Safeway

PLANT POISONS IN A TERRESTRIAL FOOD CHAIN*

By Lincoln P. Brower, Jane Van Zandt Brower, and Joseph M. Corvino

DEPARTMENT OF BIOLOGY, AMHERST COLLEGE, AMHERST, MASSACHUSETTS

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Abundant evidence indicates that the primary adaptive role of secondary plant substances is the defense against herbivorous vertebrates and invertebrates.[1, 2] Of even greater evolutionary interest, however, is the important ecological theory that certain herbivores have evolved the ability to sequester noxious molecules from their food plants which they in turn use to deter their own predators.[1] This paper will present the results of experiments designed to test this theory for a food chain involving milkweed plants (Asclepiadaceae), the monarch butterfly (*Danaus plexippus* L.), whose larvae eat the leaves of these plants, and the blue jay (*Cyanocitta cristata bromia* Oberholser), an omnivorous bird.

1. *Experimental Design.*—The monarch butterfly was used because of its unpalatability to avian predators, and because of the close larval food plant association of the entire subfamily, Danainae, with the classically poisonous plant families, Asclepiadaceae and Apocyanaceae.[1–3] These plants contain digitalis-like cardiac glycosides which are of extreme potency as vertebrate heart toxins.[1, 4–6]

In order to test the molecular-sequestering hypothesis, it is of great importance that the insect be holometabolous to show definitively that the plant poisons are assimilated by the adult. In contrast, hemimetabolous insects such as grasshoppers could utilize gut storage of the plant material. Furthermore, in the absence of complete metamorphosis in terrestrial insects, the adult food is likely to be the same as or similar to that of the nymph, with the result that the imago is not a pristine entity as far as food intake is concerned. Use of the monarch butterfly avoided these complications.

Our rationale in attempting to demonstrate that the food plant is the source of unpalatability involved the selection of a strain of monarch butterflies that would feed upon a plant belonging to a nonpoisonous group. Cabbage (*Brassica oleraceae* L., Cruciferae[7]) was chosen because it is not known[4, 8] to contain the characteristic cardiac glycosides of the milkweeds and because it is easily grown in a greenhouse. It would be possible, then, to compare the effect upon individual birds of first feeding them cabbage-reared insects, and then milkweed-reared ones. Thus each bird would serve as its own control. Our prediction was that the cabbage-reared butterflies would be palatable, whereas those reared on milkweed would not. Our first experiment (birds 1–5) compared the palatibility of the cabbage-reared monarchs with that of monarchs reared on *Asclepias curassavica* L., a species known to contain several cardiac glycosides.[9–11]

The second experiment (birds 6–8) was carried out to test the palatability of monarchs reared on a taxonomically distant species of the asclepiadaceous plant *Gonolobus rostratus* (Vahl), Roemer and Schultes.[12] Climbing in habit, this plant genus is very different from *Asclepias*, and occurs widely in the New World tropics.[13] One species (*Gonolobus laevis* Michx.), upon which the monarch has been reported,[14] occurs as far north as Pennsylvania.[15] (In Trinidad, West Indies, August 5, 1964, Arima Valley, we found a third instar larva of the monarch on *G. rostratus*, though here *Asclepias curassavica* is clearly its preferred food plant.) The plan was to feed the birds as before on cabbage-reared monarchs and then to test them on the *Gonolobus*-reared ones,[16] our prediction being that the latter would be extremely unpalatable.

2. *Methods and Materials.*—The original stock of monarch butterflies was obtained in Florida[17] during March 1965, and was maintained for 20 generations on *Asclepias curassavica* at our Amherst College laboratory. This stock was crossed with another from Florida after 8 generations, and the material used in the experiment was accumulated from the ninth through the fifteenth generations of this amalgamated stock.

