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The Influence of Eastern North American Autumnal Migrant Monarch Butterflies (*Danaus plexippus* L.) on Continuously Breeding Resident Monarch Populations in Southern Florida

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Abstract In Florida, the eastern North American population of the monarch butterfly exhibits geographic variability in population structure and dynamics. This includes the occurrence of migrants throughout the peninsula during the autumnal migration, occasional overwintering clusters that form along the Gulf Coast, remigrants from Mexico that breed in north-central Florida during the spring, and what have been assumed to be year-round, resident breeding populations in southern Florida. The work reported here focused on two monarch populations west of Miami and addressed four questions: Are there permanent resident populations of monarchs in southern Florida? Do these breed continuously throughout the year? Do they receive northern monarchs moving south during the autumn migration? Do they receive overwintered monarchs returning via Cuba or the Yucatan during the spring remigration from the Mexican overwintering area? Monthly collections and counts of spermatophores in the bursa copulatrices of females established that a resident population of continuously breeding monarchs exists year-round in southern Florida. It was determined through cardenolide fingerprinting that most of the butterflies had bred on the local southern Florida milkweed species, *Asclepias curassavica.* During the autumn migration period, however, some monarchs had fed on the northern milkweed, *Asclepias syriaca*. It appears that instead of migrating to Mexico, these individuals travel south through peninsular Florida, break diapause, mate with and become incorporated into the resident breeding populations. None of the monarchs captured in spring had the *A. syriaca* cardenolide fingerprint, which is evidence against the southern Florida populations receiving overwintered remigrants from Cuba, Central America or Mexico.

Keywords *Asclepias syriaca* · *A. curassavica* · Biogeography · Cardenolide · Thin layer chromatography fingerprints · Danainae · Migration · Milkweed · Population biology · Resident breeding populations · Everglades · Lepidoptera

Introduction

In North America, monarch butterflies *Danaus plexippus* L. (Nymphalidae) breed east of the Rocky Mountains to the Atlantic Coast and undergo a southward migration of up to 3,900 km from the northern United States and Canada to overwintering sites in central Mexico, followed by a spring remigration back into the U.S.A. of up to an additional 2,100 km (Brower et al. 2006). A smaller population breeds west of the Rockies and migrates to numerous overwintering sites along the California coast (Frey and Schaffner 2004). Monarchs also migrate in eastern Australia (review in James 1993), where they were introduced in the 1870s (Ackery and Vane-Wright 1984). In contrast, non-migratory, continuously breeding populations occur throughout the Neotropics and the West Indies (Urquhart 1960; Barcant 1970; Brower 1985) and in introduced populations on several Pacific islands and in parts of Australia (James 1993).

A long debated question that bears on the evolutionary exploitation of a temperate flora by an essentially tropical insect (Brower 1995; Ackery and Vane Wright 1984) is whether continuously breeding, non-migratory monarch

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butterfly populations occur in the southern United States. Reasons for this debate were Williams' (1958) contention that North American monarchs do not reproduce in their overwintering areas, and the subsequent confusion between overwintering and breeding populations reported from southern Texas (Urquhart 1960), southwestern Arizona (Funk 1968), southern California (Urquhart et al. 1970), and Florida (Urquhart 1960). Brower (1962) added to the debate by reporting what he considered local breeding populations during the spring in south-central Florida. Subsequent research determined that these populations were most likely a new spring generation established by remigrants returning from overwintering sites in Mexico (Knight 1998; Knight et al. 1999).

Limited evidence of breeding during the winter in southern Florida at one of the sites described in this study (Site 1) was presented by Brower (1985). He determined that 15 of 17 females collected on 5 December 1975 had mated, and also noted five mating pairs. Farrey and Davis (2004) reported monarch larvae on milkweeds growing in a southern Florida garden in January. The possibility exists then, that populations of monarchs in southern Florida may breed year-round. Other unresolved issues are the extent to which southern Florida monarch populations may be derived from the eastern North American migratory population, the possibility that they originated as immigrants from resident breeding populations in the West Indies, or that introgression may occur from both sources.

