

Queen number and raiding behavior in the ant genus *Myrmecocystus* (Hymenoptera: Formicidae)

Bert HÖLLDOBLER, Bernd GRILLENBERGER & Jürgen GADAU



Abstract

An experimental field study demonstrates that mature colonies of *Myrmecocystus mimicus* WHEELER, 1908 raid neighboring conspecific small colonies without preceding territorial tournament actions. We also report a total of 17 complete brood raids that did not originate from territorial tournaments, collected during 10 field research seasons. The number of captured brood and booty varied greatly: 6 - 137 larvae, 9 - 152 pupae, 0 - 4 callows, 0 - 23 honeypots. We also observed raiding ants transporting liquid food in their crops when they left the raided nest (49 - 409). Most likely, this food was solicited from honeypots inside the foreign nest. In general, the captured booty during these raids is considerably smaller than that retrieved during raids that originated from tournaments.

The socio-genetic analyses provided evidence that workers eclosing from raided brood become part of the work force of the raider colony. This was shown for *Myrmecocystus mimicus* and *M. depilis* FOREL, 1901. In *M. depilis*, we confirm previous findings by KRONAUER & al. (2003) of interspecific raiding (i.e., *M. depilis* raids *M. mimicus* but not vice versa). In addition, we provide genetic evidence for facultative polygyny in *M. mimicus*, and obligatory monogyny and occasional polyandry in *M. depilis*.

Key words: *Myrmecocystus mimicus*, *Myrmecocystus depilis*, intraspecific raiding, territoriality, polygyny, polyandry.

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Introduction

The honey ant species of the genus *Myrmecocystus* owe their trivial name to a special storage caste, the so-called honeypots or repletes. The crop in the gaster of these individuals is filled with sugar solutions and proteins (CONWAY 1977, 1990) and, depending on the species, in the most extended condition the gaster can reach sizes of a pea to a cherry. During times of scarcity, the honeypots regurgitate the contents of their crops to colony members. This food storage strategy is a special adaptation to life in arid regions. The genus *Myrmecocystus* has been the focus of several behavioral (e.g., HÖLLDOBLER 1976, HÖLLDOBLER & LUMSDEN 1980, HÖLLDOBLER 1981, BARTZ & HÖLLDOBLER 1982, LUMSDEN & HÖLLDOBLER 1983, HÖLLDOBLER 1986, LLOYD & al. 1989), ecological (e.g., HÖLLDOBLER 1982, DUNCAN & LIGHTON 1994, CHEW 1995, BEDIR 1998, SANDERS & GORDON 2000, COLE & al. 2001), and genetic studies (e.g., KRONAUER & GADAU 2002, KRONAUER & al. 2003).

Myrmecocystus mimicus WHEELER, 1908 and *M. depilis* FOREL, 1901 are sister species and belong to the subgenus *Endiodictes* (see SNELLING 1976, KRONAUER & al. 2004).

Both species are active during the day and forage on termites and dead insects, harvest floral nectar, and collect honeydew from hemipterous plant-sap feeding insects (SNELLING 1976). To defend these patchy and unpredictable food sources, *M. mimicus* and *M. depilis* establish spatio-temporal territories by conducting ritualized tournaments (HÖLLDOBLER & LUMSDEN 1980, HÖLLDOBLER 1981). If there is a substantial imbalance in strength (colony size) between opposing colonies, the stronger colony interferes with the foraging activity of the weaker colony by engaging the latter in tournament interactions directly at its nest area. These interference actions can still further escalate to all-out attacks, whereby the stronger colony raids the smaller nest, enslaves the worker brood and callows, pillages the honeypots and kills or drives off the queen (HÖLLDOBLER 1976, 1981). Such intraspecific raids also occur without preceding territorial interactions. Mature colonies literally scout for small incipient colonies in their neighborhood and raid them.

We present here the quantitative raiding data gathered during 10 field research seasons. These behavioral data are

compared with previous genetic results obtained by employing microsatellites and mitochondrial DNA sequences (KRONAUER & al. 2004) and new genetic data reported in this paper. The analysis of changes in genetic patterns of colony populations due to raiding has to take into account that in mature *Myrmecocystus mimicus* colonies occasionally more than one queen can be found (R. Mendez, pers. comm.). Most likely, these are instances of primary polygyny originating from pleometrotic colony founding, common in *M. mimicus*. Often, however, in such founding associations the queen number is reduced to monogyny during colony development (BARTZ & HÖLLDOBLER 1982). The situation is different for *M. depilis*. We never found pleometrotic founding groups in this species and we could never enforce pleometrotic colony founding in the laboratory.

