Evolutionary and ecological implications of cardenolide sequestration in the monarch butterfly

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Summary. Monarch butterflies sequester cardenolides from their larval host plants in the milkweed genus *Asclepias* for use in defense against predation. Of 108 *Asclepias* species in North America, monarchs are known to feed as larvae on 27. Research on 11 of these has shown that monarchs sequester cardenolides most effectively, to an asymptote of approximately 350 μg/0.1 g dry butterfly, from plants with intermediate cardenolide contents rather than from those with very high or very low cardenolide contents. Since *Asclepias* host plant species are distributed widely in space and time across the continent, monarchs exploit them by migration between breeding and overwintering areas. After overwintering in central Mexico, spring migrants east of the Rocky Mountains exploit three predominant *Asclepias* species in the southern USA that have moderately high cardenolide contents. Monarchs sequester cardenolides very effectively from these species. First generation butterflies are thus well protected against predators and continue the migration north. Across the northern USA and southern Canada most summer breeding occurs on a fourth *Asclepias* species and in autumn most of these monarchs migrate back to Mexican overwintering sites. The ecological implications of this cycle of cardenolide sequestration for the evolution of monarch migration are discussed.

Key words. Annual cycle; *Asclepias*; cardenolide; Danaidae; *Danaus plexippus*; defense; ecological chemistry; evolution; herbivory; host plant; life history; migration; milkweed; monarch butterfly; overwintering; predation; repellent allomone; sequestration; storage.

Introduction

Although Poulton[39] first suggested as long ago as 1914 that chemists could help determine why monarch butterfly larvae feed only on milkweeds, only now are we beginning to understand the evolutionary and ecological implications of this selective herbivory. Early experiments by Jones[28] showed that aqueous extracts of monarch larval host plants repelled ants and that bird predators partially rejected adult monarchs. However it was not until 1965 that Parsons[38] implicated cardiac-active steroids, or cardenolides, as the active repellent allomones that make monarchs toxic and bitter-tasting to predators. Soon thereafter, Reichstein[40] published structures of some cardenolides found in adult monarchs that were also present in their larval host plants[41]. At the same time Brower and colleagues[4-6] showed that various milkweed host plant species had different quantities of cardenolides and that these variable quantities influenced the toxicity of monarchs to bird predators.

Through the 1970s Brower, Rothschild, and their associates[7-10,19,23,43,44] showed that monarchs sequester cardenolides from their larval host plants, that wild-caught adult monarchs also contain cardenolides, and that monarchs are toxic to various vertebrate predators. The first evidence that milkweed cardenolides vary qualitatively and that monarchs reared on different milkweed species reflect these qualitative differences was also published at this time[42]. The discovery of such differences among milkweed species led to the current series of papers[11-14,22,29,30,34-37,45,46] that describe variation in both the amounts and kinds of cardenolides that monarchs sequester from their *Asclepias* milkweed host plants.

Monarchs reared from most *Asclepias* species examined to date have different quantitative and/or qualitative patterns (chemical fingerprints or chemoprints) and these can be used to identify the host origin of wild-caught adult monarchs[34]. Furthermore, because each of the 108 North American milkweeds in the genus *Asclepias* has a characteristic distribution in time and space[50] it is usually possible to assign the larval origin of wild-caught monarchs to a particular host plant species' distribution[34]. Thus host-derived cardenolide fingerprints are now telling us much about the annual life history cycle of monarch butterflies.

Here we first review the concentrations and total amounts of cardenolides sequestered by monarch larvae from 11 common *Asclepias* species in North America. The emergent patterns are then compared with those of monarchs captured in the wild throughout their annual cycle. Finally, we discuss the evolutionary and ecological implications of cardenolide sequestration by the monarch.

Biology of the annual cycle

Monarch butterflies, *Danaus plexippus* (L.), have a life history that cycles from breeding in areas of milkweed abundance, through migration to overwintering locations and a return migration of the same individuals back to breeding areas of the previous year[15,48]. In North America monarchs exist as two populations that are separated east and west of the Rocky Mountains. Late summer monarchs in Canada and the USA, migrate in September and October to form dense aggregations in central Mexico[20] (eastern population) or along the coast

of central and southern California[47] (western population). Monarchs remain at these sites during winter and remigrate in spring back to areas of milkweed abundance. Californian monarchs are thought to migrate in February from the coast into the coastal ranges, central valley and Sierra Nevada[32]. The eastern population remigrates north from Mexico in March to the Gulf states of the southern USA[21,33,34]. In the southern USA these remigrants reproduce and two early spring generations can be completed which continue the migration north[33]. A further three summer generations are then produced in the northern USA and southern Canada to complete the annual cycle[3,21,33–35].

Breeding monarchs have been recorded feeding naturally on only 27 of the 108 species in the milkweed genus *Asclepias*[31]. Thus breeding during the annual cycle is likely to be contained primarily within the temporal and spatial distributions of these *Asclepias* species.

Host-specific patterns of cardenolide sequestration

Patterns of cardenolide sequestration by monarchs reared on 7 *Asclepias* species in California, and 3 southern *Asclepias* species and 1 northern species are summarized in table 1. These represent some of the most abundant milkweeds available to monarchs and are all known larval hostplants[31]. *A. syriaca* occurs in great abundance during the summer throughout the northern USA and southern Canada east of the Rocky Mountains and is highly exploited by migratory monarchs[35,50]. The 3 southern milkweeds are early spring species that occur in greatest abundance from west Texas east through the Gulf states to Florida[29,30,34,50].

The *Asclepias* species of table 1 are ranked according to the mean concentrations of sequestered monarch cardenolide. These range from a low of 21 µg/0.1 g for monarchs reared on the Californian milkweed *A. fascicularis*, to a maximum of 438 µg/0.1 g for monarchs from the southern milkweed *A. viridis* in Florida. Although the order of butterfly cardenolide concentrations is similar to that for plants in California, the butterflies vary greatly in how much plant cardenolide they concentrate. The ratios of butterfly to plant mean cardenolide concentrations (table 1) indicate that monarchs from high cardenolide plants do not concentrate the available titers of cardenolide as effectively as do monarchs from intermediate and low cardenolide plants. Of these 11 host species, monarchs concentrate cardenolides most effectively from *A. syriaca* and *A. californica*.

For *Asclepias* species with wide variation in cardenolide concentrations and mean concentrations above 376 µg, monarchs markedly reduced the ranges of cardenolide concentrations (table 1, fig. 1). Thus monarchs reared from *A. erosa, A. eriocarpa, A. vestita, A. viridis* (Florida), *A. asperula* and *A. humistrata* reduced the variability of their host cardenolide concentrations to narrow, normal distributions. In contrast, *Asclepias* species with lower mean cardenolide concentrations, over narrower ranges (*A. californica, A. cordifolia, A. speciosa, A. fascicularis, A. viridis* (Louisiana) and *A. syriaca*), produced monarchs with cardenolide concentrations more variable than in their host plants (table 1, fig. 1).

When variation in butterfly cardenolides is compared with that in plant cardenolides by regression analysis for individual plant species, logarithmic curves describe the data (table 2) for 7 of the species from table 1. The remaining data do not fit significant regressions whether or not the plant data are transformed. For all significant regressions, transformation of the plant data increased the regression significance because distributions of plant

Table 1. Cardenolide concentrations (µg equivalent to digitoxin[10]/0.1 g dry weight) of monarchs reared from 7 *Asclepias* species in California, and east of the Rocky Mountains, of monarchs reared on 3 species in the southern USA and 1 species in the northern USA. Within each category the species are ranked according to butterfly cardenolide concentrations. The ratio of butterfly to plant mean cardenolide concentrations is also given as the B/P ratio.

