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Social Organization in two Primitive Attine Ants, *Cyphomyrmex rimosus and Myrmicocrypta ednaella,* with Reference to Their Fungus Substrates and Food Sources

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Abstract — In the Neotropical rainforest of Barro Colorado Island, Panama, social organization and behavior were observed in 2 primitive attine ant species, *Myrmicocrypta ednaella* and *Cyphomyrmex rimosus*. Both species took nutrients from mycelia on fungus (i.e. mycophagy), and from plant nectar and sap which they collected outside the nest (i.e. phytophagy). They also obtained alimentary liquid by soliciting nestmates (i.e. stomodeal trophallaxis). Queens and larvae were wholly mycophagous, while older workers were much dependent on nectar, sap and alimentary liquid and younger workers were mostly mycophagous but only partly phytophagous. *M. ednaella* used wood chips as substrate for the fungus garden. Its fungus-growing behavior was similar to those hitherto observed in other primitive attine species. In contrast, the behavior of *C. rimosus* was unique in its utilization of crop liquid as a substrate. In the rainforest, *C. rimosus* workers frequently forage outside the nests to collect nectar and sap, most of which is probably regurgitated for fungus cultivation.

Introduction

The tribe Attini is NewWorld, mainly Neotropical, in distribution. All ants of this tribe cultivate fungus gardens and depend upon them for food. Therefore, many myrmecologists have been attracted to these ants, and there have been some specific hypotheses for the origin of the fungus-growing behavior (von Ihering 1894; Forel 1902; Weber 1956a, 1958 and 1972; Garling 1979). However, none of these hypotheses have been tested, and the evolution of mycophagy in attine ants remains controversial. This is partly due to the scarcity of studies on the behavior of primitive attine species, most of which occur cryptically in Neotropical rainforests. Among 12 genera of Attini, Cyphomyrmex has long been considered as the most primitive because the shape and position of the antennal scrobes link it with various non-attines (e.g. Forel 1885 and 1892). Moreover, Weber (1941, 1958 and 1972), Wilson (1971) and Hölldobler and Wilson (1990) regarded *C. rimosus* as the most primitive species of the genus because of its yeast-cultivating behavior. A largely ignored alternative hypothesis, that *Myrmicocrypta* is the most primitive attine genus, was first proposed by Wheeler (1910) on the basis of queen / worker polymorphism, as well as polymorphism within the worker caste. Kusnezov (1961 and 1963) supported this idea mostly by the characters of wing venation and male antennae. Furthermore, Schultz and Meier (1995) recently reconsidered the phylogeny of attine genera based on the morphology of larvae and belive *Myrmicocrypta* to be the most

primitive genus. In the present study, therefore, we aimed to compare the behavior of *Cyphomyrmex rimosus* and *Myrmicocrypta ednaella*, with special reference to their fungus substrates and food sources.

Methods

In October and November of 1993 and 1994, colonies of *C. rimosus* and *M. ednaella* were collected in the rainforest of Barro Colorado Island, Panama. Immediately after collection, the numbers of queens, workers, pupae, larvae, and eggs were counted in the laboratory. For some colonies, ants were fixed and preserved in 80% alcohol to examine the conditions of the ovary, spermatheca and yellow bodies, which are egg remnants that indicate oviposition. For some other colonies, ants were kept alive in an artificial nest (50 x 25 x 5 mm) which was covered by a glass pane in order to allow observation of ant behavior. During observation, food and nest materials such as nectar, bananas, dead vegetables and insect feces were always present in the foraging arena adjacent to the artificial nest.

Behavioral repertory and polyethism were studied in 2 *M. ednaella* colonies (M1 and M2) and 5 *C. rimosus* colonies (C1-C5). Workers were divided into 3 age groups according to their body color (yellow as young; brown as middle-aged; and dark as old). In each colony, the workers and queen were individually marked with paint, and their behavior was observed in the artificial nest for totals of 10 to 65 h. Activities of each individual were scanned for 15 min. After the observation of all workers, the queen and larvae were also observed for 10 h in an *M. ednaella* colony (M3) and for 15 h in a *C. rimosus* colony (C6) with particular attention to their feeding.

