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Nestmate relatedness in the Australian ant *Myrmecia pyriformis* SMITH, 1858 (Hymenoptera: Formicidae)

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Abstract

We investigated the genetic structure of 19 colonies of the myrmeciine ant *Myrmecia pyriformis* SMITH, 1858, using five microsatellite loci. Nestmate relatedness of $b = 0.51 \pm 0.04$ significantly deviated from the theoretical value of 0.75, which is expected in a monogynous monandrous social system. The occurrence of multiple mating by queens was the most likely explanation in the majority of colonies, and the effective number of male mates was estimated to be 2.60. However, multiple female reproductives were also inferred in some cases. The results correspond well both to observations on promiscuous mating behaviour in the winged sexuals of some species of *Myrmecia* and to the occurrence of gamergates (mated workers) in *M. pyriformis*. The finding of high levels of polyandry in a species with totipotent workers is discussed with regard to the evolution of eusociality.

Key words: Colony structure, gamergates, microsatellites, multiple mating, Myrmeciinae, paternity.

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Introduction

The debate about whether the origin of eusociality in ants has evolved by the prevalence of monogamy (having only one mate during a lifetime) and therefore is based on high nestmate relatedness has yet to be fully resolved (e.g., WILSON & HÖLLDOBLER 2005, WEST & al. 2007, HEINZE 2008, HUGHES & al. 2008). Relatedness-based concepts are well known as inclusive fitness or kin selection theory (HAMILTON 1964), on which most of the research focusing on social insects was almost uniformly based for decades (e.g., ANDERSSON 1984, CROZIER & PAMILO 1996, HAMMOND & KELLER 2004). An alternative group selection view proposes that social life resulted from direct benefits of cooperation between related or unrelated individuals, where close relatedness may be more a consequence than the immediate cause of eusociality (WILSON & HÖLLDOBLER 2005, NOWAK & al. 2010), but this has not been accepted by many authors (e.g., FOSTER & al. 2006, WEST & al. 2007). However, it is not as obvious as often suggested that both hypotheses must be considered mutually exclusive (see HEINZE 2008, BOOMSMA 2009). A recent study by HUGHES & al. (2008) endorses the view that kin selection has been crucial to the evolution of eusociality in bees, wasps, and ants, because mating with a single male was found ancestral in all eight investigated eusocial lineages. On the other hand, mating with multiple males appeared to be always derived, with high polyandry (> 2 effective mates) occurring only in species that have sterile workers. In the light of these findings, additional genetic data on social organization and nestmate relatedness, especially of those species whose workers have not lost totipotency (i.e., the potential to reproduce sexually), remain valuable tools for the reconstruction of early ant evolution.

Ants of the genus *Myrmecia*, commonly known as "bulldog ants", are strictly confined to the Australian zoogeographical region comprising more than ninety described species. These ants exhibit a wealth of morphological diversity ranging from very large to small species, additionally showing an extraordinary karyotypic diversity. A comprehensive morphological study led to the recognition of nine *Myrmecia* species groups (OGATA 1991), while a recent molecular phylogenetic treatment of those species groups has been provided by HASEGAWA & CROZIER (2006). *Myrmecia* is considered relatively "primitive" among living ants due to both morphology and social organization, for which a limited queen-worker divergence is a prominent trait (HÖLLDOBLER & WILSON 1990). However, according to molecular phylogenetic analysis the ant subfamily Myrmeciinae appears nested within the formicoid clade, which also includes some of the morphologically more derived ant subfamilies (BRADY & al. 2006). The tribe Prionomyrmecini is the accounted sister group to *Myrmecia* (see WARD & BRADY 2003), being represented only by one extant species, *Nothomyrmecia macrops* CLARK, 1934, and two fossil species of the genus *Prionomyrmex*.

The social organization of *Myrmecia* differs considerably among species in that colonies can be mono- or polygynous and are established independently or by colony fission. Reproductive females may be fully winged before nuptial flight, but different kinds of intermorphs from subapterous to ergatoid are also found (CLARK 1951). In addition, *Myrmecia* is one of several ant genera that possess gamergates, i.e., female worker ants that are able to mate and reproduce (DIETEMANN & al. 2004). We studied *Myrmecia pyriformis* SMITH, 1858 as an example of the typical "large bulldogs" belonging to the *M. gulosa* group with over forty described species (OGATA 1991). Worker size in *M. pyriformis* varies substantially. Major workers (Fig. 1a) are almost twice as large as minors within single colonies (DIETEMANN & al. 2004), which can contain up to a few thousand workers. Available observations of nuptial flights in *M. pyriformis* and closely related species suggest promiscuous mating behaviour of the sexes (WHEELER 1916, CLARK 1951, CROSLAND & al. 1988). After mating, young queens found colonies independently in a semiclaustral manner. The single dealate queen (Fig. 1b) is most conspicuous inside the nest and may be accompanied by two or three wingless intermorphs (CLARK 1951), and later on gamergates can serve as replacement reproductives (DIETEMANN & al. 2004).

Genetic data on colony structure in the subfamily Myrmeciinae are scant. In the polygynous species *Myrmecia pilosula* SMITH, 1858, pertaining to the smaller "jack jumpers", within-colony relatedness was estimated 0.20 on average using isozyme electrophoresis (CRAIG & CROZIER 1979). *Nothomyrmecia macrops* has more recently been investigated with microsatellites, showing worker relatedness in the monogynous colonies of 0.65 under moderate degrees of multiple mating by queens and replacement of the mother queen by one of her daughters (SANETRA & CROZIER 2001, 2002). Here I describe the genetic structure of colonies in the ant *Myrmecia pyriformis* using microsatellite analysis. In particular, it is addressed whether queens mate singly or multiply, whether there can be multiple reproductive females in a colony, and how the mode of reproduction affects the relatedness of nestmate workers.