To resolve these issues, this study was carried out on two monarch populations in southern Florida immediately west of Miami, on the eastern edge of the Everglades. Four questions are addressed: Are the populations present throughout the year? Do they breed during all months of the year? Do they receive northern monarchs moving south during the autumn migration? Do they receive monarchs remigrating northward through the Florida peninsula during the spring?

To answer the first two questions, monarchs were censused nearly every month from September 1994 to November 1995 and their breeding status was determined by dissecting females and counting the number of spermatophores in their bursa copulatrices. The last two questions were addressed by analyzing chemical fingerprints of cardenolides present in individual monarchs collected from the two Miami populations throughout the same period. This technique utilized thin-layer chromatography (TLC) to visualize the arrays of cardenolides extracted from individual butterflies that reflect the species-specific pattern of these poisons that are present in the milkweed species (Asclepiadaceae) on which the monarch fed while a larva (Brower et al. 1982). Because different milkweed species have distinct thin-layer chromatography patterns, it is often possible to determine the general geographic area where a monarch adult was born, based on the milkweeds' discreet geographic distributions (Woodson 1954).

This method allows differentiation of autumn migrant monarchs that would have fed as larvae on northern milkweeds, predominantly *Asclepias syriaca* L. (Malcolm et al. 1993; Wassenaar and Hobson 1999), from monarchs that would have fed on southern Florida milkweeds, principally *A. curassavica* L. *Asclepias syriaca* is the most abundant eastern North American milkweed species and is distributed from southern Canada to Virginia (Woodson 1954). Because of human influence, it recently has extended its range to north central Georgia, but not Florida (Wyatt 1996). *Asclepias curassavica* occurs widely in the Neotropics and on the Caribbean islands (Woodson 1954). It is also abundant in cattle pastures in southeastern Florida (Cohen 1983, 1985). Several other species of milkweed occur in southern Florida and are discussed below.

Methods and Materials

Monarch Collections and Sampling Sites Adult monarchs were collected from two field sites near Miami, Florida. The butterflies were netted, placed in glassine envelopes, and kept continually on ice in a cooler, then frozen in a standard laboratory freezer until analyzed.

Site 1, the main collecting area was a ≈ three ha pasture in Hialeah, Florida (25°53′N, 80°22′W) and surrounded a water filled limestone quarry. The pasture contained large patches of *A. curassavica* as well as two other less abundant milkweeds, *A. incarnata* Walt. and *Sarcostemma clausum* (Jacq.) Schult. The site was bordered to the south by a stand of large live oaks, *Quercus virginiana* L. (Fagaceae), in which monarchs were observed roosting and as resting mated pairs. *Sida* sp. (Malvaceae) also grew in the pasture and was a potential nectar source. Censuses were made in March–April 1990 and monthly from September 1994 to November 1995, except for November 1994 and June and August 1995. Reference to Google Earth, "Graham Baptist Church", access date 9 May 2009, indicates that the old field vegetation surrounding the quarry was destroyed, probably around 2002.

Site 2 (25°48′N, 80°20′W) was ≈ three ha area 10 km south of Site 1. It consisted of a fenced cattle pasture containing abundant but scattered *A. curassavica* plants, and a larger, ungrazed old-field that contained a few *A. curassavica* and a variety of composites (Asteraceae) that served as nectar sources. Monarchs were monitored from February to May 1995, and one sample was collected here for thin-layer chromatography analyses on 7 Nov 1995.

Wing Condition and Mating Status Wing condition for each butterfly was rated visually from 1 (fresh, virtually no scales missing) to 5 (worn, many scales missing) in increments of 0.5. Mating frequency was determined for

all females collected in 1994 and 1995 by dissecting their bursa copulatrices and counting spermatophores, following the method of Van Hook (1999). Butterflies collected in 1990 were too desiccated for bursa copulatrix dissections.

