

# Ant gardens of *Camponotus (Myrmotarsus) irritabilis* (Hymenoptera: Formicidae: Formicinae) and *Hoya elliptica* (Apocynaceae) in Southeast Asia

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**ABSTRACT.** *Camponotus irritabilis* (Formicidae: Formicinae) and *Hoya elliptica* (Apocynaceae) are very closely associated in ant gardens in Malaya and Sumatra. Ants and epiphyte partners have some characteristics that make them especially suitable for this association: The ants selectively retrieve the seeds of their epiphyte partners, and they fertilize their carton nests on which the plants are growing. In comparison to non-myrmecophytic *Hoya coriacea*, *Hoya elliptica* performs an extensive root growth as long as growing on moist substrate. The roots stabilize the ants' nests and anchor them to the host tree. *Camponotus irritabilis* initiate ant gardens by constructing carton buildings on branches, which serve as substrate for incorporated seeds and climbing parts of already established *Hoya elliptica*. *Camponotus irritabilis* influence actively the available chamber size within their nests, by biting off roots, fertilizing only certain parts of the nests and retrieving seeds into the 'growing zone' of the nest building. Ants thereby prevent uninhibited, space-consuming root growth but influence stability and architecture of the ant garden by guiding the spread out of the roots. As additional partners of the ant garden system, trophobionts, undetermined fungi on the inner nest substrate, several parabiogenic *Crematogaster* spp. and a probably lestoprobiotic *Solenopsis* sp. were found. Similarity in genus composition of the three co-occurring ants, as well as behaviors of *Camponotus irritabilis*, degree of the mutual benefits with the epiphytes and phenology of this ant garden association might represent a remarkable case of convergence with neotropical ant gardens.

**Keywords:** ant-plant interactions, epiphyte, myrmecochory, nest building behavior, paleotropic

## INTRODUCTION

Although neglected for a long time (Van Leeuwen, 1913, 1929a,b,c), ant gardens are highly important elements of Southeast Asian tropical rain forest canopies (Kiew & Anthonysamy 1995, Kaufmann *et al.* 2001, Kleijn & Donkelaar 2001, Kaufmann & Maschwitz 2006, Maschwitz 2010 *et al.*). A large majority of vascular epi-

phytes in lowland forests (except for ferns and orchids) are totally dependent on ants for their establishment and proliferation (Kaufmann & Maschwitz 2006). Generally, the establishment of ant gardens follows the same behavioral patterns in all ant garden systems that have already been described by Ule (1901): Ants construct small carton nests, into which they then retrieve seeds of their epiphyte partners. However, de-

**Table 1.** Behavior towards dead colony members that were experimentally offered at 50 to 100 cm distance from a nest entrance (n=10 in each experiment). In the carton nests of *Camponotus (K.) belumensis* Dumpert, 1995, and *Crematogaster cf. artifex* no plants were cultivated.

Ant species	<i>Camponotus (M.) irritabilis</i>				<i>Camponotus (K.) belumensis</i>				<i>Crematogaster cf. artifex</i>			
Experiment no.	I	II	III	IV	I	II	III	IV	I	II	III	IV
Time until discovery	12	2	4	9	15	23	8	27	2	4	2	8
#Discarded	0	0	0	0	2	4	4	4	8	6	6	8
#Retrieved	10	10	10	10	0	0	0	0	0	0	0	0

tails on ontogenetic development of ant gardens, specificity of ant and epiphyte partners, colony structure and ants' behavior vary greatly depending on the involved species (e.g. Belin-Depoux *et al.* 1987, Davidson & Epstein 1989; Orivel *et al.* 1997, Corbara & Dejean 1996, Cedeño *et al.* 1999, Orivel & Leroy 2011).

As a rule, ant garden associations are beneficial for ant and epiphyte partners. Ants provide reliable short distance seed dispersal, and a highly nutritional growth substrate with good water storing capacity. They prevent water loss of the root substrate and possibly protect the plants from herbivores (e.g. Longino 1986, Davidson 1988, Kleinfeldt 1978, 1986, Schmidt-Neuerburg & Blüthgen 2007). In addition to rain water the garden is provided with honeydew from trophobionts housed within the nest (Maschwitz *et al.* 2010). The epiphytes stabilize the ants' nests with their roots and might additionally sometimes offer food in form of edible fruit pulp, seed appendages or floral and/or extra-floral nectaries (e.g. Yu 1994, Davidson 1988; Kleinfeldt 1978, 1986). Again, details and degree of the mutual benefits depend on epiphyte and ant species (Weissflog *et al.* 1999, Kaufmann & Maschwitz 2006).

Here we report on a so far unknown ant garden association from the palaetropical region and present data on *Camponotus (Myrmotarsus) irritabilis* Smith F., 1857 which is strongly, though not exclusively, associated to a single epiphyte species, *Hoya elliptica* Hook. f., 1885 (Apocynaceae; Endress & Bruyns 2000, Endress & Stevens 2001). Besides the phenological description, experiments on nest building behavior, seed retrieval and the suitability of ants and epiphytes for the association were the main topics of our study.

## MATERIAL AND METHODS

Altogether, 18 colonies of *C. irritabilis* were discovered in Peninsula Malaysia (n = 16, Ulu Gombak Field Studies Centre, Genting Highlands, and North Selangor Peat Swamp Forest), Thailand (n = 1, Klong Thom National Park) and Sumatra / Indonesia (n = 1, Gunung Leuser National Park). Generally, all nests that could be discovered from the ground (using binoculars) were counted and plants growing on the nests were determined. Additionally, we felled several nest trees for more detailed examinations of the epiphytes and the ants, position and size of nests and for behavioral experiments.

