# *Macaranga* ant-plants hide food from intruders: correlation of food presentation and presence of wax barriers analysed using phylogenetically independent contrasts

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Many tropical ant-plants provide specialized ant partners with food, which may attract foreign ants parasitizing the mutualism. We present evidence for the ant-plant genus *Macaranga*, showing that ant competition has forced host plants to hide food resources and restrict access to the mutualists. In *Macaranga* myrmecophytes, the influence of ant competition strongly depends on the presence of slippery 'wax barriers'. Of all *Macaranga* ant-plant species, 50% have waxy stems that can be climbed only by the specific ant partners and not by other ant species. We compared the presentation of food (food bodies and extrafloral nectar) between waxy and non-waxy *Macaranga* host plants using traditional and phylogenetic comparative methods. Consistent with the hypothesized effect of ant competition, wax-free *Macaranga* host species had fewer extrafloral nectaries and more often produced food bodies under recurved or tubular stipules inaccessible to other ants; closed stipules were less persistent in waxy hosts. Several traits showed phylogenetic signal, but our finding of a more promiscuous food presentation in waxy *Macaranga* hosts was still supported by phylogenetic comparative analyses. We conclude that competition among ants is an important factor in the evolution of myrmecophytism, and that it has given rise to traits acting as protective filter mechanisms.   © 2005 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2005, **84**, 177–193.

ADDITIONAL KEYWORDS: adaptation – ant competition – biotic defence – coevolution – comparative method – epicuticular wax – extrafloral nectaries – mutualism – myrmecophyte.

## INTRODUCTION

Coevolution may have played a key role in the emergence of biological diversity. Even though interactions influencing the fitness of organisms are widespread, demonstration of coevolution in its strictest sense, as 'reciprocal evolutionary change in interacting species' (Thompson, 1994), has proven difficult (Janzen, 1980). Ant-plant mutualisms have often been considered as examples of diffuse or specific coevolution (Janzen, 1966; Jolivet, 1996; Brouat *et al*., 2001; Itino *et al*., 2001). However, as Janzen (1980) pointed out, a perfect match of congruent traits between two mutualists does not necessarily imply that these traits have evolved as adaptations in response to the other organism. Traits that are considered adaptations to the mutualism could represent preadaptations evolved in the context of other functions and reflect phylogenetic history rather than current functional relationships. To test whether a trait can indeed be considered 'adaptive', a comparative approach is necessary that integrates functional and phylogenetic information (Losos, 2000; Irschick & Garland, 2001; Autumn, Ryan & Wake, 2002).

The phylogenetic independent contrast method devised by Felsenstein (1985) incorporates phylogenetic data in across-species comparisons of continuous traits. Independent contrasts continue to be the most widely used phylogenetic comparative method for continuous data and compare well to a variety of more

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recently proposed mathematical procedures (Martins, Diniz-Filho & Housworth, 2002). Other methods, such as Maddison's (1990) 'concentrated changes' test, have been developed for discrete characters.

It has been argued that rapidly evolving traits may not be correlated with their phylogenetic history and that application of a phylogenetically based comparative method to such traits can create problems of statistical non-independence because of the potential lack of phylogenetic autocorrelation (Gittleman et al., 1996; Rosenzweig, 1996). Measuring the amount of phylogenetic signal in comparative data may be a crucial prerequisite for correctly interpreting and understanding evolutionary patterns (Gittleman et al., 1996; Abouheif, 1999; Blomberg & Garland, 2002). Abouheif (1999) suggested that the assumption of phylogenetic independence be tested empirically before applying a phylogenetically based comparative method. He presented a statistical diagnostic (called the test for serial independence (TFSI) for continuous data and RUNS test for discrete data) to verify whether one's dataset needs phylogenetic correction in the first place, and whether one has achieved phylogenetic independence after the application of a phylogenetically based comparative method. This method has the advantage that it does not assume that the branch lengths and the model of evolutionary change are known.

We study adaptation and coevolution in ant–plant associations in the genus *Macaranga* (Euphorbiaceae) in South-east Asia. This complex mutualism comprises 29 host plant species (recent revision by Davies, 2001), at least 12 morphospecies of specialized ant (nine *Crematogaster* and three *Camponotus* species; Fiala et al., 1999; Feldhaar et al., 2003a) and endophytic coccids as a third partner of the association (Heckroth et al., 1998). Based on the extensive knowledge available on its ecology (review in Fiala et al., 1999; more recent work: Heil et al., 1999; Feldhaar et al., 2000; Markstädter et al., 2000; Federle et al., 2001; Federle, Maschwitz & Hölldobler, 2002; Moog et al., 2002; Murase et al., 2002; Feldhaar et al., 2003b) and phylogeny (Blattner et al., 2001; Davies et al., 2001; Davies, 2001; Feldhaar et al., 2003a), the *Macaranga* ant-plant system has emerged as an excellent model for the study of mutualism. The obligate *Crematogaster/Camponotus* ant partners inhabit the hollow stems of their *Macaranga* host plants. Their diet consists primarily of food bodies produced on the surface of the host plant, which are rich in lipids and proteins. In exchange, the ants protect their hosts against herbivory and climber overgrowth (Fiala et al., 1989; Heil et al., 2001a).

Fourteen *Macaranga* host plant species have stems covered with a waxy bloom of epicuticular wax crystals. These surfaces function as slippery mechanical barriers that make the trees virtually inaccessible to most non-flying insects. Only the ant partners of waxy *Macaranga* hosts are skilful 'wax-runners' and thus escape predation and competition by generalist ants (Federle et al., 1997). Slippery, waxy stems without hairs (which neutralize the barrier effect) occur exclusively among the myrmecophytic *Macaranga* species, which strongly indicates that the host plants benefit from protecting their specific ant associates (Federle et al., 1997). However, more than 50% of all *Macaranga* ant-plant species are not waxy and lack this protective mechanism. These *Macaranga* host plants are associated with ant partners unable to climb waxy stems (Federle et al., 1997; Feldhaar et al., 2003a). How do these associations survive in the absence of beneficial wax barriers?

We discovered that several other protective mechanisms may compensate for the lack of wax barriers. For example, *Crematogaster (Decacrema)* ant partners of waxy hosts prune neighbouring vegetation less intensely than do their congeners inhabiting wax-free *Macaranga* hosts (Federle et al., 2002). Stronger pruning behaviour does not only prevent host plants from being overgrown but also provides an earlier and more effective protection against invasions of foreign ants (Davidson, Longino & Snelling, 1988; Federle et al., 2002). Moreover, host plant traits other than waxy stems can act as protective mechanisms, such as the thin-walled prostomata that occur more often among non-glaucous *Macaranga* (Federle et al., 2001). In this study, we tested another hypothetical protective mechanism, the limited access to food provided by the host plant. Preliminary observations indicated that in most non-glaucous *Macaranga* host plants, food bodies are presented in a secluded space under the recurved stipules, whereas many waxy hosts offer them openly on the plant surface. Similarly, extrafloral nectaries (EFN) along the leaf margins appear to be largely absent in wax-free *Macaranga* hosts but not in many glaucous members of the genus. We used a comparative approach that accounts for phylogenetic history to test whether food body and nectar presentation differ between waxy and non-waxy host plants.

## MATERIAL AND METHODS

### STUDY PLANTS

To assess the morphology of stipules and leaf margin glands, we studied 25 *Macaranga* ant-plant species in the field at different sites in West Malaysia and Borneo (Sarawak and Sabah). Investigation of fresh material was necessary, because in many species, stipules and stalked leaf margin glands fall off easily and may no longer be present in herbarium speci-

mens. *Macaranga* taxonomy follows Whitmore (1975), Davies (1999, 2001) and Davies *et al*. (2001).

