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Effects of food rewards offered by ant–plant *Macaranga* on the colony size of ants

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Myrmecophytes (ant–plants) have special hollow structures (domatia) in which obligate ant partners nest. As the ants live only on the plants and feed exclusively on plant food bodies, sap-sucking homopterans in the domatia, and/or the homopteran's honeydew, they are suitable for the study of colony size regulation by food. We examined factors regulating ant colony size in four myrmecophytic *Macaranga* species, which have strictly species-specific association with *Crematogaster* symbiont ants. Intra- and interspecific comparison of the plants showed that the ant biomass per unit food biomass was constant irrespective of plant developmental stage and plant species, suggesting that the ant colony size is limited by food supply. The primary food offered by the plants to the ants was different among *Macaranga* species. Ants in *Macaranga beccariana* and *Macaranga bancana* relied on homopterans rather than food bodies, and appeared to regulate the homopteran biomass and, as a consequence, regulate the ants' own biomass. In contrast, ants in *Macaranga winkleri* and *Macaranga trachyphylla* relied primarily on food bodies rather than homopterans, and the plants appeared to manipulate the ant colony size. Per capita plant investment in ants (ant dry weight plant dry weight^{-1}) was different among the four *Macaranga* species. The homoptera-dependent *M. beccariana* and *M. bancana* harbored lower biomass of ants than the food-body dependent *M. winkleri*, suggesting that energy loss is involved in the homoptera-interposing symbiotic system which has one additional trophic level. The plants' investment ratio to the ants generally decreased as plants grew. The evolution of the plant reward-offering system in ant–plant–homopteran symbioses is discussed with an emphasis on the role of homopterans.

Key words: ant–plant–homopteran symbiotic mutualism; coccids; food body; ontogenetic change in ant defense; population regulation.

INTRODUCTION

Ants are everywhere on earth. When combined, all ants in the world weigh about as much as all humans (Hölldobler & Wilson 1994). What factors limit the upper end of ant numbers globally, locally, and within the colony? Although territorial interference (Levings & Traniello 1981), climate (Greenslade 1975a, 1975b; Kaspari & Vargo 1995), predation (Franks & Fletcher 1983), disease (Schmid-Hempel 1998), food (Boomsma *et al*. 1982) and nest-site availability (Brian 1956) have been occasionally documented to affect the local population size or colony size of ants, few systematic works have been performed so far to evaluate the limiting factor for ant colony size.

Myrmecophytes (ant–plants) prepare special nesting space (domatia) for obligate ant partners, and often provide the ants with food. The ants, in return, protect the plant from herbivores and vines. As the plant-ants usually do not forage outside of the host plant but stay on the plant throughout, they are suitable for the study of colony size regulation. Fonseca (1993) examined

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factors limiting ant colony size in the South American myrmecophyte *Tachigali* and concluded that nesting space availability, rather than food supply, delimited the ant colony size. For plants, however, the regulation of ant population by food supply seems better to keep the ant population flexibly at the level that maximizes plant fitness. Here, we examine factors regulating ant colony size in four myrmecophytic *Macaranga* species. We found that food resource supply by the plants delimited the ant colony size.

The second focus of this paper is to show the interspecific variations in the reward-offering system in *Macaranga* and discuss its implication in the evolution of ant–plant–homopteran mutualism. In ant–myrmecophyte symbioses, ants consume sap-sucking homopterans, their honeydew and/or plant exudates (extrafloral nectar or food bodies) (Davidson & McKey 1993). The rewarding system varies considerably among myrmecophytes, from the exclusively homoptera-dependent *Tachigali* (Fonseca 1993) to the South American *Acacia* that lacks the third partner homopterans (i.e. the ants being wholly dependent on plant food bodies (Janzen 1966)). The ant–myrmecophyte mutualism is thought to originate from interactions between ants and homopterans (Benson 1985; Ward 1991). This is supported by the observation that most myrmecophytes harbor homopterans as a third partner while only some produce specialized food bodies (Davidson & McKey 1993). Because the presence of homopterans imposes an additional trophic level consuming extra energy (Gaume *et al*. 1998), selective interests from the plants and ants would tend to abort the homopterans from the tripartite system. Few attempts, however, have been made to assess the costs in homoptera-involving systems, and relate them to the evolution of ant–plant–homopteran symbiotic mutualism. Who manipulates the tripartite system and takes the initiative in the system's coevolution?

Regulation of the ant and homopteran populations is essential in the maintenance of this tripartite symbiosis because excessive numbers of ants or homopterans would cause overconsumption of plant sap and hence weaken the plant, while insufficient number of ants would reduce herbivore deterrence and consequently decrease plant fitness. In the regulations of the ant and homopteran populations, the fitness interests of ants, plants and homopterans often conflict. We can assume an extreme case where cheater parasitic ants breed a tremendous number of homopterans in the domatia and quickly produce ant reproductives that disperse while the plant withers. Such a case of cheater ants was actually documented by Yu and Pierce (1998). Thus, each of the three participants is assumed to struggle for controlling the ant and homopteran populations in order to maximize its own fitness.

