens of monogynous ants are often long-lived (e.g., PAMILO 1991) and intra-colonial worker relatedness can remain constant for a long period of time unless there are temporal changes in the sperm use by a polyandrous queen (WIERNASZ & COLE 2010). In polygynous species, on the other hand, the number of queens as well as the levels of queen relatedness may fluctuate as the colony enters different stages of life cycle. Thus, single sampling provides only a snap shot of the kin structure of a given colony. Furthermore, queen turnover (i.e., death of resident and recruitment of new queens), colony fragmentation, and queen and worker migration between nests complicate population and geographical structures (HEINZE 1995) and colony-level characteristics. Because of the close interaction between sociogenetic structure, phase of colony cycle and ecological history, investigation of intra-colonial demography requires multiple temporal samples (BOURKE & FRANKS 1995, CHAPUISAT & al. 2004, ANDRÉ & al. 2006) based on highly informative molecular markers.

Nest sites of the Australian queenless ant *Rhytidoponera* sp. 12 A.N.I.C. (CROZIER & al. 1986) are long lasting, making it a good model to study changes in colony kin structure (CROZIER & al. 1984, PAMILO & al. 1985, CROZIER & PAMILO 1986). Offspring are produced by multiple mated workers (= gamergates, PEETERS & CREWE 1984), even though all workers possess functional ovaries and a spermatheca and are able to mate and reproduce. Allozyme studies (CROZIER & al. 1984, CROZIER & PAMILO 1986) showed that relatedness among worker nestmates was low but significantly larger than zero, although it was suspected that relatedness could fluctuate greatly among colonies (CROZIER & al. 1984). DNA microsatellite studies showed also that co-existing gamergates in *R.* sp. 12 colonies were closely related and that gamergate numbers varied greatly among colonies (TAY & CROZIER 2000a, b). Based on these patterns, colonies were classified into either Type 1, with all gamergates being full sisters, or Type 2, with less related gamergates breeding in nests (TAY & CROZIER 2000a). Finally, allozyme (e.g., CROZIER & al. 1984) and mtDNA studies (TAY & al. 1997) showed that *R.* sp. 12 populations are genetically viscous, indicating that colony founding in *R.* sp. 12 most likely takes place by fission of the parental colonies (CROZIER & al. 1984, PAMILO & al. 1985, PEETERS 1987, 1988).

Our earlier studies inferred the general population structure of *Rhytidoponera* sp. 12 from samples in 1995 and 1996 (TAY & CROZIER 2000a), and worker migration was investigated in 1993 (TAY & al. 1997). However, temporal stability of nestmate relatedness and reproductive dominance were not investigated even though the same colonies were sampled in different years. Here we aim to study the temporal genetic structure of *R.* sp. 12 colonies on the basis of the samples over the three-year period, and to infer gamergate reproductive dominance by calculating reproductive turnover rates within these colonies. *Rhytidoponera* sp. 12 has extremely long-lived colonies (CROZIER & al. 1984, TAY & CROZIER 2000a) due to its idiosyncratic life history patterns, i.e., colony founding involves a group of mated workers accompanied by unmated workers leaving the parental colony; all *R.* sp. 12 workers are able to mate, mating occurs near colony's entrance and mated workers are accepted back to natal colonies to further contribute to colony growth. Here we show that the colony kin structure is not temporally stable. Our findings further show that reproductive dominance among gamergates and gamergate turnover affect worker nestmate relatedness. Type 2 colonies can arise from workers accepting mated nestmates as new breeders, which would lead to gamergates of multiple overlapping generations and lower relatedness breeding in a colony, but the origin of Type 1 colonies is more difficult to explain as no monogynous colony founding stage is known in *R.* sp. 12.

Materials and methods

Sampling and laboratory procedures

Workers were sampled from 17 *Rhytidoponera* sp. 12 colonies from Conservation Paddock Site 1, Fowlers Gap Arid Zone Research Station (31˚ 6' S, 141˚ 44' E), New South Wales, Australia. Same colonies were sampled over three years, in November 1993 (13 colonies sampled), April 1995 (15 colonies) and May 1996 (15 colonies). Eleven colonies were sampled each year, four colonies twice (one of them in the first and last year), and two colonies only once (Tab. 1). Samples were stored in absolute ethanol and then stored in -20˚C until needed. We also excavated five of the colonies and collected all the gamergates found at the end of the study.

We analysed usually 20 workers (range 15 - 20, total 843, Tab. 1) and all gamergates found (range 7 - 62, total 112, Tab. 2) from each nest by using five DNA microsatellite loci: Rh12-07032, Rh12-13073, Rh12-13579, Rh12-06075 and Rh12-12003 (TAY & CROZIER 2000a). The identity of gamergates was confirmed by the presence of sperm-filled spermathecae, the presence of yellow bodies at the basal of nurse-cells in the ovaries, and advanced oocytes (TAY & CROZIER 2000b, see also PAMILO & al. 1985, PEETERS 1987). Details of DNA extraction, PCR, as well as separation of PCR products on 6% polyacrylamide denaturing gels are given elsewhere (TAY & CROZIER 2000a, 2001).

