

Cospeciation of ants and plants

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Cospeciation, in which both parties of an ecological interaction speciate in parallel with each other, has rarely been reported in biotic associations except the cases for host–parasite interaction. Many tropical plants house ants and thereby gain protection against herbivores. Although these ant–plant symbioses have been regarded as classical cases of coevolved mutualism, no evidence of cospeciation has been documented. The Asian ant–plant association between *Crematogaster* ants and *Macaranga* plants is highly species specific and the molecular phylogeny of the ants parallels the plant phylogeny, reflecting history of cospeciation. Evidence is presented that this association has been maintained over the past seven million years. Phylogeographic patterns of 27 ants from two *Macaranga* species suggest that allopatric cospeciations are still in progress in Asian wet tropics.

Key words: coevolution; *Crematogaster*; *Macaranga*; molecular phylogeny; myrmecophyte.

INTRODUCTION

Ecological interactions between distantly related organisms, including parasitism, predation and mutualism, have generated major diversification in the evolution of organic complexity. The reconstruction of the evolutionary history of these interactions requires phylogenies for both parties of the interaction. They provide a test of whether a particular association was inherited by descent ('cospeciation'), or produced by 'colonization' in which a member of one lineage forms a new association with another lineage. Colonization has been reported to predominate in most biotic associations (Mitter *et al.* 1991), while cospeciation has rarely been documented in animal–plant interac-

tions. The few rare examples of partially cospeciated interaction include plant–herbivore interaction (Farrell & Mitter 1990) and fig–pollinator wasp interaction (Herre *et al.* 1996). Cospeciation is, however, not uncommon in animal host–parasite interactions (Hafner *et al.* 1994; Page *et al.* 1998).

The symbiotic association between ant plants (myrmecophytes) and their inhabitant ants is one of the classical cases of mutualism. The ants nest obligatorily in plant cavities and benefit from foods supplied by the host plants and, in return, the plants benefit from protection offered by the ants against herbivores and vines (Janzen 1966). Mutual dependence of plants and their partner ants has led to ultimate specialization, so in some cases neither party can successfully reproduce without the partner (Schupp 1986; Fiala *et al.* 1989; Treseder *et al.* 1995). These symbioses have been reported to be seldom highly species specific and, even in cases where one-to-one species specificity occurs, phylogenetic patterns in the

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American (Ward 1991; Ayala *et al.* 1996) and African (Chenuil & McKey 1996) ant–plant associations do not support the hypothesis of cospeciation, rather they suggest that various types of colonization by ants have determined these associations (Davidson & McKey 1993a, 1993b).

In Asia, the tree genus *Macaranga* (Euphorbiaceae) includes many ant plant species that have symbiotic association with *Crematogaster* ants. Historically, the specificity of ants to *Macaranga* has been poorly understood, and *Crematogaster borneensis* has been tentatively considered to be a phenotypically very variable ant inhabitant of *Macaranga* (Fiala *et al.* 1989; Fiala & Maschwitz 1990). There is, however, growing recognition that several *Crematogaster* species are involved and they are associated with *Macaranga* in a fairly species-specific manner (Maschwitz *et al.* 1996; Fiala *et al.* 1999). The main questions addressed in the present study are: (i) to what extent is the association of ants and plants specific? (ii) are there any ant ‘host races’ which correspond to particular *Macaranga* species? (iii) are the topologies of ant and plant phylogenies concordant and the timing of divergence simultaneous (both of which would be evidences for cospeciation)?

METHODS

Sampling

For seven of the nine *Macaranga* species two to four plants were surveyed and the inhabitant ants were collected at Lambir Hills National Park (N. P.) (North Borneo, Malaysia). For *Macaranga bancana* and *Macaranga hypoleuca*, inhabitant ants were sampled from seven sites (North Borneo: Lambir Hills N. P. and Mulu N. P.; West Borneo: Semongok Forest Reserve, Kubah N. P., Bau limestone area and Mt. Santubong; Peninsular Malaysia: Kuala Tenbeling).