The construction of the fungus garden was observed for a total of 30 and 40 h in 3 *M. ednaella* colonies (M4, 5 and 6) and 4 *C. rimosus* colonies (C7-C10), respectively. These observations were focused on the materials which workers used for the substrates of the fungus garden.

Finally, we also collected foraging *C. rimosus* workers in the rainforest in late November, 1994. The captured workers were dissected in the laboratory and their crop examined for liquid or not. Some species of flowers were also collected in the field and examined in the laboratory to see whether or not *C. rimosus* workers were in these flowers to collect nectar from them.

Results

Myrmicocrypta ednaella

We collected 17 *M. ednaella* colonies which had nested about 10 cm below ground. In each nest, an almost spherical-shaped fungus garden was found. It was about 50 mm in diameter and composed of many wood chips with occasional pieces of insect corpses. The number of adult workers ranged from 10 to 190 with an average of 60.7 ± 42.3. Dissection revealed that the workers lacked spermathecae and had only 1 pair of atrophied ovarioles containing no eggs. This species was always monogynous, with a single queen which was inseminated and had $3+3$ developed ovarioles.

Behavioral repertories

In the laboratory observation of *M. ednaella*, we distinguished 12 activities. Since there was no significant difference in the characteristics of polyethism between colonies M1 and M2, the data of the 2 colonies were combined (Table 1). The queens were always inside the nest, spending 53.7% of their time in doing nothing (=repose). It was followed by mycophagy (20.0%) in which the queens stayed on the fungus garden and fed on the mycelium. During the entire observation of 78 hours, oviposition by the queen was observed only once. On the other hand, workers accomplished a variety of tasks, including fungus care, grooming, nest defense and extranidal foraging. Unlike workers of other myrmicine tribes, *M. ednaella* workers rarely took care of brood, probably because the larvae of attine species are placed in the fungus garden and can eat mycelium without assistance of workers.

Polyethism

Based on the body color, workers of colony

Table 1. Behavioral repertory of *M. ednaella*. Figures in columns indicate the percentage of frequency of each activity. Data from 2 colonies were combined. Workers were divided into 3 classes based on their pigmentation. The number of ants observed is given in parentheses.

	Queens	Workers		
	(n=2)	yellow (n=10)	brown (n=22)	black (n=9)
Intranidal activities				
Repose	53.7	11.5	6.1	4.4
Walk	8.7	4.9	7.2	3.2
Fungus care	1.2	58.2	30.5	7.9
Grooming	8.5	16.2	24.0	21.9
Antennation	7.5	4.4	4.6	2.0
Mycophagy	20.0	1.8	1.1	0.2
Trophallaxis	0.0	1.3	2.6	2.1
Phytophagy	0.0	0.1	0.0	0.1
Defense	0.0	1.2	3.9	1.5
Brood care	0.2	0.1	0.1	0.1
Oviposition	0.2	0.0	0.0	0.0
Subtotal	100.0	99.8	80.2	43.5
Extranidal activities				
Repose	0.0	0.0	1.8	6.2
Walk	0.0	0.1	11.4	37.2
Collect wood chi	0.0	0.0	2.8	5.1
Trophallaxis	0.0	0.0	0.1	0.0
Phytophagy	0.0	0.0	2.2	2.8
Grooming	0.0	0.0	1.4	4.7
Antennation	0.0	0.1	0.1	0.5
Subtotal	0.0	0.2	19.8	56.5
Total	100.0	100.0	100.0	100.0
Total number of act	425	2281	3950	1631

M1 were divided into 6 young, 12 middle-aged and 5 old individuals. They showed a remarkable age-polyethism (Table 1). Young workers were most frequently engaged in fungus care (percentage of acts=58.2), while old workers performed extranidal activities such as collecting wood chips for the fungus garden (percentage of acts=56.5). Middle-aged workers showed a broad range of activities, their characteristics being intermediate between the young workers and the old workers. These 3 groups were clearly distinguished from each other by discriminant analysis (Fig. 1). A similar result was also obtained in the discriminant analysis of colony M2.