Parts of five colonies were studied in detail: The epiphytes were determined to genus or if possible species (Burt & Woods 1974, Rintz 1978, Pigott 1988; Herbarium of the Forest Research Institute of Malaysia), parabioc and lestopbioc ants were determined to morpho-species (Forel, 1911, Bolton 1994). *Camponotus irritabilis* has been determined by Seiki Yamane (Kagoshima University, Japan, personal communication). Type specimens were deposited in the State Museum of Natural History Karlsruhe (Germany) and in the collection of the authors (AW, EK). The host trees were not determined to species, but it was recorded that they belonged to several different taxa.

Additionally, experimental studies were performed at the Ulu Gombak Field Studies Centre, Peninsula Malaysia (3°19'N 101°45'E). Three major topics have been part of these experiments:

**Table 2.** Seed carrying behavior of *C. irritabilis* towards various seeds. AG=ant garden epiphyte, non-AG=terricolous non-ant garden plant.

Tested item	n	Retrieved [%]	Ignored [%]	Disposed [%]
Fresh seed of <i>Hoya elliptica</i> Hook f. (AG)	110	92	8	
Seed of <i>Hoya elliptica</i> , heated to 110°C (1 hr)	20	80	20	
Seed of <i>Hoya elliptica</i> , heated to 200°C (1hr)	20	10	90	
Seed of <i>Dischidia nummularia</i> R. Brown (AG)	20		100	
Seed of <i>Aeschynanthus fecundus</i> Woods (AG)	20		100	
Seed of <i>Pachycentria constricta</i> Blume (AG)	20	100		
Seed of <i>Pachycentria constricta</i> (AG) after passage through a bird's gut	20	100		
Seed of <i>Neonauclea</i> sp. (non-AG)	10		18	82
Seed of <i>Ageratum conyzoides</i> L., 1753 (non-AG)	60	22	78	
Seed of <i>Pterocymbium javanicum</i> R.Br., 1844 (non-AG)	15		12	88
Seed of <i>Acacia mangium</i> Willd.1806 (non-AG)	10	45	55	
Seed of <i>Helianthus annuus</i> L., 1753 (non-AG)	10		21	79
Porcelain baits treated with acetone	25	0	100	
Porcelain baits treated with acetone extract of <i>H elliptica</i>	25	84	16	
Porcelain baits treated with acetone extract of <i>Aeschynanthus fecundus</i>	25	60	40	

**A) Nest building behavior of *C. irritabilis*, with special respect to the suitability of the carton material as substrate for epiphyte growth.**

Three experiments were performed on this topic:

1. The nest building behavior was induced experimentally and described in detail. 500 individual workers of all subcastes of *C. irritabilis* as well as larvae and pupae were placed on a small tree of *Castanopsis nephelioides* King ex Hook. f., 1888 (Fagaceae). A small plastic box was provided as shelter. A plastic cover shaded the tree and tangle trap was used to prevent the ants from fleeing. After 24 hrs, potential building material was offered (moist soil particles, moist bark fragments, woody fibers, cuticular fragments of dead insects, dry grass, seeds of *H. elliptica*, root cuttings of *H. elliptica*). The nest building behavior was observed for five days at different times of the day, using video and photo camera for documentation.

2. The behavior of *C. irritabilis* towards fragments of workers carcasses has been observed in comparison to other arboreal carton nest building ant species (Table 1). For these experiments, ten freshly killed members of the colony were offered in 0.5 to 1 m distance from the nest entrance. The behavior of workers finding their dead sisters was documented either until all dead ants had been removed, or for a maximum period of 60 min. Each experiment was repeated four times.

3. The substrate was analyzed a) macroscopically to determine material used for carton building, b) for its water storing capacity and c) chemically for its nitrogen, ammonium and phosphate content.

For the analysis of these major plant nutrients, a reflectometric method (Merck Reflectoquant®) was used. The original method has been designed for larger soil samples, therefore we had to adapt it to our demands (i.e. very small sample

size, poorly equipped field laboratory). Thus our results might not be comparable to results from other studies. To evaluate the measured contents, these were compared with the same method to those of other potential growth substrates like carton material of arboricolous ants and termites, forest soil and bark from host trees.

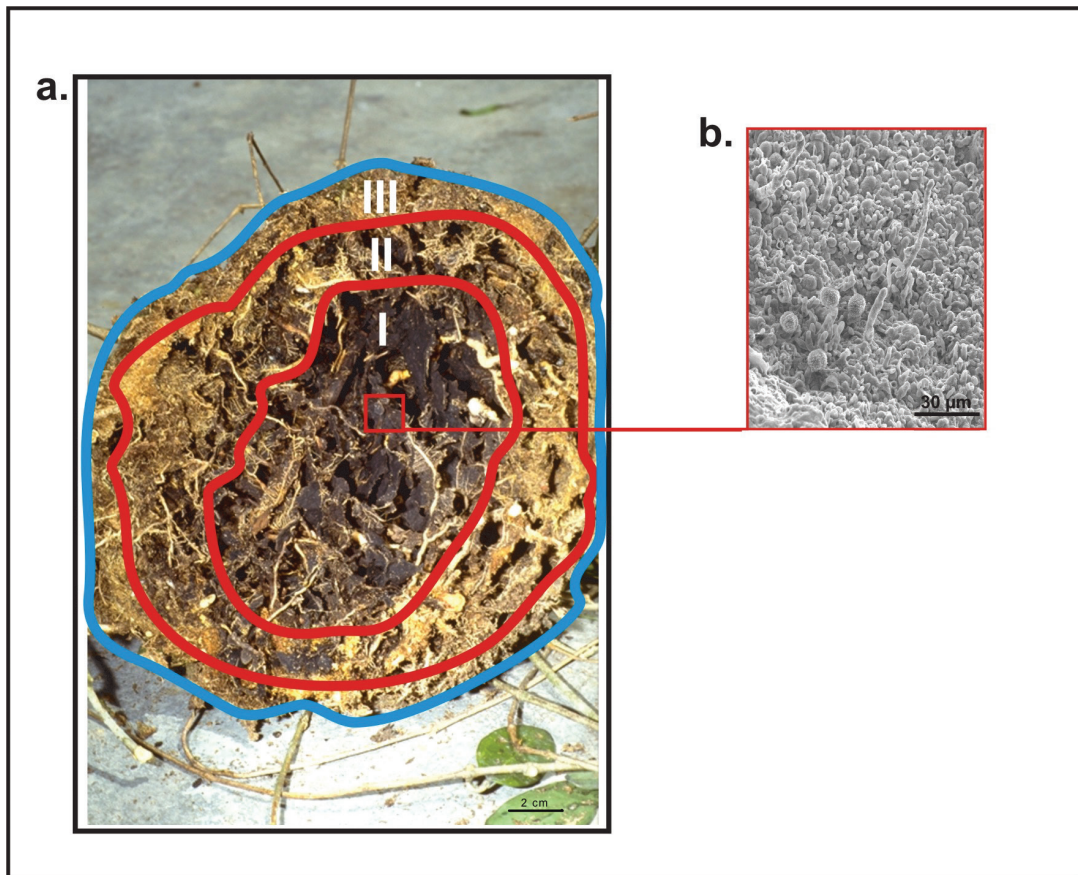
### B) Seed carrying behavior of *C. irritabilis* and associated ants.

