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Genetic evidence for intra- and interspecific slavery in honey ants (genus *Myrmecocystus*)

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The New World honey ant species *Myrmecocystus mimicus* is well known for its highly stereotyped territorial tournaments, and for the raids on conspecific nests that can lead to intraspecific slavery. Our results from mitochondrial and nuclear markers show that the raided brood emerges in the raiding colony and is subsequently incorporated into the colony's worker force. We also found enslaved conspecifics in a second honey ant species, *M. depilis*, the sister taxon of *M. mimicus*, which occurs in sympatry with *M. mimicus* at the study site. Colonies of this species furthermore contained raided *M. mimicus* workers. Both species have an effective mating frequency that is not significantly different from 1. This study provides genetic evidence for facultative intra- and interspecific slavery in the genus *Myrmecocystus*. Slavery in ants has evolved repeatedly and supposedly by different means. We propose that, in honey ants, secondary contact between two closely related species that both exhibit intraspecific slavery gave rise to an early form of facultative interspecific slavery.

Keywords: dulosis; brood raiding; mating frequency; sociogenetics; intracolony relatedness

1. INTRODUCTION

Slavery is a common feature in the life histories of several ant species, ranging from the occasional capture of foreign brood as a by-product of intraspecific territorial conflicts to highly specialized obligate interspecific slavery (Hölldobler & Wilson 1990). Interspecific slavery in ants (where it is referred to as dulosis) is possible because a brood that emerges in a foreign nest acquires and learns the recognition label of the foster colony (Lenoir *et al.* 2001). Therefore, raided workers normally function like regular colony members. The New World honey ants of the genus *Myrmecocystus* inhabit arid and semi-arid environments and owe their trivial name to a specialized caste, the so-called honey-pots or repletes. These sweet-tasting individuals store food in their highly expanded gasters and can nourish the colony during times of scarcity. *Myrmecocystus mimicus* is the first species in which intraspecific slavery in ants was documented (Hölldobler 1976). Frequent shifts in a colony's spatio-temporal territory result in regular home-range overlaps with neighbouring colonies (Hölldobler & Lumsden 1980), which often lead to highly stereotyped display fights at temporary territorial boundaries. These tournaments can last for several days, but once a colony demonstrates its superiority the inferior one is constrained in its foraging activity and in the extreme outcome is raided and larvae, pupae, callow workers and honeypots are captured by the superior colony (Hölldobler 1976, 1981). However, mature colonies also appear to scout out incipient or smaller colonies in their neighbourhood and subsequently raid and destroy them. Raiding behaviour in *M. mimicus* may be rooted in the colony founding phase. Bartz & Hölldobler (1982) reported dense aggregations of founding nests for a population from Cochise County (AZ, USA), and laboratory experiments revealed intercolony raiding, during which those colonies that acquired the largest numbers of workers emerged as final victors. In the *M. mimicus* population studied, fast initial colony growth is also accomplished by pleometrosis (association of several founding queens). Both strategies have also been reported for other species, which do not conduct slave raids at a mature colony stage (Tschinkel & Howard 1983; Rissing & Pollock 1987). It has to be stressed that slavery in *Myrmecocystus* is facultative and colonies are in no way dependent on enslaved workers as is the case in obligate slave-making species.

Myrmecocystus depilis has been proposed to be the sister taxon of *M. mimicus* by Snelling (1976). The two species occur in sympatry at the study site and resemble each other considerably in ecology and foraging behaviour. Hölldobler (1981) also reports tournament interactions for *M. depilis*, but these differ considerably from those of *M. mimicus* in being temporally less stable and in tending to escalate more easily into physical fights which is rarely the case in *M. mimicus* tournaments. Furthermore, it has never been shown that these combats culminate in brood raids. Although tournaments between the two species have not been observed under natural conditions, *M. depilis* has been reported to pull workers of *M. mimicus* into the resident's colony, while *M. mimicus* tended to drag workers of the other species away from the nest entrance, when workers of the two species were artificially confronted with each other (Hölldobler 1981).

Although *M. mimicus* from the study site often founds colonies pleometrotically, mature colonies of *M. mimicus* and *M. depilis* appear to be exclusively monogynous (Bartz & Hölldobler 1982; Bedir 1998); supernumerary queens are eliminated early during colony development (Bartz & Hölldobler 1982).

