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Ecological studies on coccids inhabiting nests of the plant-ants on *Macaranga* myrmecophytes

Chihiro HANDA

Summary

Chapter 1. General introduction

The genus *Macaranga* (Euphorbiaceae) that is distributed mainly in the Southeast Asian tropics includes over 20 species of myrmecophytes (ant-plants). Myrmecophytes are defined as plants that have special organs that are used by symbiotic ants (plant-ants) as nest spaces. *Macaranga* myrmecophytes harbor their plant-ants inside their hollow stems, and provide them with nutritious lipid and protein-rich food bodies. The plant-ants, in return, attack herbivores to protect their host plants. A large number of studies have clarified details of the relationship between *Macaranga* myrmecophytes and their plant-ants. In some *Macaranga* myrmecophyte species, the relationships between plant-ants and myrmecophytes are so close that each cannot survive without the other. Thus, there are mutualistic relationships between *Macaranga* myrmecophytes and their symbiotic plant-ants.

Almost all *Macaranga* myrmecophytes also harbor coccids, or soft-scale insects, classified as *Coccus* (Hemiptera; Coccidae), in their hollow stems that are used by plant-ants as nest spaces. Coccids in *Macaranga* myrmecophytes are thought to feed on phloem sap inside the hollow stems of *Macaranga* myrmecophytes. Plant-ants on *Macaranga* myrmecophytes have been observed to consume the honeydew excreted by the coccids. However, basic characteristics of such coccids inhabiting *Macaranga* myrmecophytes, such as life history and ecological traits in relation to interactions with their plant-ants and those with their host plant, have not been well elucidated. In this study, I aimed to elucidate the basic ecological characteristics of the coccids that are symbiotic with plant-ants of *Macaranga* myrmecophytes for better understanding of the relationships between coccids and symbiotic plant-ants and the relationships between coccids and *Macaranga* myrmecophytes.

Chapter 2. Study site and materials

The study was conducted in Lambir Hills National Park, Sarawak, Malaysia. The park is mainly covered with primary lowland mixed dipterocarp forest. The uppermost canopy layer ranges from 30 to 40 m, with emergent trees penetrating the layer to heights of >70 m. Clear annual rhythms of rainfall and temperature do not occur. More than 16 species of *Macaranga* plants, including at least 10 species of myrmecophytes that can reach 20 m in height, are found around the study site. In almost all *Macaranga* myrmecophytes, coccids live in ant nests inside their hollow stems. In Chapter 3 and Chapter 5, I targeted a myrmecophyte species, *Macaranga bancana* and its specific symbiotic plant-ants, *Crematogaster* (subgenus *Decacrema*) spp.. In Chapter 4, I targeted a myrmecophytic species *Macaranga beccariana* and its specific ant partner species, *Crematogaster decamera*. By the time these plants reach 50 cm in height, coccids start symbioses in the domatia.

Chapter 3. How do coccids settle into the nests of plant-ants on *Macaranga* myrmecophytes? Dispersal by wind and selection by plant-ants

In this chapter, I elucidated the process of settlement by coccids into *Crematogaster* plant-ant nests formed inside the hollow stems of a myrmecophytic species, *Macaranga bancana*. I collected wafting scale insect nymphs from the canopy using sticky traps and characterized the DNA sequence of the trapped nymphs. In addition, I experimentally introduced first-instar nymphs of both symbiotic and nonsymbiotic scale insects to *M. bancana* seedlings with newly formed plant-ant colonies. Nymphs of symbiotic species were generally carried by ants into their nests within a few minutes of introduction. Most nymphs of nonsymbiotic species were thrown to the ground by ants. These results suggest that in *Crematogaster–Macaranga* myrmecophytism, symbiotic coccids disperse by wind onto host plant seedlings at the nymphal stage, and plant-ants actively carry the nymphs landing on seedlings into their nests in discrimination from nonsymbiotic scale insects.

