

Strict monandry in the ponerine army ant genus *Simopelta* suggests that colony size and complexity drive mating system evolution in social insects

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Abstract

Altruism in social insects has evolved between closely related full-siblings. It is therefore of considerable interest why some groups have secondarily evolved low within-colony relatedness, which in turn affects the relatedness incentives of within-colony cooperation and conflict. The highest queen mating frequencies, and therefore among the lowest degrees of colony relatedness, occur in *Apis* honeybees and army ants of the subfamilies Aenictinae, Ecitoninae, and Dorylinae, suggesting that common life history features such as reproduction by colony fission and male biased numerical sex-ratios have convergently shaped these mating systems. Here we show that ponerine army ants of the genus *Simopelta*, which are distantly related but similar in general biology to other army ants, have strictly monandrous queens. Preliminary data suggest that workers reproduce in queenright colonies, which is in sharp contrast to other army ants. We hypothesize that differences in mature colony size and social complexity may explain these striking discrepancies.

Keywords: colony fission, eusociality, Formicidae, mating frequency, microsatellites, male parentage

Received 12 July 2010; revision received 29 October 2010; accepted 4 November 2010

Introduction

Single mating (monandry) by females was the ancestral condition in all major lineages of eusocial insects (Boomsma 2007; Hughes *et al.* 2008). This is important for two reasons: first, it means that relatedness among a female's offspring was high, which favoured the evolution of eusociality (Crozier & Pamilo 1996; Boomsma 2007, 2009; Crozier 2008), and second, that females minimized the cost of mating. Multiple mating (polyandry) is thought to be costly for females because it increases the risk of predation and contracting diseases, and it augments time and energy expenditure. Furthermore, males can physically harm females during copulation (e.g. Kamimura 2008) and storing ejaculates from multi-

ple males can be associated with immunity costs (Baer *et al.* 2006) and reduced sperm viability because of sperm competition (den Boer *et al.* 2010). In line with these risks, monandry or low levels of facultative polyandry prevail in the social Hymenoptera (Strassmann 2001; Hughes *et al.* 2008; Boomsma *et al.* 2009). Some notable exceptions exist, however, and substantial effort has been directed towards understanding the adaptive value of the high levels of obligate polyandry in these systems (Crozier & Page 1985; Boomsma & Ratnieks 1996; Palmer & Oldroyd 2000; Strassmann 2001; Crozier & Fjerdingstad 2001; Boomsma *et al.* 2009). Most prominent among these are the army ants of the dorylomorph subfamilies Aenictinae, Ecitoninae, and Dorylinae (abbreviated as the AenEcDo army ants throughout this manuscript; Kronauer *et al.* 2007a; Kronauer 2009), the *Atta* and *Acromyrmex* leaf-cutting ants (Villesen *et al.* 2002; Sumner *et al.* 2004), the *Pogonomyrmex* harvester

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ants (Cole & Wiernasz 2000; Pol *et al.* 2008), the *Vespula* wasps (Foster & Ratnieks 2001), and the *Apis* honeybees (Oldroyd *et al.* 1998; Tarpay *et al.* 2004).

While the precise parameters in each case are still not fully understood, four factors seem particularly important in explaining the evolution of polyandry in social Hymenoptera, three of which are directly related to increased genetic diversity within colonies: first, genetically diverse colonies might be more resistant to parasite and pathogen infections (Hamilton 1987; Sherman *et al.* 1988); second, genetic diversity might increase homeostasis via more effective task allocation (Oldroyd & Fewell 2007); third, multiple mating might, under certain circumstances, alleviate the cost of producing diploid, sterile males when queens mate with males that carry an identical sex-locus allele (Page 1980). Finally, queens of species with large reproductive output might mate with numerous males because a single male may not provide sufficient sperm (Cole 1983). The overall trend suggests that obligate polyandry has mainly evolved in species with large and complex societies, which is in accordance with hypotheses one, two, and four (Boomsma *et al.* 2009). The application of hypothesis three, on the other hand, has largely been limited to explaining the idiosyncratic mating system of honeybees (Page 1980; Tarpay & Page 2001, 2002; but see Kronauer *et al.* 2007a for army ants).