Statistical methods

Relatedness and inbreeding. Worker nestmate relatedness (r_{ww}) for the whole population and for individual colonies, as well as inbreeding coefficient (F) were estimated using the program RELATEDNESS 4.2c (QUELLER & GOODNIGHT 1989). Standard errors associated with the estimates were obtained by jackknifing over loci. As RELATEDNESS 4.2c limits the number of alleles in each locus to 25 alleles, we grouped four alleles with the smallest frequency in the most variable locus (Rh12-07032, 28 alleles detected) prior to calculations. Relatedness patterns were previously assessed for the samples of the two last years of the study (TAY & CROZIER 2000a, 2001), and here we re-analyse the data to also include samples from the 1993 sampling period.

The effective number of gamergates and reproductive dominance. We estimated the effective number of breeding gamergates (N_e) and the extent of reproductive dominance by using information from the five excavated study colonies (TAY & CROZIER 2000a). The effective number of breeding gamergates refers to the number of gamergates needed to account for the observed genetic relatedness among the offspring generation, assuming equal repro-

Tab. 1: Worker nestmate relatedness (r_{ww} ± standard error) in individual nests and in the whole sample. Average population inbreeding (F) levels in all three sample years were also estimated. Deviations from zero were tested using two-tailed t-tests, P indicates significant differences from zero: n.s.: $P > 0.05$; *: $P < 0.05$; **: $P < 0.01$. Sample size (n) is given as the number of individuals for each nest, and number of nests for each year. Colonies marked with an asterisk (*) were excavated and sampled for gamergates (TAY & CROZIER 2000a, 2001).

Colony	1993			1995			1996		
	n	r_{ww}	*P*	*n*	r_{ww}	*P*	*n*	r_{ww}	*P*
065*				20	0.268 ± 0.061	*			
066	20	0.091 ± 0.017	*	20	0.145 ± 0.034	*	20	0.069 ± 0.019	*
068	20	0.019 ± 0.008	n.s.	20	0.055 ± 0.034	n.s.	20	0.119 ± 0.056	n.s.
069	20	0.079 ± 0.045	n.s.	19	0.112 ± 0.045	n.s.	20	0.124 ± 0.021	**
070	20	0.279 ± 0.037	**				20	0.119 ± 0.014	**
072	20	0.340 ± 0.075	*	20	0.319 ± 0.059	*	19	0.221 ± 0.081	n.s.
074*	20	0.279 ± 0.096	n.s.	20	0.250 ± 0.055	*	20	0.271 ± 0.049	*
078	20	0.013 ± 0.028	n.s.	20	0.107 ± 0.046	n.s.	20	0.105 ± 0.030	*
080*	20	0.197 ± 0.049	*	20	0.217 ± 0.020	**	15	0.261 ± 0.054	*
081							19	0.071 ± 0.026	n.s.
094				20	0.093 ± 0.053	n.s.	20	0.146 ± 0.048	n.s.
097				20	0.085 ± 0.040	n.s.	20	0.218 ± 0.056	*
099*	18	0.039 ± 0.036	n.s.	20	0.045 ± 0.047	n.s.	20	0.182 ± 0.098	n.s.
100	20	0.292 ± 0.060	*	19	0.175 ± 0.046	*	19	0.079 ± 0.032	n.s.
101*	20	0.041 ± 0.038	n.s.	20	0.100 ± 0.048	n.s.	20	0.045 ± 0.025	n.s.
112	20	0.063 ± 0.014	*	20	0.098 ± 0.038	n.s.	15	0.206 ± 0.064	*
154	20	0.137 ± 0.046	n.s.	20	0.070 ± 0.037	n.s.			
Mean	13	0.142 ± 0.011	< 0.001	15	0.144 ± 0.007	< 0.001	15	0.149 ± 0.017	< 0.002
F		-0.042 ± 0.016	*		0.008 ± 0.010	n.s.		0.019 ± 0.020	n.s.

Tab. 2: The observed (r_{ww}) and expected (Type 1: r_{EXP-1}, Equation 2; Type 2: r_{EXP-2}, Equation 3) worker nestmate relatedness assuming equal reproductive shares of coexisting gamergates. $r_{ww-MEAN}$ is the simulated relatedness value of r_{EXP-1} and its upper and lower 95% confidence intervals (CI) for the Type 1 colonies. P_{EQUAL} is the reproductive share of each gamergate (= 1 / #G, #G is the observed number of gamergates in colonies), $P_{DOMINANT}$ is the reproductive share of the putative dominant egg-layer required to attain the observed worker nestmate relatedness (r_{ww}) in colonies (n.a. indicates that the observed relatedness is smaller than predicted by no dominance). Note: Type 1 and Type 2 colony classifications are based on gamergates being related (rG) as full-sisters or as significantly related but not as full sisters (TAY & CROZIER 2000a).