All the trees investigated were between 2.0 and 4.0 m tall ($N = 5$–20 trees per species). We recorded the presence of wax blooms covering the stem, the morphology of the stipules, the number of stipules present per (main) shoot and the number and relative frequency of leaf margin EFN (see below). For each host plant species, mean values were used for further analysis.

## INFORMATION ON FOOD PRESENTATION IN *MACARANGA* ANT-PLANTS

Most *Macaranga*-colonizing plant-ants never forage off their host plants and feed almost exclusively on host plant resources. Their major food source are food bodies rich in lipid and protein (Fiala & Maschwitz, 1990; Heil *et al*., 1998; Heil *et al*., 2001b), whose production requires up to 5% of the plant's total energy budget (Heil *et al*., 1997). Food bodies generally grow on the primary surface of all above-ground *Macaranga* plant parts but are most abundant near the shoot tips (Fiala & Maschwitz, 1991; Fiala & Maschwitz, 1992; Heil *et al*., 1998). In many *Macaranga* ant-plants, food body production is strongly concentrated on the stipules. For example, more than 99% of the food bodies in *M. bancana* are produced on the stipules (Heil *et al*., 1998). In addition to food bodies, the ants may obtain carbohydrates either indirectly through endophytic coccids inside the hollow stems (Heckroth *et al*., 1998) or directly from EFN on the leaves.

EFN in *Macaranga* occur in two different forms: (1) flat, disk-shaped nectaries on the leaf blade, and (2) glands on the leaf margins (Fiala & Maschwitz, 1991). Leaf blade nectaries are common among non-myrmecophytic *Macaranga* species. In *Macaranga* ant-plants, they occur only rarely, on leaves of young saplings that are not yet inhabited by the specific ant associates (Davies *et al*., 2001; Davies, 2001). In contrast, glands on the leaf margins are conspicuous and abundant in most *Macaranga* myrmecophytes (Fiala & Maschwitz, 1991; Davies *et al*., 2001). However, Fiala & Maschwitz (1991) found no or very low sugar contents in the leaf margin gland secretions of four myrmecophytic *Macaranga* species. They concluded that nectar production has generally been reduced in *Macaranga* ant-plants to save assimilates and to minimize competition with foreign ant species.

## ASSESSMENT OF THE NATURE OF LEAF MARGIN GLANDS

Spot checks of leaf margin gland nectar secretions were performed in the greenhouse for selected *Macaranga* species (*M. bancana, M. constricta, M. hosei,*

*M. hullettii, M. hypoleuca, M. lamellata*, *M. pruinosa*, $N = 3$ plants per species; with four measurements per plant). In addition to the greenhouse experiments, nectar secretion was investigated in the field. Due to the exposed location of the glands, it was not possible to keep away nectar-collecting, flying insects by bagging the leaves. As the results may thus underestimate the amount of nectar produced, field measurements were only included if no greenhouse data were available (in *M. beccariana, M. havilandii, M. trachyphylla* and *M. umbrosa*).

To measure nectar from plants inhabited by ants, we made individual branches ant-free by injecting a 1% solution of pyrethroid insecticide (Spruzit, Neudorff, Emmerthal, Germany) into the domatia, removed all contacts with other branches or plants, and applied rings of sticky resin (Tangletrap, Tanglefoot Corp., USA) around the stem. Leaves were washed carefully with pure water 24 h before leaf margin secretions were collected. Nectar droplets were collected with 5-µL micropipettes, allowing quantification of volume. For each leaf we pooled droplets from all the margin glands present. Nectar concentration was measured with a portable, temperature-compensated hand refractometer (ATAGO, L. Kübler, Karlsruhe, Germany). To remove any nectar remaining on the glands, we carefully sprayed the leaves from underneath with pure water using an atomizer and again collected the droplets from the glands. The total production of solid sugary compounds (given as µg sucrose equivalents) was determined by summing all collections for each leaf.

Leaf margin glands were classified as 'Type A' or 'Type B' if a central depression was absent or present, respectively. To evaluate the function of the two co-occurring, distinct gland types, we compared the sugar contents of the secretion of Type A and Type B glands in the host plant *M. pruinosa*: separate samples were taken from the triangular (Type A) and the cup-shaped (Type B) glands of the same leaf. Additionally, we compared the frequency of ant visits at the two types of glands in *M. pruinosa*.

To measure the distribution of the two types of leaf margin gland among *Macaranga* ant-plant species, their frequency was counted in the youngest, fully unfolded leaf of the main shoot using a pocket microscope (one leaf per tree; $N = 5$–20 trees per species).

## STATISTICAL AND PHYLOGENETIC COMPARATIVE ANALYSIS

In addition to testing hypotheses using the traditional comparative method, which treats each species as an independent observation, we conducted statistical tests based on phylogenetic information for the genus *Macaranga* published recently by Davies *et al*.

(2001) and Blattner *et al*. (2001). As the two published phylogenetic hypotheses are similar but not identical, all tests were performed separately for both phylogenies. Both phylogenetic trees are based on molecular characters of internal transcribed spacer (ITS) regions of nuclear ribosomal DNA. As the ITS analysis alone yielded unresolved polytomies within the section *Pachystemon,* Blattner *et al*. (2001) included information from random amplified polymorphic DNA (RAPD) and microsatellite-primed PCR (MP-PCR) techniques, whereas Davies *et al*. (2001) conducted a combined analysis of molecular (ITS) and morphological characters. We modified the working phylogeny to include three species (*M. aetheadenia*, *M. lamellata* and *M. pruinosa*) that comprise distinct forms with and without wax barriers (a densely glaucous form of *M. aetheadenia* occurs in Sarawak, a non-waxy form in Brunei; *M. lamellata* was found to be waxy in Sarawak and Brunei, but not in Sabah (Tawau); *M. pruinosa* is waxy and ant-inhabited in West Malaysia and Sumatra, but non-myrmecophytic and non-waxy in Borneo). As branch lengths were only given for the non-polytomous nodes of both trees, we used constant branch lengths in our working phylogenies. The morphological data to be tested consisted of both continuous (number and relative frequency of EFN, number of persistent stipules) and discrete characters (presence/absence of waxy stems, open/closed stipule morphology). The TFSI and the RUNS tests (both with 5000 replicates) were carried out with the computer program 'phylogenetic independence' (Reeve & Abouheif, 2003; freely available on the Internet). Standardized independent contrasts (Felsenstein, 1985) of continuous characters were computed with the program PDTREE (Garland *et al*., 1993; freely distributed by T. Garland). Standardized independent contrasts were positivized and the adequacy of the branch lengths for statistical purposes was verified as suggested by Garland, Harvey & Ives (1992). Log transformations were conducted whenever required for proper standardization (Garland *et al*., 1992). The discrete characters (presence/absence of wax barriers and open/closed stipule morphology) were investigated using Maddison's (1990) concentrated changes test, implemented in the program MacClade 4.0 (Sinauer Associates, Inc., Sunderland, Massachusetts). This test investigates whether gains or losses of one trait are significantly concentrated on those branches of a phylogenetic tree on which a second trait has a specified state. Ancestral character states were reconstructed by parsimony using MacClade 4.0. In cases where more than one most parsimonious reconstruction was possible, we applied the concentrated changes test to reconstructions based on both the ACCTRAN (favours losses over independent gains of character states) and DELTRAN (favours independent gains over losses) algorithms.