Itioka *et al*. (2000) and Nomura *et al*. (2000) documented that three coexisting myrmecophytic *Macaranga* species differed in their biotic and chemical defenses; from highly ant-defended but chemically defenseless species (*Macaranga winkleri*) to less ant-defended but chemically well-defended species (*M. beccariana*). The herbivore fauna on each *Macaranga* species varied accordingly (Itino & Itioka 2001). These observations suggest that the food-rewarding system of *Macaranga* differs in quantity (for example, highly ant-defended plants may offer more rewards to ants) and/or quality (homopterans or food bodies?) from species to species. Here, we examine the interspecific variation in food-rewarding system in four ant-defended *Macaranga* species and discuss the evolution of the ant–plant–homopteran symbiotic mutualism.

METHODS

Study area

The field survey was conducted in a lowland mixed dipterocarp forest in Lambir Hills National Park, Sarawak, Malaysia (4°20′N, 113°50′E, altitude approx. 60 m). The park receives approximately 4000 mm of rainfall annually with no pronounced dry season (Sakai *et al.* 1997). Inoue and Hamid (1994) give a detailed description of the park.

Macaranga and ants

Among Bornean myrmecophytic *Macaranga* species (approx. 20 spp.), *M. beccariana, M. bancana* (formerly treated as *M. triloba*), *M. trachyphylla* and *M. winkleri,* all of which are closely related to or included within section *Pachystemon*, were selected

for this study. They are dominant in open habitat and it is not uncommon to find all four species within a small forest gap or a narrow riverbank in the study area.

A queen ant colonizes a small *Macaranga* seedling and nests inside the swollen hollow stem. Nearly 100% of 50-cm tall saplings of the four *Macaranga* species are occupied by obligate ant colonies (Itino & Itioka 2001). Workers actively patrol the leaves of *Macaranga,* protecting the plants against herbivores and vines (Fiala *et al.* 1989; Itioka *et al*. 2000). They exclusively consume the food bodies secreted by the plants, the honeydew of small coccids (*Coccus* spp.) living inside the hollow stem, and the coccids themselves. The skewed age distribution of the coccids strongly suggests that the ants selectively eat younger coccid nymphs (T. Itioka, unpubl. obs., 1995).

The four *Macaranga* species have close associations with the following morphospecies of ants: *M. beccariana* with *Crematogaster decamera*, *M. bancana* and *M. trachyphylla* with *Crematogaster borneensis*, and *M. winkleri* with *Crematogaster* sp. 2 (Itino *et al*. 2001). Each *Macaranga* species has its own unique herbivore fauna as well. When not occupied by ants, *M. beccariana* becomes very much damaged by gall-making flies, *M. bancana* and *M. trachyphylla* by leaf-eating insects, and *M. winkleri* by leaf eaters (and ant-feeding woodpeckers when occupied by ants) (Itino & Itioka 2001).

According to Davies (1996), *Macaranga triloba* (*bancana*) and *M. trachyphylla* are very close relatives within the *triloba* (*bancana*) clade. *Macaranga beccariana* is outside of the *triloba* (*bancana*) clade although the three species, *M. bancana, M. beccariana* and *M. trachyphylla,* are within the *Pachystemon* clade. *Macaranga winkleri* is out of the *Pachystemon* clade. *Macaranga bancana, M. trachyphylla* and *M. winkleri* produce food bodies mostly under recurved stipules, while *M. beccariana* produces them on new leaves (see also Fiala & Maschwitz 1992). *Macaranga winkleri* is light demanding while the other three species are moderately light demanding (Davies *et al*. 1998).

Colony collection

The census was carried out on a 1.5-km transect along riverbanks in the lowland dipterocarp forest. Along the transect, we randomly selected 40 ant-occupied trees with little leaf damage (0.5–5 m in height, 10 trees for each of the four *Macaranga* species). Between 17 and 19 August 1995, we cut the trees and collected the ant colonies. Each tree was covered with plastic bags and cut into approximately 25-cm-long pieces at the collection site. Each tree piece was sealed in a plastic bag individually. Workers outside the domatia were collected and counted into the colony size. The bags were kept at 60°C for 2 h in an oven to kill the insects.

Each tree piece was dissected and the contents examined. For each piece, number of queens, alate females/males, queen larvae/pupae (when detectable by their body size larger than the worker larvae/pupae) and coccids (together with the information on their body length), and the presence or absence of ant workers, pupae, larvae and eggs were recorded. For the whole colony, the dry weights of ant adults and ant immatures were measured after 48-h drying at 55°C in an oven. The volume of the coccids in the plants was estimated by cubing the body length. The dry weight (mg) of coccids was estimated by volume (mm^3)/20, which was empirically obtained from the correlation between the dry weight and the volume of coccids. The ant species were identified based on worker and queen morphology.

Plant morphology

For each plant, height (cm) was measured and the numbers of leaves and stipules were counted. Stems, leaves and food bodies were weighed after 72-h drying at 80°C (stems and leaves) or 48-h drying at 55°C (food bodies) in an oven. The standing crop of food bodies is assumed proportional to their rate of production in this study. As immature food bodies are usually left unharvested by the ants and quickly consumed when matured (A. Hatada, unpubl. obs., 1999), the assumption is reasonable given that time needed for the food body maturation is constant irrespective of the plant species.

