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24 February 1987; accepted 9 June 1987

Vein-Cutting Behavior: Insect Counterploy to the Latex Defense of Plants

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Many mandibulate insects that feed on milkweeds, or other latex-producing plants, cut leaf veins before feeding distal to the cuts. Vein cutting blocks latex flow to intended feeding sites and can be viewed as an insect counteradaptation to the plant's defensive secretion. Experimental vein severance renders milkweed leaves edible to generalist herbivores that do not show vein-cutting behaviors and ordinarily ignore milkweeds in nature.

WHEN INJURED, MANY PLANTS EXude latex, a viscous, often milky secretion. The suggestion, advanced a century ago, that the fluid is defensive (*1*) received early support from the pioneering experiments of Kniep (*2*). Working with Euphorbiaceae, Kniep showed that if such plants were drained of latex by repeated puncturing of their leaves, they were rendered edible to slugs. Untreated plants kept as controls remained uneaten.

We have found that Kniep's experiment is performed as a matter of dietary routine by a diversity of insects that feed on latex-producing (laticiferous) plants. These insects first treat their hosts by inflicting localized bites, thereby inducing latex drainage and blockage of latex flow to intended feeding sites. We view this behavior, which appears to have evolved independently in a number of phyletic lineages, as a major adaptation enabling insect herbivores to circumvent the latex defenses of plants. We now describe vein-cutting behavior for a number of insects and provide experimental evidence for its function.

In many of the major laticiferous plants, including milkweeds (Asclepiadaceae), latex is stored under pressure within elongate cellular tubes (laticifers) that follow the venational system of the leaves (*3*). Thus, to sever laticifers and induce latex emission, a herbivore need only cut into the veins of its host. We first observed such vein-cutting behavior in *Labidomera clivicollis*, a chrysomelid beetle commonly found on field milkweed, *Asclepias syriaca*. *Labidomera* adults (Fig. 1, A to D) and larvae (Fig. 1G) typically initiate the behavior by biting repeatedly into several adjacent branches of a leaf midrib, inducing latex emission with each bite (Fig. 1, A, B, and G). They then move distal to the cuts and, commencing at the edge of the leaf, consume the area of leaf blade delimited by the incisions (Fig. 1, C and D). As they feed, there is no visible latex emission from the tissue being eaten. By severance of veins, *Labidomera* evidently render leaf tissue distal to the cuts relatively latex-free and block further latex flow to the site. Experimental replication of the procedure confirms this process. Although initial vein transections invariably induce latex outflow, subsequent transections made distal to the first result in little or no emission (Fig. 1J).

Other insects that feed on milkweeds show similar ingestive strategy. The cerambycid beetle *Tetraopes tetrophthalmus* characteristically bites repeatedly into the midrib before feeding at the leaf tip (Fig. 1, E and F), whereas caterpillars of certain moths (*Euchaetes egle* and *Cycnia tenera*) and butterflies (*Danaus gilippus* and *Danaus plexippus*) first treat leaves by chewing furrows across the midrib or the petiole itself (Fig. 1H). In *Danaus plexippus* such behavior had previously been noted (*4*). Young caterpillars of *Danaus gilippus* cut a circular trench (Fig. 1I) and then feed on leaf tissue within the trench. In all these cases we noted that latex outflow was profuse only in conjunction with the initial vein severances and was minimal from tissue being subsequently ingested.

Leaf tissue distal to vein cuts is not always completely consumed by milkweed insects. Such tissue may be exploited secondarily by other insects, including general feeders that ordinarily ignore milkweed leaves. We observed one such generalist, a Japanese beetle (*Popillia japonica*), feeding distal to vein cuts seemingly produced by *Labidomera*. This led us to compare the acceptability to both specialist and generalist herbivores of milkweed leaves with either intact or experimentally transected veins.

