

STUDIES ON THE SOUTH EAST ASIAN
ANT-PLANT ASSOCIATION *CREMATOGASTER*
BORNEENSIS/MACARANGA : ADAPTATIONS
OF THE ANT PARTNER

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SUMMARY

C. borneensis (Myrmicinae) lives in close association with several myrmecophytic species of the South East Asian pioneer tree genus *Macaranga* (Euphorbiaceae). The ants are adapted to the plants so closely that they do not survive away from it. The only food they utilize is provided as food bodies by the plant and honeydew from specific scale insects kept inside the hollow internodes. The anatomy of the digestive tract is also adapted to life on the host plant: the crop is very small and can store only minute food quantities.

C. borneensis exclusively colonizes certain *Macaranga* species. Queens as well as workers are able to recognize their host plant species, probably by chemical cues. Colony founding queens swarm throughout the year, mostly during darkness. There is strong competition among queens for host plants. Queens do not carry scale insects on their nuptial flight. Worker ants are active day and night. Most of them patrol and collect food bodies on the younger parts of the host plant. An important characteristic is their cleaning behaviour, which results in removal of all foreign objects. Even though they are rather small, workers respond very aggressively to certain kinds of disturbance of the host plant. The ants attack most phytophagous insects and are especially effective in killing and removing small, softbodied herbivores (e.g. caterpillars). They do not possess a functional sting, but apply defensive secretion and—once biting an intruder—will not let go. Their effective alarm system results in a mass attack, which provides adequate defence for the colony and the host plant. A comparison with another *Crematogaster* species further illustrated the special adaptations of *C. borneensis* to its host plant.

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ZUSAMMENFASSUNG

**Untersuchungen über die südostasiatische Ameisen-Pflanzen-Vergesellschaftung
Crematogaster borneensis Macaranga : Anpassungen des Ameisenpartners**

C. borneensis (Myrmicinae) lebt in enger Gemeinschaft mit myrmekophytischen Arten der südostasiatischen Pionierbaumgattung *Macaranga* (Euphorbiaceae). Die Ameise ist so eng an die Pflanze adaptiert, daß sie getrennt von ihr nicht lebensfähig ist. Die Nahrung bezieht *C. borneensis* in Form von Nährkörperchen ausschließlich von der Pflanze. Im Sproßachseninnern gehaltene spezifische Schildläuse bieten eine weitere Nahrungsquelle. Die Adaptationen erstrecken sich bis auf die Anatomie des Verdauungstraktes: Der Kropf ist sehr klein und kann nur geringe Nahrungsmengen speichern.

C. borneensis besiedelt spezifisch nur *Macaranga*-Pflanzen. Sowohl Königinnen als auch Arbeiterinnen sind in der Lage, die Wirtspflanze zu erkennen, wobei offenbar chemische Reize eine Rolle spielen. Die koloniegründenden Königinnen schwärmen das gesamte Jahr über, das Schwärmen erfolgt überwiegend während der Dunkelheit. Um die besiedlungsfähigen *Macaranga*-Pflanzen herrscht ein starker Konkurrenzdruck. Die beteiligten Schildlausarten sind spezifisch für die Assoziation. Sie werden nicht von der Königin beim Hochzeitsflug mitgenommen. Die Arbeiterinnen sind tag- und nachtaktiv. Die meisten Tiere halten sich im jüngsten Drittel der Pflanze auf, wo sie patrouillieren und Nährkörperchen sammeln. Mittels eines spezifischen Säuberungsverhaltens entfernen die Arbeiterinnen alle Fremdobjekte von der Pflanze.

Trotz ihrer geringen Größe attackieren die Ameisen eine Vielzahl phytophager Insekten und sind dabei besonders effektiv in der Abwehr kleiner, wenig sklerotisierter Tiere wie z.B. Raupen. Sie verfügen zwar nicht über einen funktionsfähigen Stachel, setzen aber Wehrsekrete ein und beißen sich hartnäckig fest. Mit Hilfe eines effektiven Alarmierungssystems, das einen Massenangriff ermöglicht, gewährleisten sie eine Verteidigung ihrer Kolonien und damit gleichzeitig ihrer Wirtspflanze. Eine Vergleich mit einer anderen *Crematogaster*-Art demonstriert die besonderen Adaptationen von *C. borneensis* an ihre Wirtspflanze.

INTRODUCTION

Many ants in the tropics have developed close relationships with vascular plants. Several of these myrmecophytes are inhabited by ant species that are in their occurrence restricted to their respective plant partner (review in BUCKLEY, 1982; BEATTIE, 1985). In the central Malesian region trees of the pioneer genus *Macaranga* are common colonizers of secondary habitats like forest gaps and road sides. Nine of the 27 *Macaranga* species of Peninsular Malaysia live in close association with ants (FIALA *et al.*, in press).

The importance of the plants for the ants is evident: They provide nesting space within the hollow stems and food as food bodies. Plant sap can be utilized via specific coccids which are kept by the ants in the interior of the stem.

In a functional analysis of the myrmecophytic system we showed that the small, non-stinging *C. borneensis* ants, although seemingly rather defenceless, were able to protect their host plant effectively against certain herbivore damage and vine cover (FIALA, 1988; FIALA *et al.*, 1989). However, the extent

of their dependence on the plant was unexpectedly great: *C. borneensis* was not capable of living isolated from its host plant.

Here we report in detail the dependence and adaptations of the ants with respect to their host plant. We document the specificity of the association, host plant finding and recognition, colony foundation and development, nutrition, activity, territoriality, and aggressive behaviour of the ants.

MATERIALS AND METHODS

Study sites

The study was carried out in the Malay Peninsula during 4 stays covering a total of 15 months (Dec 84-Feb 85; Dec 85-April 86; Jan 87-March 87; Sep 88-Feb 89). We studied the behaviour of *C. borneensis* colonies which were living in *Macaranga* plants growing along forest trails in different areas of the Peninsula. Dominant species were *M. triloba*, *M. hulletti* and *M. hypoleuca*. More than 200 colonies were investigated with respect to ant species and developmental stage and ant samples from more than 400 plants were taken. In addition we studied 10 colonies which were kept on intact plants in the greenhouse in Germany.