Sequencing and alignment

A 496-bp mitochondrial (mt)DNA fragment corresponding to positions 1721–2216 in the *Drosophila yakuba* mtDNA genome (partial cytochrome oxidase I (COI) gene) (Clary &

Wolstenholme 1985) was amplified by polymerase chain reaction (PCR) and both strands were directly sequenced. With the outgroup species from Formicinae (*Oecophylla smaragdina* and *Formica fusca*) obtained from DNA Data Bank of Japan (DDBJ, AB019426 and AB010925), the sequences were aligned by CLUSTAL W (Thompson *et al.* 1994) (polymerase chain reaction primer sequences and details of the protocol available from corresponding author). Sequences have been deposited in DDBJ under accession numbers AB030518–AB030564.

Phylogenetic analysis

Phylogenetic analyses of all sample ants were carried out using PAUP* 4.0 (Swofford 1998). Maximum parsimony analysis was performed using the heuristic search with 10 random-addition replications and bisection-reconnection branch swapping, keeping two trees per each replicate search. Maximum likelihood analysis was performed using the Hasegawa–Kishino–Yano (HKY) model (Hasegawa *et al.* 1985).

The phylogeny of *Macaranga* plants from Davies *et al.* (2001) was used, to compare with that of *Crematogaster* ants. This plant phylogeny was derived from combined analyses of morphology and the nuclear ribosomal internal transcribed spacer region (nrITS) DNA of 34 *Macaranga* species with 74 informative morphological characters and 34 informative ITS sites.

To compare the topologies of the ant and plant phylogenies statistically, we used tree mapping with the computer program COMPONENT (Page 1993). Tree mapping measures the overlap between two trees by creating a reconciled tree of the ants and plants, under the assumption that their association is due to association by descent. To construct a reconciled tree, COMPONENT modifies one of the cladograms by duplicating branches as necessary until it includes the topology of the other cladogram. There are two measures of fit that can be tested statistically by comparing the ant tree with many random plant trees. ‘Leaves added’ is the difference between the number of nodes in the ant tree and the reconciled tree, and ‘losses’ is the number of instances in which an ant is absent where it is predicted to occur on the reconciled tree. Both parameters

decrease with increasing similarities of ant and plant trees [see Page (1993) for details].

RESULTS AND DISCUSSION

Our survey in a Bornean rainforest confirmed high specificity between nine species of *Macaranga* and four species of *Crematogaster* (three species of subgenus *Decacrema* and a species of subgenus *Crematogaster*; Table 1). Yet, the specificity looks one-sided: every *Macaranga* species is principally associated with a single ant species, whereas two of the four *Crematogaster* species (*C. (Decacrema) borneensis* and *C. (D.) decamera*) had several plant associates. We hypothesized that such ant species might be a mixture of morphologically similar but reproductively isolated sibling species or races that correspond to particular *Macaranga* species.

To test this, we examined DNA sequence variation of *Crematogaster* ants living in the nine *Macaranga* species. The 496 nucleotide sites yielded 179 parsimony-informative characters. Three equally parsimonious trees were produced, with tree lengths of 389, consistency index (CI) of 0.7275, and retention index (RI) of 0.9274; these trees differed only in sister taxa relations within the clade that includes the uppermost 14 opera-

tional taxonomic units (OTUs) in Fig. 1 (from m-L to c-B, all belonging to *C. borneensis*). Maximum likelihood (ML) analysis identified a single most likely tree, which resembles the parsimony tree in all key features (including the branch length) except for the placement of the *M. hosei* clade (in ML analysis, it is regarded as a sister clade of the uppermost 22 OTUs in Fig. 1).

The ant phylogeny revealed six primary mtDNA lineages (Fig. 1), suggesting that the previously detected two morphological species, *C. (D.) borneensis* and *C. (D.) decamera*, are in fact, each divided into two or more genetically differentiated lineages with different mtDNA types. Four of the six ant lineages (L2, L3, L5 and L6) have basically one specific partner plant species while each of the other two ant lineages (L1 and L4) have as many as three associates (Fig. 1). Despite the insufficiency of free-living and non-symbiotic *Crematogaster* species sampled, the ant phylogeny suggests that *Macaranga*-associated *Crematogaster* have arisen at least twice, namely in subgenera *Decacrema* and *Crematogaster*. Based on the assumption that the mean rate of divergence in mtDNA sequences is 2.3% per million years in arthropods (Brower 1994), the age of diversification in plant-ant subgenus *Decacrema* is estimated to be less than seven million years (Fig. 1).

