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Research article

The triple alliance: how a plant-ant, living in an ant-plant, acquires the third partner, a scale insect

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Summary. Mutualistic associations between ants and plants often involve a third partner, scale insects (Hemiptera, Coccoidea). In southeast Asia, plant-ants of the genus *Cladomyrma* live together with coccoids in hollowed twigs of a wide range of ant-plants (myrmecophytes). Established colonies never lack sap-sucking scale insects and the ants appear to be dependent on the honeydew excretions of their trophobionts. Acquisition of scale insects thus seems to be an important step in the successful establishment of a new colony on a host plant. Coccoids may either be transported by a foundress ant on her nuptial flight (vertical transmission via co-dispersal) or ant and coccoid disperse on their own and associate anew during colony initiation (horizontal transmission). Here we test the assumption that founding gynes of *Cladomyrma* do not transport scale insects during their dispersal flight, and we determine when and how trophobionts are acquired. The results obtained in this study show that although coccoids are not carried by foundresses, their numbers within founding chambers of *Cladomyrma* increased with the founding stage. In all *Cladomyrma* species, colony-founding is claustral and the entrance hole into the nest chamber is first plugged with pith debris and later grows over by wound callus. However, access of trophobionts into hollow twigs is facilitated either by additional small openings ('secondary slits') produced by the foundress ant after hollowing out a nest chamber or by the maintenance of an orifice at the entrance hole during the founding period. Immature pseudococcids experimentally placed onto twigs close to a *Cladomyrma* foundation readily entered these slits. Exclusion experiments suggest that foundresses of *C. petalae* are less successful in rearing their first offspring if entry of coccoids into founding chambers is prevented.

Key words: Ant-plant-hemipteran mutualism, *Cladomyrma*, colony foundation, Coccoidea, Malaysia.

Introduction

Most of the obligate mutualisms between ants and myrmecophytes (ant-plants) involve scale insects (Hemiptera: Sternorrhyncha: Coccoidea), they thus represent three-partner systems. These coccoids feed in plant phloem and, after partial digestion of the plant sap, emit droplets of excreta known as honeydew (Williams and Williams, 1980). The honeydew is a complex mixture of sugars and other nutrients such as amino acids, and it has been proposed that honeydew of coccoids might play a vital part in the nutrition of many arboreal ant species (Delabie, 2001; Davidson et al., 2003; Blüthgen et al., 2004).

The species of the Southeast Asian plant-ant genus *Cladomyrma* appear to essentially depend on honeydew eliminated by their associated trophobiotic coccoids. Neither during field observations nor in staged encounters with herbivorous insect larvae placed onto young leaves *Cladomyrma* ants were ever observed to feed on the insects they killed, rather the ants simply chased them off or discarded them from the leaves.

Several other factors also support the hypothesis that honeydew is sufficient to supply a (nearly) complete diet for *Cladomyrma* ants: (i) populations of coccoids are maintained in hollow plant internodes throughout the life of a colony; (ii) *Cladomyrma* workers do not forage off their host; (iii) plant-derived food sources such as extrafloral nectar (EFN) or food bodies are not provided by host plants of *Cladomyrma*, except in *Saraca thaipingensis* where foliar nectaries are active on young developing leaves. However, the ants were observed to exploit EFN of *Saraca* only under starving conditions; (iv) when disturbed workers carry trophobiotic mealybugs into safety; and (v) the ratio of the nitrogen isotopes $^{15}N/^{14}N$ obtained from two *Cladomyrma* species, *C. andrei* and *C. dianeae*, was among the lowest measured for a wide set of arboreal ant species and did not differ from those of sap-feeding insects (Davidson et al., 2003).

Honeydew thus appears to be an essential source of colony nutrition in *Cladomyrma* ants. One question immediately arising from this fact is, how the coccoids enter newly founded nests and thus secure the successful establishment of the ant colony on the host plant. In obligate ant-plant mutualism the partners are horizontally transmitted, that is, descendants of both plant and ant disperse independently from each other and must associate anew in each generation. The third partner, the trophobionts, may also disperse on their own, e.g., are dispersed by wind (Washburn and Washburn, 1984; Heckroth et al., 1998).

Another possibility is that founding ant gynes carry the coccoid symbionts in their mandibles during the nuptial flight and colony foundation, thus establishing a honeydew source for the newly founded nest (Gullan, 1997; Williams, 1998). This mode of vertical transmission of trophobionts has been recorded for ant species of the genus *Acropyga* (e.g., Johnson et al., 2001; LaPolla, 2004), for the bamboo-dwelling *Tetraponera binghami* (Klein et al., 1992), and once for an unidentified *Cladomyrma* species (Roepke, 1930). According to Reyne (1965, p. 150), however, this *Cladomyrma* has been wrongly identified and is an *Acropyga* species instead.

