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Geographical variation of mutualistic relationships between *Macaranga* myrmecophytes and their ant partners: research plans in Sarawak

Usun Shimizu-kaya [1,2,6], Yoko Inui [3], Shouhei Ueda [4,5], Takao Itino [4] and Takao Itioka [1]

[1] Graduate School of Human and Environmental Studies, Kyoto University, Kyoto, Japan
[2] Center for Ecological Research, Kyoto University, Otsu, Japan
[3] Osaka Kyoiku University, Kashiwara, Osaka, Japan
[4] Sinshu University, Matsumoto, Japan
[5] Osaka Prefecture University, Sakai, Japan
[6] Author for correspondence (e-mail: shimizu.kaya@gmail.com)

Abstract Myrmecophytes have mutualistic associations with plant-inhabiting ants (so-called plant-ants). They provide plant-ants with nest space and sometimes foods, and in return, the plant-ants protect their host-plants against herbivores, pathogens and climbing plants. In the tree genus *Macaranga* (Euphorbiaceae), 26 species are myrmecophytic, among which 18 species are distributed in Sarawak. The relationships between *Macaranga* myrmecophytes and plant-ants are remarkable for their high species-specificity and strong interdependency. For 20 years, we have studied the myrmecophyte-plant-ant relationships and their effects on the herbivorous insect assemblage associated with *Macaranga* plants at Lambir Hills National Park (LHNP), where 17 *Macaranga* species, including 12 myrmecophytic species, are distributed. Here, we describe 1) some ecological characteristics of *Macaranga* myrmecophytes, 2) the main results of our studies on the character of the mutualisms and their ecological consequences, and 3) our new research plans in Sarawak to investigate the geographical variations in the mutualisms. In our future research, we will enhance collaboration with Sarawakian researchers.

Keywords Ant-plant interactions, Bornean tropical rainforests, Mutualism, Southeast Asian tropics

What are *Macaranga* myrmecophytes?

Myrmecophytes are plants that harbor ant colonies within their hollow structures known as domatia, such as hollow stems and thorns (Davidson and McKay 1993). Ants colonizing myrmecophytes protect their host-plants from herbivory. The associations between myrmecophytes and their ant partners, which are called ‘plant-ants’, have been addressed as a model system of mutualism (Bronstein 1998; Bronstein et al. 2006).