Among the groups with obligate polyandry, army ants and honeybees stand out in that their queens have by far the highest mating frequencies, with typical mate numbers somewhere between 10 and 40 (Tarpay *et al.* 2004; Kronauer *et al.* 2007a). Two strikingly convergent aspects of the army ant and honeybee mating system might at least partially account for the extreme queen-mating frequencies: reproduction by colony fission, and highly male biased numerical sex-ratios (Kronauer *et al.* 2004, 2007a; Kronauer 2009). In monogynous species with colony fission, a mother colony invests substantially in a single or very few daughter queens, which retain a large part of the colony's worker force. This mode of reproduction is rare among most groups of eusocial Hymenoptera, but is the rule in army ants, honeybees, stingless bees, and many ponerine ants (Peeters & Ito 2001; Boomsma *et al.* 2009). Dependent colony founding, where workers accompany young queens, also occurs in many polygynous ant species (Keller 1991) and epiponine wasps (Strassmann *et al.* 1997), but the social dynamics in such systems are very different from monogynous species with colony fission (Peeters & Ito 2001). Models suggest that under colony fission in monogynous species, it might be advantageous for queens to avoid extreme colony genotypes by mating multiply and thereby decreasing the genetic variance between colonies (Page 1980; Sherman *et al.* 1988;

Kronauer *et al.* 2007a; Rüppeil *et al.* 2008). In species with independent colony founding, on the contrary, competition between the many incipient colonies will be intense so that queen-male combinations with even slightly genetically deleterious effects will probably be eliminated from the population. This idea was originally developed when evaluating the consequences of diploid male load in honeybees (Page 1980) but similar models have later been developed for parasite and pathogen load (Sherman *et al.* 1988), showing that the general idea can be applied to any trait with a genetic basis (Rüppeil *et al.* 2008).

Because a large proportion of a colony's worker force joins or remains with the new queen during colony fission, these workers have to be counted as investment into that queen. Because of expected equal investment into both sexes (Fisher 1930), an evolutionary corollary of colony fission is a highly male-biased numerical sex-ratio, with hundreds or thousands of males per queen (Macevitz 1979; Bulmer 1983). This suggests that queens of fissioning species are not limited in their access to males and that mating costs are comparatively low, which allows mating frequencies in honeybees and army ants to reach such extreme values (Kronauer *et al.* 2004, 2007a).

In this study, we used newly developed microsatellite loci to analyse the colony pedigree structure and mating frequency of queens in the ponerine army ant genus *Simopelta*, which is only distantly related to the *AenEcDo* army ants, but strikingly resembles them in overall life history and ecology (Gotwald & Brown 1966; Brady 2003; Mackay & Mackay 2008; Kronauer 2009). We use these data to evaluate the hypothesis that high levels of polyandry are an integral part of the army ant adaptive syndrome, together with group predation, nomadism, and dependent colony founding, and, more generally, that species with colony fission should be particularly likely to evolve high queen-mating frequencies (Kronauer *et al.* 2007a; Kronauer 2009).

Natural history of Simopelta army ants

The genus *Simopelta* is widely distributed throughout the Neotropics, ranging from Guatemala to southern Brazil, and is most often found in moist montane tropical forests (O'Donnell & Kumar 2006; Mackay & Mackay 2008). Colonies consist of *c.* 1000 workers and a single queen (Gotwald & Brown 1966). However, entire colonies are rarely collected and *Simopelta* males are hitherto unknown. The striking convergence with *AenEcDo* army ants in overall life history and ecology has been noted repeatedly (Gotwald & Brown 1966; Brady 2003; Mackay & Mackay 2008; Kronauer 2009). Queens are highly specialized dichthadiiforms, i.e. they