# RESULTS

## STIPULE MORPHOLOGY

The stipules in many *Macaranga* ant-plant species are conspicuously recurved and tightly abutting the stem so that they enclose a hemispherical cavity (Fig. 1A, B). The food bodies are produced on the inner (abaxial) side of these stipules so that they are well secluded. The hemispherical cavities are usually accessible only through a small opening just large enough for the passage of a single *Crematogaster* (*Decacrema*) ant worker (diameter *c*. 0.4–1.3 mm; Fig. 1B). This opening can be either formed by the pointed, asymmetrically curved stipule tip or located at the base of the stipule. We observed that the inhabitant *Crematogaster* (*Decacrema*) ants often built carton-like structures between the stem surface and the stipule margin to 'seal' the cavities beneath the stipule and to keep the access openings as small as possible (Fig. 1B,C). Obviously, the recurved stipule morphology makes food bodies inaccessible to larger ant species and more easily defensible against smaller generalist ants that are potential intruders.

The production sites of food bodies can be protected not only by recurved but also by horn-shaped, tubular stipules (in *M. kingii, M. umbrosa* and *M. lamellata*, see Fig. 1C), which represent an evolutionarily independent development. In this group of species, the stipules are folded longitudinally creating an enclosed tubular space where most of the food bodies are produced. The entrance openings at the base and the tip of the stipule are kept small by a median keel on the stipule underside and by ant cartons (Fig. 1C).

In many *Macaranga* ant-plant species, however, stipule morphology is relatively open and no protected cavities are formed. These stipules are either straight (horizontal or upright, Fig. 1D) or saccate (Fig. 1E; Davies, 2001).

## NATURE OF LEAF MARGIN GLANDS

There are two structurally different types of *Macaranga* leaf margin gland: (1) flattened, triangular, blunt-tipped glands pointing outward in the leaf plane, directly innervated by large leaf veins ('Type A', Fig. 1F, H) and (2) cup-shaped glands with a central depression, mostly orientated toward the leaf underside ('Type B', Fig. 1G). Type B glands often are not directly innervated by large leaf veins; they have short stalks in some species. Type B glands vary considerably in size ('cup' diameter ranging from 0.25 mm
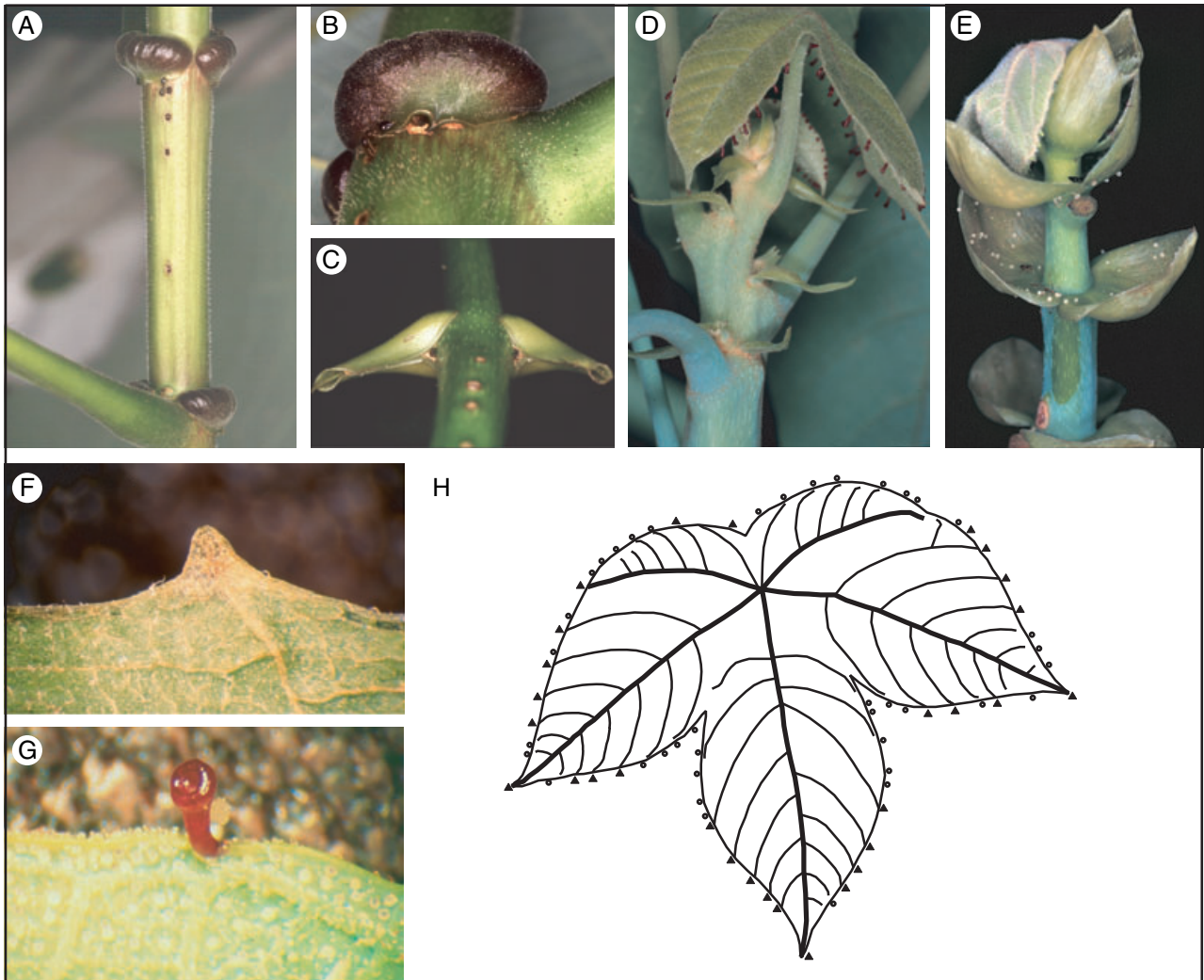
**Figure 1.** Stipule and leaf margin nectary morphology in *Macaranga* ant-plants. A, B, recurved stipules (*M. trachyphylla*). Note the carton-like material sealing the cavity and the small ant entrance opening at the stipule tip. C, tubular stipules (*M. umbrosa*). Note the opening at the stipule base made smaller by ant carton. D, E, open stipules [D, straight, horizontal in *M. hypoleuca*; E, Saccate in *M. pruinosa*]. F, triangle-shaped 'Type A' leaf margin gland in *M. hullettii*. G, cup-shaped 'Type B' gland in *M. hypoleuca*. H, schematic of the location of Type A (solid triangles) and Type B glands (open circles) on a *M. pruinosa* leaf.

in *M. bancana* to 1.3 mm in *M. aetheadenia*). Both types of leaf margin gland typically co-occur side-by-side on leaves of most *Macaranga* ant-plant species (Fig. 1H), although in some species, all glands belong to the same type (see Fig. 4).

Type A and Type B glands do not only differ morphologically; they also have different functions. We compared the nectar secretion of Types A and B glands on leaves of *M. pruinosa* (Fig. 2A). Even though liquid droplets were often visible on both gland types in the field, only Type B gland secretion contained significant amounts of sugar. Type B glands secreted 65 times more sucrose equivalents (per gland) than did Type A glands from the same leaves,

the difference being highly significant (paired *t*-test: $N = 10$, $P < 0.001$).

