Journal of Chemical Ecology, Vol. 11, No. 1, 1985

DIFFERENCES AND SIMILARITIES IN CARDENOLIDE CONTENTS OF QUEEN AND MONARCH BUTTERFLIES IN FLORIDA AND THEIR ECOLOGICAL AND EVOLUTIONARY IMPLICATIONS

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(Received February 22, 1984; revised May 21, 1984)

Abstract—Florida queen butterflies are highly variable in cardenolide content and, in three populations studied, contained less cardenolide than did a sample of sympatric Florida monarchs. The possibility that queens stored a more potent set of cardenolides from their host plants (and therefore were as well protected as monarchs, even at lower concentrations) is refuted by chromatographic analysis of wild butterflies, as well as controlled laboratory rearings. It therefore appears that, with respect to cardenolides, monarchs are better defended than are queens. Consequently, cardenolides are unlikely to explain the apparent shift in Florida viceroy mimicry away from resemblance of the monarch, toward mimicry of the queen. Other hypotheses to explain this mimetic phenomenon are suggested. Adult monarchs exhibit significant negative correlations between the concentration of cardenolide stored in their tissues and both body size and weight, whereas queens show no such correlations. The implications of these results for the study of "metabolic costs" of allelochemic storage are discussed. Chromatographic evidence is provided that monarchs do breed in south Florida during the winter months and that the likely host plant employed by the population studied was *Asclepias curassavica.* This represents the first practical application of cardenolide "fingerprinting" to identify the larval host plants of wild danaid butterflies.

Key Words—*Danaus gilippus, Danaus plexippus,* Lepidoptera, Danaidae, cardiac glycosides, cardenolides, *Asclepias,* Asclepiadaceae, allelochemics, plant secondary chemistry, chemical ecology, chemical defense, mimicry, *Limenitis archippus.*

INTRODUCTION

The butterfly family Danaidae has figured prominently in the development of the theory of plant–herbivore coevolution (Brower and Brower, 1964; Ehrlich

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and Raven, 1964) and has provided one of the best examples of the chemical defenses of insects against vertebrate predators (Brower, 1969; Brower et al., 1982; Dixon et al., 1978; Reichstein et al., 1968). However, much of our understanding of the ecology and evolution of plant–danaid–predator interactions is based upon only one species in this tropical family, the monarch butterfly (*Danaus plexippus* L.; review in Brower, 1984). Because this species is known to be unique among danaids in certain other respects (e.g., migration: Urquhart, 1960; lack of plant-derived sex pheromones: Edgar et al., 1976; Boppré, 1978), one must question whether monarchs are representative of the Danaidae with respect to host plant adaptation and chemical defense, or whether they represent just one point (or range) within a broader spectrum of danaid adaptations. If such a spectrum is found to exist, it may ultimately become possible to reconstruct some of the evolutionary steps leading to the close association between danaids and their highly toxic milkweed host plants (Asclepiadaceae).

Danaid species differ in the amounts of toxic cardenolides which they sequester from their food plants. For example, Brower et al. (1975) showed that the cardenolide concentrations of laboratory-reared African queen butterflies (*Danaus chrysippus* L.) were only about 30% that of monarchs simultaneously reared on the same milkweed host plants (see also Brower et al., 1978; Rothschild et al., 1975). The cardenolide concentrations of queen butterflies (*D. gilippus berenice* Cramer) from Florida were, on average, about 75% that of monarchs reared in the laboratory on the same host plants. Other genera of danaids (e.g., *Amauris, Euploea*) appear to prefer as larval host plants those milkweed species lacking cardenolides (Rothschild and Marsh, 1978).

Does this diversity in the storage of cardenolides reflect differences among danaid species in their degree of adaptation to these defensive plant allelochemics? Do queens, for example, store less cardenolide than monarchs because they are less well adapted and can tolerate these compounds less readily? Do the cardenolide contents of various danaid species differ only quantitatively, as described above, or are there also qualitative differences in the particular set of host plant cardenolides sequestered?

Here these questions are addressed through a comparison of the cardenolide contents of wild queen butterflies (*D. gilippus berenice*) from three populations in Florida and of monarchs (*Danaus plexippus*) from one of these populations. Following Brower and Moffitt (1974), the "cost" of storing cardenolides will be assessed by searching for correlations between body size or weight and cardenolide concentration of the butterflies. Since body size and weight are typically correlated with fecundity in Lepidoptera (see Hinton, 1981), a negative correlation with cardenolide concentration would suggest that one component of fitness (i.e., fecundity) has been traded for another (e.g., higher survival due to chemical defense from cardenolides). While such a trade may well be of positive net value to the insect, it nevertheless would require an investment or cost which, it is assumed, should be lessened as adaptation to allelochemics evolves.

The subspecies of queen studied here is of further interest because it is the apparent model for mimicry by the southern subspecies of the viceroy butterfly (*Limenitis archippus floridensis* Strecker), which throughout the remainder of North America mimics the monarch (Brower, 1958a,b; Klots, 1951; Remington, 1968). Thus, a comparison of the chemical basis for defense in the queen and monarch should aid in understanding both the selective rationale for the switch in viceroy mimicry and the broader issues of herbivore adaptation to host plants mentioned above.