Light environment

Exposure to the sun was assessed as canopy openness (%) for each tree. Hemispherical photographs

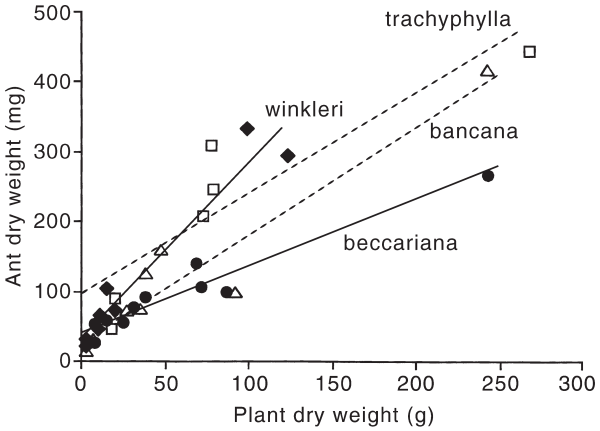

Fig. 1. Relationship between dry weight of the symbiotic ants and the plants. ●, *M. beccariana*; △, *M. bancana*; □, *M. trachyphylla*; ◆, *M. winkleri*.

were taken with a Nikon (Nikon Co., Tokyo, Japan) 8 mm fish-eye lens from the canopy of each tree. The black and white prints were scanned and analyzed using LIA 32 ver. 0.37 (Yamamoto 1997).

RESULTS

Limiting factors for ant colony size

The correlation of dry weight between ants and plants was highly significant in the four *Macaranga* species (Fig. 1; $r^2 = 0.939$, 0.906, 0.812 and 0.935; $n = 10$, 8, 6 and 8; $P < 0.02$ in *M. beccariana, M. bancana, M. trachyphylla* and *M. winkleri*, respectively). The slopes (the regression coefficients of the ant biomass to the plant biomass) were, however, heterogeneous among the four *Macaranga* species (ANCOVA with the assumption that Y-intercepts of the regression lines are zero; $F = 3.60$, $P = 0.027$), indicating that ant biomass per unit plant biomass was significantly different among the plant species. These results suggest that plant biomass certainly affects ant biomass in some way but it does not wholly explain the variation in ant biomass, especially the variation between *Macaranga* species. In addition to the plant biomass, some other factors specific to plant species seem to affect the ant biomass. We hypothesize that food resource availability would wholly explain the intra- and interspecific variation in ant biomass.

Ant biomass (dry weight) was significantly or highly correlated with food body dry weight (FBW)

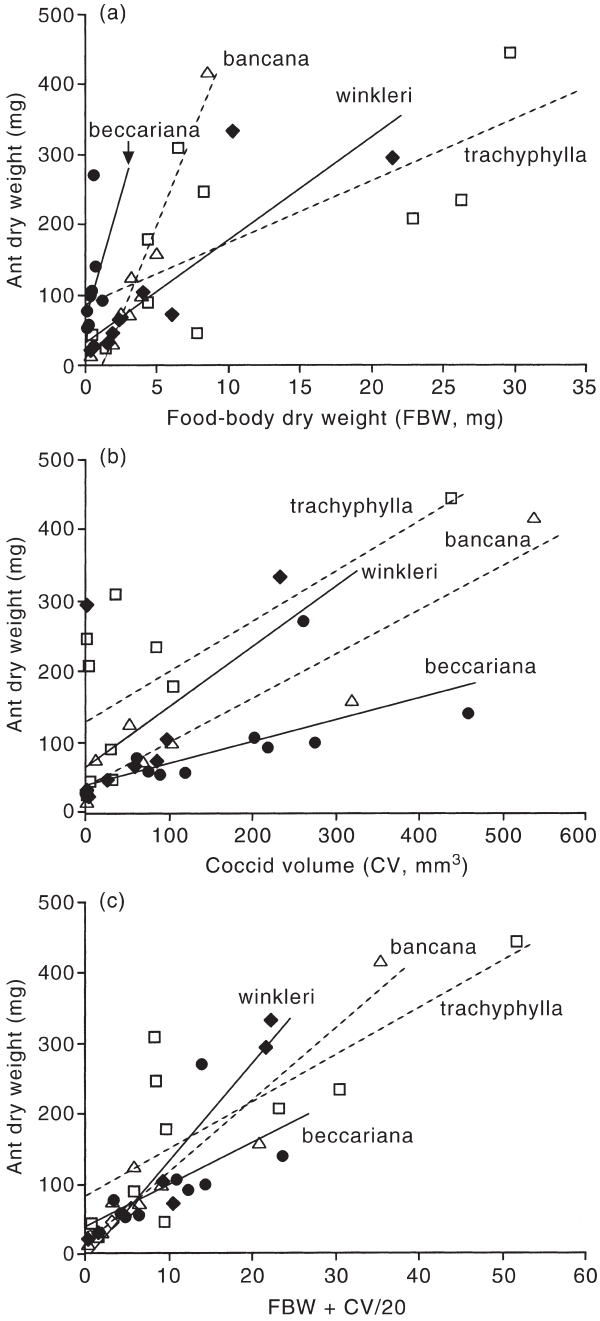

Fig. 2. Relationship between dry weight of the symbiotic ants and (a) dry weight of food bodies (FBW), (b) coccid volume (CV) and (c) FBW plus CV/20 (the estimation of dry weight of the coccids) in the four *Macaranga* species. ●, *M. beccariana*; △, *M. bancana*; □, *M. trachyphylla*; ◆, *M. winkleri*.