Vertical transmission of ant and trophobiotic coccoid from one generation to the next may also be achieved by colony budding or phoresis. In the former, newly mated ant gynes return to their maternal nest (or to other conspecific nests), and leave it with a part of the colony's workers which carry brood and trophobionts to the new nest site (Dill et al., 2002; Kaufmann et al., 2003). In the latter, trophobionts *actively* mount and cling to the ant's body. [Note that the term phoresis is used here in a strict sense, excluding cases in which trophobionts are picked up by an ant.] Several mealybug species of the tribe Allomyrmococcini have been described to ride on workers of Asian *Dolichoderus* 'herdsmen' ants in different functional contexts (Williams, 1978; Maschwitz and Hänel, 1985; Dill et al., 2002). Co-dispersal of trophobionts and ant foundresses via phoresis apparently is rare, it has only once been suggested for the African plant-ant *Aphomomyrmex afer* associated with the mealybug *Paraputo anomala* (Gaume et al., 2000).

In a previous study on two *Cladomyrma* species inhabiting the trees *Saraca thaipingensis* and *Crypteronia griffithii*, Maschwitz et al. (1991) hypothesized that coccoids are not carried by founding gynes and they assumed that the trophobionts arrive at the host plant via wind dispersal. The authors also observed conspicuous tiny slits along the outside of the founding chamber in *Saraca* which are chewed by the founding female, presumably enabling mealybug crawlers to enter the nest.

The aim of this study is to clarify these preliminary results and to assess if the (early) arrival of scale insects in founding chambers is an indispensable condition for the formation of a *Cladomyrma*-plant association. Specifically, the objectives are (i) to determine when and how trophobionts are acquired and (ii) to assess the role of trophobionts for the successful establishment of *Cladomyrma* on its host.

Materials and methods

Study sites and species investigated

The study was primarily conducted in Peninsular Malaysia at the Ulu Gombak Field Station (3°19′N, 101°45′E, 250 m a.s.l.) and in the hill regions of the Gombak valley, (400–800 m a.s.l.). Additional data were obtained from Lambir Hills NP (4°20′N, 113°50′E, Sarawak) and Poring Hot Springs (6°02′N, 116°42′E, Sabah) in Borneo. Information about the sites and climate is provided in Davis et al. (1995).

All eleven known species of the plant-ant genus *Cladomyrma* Wheeler inhabit live stems of a wide range of plant taxa (Agosti et al., 1999; Moog et al., 2003), and the ants are known to protect their host plants against herbivorous insects (Moog et al., 1998; unpublished results). In the present study data were obtained from seven (eight) *Cladomyrma* species: *C. andrei* (Emery), *C. aurochaetae* Agosti, Moog & Maschwitz, *C. crypteroniae* Agosti, Moog & Maschwitz, *C. dianeae* Agosti, Moog & Maschwitz, C. *hewitti* (Wheeler), *C. maschwitzi* Agosti, *C. petalae* Agosti, and probably *C. maryatiae* Agosti, Moog & Maschwitz. Host plants include the woody climbers *Callerya nieuwenhuisii* (J.J. Sm.) Schot and *Spatholobus oblongifolius* Merrill, and the trees *Crypteronia griffithii* Clarke in Hook. f., *Saraca thaipingensis* Cantley ex Prain, and several species of *Neonauclea* Merrill.

The trophobionts utilized by *Cladomyrma* belong to a wide range of taxa, mainly Pseudococcidae (Williams, 2004). They include *Crenicoccus cladomyrmae* Williams, *C. gullanae* Williams, *Crisicoccus theobromae* Williams, *Exallomochlus hispidus* (Morrison), *Hordeolicoccus crypteroniae* Williams, *H. heterotrichus* Williams *H. nephelii* (Takahashi), *Maconellicoccus multipori* (Takahashi), *Paracoccus interceptus* Lit, *Paraputo areolatus* Williams, *P. capillulatus* Williams, *P. cubicus* Williams, *P. danzigae* Williams, *P. domatium* Williams, *P. drypetis* Williams, *P. latebrae* Williams, *P. limitaneus* Williams, *P. lisponotus* Williams, *P. malesicus* Williams, *P. odontomachi* (Takahashi), *P. ranauensis* Williams, *P. sekayuensis* Williams, *P. spatholobi* Williams, *P. specicola* Williams, *Planococcus angkorensis* (Takahashi), *P. lilacinus* (Cockerell), *P. minor* (Maskell), *Pseudococcus aurantiacus* Williams, and *P. cryptus* Hempel. The dominant associates of *Cladomyrma* appear to be several *Paraputo* species.

Coccidae are rare in most *Cladomyrma*-plant associations. Established colonies of *Cladomyrma* usually tend pseudococcids as the sole trophobiont but occasionally coccids occur within the same colony in addition. In contrast, colonies of *Cladomyrma maschwitzi* often utilize a higher proportion of coccids relative to pseudococcids. Coccids as sole trophobionts have been found rarely in *Cladomyrma* nests. The taxonomic identity of the coccids is unknown in most cases. Identifications of a small sample by P. Gullan and H.-P. Heckroth revealed that several "*Coccus*" species are involved, e.g., *C. macarangae* Morrison and *C. secretus* Morrison.

