Chemical ecology of ant-plant dwellers and interactions with the ants

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Abstract Two myrmecophilous insect taxa on myrmecophytes (ant-plants) were studied, *Arhopala* (Lycaenidae) butterfly larvae feeding on *Macaranga* ant-plants and a monotypic cockroach genus inhabiting epiphytic ant-ferns. The chemical profiles of cuticular hydrocarbons did not always match between these myrmecophilous insects and the associated ants on the ant-plants. Chemically, *Arhopala dajagaka* showed good mimicry of the host ant species, *A. amphimuta* does not mimic, and *A. zylda* lacked hydrocarbons and thus appeared to be chemically insignificant. The cuticular hydrocarbons of *Pseudoanaplectinia yumotoi* cockroach were propagated among the host ant colonies. It is suggested that considerable variations exist in the chemical strategies that the myrmecophilous insects on ant-plants use in order to avoid ant attack and profit from the ant–plant associations.

Keywords Chemical mimicry, Cuticular hydrocarbons, Myrmecophyte, Myrmecophile

What are myrmecophytes?

Myrmecophytes, or ant-plants, are plant species that are specialized for mutualistic associations with specific ant species (Janzen 1966; Davidson and McKey 1993). What makes a plant species myrmecophytic is the presence of domatia, which are hollow structures inside plants (Davidson and McKey 1993; Jolivet 1996). When domatia are occupied by ants, they are referred to as myrmecodomatia. Myrmecodomatia are essential structural adaptations of ant-plants for forming mutualistic interactions with ants. For example, *Macaranga* ant-plants (Euphorbiaceae) possess hollow bamboo-like stems that are colonized by ants (Fiala et al. 1989). Similarly, *Acacia* ant-plants (Fabaceae) have enlarged hollow thorns (Janzen 1966), and *Hydnophytum* epiphytes (Rubiaceae) have an enlarged caudex (Huxley 1980). All of these domatia can provide moderate and stable microenvironments for the symbiont ants. Many ant-plants also provide the ants with food, such as extrafloral nectaries and lipid-rich food bodies that are harvested by their ant

symbionts and used as a primary source of food. In some cases, ant-plants are almost exclusively associated with hemipterans, for example *Coccus* scale insects. In addition to the plant secretions, the scale insects provide the ants with sugars in the form of honeydew (Heckroth et al. 1998; Ueda et al. 2012). As a result of being housed and fed by the host ant-plant, some symbiotic ants never need to leave the host plant. In return, the resident ant colony competently provides an anti-herbivore defense service. The ant workers patrol their host plant and attack and remove any intruders, such as herbivorous insects or clinging vines. In this way, the ants fulfill the role of a live-in security guard and they are typically quite aggressive.

While ant-plants are generally well protected by their ant symbionts, some insects still manage to survive and develop on the ant-plants without being attacked by the ants. These myrmecophilous insects have adaptations specialized to life on the host ant-plants; one of which is the use of chemical compounds to penetrate the ant colonies.

Ant chemistry and chemical mimicry

In principle, all insects have epidermal layers covered with cuticular lipids whose primary properties are water-proofing and preventing desiccation (Blomquist and Bagnères 2010). The insect cuticular lipids are composed predominantly of hydrocarbons. In ants, the compositions of these cuticular hydrocarbons are known to be species-specific, and the relative proportions of these compounds are colony-specific. Importantly, the profile of these cuticular hydrocarbons in ants plays a central role in colony-mate recognition (Bonavita-Cougourdan et al. 1987; Howard and Blomquist 2005). Thus, in order to survive in an ant colony, an intruder insect needs a cuticular hydrocarbon profile that is either similar to the profile of, or acceptable to, the host ants. In fact, some myrmecophilous insects have been reported to have cuticular hydrocarbon profiles that closely match those of their host ants. This strategy of matching their cuticular chemicals with those of their hosts is referred to as chemical mimicry (*de novo* biosynthesis) or chemical camouflage (acquisition by passive transfer). Chemical mimicry or camouflage has long been considered to be a prevalent, and even essential, chemical strategy of myrmecophilous insects (Dettner and Liepert 1994; Akino 2008). However, among the ant-plants investigated in the forests of Sarawak, we found two myrmecophilous insect taxa that do not employ chemical mimicry to evade ant attack. These taxa are *Arhopala* caterpillars, which feed on *Macaranga* ant-plants, and a cockroach that inhabits epiphytic ant-ferns. Both of these ant-plants are protected by *Crematogaster* ants in a species-specific manner. Interestingly, these myrmecophiles avoid being attacked by the ants, even though they do not have matching cuticular hydrocarbons.

