Correlation between the leaf turnover rate and anti-herbivore defence strategy (balance between ant and non-ant defences) amongst ten species of *Macaranga* (Euphorbiaceae)

Masahiro Nomura[1], Aya Hatada[1, 3] and Takao Itioka[2*]

[1] *Center for Ecological Research, Kyoto University*
Otsu 520-2113, Japan

[2] *Graduate School of Human and Environmental Studies, Kyoto University*
Kyoto 606-8501, Japan

[3] Present address: *Kyoto University of Foreign Studies*
Kyoto 615-8558, Japan

[*] *Corresponding author*: *E-mail*: itioka@z04.mbox.media.kyoto-u.ac.jp

Abstract

We measured variation in the intensities of ant and non-ant anti-herbivore defences amongst ten *Macaranga* species in Sarawak, Malaysia. Intensities of non-ant defences were estimated by measuring effects of fresh leaves (provided as food) of these *Macaranga* species on survival of common cutworm larvae [*Spodoptera litura* (Fabricius), Lepidoptera: Noctuidae]. Intensities of ant defences were estimated by measuring ant aggressiveness in the presence of artificial damage inflicted on plants. As part of our examination of non-ant defences, we measured leaf toughness (punch strength, by penetrometry), and the contents of total phenols and condensed tannin. We demonstrated interspecific variation in intensities of both ant and non-ant defences amongst ten *Macaranga* species and showed that the rank order of ant defence intensity was negatively correlated with the intensity of non-ant defence. We also found that the balance between ant and non-ant defence intensity was correlated with the rates of leaf turnover and shoot growth. Species investing more in ant defence tended to have higher leaf turnover rates. *Macaranga* species that occur preferentially in shadier microhabitats had lower leaf turnover rates, suggesting that non-ant defences are more cost-effective in more shade-tolerant species. Our results also suggest that the total intensity of non-ant defences is positively correlated with both leaf toughness and total phenol content.

Keywords: ant-plant mutualism, herbivore-plant interactions, myrmecophyte, Sarawak, Borneo, South East Asian tropics

Introduction

Plants have evolved various mechanisms of anti-herbivore. Many attract or nourish other organisms that repel grazers (Buckley 1987; Farmer 1997; Huxley and Catler 1991; Williams et al. 1988). Some plants form mutualistic relationships with ants, which in turn provide the anti-herbivore defences (Beattie 1985; Benson 1985; Davidson and Epstein 1989; Davidson and McKey 1993; Fiala and Linsenmair 1995; Huxley and Catler 1991; Oliveira and Oliveira-Filho 1991). These ants are usually rewarded with food and/or nest sites by the plants (Buckley 1987; Davidson and McKey 1993; Huxley and Catler 1991; Janzen 1966; Lincolon et al. 1982; McKey et al. 1993). Here, we refer to anti-herbivore defence through ants as "ant defences".

Almost all plants (including those that use ants for anti-herbivore defence) defend themselves with innate mechanisms that do not involve other organisms (Harborne 1997; Pollard 1992; Rosenthal and Berenbaum 1991). The mechanisms include production of various secondary compounds such as toxins and repellents, as well as physical obstacles such as hairs, spines, and thick cell walls. We refer to these mechanisms as "non-ant defences".

Both ant and non-ant defences require metabolic resources. To maintain non-ant defences, plants synthesise secondary metabolites such as phenolics and alkaloids, and/or fibres (Barker 1989; Simms 1992). To maintain ant defences, plants provide food and/or nest sites for their mutualistic partners. Investment in anti-herbivore defence may increase the efficacy of the mechanism, but it does not necessarily increase plant fitness. Plants allocate resources to anti-herbivore defences and to growth, reproduction and dispersal (Briggs and Schultz 1990; Herms and Mattson 1992; Simms 1992; Simms and Rausher 1987). Because resources for essential functions are limited, trade-offs in resource allocation must occur between ant and non-ant anti-herbivore defence mechanisms (Eck et al. 2001; Heil et al. 2000a, b; Janzen 1966; Linsenmair et al. 2001; Nomura et al. 2000, 2001; Rehr et al. 1973).

How do plants strategically allocate resources between these competing demands? Various hypotheses have been applied to seek explanations in understanding the effects of environmental variations on the balance amongst anti-herbivore defence mechanisms (Coley et al. 1985; McKey 1984). McKey (1984) proposed that carbon-based non-ant defences, such as formation of thick leaves and constitutive defence chemicals, may be amortised only in plants with low leaf turnover rates and that ant defences may be profitable in plants with higher leaf turnover rates ("leaf life span hypothesis"). To maintain ant defences, plants must provide their mutualistic partner with food rewards containing large amounts of lipids, amino acids and carbohydrates (Hatada et al. 2002; Heil et al. 1997, 1998; Rickson 1980). Although relatively high maintenance costs are required for ant defences, plants can relocate ants to particular plant parts in which an immediate need exists for anti-herbivore defence; the relocation of ants is achieved by shifting food rewards to sites where anti-herbivore defence is required. Accordingly, McKey (1984) characterised ant defences as adjustable defence mechanisms with relatively low construction costs and high maintenance costs. Here, 'adjustable' defence indicates that the intensity of ant anti-herbivore deterrence can be shifted amongst parts of a single plant on a needs basis. Rewards for ant agents of defence are moved selectively to plant parts that are currently under attack by herbivores.

To maintain carbon-based non-ant defences, plants must invest in initial costs for synthesising and stocking a complex suite of carbon-based compounds, including structural material like fibres and/or defensive chemicals such as phenolic compounds. While there are no maintenance costs or modest costs for non-ant defences after synthesis, plants are obviously unable to access invested resources from leaves that have been shed. Hence, McKey (1984) characterised carbon-based non-ant defences as non-adjustable mechanisms with relatively high construction costs but low maintenance costs.

Members of the genus *Macaranga* (Euphorbiaceae) are mostly pioneer trees distributed from West Africa to the South Pacific islands (Davies 2001; Whitmore 1969, 1975, 2008). The genus has a wide variety of insect mutualisms, ranging from non-myrmecophytes to facultative, transitional and obligate myrmecophytes (Fiala and Maschwitz 1990; Fiala et al. 1989, 1994, 1999; Itioka et al. 2000). There are fragmentary descriptions of interspecific variation in intensities of ant and non-ant defences in *Macaranga* (Eck et al. 2001; Fiala and Maschwitz 1990, 1991, 1992; Fiala et al. 1989, 1994; Heil et al. 1999; Hnawia et al. 1990; Itioka et al. 2000; Lin 1993, 1994; Lin et al. 1990a, b; Nomura et al. 2000, 2001; Sultana and Ilyas 1986). Itioka et al. (2000) and Nomura et al. (2000) demonstrated wide variations in defence intensities, even amongst sympatric species of *Macaranga,* and showed that the intensities of non-ant defences are negatively correlated with the intensities of ant defences.

Considering the predictions and patterns of earlier studies, we have hypothesised that the ratio of ant defence intensity to non-ant defence intensity in the genus *Macaranga* would be higher in species with elevated leaf turnover rates. To test this hypothesis, we measured relationships between (1) variation in intensities of ant and non-ant defences and (2) leaf turnover rates amongst ten *Macaranga* species. Our hypothesis is based on the premise that interspecific variation in the balance between intensities of the two types of defence should reflect interspecific variation in the balance of strategic resources allocated to one or the other type of anti-herbivore defences. There is an underlying assumption that the the effectiveness of a given form of defence increases in proportion to the quantity of resources invested in it. With the data collected, we examined correlations between leaf turnover rates and the balance between different anti-herbivore defence mechanisms. For non-ant defence mechanisms, we separately measured leaf toughness (physical defence) and concentrations of condensed tannin and total phenolics (chemical defences) in order to estimate the relative contributions to intensity of total non-ant defence.

Materials

Study site

This study was conducted in Lambir Hills National Park, Miri, Sarawak, Malaysia (4°2'N, 113°50'E, altitude 50–150 m). Most of study site is covered with a primary lowland mixed dipterocarp forest. The park is located in the humid tropics and has no pronounced dry season (Kato et al. 1995). At least 15 species of *Macaranga* occur at the study site and many of them are dominant pioneer trees (Itioka et al. 2005).

Macaranga

We focused on the following ten *Macaranga* species: *Macaranga winkleri* Pax. and Hoffm., *M. trachyphylla* Airy Shaw, *M. bancana* (Miq.) Muell. Arg., *M. hypoleuca* (Reichb. F. and Zoll.) Muell Arg., *M. beccariana* Merr., *M. lamellata* Whitmore, *M. umbrosa* Davies., *M. hullettii* King ex Hook., *M. gigantea* (Reichb. F. and Zoll.) Muell. Arg. and *M. praestans* Airy Shaw. The first eight are obligate myrmecophytes, whilst the other two are non-myrmecophytes.