Material and methods

Sample collection: Colonies of *Myrmecia pyriformis* were collected in the Wildlife Reserve of La Trobe University located in the northern suburbs of Melbourne (Victoria, Australia; 37.7166º S / 145.0533º E) in September 1999. This area serves the restoration and management of indigenous flora and fauna, providing an example of the original River Red Gum (*Eucalyptus camaldulensis*) grassy woodlands also with diverse shrub layers.

Microsatellites: Primers were developed as described by QIAN & al. (in press). Since some of the primer sequences are not listed as polymorphic in that study, details on the five microsatellites loci used are given here: Mfor697 – approx. size 190 bp, Fw–atcgtagctgatatgaacgtg, Rv–gcatgaaaatcaatatgcagt. Mpyr28 – approx. size 180 bp, Fw–gctcgctatacatctcgtcc, Rv–acaaagtggaacgataaaag. Mpyr38 – approx. size 220 bp, Fw–gaggcagaccgagtgacgag, Rv–cgaagaatggaggcgaagtg. Mpyr57 – approx. size 300 bp, Fw–gtgtgtacctggggctgc, Rv–ttctctacctttctccttcc. Mpyr81 – approx. size 200 bp, Fw–cccttttccgtttctcg, Rv–ttcaggcggcgtaccaag. Annealing temperatures for all primer pairs were 50°C, except Mfor697 for which 40 - 45°C were used. Primers were labelled with fluorescent Hex, Tet and Fam dyes and the amplifed PCR products were electrophoresed and visualized on an ABI Prism 377 DNA Sequencer. Alleles were scored using the GENESCAN 2.0.1.^{TM} (Perkin Elmer / ABI) software.

Data analysis: Worker offspring were assigned to different patri- and matrilines by manual inspection of multilocus genotypes. To this end, a rather conservative approach, aiming to reduce the number of inferred females, was used given the knowledge that *Myrmecia pyriformis* usually has only one dealate queen though additional gamergates may occur (DIETEMANN & al. 2004). In one instance (colony MpA), the genotype of the resident queen was known, while the others were inferred from genotypes of workers (see Tab. 2 for examples). The pedigree-effective mate number could be directly obtained from the genotype data as $m_e = 1 / \sum p_i^2$, where p_i^2 is the proportional paternity contribution of the *i*th male (STARR 1984). The unbiased estimate of $\sum p_i^2$ corrected for sampling error after PAMILO (1993) with the observed male contributions y_i^2 is given by $\sum p_i^2 = (N \sum y_i^2 - 1) / (N - 1)$. The pedigree relatedness (g) among daughters was estimated from the effective paternity as $g_{dd} = 0.25 + 1 / (2m_e)$.

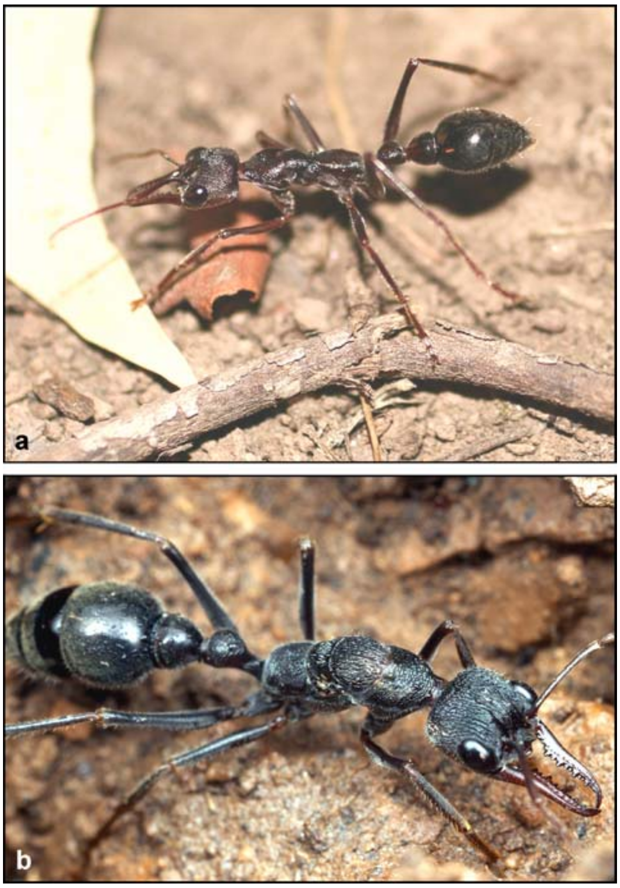

Fig. 1: Queen-worker polymorphism in *Myrmecia pyriformis*. (a) Major worker – Photo: R. Lowe; (b) dealate queen – Photo: A. Wild.

Regression relatedness (b) was calculated from worker genotypes using the program RELATEDNESS 5.0.5 (GOODNIGHT 2000), which employs the methods described by QUELLER & GOODNIGHT (1989). Colonies were weighted equally and standard errors were estimated by jackknifing over colonies. Expected heterozygosities (H_e) were obtained from the squared sum of allele frequencies.