Seeds of several epiphyte and non-epiphyte species (Table 2) were offered to *C. irritabilis* along a main foraging trail. The seeds were observed for a period of ten minutes and the behavior of the

ants towards the seeds was recorded. Seeds that had not been removed by the ants were replaced with fresh seeds after ten minutes. Additionally, porcelain baits were treated with seed extracts and offered in the same way in simultaneous experiments with seeds of *H. elliptica* as positive control and porcelain baits treated with pure solvent as negative control.

### C) Secondary root growth of *H. elliptica* in comparison with the non-myrmecophyte *Hoya coriacea* Blume, 1826.

*H. elliptica* and *H. coriacea* cuttings were placed on five different types of substrate: carton mate-



**Fig. 1.** (a) Free cross section trough matured *C. irritabilis* nest (volume: 11,494 cm<sup>3</sup>). In the centre (Area I, ca. 17 x 15 cm) very few epiphyte roots penetrated deeply black, paper like materials. (b) An undetermined fungus that was only found in this area of the nest caused the colour. **Area II** was characterized by a dense network of roots structuring the walls (up to 2.2 cm thick), and irregularly shaped chambers. Almost no carton material was found between the roots in this part of the nest. **Area III**, the growing area of the nest, consisted of one layer of smaller chambers with thinner walls (0.3-0.5 cm) that was covered with a layer of building material penetrated by young roots of *H. elliptica*.

**Table 3:** Epiphyte species occurring on the nests of *C. irritabilis*. We present the total number of *C. irritabilis* nests on which each epiphyte species was growing (# nests) and the number of colonies with which it occurred (# colonies). Data are based on 18 colonies.

Plant family	Plant species	# Nests	# Colonies
Araliaceae	<i>Schefflera</i> sp. <sub>KfmE</sub> 75	3	2
Apocynaceae	<i>Hoya</i> sp. <sub>KfmE</sub> 94	1	1
Apocynaceae	<i>Hoya elliptica</i> Hook f., 1885	38	16
Gesneriaceae	<i>Aeschynanthus albidus</i> Blume, 1840	9	3
Melastomataceae	<i>Pachycentria constricta</i> Blume, 1881	1	1
Melastomataceae	<i>Pachycentria glauca</i> subs. <i>maingayi</i> C.B. Clarke, 1879	1	1
Orchidaceae	<i>Dendrobium</i> sp.	1	2
Polypodiaceae	<i>Asplenium nidus</i> L., 1753	1	1
Polypodiaceae	<i>Lepisorus longifolius</i> Holtt., 1955	19	3
Polypodiaceae	<i>Pyrossia</i> sp.	1	1
Rubiaceae	<i>Myrmecodia tuberosa</i> Jack, 1823	1	1
Urticaceae	<i>Poikilospermum microstachys</i> (Barg.-Petr.) Merr., 1934	4	3
Urticaceae	<i>Poikilospermum cordifolium</i> (Barg.-Petr.) Merr., 1934	3	2
Zingiberaceae	<i>Hedychium longicornutum</i> Griff. ex Baker, 1892	12	1

rial of *C. irritabilis*, carton material of arboreal *Crematogaster* cf. *artifex* Mayr, 1879 and of *Technomyrmex* sp., moist forest soil, and water. Two carton samples (5x5 cm<sup>2</sup>) were taken from each of the ant nests, one of which was kept moist while the other one dried. Five cuttings of each epiphyte species were tested on any of the substrate types. Root development was measured after 1, 7, and 14 days respectively.

## RESULTS

### Phenology of the ant gardens of *C. irritabilis*

*Camponotus irritabilis* is an obligate ant garden ant. The workers of this very aggressive formicine are highly polymorphic (alitrunk length: 2.1-5.0 mm). Major workers can easily penetrate vertebrates' skin with their mandibles and apply formic acid into the wounds - a highly effective defense.

All 18 colonies that were found during our investigations in Malaysia, Thailand and Sumatra were located on one to four nest trees, belonging to various tree species, often along riverbanks (n = 14). Each colony comprised many

nests, the largest colony had at least 86 nest buildings. The size of a nest ranged between 4 and 29,300 cm<sup>3</sup> (median: 470 cm<sup>3</sup>). On all larger nests of *C. irritabilis*, epiphytes were growing. Most commonly found was *H. elliptica* (Apocynaceae), occurring on at least some nests of 16 of the 18 colonies and altogether on 86 % of all recorded nests. All other epiphytes occurred on some nests only, none of them being as predominant as *H. elliptica* (Table 3).

*H. elliptica* was often climbing up a branch towards the next nest building, in which it developed an extensive secondary root growth. Outside the nests, roots seemed to serve as hold-fasts rather than as nutritional organs and reached a maximum length of 0.5 cm without any branching. Inside the nests, roots were strongly branching and sometimes more than 20 cm in length.