have a greatly enlarged gaster, and are permanently wingless. Winglessness in turn implies that queens never go on a mating flight, that mating occurs inside or close to the nest and, in combination with monogyny, that colonies reproduce by fission, as do *AenEcDo* army ants. In fact, Borgmeier (1950) observed a *Simopelta pergandei* colony approaching fission, as it had both an old queen and a single young (callow) queen. As has been outlined above, strongly male-biased numerical sex-ratios are a corollary of reproduction by colony fission in monogynous species (Macevicz 1979; Bulmer 1983). Accordingly, although *Simopelta* males are presently not known, we would expect that, in the case that males exist and sexual reproduction occurs, numerical sex-ratios are highly male-biased in *Simopelta*. As in many dorylomorph army ants, brood is produced in distinct cohorts, and colonies therefore probably show cyclic behaviour (Gotwald & Brown 1966; D. Kronauer, pers. obs.). *Simopelta* workers forage in columns and are specialized on raiding the nests of other ants (Gotwald & Brown 1966; Mackay & Mackay 2008). In addition, this aspect of their lifestyle is consistent with reproduction by colony fission because small incipient colonies would not be able to attack other ant societies (Gotwald 1995; Kronauer 2009). Colonies of different *Simopelta* species have been observed on emigrations, suggesting a nomadic lifestyle (Kronauer 2009; S. O'Donnell, pers. obs.; J. T. Longino, pers. comm.). Taken together, this suggests that *Simopelta* shows the most complete convergence on the army ant adaptive syndrome outside the dorylomorph clade of ants (Brady 2003).

Materials and methods

Study species

In this study, we focused on two species, *S. pergandei* and *S. pentadentata*. In a recent taxonomic revision, Mackay & Mackay (2008) divided the 21 described species of *Simopelta* into two distinct species groups, the *curvata* species complex, with five species including *S. pergandei*, and the *williamsi* species complex, with 16 species including *S. pentadentata*. Our choice of species should therefore be representative of the genus as a whole.

Sample collection, DNA extraction and genotyping

We sampled and analysed 12 colonies of *Simopelta pentadentata* from Costa Rica, and three colonies of *S. pergandei* from Venezuela. *S. pentadentata* colonies were collected during four separate field trips in 2005, 2007 and 2009. Two *S. pergandei* colonies were collected during a six-week field season in 2003, and the third colony was collected opportunistically at the same site in 2002. Collection details are given in Table 1. All samples were collected directly into 96% ethanol.

DNA was extracted by first freezing entire individuals in a plastic tube in liquid nitrogen and crushing them with a pestle. We then added 200 µL of 5% Chelex 100 (Bio-Rad), heated samples to 96 °C for 15 min, vortexed, and centrifuged briefly. The supernatant was used as template in subsequent PCR amplifications.

Simopelta pergandei samples were genotyped for nine polymorphic microsatellite loci with the following

Table 1 Collection details for the *Simopelta pentadentata* (Spt) and *S. pergandei* (Spg) samples