Material and methods

The study site for collecting raiding data for *Myrmecocystus mimicus* was located in a mesquite-acacia community near Portal, Arizona, and Rodeo, New Mexico. Field data on raiding were collected during the summer months of 10 years spanning 1984 to 2003. Unfortunately, many raiding events we witnessed were useless for this paper, because we encountered them when they were already in progress and therefore it was impossible to determine the amount of brood captured during one raid. However, over a total of 10 field seasons we were able to observe 17 raids in their entirety. These raids occurred without any signs of preceding tournaments.

To test whether raiding behavior is initiated by scouts that discovered colonies within the home range of a resident colony, we conducted experiments with artificial incipient nests. To accomplish this, we excavated two mature colonies with the aid of a backhoe. From these colonies, small colonies consisting of approximately 100 to 200 workers, 30 larvae, 20 pupae and 5 - 10 honeypots, were housed in a test-tube nest. Each tube measured 15 × 2.2 cm, with water supply in the tube trapped at the bottom by cotton plugs. The tubes were closed with a cork stopper through which a plastic tube (diameter 0.6 cm) reached into the tube nest and served as nest entrance. After two days of acclimatization, the artificial incipient nests were buried 6 to 10 cm deep in the soil with the exit tube reaching the surface. The exit tube opening was surrounded by sand and little stone pebbles. Five such test nests each were placed in the surroundings of four mature *Myrmecocystus mimicus* nests in distances from four to 20 meters in arbitrarily chosen directions. We checked four times a day for a period of 18 days whether or not there was still activity at the entrance of the test-tube nests, and recorded any interactions between the mature nests and the surrounding test-tube nests.

Unfortunately, the original study site near Rodeo (used in the study KRONAUER & al. 2003) was not accessible when we conducted our second series of genetic analyses. Therefore, we had to choose a collection site a few kilometers away in the Chihuahuan desert (Cochise County, Arizona) at the Foothill Road. The plot follows the road for about 73 m and stretches for about 142 m to the northwest. The total area of the plot covers about 9700 m². We attempted to detect every *Myrmecocystus mimicus* and *M. depilis* colony by systematically surveying the terrain repeatedly.

Overall foragers of 12 *M. mimicus* colonies and 25 *M. depilis* colonies were collected in 2002 and 11 colonies of *M. mimicus* and 24 *M. depilis* in 2003. Of each colony between 10 and 50 individuals were collected directly at the entrance to avoid contamination with stray foragers of foreign colonies. Workers were immediately conserved in 95% ethanol.

On average, 20 individuals per colony (range 10 - 44) were analyzed using the *Myrmecocystus* microsatellite markers developed by KRONAUER & GADAU (2002). After testing the variability of these markers in *M. mimicus* and *M. depilis* with five individuals from five colonies of each species (n = 50), we decided that the following markers would provide a good resolution for our questions (*M. mimicus*: Mm1, Mm2, Mm3, Mm4 and Mm5; *M. depilis*: Mm1, Mm2, Mm4 and Mm5). DNA extraction was performed following a standard chloroform-phenol protocol employed by GADAU & al. (1996). The quality and amount of DNA was assessed on 0.8% agarose minigels. The extracted DNA solution was diluted 1:10 with purified water (HPLC-grade). The PCR conditions are based on the recommended protocols from LI-COR® and KRONAUER & GADAU (2002), but have been slightly modified. They are similar for all loci and species, and differed only in the concentration of primer and template in the master mix. The general reaction solution in a 10µl volume was: 1× PCR-buffer (10 mM Tris-HCL, 50 mM KCL, 0.08% Nonidet P40), 0.2 mM dNTPs, 1.75 mM MgCl₂, 0.5 U of *Taq* DNA Polymerase (MBI Fermentas), the suitable concentration of forward and reverse Primer (ranging from 0.05 to 0.10 pmol/µl) and 1 or 2 µl of the 1:10 diluted DNA solution. The reaction was performed in a "Biometra® T1 Thermocycler", an "Eppendorf® Mastercycler", or "Eppendorf® Mastercycler gradient" thermocycler with the following parameters: 3 min at 94°C, followed by 30 cycles of 94°C for 1 min, 50°C for 1 min, 72°C for 1.5 min, and a final extension time of 5 min at 72°C (KRONAUER & GADAU 2002).

The PCR products were analyzed using a LI-COR® 4300 DNA Analyser and the SAGA Generation 2 (LI-COR®, 2001) software. The SAGA Generation 2 (LI-COR®, 2001) software is supposed to be able to score the molecular weight of the samples down to the scale of one base pair. But as the results are influenced by factors like gel irregularities, the variation between different gels is not negligible. Therefore, as a confirmation of the software's results we run some samples from different gels on a control gel to confirm allele identities. GENEPOP ON THE WEB (Raymond & Rousset 2004) was employed to determine inbreeding coefficients and linkage disequilibrium for the derived queen genotypes.