When calculating the total number of male mates, two potential sources of error can occur, either from non-detection caused by limited marker variation or from effects of non-sampling. Non-detection errors were assessed through the probability of being homozygous at a given locus denoted by $1 - H_e$, which equals the situation of two males sharing the same allele by chance. The final probability is

Tab. 1: Parameters of colony structure in a suburban Melbourne population of *Myrmecia pyriformis*: total number of male mates detected (m_t), pedigree-effective mate number (m_e), sum of squared paternity contributions (Σy^2), daughter-daughter relatedness inferred from pedigree analysis (g_{dd}), regression relatedness among nestmates (b_{ww}), number of colony members assigned to a different reproductive (n_{dr}), and number of individuals sampled (n_s); * dealate queen sampled; † mean includes only multiply mated females.

Colony	m_t	m_e	Σy^2	g_{dd}	b_{ww}	n_{dr}	n_s
MpA	3*	2.88	0.35	0.43	0.52		12
MpB	2	1.95	0.51	0.51	0.50		12
MpC	1	1.00		0.75	0.88		12
MpD	4	3.43	0.29	0.40	0.46		12
MpE	4	2.88	0.35	0.43	0.31		12
MpF	4	3.43	0.29	0.40	0.28		12
MpG	4	3.67	0.27	0.39	0.36	1	12
MpH	1	1.00		0.75	0.78		10
MpI	3	2.67	0.38	0.44	0.42		4
MpJ	2	1.60	0.63	0.57	0.21	2	10
MpK	2	1.22	0.82	0.66	0.61		10
MpL	3	1.85	0.54	0.52	0.55		10
MpM	2	1.47	0.68	0.59	0.42		10
MpN	1	1.00		0.75	0.40	3	10
MpO	3	2.63	0.38	0.44	0.47		10
MpP	1	1.00		0.75	0.89		10
MpT	2	1.36	0.73	0.62	0.68		19
MpW	2	1.74	0.57	0.54	0.60		13
MpX	4	2.88	0.35	0.43	0.41		14
Average	2.53	2.09	0.48†	0.55	0.51	0.32	11.26

then given by the product of individual homozygosity estimates across all loci. Non-sampling errors mainly occur because some males contribute only very little to the total number of offspring. In a sample of *n* offspring, individuals from minority males occurring at a proportion *p* will escape sampling with probability $(1 - p)^n$.

Results

Genetic marker variation: The studied population of *Myrmecia pyriformis* in suburban Melbourne showed 30 alleles at the most variable locus Mfor697. There were 19 alleles at locus Mpyr57, 18 alleles at locus Mpyr81, 17 alleles at locus Mpyr38, and five alleles at the least variable locus Mpyr28. Expected heterozygosities (H_e), which indicate the amount of genetic variation, ranged from 0.40 - 0.93, with an average heterozygosity value of 0.80.

Colony structure: In the 19 investigated colonies of *Myrmecia pyriformis*, a number of 1 - 4 males were detected in the worker offspring (Tab. 1, Fig. 2). Principal reproductive females were singly mated in four colonies, doubly mated in six colonies, triply mated in four colonies, and had mated with four males in five of the colonies. Thus, the number of male mates was relatively evenly distributed (Fig. 2), resulting in a proportion of multiple-paternity colonies of 79%. Paternity shares did not vary greatly from equality, however this needs to be examined in more detail with larger sample sizes. For example, colonies *MpA* and *MpF* had male contributions of 5 / 4 / 3 and 5 / 3 / 2 / 2 workers, respectively (see Tab. 2).

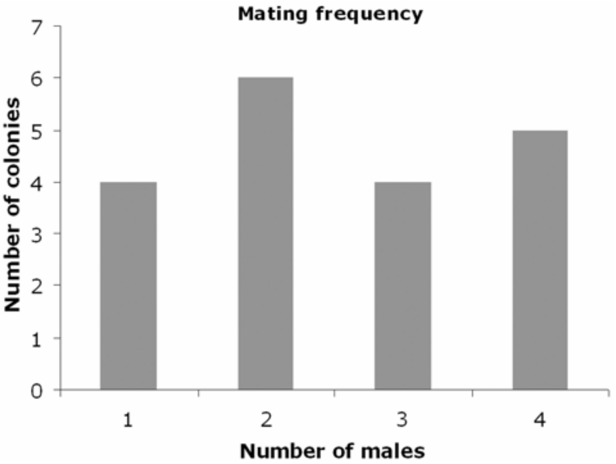

Fig. 2: Absolute numbers of singly and multiply mated females in a suburban Melbourne population of *Myrmecia pyriformis*.

Tab. 2: Worker genotypes of five selected colonies of *Myrmecia pyriformis* at four microsatellite loci (Mpyr28 not shown due to low resolving power). Examples for multiple mating by females: (a) 3 males detected with dealate queen's genotype known and (b) 4 males detected with female's genotype inferred from workers' genotypes. Examples for incongruence of worker genotypes with only one reproductive female: (c) 1 female mated with 4 males plus 1 worker from second female, (d) 1 female mated with 2 males plus 2 workers from second female, and (e) offspring of 2 females singly mated with ratio 7:3. Genotypes are written in the format Allele female / Allele male, except for second females where this could not be deduced. Numbering of alleles refers to their size and observed genotype counts are given in parentheses.