The functional difference between both types of leaf margin gland was also evident from the frequency of ant visits (Fig. 2B). When ants collect nectar, they usually stand for several seconds, their mouthparts in contact with the nectary (Josens, Farina & Roces, 1998). We observed foragers of a generalist *Cremato-gaster* species that had access to an ant-free *M. pruinosa* twig with seven leaves containing 112 Type B and 175 Type A glands. During a 1-h observation period, we counted 19 ant visits (of >2 s) at the Type B glands but only one visit at the Type A one (Fig. 2B). The ants' preference of Type B glands was
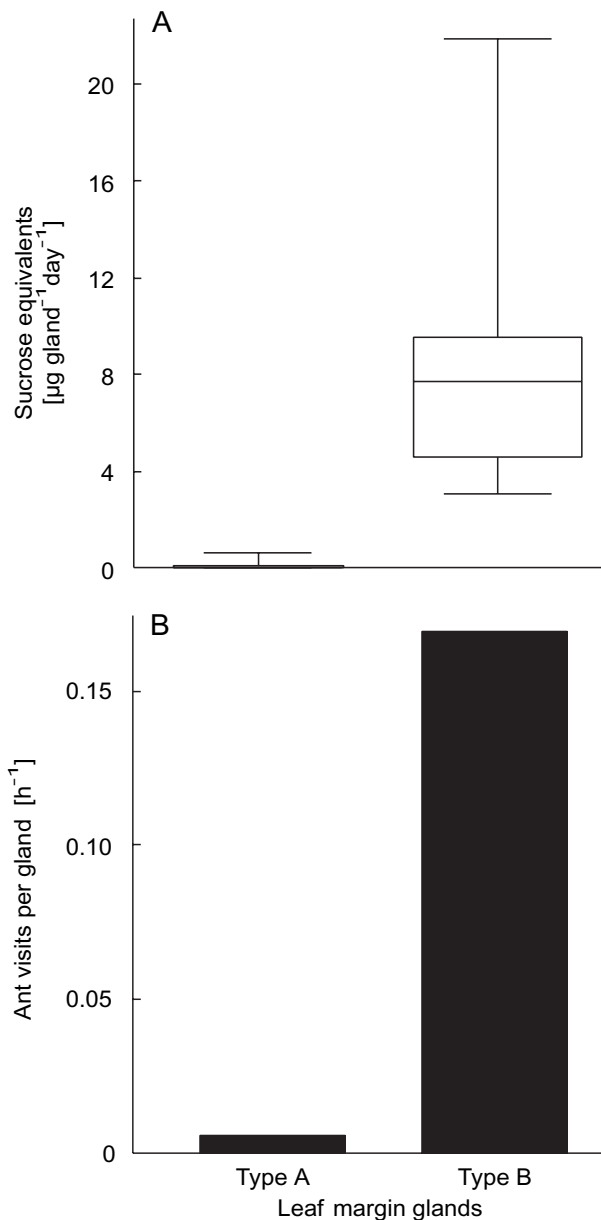
**Figure 2.** A, nectar secretion of Type A and B leaf margin glands in *Macaranga pruinosa*. Data from ten leaves (belonging to different trees, 2.0 m in height). For each leaf, the daily production was pooled for glands of one type and divided by the number of glands present on the leaf (mean: 25.6 Type A and 22.9 Type B glands per leaf). B, frequency of ant visits at Type A and B glands of *M. pruinosa* leaves.

highly significant (Yates corrected chi-square test: $\chi^2 = 21.71$, d.f. = 1, $P < 0.001$). Thus, only the cup-shaped Type B glands represent functional EFN, whereas the triangular Type A glands may be considered hydathodes.

The number of EFN per leaf and their proportion of all leaf margin glands strongly varied between species

(see table in Fig. 4). The results obtained for *M. pruinosa* suggested that plants with a higher proportion of Type B glands secrete more extrafloral nectar. To test whether this conclusion is justified, we measured nectar secretion in selected *Macaranga* ant-plant species. The results were in good agreement with the frequency of Types A and B glands for different species (Fig. 4). Considerable amounts of nectar were produced in many species with Type B glands, but only traces of sugar were found in the secretions of species with predominantly Type A glands (e.g. *M. hullettii*, *M. umbrosa*, Fig. 4). Some species produced only small amounts of nectar despite the presence of Type B glands (e.g. *M. bancana*). Weak nectar secretion appears to be related to the particularly small size of Type B glands in this species.

## CORRELATION BETWEEN THE PRESENCE OF WAX BARRIERS AND FOOD PRESENTATION

To test for a possible functional relationship between the presence of slippery wax barriers and the presentation of food in *Macaranga* ant-plants, we performed statistical tests (a) by using the traditional approach, treating each species as an independent sample, and (b) by applying phylogenetically based comparative methods. Additionally, we examined whether the traits investigated exhibited phylogenetic signal in the first place, and whether the application of phylogenetically based comparative methods had removed this signal from the dataset. Five parameters with possible functional significance were investigated: the number of EFN per leaf, the proportion of EFN among all leaf margin glands, the secretion of nectar (sucrose equivalents per leaf and day), the open/closed morphology of the stipules and the number of stipules persistent at the main shoot. Figure 3 shows the distribution of these traits in *Macaranga* host plants with and without slippery wax barriers. The observed effects will be presented separately for stipules and for EFN.

### Distribution of extrafloral nectaries (EFN) in *Macaranga* ant-plants

Figure 3A indicates that host plants with wax barriers had a greater number of EFN per leaf than plants without wax barriers. When treating species as independent observations, both the number and percentage of EFN were significantly higher in the waxy species (Table 1). The same result (with increased significance for the percentage of EFN) was found when only the host plants inhabited by *Crematogaster* (*Decacrema*) ants were considered (*M. caladiifolia*, *M. puncticulata* and *M. winkleri* excluded, Table 1). In agreement with the greater number of EFN present, *Macaranga* host plants with wax barriers secreted sig-
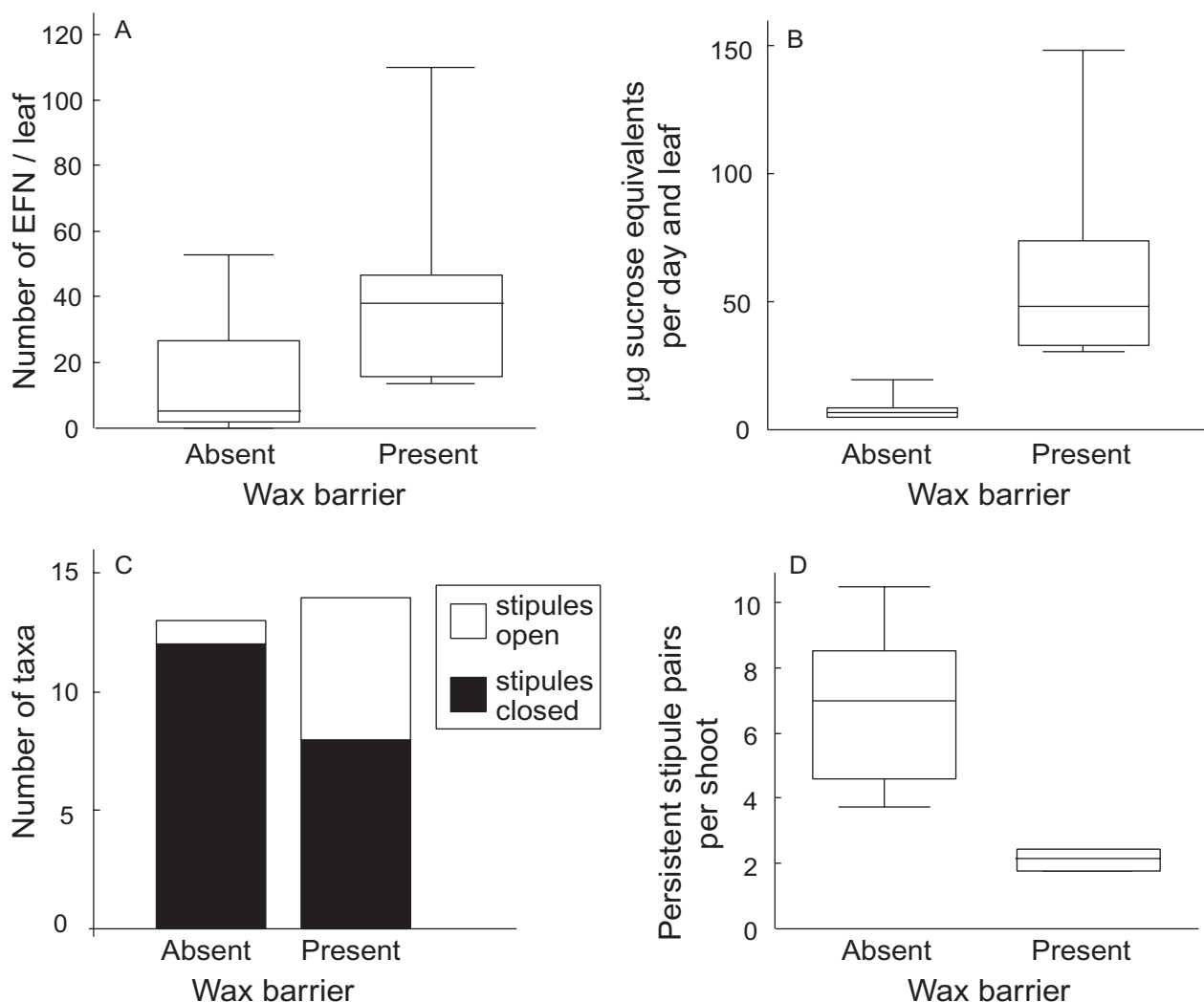
**Figure 3.** Comparison between *Macaranga* host plants of *Crematogaster* (*Decacrema*) ants with and without slippery wax barriers, regarding: A, number of extrafloral nectaries (EFN) per leaf; B, nectar secretion (sucrose equivalents per day and leaf); C, frequency of open and closed stipule morphology; and D, number of persistent stipules per shoot tip (only species with recurved stipules). Data in A, B and D represent mean values for each species (all data from table in Fig. 4).