(Fig. 2a; $r^2 = 0.121$, $n = 10$, $P = 0.325$ in *M. beccariana*, $r^2 = 0.921$, $n = 8$, $P = 0.0002$ in *M. bancana*; $r^2 = 0.503$, $n = 10$, $P = 0.022$ in *M. trachyphylla* and $r^2 = 0.726$, $n = 8$, $P = 0.0073$ in *M. winkleri*), and coccid volume (CV) (Fig. 2b;

$r^2 = 0.390$, $n = 10$, $P = 0.054$ in *M. beccariana*; $r^2 = 0.889$, $n = 8$, $P = 0.0004$ in *M. bancana*; $r^2 = 0.512$, $n = 10$, $P = 0.020$ in *M. trachyphylla* and $r^2 = 0.318$, $n = 8$, $P = 0.146$ in *M. winkleri*). Despite these correlations, either FBW or CV does not sufficiently explain the interspecific variation in ant biomass because different *Macaranga* species harbored significantly or nearly significantly different amounts of ants given that the overall FBW (or CV) is held constant at the mean value (Fig. 2a,b; ANCOVA with separate slopes: $F = 2.22, P = 0.108$ for FBW; $F = 4.45$, $P = 0.011$ for CV).

In order to see the overall effect of food resources on ant biomass, ant dry weight was plotted against FBW + CV/20 (estimation of coccid dry weight) (Fig. 2c). The correlations were significant or nearly significant for the four *Macaranga* species ($r^2 = 0.391$, $n = 10$, $P = 0.053$ in *M. beccariana*; $r^2 = 0.913$, $n = 8$, $P = 0.0002$ in *M. bancana*; $r^2 = 0.614$, $n = 10$, $P = 0.0074$ in *M. trachyphylla* and $r^2 = 0.935$, $n = 8$, $P < 0.0001$ in *M. winkleri*). More importantly, the standardized least squares means (± SE, mg) of the ant biomass (100.0 ± 20.1 in *M. beccariana,* 115.2 ± 21.7 in *M. bancana,* 141.8 ± 19.6 in *M. trachyphylla* and 135.6 ± 22.8 in *M. winkleri*) were very similar among the four plant species (ANCOVA with the assumption that Y-intercepts of the regression lines are zero; $F = 0.80$, $P = 0.502$), indicating that ant biomass per capita food resource availabilitiy was similar irrespective of plant species. This means that overall food dry weight explains the intra- and interspecific variation in ant dry weight and, therefore, is regarded to be the determinant of ant biomass.

Variation of reward-offering system among *Macaranga* species

Macaranga winkleri, for example, harbored more ants than *M. beccariana* per unit plant dry weight (Fig. 1). This leads us to predict that *M. winkleri* provides more food bodies and/or coccids to the ants than *M. beccariana.* The slopes of the regression of food body dry weight (FBW) on plant dry weight (PW) were heterogeneous among the four *Macaranga* species (Fig. 3a; ANCOVA with the assumption that Y-intercepts of the regression lines are zero; $F = 10.55$, $P = 0.0001$). The standardized least squares means (± SE) of FBW (mg) were, in descending order, 8.69 ± 1.36 in *M. winkleri*, 8.54 ± 1.25 in *M. trachyphylla*, 2.38 ± 1.13 in *M. bancana*, and – 0.40 ± 1.03 in *M. beccariana*. The interspecific differences were significant in the following pairs: *M. winkleri–M. bancana, M. winkleri–M. beccariana, M. trachyphylla–M. bancana* and *M. trachyphylla–M. beccariana*.