Colony	Locality with coordinates and elevation if available	Collection date
Spt1	San Gerardo Station, Monteverde, Costa Rica; 1050 m asl 10°22.075'N, 84°46.801'W	27 Dec 2007
Spt2	San Gerardo Station, Monteverde, Costa Rica; 1200 m asl 10°21.826'N, 84°47.413'W	27 Dec 2007
Spt3	Sanctuário Ecológico, Cerro Plano de Monteverde, Costa Rica; 1260 m asl 10°18.469'N, 84°49.464'W	20 Dec 2007
Spt4	Reserva Forestal Peñas Blancas, Monteverde, Costa Rica; 985 m asl 10°18.290'N, 84°44.199'W	31 Dec 2007
Spt5	San Gerardo Station, Monteverde, Costa Rica; 1090 m asl 10°22.112'N, 84°46.764'W	27 Dec 2007
Spt6	Ecolodge, San Luis de Monteverde, Costa Rica; 1005 m asl 10°16.82'N, 84°47.27'W	24 Dec 2007
Spt7	Reserva Forestal Peñas Blancas, Monteverde, Costa Rica; 919 m asl 10°18.179'N, 84°44.466'W	31 Dec 2007
Spt8	San Gerardo Station, Monteverde, Costa Rica; 1200 m asl 10°22'N, 84°47'W	11 Jan 2005
Spt9	San Gerardo Station, Monteverde, Costa Rica; 1200 m asl 10°22'N, 84°47'W	28 Jan 2005
Spt10	San Gerardo Station, Monteverde, Costa Rica; 1250 m asl 10°22'N, 84°47'W	24 Feb 2005
Spt11	Las Cruces Station, San Vito, Costa Rica; 1175 m asl 8°47.073'N, 82°57.728'W	21 Mar 2009
Spt12	Rockwell Farm, Monteverde, Costa Rica; 1387 m asl 10°18'N, 84°48'W	25 Jul 2009
Spg1	Valle Sta. Maria, Parque Nacional Henri Pittier, Venezuela; 685 m asl 10°21'N, 67°49'W	13 Aug 2003
Spg2	Valle Sta. Maria, Parque Nacional Henri Pittier, Venezuela; ca. 700 m asl 10°21'N, 67°49'W	11 Aug 2003
Spg3	Valle Sta. Maria, Parque Nacional Henri Pittier, Venezuela; 700 m asl 10°21'N, 67°49'W	07 Jul 2002

All samples were collected from raiding or emigration columns on the forest floor with the exception of colony Spg3, for which the nest was found under a stone. Reference specimens have been deposited in the ant collection at the Museum of Comparative Zoology, Harvard University.

observed heterozygosities (H_O) and numbers of alleles (A): SpgA12 ($H_O = 0.867$, $A = 6$), SpgA37 ($H_O = 0.852$, $A = 4$), SpgA40 ($H_O = 0.500$, $A = 5$), SpgB6 ($H_O = 0.750$, $A = 7$), SpgB11 ($H_O = 0.133$, $A = 2$), SpgB20 ($H_O = 0.597$, $A = 5$), SpgB27 ($H_O = 1.000$, $A = 8$), SpgB28 ($H_O = 0.633$, $A = 5$), and SpgB33 ($H_O = 0.782$, $A = 5$). The properties of these loci and technical details are given in Kronauer *et al.* (2010a). Four of these loci were also polymorphic in *S. pentadentata*: SpgA37 ($H_O = 0.653$, $A = 7$), SpgB20 ($H_O = 0.192$, $A = 4$), SpgB27 ($H_O = 0.682$, $A = 13$), and SpgB28 ($A = 16$). The *S. pentadentata* data for locus SpgB28 were in full accordance with the remaining data set in that they did not uncover any additional queen-mates, but we discarded this locus from all formal population genetic analyses because of the high frequency of null alleles in this species.

The numbers of genotyped offspring (workers and diploid brood) per queen are given in Table 2. Brood was genotyped in addition to adult workers whenever available. This can be helpful to disentangle pedigree structures in army ants, because shortly after a colony fission event, workers, but not brood, will be mixed offspring of both the mother and daughter queen (Kronauer *et al.* 2004). Additionally, we genotyped the following samples, which are not listed in Table 2: (i) the queen of colony Spg1, which was used to reconfirm the pedigree reconstruction for that colony based on worker genotypes; (ii) two workers of *S. pergandei* from a pitfall sample collected at the same location as the colony series (these genotypes were only used to increase

the sample for estimating background allele frequencies for *S. pergandei*); (iii) one haploid (male) larva from colony Spg3; and (iv) six separately collected *S. pentadentata* workers from Costa Rica (representing six different colonies; these genotypes were only used to estimate background allele frequencies for *S. pentadentata*).