The most probable queen genotype(s) was / were estimated based on the worker genotypes and allele frequency for all tested loci. First, we identified and homologized alleles and genotypes for each colony and locus separately. Then we added one locus at a time for each colony until we had the final multilocus queen genotype or all possible multilocus genotypes. In case there were multiple possible queen genotypes we analyzed all of them. All workers sharing the same father inherit the same paternal allele because male ants are haploid. Hence, workers of a colony headed by a single monoandrous queen can have at most two genotypes or three alleles at any given locus. If there were more than three alleles present in the workers from

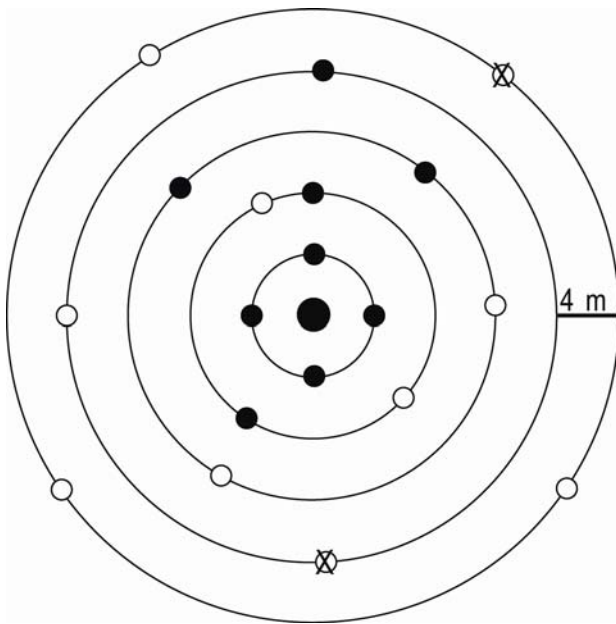


Fig. 1: Lumped data of experimentally staged raids in four colonies of *Myrmecocystus mimicus*. Five artificial incipient nests were placed in the surroundings of each of four mature colonies in distances from four to 20 meters in arbitrarily chosen directions. Open circles are artificial incipient nests alive after 18 days, filled circles are artificial incipient nests that were raided, and crossed circles are artificial incipient nests that were not raided but perished within 18 days.

one colony in at least two loci, a second or third father was taken into account. If worker genotypes could not be explained by a single polyandrous queen, we assumed additional reproducing queens (polygyny); or we postulated raided workers, if we had further evidence that allowed us to reject polygyny (following GADAU & al. 2003, see below). Workers were categorized as raided workers if a matriline was present only in one of the two sampled years, or if the worker belonged to a different species (*Myrmecocystus depilis* also raids *M. mimicus* workers, KRONAUER & al. 2003). In two cases in *M. mimicus* it was not possible to decide whether individuals belonged to a rare matriline or were raided because these colonies were only sampled in one year. This is, however, not an issue for *M. depilis*, which appears to be obligatorily monogynous (see results). This is a conservative approach in the sense that we might underestimate the number of colonies with raided workers because colonies we classified as polyandrous or polygynous could represent cases of raided workers from foreign colonies with similar genotypes. Note, the vast majority of colonies and worker genotypes could be explained by postulating a monoandrous and monogynous colony structure.

To judge the influence of additional patriline, the effective mating frequency (m_e) was calculated following STARR (1984). This parameter takes the unequal representation of patriline into account. To estimate the influence of additional matrilines on the within-colony relatedness (r -value) between workers, the software RELATEDNESS 5.0.8 (GOODNIGHT & QUELLER 1999) was used. To do so, the intracolony r -value between workers including all matrilines and from single matrilines were calculated. The

mean over all colonies of one species in one year was compared with the theoretical mean r -value that would be assumed under monogyny and monoandry in a Hymenoptera colony, 0.75. For this comparison, a two-tailed t -test was performed using SPSS (release 10.0.1, SPSS Inc. 1999). In order to test whether putatively raided workers could gain some indirect fitness, we calculated their relatedness to the slave maker colony's queen using again RELATEDNESS 5.0.8.

Results

Our field studies demonstrate that mature colonies of *Myrmecocystus mimicus* raid conspecific small colonies without preceding tournament actions. Of a total of 20 artificial incipient nests, nine were discovered and raided within the observation time of 18 days (Fig. 1). Not surprisingly, the closest to the nest exit of the mature colonies were all discovered and raided, but even one artificial incipient nest 16 m distant from the mature colony's entrance was raided. Two artificial incipient nests perished from unknown causes during the observation period.