The myrmecophytes provide their mutualistic ant partners with nest sites inside hollow stems and food bodies inside dome-shaped stipules or on leaf surfaces. Ants patrol leaves of host plants and protect them against herbivores and vines (Fiala and Maschwitz 1991, 1992; Fiala et al. 1989, 1994; Heil et al. 1999, 2001; Itioka et al. 2000). The association between obligate myrmecophytes and their ants is highly species-specific, and almost all of the plants are colonised by only one or two ant species (Fiala et al. 1999; Itino et al. 2001).

The non-myrmecophytic species *M. gigantea* and *M. praestans* often attract nonspecific ants with extrafloral nectar (EFN) produced on leaf surfaces. Ants that are attracted to EFN also defend hosts against herbivores, although the effectiveness of defence is much weaker than in myrmecophytes (Fiala and Maschwitz 1991; Heil et al. 2001). In *M. praestans*, only newly developing leaves produce EFN; the EFN deteriorates as leaves mature, which is not the case for *M. gigantea*. Hence, we consider the intensity of ant defence in mature leaves of *M. praestans* to be weaker than that in *M. gigantea*. Taxonomic fidelity is low amongst EFN-collecting ants associated with the two non-myrmecophyte *Macaranga* species; to date, more than ten ant species have been observed collecting EFN (T. Itioka et al. *unpublished data*).

All myrmecophytic species occur preferentially in moderate shade, found, for example, in forest treefall gaps and by riversides. Although there is considerable habitat overlap, five of the eight myrmecophytic species (*M. winkleri, M. trachyphylla, M. bancana, M. hypoleuca* and *M. beccariana*) are more often found in slightly better-illuminated habitats than the other three (*M. hullettii, M. umbrosa* and

M. lamellata) [Davies et al. 1998; *M. bancana* and *M. umbrosa* as *M. triloba* and *M. kingii*, respectively, in Davies et al 1998 (Davies, SJ personal communication, Davies 2001)]. Hereafter, we refer to the first five species as “less shade-tolerant myrmecophytes” and the remaining three as “more shade-tolerant myrmecophytes”. Of the nine species (other than *M. praestans*), *M. lamellata* prefers sand-rich soils, *M. hulletti* is not strongly affected by soil texture, and the other six species prefer clay-rich soils (Davies et al 1998). Of the nine species, *M. gigantea* grows to 25-30 m tall whearas the other species rarely reach 20 m tall (Davies et al 1998). The maximum tree height tends to be lower in the three more shade-tolerant myrmecophytes than in the less shade-tolerant myrmecophytes although the maximum tree height of *M. beccariana* is lower than that of *M. hullettii* (Davies et al 1998). Although *M. gigantea* preferentially occurs in slightly brighter conditions than these myrmecophytic species (Davies et al 1998), its habitat broadly overlaps those of myrmecophytic species. *M. praestans* occurs more often than any other species in the shadiest conditions, e.g., forest floor, and its maximum tree height seems to be similar as those of the more shade-tolerant myrmecophytes (*personal observation*).

Assessment of ant defence level

When myrmecophytic species are injured by herbivores, aggressive behaviour is induced in mutualistic ants (Itioka et al. 2000); ant workers aggregate at the locations of injury and attack herbivores, if any are present. We used this ant behaviour to measure interspecific differences in ant defence level amongst myrmecophytes. For each of the *Macaranga* species, we randomly selected 1.5–2.2-m-tall saplings without noticeable damage. We cut off a leaf tip (1cm^2) collected from a selected sapling and held it with forceps in contact with the second node of another sapling of the same species. We recorded the numbers of ants that aggregated around the leaf tip, climbed onto the forceps and/or hand of the experimenter holding the leaf tip, or bit the experimenter's hand during a 90-s period. We calculated the average numbers of ants engaging in these four types of aggressive behaviour.

Assessment of non-ant defence level

To assess interspecific variation in intensity of non-ant defences, we measured inhibitory effects of each *Macaranga* species on survival and growth performance of common cutworm larvae (*Spodoptera litura*, Lepidoptera: Noctuidae); fresh leaves of *Macaranga* were provided as the only food source. The cutworm is an extremely polyphagous herbivore (Okamoto and Okada 1968), and the larva have been used to assess levels of tannin-related anti-herbivore defences in target plants (Nomura and Itioka 2002). Hence, we assumed that non-ant defensive effects against generalist herbivores that feed on *Macaranga* can be represented by effects on cutworm larvae. The larvae can complete their growth even when fed an artificial diet (Insecta LFS; Nihon Nosankougyou, Tokyo, Japan). The strain used in this study had been maintained on an artificial diet in the laboratory for more than 25 years.

To collect leaves for use as experimental diet, we randomly selected 1.5–2.2 m tall saplings (of all species except *M. gigantea*) that had no noticeable damage. For *M. gigantea*, we selected 3–8 m tall saplings. One undamaged leaf located near the apical part of the stem, but fully expanded and fully toughened was collected from each sapling. However, the leaves might be softer than the older leaves that were farther away from the apical parts.

Cutworm larvae that had hatched within a 12-h period were used for the assessment. We prepared 120 plastic boxes (22 × 14 × 3.7 cm) and placed in each box ten first-instar larvae together with a fresh leaf of one *Macaranga* species. The 120 boxes were assigned to ten treatments (12 replicates per treatment) and larvae were reared at 28 °C in the laboratory. Every second day, larval survival was checked and leaves were replaced.

Nomura et al. (2000) measured interspecific variation in intensity of non-ant defences amongst six *Macaranga* species. Their method of assessing anti-herbivore defences was almost identical to ours. We therefore included their data for six species in our comparisons of non-ant defences amongst plants.

Assessment of leaf toughness

To assess the strength of physical defence, we determined leaf toughness (punch strength). Since the leaves used in this study bore very few trichomes or spines, we assumed that leaf toughness would be a good measure of the strength of physical defence. Leaves were sampled from 20 saplings of each species randomly selected in a manner identical to that for our larval cutworm assessment of non-ant defence. Leaf toughness represented by punch strength was quantified by penetrometry, i.e., the weight needed to push a 3-mm-diameter column through a fresh leaf (CPU; Aikoh Engineering, Tokyo, Japan).

Nomura et al. (2000) measured interspecific variation in leaf toughness (punch strength) amongst five *Macaranga* species using a method similar to ours. We supplemented our own data with those from the earlier study (Nomura et al. 2000) to achieve an adequate sample size.

Quantification of total phenol and condensed tannin

Leaves were sampled via a standard procedure (see "Assessment of ant defence level"). The leaves were vacuum-dried in a freeze-dryer (FDU-810; Tokyo Rikakikai, Tokyo, Japan), and the major veins were excised from the dry leaves, which were ground in an electric mill. Dry leaf powder was stored in Ziploc^{TM} bags over silica gel until analysis. Dry leaf powder (0.2 mg) was extracted at 40 °C with 5 ml of 50 % methanol for 1 h using an ultrasonicator. The extract was centrifuged at 3000 g for 15 min and the supernatant, which used for quantifying the condensed tannin and total phenol, was collected. The extraction was performed in duplicate.

Condensed tannin content was measured by the proanthocyanidin method (Waterman and Mole 1994). One millilitre of extract was added to 4 ml of *n*-butanol-conc. HCl (95 : 5). The mixture was hydrolysed at 100 °C for 2 h. After cooling for 15 min, the absorbance of the reaction was measured at 550 nm. The reference reagent was *n*-butanol-conc. HCl (95 : 5) with HCl replaced by distilled water. Condensed tannin content was determined using cyanidin chloride.

Total phenol content was measured by the Folin–Ciocalteu method (Waterman and Mole 1994). Two hundred microlitres of extract was diluted with 2 ml of distilled water, and 1 ml of Folin–Ciocalteu reagent was mixed into the solution. One to 8 min later, 5 ml of $NaCO_3$ solution (20 %) was added, and the mixture was shaken. This solution was made up to a volume of 10 ml with distilled water. After incubation at room temperature for 20 min, absorbance of the reaction was measured at 760 nm. Distilled water replaced normal reagent in the reference tubes. Total phenol content was determined using tannin acid.