Colony	#G	r_G	r_{ww}	$r_{EXP-1/2}$	$r_{ww-MEAN}$	95% CI Lower	95% CI Upper	P_{EQUAL}	$P_{DOMINANT}$
Type 1									
065	14	0.77 ± 0.10	0.268	0.23	0.224	0.208	0.247	0.071	0.33
074	16	0.75 ± 0.06	0.271	0.22	0.229	0.211	0.256	0.063	0.35
080	7	0.75 ± 0.08	0.261	0.27	0.259	0.230	0.300	0.143	n.a.
Type 2									
099	13	0.37 ± 0.07	0.182	0.075	–	–	–	0.077	0.4
101	62	0.18 ± 0.03	0.050	0.016	–	–	–	0.016	0.2

Tab. 3: The observed (h_o) and expected (h_e) levels of heterozygosity, as well as numbers of alleles detected in the *Rhytidoponera* sp. 12 populations during 1993, 1995 and 1996 using five *R.* sp. 12 microsatellite loci.

Locus	1993			1994			1996		
	h_o	h_e	Alleles	h_o	h_e	Alleles	h_o	h_e	Alleles
Rh12-07032	0.9140	0.9214	28	0.9097	0.8761	26	0.9349	0.9002	25
Rh12-13073	0.8924	0.9025	24	0.8836	0.8815	22	0.8730	0.8852	20
Rh12-06075	0.8952	0.8652	15	0.8520	0.8583	14	0.8860	0.8810	16
Rh12-13579	0.8726	0.8515	13	0.8645	0.8221	9	0.8632	0.8060	11
Rh12-12003	0.7468	0.6841	8	0.7204	0.7060	8	0.6319	0.6590	7

duction by gamergates and random mating. We estimated N_e separately for Type 1 and 2 colonies as:

$$N_e = \frac{4r_s - r_q - 2r_{m1}}{4r_f - r_q - 2r_{m1}} \quad (1)$$

(ROSS 1993, SEPPÄ 1994). From previous work we know that almost all *Rhytidoponera* sp. 12 gamergates mate just once (the effective number of mating $M_e = 1.02$), relatedness among sisters being $r_s = 0.50 / M_e + 0.25 = 0.74$, and that relatedness among males breeding in the same colony is $r_{m1} = 0.032$ (TAY & CROZIER 2001). Furthermore, the average relatedness among nestmate gamergates (r_q) has been estimated as $r_q = 0.76$ in Type 1 and $r_q = 0.27$ in Type 2 (TAY & CROZIER 2000a). Averages of relatedness estimates between nestmate workers (r_f) were entered in the equation separately for Type 1 and Type 2 colonies to obtain N_e.

In Type 1 colonies (gamergates full sisters), the expected worker nestmate relatedness (r_{EXP}) is:

$$r_{EXP} = \frac{3}{16} \times \left[\frac{3nP^2 - 6P + n + 2}{n - 1} \right] \quad (2)$$

and in Type 2 colonies (gamergates less related):

$$r_{EXP} = 3 - \frac{9(n-1)}{nP^2 - 2P + 3n - 2} \quad (3)$$

where n is the number of gamergates breeding in the colony, and P is the proportion of daughter workers produced by one dominant gamergate (PAMILO & VARVIO-AHO 1979; note the typing error in the form given in the original paper for equation 2). If the gamergates share reproduction equally, equations (2) and (3) give the expected worker nestmate relatednesses when setting $P = 1/n$. However, these equations can also be used to estimate what fraction the dominant gamergate must produce in order to obtain the observed worker nestmate relatedness. This is done by substituting r_{EXP} with the observed worker nestmate relatedness (r_{ww}) and solving the equations for P by using the observed gamergate numbers (n). This assumes that the remaining reproduction is shared equally by the rest of gamergates, with a share $(1 - P) / (n - 1)$ for each.

In reality, the representation of the maternal lineages in the samples varies stochastically and frequencies of the full-sister groups in small samples depart from the actual proportions in the colony. To explore the effects of such sampling errors, we simulated sampling from Type 1 colonies by using a program "SampRel" (written by Ross H. Crozier) with 1,000 iterations. Simulation results (mean and confidence intervals) were used to test whether the observed worker nestmate relatedness (*r*ww) agrees with the expected worker nestmate relatedness. Sample sizes in the simulations were the same as in the actual genetic analyses (Tab. 1).