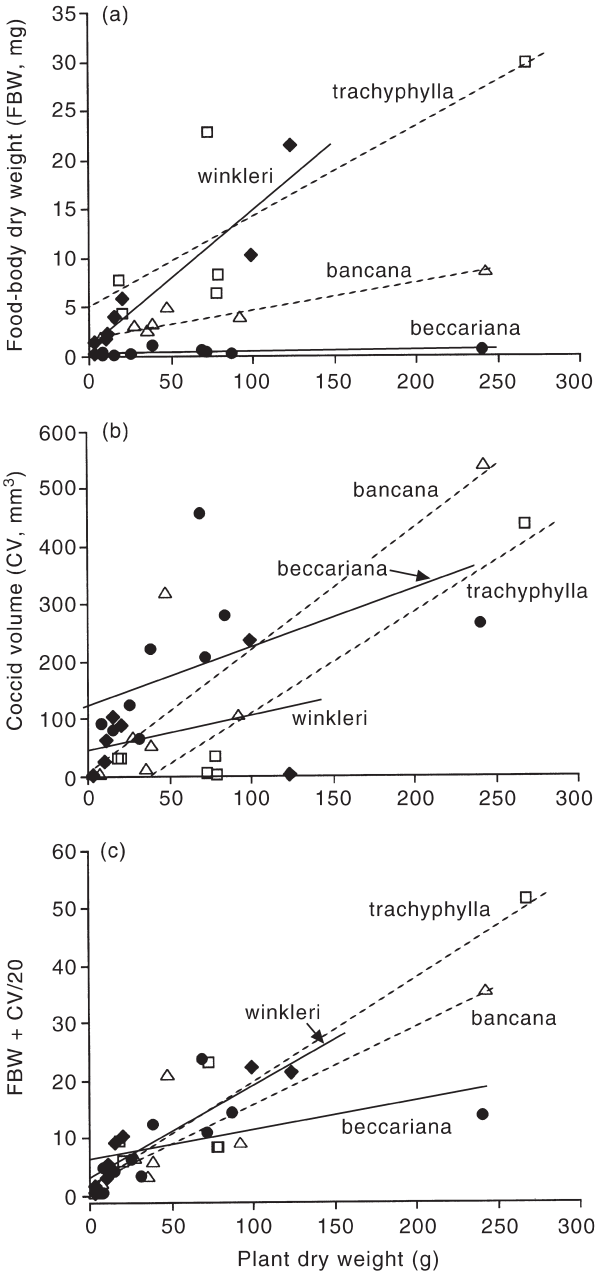

Fig. 3. Relationship between (a) dry weight of food bodies (FBW), (b) coccid volume (CV), and (c) FBW plus CV/20 (the estimation of dry weight of the coccids), and the plant dry weight in the four *Macaranga* species. ●, *M. beccariana*; △, *M. bancana*; □, *M. trachyphylla*; ◆, *M. winkleri*.

For coccid volume (CV), although the slopes of the regressions were not detected heterogeneous

among the four *Macaranga* species (Fig. 3b; ANCOVA with the assumption that Y-intercepts of the regression lines are zero; $F = 1.45$, $P = 0.25$), the standardized least squares means (± SE) of CV (mm^3) were significantly different among species ($F = 3.24$, $P = 0.039$: 169.7 ± 30.0 in *M. beccariana*, 118.6 ± 32.9 in *M. bancana*, 72.7 ± 39.6 in *M. winkleri*, and 13.8 ± 36.5 in *M. trachyphylla,* where the significant difference was only detected between *M. trachyphylla* and *M. beccariana*).

To see the overall food investment by plants towards ants, we adopted the regression of the FBW + CV/20 value against PW (Fig. 3c). The slopes of the regressions were significantly heterogeneous among the four *Macaranga* species (ANCOVA with the assumption that Y-intercepts of the regression lines are zero; $F = 4.04$, $P = 0.018$) although the standardized least squares means of FBW + CV/20 were not significantly different among species ($F = 0.69$, $P = 0.57$). These results indicate that there is variation in the reward-offering system among *Macaranga* species.

Ontogenetic change in the plant's investment towards ants

There was a clear trend that younger plants invested more towards ants. The regression of per capita plant's investment to ants (ant dry weight plant dry $weight^{-1}$) on plant dry weight indicates that investment ratio to ants was larger when plants were smaller (Fig. 4; $\beta = -0.014$, $F_{[1,8]} = 4.36$, $P = 0.070$ in *M. beccariana,* $\beta = -0.008$, $F_{[1,6]} = 3.87$, $P = 0.097$ in *M. bancana*, $\beta = -0.008$, $F_{[1,4]} = 3.45$, $P = 0.137$ in *M. trachyphylla* and $\beta = -0.040$, $F_{[1,6]} = 6.81$, $P = 0.040$ in *M. winkleri*). The trend was especially pronounced in *M. winkleri*. This result is consistent with Heil *et al.* (1997) that found that smaller seedlings of *M. triloba* produced more food bodies per plant weight than mature trees. These developmental decreases in ant defense seem to be compensated by the developmental increases in chemical defense in *Macaranga* species (M. Nomura, unpubl. obs., 2000).

Ant colony structures

Each plant usually harbored one ant queen and many workers, immatures (eggs, larvae and pupae) and coccids (Table 1). Three ant species were involved and they inhabited the *Macaranga* species in a strict species-specific manner with the exception of *M. winkleri* tree no. 6 that *Crematogaster borneensis* inhabited rather than the specific partner ant *Crematogaster* sp. 2. In this mismatched case, when compared with other same-sized trees, the ant colony contained far fewer workers and immatures, and also the biomass of food bodies and homopterans was less (Table 1). As for the coccids, they consisted of at least several species (mostly *Coccus* spp.) that are not within a monophyletic group (S. Takagi, pers. comm., 1996). Their host association is to be investigated.

