Predator Avoidance Effects of Southeast Asian Danaid Butterflies in the Wild

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Chemical defenses, a typical butterfly defense strategy, have been well studied (Turner 1984), especially for danaid butterflies. The monarch butterfly, *Danaus plexippus* (Danaidae), which ranges mainly in North and Central America, is the best studied species in this family. Monarchs become unpalatable to birds, the major predators of adult butterflies (Edmunds 1974), when their larvae feed on poisonous milkweed (*Asclepias* spp., Asclepiadaceae) or other plants that contain cardiac glycosides (cardenolides). Birds learn to reject monarch butterflies after they have eaten one and vomited (Brower 1958; Brower 1969). Not all of the monarch butterfly's food plants contain cardenolides, however, and birds repeatedly ate monarchs reared on plants that lacked cardenolides (e.g., *Asclepias syriaca*, *A. tuberosa*, and *A. incarnata*). These studies indicate that the palatability of monarch butterflies was directly related to the species of plant ingested by the larvae (Brower 1969).

Other studies have raised doubts about predator avoidance of danaid butterflies in Southeast Asia. Several species of the danaid butterflies in this area are polyphagous and have been observed feeding on a wide range of seven plant families (Robinson et al. 2001), and there is no evidence of cardenolides in these plants. Furthermore, chemical analyses have shown that *Euploea core* (Danaidae), which is one of the most common species of danaid butterfly in this area, stores emetic cardenolides only as larvae and not as pupae and adults when reared on *Nerium oleander* (Apocynaceae) (Malcolm and Rothschild 1983).

We found no field studies of predator avoidance of danaid butterflies in Southeast Asia except for Ohsaki (1995)'s examination of predator avoidance of danaid butterflies. He used "beak marks" (certain types of wing damage that represent an unsuccessful attack by birds; Johki 1985) on butterfly wings as an indicator of predation pressure by birds. A high frequency of beak-marked species should reflect a high attack rate by birds (Edmund 1974). Ohsaki showed that the proportion of individuals with beak marks in the Danaidae family was significantly lower than it was for other nonpoisonous families of butterflies. However, beak-mark frequency is only indirect evidence of bird predation pressure (Edmunds 1974). Thus, we need to evaluate avoidance effects with a more direct method.

Our objective was to investigate the avoidance effects of Southeast Asian danaid butterflies in the wild. We evaluated how much these butterflies were avoided by predators compared with putative nonpoisonous butterflies (mostly Nymphalidae, Satyridae, Pieridae, and Papilionidae) in a tropical rainforest in Lambir National Park (4°20'N, 113°50'E), Sarawak, Malaysia. First, we regularly collected the butterflies and scored them for the presence of beak marks on their wings. Then, as a more direct method of observation, we conducted field presentation experiments employing the butterfly specimens.

Materials and Methods

Beak-mark rate

To determine the proportion of individuals with beak marks (the beak-mark rate), we collected butterflies on an approximate 3-km transect across the inside and outside of a primeval forest in Lambir National Park from 2004 to 2006. We walked the transect in both directions twice a week and captured butterflies that were within 3 m on either side and 5 m in the front with a net (for details on the method, see Pollard 1975). We then recorded the presence or absence of beak marks on the butterfly's wings (for details on the method, see Johki 1985).

We compared the beak-mark rate of danaid butterflies with those of butterflies from four nonpoisonous families (Nymphalidae, Satyridae, Pieridae, and Papilionidae). We omitted some genera (e.g., *Delias, Cethosia, Elymnias,* and *Chilasa*) from this analysis because most of them are thought to be poisonous or to mimic other unpalatable butterflies.

Field presentation experiment

For the field presentation experiment, we selected four abundant species of danaid butterfly (*Euploea diocletianus*, *E. mulciber*, *E. crameri,* and *Parantica aspasia*) and two species of nymphalid butterfly (*Lexias pardalis* and *Tanaecia munda*), which had roughly the same wing size (Otsuka 1998) and sometimes shared the same habitat (personal observation). Food plants of these butterflies at this study site are unknown, but there are several reports that *Euploea* and *Parantica* in Southest Asia feed mainly on Asclepiadaceae, Apocynaceae, and Moraceae (many species in these families are inferred to be poisonous), whereas *Tanaecia* and *Lexias* probably feed on plants that are not poisonous (e.g., Lecythidaceae and Hypericaceae, respectively) (Robinson et al. 2006).

We carried out a field presentation experiment during the day in March, May-June, and August-October 2003 and April 2004. We caught the butterflies with a net and stored them in a freezer. One day before the experiments, we took them out of the freezer and then unfolded the specimen's wings at about a 90° angle and fixed them in order to make the specimens imitate the natural state of a butterfly's wings.

We paired one specimen from each of the four Danaidae species with one from each of the two Nymphalidae species to make eight different types of butterfly pairs. We selected a pair from among the eight and tied the thorax of each butterfly specimen with a fishing line. The line was attached to a fishing rod, and two rods were used for the presentation of one pair.

We simultaneously passed the ends of lines holding the specimen pair from the canopy walkway to the subcanopy layer (roughly 10 m below). We defined a wild bird's response as an "approach" when a bird came within 2 m of either specimen. We recorded the species of the approached butterfly specimen and the bird whenever possible. If no bird approached the offered specimens within 20 minutes, we removed them and presented them again at another point. We compared the approach frequency between the danaid and nymphalid butterfly specimens for each of the eight pairs.