In interspecific slavery, the slave ant species can easily be identified in mixed colonies, but this is not possible in cases of intraspecific slavery. Therefore, it remains unclear, for intraspecific slavery, to what extent the raided

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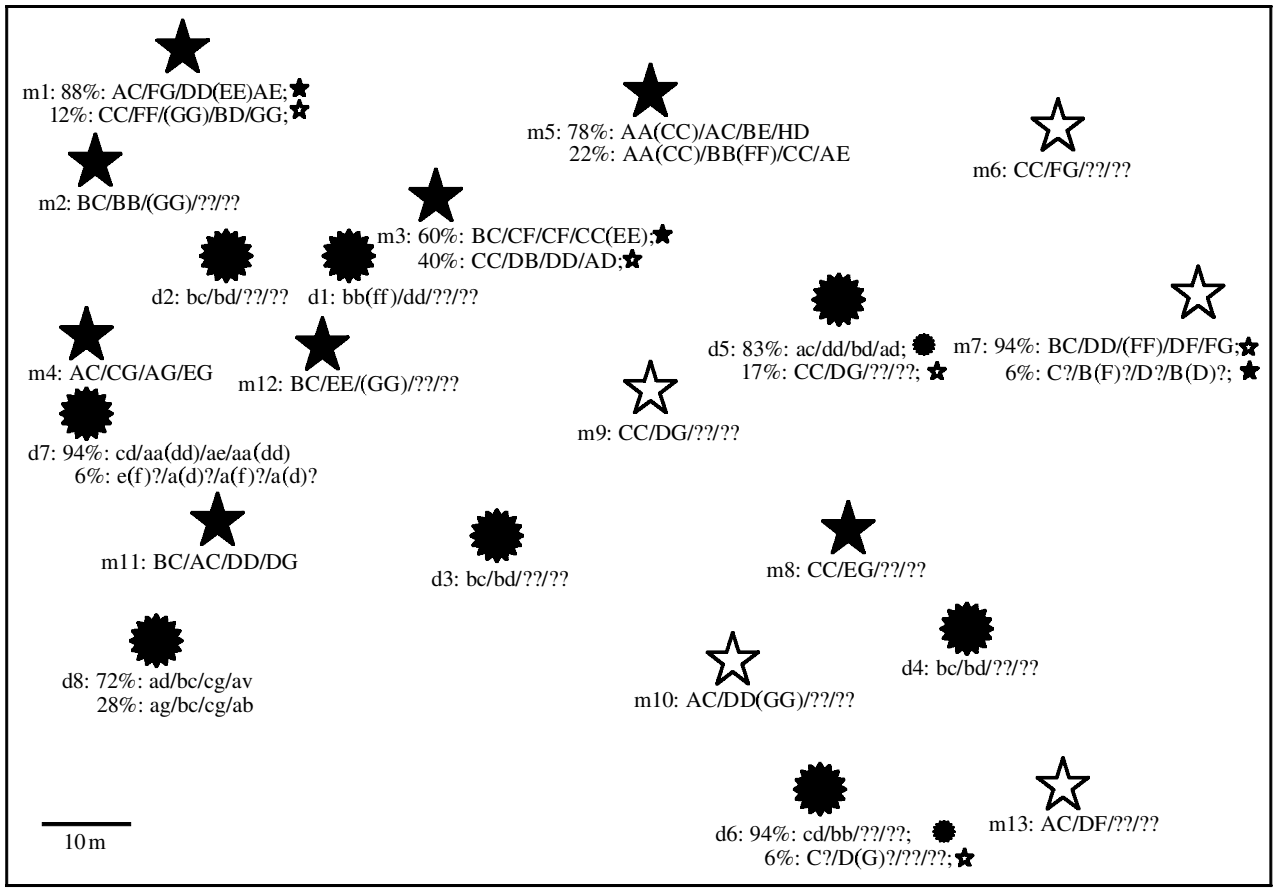

Figure 1. Schematic map of the study site in Cochise County (AZ, USA). Large symbols represent the location of the colony and the mitochondrial haplotype of the colony's queen. Filled and empty pentagrams are haplotype M1 and M2, respectively and the 16-point star symbols represent haplotype D1 (see table 2). Upper-case letters represent *M. mimicus* alleles and lower-case letters stand for *M. debilis* alleles. Also given is the percentage of queen's offspring (see the first line below the large symbol) and raided workers (second line) as well as the mitochondrial (small symbols if different from the colony's queen) and nuclear haplotype of the queen. Undetermined genotypes are indicated by question marks.

brood ecloses under natural conditions. We used mitochondrial and nuclear DNA markers to study and quantify the effect of intraspecific (and potentially interspecific) brood raiding on colony structure in the populations of *M. mimicus* and *M. depilis* from Cochise County, AZ, USA, mentioned earlier.