Colony		**Mfor697**	**Mpyr38**	**Mpyr57**	**Mpyr81**
a) *MpA*					
Female 1	Male 1	214 / 283 (5)	216 / 236 (4) 220 / 236 (1)	298 / 302 (5)	194 / 200 (2) 200 / 200 (3)
	Male 2	214 / 220 (4)	216 / 210 (2) 220 / 210 (2)	298 / 310 (4)	194 / 194 (2) 200 / 194 (2)
	Male 3	214 / 190 (3)	216 / 230 (2) 220 / 230 (1)	298 / 298 (3)	194 / 200 (1) 200 / 200 (2)
b) *MpF*					
Female 1	Male 1	180 / 188 (1) 202 / 188 (2)	216 / 212 (2) 226 / 212 (1)	298 / 292 (3)	206 / 202 (2) 218 / 202 (1)
	Male 2	180 / 202 (1) 202 / 202 (1)	216 / 214 (2)	298 / 308 (2)	206 / 196 (2)
	Male 3	180 / 220 (3) 202 / 220 (2)	216 / 232 (3) 226 / 232 (2)	298 / 320 (3) 300 / 320 (2)	206 / 212 (2) 218 / 212 (3)
	Male 4	180 / 230 (2)	216 / 212 (1) 226 / 212 (1)	298 / 290 (1) 300 / 290 (1)	206 / 194 (1) 218 / 194 (1)
c) *MpG*					
Female 1	Male 1	198 / 202 (4)	222 / 210 (2) 236 / 210 (2)	302 / 302 (1) 296 / 302 (3)	188 / 208 (1) 202 / 208 (3)
	Male 2	202 / 198 (2)	222 / 212 (2)	296 / 306 (1) 302 / 306 (1)	188 / 202 (2)
	Male 3	202 / 198 (3)	222 / 210 (1) 236 / 210 (2)	296 / 318 (1) 302 / 318 (2)	188 / 214 (1) 202 / 214 (2)
	Male 4	198 / 214 (1) 202 / 214 (1)	222 / 194 (2)	296 / 312 (1) 302 / 312 (1)	188 / 202 (1) 202 / 202 (1)
Female 2	Male 1	202 / 204 (1)	218 / 220 (1)	304 / 300 (1)	194 / 206 (1)
d) *MpJ*					
Female 1	Male 1	186 / 202 (1) 208 / 202 (5)	220 / 216 (3) 230 / 216 (3)	298 / 304 (2) 306 / 304 (4)	198 / 194 (5) 206 / 194 (1)
	Male 2	186 / 226 (1) 208 / 226 (1)	– / –	306 / 304 (2)	198 / 198 (1) 206 / 198 (1)
Female 2	Male 1	196 / 198 (1) 208 / 198 (1)	214 / 218 (2)	302 / 309 (2)	200 / 208 (2)
e) *MpN*					
Female 1	Male 1	182 / 212 (5) 208 / 212 (2)	224 / 216 (3) 228 / 216 (4)	302 / 302 (4) 304 / 302 (3)	190 / 198 (3) 194 / 198 (4)
Female 2	Male 1	202 / 198 (2) 208 / 198 (1)	220 / 216 (1) 232 / 216 (2)	304 / 300 (3)	206 / 210 (3)

Three colonies were different from the others in that worker genotypes could not be explained with one reproductive female only. Specifically, there were between one and three individuals per colony clearly derived from a second reproductive (Tab. 1). In colony *MpG*, 11 workers out of 12 could be assigned to a single female that had mated with four males. The remaining worker, however, showed only one allele at four microsatellite loci that was shared with the remainder of the colony. Hence it did not belong to that half-sib family and must have resulted from a different female (see Tab. 2). Colony *MpJ* consisted of eight workers most likely produced by a doubly mated female and two workers from another female. Between these two groups of workers, allelic overlap was again confined to a single allele. The presence of multiple female reproductives in *Myrmecia pyriformis* was probably best exemplified by colony *MpN*, in which two singly mated females were inferred from workers' genotypes. Seven workers were assigned to one female and seven workers to the other. Although these workers shared some alleles (Tab. 2), their pedigree relationship to other members of the colony was not obvious (see discussion).

Estimates of paternity and relatedness: The total number of males (± standard deviation) detected per colony by genotype inspection was 2.53 ± 1.13, while the pedigree-effective mate number was estimated to be 2.09 ± 0.92. The population-wide non-detection error due to limited variation of the marker loci was 4.4×10^{-5}, showing that the number of males that might have escaped detection because of genetic identity at all loci combined should be of little effect. However, sample sizes were fairly limited with an average of 11.3 workers per colony. The non-sampling error was therefore potentially significant but its influence depends largely on the amount of paternity skew among males. For instance, a sample of ten individuals would include offspring of a male with > 95% probability if it had a paternity share of more than 26%. Patterns of paternity variation were difficult to explore in our limited samples, but males that appeared to contribute as little as 10 - 20% to the offspring did occur in a number of cases. Thus, we applied PAMILO's (1993) probabilistic sample size correction, which increased the effective paternity estimate to 2.60 ± 1.54.

Using the effective number of male mates, the pedigree-relatedness among daughters was estimated as $g_{dd} = 0.55 \pm 0.13$, which, however, did not include offspring that originated from different matrilines. Even so, this estimate was not considerably different from the average genetic relatedness among all nestmates, $b_{ww} = 0.51 \pm 0.04$ (Tab. 1), as calculated by regression analysis of microsatellite genotypes. The non-overlapping confidence interval of the genetic relatedness (CI = 0.42 - 0.60) showed that the calculated mean did differ significantly from 0.75, which is the theoretically expected value for workers produced by queens mated with a single male.