Assessment of growth rate and leaf turnover

We randomly selected 1.5–2.2-m-tall saplings without noticeable damage in the field. We measured height and stem radius at the ground level, and numbered the leaves from oldest to youngest with a felt-tipped pen. We re-measured height and stem radius, and counted the number of fallen leaves and newly developed leaves on four or five occasions ca 1, 3, 12 and 36 months later. Based on the volume of plants, we calculated the growth rate as follows:

$$\text{Growth rate} = \left\{ \ln (S_{\text{final}} \times S_{\text{final}} \times H_{\text{final}}) - \ln (S_{\text{initial}} \times S_{\text{initial}} \times H_{\text{initial}}) \right\} / \text{Days},$$

where S_{initial} and S_{final} are stem radii of the sapling at times of first and second measurements, respectively, and H_{initial} and H_{final} are height of sapling at times of first and second measurements, respectively. We calculated leaf turnover rate as follows:

$$\text{Leaf turnover rate} = 1 / 2\ (\text{NFL} + \text{NNL}) / \text{MNL} / \text{Days},$$

where NFL and NNL are the numbers of fallen leaves and newly developed leaves, respectively, and MNL is the mean number of leaves between first and second measurements.

Results

Intensity of ant aggressiveness

Significant differences were detected in all measures of ant aggressive behaviour amongst the eight *Macaranga* myrmecophytes (Kruskal–Wallis test: adjusted $H > 41.65$, $P < 0.0001$ for each behaviour; Table 1). The numbers of ants engaged in the four types of aggressive behaviours tended to be higher on the five less shade-tolerant myrmecophytes than on the three more shade-tolerant myrmecophytes, and were different amongst the five less shade-tolerant myrmecophytes. The numbers of ants engaged in the four types of aggressive behaviours on *M. winkleri* were significantly higher than those on the other less shade-tolerant myrmecophytes, and the numbers of ants that climbed on the forcep and/or hand on *M.*

trachyphylla were significantly higher than those on *M. beccariana* and *M. hypoleuca* (multiple comparisons based on Mann–Whitney *U*-test, $P < 0.05$). Significant differences were observed amongst the eight plant species in the sum of average numbers of ants engaged in the four types of aggressive behaviour (Kruskal–Wallis test: adjusted $H = 48.616$, $P < 0.0001$; Table 1). The sum of the average numbers was significantly higher on *M. winkleri* than on the other species; significantly higher on *M. trachyphylla* than on *M. hypoleuca, M. lamellata* and *M. umbrosa*; and significantly higher on *M. beccariana* than on *M. lamellata* and *M. umbrosa* (multiple comparisons based on Mann–Whitney *U*-test, $P < 0.05$).

Effects of eating fresh leaves on the growth of cutworm larvae

The hazard curves of the cumulative cutworm larval survival rates were significantly different amongst the ten species of leaves (logrank test, $\chi^2 = 1135.818$, $P < 0.0001$; Fig. 1). The hazard curves were higher for larvae on the five less shade-tolerant myrmecophytes than on the three more shade-tolerant myrmecophytes, and lower on *M. praestans* than on any other species except *M. hullettii*. The larval mandibles were able to penetrate leaves of all species except *M. praestans* (all larvae died of starvation when fed this species, probably due to their inability to break the leaf surface). The larval hazard curve on *M. gigantea* was intermediate between those on the more shade-tolerant myrmecophytes and those on the less shade-tolerant myrmecophytes (except *M. hypoleuca*). Although the larval hazard curves were not significantly different between *M. gigantea* and *M. beccariana*, some larvae on *M. beccariana* were able to survive until the third instar, whereas all larvae on *M. gigantea* died during the second instar. The larval hazard curves were different amongst the five less shade-tolerant myrmecophytes and amongst the three more shade-tolerant myrmecophytes. The larval hazard curves were highest on *M. lamellata* and lowest on *M. hullettii* amongst the three more shade-tolerant myrmecophytes .

Some larvae grew to the adult stage, but only on leaves of *M. winkleri* and *M. trachyphylla*; larvae died on the leaves of other *Macaranga* species.

Leaf toughness

Significant differences in leaf toughness were observed amongst the ten species (ANOVA, $F = 84.033$, $P < 0.0001$; Fig. 2). Leaf toughness tended to be lower in the five less shade-tolerant myrmecophytes than in other species. Leaf toughness in *M. praestans* was significantly higher than in the other species (Scheffé's *F* test, $P < 0.05$). *M. gigantea* had a leaf toughness intermediate between those of the two types of myrmecophytic species; its leaf toughness was significantly higher than those of *M. winkleri, M. trachyphylla* and *M. bancana*, and significantly lower than those of *M. hullettii* and *M. praestans* (Scheffé's *F* test, $P < 0.05$). Leaf toughness differed amongst the five less shade-tolerant myrmecophytes, and amongst the three more shade-tolerant myrmecophytes. Leaf toughness was significantly lower in *M. winkleri* than in *M. hypoleuca*, *M. beccariana* and *M. bancana*, and significantly higher in *M. hullettii* than in *M. umbrosa* and *M. lamellata* (Scheffé's *F* test, $P < 0.05$).

Contents of total phenol and condensed tannin

A significant difference in total phenol content was seen amongst the ten species (ANOVA, $F = 31.164$, $P < 0.0001$; Fig. 3a). Total phenols content tended to be lower in the five less shade-tolerant myrmecophytes than in the other species, and was intermediate in the two non-myrmecophytic species between those in less shade-tolerant myrmecophytes and those in more shade-tolerant myrmecophytes. Total phenols content was significantly lower in *M. praestans* than in *M. umbrosa* (Scheffé's *F* test, $P < 0.05$), significantly higher in *M. gigantea* than in *M. winkleri*, *M. trachyphylla* and *M. beccariana,* and significantly lower in *M. gigantea* than in *M. umbrosa* (Scheffé's *F* test, $P < 0.05$). Total phenols content differed amongst the three more shade-tolerant myrmecophytes, being significantly higher in *M. umbrosa* than in *M. hullettii* and *M. lamellata,* and significantly higher in *M. lamellata* than in *M. hullettii* (Scheffé's *F* test, $P < 0.05$).

A significant difference in condensed tannin content was detected amongst the ten species (ANOVA, $F = 24.575$, $P < 0.0001$; Fig. 3b), but no consistent difference was observed between the less shade-tolerant and more shade-tolerant myrmecophytes. The condensed tannin contents for the two non-myrmecophytic species tended to be higher than those for the myrmecophytic species. The contents were significantly higher in *M. gigantea* than in the other species, and significantly higher in *M. praestans* than in *M. winkleri, M. trachyphylla, M. bancana, M. lamellata, M. umbrosa and M. hullettii* (Scheffé's *F* test, $P < 0.05$).

Leaf turnover and growth rates

A significant variation in the leaf turnover rate amongst the ten species was seen (ANOVA, $F = 70.293$, $P < 0.0001$; Fig. 4a). Leaf turnover rates of the five less shade-tolerant myrmecophytes tended to be higher than those of the three more shade-tolerant myrmecophytes. Leaf turnover rate was significantly lower in *M. praestans* than in the other species (Scheffé's *F* test, $P < 0.05$); that in *M. gigantea* was intermediate amongst those of the eight myrmecophytic species, significantly lower than that in *M. winkleri* (Scheffé's *F* test, $P < 0.05$), and significantly higher than those in *M. lamellata*, *M. umbrosa* and *M. praestans* (Scheffé's *F* test, $P < 0.05$). Leaf turnover rate differed amongst the five less shade-tolerant myrmecophytes, and amongst the three more shade-tolerant myrmecophytes. The rate was significantly higher in *M. winkleri* than in the other less shade-tolerant myrmecophytes, and significantly lower in *M. umbrosa* than in the other more shade-tolerant myrmecophytes (Scheffé's *F* test, $P < 0.05$).

Significant variation in growth rate amongst the ten species was seen (ANOVA, $F = 17.241$, $P <$

0.0001; Fig. 4b), although no consistent difference was seen between less and more shade-tolerant myrmecophytes. Growth rate was significantly lower in *M. praestans* than in species other than *M. umbrosa* (Scheffé's *F* test, $P < 0.05$). The growth rate of *M. gigantea* was intermediate amongst those of the five less shade-tolerant myrmecophytes, and significantly lower than that of *M. winkleri* (Scheffé's *F* test, $P < 0.05$). Growth rate differed amongst the five less shade-tolerant myrmecophytes, and was significantly higher in *M. winkleri* than in the other less shade-tolerant myrmecophytes (Scheffé's *F* test, $P < 0.05$).

Correlation between anti-herbivore defences and life history traits

The rank order of ant defence intensity was significantly negatively correlated with those of non-ant defence intensity, leaf toughness and total phenol content (Kendall's coefficient of rank correlation, adjusted τ = –0.644, –0.778 and –0.644 for non-ant defence intensity, leaf toughness and total phenols content, respectively, $P < 0.01$ for all comparisons; Table 2). The rank order of non-ant defence intensities was significantly positively correlated with those of leaf toughness and total phenols content (Kendall's coefficient of rank correlation, adjusted τ = 0.778 and 0.556, $P < 0.01$ and 0.05, for leaf toughness and total phenol content, respectively; Table 2), and significantly negatively correlated with that of leaf turnover rate (Kendall's coefficient of rank correlation, adjusted τ = –0.511, $P < 0.05$; Table 2). The rank order of leaf turnover rates was significantly positively correlated with that of growth rate (Kendall's coefficient of rank correlation, adjusted τ = 0. 689, $P < 0.01$; Table 2).