Six nests had no epiphytes growing on them. These were significantly smaller (range: 4-66 cm<sup>3</sup>, median 24 cm<sup>3</sup>; U-test: U = 0.0; p < 0,001) than those with epiphytes (range: 255-29,300 cm<sup>3</sup>; median: 1,335 cm<sup>3</sup>). Underneath the nest buildings as well as on other parts of the host trees and adjacent plants, the ants

kept trophobiotic scale insects, covered by rough carton shelters. All the nests and the trophobiosis sites were connected by an extensive trail system, on which *C. irritabilis* was foraging both night and day. The foraging area of one colony of *C. irritabilis* comprised up to 380 m<sup>2</sup>.

Mature *C. irritabilis* nests with *H. elliptica* growing on them were distinctly structured (Fig. 1): In the center, very few epiphyte roots penetrated the deeply black, paper like thin walls. An undetermined fungus that was only found in this area and only in mature ant gardens (volume > 10.000 cm<sup>3</sup>, n = 10) but not in the outer parts of the nest, caused the black color. Around this central area, a dense network of roots structured the walls (up to 2.2 cm thick), producing a system of irregularly shaped chambers. Almost no carton material or soil was found between the roots in this area. The third area, the growing area of the nest, consisted of one layer of smaller chambers with thinner walls (0.3-0.5 cm) that was covered with a layer of building material penetrated by young roots of *H. elliptica* that formed a close-meshed net-structure. A similar principal structure was also found in nests with other epiphytes. In that case the outer network of roots was less dense than in nests with *H. elliptica*.

In the nests of *C. irritabilis* and on its foraging trails, several other ant species were encountered. Workers of *Crematogaster* sp. 11 (n=5) were found in great numbers inside the ant gardens but nested in small cavities in dead wood or underneath bark. The nest entrance was often covered with carton material; seedlings of different epiphytes were growing on them. The same type of association was found for *Crematogaster* sp. 39 (n = 1) and *Crematogaster* sp. 47 (n = 1).

*Solenopsis* sp. 1 was nesting inside the ant gardens of *C. irritabilis*, workers and brood have been found in small chambers in the outer walls of the nests. This species is several times smaller than *C. irritabilis* and moved cryptically in tiny runways inside the nest walls of the ant garden. Details on what *Solenopsis* is doing inside the nest, and if it is feeding on the ant brood as it is known for other species of *Solenopsis*, have not been subject of this study and remain unknown. *Camponotus irritabilis* mostly ignored workers of both *Crematogaster* species but attacked *Solenopsis*.

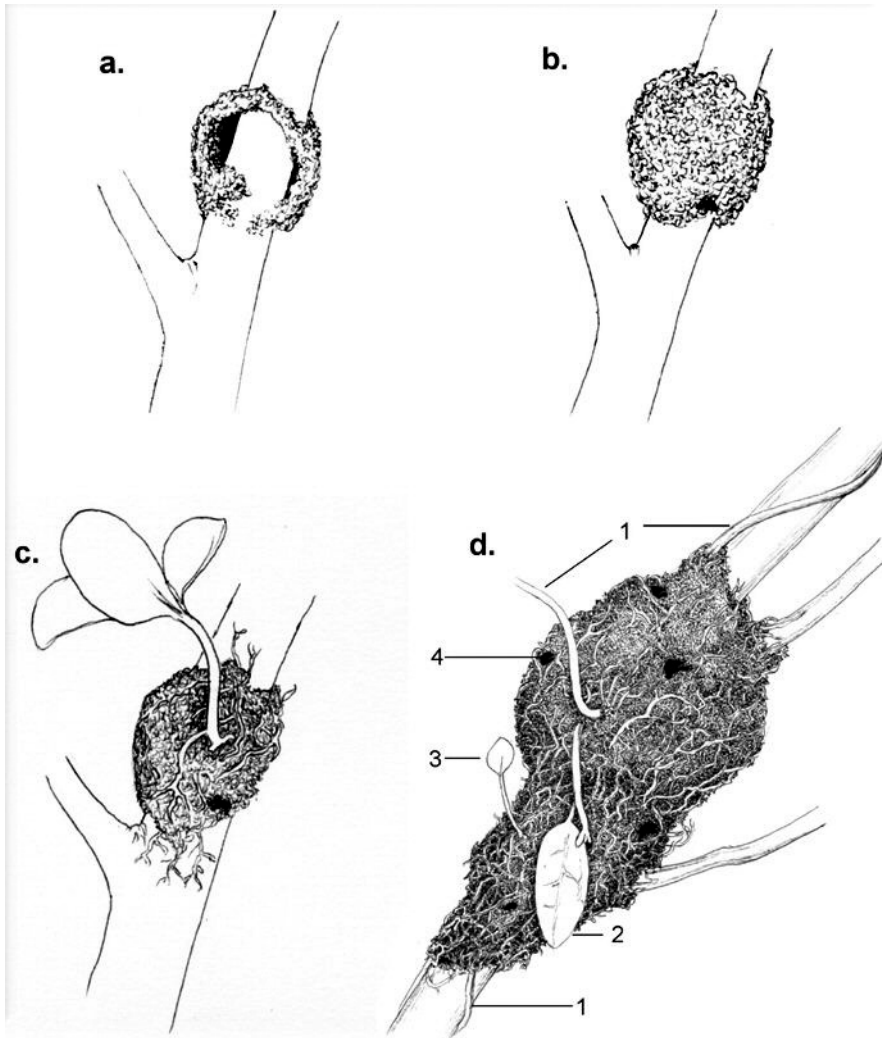
Trophobionts were found in low numbers of individuals within the carton nests, but only feeding on the roots of *H. elliptica*. On the various host trees we found mealybugs (Pseudococcidae; *Drepanococcus chiton* Green, 1909) and coccids (*Coccus hesperidum* Linnaeus, 1758, *Pulvinaria urbicola* Cockerell, 1893) within the nests and under sheltered pavilions. As a rule, they were feeding on the phloem of the host tree, thus providing a source of moisture, nutrients and minerals for the ant garden system.