The queens and young workers always groomed themselves before stepping into the fungus garden and frequently did so when feeding on mycelium. Workers guarding at the nest entrance groomed the nestmates that returned from extranidal tasks. Returning workers also did self-grooming in the nest.

Feeding

For the queens and larvae, the feeding behavior was observed 85 and 29 times, respectively, indicating that they depended exclusively on mycelia as nutrition (Fig. 2). No trophallaxis was observed between worker and larva. However, mycophagy is much less nutritionally important for workers. In particular, the old workers rarely fed on mycelia. Instead, they depended much more on nectar and saps of plants (54.8% of all feeding acts) and on alimentary li-

quid regurgitated from other old workers (38.1%).

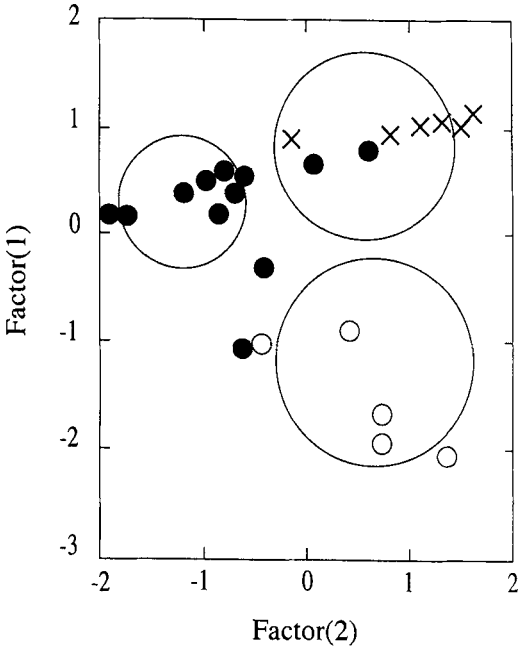

Fig. 1. Discriminant analysis of behavioral differences between 3 age groups in *M. ednaella* No.1 colony. Canonical discriminant scores are shown for Factors 1 and 2. Each circle indicates 95% confidence range. ○: old worker; ●: middle-aged worker; ×: young worker

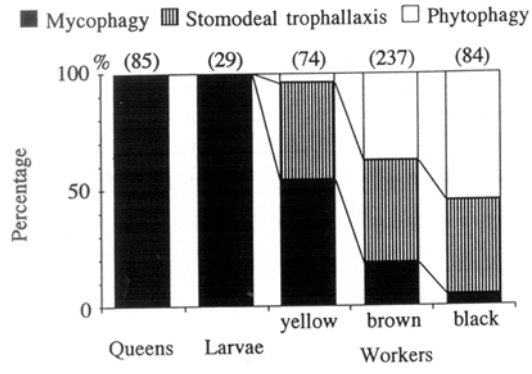

Fig. 2. Food habits of *M. ednaella*. Total frequency of each activity is shown in parentheses.

Fungus growing

Observation with colonies M4, M5 and M6 clarified the following characteristics of the fungus-growing behavior of *M. ednaella*. The first stage of fungus growing was cleaning of the nest floor by a worker's licking with the mouthparts. At the same time workers outside the nests collected wood chips by crushing dead twigs with their mandibles. They retrieved and piled up the chips on the nest floor which had previously been cleaned. Then, other workers plucked hyphae from the old part of the fungus garden and planted them onto the new wood chips. These workers subsequently fertilized the fungi by secreting fecal liquid. As the mycelia grew, workers often cut off long hyphae with their mandibles and replaced old substrates with newly collected wood chips. The new substrate was planted with hyphae which were subsequently fertilized with fecal secretion.

