

The Morphological and Ecological Characteristics of *Arhopala* Species (Lycaenidae) Utilizing *Macaranga* Species (Euphorbiaceae) as Host Plants in a Bornean Dipterocarp Forest

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Introduction

The Asian butterfly genus *Arhopala* (Lycaenidae) includes 160 species that are distributed from India to Australia. The *amphimuta* subgroup of this genus is known to utilize *Macaranga* species (Euphorbiaceae) as its host plants (Maschwitz et al. 1984; Megens et al. 2005). Genus *Macaranga* includes more than 20 species of myrmecophytes (Whitmore 1969; Quek et al. 2004). These myrmecophytic species develop mutualistic relationships with species-specific specialist ants of the genera *Crematogaster* or *Camponotus* (Fiala and Maschwitz 1991, 1992). These plants provide nesting space for ants in the internodes and produce food bodies in the leaves and stipules that provide a food resource for the ants. The symbiotic ants exclude herbivores by responding to volatile chemicals from injured tissues of the host plant; they quickly aggregate at the injured points, where they attack the herbivores.

Arhopala larvae could feed on such myrmecophytic *Macaranga* if they are capable of evading the aggressive ants. However, studies of the utilization of *Macaranga* as a host plant by *Arhopala* are limited. In the Malayan peninsula, only three *Macaranga* species have been investigated. Each of these species serves as a host for the larvae of one *Arhopala* species, and symbiotic ants do not appear to attack the *Arhopala* larvae (Maschwitz et al. 1984). There is wide variation in the intensity of the mutual dependency in ant–*Macaranga* interactions, ranging from non-myrmecophytes to facultative, transitional, and obligate myrmecophytes (Fiala and Maschwitz 1990; Fiala et al. 1994). In addition, the defenses of *Macaranga* include defense by ants and chemical or physical defenses (non-ant defenses). The balance between ant and non-ant defenses also differs among *Macaranga* species (Itioka et al. 2000; Nomura et al. 2000; Itioka 2005).

Each *Arhopala* species that utilizes *Macaranga* may overcome one or more components of the variation in anti-herbivore defenses by *Macaranga*. However, the relationship between *Macaranga* and *Arhopala* has not been investigated, and as a result, the survival strategy of each *Arhopala* species that utilizes *Macaranga* has not yet been revealed. In the present study, we investigated the relationships between *Macaranga* and *Arhopala* in Borneo, where the highest species richness of *Macaranga* is found. In addition, we describe the morphology and ecology of the *Arhopala* larvae, and especially the interaction between the larvae and symbiotic ants.

Methods

Study site

Our study was conducted in Lambir Hills National Park, Sarawak, Malaysia (4°2'N, 113°2'E, 150 to 200m

a.s.l.), from the end of May 2006 to the beginning of March 2007. This park is covered primarily by lowland mixed dipterocarp forest. More than 16 *Macaranga* species, including at least 11 myrmecophytic species, occur in the park.

Sampling and rearing

We investigated 11 myrmecophytic and 5 non-myrmecophytic *Macaranga* species to obtain *Arhopala* eggs and larvae. During this collection, we recorded the parts of the plant on which the larvae fed, the location of the larvae, ant behavior in response to the larvae, and the number of attendant ants. The collected eggs and larvae were taken to the laboratory on their host plants for rearing and observation. In the laboratory, the eggs and larvae were maintained in plastic boxes with their host plants until they reached the adult stage. We observed any myrmecophilic organs on the larvae and recorded. The host plants were maintained by cutting the stems and inserting them in sponges used in floral arrangements (i.e., in floral oases).

Results

In total, we recovered 18 eggs and 93 larvae of *Arhopala* from four myrmecophytic and two non-myrmecophytic *Macaranga* species. In the myrmecophytic plants (*M. trachyphylla*, *M. bancana*, *M. beccariana*, and *M. hosei*), each plant hosted the larvae of a single *Arhopala* species; two closely related species (*M. trachyphylla* and *M. bancana*) hosted conspecific *Arhopala*. Two non-myrmecophytic plants (*M. gigantea* and *Macaranga* sp. A) were also utilized by conspecific *Arhopala*. The morphological and ecological characteristics differed among the *Arhopala* species (Table 1).