Colony founding

Foundresses of *Cladomyrma* chew their entrance holes into young internodes (Fig. 1A–B). After creating a founding chamber by removing the pith, the entrance hole is fully (or partially) blocked by debris (Fig. 1C). Most *Cladomyrma* species, except *C. maschwitzi* and *C. crypteroniae*, produce additional tiny slits (0.5–1.0 mm long, on average 0.7 mm) from within the founding chamber (Fig. 1C–D). These slits are termed '*secondary slits*' to distinguish them from the '*primary hole*' through which the foundress gained access into the internode. These secondary slits are never created by workers when enlarging their nest into young plant parts, hence they are an excellent character signifying founding chambers on host plants.

We dissected internodes containing founding chambers to determine when trophobionts are acquired relative to the presence of brood or the first cohort of workers (nanitics). For each such internode encountered, we recorded whether the founding chamber contained a foundress, brood, trophobionts, or workers. We also measured the number of secondary slits and the status of the primary hole (open or closed).
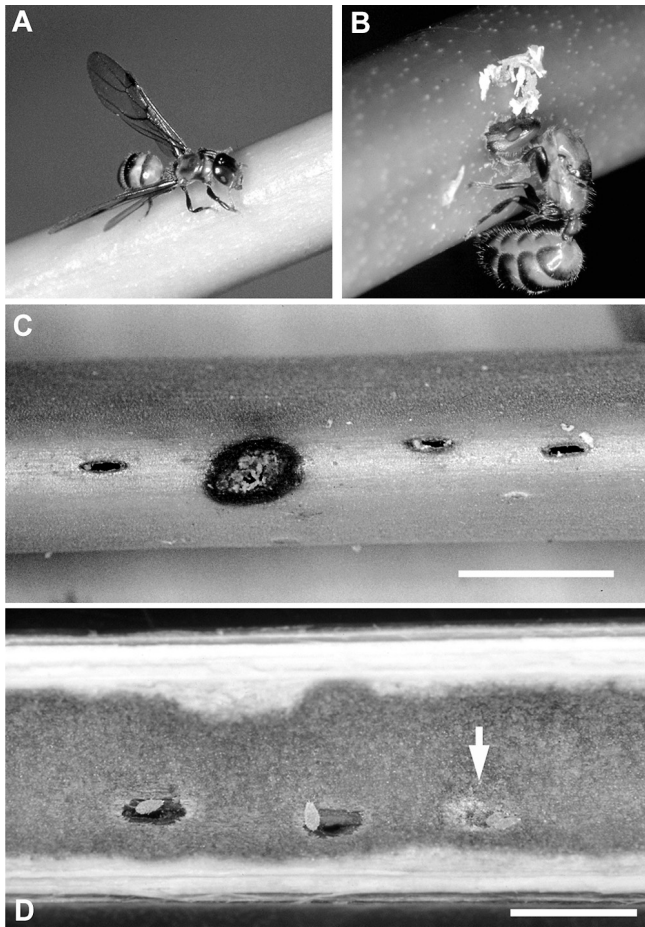
Figure 1. Colony-founding in *Cladomyrma*. **A**: alate *C. petalae* gyne checking an internode of *Saraca thaipingensis* for its suitability as founding site. **B**: *C. petalae* chewing into a *Saraca* internode. **C**: lateral side of domatium of *Neonauclea gigantea* showing 'primary hole' plugged with pith debris and 3 'secondary slits'. Scale bar: 5 mm. **D**: mealybug crawlers inside a founding chamber of *C. petalae*. They entered the nest through the secondary slits and settled in depressions gnawed by the foundress (arrow). Scale bar: 3 mm

Experimental exclusion of trophobionts

To assess (i) when trophobiotic coccoids arrive at newly founded nests and (ii) whether arrival of these scale insects in founding chambers is an indispensable condition for the successful rearing of the first offspring, we first located in the field colony-founding gynes of *Cladomyrma petalae* which were in the process of chewing an entrance hole into internodes of *Saraca thaipingensis*. The foundresses were carefully checked for any accompanied trophobiont without disturbing them in their task. Once the founding gynes entered the hollowed-out internode, a barrier of sticky resin (Tangle-trap, Tanglefoot Corp., Grand Rapids, Mich., USA) was applied around the internode in half of the new foundings (n=11), thus preventing coccoids from entering the nest chamber. The other foundings served as controls (n=11). After 19 to 108 days the nest chambers were collected. As each internode was dissected, we recorded foundress survival (dead or alive), number of eggs, larvae, pupae, workers and coccoids, as well as number of secondary slits and length of founding chamber. In addition, we monitored four unmanipulated foundings of *C. petalae* after 1, 3, 6, and 8 days to better observe the arrival time of trophobionts in the founding chambers.