nificantly more nectar than did non-waxy species (measured in 11 *Macaranga* species regularly associated with *Crematogaster* (*Decacrema*) ants, Fig. 3B and Table 1).

The presence of wax barriers and the traits related to food presentation were tested for phylogenetic independence (Table 2). The distribution of slippery wax barriers was found to be independent of phylogeny, based on the phylogeny of Blattner *et al*. (2001), but not based on that of Davies *et al*. (2001). In the EFN-related traits, we found phylogenetic autocorrelation only in the proportion, and not in the number, of EFN and nectar secretions (in both phylogenetic trees). The TFSI test showed that the application of Felsenstein's (1985) independent contrast method successfully

removed phylogenetic signal wherever it occurred with the exception of '%EFN' in the phylogeny of Blattner *et al*. (2001).

We tested the correlation of EFN characters with the presence of slippery waxy barriers using Felsenstein's (1985) phylogenetically independent contrasts. Table 3 shows that most of the observed effects were significant. Again, significance increased when only the host plants of *Crematogaster* (*Decacrema*) ants were included.

*Distribution of stipule morphology and stipule persistence in Macaranga ant-plants*
Figures 4 and 5 show the distribution of closed and open stipule morphology among *Macaranga* ant-

**Table 1.** Statistics of the correlation between slippery wax barriers and traits related to food presentation using the traditional comparative method (treating species as independent samples)

| Trait | d.f. | $t$ | $P$ |
|---|---|---|---|
| n(EFN) | 23 | 2.593 | 0.016* |
|  | (20) | (2.609) | (0.017*) |
| %(EFN) | 23 | 3.129 | 0.005** |
|  | (20) | (4.489) | (< 0.001***) |
| Nectar production | 9 | −2.624 | 0.028* |
| Stipule persistence | 8 | −3.714 | 0.006** |
| Closed stipule morphology | $N = 31$ | 0.478 |  |
|  | ($N = 27$) | (0.077) |  |

Values in brackets show results for *Macaranga* ant-plants inhabited by *Crematogaster* (*Decacrema*) ants (*M. caladiifolia*, *M. puncticulata*, *M. winkleri* and *M. winkleriella* excluded). *t*-test for independent samples for all traits except stipule morphology, where Fisher's exact test was performed (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$).
'nEFN': number of Type B leaf margin glands per leaf; '%EFN': percentage of Type B glands among leaf margin glands.

plants. It can be seen that almost all of the non-waxy host plants with open stipule morphology are not inhabited by *Crematogaster* (*Decacrema*) ants (with the exception of *M. puberula*). When these species (*M. caladiifolia*, *M. puncticulata, M. winkleri* and *M. winkleriella*) are excluded, a trend becomes visible, with open stipule morphology being more frequent among waxy *Macaranga* ant-plants (six vs. one species, Fig. 3C). However, this effect was only marginally significant (Fisher's exact test, $P = 0.077$, Table 1).

The results of the RUNS test (Table 2) indicate that the distribution of stipule morphology exhibits a highly significant phylogenetic autocorrelation. This implies that correction with a phylogenetically based comparative method is necessary. Phylogenetic correction in discrete data can be accomplished using the concentrated changes test (Maddison, 1990), which explores whether gains or losses of two or more traits are concentrated on any particular branch of a tree. The results of this test strongly depend on the ancestral states that the concentrated changes test reconstructs using parsimony. The present analysis is focused on only a small subgroup of the genus *Macaranga* (i.e. the ant-plants). Reconstruction of ancestral character states on such a 'reduced' tree carries the

**Table 2.** C-statistics for phylogenetic autocorrelation and their *P*-values based on the phylogenies of Davies *et al*. (2001) and Blattner *et al*. (2001)

| Continuous traits | $N$ | Blattner *et al*. (2001) | | | | Davies *et al*. (2001) | | | |
|---|---|---|---|---|---|---|---|---|---|
|  |  | Raw data | | Independent contrasts | | Raw data | | Independent contrasts | |
|  |  | C-stat | $P$ | C-stat | $P$ | C-stat | $P$ | C-stat | $P$ |
| n(EFN) | 25 | 0.072 | 0.279 | 0.188 | 0.123 | 0.138 | 0.159 | −0.050 | 0.395 |
| %(EFN) | 25 | 0.403 | 0.005** | 0.346 | 0.014* | 0.429 | 0.003** | 0.125 | 0.230 |
| Nectar secretion | 11 | 0.115 | 0.252 | −0.137 | 0.267 | 0.087 | 0.299 | 0.257 | 0.106 |
| Stipule persistence | 10 | 0.053 | 0.361 | 0.220 | 0.246 | 0.249 | 0.113 | −0.300 | 0.131 |

| Continuous traits | $N$ | Raw data | | Independent contrasts | | Raw data | | Independent contrasts | |
|---|---|---|---|---|---|---|---|---|---|
|  |  | C-stat | $P$ | C-stat | $P$ | C-stat | $P$ | C-stat | $P$ |
| Stipule morphology | 25 | 4.28 | < 0.001*** | – | – | 3.68 | < 0.001*** | – | – |
| Slippery wax barriers | 25 | 12.13 | 0.218 | −0.063 | 0.371 | 10.06 | 0.029* | −0.171 | 0.203 |

Discrete characters were computed with the RUNS test while continuous traits and independent contrasts were subjected to the test for serial independence (TFSI). Significant *P*-values indicate phylogenetic non-independence. All traits were analysed before ('raw data') and after applying Felsenstein's (1985) independent contrasts (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$).
'nEFN': number of Type B leaf margin glands per leaf; '%EFN': percentage of Type B glands among leaf margin glands.