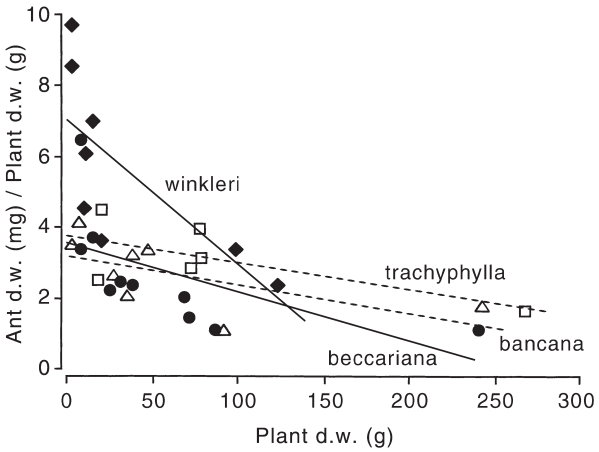

Fig. 4. Relationship between per capita plant investment to ants (ant dry weight (d.w.) per unit plant dry weight) and plant dry weight in the four *Macaranga* species. ●, *M. beccariana*; △, *M. bancana*; □, *M. trachyphylla*; ◆, *M. winkleri*.

Males, alate females and queen larvae/pupae occurred in four, six and seven trees, respectively, out of the 40 trees (Table 1). The trees containing these reproductives were mostly over 1 m in height. In the younger saplings (<2.5 m) of *M. bancana* and *M. winkleri* no reproductives were found. On the other extreme, in *M. beccariana* the reproductives were produced much earlier (among approx. 1–2.5 m tall saplings, 86% trees had reproductive ants inside).

Another interspecific difference was detected in the frequency of dead queens in domatia. A single dead queen was found in each of one *M. beccariana,* one *M. bancana* and seven *M. winkleri* trees at the bottom end of a hollow stem. All the dead queens belonged to 'correct' partner species (i.e.

Table 1 Information on the dissected *Macaranga* plants and their inhabitant ant colonies[1]

[2]Plant code	Height (cm)	No. leaves	No. stipules	Stem dry weight (g)	Leaf dw (g)	Stem+ leaf dw (g)	[3]Ant species	Ant adults dw (mg)	Ant immatures dw (mg)	Food bodies dw (mg)	Coccids biomass (mm^3)	Position of coccids (height above the ground, cm)	[4]No. queens	Position of queen (height above the ground, cm)	No. alate males/ females	No. queen larvae/ pupae	Canopy openess (%)
Macaranga beccariana																	
4	76	15	22	3.1	4.6	7.7	Cd	9.0	17.0	0.56	0	–	1	33			13.7
9	83	15	34	3.9	4.2	8.1	Cd	37.3	15.0	0.12	94	40–70	1	12			2.1
3	94	18	37	7.5	7.7	15.2	Cd	38.0	18.9	0.21	81	30–80	1	13			11.0
2	122	18	33	11.9	13.1	25.0	Cd	35.0	20.1	0.27	125	10–110	1	13			10.1
8	98	25	50	10.8	20.3	31.1	Cd	45.4	31.2	0.07	67	70–90	1	11		3/1	5.1
10	101	24	25	11.6	26.7	38.3	Cd	47.6	43.7	1.15	224	10–90	1	13	0/1	2/8	9.3
1	182	30	119	45.6	23.3	68.9	Cd	102.0	37.8	0.73	459	120–170	1	35	0/7	4/8	10.8
6	158	65	182	37.5	34.5	72.0	Cd	65.8	39.4	0.49	208	110–150	1	38	2/1	3/0	13.9
5	184	44	118	48.6	38.0	86.6	Cd	57.8	40.1	0.30	281	50–170	1	15		11/0	4.2
7	253	82	190	155.8	84.6	240.4	Cd	201.0	69.7	0.63	267	190–240	1	36	0/10	7/8	7.9
Macaranga trachyphylla																	
3*	37	7	8	–	3.1	–	Cb	9.4	15.8	1.40	3	20–30	1	9	6/4		9.3
7*	56	7	10	–	5.0	–	Cb	36.0	7.2	0.50	7	40–50	2	13			10.4
4	115	7	10	10.3	8.2	18.5	Cb	15.9	30.6	7.80	33	60–110	1	13			9.0
2	97	9	8	10.3	9.9	20.2	Cb	65.1	25.3	4.40	31	40–90	1	14			6.7
8*	93	10	12	–	11.8	–	Cb	137.0	41.0	4.40	105	10–90	1	12			10.5
9	161	12	19	50.4	22.3	72.7	Cb	177.1	29.6	22.90	5	60–150	1	13			8.8
5	123	14	18	34.3	43.5	77.8	Cb	187.8	120.9	6.50	36	40–90	1	13			10.6
10	225	9	18	55.8	22.8	78.6	Cb	186.8	59.7	8.30	3	190–220	1	14	1/0		9.0
6*	164	14	16	–	32.2	–	Cb	180.9	54.1	26.30	85	90–160	1	13			11.6
1	374	10	18	236.9	30.4	267.3	Cb	341.4	102.9	29.70	439	200–370	1	12	1/0		7.5