Data analysis

All analyses were conducted separately for the two species. Measures of observed heterozygosity (H_O) were based directly on the observed worker genotypes and were calculated in the program FSTAT version 2.9.3.2 (Goudet 2002). We initially used the program MATESOFT version 1.0 (Moilanen *et al.* 2004) to deduce the genotypes of all colony queens and their mates with all microsatellite alleles set to equal frequencies. Together with the independently collected samples described above, the deduced queen and male genotypes were then used to estimate population allele frequencies and expected heterozygosities (H_S) in FSTAT. The additional initial step is necessary because workers within social insect colonies are related and therefore do not constitute independent samples for population genetic analyses (e.g. Berghoff *et al.* 2008). Using unbiased population allele frequencies we then calculated mating frequency statistics in MATESOFT. These included the observed number of patriline in each colony (k_{obs}), the observed (D_{obs}) and estimated (D_{est}) proportion of double mated queens, the average pedigree effective mate number ($m_{e,p}$), and the average weighted nonidentification error (f'), i.e. the probability of not detecting a second male because of sample size or genetic marker limitations (Pedersen & Boomsma 1999). Bootstrapping over groups was used to estimate 95% confidence intervals (95% CI) for f' .

Average regression relatedness between the workers within each colony was calculated in the program RELATEDNESS version 5.0.8 (Goodnight & Queller 1998), using the unbiased background allele frequencies from FSTAT. 95% CIs were calculated by bootstrapping over loci. If diploid queens mate only with a single, unrelated, haploid male, then workers within colonies should be exclusively full-sisters and related to each other by $r_{\text{ww}} = 0.75$. If queens mate once with a related male and inbreeding occurs, then r_{ww} should be >0.75 . We also calculated an inbreeding coefficient $G_{IS} = 1 - H_O/H_S$ (Nei 1987) with 95% CI from jackknifing over loci.

Results

All *Simopelta* colonies studied were headed by one singly mated queen ($k_{\text{obs}} = 1$). Accordingly, D_{obs} and D_{est} were 0 for both species, and $m_{e,p}$ was 1. The average

Table 2 Observed mating frequencies for 12 *Simopelta pentadentata* (Spt) and three *S. pergandei* (Spg) queens: n is the number of offspring (workers and diploid larvae) genotyped for microsatellite markers; k_{obs} is the observed number of patrilines among the genotyped offspring; r_{ww} is the average relatedness between colony workers

Queen	n workers	n diploid larvae	k_{obs}	r_{ww}
Spt1	15	0	1	0.764
Spt2	15	0	1	0.618
Spt3	15	0	1	0.739
Spt4	25	0	1	0.876
Spt5	15	0	1	0.918
Spt6	15	21	1	0.776
Spt7	15	0	1	0.786
Spt8	13	0	1	0.614
Spt9	15	0	1	0.592
Spt10	16	0	1	1.000
Spt11	15	0	1	0.780
Spt12	14	1	1	0.786
Spg1	10	21	1	0.607
Spg2	15	0	1	0.683
Spg3	17	2	1	0.669

weighted nonidentification error was low (*S. pentadentata*: $f = 0.02339$ (95% CI: 0.00983–0.03894); *S. pergandei*: $f = 0.00005$ (95% CI: 0.00001–0.00008), showing that we are unlikely to have missed cases of double mating due to limitations in sample size or marker resolution.

Within colony regression relatedness between workers was high as expected for both species, and 95% CI included $r_{ww} = 0.75$, the expected value under single queen mating and outbreeding [*S. pentadentata*: $r_{ww} = 0.788$ (0.661–0.914); *S. pergandei*: $r_{ww} = 0.660$ (0.569–0.751)]. Similarly, the 95% CI for the inbreeding coefficient G_{IS} included zero (*S. pentadentata*: $G_{IS} = 0.261$ (–0.078 to 0.601); *S. pergandei*: $G_{IS} = 0.050$ (–0.086 to 0.186)). Overall, there was no evidence for inbreeding in *Simopelta*, which suggests that queens are normally inseminated by unrelated males, as is the case in dorylomorph army ants (Kronauer *et al.* 2007a; Berghoff *et al.* 2008). However, given the large CI of our estimates, this result should be treated with caution.