The ants belong to the subgenus *Decacrema* which is easily identified by its 10-segmented antennae (WHEELER, 1922). Within the subgenus, however, the taxonomic relations are obscure. Identifications are based on a comparison with the original descriptions cited by CHAPMAN and CAPCO (1951). Details concerning colony structure have been treated by ONG (1978).

Diet of the ants

The laboratory colonies were kept without any additional food. 5 starving colonies on dead plants received dilute honey solution and mealworms for a period of four weeks. Seventy times we offered animal protein with a spectrum from insect eggs, dead dipterans, cockroaches to pieces of fish and butter.

We tried to keep ants alive in artificial nests isolated from the plant. 25 occupied plants were cut into pieces of 10 cm and put in plastic containers which were lined with moist paper. As new nesting space we offered glass tubes with the same diameter as the plant stems. We gave honey solution and pieces of freshly killed insects as well as food bodies to some of the colonies.

Host plant specificity and host plant recognition

a) In the laboratory 20 foreign plants were brought in contact with inhabited *M. triloba*. We tested plants from different systematic groups as well as saplings of *M. triloba*. b) Different plants were offered for colonization to three colonies whose host plants had died. We used *M. triloba*, *M. tanarius* and a *Ficus benjamini* which were accessible over wooden bridges. c) Aqueous extracts of young leaves from 3 *Macaranga* species as well as from 3 plants out of randomly chosen other genera (*Passiflora* sp., *Dioscorea* sp., *Phaseolus* sp.) were tested on 4 laboratory colonies on *M. triloba*. With a pipette a droplet of each filtrate was cautiously put onto the leaf surface. We chose leaves in the middle part of the plant which were at about the same height. The leaf positions for the extracts of the different plant species changed in rotation. A drop of water served as control in each experiment. The number of ants on the leaves was

counted 15 minutes before starting the experiment and every 5 minutes thereafter for a period of 1 h.

Colony foundation and development

During the entire study period we checked for the presence of alates and for swarming activity of queen ants. The surface of larger *Macaranga* trees (mainly *M. triloba*) was checked several times a day (5 a.m. - 1. a.m.). The frequency pattern of colony foundation was obtained by combining all surveys of *M. triloba*, *M. hulletti*, *M. hypoleuca* and *M. hosei*. Twice a month we checked *Macaranga* plants of different sizes for the occurrence of ant colonies, their age structure and composition.

Activity

We recorded the number of ants on the upper and lower sides of the leaves of *M. triloba* and *M. hypoleuca* at different times of the day. We checked 18 unbranched plants with a total of 180 leaves. The plants had a size from 1-2 m and 9-15 leaves, all grew in halfshady places. The distribution of the ants was summarized for the upper, middle and lower part of the crown resp. On 4 subsequent sunny days (15.2. - 20.2.87) we recorded the number of ants on the leaves of seven plants. To facilitate comparison of data only the 8 top leaves were included. These usually supported more than 90 % of all ants active on the surface. The first 2 days we censused hourly, later only every 2 hours with longer breaks during the night.

We observed naturally occurring encounters between workers of *C. borneensis* and various insects. In addition we placed 50 times live insects of different groups on occupied plants.

RESULTS

Specificity

In W Malaysia we have never found *C. borneensis* nesting away from *Macaranga* plants. The ants in our samples varied in size as well as in colour but were otherwise structurally rather uniform. They were found sympatrically in all *Macaranga* species in the same study area. There was no recognizable differentiation in their way of living. Probably most samples from the investigated *Macaranga* plants in W Malaysia belong to *C. borneensis* which seems to be highly variable. Based on these samples, however, several additional, closely related *Crematogaster* species may be involved (LONGINO, pers. com.). Our results did not confirm that *C. deformis* (*) inhabits mainly *M. hypoleuca* as stated by ONG (1978). In Borneo we found an other as yet undetermined *Crematogaster* species on *M. kingii* which can easily be distinguished by its 11 - segmented antennae, indicating a different subgenus.

(*) This species is listed by ONG (1978) as *C. difformis* (after BINGHAM, 1903). The correct name is *C. deformis*, it belongs to the subgenus *Physocrema* (CHAPMAN and CAPCO, 1951).

Diet

The ants show a close adaptation to their host plants with respect to their diet. The workers were never observed foraging away from the plant. In the laboratory we kept several *C. borneensis* colonies over a period of 4 years without any additional food. Nevertheless the colonies grew and had no apparent deficiencies. Ants on intact *Macaranga* plants obtain carbohydrates mainly from plant sap solicited from coccids which are cultivated inside the stem. The 7 coccid species so far described occur specifically only in inhabited *Macaranga* plants (MORRISON, 1921; TAKAHASHI, 1952).

Only in 7 out of 35 experiments some workers accepted the standard laboratory food for ants (maximum number of ants feeding on honey solution: 25 workers). No food recruitment took place. Animal protein was only accepted in 5 out of 70 trials (pieces of: 2x butter, once tuna, once yolk and 1 *Drosophila*). In all other trials these objects were ignored.

Colonies living on *M. triloba* did accept food bodies of different *Macaranga* species offered to them, but rejected food bodies from other plant genera (table I). The rejection of food bodies from foreign genera was highly significant ($\chi^2 = 29.3$; $p < 0.001$) whereas there was no significant difference between the *Macaranga* species ($\chi^2 = 1.3$; n.s.).

Table I. — Responses of *C. borneensis* workers toward different kinds of food bodies ((number of trials).

Tabelle I. — Verhalten von *C. borneensis* auf das Angebot verschiedener Nährkörperchen (Anzahl der Versuche).