Asclepias species[a]	N[b]	Plant Mean	SD	Range	Butterfly Mean	SD	Range	B/P Ratio
Californian								
1) *A. erosa*[37]	71	562	338	79–2102	347	85	181–567	0.62
2) *A. eriocarpa*[11]	172	421	170	102–919	318	79	136–606	0.76
3) *A. vestita*[37]	63	750	516	88–1718	250	51	153–352	0.33
4) *A. californica*[14]	85	66	45	9–199	234	74	59–410	3.55
5) *A. cordifolia*[37]	112	73	44	19–238	182	64	38–321	2.49
6) *A. speciosa*[13]	111	90	65	19–344	179	104	41–547	1.99
7) *A. fascicularis*[c]	105	17	10	2–59	21	16	0–63	1.24
Southern								
8) *A. viridis* (Florida)[34]	18	376	203	148–972	438	63	337–548	1.16
9) *A. asperula* (Texas)[30]	41	886	255	341–1616	363	77	231–515	0.41
10) *A. viridis* (Louisiana)[29]	60	245	70	95–433	337	105	73–591	1.38
11) *A. humistrata* (Florida)[34]	22	389	141	71–639	337	65	243–478	0.87
Northern								
12) *A. syriaca*[35]	158	50	39	4–229	234	150	0–792	4.68

[a] Superscript denotes reference source for data; [b] Number of plant-butterfly, paired samples; [c] Brower, Seiber, Nelson, Lynch and Holland (in preparation).

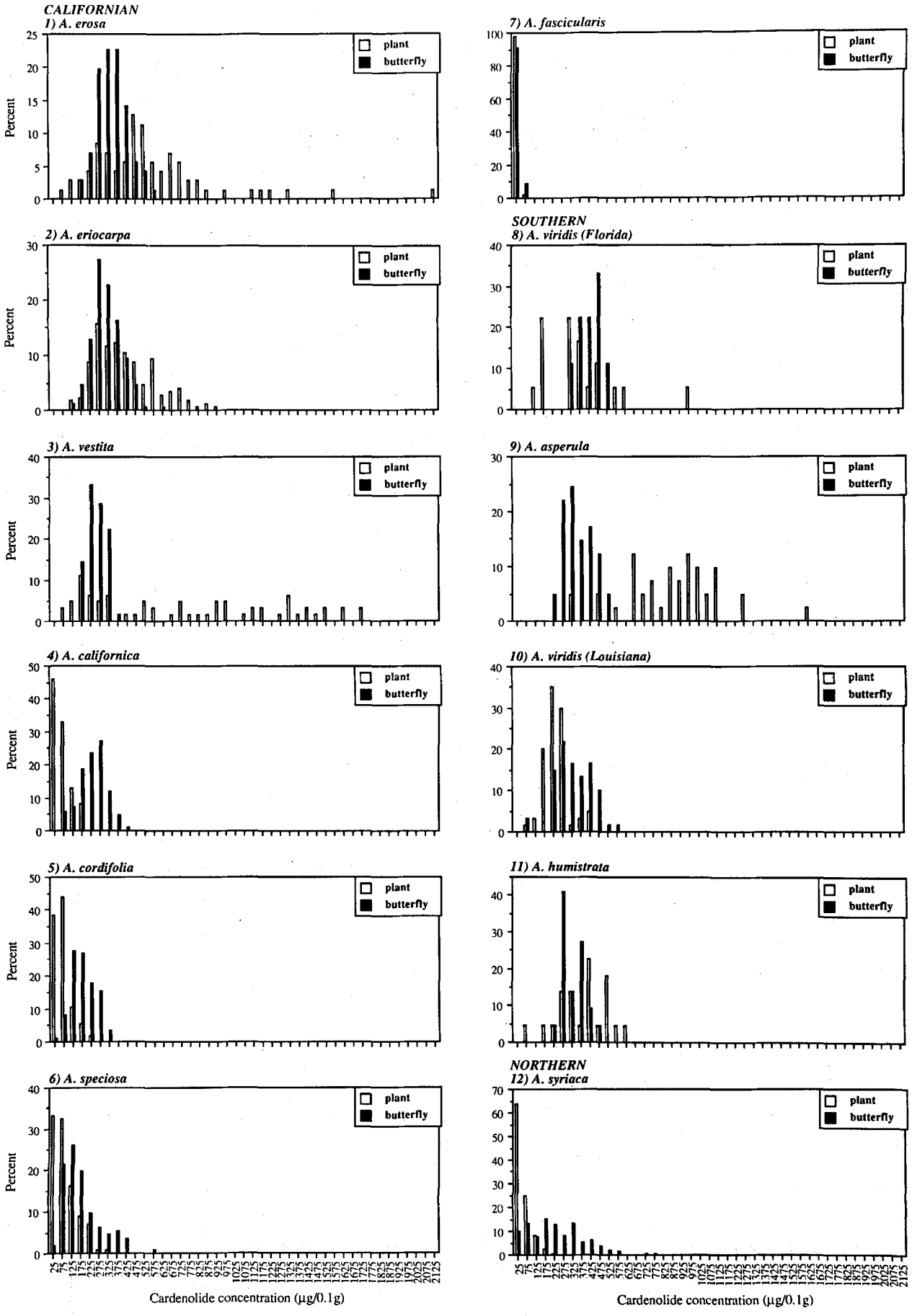

Figure 1. Frequency distributions of cardenolide concentrations (µg/0.1 g dry weight) in 11 *Asclepias* species and monarch butterflies reared from them. Concentration intervals are set at 50 µg/0.1 g and range from 0 to 2200 µg.

Table 2. Regression analyses of the plant-butterfly paired cardenolide concentration data from table 1. Linear regressions of untransformed butterfly cardenolide concentrations versus both their untransformed and logarithmically transformed plant cardenolide concentrations are shown. Using the Kolmogorov-Smirnoff one-sample normality test all samples were normally distributed except for *A. cordifolia* and *A. syriaca* plant samples. However, published analyses of normality (see table 1 for references) show that samples of *A. eriocarpa* plants, *A. vestita* plants and butterflies, *A. californica* plants, *A. speciosa* plants and butterflies, *A. asperula* butterflies, *A. viridis* plants, and *A. syriaca* plants, were all non-normal. Regressions that were not significant at 5% are shown as NS.