### A) Development of an ant garden

*C. irritabilis* constructs carton shelters of small sizes on branches which serve as initial buildings for new ant gardens (Fig. 2).

1. Our behavioral experiments concerning the early development of ant gardens showed that *C. irritabilis* started constructing a new nest right after building material was offered. Mainly medium sized workers participated in the building process, while major and minor workers mostly remained inside the artificial nest. First, the ants took root fragments of *H. elliptica* and moist soil particles and established a small cover around a branch. They stacked up building material and condensed it by pressing with closed mandibles. Other building material was included after the very first period. Dry soil particles and grass were not used. Offered seeds of *H. elliptica* were incorporated in the carton nest and germinated after 48 hrs. After 17 days, one young plant had developed four leaves and was 3.5 cm high. The root system was dense with an average length of 6 cm. It penetrated the carton material and anchored the nest to the branch on which it was established (Fig. 2, Fig. 3).

Generally, two different ways of nest establishment could be distinguished. The one observed in the experiment represented the type in which a seed of *H. elliptica* was used as building material in a small nest construction. In the second type, a climbing part of an already established plant (in an older nest) reached a small nest and roots started spreading into the new carton substrate. Once the epiphyte's roots were spreading inside the nest, new building material was added on the surface and the nest was thus enlarged (Fig. 2).



**Fig. 2.** Development of *C. irritabilis* ant-garden. **(a)** Nest walls were built by the workers around a branch. **(b)** A spherical pavilion was constructed from soil- and wooden particles (3 x 2 cm). The settlement of *H. elliptica* in the nests of *C. irritabilis* occurs in two ways: **(c)** Seeds incorporated during nest building germinated within two days. The seedling reaches a length of 3.5 cm within 17 days and the roots covered and penetrate the new nest material **(d)** **(1)** Shoots and **(2)** matured *H. elliptica* with their origin in other nest buildings reached the new nest pavilion and finally small rootlets infiltrated the whole nest. The workers incorporated additional materials and nutrients (e.g. bird droppings, insect fragments) on the nest surface, which were penetrated by the roots and resulted in growing nests. **(3)** Built-in seeds of *H. elliptica* germinate within the nest. **(4)** The workers remove partly rootlets and form chambers and create nest entrances.

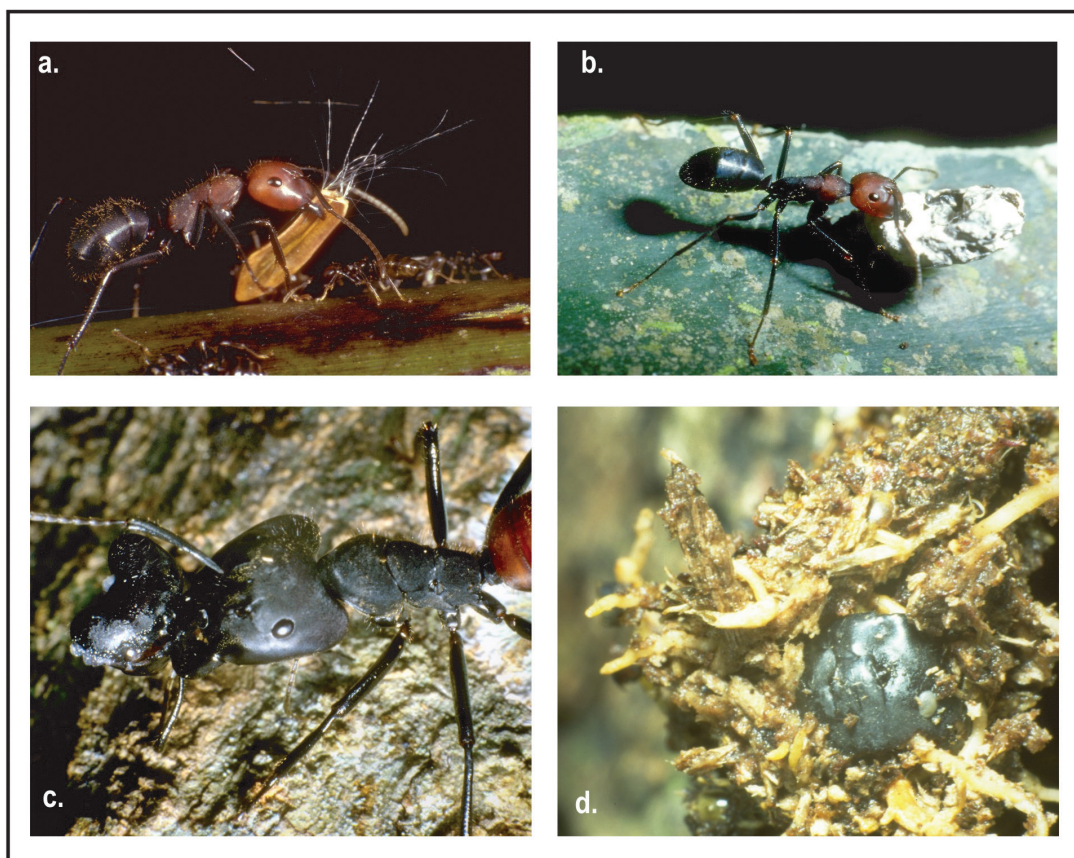
Unlimited root growth would have restricted the nesting space, and probably would even have excluded ants from their own nest. The ants controlled root growth in three different ways. Firstly, they selectively bit off certain parts of the roots that were penetrating their nesting space, and they pressed tiny root parts to the

carton walls so that they further strengthened the walls rather than limiting nest space. The ants also cleared larger areas in the walls of the nest and thus formed chambers, runways and entrance holes. Secondly, the ants stored bird droppings and insect fragments as a rule in the outer parts of the nest, not in the inner chambers. This



**Fig. 3.** Ant garden of *C. irritabilis*. The roots of the epiphytic *H. elliptica* have completely intersected the outer regions of the nest. The plant continues to grow along the stem and branches, without developing further roots on the host tree surface. In this way it reaches other nutrient rich new nests buildings, in which it then rapidly forms an extensive root system. Ant garden dimension: 26 cm x 27 cm.