Cyphomyrmex rimosus

A total of 40 colonies were collected from fallen twigs on the forest floor. The number of workers ranged from 7 to 334, with an average of 66.9±71.1. Although most colonies were monogynous, there were 2 queens in 2 colonies and no queen in 4 colonies. All the queens collected were found to be inseminated and had well-developed 3+3 ovarioles. Workers were also dissected. They had no spermatheca but had a pair of atrophied ovarioles without any developed eggs. This suggests that the queens monopolize oviposition in this species. In the colonies with 2 queens, both queens had yellow bodies, indicating that they were equally functional. In the 4 queenless colonies, there was no egg, indicating that the absence of a queen was not due to mis-sampling but probably due to natural death.

Behavioral repertories

The behavioral repertory of *C. rimosus* consisted of 12 activities (Table 2). Although the queens spent 66.0% of their time in repose, their repertory was more worker-like than *M. ednaella* queens, which rarely performed tasks other than grooming and mycophagy (cf. Table 1). The inseminated queens of *C. rimosus* spent 4.1 and 3.1% of their acts in fungus care and brood care, respectively. Worker behavior was also different between *C. rimosus* and *M. ednaella*. *C. rimosus* workers frequently licked the body surfaces of larvae and often trans-

Table 2. Behavioral repertory of *C. rimosus*. Figures in columns indicate the percentage of frequency of each activity. Data from 2 colonies were combined. Workers were divided into 3 classes based on their pigmentation. The number of ants observed is given in parentheses.

	Inseminated queens (n=5)	Virgin queens (n=4)	Workers yellow (n=36)	Workers brown (n=50)	Workers black (n=22)
Intranidal activities					
Repose	66.0	61.4	27.8	24.8	9.6
Walk	6.1	8.6	8.1	9.5	8.3
Fungus care	4.1	6.4	24.6	11.2	9.9
Grooming	6.6	7.5	12.0	16.6	20.2
Antennation	4.3	6.4	3.4	4.5	3.4
Mycophagy	8.7	4.3	3.0	1.4	0.4
Trophallaxis	0.0	0.0	0.6	0.7	1.2
Phytophagy	0.0	0.0	0.0	0.0	0.0
Defense	0.0	0.4	5.0	10.0	4.9
Brood care	3.1	4.6	14.6	7.7	1.0
Oviposition	1.0	0.0	0.0	0.0	0.0
Subtotal	100.0	99.6	99.1	86.3	58.9
Extranidal activities					
Repose	0.0	0.0	0.0	1.1	2.4
Walk	0.0	0.4	0.3	8.6	24.8
Collect feces	0.0	0.0	0.5	2.1	5.2
Trophallaxis	0.0	0.0	0.0	0.0	0.0
Phytophagy	0.0	0.0	0.0	0.8	6.2
Grooming	0.0	0.0	0.1	1.0	2.5
Subtotal	0.0	0.4	0.9	13.7	41.1
Total	100.0	100.0	100.0	100.0	100.0
Total number of acts	391	280	2422	4051	1911

ported them to fresh areas of the fungus garden where yeast growth more vigorous.

Polyethism

Figure 3 shows the result of discriminant analysis done for colony C1, which contained 17 young, 32 middle-aged and 10 old workers. Young workers were engaged more often in the care of fungi and larvae, while older workers performed most extranidal activities (Table 2). In the discriminant analysis, 3 age groups were separated from each other with a probability of 82.8%. A similar result was obtained also for the other 4 colonies (C2-C5). Like *M. ednaella*, *C. rimosus* frequently groomed themselves or nestmates, probably to prevent the fungus garden from being contaminated.

Feeding

Food sources in *C. rimosus* were the yeast growing on the substrate, the nectar and sap of plants, and the alimentary liquid regurgitated from nestmates. The queen and larvae were exclusively dependent on the yeast as their nutrient (Fig. 4). In young and middle-aged workers, mycophagy accounted for 81.8% and 48.3% of feeding, respectively (Fig. 4). Since these workers frequently engaged in trophallaxis with old workers (22.7% in young workers and 35.3% in middle-aged workers), they probably also obtained nutrient in this way. The old workers, who mostly depended on nectar and sap (79.2%). Trophallaxis among old workers was frequent (15.4%) but they have never been

observed to receive nutrient from younger workers. This suggests that the old workers do not get nutrient from the yeast even in an indirect manner via younger workers. Larval mycophagy was recorded 39 times during 15 h of observation with 6 larvae.

