

Research article

Social parasitism involving ants of different subfamilies: *Polyrhachis lama* (Formicinae) an obligatory inquiline of *Diacamma* sp. (Ponerinae) in Java

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Summary. Socially parasitic ants usually live in dependence on closely related host species. An instance of obligatory social parasitism by a formicine ant, *Polyrhachis lama*, on colonies of a ponerine ant, *Diacamma* sp., from Java is described. Reproductive females of both species coexist in the nests, in which one gamergate queen of the *Diacamma* host is always found. Dealate females (gynomorphs) of the parasite species are rare (7 in a total of 48 mixed colonies), and only 2 out of 4 dissected individuals were inseminated. Parasitized nests comprise a very low number of *P. lama* “workers” (ergatomorphs), between one and rarely more than 10 (Table 2). Both virgin and mated dealate gynomorphs, and also the ergatomorphs, which always lack a spermatheca, are fully reproductive, probably having males, ergatomorphs and alate gynomorphs as offspring. The broods of host and parasite are mixed, and the *Diacamma* workers take over, at least in part, the brood care for the parasite. *Polyrhachis lama* is very rarely found outside the nests and its ergatomorphs do not engage in foraging. Parasitizing distantly related species may be more common in ants, particularly in the genus *Polyrhachis*, though the few reported instances are anecdotal. We suggest an evolutionary origin of this kind of parasitism from mimetic relations between *Polyrhachis lama* and the pugnacious *Diacamma* species.

Key words: Formicidae, *Polyrhachis lama*, *Diacamma*, social parasitism, Emery’s rule.

Introduction

Social parasitism among ants is a frequent phenomenon, almost one third of all species in well-investigated faunas of Europe and North America being social parasites (Hölldobler and Wilson, 1990). In various modes such as temporary parasitism (colony foundation with the aid of a foreign species), dulosis (slave-making), or inquilinism (permanent parasitism, the parasite species often being workerless), a parasitic species obligatorily exploits the brood rearing and other social capacities of a free-living host species. “True” social parasites coexist in the nest together with the workers and sometimes with the queens (most inquilines) of their host species, whose workers rear the parasites’ brood.

Practically all such parasites are close relatives of their hosts, from whom they were believed to have derived (Emery, 1909: 361). However, “Emery’s rule” (Le Masne, 1956) in its strict sense, which states that the host species is always the direct ancestor and closest relative of its parasite, is obviously incorrect (Wasmann, 1909: 627; Buschinger, 1970). This is because dulotic species usually enslave a range of host species belonging to one genus, and because monophyletic genera of social parasites exist, e.g., *Polyergus*, *Strongylognathus*, *Epimyrmica* or *Harpagoxenus*, the species of which are clearly more closely related to one another than to any of their host species (Buschinger, 1986).

More recently, a few other instances of inquilinism have been described, where host and parasite are congeners. Nevertheless they are believed not to be very closely related, belonging to different “species complexes” within the host/parasite genus (the “putatively inquiline” *Cataglyphis hananensis*: Agosti, 1994; *Pseudomyrmex inquilinus* and *P. leptosus*: Klein, 1987; Ward, 1985, 1989, 1996). In all three instances only very few parasite colonies have been found, and the phylogenetic relationships of hosts and parasites were esti-

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mated by cladistic analysis of morphological characters only. These instances are properly also regarded as contradictory to Emery's rule in its strict sense.

On the other hand, morphology, physiology, behavior, and molecular genetics have unanimously confirmed Emery's rule in its wider sense, i.e., that hosts and their respective parasites belong to related groups, and that the parasites have originated from the group (genus or tribe) to which their hosts belong (Baur et al., 1995, 1996; Bolton, 1988; Buschinger, 1986, 1990; Heinze, 1991; Hölldobler and Wilson, 1990; Sanetra et al., 1994). It is a matter of debate whether the parasites have evolved via sympatric speciation from independent species which then became their host species, or whether independent species under certain circumstances switched over to parasitizing sister species (Agosti, 1994; Bourke and Franks, 1991; Buschinger, 1990; Ward, 1996; Wilson, 1971). In any case, later radiation of both host and parasite species, transfer of a parasite from one host species to another closely related one, or extension of the host species range, may have occurred (Buschinger, 1986, 1990; Lowe and Crozier, 1997 for allodapine bees).

Other obligatory associations of different ant species are called xenobiosis, where the "guest ant" lives within, or at the periphery of, the host nest, but rears its own brood in separate chambers. Guest ants usually depend on the host species merely for obtaining food and perhaps shelter. Even less close and often facultative relationships characterize parabiosis (two species share the same odor trails and sometimes nest together, but keep their broods separate); lestobiosis (the parasite lives in the nest walls of the host, predating its brood); cleptobiosis (a "parasite" robs prey from the host workers); and plesiobiosis (two species live in close vicinity but engage in little or no direct communication) (Hölldobler and Wilson, 1990).