***Arhopala* larvae on *Macaranga* ant-plants**

We studied three *Arhopala* species, *A. dajagaka*, *A. amphimuta*, and *A. zylda*. The larvae of each species feed on different *Macaranga* species (Okubo et al. 2009), and each *Macaranga* species is habitually defended by a different species of *Crematogaster* ant (Itioka et al. 2000). We collected *Arhopala* larvae and the ants and extracted their cuticular hydrocarbons for analysis with a gas chromatograph-mass spectrometer.

Among the three *Arhopala* species, *A. dajagaka* larvae shared many hydrocarbons with their host ant species. The proportions of the hydrocarbons in both taxa were also very similar. In addition, multi-dimensional scaling revealed that cuticular hydrocarbon profiles were more closely matched between the ant hosts and *A. dajagaka* larvae collected from the same *Macaranga* tree.

This means that *A. dajagaka* larvae can precisely mimic the host-ant hydrocarbons at the colony-level (Inui et al. 2015). This finding was not surprising because similar close matches in the cuticular hydrocarbon profiles between ants and lycaenid butterflies have already been reported (Akino et al. 1999; Hojo et al. 2008).

On the other hand, most of the hydrocarbons extracted from *A. amphimuta* larvae differed from those of their host ants (Inui et al. 2015). Specifically, the hydrocarbons of the larvae were heavier (with higher boiling point) than those found in the ants. This pattern is similar to that of non-myrmecophilic lycaenid species that are facultatively attended by several ant species. Such facultative species are considered to employ specialized organs, such as tentacle organs (TOs), in order to appease ants. However, the function and mechanism of TOs are still unclear. *A. amphimuta* larvae do not mimic the host ants, possibly because *A. amphimuta* larvae feed on several *Macaranga* species, all of which harbor different ant species. Consequently, it is conceivable that chemical mimicry of multiple ant models would involve greater physiological costs to the larvae. Instead, the larvae of *A. amphimuta* may depend largely on myrmecophilous organs to avoid ant attacks.

More surprisingly, in the third species, *A. zylda*, only trace amounts of a few hydrocarbons were detected. In fact, the trace amounts of these components were so low that we considered them to be the result of contamination from the host ants. Thus, it seems that the larvae of *A. zylda* lack cuticular hydrocarbons (Inui et al. 2015). In addition, they also lack myrmecophilous organs, such as dorsal nectary organs (Shimizu-kaya et al. 2013). However, they were almost entirely ignored by the ants. It is presumed that the larvae could achieve this if they are chemically unrecognizable and do not produce any hydrocarbons at all. At present, however, it is almost inconceivable that an insect does not have any cuticular hydrocarbons because these cuticular lipids generally play important roles in insects' survival (Blomquist and Bagnères 2010).

Further analysis revealed the existence of compounds other than hydrocarbons, and these were identified as triterpenoids. Surprisingly, these compounds have not previously been reported in insects but have been reported in epicuticular wax of various plants that have a glaucous surface, such as sedum, common laurel, wax gourd, and *Macaranga beccariana*, the host species of *A. zylda* (Barthlott et al. 1998; Buschhaus and Jetter 2011). The larvae of *A. zylda* do not feed on leaves of the host plant itself, but rather on food bodies that are produced on young leaves, before reaching the final instar of the larval stage (Shimizu-kaya et al. 2014). We analyzed the surface chemicals of these food bodies and found that they contained large amounts of the same triterpenoid compounds. This suggests that *A. zylda* larvae obtain these terpenoids from their food (food bodies) and that they use these compounds to mimic their host plant. We could call this form of mimicry "*phytomimesis*". This strategy is presumed to have arisen because *Macaranga beccariana* has a relatively weak ant-defense system, but a relatively strong chemical defense system, and because the food bodies are produced on the leaf surface, not enclosed by the stipules, and hence are highly accessible.