Food bodies from	Acceptance	Rejection
<i>M. triloba</i> (myrmecophyte)	20	10
<i>M. hypoleuca</i> "	10	0
<i>M. tanarius</i> (non-myrmecophyte)	15	10
<i>M. conifera</i> "	6	4
<i>Cecropia</i> sp.	1	14
<i>Nephelium</i> sp.	0	10

Food bodies were the most important food source and ants collected them continuously. So far it has not been proven whether the workers eat the food bodies themselves or feed them to the larvae. Despite several hours of observation and frequent controls we never saw the ants eat food bodies on the plant surface. The ants always carried them into the stem where they were deposited near the larvae. We did not find food bodies in the mid gut of 20 *C. borneensis* workers, but ONG (1978) found them in the gut of queen ants. We have no evidence for utilization of coccids as a protein source.

In the course of the experiments concerning the defensive behaviour (FIALA *et al.*, 1989) the ants often killed caterpillars and other small insects. Even this potential source of protein was not used as food but removed from

the plant. In conclusion we find that *C. borneensis* refuses almost all food which does not originate from its host plant.

Adaptations to the life on the plant seem to manifest themselves even in the morphology of the ants. The crops of 28 out of 30 dissected workers (from the interior and exterior of the plant) were very small and only poorly filled: they attained only a third to maximally one half of the size of the midgut. This is in contrast to other myrmicines, e.g. *C. scutellaris* which was dissected for comparison. After uptake of honey solution in this species the crop is at least 5 times bigger than the midgut. The crop content of *C. borneensis* was liquid, clear as water and exhibited a high sugar concentration (test for glucose with dextrostix (MERCK) yielded the max. possible reading of $\geq 2,5$ g/l). The small crop of *C. borneensis* which cannot store large amounts of food could explain why isolated workers die so quickly. The reason seems to be a lack of energy reserves. Therefore the ants cannot leave the host plant—their only source of food—for extended periods.

All experiments to keep small ant colonies with some hundred workers isolated from their host plants failed. The ants always died after a few days to 2 weeks. They did not move into the glass tubes offered as alternative shelter but remained in or under the stem pieces.

Host plant recognition

Since *C. borneensis* colonizes only certain species of *Macaranga* the ants must be able to find and recognize their host plant species. This is first of all a task of the colonizing queen. But it is also important for the workers to recognize the host plant and to discriminate it from other plants. The ants prune all foreign plant material e.g. vines that come in contact with their host plant (FIALA *et al.*, 1989).

To test whether the workers know their host plant we put various unoccupied plants in contact to a colony on a *M. triloba* host plant. The ants displayed different reactions depending on the plant species involved (table II).

Table II. — Reaction of *C. borneensis* workers from a colony on *M. triloba* against foreign plants in contact with their host plant.

Tabelle II. — Verhalten der Arbeiterinnen einer *C. borneensis*-Kolonie auf *M. triloba* gegen mit der Wirtspflanze in Kontakt gebrachte fremde Pflanzen.

Test subject	Pruned	Not pruned
<i>M. triloba</i>	2	18
<i>M. hypoleuca</i>	4	6
<i>M. tanarius</i>	8	2
<i>M. conifera</i>	3	0
<i>Sedum</i> sp., <i>Plantago</i> sp., <i>Phaseolus</i> sp. <i>Silene</i> sp., <i>Passiflora</i> sp., <i>Nephelium</i> sp., <i>Hibiscus</i> sp., <i>Ficus</i> sp. (4 each)	32	0

All plants that did not belong to the genus *Macaranga* were bitten off without exception ($\chi^2 = 18.7$, $p < 0.001$). Within the genus *Macaranga* non-myrmecophytes were significantly more heavily pruned than myrmecophytes ($\chi^2 = 13.25$, $p < 0.001$). The results for the myrmecophytic *M. hypoleuca* are not clear - only small spots were pruned. *M. triloba* was almost never attacked (no significant difference to *M. hypoleuca*: $\chi^2 = 2.11$; n.s.).

The host plants of 3 colonies died after transport from Malaysia to Frankfurt. Each colony was simultaneously offered an unoccupied *M. triloba* (myrmecophyte), an *M. tanarius* (nonmyrmecophyte) and a *Ficus benjamini*. A few hours after the start of the experiment the workers of all 3 colonies started to bite entrance holes in the stem of the new *M. triloba*. After 1-2 days all colonies were completely settled in the *M. triloba* plants. Both other plant species were ignored after a brief investigation.

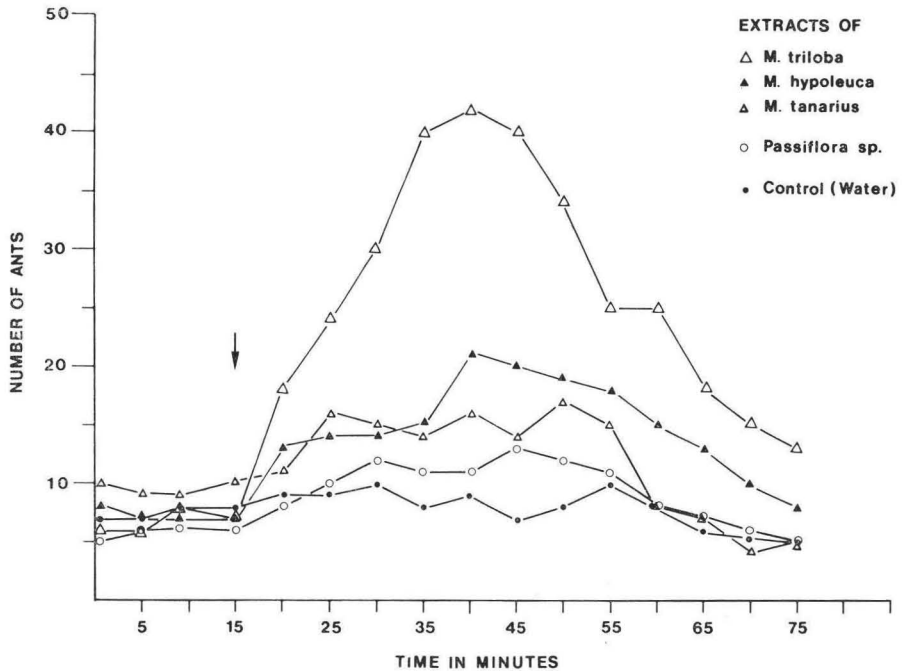


Fig. 1. — Mean increase of the number of ants on the leaf after application of different leaf extracts. Values shown are averages out of 10 trials. The arrow indicates the start of the experiment. For better comparison data are connected with lines. For significance see text.