Asclepias species	Untransformed			Transformed			Regression equation
	r	F	P	r	F	P	
Californian							
A. erosa	0.22	3.62	0.061	0.28	5.87	0.018	y = 93.4 (log x) + 96.5
A. eriocarpa	0.15	3.67	0.057	0.14	3.40	0.067	NS
A. vestita	0.04	0.08	0.773	0.03	0.06	0.810	NS
A. californica	0.57	39.14	0.0001	0.65	60.96	0.0001	y = 144.7 (log x) − 12.9
A. cordifolia	0.64	78.13	0.0001	0.65	81.43	0.0001	y = 172.8 (log x) − 128.1
A. speciosa	0.56	49.80	0.0001	0.56	50.59	0.0001	y = 200.0 (log x) − 191.9
A. fascicularis	0.13	1.65	0.202	0.12	1.56	0.214	NS
Southern							
A. viridis (FL)	0.19	0.61	0.447	0.07	0.09	0.773	NS
A. asperula (TX)	0.01	0.004	0.949	0.04	0.07	0.792	NS
A. viridis (LA)	0.45	14.52	0.0003	0.48	19.97	0.0001	y = 402.0 (log x) − 616.0
A. humistrata (FL)	0.28	1.75	0.201	0.18	0.69	0.417	NS
Northern							
A. syriaca	0.75	201.86	0.0001	0.78	243.08	0.0001	y = 334.4 (log x) − 292.0
Pooled data (N = 1018 plant-butterfly pairs)							
All 12 data sets	0.45	261.09	0.0001	0.70	988.93	0.0001	y = 153.9 (log x) − 75.7

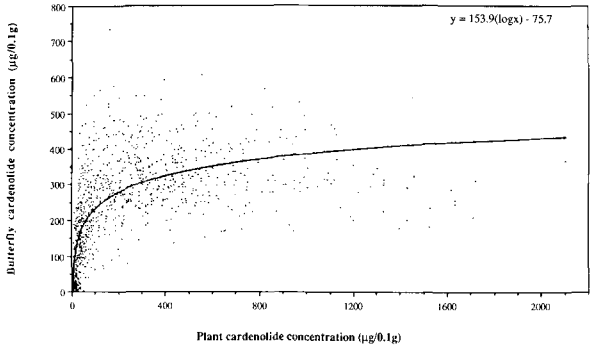

Figure 2. Scatterplot of plant against butterfly cardenolide concentrations (µg/0.1 g dry weight) for all 1018 plant butterfly pairs from the 11 *Asclepias* species shown in figure 1 and tables 1 and 2. The overall regression of $\log_{10}$ plant concentration against butterfly concentration from table 2 is also shown.

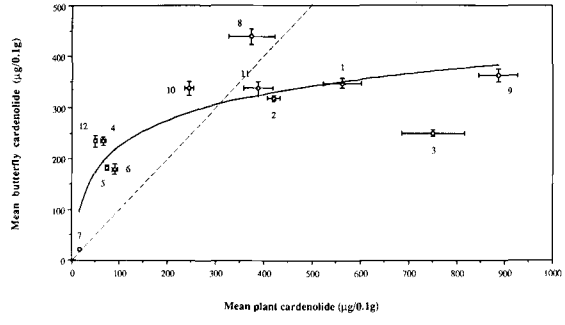

Figure 3. Scatterplot of mean plant against mean butterfly cardenolide concentrations (µg/0.1 g dry weight, with SEM) and the fitted regression, y = 167.1 (log x) − 110.5 (r = 0.82, F = 20.78, df = 11, p = 0.001). The line of equal cardenolide concentrations in plants and butterflies is also shown. Plant-butterfly samples (4, 5, 6, 7, 8, 10, 12) above this line are equivalent to the samples in table 1 with B/P ratios greater than 1.00.

cardenolide concentrations (fig. 1) were skewed toward higher concentrations with a higher preponderance of low cardenolide plants.

The data for all 1018 plant-butterfly paired samples, across the 12 sets of data in table 1, show a similar, highly significant logarithmic relationship between plant and butterfly cardenolide concentrations (table 2, fig. 2). Although the logarithmic relationship increases to above butterfly concentrations of 400 µg cardenolide/0.1 g, most data are clustered around butterfly concentrations of approximately 250–350 µg. A very similar regression is produced by plotting the mean plant cardenolide concentrations against those of butterflies for each species (fig. 3). This relationship also shows the butterfly means clustered around 300–350 µg. In contrast, cardenolides sequestered by the seed-feeding milkweed bug, *Oncopeltus fasciatus* increase linearly with increasing cardenolide concentrations of different *Asclepias* species [24]. However, like the monarch, *O. fasciatus* sequesters more cardenolide than expected from low cardenolide *Asclepias* seeds and less from high cardenolide seeds [49].

With the exception of *A. erosa*, it is those species with the highest ratio of butterfly to plant cardenolide (table 1) that show the most statistically significant relationship between plant and butterfly cardenolide. Thus the 5 species with ratios from 1.38 to 4.68 produce highly significant regressions whereas the 6 species with ratios of 1.24 to 0.33 (except *A. erosa* at 0.62) show no significant relationships between plant and butterfly cardenolide concentrations. Furthermore it is the 2 species with the highest plant cardenolide concentrations (*A. asperula* and *A. vestita*) and the species with the lowest (*A. fascicularis*) that show no relationship between plant and butterfly cardenolide concentrations. Perhaps not sur-

prisingly, the 3 species with mean plant cardenolide concentrations close to the approximate butterfly cardenolide asymptote of 350 μg (*A. eriocarpa*, *A. viridis* (Florida) and *A. humistrata*) similarly lack a significant relationship between plant and butterfly cardenolide concentrations. These 6 species have plant cardenolide means at each end of the regression relationship (fig. 3) which suggests that monarchs can sequester cardenolides more effectively from milkweed species of intermediate cardenolide content than milkweed species with either high or low cardenolide contents.

Thus it seems that monarchs are able to sequester cardenolides at least partially irrespective of the cardenolide content in their larval host plants by either reducing or increasing available concentrations. If monarchs simply reflected the cardenolide concentrations of their host plants, their concentrations should fall along the line of equality in figure 3. That 5 of the 11 species produce monarchs with higher than expected cardenolide concentrations suggests that cardenolide sequestration in monarchs is an adaptive feature maintained by selection. Likewise, monarchs that reduce the cardenolide concentrations of plants rich in cardenolide also suggests adaptive sequestration since plant variation is reduced to a narrow range near 350 μg.

Patterns of cardenolide sequestration during the annual cycle

With the exception of monarchs overwintering in California in 1971 [8, 25] few data are available for the cardenolide contents of wild-caught western monarchs. Thus we confine the present discussion to a consideration of annual cardenolide cycling in wild-caught monarchs of the eastern population.

Cardenolide analyses of 25 samples of monarchs caught at various times of the year from 1970 to 1985 are available for comparison (table 3). The additional 5 samples

Table 3. Date, location and source of each monarch sample assayed for cardenolide content, listed in chronological order. Unpublished data are from the laboratory of L. P. Brower.

Sample	Date	Year	Reference
Winter aggregation (Mexico)			
1) Sierra Chincua, Michoacan	15 January	1978	unpublished
2) Sierra Chincua, Michoacan	15 January	1980	25
3) Sierra Chincua, Michoacan	23 January	1979	unpublished
4) Sierra Chincua, Michoacan	26 January	1977	unpublished
5) Cerro Pelon, México	5 February	1978	unpublished
6) Altamirano, Michoacan	15 February	1978	unpublished
7) Sierra Chincua, Michoacan	20 March	1978	unpublished
Spring migration (south)			
8) Southern (Texas, Louisiana, Florida)	30 March–12 May	1985	34
Spring breeding			
9) *A. viridis*-reared, Louisiana	26 April–15 May	1981	29
10) *A. viridis*-reared, Florida	10 May–13 May	1983	34
11) *A. humistrata*-reared, Florida	10 May–2 June	1983/84	34
12) *A. asperula*-reared, Texas	27 May–3 June	1983	30
Spring migration (north)			
13) Northern (North Dakota, Minnesota, Wisconsin, Michigan, Ohio, Pennsylvania, Massachusetts)	22 May–24 June	1985	34
Summer breeding			
14) Salmon Point, Ontario	31 Aug.–1 Sept.	1970	7
15) *A. syriaca*-reared, North Dakota east to Vermont and south to Virginia	16 Aug.–26 Sept.	1983	35
16) Plainfield, Massachusetts	2–10 September	1977	unpublished
17) Baltimore, Maryland	4–26 September	1970	7
18) Hockanum, Massachusetts	19–26 September	1970	7
Autumn migration			
19) Lawrence, Kansas	23 September	1979	unpublished
20) Cape May, New Jersey	29 September	1979	unpublished
21) Beach Haven, New Jersey	30 September	1979	unpublished
22) Eastern Point, Massachusetts	4 October	1979	unpublished
23) Austin, Texas	10 October	1979	unpublished
24) Windermeyer, Texas	11 October	1979	unpublished
25) Lighthouse Point, Florida	28–29 October	1979	unpublished
26) San Javier, Mexico	31 October	1977	unpublished
27) Sapelo Island, Georgia	22 November	1980	unpublished
28) Flamingo, Florida	27 Nov.–1 Dec.	1970	7
29) Miami, Florida	4 December	1981	22
Winter aggregation (Mexico)			
30) Sierra Chincua	11 December	1977	unpublished