Discussion

Analysis of microsatellites has shown that up to four males contributed to the worker offspring in colonies of *Myrmecia pyriformis*. This suggests rather promiscuous mating behaviour, which has been documented in the giant bulldog ants of the *M. gulosa* group as an outstanding spectacle. The first account by WHEELER (1916) concerns a mating flight of *M. sanguinea* SMITH, 1858 (now a junior synonym of *M. pyriformis*) near Armidale in New South Wales: "As soon as a male (and there were hundreds of males to every female) captured a female on a bush, other males surrounded the couple till there was a struggling mass of ants forming a ball as large as one's fist. As many as half a dozen of these balls would keep forming on every little bush and this went on throughout the morning". Further, CLARK (1951) provides a captivating report on a nuptial flight in *M. forficata* (FABRICIUS, 1787), a close relative of *M. pyriformis*, at Fern Tree Gully, Victoria: "The flight lasted until about 5 p.m., and on this occasion both sexes were from several large nests in the vicinity. Many of the females flew to a height of thirty feet but generally long before a female reached that height she was pursued and surrounded by numerous males who pounced on her, their combined weight immediately bringing the cluster of winged forms to the ground with a discernible thud, the struggling mass resembling a free fight more than anything else". Another nuptial flight with similar features was recorded by CROSLAND & al. (1988) on a hillock in New South Wales in a species resembling *M. pyriformis*. Given those three reports the mating behaviour of the giant bulldog ants most closely resembles that of some more derived ant species, best described in the myrmicine genus *Pogonomyrmex* where large mating clusters are typically formed and strong male competition exists (HÖLLDOBLER 1976).

The pedigree effective mate number in *Myrmecia pyriformis* was 2.60, representing the highest value hitherto recorded in a species that has totipotent workers, which can mate and reproduce sexually. Only a few species of ponerine ants with gamergates exhibit facultative low polyandry with the number of effective mates being only slightly larger than one (TAY & CROZIER 2001 for *Rhytidoponera* sp. 12, GIRAUD & al. 2000 for *Gnamptogenys striatula*), while the remaining species investigated thus far show no evidence of multiple mating at all (WARD 1983 for *Rhytidoponera chalybea* and *R. confusa*, ANDRÉ & al. 2001 for *Diacamma cyaneiventre*, KIKUTA & TSUJI 1999 for *Diacamma* sp., SCHLÜNS & al. 2006 for *Streblognathus aethiopicus*, MONNIN & PEETERS 1998 for *Dinoponera quadriceps*). Species that are most similar to *M. pyriformis* with respect to their effective mate number are *Atta colombica*, 2.31 (FJERDINGSTAD & al. 1998), *Plagiolepis pygmaea*, 2.37 (TRONTTI & al. 2007), *Pachycondyla inversa*, 2.38 (KELLNER & al. 2007), *Cardiocondyla batesii*, 2.63 (SCHREMPF & al. 2005), and *Pogonomyrmex barbatus*, 3.13 (CAHAN & al. 2002). The phylogenetically closest relative to *Myrmecia* spp., which is the Australian endemic *N. macrops*, has an effective mate number of 1.33 (SANETRA & CROZIER 2001). In that regard, the relatively high level of polyandry in *M. pyriformis* comes as a surprise, especially when considering the small difference to some of the very advanced species like *A. colombica* and *P. barbatus*. Although the phylogenetic position of the Myrmeciinae is not entirely basal within the ants, the origin of myrmeciomorphs (Myrmeciinae + Pseudomyrmecinae) near the base of the formicoid clade probably dates back some 90 - 100 million years ago (BRADY & al. 2006). This long independent evolutionary history may well serve as an explanation why species in this subfamily encompass relatively "primitive" as well as more derived features.

Multiple-paternity colonies in *Myrmecia pyriformis* occurred at a frequency of about 80%, which is in good accord to other ant species where queens commonly mate with two or more males. For instance, proportions of multiply mated females between 80 - 95% have been reported in two species of *Pachycondyla* (see KELLNER & al. 2007), *Plagiolepis pygmaea* (see TRONTTI & al. 2007), and *Atta colombica* (see FJERDINGSTAD & al. 1998). In comparison, *Nothomyrmecia* containing the only extant species in the sister group of *Myrmecia*, has a proportion of multiply mated queens of only 35% (SANETRA & CROZIER 2001).

Nevertheless, the latter evaluation bears little evolutionary insights given the large diversity in the genus *Myrmecia* comprising around ninety species in nine distinct species groups, and hence more species have yet to be examined in order to elucidate general patterns of mating strategies.

Nestmate worker relatedness in *Myrmecia pyriformis* was 0.51, thus being roughly in agreement with the estimated number of 2.6 effective male mates. This indicates that males siring offspring in multiple paternity colonies were mostly unrelated, which also follows from the reports on nuptial flights to some extent (e.g., Clark 1951). In several other ant species that mate multiply and have more than two effective mates, males are related due to inbreeding, such as in *Plagiolepis pygmaea* (see Trontti & al. 2007), *Pachycondyla inversa* (see Kellner & al. 2007), and *Cardiocondyla batesii* (see Schrempf & al. 2005), but not so in *Atta colombica* (see Fjerdingstad & al. 1998) and *Pogonomyrmex barbatus* (see Cahan & al. 2002). Therefore, relatedness and inferred social structure of *M. pyriformis* in most colonies come closest to the last mentioned species, with a relatedness of 0.52 (Fjerdingstad & al. 1998) and 0.41 (Cahan & al. 2002), respectively.

