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Chemoecological study of host selection and host shifts in papilionid butterflies

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Phytochemical constraints are thought to be one of the major determinants of host range evolution and host shifts in phytophagous insects. Papilionid butterflies comprising over 560 species utilize, for larval growth, a limited range of plants in the families of Aristolochiaceae, Annonaceae, Lauraceae, Magnoliaceae, Rutaceae or Apiaceae. Oviposition stimulants so far identified for papilionids are structurally quite diverse and include amino acid derivatives, sugar-related acids, cyclitols, alkaloids, flavonoids and hydroxycinnamates. Some compounds, however, are common stimulants for certain species, and others are structurally related with one another within a given class of compounds, thereby strongly suggesting that phytochemicals mediate host specialization on one hand, and expansion of host range on the other hand. Even so, there still remain several missing links in view of the phytochemical mechanisms underlying the pathway of their host shifts. Comprehensive investigations on plant chemicals that govern their host recognition system are therefore needed to clarify how they have accomplished drastic host shifts, probably from Aristolochiaceae, *via* Magnoliaceae, Annonaceae, Lauraceae and Rutaceae, eventually to Apiaceae and Asteraceae.

Papilio polytes inhabiting the Southwestern Islands of Japan utilizes only a few plant species of Rutaceae as hosts in the field. We examined in detail the acceptability of its major host plant, *Toddalia asiatica*, and four other potential rutaceous hosts, *Glycosmis citrifolia*, *Murraya paniculata*, *Melicope triphylla* and *Phellodendron amurense*, for ovipositing females of the butterfly. Female responses to the foliage, methanol extracts and partitioned fractions from these plants were assayed for the presence of oviposition stimulants and/or deterrents. Larval survivorship on these plant species was also compared as an estimate of fitness. The oviposition bioassays revealed that *T. asiatica* and *G. citrifolia* contain potent oviposition stimulant(s) and that weak stimulant(s) are present in *P. amurense*. Poor or negative oviposition responses to both *Mu. paniculata* and *Me. triphylla* proved to be attributable to strong deterrent(s) present in these plants. While larvae performed very well on *T. asiatica*, *G. citrifolia*, and *P. amurense*, larval mortality was very high on both *Mu. paniculata* and *Me. triphylla*, suggesting the involvement of antifeedant(s) or toxic substance(s) present in these plants.

We attempted to identify oviposition stimulants for the butterfly from *T. asiatica* and *G. citrifolia*. Bioassay-guided fractionation and isolation procedures based on column chromatography, preparative TLC, and HPLC led to the identification of active substances. Two compounds [*trans*-4-hydroxy-*N*-methyl-L-proline and 2-*C*-methyl-D-erythronic acid] were identified as constituents that synergistically stimulate egg-laying by *P. polytes*. A mixture of the two compounds was found to be as active in stimulating oviposition as was the plant foliage. On the other hand, similar procedures led to the identification of an oviposition deterrent [trigonelline] from *Mu. paniculata*. However the deterrent activity of trigonelline was moderate.

Although a number of oviposition stimulants have been reported for some papilionid butterflies, no publication has dealt with oviposition stimulants for Magnoliaceae-feeding papilionid butterflies. We thus attempted to identify oviposition stimulants for *Graphium doson* present in *Michelia compressa* (a primary host plant) and *Magnolia grandiflora* (Magnoliaceae). By analogous methods used for *P. polytes*, an oviposition stimulant was identified as D-pinitol. The compound displayed moderate oviposition-stimulatory activity in itself.

In this study, two oviposition stimulants [*trans*-4-hydroxy-*N*-methyl-L-proline (HMP) and 2-*C*-methyl-D-erythronic acid (MEA)] and a deterrent [trigonelline] were identified as semiochemicals that impart crucial cues to ovipositing *P. polytes* females. Since the chemical structure of HMP is similar to trigonelline, the deterrent activity of trigonelline may possibly be due to the antagonistic effect exerted by the compound acting on the same receptor site.

HMP is similar in chemical structure to L-stachydrine (L-proline *N*, *N*-dimethylbetaine), already shown to be one of the oviposition stimulants for both *P. protenor* and *P. xuthus*, identified from *Citrus unshiu*. MEA is a compound closely related to 2-*C*-methyl D-*erythtono*-1,4-lactone (γ -lactone of MEA), which has been reported to be an oviposition stimulant for *P. bianor* feeding on another rutaceous plant, *Orixa japonica*. Moreover, MEA as well as D-quinic acid, which is also an important oviposition stimulant for *P. protenor* present in *C. unshiu*, is polyhydroxycarboxylic acids with short (5-7) carbon chains. These finding may provide useful clues to the elucidation of sensory mechanisms by which differential acceptance of potential hosts and shifts in host affiliation took place.

On the other hand, pinitol (a cyclitol) was found to be one of the key stimulants responsible for host recognition by *G. doson*. It is very interesting that certain cyclitols are also involved in stimulation of oviposition by some Aristolochiaceae- and Rutaceae-feeders. We propose a possible phytochemical-mediated pathway of host shifts in papilionids in relation to evolution of biosynthesis of cyclitols in plants from *myo*-inositol, *via* sequoyitol and pinitol, to *chiro*-inositol. Since extreme synergism of multiple compounds features the stimulatory system of oviposition by papilionids, particularly *Papilio* butterflies, how other co-occurring compounds are implicated in their host recognition and host range expansion still remains to be studied. Although a hypothesis has been propounded that positive responses to hydroxycinnamic acid derivatives may have been a primitive feature of Papilionidae, we argue that female responses to relatively simple plant primary metabolites such as polyhydroxylated compounds are more likely to be a plesiomorphic trait of the family Papilionidae, for most compounds crucial for eliciting egg-laying are closer to primary metabolites in their structures, rather than being categorized as secondary metabolites.

Key words: Papilionidae, host selection, host shift, oviposition stimulants and deterrents, *Papilio polytes, Graphium doson*, Rutaceae, Magnoliaceae, *trans*-4-hydroxy-*N*-methyl-L-proline, 2-*C*-methyl-D-erythronic acid, trigonelline, D-pinitol