Localization of Heart Poisons in the Monarch Butterfly

Sequestered cardenolides are concentrated to different degrees in various body parts as an antipredator strategy.

Lincoln P. Brower and Susan C. Glazier

Chemical defense in arthropods involves a myriad of sophisticated mechanisms (*1*). One of the most thoroughly investigated is found in butterflies of the family Danaidae. During their larval stages, these butterflies sequester cardiac glycosides from their foodplants, milkweeds of the family Asclepiadaceae (*2–7*). Cardiac glycosides, also known as cardenolides, are powerful heart drugs when used in vertebrates, and in addition cause severe vomiting. As a consequence, danaine butterflies are poisonous to bird predators, which rapidly learn to reject the prey after one or more emetic experiences (*7, 8*). Several other milkweed-feeding insects have recently been shown to employ this same antipredator strategy (*9, 10*). The general phenomenon of drugs being sequestered from a lower level in the food chain for use as predator deterrents to organisms of a higher level in the chain appears increasingly widespread as our knowledge of community interactions increases (*11–13*).

In this article we present experimental evidence that the monarch butterfly selectively concentrates cardiac glycosides of differing emetic potencies in various parts of its body in a manner which appears to maximize the effectiveness of these compounds as predator deterrents. We also argue that the evolution of sequestering emetic poisons can be explained without invoking theories of either kin or group selection, but that such evolution has occurred at a physiological cost.

Dr. Brower is professor of biology and Ms. Glazier is a research associate at Amherst College, Amherst, Massachusetts 01002.

Experimental Materials

Monarch butterflies (*Danaus plexippus* L., Lepidoptera, family Danaidae) were reared to the adult stage in the laboratory on the neotropical milkweed, *Asclepias curassavica* L., frozen, oven dried, and weighed individually (*14*). Fifty individuals of each sex were dissected to separate the wings, abdomen, thorax, and legs and head, and the parts were separately ground to provide four pools of dry powder from which to obtain material for chemical and bioassays. The mean dry weights of the whole butterflies and of their body parts are shown in Table 1. A separate group of 12 males and 12 females from the same stock was used to provide material for bioassays of the whole butterflies.

Wet weights of whole butterflies and their component parts (Table 1) were subsequently determined on a separate set of 50 males and 50 females reared in September 1973 (Massachusetts stock H-1). These butterflies were stored individually in glassine envelopes inside two plastic bags in a frost-free freezer until February 1974. To prevent desiccation, one butterfly at a time was removed from the freezer, weighed while wet, and then dissected into the component parts which were also weighed while wet, and then dried in a forced draft oven at 60°C for 16 hours, and subsequently weighed in the dry state. These butterflies weighed slightly more than the Florida stock, but the differences in dry weights of the parts as percentages of the whole for the two stocks differed only slightly (0 to 4 percent).

Differential Concentrations

By means of a spectrophotometric assay specific for cardenolides [(*15*), as modified in (*16*)] absorbance values were determined for 0.1-gram samples of the dry powder obtained from the wings, abdomen, and thorax (Table 2). Based on the original proportions of the powders, including head and legs, absorbance values were also determined for the reconstituted whole butterfly material. The mean absorbances of the separate group of 12 males and 12 females used in the whole butterfly bioassay were 0.490 and 0.630. From the absorbance values, we calculated the respective molar concentrations of the gross cardenolide contents of the body parts on the now partially verified assumption that these cardenolides have molar extinction coefficients similar to digitoxin (*17*). The data in Table 2 show that the concentration of cardiac glycosides was highest in the wings, intermediate in the abdomen, and lowest in the thorax (see also Fig. 1), and they thus confirm Parsons' findings (*2, 18*). These differences in concentration are much greater in the wet than the dry weight samples. For example, in dried male wings the cardiac glycosides are 3.54 times more concentrated than they are in the thorax; in the wet weight samples this difference increases to 7.75. This is undoubtedly of importance to bird predators learning to reject the insects, as we will discuss below. In two previous studies of wild monarch butterflies captured in California, Ontario, Massachusetts, Maryland, and Florida (*15, 16*), the concentration of cardiac glycosides was substantially higher in females compared to males, and these new data confirm this for each of the body parts as well.

Table 2 also shows the digitoxin equivalent of cardiac glycosides, expressed as milligrams, present in the different parts of the butterfly. It is remarkable that each monarch butterfly reared on *A. curassavica* contains more cardiac glycosides than the equivalent dose of digitoxin initially administered to adult human beings suffering from acute congestive heart failure (0.5 milligram), and from three to nearly nine times the daily oral maintenance dose (0.1 to 0.2 mg) (*19*). Because the therapeutic dosage is not much less than the lethal dosage (*20*), we agree with Parsons (*2*) that participants in entomological tasting parties should be cautious.

Differential Emetic Potencies

By means of the bioassay described previously (*5*) we determined the dosages of dried butterfly material necessary to evoke emesis in the blue jay (*Cyanocitta cristata bromia* Oberholser) with a probability of 50 percent, that is, the emetic dose 50, hereafter referred to as the ED_{50} (*21*). From the ED_{50} values and from the 95 percent confidence limits (see Table 3), we conclude that the three body parts as well as the two sexes are substantially different in their emetic potencies. The most emetic (that is, requiring the smallest dose) is the abdomen, followed by the wings, and then the thorax. This same order holds for both sexes, but the females are much more emetic in each of their respective parts. From the point of view of the predator, the extent to which each part of the butterfly is unsuitable as food must be considered. By dividing the respective mean dry weights (see Table 1) by the ED_{50}'s and adjusting for the mean weight of the jays (85 g), we obtained the number of ED_{50} units in each body part (Table 3 and Fig. 1). The least emetic is the thorax, which contains 0.49 and 0.74 ED_{50} units for the male and female, respectively. In other words, a blue jay could eat the thorax without vomiting. However, if a bird ate the wings it would get sick and if it ate the abdomen, it would get extremely sick. The three parts of the female range from 1.32 to 1.95 times as emetic as the corresponding parts of the male and, for the whole insect, the females are 1.70 times as emetic.

The different emetic potencies of the various body parts and sexes cannot be explained by differences in concentration. For example, the male wings have twice the concentration of cardiac glycosides as the male abdomen (Table 2). Consequently, they should be correspondingly more emetic. However, exactly the opposite is true (see Table 3). To quantify this, we took the ratio of ED_{50} units per 0.1 g of dry material (Table 3) to the concentration of cardenolides per 0.1 g of the same dry material (Table 2). When each of the eight resultant values is divided by the lowest (the male wings), a measure of the relative emetic potency of the different parts is obtained (see Table 3 and Fig. 1). It can be seen that the male abdomen is 4.37 times as emetic as the wings and 3.77 times (4.37 divided by 1.16) as emetic as the thorax. For the whole insect, the females are 1.24 times more emetic than the males.