Chemical Procedures In order to perform TLC analyses and determine the foodplants that the butterflies had fed upon as larvae, butterflies were assayed for cardenolide contents as follows. They were dried at 60°C for 16 h in a forced draft oven and weighed on a Mettler AK 160 balance (Mettler-Toledo, Inc., Columbus, OH, USA). Following Alonso-Mejia et al. (1997), each dried butterfly was ground in petroleum ether to extract the lipids. Following Malcolm et al. (1989), cardenolides were ethanol-extracted from the individually defatted butterflies in 10 ml volumetric flasks and spectroassayed to determine gross concentration as µg/0.1 g of dry butterfly material. A Lambda IIs dual beam spectrophotometer (Perkin Elmer Corporation, Norwalk, CT, USA) was used for all spectroassays.

Seven ml of each remaining ethanol extract were cleaned of plant pigments for TLC in order to visualize the component cardenolides by using the tetranitrodiphenyl (TNDP) reagent (Brower et al. 1982; Malcolm et al. 1989; Moranz and Brower 1998). Chromatography was performed on the extracts of a total of 408 monarchs. Each TLC plate was spotted with 14 monarch extracts along with 10 µg of digitoxin and digitoxigenin cardenolide standards spotted on the center and sides of each plate (Fig. 1a and b). Following visualization, each plate was photographed on Kodachrome 25 film, and the images were transferred digitally to a compact disc. Adobe Photoshop (ver. 2.0) was used to sort individual butterflies by cardenolide pattern, as described in Moranz and Brower (1998).

Discriminating Cardenolide Fingerprints of Monarch Adults that Fed as Larvae on Various Milkweed Species The various fingerprints were sorted by comparing the cardenolide spot intensities and mobilities with published fingerprints of the monarch and/or queen butterfly (*Danaus gilippus* Cramer) reared on several milkweed species. The two most critical fingerprints are those found in monarchs that fed as larvae either on the southern Florida *A. curassavica* (Fig. 1a and b) or the northern *A. syriaca* (Fig. 1b). Published *A. curassavica* fingerprints are in Cohen 1983; Brower 1984; Malcolm et al. 1989; Knight 1998, while published *A. syriaca* fingerprints are in Brower 1984; Seiber et al. 1986; Malcolm et al. 1989; Knight 1998. Several other species of milkweeds occur in central to