A low level of worker policing has been reported in a close relative of *Myrmecia pyriformis*, *Myrmecia gulosa* (Fabricius, 1775), in which reproductive workers were seized and immobilized in laboratory colonies (Dietemann & al. 2005). According to kin selection theory, policing behaviour, which here refers to the prevention of worker reproduction, should be associated with polygyny and polyandry, and monogyny and monandry with worker reproduction (Ratnieks 1988). However, these predictions earned little support in *Formica* ants (Helanterä & Sundström 2007) and the occurrence of policing in the monogynous monandrous ant *Diacamma* sp. (Kikuta & Tsuji 1999) contradicts the theory. Heinze (2008) thus reached the conclusion that worker reproduction is usually prevented in ant societies regardless of their genetic structure.

In addition to multiple mating, the presence of different reproductive females in 3 of 19 investigated colonies was inferred from microsatellite genotypes (Tabs. 1, 2). Since the single dealate queen may be accompanied by several wingless intermorphs (Clark 1951), and gamergates have been described in *Myrmecia pyriformis* (see Dietemann & al. 2004), there is much potential for alternative reproductive strategies in this species. Apparently, the giant bulldog ants do not exemplify a type of polygyny where multiple queens of the same kind co-occur inside a nest and reproduce, which is, for instance, the case in *M. pilosula* showing low relatedness of 0.17 among workers (Craig & Crozier 1979). In *M. pyriformis*, the most likely scenario is that colonies are founded by a single queen, which then gets replaced by gamergates (perhaps sometimes also by intermorphic queens) after senescence. A similar type of queen supersedure by gamergates has been described in the ponerine ant *Harpegnathos saltator* (see Peeters & al. 2000), and *Rhytidoponera* workers may also differentiate into gamergates after queen death in some species (Ward 1983, 1986). In general, queen replacement results in an extension of colony lifespan and inheritance of nest site as a valuable resource, a life history trait which is also found in the sister group of *Myrmecia*, the strictly monogynous *Nothomyrmecia macrops*. Due to the lack of gamergates, orphaned colonies of *N. macrops* are taken over by a daughter queen, thus leading to serial polygyny (Sanetra & Crozier 2002).

Workers that descended from different female reproductives in *Myrmecia pyriformis* did not appear to be closely related, as only few alleles were shared (Tab. 2). This pattern of low allelic overlap could be explained by the occurrence of successive gamergate generations, but without genotyping the larvae present in the nest one cannot distinguish between true polygyny and serial polygyny. Furthermore, the resolving power with such small samples to establish pedigree relationships is very limited despite high heterozygosity measures of 80%. The degree of microsatellite variation is comparable to those of other ant species, and particularly similar to the estimate in the related Australian genus *Nothomyrmecia* (see Sanetra & Crozier 2001). For future research, *Myrmecia* might be an interesting study group in order to gain insights into early ant evolution, specifically because its constituent species cover a wide range of social strategies as well as morphological diversity.

The discovery of an ant species with gamergates having more than two effective mates renders the conclusion that high polyandry occurs only in lineages whose workers have lost reproductive totipotency (Hughes & al. 2008) premature. High polyandry significantly reduces the large inclusive-fitness benefits assumed for workers in the Hymenoptera (reviewed by Crozier & Pamilo 1996), however, some authors have pointed out that the "haplodiploidy" or "$^3/_4$ relatedness" hypothesis is only one idea arising from kin selection theory (e.g., Foster & al. 2006, Heinze 2008). The recent focus on ancestral monogamy as key factor to the evolution of eusociality (Hughes & al. 2008) underscores the perception that "a number of researchers erroneously adopted the idea that haplodiploidy with its 0.75 relatedness among fullsisters is the explanation of eusociality" (Heinze 2008).

The social system of *Myrmecia pyriformis* with a differentiated queen caste and gamergates as a form of supplementary reproductives is quite conspicuous in social Hymenoptera. Sexually reproducing workers are otherwise mostly present in some of the poneromorph subfamilies, and many features of those species are shared with the neotenics in termites and the non-breeding helpers in birds (e.g., Korb & Heinze 2008). Hence, a unifying theory of social evolution, which encompasses both social arthropods and vertebrates, is greatly needed, where more of the differing concepts from social taxa other than bees, wasps, and ants should be adopted (e.g., Avilés 1993, 1997 for social spiders, Korb 2009 for termites). The widespread application of reproductive-skew models in the late 1990s commonly acknowledged that factors other than relatedness, for example ecological constraints and competitive abilities, are important for shaping cooperative behaviour (e.g., Jamieson 1997, Reeve & Keller 2001). In the case of monogamy, Hamilton's equation becomes reduced to costs and benefits for helping behaviour (Boomsma 2009), but these factors have been widely neglected in studies on social insects (e.g., Korb & Heinze 2004).

Focusing on costs and benefits instead of relatedness, it becomes conceivable that group selection has been a major driving force for social evolution (e.g., Wilson & Hölldobler 2005, Nowak & al. 2010). The latter theory was discredited early on in favour of pure kin selection, similar to the long-lasting refusal of sympatric speciation (Via 2001). Nevertheless, as is true for other scientific disputes the two hypotheses are not mutually exclusive (Korb & Heinze 2004, West & al. 2007, Heinze 2008), and Boomsma (2009) states that transitions towards eusociality require kin selection but are ultimately driven by benefits obtained from group (colony)-level selection. Similarly, a multilevel selection approach by Korb & Heinze (2004) showed that group-level adaptations are able to prevent or resolve intracolonial conflicts for the maintenance of eusociality. Social Hymenoptera thus most likely represent an example of group selection of kin groups, whose particular preadaptation, the prevalence of maternal care in their solitary ancestors, deserves to be accentuated (see also Wilson & Hölldobler 2005, Nowak & al. 2010).