From this it is apparent that the larvae may be selective in the amounts and kinds of cardenolides that they sequester from the plant leaves. Up to 22 different cardiac glycosides have been found in *Asclepias curassavica* (*4, 6, 20, 22*), and ten of these have been isolated from adult monarchs reared on this plant (*4, 6, 23*). Alternatively, the larvae (or later metamorphic stages) may convert the molecular structures of those cardenolides that they do sequester to more or less emetic ones (*24, 25*). Analagous sorting and molecular changing are known to occur in carotenoids which are sequestered by other insects (*26*). In either case, or both, the data indicate that during the metamorphosis of the monarch butterfly to the adult stage, cardiac glycosides of differing emetic potencies are localized with respect to body part.

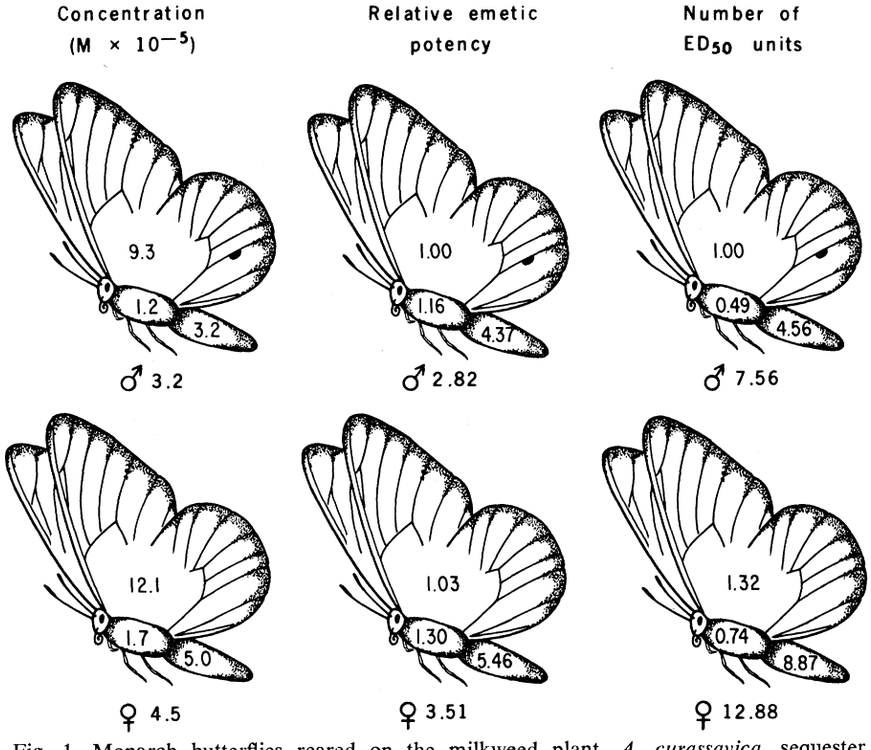

Fig. 1. Monarch butterflies reared on the milkweed plant, *A. curassavica*, sequester and differentially concentrate cardiac glycosides of different emetic potencies in the three body parts and the two sexes. Values for the whole butterfly (including the legs and head) are shown below each insect next to the male and female sex symbols. The wings have the highest concentration and the thorax the least, and females have higher concentrations than males (left). The poisons in the wings are the least emetic, whereas those in the abdomen are the most, and the females contain cardiac glycosides which are more emetic than those in the males (middle). Because the females have higher concentrations of the more potent cardiac glycosides, they contain more ED_{50} units in each body part than do the males (right). The highest concentration of the least emetic glycosides in the wings, together with the differences in the sexes, suggest that the butterflies incorporate the poisons at a physiological cost but, nevertheless, maximize their effectiveness in deterring predation by birds.

Heads and Legs

The head and legs (less coxae and trochanters) were assayed together, and the results were as follows: for males and females, respectively, the mean dry weights were 0.015 g and 0.014 g. The mean wet weights were 0.032 g and 0.027 g. The absorbance per 0.1 g of dry weight material for the males was 0.420 and for the females 0.580. For the males, the ED_{50} was 0.078 g and the 95 percent confidence limits were 0.061 g to 0.100 g (*21*). The ED_{50} test for the females was incomplete because we ran out of material. The number of ED_{50} units for the male head and legs was 0.23, and the total for the separate parts was 6.28. This value is 83 percent of the total number of ED_{50} units determined for the whole butterfly. Based on the relative emetic potencies of the whole male compared to

the whole female (80 percent), the ED_{50} for the female is estimated to be 0.062 g, from which we calculated 0.27 ED_{50} unit. The total number of ED_{50} units for all the female parts assayed separately is therefore approximately 11.20, and is 87 percent of the total determined on the whole butterfly material.

The unaccountability of 17 percent and 13 percent of the ED_{50} units in the male and female parts, respectively, is unlikely to be due to experimental error. The head and legs of the males contain an equivalent of 0.039 mg of digitoxin, giving a total digitoxin equivalent of 0.627 mg for all the parts. This value is 98 percent of that measured in the whole intact butterfly. Similarly, the head and legs of the females contain an equivalent of 0.051 mg of digitoxin, giving a total of 0.879 mg. This value is 100.7 percent of that measured in the whole intact butterfly. Thus, in terms of amounts of cardenolides, the sum contained in the parts is effectively equal to the whole for both males and females.

The difference in numbers of ED_{50} units therefore raises the possibility that there is a synergistic increase in emetic potency when the cardenolides from all the body parts are administered simultaneously to a blue jay.

Metabolic Costs

Previous evidence suggested that the monarch butterflies sequester cardiac glycosides at a physiological cost. In East Coast migratory populations of butterflies, those with the lower concentrations of cardenolides appeared to be more successful migrants than those with the higher concentrations (*27, 28*). Among butterflies collected in Massachusetts, the females with extremely high concentrations were smaller and weighed less than those with lower concentrations (*15*) and, in both previous studies (*15, 16*) and the present one, females have always had a sub-

Table 1. Mean dry and wet weights of the body parts of 50 male (M) and 50 female (F) adult monarch butterflies reared on the milkweed plant *Asclepias curassavica*. Mean weights of the whole butterflies (before dissection) also are shown.

Body part	Dry weight*				Wet weight†				Dry weight as percentage of wet weight	
	In grams		As percentage of whole		In grams		As percentage of whole			
	M	F	M	F	M	F	M	F	M	F
Wings	0.045	0.045	20	21	0.073	0.072	14	14	67	68
Abdomen	0.097	0.098	43	45	0.186	0.184	36	36	48	49
Thorax‡	0.068	0.062	30	28	0.221	0.219	43	43	30	29
Whole§	0.225	0.219			0.516	0.507			43	43

* Determined on Florida stock B-3, as described in (*14*). † Determined on Massachusetts stock H-1, as described in text. ‡ The dissection left the coxae and trochanters attached to the thorax. § Includes the head and legs (see text).