listed in table 1 are for monarchs reared from the 4 most important milkweed species for eastern monarchs and are the same samples given in tables 1 and 2. The complete set of 30 samples represents 2989 monarchs and they are listed in chronological order in tables 3 and 4. Table 4 shows the sample sizes, with data for total cardenolide per butterfly, cardenolide concentrations for the whole butterfly dry weights and, where possible, also for lean (defatted) butterfly dry weights. The different data sets for total cardenolide per butterfly are plotted against time in figure 4.

Winter aggregation of monarchs at small overwintering sites in central Mexico provides a convenient point to begin the monarch's annual cycle (table 4, fig. 4). It is during these winter aggregations that monarchs carry little sequestered cardenolide which may explain the high incidence of bird and mouse predation on overwintering monarchs[15-19,25-27]. The 8 samples of these aggregated monarchs contained mean total cardenolide amounts per butterfly of between 73 and 165 µg and the pattern of low cardenolide content is consistent among both years and overwintering sites (table 4). The frequency distributions of cardenolides are also remarkably consistent among both years and sites with all sample distributions strongly skewed to the right (fig. 5).

Although most overwintering monarchs carry little cardenolide they do contain sufficient amounts to show that they derived cardenolides from the common northern milkweed *A. syriaca*[46]. Thus 92% of monarchs overwintering in Mexico have now been shown to have the qualitative cardenolide chemoprint characteristic of monarchs reared from *A. syriaca*[34,35].

Similarly 84% of the spring migrants caught in the southern states of Texas, Louisiana and Florida in April have the same *A. syriaca*-derived chemoprint. Like overwintering monarchs these also have low cardenolide contents with strongly skewed distributions (fig. 5) that are not significantly different[34]. These similarities are good evidence that spring arrivals in the southern USA are the same butterflies that overwintered in Mexico. After migrating north from Mexico to the southern USA these monarchs lay eggs on the abundant southern milkweeds, such as *A. asperula* in western Texas, *A. viridis* from central Texas and Oklahoma east to Florida and Georgia, and *A. humistrata* over the southeastern coastal plain in Florida and Georgia[33,34]. Field observations show that monarchs breed more extensively on these three southern milkweed species in the spring than on any other available species[21,29,30,33,34]. Hence the cardenolide content of first generation spring monarchs that fed on these milkweeds increases to between 523 and 754 µg/butterfly from a mean of 89 µg/butterfly in their parents that had migrated from Mexico north to the southern USA (table 4, fig. 4). The distributions of cardenolides in these freshly emerged first generation spring monarchs are also very different from their parents. Butterflies reared from the southern milkweeds *A. viridis*, *A. asperula* and *A. humistrata* all had similar, normal distributions of total cardenolide (fig. 5).

In early May the newly emergent first generation monarchs with high cardenolide contents continue the migration north[21,34]. Thus in contrast to migrants in early spring, 84% of the monarchs arriving in the northern USA in late spring (May–June) had almost certainly fed

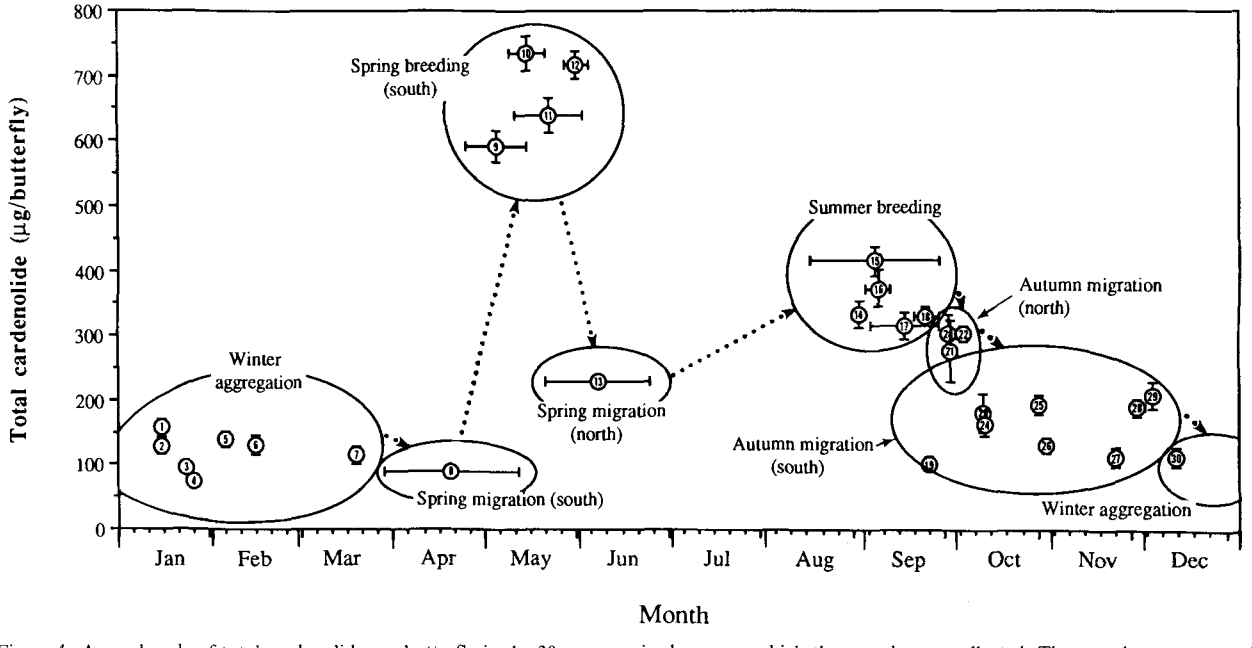

Figure 4. Annual cycle of total cardenolide per butterfly in the 30 monarch butterfly samples listed in tables 3 and 4. Sample numbers refer to the sample numbers of these tables. Vertical error bars represent standard errors of the mean for each sample and horizontal bars show the period in days over which the sample was collected. The samples are grouped according to the table classification to highlight similarities within groups and dissimilarities among groups. The time scale is divided into months and 5-day intervals.

Table 4. Sample sizes, total cardenolide (mean ±SD μg/dry butterfly) and cardenolide concentrations (μg/0.1 g) for both fat and lean dry weights (means and SD's) of the 2989 monarchs of both sexes from the samples listed in table 3. Some of the samples were not defatted and so the data for lean concentrations are unavailable.