Xenobiosis and the other, looser associations, occur not only between more or less closely related species or genera, but even between members of different ant tribes or subfamilies. For example, the guest ant genus *Formicoxenus* (Myrmicinae) coexists with *Myrmica* or *Manica* (Myrmicinae), and a few species live in *Formica* mounds (Formicinae) (Francoeur et al., 1985). Parabiosis has been claimed to occur between a *Crematogaster* (Myrmicinae) and a *Camponotus* (Formicinae) species, with the workers sometimes even exchanging liquid food, and in an Australian *Camponotus* living within the mounds of *Iridomyrmex* ants (Dolichoderinae) (Hölldobler and Wilson, 1990).

However, truly mixed nests of two species, dependent colony foundation of the parasite within the host nests, and the tending of parasite brood by host workers, was previously thought to occur exclusively between close relatives, in accord with Emery's rule in the wider sense. A few, mostly questionable exceptions have been reported: e.g., *Rhoptryrmex schmitzi* (Myrmicinae) was claimed to live as an inquiline in nests of *Tapinoma erraticum* (Dolichoderinae), but only one single specimen of the "parasite" has ever been found (Bolton, 1986; Forel, 1910). Several *Oxyepoecus* species (Myrmicinae) are said to coexist with *Pheidole* and *Solenopsis* (both Myrmicinae, but belonging to different

tribes) (Ettershank, 1966; Kusnezov, 1952), though nothing exact is known of their life histories. The genus *Polyrhachis* itself comprises a couple of species for which observations have been published suggesting parasitic relations with other Formicinae and with Myrmicinae and Ponerinae (see discussion).

Here we report the first well-documented instance of inquilinism involving species belonging to different ant subfamilies, the parasitism of *Polyrhachis lama* (Formicinae) on colonies of a *Diacamma* sp. (Ponerinae) from Java, Indonesia. The observations suggest that inquilinism here has evolved via a particular route, leading to relationships not obeying Emery's rule even in the widest sense.

Materials and methods

Polyrhachis lama belongs to a *P. viehmeyeri* species group which mainly occurs in Australia. It was described by Kohout (1994) from a dealate female and 5 ergatomorphs which had been collected during the Deutsche Tibet-Expedition 1938/39 "in Tibet", and which were deposited in the Senckenberg Museum at Frankfurt/Main (1 paratype worker is also in Queensland Museum, Brisbane). Any further data on the type locality and details of the mode of collection and the ant's behavior are lacking. R. Kohout (pers. comm.) confirmed that our material corresponds to the type specimens, though some doubts remain over whether and how this species can have such a wide range. We have placed voucher specimens from Java (2 males, 2 gynomorphs and 3 ergatomorphs) in the Senckenberg Museum. Others were sent to R. Kohout, and to MCZ at Harvard University (2 males, 2 gynomorphs and 3 ergatomorphs).

The host species of *P. lama* clearly belongs to the genus *Diacamma*, however, an identification of the species was not yet possible.

Collecting of ants and colony censuses

The ants were detected in a pasture in central Java, near Bandung (7°13'S, 110°28'E), at an elevation of ca. 1000 m. We found the nest entrances of *Diacamma* sp. either visually or by following the path of a *Diacamma* worker carrying a bait (insect pieces offered to foraging individuals). The nest entrance was then stoppered, and a trench was dug around it. When gently digging down the entrance tunnel practically all the ants trying to escape could be aspirated or picked up and transferred into a plastic bag. Only a few *Diacamma* foragers were lost, and all the brood was collected. The presence of *P. lama* in a nest was not recognizable from outside, but was usually noticed during the digging because of the more erratic movements of *P. lama* and because of the different shape and color of its cocoons.

The site was visited twice. During a visit in March, 1997, when the occurrence of *Polyrhachis* in nests of *Diacamma* sp. was observed for the first time, 11 mixed colonies were found but not completely collected. In January, 1998, we studied the population more closely. During this stay we excavated 120 *Diacamma* nests without, and another 36 nests with, *P. lama* (Tables 1, 2). We searched for *P. lama* in a slope to the W of Bandung, beginning at the original site and making a transect of ca. 2 km up to an elevation of 1500 m, where the parasite was still found. In a second place, on an eastern slope of Mt. Merbabu (7°15'S, 110°36'E), in a degraded forest area, only one dealate female of *P. lama* was found in one out of ca. 30 *Diacamma* nests (# 1 in Table 2). This site is located in about 20 km linear distance from the first one. In total, we thus have data on 48 mixed nests of *P. lama* with *Diacamma* sp.