Discussion

Since herbivorous insects that specialise on or constantly utilise a particular plant species in the field tend to overcome plant non-ant defences through coevolutionary processes (Ehrlich and Raven 1964; Feeny 1977), the strength of general non-ant defences of the plant cannot be assessed with bioassay using these specialist herbivorous insects. In contrast, the common cutworm has a broad host range, making it a suitable choice for bioassays of general *Macaranga* non-ant defences. For the same reason, the strength of ant defences in a particular plant species cannot be assessed with a bioassay using herbivorous insects that specialise on *Macaranga* species. Therefore, we measured ant aggressiveness toward artificial leaf damage to estimate the intensity of ant defences against the generalist herbivores that may attack *Macaranga* trees.

In this study, we determined leaf puch strength as an idicator of leaf touness and as an indicator of resistance to invertebrate herbivory. Recently, fracture toughness has been used as another measure of leaf toughness, and there is uncertainty as to which of the two measures is better correlated with resistance to herbivory (Choong et al. 1992, Dominy et al. 2008, Grubb et al. 2008, Read and Stokes

2006). This uncertainty requires resolution in future studies on *Macaranga* plants, but at this point, we believe that punch strength is a good indicator of resistance to herbivory. The rank orders of species presumably differed little between the two types of variables because our comparison were restricted to members of a single genus, whose species probably have similar cell wall structures leaf textures, and materials in and around cell walls.

Wide interspecific variations were seen in the intensities of both ant and non-ant defences amongst the ten species, and there was a tendency for species with more intensive ant defences to have less intensive non-ant defences, supporting Janzen’s (1966) proposal of a trade-off between ant and non-ant defences. As Janzen (1966) argued, high investments into both ant and non-ant defences must place metabolic burdens on plants under resource-limited conditions.

We demonstrated interspecific variation in leaf turnover rate amongst the ten *Macaranga* species, and the rank order of this rate was positively correlated with that of ant defence intensity and negatively correlated with that of non-ant defence intensity. Assuming that intensities of the two types of anti-herbivore defences increase in proportion to plant investment in defences, and that the relationship between intensity (or effectiveness) and investment does not differ significantly amongst *Macaranga* species, the balance between the intensities of the two types of defence probably reflects interpecific difference in the investment balance between ant and non-ant defences. If these assumptions are indeed correct, our results are consistent with McKey's (1984) hypothesis; the balance between ant and non-ant defences was closely associated with leaf turnover rate (except in one *Macaranga* species), as McKey (1984) hypothesised. The order of species in the *Macaranga* leaf turnover rate was closely related to variation in microhabitat light availability amongst the species. As McKey (1984) argued, we can infer that ant defences would be more profitable for *Macaranga* species with high leaf turnovern rates. Under constraints of limited resources, each *Macaranga* species may pay metabolic costs for an optimal balance not only between the two modes of anti-herbivore defence, but also between anti-herbivore defence and growth, including leaf turnover. The relationships between balancing ant and non-ant defences, the total costs of anti-herbivore defence and leaf turnover as components of life history resource allocation strategy have yet to be investigated in *Macaranga*.

The assumption on a linear relation between investment and effectiveness (intensity) for each defence mechanism seems reasonable (1) because there is unlikely to be significant variation in mechanisms of ant defence and non-ant defence amongst the target species of the genus *Macaranga*, and (2) because it is generally believed that effectiveness of a defence mechanism is higher in plants that invest more resources in this mechanism (and different species use the same mechanism). However, the validity of the assumption should be examined more rigorously in the future because different *Macaranga* species may use different component mechanisms in the suite of non-ant defence mechanisms. Since some of non-ant defences (mainly physical and chemical defences) may be related to other life history strategy functions, such as anti-pathogen defences and resistance to drought (Edwards 1989), exact estimation of investment in non-ant defences would be difficult. In spite of this caveat, the negative correlation amongst species between the rank order of ant defence intensity and that of non-ant defence suggests that McKey's (1984) hypotheses are correct.

The strength of non-ant defences in *M. gigantea* was much lower than would have been predicted by the across-species negative correlation between ant and non-ant defence strengths. The intensities of ant and non-ant defences in this non myrmecophyte species were lower than those in the three more shade-tolerant myrmecophytes. Furthermore, *M. gigantea* was an outlier amongst the ten *Macaranga* species in the correlation between ant/non-ant defence ratio and leaf turnover rate; its ratio and turnover rate were intermediate amongst those in myrmecophytic species. These exceptional properties suggest that *M. gigantea* does not trade-off resources between ant and non-ant defences in the manner predicted by McKey's (1984) leaf life span hypothesis, whereas the other nine congeners fit the predictions. The preferential occurrence of *M. gigantea* in microhabitats that are much better-illuminated than those of other species (Davies et al. 1998, T. Itioka et al. *unpublished data*) might help explain its exceptional attributes. Coley et al. (1985) predicted that plants inhabiting favourable environments (*e.g.*, well lit with fertile soil and appropriate water supply) would allocate relatively large resource quotas into growth and relatively small quotas into anti-herbivore defences (resource availability hypothesis). With abundant resources, plants in more favourable environments are easily able to replace tissues lost to herbivory, reducing the necessity for defences against tissue loss. Thus, Coley et al. (1985) predicted that plants inhabiting resource-poor environments would have stronger anti-herbivore defences than those in resource-rich habitats. Many studies provide corroborative evidence for this prediction (Beadle 1966; Chabot and Hicks 1982; Coley 1983, 1987, 1988; Givnish 1987; Herms and Mattson 1992; Janzen 1974; Reich et al. 1992; Turner 1994) but difficulties raised, *e.g.* by Grubb (1992). We propose that *M. gigantea* falls into the category of plants living in a resource-rich habitat (light is the resource in this case) and investing more into growth than defence. The postulate awaits further testing.

Davies et al. (1998) demonstrated that eight of the ten species targeted in our study are spatially distributed across gradients of light conditions and soil characteristics. Moreover, Davies (1998) showed that ecophysiological and life history traits of *Macaranga* species are concordant with spatial distributions on the resource gradients. Our measurements of interspecific variation in the mode of anti-herbivore defence are also consistently reflected in species habitat differentiation, probably because leaf turnover rate is closely associated with the tree growth rate (Fig. 4), other leaf traits and preferred light conditions of individual species. However the distribution of most of the target *Macaranga* species in relation to sand-richness in the soil (Davies et al. 1998) were not clearly correlated with the interspecific variation in the balance between the two modes of anti-herbivore defences. Davies et al. (1998) did not detect significant differences in tolerance of drier conditions and upper altitudinal limit amongst the eight species. We were also unable to find the clear difference in the traits amongst the target species in this study. Effects of these factors on the variation in anti-herbivore defences remain to be examined.

Non-ant defences have chemical and physical components. We measured leaf toughness as a plausible correlate of physical defence against generalist herbivores, and total phenols and condensed tannin contents as plausible major agents of chemical defence. Species with tougher leaves tended to have more intensive total non-ant defence. Similarly, there was a tendency for species with higher total phenols content to have elevated total non-ant defences. In contrast, species with higher condensed tannin contents did not necessarily have elevated total non-ant defences. The correlation between leaf toughness and the intensity of total non-ant defence was slightly stronger than that between total phenols content and the intensity of total non-ant defence. Leaf toughness contributed slightly more than total phenols content to the interspecific variation in intensity of non-ant defences. Physiochemical and histological mechanisms that affect the efficiency of anti-herbivore defences constitute an issue.

Acknowledgments

We thank Hua Seng Lee and the Forest Department of Sarawak for providing opportunities to conduct this study at Lambir Hills National Park. We also thank Takayuki Ohgushi, Tohru Nakashizuka and Takao Itino for their helpful comments. We also thank Satoshi Tahara for assisting with the chemical analyses. This work was funded by the Japan Ministry of Education, Science and Culture for International Scientific Research (no. 10041163 and no. 21255004) and by Japan Science and Technology Corporation for CREST.