Abb. 1. — Durchschnittlicher Verlauf der Zunahme der Anzahl Ameisen auf dem Versuchsblatt nach Zugabe von verschiedenen Blattextrakten. Werte aus 10 Versuchen. Der Pfeil markiert den Versuchsbeginn. Wegen besserer Vergleichbarkeit wurden die Daten mit einer Linie verbunden. Statistische Auswertung vgl. Text.

Leaf extract

The reaction of *C. borneensis* to chemical signals was tested by using aqueous extracts of different plant species. The number of ants on the leaves increased already after 5 minutes. A shaking of the leaf or the control droplet of water caused no ant activity. Generally all plant extracts seemed to attract the attention of the ants but the response was much stronger to extracts from *Macaranga* leaves, esp. *M. triloba* (fig. 1).

The increase of the number of ants in response to all plant extracts was significant (WILCOXON-test: *M. triloba*, *M. hypoleuca*, *Passiflora* sp.: $p < 0.001$; *M. tanarius* $p < 0.05$; water control n.s.). *M. triloba* caused the the strongest reaction (*M. triloba* compared to *M. hypoleuca*, *M. tanarius*, *Passiflora* sp. and water: all $p < 0.001$). *M. hypoleuca* and *M. tanarius* caused stronger reaction than *Passiflora* sp. ($p < 0.001$ and $p < 0.05$ resp.). Between these two *Macaranga* species there was no significant difference. (*Dioscorea* sp. and *Phaseolus* sp. always caused reactions intermediate between those in response to *Passiflora* and water and were not included in figure 1).

In summary, chemical substances can play an important role. *Macaranga* substances were differentiated from those of other plants and clearly caused stronger reactions. Possibly this is so far a matter of imprinting as the ants responded most strongly to the odour of their host plant.

Leaf injury

When we cut into the leaves of inhabited *Macaranga* plants the ants quickly gathered around the cut. The assembly seemed to be a result of recruitment but the olfactory stimuli played a role at least for the initial recognition. We avoided that the ants localised the cut by vibrations. Observations showed that the ants ran directly and straightforward to the cut. Workers which ran back alerted others in the nest whereupon a rapid increase in number took place at the cut (fig. 2).

Colony foundation and development

Despite of numerous checks during day and night we never saw a nuptial flight. We did, however, observe 31 queens colonizing their host plants. *C. borneensis* colonies are founded by a single queen which usually drops her wings after alighting on the plant. The queen chews an entrance hole into an internode of a *Macaranga* plant, crawls into the internodal chamber and then seals the entrance with small pieces of pith. Chewing an entrance hole into the stem takes 3-4 hours during which the queen is unprotected and an easy prey for visually hunting predators. After 2-4 weeks the first small workers emerge, reopen the entrance or chew new holes.

Our observations of newly colonizing queens indicate that swarming occurs during darkness. We found most of the queen ants before sunrise

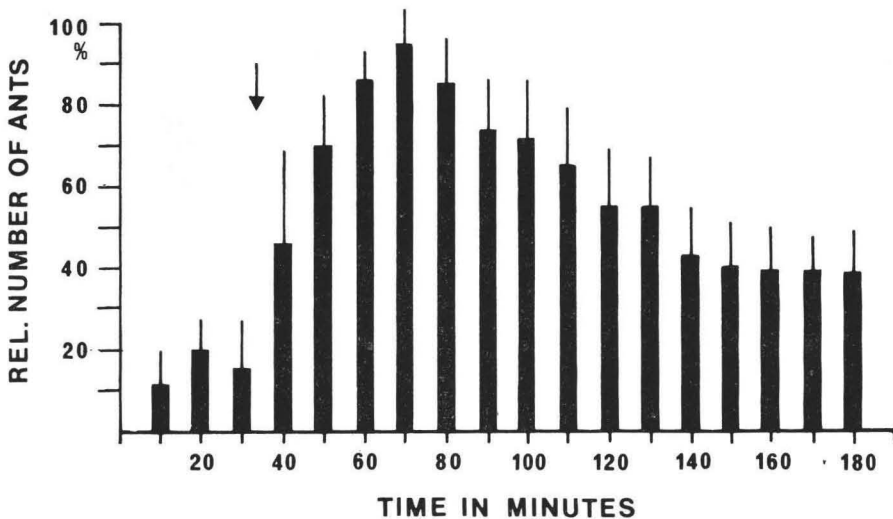


Fig. 2. — Mean number of ants on the leaf after leaf injury of *M. triloba*. Mean and standard deviation out of 12 experiments (4 trials on 3 colonies each). A piece of 9 cm² was removed out of the 2nd leaf from the top. To obtain comparable results the relative number of ants is given. The maximum number per trial was taken as 100 %.

Abb. 2. — Durchschnittlicher Verlauf der Ameisenreaktion auf eine Blattverletzung bei *M. triloba*. Mittelwerte und Streuung aus 12 Versuchen (je 4 Versuche von 3 Kolonien). Es wurde jeweils ein Blattstück von 9 cm² von der Blattspitze des zweitobersten Blattes entfernt. Um vergleichbare Werte zu erhalten, ist die relative Anzahl Ameisen angegeben; die maximale Anzahl in jedem Versuch = 100 %.

(5 before 7 a.m.), in the morning (22 from 7 a.m. to 11 a.m.) and evening (3 from 9-12 p.m.). On over 30 survey walks in the afternoon we found only one queen ant colonizing a host plant.