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References

Andersson, M. 1984: The evolution of eusociality. – Annual Review of Ecology and Systematics 15: 165-189.

André, J.B., Peeters, C. & Doums, C. 2001: Serial polygyny and colony genetic structure in the monogynous queenless ant *Diacamma cyaneiventre*. – Behavioral Ecology and Sociobiology 50: 72-80.

Avilés, L. 1993: Interdemic selection and the sex ratio: a social spider perspective. – The American Naturalist 142: 320-345.

Avilés, L. 1997: Causes and consequences of cooperation and permanent-sociality in spiders. In: Choe, J. & Crespi, B. (Eds.): The evolution of social behavior in insects and arachnids. – Cambridge University Press, Cambridge, UK, pp. 476-497.

Boomsma, J.J. 2009: Lifetime monogamy and the evolution of eusociality. – Philosophical Transactions of the Royal Society B-Biological Sciences 364: 3191-3207.

Brady, S.G., Schultz, T.R., Fisher, B.L. & Ward, P.S. 2006: Evaluating alternative hypotheses for the early evolution and diversification of ants. – Proceedings of the National Academy of Sciences of the United States of America 103: 18172-18177.

Cahan, S.H., Parker, J.D., Rissing, S.W., Johnson, R.A., Polony, T.S., Weiser, M.D. & Smith, D.R. 2002: Extreme genetic differences between queens and workers in hybridizing *Pogonomyrmex* harvester ants. – Proceedings of the Royal Society B-Biological Sciences 269: 1871-1877.

Clark, J. 1951: The Formicidae of Australia. Volume I. Subfamily Myrmeciinae. – CSIRO, Melbourne, 230 pp.

Craig, R. & Crozier, R.H. 1979: Relatedness in the polygynous ant *Myrmecia pilosula*. – Evolution 33: 335-341.

Crosland, M.W.J., Crozier, R.H. & Jefferson, E. 1988: Aspects of the biology of the primitive ant genus *Myrmecia* F. (Hymenoptera: Formicidae). – Journal of the Australian Entomological Society 27: 305-309.

Crozier, R.H. & Pamilo, P. 1996: Evolution of social insect colonies. – Oxford University Press, Oxford, 306 pp.

Dietemann, V., Liebig, J., Hölldobler, B. & Peeters, C. 2005: Changes in the cuticular hydrocarbons of incipient reproductives correlate with triggering of worker policing in the bulldog ant *Myrmecia gulosa*. – Behavioural Ecology and Sociobiology 58: 486-496.

Dietemann, V., Peeters, C. & Hölldobler, B. 2004: Gamergates in the Australian ant subfamily Myrmeciinae. – Naturwissenschaften 91: 432-435.

Fjerdingstad, E.J., Boomsma, J.J. & Thoren, P. 1998: Multiple paternity in the leafcutter ant *Atta colombica* – a microsatellite DNA study. – Heredity 80: 118-126.

Foster, K.R., Wenseleers, T. & Ratnieks, F.L.W. 2006: Kin selection is the key to altruism. – Trends in Ecology & Evolution 21: 57-60.

Giraud, T., Blatrix, R., Poteaux, C., Solignac, M. & Jaisson, P. 2000: Population structure, mode of colony foundation and mating biology of the polygynous ponerine ant *Gnamptogenys striatula* in Brazil. – Molecular Ecology 9: 1835-1841.

Goodnight, K.F. 2000: Relatedness 5.0.5. Goodnight Software, Houston, Tex. – <http://www.gsoftnet.us/GSoft.html>, retrieved on 16 September 2010.

Hamilton, W.D. 1964: The genetical evolution of social behaviour, I, II. – Journal of Theoretical Biology 7: 1-52.

Hammond, R.L. & Keller, L. 2004: Conflict over male parentage in social insects. – Public Library of Science Biology 2: e248.

Hasegawa, E. & Crozier, R.H. 2006: Phylogenetic relationships among species groups of the ant genus *Myrmecia*. – Molecular Phylogenetics and Evolution 38: 575-582.

Heinze, J. 2008: The demise of the standard ant (Hymenoptera: Formicidae). – Myrmecological News 11: 9-20.

Helanterä, H. & Sundström, L. 2007: Worker reproduction in *Formica* ants. – The American Naturalist 170: E14-E25.

Hölldobler, B. 1976: The behavioral ecology of mating in harvester ants (Hymenoptera: Formicidae: *Pogonomyrmex*). – Behavioral Ecology and Sociobiology 1: 405-423.

Hölldobler, B. & Wilson, E.O. 1990: The ants. – Belknap Press of Harvard University Press, Cambridge, MA, 732 pp.

Hughes, W.O.H., Oldroyd, B.P., Beekman, M. & Ratnieks, F.L.W. 2008: Ancestral monogamy shows kin selection is key to the evolution of eusociality. – Science 320: 1213-1216.

Jamieson, I.G. 1997: Testing reproductive skew models in a communally breeding bird, the pukeko, *Porphyrio porphyrio*. – Proceedings of the Royal Society B-Biological Sciences 264: 335-340.