Results

Beak-mark rate

Figure 1 shows the mean beak-mark rate of each family. The beak-mark rate of family Danaidae was significantly lower than those of the Satyridae, Nymphalidae, and Pieridae families (Ryan's

multiple-comparison method, $P < 0.05$ in each case) but not for Papilionidae. In contrast, we found no significant differences among the four nonpoisonous families ($P > 0.05$ in each case).

Field presentation experiment

In total, 399 pair-wise presentations were conducted, and 53 specimens were approached by birds. All specimens approached were Nymphalidae butterflies—no Danaidae butterflies were approached. The approach responses of birds were significantly less frequent to Danaidae specimens than to Nymphalidae ones for five of the eight pairs (Fisher's exact test, $P<0.01$) (Fig. 2). The total approach frequency was insufficient for statistical analyses for the other three pairs. In some cases, we could identify the bird species approaching the offered specimen, but precise determination was often difficult. We recorded birds from at least three families (Pycnonotidae, Timaliidae, and Irenidae).

Discussion

Beak marks have been interpreted as evidence of active escape by the prey (Edmunds 1974). If danaid butterflies are unpalatable, then birds that have sampled them may learn to avoid them. Thus, there should be fewer attacks and beak marks on danaid butterflies than on other butterflies. The low beak-mark rate we observed for danaid butterflies suggests that they were attacked less frequently by birds than were other putative nonpoisonous butterflies at this study area. These results are in agreement with those found by Ohsaki's (1995) study conducted in Sabah, Malaysia. However, beak-mark rates in natural populations of butterflies are not only affected by palatability (Edmunds 1974). Thus, we must be careful when interpreting these results.

The low approach frequency of birds to the offered danaid specimens more directly showed that they were avoided by some birds in the wild. Among the birds, most species of Pycnonotidae and some of Timaliidae are known to forage on some insects on trees (Smythies 1999). Therefore, we interpreted the bird's approach behavior as an attempt to prey on the offered specimen. Indeed, the bird caught the specimen in some cases. In many of the cases, the bird most likely noticed the fishing line just before attacking the butterfly and left.

Our results suggest that some insectivorous birds were more reluctant to attack danaid butterflies than other butterflies at this study site. This rejection may be due to unpalatability caused by the presence of some chemical substances in the Danaid butterflies.

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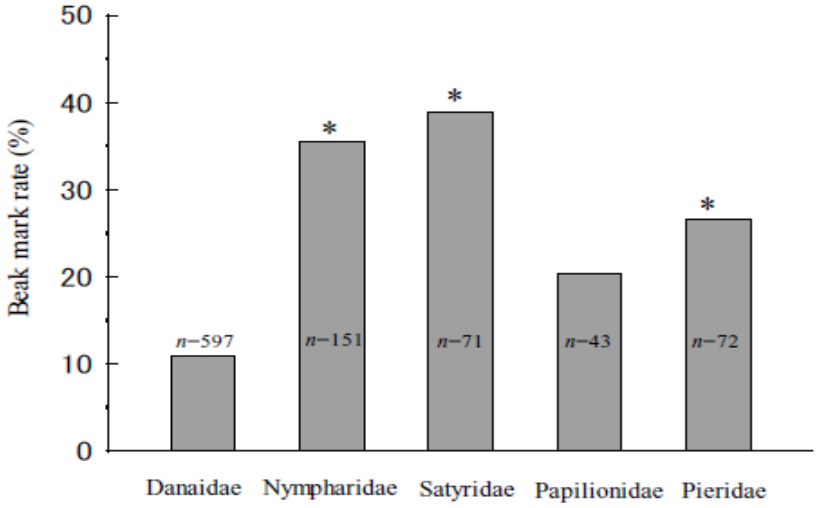

Fig. 1. Percentage of butterflies with beak marks in each families.
* indicates the beak mark rate differes siginigicntly from its Danaidae (Ryan's multiple comparison test $P<0.05$).

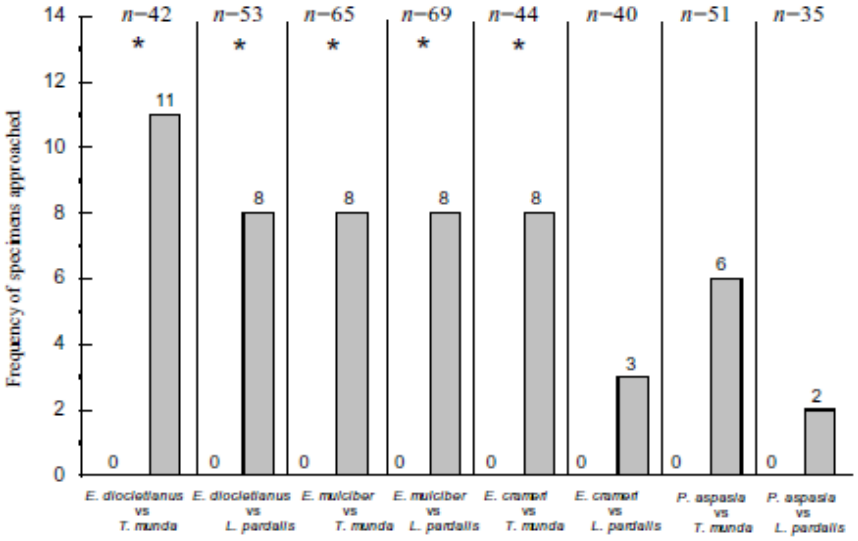

Fig. 2. Number of butterfly speciemens attacked in each pair wise presentation experiment. * indicates significant difference (Fisher's exact test $P<0.01$).