Colonizing queens were present during the entire study period (dry and rainy season), indicating that there is no distinct swarming season in *C. borneensis*, but that alates are produced year-round. The fact that colonies of all developmental stages were found at any time during our stay provides further evidence for nonseasonal swarming and colonization. Also the frequency distribution of the developmental stages did not show a seasonal pattern (table III). From Sep 88 to Feb 89 we did not thoroughly analyse the percentage of the different stages but again all stages were found over the entire period of 5 months. Young *Macaranga* plants start being occupied by ants when they are about 10 cm tall. At this size they usually have only one internode suitable for colonization. We investigated a total of 496 *M. triloba* plants for their degree of occupation according to the plant size. A plant was classified as occupied when at least one queen was present in the interior.

Table III. — Relative frequencies of colonies in various developmental stages on all *M. triloba* plants investigated. (Only plants 10-70 cm tall. Dead queens were not counted. Q = queen, E = eggs, L = larvae, P = pupae, W = workers). Note the rel. uniformity of percentages across all months.

Tabelle III. — Prozentsatz der einzelnen Koloniestadien aller untersuchten Pflanzen von *M. triloba* (n = Anzahl Pflanzen). (Berücksichtigt wurden nur Pflanzen mit einer Höhe von 10 bis 70 cm. Tote Königinnen wurden nicht mitgezählt. Q = Königin, E = Eier, L = Larven, P = Puppen, W = Arbeiterinnen). Die Verteilung über alle Monate ist rel. gleichförmig.

	1984/85		1985/86				1987		
	Jan	Feb	Dec	Jan	Feb	Mar	Jan	Feb	Mar
n	14	11	19	94	48	26	23	17	40
Colony stage	percentage								
Q	17	24	21	16	22	20	26	29	25
Q + E	3	17	15	18	24	23	17.5	18	17.5
Q + L	18	10	9	8	11	15	13	12	15
Q + P	25	14	11	16	10	19	13	12	17.5
Q + W	37	35	44	42	33	23	30.5	29	25

At a height of 10-20 cm (corresponding to an age of about 3-4 month) 62,2 % were already inhabited by ants, at 20-30 cm the percentage was 78,7. Similar results were obtained for *M. hypoleuca* and *M. hulletti*, whereas other *Macaranga* species were usually colonized at a later stage (FIALA, in prep.).

There is strong competition among queens for suitable *Macaranga* plants: Almost all plants were initially colonized by several queens (maximum: one queen in each of the ten internodes of a plant of 20 cm). Sometimes several colonies (up to 5 per plant) with surface active workers co-existed on single host plants as long as the number of ants did not exceed 20 per colony. Once colonies reached a size of more than 50 workers we never found more than one live queen per plant. Sometimes we found dead queens in the internodes. This indicates that after colonizing the same plant the members of different colonies which were separated by the nodal septa, attacked each other when they came in contact by opening the stem and killed the other queens. Larger plants with well developed colonies can no longer be colonized by newly arriving queens. This increases the competition for young *Macaranga* plants.

During the entire study period sexual brood and alate sexuals occurred. On most of the checked inhabited *M. triloba* trees taller than 3 m (50 out of 70) there were different stages of the reproductive caste (table IV). Other *Macaranga* species were only checked casually. In all of them sexual brood and alates occurred. The plants with females always contained all stages from small larvae to fully pigmented alates. Alate production was not strictly dependent on colony size. We found alates in a plant only 60 cm tall and in

Table IV. — Frequency of sexuals on large *Macaranga trees* (> 3 m tall).Tabelle IV. — Häufigkeit der Präsenz männlicher und weiblicher Geschlechtstiere in großen *Macaranga*-Bäumen (> 3 m hoch).

Species	n	Number of plants with sexuals		
		Only females	Only males	Both sexes
<i>M. triloba</i>	50	24	20	6
<i>M. hypoleuca</i>	8	6	2	—
<i>M. hulletti</i>	7	3	2	2
<i>M. hosei</i>	4	2	2	—

several plants of about 1.50 m height. However, they were most common in trees larger than 3 m.

Coccids

Since the scale insects involved in the association between *Macaranga* and *C. borneensis* have so far never been found outside *Macaranga* plants they seem to be obligatory partners of the symbiosis. However, we have no indications that the coccids are carried by the queens during the nuptial flight. The queens which had just landed on a plant and were chewing the entrance hole had not put down visible scales besides them. Also in freshly colonized plants we never found any scales. The degree of occupation with scales increases with increasing size of the plant or ant colony, resp. (table V).

Behaviour of *C. borneensis* on the plant surface

Undisturbed behaviour

Most of the workers patrol or collect food bodies. Important for the association is the ants' cleaning behaviour. Any material on the surface will be dragged away until it can be dropped off the plant. The interior of the

Table V. — Percentage of infestation with coccids of 3 myrmecophytic *Macaranga* species of different heights.Tabelle V. — Prozentsatz der Besiedlung verschiedener Größenklassen von 3 myrmekophytischen *Macaranga*-Arten mit Schildläusen.

Species		Plant height		
		≤ 1 m	1 - 2 m	> 2 m
<i>M. triloba</i>	n	155	27	61
	%	22	59	97
<i>M. hulletti</i>	n	36	21	10
	%	61	95	100
<i>M. hypoleuca</i>	n	41	15	15
	%	46	69	100

Table VI. — Mean spatial distribution of the ants in different crown regions of 18 *Macaranga* trees. Observed versus expected values assuming uniform distribution over total leaf area.

Tabelle VI. — Die räumliche Verteilung der Arbeiterinnen in verschiedenen Kronenbereichen der Pflanze.

