## Ecological Chemistry and the Palatability Spectrum

Abstract. *A new bioassay for comparing the palatability to avian predators of monarch butterflies reared on various asclepiadaceous food plants containing cardiac glycosides indicates a palatability spectrum. The monarchs reared on one plant species are six times as emetic as those fed another, while those raised on an asclepiad which lacks cardiac glycosides are not emetic at all.*

Recent studies have shown that two insects, one holometabolous (the monarch butterfly, *Danaus plexippus* L., subfamily Danainae) and the other hemimetabolous [the grasshopper, *Poekilocerus bufonius* (Klug), family Pyrgomorphidae], which feed upon cardiotoxic milkweed plants (Asclepiadaceae) contain cardiac glycosides identical to those present in the plants (*1*). Although the possibility of synthesis has yet to be ruled out, chemical, pharmacological, and experimental evidence make it virtually certain that these insects sequester the toxic molecules from the plants (*2*).

The presence of the cardiac glycosides in the insects is a highly effective defense mechanism against certain predators. Blue jays (*Cyanocitta cristata bromia* Oberholser, Corvidae) which ate monarch butterflies reared upon *Asclepias curassavica* L. exhibited typical effects of cardenolide poisoning (*3*) which included repeated vomiting (Fig. 1). Recovery followed within about one-half hour and the birds subsequently rejected further monarchs on sight. However, not all asclepiad plants contain cardiac glycosides, and blue jays repeatedly ate monarchs reared on two of these without sickness or indications of unpalatability (*2*, *3*).

The present investigation explored the further possibility that a spectrum of insect palatability exists, determined by the species of asclepiad food plant ingested by the insects during their development.

To obtain an objective and ecologically meaningful criterion of palatability, we developed a new technique. Male monarch butterflies (*4*) were reared (*5*) on milkweed species belonging to four different genera (*6*). Sixteen to 48 hours after emergence the adult butterflies were killed by freezing. Individually placed in glassine envelopes, they were accumulated in plastic bags in a deep freezer and kept there from 3 days to 5 months. To prepare the butterflies, four lots of ten males reared on each species of plant were dried for 15 hours at 60°C in a forced-draft oven. Each lot was then ground separately to a fine homogeneous powder with a mortar and pestle. The powder was stored from 0 to 12 days in a desiccator containing Drierite (*7*) and kept in a refrigerator (at about 5°C) when not in use. When any one of the four lots was used up, a new set of four lots was ground. In total, three sets were used, ground on 13 and 21 March and 3 April. Six, eight, and six feeding sessions resulted from the three sets, respectively.

Blue jays (*8*) were again selected as subjects and the "up and down" sequential analysis (*9*) was used to determine and compare the dosages of powdered butterfly material necessary to produce emesis with a probability of 50 percent [that is, emetic-dose-50 ($ED_{50}$) determinations]. At the beginning of an experimental run, four jays were weighed (Mettler P-1000 balance), deprived of food (water was available at all times) for approximately 2 hours, and then weighed again immediately before being force-fed. Weight loss was about 1 g/hr during deprivation, which was taken into account in calculating each bird's dosage. The largest dosage error in this experiment was +3 percent.

Dosages were administered as follows. A No. 2 gelatin capsule (Eli Lilly Co.) was loaded with the desired dry weight (Mettler H-16 balance) of pow-

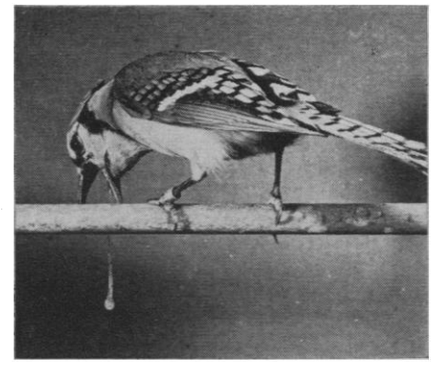

Fig. 1. Emesis reaction of a blue jay that ingested a monarch butterfly reared as a larva on *Asclepias curassavica*. Recovery followed in approximately ½ hour.