METHODS AND MATERIALS

Wild queen butterflies were collected from three populations in Florida, listed from north to south as follows: Lake Istokpoga, Highlands County, September 7, 1981; Corkscrew Swamp Sanctuary, Collier County, September 6, 1981; and Miami, Dade County, December 4, 1981. In addition, monarchs were collected from the Miami site where they were sympatric with queens, and where only the milkweed *Asclepias curassavica* grew abundantly. (However, a few *A. incarnata* plants were also located.) These collections therefore permit a study of population variation in cardenolide content of queens, as well as a comparison of the relative value, with respect to cardenolides, of monarchs and queens as models for viceroy mimicry. All butterflies were placed on ice immediately following capture, killed by freezing, and later dried for 16 hr at 60°C. Dry weights were determined using a Mettler AK-160 electronic balance. The right wing was removed with forceps, and the distance from the apex to the anterior notal process was measured to the nearest 0.5 mm with hand calipers. Fat was removed from the butterflies by petroleum–ether extraction of each entire insect for 1 hr (methods in Walford and Brower, 1985). This procedure removes only negligible amounts of cardenolide from the insect (Nelson and Brower, unpublished data; see also Nishio, 1980). Lean weights were calculated by subtracting the weight of extracted fat from the total dry weight of each insect.

Cardenolide content was determined by standard spectrophotometric methods (Brower et al., 1972, 1975), with one modification. Soon after beginning the spectroassay of queens from Corkscrew Swamp, it became evident that many of the butterflies contained very little cardenolide. In such cases it is frequently difficult to achieve a stable absorbance reading. Consequently, 0.3 ml of a 12.5×10^{-5} M ethanolic solution of digitoxin was added to each butterfly extract in the cuvette (replacing 0.3 ml of 95% ethanol; see Brower et al., 1972) in order to artificially "boost" absorbance readings to a more stable mid-range. A pilot test demonstrated that the absorbance of the "boost" digitoxin alone was 0.400 ± 0.009 ($\bar{X} \pm$ SD; $N = 9$). This mean value was therefore subtracted from the total absorbance read for a sample, the remainder being the absorbance due to the butterfly extract alone. In order for this remainder to be considered signifi-

cantly different from zero, it had to exceed background level (0.400) by at least two standard deviations (i.e., 0.018); thus, the minimum detectable cardenolide concentration, using this method was 3 μg/0.1 g.

In order to compare qualitatively the cardenolides present in sympatric monarchs and queens from the Miami sample, those butterflies containing a total of at least 30 μg equivalents of digitoxin (as determined from the spectroassay) were subjected to a lead acetate clean-up procedure in preparation for thin-layer chromatography (TLC). The procedure used was that described by Brower et al. (1982) with the exception that the final solution was filtered through a Millipore filter (Millipore Corp., Bedford, Massachusetts) rather than through a funnel of glass wool and anhydrous sodium sulfate. This clean-up procedure removes much of the interfering pigments and other noncardenolide compounds from the butterfly samples. TLC was then performed and plates developed four times in a chloroform–methanol–formamide solvent system (90:6:1). Further details of the TLC procedure are available in Brower et al. (1982).

In addition to the wild-caught butterflies, eggs and first instar larvae of both monarchs and queens were collected from milkweed plants growing in La Vega province, Dominican Republic, during July 1981. These were brought back to the laboratory and reared to maturity on an exclusive diet of *A. humistrata* leaves, collected wild in the vicinity of Gainesville, Florida. The leaves of this species contain relatively high concentrations of cardenolide (Cohen and Brower, 1982). From these rearings, 10 adult monarchs and seven queens were compared for quantitative (via spectrophotometry) and qualitative (via TLC) differences in cardenolide storage. For comparison, two arctiid moth species (*Cycnia tenera,* an apocynale specialist; and *Estigmene acraea,* a highly polyphagous species; Tietz, 1972) were reared on *A. humistrata* and chromatographed along with the danaids.

RESULTS

Wild-Caught Butterflies from Florida. The frequency distributions of cardenolide concentration and total cardenolide per insect for the the wild-caught butterflies are shown in Figures 1 and 2, respectively. Each distribution departs significantly from normality [Shapiro-Wilk (W) tests; $P < 0.01$; Helwig and Council, 1979]. Thus, nonparametric statistical analyses were used.

Males and females did not differ significantly either in cardenolide concentration or total content in any of the queen or monarch populations studied (Table 1; Wilcoxon two-sample tests, $P > 0.05$ for all pairwise comparisons). Consequently, the data from both sexes of each population were pooled for all subsequent analyses.