Most of the *Diacamma* colonies were set free after careful checking for presence of *P. lama*. The parasitized colonies were censused a few hours after collecting, and a number of them were kept in plastic jars for

Table 1. Composition of 9 non-parasitized *Diacamma* sp. colonies from Java. Ø: Mean

Col. #	gamergate queen	workers	cocoons	larvae	eggs
1	1	98	23	12	28
2	0	28	4	0	16
3	1	33	1	3	14
4	1	89	22	9	12
5	1	46	1	2	3
6	1	86	6	1	7
7	1	41	25	6	2
8	1	18	0	3	3
9	1	54	8	3	4
Σ	8	493	90	39	89
Ø	1	54.8	10	4.3	9.9
± s.d.		25.9	10.3	3.9	8.6

at least several days. They were fed with insect pieces and diluted honey. Colonies kept for a longer time had access to plastic arenas of various sizes. The *P. lama* gynomorphs and ergatomorphs of other colonies were dissected, and the *Diacamma* specimens then released in the field.

Dissection of ants

Dissections were made under a dissecting microscope as described by Buschinger and Alloway (1978), and the contents of spermathecae were checked with a compound microscope. Specimens were rated "fertile" when the ovarioles contained yolky oocytes and/or corpora lutea.

Caste terminology

We apply the functional caste definition of Michener (1974), which is based on the reproductive division of labor (cf. Buschinger and Winter, 1976; Buschinger, 1987). "Queen" is a reproductive, inseminated female, irrespective of its morphology. "Worker" is a normally non-laying female that is engaged in brood rearing or foraging, also irrespective of morphology.

Morphological differences among the females are described by the terms "gynomorph" (alate or dealate female with fully developed thoracic structures), "ergatomorph" (wingless specimens with reduced thoracic skeleton, looking like the usual "worker" of ants in the morphological caste definition), and "intermorph" for the range of specimens which are morphologically in between (= "intercaste" according to Peeters, 1991; cf. also Heinze, 1998).

In the particular instance of *Polyrhachis lama* any caste definition becomes somewhat problematic. As will be described below, there are two clearly distinct morphs, alate/dealate gynomorphs, and ergatomorphs which look like ordinary *Polyrhachis* "workers". However, among the dealate *P. lama* gynomorphs we found specimens with and without sperm in their spermathecae, but in both instances these females were egg-laying. And all the *P. lama* ergatomorphs except for a few very young ones had long ovarioles containing yolky oocytes and corpora lutea, though they lack a spermatheca. Our field observations suggest that both virgin gynomorphs and the ergatomorphs of *P. lama* reproduce by amphitoky (parthenogenetic production of males and females), whereas only few mated gynomorphs can reproduce sexually.

In the ponerine genus *Diacamma* gynomorphs are lacking (Peeters and Higashi, 1989), which holds true also for the host species of *P. lama*. All female specimens at birth are identical, looking like ergatomorphs, but bear a pair of bladder-like appendages in the place where the gynomorphs would have wings. Only specimens retaining these "gemmae" can mate and reproduce sexually; they are called "gamergates" (= mated workers). The gamergates bite off the gemmae of most of their younger nestmates which then take over the role of workers. In this

paper we consequently term "gamergate queen" the single mated, re-productive female in a *Diacamma* colony, and "workers" the actually working individuals lacking the gemmae.

Laboratory experiments and observations

As we were unable to stay long enough at Bandungan to complete all the desired experiments, we transferred a few *P. lama*/*Diacamma* colonies to Frankfurt and to the field studies centre of the University of Malaya at Ulu Gombak, Malaysia. For clarity we present the respective experiments and results separately for four "observation periods".

Observation period I: A first sample of several colonies of *Diacamma* with *P. lama* was brought to Frankfurt in March, 1997. Unfortunately most of the *Polyrhachis* adults, and all their brood, did not survive transport. However, with the remaining specimens from 3 colonies we could form a colony comprising 53 *Diacamma* workers, their brood, and 5 *P. lama* ergatomorphs. Surprisingly, no aggression occurred between the specimens from different colonies. This observation, however, matches a report of Goodloe et al. (1987) who were able to mix colonies of the slave-making ant *Polyergus lucidus* without difficulties. The *Polyrhachis lama*/*Diacamma* colony was housed in a Petri dish with a plaster of Paris floor. The Petri dish was put into a small plastic box within a larger arena (20 × 60 cm) where food was provided. Over two months on random occasions we recorded the position of the *Polyrhachis* ergatomorphs within the nest and we observed which ants left the Petri dish. In a number of nest relocation experiments we tried to characterize the behavioral capacities of *P. lama*.