Table 1. Event record for 20 sequential forced feedings to blue jays of powdered male monarch butterflies reared as larvae on four food plants in the family Asclepiadaceae. X denotes emesis; O denotes no emesis.

| Dosage (in grams) of powdered butterfly per 100 grams of bird | | Four larval food plants | |
|---|---|---|---|
| Actual dosage | Normalized ($\log_N$) dosage | *Calotropis procera* | *Asclepias curassavica* |
| 0.017 | −4.049 | O | |
| .021 | −3.849 | O X | |
| .026 | −3.649 | O O O X O O X | O O |
| .032 | −3.449 | O X X O X X X X | O O O X O X X |
| .039 | −3.249 | X X | X O X X O O X X |
| .047 | −3.049 | | X X X |
| .058 | −2.849 | | |
| .071 | −2.649 | | |
| .086 | −2.449 | *Gomphocarpus sp.* | *Gonolobus rostratus* (control) |
| .105 | −2.249 | | |
| .129 | −2.049 | O O O O | O O O O |
| .157 | −1.849 | X X O O O X X O | O O O O O O O O |
| .192 | −1.649 | X O X X X O | O O O O O O |
| .235 | −1.449 | X X | O O |
| .287 | −1.249 | | |

dered butterfly material and sealed with a dab of water. The narrow end of the capsule was then forced into the fire-polished end of a glass tube, and the tube and capsule were coated lightly with mineral oil. The bird was hand-held, its beak opened, and the tube with the protruding capsule was forced down its esophagus into the gizzard, a distance of about 12 cm. A glass plunger inside the tube served to release the capsule. High dosages (0.235 g and occasionally 0.192 g) were administered by one No. 2 and one No. 3 capsule; the latter fit inside the tube and both could therefore be administered simultaneously.

Following a forced-feeding, the jay was placed in a clean cage (a 17-inch cube of ½ inch by 1 inch mesh wire) (1 inch = 2.54 cm) suspended behind one-way glass. Observations were made continuously by two of us on pairs of visually isolated birds for a period of 1 hour. All four categories were represented in each observation period.

In all, 80 forced-feedings were carried out on 51 different birds from 13 March through 5 April 1968. Twenty-two were tested once and 29 twice. Of the 51 birds, 27 had been force-fed once in an earlier experiment and 3 twice. A minimum of 10 days intervened between successive feedings. The birds were maintained on cracked corn, sunflower seeds, and pigeon pellets (Ralston Purina Co.).

Although other indications of unpalatability can be observed when a bird has ingested a poisonous monarch (*3*), the emesis criterion is the most objective. Butterflies reared on *Gonolobus rostratus*, previously shown to be non-emetic [this plant lacks cardiac glycosides (*3*)] were fed in dosages matched to the *Gomphocarpus* dosages as a control against a large amount of material per se causing emesis. In this experiment no *Gonolobus*-fed birds vomited, although one out of 33 did so in another study.

The record of events for each category is given in Table 1 and the data are summarized in Table 2. Based on the mean weight of the birds (*10*) and the mean dry weight of a sample of wild monarchs (*11*), the $ED_{50}$ data were converted into the mean number of $ED_{50}$ units per butterfly (Table 2). In other words, a single male monarch butterfly which had fed as a larva upon *Calotropis procera* contains sufficient poison to cause 4.8 blue jays to vomit 50 percent of the time; but a single individual reared on *Gomphocarpus* sp. did not cause emesis, although if a bird ate two it would have vomited. The *Asclepias curassavica*–reared monarchs were not quite as emetic as the *Calotropis*–reared ones, but nearly five times as emetic as the *Gomphocarpus*–reared individuals.