2. MATERIAL AND METHODS

(a) *Sample collection and DNA extraction*

Samples from 13 and eight mature colonies with totals of 324 and 135 individuals of *M. mimicus* and *M. depilis*, respectively, were obtained from the study site in Cochise County (32°08′ N, 109°05′ W). The spatial distribution of the colonies is shown in figure 1. Foraging workers were sampled directly from the colony entrance. Specimens were stored in 95% ethanol and DNA was extracted following a standard phenol–chloroform protocol (Gadau *et al.* 1996).

(b) *Mitochondrial markers*

We designed primers to amplify a 241 bp fragment of mitochondrial DNA, including parts of *COI*, the intergenic spacer region, and parts of *tRNA Leu*. Using Poland analysis (Steger 1994), a short GC-clamp of 23 bp was added to the 5′ end of the reverse primer to obtain multiple melting domains (primer sequences are given in table 1). PCR amplification was performed on a T1 Thermocycler (Biometra) in a total reaction volume of 25 μl containing *ca.* 10 ng of template DNA, 1 × PCR buffer (10 mM of Tris–HCl, 50 mM of KCl, 0.08% Nonidet P40), 2 mM of $MgCl_2$, 240 μM of deoxynucleotide triphosphates, 800 μM of each primer and 1.25 U of Taq DNA Polymerase (MBI Fermentas). Cycle parameters were as follows: 3 min at 94 °C, followed by 30 cycles of 94 °C for 1 min, 50 °C for 1 min and 72 °C for 1.5 min, and a final extension time of 5 min at 72 °C.

PCR products were mixed in equal volumes with a standard PCR product of known genotype. The mixture was denatured at 95 °C for 5 min and subsequently renatured at 50 °C for 5 min.

A total of 2.5 μl of loading buffer (0.1% Triton-X 100, 0.01% bromophenol blue dye and 0.01% xylene cyanol dye in 0.1 × TBE) was added to 2.5 μl of the sample. The resulting solution (total of 5 μl) was loaded onto a polyacrylamide gel (8% acrylamide, 7 M urea, 0.1 × TBE, 2% glycerol, 0.22% TEMED (N,N,N′,N′-tetramethylethylenediamine) and 0.016% ammonium persulphate in aqua bidest.) and run on a TGGE (temperature gradient gel electrophoresis) MAXI System (Biometra) at 300 V for 6 h over a temperature gradient from 46 °C to *ca.* 50 °C. Gels were stained with SYBR Green. As partial melting occurs at a lower temperature in heteroduplices, velocity is decreased owing to the resulting fork-like structure of the DNA fragment. Sequence differences between the sample and the standard result in one additional visible band (figure 2).

Table 1. Primer sequences for temperature gradient gel electrophoresis analysis.
(A 241 bp fragment including parts of *COI*, the intergenic spacer region and parts of *tRNA Leu* was amplified using the primers VARf and LeuClamp.)

primer	direction	sequence 5′–3′	reference position[a]
VARf	forward	GAATCTTTATCTTCTAAACG	3209–3229
LeuClamp	reverse	CCCGCCGCGCCCCGCCGCCCGCCGGGGTTTAAATCCAATGCAC	3373–3392

[a] Reference positions are relative to the *Apis mellifera* mitochondrial genome (Crozier & Crozier 1993).