Table 2. Spectrophotometric absorbances, equivalent digitoxin concentrations, and equivalent amounts of cardiac glycosides as digitoxin in the body parts of male (M) and female (F) monarch butterflies reared on *Asclepias curassavica*.

Body part	Sample absorbance (at 626 nm)*		Digitoxin equivalent of glycosides in 0.1-g samples in solution ($10^{-5}M$)						Digitoxin equivalent of glycosides per butterfly (mg)	
			Dry weight sample			Wet weight sample†				
	M	F	M	F	F/M	M	F	F/M	M	F
Wings	0.840	1.075	13.8	17.8	1.3	9.3	12.1	1.3	0.238	0.306
Abdomen	0.412	0.628	6.7	10.3	1.5	3.2	5.0	1.6	0.248	0.386
Thorax	0.245	0.355	3.9	5.7	1.5	1.2	1.7	1.4	0.102	0.136
Whole‡	0.455	0.635	7.4	10.4	1.4	3.2	4.5	1.4	0.638	0.873

* The sample absorbances were determined spectrophotometrically from a color reaction with 0.5 ml of the original butterfly material extract (0.1 g of dry powder in 5.0 ml of ethanol) and diluting this 0.5-ml sample to 2.0 ml with reagents in the cuvette (*15–17*). † Determined through calculation by multiplying the molar concentration of the digitoxin equivalent of glycosides in the solution containing 0.1 g (dry weight) of sample by the dry weight (as a percentage of the wet weight) of the respective butterfly part. The last-mentioned values are from Table 1. ‡ Includes the head and legs.

Table 3. Emetic potency (ED_{50}) of cardiac glycosides in monarch butterflies reared on *Asclepias curassavica*. Potencies were tested on blue jays (*21*).

Body part	Grams of dry butterfly powder inducing emesis per 100 g of bird (ED_{50})						Number of ED_{50} units per 85-g blue jay				Relative emetic potency‡		
	Male butterflies			Female butterflies			In body parts and whole butterfly*		Per 0.1 g of butterfly powder†				
	ED_{50}	Birds tested (No.)	95% confidence limits	ED_{50}	Birds tested (No.)	95% confidence limits	M	F	M	F	M	F	F/M
Wings	0.053	13	0.048–0.060	0.040	21	0.035–0.045	1.00	1.32	2.22	2.94	1.00	1.03	1.03
Abdomen	0.025	14	0.023–0.027	0.013	15	0.012–0.015	4.56	8.87	4.71	9.05	4.37	5.46	1.25
Thorax	0.162	13	0.138–0.190	0.099	13	0.087–0.112	0.49	0.74	0.73	1.19	1.16	1.30	1.12
Whole§	0.035	9	0.030–0.041	0.020	13	0.019–0.021	7.56	12.88	3.36	5.88	2.82	3.51	1.24

* Determined by dividing the dry weight values in Table 1 by the respective ED_{50} values in this table, and then correcting for the mean weight of the blue jays. (85 g). † Determined by dividing 0.1 by the ED_{50} values in this table, and correcting for the mean weight of the blue jays (85 g). ‡ Determined by dividing the number of ED_{50} units per 0.1 g of dry butterfly material per 85-g blue jay by the concentration of the 0.1-g sample (dry weight) for the respective body part from Table 2, and then dividing the resultant values by 0.161. This last-mentioned value is the lowest of these ratios, and is that obtained for the wings. § Includes the head and legs (see text).

stantially higher concentration of cardenolides than the males. It is unlikely that this sex difference is an accidental by-product of, for example, lipid mobilization in the female from fat body to ovary (*29*) because in both studies with the wild butterflies, specimens were captured during the fall migration when the ovaries (as well as sexual behavior) are inactive (*30*). Furthermore, all sequestering is done in the larval stage prior to development of adult tissues.

Perhaps most indicative of the physiological cost hypothesis is the evidence in the present study that the females store cardiac glycosides which overall are 24 percent more potent (emetic) than those in the male (Table 3). Genetically determined characters expressed to a greater degree in one sex or in one sex only (that is, sex-limited traits) are controlled by a balance of opposing factors (*31*). In the monarch butterfly, the ability of both sexes to incorporate the cardenolides is probably subject to some negative selection because of a metabolic cost of handling them, but it is selected because of the deterrent effect of cardenolides on predators. Females are more vulnerable than males because of their exposure to attack while depositing eggs over a long period of time, and they are thus subject to a greater selective pressure which may have favored the evolution of a mechanism allowing them to tolerate the higher concentration of the more emetic cardiac glycosides. There is a remarkable parallel between the reduced concentration of cardenolides, resulting in lowered protection in male monarch butterflies, and the sex-limited mimicry in butterflies in which only the females are mimetic. The evolution of sex-limited mimicry involves a selective balance too (*32*), and this mechanism probably also accounts for the greater amount of poisons reported in female moths of several species (*9*).

The argument that cardiac glycosides are incorporated by the butterflies at a metabolic cost is supported by more general considerations of the adaptive role of these chemicals in the plants. Historically they, as well as a host of other organic chemicals present in the tissues of plants, have been called secondary plant products and have been considered as having originated as metabolic waste products without an ecological raison d'être. This is principally because most organic chemists and physiologists have been reluctant to include evolution in their reasoning, which they generally dismiss as resulting in teleological explanations (*33, 34*). It is rapidly emerging, however, that plants synthesize these chemicals at a substantial metabolic expense, and that this cost is more than offset by benefits to a variety of ecological functions, including regulating competition, resisting disease, protecting the photosynthetic factory against invertebrate and vertebrate herbivores, promoting seed survival, and facilitating cross-pollination (*34, 35*). Many of these chemicals are biologically active and possess remarkable pharmacological properties which are medicinally useful if administered knowledgeably, but extremely toxic if not (*11, 36*). Just as it is costly for the plants to produce these substances, so also are they metabolically expensive for animals to process following ingestion (*37, 38*).

In vertebrates, the liver is the principal organ responsible for detoxifying poisonous plant substances (*39*) while in many insects, including most Lepidoptera that have been studied, the detoxification is effected by analogous microsomal oxidase systems located in the cells either of the digestive tract or the fat body (*40*). These systems, however, are somewhat selective because they must leave intact certain dietary sterol molecules which, as far as is known, all insects must have as precursors, not only for constructing subcellular membranes, but also for the synthesis of steroid metabolites including hormones (*41*) that regulate growth, metamorphosis, ovarian development, and sexual behavior (*42*). Experiments have shown that certain enzymes or intermediates in the metabolic pathways by which these essential dietary sterols are transformed to higher or lower derivatives are affected adversely by other steroids (*41*). Significantly, digitonin, a steroid glycoside (not a cardenolide) from *Digitalis purpurea* L. (Scrophulariaceae) precipitates cholesterol (*43*) and is toxic when added to a chemically defined diet of the grasshopper, *Melanoplus bivitatus* (Say) (*38*). It therefore seems likely that the ingested milkweed cardenolides (carbon-23 steroids) may produce detrimental or at least energy-consuming interactions either directly, or indirectly, by requiring special enzymes to handle them.