Gamergate turnover. We estimated gamergate turnover rate (t) in individual colonies as:

$$t = 1 - \frac{r_{w1-w2}}{r_{w1} + r_{w2} - r_{w1-w2}} \quad (4)$$

where r_{W1}, and r_{W2} are worker nestmate relatednesses within two consecutive cohorts W1 and W2, and $r_{W1\text{-}W2}$ is the relatedness between these two cohorts (PEDERSEN & BOOMSMA 1999). We used all temporal samples to calculate turnover estimates (i.e., 1993 to 1995, 1993 to 1996 and 1995 to 1996), and used them to calculate an average annual turnover rate for each colony.

Results

The observed and expected heterozygosity for the five microsatellite DNA loci used in this study, as well as the number of alleles detected for each locus over the three sampling periods are presented in Table 3. Across populations inbreeding coefficients (F) were not significantly different from zero for 1995 and 1996 but significantly smaller than zero for 1993 (Tab. 1). This is in agreement with analyses of sperm present in gamergates' spermathecae that also suggested that gamergates and males are in general not related (TAY & CROZIER 2001).

In all three years, the average worker nestmate relatedness was low but significantly different from zero (Tab. 1). Over the sampling period, worker nestmate relatedness in individual colonies was highly variable (Tab. 1, Fig. 1). In some colonies it remained relatively stable (e.g., colonies 066, 101), but in many colonies it increased consistently from initially low values (even close to zero) (e.g., 078, 112) or decreased from higher values (e.g., 072, 100). The long-term change indicated by the relatedness difference between the first and the last sample was significant in four colonies (070, 078, 100, 112), and remained statistically significant in two colonies (070 and 100, Fig. 1) after correcting for multiple tests. The effective number of gamergates (N_e, Equation 1) was smaller in Type 1 colonies (N_e = 9.0) than in Type 2 colonies (N_e = 22.3). These estimates of N_e were within the range of the counted numbers of gamergates, though on average somewhat smaller (Tab. 2). The small number of colonies prevented a critical comparison.