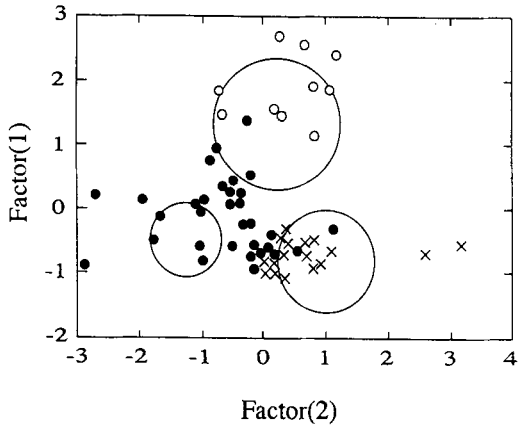

Fig. 3. Discriminant analysis of behavioral differences between 3 age groups in *C. rimosus* No.1 colony. Canonical discriminant scores are shown for Factors 1 and 2. Each circle indicates 95% confidence range. ○: old worker; ●: middle-aged worker; ×: young worker

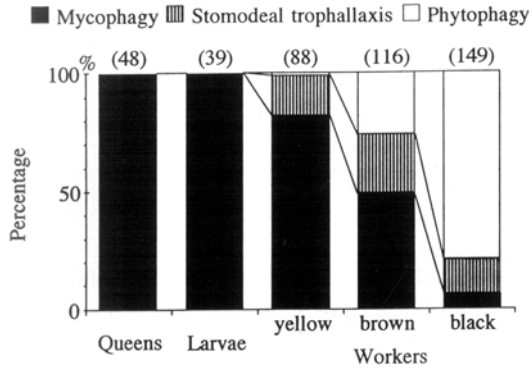

Fig. 4. Food habits of *C. rimosus*. Total frequency of each activity is given in parentheses.

Fungus growing

The fungus-growing behavior of *C. rimosus* was quite different from those known so far in other attines. As shown in Fig. 5, an intranidal *C. rimosus* worker licked and cleaned a part of the nest floor and put there a piece of insect or millipede feces that had been previously carried into the nest by an extranidal worker. Then another extranidal worker regurgitated alimentary liquid from her crop onto the surface of the feces. She occasionally mixed her fecal secretion into the alimentary liquid and then left it for 1 day. When the liquid had dried and became a nodule, another worker again added her 2 types of liquid to it. Out of 93 secretions observed in 5 colonies, 83 were the regurgitation from mouthparts and 10 were anal secretion. Thus it seems that the material for the substrate of the fungus garden is mostly crop liquid.

After secretion and desiccation were alternately repeated, the brown nodule grew into a ball of about 0.8-mm diameter. At this stage the nodule was removed and transferred to the surface of insect or millipede feces on which some nodules that were already covered with yeast were present. The transporting worker transplanted the yeast by rubbing the new nodule against the neighboring old ones. Within 24 h, the newly placed nodule became densely covered with yeast. The intranidal workers maintained this fungus garden by licking and plucking the yeast. When the nodule became too old, workers gnawed off its surface and regurgitated the alimentary liquid onto another newly exposed area and again rubbed it against an yeast-covered nodules again.

In the field, we collected 59 *C. rimosus* workers that were foraging in the rainforest: 18 of them were found on trees, 37 on the ground and 4 in flowers that had fallen on the forest floor. Dissection revealed that 20 of these workers had crops filled with a liquid. This suggests that the workers of *C. rimosus* frequently forage for nectar and sap of plants. Laboratory experiments showed that *C. rimosus* collected nectar from the flowers of 4 plant species: *Psychotria marginata, Erythrin erythrinum, Quassia* sp., and Rubiaceae sp.