Kellner, K., Trindl, A., Heinze, J. & d'Ettorre, P. 2007: Polygyny and polyandry in small ant societies. – Molecular Ecology 16: 2363-2369.

Kikuta, N. & Tsuji, K. 1999: Queen and worker policing in the monogynous and monandrous ant *Diacamma* sp. – Behavioral Ecology and Sociobiology 46: 180-189.

Korb, J. 2009: Termites: an alternative road to eusociality and the importance of group benefits in social insects. In: Gadau, J. & Fewell, J. (Eds.): Organization of insect societies: from genome to sociocomplexity. – Harvard University Press, Cambridge, MA, pp. 128-147.

KORB, J. & HEINZE, J. 2004: Multilevel selection and social evolution of insect societies. – Naturwissenschaften 91: 291-304.

KORB, J. & HEINZE, J. 2008: The ecology of social life: a synthesis. In: KORB, J. & HEINZE, J. (Eds.): Ecology of social evolution. – Springer Press, Heidelberg, pp. 245-259.

MONNIN, T. & PEETERS, C. 1998: Monogyny and regulation of worker mating in the queenless ant *Dinoponera quadriceps*. – Animal Behaviour 55: 299-306.

NOWAK, M.A., TARNITA, C.E. & WILSON, E.O. 2010: The evolution of eusociality. – Nature 466: 1057-1062.

OGATA, K. 1991: Ants of the genus *Myrmecia* FABRICIUS: a review of the species groups and their phylogenetic relationships (Hymenoptera: Formicidae: Myrmeciinae). – Systematic Entomology 16: 353-381.

PAMILO, P. 1993: Polyandry and allele frequency differences between the sexes in the ant *Formica aquilonia*. – Heredity 70: 472-480.

PEETERS, C., LIEBIG, J. & HÖLLDOBLER, B. 2000: Sexual reproduction by both queens and workers in the ponerine ant *Harpegnathos saltator*. – Insectes Sociaux 47: 325-332.

QIAN, Z.-Q., CECCARELLI, S.F., CAREW, M.E., SCHLÜNS, H., SCHLICK-STEINER, B.C. & STEINER, F.M. (in press): Characterization of polymorphic microsatellites in a bulldog and a jumper ant species (*Myrmecia brevinoda* and *M. pilosula*). – Journal of Insect Science.

QUELLER, D.C. & GOODNIGHT, K.F. 1989: Estimating relatedness using genetic markers. – Evolution 43: 258-275.

RATNIEKS, F.L.W. 1988: Reproductive harmony via mutual policing by workers in eusocial Hymenoptera. – The American Naturalist 132: 217-236.

REEVE, H.K. & KELLER, L. 2001: Test of reproductive skew models in social insects. – Annual Review of Entomology 46: 347-385.

SANETRA, M. & CROZIER, R.H. 2001: Polyandry and colony genetic structure in the primitive ant *Nothomyrmecia macrops*. – Journal of Evolutionary Biology 14: 368-378.

SANETRA, M. & CROZIER, R.H. 2002: Daughters inherit colonies from mothers in the "living-fossil" ant *Nothomyrmecia macrops*. – Naturwissenschaften 89: 71-74.

SCHLÜNS, E.A., MORITZ, R.F.A., NEUMANN, P., SCHLÜNS, H. & HEPBURN, H.R. 2006: Nestmate recognition and genetic composition of nests in the queenless ponerine ant *Streblognathus aethiopicus* SMITH. – African Entomology 14: 95-102.

SCHREMPF, A., REBER, C., TINAUT, A. & HEINZE, J. 2005: Inbreeding and local mate competition in the ant *Cardiocondyla batesii*. – Behavioural Ecology and Sociobiology 57: 502-510.

STARR, C.K. 1984: Sperm competition, kinship, and sociality in aculeate Hymenoptera. In: SMITH, R.L. (Ed.): Sperm competition and the evolution of animal mating systems. – Academic Press, Orlando, FL, pp. 428-459.

TAY, W.T. & CROZIER, R.H. 2001: Mating behaviour of *Rhytidoponera* sp. 12 ants inferred from microsatellites. – Molecular Ecology 10: 167-173.

TRONTTI, K., THURIN, N., SUNDSTRÖM, L. & ARON, S. 2007: Mating for convenience or genetic diversity? Mating patterns in the polygynous ant *Plagiolepis pygmaea*. – Behavioural Ecology 18: 298-303.

VIA, S. 2001: Sympatric speciation in animals: The ugly duckling grows up. – Trends in Ecology & Evolution 16: 381-390.

WARD, P.S. 1983: Genetic relatedness and colony organization in a species complex of ponerine ants. I. Phenotypic and genotypic composition of colonies. – Behavioural Ecology and Sociobiology 12: 285-299.

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

WARD, P.S. & BRADY, S.G. 2003: Phylogeny and biogeography of the ant subfamily Myrmeciinae (Hymenoptera: Formicidae). – Invertebrate Systematics 17: 361-386.

WEST, S.A., GRIFFIN, A.S. & GARDNER, A. 2007: Evolutionary explanations for cooperation. – Current Biology 17: R661-R667.

WHEELER, W.M. 1916: The marriage-flight of a bull-dog ant (*Myrmecia sanguinea* F. SMITH). – Journal of Animal Behaviour 6: 70-73.

WILSON, E.O. & HÖLLDOBLER, B. 2005: Eusociality: origin and consequences. – Proceedings of the National Academy of Sciences of the United States of America 102: 13367-13371.