Active entry of immature mealybugs into founding chambers

The three nymphal instars of most female pseudococcids are relatively mobile and the first-instar nymphs (called 'crawlers') are usually the main dispersal agents. To test whether pseudococcids are capable of entering founding chambers on their own, nymphs (first and second instars) were carefully placed onto an intact, unmanipulated twig of *Saraca thaipingensis* at a distance of 3 centimetres from a founding chamber of *Cladomyrma petalae* with a total of eight open secondary slits. The twig was fixed in a horizontal position to reduce a possible geotactic response of the immature mealybugs. However, we placed the nymphs onto the twig with their head pointing into the direction of the founding chamber in case they walked in the opposite direction. In total, we tested 10 nymphs ranging in length from 0.5 to 1.5 mm. Behaviour of each nymph was recorded for a 5-min period.

Primary hole

In contrast to other *Cladomyrma* species, *C. maschwitzi* does not create secondary slits. The entrances or 'primary holes' of founding chambers inhabited by *C. maschwitzi* remain open as slit-like orifices for several weeks after foundation although callus growth had begun. By measuring the diameter of the primary hole in founding chambers with and without foundresses during a 40-day period, we tested the hypothesis that *C. maschwitzi* foundresses chew callus tissue and keep open the primary hole, thus potentially allowing the passage of coccoid crawlers. We selected 8 young founding chambers with no or little callus growth and removed the foundresses from 4 chambers, the other half served as controls (foundress maintained). The apex and base of each internode containing a founding chamber was coated with a sticky resin in order to prevent the arrival of other colony-founding gynes which may recolonize empty chambers or overtake existing foundations.

Results

Dispersal flight

Alate host-searching females of *Cladomyrma crypteroniae* (n=1), *C. dianeae* (n=1), *C. hobbyi* (n=1), *C. maschwitzi* (n=3) and *C. petalae* (n=7) were witnessed during their dispersal flight. They had just landed on a host plant to check the suitability of a young twig for nest founding (Fig. 1A). None of these gynes carried a trophobiont in their mandibles nor did we detect any phoretic trophobiont on the ant's body. In addition, dealate founding gynes of *C. crypteroniae* (n=2), *C. dianeae* (n=5), *C. hobbyi* (n=1), *C. maschwitzi* (n=6), and *C. petalae* (n~40) wandering along an internode or chewing an entrance hole likewise were free of accompanied trophobionts (Fig. 1B).

Colony founding

The dissection of 338 founding chambers of eight *Cladomyrma* species showed that coccoid abundance strongly increased with founding age in all species (Fig. 2). For the pooled data, the majority of founding chambers lacking brood contained no trophobionts (81.7%, 85 of n=104) whereas founding chambers with nanitic workers (minims) usually contained coccoids (93.6%, 44 of n=47). In the three cases in which the natal colony lacked coccoids (1× in *C. crypteroniae* and 2× in
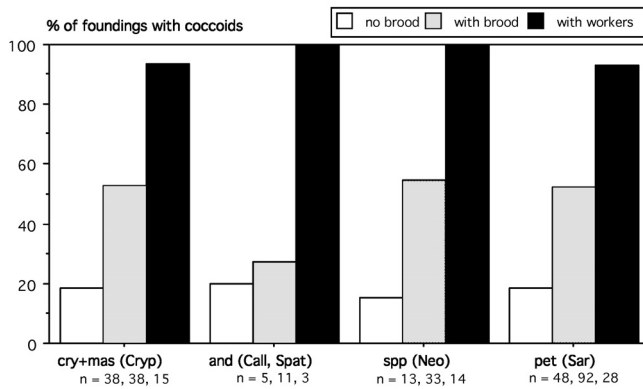
Figure 2. Distribution of coccoids in colony-founding chambers of *Cladomyrma* spp. according to founding stage. *No brood* = neither brood nor workers present. *With brood* = eggs, larvae or pupae, but no workers. *With workers* = first cohort of workers (nanitics), initial founding chamber excavated by the foundress not yet enlarged by workers. *Cladomyrma* species: cry = *C. crypteroniae*; mas = *C. maschwitzi*; and = *C. andrei*; spp = species include *C. aurochaetae*, *C. dianeae*, *C. hewitti*, and probably *C. maryatiae*; pet = *C. petalae*. Host plant species in parentheses: Cryp = *Crypteronia griffithii*; Call = *Callerya nieuwenhuisii*; Spat = *Spatholobus oblongifolius*; Neo = *Neonauclea* spp.; and Sar = *Saraca thaipingensis*. Sample size (n) is given for each founding stage

C. petalae), worker and brood number was low (1–2 workers, 0–7 eggs, 0–4 larvae, 0 pupae), probably indicating that resources were in short supply.