**Table 3.** Phylogenetically based statistics for the correlation of continuous traits with the presence of slippery wax barriers, for the phylogenies of Davies *et al.* (2001) and Blattner *et al.* (2001)

| Trait*t* | d.f. | Davies *et al.* (2001) | | Blattner *et al.* (2001) | |
|---|---|---|---|---|---|
| | | *t* | *P* | *t* | *P* |
| n(EFN) | 23 | 1.805 | 0.084 | 2.876 | 0.009** |
| | (20) | (1.464) | (0.159) | (3.205) | (0.004)** |
| %(EFN) | 23 | 2.623 | 0.015* | 1.902 | 0.070 |
| | (20) | (2.825) | (0.010)** | (3.042) | (0.006)** |
| Nectar secretion† | 9 | 2.230 | 0.0473* | 2.205 | 0.0549 |
| Stipule persistence† | 8 | −1.684 | 0.131 | −3.431 | 0.009** |

Values in parentheses give results for the subset of *Macaranga* ant-plants that are inhabited by *Crematogaster* (*Decacrema*) ants (*M. caladiifolia, M. puncticulata* and *M. winkleri* excluded).
*$P < 0.05$, **$P < 0.01$.
†Tested species include host plants of *Crematogaster* (*Decacrema*) only.
'nEFN': number of Type B leaf margin glands per leaf; '%EFN': percentage of Type B glands among leaf margin glands.

**Table 4.** Phylogenetically based statistics for the correlation of discrete traits (myrmecophytism, open/closed stipule morphology, presence/absence of wax barriers)

| Distinguished/dependent trait | Ancestral state | *N* | Total number of gains/losses | Gains/losses in 'distinguished' branches | *P* |
|---|---|---|---|---|---|
| Davies *et al.* (2001) | | | | | |
| Myrmecophytism /wax barriers | No wax barrier | 55 | 8/1[DELT.] | 8/0 | <0.001*** |
| | | | 6/3[ACCT.] | 6/2 | 0.004** |
| Myrmecophytism /closed stipule morphology | Open stipules | 55 | 2/2 | 2/0 | <0.001*** |
| Closed stipule morphology /wax barriers | No wax barrier | 55 | 8/1[DELT.] | 5/0 | 0.999 |
| | | | 6/3[ACCT.] | 3/1 | 0.675 |
| Blattner *et al.* (2001) | | | | | |
| Myrmecophytism /wax barriers | No wax barrier | 51 | 10/0[DELT.] | 10/0 | <0.001*** |
| | | | 9/1[ACCT.] | 9/1 | <0.001*** |
| | | | 2/4[DELT.] | 2/1 | <0.001*** |
| Myrmecophytism /closed stipule morphology | Open stipules | 51 | | 2/0† | <0.001*** |
| | | | 4/2[ACCT.] | 4/1 | 0.028* |
| | | | | 4/0† | 0.003** |
| Closed stipule morphology /wax barriers | No wax barrier | 51 | 10/0[DELT.] | 6/0 | 0.987 |
| | | | 9/1[ACCT.] | 5/1 | 0.367 |

Probabilities calculated by the concentrated changes test (Maddison, 1990); gains and losses of traits inferred from DELTRAN ([DELT.]) and ACCTRAN ([ACCT.]) reconstruction algorithms.
*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$
†*M. caladiifolia* treated as 'non-myrmecophytic' (see text).

risk of underestimating the real number of gains and losses, because convergently acquired traits may be misinterpreted as being ancestral. We thus used the largest available phylogenetic trees (which also include non-myrmecophytes) to locate transitions of the characters 'wax barriers' and 'stipule morphology' (Fig. 5).

We first investigated whether stipule morphology and slippery wax barriers are correlated with myrmecophytism. The concentrated changes test (Table 4)

| | ant partner | n EFN | % EFN | nectar secretion | stipule persistence |
|---|---|---|---|---|---|
| M. angulata | Cr.(Dec.) | 0.0 | 0.0 | -- | 10.5 |
| M. hullettii | Cr.(Dec.) | 2.8 | 7.7 | 4.9 | 6.5 |
| M. aetheadenia_{Sarawak} | Cr.(Dec.) | 15.8 | 28.0 | -- | -- |
| M. aetheadenia_{Brunei} | Cr.(Dec.) | 9.4 | 12.9 | -- | 5.5 |
| M. indistincta | Cr.(Dec.) | 52.8 | 60.4 | -- | 7.0 |
| M. petanostyla | Cr.(Dec.) | 0.0 | 0.0 | -- | 3.7 |
| M. glandibracteolata | Cr.(Dec.) | 109.7 | 83.0 | -- | 2.2 |
| M. bancana | Cr.(Dec.) | 37.1 | 56.0 | 8.5 | 9.1 |
| M. calcicola | Cr.(Dec.) | 4.7 | 13.2 | -- | -- |
| M. trachyphylla | Cr.(Dec.) | 5.0 | 5.3 | 19.8 | 7.9 |
| M. havilandii | Cr.(Dec.) | 57.7 | 99.5 | 48.0 | 2.4 |
| M. motleyana | Cr.(Dec.) | 37.8 | 73.4 | -- | -- |
| M. griffithiana | Cr.(Dec.) | 19.3 | 82.4 | -- | 1.8 |
| M. constricta | Cr.(Dec.) | 29.0 | 100.0 | 148.2 | -- |
| M. lamellata_{Brunei} | Cr.(Dec.), Camp. | 38.0 | 74.7 | 44.1 | (tubular) |
| M. umbrosa | Cr.(Dec.) | 0.6 | 0.8 | 5.0 | (tubular) |
| M. caladiifolia | diverse | 3.5 | 20.0 | -- | (open) |
| M. puncticulata | Camp. | 28.3 | 100.0 | -- | (open) |
| M. hypoleuca | Cr.(Dec.) | 51.3 | 100.0 | 30.5 | (open) |
| M. beccariana | Cr.(Dec.) | 46.7 | 100.0 | 35.0 | (open) |
| M. hosei | Cr.(Dec.) | 13.4 | 37.2 | 81.1 | (open) |
| M. pruinosa | Cr.(Dec.) | 46.6 | 62.8 | 66.5 | (open) |
| M. puberula | Cr.(Dec.) | 24.7 | 52.0 | -- | (open) |
| M. pearsonii | Cr.(Dec.) | 13.4 | 25.7 | -- | (open) |
| M. winkleri | Cr.(Cre.) | 41.5 | 89.2 | -- | (open) |

**Figure 4.** Stipule morphology and presence of a slippery (i.e. not pubescent) waxy stem surface mapped on the phylogenetic tree of Blattner *et al*. (2001) compiled with a data table of traits related to food presentation. ('nEFN': number of Type B leaf margin glands per leaf; '%EFN': percentage of Type B glands among leaf margin glands; 'nectar secretion': μg sucrose equivalents per day and leaf; 'stipule persistence': number of stipules present at the main shoot; only species with closed, recurved stipule morphology included). Stipule morphology: black lines = open; grey lines = closed, tubular; thin black double line = closed, recurved. Wax barriers: ◆, present; ◇, absent. *Camp*, *Camponotus*.

shows that slippery wax barriers (i.e. waxy stems without hairs) originated significantly more often within the ant-plants of the genus, which confirms earlier conclusions based on the traditional method (Federle *et al*., 1997). The association of closed stipule morphology and myrmecophytism was also clearly supported by the concentrated changes test (Table 4). Closed stipules consistently disappeared at least twice together with a loss of myrmecophytism (*M. ashtonii, M. depressa, M. rostrata* and *M. triloba*). The only loss within a myrmecophytic clade occurred in *M. caladiifolia*, the only *Macaranga* ant-plant characterized by a non-specific colonization by generalist ants (Fiala, Maschwitz & Linsenmair, 1996).