Table 1 Species specificity between *Macaranga* plants and *Crematogaster* ants*

Plant species	No. plants examined	Ant species (%)				
		<i>C. (Decacrema) decamera</i> group	<i>C. (D.) borneensis</i> group	<i>C. (D.)</i> sp. 4	<i>C. (Crematogaster)</i> sp. 2	<i>Camponotus macarangae</i>
<i>M. beccariana</i>	51	100				
<i>M. havilandii</i>	12	100				
<i>M. hypoleuca</i>	9	100				
<i>M. lamellata</i>	7	43				57
<i>M. trachyphylla</i>	50		100			
<i>M. bancana</i>	42		100			
<i>M. bullettii</i>	6		100			
<i>M. hosei</i>	6			100		
<i>M. winkleri</i>	31		3		97	

*Percent occupation of *Macaranga* (*M.*) plants by obligate ants is indicated. Every *Macaranga* plant harbored only one ant colony with a single queen. Plants >50 cm tall were investigated for all species. In *Macaranga hosei*, plants >250 cm were sampled. The field census was conducted within an area of 1 km² in a tropical lowland dipterocarp forest of Lambir Hills National Park, Sarawak, Borneo. The ant identification was based on worker morphology. *Camponotus macarangae* is one of the two alternative obligate ants of *M. lamellata* (Maschwitz *et al.* 1996).