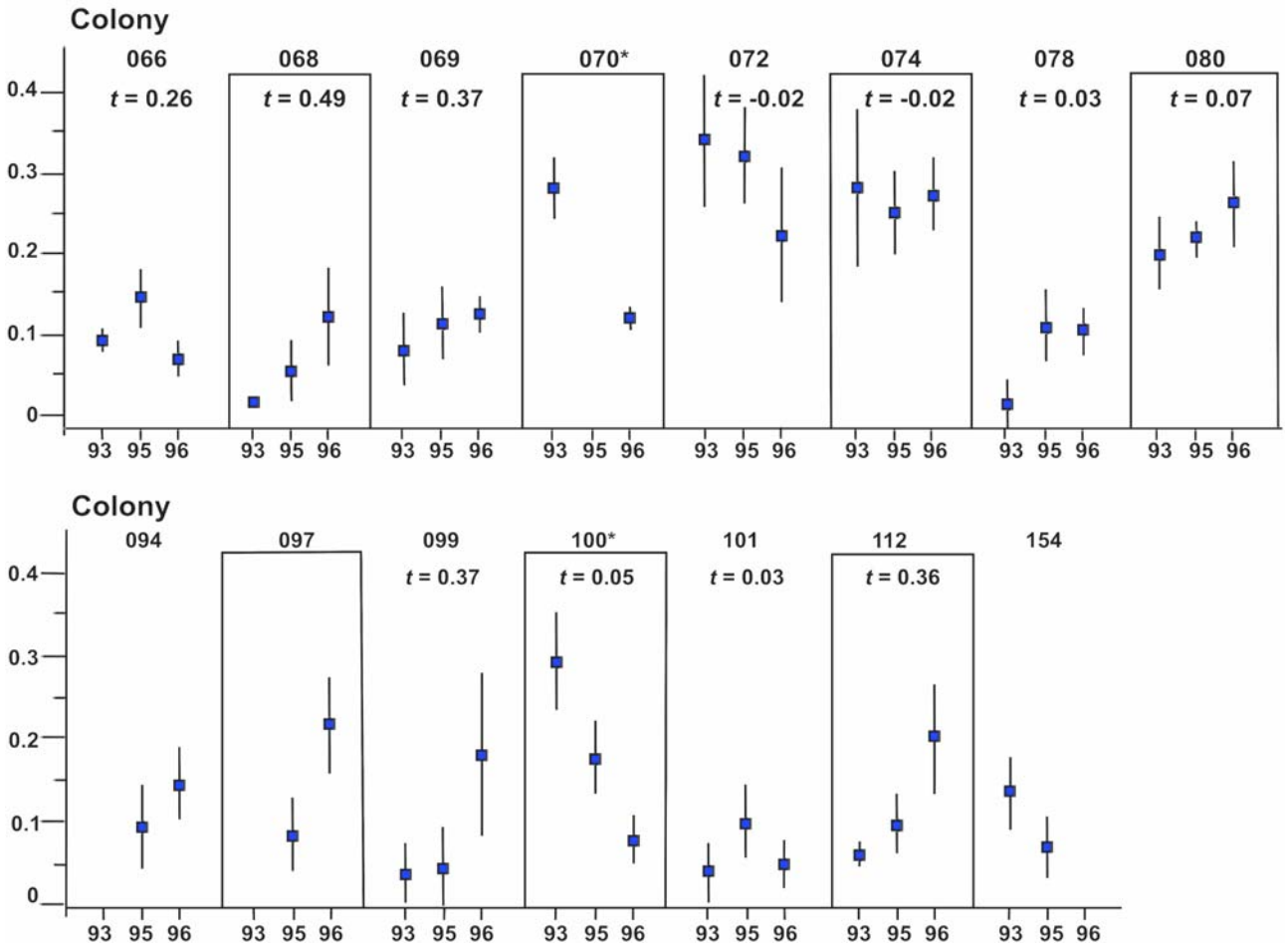

Fig. 1: Worker nestmate relatedness (r_{ww} ± standard error, Y-axes) over different sampling years (X-axes). Colonies 065 and 081 (sampled only once) are excluded. Average annual gamergate turnover rates (*t*, Equation 4) are given for those nests where relatedness for all three years was available. Two colonies (070, 100) have significant temporal relatedness variation after Bonferroni correction for multiple tests and are here indicated by "*".

The observed worker nestmate relatedness in two Type 1 colonies (065, 074) was significantly higher than the expected relatedness based on the gamergate number (r_{EXP-1}) and assuming equal reproduction by them, whether the expected value was calculated with Equation 2 or simulated by random sampling of full-sister groups (Tab. 2). Because the observed relatedness in these two colonies was larger than the upper 95% limit among the simulated values (Tab. 2), the result indicates unequal shares of reproduction in these colonies. The results are consistent with one gamergate producing about one third of the offspring. In the third Type 1 colony (080) with seven gamergates, the observed genetic relatedness agrees with the assumption of no reproductive skew among the gamergates (Tab. 2). In Type 2 colonies the observed nestmate relatedness was clearly higher than that expected with equal reproduction among the gamergates, and suggests considerable reproductive skew, especially in colony 099 (Tab. 2).

We estimated the turnover rates only for colonies with data from all three years. The average turnover of gamergates (Equation 4) in the colonies was 17%. However, turnover was highly variable across colonies, with several colonies showing no turnover at all and some with turnover rates approaching 50% (Fig. 1).

Discussion

Worker nestmate relatedness and the effective number of gamergates

The average worker nestmate relatedness remained similar throughout the study period and was also similar to the estimates in previous allozyme studies on *Rhytidoponera* sp. 12 made at the same site (CROZIER & al. 1984, CROZIER & PAMILO 1986). Thus, the average colony kin structure has remained remarkably constant in the population over long period of time. However, variation of single-colony relatedness was extensive among colonies within a year and among years within a single colony. The largest and significant changes in relatedness were the drops from *r* close to 0.30 in 1993 to less than or close to 0.1 in 1996 in colonies 070 and 100. Large variation in single-colony estimates at any given time is common and inevitable due to variation in the level of polygyny and also to some extent to sampling-error effects. Temporal changes of relatedness have been rarely monitored in same field colonies in polygynous ants. Fluctuation of relatedness can result from: (I) recruitment of new gamergates, (II) changes in reproductive dominance among coexisting gamergates, (III) changes in the composition of gamergate pool (i.e., gamergate turnover), (IV)

changes in the gamergate pool following colony fission, and / or (V) incomplete sampling of worker cohorts. We will discuss these in the end of the article.

A major factor contributing to low worker nestmate relatedness is the presence of multiple egg-layers in the nest (e.g., CRAIG & CROZIER 1979, PAMILO 1982, SEPPÄ 1992, 1994, HEINZE 1995), which is true for *Rhytidoponera* sp. 12 as well. In this species, gamergates mate almost always just once (TAY & CROZIER 2001) and mating seems to be random (i.e., mating males and females are generally unrelated). The only factor besides multiple egg-layers introducing genetic heterogeneity in the colonies is occasional migration of workers between colonies (e.g., TAY & al. 1997). Temporal variation in worker nestmate relatedness has been reported also in some other ants (*Lasius flavus*, see BOOMSMA & al. 1993), but not in all studies (*Myrmica tahoensis*, see EVANS 1996). Relatedness fluctuation in *L. flavus* samples was attributed to the tendency of both workers and gynes belonging to the same matriline clumping together, either in time or in space (BOOMSMA & al. 1993).