Cardenolide concentration varied significantly among the three queen populations (Kruskal-Wallis $H = 31.60$, $df = 2$, $P < 0.0001$), as did the total

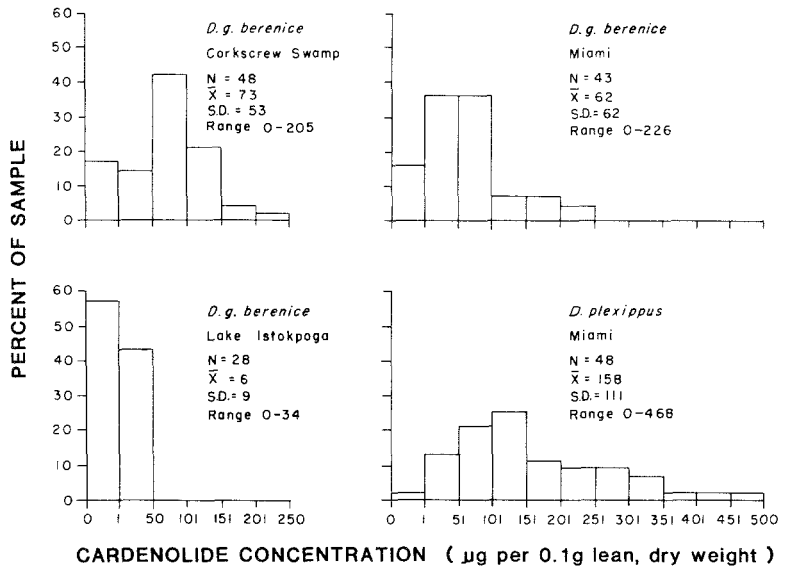

FIG. 1. Frequency distributions of cardenolide concentration (sexes pooled) for three populations of Florida queen butterfly, and one population of monarchs. Units in digitoxin equivalents.

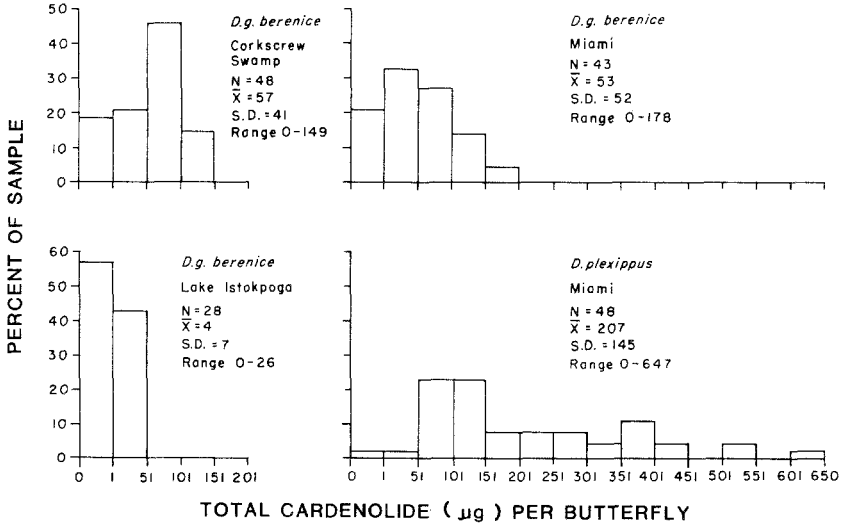

FIG. 2. Frequency distributions of total cardenolide per individual butterfly (sexes pooled).

TABLE 1. RIGHT WING LENGTHS, WEIGHTS, FAT, AND CARDENOLIDE CONTENTS OF WILD-CAUGHT QUEEN AND MONARCH BUTTERFLIES FROM THREE SITES IN FLORIDA: LAKE ISTOKPOGA (LI), CORKSCREW SWAMP (CS), AND MIAMI (MI)

Species (site)	Sex	N	Wing length (cm)		Dry weight (mg)		Lean weight (mg)		Percent fat		Cardenolide: Concentration (μg/0.1 g)		Cardenolide: Total per insect (μg)	
			$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD
Queens														
LI	M	15	4.22	0.19	94.9	22.8	84.4	19.4	10.9	3.0	8	11	6	9
	F	14	3.99	0.27	87.8	22.6	78.9	20.4	10.1	3.6	4	5	3	4
CS	M	21	4.27	0.22	95.2	16.4	88.8	15.3	6.7	2.4	64	44	57	42
	F	27	4.08	0.25	84.5	15.8	77.1	14.0	8.6	1.8	79	59	58	42
MI	M	26	4.26	0.18	92.4	15.1	82.9	12.1	10.0	3.2	78	71	66	58
	F	19	4.24	0.21	107.4	31.6	87.2	16.0	16.4	10.2	37	35	34	31
Monarchs														
MI	M	25	4.92	0.29	146.6	28.1	136.6	23.9	6.4	2.6	150	90	198	116
	F	23	4.89	0.29	158.8	42.4	133.5	25.4	14.0	9.6	166	131	216	173

cardenolide content per butterfly ($H = 30.03$, $df = 2$, $P < 0.0001$). Pairwise comparisons revealed that the queens from Lake Istokpoga had significantly lower cardenolide concentrations than either those from Corkscrew Swamp (Wilcoxon two-sample test; $Z = 5.17$, $P < 0.0001$) or those from Miami ($Z = 4.72$, $P < 0.0001$), but that the latter two populations did not differ significantly from one another ($P > 0.05$). Similar results were found for total cardenolide content per butterfly.