Reference

Barker GM (1989) Grass host preference of *Listronotus bonariensis* (Coleoptera: Curculionidae. J Econ Entomol 82:1807-1816

Beadle NCW (1966) Soil phosphate and its role in molding segments of the Australian flora and vegetation, with special reference to xeromorphy and selerophylly. Ecology 47:992-1007

Beattie AJ (1985) The evolutionary ecology of ant-plant mutualism. Cambridge University Press, Cambridge

Benson WW (1985) Amazon ant-plants. In: Prance GT, Lovejoy TE (eds) Amazonia. Pergamon Press, Oxford, pp 239-266

Briggs MA, Schultz JC (1990) Chemical defence production in *Lotus corniculatus*. II. Trade-offs among growth, reproduction, and defence. Oecologia 83:32-37

Buckley R (1987) Ant-plant-homopteran interactions. Adv Ecol Res 16:53-85

Chabot BF, Hicks DJ (1982) The ecology of leaf life spans. Annu Rev Ecol Syst 13:229-259

Choong MF, Lucas PW, Ong JSY, Pereira B, Tan HTW, Turner IM (1992) Leaf fracture-toughness and sclerophylly - their correlations and ecological implications. New Phytologist 121:597-610

Coley PD (1983) Herbivory and defensive characteristics of tree species in a lowland tropical rain forest. Ecol Monogr 53:209-233

Coley PD (1987) Interspecific variations in plant anti-herbivore properties: the role of habitat quality and rate of disturbance. New Phytol 106:251-263

Coley PD (1988) Effects of plant growth rate and leaf lifetime on the amount and type of anti-herbivore defence. Oecologia 74:531-536

Coley PD, Bryant JP, Chapin FS III (1985) Resource availability and plant antiherbivore defencescience 230:895-899

Davidson DW, Epstein WW (1989) Epiphytic associations with ants. In: Lüttge U (ed) Vascular plants as epiphytes. Springer-Verlag, Berlin, pp 200-233

Davidson DW, McKey D (1993) The evolutionary ecology of symbiotic ant-plant relationship. Journal of Hymenoptera Research 2:13-83

Davies SJ (1998) Photosynthesis of nine pioneer *Macaranga* species from Borneo in relation to life history. Ecology 79:2292-2308

Davies SJ (2001) Systematics of *Macaranga* sects. *Pachystemon* and *Pruinosae* (Euphorbiaceae). Harvard Papers in Botany 6:371-448

Davies SJ, Palmiotto PA, Ashton PS, Lee HS, Lafrankie JV (1998) Comparative ecology of 11 sympatric species of *Macaranga* in Borneo: tree distribution in relation to horizontal and vertical resource heterogeneity. J Ecol 86:662-673

Dominy NJ, Grubb PJ, Jackson RV, Lucas PW, Metcalfe DJ, Svenning JC, Turner IM (2008) In tropical lowland rain forests monocots have tougher leaves than dicots, and include a new kind of tough leaf. Annals of Botany 101:1363-1377

Eck G, Fiala B, Linsenmair KE, Hashim RB, Proksch P (2001) Trade-off between chemical and biotic antiherbivore defence in the South East Asian plant genus *Macaranga*. J Chem Ecol 27:1979-1996

Edwards PJ (1989) Insect herbivory and plant defence theory. In: Grubb PJ, Whittaker JB (eds) Toward a more exact ecology: 30th Symposium of the British Ecological Society (Symposia of the British Ecological Society). Cambridge University Press, Cambridge, pp 275-297

Ehrlich PR, Raven PH (1964) Butterflies and plants: a study in coevolution. Evolution 8:586-608

Farmer EE (1997) New fatty acid-based signals: a lesson from the plant world. Science 276:912-913

Feeny P (1977) Defensive ecology of the Cruciferae. Annual Missourii Botanical Garden 64:221-234

Fiala B, Linsenmair KE (1995) Distribution and abundance of plants with extrafloral nectaries in the woody flora of a lowland primary forest in Malaysia. Biodivers Conserv 4:165-182

Fiala B, Maschwitz U (1990) Studies on the South East Asian ant-plant association *Crematogaster borneensis / Macaranga*: adaptations of the ant-partner. Insect Soc 37:212-231

Fiala B, Maschwitz U (1991) Extrafloral nectaries in the genus *Macaranga* (Euphorbiaceae) in Malaysia: comparative studies of their possible significance as predispositions for myrmecophytism. Biol J Linn Soc 44:287-305

Fiala B, Maschwitz U (1992) Food bodies and their significance for obligate ant-association in the tree genus *Macaranga* (Euphorbiaceae). Bot J Linn Soc 110:61-75

Fiala B, Grunsky H, Maschwitz U, Linsenmair KE (1994) Diversity of ant-plant interactions: protective efficacy in *Macaranga* species with different degrees of ant associations. Oecologia 97:186-192

Fiala B, Jakob A, Maschwitz U, Linsenmair KE (1999) Diversity, evolutionary specialisation and geographic distribution of mutualistic ant-plant complex: *Macaranga* and *Crematogaster* in South East Asia. Biol J Linn Soc 66:305-331

Fiala B, Maschwitz U, Pong TY, Helbig AJ (1989) Studies of a South East Asian ant-plant association:

protection of *Macaranga* trees by *Crematogaster borneensis*. Oecologia 79:463-470

Givnish TJ (1987) Comparative studies of leaf form: assessing the relative roles of selective pressures and phylogenetic constraints. New Phytol 106:131-160

Grubb PJ (1992) A positive distrust in simplicity – lessons from plant defenses and from competition among plants and among animals. J Ecol 80:585-610

Grubb PJ, Jackson RV, Barberis IM, Bee JN, Coomes DA, Dominy NJ, De la Fuente MAS, Lucas PW, Metcalfe, DJ, Svenning JC, Turner IM, Vargas O (2008) Monocot leaves are eaten less than dicot leaves in tropical lowland rain forests: Correlations with toughness and leaf presentation. Annals of Botany 101:1379-1389

Harborne JB (1997) Plant secondary metabolism. In: Crawley MJ (ed) Plant ecology. Blackwell Scientific Publications, Oxford, pp 132-155

Hatada A, Itioka T, Yamaoka R, Itino T (2002) Carbon and nitrogen contents of food bodies in three myrmecophytic species of *Macaranga*: implications for antiherbivore defence mechanism. J Plant Res 115:179-184

Heil M, Fiala B, Baumann B, Linsenmair KE (2000a) Temporal, spatial and biotic variations in extrafloral nectar secretion by *Macaranga tanarius*. Funct Ecol 14:749-757

Heil M, Fiala B, Boller T, Linsenmair KE (1999) Reduced chitinase activities in ant-plants of genus *Macaranga*. Naturwissenschaften 86:146-149

Heil M, Fiala B, Kaiser W, Linsenmair KE (1998) Chemical contents of *Macaranga* food bodies: adaptations to their role in ant attraction and nutrition. Funct Ecol 12:117-122

Heil M, Fiala B, Zotz G, Menke P, Maschwitz U (1997) Food body production in *Macaranga triloba* (Euphorbiaceae): a plant investment in antiherbivore defence via symbiotic ant partners. J Ecol 85:847-861

Heil M, Staehelin C, McKey D (2000b). Low chitinase activity in *Acacia* myrmecophytes: a potential trade-off between biotic and chemical defences? Naturwissenschaften 87:555-558

Heil M, Fiala B, Maschwitz U, Linsenmair KE (2001) On benefits of indirect defenceshort- and long-term studies of antiherbivore protection via mutualistic ants. Oecologia 126:395-403

Herms DA, Mattson WJ (1992) The dilemma of plants to grow or to defend. Q Rev Biol 67:283-335

Hnawia E, Thoison O, Guéritte-Voegelein F, Bourret D, Sévenet T (1990) A geranyl substituted flavonol from *Macaranga vedeliana*. Phytochemistry 29:2369-2368

Huxley CR, Cutler DF (eds) (1991) Ant-plant interactions. Oxford University Press, Oxford

Itino T, Davies SJ, Tada H, Hideda Y, Inoguchi M, Itioka T, Yamane S, Inoue T (2001) Cospeciation of ants and plants. Ecol Res 16:787-793

Itioka T, Nomura M, Inui Y, Itino T, Inoue T (2000) Difference in intensity of ant defence among three species of *Macaranga* myrmecophytes in a Southeast Asian dipterocarp forest. Biotropica 32:318-326

Itioka T (2005) Diversity of anti-herbivore defences in *Macaranga*. In: Roubik DW, Sakai S, Karim AAH (eds) Pollination ecology and the rain forest: Sarawak studies. Springer, New York, pp 158-171

Janzen DH (1966) Coevolution of mutualism between ants and acacias in Central America. Evolution 20:249-275

Janzen DH (1974) Tropical black water rivers and mast fruiting by the Dipterocarpaceae. Biotropica 6:69-103