The herbivores included three milkweed specialists (*L. clivicollis*, *T. tetrophthalmus*, and *Danaus plexippus*) and four generalists not ordinarily found on milkweeds (Japanese beetles, *Popillia japonica*; woolly bear caterpillars, *Pyrrharctia isabella*; southern armyworm caterpillars, *Spodoptera eridania*; and the slug *Deroceras reticulatum*). Each animal was enclosed within a mesh sleeve with a single live milkweed leaf (*A. syriaca*) and allowed to feed for a set period (*5*); it

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was then transferred to a second leaf (similarly enmeshed) on another plant. The two leaves (Fig. 2) were an intact control leaf and an experimental leaf that was damaged on one side of the midrib to simulate vein cutting by *Labidomera* (*6*). Half the individuals of each species were presented with the control leaf first, the other half with the experimental leaf. We recorded whether feeding took place (area consumed was measured from photocopies of leaf remains), whether it involved one or both sides of the experimental leaf, and whether there had been vein severance (evidence of scars).

Both specialists and generalists (n = 20 per species) showed distinct patterns in their feeding preferences (Table 1). Both groups fed on the treated halves of experimental leaves. However, only the specialists fed extensively on the control leaf and on the untreated half of the experimental leaf, where veins were initially intact (*7*). The specialists always cut veins and fed distal to the cuts, just as they do in nature. In contrast, none of the leaves offered to generalists bore signs of vein cutting inflicted by the feeders themselves. Dietary access to undamaged milkweed tissue is evidently open only to herbivores with a propensity for cutting veins. To the noncutting generalist, the laticiferous system is a barrier.

Two of the specialists, *Labidomera* and *Tetraopes*, fed preferentially on the treated

Fig. 1. (**A** to **D**) *Labidomera clivicollis* cutting veins (A and B) of a milkweed leaf (*A. syriaca*), preparatory to feeding distal to the cuts (C). After consuming most tissue beyond these initial cuts, the beetle severed three additional veins and fed beyond these cuts as well (D). (**E** and **F**) *Tetraopes tetrophthalmus* inflicting one of several sequential bites into the midrib of an *A. syriaca* leaf (E), before feeding at the leaf tip (F). (**G**) *Labidomera clivicollis* larva biting into a leaf vein (*A. syriaca*) before feeding distal to the bite. (**H**) Caterpillars of the queen butterfly, *Danaus gilippus*, chewing into the midrib of an *Asclepias curassavica* leaf (top larva), and feeding (bottom larva) on a leaf after severing the midrib. (**I**) First-instar queen caterpillar on an *A. syriaca* leaf, in the process of cutting a circular trench (denoted by latex outflow) around its eventual feeding site. (**J**) Live leaf of *A. syriaca* experimentally treated to show how vein severance blocks latex flow. Four veins to the right of the midrib have been transected, eliciting localized latex effluence. Subsequent lengthwise bilateral trimming of the leaf with scissors resulted in latex emission from all veins except those previously severed. (**K**) Milkweed beetle, *T. tetrophthalmus*, with drop of fresh *A. syriaca* latex experimentally applied to its mouthparts. Upon drying, the drop becomes sticky (**L**), effectively gumming up the beetle's mandibles. Reference bars: (A, E, and G) 0.5 cm; (H) 2.0 cm; (I and K) 0.1 cm; and (J) 1.0 cm.

Table 1. Leaf tissue preference of test herbivores on control and experimentally altered *A. syriaca* leaves. The number of herbivores feeding on control versus experimental leaves and on treated versus untreated halves of experimental leaves was compared by the Sign test; the number of feeders that cut veins on control versus experimental leaves was compared by Fisher's exact test (*16, 17*). NS, not significant.

Herbivore	Insects (n = 20) feeding on leaves			Insects feeding on experimental leaf			Feeders that cut veins on leaves		
	Control (%)	Experimental (%)	P	Treated (%)	Untreated (%)	P	Control (%)	Experimental (%)	P
Milkweed specialists									
Labidomera clivicollis									
Larvae	15	100	<0.01	100	5	<0.01	100	35	NS
Adults	25	100	<0.01	90	15	<0.01	100	35	<0.05
Tetraopes tetrophthalmus	65	100	<0.05	100	45	<0.01	100	65	<0.05
Danaus plexippus	95	100	NS	95	85	NS	>84	95	NS
Generalists									
Popillia japonica	0	100	<0.01	100	0	<0.01	0	0	
Pyrrharctia isabella	15	95	<0.01	100	5	<0.01	0	0	
Spodoptera eridania	25	100	<0.01	100	0	<0.01	0	0	
Deroceras reticulatum	0	100	<0.01	100	10	<0.01	0	0	