Discussion

Weber (1958 and 1972) and Schultz and Meier (1994 and 1995) proposed different phylogenies for attine genera. Their phylogenies are different particularly in the phyletic position of *Cyphomyrmex* and *Myrmicocrypta*. Schultz

and Meier carried out a cladistic analysis on 51 attine species using 44 morphological characters of larvae, and found that *Myrmicocrypta* larvae have some traits common with the other myrmicine tribes, while *Cyphomyrmex* larvae are more specialized. In contrast, Weber adopted morphological and behavioral characters of adults, and his conclusion was that *Cyphomyrmex*, especially *C. rimosus*, is the most primitive. Hölldobler and Wilson (1990) discussed the evolution of mycophagy based on Weber's idea. On the other hand, Chapela et al. (1994) and Hinkle et al. (1994) emphasized Schultz and Meier's phylogeny. We believe, however, that more information is needed; in particular, DNA sequence data are essential.

If the phylogeny of Schultz and Meier is correct, phylogenetic proximity would not be correlated with the behavioral similarity in the Attini, because the fungus-growing behavior of *M. ednaella* is not greatly different from those of more advanced genera, e.g. *Sericomyrmex* and *Trachymyrmex*, which construct mycelial fungus gardens with fragments of dead plants (Weber 1954, 1956b and 1972). In contrast, even when compared with other congeneric species, the behavior of *C. rimosus* is unique in the Attini in 3 ways. First, *C. rimosus* does not form mycelial fungus gardens but cultures a yeast on small substrates which are placed on the feces of herbivorous insects or millipedes. Second, the workers frequently take care of larvae by licking or transporting them. Finally, unlike Weber's description (1962 and 1972) of the worker's feces is importance of worker's feces in cultivating the mutualistic yeast, our study demonstrated that the anal liquid is much less important for the nutrition of the yeast than the crop liquid, or that the development of the yeast is limited to the surface on the desiccated nodule of alimentary liquid. In *C. rimosus*, thus, the main substrates for the yeast are apparently the alimentary liquids regurgitated by the ants.

It was long believed that in the Attini all colony members get nutrients exclusively by eating mycelia. However, Quinlan and Cherrett (1979) found that *Atta cephalotes* workers obtain 95% of their carbohydrates from plant products that are collected outside the nests, and only 5% from mycophagy. Our study revealed that the workers, particularly, old individuals of the primitive attines such as *M. ednaella* and *C. rimosus*, are also more dependent on plant products than on mycelium.

In the present study of *M. ednaella* and *C. rimosus*, trophallaxis from workers to larvae was not observed and mycophagy was the unique mode of nutrition for larvae. Absence of trophallaxis between worker and larva is common in the Attini (Weber 1972; Littledyke and Cherrett 1976; Quinlan and Cherrett 1979). On the other hand, the larvae of most myrmicines are given alimentary liquid by stomodeal trophallaxis from workers (Wilson 1971; Hölldobler and Wilson 1990). Then a question arises: how has mycophagy evolved from stomodeal trophallaxis in nutrition of attine larvae? Moreover, the fact that ant larvae are very vulnerable to coprophilous or saprophytic fungi (Wilson 1971) leads to a second question: how has the mutualistic fungus been selected from a variety of fungi, most of which seem to be harmful to ant larvae? Although various hypotheses have been proposed for the evolution of mycophagy in attines (von Ihering 1898; Forel 1902; Weber 1956a, 1958 and 1972; Garling 1974), none refers to these questions. In this regard, our finding in *C. rimosus* that the worker's crop liquid is necessary for the growth of the mutualistic fungus is suggestive. It may be that the ancestor of the mutualistic fungus first grew on the substance regurgitated by workers and the larvae fed on such fungus-infested crop liquid. In this beginning stage of mycophagy (and also presently in *C. rimosus*), chemical substances from worker's exocrine glands might be capable for selection of the edible fungi.