One to two days after placing the artificial nests in the soil around the vicinity of the mature nests, workers of the artificial nests were seen leaving the nests, roaming around and returning. The first artificial nest (4 m distant) was raided after four days. We were able to observe the entire process on three occasions. Few *Myrmecocystus mimicus* workers, most likely from the mature nest, passed the nest entrance of an artificial nest, and some briefly inspected the hole, moved away, returned and inspected the hole again, moved into the exit, came out again, and this happened repeatedly. Suddenly, a number of ants rushed out from artificial nest. Some remained outside at the entrance, and some walked on "stilted" legs, assuming a tournament display position (see HÖLLDOBLER 1981). In a few cases, a *Myrmecocystus* worker emerged from the artificial nest with another ant held in its mandibles by a leg or antenna. This was a clear indication that foreign *Myrmecocystus* workers had entered the artificial nest and were attacked by resident ants. Invariably, one or a few ants moved to the mature nest with their abdominal tip touching the ground in frequent intervals (obviously laying a chemical trail: see HÖLLDOBLER 1981). After arriving at their own nest, these ants exhibited the typical jerking display of recruiting workers described and analyzed in previous studies (HÖLLDOBLER 1981). Soon afterwards, a swarm of apparently recruited ants traveled in a loosely organized column to the artificial incipient nest. At the front of the raiding swarm moved the recruiting ant, clearly visible because it continued to exhibit trail-laying and jerking movements when it encountered nest mates. The raiding column swarmed around the artificial incipient nest which was quickly followed by physical fighting and stilted-legs displays between the artificial-incipient-nest ants and the invaders. Many invaders then moved into the artificial incipient nest and soon emerged again with pupae or larvae held in their mandibles. Lastly, honeypots were pulled out and all the booty was carried or dragged to the invaders' nest.

These experimentally initiated raids closely resembled the natural occurring raids we were able to observe, although we had never witnessed the scouting and very first recruitment phase during naturally occurring raids. We are, however, quite certain that in 17 occasions we were able to

Tab. 1: Complete raids without tournaments. The number and class of raided individuals for all raids that were initiated without a previous tournament. Note that raids developing from tournaments are documented in HÖLLDOBLER (1981).

Date	Year	Larvae	Pupae	Honeypots	Callows	Ants with filled crops
23.IV.	1984	6	9	0	0	51
14.VII. to 16.VII.	1984	26	21	3	1	49
11.VIII. to 13.VIII.	1984	67	48	9	3	109
18.VIII. to 19.VIII.	1986	21	15	5	0	57
19.VII. to 27.VII.	1988	137	76	18	4	226
12.VIII. to 14.VIII.	1988	10	36	6	0	94
10.VIII. to 11.VIII.	1990	11	20	0	0	40
21.VII. to 26.VII.	1992	86	107	12	5	221
22.VIII. to 24.VIII.	1992	22	39	6	2	102
03.VII. to 08.VII.	1993	119	67	9	3	228
14.VII. to 15.VII.	1993	8	12	2	0	49
02.VIII. to 03.VIII.	1993	11	21	1	0	56
15.VIII. to 16.VIII.	1993	31	43	4	0	63
16.VIII. to 19.VIII.	1994	61	65	12	3	203
03.IX. to 08.IX.	1997	102	152	23	3	409
29.VIII. to 01.IX.	2001	29	69	11	0	316
12.VIII. to 15.VIII.	2002	48	85	7	2	114

record raids from the beginning of the brood retrieval. It also seems clear that these raids did not emerge from previous territorial tournaments, because we regularly surveyed all the nests in our study area and did not see any signs of preceding tournament behavior between raiders and the raided colony. In fact, the raided nests were so inconspicuous that in most cases we did not even notice them before the commencement of the raid.

Table 1 presents the number of captured brood, callow workers, honeypots, and raiding workers with filled crops emerging from the raided nest. The latter phenomenon is quite conspicuous; nevertheless we missed it in our previous studies (HÖLLDOBLER 1981). Raiding ants did not exhibit an extended gaster when entering the raided nest, but a considerable number of them left the raided nest with swollen gasters and traveled towards the raiders' nest. Most likely, these ants enticed the honeypots inside the nest to regurgitate food which they then carried in their crops to their own nest. Indeed, honeypots that were pulled out of their nest often did not exhibit well-rounded gasters typical for honeypots inside the nest. Instead, some of them appeared rather shriveled.

All observations previously made of this sequence in the laboratory indicate that the captured brood in *Myrmecocystus mimicus* was incorporated into the labor force of the raiding colony (BARTZ & HÖLLDOBLER 1982; B. Hölldobler, unpubl.). There were incidences of brood cannibalism, however, in most cases such brood appeared to be injured. More frequently, honeypot ants were injured during raiding process. Such individuals are invariably eaten in the raiders' nest, at least this is what we observed in laboratory nests. However, intact honeypots appear to be incorporated into the raiders' colonies. We have no data on the

survival of callows after being captured by a raiding colony, and there is no indication that adult workers of the raided nest join the raider colony. We also observed brood raids in *M. depilis*, but we did not collect quantitative data for this species. We did, however, include *M. depilis* in our genetic studies.