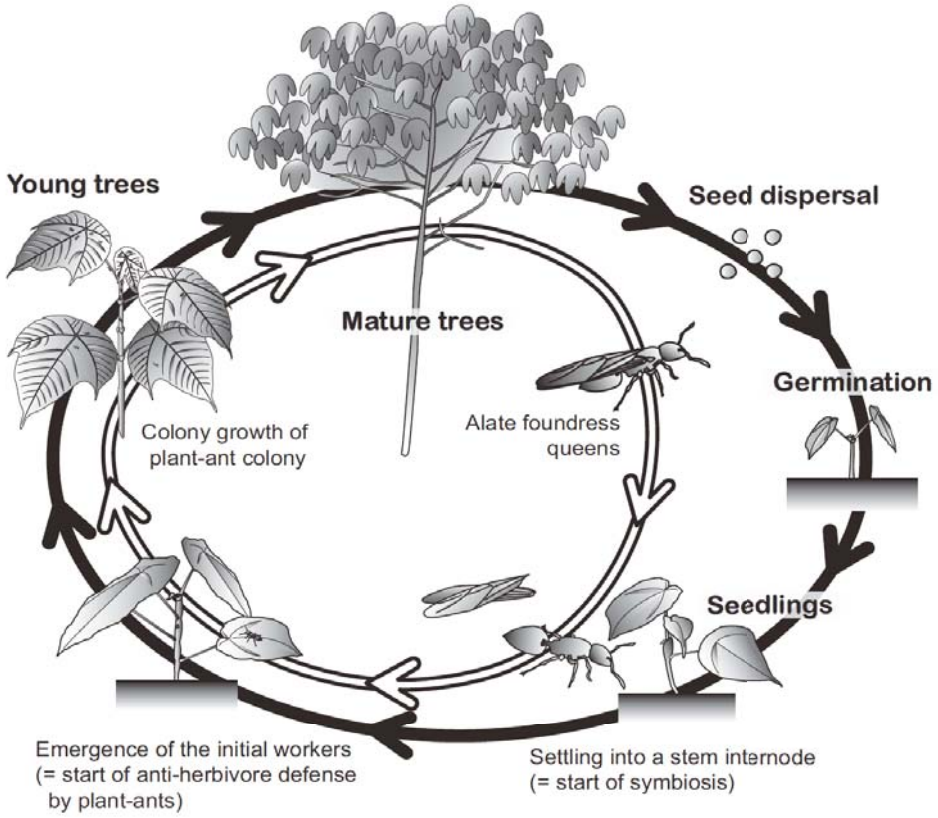

Fig. 1 Life cycles of *Macaranga* myrmecophytes (*black line*) and plant-ant colonies (*white line*).

In the tree genus *Macaranga* (Euphorbiaceae), 26 myrmecophytic species are found in the Malayan Archipelago, and 18 of these species are distributed in Sarawak (Davies 2001; Davies et al. 2001; Whitmore 2008). The myrmecophytic species are mostly inhabited by *Crematogaster* ants (Baker 1934; Fiala et al. 1999). The relationships between *Macaranga* myrmecophytes and *Crematogaster* ants are remarkable for their high species-specificity and strong interdependency (Fiala et al. 1999; Itino et al. 2001a; Quek et al. 2004, 2007).

In most myrmecophytic *Macaranga* species, the plants keep symbiotic interactions with their plant-ants throughout their life cycles except for the periods of seed dispersal and seedling at the early stage (Fig. 1, Fig. 2a). One or a few internodes of stem swell at the stage of young seedling and then become hollow due to degeneration of the pith (Fiala and Maschwitz 1992a; Fig. 2b). The symbiotic relationship between a *Macaranga* myrmecophyte and its plant-ants begins at the time when a foundress queen flies to the seedling with such a hollow stem, a so-called domatium (Fig. 1, Fig. 2c). The queen makes an entrance hole on the stem surface and settles singly into the domatium (Fig. 2c), and begins to rear her initial workers with nutrients that she regurgitates inside the domatium inside which she has confined herself (Fig. 2d). By the time the initial workers emerge from the domatium, the seedling starts to produce nutrient-rich 'food bodies' and 'extra-floral nectaries' (Fiala and Maschwitz 1991, 1992b; Fig. 3). Thus, a mutualistic association begins between them; plant-ants begin to feed on such products provided by their host-plants and in return they protect their hosts from herbivores, pathogens and climbing plants

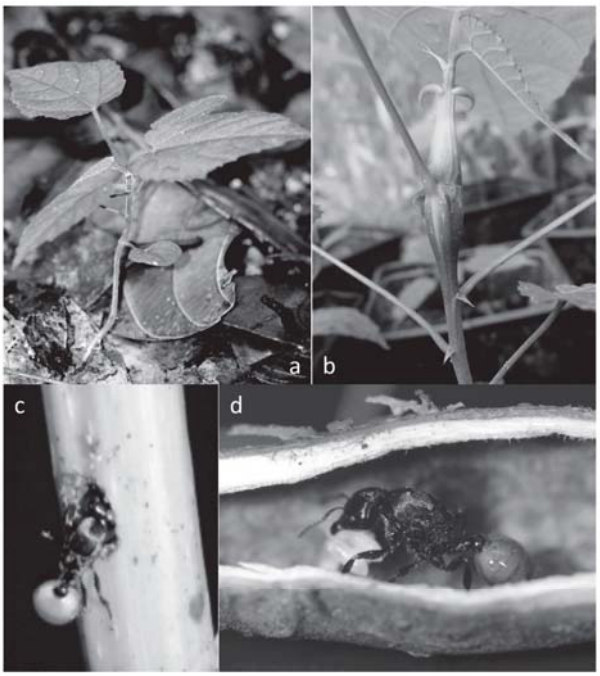

Fig. 2 Before and after the beginning of symbiosis: a) a young seedling of *Macaranga trachyphylla* before the stem swells, b) a young seedling of *M. winkleri* with two swelling internodes of stem, c) a foundress queen of plant-ants making an entrance hole on the hollow stem of a young seedling of *M. winkleri* just after she landed, and d) a foundress queen feeding her initial workers inside a domatium in the hollow stem (dissected) of *M. winkleri.*