Observation period II: During our stay at Bandungan in January, 1998, ca. 25 mixed colonies were kept in plastic jars for at least several days. We tried to optimize the rearing and feeding conditions, identified the brood stages of *P. lama* and *Diacamma* sp., and checked the offspring hatching from the *Polyrhachis* cocoons. We also ran several additional nest relocation experiments.

Observation period III: Three mixed colonies were brought from Java to Malaysia on 15 Jan., 1998, where they were observed until mid-March, 1998. Mainly egg-laying and brood care of *Polyrhachis* ergatomorphs were watched during this time.

Observation period IV: Finally, these three mixed colonies were transferred to Frankfurt on 12 March, 1998. There, nest relocation behavior was again studied, to investigate interspecific communication involving tandem running between *Diacamma* and *P. lama* (to be described elsewhere; Liefke et al., in prep.). In contrast to observation period I, the colonies now comprised both *Polyrhachis* and *Diacamma* brood.

Results

Field observations and colony composition

Polyrhachis lama is a grey ant with workers ca. 8 mm long. It is very similar to its ponerine host in size and coloration. *P. lama* larvae are characterized by a typical formicine shape and a distinct dark and dense pilosity, as compared to the typical ponerine *Diacamma* larvae. The eggs are ovoid and whitish, thus easily distinguished from the rod-shaped brownish *Diacamma* eggs. The light brown *P. lama* cocoons are also different from dark brown *Diacamma* cocoons, being somewhat shorter and stouter. As yet, in Java, we have found *P. lama* exclusively within the nests of *Diacamma* sp., and only twice in a period of several weeks was a *Polyrhachis lama* ergatomorph seen outside a nest in the field.

The locality near Bandungan is a pasture of short grass where *Diacamma* sp. abounds, and where workers of this

Table 2. Composition of 37 mixed colonies of *Diacamma* sp. and the parasitic ant, *Polyrhachis lama* from Java. In colonies with (+G) in the second column the gamergate queen of the host species was identified, in colonies with (–G) it could not be found, all others were not checked for its presence. \bar{O}_{36} : Mean of 36 colonies (#1 excluded). *Polyrhachis lama* dealate ♀ **M**: mated, reproductive gynomorph (queen); **V**: virgin reproductive gynomorph; inc: colonies not completely collected

<i>Diacamma</i> sp.					<i>Polyrhachis lama</i>					
col. #	workers	cocoons	larvae	eggs	dealate ♀	ergato-morphs	cocoons	larvae	eggs	♂/♀ alate
1 inc.	?	?	?	?	1M	0	0	?	?	0/0
2	62 +G	2	10	13	1M	2	4	0	0	0/0
3	58	17	8	15	1V	0	0	0	0	0/0
4	46 –G	0	7	10	1V	6	1	0	0	0/0
5	66 +G	5	9	10	–	9	10	7	0	3/1
6	68 +G	7	3	5	–	14	3	1	0	0/0
7	65 +G	2	3	3	–	5	0	0	0	0/0
8	56 +G	4	10	9	–	5	1	1	0	0/0
9	32 +G	3	10	7	–	5	4	0	0	1/0
10	81 +G	3	16	15	–	11	0	2	0	0/0
11	41 +G	0	10	8	–	1	1	0	0	0/0
12	64 +G	4	11	0	–	11	0	3	0	1/0
13	12 –G	6	4	1	–	2	0	0	0	0/0
14	76 –G	4	13	9	–	7	1	1	0	0/0
15	68	4	8	15	–	6	2	0	4	0/0
16	22	2	8	16	–	3	2	7	0	0/0
17	54	8	10	26	–	5	4	0	0	0/0
18	41	4	12	18	–	5	8	1	10	2/1
19	66	19	9	6	–	7	0	0	1	1/0
20	58	32	6	3	–	1	0	0	0	0/0
21	43	11	9	2	–	3	3	0	0	0/0
22	92	18	11	7	–	3	1	0	1	0/0
23	81	11	16	14	–	7	6	6	2	0/0
24	44	0	0	10	–	1	0	0	4	0/0
25	26	9	7	9	–	4	4	0	6	0/0
26	52	8	19	6	–	4	4	16	0	1/1
27	65	19	17	6	–	13	7	0	0	1/1
28	100	19	11	5	–	10	5	1	0	1/0
29	56	4	14	13	–	7	2	10	13	0/0
30	111	20	12	10	–	5	2	0	0	0/0
31	95	26	21	8	–	5	1	2	0	0/0
32	129	4	14	18	–	6	10	10	0	0/0
33 inc.	24	0	5	5	–	1	0	0	0	0/0
34 inc.	28	0	1	0	–	6	0	0	0	0/0
35	58	10	13	3	–	7	0	6	1	2/0
36	58	3	13	4	–	9	4	0	3	0/1
37	69	8	11	10	–	4	0	1	0	0/1
Σ	2167	296	367	319	4	200	90	75	45	13/6
\bar{O}_{36}	60.2	8.2	10.0	8.9	–	5.5	2.5	2.1	1.25	–
± s.d.	25.2	8.0	4.7	5.8	–	3.4	2.8	3.7	2.9	–