Comparisons of the alleles present in *Myrmecocystus mimicus* and *M. depilis* at loci Mm3 and Mm5 proved that it is possible to distinguish the two species using these microsatellites. The *M. depilis* alleles of locus Mm5 did not overlap with *M. mimicus* alleles. Additionally, locus Mm3 had many stutter bands in *M. depilis* but almost none in *M. mimicus*. This continued to be very helpful, because small individuals of *M. depilis* and *M. mimicus* can often not be distinguished using morphological markers.

Genetic evidence alone does not allow us to distinguish between the offspring of additional resident queens (polygyny), and raided individuals. To do so, we needed to invoke other circumstantial evidence. Foraging workers usually are relatively short lived. Hence, if workers from an additional matriline are present only for one of the two years we have assayed a colony, we assumed that these are raided workers and not the offspring of an additional resident queen.

Employing these criteria we found putative raided workers in colonies of both species (Tab. 2, Fig. 2). In *Myrmecocystus mimicus*, we found additional evidence for polygyny in four and evidence for polyandry in one out of 14 colonies (Tab. 2, Fig. 2). In *M. depilis*, we found no evidence for polygyny but evidence for polyandry in ten out of 27 colonies (Tab. 2). Thus, *M. depilis* appears to be obligatorily monogynous. This assumption was further supported by our field data: In a total of 397 incipient colonies and