Arrival of trophobionts prior to the production of brood is not uncommon. About 18% of the foundations without brood already harboured coccoids (Fig. 2). In *Cladomyrma petalae*, chewing of both the founding chamber and the secondary slits is completed at the latest after 5 to 6 days and egg-laying follows completion of the nest (Moog, 1991). It appears from these data that arrival of trophobionts can occur within the first days after foundation, although absence of brood does not necessarily indicate recent founding (egg-laying may be delayed under unfavourable conditions or trophic eggs are consumed by the foundress).

The total number of secondary slits and their distribution around the founding chamber differs among *Cladomyrma* species. In the *Cladomyrma-Neonauclea* associations the slits are always located in a straight line on both lateral sides of the swollen internodes (Fig. 1C). On average, 8.1 (SD ± 4.1) slits were produced by the *Cladomyrma* gynes inhabiting *Neonauclea* trees (n=33). In contrast, the secondary slits chewed by *Cladomyrma petalae* are more or less evenly distributed in two or three rows around the twig and their mean number was 4.0 (SD ± 2.9; n=92). The lowest number of secondary slits were produced by *C. andrei* (mean 1.6 ± 1.4 SD, n=11); this possibly explains the comparatively low percentage (27.3%) of founding chambers at the 'brood stage' that contain mealybugs (Fig. 2). The high proportion of chambers with mealybugs in the final founding stage ('with workers') might partially be explained by the foraging of the first nanitic workers which assumably collect coccoid nymphs from the plant surface and transport them into the nest. Species of *Cladomyrma* never tend coccoids on the surface

of their host plant and, on occasion, we have observed workers of a mature colony of *C. petalae* carrying mealybugs found on the plant surface into the stem interior.

Experimental exclusion of trophobionts

Within the first 5 to 6 weeks after founding, all foundations dissected of both the experimental (coccoids excluded) and the control group (coccoid access allowed) contained eggs and/or larvae (Table 1). In the foundations examined after the 6th week, however, the two groups showed contrasting patterns of colony development. In the experimental group, none of the foundresses had produced pupae and workers and although a few larvae were present until the 8th week, eggs were not found after the 6th week. This was in striking contrast to the control group (Table 1). Workers and pupae were present in all but two foundations examined after the 6th week. Of the two lacking workers and pupae one was also devoid of pseudococcids.

Overall, the two groups differed significantly in the number of pseudococcids, eggs, pupae and workers ($P = 0.0003$,

Table 1. Developmental stage of colony foundings of *Cladomyrma petalae* in *Saraca thaipingensis* collected after 19 to 108 days. Experimental group (coccoid access to founding chambers prevented by sticky resin applied around twigs): n = 11. Control group (unmanipulated foundings): n = 11. Mann-Whitney U-test: $P_{pseudococcids} = 0.0003$, $P_{workers} = 0.006$, $P_{eggs} = 0.0003$, $P_{larvae} = 0.23$, $P_{pupae} = 0.006$

Experimental group

days after founding	gynes († = dead)	no. pseudo-coccids	no. workers	brood: eggs + larvae + pupae
19	1	0	0	0 + 3 + 0
19	1	0	0	8 + 0 + 0
29	1	0	0	2 + 1 + 0
36	1	0	0	4 + 4 + 0
36	1	0	0	0 + 3 + 0
52	1	0	0	0 + 3 + 0
53	1	0	0	0 + 1 + 0
75	1†	0	0	0 + 0 + 0
75	1†	0	0	0 + 0 + 0
75	1†	0	0	0 + 0 + 0
91	1†	0	0	0 + 0 + 0

Control group

days after founding	gynes	no. pseudo-coccids	no. workers	brood: eggs + larvae + pupae
19	1	1	0	3 + 0 + 0
21	1	1	0	14 + 0 + 0
22	1	0	0	2 + 0 + 0
48	1	3	3	32 + 4 + 2
49	1	0	0	6 + 0 + 0
50	1	6	4	54 + 7 + 1
51	1	5	3	18 + 11 + 3
62	1	4	7	35 + 8 + 6
65	1	3	0	50 + 0 + 0
72	1	2	3	7 + 13 + 1
108	1	4	6	24 + 15 + 8

$P = 0.0003$, $P = 0.006$ and $P = 0.006$, respectively) but not in the number of larvae ($P = 0.23$; Mann-Whitney U-test). The two groups did not differ in the number of secondary slits ($P = 0.66$), chamber length ($P = 0.37$) or collection time ($P = 0.82$), indicating that differences are due to the absence/presence of coccoids.

Strikingly, all four foundresses of the experimental group collected after the 10th week (day 75 to 91) were found dead. The snapshot method applied in this study does not allow us to determine the date of foundress death. Small chamber length and low number of secondary slits usually indicate early death of a foundress shortly after founding, however, the two parameters were obviously not very different from those of the control group (*secondary slits*: $mean_{dead}$ 3.0 ± 1.4 SD versus $mean_{control}$ 4.4 ± 2.8 SD; *chamber length*: $mean_{dead}$ 2.85 cm ± 0.35 SD versus $mean_{control}$ 3.4 cm ± 0.6 SD). The founding chambers containing the dead foundresses were intact but the primary holes and the secondary slits were fully closed by callus tissue. Combined with the degree of decomposition of the ants we assume that death had occurred at least 1 or 2 weeks prior to collection.