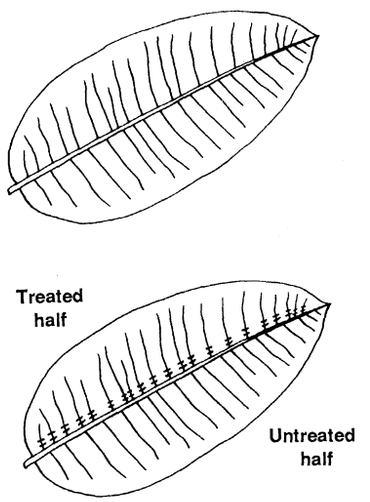

Fig. 2. (Top) Control leaf; (bottom) experimental leaf showing treated and untreated halves.

half of the experimental leaves. Both evidently recognize, and favor, leaf tissue that can be consumed without preparatory vein cutting. In nature, we have repeatedly observed both these beetles feeding without prior vein cutting on damaged leaf tissue, or distal to older vein cuts made by previous feeders. The slug *Deroceras reticulatum* may also be an opportunist in nature. We have commonly found it, in the fall, feeding on senescent *A. syriaca* leaves from which we could elicit little or no latex emission.

Evidence was obtained attesting to the effectiveness of milkweed latex as a deterrent to feeding. Deterrency had previously been demonstrated for ants (*8*), but not for insect herbivores. Fresh latex droplets (1 μl) from punctured *A. syriaca* leaf veins placed beside the mandibles of *S. eridania* caterpillars (n = 10) that were feeding on *A. syriaca* leaves (treated by severance of veins) induced the larvae to cease feeding and engage in oral cleansing activities when they contacted the latex and to redirect their feeding to another site. Droplets of water of comparable size offered before and after each latex presentation had no effect. *Asclepias* latex contains toxins and other potentially unpalatable factors (*9*), but it may also act mechanically because it coagulates upon exposure to air. Droplets of *A. syriaca* latex placed on the mouthparts of tethered *T. tetrophthalmus* eventually hardened to the point of virtually muzzling the beetles (Fig. 1, K and L).

We predicted and confirmed that vein-cutting behavior, as a strategy for blocking latex flow to feeding sites, is practiced also by insects on laticiferous plants other than Asclepiadaceae. These insects include a cerambycid beetle, a katydid, and caterpillars of several families (Arctiidae, Noctuidae, Pyralidae, and Sphingidae) that feed on assorted Apocynaceae, Asteraceae, Caricaceae, and Moraceae (*10*). Observations by others supplement this list (*4, 11*). An exception to the rule appears to be *Chrysochus auratus*, a chrysomelid beetle that feeds on leaves of *Apocynum cannabinum* without prior vein cutting, apparently undeterred by the copious latex emission elicited by its bites. Also of interest is the seemingly refined strategy of certain caterpillars, such as *Erinnyis alope* on *Carica papaya*, which in addition to biting into the midrib of a leaf, trench or cut across the leaf blade on each side of the bite (*10*). *Carica* leaves have a venational network laid out in loops, such that latex can bypass severed veins by flowing through neighboring veins. Only through total transection of the network can an insect block latex flow to feeding sites on such leaves.

Vein-cutting behavior may also occur in insects that feed on nonlaticiferous plants (*10, 12–14*). On such plants the behavior may serve against other canalicular defenses (we have noted vein cutting by insects on Anacardiaceae and Apiaceae that have resin canals) (*10*), or against chemical deterrents mobilized by plants in response to insect attack. Leaf trenching by *Epilachna* beetles on squash, for instance, is said to block the plant's rapid translocation (within 40 minutes) of deterrent cucurbitacins to tissue beyond the trench (*13*). It has also been suggested that vein cutting benefits insects by leading to local nutrient or metabolite changes or to changes in leaf position or turgidity (*4, 14*). Whatever the role or roles of vein-cutting behavior in these situations, for insects on laticiferous plants it serves specifically, if not necessarily exclusively, as a counterdefense to latex.