Second, we tested the correlation of open/closed stipule morphology and the presence of wax barriers in *Macaranga* ant-plants. For both phylogenetic trees, the concentrated changes test did not reveal any significant effect (Table 4). The difference from the result of the traditional analysis (Table 1) is apparently due

to the fact that most non-waxy host plants of *Cremato-gaster* (*Decacrema*) with closed stipules belong to a single clade of the *Macaranga*-section *Pachystemon* (Fig. 5).

*Macaranga* species differ strongly with regard to the persistence of their stipules. Stipule persistence is probably a critical parameter when food body production is restricted to the stipules, but it may be less important when food bodies are also produced at other locations on the plant surface. When stipules are caducous, most food bodies are probably produced elsewhere on the plant surface, where they are openly accessible. We compared stipule persistence within the (probably monophyletic, Figs 4, 5) group of *Macaranga* host plants with recurved stipules. Stipules were less persistent in the waxy *Macaranga* host plants (Fig. 3D). This effect was found to be highly significant using the traditional comparative method (Table 1). We observed considerable food body production outside the stipules (on the stems, petioles, and leaf undersides) in several species with caducous stipules (e.g. *M. constricta, M. griffithiana, M. glandibracteolata*). Stipule persistence exhibited a weak (non-significant) correlation with phylogeny (Table 2). When testing the association of wax barriers and stipule persistence using phylogenetically independent contrasts, the effect was significant for the phylogeny of Blattner *et al.* (2001) (Table 3).

## DISCUSSION

Food rewards such as food bodies and extrafloral nectar offered by plants generally attract a variety of foraging ant species. In most non-symbiotic ant–plant interactions (i.e. plants only visited by generalist foragers), these ants have a beneficial effect, because they protect against herbivores (for reviews, see Buckley, 1982; Koptur, 1992). However, generalist ant foragers may have a reversed, negative effect in specific ant–plant mutualisms. These ants may deplete the ant partner's food resources and behave aggressively against or even prey on the competitively inferior plant-ants. Obligate plant-ants permanently inhabiting their host plants may be better mutualists than are generalist foragers because they defend more effectively against herbivores (for *Macaranga* see Fiala *et al.*, 1994; Nomura, Itioka & Itino, 2000; Heil *et al.*, 2001a). Moreover, many obligate plant-ants prune neighbouring vegetation and protect their host plants against overgrowth (Davidson & McKey, 1993). Myrmecophytes often have a reduced chemical defence arsenal (e.g. Rehr, Feeny & Janzen, 1973; Eck *et al.*, 2001) so that their survival depends strongly on the presence of their specific ant partners (e.g. *Acacia*, Janzen, 1966; *Barteria*, Janzen, 1972; *Leonardoxa*, McKey, 1984; *Maieta*, Vasconcelos, 1991; *Macaranga*,

Heil *et al.*, 2001a). As a consequence, host plants may benefit from protecting their specific ant partners against competition and predation by other ants.

Our findings provide evidence that competition among ants has played a central role in the evolution of ant–plant associations in the genus *Macaranga*. First, the phylogenetically based comparative analysis supports our earlier hypothesis that slippery wax barriers evolved in myrmecophytic *Macaranga* as an adaptation to protect specific ant partners (Federle *et al.*, 1997). Second, several traits of *Macaranga* ant-plants related to the presentation of food vary systematically with the presence or absence of slippery wax barriers. Host plants without wax barriers are largely free of ant food rewards on their outer surface. They mainly produce food bodies within cavities enclosed by recurved or tubular stipules and secrete less extrafloral nectar than do species with wax barriers. The correlation of these characters with the presence of wax barriers suggests that they act as alternative, protective mechanisms.

We investigated traits by using both tradtional and phylogenetically based comparative methods. Application of the traditional approach (treating species as independent data points) is only appropriate if both traits in consideration exhibit no phylogenetic autocorrelation. As our result for the wax barriers was equivocal (phylogenetically autocorrelated for the tree of Davies *et al.* (2001) but not for that of Blattner *et al.* (2001)), it is unclear whether using the traditional method is appropriate. Based on Blattner's phylogeny, testing the correlation between wax barriers and the number of EFN, nectar secretion and stipule persistence using the traditional method is justified.

The results of our statistical tests differed according to which phylogenetic tree was used for the analysis. Table 3 shows that EFN frequency ('%EFN') was significantly correlated with slippery wax barriers for both phylogenetic trees, whereas number of EFN ('nEFN'), stipule persistence and nectar secretion yielded significant correlations for only one of the trees. Even though the phylogenetic studies by Blattner *et al.* (2001) and Davies *et al.* (2001) arrived at largely similar tree topologies, they differ in their evolutionary reconstruction of the genus section *Pachystemon*, where rapid speciation has led to unresolved polytomies of the ITS analysis. It is difficult to decide whether one of the two phylogenetic hypotheses should be given priority. The phylogenetic tree by Davies *et al.* (2001) was constructed based on molecular (ITS) and morphological characters. Of the 81 morphological characters used by Davies *et al.* (2001), 12 are traits related to wax barriers, EFN and stipules. Reconstructing changes of the same traits from which a phylogeny is (partly) inferred may result in circularity (Felsenstein, 1985). On the other

**Figure 5.** Myrmecophytism, stipule morphology and presence of slippery wax barriers mapped on the phylogenetic tree of Davies *et al.* (2001). Ancestral character states reconstructed by parsimony and DELTRAN algorithm using MacClade 4.0. Gains/losses of closed stipule morphology and slippery wax barriers are indicated with bars as '+/–CS' and '+/–W', respectively. Myrmecophytism: black lines = ant-plant; grey lines = non-myrmecophytic. Stipule morphology: □, open; ▨, closed, tubular; ■, closed, recurved. Wax barriers: ◆, present; ◇, absent.

hand, the reliability of the phylogenetic tree of Blattner *et al.* (2001) may be challenged due to the use of RAPD and MP-PCR analyses. This approach relies on comigrating bands for the estimation of relatedness among taxa; it has been criticized because of the possible non-homology of the comigrating bands (Rieseberg, 1996).

The exclusion of *Macaranga* host plant species not inhabited by *Crematogaster* (*Decacrema*) ants resulted in a more significant correlation of wax barriers with traits related to food presentation. The biology of the excluded species, i.e. of *M. caladiifolia, M. winkleri, M. winkleriella* and *M. puncticulata*, differs in several respects from the other associations. First, *M. caladiifolia* is colonized by generalist stem-nesters (Fiala *et al.*, 1996), which may be lured by extrafloral nectar and might have problems harvesting food bodies from narrow stipule cavities. Second, the *Crematogaster* ant partners of *M. winkleri* and *M. winkleriella* appear much more aggressive than are *Crematogaster* (*Decacrema*) ants and may easily defend their food resources (Itino & Itioka, 2001). Third, the *Camponotus (Colobopsis)* ant partners of *M. puncticulata* also have superior defensive capabilities because of their specialized bursting behaviour (Federle, Maschwitz & Fiala, 1998b). Moreover, extrafloral nectar makes up an important part of their diet because of the absence of endophytic scale insects in this association (Federle *et al.*, 1998b; Federle, Fiala & Maschwitz, 1998a).