Sample	N	Total cardenolide mean	SD	Concentration (fat) mean	SD	Concentration (lean) mean	SD
Winter aggregation (Mexico)							
1) Sierra Chincua, Michoacan (15 January 1978)							
Males	58	150	152	59	58	89	88
Females	47	165	118	60	46	105	75
Total	105	157	137	60	53	97	82
2) Sierra Chincua, Michoacan (15 January 1980)							
Males	50	132	107	56	46	77	64
Females	49	125	134	50	55	77	80
Total	99	129	121	53	51	77	72
3) Sierra Chincua, Michoacan (23 January 1979)							
Males	79	90	100	39	47	57	66
Females	78	103	108	43	47	68	74
Total	157	96	104	41	47	62	70
4) Sierra Chincua, Michoacan (26 January 1977)							
Males	50	73	91	29	39	48	60
Females	51	75	97	31	41	52	68
Total	101	74	93	30	40	50	64
5) Cerro Pelon, México (5 February 1978)							
Males	50	134	117	62	58	84	74
Females	50	148	129	66	58	98	86
Total	100	141	123	64	58	91	80
6) Altamirano, Michoacan (15 February 1978)							
Males	50	143	149	67	67	85	86
Females	50	119	121	56	59	76	78
Total	100	131	136	61	63	81	82
7) Sierra Chincua, Michoacan (20 March 1978)							
Males	51	97	112	48	60	61	72
Females	50	134	152	58	62	84	91
Total	101	115	134	53	61	72	82
Spring migration (south)							
8) Southern (Texas, Louisiana, Florida; 30 March–12 May 1985)							
Males	75	73	81	45	45	50	49
Females	58	109	129	70	69	78	79
Total	133	89	106	56	58	62	65
Spring breeding							
9) *A. viridis*-reared, Louisiana (26 April–15 May 1981)							
Males	29	523	179	287	99	–	–
Females	31	655	167	385	89	–	–
Total	60	591	184	337	105	–	–
10) *A. viridis*-reared, Florida (10–13 May 1984)							
Males	6	737	99	387	50	–	–
Females	12	734	122	463	53	–	–
Total	18	735	112	438	63	–	–
11) *A. humistrata*-reared, Florida (10 May–2 June 1983/84)							
Males	17	615	101	313	49	–	–
Females	5	728	177	419	43	–	–
Total	22	640	127	337	65	–	–
12) *A. asperula*-reared, Texas (27 May–3 June 1983)							
Males	19	675	134	321	55	–	–
Females	22	754	130	399	76	–	–
Total	41	717	136	363	77	–	–
Spring migration (north)							
13) Northern (North Dakota, Minnesota, Wisconsin, Michigan, Ohio, Pennsylvania, Massachusetts; 22 May–24 June 1985)							
Males	447	218	121	132	66	143	72
Females	199	253	141	166	79	187	91
Total	646	229	129	142	72	157	81
Summer breeding							
14) Salmon Point, Ontario (31 August–1 September 1970)							
Males	38	330	187	168	91	–	–
Females	38	334	186	170	96	–	–
Total	76	332	185	169	93	–	–

Table 4. (continued)

Sample	N	Total cardenolide mean	SD	Concentration (fat) mean	SD	Concentration (lean) mean	SD
15) *A. syriaca*-reared, North Dakota east to Vermont and south to Virginia (16 August–26 September 1983)							
Males	77	354	258	204	151	–	–
Females	81	472	287	262	145	–	–
Total	158	415	279	234	150	–	–
16) Plainfield, Massachusetts (2–10 September 1977)							
Males	19	357	118	165	56	191	65
Females	13	398	217	204	121	238	137
Total	32	373	163	181	89	210	101
17) Baltimore, Maryland (4–26 September 1970)							
Males	25	296	178	119	77	–	–
Females	31	333	140	140	69	–	–
Total	56	316	158	130	73	–	–
18) Hockanum, Massachusetts (19–26 September 1970)							
Males	71	334	172	169	84	–	–
Females	51	327	162	177	85	–	–
Total	122	331	167	173	84	–	–
Autumn migration							
19) Lawrence, Kansas (23 September 1979)							
Males	61	82	105	44	52	48	59
Females	60	122	126	69	76	79	87
Total	121	102	117	56	66	64	75
20) Cape May, New Jersey (29 September 1979)							
Males	23	318	183	154	84	172	91
Females	13	277	183	150	95	168	108
Total	36	304	181	153	87	171	96
21) Beach Haven, New Jersey (30 September 1979)							
Males	12	240	129	104	65	137	74
Females	2	497	342	189	148	277	219
Total	14	277	178	116	79	157	105
22) Eastern Point, Massachusetts (4 October 1979)							
Males	67	298	109	151	54	171	62
Females	37	315	148	162	72	186	82
Total	104	304	124	155	61	176	70
23) Austin, Texas (10 October 1979)							
Males	20	224	197	84	79	129	114
Females	22	141	173	52	72	85	108
Total	42	181	187	67	76	106	111
24) Windermeyer, Texas (11 October 1978)							
Males	33	151	162	54	59	81	85
Females	33	178	151	66	56	105	94
Total	66	165	156	60	57	93	90
25) Lighthouse Point, Florida (28–29 October 1979)							
Males	56	153	134	77	68	89	78
Females	52	236	193	116	93	138	111
Total	108	193	169	96	83	112	98
26) San Javier, Mexico (31 October 1977)							
Males	39	137	133	47	48	82	82
Females	62	126	116	39	37	77	74
Total	101	130	123	42	42	79	77
27) Sapelo Island, Georgia (22 November 1980)							
Males	29	118	110	64	58	72	65
Females	16	104	108	63	69	70	75
Total	45	113	109	64	61	71	68
28) Flamingo, Florida (27 November–1 December 1970)							
Males	80	168	114	86	60	–	–
Females	21	266	190	125	83	–	–
Total	101	189	138	94	67	–	–
29) Miami, Florida (4 December 1981)							
Males	25	198	116	142	87	150	90
Females	23	216	173	144	116	166	131
Total	48	207	145	143	101	158	111
Winter aggregation (Mexico)							
30) Sierra Chincua (11 December 1977)							
Males	37	95	100	33	37	63	69
Females	39	133	149	45	54	87	99
Total	76	114	128	39	46	75	86