Note added in proof: Vein-cutting behavior of additional insects is described in two recent papers (*15*).

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5. Test animals were enclosed with each leaf for 24 hours, except for *Popillia* (48 hours), *Deroceras* (16 hours), and *Spodoptera* (6 hours). All animals were tested in the field on mature, previously undamaged leaves of *A. syriaca*, except *Spodoptera* larvae, which were tested in a greenhouse on potted milkweeds.
6. Major veins on one side of the experimental leaf were transected twice near the midrib with forceps. This resulted in momentary outflow of latex from the cuts and in blockage of latex supply to the treated side of the leaf.
7. These differences in acceptability were reflected also in differences of leaf area ingested. The specialists consumed substantial portions of both the control and experimental leaves ($\bar{x}$ ± SD cm^2 leaf area; n = 3 to 20 individuals; including only actual feeders); *Labidomera* larvae (2.11 ± 0.92 cm^2 on control versus 1.75 ± 1.91 cm^2 on experimental), *Labidomera* adults (1.48 ± 1.29 cm^2 versus 1.78 ± 1.43 cm^2), *Tetraopes* (1.44 ± 1.12 cm^2 versus 160 ± 0.78 cm^2), and *Danaus* (37 ± 18 cm^2 versus 30 ± 17

cm^2). In contrast, the generalists, with the exception of two *Pyrrharctia* larvae, consistently consumed only minimal areas (<0.25 cm^2) of the control leaf. For example, the *Spodoptera* larvae ingested 0.13 ± 0.07 cm^2 (n = 5) of the control leaf versus 1.97 ± 1.35 cm^2 (n = 20) of the experimental leaf. Evidently, milkweed tissue with intact veins is inherently unacceptable to these herbivores. The preference of generalists for the treated half of the experimental leaf can therefore be attributed to increased acceptability of this half, rather than to a possible decrease in acceptability of the untreated half.

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16. The sequence of presentation of control and experimental leaves had no detectable effect on the number of individuals feeding on either leaf.
17. Only some of the milkweed specialists fed on both the control and experimental leaves. Thus, in order to compare the total number of feeders that cut veins on these leaves, the paired experimental design must be ignored. We used Fisher's exact test for 2 by 2 tables in preliminary tests for independence between cutting veins on one leaf, and cutting and feeding on the other leaf. The lack of any statistically significant departure from independence was regarded as justification for ignoring the paired design.
18. Supported in part by the Bache Fund (National Academy of Sciences) and NIH grant AI-02908. We thank J. Boggan, W. A. Hoose, and E. Jakob for technical assistance; D. S. Robson for statistical advice; and E. W. Lawson, I. Baldwin, J. Ballarino, R. F. Denno, and J. G. Shepherd for helpful discussions.

25 March 1985; accepted 28 May 1987

Ouabain Resistance Conferred by Expression of the cDNA for a Murine Na^+,K^+-ATPase α Subunit

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The molecular basis for the marked difference between primate and rodent cells in sensitivity to the cardiac glycoside ouabain has been established by genetic techniques. A complementary DNA encoding the entire α_1 subunit of the mouse Na^+- and K^+-dependent adenosine triphosphatase (ATPase) was inserted into the expression vector pSV2. This engineered DNA molecule confers resistance against 10^{-4} M ouabain to monkey CV-1 cells. Deletion of sequences encoding the carboxyl terminus of the α_1 subunit abolish the activity of the complementary DNA. The ability to assay the biological activity of this ATPase in a transfection protocol permits the application of molecular genetic techniques to the analysis of structure-function relationships for the enzyme that establishes the internal Na^+/K^+ environment of most animal cells. The full-length α_1 subunit complementary DNA will also be useful as a dominant selectable marker for somatic cell genetic studies utilizing ouabain-sensitive cells.