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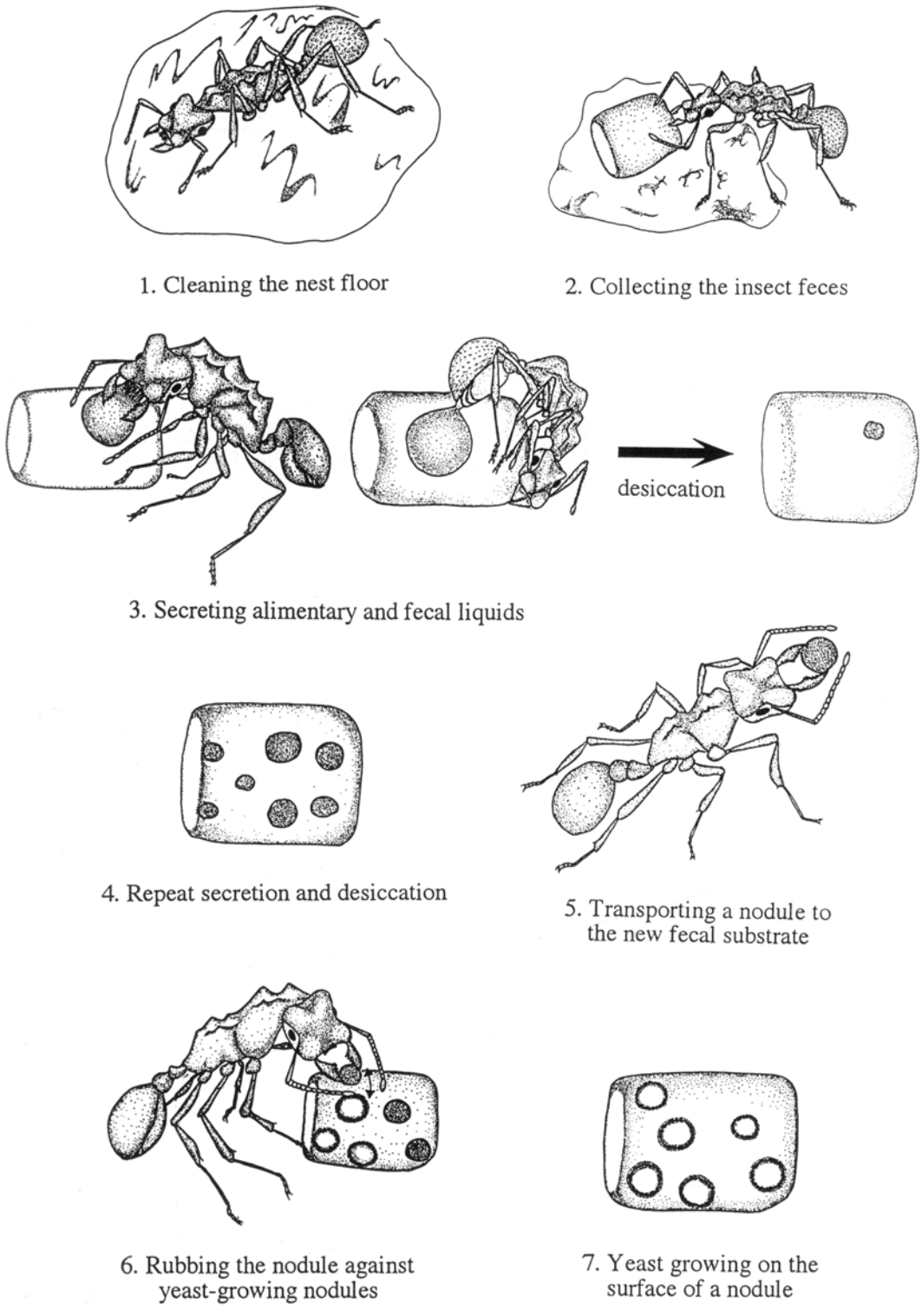

Fig. 5. Fungus-growing behavior of *C. rimosus*

References

Chapela, I.H., S.A. Rehner, T.R. Schultz and U.G. Muellerk 1994 Evolutionary history of the symbiosis between fungus-growing ants and their fungi. *Science 266* : 1691-1694.