Crown region	% of total leaf area	Number of ants	
		expected	observed
Upper third	39.2	359	500
Middle third	31.8	291	304
Lower third	29.0	265	111
Total	100.0	915	915

$\chi^2 = 146,4$; $p < 0.001$

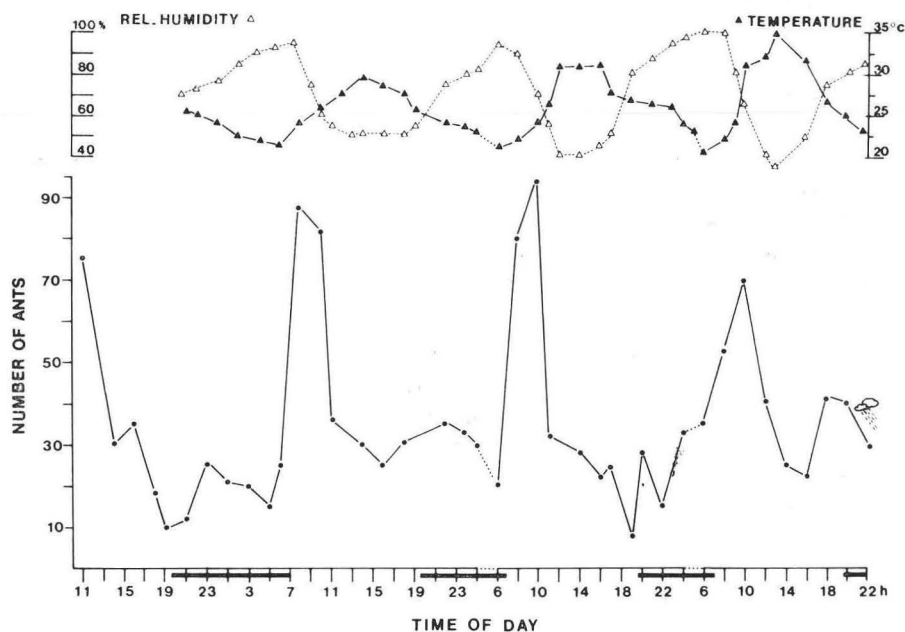


Fig. 3. — Mean diel activity pattern of two representative *C. borneensis* colonies on four successive days with similar weather conditions (sunny; rain only starting at night of the last day). Control counts every two weeks showed no principal change of the pattern. Black bars indicate darkness.

Abb. 3. — Durchschnittlicher Verlauf der Tagesaktivität zweier repräsentativer *C. borneensis*-Kolonien an 4 aufeinanderfolgenden Tagen mit ähnlichem Witterungsverlauf (sonnig, Regen setzt in der letzten Nacht ein). Kontrollzählungen alle 2 Wochen ergaben keine prinzipielle Änderung. Schwarze Balken weisen auf Dunkelheit hin.

stem is cleared of debris by removing it through the entrance hole. *C. borneensis* is active day and night. Although the number of ants active on the surface at night is lower than during the day, patrolling workers were found at any time (fig. 3). Intense sunshine reduced the ants' surface activity.

The 915 workers counted were not evenly distributed over the plant but preferred the upper crown region (table VI). The empirical data were compared with a random distribution. As expected, ants preferentially patrol the young and fresh leaves which can be assumed to be least protected against herbivores by structural and/or chemical means.

Response to experimental interference

Territoriality and defensive behaviour.

C. borneensis is strongly territorial and members of foreign conspecific colonies were attacked immediately upon contact (80 % of 30 encounters). The workers attacked almost every insect. Many animals left the plant immediately after the first encounter with *C. borneensis* workers. Small insects were removed in solitary actions with no distinct alarm. The ants took small insects like plant-lice or small caterpillars with their mandibles and dropped them off the plant. Larger caterpillars often let themselves fall off following a contact with the ants ($n = 30$). Those which did not leave the plant were attacked by many workers simultaneously. Some workers applied defensive secretions and bit so deeply into the soft bodies that sometimes the victim's gut was perforated and its contents spilled.

The much bigger, strongly sclerotized and very agile beetles and grasshoppers were attacked by applying defensive secretions and by biting into less protected body parts. Large intruders raised the attention of many workers so that the insect was eventually attacked from all sides. This usually caused them to move to more peripheral parts of the plant or to leave altogether. At the apex and on the younger leaves they attacked within a few seconds. The fact that not all insects were attacked was due to the number of ants active at the site and to the defensive attributes of the intruder (secretions, spines, urticating hairs).

Defensive secretion and trail pheromone

In *C. borneensis* the Dufour's gland is enlarged, whereas the poison gland is small and inconspicuous. The defensive secretion is lipophilic, viscous and sticky. A preliminary chromatographic investigation showed that it contained no volatile substances and mainly consisted of long-chained, saturated hydrocarbones (ATTYGALLE *et al.*, in prep.) which are common in Dufour's glands of myrmicines (BLUM and HERMANN, 1978).

Because colonies of *C. borneensis* are restricted to single host plants the trail marking system is certainly not such an important factor as in species

foraging far away from the nest. However, in the laboratory we observed *C. borneensis* following trails when collecting food bodies which had fallen off the host plant.

In *C. borneensis* as well as in other *Crematogaster* species so far investigated the gland producing the trail pheromone was found in the hind tibiae. Dr. MORGAN (Univ. Keele, GB; in litt.) tested the trail pheromone of *C. borneensis* workers on different *Crematogaster* species from various geographic regions (Europe, N- and S-America, Borneo). All species followed the trails of *C. borneensis* pheromone so it seems that the pheromone is not species- but genus-specific.

Comparison of *C. borneensis* with another *Crematogaster* species observed on *M. triloba*

The specific adaptations of *C. borneensis* to the host plant were demonstrated by a comparison with an as yet undetermined *Crematogaster* sp. A., probably subgenus *Paracrema* (LONGINO, pers. comm.). This was found on *M. triloba* in an area where also *C. borneensis* occurred. On 32 unoccupied *M. triloba* plants *Crematogaster* A visited scales which were always sucking at the exterior of the plant and never inside as with *C. borneensis*. Only 6 times we found brood of C. A. inside the stem of plants which had been abandoned by *C. borneensis*. They entered the stems through holes which had probably originally been chewed by *C. borneensis* and were later enlarged by C. A. The foraging range of C. A was much larger than that of *C. borneensis* because not only the *Macaranga* plant was visited but many different plants in the neighbourhood. C. A probably also fed on food bodies. On 18 of the plants the stipules under which the food bodies are produced, were totally empty or only a few small food bodies were found. This was in distinct contrast to plants occupied by *C. borneensis*, where in spite of the regular collecting by the ants always many food bodies were present under the stipules. Probably the colony size of *C. borneensis* parallels more closely the amount of food bodies available. C. A was also active day and night and was therefore potentially able to protect the plant against nocturnal herbivores, but it was much less aggressive than *C. borneensis*. Beetles and grasshoppers on the plant were usually ignored, but caterpillars were attacked, killed and taken as prey whereas *C. borneensis* never fed on intruders they attacked.