The census of four additional, unmanipulated colony foundings of *Cladomyrma petalae* after 1, 3, 6 and 8 days revealed that pseudococcids may arrive as early as the first day after foundation. All four foundresses were witnessed during their founding process and were not accompanied by mealybugs. However, in the founding chambers collected after 1 and 3 days we found a mealybug crawler each. The two remaining nest chambers were free of trophobionts. In most cases the primary hole was fully plugged with pith debris, except in the founding dissected after one day. Here, the pith plug left a slit big enough for the passage of a mealybug crawler. This founding still lacked secondary slits but they were present in the other three foundations. This observation illustrates that even a single tiny slit may be detected and entered by a crawler shortly after colony founding.

Active entry of mealybug nymphs into founding chambers

The first arrivals in *Cladomyrma* founding chambers are pseudococcids rather than coccids (see above, Fig. 1D). We therefore tested the capability of immature pseudococcids to enter founding chambers on their own. Because species of Pseudococcidae could not be identified in the field, they were pooled and referred to as 'pseudococcids' or 'mealybugs' in the following analysis.

The results show that active entry of pseudococcids into founding chambers of *Cladomyrma petalae* inhabiting a young shoot of the tree *Saraca thaipingensis* is a regular phenomenon under the conditions chosen for the experiment. Of the 10 mealybugs tested, 5 successfully located and entered the foundations. On average, the mealybugs needed 66.8 seconds (± 10.0 SD; n = 5) to cover the distance from their starting point to the foundation and to disappear into one of the eight slits produced by the ant foundress. On reaching a slit the pseudococcids all appeared to enter it without hesitation. However, the pseudococcids were often observed to narrowly

pass slits (*c*. 1 mm), apparently without recognizing their proximity. Of the five remaining mealybugs tested two settled in depressions on the external surface of the plant (closed secondary slit, leaf axil) and two hid under plant structures (stipule, leaf base). Thus, 'hiding' was the predominant behavioural response of immature mealybugs within the 5-min observation period. Only a single mealybug failed to find a hiding place and fell to the ground due to an unknown cause.

Primary hole

The abundance of coccoids inside the founding chambers of *Cladomyrma maschwitzi* increased with the founding stage much in the same way as in *Cladomyrma* species producing secondary slits (Fig. 2). How do the coccoids find their way into the foundations lacking secondary slits? It is hypothesized that foundresses of *C. maschwitzi* prevent the closing of the entrance (primary) hole by chewing the wound callus. The effect of foundress exclusion on primary hole diameter relative to that of the controls (foundresses maintained) is presented in Figure 3. After about 4 to 5 weeks the primary hole was fully closed by callus tissue in the experimental group (n=4). In contrast, the diameter of the primary holes of inhabited founding chambers (n=4) decreased more slowly and then remained more or less constant during the last week of the 40-day study period.

Prior to callus growth, foundresses of *C. maschwitzi* plug the primary hole during the first days after founding with pith debris as do other *Cladomyrma* species. The nest entrances are, however, rarely fully plugged; the founding gynes usually maintain an orifice by incompletely blocking the primary hole. As a rule, secondary slit-producing *Cladomyrma* foundresses completely plug the primary hole and allow them to grow over whereas those of *C. maschwitzi* maintain an orifice at the primary hole throughout the founding stage.

Discussion

Co-dispersal

Vertical transmission via co-dispersal of ant foundresses and coccoid trophobionts has been suggested for the African plant-ant *Aphomomyrmex afer* and its mealybug partner (Gaume et al., 2000), and may occur more frequently in plant-ants and associated throphobionts than currently reported. However, one main result emerging from this study is that *Cladomyrma*-founding gynes do not carry trophobiotic coccoids during their dispersal flight. The *Cladomyrma* foundresses neither carried coccoids in their mandibles nor were coccoids carried phoretically on the ants' bodies. Thus the previous hypothesis of Maschwitz et al. (1991) that coccoids arrive independently of the ants on the host plant is confirmed by this study.

Generally, coccoid-carrying by plant-specialist ants during the nuptial flight is not well understood. Although Stout (1979) and Schremmer (1984) suspect mandible-transport of trophobiotic coccoids by plant-ant foundresses of *Myrme-*

lachista and *Pseudomyrmex*, respectively, it has only been confirmed once for *Tetraponera binghami* (Klein et al., 1992). What could explain the rarity of such behaviour in plant-ants? With the exception of *Tetraponera binghami* which gains access through holes already present in bamboo internodes, plant-ant foundresses usually have to cut an entrance hole into the domatium of the host plant and, therefore, need unencumbered mandibles to chew into plant tissue (Moog, 1991; Gullan et al., 1993). If coccoids are indeed carried in the mandibles the founding female must release the trophobiont for the whole period of entrance chewing and nest excavation (taking minutes to hours). It seems unlikely that the coccoid would stay during the whole excavation process at the place where it has been dropped.