The *A. curassavica* plants were grown from seeds obtained in the vicinity of Mayaro, Trinidad, West Indies, and also from cuttings of these plants. It is possible that some of the seedlings came from three plants of unknown origin, although all are unquestionably *A. curassavica.*

Selection of a cabbage-eating strain in early 1966 was difficult. Young first instar larvae were removed from *A. curassavica* plants upon which the females had oviposited and were placed in small plastic containers[18] on cabbage leaves taken from 1- to 3-in.-high seedlings. All the larvae refused to eat, and died. A second approach was made by painting the cabbage leaves with a crude ether extract of *A. curassavica* leaves. Some first instar larvae ate these leaves, and three ultimately reached the fourth instar but ceased feeding. They were returned to *A. curassavica,* upon which they completed development. These three were mated *inter se* but produced no fertile eggs and were therefore bred to individuals that had been reared on *A. curassavica.* Numerous fertile eggs resulted and upon hatching were divided into two groups. Group 1 was left on *A. curassavica* and reared through all stages on it. Group 2 was transferred onto cabbage which was now (and subsequently) *not* painted with the ether extract. Larvae in this group developed slowly on the exclusive cabbage diet, with high mortality particularly from the fourth instar to the pupal stage. Although a few adults were obtained, none was fertile.

To continue the line, fourth instar cabbage larvae were transferred back to *A. curassavica* to complete their development normally. Matings of these transfers among themselves and also with siblings from group 1 produced eggs for the next generation that were similarly subdivided, and so on, allowing the maintenance of the cabbage-eating strain for five generations.

Exclusively cabbage-fed (except for the possible ingestion of a small amount of *A. curassavica* during the first instar prior to putting the insects on cabbage) larvae, prepupae, and adults were frozen in a deep freezer and accumulated over the five generations along with comparable material fed on an exclusive *A. curassavica* diet. The only difference between the two was that because of continued attempts to breed them, the cabbage-reared adults were one to several days old, whereas the *Asclepias*-reared ones were always frozen before they were 48 hours old.

In the experiments, we fed each jay one of three stages of monarchs: fifth instar larvae, prepupae (very late fifth instar larvae, which had evacuated their gut contents), or adult males. All material was thawed to room temperature before being presented to the birds.

The blue jays were caught in the wild (in Franklin County, Massachusetts) by mist netting and were stored in our aviary for a few days to several weeks before use.

The sex of the birds was not determined. The four jays of the first experiment were placed in individual cages in the laboratory and given the experimental food manually, as described previously.[3] Preliminary experiments established that although the birds freely accepted mealworms, all initially refused cabbage-reared monarch larvae on sight. This reluctance was overcome by an extreme food deprivation schedule: birds were restored to their regular diet only when they accepted the cabbage-reared monarchs. Of six birds, two died of starvation before eating and four (birds 1–4) were tested. With birds 5–8, new automatic feeders[19] were employed which obviated the long deprivation period.

The experiments with birds 1–4 were conducted from March 19 to April 13, 1966, using fourth instar larvae for two birds, prepupae for one bird, and the bodies of adult male butterflies (from which wings and legs had been removed) for one bird. The experiments with birds 5–8, using adult male butterflies, were carried out in December 1966. All five birds receiving adult male monarchs were initially broken in on similarly prepared adult males of *Anartia amalthea* (L.), a known palatable Nymphaline species.[1] The visible behavior of each bird was recorded in writing for at least 30 minutes after it had eaten all the experimental insects, or, if some material was left, for 30 minutes after it stopped eating.

Birds 1–5 were given one half of their total cabbage-reared monarchs on day 1, followed by the remaining half on day 2. On day 3 they were given the *Asclepias* material. On a final day, all five birds were given mealworms as a terminal control insect. Birds 6–8 were fed all individuals in each monarch category on separate days. Bird 6 received its mealworms on a final day, whereas birds 7 and 8 were given theirs three hours after receiving the *Asclepias*-reared monarchs.