Analysis of the Miami samples shows that monarchs had significantly greater cardenolide concentrations (Wilcoxon two-sample test; $Z = 4.82$, $P < 0.0001$) and total contents ($Z = 6.12$, $P < 0.0001$) than the sympatric queens. Chromatography reveals a single cardenolide profile common to both species (Figure 3). This consists of nine spots, including a major one at the approximate R_f of digitoxin, and one of higher R_f. A few individuals (of both species) exhibit all of these spots plus an additional two faint spots of still higher R_f (numbered spots 10 and 11 in Figure 3). This chromatogram is virtually identical to that of monarchs reared in the laboratory on *Asclepias curassavica* (Figure 3 inset), providing strong evidence that this was the host plant utilized by the Miami monarchs and queens. To date, no other milkweed species is known to produce this particular chromatographic profile in danaid butterflies (Brower et al., 1982, 1984a,b).

The wing lengths, dry and lean weights, and fat contents of the butterflies are summarized in Table 1. The correlations between these variables and cardenolide concentration are indicated in Table 2. For both male and female monarchs, there was a highly significant negative correlation between wing length and cardenolide concentration (Table 2C). Moreover, males also showed negative correlations between cardenolide concentration and both body weight and fat content. For queens, none of the correlations was significant in either sex (all three populations pooled; Table 2A).

These statistical differences between monarchs and queens could reflect true species differences. However, the monarchs were all collected in the Miami region, while the queens for this analysis were pooled from three areas. It is possible that an unknown geographic effect is operating here, such that butterflies from the Miami region, regardless of species, would show these negative correlations (e.g., due to a common host plant). To test this, the queens from Miami were also analyzed separately from the other two populations (Table 2B). However, as before, no significant correlations emerged ($P > 0.10$ for all tests). Thus, the observed species difference is not attributable to a geographic difference.

It is possible that monarchs show negative correlations between body size and cardenolide concentration, while queens do not, simply because they store greater concentrations of these chemicals (i.e., queens may not store sufficient cardenolide to be adversely affected by it). If this is the case, then the negative correlations should vanish for that subset of monarchs having concentrations

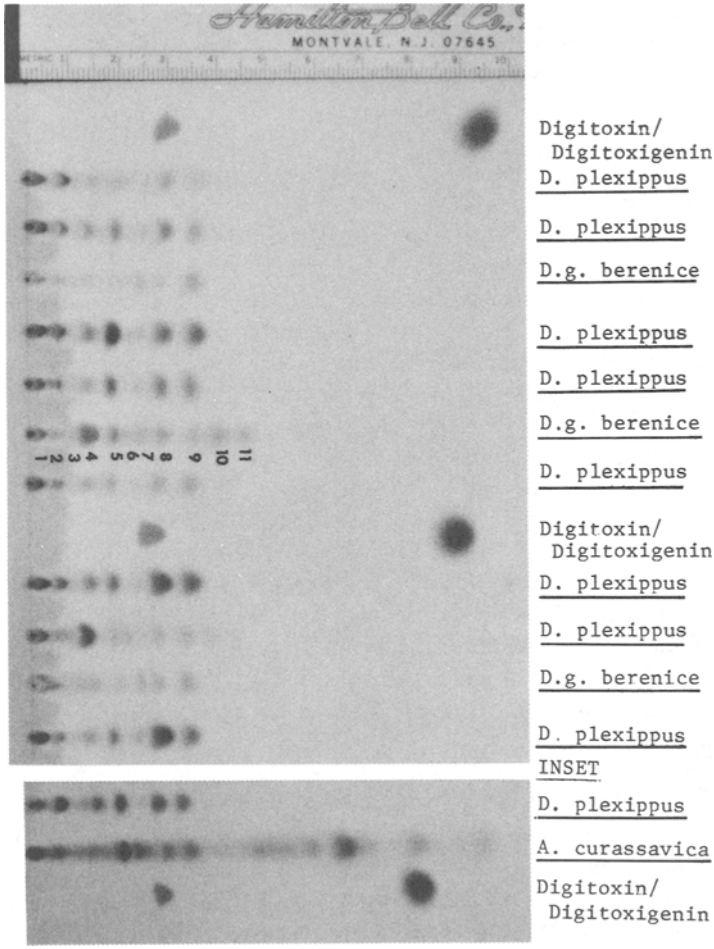

FIG. 3. Thin-layer chromatogram of wild-caught monarchs and queens from Miami area. Developed four times in chloroform–methanol–formamide (90:6:1 v/v/v). The great concordance of all profiles below spot 10 suggests that these animals had all fed upon the same larval host plant species. Note the agreement of this profile with that of monarchs reared in the laboratory on *Asclepias curassavica* (inset), a milkweed growing abundantly in the collection area. Since nearly all milkweed species studied to date produce qualitatively different TLC profiles in danaids (Brower et al., 1982, 1984a,b; see also Figure 4), *A. curassavica* is the likely larval host plant of the Miami butterflies. For reference, a 1:1 (v/v) mixture of digitoxin and digitoxigenin was spotted in three channels. Numbers to the right of each channel indicate the micrograms of cardenolide spotted, calculated prior to lead acetate clean-up. (inset from Brower, 1984)

TABLE 2. SPEARMAN CORRELATION COEFFICIENTS FOR CARDENOLIDE CONCENTRATION VS. BODY SIZE, WEIGHT, AND FAT CONTENT OF WILD-CAUGHT QUEEN AND MONARCH BUTTERFLIES FROM FLORIDA[a]