A single mother queen could account for all sampled workers and diploid larvae in each of the 15 studied colonies. In social insects with reproduction by colony fission, such as AenEcDo army ants, worker offspring from both the mother- and the daughter-queen are expected to be present shortly after a fission event (e.g. Kronauer *et al.* 2004). Unfortunately, the present study did not provide additional information regarding the possibility of reproduction by colony fission in *Simopelta* because mixed offspring were never found.

Of the three late instar larvae sampled and genotyped from colony Spg3, one had a single allele at each of the nine microsatellite loci, while all workers from that colony were heterozygous at four to seven loci. We therefore concluded that this was a haploid male larva. Interestingly, while this male carried an allele that was also found in the colony queen at seven loci, it had the allele of the queen's mate at two loci (SpgB6 and SpgB27), which means that it was a worker son. Because two queen-produced female larvae of similar age were simultaneously present in the sample, worker reproduction in that colony must have occurred in the presence of the queen.

Discussion

All studied queens of the ponerine army ant genus *Simopelta* were inseminated by a single male, which is in sharp contrast to AenEcDo army ants (Kronauer *et al.* 2007a). This means that, while the life histories of the two groups are strikingly convergent, the mating systems represent opposite extremes (Boomsma *et al.* 2009). This implies that a high level of multiple mating by queens is not an integral part of the functionally defined army ant adaptive syndrome (Kronauer *et al.*

2007a; Kronauer 2009), but may specifically characterize AenEcDo army ants.

A similar discrepancy occurs between honeybees and stingless bees. The two groups resemble each other in many aspects of their ecology and life history: both are highly eusocial with a sophisticated division of labour, morphologically differentiated queen and worker castes, and large perennial societies that are headed by a single queen and reproduce by colony fission (e.g. Peters *et al.* 1999; Palmer & Oldroyd 2000). However, honeybee queens are highly polyandrous (Tapy *et al.* 2004), whereas queens of stingless bees have low mating frequencies and are typically monandrous (Peters *et al.* 1999; Green & Oldroyd 2002; Palmer *et al.* 2002; Tóth *et al.* 2002, 2004; Paxton *et al.* 2003). One of the leading hypotheses that could explain the evolution of multiple mating in honeybees is that polyandry reduces the genetic variance between colonies and thereby the average negative effect of diploid male production (Page 1980). In honeybees (and possibly many other social Hymenoptera) sex is determined by a single locus, where heterozygous individuals develop into females, hemizygous individuals develop into functional, haploid males, and individuals homozygous for the sex determining locus develop into sterile, diploid males, which impose a cost on colony fitness (Woyke 1980, 1981; Cook & Crozier 1995; Beye *et al.* 2003). If the relationship between colony fitness and the proportion of diploid male brood produced by a queen is concave, polyandry will be adaptive (Page 1980).

A concave relationship between colony fitness and the trait in question is particularly likely in species that reproduce by colony fission, such as honeybees, stingless bees, and army ants (Page 1980; Ratnieks 1990; Kronauer *et al.* 2007a). The difference in mating frequency between honeybees and stingless bees may have arisen because, unlike honeybees, stingless bees cannot detect and remove diploid male larvae at an early developmental stage, because brood cells are mass provisioned and sealed (Camargo 1982; Ratnieks 1990). This implies that the abovementioned relationship will not be concave and that any amount of diploid male load would fatally drain a colony's resources. Therefore, selection favours monandry, which maximizes the probability of having zero diploid male load. Furthermore, stingless bee workers are predicted to execute their queen if she has mated with a male that carried an identical sex-locus allele and therefore produces diploid males, and to rear a replacement queen (Ratnieks 1990; Page & Kerr 1990).