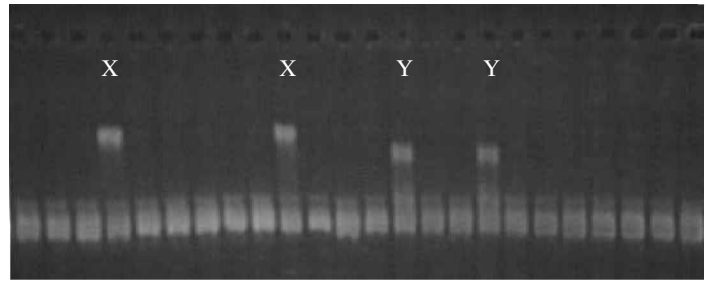

Figure 2. The TGGE analysis of 10 and 14 workers of a *M. depilis* colony (lanes 1–10) and a *M. mimicus* colony (lanes 11–24), respectively, as described in § 2b. Lanes 4 and 10 (X) represent two *M. mimicus* workers incorporated into a *M. depilis* colony (interspecific raiding); lanes 14 and 17 (Y) represent two *M. mimicus* workers of a different matriline in a *M. mimicus* colony (intraspecific raiding).

Table 2. Sequence differences of the three mitochondrial haplotypes that could be distinguished by TGGE (see table 1 and figure 2).
(Deletions are represented by '–'.)

haplotype	34	35	44	110	115	157	176	177	178	190	191	203	GenBank accession number
M1	A	T	T	T	C	A	–	–	T	T	C	T	AF529216
M2	C	C	C	T	C	A	–	–	–	T	C	–	AF529217
D1	A	T	C	C	T	T	A	A	T	C	T	T	AF529218

A total of 89 samples from 13 *M. mimicus* colonies and 69 samples from eight *M. depilis* colonies were analysed. Two *M. mimicus* (M1 and M2) and one *M. depilis* (D1) haplotypes were distinguished using TGGE. All three haplotypes were sequenced. The sequence differences are shown in table 2. The distribution of the three haplotypes in the studied population is given in figure 1.

(c) *Nuclear markers*

Samples from both species were initially genotyped for the two nuclear microsatellite markers Mm1 and Mm2 (Kronauer & Gadau 2002). When worker genotypes were incompatible with a colony structure of a single mated queen, individuals were genotyped for two additional microsatellite loci, Mm3 and Mm4 (Kronauer & Gadau 2002). See table 3 for numbers of analysed individuals and figure 1 for the queens' nuclear haplotypes. The molecular methods and properties of these loci in the studied population are described in Kronauer & Gadau (2002). Regression worker–worker relatedness, R, and Wright's inbreeding coefficient, F_{is}, were calculated from the microsatellite data, weighting colonies equally, with the program Relatedness v. 4.2c (Goodnight 1994), which uses algorithms developed by Queller & Goodnight (1989). The effective mating frequency, m_e, was calculated according to Pamilo (1993) and Crozier & Pamilo (1996), using the formula $m_e = 0.5/(R - 0.25)$ and assuming that the males are unrelated. Standard errors were estimated by jack-knifing over the colonies. All values in § 3 are expressed as mean ± s.e.

Whenever more than one matriline could be identified in a colony, workers from the most common matriline were assumed to be descendants of the colony's queen, while all other workers were denoted as raided.

3. RESULTS

(a) *Inbreeding, mating frequency and average worker–worker relatedness*

In *M. mimicus*, no evidence of inbreeding could be detected in the study population ($F_{is} = -0.004 \pm 0.067$) and the effective mating frequency was not significantly different from 1 ($m_e = 1.154 \pm 0.186$). Out of the 13 colonies studied, 11 were monandrous, one (m4) was double mated and one (m11) was triple mated. Average worker–worker relatedness (R_{pop}) rose from 0.603 ± 0.069 to 0.683 ± 0.060 when raided workers were treated as such (if fewer than four raided workers were detected in a colony sample, they were excluded from the analysis, otherwise the raided workers from a colony were treated as an additional colony).

Table 3. Colony structure and the distribution of raided individuals in 13 and eight colonies of *M. mimicus* (m1–m13) and *M. depilis* (d1–d8), respectively.

