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DO NORTH AMERICAN MONARCH BUTTERFLIES TRAVEL TO CUBA? STABLE ISOTOPE AND CHEMICAL TRACER TECHNIQUES

CRISTINA DOCKX,[1,5] LINCOLN P. BROWER,[2] LEONARD I. WASSENAAR,[3] AND KEITH A. HOBSON[4]

[1]*277-14 Corry Village, University of Florida, Gainesville, Florida 32603 USA*
[2]*Department of Biology, Sweet Briar College, Sweet Briar, Virginia 24595 USA*
[3]*Environment Canada, 11 Innovation Blvd., Saskatoon, Saskatchewan, Canada S7N 3H5*
[4]*Environment Canada, 115 Perimeter Road, Saskatoon, Saskatchewan, Canada S7N OX4*

Abstract. Since the discovery of monarch butterfly (*Danaus plexippus*) overwintering colonies in Mexico in the 1970s, it was assumed that monarchs from eastern North America migrated only to Mexico. This paper reveals that monarchs from Canada and the east coast of the United States also regularly travel to Cuba during the migration period. The natal grounds of Cuban monarchs were determined through the combined use of stable hydrogen (δD) and stable carbon (δ^{13}C) isotope measurements and by cardenolide fingerprint analysis using thin-layer chromatography (TLC). The TLC data revealed that there was an influx of migrants in November to Cuba, and the stable isotope data revealed that migrant Cuban monarchs originated from southeastern Canada and the eastern United States. Our findings suggest that North American migrant monarchs that move to Cuba hybridize with resident populations there and do not return to the continent. The differences in the natal grounds, migratory route, and reproductive stages between monarchs wintering in Mexico and Cuba suggest that there are at least two subpopulations of eastern North American monarchs. The extent to which Cuba may act as a bridge for monarch movement to the Yucatan and other Caribbean islands and the genetic impact of this newly revealed flux in monarch movements remain to be determined.

Key words: butterfly; cardenolide fingerprint analysis; Cuba; Danaus plexippus; Danaus plexippus megalippe; Danaus plexippus plexippus; *hybridization; Mexico; migration; monarch butterfly; stable isotope; thin-layer chromatography.*

INTRODUCTION

The monarch butterfly (*Danaus plexippus*) is a cosmopolitan migratory species in the Americas with two subspecies: *Danaus plexippus plexippus* (Linnaeus) in North America and *Danaus plexippus megalippe* (Hübner) in the Caribbean and northern South America (Ackery and Vane-Wright 1984; see Appendix). The subspecies *D. p. plexippus* has two distinct populations that are geographically separated by the Rocky Mountains. The eastern monarchs undergo an extraordinary fall migration of up to 4000 km from their breeding grounds to discrete wintering colonies in the volcanic mountains of central Mexico (Brower 1995, Wassenaar and Hobson 1998). They arrive at these colonies between the end of October and November, overwinter, and then return to the southern United States in late March. A fall migration of the western monarchs, on a lesser scale, occurs west of the Rocky Mountains, mainly to smaller, dispersed overwintering sites along the California coastline (Brower 1995).

By contrast, monarchs in the neotropics such as *D. p. megalippe* (see Plate 1 and Appendix) do not migrate, but instead breed year-round in open grasslands where their natal host plants (milkweeds, *Asclepias* spp.) are present, especially *Asclepias curassavica* (Williams et al. 1942, Alayo and Hernandez 1987; J. Salazar, *personal communication*; C. Dockx, *personal observations*). These differences in behavior between temperate and neotropical monarchs, as well as size, color, and wing pattern differences, have resulted in their recognition as two subspecies (Clark 1941, Brown and Heineman 1972, Urquhart and Urquhart 1976). Phenotypic differences between them are less obvious in the Caribbean islands. Brown and Heineman (1972) state: ''The islands of Cuba, Jamaica, and Hispaniola undoubtedly harbor indigenous sedentary subspecies that have not yet been recognized because of the confusing mixtures present on the islands. Much of this mixing is undoubtedly the result of the southward movement of *D. p. plexippus* deep into the northern parts of the tropics, probably during the peak of the Wisconsin [sic] glacial stage.'' During the 1970s, a single monarch tagged in the vicinity of Lake Ontario was recaptured near Havana (Urquhart and Urquhart 1976). Since then, although observations have been made of monarchs flying southward toward the Caribbean islands between September and November (Williams et al. 1942), this single tag recovery has been the only real scientific evidence of migrant monarchs reaching Cuba.