It is possible that there are measurable differences in developmental rates, ovarian development, mating ability, fertility, and fecundity resulting from cardenolide metabolism. It is surprising, therefore, that a recent study in which monarch larvae were reared on four species of *Asclepias* of differing cardenolide content provided no evidence for such differences (*44*). The experiment was, however, limited both in replication and design. Feeding efficiencies were determined only on fourth instar larvae although major food consumption occurs during the fifth instar (*45*). It is also possible that the most critical effects of cardenolide ingestion occur very early in larval development (*46*). In this experiment fecundity was not determined with reference to the normal life span and was low for all categories, and any differential effects of cardenolides could have been masked by other differences in the four plant species, both physical and chemical.

In a related investigation on the tobacco hornworm (*Protoparce sexta* Johan., Lepidoptera, family Sphingidae), a species adapted to feed on alkaloid-bearing plants (Solanaceae), large amounts of injected nicotine resulted only in temporary sluggish behavior (*47*), whereas some other (but not all) non–tobacco-feeding species are extremely sensitive to nicotine, either when it is ingested or injected (*48*). It was similarly reported that a grasshopper, *Poekilocerus bufonius* (Klug) (Orthoptera, family Pyrgomorphidae), which feeds on milkweeds and sequesters cardiac glycosides, is 300 times less sensitive (in terms of lethal dosage) to injections of the cardenolide ouabain than are two other species which do not eat milkweeds (*25*). In Erickson's study (*44*) it was undoubtedly difficult to measure the physiological cost of sequestering cardenolides, both because of the probable long-term coevolution of the Danaidae with the Asclepiadaceae (*49*) and because of the fact that the monarch butterfly in the wild feeds on all four of the *Asclepias* species (*28, 50*). Future research on the metabolic cost of sequestering cardenolides should be done, particularly in view of the fact that individual butterflies from populations in California and Massachusetts not only contain concentrations of cardenolides that range from none to large amounts, but also contain cardiac glycosides of very different emetic potencies (*16*).

Antipredator Strategy

To deduce the probable adaptive significance of the differential cardenolide distribution in the various body parts, it is important to observe the laboratory behavior of blue jays toward monarch

butterflies. In a typical response to a palatable monarch [that is, a nonemetic one reared on an asclepiad lacking cardenolides (*7*)], the jay seizes a wing in its bill, carries the butterfly to a perch, and rapidly pecks off all the wings and legs, which it almost never eats. The bird then swallows the wingless butterfly, will eat up to six in a row, and will continue to eat them day after day. The first toxic monarch offered to the bird will be treated in essentially the same manner. However, after the ensuing emetic experience, the jay's behavior toward the next monarch, if it attacks it at all (*7, 8, 51*), is to take the butterfly to the perch and much more slowly manipulate it with bill and feet. Under restrictive feeding conditions, jays can learn after a single emetic experience to discriminate between monarchs containing cardiac glycosides and those lacking them (*52*).

Behavioral and neurophysiological studies on a limited number of bird species have indicated gustatory sensibility and aversiveness to bitter compounds (*53*), and quinine salts have been used effectively to render insects and other experimental food unpalatable to several species of wild birds both in the laboratory and in the wild (*54*). Unpalatability was demonstrated when the birds, after one or more taste experiences, associated a color or pattern with the quinine-treated food and thereafter rejected it, while continuing to accept alternative food without quinine and of a different color pattern.

Although comparative quantitative studies are lacking, most naturally occurring plant glycosides (*55*), including cardenolides (*56*), are bitter tasting (*53, 57*). It is reasonable, therefore, that in at least some birds the bitter flavor of the cardenolides acts as a conditioned aversive stimulus enabling them to discriminate the emetic from nonemetic monarchs (*58*). There is a substantial literature on Lepidoptera collected in the wild showing wing damage definitely attributable to attack by birds (*59*). Of particular relevance are two statistical studies indicating that among unpalatable butterfly species, including danaines, there is a higher frequency of beak-marked individuals than there is in palatable species (*60*). This is consistent with the idea that previously conditioned birds in the wild capture both emetic and nonemetic monarchs, taste and eat the latter, but taste and reject the bitter tasting cardenolide-laden ones, leaving them unharmed, but with the telltale beak marks upon their wings.

Evolution of Unpalatability

These observations help elucidate the selective basis for the evolution of unpalatability based on emesis without the necessity of invoking theories of kin selection (*61*). Kin selection has generally been thought necessary for such evolution because the predator must ingest and thereby kill the initial carrier of the gene which allows the butterfly to be unpalatable (*31, 62*). According to this reasoning, the gene confers an advantage upon the descendants of the original emetic butterfly in that they are subjected to lowered predation because the individual predator remembers the unpleasant experience and associates it with the color or pattern of the prey, that is, because of conditioned visual avoidance. However, since all the monarch butterflies look alike, the predator is unable visually to distinguish between palatable and unpalatable individuals in the population. In other words, the advantage accruing from the predator's learned avoidance is divided equally among all the individuals in the population with no differential advantage to the original mutant individual's descendants.

While it is true that a population containing emetic individuals will be slightly less subject to attack as a whole (thus raising the remote possibility of group selection) and therefore might increase in size, whenever members of such a population intermingled and intermated with members of another population lacking the gene, the frequency of the gene would automatically decrease. If, however, the surviving emetic offspring on subsequent capture by the previously sickened predator *tended to be rejected without being killed,* then the gene would increase in frequency from generation to generation.

It therefore seems of great significance that the concentration of cardenolides in the wings of the monarch butterfly is so very much higher than in any other part of the body (Fig. 1, left). Such a distribution should enhance the likelihood of the predator recalling the association of the bitter flavor of the cardenolides with the prior emetic experience. However, if the bird were to forget the experience, or were extremely hungry and proceeded to peck off the wings, it would be discarding the *least* emetic of the sequestered glycosides (Fig. 1, center). Once the wings of an individual butterfly have been removed, its survival is no longer possible, and in such a situation the best strategy for the insect would be to keep the bird eating so as to ensure the ingestion of at least one emetic dose. Almost invariably, after the bird removes the wings the next part of the butterfly that it pecks apart and eats is the thorax. Since this portion of the body has the lowest concentration of cardenolides, as well as the smallest amounts (Fig. 1, left and right), it is the least deterrent and leads the bird into eating the abdomen. Since the abdomen has nearly ten times the number of ED_{50} units as the thorax (Fig. 1, right), emesis is likely even if only part of it is eaten, and this reaction will reinforce the bitter flavor as the conditioned aversive stimulus.