Kato M, Inoue T, Hamid AA, Nagamitsu T, Merdek MB, Nana AR, Itino T, Yamane S, Yumoto T (1995) Seasonality and vertical structure of light-attracted insect communities in a dipterocarp forest in Sarawak. Res Popul Ecol 37:59-79

Lin JH (1993) Studies on tannins of the bark of *M. tanarius* (L.) Muell. Et Arg. Journal of Food Drug Analysis 1:273-280

Lin JH (1994) Studies on tannins of the bark of *M. sinensis* (Baill.) Muell.-Arg. Journal of Food Drug Analysis 2:201-209

Lin JH, Nonaka GI, Nishioka I (1990a) Tannins and related compounds XCIV isolation and characterization of seven new hydrolizable tannins from the leaves of *Macaranga tanarius* (L.) Muell. Et Arg. Chemical and Pharmaceutical Bulletin (Tokyo) 38:1218-1223

Lin JH, Ishimatsu M, Tanaka T, Nonaka GI, Nishioka I (1990b) Tannins and related compounds XCIV structures of macarangins and macarinins. New hydolyzable tannins possessing macaronyl and tergalloyl ester group from the leaves of *M. sinensis* Baill. Muell.-Arg. Chemical and Pharmaceutical Bulletin (Tokyo) 38:1844-1851

Lincolon RJ, Boxshall GA, Clark PF (1982) A dictionary of ecology. Cambridge University Press, Cambridge

Linsenmair KE, Heil M, Kaiser WM, Fiala B, Koch T, Boland W (2001) Adaptations to biotic and abiotic stress: *Macaranga*-ant plants optimize investment in biotic defence. J Exp Bot 52:2057-2065

McKey D (1984) Interaction of the ant-plant *Leonardoxa africana* (Caesalpiniaceae) with its obligate inhabitants in a rainforest in Cameroon. Biotropica 16:81-99

McKey D, Davidson DW, Gay H (1993) Ant-plant symbiosis in Africa and neotropics: history, biogeography, and diversity. In: Goldblatt P (ed) Biological relationships between Africa and South America. Yale University Press, New Haven, Connecticut, pp 568-606

Nomura M, Itioka T (2002) Effects of sysnthesized tannin on the growth and survival of a generalist herbivorous insect, the common cutworm, *Spodoptera litura* (Lepidoptera: Noctuidae). Appl Entomol Zool 37:285-289

Nomura M, Itioka T, Itino T (2000) Variation in abiotic defence within myrmecophytic and non-myrmecophytic species of *Macaranga* in a Bornean dipterocarp forest. Ecol Res 15:1-11

Nomura M, Itioka T, Murase K (2001) Non-ant anti-herbivore defences before plant-ant colonization in *Macaranga* myrmecophytes. Popul Ecol 43:207-212

Okamoto D, Okada S (1968) Studies on the tabacco cutworm, *Sprodenia litura*, Fabricius, as an insect pest of the forage crops. Bulletin of the Chugoku National Agricultural Experiment Station Series E. 2:111-144. [In Japanese with English summary]

Oliveira PS, Oliveira-Filho AT (1991) Distribution of extrafloral nectaries in the woody flora of tropical communities in Western Brazil. In: Price PW, Lewinsohn TM, Fernandes GW, Benson WW (eds) Plant-animal interactions. John Wiley & Sons, New York, pp 163-178

Pollard AJ (1992) The importance of deterrence: responses of grazing animals to plant variation. In: Fritz RS, Simms EL (eds) Plant resistance to herbivores and pathogens. The University of Chicago Press, Chicago, pp 216-239

Read J, Stokes A (2006) Plant biomechanics in an ecological context. Am J Bot 93:1546-1565

Rehr SS, Feeny PP, Janzen DH (1973) Chemical defence in Central American non-ant-acacias. J Anim Ecol 42:405-416

Reich PB, Walters MB, Ellsworth DS (1992) Leaf lifespan in relation to leaf, plant and stand characteristics among diverse ecosystem. Ecol Monogr 62:365-392

Rickson FR (1980) Developmental anatomy and ultrastructure of the ant-food bodies (Beccarian bodies) of *M. triloba* and *M. hypoleuca* (Euphorbiaceae). Am J Bot 67:285-292

Rosenthal GA, Berenbaum MR (eds) (1991) Herbivores-their interactions with secondary plant metabolites. Vol. 1: The chemical participants. Academic Press, New York

Simms EL (1992) Cost of plant resistance to herbivory. In: Fritz RS, Simms EL (eds) Plant resistance to herbivores and pathogens. The University of Chicago Press, Chicago, pp 216-239

Simms EL, Rausher MD (1987) Costs and benefits of the evolution of plant defence against herbivory. Am Nat 130:570-581

Sultana S, Ilyas M (1986) Chromenoflavones from *Macaranga indica*. Phytochemistry 25:953-954

Turner IM (1994) Sclerophylly: primarily protective? Funct Ecol 8:669-675

Waterman PG, Mole S (1994) Methods in ecology: analysis of phenolic plant metabolites. Blackwell Scientific Publications, Oxford

Whitmore TC (1969) First thoughts on species evolution in Malayan *Macaranga* (Studies in *Macaranga* III). Biol J Linn Soc 1:223-231

Whitmore TC (1975) *Macaranga*. In: Airy Shaw HK (ed) The Euphorbiaceae of Borneo. Kew Bulletin Additional Series 4, Kew Gardens, UK, pp 140-159

Whitmore TC (2008) The Genus *Macaranga*. A Prodromus. Royal Botanic Garden, Kew, UK

Williams HJ, Elzen GW, Vinson SB (1988) Parasitoid-host-plant interactions, emphasizing cotton (*Gossypium*). In: Barbosa P, Letourneau DK (eds) Novel aspects of insect-plant interactions. Wiley, New York, pp 171-200

Figure Legends

Fig. 1 Cumulative survival rate hazard curves for common cutworm (*Spodoptera litura*) larvae fed fresh leaves of ten *Macaranga* species: *M. winkleri* (solid squares), *M. trachyphylla* (open stars), *M. bancana* (solid circles), *M. beccariana* (open circles), *M. hypoleuca* (solid triangles), *M. lamellata* (open triangles), *M. umbrosa* (open squares), *M. hullettii* (open diamonds), *M. gigantea* (black crosses) and *M. praestans* (solid stars). Hazard curves end on the day when all larvae had died or reached the pupal stage. Curves were significantly different amongst the ten species (logrank test, $\chi^2 = 1135.818$, $P < 0.0001$). Data for *M. winkleri*, *M. trachyphylla*, *M. beccariana*, *M. gigantea* and *M. praestans* are from Nomura et al. (2000).

Fig. 2 Leaf toughness in ten *Macaranga* species: *M. winkleri* (win), *M. trachyphylla* (tra), *M. bancana* (ban), *M. beccariana* (bec), *M. gigantea* (gig), *M. hypoleuca* (hyp), *M. lamellata* (lam), *M. umbrosa* (umb), *M. hullettii* (hul) and *M. praestans* (pra). Leaf toughness was measured as the weight (mean kg ± SD) required to puncture fresh leaves with a 3-mm-diameter penetrometer column. Means sharing lower case letters are not significantly different from each other (Scheffé's *F* test, $P < 0.05$). Data for *M. winkleri, M. trachyphylla, M. beccariana, M. gigantea and M. praestans* are from Nomura et al. (2000), with additional measurements to increase sample size.

Fig. 3 Total phenol (a) and condensed tannin (b) contents in leaves of ten *Macaranga* species. Phenol content is mg tannic acid/g dry wt, and condensed tannin content is mg cyanidin chloride/g dry wt. See Fig. 2 for the abbreviations of species names. Means sharing lower case letters are not significantly different from each other (Scheffé's *F* test, $P < 0.05$). Means ± SD.

Fig. 4 Leaf turnover (a) and growth (b) rates of ten *Macaranga* species. See text for calculation methods and Fig. 2 for the abbreviations of species names. Means sharing lower case letters are not significantly different from each other (Scheffé's *F* test, $P < 0.05$). Means ± SD.