**Fig. 4.** (a) *C. irritabilis* harvesting seed of *H. elliptica*. (b) Worker collected nutrients like bird droppings and (c.) insect fragments (e.g. ant-head). (d.) The nitrogen rich plant nutrients are stored by the workers within the nest chambers and later are penetrated by the roots of *H. elliptica*.

**Table 4** Chemical analysis of nesting substrate of several ant garden species in comparison to a non-ant garden ant (*Cr. cf. artifex*), arboricolous termites, forest soil and bark of a variety of different trees. For the tests, dry, pure substrate was homogenized and filled to tubes. For nitrate and ammonium tests, 5g of substrate were used for each test, for phosphate only 2.5g. For ‘bark’ it was difficult to get that much substrate. Therefore, bark from several trees was combined for each test.

Substrate	Ammonium			Nitrate			Phosphate		
	n	Mean	SD	n	Mean	SD	n	Mean	SD
Carton of <i>C. (M.) irritabilis</i>	23	15.3	6.6	24	22.0	15.8	20	107.2	167.2
Carton of <i>Cr. cf. artifex</i>	6	7.3	2.9	6	22.2	7.2	6	115.9	226.0
Nest of arboricolous termite	2	2.2	1.4	2	28.0	5.7	2	30.0	28.3
Forest soil	6	15.0	14.5	3	27.9	13.9	3	27.5	31.8
Bark	2	1.9	0.4	2	31.9	16.9	2	175.0	35.4

supported root growth in the superficial areas, and restricted proliferation of roots into the nest center. The third mechanism was the storing of incorporated seeds in the superficial chambers rather than inside the nest, thus promoting root development in this area (Fig. 4).

2. The storing of insect parts and bird droppings in the outer layers of the nest was very remarkable, as *C. irritabilis* even carried corpses of its own colony members back into the nest - a behavior relatively uncommon in ants, as most ants remove corpses from the nest (Table 1).

3. The obvious “fertilization” of *C. irritabilis* ant gardens suggests that these should contain relatively large amounts of plant nutrients. Ammonium, nitrate and phosphate contents of *C. irritabilis* ant gardens were compared to nest cartons of *Cr. cf. artifex*, nest material of an arboricolous termite, forest soil and bark (Table 4).

*Camponotus irritabilis* nests contained about as much ammonium as did forest soil (T-test,  $t = 0.06$ ,  $p = 0.94$ ), and significantly more than nest substrate of the arboricolous *Cr. cf. artifex* (T-test,  $t = 2.9$ ,  $p < 0.01$ ). There was no difference in nitrate (T-test,  $t = 0.03$ ,  $p = 0.97$ ) and phosphate (T-test,  $t = 0.10$ ,  $p = 0.93$ ) concentration compared to *Cr. cf. artifex*. However, considering that nutrients were constantly taken from *C. irritabilis* nests, while this was not the case in any of the other tested material, the quality of nest substrate becomes more evident. The water storing capacity of the carton material in the ant gardens (collected after several days of heavy rain), estimated as factor  $x = \text{wet weight of nest-substrate} / \text{dry weight of nest-substrate}$ , was  $5.6 \pm 0.6$  ( $n = 6$ ). For comparison: water storing capacity of *Cr. cf. artifex* nests, which are made out of long-stranded plant fibers but without soil and detritus, was conspicuously lower ( $2.6 \pm 0.9$ ,  $n = 6$ ; T-test,  $t = 6.79$ ,  $p < 0.01$ ).

**Table 5.** Root growth of the epiphytic *H. elliptica* (ant garden species) and *H. coriacea* (non-ant garden species) on various types of substrate. Growth values represent the mean of five plants of each species that were tested on each type of substrate. Total length in mm is given, with indication that there was no further root growth compared to the last measured value. The standard deviation is given for each value (s).

Substrate	Root growth [mm]											
	Hoya elliptica						Hoya coriacea					
	1 day	s	7 days	s	14 days	s	1 day	s	7 days	s	14 days	s
<b>Carton of <i>C. irritabilis</i></b>												
Moist	2.2	1.3	3.9	0.94	16.1	4.52	1	0.84	1.3	0.6	-	-
Dry	1.6	0.65	-	-	-	-	-	-	-	-	-	-
<b>Carton of <i>Cr. cf. artifex</i></b>												
moist	2.4	1.14	5.3	0.76	10.9	2.45	-	-	-	-	-	-
dry	-	-	-	-	-	-	-	-	-	-	-	-
<b>Carton of <i>Technomyrmex</i> sp.</b>												
Moist	-	-	4.2	0.78	12.7	1.42	-	-	-	-	-	-
Dry	-	-	-	-	-	-	-	-	-	-	-	-
Moist forest soil	3.1	0.74	4.8	1.27	15.1	1.96	2.1	0.63	-	-	-	-
Water	4.1	0.71	10.1	3.51	25.2	2.25	2.4	0.94	-	-	-	-

## B) Seed carrying behavior

It has already been shown that *C. irritabilis* incorporated seeds of *H. elliptica* into its nests. However, our experiments using seeds of different plant species as well as baits and seeds of *H. elliptica* after various treatments revealed some interesting results (Table 2). Seeds of *H. elliptica* were retrieved to 92% (n = 110), while the much smaller but also plumed seeds (with featherlike hairs) of two other ant garden epiphytes, *Dischidia nummularia* R. Brown, 1810 (Apocynaceae) and *Aeschynanthus fecundus* Woods, 1975 (Gesneriaceae), as well as most seeds of the tercolous (ground rooting) plants, were always ignored or even discarded.