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[5] Present address: Leiden University, P.O. Box 9516, Kaiserstraat 63, 2300 RA, Leiden, Netherlands.
E-mail: cristinadockx@yahoo.com

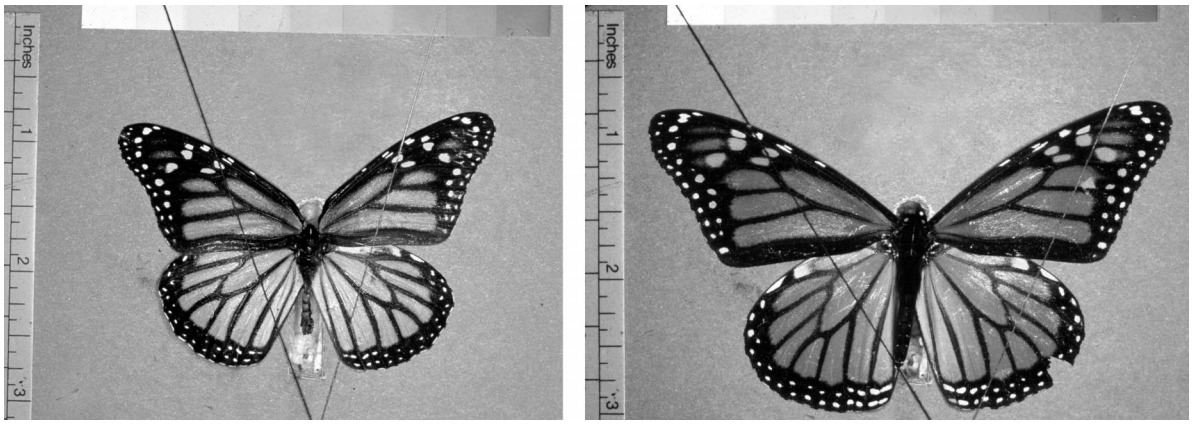

PLATE 1. (Left) Resident female monarch, *Danaus plexippus megalippe*. (Right) Migrant female monarch, *Danaus plexippus plexippus*. These two specimens were captured during November in Cuba. Notice the differences in general size, forewing shape, and the close absence of the inner line of white spots at the apex of the forewing in *D. p. megalippe*. Photo credit: C. Dockx.

Here we test the hypothesis that eastern North American monarchs move regularly to Cuba during their fall migration. We determined the natal origins of butterflies captured in Cuba using two independent chemical techniques: cardenolide fingerprinting using thin-layer chromatography, TLC (Roeske et al. 1975, Brower et al. 1984, Malcolm et al. 1989), and stable isotopic measurements of carbon, $\delta^{13}C$, and hydrogen, δD (Wassenaar and Hobson 1998). These analyses allowed us to distinguish native from migrant individuals.

TLC fingerprinting defines cardenolide spot patterns unique to the milkweed species on which a monarch larva has fed. By matching the cardenolide spot patterns to a particular milkweed species, and knowing the geographical distribution of that larval host plant (Woodson 1954), it is possible to broadly estimate the natal origin of the butterfly (Seiber et al. 1986, Malcolm et al. 1992).

Relative abundance of stable isotopes, such as ^{13}C (expressed as $\delta^{13}C$) and deuterium (δD), follows a distinctive pattern across the North American continent: higher values for δD occur toward lower latitudes, and lower values for $\delta^{13}C$ occur toward southwestern areas (Wassenaar and Hobson 1998). The δD rainfall values on the continent are controlled by latitude, temperature, season, and inland distance from the coast to the point of precipitation (White 1989, Hobson et al. 1999). In turn, the isotopic values for $\delta^{13}C$ in plants are controlled by climatic, altitudinal, and photosynthetic patterns (Hobson 2002). These isotopic patterns in turn control the isotopic composition of the monarch larval food plant. These two isotopes are taken in by the monarch larva when it feeds on its host plant, and then become incorporated in the butterfly wing keratin. Because wing keratin is metabolically inert after its synthesis during the pupal stage, it reflects the δD and $\delta^{13}C$ values at the natal site of the larvae (Hobson et al. 1999*a*). The δD and $\delta^{13}C$ values in monarch wing membranes are each highly correlated ($r = 0.99$) with the isotopic composition of the larval food source (Hobson et al. 1999*b*). Thus, stable isotope analysis of monarch wing keratin yields general latitudinal (δD) and longitudinal ($\delta^{13}C$) information to help further narrow down the geographical area in which the butterfly emerged (Wassenaar and Hobson 1998).