1. *Macaranga trachyphylla*, *M. bancana* – *Arhopala amphimuta*

Arhopala amphimuta utilized *M. trachyphylla* and *M. bancana* (both myrmecophytes) as its host plant. These two species are closely related. One or two eggs covered with many warts were laid on the underside of fresh leaves (Fig. 1). The emerging light-green larvae rested on the underside of fresh leaves (Fig. 2). Some leaves of *M. trachyphylla* and *M. bancana* developed a red color on their underside. When larvae fed on these red leaves, they tended to develop a red-brown band in the middle part of the green body of the middle-instar larvae (Fig. 3). Larvae that fed on green leaves did not develop this band. All larvae developed myrmecophilic organs (a dorsal nectary organ and tentacle organs; Fig. 4). Symbiotic ants of *Macaranga* did not attack the larvae and instead appeared to tend them (Figs. 2–4). The number of ants that tended a last-instar larva averaged 6.8 ± 0.97 (mean \pm SE, $N = 5$). The pupal body color was similar to that of the stipules of the host plant (Fig. 5). A parasitic fly emerged from the body of a prepupa, and an ichneumonid fly emerged from the body of a pupa. 24% of the collected larvae were parasitized by either a parasitic fly or an ichneumonid fly.

2. *Macaranga beccariana* – *Arhopala zylda*

Arhopala zylda utilized *M. beccariana*, a myrmecophyte host plant. The whitish-green larvae rested on the underside of fresh leaves, and their body color was similar to that of these fresh leaves (Figs. 6–8). The larvae only developed tentacle organs and did not exhibit a dorsal nectary organ. Symbiotic ants of

Macaranga did not tend these larvae, but they also did not attack the larvae. The pupal body color was similar to that of fresh leaves of the host plants (Fig. 9). 23% of the collected larvae were parasitized by either a parasitic fly or an ichneumonid fly.

3. *Macaranga hosei* – *Arhopala dajagaka*

Arhopala dajagaka utilized *M. hosei*, a myrmecophyte host plant. One or two eggs covered with many warts were laid on the underside of fresh leaves (Fig. 10). The larvae had a greenish-yellow color that is similar to that of fresh leaves of the host plant (Figs. 11–13). This species developed two myrmecophilic organs (a dorsal nectary organ and tentacle organs). The larvae of this species appeared to exude more honeydew from their dorsal nectary organ than the larvae of *A. amphimuta*. The number of ants that were tending a last-instar larva averaged 17.5 ± 2.72 (mean \pm SE, $N = 4$). The pupal body color was similar to that of the fresh leaves of the host plants (Fig. 14). 25% of the collected larvae were parasitized by either a parasitic fly or an ichneumonid fly.

4. *Macaranga gigantea*, *Macaranga* sp. A – *Arhopala major*

Arhopala major utilized *M. gigantea* and *Macaranga* sp. A, both non-myrmecophytes, as its host plant. Only one larva was discovered on *Macaranga* sp. A, thus most of the larvae were discovered on *M. gigantea*, which has very large leaves. One to three eggs covered with many warts were laid on the underside of fresh leaves (Fig. 15). The larvae developed two myrmecophilic organs (a dorsal nectary organ and tentacle organs). Their body color ranged from light green to pale yellow and was similar to that of fresh leaves of the host plant (Figs. 16–18). Many fresh leaves had feeding damage caused by these larvae, but the larvae were rarely discovered on fresh leaves. This is because they usually rested inside stipules during the day (Fig. 17). The pupal body color was very similar to that of the stipules of the host plant (Fig. 19). A braconid wasp emerged from the body of a middle-instar larva, and a parasitic fly emerged from the body of a prepupa. 37% of the collected larvae were parasitized by either a parasitic fly or a braconid wasp.

Discussion

In the Malayan peninsula, three *Arhopala* species have been reported to utilize *Macaranga* species (Maschwitz et al. 1984). These species formed three associations: *A. amphimuta*–*M. triloba*, *A. moolaiana*–*M. hulletti*, and *A. zylde*–*M. hypoleuca*. In the present study (in Borneo), *A. amphimuta* utilized *M. trachyphylla* and *M. bancana*, which are closely related to *M. triloba*. *M. triloba* was not found in Lambir Hills National Park. *Arhopala zylde* utilized *M. beccariana*, which is closely related to *M. hypoleuca*, but *M. hypoleuca* was not utilized by larvae of *A. zylde* in Lambir Hills National Park. The relationship between *Macaranga* and *Arhopala* species may thus exhibit a certain degree of species specificity. The larvae of *A. moolaiana* were not found on *Macaranga* in the present study, but many adults of this species were captured. This suggests that *A. moolaiana* larvae will be found on other *Macaranga* species in the Lambir Hills National Park.