Table 1 *Continued*

[2]Plant code	Height (cm)	No. leaves	No. stipules	Stem dry weight (g)	Leaf dw (g)	Stem+ leaf dw (g)	[3]Ant species	Ant adults dw (mg)	Ant immatures dw (mg)	Food bodies dw (mg)	Coccids biomass (mm^3)	Position of coccids (height above the ground, cm)	[4]No. queens	Position of queen (height above the ground, cm)	No. alate males/ females	No. queen larvae/ pupae	Canopy openess (%)
Macaranga bancana																	
10	51	8	9	1.1	2.1	3.2	Cb	7.5	3.6	0.30	2	30–40	1	12			4.9
6	65	10	10	2.8	4.2	7.0	Cb	17.4	11.4	1.90	3	40–50	1	13			8.4
8†	105	11	14	5.2	7.6	12.8	Cb	26.4	10.0	0.90	4	60–70	0	–			8.4
2†	101	14	14	8.4	8.7	17.1	Cb	36.7	11.2	2.40	4	80–90	0	–			7.1
9	160	8	16	22.1	4.9	27.0	Cb	50.4	20.1	3.10	68	40–150	1	13			5.4
7	147	15	13	18.2	17.0	35.2	Cb	55.9	16.1	2.50	13	70–140	1	28			11.0
1	125	16	23	18.7	19.9	38.6	Cb	99.1	24.8	3.20	52	40–100	1	38			7.4
4	181	12	18	34.5	13.0	47.5	Cb	134.8	22.5	4.90	319	60–170	1	39			5.5
5	251	17	16	67.7	24.5	92.2	Cb	64.3	32.0	3.90	104	100–210	1	127			11.6
3	287	30	45	216.7	25.5	242.2	Cb	336.0	79.4	8.50	538	180–280	1	89	0/12	2/10	8.2
Macaranga winkleri																	
7	39	6	8	1.1	1.5	2.6	Csp	16.7	5.5	0.30	4	20–30	1	27			5.4
9	33	9	8	1.2	2.1	3.3	Csp	19.9	12.1	1.50	2	20–30	1	25			7.6
3†	57	9	10	4.2	2.6	6.8	Csp	9.6	0.0	1.80	11	40–50	0	–			6.6
8	88	8	10	4.7	5.3	10.0	Csp	29.6	15.6	1.90	26	10–80	1	35			11.0
4	97	9	12	4.7	6.1	10.8	Csp	43.8	21.7	2.30	65	10–90	1	13			11.0
1	100	15	17	6.1	8.6	14.7	Csp	80.0	23.0	4.05	104	10–90	1	33			3.2
5	114	12	15	10.3	9.5	19.8	Csp	35.3	36.3	6.00	90	40–100	1	49			7.4
10	212	15	28	63.6	35.6	99.2	Csp	228.3	105.0	10.30	238	10–200	1	63			13.9
6‡	189	13	14	60.0	56.5	116.5	Cb	49.0	21.5	4.20	15	100–180	1	45			4.7
2	206	17	22	80.1	43.5	123.6	Csp	150.9	143.0	21.40	3	170–180	1	134			8.9

[1]Each row represents an individual plant (ordered from lighter to heavier individuals).

[2]Some plants lacked the data of stem dryweight (dw) (*), harbored no queen ants (†), or were not occupied by the specific ant species (‡) so that they are excluded in some (*) or all († and ‡) of the analyses.

[3]Cd, Cb and Csp indicate *Crematogaster decamera*, *C. borneensis* and *C.* sp., respectively.

[4]In *M. trachyphylla* No. 7, other than the reproducing queen, one solitary alated queen was found in a separated hollow stem at the bottom of the tree trunk.

Crematogaster decamera in *M. beccariana, C. borneensis* in *M. bancana* and *C.* sp. 2 in *M. winkleri*).

The coccids generally occurred at the upper part of hollow stems irrespective of the plant species (Table 1). On the other hand, the mean vertical position of queen ants was different among plant species ($F_{[3,32]} = 3.757, P = 0.020$). In *M. beccariana* and *M. trachyphylla*, queens always stayed at the bottom part of domatia, while queens living in *M. bancana* and *M. winkleri* moved upward as the plants grew (Table 1).

DISCUSSION

Food limitation of ant colony size

The ant biomass per unit food biomass was constant irrespective of the plant species and developmental stage (Fig. 2c), suggesting that the ant colony size is limited by food supply. Limitation of the two resources, space or food, could potentially explain the variation in ant biomass. No other limiting factors for ant colony size were plausible in this *Macaranga* system because the ant colony, once established, seldom suffers limitation by climatic disturbance, predation, disease, or intra- and interspecific interference. As for space limitation, although domatia space was not measured directly in this study, the large-sized hollow stem of myrmecophytic *Macaranga* generally harbored relatively small ant populations inside (usually a few percent of the volume in relation to the domatia volume), suggesting an excess of nesting space. Furthermore, in trees taller than 2 m, the resident ant workers and immatures tended to stay in the stem domatia of the upper parts, leaving the lower domatia unused, again suggesting space excess. The food-limited ants in *Macaranga* trees present a striking contrast to the case of South American *Tachigali* whose inhabitant ant colony size was space-limited (Fonseca 1993).