In *Rhytidoponera* sp. 12, the effective number of gamergates was more than two times greater in Type 2 than in Type 1 colonies. The effective numbers were smaller than the average observed numbers of gamergates in the colonies, suggesting some reproductive dominance among the gamergates, especially within Type 2 colonies. The difference in the effective gamergate numbers in the two types reflects the life history stage of the colonies, with Type 2 colonies having multiple generations of gamergates with lower relatedness levels than Type 1 colonies (TAY & CROZIER 2000a). Even though the nestmate relatedness in the present study was very similar to that detected earlier in an allozyme study (CROZIER & al. 1984), the previous study suggested a smaller number of egg-laying gamergates per colony. This difference arises, because the estimates by CROZIER & al. (1984) assumed a uniform colony type with no reproductive skew. CROZIER & al. (1984) also assumed that gamergates are as related as workers ($r = 0.19$) and this gave an estimate of approximately six gamergates. The highly polymorphic microsatellite markers allowed more accurate estimates of the colony kin structure.

Reproductive dominance

For the five colonies with known gamergate numbers (TAY & CROZIER 2000a), the expected worker nestmate relatedness (r_{EXP}) based on the number of gamergates was consistently lower than the observed relatedness in both types of colonies (Tab. 2). This suggests some level of reproductive skew among the gamergates. The skew could be explained with reproductive dominance by a single gamergate. This assumption would imply that the dominant gamergate should produce about one third (from 20% to 40%) of the offspring (Tab. 2). Her reproductive output would thus need to be 6.5 - 15 times higher than the average of the others (6.5 - 8 times higher in Type 1 colonies and 8 - 15 times higher in Type 2). It should also be noted that the genetic relatedness in colony 080 (Type 1) agreed well with assumption of equal reproduction by the sister gamergates. The results indicate that skew could be smaller when the gamergates are full sisters, but reliable estimates of reproductive dominance are difficult to obtain because of possible temporal changes in the gamergate pool (and consequently in the proportions of full-sister groups among workers).

Reproductive dominance among gamergates has been found in various ants, implemented by behavioural interactions (e.g., NONACS 1992, HEINZE 1995, 1996), inhibition of ovarian development and sex-attractant production in the presence of gamergates (e.g., *Streblognathus aethiopicus*, see WARE & al. 1990, *Pachycondyla sublaevis*, see ITO & HIGASHI 1991) and social mutilation (MONNIN & RATNIEKS 2001). These indicate either reproductive control by the gamergates or an adaptive response and self-restraint by the workers (CROZIER 1992, KELLER & NONACS 1993, HEINZE & al. 1994). To attain partial or complete reproductive dominance is energetically costly, and a female may risk being injured, killed or expelled from the colony (HEINZE & al. 1992). However, pheromone production by gamergates or mutilation of potential new gamergates by the dominant gamergate may be the preferred method in queenless ants (PEETERS & HIGASHI 1989, WARE & al. 1990, PEETERS & al. 1991, 1992).

Reproductive dominance among gamergates has also been suggested in *Rhytidoponera chalybaea* and *R. cofusa*, but its exact mechanism is not known and its effect diminishes over time if the number of gamergates increases within colonies (WARD 1983). In *R.* sp. 12, gamergates are not aggressive towards each other in laboratory colonies and tolerate each other's presence (PEETERS 1987, TAY & CROZIER 2000b). Thus, gamergates probably suppress the ovarian development in unmated workers by pheromone inhibition or through physical interactions (TAY & CROZIER 2000b) leading to dominant egg-laying behaviour, as in *Diacamma ceylonense* (CUVILLIER-HOT & al. 2002, 2005).

Gamergate turnover

Turnover rate, i.e., a decrease in relatedness between consecutive age cohorts compared to within-cohort relatedness, is caused either by an actual change in the breeder composition of the colony, but can also be a result of a dominance change across cohorts (i.e., a change in reproductive shares of same females breeding in the colony during a longer time). Changes in the reproductive skew between generations lead to an increase in the turnover rate, and the level of relatedness within a generation can remain stable or go up or down, depending on the way the skew changes. Turnover associated to removal of old reproductives increases the relatedness, while recruitment of new reproductives decreases relatedness. The effect on the relatedness between successive generations, and thus on the turnover rate, depends also on the level of gamergate relatedness. If they are closely related and newly recruited gamergates are daughters of some of them, the turnover rate is low because the genetic change in the pool of reproductives is small. If the relatedness among the gamergates is initially low, recruitment of new gamergates from the pool of their daughters has a large effect on the turnover rate. The turnover rate thus reflects the genetic change rather than the change in the actual number of reproductives.

The observed patterns of annual turnover rate and relatedness change could be grouped into five combinations (Tab. 4). As explained above, the turnover associated with an increase of relatedness can result from an increased skew among the existing reproductives, from a reduced number of them, or from recruitment of new reproductives that are closely related. If the initial gamergates were highly related, the turnover rate and the increase in related-

Tab. 4: Combinations of annual turnover rates and relatedness trends in the study colonies, and how they can be explained in terms of the actual gamergate turnover or reproductive dominance (Skew). Figures on the bottom line of each definition are colonies that comply with each definition. Reproductive dominance cannot explain a combination of increasing relatedness and no / low turnover.