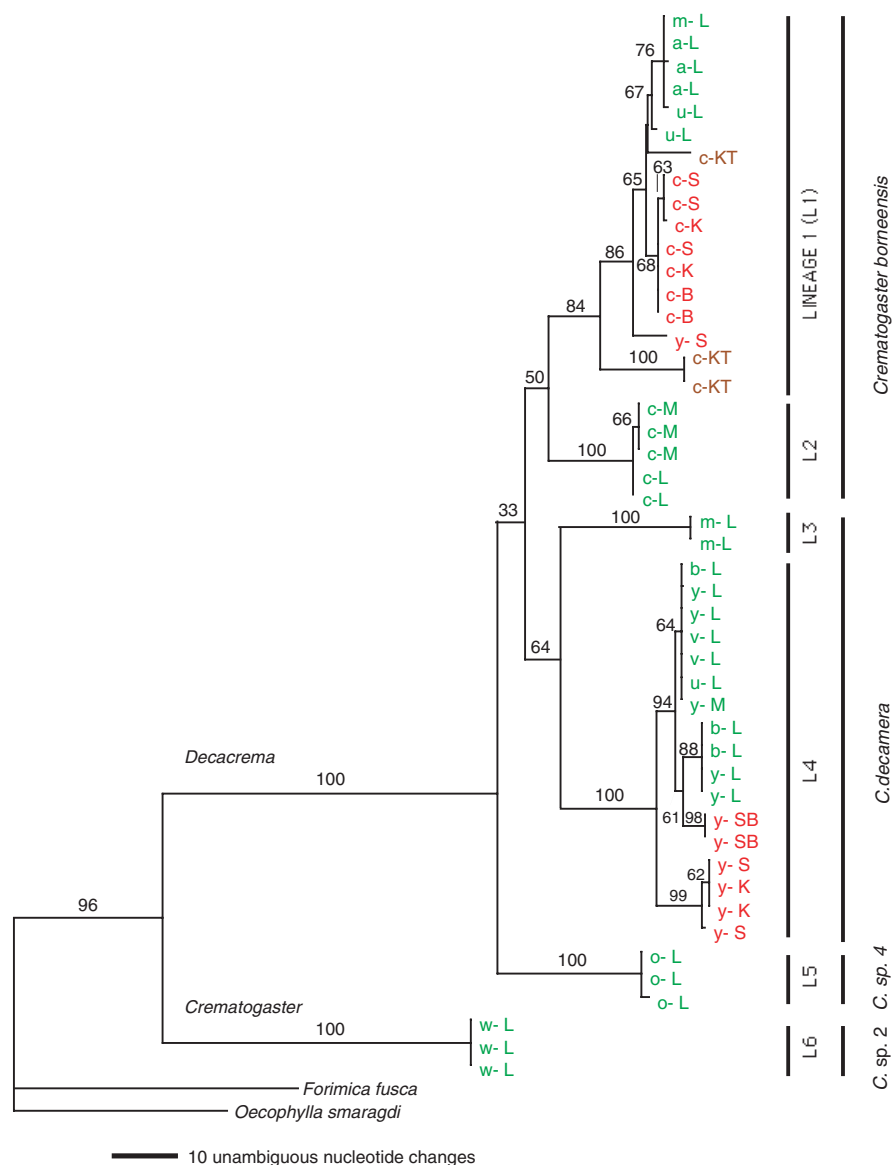


Fig. 1. Phylogeny for 47 *Macaranga*-inhabiting *Crematogaster* ants, as estimated from 496-bp mitochondrial (mt)DNA sequences (cytochrome oxidase I, COI). The host *Macaranga* species and the collection localities are indicated by codes (host plant species – locality). Host plant species codes: a = *M. trachyphylla*, b = *M. beccariana*, c = *M. bancana* (formerly treated as *M. triloba*, Davies *et al.* 2001), m = *M. lamellata*, o = *M. bosei*, u = *M. bullettii*, v = *M. havilandii*, w = *M. winkleri*, and y = *M. hypoleuca*. Localities: B = Bau limestone area, K = Kubah National Park (N. P.), KT = Kuala Tenbeling, L = Lambir Hills N. P., M = Mulu N. P., S = Semonggok Forest Reserve, and SB = Mt Santubong. Region: green = North Borneo, red = West Borneo, and brown = Peninsular Malaysia. Two Formicine species (*Oecophylla smaragdina* and *Formica fusca*) were used as outgroups. Two *Crematogaster* subgenera, *Decacrema* and *Crematogaster* are shown in the figure. The four morphological species and the six mtDNA lineages are indicated. The tree shown is one of three equally parsimonious trees that are almost identical and resemble the tree found using maximum likelihood in all important details (see text for the explanation). Bootstrap percentages from 1000 replications are shown on key branches. Branches are drawn to scale, with the bar representing approx. 2% divergence.