The advantage of using stable isotope and TLC signatures together was that in cases where the isotope or TLC fingerprints were unavailable or inconclusive, the other technique could fill this information gap (with the exception of TLC fingerprints of milkweed species found in both the United States and Cuba). Another advantage of using multiple tracer techniques is that their results can be used to verify the extent to which these techniques agree. This research employs, for the first time, two independent chemical tracer techniques simultaneously to determine origins of a migrant organism.

METHODS

Monarchs were collected in Cuba at three different locations: San Antonio de los Baños, Zapata Swamp, and Guanahacabibes Peninsula in the most western part of Cuba (Fig. 1, Table 1). Collections were made in November 1993 and 1995–1997 because migrant monarchs are known to arrive in southern Florida at this time (Knight 1998). Butterflies were collected, placed in glassine envelopes, and transferred to a refrigerator for ~20 days to keep them alive. Afterward, all butterfly samples were brought to the University of Florida and were stored in a standard freezer at −20°C before being analyzed. Vouchers of *Asclepias* species observed in Cuba were identified and stored at the herbarium at the University of Florida.

Field sites

The San Antonio de los Baños field site (Fig. 1) is a pasture located at a government-owned dairy farm

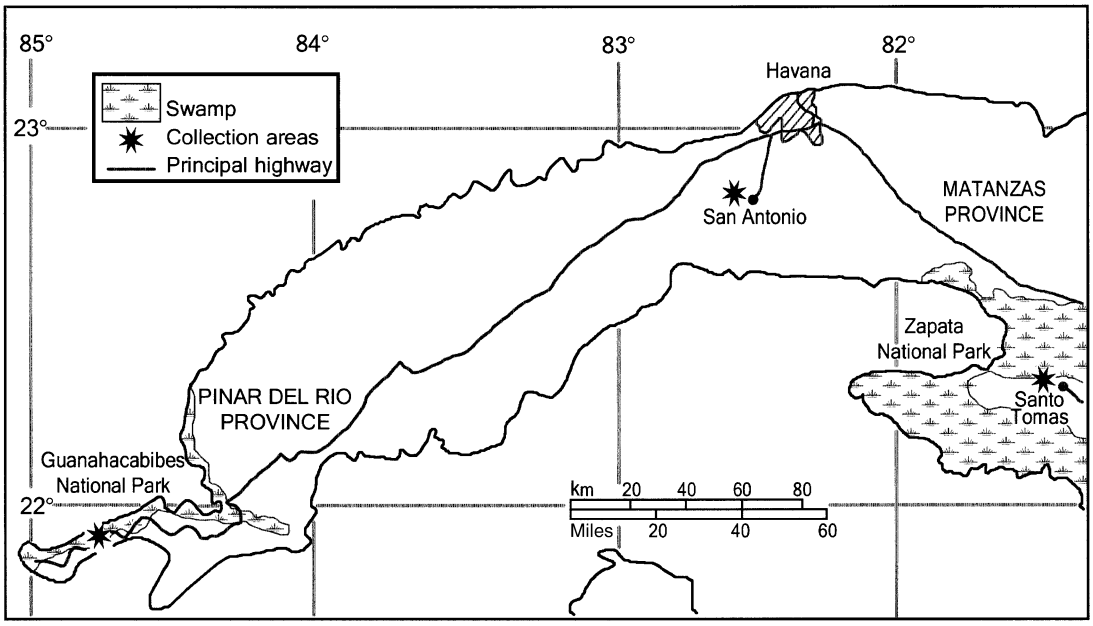

FIG. 1. Map of western Cuba showing the three monarch sample collecting localities: San Antonio, Zapata Swamp, and Guanahacabibes.

located ~33 km southwest of La Havana and 1 km from San Antonio and Guira (22°8′ N, 82°4′ W). The field had a large patch of *Asclepias curassavica*, the only *Asclepias* species where the monarchs were captured and larvae were observed.