species are often met with in the field. Their nests have an entrance tunnel leading about 20–40 cm deep into the soil and ending in one or two chambers. In 1997, among an undetermined number of pure *Diacamma* colonies, a total of 11 mixed colonies were found. Three of them contained a dealate *Polyrhachis* gynomorph each, and 1, 5, 8 *P. lama* ergatomorphs, respectively. In eight other colonies no gynomorph was found. Four of these colonies contained 1, 1, 3 and 6 *P. lama* ergatomorphs, in the remaining four colonies the *P. lama* were not censused.

In 1998 we excavated a total of 120 *Diacamma* nests in the same site and censused the ants of nine colonies (Table 1).

They comprised between 18 and 98 workers, practically always one inseminated and fully reproductive gamergate queen with gemmae, a brood, and often a few males. An additional 36 *Diacamma* nests contained specimens of *Polyrhachis lama* (Table 2). These mixed colonies also usually had a *Diacamma* gamergate queen (found in 9 out of 12 colonies which were checked for its presence; Table 2), and the number of *Diacamma* workers was not significantly different from that in unparasitized colonies (pure *Diacamma* colonies: 54.8 ± 25.9 workers, $n = 9$; mixed colonies: 60.2 ± 25.2 *Diacamma* workers, $n = 36$). In total, nearly one fourth of all *Diacamma* colonies in this site was parasitized by *P. lama*.

In the second site, on Mt. Merbabu, only one dealate *P. lama* gynomorph was found in one out of ca. 30 *Diacamma* nests which we excavated there. This single find, however, indicates that the parasite is not restricted to the first place, but may have a wider range in Java, and that it occurs in elevations of at least up to 1700 m.

Strikingly the numbers of *P. lama* adults in the mixed colonies were generally low, from one to a maximum of 15 specimens (mean 5.5 ± 3.4 s.d. ergatomorphs), and only seven out of 48 nests contained a dealate gynomorph. Dissection of 4 dealate and 4 alate gynomorphs revealed that they possess a spermatheca and 8–10 ovarioles. But only in two out of the four dealate females was the spermatheca filled with sperm, whereas it was empty in the remaining two as well as in the alate females. Nevertheless, all four dealate females had well developed ovaries with maturing eggs and corpora lutea, demonstrating previous egg-laying. We also dissected a total of 32 *P. lama* ergatomorphs. Practically all of them, including those from colonies with a dealate gynomorph, had well-developed ovaries of 4–8 ovarioles with maturing eggs and ample corpora lutea. However, the ergatomorphs lacked a spermatheca. Two individuals were apparently young and not yet laying, among them one of the two specimens collected in the field outside a nest (the other one was not dissected).

The numbers of *P. lama* brood items, eggs, larvae and cocoons, were also surprisingly low, with means of 2.5 cocoons, 2.1 larvae and 1.3 eggs (Table 2). Brood of *Diacamma* was found in all, and brood of *P. lama* in 30 out of 36 mixed colonies.

Behavioral observations in laboratory colonies:

Outdoor behavior, foraging and food distribution

Except during nest relocations, the *P. lama* specimens were never seen outside the nest in any observation period. Only workers of *Diacamma* sp. left the nest chamber for foraging in the arena. In observation period I (the colony consisting of 53 *Diacamma* workers and 5 *P. lama* ergatomorphs), we recorded on random occasions over two months which ants left the nest. Among a total of 1172 ants leaving the Petri dish chamber (648 during 66 daytime sessions and 524 during 34 night observations) there were no *P. lama* but exclusively *Diacamma* workers.

Food, i.e., insect pieces and honey water, was brought into the nest only by *Diacamma* workers. As in other Ponerinae, which do not regurgitate liquid food for trophallaxis (Dejean and Suzzoni, 1997; Hölldobler and Wilson, 1990: 292), droplets of honey water were carried beneath the head and between the mandibles. Droplets were offered directly, or after some time, to the nestmates. In several instances such droplets also were deposited on the nest floor, without previous offering to nestmates. *P. lama* workers contacted *Diacamma* from time to time with their antennae and mouthparts in a way resembling food-begging behavior. On five occasions we observed a *Diacamma* worker opening its mandibles and offering liquid food, which was apparently licked

off by a *Polyrhachis* worker. We could not observe protein feeding in *P. lama* ergatomorphs because they always sat in the densest group of *Diacamma* which had formed on the *Diacamma* brood. The *P. lama* ergatomorphs were often located within antennal contact distance of each other.