Fig. 1

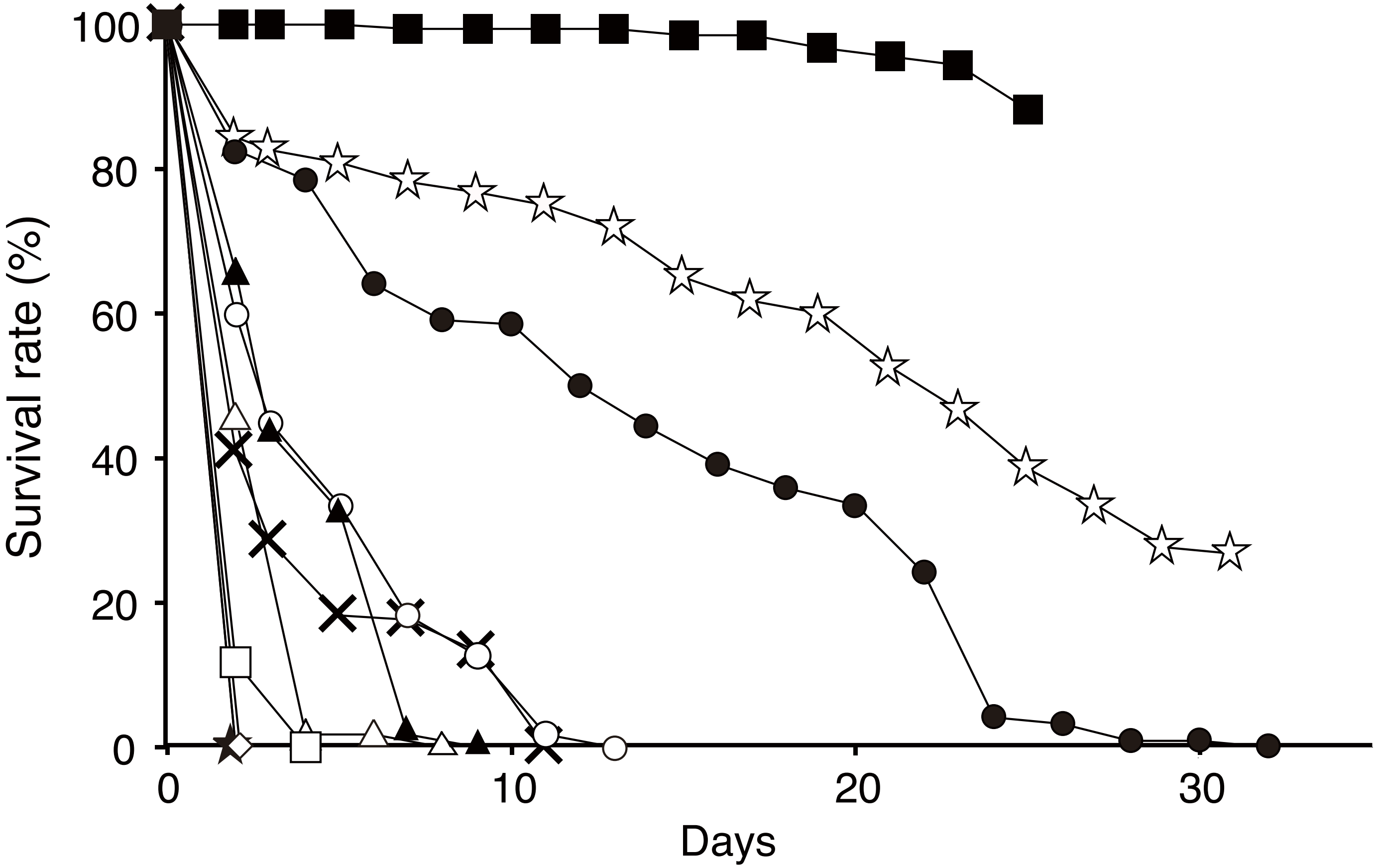


Fig. 2

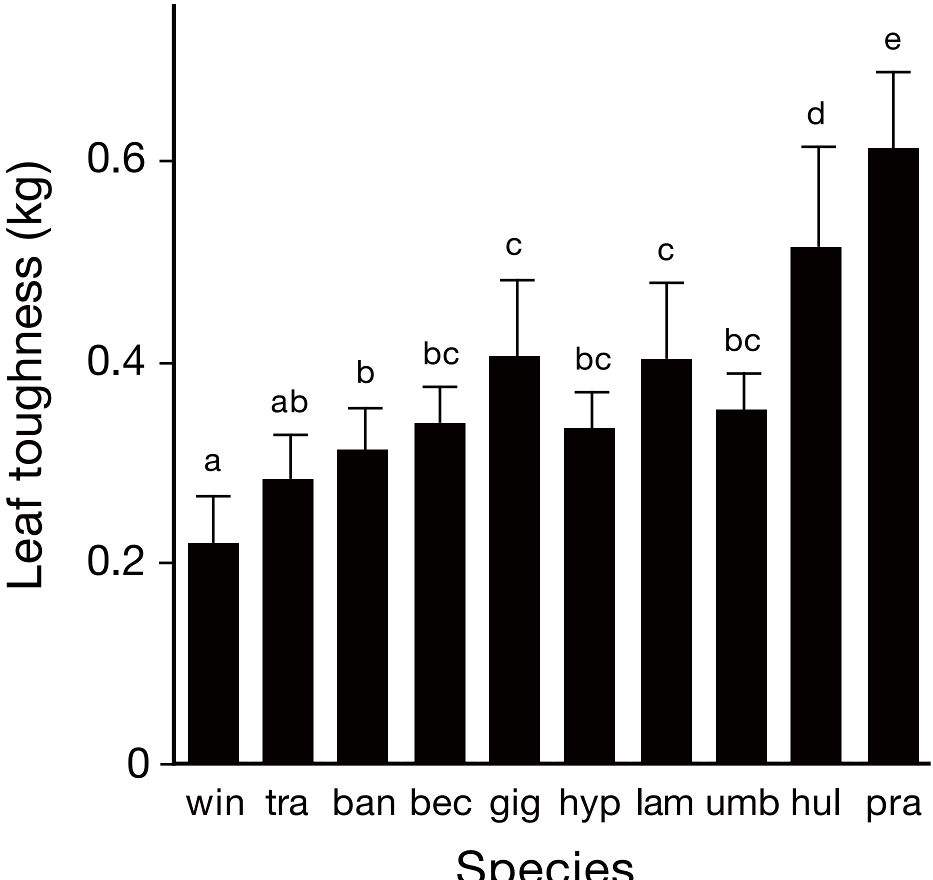
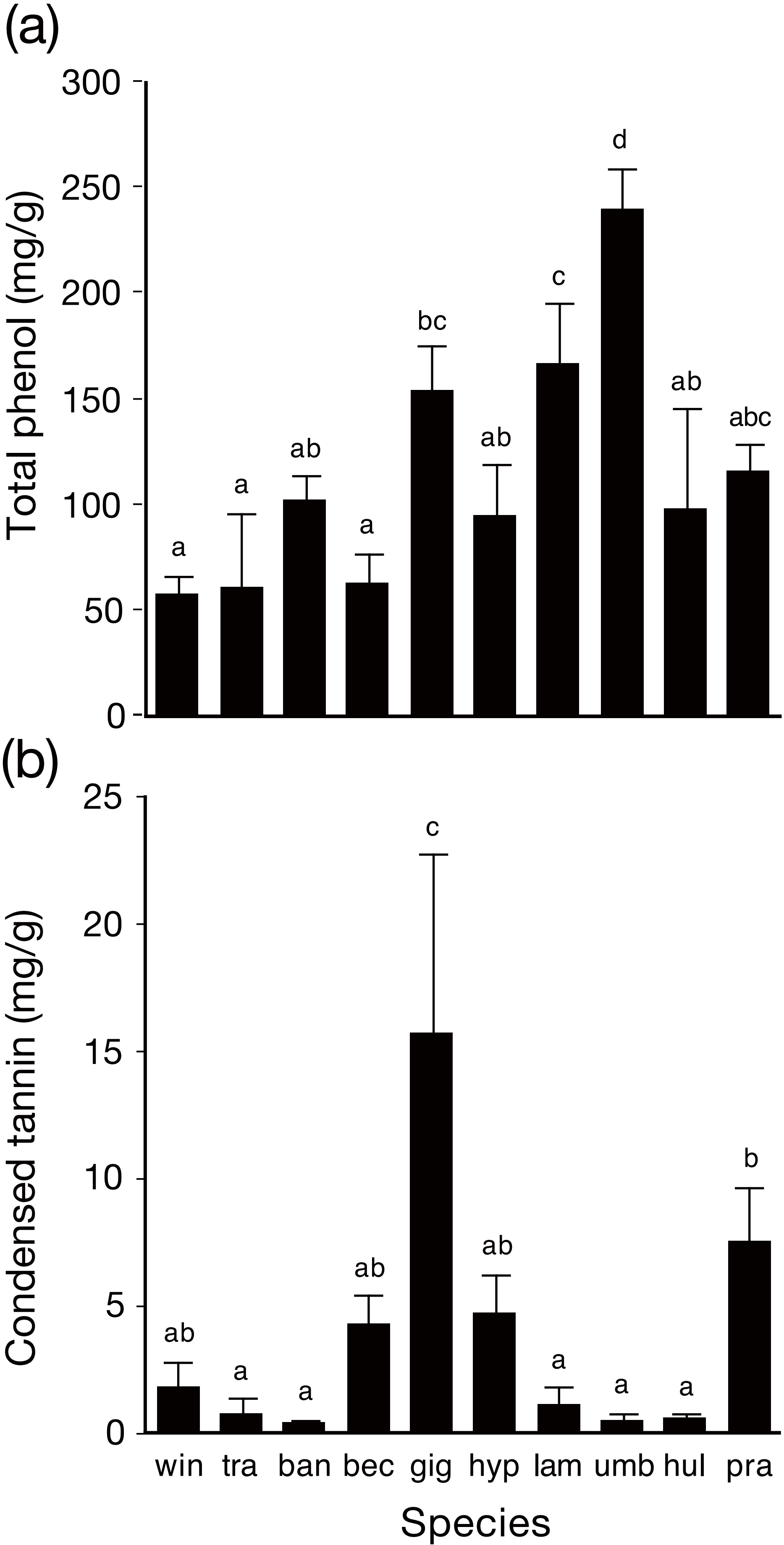