Forel, A. 1885 [1884] Études myrmécologiques en 1884 avec une description des organes sensoriels des antennes. *Bull. Soc. Sci. Natur. 20* : 316-380.

Forel, A. 1892 Attini und Cryptocerini. *Mit. Schweiz. Entomol. Gesell. 8* : 344-349.

Forel, A. 1902 Beispiele phylogenetischer Wirkungen und Rückwirkungen bei den Instinkten und dem Körpenbau der Ameisen als Belege für die Evolutionslehre und die psychophysiologische Identitätslehre. *Psychol. Neurol. 1* : 99-110.

Garling, L. 1979 Origin of ant-fungus mutualism: a new hypothesis. *Biotropica 11* : 284-291.

Hinkle, G., J.K. Wetterer, T.R. Schultz and M.L. Sogin 1994 Phylogeny of the Attine ant fungi based on analysis of small subunit ribosomal RNA gene sequences. *Science 266*: 1695-1697.

Hölldobler, B. and E.O. Wilson 1990 *The Ants*. The Belknap Press of Harvard University Press, Cambridge, Mass. 732 pp.

Ihering, H. von 1898 Die Anlage neur Colonien und Pilzgärten bei *Atta sexdens*. *Zool. Anzeig. 21* : 238-245.

Kusnezov, N. 1961 Evolution der Ameisen. *Symp. Genet. Biol. Italica 12* : 103-121.

Kusnezov, N. 1963 Zoogeografica de las hormigas en Sudamerica. *Acta Zool. Lilloana 19*: 25-186.

Littledyke, M. and J.M. Cherrett 1976 Direct ingestion of plant sap from cut leaves by the leaf-cutting ants *Atta cephalotes* (L.) and *Acromyrmex octospinosus* (Reich) (Formicidae, Attini). *Bull. ent. Res. 66* : 205-217.

Quinlan, R.J. and J.M. Cherrett 1979 The role of fungus in the diet of the leaf-cutting ant *Atta cephalotes* (L.). *Ecol. Entomol. 4* : 151-160.

Schultz, T.R. and R. Meier 1994 The phylogeny of the fungus-growing ants (Myrmicinae: Attini) and their fungi. In: A. Lenoir A, Arnold G, Lepageles M (eds) Insectes Sociaux. Univ Paris Nord, 177 pp.

Schultz, T.R. and R. Meier 1995 A phylogenetic analysis of the fungus-growing ants (Hymenoptera: Formicidae: Attini) based on morphological characters of the larvae. *Syst. Entomol. 20* : 337-370.

Weber, N.A. 1941 The biology of the fungus-growing ants. Part VII. The Barro Colorado Island, Canal Zone, species. *Revista Entomol. 9* : 93-130.

Weber, N.A. 1954 Fungus-growing ants and their fungi. *Anat. Rec. 120* : 735.

Weber, N.A. 1956a Symbiosis between fungus-growing ants and their fungus. *Year book Amer. Philos. Soc. for 1955* : 153-157.

Weber, N.A. 1956b Treatment of substrate by fungus-growing ants. *Anat. Rec. 125* : 604-605.

Weber, N.A. 1958 Evolution in fungus-growing ants. *Proc. 10th Internat. Congr. Entomol. (1956) (Montreal) 2* : 459-473.

Weber, N.A. 1962 The fungus-growing ants. *Natural History (New York) 61* : 45-51.

Weber, N.A. 1972 Gardening Ants: the Attines. Memory of The American Philosophical Society, vol.92. The American Philosophical Society, Independence Square Philadelphia. 146 pp.

Wheeler, W.M. 1910 *Ants*: their Structure, Development and Behavior. Columbia University Press, New York. 663 pp.

Wilson, E.O. 1971 *The Insect Societies*. Belknap Press of Harvard University Press, Cambridge, Mass. 548 pp.

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