Nest relocation and interspecific communication

In eight experiments during observation period I we initiated a nest relocation. The ants were taken out of the Petri dish and placed in the arena about 20 cm from the now empty nest. The ants first assembled in one corner of the arena. Then it was invariably the *Diacamma* workers who searched for the nest and organized the return of their nestmates by tandem leading (Maschwitz et al., 1986). The *P. lama* ergatomorphs always waited in the assemblage until a *Diacamma* invited them with mandible jerking and led them back into the Petri dish. When lost during tandem running, workers of *P. lama* did not find their way back to the ant assembly or reach the nest independently over distances of more than a few centimeters.

In a series of further colony relocation experiments during observation period IV again all *P. lama* were led to the new nest by the host ants. The tandem following behavior of both species is quite similar. However, single *P. lama* ergatomorphs in these experiments could be observed during nest relocation running back over a wooden bridge to their old nest over a distance of almost one meter. There they searched for *P. lama* brood items which they carried to the new nest without any assistance of the *Diacamma*.

Brood care and brood rearing in mixed colonies of Diacamma sp. and Polyrhachis lama

Field collected colonies with dealate gynomorphs of *P. lama*, as well as those exclusively comprising one or several ergatomorphs, had *P. lama* eggs, larvae, cocoons and sometimes alates of both sexes (Table 2). From the cocoons both ergatomorphs and a few alate sexuals hatched in the following days (observation period II).

During observation period III (in Malaysia) numerous *P. lama* eggs appeared in the three colonies which contained only *P. lama* ergatomorphs but no gynomorph. A *Diacamma* gamergate queen was present in two of the colonies, whereas the third one comprised only *Diacamma* workers. The *Diacamma* also produced many eggs in all 3 colonies. *P. lama* egg clumps of up to ca. 20 eggs each were carried around exclusively by *P. lama* ergatomorphs. *Polyrhachis* larvae hatched about 20 days after the first eggs had been seen. The young larvae also formed clumps, and were carried around nearly exclusively by the *P. lama* ergatomorphs. Both *Diacamma* and *P. lama* only rarely deposited eggs or small larvae on the nest floor. Instead they usually kept them between their mandibles even when not disturbed by an observer. Cocoons and large larvae were kept lying on the ground more frequently, and the brood of the two species then was mixed.

Larger *P. lama* larvae were carried singly, usually also by *P. lama*, whereas *Diacamma* brood was exclusively handled by *Diacamma* workers. Less frequently we saw *Diacamma* workers carry around single large *Polyrhachis* larvae, and rarely also groups of smaller larvae and eggs. Regularly however, *Diacamma* carried *Polyrhachis* cocoons. In one experiment a mixed colony with larvae of both species was placed in an arena (20 × 20 cm) with a small shelter. The *Diacamma* workers first carried their own larvae into the shelter, simultaneously leading *Diacamma*- and *Polyrhachis* nestmates there by tandem running, and finally, after about 2½ hrs, the seven *Polyrhachis* larvae also were carried away by *Diacamma* workers.

In a series of random observations over 3 days (observation period II) we saw in one mixed colony 43 *Diacamma* workers carrying *Diacamma* larvae and two *Diacamma* workers carrying *Polyrhachis* larvae. 61 *Polyrhachis* workers carried *Polyrhachis* larvae, and none carried a host larva.

In another series of 19 observations, the numbers of carried cocoons were censused during the first 10 seconds after a disturbance of a mixed colony. Both the *Diacamma* workers and *P. lama* ergatomorphs carried only one cocoon at a time. We counted 63 *Diacamma* workers with a *Diacamma* cocoon and 53 *Diacamma* workers with a *Polyrhachis* cocoon. No *Polyrhachis* ergatomorphs were observed carrying *Polyrhachis* or *Diacamma* cocoons.

Only a few observations were possible with respect to the nutrition of larvae (observation periods II and III). *Diacamma* workers in the field carry dead small insects (e.g., termites) and pieces of arthropods into the nest. In the laboratory, *Diacamma* larvae were seen feeding at such items. Since the *Polyrhachis* carrying clumps of larvae were often close by, we assume that the larvae may also feed on these food particles. Sometimes a *Polyrhachis* worker bent over a larger *P. lama* larva for a few seconds (up to 30), as if regurgitating food to the larva. Exact observations, however, were difficult because the ants and their broods usually form a dense clump. In an experiment we were able to feed isolated large *Polyrhachis* larvae with insect pieces. *P. lama* eggs laid by ergatomorphs during observation periods III and IV developed into males, but we could not obtain conclusive results on whether ergatomorphs and gynomorphs can develop from these eggs, too. Most of the larvae disappeared successively during larval development or after pupation. This problem will be studied in more detail in the future.

Discussion

The parasitic relations of Polyrhachis lama and Diacamma sp.