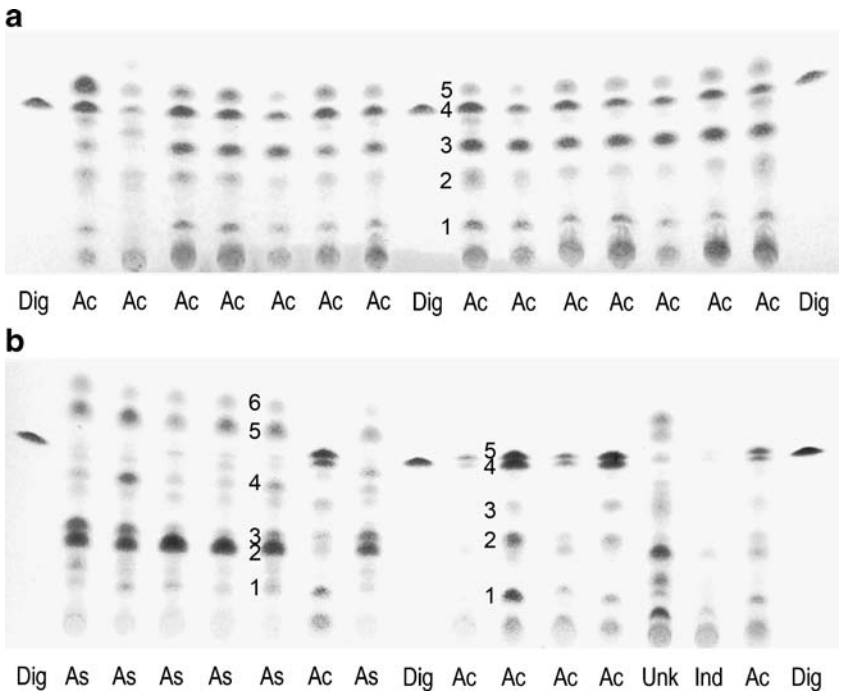

Fig. 1 **a** Thin-layer chromatogram plate of 14 monarchs captured in southern Florida at Site 1 on 1 January 1995, with 10 µg of digitoxin (Dig) standards. The butterflies were all females from which 75 µg of extracted cardenolide were applied to the single plate. Unnumbered spots are not cardenolides. All butterflies have the *Asclepias curassavica* (Ac) cardenolide fingerprint. **b** Thin-layer chromatogram plate of 14 monarchs captured in southern Florida at Site 2 on 7 November 1995, with 10 µg of digitoxin (Dig) standards. Unnumbered spots are not cardenolides. From left to right, the first three channels are from males, and the rest from females; only 7 µg were available for nos. 8 and 10, 3 µg for no. 13, and 32 µg for no. 14; the applied amount for the rest of the channels was 75 µg. Six butterflies exhibit the *Asclepias curassavica* (Ac) cardenolide fingerprint, seven the *A. syriaca* (As) fingerprint, one butterfly is *indeterminate* (Ind), and one has an as yet unknown (Unk) fingerprint

southern Florida (Woodson 1954). These include *A. perennis* Walt., *A. longifolia* Michx., *A. viridis* Walt., *A. humistrata* Walt., *A. lanceolata* Walt., and three vinaceous milkweeds, *Matelea gonocarpa* Walt. (Shinners), *Cynanchum scoparium* Nutt., and *C. angustifiolium* Pers. All of these produce monarchs and/or queens with fingerprints that are qualitatively distinct from monarchs that feed on *A. curassavica* or *A. syriaca* (Martin et al. 1992; Moranz and Brower 1998). Two other common Florida species are *A. incarnata* L. with only small amounts of cardenolide, and *A. tuberosa* L. that has virtually none (Malcolm 1991; Brower, Seiber, and Nelson, unpublished data). Both produce faint to no monarch fingerprints that are distinct from *A. curassavica* and *A. syriaca*. Cardenolide fingerprints have not been determined for three other southern Florida milkweeds: *A. verticillata* L., *A. pedicellata* Walt., and the vine, *Sacrostemma clausum* (Jacq.) Schult. The first two are relatively uncommon, and *A. pedicellata* is Florida's smallest milkweed that has little foliage to support a monarch larva. It is probable that *Sarcostemma*, like *Cynanchum* and *Matelea* (Moranz and Brower 1998), has similar weak fingerprints, vividly distinct from both *A. syriaca* and *A. curassavica.*

The monarch fingerprints were designated as indeterminate when there was an insufficient amount of cardenolide on the TLC plate to determine a clear pattern, or as unknown when a pattern was clear but could not be matched to a known one.

Results

Cardenolide Fingerprints Thin-layer chromatography revealed that at least 84% of the 379 adult monarchs collected in 12 of the 13 months at Site 1 had the *A. curassavica* cardenolide fingerprint, with 100% having this fingerprint in six of the months (Table 1; Fig. 1). In November 1995, eight of 11 individuals (73%) had the *A. curassavica* fingerprint, and three (27%) had the *A. syriaca* fingerprint. In total, 11 monarchs from Site 1 had fed as larvae on *A. syriaca*. Significantly, all *A. syriaca* monarchs were collected in autumn: six in October 1994, three in November 1995, and one each in December 1994 and October 1995. Three had fingerprints that were indeterminate, and two had unknown fingerprints.

TLC results for the Site 2 collection of 29 monarchs on 7 November 1995 had a higher proportion of *A. syriaca* monarchs (58%) than did the collection made at Site 1 one day earlier (28%); Table 1; Fig. 1). Excluding the two indeterminate individuals and the one unknown, this difference is significant (Site 2, 17/26=65%; Site 1, 3/11=27%; $\chi_1^2=4.90$, $P<0.05$). The indeterminate monarchs probably had also fed on *A. syriaca* plants that had low cardenolide concentrations. Monarchs that feed on this milkweed vary from low to high concentrations both in migrants (Knight 1998) and in those collected from across the northern summer breeding range (Malcolm et al. 1989). Overall, only eight of the 408 monarchs analyzed could not be fingerprinted to a known milkweed species; 91% had fed on *A. curassavica*, and 7% on *A. syriaca*.