3. *Results.*—Once the birds overcame their initial hesitancy to attack the cabbage-reared monarchs, they ate rapidly with no signs of unpalatability or sickness whether larvae, prepupae, or adult males (birds 1–6, Table 1). Their reaction to these, following ingestion, was virtually indistinguishable from that after eating mealworms.

In great contrast to the cabbage-fed monarchs, those reared on *Asclepias curassavica* caused all eight birds to become sick. Ingestion of these was followed uniformly by violent retching and vomiting of the partially digested insects and fluid (Table 1). Other less objective indications of unpalatability included excessive bill-wiping, crouching, alternate fluffing and flattening of the feathers, erratic movements about the cage, jerky movements of head, wings, and thoracic regions, partial closure of the eyes, eating of sand, twitching, and a generally sick appearance. The range of time for vomiting to occur following ingestion of the first *Asclepias*-reared insect was from 8 minutes and 10 seconds to 14 minutes and 30 seconds (Table 1). Recovery from the vomiting and return to a normal appearance seemed complete from approximately 20 minutes to 1 hour following ingestion of the first insect.

The results obtained with *Gonolobus*-reared butterflies were contrary to prediction: bird 6 found them as palatable as the cabbage-reared ones. Because of this, it was possible to substitute *Gonolobus*-monarchs for cabbage ones that were in short supply (birds 7 and 8). Both of these birds likewise avidly ate four *Gonolobus*-monarch males with no ill effects.

It is shown in Table 1 (bird 5) that vomiting was even caused by the ingestion of less than one *Asclepias*-reared male. The minimum number of cabbage-reared

TABLE 1

FREQUENCY AND TIME ELAPSED BEFORE VOMITING IN BLUE JAYS FOLLOWING INGESTION OF MONARCH BUTTERFLIES REARED ON THREE LARVAL FOOD PLANTS

	No. offered	No. eaten	Vomiting frequency*	Time before vomiting†
Bird 1. (larvae)				
Cabbage-fed‡	10	10	0	—
Asclepias curassavica-fed	5	3	9	9′ 20″
Mealworms	5	5	0	—
Bird 2. (larvae)				
Cabbage-fed‡	10	9	0	—
Asclepias curassavica-fed	5	2	5	14′ 30″
Mealworms	5	5	0	—
Bird 3. (prepupae)				
Cabbage-fed‡	8	8	0	—
Asclepias curassavica-fed	4	1	3	14′ 00″
Mealworms	4	4	0	—
Bird 4. (adults)				
Cabbage-fed‡	4	4	0	—
Asclepias curassavica-fed	2	1.5	9	11′ 30″
Mealworms	2	2	0	—
Bird 5. (adults)				
Cabbage-fed‡	4	4	0	—
Asclepias curassavica-fed	2	0.75	5	12′ 00″
Mealworms	4	4	0	—
Bird 6. (adults)				
Cabbage-fed	2	2	0	—
Gonolobus rostratus-fed	4	4	0	—
Asclepias curassavica-fed	2	1.5	4	13′ 00″
Mealworms	>5	>5	0	—
Bird 7. (adults)				
Gonolobus rostratus-fed	4	4	0	—
Asclepias curassavica-fed	2	2	7	8′ 10″
Mealworms	>5	>5	0	—
Bird 8. (adults)				
Gonolobus rostratus-fed	4	4	0	—
Asclepias curassavica-fed	2	1.25	6	10′ 00″
Mealworms	>5	>5	0	—

* During 30-min period following ingestion.
† Measured from time first insect was eaten.
‡ First half given on day 1, second half on day 2.

males ingested on one day was nearly three times this amount, while for the *Gonolobus*-reared ones, this difference was more than fivefold. Because of this, and because of the uniformity of the results for all eight birds, statistical treatment of the data is obviated.