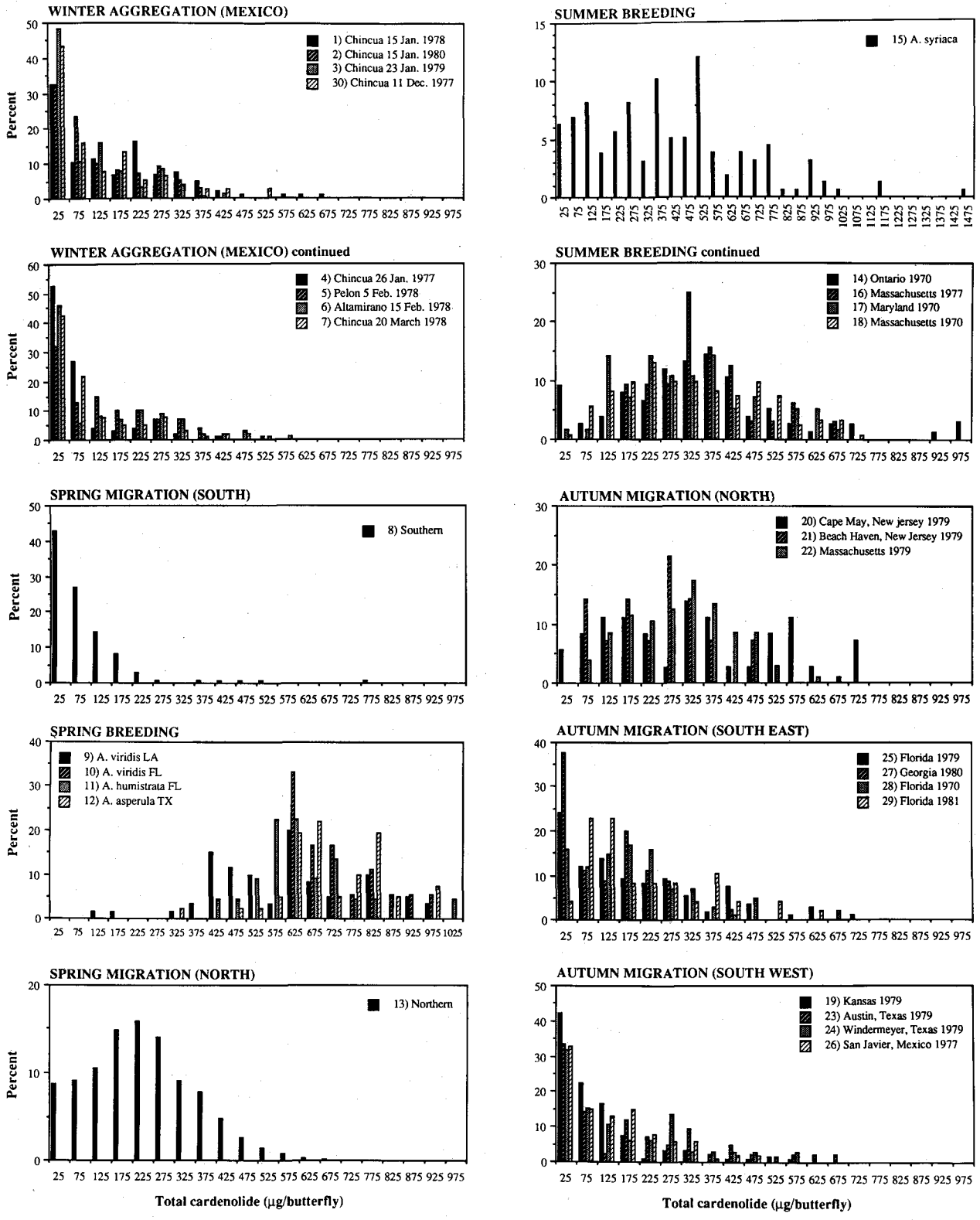

Figure 5. The frequency distributions of total cardenolide per butterfly in the 30 monarch butterfly samples shown in figure 4 and listed in tables 3 and 4. Sample numbers in the legend refer to the tabulated sample numbers.

solely on *A. viridis* as larvae[34]. Consequently, these northern arrivals have significantly more cardenolide (229 µg) than did the southern arrivals from Mexico (89 µg, table 4)[34]. Furthermore the distribution of cardenolides is normal in northern arrivals. However, although most northern arrivals are derived from *A. viridis* in the south, they show a marked reduction in total cardenolide from newly emerged *A. viridis*-derived butterflies. With means of 591 and 735 µg/butterfly for monarchs reared from *A. viridis* in Louisiana and Florida and the mean of 229 µg/butterfly for northern arrivals approximately two weeks later (table 4, fig. 4), spring migration by first generation monarchs appears to result in the loss of between 61% and 69% of their plant-derived cardenolide.

Once monarchs arrive in the northern USA and southern Canada in early June they are confronted with an extremely abundant milkweed resource, represented almost entirely by the 'common milkweed', *A. syriaca*[35]. Monarchs sequester cardenolides from this milkweed more effectively than from any other species. Thus although most *A. syriaca* plants have low cardenolide concentrations (table 1), the mean total cardenolide of butterflies reared on it is high at 415 µg (table 4, fig. 4) and the frequency distribution is normal (fig. 5).

Four samples of wild-caught summer breeding monarchs from Ontario, Massachusetts and Maryland show similar patterns of total cardenolide sequestration to monarchs reared from *A. syriaca*. Although the qualitative cardenolide chemoprints are not available for these butterflies their proximity in time, their normal distributions and similar means (table 4, figs 4 and 5) to *A. syriaca*-derived monarchs, suggests very strongly that all of these butterflies fed as larvae on *A. syriaca* – probably in late August and early September.

As the year progresses into late September and early October monarchs begin to coalesce into migratory groups and migrate south. Samples 20, 21 and 22 from New Jersey and Massachusetts (table 4) represent autumn migrants as they begin their southward migration. Like summer breeding monarchs they have normally distributed total cardenolide contents and slightly lower means (table 4, figs 4 and 5).

However, once monarchs have flown a considerable distance south to Georgia and Florida in the southeast and Kansas, Texas and northern Mexico in the southwest, the cardenolide contents change dramatically. Mean cardenolide contents drop to the low levels of overwintering monarchs and the frequency distributions shift to include high proportions of monarchs with very little cardenolide. This makes the strongly skewed distributions almost indistinguishable from those of overwintering monarchs in Mexico. Thus, like northward spring migrants, monarchs lose cardenolide on their southward, autumn migration. One explanation for this loss is differential mortality of the higher cardenolide individuals. However, this cannot be the case because such mortality would produce distributions skewed to the left, with a similar mode but without moving to the left. This clearly is not the case in autumn migrants from the southeast or southwest (fig. 5) with distributions skewed to the right, nor in the northern spring migrants with a normal but shifted distribution. Therefore the most likely explanation is that, irrespective of food plants, freshly hatched monarch individuals all lose substantial amounts of cardenolide during migration. The physiological basis for this loss and its implications for reduced chemical defense are intriguing areas for further study.

By the time monarchs have reached the southern USA and northern Mexico in late October and November, they have the cardenolide contents characteristic of monarchs overwintering in Mexico. Since overwintering monarchs are almost exclusively derived from larvae that fed on *A. syriaca* in the northern USA[34,46] it is very likely that most, if not all of the 'autumn migration' monarchs of table 4 were derived from *A. syriaca.*

Evolutionary and ecological implications of cardenolide sequestration

Migration of the monarch butterfly enables the species to exploit an entire continental flora. No other member of the essentially tropical, nymphalid subfamily Danainae has evolved such remarkable migratory behavior. Although other danaines migrate, coalesce in overwintering aggregations, and sequester cardenolides, none has evolved these adaptations to the extent of the monarch[1,51]. The best evolutionary explanation for the extensive and complex migration of monarchs implicates geographical diversification of the North American species of *Asclepias*[51]. Of 108 species in the genus, 75 occur north of Mexico[50] and monarch larvae have been reported feeding on 27 of these[31]. However, only 3 *Asclepias* species accounted for the host plant origin of 94% of overwintering and spring migrant monarchs, east of the Rocky Mountains[34]. Together these 3 species, *A. viridis, A. humistrata* and *A. syriaca*, cover almost the entire continental area from the Great Plains to the Atlantic coast and from Mexico to Canada. They also span the temporal distribution of monarch breeding from the end of March to late September. Since southern milkweeds appear each spring before northern species[21,33,34], the most obvious explanation for monarch migration is that it is an evolved foraging response to the variable spatial and temporal distributions of predominant milkweed resources. However, the variation in cardenolide contents of exploited milkweeds may provide an additional, ecological explanation for the evolution of monarch migration that emphasizes the role of defense against predation in maintaining selection for migration.