Frequency of Female Mating Of the 139 females dissected from Site 1, 133 (96%) had mated (Table 2). Five of the six virgins had excellent wing condition (1 or 1.5), suggesting that they had bred locally, and that enough time had not elapsed since eclosion for them to have mated. Of the 133

Table 1 Summary of thin-layer chromatography fingerprints of 408 adult monarch butterflies collected in March and April, 1990 and from September 1994 through November 1995 from Site 1, and in November 1995 at Site 2

Site	Date	Year	N	Butterfly cardenolide fingerprint			
				Indet[a]	Unknown[b]	A. syriaca	A. curassavica
1	19 Mar-17 Apr	1990	46	0	0	0	46 100%
1	8 Sep	1994	26	0	0	0	26 100%
1	22 Oct	1994	38	0	0	6	32 84%
1	17 Dec	1994	45	0	1	1	43 95%
1	1 Jan	1995	41	1	0	0	40 98%
1	10 Feb	1995	35	0	0	0	35 100%
1	12 Mar	1995	28	0	1	0	27 96%
1	8 Apr	1995	31	0	0	0	31 100%
1	8–9 May	1995	39	0	0	0	39 100%
1	20 Jul	1995	10	0	0	0	10 100%
1	10 Sep	1995	19	2	0	0	17 90%
1	26 Oct	1995	10	0	0	1	9 90%
1	6 Nov	1995	11	0	0	3	8 73%
2	7 Nov	1995	29	2	1	17	9 31%
Total			408	5 (1.2%)	3 (0.7%)	28 (6.9%)	372 (91.2%)

[a] Fingerprint is indeterminate

[b] Fingerprint is distinct but unknown

Table 2 Mating frequency determined by spermatophore counts for 155 female monarchs collected in 1994 and 1995 from Site 1, and in November 1995 from Site 2

Site	Date	Year	N	Numbers of spermatophores per bursa						
				Mean	Range	0	1	≥2	% mated	% multiply-mated
1	8 Sep	1994	7	2.4	1–5	0	3	4	100	57
1	22 Oct	1994	21	2.8	1–5	0	3	18	100	86
1	17 Dec	1994	24	2.7	0–8	1	3	20	96	83
1	1 Jan	1995	25	4.3	0–10	2	3	20	92	80
1	10 Feb	1995	11	5.5	2–9	0	0	11	100	100
1	12 Mar	1995	10	4.3	1–9	0	1	9	100	90
1	8 Apr	1995	13	3.3	1–7	0	2	11	100	85
1	8–9 May	1995	6	2.2	0–4	2	0	4	67	67
1	20 Jul	1995	6	2.8	1–4	0	1	5	100	83
1	10 Sep	1995	6	3.0	0–6	1	1	4	83	67
1	26 Oct	1995	5	2.6	1–4	0	1	4	100	80
1	6 Nov	1995	5	2.4	1–4	0	2	3	100	60
2	7 Nov	1995	16	2.9	0–10	2	5	9	88	56
Total			155	3.3	0–10	8	25	122	95	79

females that had mated, 113 (85%) were multiply mated, with a maximum of ten spermatophores counted in one individual. The mean number of spermatophores ranged from 2.2 in May 1995 to 5.5 in February 1995. The high frequency of multiple matings in all months indicates that mating occurs throughout the year in this population.

Of the nine *A. syriaca* females collected at Site 1, all had mated, and seven (78%) had multiply mated. These northern migrants had thus broken diapause and must be exchanging genes with the Miami population. Mating frequency also was high for the 16 females collected at Site 2, with 88% mated and 56% multiply mated. Twelve of these 16 females (75%) had the *A. syriaca* cardenolide fingerprint, ten (84%) were mated, and six (50%) were multiply mated. The two unmated females had wing conditions of 2.0, suggesting that they may have been recently arrived migrants from the north that still were in reproductive diapause.

Wing Condition Mean wing condition for all monarchs captured was 2.6 (SD=1.0). Monarchs with excellent (1.0 or 1.5) wing condition were captured in every month sampled except November 1995, Site 1, where the best condition was 2.0.