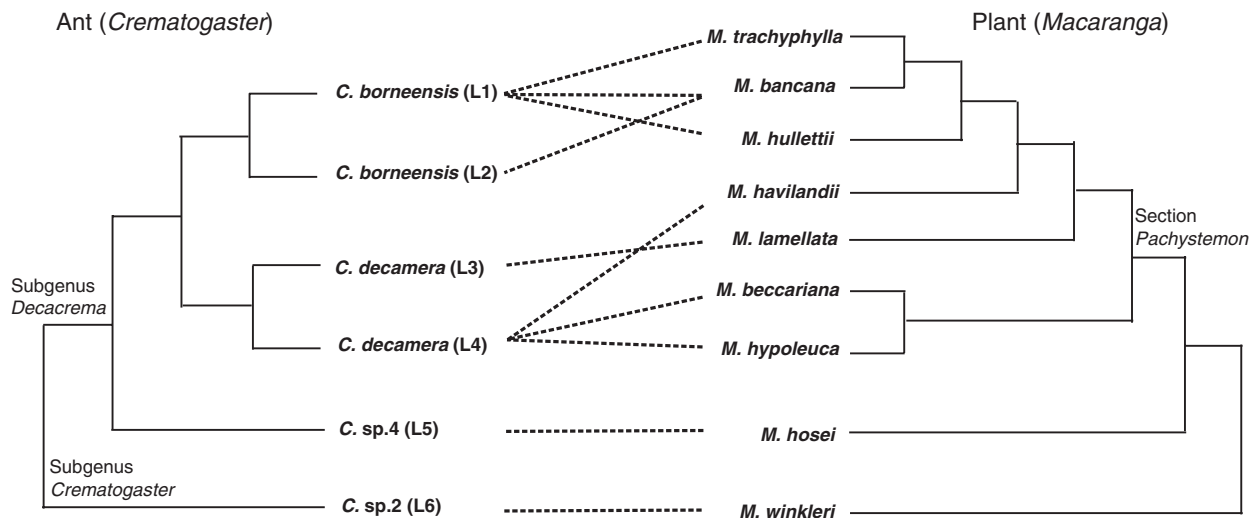


Fig. 2. Phylogeny of symbiont *Crematogaster* ants and phylogeny of the corresponding *Macaranga* hosts. For *Crematogaster*, the morphological species names with mitochondrial (mt)DNA lineage no. in parentheses are indicated (with the codes as in Fig. 1). Dashed lines indicate associations. The ant phylogeny is based on mtDNA sequences (Fig. 1), while the plant phylogeny is based on combined analyses of morphological characters and the nuclear ribosomal internal transcribed spacer region (nrITS) DNA sequences (Davies *et al.* 2001). Only nodes with >50% bootstrapping support are presented as resolved.

Given evidence for the high specificity between ants and plants (Table 1), it is possible to test for cospeciation. The cospeciation hypothesis predicts the topology of ant and plant phylogenies to be concordant and the timing of divergence to be simultaneous. The branching structures of the ant and plant phylogenies are graphically well congruent (Fig. 2) although there is a major disagreement: a member of the *C. decamera* group (L4) seems to have colonized to *Macaranga havilandii* which is presumed to have been an associate of some member of the *C. borneensis* group.

With tree mapping, the congruence of the two phylogenies is statistically significant ($P < 0.0013$ for 'leaves added'; $P < 0.0012$ for 'losses'). In addition to the phylogenetic congruence, Tertiary climatic patterns in Borneo and the restriction of myrmecophytic *Macaranga* to aseasonal forests suggest that this clade of *Macaranga* diversified in the late Tertiary (Morley 1998), which corresponds to the diversification period of the plant ant *Decacrema* (above). These results suggest that the *Macaranga* and *Crematogaster* have been rapidly cospeciating and codiversifying over the past six to seven million years.

The phylogeny of ants from *M. bancana* (abbreviation in Fig. 1 = c) and *Macaranga hypoleuca* (y)

from different geographic areas further suggests a mechanism of cospeciation in the *Macaranga-Crematogaster* mutualism (Fig. 1). The ants typically do not shift hosts between regions, indicating conservatism of the association, although in North Borneo, the ants inhabiting *Macaranga trachyphylla* (a) and *Macaranga hullettii* (u) seem to have made a host shift from *M. bancana* (c) (or recently speciated in parallel with the plants). The subdivision of ant mtDNA types into North-Bornean, West-Bornean and Peninsular-Malaysian lineages indicates recent geographic differentiation of the ants, which is presumably linked with plant allopatric differentiation.

The intimate, one-to-one coevolution of *Macaranga* and *Crematogaster* is unique and presents a striking contrast to the ant-plant associations known from American and African tropics. The associations in American and African regions are less specific (Fonseca & Ganade 1996) and show no evidence for cospeciation (Ward 1991; Ayala *et al.* 1996; Chenuil & McKey 1996). In addition, *Macaranga* is ecologically unique among myrmecophytes in forming diverse communities consisting of up to eight locally sympatric species, with each species inhabiting finely differentiated microhabitats (Davies *et al.* 1998). This kind of

sympatric differentiation within a myrmecophytic plant genus has not been reported from American and African tropical forests. It seems that the coexistence of multiple *Macaranga* species on a small spatial scale has been promoted by the species-specific ant guard system. The symbiont ant species differ in their intensity and way of defense against herbivores (Itioka *et al.* 2000; Itino & Itioka 2001; Itino *et al.* 2001), thus each ant species may create a novel enemy-free space for its host *Macaranga*. We hypothesize that the tight, cospeciating partnership between *Macaranga* and *Crematogaster* is the consequence of their fine-tuned and obligatorily dependent mutualism in long-stable South-East Asian tropical forests.

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