The Zapata Swamp location is southeast of La Havana (Fig. 1) in Zapata National Park. Monarchs were collected in a grassy area around the small village of Santo Tomas (22°3′ N, 81°5′ W) where the vine *Sarcostemma clausum* (Jacq.) was present. No eggs or monarch larvae were observed.

The Guanahacabibes peninsula is the most western portion of Cuba and its northern region is the government-managed Guanahacabibes National Park and Biosphere Preserve (Fig. 1) which includes mangroves, swamp vegetation, and forest. Monarchs were collected along the northern portion of the peninsula and around the lighthouse in the western portion of this peninsula. Two individual plants of *A. curassavica* were seen in both years without eggs or larvae.

Isotopic and chemical analysis

The right fore- and hind wing of each butterfly were saved and processed for $\delta^{13}C$ and δD analyses using the procedure in Hobson et al. (1999*a*). The monarch wings (keratin) were cleaned of surface oils and lipids by rinsing three times with a solution of 2:1 chloroform:methanol, air-dried, and stored.

Stable hydrogen analyses of non-exchangeable hydrogen were determined on 350-μg wing subsamples. Keratin standards were used such that the values reported here are for non-exchangeable hydrogen (Wassenaar and Hobson 2003). This approach involves concurrent analyses of unknown wing samples with three different keratin standards whose non-exchangeable δD values are known and span the range of expected values. Stable hydrogen isotope measurements of wing samples and keratin standards were performed on H_2 gas derived from online high-temperature flash pyrolysis of wings (Wassenaar and Hobson 2003). A Eurovector 3000 high-temperature elemental analyzer (EA; Eurovector, Milan, Italy) with autosampler was used to automatically pyrolyze samples to a single pulse of H_2 gas (and N_2 and CO gas). The resolved H_2 sample pulse was introduced to the isotope ratio mass spectrometer (GV Instruments Isoprime, Manchester, UK, with electrostatic analyser) via an open split capillary. All δD results are expressed in the typical delta notation, in units of per mil (‰), and are normalized on the VSMOW-SLAP standard scale (Vienna Standard

TABLE 1. Sites, collection dates, and numbers of individual monarch butterflies collected (*N*) at locations in Cuba from 1993 through 1997.

Sites	Date	Year	*N*
Guanahacabibes Peninsula	10–25 Nov	1993	15
San Antonio	6–9 Mar	1995	10
San Antonio	6–11 Nov	1995	18
Zapata Swamp	14–15 Nov	1995	2
Guanahacabibes Peninsula	18–24 Nov	1995	3
San Antonio	15–23 Nov	1996	78
Guanahacabibes Peninsula	23–28 Nov	1996	1
San Antonio	11–26 Nov	1997	42
Total			169

TABLE 2. Summary of TLC fingerprint results of 169 monarchs collected in Cuba in Guanahacabibes, San Antonio, and Zapata Swamp, during 1993 and 1995–1997.

Monarch samples		*Asclepias* cardenolide patterns				
Site and date	*N*	Invisible or unknown	*A. curassavica* (unknown origin)	*A. curassavica* (resident)	*A. syriaca*	Lost†
Guanahacabibes						
Nov 1993	15	3	1	0	11	0
Nov 1995	3	0	2	0	1	0
Nov 1996	1	0	0	0	0	1
San Antonio						
Mar 1995	10	0	0	10	0	0
Nov 1995	18	0	18	0	0	0
Nov 1996	78	4	40	15	3	16
Nov 1997	42	3	19	18	2	0
Zapata Swamp						
Nov 1995	2	0	1	0	1	0
Total, all sites	169	10 (6%)	81 (48%)	43 (25%)	18 (11%)	17 (10%)

† Samples that were lost at any point in the chemical analyses.

Mean Ocean Water-Standard Light Antarctic Precipitation). Repeated analyses of hydrogen isotope intercomparison material IAEA-CH-7 (−100‰; International Atomic Energy Agency, Vienna, Austria), routinely included as a check, yielded an external repeatability of better than ± 1.0‰.