Discussion

This study addressed whether resident monarch populations occur in southern Florida, and, if so, whether they breed continuously through the year. Monthly collections confirmed that two populations on the eastern edge of the Everglades near Miami occur year-round, and periodic research at one of these sites indicates that monarchs as well as abundant *A. curassavica* and *A. incarnata* plants have occurred there for at least 20 years, in December 1975 (Brower 1985), December 1981 (Cohen 1983), September 1984 (Malcolm and Brower 1986), March–April 1990 (this paper), and September 1994–November 1995 (this paper).

Monarchs were collected in every month visited from September 1994 to November 1995. Although no visits were made in June and August of 1995, the population appeared stable throughout the study, as indicated by the numbers of monarchs captured during each visit. The capture and bursa dissection results, along with the constant presence of *A. curassavica* as a food plant and the *A. curassavica* cardenolide fingerprint in the butterflies collected at both sites are evidence for a viable, year-round continuously breeding resident monarch population in this part of southern Florida.

Bursa copulatrix dissections of female monarchs confirmed that the monarchs breed year-round. Sixty-seven to 100% of the females were mated in all months, with most multiply mated. Similarly, Brower (1985; unpublished data) found that 15 of 17 females captured at Site 1 on 5 December 1975 had mated from two to seven times, saw four mating pairs, and also determined that two fresh females had not mated. These results are similar to those found in resident populations of monarchs in Trinidad, West Indies, where mating frequency in the summer was 96%, with 52% multiply mated (Pliske 1973), and for summer breeders both in Massachusetts, U.S.A. and in Australia where 98% of the females were mated, with 80 and 95% multiply mated, respectively (Brower 1985; Suzuki and Zalucki 1986). Finally, the wing condition also provided evidence that new generations are being produced

throughout the year: at least some individuals in each non-autumn month had wing conditions of 1.0 or 1.5, indicating recent eclosion.

Year-round breeding of monarchs may not be possible throughout much of the southeastern U.S. because of warm summer temperatures. Based on the laboratory findings of Zalucki (1982) and Rawlins and Lederhouse (1981) that constant temperatures between 31 and 35.5°C are lethal for monarch larvae, Malcolm et al. (1987) deduced that high early summer temperatures in the Gulf coastal states force the new spring generation produced during late March and April to move north, which they do in early May (Knight et al. 1999). It seems likely, however, that maximum summer temperatures in the Miami area are slightly below this threshold most of the time. The mean monthly maximum temperature for 30 years (1961–1990) from April to September at the Miami airport was 31.7°C (Anon. 1999). Further north in Florida, however, temperatures may exceed the threshold sufficiently often to be lethal. For example, in Gainesville approximately 480 km north of Miami, the mean monthly maximum temperature for 30 years (1961–1990) from April to September was 32.6°C (Anon. 1999).

In contrast, warm temperatures are required for monarchs to breed year-round and thus sustain a population in southern Florida because neither the butterflies nor their larval food plants can survive severe freezing temperatures (Ackery and Vane-Wright 1984; Larsen and Lee 1994; Anderson and Brower 1996) that periodically affect north Florida and the Gulf Coast (Brower 1995). Although warm temperatures also occur throughout the winter in the southwestern United States, where winter breeding has been reported, these areas probably cannot sustain a permanent year-round breeding population due to the dieback of milkweeds during the autumn and winter (Funk 1968; Urquhart et al. 1970). This is not the case, however, in southern Florida, where the climate is sufficiently warm and wet to sustain year-round populations of *A. curassavica*, a nearly ubiquitous neotropical milkweed (Woodson 1954) that is the principal food plant of monarchs throughout the West Indies, Central and South America (Barcant 1970; Haber 1993). Thus, the combination of warm temperatures and a constant host plant supply, which promotes year-round reproduction in southern Florida, resembles conditions in the tropics where continuously breeding, resident monarch populations occur widely.