Whenever complete plumed seeds of *H. elliptica* were offered, *C. irritabilis* attacked them first. The ants calmed down after a short while and often bit off the appendage before retrieving the seed. Seeds from which the hairy appendage had been removed were taken without attack and carried into the nest. After dissection of five ant gardens we found seeds only on the surface of nests (n = 5) and placed in the outer nest chambers (n = 45) five days after carrying out the tests.

## C) Secondary root growth

The results of the experiments on the root growth of *H. elliptica* in comparison to *H. coriacea* on various types of substrate are presented in Table 5. Comparing the two species, the root growth of *H. elliptica* was in any case distinctly higher than that of *H. coriacea*. Concerning the substrate, the water content seems to be most important. Pure water produced the longest roots within the two-week period, followed by moist nesting material of *C. irritabilis* and moist forest soil. The root growth on dry substrate was insignificant. At least in the first 14 days there was no significant difference in root growth on the carton material of the three ant species (but compare results: see paragraph A).

## DISCUSSION

Ant gardens are among the most complex ant-plant associations, because they usually include more than two species and always need a host tree as third partner. In most cases, even more part-

ners are involved. In *C. irritabilis* ant gardens, the host tree played an important role not only as mechanical support, but particularly as a food source for the fourth partner, the hemipteran trophobionts. These were numerous and assumed to be one main nutrient source for their partner ants. Moreover, they might provide the partners of the association with moisture tapped by the trophobionts from the host tree. The significance of the fifth partner, the undetermined fungus growing on the carton material in the very interior of the nest, remains unknown. As these fungi were only detected in well-developed ant gardens and only in the innermost part, it seems unlikely that they stabilize the nests, as it is the case in other carton building ant species (Weissflog 2001). One can speculate that these fungi may facilitate plant uptake of nutrients by forming an ektomykorrhiza, as it is described by Belin-Depoux (1991) in the neotropical ant-garden association with the epiphyte *Philodendron melinonii* Brongn. ex Regel, 1874 (Araceae).

The similarity in genus composition of the three co-occurring ant garden ants is a striking convergence in the neotropical and oriental zoogeographical regions: In neotropical ant gardens the highly aggressive *Camponotus femoratus* Fabricius, 1804 (the world's most aggressive ant, according to D. Davidson (cited in Hölldobler & Wilson 1990)) interacts with the parabiocytic *Crematogaster limata* cf. *parabiocica* Forel, 1904 and *Solenopsis parabiocicus* (Jolivet 1998) as a thief ant (lestobiosis) living in the nest walls of *C. femoratus*. *Crematogaster* cf. *limata parabiocica* frequently nests in small natural cavities or even in the soil, i.e. not in ant gardens (Orivel *et al.* 1997, Cedeno *et al.* 1999). Although we do not know whether *Solenopsis* sp. found in *C. irritabilis* ant gardens also is a thief ant, this seems likely because this is a widespread phenomenon in that genus (e.g. *Solenopsis fugax*, Forel, 1869). The interactions between *C. irritabilis* and *Crematogaster* sp.<sub>KimA</sub> 11 and *Crematogaster* sp.<sub>KimA</sub> 21 in the oriental region strongly resemble the ones of *C. femoratus* and *Cr. limata* cf. *parabiocica*, as both *Camponotus* species were exclusively nesting in ant gardens and occurred also without any parabiocytic species, while the *Crematogaster* spp. frequently nested in preformed cavities of the trees. As we have not studied details of the

*Crematogaster* spp. - *C. irritabilis* interaction in Southeast Asia, further comparisons would be too speculative.

For ants, epiphytes and trophobionts, the interaction can be rated as a mutualism, because these partners clearly profit from their association.

The beneficial effects *C. irritabilis* provides for its epiphyte partners, and particularly for *H. elliptica*, are obvious: *H. elliptica* benefits not only from seed dispersal by the ants (1), but the seeds are also integrated into a nutrient-rich carton structure (2).

The highly selective seed retrieval of *H. elliptica* seeds is based on chemical cues in combination with size preferences. Most epiphytes besides *H. elliptica*, growing on *C. irritabilis* ant gardens, belong to one of the following two groups: a) Primarily bird dispersed fruits (e.g. *Pachycentria constricta* Blume, 1831) that are retrieved either for their sugary fruit pulp or for minerals obtained from birds' feces. b) 'Microdiaspore'-strategists like ferns and orchids that produce millions of tiny spores/seeds, some of which will be sure to land on an established ant garden. For the complex of selective seed carrying of ant garden ants in general, chemical cues are of special interest. While the seeds of the two ant garden epiphytes *D. nummularia* (Apocynaceae) and *A. fecundus* (Gesneriaceae) were not retrieved in our experiments, extracts of these seeds applied to larger baits were highly attractive. In tropical American ant gardens (Davidson *et al.* 1990, Seidel *et al.* 1990, Youngsteadt *et al.* 2008), and also in terricolous myrmecochores (Marshall *et al.* 1979, Brew *et al.* 1989, Hughes *et al.* 1994, Sheridan *et al.* 1996), special olfactoric attractants occur in a wide range of plant species and families. Our results suggest that something similar might also be found in Southeast Asian ant gardens.