Leaf toughness (kg)
0.6
0.4
0.2
0
a
ab
b
bc
c
bc
c
bc
d
e
win
tra
ban
bec
gig
hyp
lam
umb
hul
pra
Species

Fig. 3


(a)
Total phenol (mg/g)
300
250
200
150
100
50
0
a
a
ab
a
bc
ab
c
d
ab
abc
(b)
Condensed tannin (mg/g)
25
20
15
10
5
0
ab
a
a
ab
c
ab
a
a
a
b
win
tra
ban
bec
gig
hyp
lam
umb
hul
pra
Species

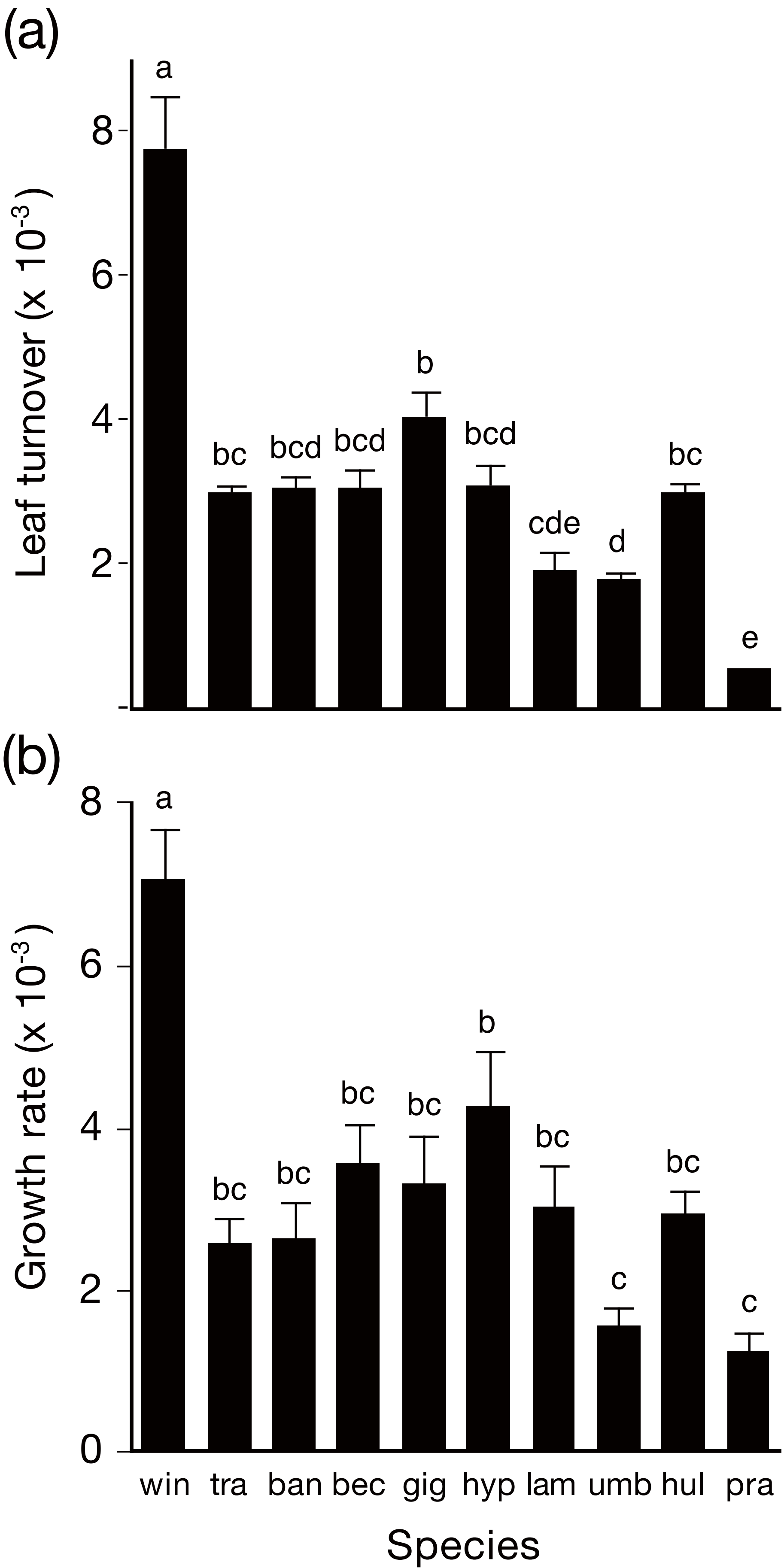

(a)
Leaf turnover (x 10^-3)
8
6
4
2
a
bc
bcd
bcd
b
bcd
cde
d
bc
e
(b)
Growth rate (x 10^-3)
8
6
4
2
0
a
bc
bc
bc
bc
b
bc
c
bc
c
win
tra
ban
bec
gig
hyp
lam
umb
hul
pra
Species

Table 1. Number of ants engaged in the four types of behaviours when a leaf tip (c. 1cm^2) from a different tree was held with forceps in contact with the second node of their nest plant (Mean ± SE).

	Sum	No. of ants (Mean ± SD)			
Species	(Mean ± SE)	Aggregated at the leaf tip	Climbers onto the forceps	Climbers onto the hand	Biters
M. winkleri	82.6^{a} ± 8.3	39.2^{a} ± 3.8	24.6^{a} ± 3.0	16.9^{a} ± 2.6	1.9^{a} ± 0.5
M. trachyphylla	31.0^{b} ± 4.1	19.7^{b} ± 2.7	8.6^{b} ± 1.7	2.3^{b} ± 0.6	0.4^{b} ± 0.2
M. beccariana	15.6^{bc} ± 4.7	7.8^{cd} ± 1.3	5.7^{bc} ± 2.4	2.0^{b} ± 1.4	0.1^{b} ± 0.1
M. bancana	13.4^{bcd} ± 4.6	9.2^{bc} ± 2.1	3.3^{bcd} ± 1.9	0.9^{b} ± 0.8	0
M. hypoleuca	11.3^{cd} ± 1.9	8.2^{c} ± 1.4	2.1^{bcd} ± 0.7	1.0^{b} ± 0.4	0
M. hullettii	10.7^{bcd} ± 3.0	8.1^{bcd} ± 2.6	2.2^{cd} ± 0.5	0.3^{b} ± 0.2	0
M. umbrosa	4.4^{d} ± 1.7	4.0^{cd} ± 1.7	0.4^{d} ± 0.2	0	0
M. lamellata	3.8^{d} ± 0.8	3.0^{d} ± 0.5	0.8^{cd} ± 0.3	0	0

Significant differences were detected amongst eight species for all types of aggressive behaviours (Kruskal-Wallis test, $P < 0.0001$). Means sharing superscript letters are not significantly different from each other in the comparisons amongst species (multiple comparisons based on Mann-Whitney U-test; $P < 0.05$).

Table 2. Kendall's coefficients of rank correlations between the two of the following seven measurements for ten *Macaranga* species: ant defence intensity, non-ant defence intensity, leaf toughness, total phenol content, condensed tannin content, growth rate, leaf turnover rate.

Source of Variation	Non-ant defence	Leaf toughness	Total phenol	Condensed tannin	Growth rate	Leaf turnover rate
Ant defence	$\tau = -0.644$**	$\tau = -0.778$**	$\tau = -0.644$**	NS	NS	NS
Non-ant defence		$\tau = 0.778$**	$\tau = 0.556$*	NS	NS	$\tau = -0.511$*
Leaf toughness				NS	NS	NS
Total phenols				NS	NS	NS
Condensed tannin					NS	NS
Growth rate						$\tau = 0.689$**

* $P < 0.05$, ** $P < 0.01$,