The difference in mating system between AenEcDo and ponerine *Simopelta* army ants at first seems difficult to reconcile. Like all ants, both are in constant contact with their brood, so there is no obvious reason to

assume that AenEcDo army ants should be able to detect and remove diploid males earlier. However, it is not clear which role diploid male load plays in the evolution of army ant mating systems, as neither the sex determining system nor diploid males are known (Kronauer *et al.* 2007a). One striking difference between the two groups, however, is colony size and complexity. The smallest AenEcDo army ant colonies are those of the genus *Aenictus* with *c.* 100 000 workers, while African *Dorylus* (*Anomma*) army ant colonies can have *c.* 15 million workers (Schneirla 1971). Such huge insect societies face immense challenges when it comes to defence against parasites, worker task allocation, and the maintenance of colony homeostasis, all of which have been suggested to favour genetic diversity and therefore polyandry (Hamilton 1987; Sherman *et al.* 1988; Oldroyd & Fewell 2007; Boomsma *et al.* 2009). Furthermore, queens of species with large colony sizes have to store large amounts of sperm, which also might promote multiple mating (Cole 1983). *Simopelta* colonies, on the contrary, only number *c.* 1000 workers (Gotwald & Brown 1966), which is two to four orders of magnitude less than the colonies of AenEcDo army ants. This overall difference in colony size and complexity might ultimately explain the difference in queen mating frequency.

With some exceptions, ant species in the subfamily Ponerinae have low levels of queen-worker dimorphism, monomorphic worker castes, small- to medium-sized colonies, and rather 'primitive' levels of social organization compared with many other ant groups, although their reproductive cycles and mechanisms of social regulation are extremely diverse (Peeters 1997; Hölldobler & Wilson 2009). This suggests that strict monandry might be particularly common in Ponerinae, although detailed genetic data to support this are presently available for only a few species (André *et al.* 2001; Hartmann *et al.* 2005). *Simopelta* might therefore be a rather typical ponerine with respect to the mating system, despite its army ant lifestyle. This is unlikely to be simply due to phylogenetic inertia, as the queens of at least two *Pachycondyla* ponerine ants often mate with multiple males (Kellner *et al.* 2007).

Probably as a corollary of the difference in mate number, honeybees and stingless bees differ in several other important aspects of sociality, many of which are related to male production (Peters *et al.* 1999; Tóth *et al.* 2004). While workers of most eusocial hymenopterans cannot mate, they have usually retained the ability to lay unfertilized, haploid eggs that can develop into males (Bourke 1988). Because a worker is more closely related to her own sons ($r = 0.5$) than to the sons of the queen (0.25), the sons of a full-sister (0.375), or the sons of a half-sister ($r = 0.125$), a worker should always pre-

fer her own reproduction over that of her nestmates. Following the same logic, the worker collective should prefer worker sons over queen sons in colonies where workers are full-sisters, which is in conflict with a queen's interests, as she would prefer her own sons. However, if the effective mating frequency of the queen is higher than two, enough workers will be half-sisters so that the worker collective should always prefer queen sons over worker sons (Ratnieks 1988). Under these circumstances, workers are expected to prevent the reproduction of other workers, normally by eating their eggs or by showing aggression towards workers with developed ovaries. This phenomenon is known as worker-policing (Ratnieks 1988) and has been demonstrated in many social insect species (overviews are given in Ratnieks *et al.* 2006; Wenseleers & Ratnieks 2006). In accordance with theory, worker oviposition is rare in polyandrous honeybees, worker-laid eggs are eaten (policed) by other workers, and males produced by queenright colonies are nearly exclusively the sons of the queen. In contrast, in monandrous stingless bees, worker oviposition is common, policing is typically exerted by the queen, and workers often produce a significant proportion of the males, even in queenright colonies (Peters *et al.* 1999; Tóth *et al.* 2002, 2004; Paxton *et al.* 2003; but see Palmer *et al.* 2002).

Similar to honeybees, in polyandrous AenEcDo army ants, males in queenright colonies are exclusively queen-sons (Kronauer *et al.* 2006, 2007b), despite workers having retained the capacity to reproduce (Whelden 1963; Raignier 1972; Kronauer *et al.* 2010b). This could be either because of active worker-policing or worker self-restraint, which in turn would probably have evolved under an efficient worker-policing system (Wenseleers *et al.* 2004; Dijkstra *et al.* 2005; Ratnieks *et al.* 2006). *Simopelta* males are not known and will probably be difficult to collect in any significant numbers, especially within their mother colonies. However, we were lucky enough to sample a single male larva which, together with our data on colony pedigree structure, proves that males and sexual reproduction are part of the *Simopelta* life cycle. While this single male *Simopelta* larva does not allow us to draw any general conclusions, it is interesting to note that it was a worker-son produced in a queenright colony. This, again, is in sharp contrast to AenEcDo army ants and suggests that, as in honeybees and stingless bees, the difference in mating system may be associated with different outcomes of the conflict over male parentage.