With their nest construction, ants provide their plant partners with a highly nutritive substrate that additionally has a good water storing capacity (Yu 1994, Schmit-Neuerburg & Blütgen 2008). The latter might be vital for *H. elliptica* mainly in the establishment phase, because tree crowns can be relatively arid habitats even in ever-wet forests (Benzing 1990), and seedlings usually have no morphological structures that protect them from drought. Trophobionts guarantee a continuous moisture supply through the

phorophytes phloem, thus contributing to a reliable microclimate in the tree crown. The high nutrient content in the ants' carton is reached through a special ant behavior: the use of bird droppings, and the deposit of parts of dead insects and even dead colony members as building material. A similar behavior is also known from *Philidris* species and their associated 'ant house' epiphytes (e.g. *Myrmecodia* spp., *Hydnophytum* spp., *Dischidia* spp.). In this case nutritive debris is stored in the domatium structures (e.g. Janzen 1974, Peeters & Wiwatwitaya 2014). However, most ants remove debris from their nests. Thus, this behavior might be regarded as a special trait of at least some epiphyte-associated ants. Quantitative data on the nutritive value of tropical American ant gardens are provided by Blütgen *et al.* (2001). They also found substrate of ant garden ants to contain significantly higher amounts of certain plant nutrients in comparison to other potential growth substrates (termite nests). However, the nutritive value of the substrate varied depending on the partner ant. Other beneficial effects that are known from terricolous ant plant interactions, like herbivore defense, have not been tested so far, but seem not unlikely considering the aggressiveness of *C. irritabilis*. However, *H. elliptica*, as a member of the milkweed family, contains poisonous latex, its leaves are thick and leathery and the stem tends to lignify quickly.

The main benefit the ants gain from *H. elliptica* is carton nest stabilization and enlargement of available nesting space. The exceptional suitability of *H. elliptica* for this purpose has been shown by comparison of root growth with its non-ant garden congener *H. coriacea*: After two weeks, the roots of *H. elliptica* were about 12 times longer than those of *H. coriacea*. The beneficial effect this has on nest stability can be estimated from the size of ant nests with and without epiphytes: The largest nest with *H. elliptica* was about 440 times the size of the largest nest without epiphytes. The roots not only stabilize the nests but also serve as a holdfast that anchors the nest to the branch.

The mutualistic contribution of the trophobiotic partners is obvious: They provide the ant garden system with nutrients, minerals and moisture from the phloem sap of the host tree. As

a reward they gain protection in the ant nests. We do not know if the ants also distribute them, as is known from other ant species (Dill *et al.* 2002).

Although *C. irritabilis* and *H. elliptica* do not form a species-specific ant garden system, a very close relationship between both species apparently exists. Almost all colonies of *C. irritabilis* had *H. elliptica* growing at least on some of their nests, some even occurred with *H. elliptica* exclusively. *Hoya elliptica* has seldom been found without *C. irritabilis*, and if so, the plants were growing on the nests of other ant garden ants (Weissflog 2001, Kaufmann & Maschwitz 2006). This high degree of specialization is untypical for ant gardens both in tropical America and Southeast Asia (e.g. Davidson 1988, Weissflog *et al.* 1999, Kaufmann *et al.* 2001). Most ant garden ants are associated with a variety of different ant garden epiphytes. The selective seed retrieval might be at least partly responsible for the preferred co-occurrence of *C. irritabilis* and *H. elliptica*. Other factors, like light and humidity demands, might additionally contribute to this pattern. Many common ant garden epiphytes have been found in the same area like *H. elliptica*, but on ant gardens of other species (Kaufmann & Maschwitz 2006). Our untested assumption is that these species are growing in sunnier microhabitats, while *H. elliptica* is relatively shade tolerant.

Orivel & Dejean (1999) also report on certain species-dependent seed preferences that were strongly correlated with the species distribution on ant nests for tropical American ant gardens of *C. femoratus* (+ parabiocic *Cr. cf. limata parabiocica*), and suspect that microhabitat or seed availability are additional factors influencing species composition.

*Camponotus irritabilis* initiate ant gardens by building carton shelters of small sizes on branches in the same or in neighboring host trees. In some cases, the ants incorporate seeds of *H. elliptica* as building material, which germinate and rapidly grow into carton buildings. Interestingly, very often a climbing part of an already established plant reaches these carton shelters and mainly because of moisture and the organic materials in the carton, roots started spreading quickly into the substrate. *Hoya elliptica* might be particularly suitable for such an ant garden

initiation, since it shows an extensive and fast secondary root growth. Once the epiphyte's roots were spreading inside these satellite nests further building material was added on the surface and the nest was thus enlarged.

In ant-house association from Thailand it has been shown that pitchers of *Dischidia major* (Vahl) Merr., 1917 are spatially divided into many compartments as a result of the ants' carton building behavior. Here workers of *Phildiris th01* use debris from outside to build walls, which causes extensive root growth and branching (Peeters & Wiwatwitaya 2014). *Camponotus irritabilis* influence and even more guide the root growth of *H. elliptica* by positioning insect remains, bird droppings, and other debris as a rule in the outer areas of the nest. Additionally, by biting off and manipulating the direction of root growing and actively digging and building walls, they obtain suitable nest chambers for the colony in the inner and outer layers of the nest. Thus they influence actively not only the available space within their nests for brood and nestmates but also prevent uninhibited, space-consuming root growth. Such an active and manipulating building behavior was so far not reported for the neotropical ant gardens, although architectural characteristics of ant gardens of *C. femoratus* implied comparable behaviors (own observation AW).

Nesting space is the main factor limiting colony size and reproductive success of ants in rain forests (Wilson 1987, Fonseca 1993, Fiala & Maschwitz 1992). This is also true for the members of the ant genus *Camponotus*. Up to now in Southeast Asia rainforests only members of the highly aberrant subgenus *Camponotus (Karaivaevia)* are known to achieve independence from pre-existing nest cavities in the canopy by actively weaving their nests with the help of larval silk (Maschwitz *et al.* 1985, Dumpert *et al.* 2006). Most of the other *Camponotus* species, however, are competing for temporal available natural nesting space in dead or rotting wood, enlarge the space slightly by digging and trench the wood before its rapid decomposition.

Because of the help of its epiphyte partner, and due to the specialized, active and manipulating building behavior, suitable preformed nest cavities are no longer a limiting factor, and this may qualify *C. irritabilis* to establish huge

and dominant colonies in the crown region. Our results regarding the specificity of ant and epiphyte partners, colony structure, ants' behavior and degree of the mutual benefits with the epiphytes show great consistency and comparability with neotropical ant-gardens, but also advanced characteristics that may be unique for paleotropical ant-garden associations.

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