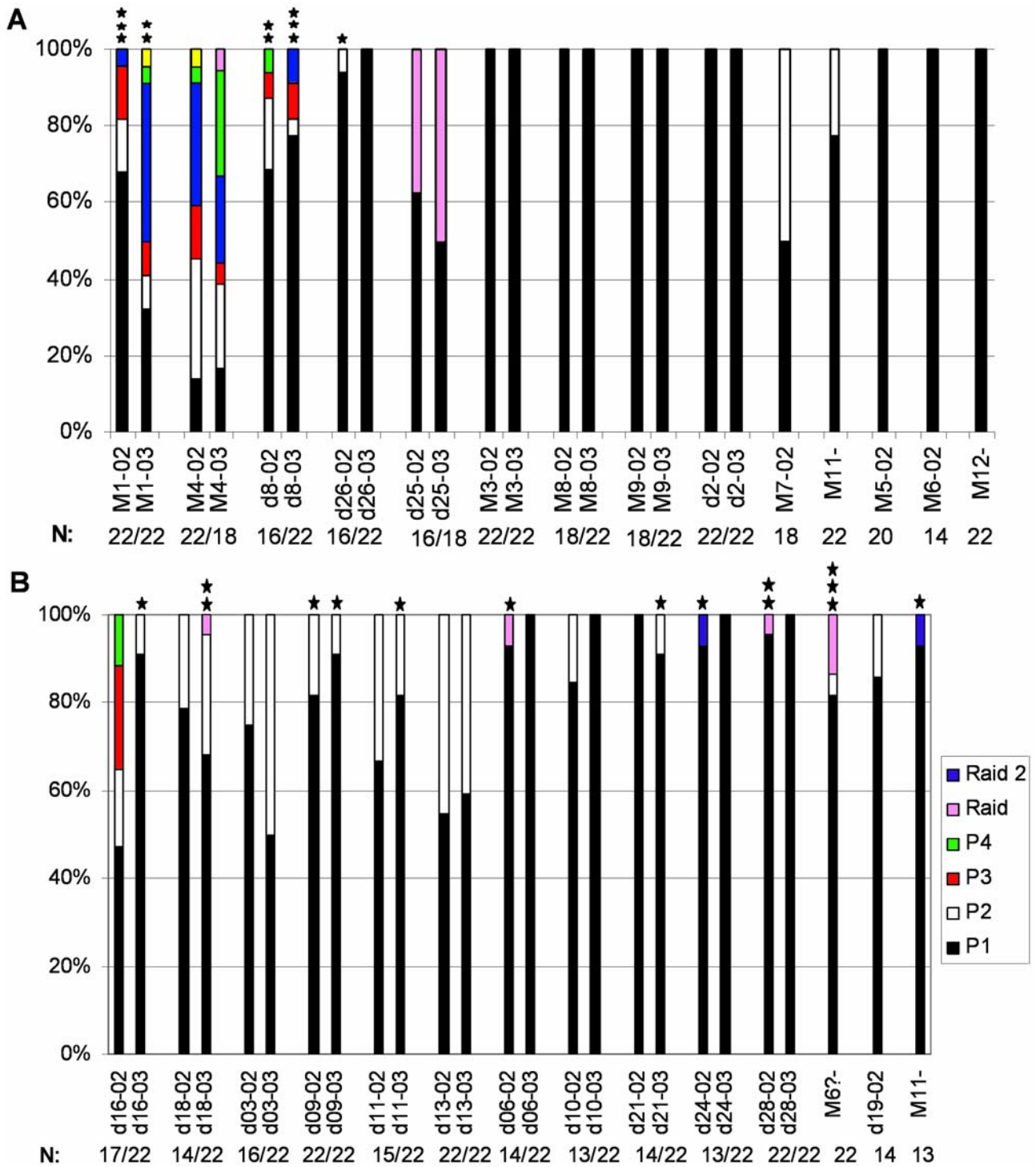


Fig. 2: Distribution of matriline or raided workers and patriline for all *Myrmecocystus mimicus* colonies (A) and all *M. depilis* colonies (B). Neighboring columns in *M. mimicus* and *M. depilis* represent the same colony of two consecutively collected years 2002 and 2003. Identical shading within one colony represents the same matri- or patriline. Black columns stand for the number of workers belonging to the resident queen's offspring. Asterisks indicate the level of significance (χ^2 -test against equal distribution of matri- / patriline; in the cases with only two matri- / patriline with Yates correction); numbers below the columns give the number of individuals analyzed. **A:** Distribution of the matriline or raided workers of *M. mimicus*; all bar colors indicate different matriline, except in colony d25 where a second patriline was detected. The first four colonies (M1, M4, d8 and d26) have matriline that are only present in one of the two sampled years (e.g., M1-03 shows two individuals from a matriline that was not present in M1-02). These are most likely raided workers. **B:** 14 out of 27 analyzed *M. depilis* colonies that violated the assumption of monogyny and / or monoandry. The values for P2 - P4 indicate additional patriline, pink (Raid) indicates an additional matriline, and blue (Raid 2) represents workers raided from *M. mimicus*.

Tab. 2: Summary of the genetic analyses in *Myrmecocystus mimicus* and *M. depilis* samples of the year 2002 and 2003. Shown are: mean effective mating frequency ($m_e \pm$ standard error), ratio of polygynous to monogynous colonies (poly / mono), number of colonies with raided individuals (# col), and the mean percentage of raided individuals in these colonies (% raided) (see also Fig. 2). Additionally, the mean F_{IS} -value and the level of significance from a 2-tailed t-test against zero are given. The last column represents the non-detection probability of additional patriline.

Species	$m_e \pm$ SE	Poly / mono	# col / % raided	F_{IS}	P-value of F_{IS}	$P_{non-det}$
<i>M. mimicus</i> 02	1.07 \pm 0.074	4 / 8	3 / 5.1%	0.12 \pm 0.080	0.196	0.009*10 ⁻³
<i>M. mimicus</i> 03	1.10 \pm 0.100	4 / 7	3 / 7.9%	0.16 \pm 0.070	0.081	0.10*10 ⁻³
<i>M. depilis</i> 02	1.24 \pm 0.093	0 / 25	4 / 6.8%	0.003 \pm 0.094	0.973	0.008
<i>M. depilis</i> 03	1.18 \pm 0.065	0 / 24	1 / 13.6%	0.062 \pm 0.117	0.630	0.006

Tab. 3: Number of alleles (N), expected (H_e) and observed (H_o) heterozygosities and mean F_{IS} -values of the used loci; two tailed t-test against zero; none of the F_{IS} -values differs significantly from zero; there is no overlap between the alleles at the locus MM5 of *Myrmecocystus mimicus* and *M. depilis*.

		MM1	MM2	MM3	MM4	MM5	Mean $F_{IS} \pm$ SE	Sig. 2-tailed
<i>M. depilis</i> 02	N	8	12	–	11	2	-0.0034 \pm 0.0935	0.973
	H_o	0.85	0.88	–	0.69	0.31		
	H_e	0.74	0.75	–	0.82	0.36		
<i>M. depilis</i> 03	N	9	12	–	10	2	0.062 \pm 0.117	0.630
	H_o	0.80	0.88	–	0.58	0.28		
	H_e	0.72	0.77	–	0.85	0.35		
<i>M. mimicus</i> 02	N	4	9	12	18	12	0.12 \pm 0.0802	0.196
	H_o	0.54	0.63	0.81	0.83	0.58		
	H_e	0.47	0.81	0.90	0.94	0.86		
<i>M. mimicus</i> 03	N	4	11	11	15	11	0.16 \pm 0.0699	0.081
	H_o	0.46	0.63	0.71	0.88	0.58		
	H_e	0.44	0.85	0.88	0.92	0.87		

27 approximately two-years-old colonies of *M. depilis* excavated in the study area during a period of 26 years, we found in each case only one queen. All attempts to initiate pleometrotic founding association, easily possible with *M. mimicus*, failed with *M. depilis*.