colony (total n)[a]	n two loci/n four loci	R[a]	excluded/ subdivided[b]	R excluded/ subdivided[c]	minimum number of patrilines[d]	minimum number of matrilines[e]	status of slaves[f]	slavery type[g]
m1 (17)	5/12	0.515	2 excluded	0.775	1 + 1	2	confirmed	intra
m2 (36)	36/0	0.847		0.852	1	1	none	
m3 (10)	0/10	0.437	subd. 60/40%	0.645/0.589	1 + 2	3	confirmed	intra
m4 (53)	41/12	0.310	subd. 55/45%	0.781/0.624	2	2	none	
m5 (36)	24/12	0.481	subd. 78/22%	0.728/0.922	7	2	suggestive	intra
m6 (18)	18/0	0.842		0.852	1	1	none	
m7 (17)	5/12	0.662	1 excluded	0.729	1 + 1	2	confirmed	intra
m8 (18)	18/0	0.833		0.837	1	1	none	
m9 (16)	16/0	0.832		0.843	1	1	none	
m10 (31)	31/0	0.739		0.757	1	1	none	
m11 (36)	24/12	0.323	subd. 61/39%	0.616/0.722	3	3	none	
m12 (18)	18/0	0.753		0.774	1	1	none	
m13 (18)	18/0	0.696		0.691	1	1	none	
d1 (10)	10/0	1.000		1.000	1	1	none	
d2 (18)	18/0	0.529		0.595	1	1	none	
d3 (18)	18/0	0.564		0.621	1	1	none	
d4 (18)	18/0	0.638		0.665	1	1	none	
d5 (18)	6/12	0.837		0.848	1	1	confirmed	inter
d6 (18)	18/0	0.892		0.895	1	1	confirmed	inter
d7 (17)	5/12	0.787	1 excluded	0.856	3	2	suggestive	intra
d8 (18)	6/12	0.371	subd. 72/28%	0.550/0.619	11	3	suggestive	intra

[a] n, number of individuals genotyped; R, intracolonial worker–worker relatedness.
[b] If fewer than four enslaved workers were detected in a colony, they were excluded from the analysis; otherwise the colony was subdivided.
[c] R values after exclusion of raided workers or colony subdivision. Note that the differences in the R values of colonies without raided workers arise from changes in population-wide allele frequencies due to the exclusion of raided workers and colony subdivisions.
[d] Minimum number of patrilines necessary to explain genotypes (assuming a single queen in colonies where the presence of additional matrilines has not been proven by the mitochondrial marker).
[e] Minimum number of monandrous matrilines necessary to explain genotypes.
[f] The status of slaves was considered to be confirmed when different mitochondrial haplotypes were found in one colony, suggestive if assuming several matrilines could better explain the microsatellite genotypes and if microsatellite genotypes appeared at unusual frequencies, and none if no unambiguous indication for enslaved workers was detected.
[g] This indicates whether slavery was intraspecific or interspecific.

In addition, in *M. depilis*, F_{is} was not significantly different from zero (0.057 ± 0.185) and m_e was not significantly different from 1 (0.976 ± 0.216). All *M. depilis* colonies could be accounted for by a single-mated queen when raided workers had been identified and discarded from the analysis. R_{pop} was 0.719 ± 0.114 with intraspecifically raided workers included, and 0.763 ± 0.093 when they were treated as foreign to a colony.

Table 3 lists the effect of raided workers on intracolonial worker–worker relatedness in both species.

(b) *Intraspecific raiding*

Multiple matrilines in a single colony were unambiguously identified by our mitochondrial marker in three *M. mimicus* colonies (m1, m3 and m7), with the second or second and third matriline accounting for 12%, 40% and 6% of the sampled worker force, respectively. In all cases, these results were confirmed by our microsatellite analysis.

In a fourth *M. mimicus* colony (m5), a second matriline, represented by 22% of sampled workers, was identified using microsatellite markers. Alternatively, one could assume in this case a single queen and seven patrilines with genotype distributions deviating from the expected.

In colonies m1, m7 and m5 the raided workers can be explained by one additional single-mated matriline, while we have to assume two additional single-mated matrilines or one additional double-mated matriline to account for raided workers in colony m3.

In *M. depilis*, intraspecifically raided workers were identified in two colonies using microsatellite markers. In colony d7, 94% of the sampled workers can be explained by one single-mated matriline. However, 6% (one individual) would add two additional patrilines, resulting in highly unexpected genotype frequencies, if we wanted to explain all workers by a single queen. Alternatively, one additional matriline can be assumed. Unexplained genotypes from 28% of sampled workers in colony d8 can be explained by assuming one additional double-mated or two additional single-mated queens. Alternatively, we could assume one queen and 11 patrilines with genotype distributions deviating from the expected.

By discarding raided workers from the analysis, we can account for all colonies that were identified to contain raided workers by one single-mated queen.

(c) *Interspecific raiding*

In two *M. depilis* colonies (d5 and d6), three (17%) and one (6%) raided *M. mimicus* workers, respectively, were detected by microsatellite analysis and confirmed by TGGE.