	Explained by	**Increasing relatedness**	**Decreasing relatedness**	**No change in relatedness**
High *t*	Turnover	#Egg-layers decreases from Y1 to Y2, or new egg-layers are recruited preferentially from one family	#Egg-layers increases from Y1 to Y2, new egg-layers come from different families randomly	#Egg-layers stable, new egg-layers recruited from a new sibship
	Skew	Increasing skew from Y1 to Y2	Decreasing skew from Y1 to Y2	Similar skew in Y1 and Y2, but the dominant gamergates change
Colonies		068, 069, 099, 112	Not found	066
No / low *t*	Turnover	#Egg-layers decreases from Y1 to Y2, egg-layers are closely related	#Egg-layers increases from Y1 to Y2, egg-layers are closely related	#Egg-layers stable, same egg-layers in Y1 and Y2
	Skew	Slight increase in skew from Y1 to Y2	Slight decrease in skew from Y1 to Y2	No change in skew
Colonies		078, 080	072, 100	074, 101

ness remain low. A high turnover rate would require large changes in the reproductive pool, and it seems more likely that this could be a result of changing skew rather than by the process of death-and-recruitment, but it is difficult to separate the alternatives in our data. All Type 1 colonies had low turnover rates (Fig. 1) as expected when the reproductives are highly related. Type 2 nests showed both high and low turnover rates, and increased and stable relatedness (Tab. 4). These patterns could have been produced either by the actual turnover of gamergates or by changes in skew. Both alternatives make biological sense: The maximum life span of the gamergates is probably short enough that some turnover must have taken place during the study period, and there are potentially more conflicts and dominance changes between less-related gamergates than there would be between full-sisters.

Several factors can contribute to the observed temporal relatedness patterns in *Rhytidoponera* sp. 12 colonies (Fig. 1). Recruitment of gamergates is expected to lead to a decrease in worker nestmate relatedness, as observed in some of our study colonies (072, 100), but it can also result from decreasing reproductive skew among resident gamergates. Decreasing relatedness was coupled with no (or low) turnover rate in our colonies (Fig. 1, Tab. 4), and this agrees with expectation when the colony recruits own daughters as new reproductives. Relatedness decreases somewhat, but the new recruits are also related to the old gamergates and the genetic turnover remains low. Relatedness increase coupled with high turnover (colonies 068, 069, 099, 112) can be explained by replacing old gamergates with a new group of highly related gamergates or by drastically reducing the number of reproductives. These alternatives would indicate a short life-span of gamergates. Thus, increasing reproductive skew among resident gamergates is a more plausible explanation for this combination. This is likely as our genetic analysis (combination of the number of gamergates and the relatedness among workers) showed that some degree of reproductive skew must be invoked to explain the observed worker nestmate relatedness.

Colony founding and the dynamic social structure in *Rhytidoponera* sp. 12

Since originally noted by BROWN (1958), there have been no direct observations and very little other information on colony founding in *Rhytidoponera* sp. 12. Colony fission is likely as worker carrying, a common behaviour during colony budding, has been reported by PAMILO & al. (1985). Since gamergates are wingless and have low fecundity (PEETERS 1987, TAY & CROZIER 2000b), and independent colony foundation has high ecological constraints (i.e., high predation risks, high temperature of the arid zone, effects of drought), it seems highly unlikely that gamergates are able to successfully found colonies independently. Thus, colony founding must generally take place dependently, with one or more gamergates leaving the parental nest accompanied by unmated workers. Low fecundity of gamergates (PEETERS 1987, TAY & CROZIER 2000b) suggests that in an incipient colony a single gamergate is most likely not able to lay sufficient amount of eggs to build up the necessary worker population and the production of new gamergates. This requires further study, however, but such colonies have not been observed in *R.* sp. 12 so far (CROZIER & al. 1984, TAY & CROZIER 2000a).

Assuming that new colonies are always founded dependently by dividing old ones, how do the two social types arise in the first place? The origin of Type 2 colonies (many gamergates with relatively low relatedness) can be explained by workers accepting mated nestmates as new breeders in established colonies. This is expected to lead to accumulation of egg-layers from multiple overlapping generations breeding in the colonies and an inevitable decrease of relatedness as new gamergates mate with unrelated males. This system resembles another ant with wingless queens, *Proformica longiseta*, which also lacks means for long-

range dispersal and independent colony founding (FERNÁNDEZ-ESCUDERO & TINAUT 1999, FERNÁNDEZ-ESCUDERO & al. 2001). Furthermore, queen accumulation and gradual decrease in worker nestmate relatedness with the age of the colony probably occurs in many polygynous ants, such as in polygynous mound-building *Formica* (see SUNDSTRÖM & al. 2005).