Colony founding

In all *Cladomyrma* species tested (Fig. 2), coccoids were more frequent in advanced than in early stages of foundations, confirming that coccoids arrive on the plant after the foundresses. [The relative ages of the foundations are indicated by the presence or absence of brood or the first cohort of workers, but the absolute ages of each of these foundations are unknown.] The alternative explanation that foundresses had carried the nymphs but failed to rear them in their chambers, is excluded by the fact that all gynes observed in the process of founding (alighting on a host or chewing an entrance hole) were free of accompanying coccoids. Thus, independent arrivals of trophobionts prior to the production of brood is not uncommon and may occur as early as within the first days after colony founding.

The sealing of entrance holes is commonly observed in claustrally founding plant-ants (e.g., Choe and Perlman, 1997; Federle et al., 1998). This 'closing behaviour' is generally thought to be an important trait to reduce intra- and inter-specific competition as well as to avoid attacks by predators and parasitoids. By this reasoning, the necessary sealing of the entrance hole and the acquisition of trophobionts appear to stand in contradiction. However, founding gynes of most *Cladomyrma* species ensure both the safety of their nest chambers and access of coccoids simultaneously by producing openings small enough to exclude alien ants but large enough to allow the passage of immature scale insects. The narrow oval slits ('secondary slits') are safely chewed by the foundress from within the initial nest chamber, and their size and shape correspond largely to those of nymphal coccoids (Fig. 1C–D). Whether the secondary slits also play a role in regulating microclimatic conditions within the founding chamber of *Cladomyrma* remains to be tested.

Secondary slits are not gnawed by gynes of *Cladomyrma crypteroniae* and *C. maschwitzi*, both inhabiting the tree *Crypteronia griffithii*. The experimental results on primary hole development during the founding stage obtained from *C. maschwitzi* (Fig. 3) provide strong evidence that potential coccoid access is ensured by the foundresses which gnaw on growing callus tissue and thus prevent the full closure of the entrance hole for many weeks.
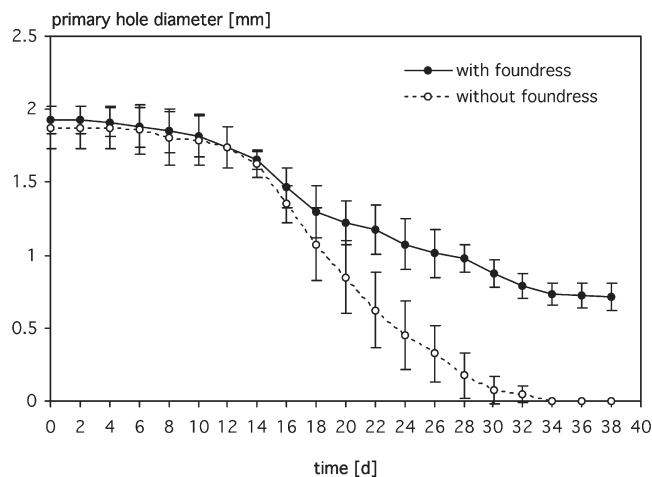

Figure 3: Time course of entrance (primary) hole size during colony-founding by *Cladomyrma maschwitzi* inhabiting the tree *Crypteronia griffithii* over a 40-day period. The diameter given refers to the hole margins and excludes pith debris that may plug the hole. Experimental group (foundress excluded): n = 4. Control group (foundress maintained): n = 4. Error bars = ± 1 SD

Experimental exclusion of trophobionts

Is the presence of trophobionts essential for the successful rearing of the first cohort of workers in *Cladomyrma*? The experimental exclusion of coccoids from founding chambers of *C. petalae* appears to support this hypothesis (Table 1). Founding chambers to which mealybug access was prevented contained a significantly lower number of brood and pupae than those of the control group. Furthermore, all foundresses of the experimental group failed to produce workers.

One striking result of the study was that all four foundresses of the experimental group collected after the 10th week were found dead. Although foundress death may be due to causes other than absence of coccoids, several reasons seem to favour the latter. First, two parameters, i.e. chamber length and number of secondary slits, that are usually low in case of early death of a *Cladomyrma* founding gyne, were similar to those of the control group. Second, each foundation was fully isolated, having no open orifice connecting it to the outside world. Third, the foundress corpses were complete and no remains of other arthropods could be detected. These observations combined suggest (i) that death had not occured within the first weeks after founding and (ii) that parasitoids or predators are not likely to be the cause of foundress death. Parasitoid infestation appears to be generally rare in *Cladomyrma* species. Among hundreds of foundations dissected (n > 600) we found only four parasitoids (unidentified flies and braconid wasps).