### EXTRAFLORAL NECTARIES (EFN)

EFN are common in many Euphorbiaceae (Elias, 1983). Leaf margin glands occur in many non-myrmecophytic *Macaranga* and their presence predates the origin of myrmecophytism (Davies *et al.*, 2001). We discovered that there are two distinct types of leaf margin gland with different morphology and function, namely triangular (Type A) hydathodes and cup-shaped (Type B) EFN, which had not been differentiated previously. The number and frequency of EFN strongly varied between *Macaranga* ant-plant species. We found that *Macaranga* host plants with slippery wax barriers had more EFN and also secreted more nectar compared with host plants without wax barriers.

Our findings modify the conclusions drawn by Fiala & Maschwitz (1991). Nectar production has not disappeared completely among myrmecophytic *Macaranga*. We found that some *Macaranga* ant-plants (e.g. *M. constricta*) produce nectar at the same rate as do the non-myrmecophytic *M. tanarius* (Heil *et al.* 2000). Only some species (e.g. *M. umbrosa, M. hullettii*) secrete very small amounts of nectar. The different results may be due to the fact that Fiala & Maschwitz (1991) investigated only a small number of species (*M. bancana, M. griffithiana, M. hullettii* and

*M. hypoleuca*), and collected droplets without spraying the leaves with water, which may result in an underestimation of nectar secretion.

In the majority of all ant-plant genera, the resident ants cultivate homoptera (Davidson & McKey, 1993) from which they obtain honeydew. In some ant-plant genera (including *Macaranga, Acacia*), EFN were present before the evolution of myrmecophytism. When EFN and homoptera co-occur on the same host plant, the ants may depend less on nectar as a source of carbohydrates. Fiala & Maschwitz (1991) concluded that the reduction of nectar production in *Macaranga* ant-plants may (1) save assimilates and (2) protect the specific association against generalist ant competition. The considerable nectar secretion found in many waxy *Macaranga* ant-plants suggests that selective pressure has been exerted mainly by the threat of foreign ant invasions.

### CLOSED STIPULES

Our study confirms that closed stipule morphology in *Macaranga* ant-plants represents an important adaptation to myrmecophytism. Recurved or tubular stipules occur exclusively among the ant-plants of the genus and probably act as mechanical filters which reduce competition for the specific ant partners by making food bodies inaccessible to other ants.

Almost all host plants of *Crematogaster* (*Decacrema*) with open stipules have slippery wax barriers. However, our data provide no definite evidence for the correlation of wax barriers and open stipule morphology. The trend observed in the traditional comparative analysis (Table 1) was not confirmed by the results of the concentrated changes test (Table 4). The concentrated changes test has been found to be fairly robust in its diagnosis of evolutionary correlations, but it has a tendency to commit type II errors (not detecting correlations where they exist, Lorch & Eadie, 1999). Phylogenetic comparative methods such as the concentrated changes test focus on evolutionary change and may ignore adaptations that have been maintained by stabilizing selection (Hansen, 1997; Martins, 2000). This situation might apply to the evolution of recurved stipule morphology, where a single gain has been retained in 15 ant-plant species (Fig. 5). The only non-waxy host plant of *Crematogaster* (*Decacrema*) with open stipules, *M. puberula*, is a submontane tree growing at an altitude of 800–1800 m. We assume that generalist ant competition is much less severe at higher altitudes, because the species richness and abundance of ants are markedly reduced compared with those of lowland forests (Brown, 1973; Brühl, Mohamed & Lins, 1999).

Recurved stipules have been repeatedly lost together with a loss of myrmecophytism (in

*M. ashtonii, M. depressa, M. rostrata* and *M. triloba*). Considering the barrier function of the waxy stem surfaces, the question arises as to why closed stipules have not also been lost in many waxy host plants. We assume that closed stipule morphology has a strong negative effect in ant-plants that have lost their specific ant partner(s) by geographical separation or other mechanisms. The food bodies of these plants would be inaccessible to foragers, and the tree would be left with no biotic protection against herbivory whatsoever. Thus, selection may act strongly against closed stipule morphology in ant-plant populations that have lost their ant partners, but not in waxy host plants. Moreover, closed stipules may retain a weakly positive effect in waxy plants, because they prevent flying insects (which are less affected by the wax barriers) from harvesting food bodies (for example, we observed cockroaches consuming openly accessible food bodies of *M. pruinosa*).

Our data indicate that the recurved stipules of waxy *Macaranga* host plants are less persistent. It has been reported for *M. bancana* that food body production strongly differs between ant-inhabited and ant-free host plants, the difference resulting mainly from lower stipule numbers in the absence of ants. The resident ants appear to regulate their host plant's food body production by making the stipules more persistent (Heil *et al*., 1997). Thus, the shorter persistence of recurved stipules in glaucous *Macaranga* ant-plants found in this study could be a consequence of a generally smaller number of ants living on waxy hosts (see Federle *et al*., 2002). However, data for the waxy *M. griffithiana* indicate that food bodies are far less concentrated on the recurved stipules than they are in the non-waxy *M. bancana*. A census of 11 *M. griffithiana* greenhouse plants showed that only 30% of all food bodies were produced beneath the stipules (Menke, 1996), whereas in *M. bancana*, the same proportion was found to be 99% (Heil *et al*., 1998). Despite the recurved stipule morphology, most food bodies are produced outside the stipules, which is functionally similar to the glaucous species with an open stipule morphology (*M. hypoleuca*, *M. hosei*). We conclude that not only the closed stipule morphology but also their persistence are traits functionally related to ant competition.

## SELECTIVE FILTERS IN ANT-PLANT MUTUALISMS

Plant characters that restrict access to resources are well known from pollination mutualisms (Faegri & van der Pijl, 1979; Proctor, Yeo & Lack, 1996), but have received less attention in ant–plant associations. However, as pointed out by Davidson & McKey (1993), evolutionary specialization of ant-plants and plant-ants has been driven largely by competition among ant species. Ant-plant traits can influence the outcome of the competition between ant species and thus act as selective filters promoting specialists and keeping away generalists. Many ant-plants have evolved specialized surfaces to restrict generalist ant access, such as the slippery wax crystal coatings in *Macaranga* (Federle *et al*., 1997) or the long and erect trichomes occcuring in at least 18 neotropical myrmecophytic genera, which selectively inhibit the movements of large-bodied ants. Other mechanical barriers control the access to domatium space and food resources. Thin-walled 'prostomata' (i.e. preformed, thin zones of the stem, where ants preferably chew their entrance holes) act as selective filters facilitating domatium entry only for some ants and not for others (*Leonardoxa,* Brouat *et al*., 2001; *Macaranga,* Federle *et al*., 2001). Similar to the function of *Macaranga* stipules, leaf or stipule pouches of ant-plants can cover EFN or provide shelter for coccids (Huxley, 1986). Ant-plants can most effectively limit access to food resources when they supply them inside the domatia, so that ant foraging occurs in seclusion and entails little risk. For example, many plant-ants obtain most of their nutrition from endophytic scale insects, and even food bodies can be supplied inside the domatia (*Piper*, Risch & Rickson, 1981; Rickson & Risch, 1984; *Maieta*, Vasconcelos, 1991).

Ant-plants can control the access to food resources not only by mechanical barriers but also chemically by making food bodies unattractive to generalists (Rickson, 1977; Davidson & McKey, 1993) or by producing most of the food at night (for nocturnal ants), when ant competition may be reduced (Davidson & McKey, 1993). To achieve a better understanding of the evolution of food presentation in ant-plants, further comparative studies should focus on the quantity and quality of host plant resources, on the temporal patterns of their production and on their palatability and attractiveness to specialist and generalist ants.

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