Without southern milkweeds all monarchs would have to migrate in spring from Mexican overwintering sites to the northern USA, instead of migrating only as far as the southern USA. They would have to live longer and fly

further before reproducing in the northern USA. This would depress their intrinsic rate of natural increase through delayed reproduction and increase both energy expenditure and mortality. Since overwintered migrants have very low cardenolide contents, they would also be vulnerable to predation for longer. Thus the costs of migration for overwintered northern arrivals would be high, perhaps even sufficiently high to select against migration. It would then only require the evolution of increased freeze tolerance[2] to allow monarchs to diapause and overwinter in situ and dispense with migration altogether. However, milkweeds are available and abundant in early spring throughout the Gulf states of the southern USA, and the species that are exploited most by monarchs are among those with the highest known cardenolide contents, *A. viridis*, *A. humistrata* and *A. asperula*[21, 29, 30, 33, 34]. Hence we suggest that monarch exploitation of southern milkweeds produces very well protected, new generation monarchs, with high cardenolide contents, which are at low predation risk as they migrate north and track further milkweed resources. These abundant northern milkweed resources are represented primarily by *A. syriaca*. The fact that monarchs sequester cardenolides more effectively from *A. syriaca* than from any other species suggests selection to improve the weak defenses of autumn migrant and overwintering, *A. syriaca*-derived butterflies. In fact, the interaction between *A. syriaca* and the monarch also suggests coevolutionary reciprocity between monarch-induced selection for reduced host cardenolide production and host plant-induced selection for increased cardenolide sequestration by monarch larvae.

The high predation on densely aggregated and vulnerable monarchs overwintering in Mexico[16–19, 25–27], that are characterized by low cardenolide contents, highlights the importance of cardenolides in the monarch's annual cycle. Such an annual cycle of cardenolide sequestration and cardenolide loss during migration suggests that while migration enables monarchs to track their milkweed hosts in space and time, it is dependent on effective cardenolide sequestration. High cardenolide, southern milkweeds may be necessary for northward spring migration because overwintered monarchs have so little cardenolide and migrants lose so much of their sequestered cardenolide during the process of migration. Although there appears to have been strong selection for monarchs to sequester cardenolides from the northern *A. syriaca* more effectively than from any other *Asclepias* species, the low cardenolide content of this host species may preclude the existence of monarch migration without the presence of southern milkweeds.

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1 Ackery, P. R., and Vane-Wright, R. I., Milkweed butterflies: their cladistics and biology. British Museum (Natural History), Comstock Publishing Associates, Cornell University Press, Ithaca, New York 1984.
2 Anderson, J. A., and Brower, L. P., The cold-hardiness of the monarch butterfly during its annual cycle of overwintering, migration and breeding, in: Biology and Conservation of the Monarch Butterfly. Eds S. B. Malcolm and M. P. Zalucki. Natural History Museum of Los Angeles County, Contributions in Science, Los Angeles, in press 1989.
3 Borkin, S. S., Notes on the shifting distribution patterns and survival of immature *Danaus plexippus* (Lepidoptera: Danaidae) on the foodplant *Asclepias syriaca*. Great Lakes Ent. *15* (1982) 199–206.
4 Brower, L. P., Brower, J. V. Z., and Corvino, J. M., Plant poisons in a terrestrial food chain. Proc. natl Acad. Sci. *57* (1967) 893–898.
5 Brower, L. P., Ryerson, W. N., Coppinger, L. L., and Glazier, S. C., Ecological chemistry and the palatability spectrum. Science *161* (1968) 1349–1351.
6 Brower, L. P., Ecological chemistry. Sci. Am. *220* (1969) 22–29.
7 Brower, L. P., McEvoy, P. B., Williamson, K. L., and Flannery, M. A., Variation in cardiac glycoside content of monarch butterflies from natural populations in eastern North America. Science *177* (1972) 426–429.
8 Brower, L. P., and Moffitt, C. M., Palatability dynamics of cardenolides in the monarch butterfly. Nature *249* (1974) 280–283.
9 Brower, L. P., and Glazier, S. C., Localization of heart poisons in the monarch butterfly. Science *188* (1975) 19–25.
10 Brower, L. P., Edmunds, M., and Moffitt, C. M., Cardenolide content and palatability of a population of *Danaus chrysippus* butterflies from West Africa. J. Ent. (A) *49* (1975) 183–196.
11 Brower, L. P., Seiber, J. N., Nelson, C. J., Lynch, S. P., and Tuskes, P. M., Plant-determined variation in the cardenolide content, thin-layer chromatography profiles, and emetic potency of monarch butterflies, *Danaus plexippus* reared on the milkweed, *Asclepias eriocarpa* in California. J. chem. Ecol. *8* (1982) 579–633.
12 Brower, L. P., Chemical defence in butterflies. Symp. R. ent. Soc. Lond. *11* (1984) 109–134.
13 Brower, L. P., Seiber, J. N., Nelson, C. J., Lynch, S. P., and Holland, M. M., Plant-determined variation in the cardenolide content, thin-layer chromatography profiles, and emetic potency of monarch butterflies, *Danaus plexippus* L. reared on milkweed plants in California: 2. *Asclepias speciosa*. J. chem. Ecol. *10* (1984) 601–639.
14 Brower, L. P., Seiber, J. N., Nelson, C. J., Lynch, S. P., Hoggard, M. P., and Cohen, J. A., Plant-determined variation in cardenolide content and thin-layer chromatography profiles of monarch butterflies, *Danaus plexippus* reared on milkweed plants in California: 3. *Asclepias californica*. J. chem. Ecol. *10* (1984) 1823–1857.
15 Brower, L. P., New perspectives on the migration biology of the monarch butterfly, *Danaus plexippus* L. Univ. Texas Contrib. Marine Sci. Suppl. *27* (1985) 748–785.
16 Brower, L. P., and Calvert, W. H., Foraging dynamics of bird predators on overwintering monarch butterflies in Mexico. Evolution *39* (1985) 852–868.
17 Brower, L. P., and Fink, L. S., A natural toxic defense system: cardenolides in butterflies versus birds. Ann. N.Y. Acad. Sci. *443* (1985) 171–186.
18 Brower, L. P., Horner, B. E., Marty, M. A., Moffitt, C. M., and Villa-R, B., Mice (*Peromyscus maniculatus, P. spicilegus,* and *Microtus mexicanus*) as predators of overwintering monarch butterflies (*Danaus plexippus*) in Mexico. Biotropica *17* (1985) 89–99.
19 Calvert, W. H., Hedrick, L. E., and Brower, L. P., Mortality of the monarch butterfly (*Danaus plexippus* L.): avian predation at five overwintering sites in Mexico. Science *204* (1979) 847–851.
20 Calvert, W. H., and Brower, L. P., The location of monarch butterfly (*Danaus plexippus* L.) overwintering colonies in Mexico in relation to topography and climate. J. Lepid. Soc. *40* (1986) 164–187.
21 Cockrell, B. J., Malcolm, S. B., and Brower, L. P., Spring migration of the monarch butterfly: spatial and temporal patterns of population distribution, in: Biology and Conservation of the Monarch Butterfly. Eds S. B. Malcolm and M. P. Zalucki. Natural History Museum of Los Angeles County, Contributions in Science, Los Angeles, in press 1989.