No raided *M. depilis* workers were detected in *M. mimicus* colonies.

The status and contributions of intra- and interspecifically raided workers to the colony structure are summarized in table 3 (columns 4 and 8) and figure 1.

4. DISCUSSION

Based on mitochondrial markers we found that three *M. mimicus* colonies out of 13 contained workers of different matrilines. In addition, we identified a second matriline in a fourth *M. mimicus* colony (m5) and in two out of eight *M. depilis* colonies (d7 and d8), using microsatellite markers. Alternative explanations for these latter cases would be a single queen per colony that was seven (*M. mimicus,* m5) and 11 (*M. depilis*, d8) times mated. Given that the effective mating frequencies of both honey ant species are not significantly different from one, as is the case in most ant species (Strassmann 2001), this alternative scenario is most unlikely. In the second case of intraspecific slavery in *M. depilis* (d7), we would need to assume one triple-mated queen instead of one single-mated queen to account for one worker. This, too, seems highly unlikely.

Intraspecifically enslaved workers were found in 31% of the *M. mimicus* and 25% of the *M. depilis* colonies studied, accounting for 6–40% (mean 20%) and 6–28% (mean 17%) of investigated workers per colony, respectively. However, this can be only a minimum estimate, especially when the studied population is viscous, or the overall genetic diversity is low. It is also not clear whether our TGGE approach was appropriate for detecting very small differences between mitochondrial haplotypes or whether additional microsatellite loci would have turned up further slave workers.

Non-detection is not a problem when it comes to interspecific slavery. Raided *M. mimicus* workers were detected in 25% of the investigated *M. depilis* nests, constituting between 6% and 17% (mean 12%) of studied workers per colony. These numbers are similar to those obtained for another facultative dulotic species, *Formica subnuda*, by Savolainen & Deslippe (1996). They estimated that one-third of the colonies contained slaves (of the species *F. podzolica*) with the proportion of slaves varying between 1% and 30%. Although our sample size for *M. mimicus* was 2.4 times greater than for *M. depilis*, no instance of interspecific slavery was observed in the reverse direction. This indicates that interspecific raiding might be unidirectional, or at least far more common in *M. depilis*.

Interspecific slavery (dulosis) occurs in several groups of ants, mainly in species of the subfamilies Formicinae and Myrmicinae. On considering phylogenetic relationships, it becomes apparent that this phenomenon has evolved independently several times (Alloway 1980; Hölldobler & Wilson 1990), with intraspecific territoriality and slavery, such as that described for *M. mimicus*, being the starting point for one of the hypothesized evolutionary pathways of dulosis. This hypothesis was promoted by Wilson (1975), Alloway (1980), Stuart & Alloway (1982) and Pollock & Rissing (1989). Once intraspecific slavery has evolved, similar territorial encounters between different species could result in functional facultative interspecific slavery, with the slave-making species retaining a completely functional worker caste. Both stages have been repeatedly reported for a variety of ant species (Hölldobler 1976; Alloway 1980; Rosengren & Pamilo 1983; Rissing & Pollock 1987; Pollock & Rissing 1989; Mori *et al.* 2001). Cases of intraspecific brood raids, probably as a consequence of territorial competition, have also been described for obligate dulotic species (Schumann 1992; Le Moli *et al.* 1993; Grasso *et al.* 1994). Finally, Buschinger (1970) proposed that brood transportation among nests within a single polygynous and polydomous colony could be extended to foreign colonies of the same or alien species and thereby eventually lead to intra- or interspecific slavery, respectively.

According to the rule of Emery (1909), interspecific slavery is much more likely to evolve between closely related species, as the communication systems of host and parasite have to be compatible. According to the strict version of the rule of Emery (1909), host and parasite have to be sister species, whereas the loose form of this rule contends that host and parasite might be more distantly related at present, although the ancestral host and parasite could have been sister species.

Different possible scenarios of an ancestral species splitting into a host and its parasite have been advanced and recently summarized by Lowe *et al.* (2002) for inquilines.