Type 1 colonies (colonies with few full-sister gamergates) could be derived via an intermediate state with a single gamergate, via strong reproductive dominance by one gamergate, or by elimination of gamergates which do not belong to this full-sister group. The alternative including colony founding with a single gamergate, and the recruitment of her daughters as new gamergates would imply that Type 1 colonies are young. However, such colony-founding stage is not known in *Rhytidoponera* sp. 12. Alternatively, Type 1 colonies could arise through a bottleneck in which only one of the gamergates in a colony survives. Even though this might happen occasionally, the low fecundity of gamergates makes this alternative unlikely as a common phenomenon. Extreme reproductive skew, where one queen / gamergate reproduces all the next-generation queens / gamergates while other co-habiting queens / gamergates reproduce no female sexuals (i.e., only produced sons) has not been reported in multi-queen eusocial hymenopteran species, although KÜMMERLI & KELLER (2007) reported extreme reproductive specialisation in *Formica exsecta* involving production of males and workers. Finally, Type 1 colonies could arise through selected recruitment of gamergates from one full-sib family or elimination of gamergates representing other families. The preferential representation of one full-sib group among the gamergates would require some type of kin discrimination within the colony or at the time of colony fission.

Non-random association of gamergates along kin lines during fission of a Type 2 colony could result in a Type 1 colony, as recently suggested in *Proformica longiseta* (see SEPPÄ & al. 2008). Social groups usually fission when they grow to a larger than optimal size (LEFEBVRE & al. 2003), and low worker nestmate relatedness associates with large gamergate and worker populations in some of Type 2 colonies (e.g., colony 101; TAY & CROZIER 2000a, b). Fission of a colony is likely to occur when the numbers of gamergates and / or workers have become large enough to be successfully divided between the mother colony and a daughter colony. With the increased number of gamergates, intra-colony relatedness has decreased, a process suggested by CROZIER (1979). Using the observed sizes of the full-sib groups among the gamergates (Tab. 2, TAY & CROZIER 2000a), it seems difficult for a colony to switch directly from Type 2 to Type 1. For example, if we sample seven gamergates (the smallest gamergate number observed by us in Type 1 colonies) from the gamergate pool of a Type 2 colony (e.g., 101, with several full-sib groups of gamergates) and use combinatorics, the probability of getting only full sisters is very small with $P \approx 0.0001$. A direct transition from Type 2 to Type 1 is thus unlikely, at least on the basis of the colony data we have. Whether colony cycles of queenless ants with gamergates are really characterized by cyclical fluctuation of the number and relatedness of gamergates associated with colony fission remains to be studied. The queen number has been shown to vary cyclically in the ant *Leptothorax acervorum* (cyclical monogyny, HEINZE & al. 1995), and some epiponine wasps are characterized by regular changes in queen numbers (cyclical oligogyny, STRASSMANN & al. 1992, HUGHES & al. 1993, STRASSMANN & al. 1997). Such cycles are expected to result in fluctuating patterns of worker nestmate relatedness. Similar relatedness effects can be expected from sporadic queen production, as in *Formica exsecta* (see BROWN & KELLER 2002) and *Myrmica sulcinodis* (see ELMES 1987), or regular queen execution in *Linepithema humile* (see KELLER & al. 1989).

The present results show that the kin structure of a single colony can change in various ways over years, and that the colonies must occasionally also switch between the Types 1 and 2 with unknown intermediate stages. Whether the origin of Type 1 colonies involves a step where gamergates preferentially associate with their full sisters, remains to be tested. Whereas many social insects are known to recognize nestmates (and discriminate non-nestmates), kin discrimination within a single society has not been demonstrated (and may not even be expected). Kin association in Hymenoptera deserves further investigation, and could potentially play an important role during *Rhytidoponera* sp. 12 colony-founding stages. CROZIER & DIX (1979) in their analysis of Gestalt versus Individualistic genetic models for the innate components of colony odour in social Hymenoptera found that the Gestalt model was generally favoured in most species, although evidence also suggested that the Individualistic model (see also CROZIER 1988) could potentially operate in some primitive ants (i.e., representing an earlier stage in the evolutionary process of the recognition system). In studying kin recognition in the congeneric *R. confusa* ant (a species with both queen-right and queenless colonies), CROSLAND (1989) found that the Individualistic component (72%) was sufficiently more important than the Gestalt component (28%), thereby suggesting that at least in *R. confusa*, nestmates possibly have sufficiently distinctive labels to enable preferential discrimination between differentially related conspecifics. Polygynous social insects, especially those lacking a well-defined queen caste and with long-lived colonies, may represent ideal candidates to look for the presence of non-random colony fission along kin-lines.

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