Table 3 gives an overview about the number of alleles found per locus and year and the observed-versus-expected heterozygosity. None of the two species showed any signs of inbreeding (Tab. 3, F_{IS} not significantly different from 0). The mean effective mating frequency (m_e) did not differ significantly from one in *Myrmecocystus mimicus* in both years (2-tailed t-test: *M. mimicus* 02: $t = 1.000$, $df = 11$, $p = n.s.$; *M. mimicus* 03: $t = 1.000$, $df = 9$, $p = n.s.$). In *M. depilis*, m_e differed significantly from one in both years (2-tailed t-test: 2002: $t = 2.644$, $df = 25$, $p < 0.014$; 2003: $t = 2.732$, $df = 23$, $p < 0.012$; Tab. 2). The non-detection probability for additional patriline was calculated following GADAU & al. (2003). For both species, $P_{non-det}$ was below 1% (Tab. 2).

The intracolony worker-worker relatedness value of 0.75 expected under monogyny and monoandry differed significantly from the observed worker relatedness in *Myrmecocystus mimicus* in both years; (2-tailed t-test: *M. mimicus* 02: $r = 0.54 \pm 0.071$, $t = -2.899$, $df = 11$, $p < 0.014$; *M. mimicus* 03: $r = 0.57 \pm 0.074$, $t = -2.427$, $df = 10$, $p < 0.036$). In *M. depilis*, only the samples from 2002 showed a significant deviation from 0.75 (2-tailed t-test: $r(02) = 0.66 \pm 0.035$, $t = -2.544$, $df = 24$, $p < 0.018$; $r(03) = 0.70 \pm 0.035$, $t = -1.450$, $df = 23$, $p = n.s.$), indicating that facultative polyandry in *M. depilis* had a smaller effect on the average worker-worker relatedness than facultative polygyny in *M. mimicus*.

A calculation of the mean relatedness between the matriline of polygynous *Myrmecocystus mimicus* colonies showed no significant deviation from zero (1-tailed t-test: 2002: $r = 0.11 \pm 0.092$, $t = 1.174$, $df = 3$, $p = n.s.$; 2003: $r = 0.03 \pm 0.019$, $t = 1.489$, $df = 2$, $p = n.s.$). This suggests that queens in polygynous colonies are not related, which is

typically the case for primary polygyny that originates from pleometrotic foundress associations.

Discussion

Field data collected during 10 fieldwork seasons confirm previous observations that the honey ant *Myrmecocystus mimicus* conducts raids on neighboring small conspecific colonies. Contrary to former assumption that raids only arise from territorial tournaments (HÖLLDOBLER 1982), we show here that many raids occur without a preceding territorial tournament. Raids are usually conducted by larger colonies onto considerably smaller colonies and are initiated by recruiting scouts. Although tournament interactions between individuals, which can escalate to physical fighting, frequently occur at the nest of the raided colony, this is different from elaborate territorial tournament behavior conducted between two larger colonies. Previous studies focused on these territorial tournaments, and the raids recorded most likely arose from such tournaments. From comparison with the new data we conclude that the raided colonies reported by HÖLLDOBLER (1982) were not all of incipient status, but some of them most likely were larger in size, because the amount of captured brood and honeypots was considerably larger than what we observed in subsequent studies. Another new finding was that the raiding colony not only capture brood and honeypots, they also collect stored food from the raided colony's honeypots. The evidence supporting this conclusion was the appearance of numerous workers from the raiding colony emerging from the raided nest with filled crops traveling to the raiders' nest.

Although our field studies were focused on *Myrmecocystus mimicus*, we also paid attention to the similar species *M. depilis*. Unfortunately, we only observed brood raids in *M. depilis* but we did not take quantitative records. The genetic findings by KRONAUER & al. (2003) confirmed intra- and interspecific brood raiding in *M. depilis*, and also strongly suggested that raided conspecific brood is incorporated into the labor force in *M. mimicus* colonies. However, the current work also revealed strong evidence for occasional polygyny or oligogyny in *M. mimicus* which made a reinterpretation of some of the results by KRONAUER & al. (2003) necessary. For *M. depilis*, however, the genetic as well as sociobiological data confirm obligatory monogyny, and workers of different matrilines therefore must be due to intra- and interspecific raiding.

We employed five (*Myrmecocystus mimicus*) and four (*M. depilis*) highly polymorphic microsatellites for the identification of additional matrilines in a colony. In case a matriline is present in one year only, or its frequency is low and significantly different from an equal intracolony distribution of matrilines, we considered this as evidence that this particular matriline derived from a foreign colony, i.e., represents a raided individual. The first criterion is a stronger indicator of foreign origin of a matriline, although even in this case we cannot entirely rule out the possibility of queen death or queen replacement. The first criterion is met by four *M. mimicus* and two *M. depilis* colonies, suggesting these workers developed from brood adopted after intraspecific raids. In addition, one *M. depilis* colony revealed an interspecific raid on a *M. mimicus* colony.

In the current study we made additional identifications of putative raided individuals employing the matriline-frequency criterion in one *Myrmecocystus mimicus* colony.

The frequency criterion is based on the assumption of no hierarchical dominance among multiple reproducing queens in a colony. However, if only one queen produces the majority of the offspring in a polygynous colony, an unbalanced pattern in the matriline frequency would be the result and an unequivocal identification of raided individuals without a sample of a second year is impossible.