Chapter 4. Abundance, within-tree distribution, and size structure of coccids in the ant nests on a myrmecophyte, *Macaranga beccariana*

In this chapter, I determined the abundance, within-tree distribution and size structure of coccids that inhabit the nests of plant-ants on a *Macaranga* myrmecophytic species, *M. beccariana.* In order to determine the attributes, I cut down a total of 26 trees of *M. beccariana*, sized between 0.52 and 13.9 m in height in the study site, and then collected all plant-ants and symbiotic coccids on the trees and measured the dry weights of the entire plant body, the whole colony of plant-ants, and all the symbiotic coccids for each of the cut-down trees. The dry weight of symbiotic coccids and that of the plant-ants increased as host plants grew, and the rates of increase for both values declined gradually after trees started to branch. These results suggest that changes in coccid biomass and plant-ant biomass along the ontogenesis of *M. beccariana* are associated with the allometric change of tree structure from the unbranched stage to the branching stage. On the other hand, the ratio of coccid biomass to ant biomass seemed to be constant regardless of growth stages of tree. This suggests that the possibility that the coccids and the plant-ants affect each other's biomass. My analysis of the size structure of symbiotic coccids showed that the density of the larger coccids was lower than that of the smaller coccids, suggesting that smaller coccids suffer higher mortality, which is presumed to be imposed by plant-ants on the population growth of symbiotic coccids. In addition, it was shown that most of the coccids were distributed at the stem area of 100-cm-long apical top of the trees, and that smaller individuals tended to distribute relatively close to the uppermost end of each tree.

Chapter 5. Effects of symbiotic coccid on the plant-ant colony growth in the myrmecophyte *Macaranga bancana*

In this chapter, I investigated how such myrmecophilous coccids, *Coccus* spp. affect the colony growth of plant-ants on the myrmecophytic species *Macaranga bancana*. I compared the growth of plant-ant colonies on seedlings from which coccids were experimentally excluded with that on seedlings into which coccids were experimentally introduced. Foundress queens in 14 of 16 coccid-excluded seedlings died by the time I dissected the seedlings 235–637 days after the seedlings were transplanted in a meshed nursery. In contrast, those on 12 of 17 coccid-introduced seedlings survived during that period, with the survival rate of queens differing significantly between treatments. This result suggests that the symbiotic coccids provide nutrients that facilitate the colony growth of plant-ants inhabiting *Macaranga* myrmecophytes.

Chapter 6. General discussion

Few ecological characteristics of coccids that symbiotically inhabit nests of the plant-ants of *Macaranga* myrmecophytes have been well elucidated so far. In this study, I described some of the ecological characteristics of the symbiotic coccids on two myrmecophytic species of *Macaranga*. Almost all of the results in this study suggest that symbiotic coccids have close relationships with their plant-ants and their *Macaranga* host plants. Selective carriage of nymphs of symbiotic coccids by plant-ants (Chapter 3) suggests the closeness of the relationships and could be considered to function as the mechanisms to maintain the specificity in the relationship between plant-ants and the coccids. Selecting species of hemipterans is expected to be important for attending ants, because the ants gain a profit in fitness if they can attend the coccids that can excrete honeydew efficiently, or those that can excrete honeydew containing essential components for them. The closeness was also suggested by the phenomenon that the ratio of symbiotic coccid biomass to plant-ant biomass rather constant, compared with the change in the ratio of plant-ant biomass to host plant biomass, along the host plant grow (Chapter 4). The observed phenomenon leads us to infer that increase in the number of symbiotic coccids might be regulated by some kind of predictable factors related to the interactions between coccids and plant-ants, such as regulation of the coccid abundance by the plant-ants. The experiments of exclusion and introduction of symbiotic coccids suggest that the growth of plant-ant colonies may decrease in the absence of symbiotic coccids (Chapter 5). Although, at the present, drawing a decisive conclusion from the results of the experiments should be withheld because of the insufficient number of replications of experiments, the results also indicate the tight interspecific interactions between the symbiotic coccids and plant-ants. If the symbioses with coccids are indispensable for the growth of plant-ant colonies, the symbiotic coccids might provide nutrients that are necessary for ovarian maturation of plant-ant queens and/or growth of plant-ant worker individuals and are not contained in the food bodies of *Macaranga* myrmecophyte. This study firstly emphasized the importance of coccids for *Macaranga* myrmecophytism, especially for the plant-ants, although further work on the symbiotic coccids is required.