An overview of relevant life history and social traits for honeybees, stingless bees, AenEcDo army ants, and *Simopelta* army ants, including the findings of this study, is given in Table 3. While striking overall similarities exist between the AenEcDo-*Simopelta* army ant

Table 3 Overview of general life history traits of honeybees, stingless bees, AenEcDo army ants, and *Simopelta* army ants

Trait	Honeybees	Stingless bees	AenEcDo army ants	<i>Simopelta</i> army ants
Queen mating frequency	High	Low	High	Low
Colony size in number of workers	5×10^3 – 5×10^4	10^3 – 6×10^4	10^5 – 10^7	10^3
Numerical sex-ratios	Male-biased	Male-biased	Male-biased	?
Diploid male load	Yes	Yes	?	?
Worker reproduction in queenright colonies	No	Yes	No	Yes

A question mark indicates missing information. All taxa reproduce by colony fission. The colony size ranges for honeybees and stingless bees are according to Oldroyd & Wongsiri (2006) and Roubik (1979). Remaining references are given in the text.

and the honeybee–stingless bee contrasts, it is also clear that the difference in social complexity, which might account for differences between the two groups of army ants, does not apply to the latter contrast.

It has proved difficult to develop a single predictive theory for the evolution of mating systems in the eusocial Hymenoptera (Crozier & Page 1985; Boomsma & Ratnieks 1996; Palmer & Oldroyd 2000; Strassmann 2001; Crozier & Fjerdingstad 2001; Boomsma *et al.* 2009). This study underlines that taxa with highly convergent overall life histories can have strikingly different mating systems. The best explanation for this contrast between AenEcDo army ants and *Simopelta* seems to be the difference in colony size and social complexity in general. This finding will be important in future comparative analyses of social insect mating systems and social conflicts. With the advent of genomic resources for a variety of ant species, we will hopefully soon have a better understanding of the role of sex-determination and diploid male load in the evolution of mating systems. Additional studies on *Simopelta* and other ponerine army ants, such as some Asian *Leptogenys* species, should further elucidate the fundamental similarities and differences between ponerine and AenEcDo army ants.

Acknowledgements

We thank John Lattke, John T. Longino, Edith Rodríguez, and Alex Wild for contributing samples. Susan Bulova, Anjali Kumar, Corina Logan, Siobhan O'Donnell, Tom Soare, and Sean Tully assisted with field observations and specimen collections. D.J.C.K. is supported by the Harvard Society of Fellows, S.O'D. by the University of Washington Royalty Research Fund and by NSF grant 0347315, and N.E.P. by NSF SES-0750480. Costa Rican specimens were collected under research permits from MINAE Costa Rica: scientific passports 0387 and 01667, and a 2009 Organization for Tropical Studies field course permit to S.O'D. The Organization for Tropical Studies assisted with logistics and research permitting in Costa Rica. This contribution is dedicated to Carl W. Rettenmeyer, one of the world's great myrmecophiles and passionate student of army ants, who sadly passed away on April 9th 2009.

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All four authors share a long-standing interest in the evolution of insect societies. DJCK wants to understand how the genetic composition of insect colonies affects social life, and how social behavior is regulated on a molecular level. Army ants are his main study systems. SO'D studies neuroecology and community ecology of Neotropical social insects, including paper wasps and army ants. He is especially interested in the biology of montane forests. JJB works on a wide array of questions pertaining to social insect biology. NEP is broadly interested in symbiotic interactions, especially those involving ants.