Though at present the life history of *Polyrhachis lama* is not completely known, there are several features which are indicative of permanently parasitic, inquiline life habits. (I) *P. lama* was found exclusively within nests of another species, here belonging to the genus *Diacamma*, a species which also has numerous nests without *P. lama* in the study area. (II) Reproductive females of both species coexist in the mixed

nests which (III) also contain the brood of the two species. (IV) The parasite does not engage in foraging outside the nest. (V) In emergency situations such as enforced nest relocation the *P. lama* workers are quite inactive and sluggish, as is common in inquiline ants. (VI) *Polyrhachis* specimens receive food from *Diacamma* workers, and (VII) *Diacamma* workers at least partially take over the care of the *Polyrhachis* brood. Though the ability of *Polyrhachis lama* to survive and to rear its brood alone was not checked, we conclude that this species represents an obligatory permanent parasite of its *Diacamma* host. Since the two species belong to different ant subfamilies, the Formicinae and Ponerinae, this parasitism does not fulfil Emery's rule even in the widest sense. It is the first well-documented instance of an obligatory, permanent parasitism between members of two ant subfamilies.

Social parasites should be somehow detrimental to their hosts, and in many instances a considerable impairment of the host colonies is obvious. For example, in slave-making ants, a queen during colony foundation destroys one host colony completely, and the colony then damages a number of other host colonies by slave-raiding. Queens of temporary parasites also destroy one host colony each during dependent colony foundation. Much less is known about the effects of inquiline ants that are found in queenright host colonies, although, in some instances, they apparently reduce the sexual production of their hosts. In the case of *P. lama* there seems to be no effect on worker number in the parasitized *Diacamma* colonies, and also the number of *Diacamma* brood items in these colonies is not significantly different whether or not they harbor *P. lama*. Our data thus do not reveal any sizeable impairment of the *Diacamma* host colonies.

The reproductive biology of Polyrhachis lama

Most of the mixed colonies (41 out of 48) lacked a dealate gynomorph. We are sure that this is not due to loss or overlooking of the gynomorphs in a majority of the colonies during collecting, and it is unlikely that such a high proportion of colonies should recently have lost their gynomorphic queens. The evidently very low production of *P. lama* alate gynomorphs and males (Table 2) is a strong argument against the assumption of frequent infestation of new *Diacamma* colonies by dealate *P. lama* females, and their early orphaning. We also rule out the possibility of a polydomous nesting system, with always one queenright *P. lama*/*Diacamma* nest surrounded by 6–7 queenless branch nests, because the single nests were usually several to many meters apart from each other, with a couple of unparasitized *Diacamma* colonies in between. Of course, we may not have found all the *P. lama* nests that were present in a given area.

On the other hand, practically all *P. lama* ergatomorphs, and also virgin dealate gynomorphs of this species, had fully developed ovaries with yolky oocytes and corpora lutea, indicating egg-laying. Most of the mixed colonies with adult *P. lama* ergatomorphs but no *P. lama* gynomorphs had *P. lama* brood, eggs, larvae and/or cocoons (Table 2), the brood thus probably being the progeny of ergatomorphs. Young

alate gynomorphs were found in six colonies, and all these colonies comprised only ergatomorphs but no dealate gynomorph of *P. lama* which could have been their mothers. The four colonies with dealate gynomorphs that were censused, had by no means more brood items than the colonies with only *P. lama* ergatomorphs. These observations seem to indicate that virgin gynomorphs and ergatomorphs of *P. lama* can produce not only males but gynomorphs and ergatomorphs as well, by parthenogenesis. Arrhenotoky (parthenogenetic male production) is very common in ant workers, whereas thelytoky (female offspring produced by unmated workers) occurs only rarely (Heinze and Hölldobler, 1995; Tsuji and Yamauchi, 1995). We hypothesize that sexual reproduction by mated gynomorphs is an exception in *P. lama*, and that ergatomorphs and virgin gynomorphs reproduce by amphitoky, but this must be studied more extensively with rearing experiments. If this hypothesis proves true, we may assume that the reproductive system of *P. lama* is somehow similar to that of other ants with queen polymorphism (Buschinger and Heinze, 1992), where alate gynomorphs are produced for long range dispersal, and intermorphic or ergatomorphic queens can colonize local patches of suitable habitat. The ergatomorphs of *P. lama*, however, both in morphology and anatomy correspond to ordinary workers of independent *Polyrhachis* species in that they lack a spermatheca.