As the frequency of the genes controlling cardenolide sequestration increases in the butterfly population as a result of the predators' learned taste discrimination, the birds will begin to reject the butterflies on sight (*63*). At this point, visual automimicry (*8*) will come into operation and greatly lower the advantage of any further increase in the frequency of emetic individuals in the population. This will promote a balanced polymorphism within which the proportion of palatable to unpalatable individuals will vary, depending upon both the extent to which the cardiac glycosides are physiologically detrimental and the availability of poisonous and nonpoisonous milkweeds (*64*).

We conclude that, given the probable metabolic costs of handling the ingested cardenolides, the evolution of sequestering and of differential placement of these emetic poisons in the body parts of the monarch butterfly has reached a remarkable state of refinement as an antipredator device.

Summary

The cardiac glycosides that monarch butterflies sequester from milkweed plants during the larval stage differ remarkably in their emetic potency and are concentrated to different degrees in the various parts of the body as well as in the two sexes (Fig. 1). The very high concentrations of these compounds in the wings probably facilitate learned taste rejection in predators and account for the relatively high frequency of Danaid butterflies with beak-marked wings in natural populations. The cardiac glycosides in the abdomen have a much higher emetic potency than those

in the rest of the body. Consequently, naive, extremely hungry, or forgetful birds which capture and peck off the wings but eat the abdomen discard the least emetic glycosides and ingest the most emetic, and thus again experience emesis. The nonrandom distribution of cardenolides in the wings, abdomen, and thorax, together with the fact that monarch males not only contain lower concentrations of cardiac glycosides than females but also contain cardenolides that are overall less emetic than those in females, is interpreted as evidence that these poisons are incorporated at a physiological cost. This cost, balanced against the benefits of protection from predation, provides a selective basis for the occurrence of both emetic and nonemetic individuals in natural populations. Since birds can discriminate emetic from nonemetic monarchs on the basis of taste, it is not necessary to invoke theories of kin or group selection to explain the evolution of this kind of unpalatability.