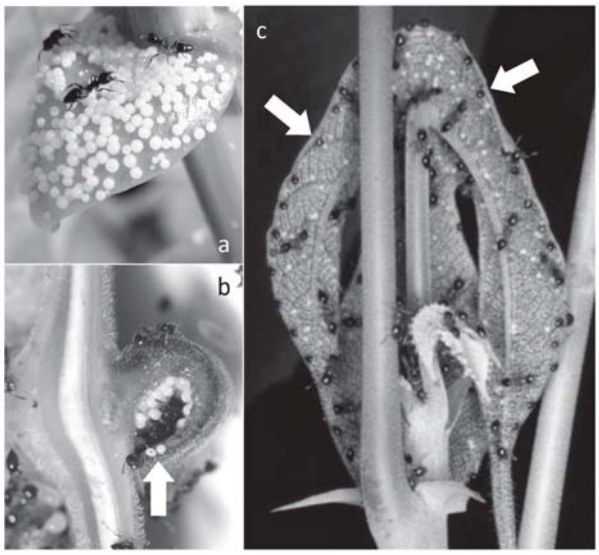

Fig. 3 Foods for plant-ants provided by *Macaranga*: a) food bodies on a flat stipule of *M. rufescens*, b) food bodies produced on the inner surface (abaxial side) of dome-shaped stipule of *M. trachyphylla* (*white arrow*, longitudinal cross-section), and c) food bodies on the undersurface (abaxial side) of the secondly youngest leaf and its marginal glands of extra-floral nectaries (*white arrows*) around the apical part of a sapling of *M. beccariana*.

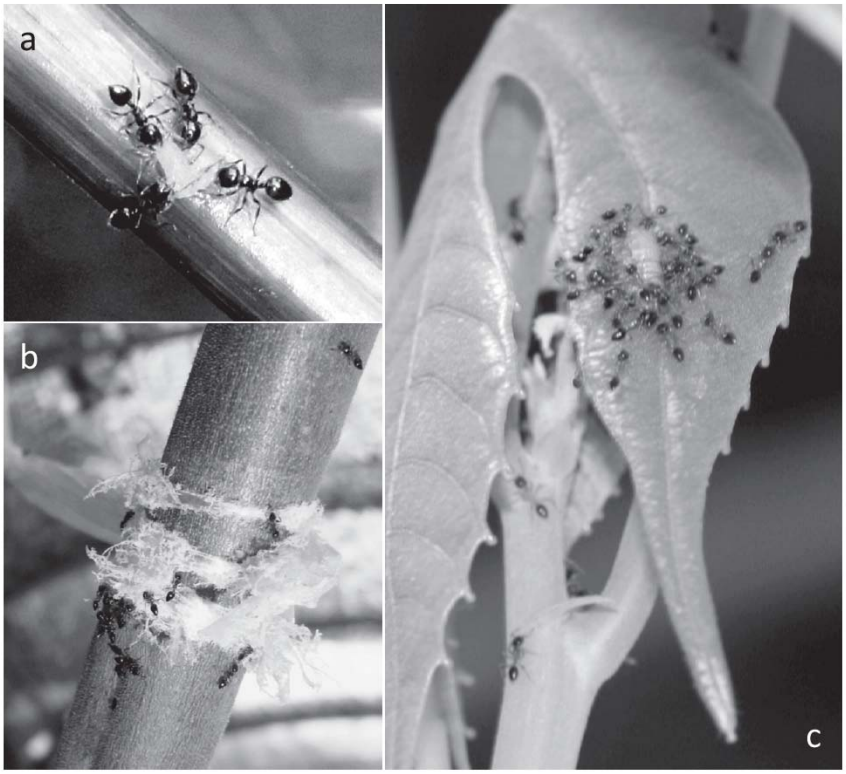

Fig. 4 Defensive behaviors by plant-ants on *Macaranga* myrmecophytes: a) plant-ant workers biting a lepidopteran larva on a stem of *M. bancana*, b) plant-ants biting a string experimentally wound on a stem of *M. trachyphylla*, and c) plant-ant workers surrounding and biting a lepidopteran larva on *M. beccariana.*

Fig. 5 Scale insects tended by a plant-ant worker inside a hollow stem (longitudinal cross-section) of *Macaranga winkleri.*

(Fiala et al. 1989, 1994; Fiala and Maschwitz 1990; Fig. 4). The colony size of plant-ants inside domatia increases with the host-plant's growth until the plant becomes mature (Itino et al. 2001b). The mutualistic association often involves a third partner, scale insects (Coccoidea, Hemiptera) that live inside domatia (Heckroth et al. 1998; Fig. 5). No scale insects live in any domatia at the

time when the initial workers emerge from the domatium, but scale insects soon thereafter settle in the ant nest inside the domatia (Handa et al. 2012). Plant-ants feed on honeydew excreted by the scale insects.