Other instances of social parasitism in the genus Polyrhachis

The occurrence of social parasitism in the genus *Polyrhachis* has perhaps been underestimated. Kutter (1968) expressed astonishment at the fact that this large genus did not comprise any social parasites, and Hölldobler and Wilson (1990) omitted the genus from their list of parasitic ants. Though obligatory parasitism in a *Polyrhachis* species has not been described before now, a couple of suggestive reports do exist. Thus, Yano (1911) discovered one mixed colony of *Polyrhachis lamellidens* and *Camponotus japonicus* in Japan. Kohribe (1963) studied the same association in an artificially established laboratory colony of one *P. lamellidens* queen and a few *Camponotus* workers, and he observed that *Camponotus* workers fed both workers and brood of the *Polyrhachis* via regurgitation. In addition, the *Polyrhachis* workers also tended their own larvae. The "parasites" sometimes left the nest, and in the winter they formed a cluster together with their brood, separate from the hosts. Kohribe (1966) found a single *Camponotus japonicus* worker in a nest of *P. lamellidens*, and in the laboratory he confirmed that the *Camponotus* was working for the colony. Another such instance was reported by Kubota (1974) who found a dealate queen of *Polyrhachis lamellidens* together with workers of *C. japonicus*, 20 cm deep in the ground in the periphery of a *C. japonicus* nest. Both authors observed some aggressive behavior of *Camponotus* workers towards the *Polyrhachis* queens, but also documented a kind of appeasement behavior by the latter. Kohribe (1966) provided a photograph of this "rubbing behavior", the *Polyrhachis* female sitting astride on

top of a *Camponotus* worker and tapping it with the antennae. Though little extra information exists on the relations of these two formicine ants, a kind of temporary parasitism, which is perhaps facultative, can be assumed.

A few more *Polyrhachis* species may be involved in parasitic relations with other ant species, according to a number of anecdotal reports. Chapman (1963) observed for many years that *Polyrhachis parabiatica* workers and wingless females joined the forays of *Myrmecaria* sp., a myrmicine. Since the nest of the *Polyrhachis* was located in the trunk of a tree fern that was surrounded by the *Myrmecaria* mound nest, this relation probably represents a parabiosis. Kohout (1990) mentions some instances where several *Polyrhachis* species were found together with *Rhytidoponera* species (running together in disarray; or found together in galleries of a common large *Rhytidoponera*; or collected under the same rock). These species, like *P. lama*, belong to the *viehmeyeri* group in the *Polyrhachis* subgenus *Myrmhopla*. Altogether, it seems promising to study more closely such instances. Our observations on *P. lama* strengthen the suspicion that parasitism of unrelated host species groups may have evolved several times in this genus.

Evolutionary aspects of social parasitism among unrelated ant species

According to Wilson (1971) and Hölldobler and Wilson (1990), permanent social parasitism (inquilinism) may have evolved via three routes, (I) via territorial aggression and enslavement of workers of the same or different species, leading to dulosis, (II) via adoption of young queens, polygyny and budding, leading to temporary parasitism, and (III) via plesiobiosis and xenobiosis; all three routes ending up in permanent inquilinism. This hypothesis has been questioned by Buschinger (1990), who suggested a separate origin of inquilinism, temporary parasitism and slavery, all evolving from a "preparasitic" state of facultative polygyny.

With respect to the direct origin of a parasite, two competing hypotheses propose either an allopatric speciation where one of two independent species after secondary overlap of their ranges becomes the parasite of its sister species (Agosti, 1994; Hölldobler and Wilson, 1990; Ward, 1996), or sympatric speciation (Bourke and Franks, 1991; Buschinger, 1990; Elmes, 1978; West-Eberhard, 1986, 1987). Both hypotheses explain the close relationships of hosts and parasites that characterize the traditional instances of obligatory social parasitism.

In such parasitic associations the partners have similar or equal communication signals and physiological properties because of direct common descent. For social parasites not obeying Emery's rule, particularly those exploiting hosts of another ant subfamily, the problem emerges that they have to adapt to the respective features of their hosts, to the communication system (tactile and/or chemical), to their diet, brood care and provisioning (e.g., with special glandular secretions) and perhaps to a different seasonal niche. Further research has to focus on such adaptations. Most probably such

parasitism begins with plesiobiosis or parabiosis, and the incipient parasite may adapt more and more to the life history features of its host. The striking morphological similarity between adult *P. lama* and *Diacamma* sp. in the case described here points to former mimetic relations of *P. lama* with the highly pugnacious *Diacamma* as a model during earlier evolution of this parasitic system. In a recent paper (Gobin et al., 1998) on *Polyrhachis rufipes* following the trails of the ponerine ant, *Gnamptogenys menadensis*, morphological similarity of the two species is also stated. A comparison of other symbiotic or parasitic *Polyrhachis* species and their free-living relatives should reveal such evolutionary processes. In addition, it will be of great interest to investigate whether parasitism between distantly related species has morphological consequences similar to the "anatomical parasitic syndrome" of Wilson (1984).

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