C. borneensis keeps its host plant free of vine growth (FIALA *et al.*, 1989), but plants visited by C. A were often overgrown with vines. 43,8 % of the plants with C. A were vine infested but only 5 % of *C. borneensis* occupied plants. Vines we wound around *M. triloba* plants were ignored by C. A workers and after a few days we found scale insects on them which were visited by C. A.

A comparison of the herbivore damage of the plants occupied by *C. borneensis* and C. A yielded a mean loss of leaf area of about 8 % and 21 %

resp. The characteristic assemblages of *C. borneensis* workers at leaf cuts did not occur with *C. A.* After experimental leaf injuries *C. A.* workers either ignored the cut or fled (after vibration).

DISCUSSION

Host specificity

The *Macaranga/Crematogaster*-system in W Malaysia apparently is dominated by one ant species—*C. borneensis*—inhabiting several host tree species. Associations which involve only a few-ant partners are so far known only for the following ant species: *Pheidole bicornis* on *Piper* (LETOURNEAU, 1983); *Pseudomyrmex triplarinus* on *Triplaris* (SCHREMMER, 1984); the mutualist *Petalomyrmex phylax* and the parasite *Cataulacus mckeyi* on *Leonardoxa africana* (MCKEY, 1984); *Aphomomyrmex afer* on *Leonardoxa letouzeyi* (MCKEY, 1989); *Azteca longiceps* and *Zacryptocerus setulifer* on *Cordia alliodora* (LONGINO, 1989); 2 *Pachysima* species on *Barteria fistulosa* (JANZEN, 1972). In most other systems several ant species are involved in the symbioses (e.g. 9 *Pseudomyrmex* spp. on *Acacia* (JANZEN, 1969); 10 (JAZEN, 1969) or 14 *Azteca*-species resp. (ANDRADE, 1981, cit. in JOLIVET, 1987), on *Cecropia*. Other *Crematogaster*-species as well as members of other formicid genera sporadically visit uninhabited *Macaranga* plants but none of them can be considered primary colonizer of the plant. Apparently they lack the important behavioural pre-adaptation which enables *C. borneensis* to chew the entrance holes into green plant tissue. Other species depend on already existing entrances. They use *Macaranga* plants abandoned by *C. borneensis* as one of many nesting possibilities but do not depend on it. This seems to be the case in such an extreme only with *C. borneensis*, which has never been found nesting outside *Macaranga* plants (no personal, literature or museum record).

The specificity of the association requires precise host recognition mechanisms by the ants. First this concerns the queens on search for suitable host plants. ONG (1978) supposed that host plant selection is based on special physical plant characteristics and mediated by optical signals. However, our observations of colonizing occurring during darkness indicate that chemical stimuli are more likely. The inhabited *Macaranga* species can be differentiated by the ants from other myrmecophytic species, these again from non-myrmecophytic congeners and *Macaranga* species from non-*Macaranga* plants. The response of the workers to chemical signals plays an important role concerning the protection of the plant since the ants rapidly and specifically react to plant odours which are released after leaf injury. The worker also recognize uninjured foreign plants in contact with their host plant and remove them.

Diet

With regard to the diet the adaptations of *C. borneensis* to its host plants are also very specific. In contrast to other *Crematogaster* species *C. borneensis* did not survive on artificial diets. It is so specialized that insects which were attacked in protecting the plant were not utilized as food but removed from the plant. So the ants seem to be totally adapted to food of plant origin. Carbohydrates are derived from plant sap via the scale insects. Our results indicate that the association with the scales is not obligatory, at least at early stages of the colony (59 % of the colonies on *M. triloba* plants 1-2 m tall were still without coccids). Also larger colonies were found without scales but it is still unclear whether colonies with and without scales develop equally well.

Workers isolated from the plant do not accept food. They die rather quickly indicating small energy reserves of the ants. Actually the crop is much reduced—in contrast to crops known of other myrmecines (EISNER, 1957). The crop reduction indicates that trophobiosis only plays a minor role. The permanent presence and easy accessibility of food on the plants makes storing food in the crop unnecessary and enables the ants to restrict foraging to the host plant. This has two advantages: a) The danger of predation during foraging is minimized and b) presence of ants on the host plant and thus protection against disturbances is maximized. Whether the crop reduction is an extreme adaptation to the symbiotic way of life can only be answered by comparative investigations within the genus *Crematogaster*.

The coccids live in obligatory symbiosis with the *Macaranga/C. borneensis* system (LEE, 1977; ONG, 1978). Queen ants do not carry young scales with them on their nuptial flights since the queens cannot hold the scales while biting the entrance hole. Probably the coccids are dispersed by winddrift at the L₁-stage like other scale species (BRAUNS, 1976) and the workers carry them into the interior of the stem. This reduces competition with other ant species for coccids and prevents them from being parasitized. Other *Crematogaster* species that live on *Macaranga* plants abandoned by *C. borneensis* did not carry around the coccids they visited. In other myrmecophytic associations scales are also kept inside the stem, but little is known about how they get there. Only SCHREMMER (1984) hypothesized that the *Pseudomyrmex* queens carry scales as described from *Acropygia* (BÜNZLI, 1935), *Cladomyrma* (ROEPKE, 1930) und *Plagiolepis* (BUSCHINGER *et al.*, 1987). We did not find carrying of scales by two *Cladomyrma* spp. which live in *Saraca thaipinensis* (Fabaceae) in Malaysia. (MASCHWITZ *et al.*, in press).