4. *Discussion.*—The results of this experiment establish that the unpalatability of the monarch butterfly is causally related to the species of plant ingested by the larvae. What is not certain, however, is whether the unpalatability is caused by molecules taken directly from the plants. It is possible that the cabbage-reared monarchs are palatable because cabbage has within it chemical substances to which the monarch butterfly is not adapted and which disrupt its metabolism, thereby preventing the insect from synthesizing its noxious properties. Cabbage is not a food plant of the monarch butterfly in its natural environment, and the ones we did manage to rear on it had high mortality in the later stages and no fertile adults were produced; clearly, their metabolism was abnormal when they were reared on this plant. Consequently, the cabbage-feeding experiment *per se* does not prove the molecular-sequestering hypothesis. However, *Gonolobus* is a genus in the Asclepiadaceae that is normally fed upon by the monarchs in nature, and it is therefore

very unlikely that the metabolism of the butterfly would be disturbed when eating this plant. Why, then, were the *Gonolobus*-reared monarchs palatable?

At the outset of our experiment, we had made the general assumption that all asclepiad plants contain cardiac glycosides.[20] We now know that this is not true: Professor Th. Reichstein, of Basel University, has chemically analyzed dried leaves of our *Gonolobus rostratus* plants and found them to be devoid of cardiac glycosides.[21] In contrast, *Asclepias curassavica* contains at least seven different cardiac poisons.[9–11] Thus our experiments narrow down the association of unpalatability in the monarch to those asclepiad plants containing these compounds, as opposed to those lacking them.

Further support for the molecular-sequestering hypothesis has been provided by Parsons.[22] With material from our Trinidad laboratory, he has produced chemical and pharmacological evidence that adult monarch butterflies reared on *Asclepias curassavica* definitely contain at least three cardiac poisons. Moreover, the case is made stronger by the fact that cardiac glycosides have a steroid nucleus,[6] and insects are, as far as is known, unable to synthesize steroids.[23] Further work on the chemical identity of the poisons in the butterflies and the plants is in progress.[24]

While it is now extremely likely that these butterflies do obtain the poisonous molecules from the milkweeds, final disproof of their synthesis by the butterflies can only be achieved by radioactive labeling studies of the biosynthetic and transfer pathways of the cardiac glycosides in both the plants and the insects.

The fact that the palatability of the monarch butterfly can vary according to the food plant that its larvae ingested may at last resolve the controversy over the theory of warning coloration. The basis of this argument has largely been the conflicting evidence on the palatability of butterflies to vertebrate predators among which the monarch has been a principal subject.[25] Our new evidence provides a basis for reinvestigating the relative frequencies of palatable and unpalatable members of the same species in the same and in different geographic areas where alternative foods are available.

Finally, on the basis of this experiment, we offer an additional theoretical category of mimicry: *automimicry*. Here, a species has a palatability polymorphism resulting from the larval food plant selected by the ovipositing female. As such, palatable insects are perfect visual mimics of the unpalatable members of their own species.

5. *Summary.*—(1) By the selection of a strain of cabbage-eating monarch butterfly larvae, *Danaus plexippus*, it has been possible to show experimentally that this nonpoisonous food plant renders larvae, prepupae, and adult monarchs palatable to blue jays. (2) Monarchs reared on *Asclepias curassavica*, a natural food plant known to contain heart poisons, caused the same birds to vomit, even following the ingestion of less than one adult. (3) Another asclepiad plant (*Gonolobus rostratus*), lacking cardiac toxins, proved to produce fully palatable adults. (4) It was concluded that the unpalatability of the monarch butterfly is causally related to the species of plant ingested by the larvae. Although direct transfer of poisonous molecules from plant to insect is strongly suggested, radioactive labeling would provide the only direct proof. (5) The discovery of an intraspecific palatability polymorphism in the monarch butterfly prompts us to advance a new theoretical category of mimicry: *automimicry*.

This paper is dedicated to Professor Harold H. Plough.

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[19] Motor-powered rotary feeders with 12 cups appearing sequentially per revolution with approximately a 1-min exposure for each cup.

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