That absence of pseudococcids in the experimental group is the most likely explanation for the death of the foundresses seems to be supported by differences in the egg number within the control group. Egg numbers were generally higher in the control than in the experimental group, except when coccoids had not yet been acquired. In these cases, egg num-

ber was as low as in the experimental group, suggesting that the presence of coccoids influences the amount or quality of resources a *Cladomyrma* foundress has for raising her first batch of brood.

It is proposed that acquisition of trophobionts within the survival limits set by internal and external factors of founding gynes (e.g., amount of body reserves, costs of host finding and nest excavation) is either an indispensable condition or at least a major advantage for the successful establishment of a new colony. Depending on arrival time, coccoids may (i) facilitate worker production itself, (ii) enhance the chance to survive until the first workers emerge, or (iii) they may serve as an immediately available resource for the first workers and thus favour rapid colony development.

In view of the strong intraspecific competition (as is the rule in *Cladomyrma*), rapid colony development is certainly a premium. The importance of an immediately available food resource for the persistence and establishment of new colonies is a general trait in plant-ants (Davidson and Fisher, 1991; Davidson and McKey, 1993). In ant-plants providing their ant partners with specialized food bodies, colonization of host saplings by founding gynes usually does not occur prior to the production of food (e.g., Fiala and Maschwitz, 1992).

Active entry of immature mealybugs into founding chambers

If trophobionts are as important as proposed above for the establishment of a new colony, all depends on the probability of their acquisition. What is the chance for *Cladomyrma* foundresses to acquire trophobionts?

The main dispersal agents in the Coccoidea are first instar nymphs, the so-called crawlers (review in Gullan and Kosztarab, 1997). The crawlers are passively dispersed by the wind, although mortality is high. To our knowledge, estimates of 'crawler rain', i.e., number of wind-dispersed mealybug crawlers arriving on a given area per unit of time, are not available for rainforest habitats. However, immature pseudococcids already present on the plant surface seem to possess a well developed ability to enter founding chambers on their own. Half of the nymphs experimentally placed close to a foundation of *C. petalae* successfully located and entered the nest chamber through one of the secondary slits gnawed by the foundress ant. If these results based on a low sample size are representative of mealybug behaviour under unmanipulated conditions, access of immatures to foundations may occur with striking ease when nymphs are in proximity to nest chambers.

Active entry of scale insects into nest chambers is possibly a rather common phenomenon in tripartite ant-plant-coccoid associations. In the African ant-plant *Cuviera*, associated with *Crematogaster* ants, coccoids can enter young domatia on their own accord, as indicated by the presence of coccoids in some domatia not yet inhabited by ants but infested by beetle larvae feeding on the remains of dried pith (Bequaert, 1922). An apparent similar case is reported for the neotropical ant-plant *Tachigali paniculata*. Pseudococcids are found in domatia occupied by beetles prior to the presence of ants (Wheeler, 1921). In *Cecropia* trees, a small percentage of

foundations of *Azteca xanthocroa* and *A. constructor* (still lacking workers) contained pseudococcids (0.21%, n=967), although founding gynes do not carry coccoids on their dispersal flight (Perlman, 1992). Mature colonies of *Azteca* plant-ants usually all tend trophobiotic coccoids (Longino, 1991). In Australia, an assumably host-specific *Camponotus* species inhabits twigs of the mangrove tree *Sonneratia alba* (Nielsen, 2000). None of several hundred founding chambers discovered contained workers, but small coccids of the genus *Myzolecanium* were present in some of the cavities known to be more than 6 weeks old. Again, virtually all mature colonies contained trophobionts. Successive arrival of trophobionts in ant nests has also been reported for the obligate associations of SE Asian *Camponotus* with *Korthalsia robusta* (Mattes et al., 1998), *Crematogaster* or *Camponotus* with *Macaranga lamellata* (Maschwitz et al., 1996), as well as in the mutualism between African *Tetraponera* and *Barteria fistulosa* (Yumoto and Maruhashi, 1999). To sum up, acquisition of trophobiotic scale insects appears to occur relatively early in all obligate tripartite ant-plant-coccoid associations for which information on trophobiont access is available.

Conclusion

The chance of scale insect acquisition during the founding stage is assumably better for many plant-ants than recognized by previous studies (Gaume et al., 2000). Although intriguing, co-dispersal, i.e., the vertical transmission of both ant and coccoid, is a rarely realized option for plant-ants to successfully establish a colony on a new host plant. Instead, plant-ants and coccoids commonly disperse independently from each other (horizontal transmission). During the founding stage, the maintenance of small slits permitting the entry of immature coccoids seems to be an important behavioural trait in *Cladomyrma* ants that increases the chance of coccoid access. Trophobionts appear to play an important, if not indispensable, role in the success of *Cladomyrma* colony foundation, at least in *C. petalae*.

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