In the context of our present study, the evolutionary scheme proposed by Wilson (1971) is of particular interest. A single 'parental' species is divided into two 'daughter' species (in the present case *M. mimicus* and *M. depilis*), supposedly by allopatric speciation. When the newly formed species reinvade one another's ranges, they might exhibit considerable interspecific competition and territoriality owing to a broad niche overlap. The more aggressive species may be preadapted to raid not only conspecific nests (as both *M. mimicus* and *M. depilis* do), but also nests of the sister species. Several behavioural and ecological indicators suggest that *M. depilis* is the more aggressive species in this case. It may, in fact, represent an early stage on the evolutionary pathway to facultative dulosis. The final step in this evolutionary process would be the transition from a facultative to an obligate dulotic species with a worker caste highly specialized on raiding and the queen dependent on its host for colony founding. However, it has been argued that for obligate dulosis to evolve, the parasitic species has to be preadapted to this dependent way of colony founding, for example through the repeated adoption of foreign conspecific queens in a polygynous colony (Buschinger 1970, 1986; Alloway 1980; Topoff 1990). If this hypothesis holds, the *Myrmecocystus* system might be a 'dead end' for the evolution of obligate dulosis, as no such preadaptation is observed in honey ants.

It would now be a rewarding task to expand this analysis to other *Myrmecocystus* species, in parallel with a study of the phylogenesis of the genus. We also have to take into account the fact that there may be differences among populations of *M. mimicus* and *M. depilis.* For instance, it has been reported that certain populations of *M. mimicus* are predominantly pleometrotic (Wheeler 1917; Bartz &

Hölldobler 1982), while others are exclusively haplometrotic (Rissing *et al.* 2000). As availability of nesting sites, pleometrosis and brood raiding between founding colonies might very well be correlated (Bartz & Hölldobler 1982; Herbers 1986; Rissing & Pollock 1987), slavery could be a variable trait within this species, as is metrosis.

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REFERENCES

Alloway, T. M. 1980 The origins of slavery in Leptothoracine ants (Hymenoptera: Formicidae). *Am. Nat.* **115**, 247–261.

Bartz, S. H. & Hölldobler, B. 1982 Colony founding in *Myrmecocystus mimicus* Wheeler (Hymenoptera: Formicidae) and the evolution of foundress associations. *Behav. Ecol. Sociobiol.* **10**, 137–147.

Bedir, M. A. 1998 Nest structure and populations of two species of the honey ants, *Myrmecocystus depilis* and *Myrmecocystus mexicanus* (Hymenoptera: Formicidae). *J. Egypt. Ger. Soc. Zool.* **25**, 53–68.

Buschinger, A. 1970 Neue Vorstellungen zur Evolution des Sozialparasitismus und der Dulosis bei Ameisen (Hymenoptera: Formicidae). *Biol. Zentralbl.* **88**, 273–299.

Buschinger, A. 1986 Evolution of social parasitism in ants. *Trends Ecol. Evol.* **1**, 155–160.

Crozier, R. H. & Crozier, Y. C. 1993 The mitochondrial genome of the honeybee *Apis mellifera*: complete sequence and genome organization. *Genetics* **133**, 97–117.

Crozier, R. H. & Pamilo, P. 1996 *Evolution of social insect colonies—sex allocation and kin selection*. New York: Oxford University Press.

Emery, C. 1909 Über den Ursprung der dulotischen, parasitischen und myrmikophilen Ameisen. *Biol. Zentralbl.* **29**, 352–362.

Gadau, J., Heinze, J., Hölldobler, B. & Schmid, M. 1996 Population and colony structure of the carpenter ant *Camponotus floridanus*. *Mol. Ecol.* **6**, 785–792.

Goodnight, K. F. 1994 RELATEDNESS v. 4.2c. Houston, TX: Goodnight Software.

Grasso, D. A., Mori, A., D'Ettore, P. D. & Le Moli, F. 1994 Intraspecific raid and territoriality in *Polyergus rufescens* Latr. (Hymenoptera: Formicidae). *Ethol. Ecol. Evol.* (Special Issue 3), 81–87.

Herbers, J. M. 1986 Nest site limitation and facultative polygyny in *Leptothorax longispinosus*. *Behav. Ecol. Sociobiol.* **19**, 115–122.

Hölldobler, B. 1976 Tournaments and slavery in a desert ant. *Science* **192**, 912–914.

Hölldobler, B. 1981 Foraging and spatiotemporal territories in the honey ant *Myrmecocystus mimicus* Wheeler (Hymenoptera: Formicidae). *Behav. Ecol. Sociobiol.* **9**, 301–314.