22 Cohen, J. A., Differences and similarities in cardenolide contents of queen and monarch butterflies in Florida and their ecological and evolutionary implications. J. chem. Ecol. *11* (1985) 85–103.
23 Dixon, C. A., Erickson, J. M., Kellett, D. N., and Rothschild, M., Some adaptations between *Danaus plexippus* and its food plant, with notes on *Danaus chrysippus* and *Euploea core* (Insecta: Lepidoptera). J. Zool., Lond. *185* (1978) 437–467.
24 Duffey, S. S., Blum, M. S., Isman, M. B., and Scudder, G. G. E., Cardiac glycosides: a physical system for their sequestration by the milkweed bug. J. Insect Physiol. *24* (1978) 639–645.
25 Fink, L. S., and Brower, L. P., Birds can overcome the cardenolide defence of monarch butterflies in Mexico. Nature *291* (1981) 67–70.
26 Fink, L. S., Brower, L. P., Waide, R. B., and Spitzer, P. R., Overwintering monarch butterflies as food for insectivorous birds in Mexico. Biotropica *15* (1983) 151–153.
27 Glendinning, J. I., Alonso Mejia, A., and Brower, L. P., Behavioral and ecological interactions of foraging mice (*Peromyscus melanotis*) with overwintering monarch butterflies (*Danaus plexippus*) in México. Oecologia *75* (1988) 222–227.
28 Jones, F. M., Insect coloration and the relative acceptability of insects to birds. Trans R. ent. Soc. Lond. *80* (1932) 345–386.
29 Lynch, S. P., and Martin, R. A., Cardenolide content and thin-layer chromatography profiles of monarch butterflies, *Danaus plexippus* L., and their larval host-plant milkweed, *Asclepias viridis* Walt., in northwestern Louisiana. J. chem. Ecol. *13* (1987) 47–69.
30 Martin, R. A., and Lynch, S. P., Cardenolide content and thin-layer chromatography profiles of monarch butterflies, *Danaus plexippus* L., and their larval host-plant milkweed, *Asclepias asperula* subsp. *capricornu* (Woods.), in north central Texas. J. chem. Ecol. *14* (1988) 295–318.
31 Malcolm, S. B., and Brower, L. P., Selective oviposition by monarch butterflies (*Danaus plexippus* L.) in a mixed stand of *Asclepias curassavica* L. and *A. incarnata* L. in south Florida. J. Lepid. Soc. *40* (1986) 255–263.
32 Malcolm, S. B., Monarch butterfly migration in North America: controversy and conservation. Trends ecol. Evol. *2* (1987) 135–138.
33 Malcolm, S. B., Cockrell, B. J., and Brower, L. P., Monarch butterfly voltinism: effects of temperature constraints at different latitudes. Oikos *49* (1987) 77–82.
34 Malcolm, S. B., Cockrell, B. J., and Brower, L. P., Spring recolonization of eastern North America by the monarch butterfly: successive brood or single sweep migration? in: Biology and Conservation of the Monarch Butterfly. Eds S. B. Malcolm and M. P. Zalucki. Natural History Museum of Los Angeles County, Contributions in Science, Los Angeles, in press 1988.
35 Malcolm, S. B., Cockrell, B. J., and Brower, L. P., The cardenolide fingerprint of monarch butterflies reared on the common milkweed, *Asclepias syriaca* L. J. chem. Ecol. (1988) in press.
36 Nelson, C. J., Seiber, J. N., and Brower, L. P., Seasonal and intraplant variation of cardenolide content in the California milkweed, *Asclepias eriocarpa*, and implications for plant defense. J. chem. Ecol. *7* (1981) 981–1010.
37 Nelson, C. J., A model for cardenolide and cardenolide glycoside storage by the monarch butterfly, *Danaus plexippus* (L.), in: Biology and Conservation of the Monarch Butterfly. Eds S. B. Malcolm and M. P. Zalucki. Natural History Museum of Los Angeles County, Contributions in Science, Los Angeles, in press 1988.
38 Parsons, J. A., A digitalis-like toxin in the monarch butterfly, *Danaus plexippus* L. J. Physiol. *178* (1965) 290–304.
39 Poulton, E. B., Mimicry in North American butterflies: a reply. Proc. Acad. natl Sci. Philad. *66* (1914) 161–195.
40 Reichstein, T., Cardenolide (herzwirksame Glykoside) als Abwehrstoffe bei Insekten. Naturw. Rdsch., Stuttg. *20* (1967) 499–511.
41 Reichstein, T., von Euw, J., Parsons, J. A., and Rothschild, M., Heart poisons in the monarch butterfly. Science *161* (1968) 861–866.
42 Roeske, C. N., Seiber, J. N., Brower, L. P., and Moffitt, C. M., Milkweed cardenolides and their comparative processing by monarch butterflies (*Danaus plexippus* L.). Rec. Adv. Phytochem. *10* (1976) 93–167.
43 Rothschild, M., Secondary plant substances and warning colouration in insects. Symp. R. ent. Soc. Lond. *6* (1973) 59–83.
44 Rothschild, M., von Euw, J., Reichstein, T., Smith, D.A.S., and Pierre, J., Cardenolide storage in *Danaus chrysippus* (L.) with additional notes on *D. plexippus* (L.). Proc. R. Soc. Lond. B. *190* (1975) 1–31.
45 Seiber, J. N., Tuskes, P. M., Brower, L. P., and Nelson, C. J., Pharmacodynamics of some individual milkweed cardenolides fed to larvae of the monarch butterfly (*Danaus plexippus* L.). J. chem. Ecol. *6* (1980) 321–339.
46 Seiber, J. N., Brower, L. P., Lee, S. M., McChesney, M. M., Cheung, H. T. A., Nelson, C. J., and Watson, T. R., Cardenolide connection between overwintering monarch butterflies from Mexico and their larval food plant, *Asclepias syriaca*. J. chem. Ecol. *12* (1986) 1157–1170.
47 Tuskes, P. M., and Brower, L. P., Overwintering ecology of the monarch butterfly, *Danaus plexippus* L., in California. Ecol. Ent. *3* (1978) 141–153.
48 Urquhart, F. A., The Monarch Butterfly. University of Toronto Press, Toronto 1960.
49 Vaughan, F. A. Effects of gross cardiac glycoside content of seeds of common wilkweed, *Asclepias syriaca*, on cardiac glycoside uptake by the milkweed bug *Oncopeltus fasciatus*. J. chem. Ecol. *5* (1979) 89–100.
50 Woodson, R. E. Jr., The North American species of *Asclepias* L. Ann. Mo. Bot. Gard. *41* (1954) 1–211.
51 Young, A. M., An evolutionary-ecological model of the evolution of migratory behavior in the monarch butterfly, and its absence in the queen butterfly. Acta Biotheor. *31* (1982) 219–37.

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Evolution of exocrine chemical defense in leaf beetles (Coleoptera: Chrysomelidae)

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Summary. In this review we speculate on possible scenarios for the evolution of the very high diversity in chemical compounds liberated by exocrine glands of adults Chrysomelidae. Shift in host plant affinities and subsequent adaptation of the beetles to the plant toxins strongly influence the nature of the beetles' chemical defense.
Key words. Cardenolides; dipeptides; isoxazolinone glucoside; pyrrolizidine alkaloids; host plant influence; toxin sequestration.

The name 'Chrysomelidae' is derived from the Greek *chrysos*, gold, and *melolanthion*, beetle, referring to the bright metallic colors characteristic of many members of this taxon. These colors are not there simply for the pleasure of amateur entomologists, but rather they have biological significance as intra- or interspecific signals. In