The variation in these chemical strategies may have arisen due to the variation in ant-plant traits and the intensity of the myrmecophiles' association with the ants.

A curious myrmecophilous cockroach inside epiphytic ant-ferns
In the canopies of *Shorea* trees, two conspicuous epiphytic fern species grow together, both of which are ant-plants that belong to the family Polypodiaceae (Tanaka and Itioka 2011). The fern

rhizomes and basal frond layers form domatia (Gay 1993a, b). The arboreal ant *Crematogaster difformis* monopolistically occupies these fern domatia (Inui et al. 2009; Tanaka et al. 2009). Workers of *C. difformis* are very aggressive and hence keep herbivore damage to the ferns and the host *Shorea* trees low (Tanaka et al. 2009; Tanaka et al. 2012). The presence of *C. difformis* on the ferns also keeps other ant species out the canopy. Nonetheless, despite the aggression of these ants, a small and slow moving cockroach species, *Pseudoanaplectinia yumotoi* Roth, is also found deep inside the domatia of the ferns. This cockroach is the only species in this genus (Roth 1995). We investigated the species composition of the insect taxa within the domatia of the ferns. There were several rare myrmecophilous insect species, with a few to a dozen individuals per fern. However, *P. yumotoi* cockroaches accounted for 10 to 20 % of the insect inhabitants, which is unusually high, and indicates that the cockroach is a myrmecophile (Inui et al. 2009). Workers of *C. difformis* were frequently observed to immediately attack intruders, such as other arboreal ants and cockroaches, but they rarely attacked *P. yumotoi*. Suspecting that the *P. yumotoi* cockroach was a chemical mimic of *C. difformis*, we analyzed the cuticular hydrocarbons of the ants and the cockroaches.

The ants and *P. yumotoi* shared almost all of their hydrocarbons and they had very similar chemical profiles. Myrmecophilous insects typically match their hydrocarbons with those of their host ants through physical contact with the host ants (Dettner and Liepert 1994; Akino 2008). To determine whether the *P. yumotoi* acquires the shared hydrocarbons from the host ant, or they synthesize these hydrocarbons themselves, we reared the ants and cockroaches separately for a week, and then reanalyzed the hydrocarbons. Contrary to our expectations, the hydrocarbon profile of *P. yumotoi* did not change, and even more surprisingly, most of the previously shared compounds were reduced in the host ant (Y. Inui, unpublished data).

This suggests that the shared hydrocarbons were originally derived from the cockroach, not from the ant, and somehow fell away from the ants through isolated rearing. It seems that the *P. yumotoi* cockroach conferred its own chemical identity to the ants on the basis of its high abundance. In general, epiphytes often take up nutrients that are derived from detritus produced by arboreal animals (Huxley 1980). Similar to the other decomposer cockroaches, the digestive enzymes of *P. yumotoi* seem to have substantial cellulase activities (Takakazu Yumoto, personal communication). Based on the consideration of the origin of the shared hydrocarbons used for nestmate recognitions and on that of the general cellulase activities in many decomposer cockroaches, the cockroach is presumed to be the first colonizer of the ferns; after colonization it begins to exploit the ferns for their domatia, forming a nutritional association in the process, and then *C. difformis* joins the association. While the details of the nutrient processing and movement in this tripartite association are still unclear, the cockroach is likely to play an important role in the survival of the ferns. We found that the ferns cannot flourish without the cockroaches, and in some cases, the ferns died when the cockroaches were removed.

Conclusion

Our findings showed that chemical mimicry or camouflage strategies in which myrmecophilous insects employ cuticular hydrocarbon profiles that closely match those of their host ants may actually be less common than was previously thought. It can also be inferred that variation in ant–plant associations may promote diversity in terms of the types of chemical strategies employed by myrmecophilous insects.

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