The experiment therefore not only confirms the previous finding (*3*) that the palatability of monarch butterflies is causally related to the species of food plant ingested by its larvae, but also establishes the theoretical possibility of a palatability spectrum in nature. This is a most important ecological discovery: individual insects with an $ED_{50}$ unit of 1 or greater are completely unsuitable as food (they would in fact be detrimental, causing loss of previously ingested food) whereas individuals with an $ED_{50}$ unit of less than 1 could serve as an emergency food supply, provided that a bird ate them at a low enough rate. In these terms, at least one category of palatability becomes an objectively measurable variable instead of the vague concept which has historically pervaded the literature and led to considerable confusion.

Table 2. Emetic-dose-50 determinations of male monarch butterflies force-fed to blue jays. The butterflies were reared on four larval food plants in the family Asclepiadaceae. *Gonolobus rostratus* lacks cardiac glycosides and is not emetic. Analysis based on data in Table 1.

| Food plant of butterflies | No. of tests | Dosage (g dry wt) of butterfly per 100 g of bird | | | | Mean No. of $ED_{50}$ blue jay units per butterfly |
|---|---|---|---|---|---|---|
| | | Dosage in $\log_N$ form | | Dosage in grams | | |
| | | $ED_{50}$ | 95% confidence limits | $ED_{50}$ | 95% confidence limits | |
| *Calotropis procera* | 20 | −3.572 | −3.723 to −3.421 | .028 | .024 to .033 | 4.8 |
| *Asclepias curassavica* | 20 | −3.327 | −3.449 to −3.206 | .036 | .032 to .040 | 3.7 |
| *Gomphocarpus* sp. | 20 | −1.790 | −1.908 to −1.671 | .167 | .148 to .188 | .8 |
| *Gonolobus rostratus* | 20 | | | | | Not emetic |

While this study is based on a terrestrial food chain involving one group of plants, a danaine butterfly, and a single corvid predator, it is likely that the palatability of many groups of insects is a variable dependent upon the kinds and amounts of noxious chemicals they assimilate from their food plants (*12*). It is not inconceivable that the concept is generally applicable to a wide variety of herbivorous invertebrates and might even include certain vertebrates (*13*).

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**References and Notes**

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2. L. P. Brower, in *Biochemical Co-evolution*, W. C. Dennison, Ed. (29th Annual Biology Colloquium, 26–27 April 1968) (Oregon State Univ. Press, Corvallis, in press).
3. L. P. Brower, J. V. Z. Brower, J. M. Corvino, *Proc. Nat. Acad. Sci. U.S.* **57**, 893 (1967).
4. The monarch butterflies were reared from two separate stocks obtained in Mayaro, Trinidad, W.I. Trinidad stock A was obtained in August 1967; the first 20 *Calotropis* and *Gonolobus* males were from the first generation of this stock, frozen from 15 October to 3 November. Trinidad stock B was obtained in December 1967. The last ten *Calotropis* males, the first 20 *Asclepias* males, and all 30 *Gomphocarpus* males were from the second generation of this stock, frozen from 4 to 11 February 1968. The last ten *Gonolobus* and *Asclepias* males were from the third generation of this stock, frozen from 5 to 31 March 1968.
5. The butterflies were reared in a constant-environment room at approximately 23°C and 60 percent relative humidity on a 16-8-hour light-dark cycle, either on potted plants or in ½-pint (¼-liter) plastic containers, provisioned with leaves and cleaned daily. Very young first instar larvae were transferred from *A. curassavica* plants (upon which the females oviposited) to the other asclepiads.
6. All plants were grown in the Amherst College greenhouse from seed which originated as follows: *Asclepias curassavica* L., Trinidad, W.I., spring 1966, staff of Beebe Research Station; *Calotropis procera* L., vicinity Kingston airport, Jamaica, W.I., August 1965, L. P. Brower; *Gomphocarpus physocarpus*, E. Meyer (or the closely allied *G. fruticosus* R. Br.), vicinity Kampala, Uganda, fall 1965, D. F. Owen; *Gonolobus rostratus* (Vahl), Roemer and Schultes, Northern Range, Trinidad, W.I., summers of 1964 and 1966, L. P. Brower. *Calotropis* was determined by L. P. Brower following R. O. Williams and E. E. Cheesman, *Flora of Trinidad and Tobago* (Guardian Commercial Printery, Port of Spain, Trinidad, 1947), vol. 2, pt. 3, p. 164. Determinations of the rest were verified by A. A. Bullock of the Royal Botanic Gardens, Kew, England.
7. Fisher Scientific Co., Medford, Mass.
8. The blue jays were captured in the wild in Franklin and Hampshire counties, Massachusetts, from November 1967 through March 1968. Neither sex nor age determination was made.
9. W. J. Dixon and F. J. Massey, Jr., *Introduction to Statistical Analysis* (McGraw-Hill, New York, ed. 2, 1957).
10. Based on the birds' weights immediately prior to being force-fed ($N = 80$, $\bar{X} = 85.43$ g, S.D. = 6.71 g). The means for the bird groups fed *Calotropis, Asclepias, Gompho-*