References and Notes

1. T. Eisner, in *Chemical Ecology*, E. Sondheimer and J. B. Simeone, Eds. (Academic Press, New York, 1970), p. 157.
2. J. A. Parsons, *J. Physiol. (Lond..)* **178**, 290 (1965).
3. L. P. Brower, J. V. Z. Brower, J. M. Corvino, *Proc. Natl. Acad. Sci. U.S.A.* **57**, 893 (1967).
4. T. Reichstein, *Naturwiss. Rundsch.* **12**, 499 (1967).
5. L. P. Brower, W. N. Ryerson, L. L. Coppinger, S. C. Glazier, *Science* **161**, 1349 (1968).
6. T. Reichstein, J. V. Euw, J. A. Parsons, M. Rothschild, *ibid.*, p. 861.
7. L. P. Brower, *Sci. Am.* **220** (No. 2), 22 (1969).
8. L. P. Brower, F. H. Pough, H. R. Meck, *Proc. Natl. Acad. Sci. U.S.A.* **66**, 1059 (1970); F. H. Pough, L. P. Brower, H. R. Meck, S. R. Kessell, *ibid.* **70**, 2261 (1973).
9. M. Rothschild, *Symp. R. Entomol. Soc. Lond.* **6**, 59 (1973).
10. G. G. E. Scudder and S. S. Duffey, *Can. J. Zool.* **50**, 35 (1972).
11. For the first comprehensive review of the involvement of secondary plant chemicals in three trophic levels see L. P. Brower and J. V. Z. Brower, *Zoologica* **49**, 137 (1964).
12. Some examples are: (i) parasitic mistletoe incorporates cardiac glycosides from the apocyanaceous *Neium oleander* [C. Boonsong and S. E. Wright, *Aust. J. Chem.* **14**, 449 (1961)]; (ii) the mussel *Mytilus californicus* and the butter clam *Saxidonus giganteus* incorporate the highly poisonous "saxitoxin" into their tissues from dinoflagellates and are the ecological foundation for paralytic shellfish poisoning [see Lane (*13*); N. M. Trieff, J. J. Spikes, S. M. Ray, J. B. Nash, in *Toxins of Animal and Plant Origin*, A. de Vries and E. Kochva, Eds. (Gordon & Breach, New York, 1972), vol. 2, p. 557; E. J. Schantz, in *Toxic Constituents of Animal Foodstuffs*, I. E. Liener, Ed. (Academic Press, New York, 1974), p. 111]; (iii) the evidence strongly suggests that on coral reefs blue green algae impart their extremely toxic "ciguatoxin" to herbivorous fish and thence up the foodchain to larger carnivorous species [see Lane (*13*); M. H. Baslow, *Marine Pharmacology* (Williams & Wilkins, Baltimore, Md., 1969); B. W. Halstead, *Poisonous and Venomous Marine Animals of the World* (Government Printing Office, Washington, D.C., eds. 1 to 3, 1965, 1967, 1970)]; (iv) some sea cucumbers (Echinodermata) contain saponins which may be transformation products of food constituents [M. H. Baslow, *Ann. Rev. Pharmacol.* **11**, 447 (1971)]; (v) poisonous mushrooms eaten by terrestrial tortoises and unknown poisons ingested by marine species may make them poisonous [C. H. Pope, *Turtles of the U.S. and Canada* (Knopf, New York, 1939); A. F. Carr, *Handbook of Turtles, the Turtles of the United States, Canada and Baja California* (Comstock, Ithaca, New York, 1952), pp. 147, 381]; (vi) the African mammal *Hyrax* is capable of ingesting poisonous shrubs and might be poisonous [J. B. Sale, *East Afr. Wildl. J.* **3**, 127 (1965)]; (vii) for several of the insect examples see Rothschild (*9*); Scudder and Duffey (*10*); M. Rothschild, J. V. Euw, T. Reichstein, in *Mitteilungen der Basler Afrika Bibliographien* **4–6**, 135 (1972); *Proc. R. Entomol. Soc. Lond. Ser. B. Taxon.* **183**, 227 (1973); (viii) some birds may also take up poisons from their food [J. Kear, *Bull. Br. Ornithol. Club* **88**, 98 (1968); H. B. Cott, *Ostrich Suppl.* **8**, 357 (1971)].
13. C. E. Lane, *Annu. Rev. Pharmacol.* **8**, 409 (1968).
14. The plants were grown in the Amherst College greenhouse from a new stock of seeds collected in Mayaro, Trinidad, West Indies, by field assistant J. Hernandez in the spring of 1966. The butterflies were reared in April 1967 as before (*5*) at approximately 20°C and 55 percent relative humidity and were the third generation of a stock obtained in southern Florida (stock FB-3).
15. L. P. Brower, P. B. McEvoy, K. L. Williamson, M. A. Flannery, *Science* **177**, 426 (1972).
16. L. P. Brower and C. M. Moffitt, *Nature (Lond.)* **249**, 280 (1974).
17. We calculated the line of best fit of absorbances against molar concentrations determined by serial dilutions (January 1974) to the linear regression equation $y = ax + c$, where y is the absorbance at 626 nanometers, a is the extinction coefficient (calculated from the Beer-Lambert law), x is the molar concentration of the cuvette solution (one-fourth the sample concentration), and c is the noise in the method. Values from this regression for digitoxin (Sigma Chemical Co., lot No. 04200450) for a and c were 23,967 and 0.0108, respectively. Values similarly determined for eight other cardenolides and genins including four isolated from Asclepiadaceae ranged from 21,589 to 26,222, and 0.008 to 0.036 (C. M. Moffitt and L. P. Brower, in preparation).
18. Our estimates of relative toxin content of the body parts to the whole butterfly and Parsons' [based on ultraviolet absorbance, see Parsons (*2*)] agree closely even though done by different methods. Respective ratios of the wings, abdomen, and thorax to the whole butterfly from his table 1 are: 3.05, 0.95, and 0.58; and from our Table 2 are: 2.78, 1.06, and 0.38 (average males and females). Differences could result from the fact that the butterflies we reared for his use were of the smaller Trinidad, West Indies, subspecies of *D. plexippus*.
19. R. P. Walton and P. C. Gazes, in *Drills Pharmacology in Medicine*, J. R. DiPalma, Ed. (McGraw–Hill, New York, ed. 3, 1965), p. 573.