Given this food-limitation of ant colony size, the food-body-dependent *Macaranga* species (e.g. *M. winkleri*) can potentially control the amount of food supplied and therefore adjust the number of ants. But what ecological pressures prevent the ants from escaping the food constraint and foraging outside the plant? Not only the *Macaranga*-associated ants, but most myrmecophytic ants forage only on their host plants. Ecological factors such as predation risk or interspecific competition may have caused the ants to specialize exclusively on the food offered by the myrmecophytes. Hashimoto *et al*. (1997) reported that *Macaranga*-associated ants rarely collected or ate dead mosquitoes or cheese particles artificially deposited on *Macaranga* leaves.

Homopterans or food bodies?

The primary food offered by plants to their partner ants differs among myrmecophytes: (i) homopterans only (homopterans themselves and their honeydew, often with extrafloral nectars); (ii) homopterans and specialized food bodies secreted by plants; or (iii) food bodies only. *Tachigali* belongs to the first group (Fonseca 1993) while most myrmecophytic *Macaranga* species belong to the second group. In the first group, the ants are able to take the initiative in maximizing their fitness because they can manipulate the homopteran population by eating or killing them, to the level that is optimal for the ants, but often not optimal for the plants. Actually, Gaume *et al*. (1998) reported that the variation in homopteran biomass in myrmecophytic *Leonardoxa* was best explained by the number of workers in the ant colony and not by food- or space resources for homopterans, suggesting that the homopteran population was regulated by the ants. However, such regulation of homopterans by ants may sometimes fail because the homopterans can potentially overcome regulation by the ants (e.g. by multiplying very fast or escaping spatially in the plant hollow stem) and increase to the ceiling level of carrying capacity. Fonseca (1993) reported that the homopteran population size in *Tachigali* was explained by plant leaf area and not by ant colony size, suggesting that the homopterans were free from ant regulation and reached the upper limit determined by food supply by the plants. Accordingly, ants in *Tachigali* have more coccids than they can consume, their population being limited by nesting space.

Ants in *M. beccariana* and *M. bancana* relied on homopterans rather than food bodies (Fig. 2a,b), and appeared to regulate the homopteran population because the homopteran biomass was well

explained by ant biomass (Fig. 2b) rather than by plant biomass (Fig. 3b), and because the homopteran population seems too small to be explained by space- or food limitation. In contrast, ants in *M. trachyphylla* and *M. winkleri* relied primarily on food bodies rather than homopterans (Fig. 2a,b). The plants, in this case, appeared to regulate the ant population by adjusting the food-body production rate (Figs 2,3a).

Despite these differences in food-offering systems, per capita plant investment towards ants (the ant biomass per unit plant biomass) was, interestingly, not so different among the four *Macaranga* species (Fig. 4). This indicates that the optimal ant colony size from the ant side (*M. beccariana* and *M. bancana*, in which ant population is primarily regulated by the ants) and that from the plant side (*M. trachyphylla* and *M. winkleri*, in which it is regulated by the plants) are basically similar. However, there was some variation (Fig. 1): in homoptera-dependent *M. beccariana* and *M. bancana*, the ant biomass per unit plant biomass was significantly ($P < 0.05$) less than that in *M. winkleri* (the standardized least squares means ($\pm$ SE) of ant dry weight (mg) were, in descending order, 165.8 ± 22.3 in *M. winkleri*, 134.6 ± 20.6 in *M. trachyphylla*, 92.6 ± 18.6 in *M. bancana*, and 77.0 ± 16.9 in *M. beccariana*). This might be due, in part, to the energy loss involved in the homoptera-interposing system that has one additional trophic level. Without homopterans, the energy and nutritional flow from plants to ants is more direct, thereby more resources are available for the ants per unit plant biomass. Interestingly, the homoptera-dependent *M. beccariana* is more chemically, rather than biotically, defended in comparison with *M. trachyphylla* and *M. winkleri* (Itioka *et al.* 2000; Nomura *et al*. 2000), possibly due to the higher cost for the plants in maintaining the ant colony.

These interspecific differences in the food-offering system in *Macaranga* species provide us with a good opportunity to consider the evolution of reward-offering systems in ant–plant symbioses. In earlier stages of the coevolution, homopterans are thought to be the primary diet for the ants (Benson 1985; Ward 1991). At this stage, plants probably regulated ant populations via the size of domatia space, as was demonstrated in *Tachigali* (Fonseca 1993), while the homopteran population was probably not well regulated by the ants. In the next stage, food bodies began to play a more important role in the evolution of the mutualism since FBs were a more stable, efficient and adjustable diet for the ants.

Given these scenarios, why does homoptera-dependent *M. beccariana* not produce more food bodies and abandon the less efficient and less adjustable homopterans? Similarly, why have many ant–plants in general not been free of homopterans, as ant–plant *Acacia* have done (Janzen 1966)? If the homopterans are just extra costs to and ultimately useless in the plant–ant symbioses, then selection would favor their abortion from the system. In fact, one species, *Macaranga puncticulata* and its partner ants, appear to have aborted the homopteran symbionts at some point in their evolutionary history and have never accepted them again (Federle *et al*. 1998a, 1998b). One explanation for the above question is that many ant–plant systems are still in the early stages of their evolutionary trajectories and so have yet to become independent of homoptera.

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