Sample	Sex	N	Cardenolide concentration versus: Wing length	Dry weight	Lean weight	Percent fat
Queens						
A. All populations	Both	119	−0.004	0.01	0.03	−0.14
	M	61	−0.13	0.14	0.13	−0.02
	F	58	0.03	−0.08	−0.08	−0.25
B. Miami only	Both	43	−0.17	0.20	0.20	0.14
	M	26	−0.25	0.26	0.25	0.32
	F	17	−0.20	0.32	0.23	0.14
Monarchs						
C. Entire Miami sample	Both	48	−0.53***[b]	−0.22	−0.21	−0.25
	M	25	−0.55**	−0.44*	−0.39	−0.53**
	F	23	−0.50*	−0.04	−0.10	−0.06
D. Concentrations less than 226 μg/0.1 g	Both	35	−0.40*	−0.33*	−0.43**	−0.18
	M	18	−0.36	−0.46*	−0.49*	−0.36
	F	17	−0.38	−0.20	−0.40	−0.06

[a]The data for queens are first shown for all three populations pooled (A), and then for the Miami sample separately (B). Monarchs were first analyzed using the entire data set (C) and then by truncating the set such that only individuals having cardenolide concentrations equal to, or less than, that of the most concentrated queen (226 μg/0.1 g) were included (D). See text for explanation.

[b]* $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.0001$.

similar to those of queens. To test this, I analyzed only those monarchs (sexes pooled) having cardenolide concentrations equal to, or less than that of, the most highly concentrated queen (i.e., 226 μg/0.1 g). In this case, significant negative correlations between cardenolide concentration and wing length, dry weight, and lean weight still occurred (Table 2D). Thus, it appears that the difference between monarchs and queens is not due merely to geographic or cardenolide concentration differences. It is also not due to differences in larval host plant species, since Figure 3 demonstrates that both species in Miami had most likely developed on *A. curassavica.* Rather, the negative correlations appear to represent inherent species differences.

Laboratory-Reared Butterflies (Dominican Republic Stock). When monarchs were reared in the laboratory on *Asclepias humistrata,* the females developed

TABLE 3. BODY SIZES, WEIGHTS, FAT, AND CARDENOLIDE CONTENTS OF QUEENS AND MONARCHS (DOMINICAN REPUBLIC STOCK) REARED IN LABORATORY ON MILKWEED, *Asclepias humistrata*

			Wing length (cm)		Dry weight (mg)		Lean weight (mg)		Percent fat		Cardenolide			
											Concentration (μg/0.1 g)		Total per insect (μg)	
Species	Sex	*N*	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD
Queen														
	Both	7	3.60	0.14	77.3	9.0	73.5	9.0	4.9	0.9	368	58	271	61
	M	3	3.63	0.12	82.2	7.7	78.6	8.0	4.5	1.1	341	34	269	55
	F	4	3.58	0.17	73.5	9.0	69.7	8.6	5.2	0.8	388	68	272	74
Monarch														
	Both	10	4.55	0.18	147.8	31.6	140.3	30.8	5.1	3.1	489	145	657	156
	M	4	4.72	0.05	177.0	28.9	167.8	29.9	5.2	4.9	385	146	615	154
	F	6	4.43	0.12	128.4	12.8	122.1	12.8	5.0	1.7	558	104	684	164

TABLE 4. SPEARMAN CORRELATION COEFFICIENTS FOR CARDENOLIDE CONCENTRATION VS. BODY SIZE, WEIGHT, AND FAT CONTENT OF QUEEN AND MONARCH BUTTERFLIES (DOMINICAN REPUBLIC STOCK; BOTH SEXES POOLED) REARED IN LABORATORY ON *Asclepias humistrata*

		Cardenolide concentration versus			
Sample	*N*	Wing length	Dry weight	Lean weight	Percent fat
Queens	7	0.33	0.18	0.25	−0.57
Monarchs	10	−0.63**[a]	−0.56*	0.05	−0.58*

[a] $*0.05 < P < 0.10$; $**P < 0.05$.

significantly higher cardenolide concentrations than did males (Wilcoxon two-sample test; $Z = 2.02$, $P < 0.05$; Table 3). No such sex difference was evident for queens reared under identical conditions ($Z = 0.53$; $P > 0.50$). As in the wild-caught samples, only the monarchs showed significant (again, negative) correlations between cardenolide concentration and other size and weight parameters (Table 4).

Thin-layer chromatography (Figure 4) demonstrates that the two danaid species (as well as two arctiid moth species) sequestered virtually identical sets of cardenolides from *A. humistrata.* This TLC profile is clearly distinguishable from that of butterflies reared on *A. curassavica* (cf. Figure 3 inset).

DISCUSSION

Body Size and Cardenolide Content. Brower and Moffitt (1974) reported a negative correlation between the body weight and cardenolide concentration of female monarchs from Massachusetts and suggested that these individuals may have suffered a "metabolic cost," in terms of growth, of sequestering cardenolides for defense (see also Brower et al., 1972). Such negative correlations were not found for males from Massachusetts (which were 9% lower than females in mean cardenolide concentration), or in either sex collected in California (which were 62% lower in mean concentration than the Massachusetts females). However, these data confound sexual and geographic differences with correlated cardenolide concentration differences. Here, I have shown that negative correlations between cardenolide concentration and various size and weight parameters, which occur in monarchs but not in queens, are independent of geographic, sexual, food plant, or correlated concentration differences.