Twenty-year study of *Macaranga* plants in Lambir Hills National Park

We have conducted ecological studies since 1994 of plant-insect interactions on *Macaranga* myrmecophytes in Lambir Hills National Park (LHNP), where 17 *Macaranga* species, including 12 myrmecophytic species, are distributed (Itino et al. 2003; Itioka 2005a; Shimizu-kaya et al. 2015). The major topics of the studies are 1) the evolutionary history of the symbiotic association between *Macaranga* myrmecophytes and their ant partners (Itino 2005; Itino et al. 2001a, 2003; Ueda et al. 2015), 2) how specificity of plant-ants to *Macaranga* myrmecophytes is maintained (Inui et al. 2001; Murase et al. 2002), 3) the diversity of anti-herbivore defense strategies in *Macaranga* (Itioka et al. 2000; Itino and Itioka 2001; Nomura et al. 2000, 2011), and 4) the ecological consequences of anti-herbivore defense in *Macaranga* on the host plant use of insect herbivores (Shimizu-kaya et al. 2013, 2014, 2015; Shimizu-kaya and Itioka 2015). The analyses based on molecular phylogeny suggested that the *Macaranga*-*Crematogaster* mutualism originated about 20 million years ago (Quek et al. 2004, 2007), and the possibility of their co-speciation (Itino et al. 2001a). Our intensive studies in LHNP for 20 years have shown that the degree of plant dependence on their partner ants for anti-herbivore defense varies among *Macaranga* species (Itioka et al. 2000; Nomura et al. 2000, 2011; Itioka 2005a), and that these variations strongly affect the host-plant utilization by insect herbivores feeding on *Macaranga* plants, and consequently their community structure (Itino and Itioka 2001; Itioka 2005b; Shimizu-kaya et al. 2015).

Our future ecological research on *Macaranga* myrmecophytism in the next two decades

Although a series of studies on ecological aspects of *Macaranga* myrmecophytes in LHNP have revealed various characteristics of the mutualisms and their ecological consequences (e.g., Shimizu-kaya and Itioka 2015), there is still a considerable lack of detailed information about the geographical variations. Ant-plant interactions vary among the locations in which the ant species and the properties of the plants are different (Shenoy and Borges 2010). Considering the species diversity in tropical rainforests and the large variation of species compositions among forests, it is strongly expected that *Macaranga* myrmecophytes would show large geographical variations in various ecological traits such as ecophysiology, abundance, species compositions of communities, relationships with their potential ant partners, herbivores, herbivores' enemies, and so on. Along with such geographical variations, the ecological characteristics and dynamics of ant-plant interactions on *Macaranga* myrmecophytes are expected to vary geographically and consequently generate geographical variations in host-plant utilizations and community structures of herbivores feeding on *Macaranga* plants.

There is no doubt that studies on the geographical variations of such ecological traits in the *Macaranga*-associated myrmecophytism will advance our understanding of evolutionary biology and the ecology of mutualisms. From this viewpoint, we are planning to expand our target geographical regions for *Macaranga* research from LHNP to multiple sites over all of Sarawak. At the early stage of our future research, we plan to address the question of whether the species-specificity of *Macaranga*-*Crematogaster* relationships varies among regions over Sarawak. As a next step, we will investigate how the structures and host plant uses in the assemblage of

insect herbivores feeding on *Macaranga* myrmecophytes varies among the regions along with possible geographical variations of the mutualistic characteristics.

The details of the geographical variation of *Macaranga* myrmecophytism remain veiled in mystery, and there are many remaining tropical rainforests suitable for the investigation in Sarawak. For the realization of our future research plan, it will be necessary to strengthen the collaboration between the research staff in Forest Department Sarawak and the Japanese researchers studying the ecology of ant-plant interactions. In addition, in order to conduct field surveys in multiple study sites, we are thinking of calling for various kinds of support from a wide group of people belonging to various organizations all over Sarawak. Especially, participation by Sarawakian researchers in our research plan is highly welcomed.

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