20. J. H. Hoch, *A Survey of Cardiac Glycosides and Genins* (Univ. of South Carolina Press, Columbia, S.C., 1961); O. Gisvold, in *Textbook of Organic, Medicinal, and Pharmaceutical Chemistry*, C. O. Wilson, O. Gisvold, R. F. Doerge, Eds. (Lippincott, Philadelphia, 1966), p. 754.
21. The ED_{50} tests were done from 28 November 1972 through 27 July 1973 on blue jays trapped in Hampshire and Franklin counties, Massachusetts, between 11 October 1972 and 27 July 1973. The birds were stored for various times in an outside aviary or inside on a 12-hour light : 12-hour dark regime. The 111 force feedings (Table 3) utilized approximately 89 birds, most of which were force fed once only. A minimum of 35 days intervened between the successive force-feedings.
22. B. Singh and R. P. Rastogi, *Indian J. Chem.* **7**, 1105 (1969).
23. Cardenolide yields were said to differ in the two sexes but, because of the mainly qualitative estimations, were considered not significant (*6*, p. 864). Parsons (*2*) did not separate the sexes in his study.
24. Conversion in vivo may have occurred in a milkweed-feeding grasshopper; see Euw *et al.* (*25*).
25. J. V. Euw, L. Fishelson, J. A. Parsons, T. Reichstein, M. Rothschild, *Nature (Lond.)* **214**, 35 (1967).
26. J. S. E. Feltwell and L. R. G. Valadon, *J. Insect Physiol.* **18**, 2203 (1972); R. S. Mummery and L. R. G. Valadon, *ibid.* **20**, 429 (1974).
27. The diminishing concentration of cardiac glycosides in the East Coast migratory populations the further south they were collected (*15*) did not occur in the West Coast study (*16*). This was probably because the migration to the over-wintering coastal colonies in California is for the most part a relatively short-distance migration westward (A. Wenner, personal communication) whereas the migration in Eastern and Central North America involves several hundred kilometers (*28*).
28. F. A. Urquhart, *The Monarch Butterfly* (Univ. of Toronto Press, Toronto, Canada, 1961).
29. A. B. Dutkowski, *J. Insect Physiol.* **19**, 1721 (1973).
30. W. S. Herman, *ibid.*, p. 1883.
31. R. A. Fisher, *The Genetical Theory of Natural Selection* (Dover, New York, ed. 2, 1958).
32. L. P. Brower, in *16th Int. Congr. Zool. Proc.* **4**, 173 (1963); G. B. Williamson and C. E. Nelson, *Am. Nat.* **106**, 525 (1972).
33. A. Stoll, *The Cardiac Glycosides* (Pharmaceutical Press, London, 1937), p. 8; W. O. James, *J. Pharm. Pharmacol.* **5**, 809 (1953); J. D. Bu'Lock, *Adv. Appl. Microbiol.* **3**, 293 (1961); I. E. Liener, in *Toxic Constituents of Plant Foodstuffs*, I. E. Liener, Ed. (Academic Press, New York, 1969), p. 1; E. E. Conn, *J. Agric. Food Chem.* **17**, 519 (1969); E. D. Weinberg, *Adv. Microb. Physiol.* **4**, 1 (1970); C. H. Muller, *Science* **164**, 197 (1969); in *Biochemical Coevolution*, K. L. Chambers, Ed. (Oregon State Univ. Press, Corvallis, 1970), p. 13; R. F. Dawson and T. S. Osdene, *Rec. Adv. Phytochem.* **5**, 317 (1972).
34. K. Mothes, *Naturwissenschaften* **13**, 317 (1966); *Experientia* **25**, 225 (1969).
35. T. Reichstein, *Naturwissenschaften* **54**, 53 (1967); E. A. Bell and D. H. Janzen, *Nature (Lond.)* **229**, 136 (1971); C. E. Boyd and C. P. Goodyear, *Arch. Hydrobiol.* **69**, 256 (1971); V. G. Dethier, in *Chemical Ecology*, E. Sondheimer and J. B. Simeone, Eds. (Academic Press, New York, 1970), p. 83; P. M. Dolinger, P. R. Ehrlich, W. L. Fitch, D. E. Breedlove, *Oecologia* **13**, 191 (1973); J. L. Harper and J. Ogden, *J. Ecol.* **58**, 681 (1970); R. Hegnauer, *Z. Arzneipflanzenforsch.* **19**, 138 (1970); *Naturwissenschaften* **58**, 585 (1971); D. H. Janzen, *Science* **165**, 415 (1969); *Evolution* **23**, 1 (1969); D. A. Levin, *Am. Nat.* **105**, 157 (1971); A. R. Mattocks, in *Phytochemical Ecology*, J. B. Harborne, Ed. (Academic Press, New York, 1972), p. 179; S. T. Pickett, II, and J. M. Baskin, *Biologist* **55**, 49 (1973); T. Robinson, *Science* **184**, 430 (1974); L. L. Rockwood, *Ecology* **54**, 1333 (1973); D. S. Van Fleet, *Rec. Adv. Phytochem.* **5**, 165 (1972); R. H. Whittaker, in *Chemical Ecology*, E. Sondheimer and J. B. Simeone, Eds. (Academic Press, New York, 1970), p. 43; R. H. Whittaker and P. P. Feeny, *Science* **171**, 757 (1971); N. H. Williams and C. H. Dodson, *Evolution* **26**, 84 (1972); M. F. Willson, *Biologist* **55**, 89 (1973).
36. N. L. Allport, *The Chemistry and Pharmacy of Vegetable Drugs* (Newnes, London, 1943); N. R. Farnsworth, *J. Pharm. Sci.* **55**, 225 (1966); K. F. Lampe and R. Fagerstrom, *Plant Toxicity and Dermatitis* (Williams & Wilkins, Baltimore, Md., 1968).
37. E. A. Bell, in *Phytochemical Ecology*, J. B. Harborne, Ed. (Academic Press, New York, 1972), p. 163; Y. Birk, in *Toxic Constituents of Plant Foodstuffs*, I. E. Liener, Ed. (Academic Press, New York, 1969), p. 169; A. H. Conney and J. J. Burns, *Science* **178**, 576 (1972); D. G. Crosby, *Agr. Food Chem.* **17**, 532 (1969); C. C. J. Culvenor, *Search* **1**, 103 (1970); V. G. Dethier, *Evolution* **8**, 33 (1954); P. Feeny, *Ecology* **51**, 565 (1970); in *Coevolution of Animals and Plants*, L. E. Gilbert and P. H. Raven, Eds., *Symp. 1st Int. Congr. Systematics and Evolutionary Biol.* (Univ. of Texas Press, Austin, 1975), p. G. Fraenkel, *Entomol. Exp. Appl.* **12**, 473 (1969); R. E. Heal, E. F. Rogers, R. T. Wallace, O. Starnes, *Lloydia* **13**, 89 (1950); D. A. Jones, in *Phytochemical Ecology*, J. B. Harborne, Ed. (Academic Press, New York, 1972), p. 103; R. F. Keeler, *J. Agric. Food Chem.* **17**, 473 (1969); A. C. Leopold and R. Ardrey, *Science* **176**, 512 (1972); E. K. McLean, *Pharmacol. Rev.* **22**, 429 (1970); I. E. Liener, in *Toxic Constituents of Plant*