While these negative correlations might well represent "metabolic costs" of

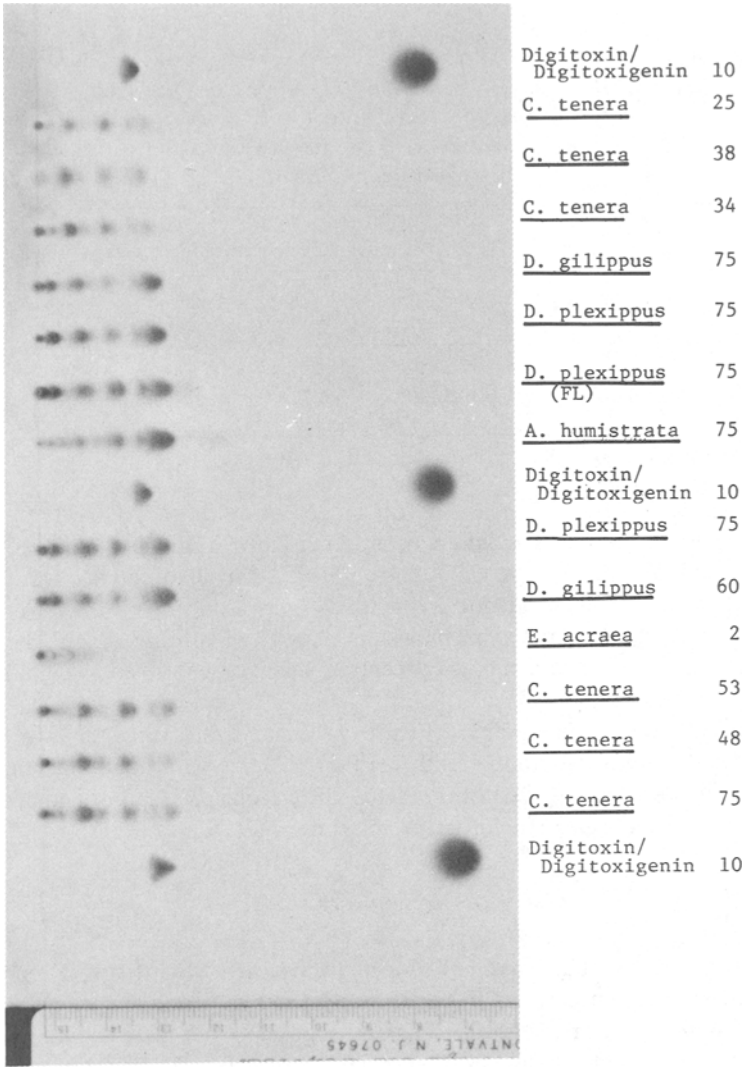

FIG. 4. Chromatograms of adult monarchs and queens reared in the laboratory on *Asclepias humistrata* (developed as in Figure 3). Note that the profiles of the two species are virtually identical, yet differ from those shown in Figure 3. All butterflies were collected as eggs or first instars in the Dominican Republic, except for the monarch labelled "FL," which was collected wild in the egg stage in Florida and shows an identical "fingerprint" pattern to those of Dominican Republic stock. For comparison, extracts of six dogbane tiger moths (*Cycnia tenera*) and one saltmarsh moth (*Estigmene acraea*), reared simultaneously on *A. humistrata,* were spotted on the same silica plate. Also spotted are an ethanolic leaf extract of *A. humistrata* and a 1:1 (v/v) mixture of digitoxin and digitoxigenin. Numbers to the right of each channel indicate the micrograms of cardenolide spotted, calculated prior to lead acetate clean-up.

cardenolide ingestion, as suggested by Brower and Glazier (1975), there is as yet no direct evidence of a causal connection between cardenolide differences and body size differences. Neither Seiber et al. (1980) nor I (Cohen, 1983) found any effect of ingested digitoxin (added to controlled diets) upon the development time, food consumption, or body weight of fourth instar monarch larvae. However, if it is true that the negative correlations do reflect metabolic costs, then we must inquire why such costs should be paid by monarchs and not by queens.

One possibility is that queens are better adapted to cardenolides than are monarchs, due to differences in population structure of these two species. Monarchs are migratory, with most individuals in eastern North America flying to a few restricted areas in Mexico to overwinter (Urquhart and Urquhart, 1977). Mating occurs during the remigration back to North America each spring and most probably results in many matings between individuals which developed as larvae on different species of milkweed, some of which lack cardenolide (see Roeske et al., 1976). Many of these matings would therefore involve butterflies that had not been subjected to selection for allelochemic tolerance. This would tend to recombine any evolving gene complexes for cost-free adaptation to allelochemics. In contrast, queens are fundamentally nonmigratory (Young, 1982), making only limited regional movements (Brower, 1961; Burns, 1983). This greater degree of philopatry should more often result in matings among individuals that fed, as larvae, on the same host plant species. In areas where high-cardenolide plants predominate, this should facilitate the evolution of a more rapid, fine-tuned adaptation to host plant allelochemics.