*carpus*, and *Gonolobus* were, respectively, 85.7, 85.7, 86.0, and 84.4 g.
11. These were collected in their natural environment in Mayaro, Trinidad, W.I., during December 1967 ($N = 50$, $\bar{X} = 0.115$ g, S.D. $= 0.028$ g).
12. See reviews in: L. P. Brower and J. V. Z. Brower, *Zoologica* **49**, 137 (1964); P. R. Ehrlich and P. H. Raven, *Evolution* **18**, 586 (1965); T. Eisner and J. Meinwald, *Science* **153**, 1341 (1966).
13. J. B. Sale [*East African Wildlife J.* **3**, 127 (1965)] has noted the mammal *Hyrax* feeding upon poisonous plants. H. B. Cott [*Proc. Zool. Soc. London* **123** (1), 123 (1953) and **124** (2), 335 (1954)] has shown that the eggs of certain bird species are unpalatable to mammals.
14. Supported by NSF grant GB4924. We are grateful to Dr. O. M. Buchanan for providing facilities at the William Beebe Tropical Research Station, Professor James Q. Denton for mathematical advice, Dr. Jane V. Z. Brower for critical comments on the manuscript, Dr. David Wetherbee for catching the birds, and Diane Ryerson, Helen Sullivan, and Warren MacAvoy for technical assistance.
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## Electrotonic Spread of Dendritic Potentials in Feline Pyramidal Cells

Abstract. *In pyramidal cells synaptic activation of the entire apical dendritic tree distal to the branch point of the major shaft can dominate the neuronal firing pattern. Uniform synaptic activation of distant parts of the dendritic tree (~ 750 microns from the soma) would produce potential changes at the soma of 2 to 3 percent of the magnitude of the dendritic potential changes. Even these small somatic potential changes could modulate the frequency of firing of neurons depolarized close to or above firing level by more proximal synaptic inputs.*

Physiologists, anatomists, and information theorists have long pondered the significance of synaptic endings upon distant dendrites of highly branched neurons. Controversy has centered upon the question of whether potentials generated upon distant dentrites would have any significant effect upon the soma and nearby axon hillock region.

We have analyzed the pyramidal cell of the cat motor cortex and estimated the upper and lower limits of attenuation of potential changes between the apical dendritic tree and the soma under "steady-state" conditions, a situation probably frequently approximated in maintained synaptic activity. The fact that the major apical dendritic shaft provides the single intracellular channel for spread of current from apical dendrites to soma simplifies analysis. Furthermore, the steady-state treatment obviates the need to consider membrane capacitances. We can calculate the potential ($V_b$) across the soma and basilar dendrites resulting from a synaptic bombardment of the dendritic tree distal to the major branch point (MBP) which sets up potential decrements ($V_2$ and $V_3$) upon the secondary and tertiary branches, respectively, resulting in a potential ($V_1$) at the MBP (Fig. 1A), where $V_3 \geqq V_2 \geqq V_1$. An equivalent resistive circuit (Fig. 1B) can be constructed from appropriately paired physiologic and new anatomic data. The only physiologic assumptions required are that the resting membrane potential is constant throughout the soma and dendritic trees and that the external resistivity between different leakage resistances and between these resistances and ground is small and can be neglected in these calculations, and the only anatomic assumption required is that any shrinkage during tissue fixation is small and can be neglected in the calculations. It is then required only to find limiting values for the ratio of apical dendritic to basilar dendritic conductance ($\alpha$) which can be done after presentation of the anatomic data. The attenuation ratios ($V_{sb}/V_1$) can then be calculated by application of Kirchoff's laws.