THE EXTREME DIFFERENCE IN OUABAIN sensitivity between rodent and primate cells has long been used as a selection strategy in somatic cell genetics (*1*). Although the Na^+- and K^+-dependent adenosine triphosphatase (Na^+,K^+-ATPase) is the apparent target of ouabain (*2*), the complex subunit structure of the enzyme (*3*) and the existence of multiple genes encoding alternative isoforms of the Na^+,K^+-ATPase (*4–6*) have left the genetic basis for differential ouabain resistance between rodent and primate cells uncertain. Furthermore, in most somatic cell genetic procedures involving the transfer of ouabain resistance from rodent to primate cells (*1*), a substantial amount of the rodent genome is transferred to recipient primate cells making it difficult to identify those rodent sequences which are directly responsible for ouabain resistance. Recently, complementary DNA (cDNA) clones encoding three rat α subunit isoforms (*4, 5*) and the rat β subunit (*7, 8*) have been identified. Hybridization of genomic DNA from monkey CV-1 cells made ouabain-resistant by transfer of metaphase mouse chromosomes to cDNAs specific for each α subunit isoform has led to the inference that the expression of the rodent α_1 subunit gene is likely to confer ouabain resistance to ouabain-sensitive primate cells (*9*). However, even in chromosome transferents, a substantial amount of rodent DNA is transferred to the recipient primate cells in addition to the gene encoding the Na^+,K^+-ATPase α_1 subunit. This consideration makes it impossible to definitively conclude that transfer of the rodent α_1 subunit gene is indeed responsible for species-specific differences in ouabain sensitivity. To resolve this issue, we have tested the capacity of a cDNA encoding the mouse α_1 subunit to directly confer ouabain resistance to monkey CV-1 cells.

To isolate full-length murine α_1 subunit cDNA, we screened a mouse pre-B cell cDNA library with a rat cDNA that encodes a portion of the rat α_1 subunit (*10*). Six clones, containing inserts of ≥3.6 kb in length, showed similar Eco RI and Bam HI restriction maps. The restriction map of one of these clones, mbα69, is shown in Fig. 1. Preliminary DNA sequence analysis indicated that clone mbα69 contained a complete coding sequence for the α_1 subunit polypeptide. A poly(A) addition consensus sequence was identified at one end of the mbα69 insert; at the opposite end we observed a continuous open reading frame containing the translation start site for the α_1 subunit polypeptide previously identified for human (*11*), sheep (*12*), pig (*13*), rat (*4, 5*), and Torpedo cDNA clones (*14*).

To test biological activity, the 3.6-kb Eco RI insert of mbα69 was introduced into the eukaryotic expression vector pSV2 (Fig. 1).

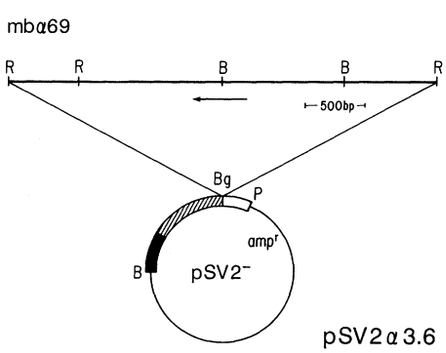

Fig. 1. Schematic representation of mouse Na^+,K^+-ATPase α_1 subunit cDNA clone mbα69 and insertion into the vector pSV2. The cDNA contains two internal Bam HI sites and one internal Eco RI site. The cDNA library containing this clone was constructed and screened with the rb5 probe (*10*) as described (*17*). Bcl I linkers were added to the mbα69 3.6-kb partial Eco RI digestion fragment for ligation to pSV2 DNA. The pSV2 vector was constructed from pSV2DHFR by excising the DHFR insert and adding Bgl II linkers (*18*). In the diagram of pSV2, the white area between Pvu II and Bgl II indicates the SV40 origin and promoter, hatched and black areas include the SV40 small T antigen splice site and poly(A) addition site, respectively. The arrow underneath mbα69 indicates direction of transcription. B, Bam HI; Bg, Bgl II; R, Eco RI; P, Pvu II.

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