Two alternative hypotheses to explain the species difference in correlations between body size and cardenolide content are that queens may either effect different metabolic conversions of cardenolides than do monarchs or that they sequester them in less sensitive tissues. Although some data are available on the metabolism (Brower et al., 1982; Marty, 1983) and tissue distribution (Brower, 1984; Brower and Glazier, 1975; Nishio, 1980) of cardenolides in monarchs, no comparable data are yet available for queens. Thus, it is not yet possible to test these alternative hypotheses.

Sexual Differences in Cardenolide Concentration. Previous studies of monarchs have found that females have higher cardenolide concentrations than males (Brower and Glazier, 1975; Brower and Moffitt, 1974; Brower et al., 1972). This was again demonstrated here for the laboratory-reared monarchs. Brower and Glazier (1975) suggested that such a dimorphism might reflect relatively stronger selection for chemical defense in females which must spend considerable time exposed to potential enemies while searching for oviposition plants. However, males also incur certain risks in searching for females, and it is not clear how these risks to males are to be compared with risks to females when predicting differences in defensive strategies. In any event, such risks would presumably apply to the congeneric queen as well. The lack of sexual difference in cardenolide content of queens shown here therefore suggests that the "differential risk"

hypothesis is not sufficient to explain the dimorphism previously observed in monarchs.

Another possible explanation is that female monarchs store more cardenolide than males as a means of providing for the defense of their eggs (Brower et al., 1982). Thomashaw (in Brower, 1984) reported that each egg of a female monarch reared on *A. curassavica* contained, on average, 0.97 μg of cardenolide. Since a female may lay from 100 (Erickson, 1973) to 400 (Urquhart, 1960) eggs, these could collectively contain as much as 97–388 μg of cardenolide. Adult monarchs reared on *A. curassavica* contain an average of 670 μg of cardenolide (Roeske et al., 1976). Thus, the amount placed by females into eggs constitutes a substantial proportion (14% to 58%) of this total. One might therefore expect cardenolides to be effective predator or parasite deterrents in monarch eggs, but this has not yet been tested. The lack of sexual difference in cardenolides of queens may suggest that females of this species are not strongly selected to store these compounds for egg defense and leads to the prediction that they should allocate proportionally less cardenolide to eggs than do monarchs. Further work should be directed at this issue.

Interestingly, while laboratory-reared monarch (but, again, not queen) females did have significantly higher cardenolide concentrations than males, this was not observed in field collections from the Miami area. This may suggest that some of the wild-caught females in the Miami sample had already laid some of their cardenolide-rich eggs, thereby reducing their (initially greater) cardenolide loads to a level similar to that of males. (This suggestion is consistent with the laboratory finding of Dixon et al. (1978) that female monarchs that had laid all of their eggs were less emetic when force-fed to pigeons than were freshly eclosed females.) Since females would likely oviposit at varying rates, this should lead to a greater variation in cardenolide concentration of females, relative to males. Indeed, females do tend to have higher variances than males for this trait (see Table 1; $F_{max} = 2.12$, $0.05 < P < 0.10$).

Cardenolide Variability and its Implications. The great intra- and interspecific uniformity in qualitative cardenolide profiles of the Miami butterflies (see Figure 3), suggests that a single host plant species had been utilized by larvae of both species but that there is individual variation in storage of certain compounds, especially those of highest R_f value. That these TLC profiles are virtually indistinguishable from those of monarchs reared in the laboratory on *A. curassavica* (Figure 3 inset) strongly suggests that this was the host plant species utilized by both danaid species in the Miami sample (cf. Figure 4 for *A. humistrata*-reared butterflies). This represents the first practical application of the cardenolide "fingerprinting technique" (Brower et al., 1982) to identify the host plants utilized, as larvae, by wild-caught danaid butterflies. It also demonstrates the potential of the technique as an aid in understanding the natural history and migration patterns of danaids. Since the monarchs studied were collected in Miami in December and developed on *A. curassavica* (an introduced milkweed

with a North American distribution restricted to southern states; Woodson, 1954), we may conclude that they were not merely migrant butterflies from northern states that had "become trapped" in peninsular Florida en route to Mexico. Rather, this is strong evidence that monarchs breed in south Florida during the winter months.

A large percentage of each queen sample consisted of butterflies containing no measurable cardenolide (57% in Lake Istokpoga, 17% in Corkscrew Swamp, and 21% in Miami). Since there were no significant sex differences, such intrapopulational variability may instead reflect localized differences in host plant species availability, intraspecific variation in plant cardenolide content (see, e.g., Nelson et al., 1981), or individual butterfly differences in cardenolide sequestration. Whatever its origin, such variability suggests the existence of a cardenolide-based palatability spectrum for queens, similar to that previously described for monarchs (Brower, 1969; Brower et al., 1968; Brower and Moffitt, 1974).