Hölldobler, B. & Lumsden, C. J. 1980 Territorial strategies in ants. *Science* **210**, 732–739.

Hölldobler, B. & Wilson, E. O. 1990 *The ants*. Cambridge, MA: Harvard University Press.

Kronauer, D. & Gadau, J. 2002 Isolation of polymorphic microsatellite markers in the new world honey ant *Myrmecocystus mimicus*. *Mol. Ecol. Notes* **2**, 540–541.

Le Moli, F., Grasso, D. A., d'Ettore, P. & Mori, A. 1993 Intraspecific slavery in *Polyergus rufescens* Latr. (Hymenoptera, Formicidae): field and laboratory observations. *Insectes Soc.* **40**, 433–437.

Lenoir, A., D'Ettore, P. D., Errard, C. & Hefetz, A. 2001 Chemical ecology and social parasitism in ants. *A. Rev. Entomol.* **46**, 573–599.

Lowe, R. M., Ward, S. A. & Crozier, R. H. 2002 The evolution of parasites from their hosts: intra- and interspecific parasitism and Emery's rule. *Proc. R. Soc. Lond.* B **269**, 1301–1305. (DOI 10.1098/rspb.2002.2008.)

Mori, A., Grasso, D. A., Visicchio, R. & Le Moli, F. 2001 Comparison of reproductive strategies and raiding behaviour in facultative and obligatory slave-making ants: the case of *Formica sanguinea* and *Polyergus rufescens*. *Insectes Soc.* **48**, 302–314.

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

Pollock, G. B. & Rissing, S. W. 1989 Intraspecific brood raiding, territoriality, and slavery in ants. *Am. Nat.* **133**, 61–70.

Queller, D. C. & Goodnight, K. F. 1989 Estimating relatedness using genetic markers. *Evolution* **43**, 258–275.

Rissing, S. W. & Pollock, G. B. 1987 Queen aggression, pleometrotic advantage and brood raiding in the ant *Veromessor pergandei* (Hymenoptera: Formicidae). *Anim. Behav.* **35**, 975–981.

Rissing, S. W., Johnson, R. A. & Martin, J. W. 2000 Colony founding behavior of some desert ants: geographic variation in metrosis. *Psyche* **103**, 95–101.

Rosengren, R. & Pamilo, P. 1983 The evolution of polygyny and polydomy in mound-building *Formica* ants. *Acta Entomol. Fennica* **42**, 65–77.

Savolainen, R. & Deslippe, R. J. 1996 Facultative and obligate slavery in formicine ants: frequency of slavery, and proportion and size of slaves. *Biol. J. Linn. Soc.* **57**, 47–58.

Schumann, R. D. 1992 Raiding behavior of the dulotic ant *Chalepoxenus muellerianus* (Finzi) in the field (Hymenoptera: Formicidae, Myrmicinae). *Insectes Soc.* **39**, 325–333.

Snelling, R. R. 1976 A revision of the honey ants, genus *Myrmecocystus* (Hymenoptera: Formicidae). *Nat. Hist. Mus. Los Angeles County Bull.* **24**, 1–163.

Steger, G. 1994 Thermal denaturation of double-stranded nucleic acids: prediction of temperature critical for gradient gel electrophoresis and polymerase chain reaction. *Nucleic Acids Res.* **22**, 2760–2768.

Strassmann, J. 2001 The rarity of multiple mating by females in the social Hymenoptera. *Insectes Soc.* **48**, 1–13.

Stuart, R. J. & Alloway, T. M. 1982 Territoriality and the origin of slave raiding in Leptothoracine ants. *Science* **215**, 1262–1263.

Topoff, H. 1990 The evolution of slave-making behavior in the parasitic ant genus *Polyergus*. *Ethol. Ecol. Evol.* **2**, 284–287.

Tschinkel, W. R. & Howard, D. F. 1983 Colony founding by pleometrosis in the fire ant, *Solenopsis invicta*. *Behav. Ecol. Sociobiol.* **12**, 103–113.

Wheeler, W. M. 1917 The pleometrosis of *Myrmecocystus*. *Psyche* **24**, 180–182.

Wilson, E. O. 1971 *The insect societies*. Cambridge, MA: Belknap.

Wilson, E. O. 1975 Leptothorax duloticus and the beginnings of slavery in ants. *Evolution* **29**, 108–119.