Stable carbon isotope ratios were determined on 350-μg monarch wing subsamples. For $\delta^{13}C$ analyses, routine continuous-flow isotope ratio mass spectrometry (CF-IRMS) was used. The CF-IRMS systems were interfaced using a Eurovector 3000 high-temperature elemental analyzer (National Water Research Institute). Stable C isotope results were expressed in standard delta (δ) notation relative to the international V-PDB (Vienna-Pee-Dee Belemnite) carbon isotope reference. We compared the stable isotope values of monarchs collected in Cuba with the extensive database for the North American continent (Wassenaar and Hobson 1998). We also collected *A. curassavica*, *A. nivea*, and *Sarcostemma clausum* to establish $\delta^{13}C$ and δD values for these plants in Cuba.

The remaining butterfly biomass (excluding the head) was dried at 60°C for 16 hours in a forced-draft oven and weighed on a Mettler AK 160 balance (Mettler-Toledo, Columbus, Ohio, USA). Dried specimens were ground in a centrifuge tube in 20 mL petroleum ether with a Janke and Kunkel SDT Ultra Turrax tissuemizer (IKA Works, Wilmington, North Carolina, USA), and the lipids were extracted following the methodology used by Alonso (1996). The defatted butterfly material was dried and weighed to determine lean mass. The remaining butterfly biomass was extracted in ethanol for determination of cardenolide concentration in μg/0.1 g of dry butterfly material using a Perkin-Elmer Lambda IIs dual-beam spectrophotometer (Perkin-Elmer, Wellesley, Massachusetts, USA). The cardenolide determination was done following the method of Brower et al. (1982) and Malcolm et al. (1989). Of the cardenolide–ethanol mixture that remained from the spectrophotometry analysis, a 7-mL sample was cleaned of contaminants and used for TLC. The clean extracts were dissolved in chloroform and spotted, along with digitoxin and digitoxigenin cardenolide standards, on a silica gel TLC plate. Each plate was developed and the cardenolides were visualized as blue spots by spraying with a saturated solution of TNDP (2,2′-4′,4′-tetranitrodiphenyl) in benzene. The developed TLC plate was immediately photographed with 35-mm Ektachrome film. Each slide was digitally scanned and transferred to a computer file.

Cardenolide fingerprint determination

The match of the TLC pattern of each butterfly and milkweed was done by visualizing spot mobilities (relative to digitoxin) from projected 35-mm color slides of TLC plates and the computer images. The TLC spot patterns were matched to a specific milkweed species through comparisons with published studies (Roeske et al. 1975, Brower et al. 1984, Seiber et al. 1986, Malcolm et al. 1989, 1992, 1993, Knight 1998, Moranz and Brower 1998). Potential difficulties of interpretation arise when using TLC in Cuban monarch populations because of the geographical overlap of *Asclepias* species in Cuba with the southern United States. In Cuba the following species are reported: *Asclepias nivea* (L.) and *Asclepias curassavica* (Brother Leon and Brother Alain 1957, Roig 1988). Other Asclepiadaceae species that we collected in Cuba were *Asclepias fruticosa*, *Calotropis procera*, and *Sarcostemma clausum* (this last one occurs in the United States as well). *Calotropis gigantea* has also been introduced to Cuba (W. D. Stevens, *personal communication*). *Asclepias curassavica* occurs in Cuba and the southern United States. Hence, if monarchs captured in Cuba had TLC patterns

of milkweed species present in both Cuba and the United States, they could not be classified as migrant or resident. On the other hand, any monarch bearing the *A. syriaca* fingerprint would have been a northern migrant because this milkweed does not naturally occur south of latitude 35° N (Woodson 1954), although anthropogenic factors are spreading it southward (Wyatt et al. 1993, Wyatt 1996). However, *A. syriaca* has never been reported in Cuba (C. Dockx, *personal observation* at the National Herbarium at Havana and in the field).