This study also addressed whether southern Florida populations receive migratory monarchs from the north during the autumn. The TLC results for October and November 1994 and 1995 show clearly that the southern Florida resident population receives an influx of autumnal migrant monarchs that have fed on the northern milkweed, *A. syriaca*. Moreover, 90% of these *A. syriaca* females were mated or multiply mated, indicating that they had broken reproductive diapause and were becoming incorporated into the resident southern Florida population. The two unmated females appeared to be recent arrivals still in reproductive diapause, which is characteristic of most autumn migrants along the Florida Gulf coast and at the overwintering sites in Mexico (Herman et al. 1989, Van Hook 1999). Mating frequencies of autumnal migrants along the northern Florida Gulf Coast have been observed at 29% (Brower 1985) and 38% (A. Knight and L. P. Brower, unpublished), which are much lower than those reported here for the migrants at the southern Florida sites.

Environmental factors strongly influence reproductive activity in monarchs. Based on physiological experiments, Barker and Herman (1976) proposed that monarchs in the Gulf coast states remain in reproductive arrest as long as average temperatures are below 28°C. Along with the evidence from the present study, this suggests that the migrants must become reproductively active when they reach the warmer, southern areas such as southern Florida and the West Indies (Urquhart 1960; Brower 1985). The incorporation of these autumnal migrants into the southern Florida population is further indicated by the fingerprint results that no monarchs that had fed on *A. syriaca* were present in March and April, 1990 or in January to September, 1995.

The final question addressed by this study is whether there is evidence for a spring remigration of monarchs that had overwintered in Cuba or the Yucatan into these southern Florida populations, as originally postulated by Urquhart and Urquhart (1976). The lack of the *A. syriaca* fingerprint in any of the southern Florida samples collected during March through May argues against this hypothesis. *A. syriaca* monarchs do appear regularly each spring in north-central Florida, but these are likely individuals returning from the Mexican overwintering sites. As far as is known, all Caribbean and southern Florida monarch populations are resident and non-migratory, and the possibility that they migrate northward is not supported by a long-term study of migration patterns of Florida butterflies (Walker 2001), nor by any evidence cited in reviews of the migration biology of the monarch butterfly (Brower 1985, 1995).

Research by Dockx et al. (2004) on monarchs collected in Cuba is in agreement. These authors combined cardenolide fingerprinting with stable isotope analyses and determined that 62% of 152 monarchs that were collected at three Cuban sites during November in 1993, 1995, 1996, and 1997 had migrated there from southeastern Canada or the northeastern United States. Of these, 18 were determined to have fed as larvae on *A. syriaca.* As in the southern Florida samples, no *A. syriaca* butterflies were found in a March sample.

The evidence from both studies provides support for the hypothesis (Brower 1985, 1995) that autumn migrants

flying south through the Florida peninsula are off the normal course to their overwintering area in central Mexico. These individuals, in effect, become physiologically trapped, break reproductive diapause, and, rather than remigrating northward the following spring, they and their offspring become assimilated into the resident breeding populations.

In summary, monthly collections confirmed that monarchs on the eastern edge of the Everglades occur year-round, and bursa copulatrix dissections of females indicated that the population is continuously reproductive. Cardenolide fingerprinting revealed that, except in the autumn, virtually all monarchs had fed as larvae on *A. curassavica*, a neotropical milkweed that grows extensively in southern Florida but does not occur naturally in the monarch's northern range. Taken together, the findings provide evidence for a year-round breeding population and for its persistence through the years.

Cardenolide fingerprints of *A. syriaca* present in butterflies collected during October–December established that northern migrants enter the Miami population during the autumn. Moreover, 90% of these *A. syriaca* females were mated or multiply mated, indicating that they had broken reproductive diapause and were becoming incorporated into the resident southern Florida population. The lack of the *A. syriaca* fingerprint in any of the spring butterflies is evidence against a spring remigration of northeastern monarchs that have migrated to and overwintered in Cuba or the Yucatan back into these southern Florida populations.

Finally, although the cardenolide fingerprints in our samples were distinct and their interpretation straightforward, multivariate analysis of fingerprint patterns could further confirm these results. We propose to conduct High Performance Liquid Chromatography (HPLC) by using remaining cardenolide extracts from these samples and to develop a statistical interpretation method.

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