The majority of lycaenids develop associations with ants that can be either facultative or obligate and

that range from mutualism to parasitism (Pierce et al. 2002). Ant association has exerted a strong selection pressure on lycaenid larval shape. Thus, the majority of lycaenid larvae develop myrmecophilic organs to protect themselves from ant attack. In the present study, the larvae of *A. amphimuta* and *A. dajagaka* developed two myrmecophilic organs and were tended by symbiotic ants on myrmecophytic *Macaranga*; however, *A. zylta* did not develop a dorsal nectary organ and was not tended by ants on myrmecophytic *Macaranga* (Table 1). Thus, larvae may be tended by the ants because they are attracted by honeydew secreted from the dorsal nectary organ. In addition, the difference in the number of ants found attending a last-instar *Arhopala* larva between *A. amphimuta* and *A. dajagaka* is likely to result from differences in the amount of honeydew provided for the ants.

Despite their lack of a nectary, the larvae of *A. zylta* were not attacked by the ants. These larvae may have evolved a chemical mimicry to penetrate symbiotic ant–*Macaranga* partnerships. The location and parasitoids of the larvae differed between the myrmecophytic and non-myrmecophytic *Macaranga* (Table 1). The larvae of *A. amphimuta*, *A. dajagaka*, and *A. zylta* on myrmecophytic *Macaranga* may protect themselves from braconid wasps by coexisting with the ants, which may defend them from the wasps. On the other hand, the larvae of *A. major* on non-myrmecophytic *Macaranga* may protect themselves from ichneumonid flies by hiding in host stipules. The larvae and pupae of the four *Arhopala* species tended to have coloration and shape similar to those of the fresh leaves or stipules that each species utilizes. The survival strategy of each *Arhopala* species may thus correspond to the specific ant–*Macaranga* symbiosis. The differences in morphological and ecological characteristics among the *Arhopala* species are likely due to differences in the anti-herbivore defense strategy adopted by the *Macaranga* species.

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Figs.1-5. *Arhopala amphimuta*. 1. Egg of *A. amphimuta* after hatching. 2. First instar larva of *A. amphimuta* underside of fresh leaf. 3. Middle instar larva of *A. amphimuta* attended by some symbiotic ants of *M. trachyphylla*. 4. Last instar larva of *A. amphimuta*. **A:** a dosal nectary organ. **B:** tentacle organs. 5. Pupa of *A. amphimuta*.



Figs.6-9. *Arhopala zylda*. 6. First instar larva of *A. zylda* underside of fresh leaf. 7. Middle instar larva of *A. zylda* on stem. 8. Last instar larva of *A. zylda*. 9. Pupa of *A. zylda* underside of fresh leaf.



Figs.10-14. *Arhopala dajagaka*. 10. Egg of *A. dajagaka* underside of fresh leaf. 11. First instar larva of *A. dajagaka* underside of fresh leaf. 12. Middle instar larva of *A. dajagaka* on leaf stem attended by some symbiotic ants of *M. hosei*. 13. Last instar larva of *A. dajagaka*. 14. Pupa of *A. dajagaka*.



Figs.15-19. *Arhopala major*. 15. Egg of *A. major* underside of fresh leaf. 16. Second instar larva of *A. major* underside of fresh leaf. 17. Middle instar larva of *A. major* inside of *M. gigantea* stipule. 18. Last instar larva of *A. major*. 19. Pupa of *A. major*.

Table 1. Summary of morphological and ecological characteristics of *Arhopala* larvae utilizing *Macaranga*

<i>Arhopala</i> species	Host plants	Morphological character			Ecological character			
		The body length of last instar larva	Myrmecophilic organs	Ant behavior to larvae	The number of attendant ants	Location of larvae	Feeding parts	Parasitoid of larvae
<i>A. amphimuta</i>	<i>M. bancana</i> and <i>M. trachyphylla</i>	16.5±0.5mm	Dorsal nectary organ and Tentacle organ	Attendant	6.8±0.97	Underside of fresh leaves	Fresh leaves	Parasitic fly and ichneumon fly
<i>A. zylida</i>	<i>M. beccariana</i>	12.5±0.5mm	Tentacle organ	Non attack	—	Underside of fresh leaves	Fresh leaves and Food-bodies	Parasitic fly and ichneumon fly
<i>A. dajagaka</i>	<i>M. hosei</i>	24.5±0.5mm	Dorsal nectary organ and Tentacle organ	Attendant	17.5±2.72	Underside of fresh leaves	Fresh leaves	Parasitic fly and ichneumon fly
<i>A. major</i>	<i>Macaranga sp.A</i> and <i>M. gigantea</i>	20±1.0mm	Dorsal nectary organ and Tentacle organ	—	—	Inside of stipules	Fresh leaves	Parasitic fly and braconid wasp