Because monarchs collected in Cuba could have potentially ambivalent TLC fingerprints, we classified them as resident or migrant also using their stable isotopic values. Isotopic data were available for butterflies collected in March 1995, November 1993, 1996, and 1997 (but not for November 1995). When stable isotope data were not available, or when monarchs did not show the *A. syriaca* TLC pattern, the butterflies could not be classified as migrant or resident and were not included in the analysis. TLC classified 18 of 152 monarchs as migrants (Table 2); isotopic analyses classified 89 of 138 monarchs as migrants (Table 3); and TLC and isotopic analyses combined classified 93 monarchs as migrants (Table 3).

TABLE 3. Summary of isotopic and TLC results of 148 monarchs collected in Cuba in November 1993, 1996, and 1997, plus 10 monarchs collected in March 1995 (see Fig. 3).

Site, collection date, and region	*N*†	Percentage of captures‡	TLC§
Guanahacabibes, November 1993			
2	9	60	*A. syriaca* (8) invisible (1)
3	3	20	*A. syriaca* (2) unknown (1)
Northeast	1	6.6	*A. syriaca* (1)
Cuba	1	6.6	invisible (1)
Undetermined	1	6.6	*A. curassavica* (1)
Guanahacabibes, November 1996			
2	1	100	lost (1)
San Antonio, November 1996			
2	31	39.7	*A. curassavica* (22) *A. syriaca* (3) lost (6)
3	1	1.3	*A. curassavica* (1)
4	23	29.5	*A. curassavica* (15) lost (6) invisible (2)
Cuba	20	25.6	*A. curassavica* (15) lost (3) invisible (2)
Undetermined	3	3.8	*A. curassavica* (2) unknown (1)
San Antonio, November 1997			
1	3	7.1	*A. curassavica* (1) *A. syriaca* (1) unknown (1)
2	12	28.6	*A. curassavica* (12)
3	3	7.1	*A. curassavica* (2) unknown (1)
4	3	7.1	*A. curassavica* (3)
Northeast	1	2.4	*A. syriaca* (1)
Cuba	18	42.8	*A. curassavica* (18)
Undetermined	2	4.8	*A. curassavica* (2)
San Antonio, March 1995			
Cuba	10	100	*A. curassavica* (2)
Zapata Swamp, November 1995			
Northeast	1	100	*A. syriaca* (1)
Guanahacabibes, November 1995			
Northeast	1	100	*A. syriaca* (1)

Note: Monarch butterflies coming from the Northeast region were classified by TLC only (see Table 2).
† Number of monarchs captured.
‡ Percentage of total captures per year.
§ In parentheses is the number of individual butterflies with that specific TLC fingerprint.

RESULTS

TLC cardenolide fingerprints

Of 169 butterflies collected in Cuba, 48% displayed *A. curassavica* patterns that could not be classified as migrant or resident, 25% showed the *A. curassavica* pattern and were also identified as Cuban residents by the isotopic technique, and 11% showed the *A. syriaca* pattern (Table 2, Fig. 2). The presence of the *A. syriaca* TLC fingerprint in butterflies collected in the four sampled years (1993 and 1995–1997) and in all three sampled areas (San Antonio, Zapata Swamp, and Guanahacabibes Peninsula) showed that monarchs do, in fact, arrive in Cuba from the northeastern United States and/or southeastern Canada. Moreover, all butterflies with the *A. syriaca* fingerprint match the expected δD and $\delta^{13}C$ values for monarchs coming from the northeastern region of the species' range in eastern North America (Table 3). By contrast, no *A. syriaca* pattern (or any TLC fingerprint from *Asclepias* species from the Northeast) was found in the 10 individuals collected in March 1995, possibly indicating the absence of migrants at this time (Table 2, Fig. 2).

Stable isotope analyses

Stable hydrogen and carbon isotope natal patterns of monarchs, here extended to include Cuba, are shown in Fig. 3. The δD isotope values are quite different between residents (−79 to −42‰) and migrant monarchs (−79 to −134‰; Fig. 4). The isotope results for monarchs collected in November 1993, 1996, and 1997 show that of 146 butterflies, 62.3% originated from the United States and southern Canada, 33.6% were Cuban residents, and 4.1% could not be classified because δD values were missing (Table 3). Like TLC, the isotopic data indicate the absence of migrant monarchs in the March 1995 sample. The stable isotope data for March 1995 agree with TLC fingerprints.