Foodstuffs, I. E. Liener, Ed. (Academic Press, New York, 1969), p. 410; M. O. Moss, in *Phytochemical Ecology*, J. B. Harborne, Ed. (Academic Press, New York, 1972), p. 1 → R. Moss and J. A. Parkinson, *Br. J. Nutr.* **27**, 285 (1972); E. Nowacki and G. R. Waller, *Flora* **162**, 108 (1973); G. H. Orians and D. H. Janzen, *Am. Nat.* **108**, 581 (1974); S. S. Rehr, D. H. Janzen, P. P. Feeny, *Science* **181**, 81 (1973); N. Sapeika, in *Toxic Constituents of Animal Foodstuffs*, I. E. Liener, Ed. (Academic Press, New York, 1974), p. 1; L. M. Schoonhoven, *Rec. Adv. Phytochem.* **5**, 197 (197 → J. M. Scriber, *Psyche* **80**, 355 (1973); V. L. Singleton and F. H. Kratzer, *J. Agric. Food Chem.* **17**, 497 (1969); M. E. Wall and K. H. Davis, Jr., in *Toxins of Animal and Plant Origin*, A. de Vries and E. Kochva, Eds. (Gordon & Breach, New York, 1972), p. 598.

38. K. L. S. Harley and A. J. Thorsteinson, *Can. J. Zool.* **45**, 305 (1967).
39. R. T. Williams, *Detoxication Mechanisms* (Chapman & Hall, London, 1959); H. Remmer, *Annu. Rev. Pharmacol.* **5**, 405 (1965); J. R. Gillette, A. H. Conney, G. J. Cosmides, R. W. Estabrook, J. R. Fouts, G. J. Manning, Eds., *Microsomes and Drug Oxidations* (Academic Press, New York, 1969); A. R. Mattocks, in *Phytochemical Ecology*, J. B. Harborne, Ed. (Academic Press, New York, 1972), p. 179.
40. C. F. Wilkinson and L. B. Brattsten, *Drug Metab. Rev.* **1**, 153 (1973); J. E. Casida, *Annu. Rev. Biochem.* **42**, 259 (1973); R. I. Krieger, P. P. Feeny, C. F. Wilkinson, *Science* **172**, 579 (1971); R. I. Krieger and C. F. Wilkinson, *Biochem. Pharmacol.* **18**, 1403 (1969).
41. R. B. Clayton, in *Chemical Ecology*, E. Sondheimer and J. B. Simeone, Eds. (Academic Press, New York, 1970), p. 235; R. H. Dadd, *Annu. Rev. Entomol.* **18**, 381 (1973); J. M. S. Forrest and B. A. Knights, *J. Insect Physiol.* **18**, 723 (1972); W. E. Robbins, J. N. Kaplanis, J. A. Svoboda, M. J. Thompson, *Annu. Rev. Entomol.* **16**, 53 (1971).
42. J. F. Barker and W. S. Herman, *J. Exp. Zool.* **183**, 1 (1973); R. M. Barth and L. J. Lester, *Annu. Rev. Entomol.* **18**, 445 (1973).
43. *Merck Index* (Merck & Company, Rahway, N.J., ed. 7, 1960), p. 358.
44. J. M. Erickson, *Psyche* **80**, 230 (1973).
→ G. P. Waldbauer, *Adv. Insect Physiol.* **5**, 229 (1968).
46. R. Hansberry, W. W. Middlekauf, L. B. Norton, *J. Econ. Entomol.* **33**, 511 (1940).
47. L. S. Self, F. E. Guthrie, E. Hodgson, *J. Insect Physiol.* **10**, 907 (1964).
48. R. S. H. Yang and F. E. Guthrie, *Ann. Entomol. Soc. Am.* **62**, 141 (1969).
→ P. R. Ehrlich and P. H. Raven, *Evolution* **18**, 586 (1965); T. R. E. Southwood, *Symp. R. Entomol. Soc. Lond.* **6**, 3 (1973).
50. L. P. Brower, *Ecology* **42**, 76 (1961).
51. When alternative palatable butterflies of a different species are offered in a random sequence with the unpalatable monarchs, the birds rapidly learn to reject the monarchs on sight alone [J. Brower, *Evolution* **12**, 273 (1958)].
52. L. P. Brower, in preparation.
53. B. M. Wenzel, in *Avian Biology*, D. S. Farner, J. R. King, K. C. Parkes, Eds. (Academic Press, New York, 1973), vol. 3, p. 389.
54. For example, starlings (*Sturnus vulgaris*) [J. V. Z. Brower, *Am. Nat.* **94**, 271 (1960)]; black-capped chicadees (*Parus atricapilli* → [J. Alcock, *Anim. Behav.* **18**, 592 (1970)]; black-capped chicadees and white-crowned sparrows (*Zonotrichia leucophrys*) [J. Alcock, *Behaviour* **40**, 1 (1971)]; starlings, blackbirds (*Turdus merula*), song thrushes (*T. philomelos*), and house sparrows (*Passer domesticus*) [M. Ikin and J. R. G. Turner, *Nature (Lond.)* **239**, 525 (1972)]; starlings, house sparrows, blackbirds, song thrushes, English robins (*Erithacus rubecula*), great tits (*Parus major*), blue tits (*P. caeruleus*), and chaffinches (*Fringilla coelebs*) [R. G. Lea and J. R. G. Turner, *Behaviour* **42**, 131 (1972); G. M. Morrell and J. R. G. Turner, *ibid.* **36**, 116 (1970)]; house sparrows and blue jays [P. O'Donald and C. Pilecki, *Evolution* **24**, 395 (1970); C. Pilecki and P. O'Donald, *ibid.* **25**, 365 (1971)].
55. J. M. Kingsbury, *Poisonous Plants of the United States and Canada* (Prentice-Hall, Englewood Cliffs, N.J., 1964), p. 23; R. S. Shallenberger and T. E. Acree, in *Handbook of Sensory Physiology* 4, *Chemical Senses*, part 2, *Taste*, L. M. Beidler, Ed. (Springer-Verlag, Berlin, 1970), p. 222.
56. For example, digitoxin [T. E. Wallis, *Textbook of Pharmacology* (Churchill, London, ed. 4, 1960), p. 147]; cymarin [see (*43*)]; for glycosides in general see R. Hegnauer, *Naturwissenschaften* **58**, 585 (1971). According to T. Reichstein (personal communication) most cardenolides are strongly bitter to the human taste, but there are some exceptions, for example, ouabain.
57. There is a correlation between the bitterness and toxicity of naturally occurring compounds [for example, see J. Z. Young, in *Biology of the Mouth*, P. Person, Ed. (AAAS, Washington, D.C., 1968), p. 21; R. Fischer and F. Griffin, *Arzneim-Forsch.* **14**, 673 (1964)]. However, this correlation needs far more ecological and evolutionary consideration than characterizes the majority of physiological and behavioral studies [for example, M. A. Amerine, R. M. Pangborn, E. B. Roessler, *Principles of Sensory Evaluation of Food* (Academic Press, New York, 1965); P. D. Sturkie, *Avian Physiology* (Comstock Publishing Association and Cornell Univ. Press, Ithaca, N.Y., ed. 2, 1965); H. L. Jacobs, in *The Chemical Senses and Nutrition*, M. R. Kare and O. Maller, Eds. (Johns Hopkins Press, Baltimore, Md., 1967), p. 187; A. N. Epstein, in *ibid.*, p. 263; M. R. Kare, in *Handbook of Sensory Physiology* 4, *Chemical Senses*, part 2 *Taste*, L. M. Beidler, Ed. (Springer-Verlag, Berlin, 1970), p. 278; J. Garcia and W. G. Hankins, in *International Symposium on Olfaction and Taste 1974*, D. Denton, Ed., in preparation.
58. Recent studies with rats as subjects established that chemical stimuli including taste and olfactory cues present in the food or water can easily be paired with delayed noxious effects so that food or water containing them is subsequently rejected [J. Garcia, F. R. Ervin, R. A. Koelling, *Psychonom. Sci.* **5**, 121 (1966); S. H. Revusky and E. W. Bedarf, *Science* **155**, 219 (1967); P. Rozin, J. W. Kalat, *Psychol. Rev.* **78**, 459 (1971); J. Garcia, B. K. McGowan, K. F. Green, in *Classical Conditioning* II: *Current Research and Theory*, A. H. Black and W. F. Prokasy, Eds. (Appleton-Century-Crofts, New York, 1972); J. Garcia, W. G. Hankins, K. W. Rusiniak, *Science* **185**, 824 (1974)]. The same has been found for coyotes [C. R. Gustavson, J. Garcia, W. G. Hankins, K. W. Rusiniak, *ibid.* **184**, 581 (1974)].
59. C. L. Collenette, *Trans. Entomol. Soc. Lond.* **1928**, 391 (1928); T. D. Sargent, *J. Lepid. Soc.* **27**, 175 (1973); A. M. Shapiro, *Am. Nat.* **108**, 229 (1974); M. Edmunds, *Oikos* **25**, 117 (1974).
60. G. D. H. Carpenter, *7th Int. Cong. Entomol., Berlin 1938*, 1061 (1939); *Proc. Zool. Soc. London* **111(A)**, 223 (1941).
→ W. D. Hamilton, *J. Theor. Biol.* **7**, 1, 17 (1964); J. M. Smith, *Am. Nat.* **99**, 59 (1965); P. J. Darlington, Jr., *Proc. Natl. Acad. Sci. U.S.A.* **69**, 293 (1972).
62. W. W. Benson, *Am. Nat.* **105**, 213 (1971); J. R. G. Turner, *Biotropica* **3**, 21 (1971); in *Ecological Genetics and Evolution*, R. Creed, Ed. (Blackwell, Oxford, England, 1971), p. 2 → I. Eshel, *Theor. Pop. Biol.* **3**, 258 (1972).
63. This contention is supported not only by theoretical and experimental investigations of mimicry (*7, 8, 51, 54*) but also by a study in which bobwhite quail (*Colinus virginianus*) were found able to associate either a gustatory or a visual cue of their drinking water solutions with drug-induced diarrhea, but when they could do both, the visual cue was preferred [H. C. Wilcoxon, W. B. Dragoin, P. A. Kral, *Science* **171**, 826 (1971); see also S. J. Shettleworth, *Adv. Stud. Behav.* **4**, 1 (1972)].
64. Once unpalatability has evolved to the point where visual automimicry is operating, mutations increasing the conspicuousness of the insects and thereby the visually conditioned avoidance will also be favored by selective predation. Thus, kin selection need not be invoked for the evolution either of emetic unpalatability or warning coloration.
65. Supported by NSF grants GB 24888 and 35317X with L.P.B. as principal investigator. I thank Homer Bessonette, Barbara Tiffany, and Billie Conley for technical help, and C. M. Moffitt for extensive assistance in data analysis together with Carolyn Roeske, James Seiber, Robert Krieger, Francisco Ayala, Arthur Shapiro, and Robert Metzger for valuable comments. I am grateful to the Division of Environmental Studies and the Department of Environmental Toxicology, University of California, Davis, for sabbatical leave hospitality.