The theoretical advances of Rall (*1*), with applications of cable theory to branching dendritic trees, provided means of determining somatic membrane electrical constants from the type of data obtainable by intracellular microelectrode techniques. These methods were applied to the pyramidal cell of the motor cortex of the cat (*2*). In the best material on identified pyramidal cells the ratio of dendritic conductance to somatic conductance ($\rho$) ranged from 3 to 6.5. By using these respective values of $\rho$, we estimated the somatic membrane specific resistivity as ranging from 1500 to 4000 ohm cm$^2$. Total neuron "input resistances" ($R_I$) have been determined by Takahashi (*3*) and by us (*2*). The average values of $R_I$ for pyramidal cells with rapidly conducting fibers (30 to 60 meter/sec) were $5.9 \pm 2.8$ and $6.7 \pm 1.9$ megohm respectively. For any given value of specific resistivity ($R_m$) and $\rho$, the larger the cell, the smaller is the input resistance. Hence the value of 4.5 megohm, which is representative of the lower values of both groups, can be taken as the input resistance of the largest neocortical pyramidal cells in the cat.

The anatomic problem was to provide measurements on a group of large pyramidal cells comparable to those studied physiologically. Sholl (*4*) in his classic study of dendritic branching had used mostly kittens and had restricted his analysis to apical dendrites branching beyond the MBP. Neither he nor any other anatomist had provided data, crucial for our purposes, on the relations of length and diameter of the major apical dendritic shaft of the mature cat. Therefore, this data is provided by one of us (S.J.).

The brains from two adult cats (2.5 kg) were impregnated by the Golgi-Cox method, dehydrated, embedded in paraffin, and sectioned frontally at 60 $\mu$. All measurements were made $\times$ 1200 with a calibrated eyepiece. The pyramidal neurons were sampled as follows: the count was initiated in the anterior sigmoid gyrus and continued into the posterior sigmoid gyrus. On the examined sections the sample was taken by starting on the lateroventral surface and moving at 1 mm intervals dorsomedially until five cells had been counted. The next section was examined, and the same procedure was followed at 180 $\mu$

Table 1. Calculated values of resistances and electronic attenuations for various values of $\alpha$ and $\rho$.

| Values of | | $R_a$ (megohm) | $R_{sb}$ (megohm) | $R_{L1}$ (megohm) | $R_{L2}$ | $R_{L3}$ | $R_{L4}$ | $R_{L5}$ | $R_{br}$ | $\frac{V_{sb}}{V_1}$ (%) |
|---|---|---|---|---|---|---|---|---|---|---|
| $\alpha$ | $\rho$ | | | | | | | | | |
| | | | | *$R_m = 1500$ ohm cm$^2$* | | | | | | |
| 0.3 | 3 | 25.6 | 5.44 | 134 | 174 | 192 | 236 | 286 | 318 | 27.5 |
| 2 | 3 | 9.0 | 9.0 | 134 | 174 | 192 | 236 | 286 | 318 | 36.2 |
| | | | | *$R_m = 4500$ ohm cm$^2$* | | | | | | |
| 0.1 | 6.5 | 60.4 | 4.88 | 357 | 463 | 513 | 627 | 765 | 849 | 28.6 |
| 1 | 6.5 | 10.5 | 7.94 | 357 | 463 | 513 | 627 | 765 | 849 | 38.1 |