Migrant monarchs and their natal grounds

Monarchs that migrate to Cuba originated from regions 1, 2, 3, and 4, or throughout all of eastern North America (Table 3, Fig. 5). In contrast, 95% of the Mex-

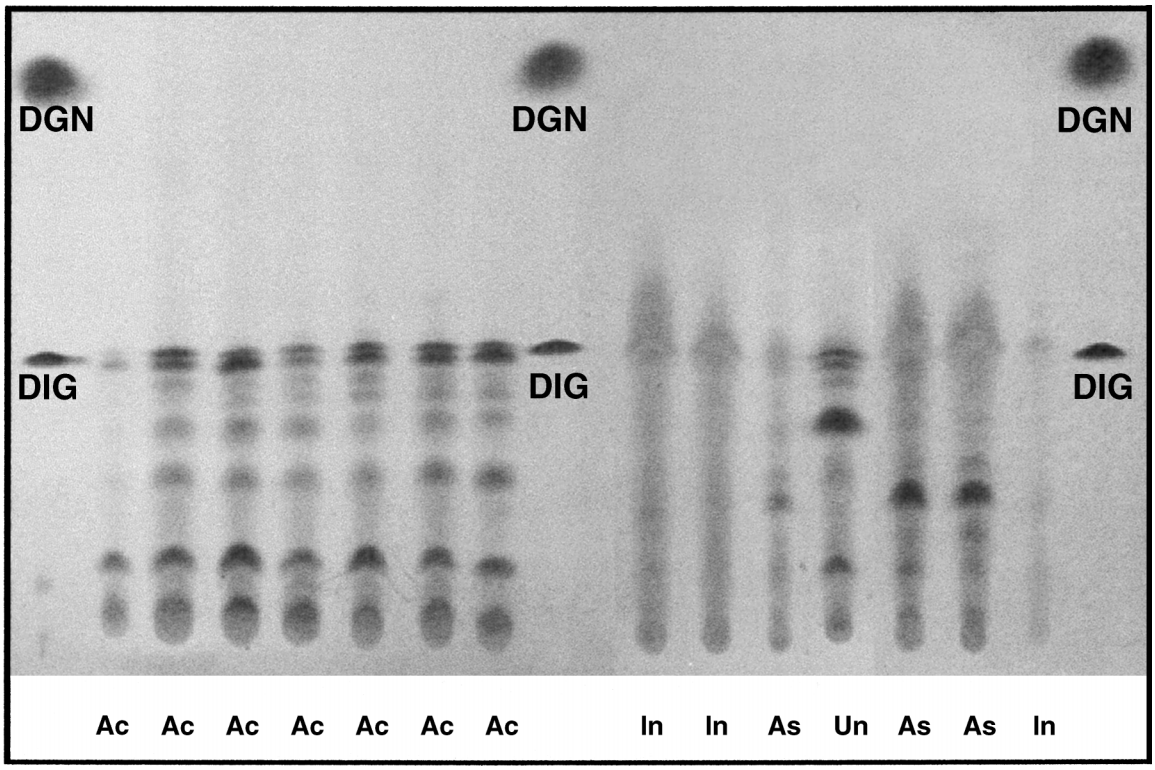

FIG. 2. Thin-layer chromatograms of 14 monarchs captured in Cuba along with digitoxin (DIG) and digitoxigenin (DGN) standards. The first seven sample columns are from monarchs collected in San Antonio, Cuba, in March 1995 (starting from the left), showing the *Asclepias curassavica* (Ac) cardenolide fingerprint. The next seven sample columns are monarchs collected in Guanahacabibes, Cuba, in November 1993. Three columns have invisible (In) patterns, three have *Asclepias syriaca* (As) cardenolide fingerprint, and one has an undetermined fingerprint (Un). This plate is composed of different channels assembled from TLC plates using Adobe Photoshop. Each channel represents a single butterfly.