This result has important implications for understanding the southern viceroy's apparent switch from mimicking the monarch (as it does elsewhere in its range), to mimicking the queen in Florida (Brower, 1958a,b). If queens had been found to contain, on average, either more cardenolide than monarchs, or a different and potentially more potent (e.g., more emetic; Brower, 1969) set of cardenolides, then such a mimetic switch might be easily understood. However, the Miami queens clearly contained lower cardenolide concentrations and total amounts than did the sympatric monarchs. Since the two species had virtually identical cardenolide "fingerprints," it cannot be argued that queens stored a more noxious array of cardenolides than did monarchs and were therefore more emetic even at lower concentrations. Thus, when fed on the same plants, monarchs and queens store the same cardenolides but monarchs concentrate these to a greater extent than do queens. This conclusion is further supported by laboratory rearings of the two species on *Asclepias humistrata* (Table 3 and Figure 4). Moreover, Brower et al. (1975) have shown that, in order for an *A. curassavica*-reared monarch (sexes pooled) to be emetic to an 85-g blue jay on 50% of test trials, it must contain at least 76 μg of cardenolide. Of the Miami butterflies analyzed here, 85% of monarchs met this criterion of unpalatability, while only 30% of queens did so. It therefore seems that, at least with respect to cardenolides, queens are poorer models for viceroy mimicry than are monarchs.

Why then should the viceroy have abandoned its usual model in favor of the queen? Since monarchs are migratory, "pulsing" through Florida in large numbers only in the spring and fall (Urquhart and Urquhart, 1976; Brower, Malcolm, and Cockrell, unpublished data), while queens are more sedentary, the latter species would be spatiotemporally more "available" than monarchs to act as models in Florida. A theoretical model developed by Pough et al. (1973) showed that mildly noxious species could serve as suitable models for mimicry if they occurred in sufficient abundance. This may provide the explanation for the switch

in viceroy mimicry. If resident Florida monarch populations have been stable and predictable in their current locations for sufficient time, then reversals in the trend of mimicry might be expected, such that viceroys in those areas should tend to be more "monarch-like" than those elsewhere in the state. A detailed geographic analysis of wing patterns and phenology is needed to test this prediction.

Alternatively, queens might, in fact, be superior models to monarchs, not because of their cardenolide content but, rather, due to sequestered pyrrolizidine alkaloids (PAs) that adults ingest from certain withering plants (Edgar, 1975; Edgar et al., 1979). While both sexes typically store these compounds as adults, male queens employ them further as precursors of their sex pheromone (Meinwald et al., 1969; Pliske and Eisner, 1969). Male and female monarchs are also somewhat attracted to PA sources and may store the alkaloids but males apparently do not use them as pheromone precursors (Edgar et al., 1976). While there is as yet no experimental verification of PA-based defense in danaid butterflies, K.S. Brown (unpublished manuscript) reports that certain neotropical spiders will release ithomiid butterflies from their webs unharmed if they contain PAs. The dependence of male queens (and not monarchs) on PAs for sexual competence suggests that, on average, queens may contain more of these compounds than monarchs, and therefore possibly serve as better models for viceroy mimicry. A comparative study of the PA concentrations of wild-caught monarchs and queens would shed further light on this intriguing problem.

Adaptation to Plant Allelochemics. With respect to the issues of herbivore adaptation to allelochemics presented in the Introduction, this study leads to the following provisional conclusions: (1) There is no evidence that the lower cardenolide concentrations sequestered by queens relative to monarchs reflects a poorer underlying tolerance for these allelochemics. On the contrary, since only monarchs demonstrate significant negative correlations between body size and cardenolide concentration, it might be argued that monarchs are less adapted than queens for handling cardenolides. However, a causative connection between cardenolide sequestration and body size has not been established (Seiber et al., 1980; Cohen, 1983). (2) Despite quantitative differences between monarchs and queens in cardenolide storage, both species appear to sequester the same individual cardenolide compounds from their host plants. Indeed, when reared on the milkweed *A. humistrata,* even dogbane tiger moths (*Cycnia tenera;* Arctiidae), also apocynad–asclepiad specialists, produced the same characteristic TLC profile (Figure 4; see also Cohen and Brower, 1983) as did the polyphagous arctiid moth, *Estigmene acraea* (although only in trace amounts; Figure 4). Moreover, Marty (1983) has shown that gut homogenates of both monarch and *E. acraea* larvae are capable of effecting a similar enzymatic transformation of one milkweed cardenolide, uscharidin, to two more polar metabolites (calactin and calotropin). Such similarity among taxonomically disparate Lepidoptera,

whether oligo- or polyphagous, suggests that there may exist only a single *qualitative* route of cardenolide processing in this insect order but that further evolution may involve a *quantitative* increase in tissue cardenolide concentration in accordance with the defensive requirements of each species. However, the basic biochemical processes shared by all these species may represent a common preadaptation to feeding on milkweeds or other cardenolide-containing plants.

Acknowledgments—This research was carried out in partial fulfillment of the requirements for the degree of Doctor of Philosophy at the University of Florida. I am grateful to my advisor, Prof. L.P. Brower, for much advice, encouragement, and stimulation, as well as to my committee members, Drs. H.J. Brockmann and F. Slansky Jr., for many helpful discussions of the work and criticisms of the manuscript. Further thanks are due to the following colleagues for a variety of forms of assistance: J.B. Anderson, B. Emmons, C.S. Hieber, R.A. Kiltie, S.B. Malcolm, P.G. May, R. Rutowski, J.N. Seiber, and N.H. Williams.

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