Overall, foragers from 12 *Myrmecocystus mimicus* colonies and 25 *M. depilis* colonies were collected in 2002 and 11 colonies of *M. mimicus* and 24 *M. depilis* in 2003 (Fig. 2; note that Fig. 2 shows only those colonies that deviated from monogyny and monoandry and have no raided workers). Occasional polyandry must be assumed for *M. depilis*, where we found an effective mating frequency significantly different from 1 in both years (Tab. 2). In addition, the data reported by KRONAUER & al. (2003) suggest polyandry in one *M. depilis* colony out of eight. In the current study we found evidence for polyandry in 10 out of a total of 27 colonies in both years (Fig. 2). The difference between both studies is still within the range of statistical variance (not significant using Fisher's exact test).

An effective mating frequency of 1.24 (or 1.18 in 2003, Tab. 2) in *Myrmecocystus depilis* is still very low and according to categories provided by BOOMSMA & RATNIEKS (1996) *Myrmecocystus depilis* does not fall into the category of an obligate multiply mated species. Why *M. depilis* shows some multiple mating is unclear and it would be interesting to explore whether this species differs also in other aspects of their mating behavior, or whether the occasional multiple mating is adaptively related to the obligatory monogyny. Although most *M. mimicus* colonies appear to be monogynous, we found in both years strong evidence for facultative polygyny (Tab. 2, Fig. 2), ranging from two to four matrilines per colony.

In general, there are two ways that a colony can become polygynous: primary and secondary polygyny. In primary polygyny, multiple queens found a colony together (pleometrosis) and remain polygynous throughout the existence of a colony. The queens of such a colony are normally unrelated (HÖLLDOBLER & WILSON 1977, 1990). In the case of secondary polygyny, a single queen founds a colony and additionally, usually related queens are adopted later after the colony became mature. Primary polygyny is relatively rare, but more cases have been reported in recent years, while secondary polygyny is widely distributed in ants (HÖLLDOBLER & WILSON 1977, 1990, HEINZE & al. 2001).

The analysis of intracolony queen relatedness of the polygynous colonies of *Myrmecocystus mimicus* was not significantly different from zero. This supports the view that polygyny in *Myrmecocystus* might be due to primary polygyny. This is further supported by the observation of pleometrosis in *M. mimicus* (see BARTZ & HÖLLDOBLER 1982). In most cases, however, aggression of workers towards less fertile queens and agonistic interactions among the co-foundresses results in monogyny. Nevertheless polygynous or oligogynous colonies do occur. In such colonies, multiple matrilines contribute equally to the offspring (e.g., colonies M1 and M4 in Fig. 2), supporting the argument that very fertile co-foundresses continued to coexist in primary polygyny in some mature colonies of *M. mimicus*.

What could be the advantages for a colony being polygynous? If the queens are closely related, kin selection theory favors the cooperation of the queens (NONACS 1988).

But if the queens are not related, like in our case, kin selection arguments fail. Rather, we have to invoke ecological or behavioral constraints (HÖLLDOBLER & WILSON 2009). One possible explanation is that there is a positive correlation between queen number and worker number during the colony-founding phase and that larger founding colonies have a higher chance of survival in the face of competitive raids (BARTZ & HÖLLDOBLER 1982, HERBERS 1986, RISSING & POLLOCK 1987, TSCHINKEL 1992, 1998). This may be one of the reasons why in some habitats pleometrosis is found regularly in *Myrmecocystus mimicus* (see BARTZ & HÖLLDOBLER 1982). However, the pleometrotic colonies described by BARTZ & HÖLLDOBLER (1982) were eventually all transformed into secondary monogynous colonies. It might very well be, however, that equally fertile queens can continue to coexist in maturing colonies (R. Mendez, pers. comm.).

ALLOWAY (1980) proposed for the evolution of obligatory interspecific slavery (dulosis) several conditions and preadaptations, mainly two or more closely related species should be present sympatrically, territorial competition should be prevalent, and the species should be preadapted for non-independent colony foundation, for example through facultative polygyny or pleometrosis.

In *Myrmecocystus*, the first two criteria are fulfilled, since the existence of facultative intraspecific slavery is known for the closely related species *M. mimicus* and *M. depilis*, and facultative interspecific slavery has been recorded in *M. depilis* (see HÖLLDOBLER 1976, HÖLLDOBLER 1981, BARTZ & HÖLLDOBLER 1982, KRONAUER & al. 2003). We have no evidence for interspecific slavery in *M. mimicus*. In the current study, we found good evidence for the presence of facultative polygyny in *M. mimicus*, and therefore the third criterion postulated for an evolutionary venue towards obligatory slavery seems to be implemented in this species, but not in *M. depilis*.

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