Little information exists concerning dietary adaptations in other ant-plant associations. With the exception of *Pseudomyrmex* on *Acacia* (JANZEN, 1967) and rarely observed casses on *Piper* (RISCH *et al.*, 1977) the ants of all myrmecophytic associations no longer seem to leave the plant for foraging.

Pseudomyrmex fulvescens could be fed artificially on honey-water and insects but did not produce eggs while on this diet (JANZEN, 1967). For *Pheidole bicornis* and *Azteca* it was stated that they were never found nesting outside their host plants (FRISCH *et al.*, 1977; JANZEN, 1973; DAVIDSON *et al.*, 1988). DAVIDSON *et al.* (1989) report rejecting of tuna and cheese baits offered to ant partners of *Cecropia*.

Activity and defensive behaviour

The cleaning behaviour of the ants effectively protects host plants because eggs of herbivores are thereby removed. In fact, such behaviour has been reported for most ant species colonizing myrmecophytes (JANZEN, 1967, 1969, 1972; RISCH, 1982; LETOURNEAU, 1983).

A small, non-stinging ant like *C. borneensis* does not seem very suitable for effective protection of their host plant, were it not for the high aggressiveness of this species. Other ants usually only fight when their nests are attacked—not in foraging area. In the case of *C. borneensis* the food plant is identical with the nest territory. *Macaranga* plants abandoned by *C. borneensis*, but inhabited by a second *Crematogaster* species, had more leaf damage than the plants occupied by *C. borneensis*. We conclude that *C. borneensis* due to its aggressiveness, "suicidal" fighting behaviour, application of defensive secretion and ability for mass attack provides effective protection of their host plants against herbivores. The *C. borneensis* workers are active day and night. Nocturnal activity is important for the plants since much herbivore damage occurs at night.

There is a distinct difference between *C. borneensis* and their congeners as to the behaviour toward plant competitors of *Macaranga* plants. The pruning behaviour displayed by *C. borneensis* has two effects: it enables the ants to get into the interior of the plant and at the same time serves the protection of the host plant by removal of vines. None of the 4 other *Crematogaster* species in the study area pruned vines on *Macaranga* plants. On the contrary these vines were often used as feeding sites of scales visited by the ants. The pruning behaviour against vines is common in ant species involved in ant-plant associations (DAVIDSON *et al.*, 1988). In the genus *Crematogaster* it is so far only known of *C. borneensis*. In other *Crematogaster* species, e.g. those associated with *Acacia*, *Tococa* and ant-garden-epiphytes, this behaviour is lacking (HOCKING, 1970; DAVIDSON *et al.*, 1988).

The origin of pruning behaviour in ants is not easily explained. It may be an extension of general aggression toward any foreign object (JANZEN, 1967, 1969). DAVIDSON *et al.* (1988) hypothesized that pruning may reduce the threat of invasions by potentially dangerous foreign ants. The removal of vines facilitates territory delimitation. As our studies of 4 Malaysian *Crematogaster* species as well as the investigation of DAVIDSON *et al.* (1988) showed, the removal of vines is not a general trait with arboreal Asiatic species of

Crematogaster and can therefore be interpreted as an adaptation to the symbiosis.

Phenology

Our field studies suggest that *C. borneensis* does not have a marked swarming season but swarms continuously and produces sexuals throughout the year. Data from the literature also indicate a year-round colonization (OH, 1973). *Macaranga* trees usually fruit twice a year. Seeds will not germinate until sufficient light is available, which may be provided by such aseasonal events as logging or treefall. The constant production of sexuals thus guarantees the rapid colonization of all *Macaranga* saplings. Competition between the newly colonizing queens is so strong that almost all saplings are at first colonized multiply. This enhances the chance for the plant that at least one colony will develop successfully. We did not find cooperation colony foundation of several *C. borneensis* queens. An example for cooperative colony foundation seems to be *Petalomyrmex phylax* (MCKEY, 1984) and *Azteca xanthochroa* (DAVIDSON *et al.*, 1989). LONGINO (1989) found chambers in *Cecropia* which contained multiple queens of *Azteca*. JOLIVET (1986) stated pleometrosis for *Pseudomyrmex* on *Triplaris americana* ("sans doute fondée par pléométrie", p. 162) whereas SCHREMMER (1984) reported fights between queens in the same association.

The monogynous colonies of *C. borneensis* never tolerate the entry of newly founding queens, which results in a continuous production of new colonies. JANZEN (1966) believes that this insures more efficient colony dispersal within the vegetation than if established colonies would absorb the newly produced or foreign queens.

In summary, *C. borneensis* ants have evolved extreme dependence on their host plant. The specificity of the relationship has been developed to such an extent that the destiny of the ant is inseparably connected to that of its host plant.

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EARLY SEX DETERMINATION IN LARVAE OF *BOMBUS TERRESTRIS*

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SUMMARY

A method is described for an easy and accurate sex determination for *all* larval stages of the bumble bee. The method is also applicable to other species of bees.

RESUME

Détermination précoce du sexe chez les larves de *Bombus terrestris*

Une méthode est décrite pour déterminer de façon aisée et précise le sexe de tous les stades larvaires du bourdon. La méthode est également applicable à d'autres espèces d'abeilles.

INTRODUCTION

To describe the morphological characters of bee larvae normally the last instar is taken. In that stage it is possible to distinguish male and female larvae by the shape and the position of the developing genital appendages, which in the male are located on the sternum of the 9th abdominal segment only and in the female on the sternum of the 7th, 8th and 9th segments. Early descriptions of these differences in the honeybee were given by ZANDER (1916) and ZANDER (1922); the first description was probably by DEWITZ (1874). He also studied a bumblebee. In females the genital opening is located at the 7th segment, and the sting apparatus is formed by outgrowths from the next two segments. In males the genital opening is located at the 9th (penultimate) segment, which also produces the penis. In larvae the imaginal discs of the genital appendages can be seen as subcuticular opaque marks.