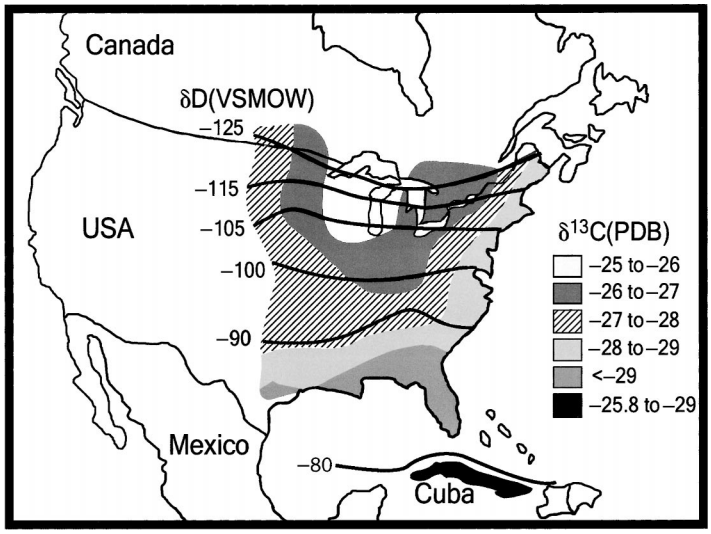

FIG. 3. Geographic patterns of δD and $\delta^{13}C$ in monarch wings from natal sites across the breeding range of eastern North America and from Cuban resident populations. The values for δD (Vienna Standard Mean Ocean Water) and $\delta^{13}C$ (Pee-Dee Belemnite) for the North American continent come from Wassenaar and Hobson (1998); for Cuba, the values come from six samples of *Asclepias* plants collected in Cuba: *A. curassavica* (3), *A. nivea* (2), and *Sarcostemma clausum* (1).

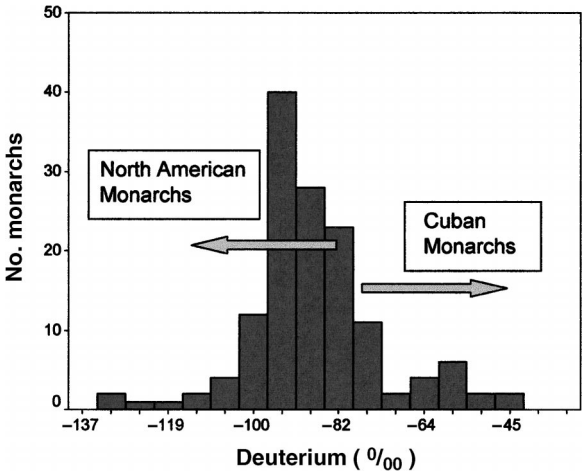

FIG. 4. Differences in isotope ratios of δD for migrant and resident Cuban monarchs. The sample size was $N = 140$ butterflies.

ican migrants from 13 wintering roost sites in central Mexico originate in regions 2 and 3 (Fig. 5; see Wassenaar and Hobson 1998). Migrants that overwinter in Mexico have natal origins that are similar to those of migrants collected in Guanahacabibes, Cuba. In contrast, monarchs collected in San Antonio came from regions 2 and 3 and also from the extremes of their breeding range: southern Canada (region 1) and southeastern United States (region 4) (Fig. 5). Only 5% of the monarchs that overwinter in Mexico came from the extremes of their breeding range (Wassenaar and Hobson 1998): regions 1 and 4. However, 38.1% (29 of 76) of the migrants in San Antonio originated from these two regions (Table 3, Fig. 5).

Monarchs with the *Asclepias curassavica* TLC fingerprint from November 1996 and 1997 samples produced contradictory results because monarchs having the *A. curassavica* fingerprint had isotopic values of geographical areas where this milkweed species is uncommon (e.g., southeastern Canada and the northeastern United States; Table 3). This may be explained by the recent use of *A. curassavica* in butterfly gardens outside of their natural range, such as southeastern Canada and the northeastern United States. Another possible explanation is that there could be another *Asclepias* species with a pattern very similar to that of *Asclepias curassavica*. In spite of this difference between results based on the TLC and the isotopic technique: (1) the two techniques agreed on the presence of migrant monarchs in November in the three Cuba locations and the four sampled years; (2) butterflies with *A. syriaca* TLC pattern had the expected isotopic values of the northeastern region, where this *Asclepias* is common (Table 3); and (3) they agreed as well in the absence of migrants in the March sample.

DISCUSSION

Movement of monarchs to Cuba and their influence on the Cuban populations

TLC cardenolide fingerprints (November 1993, 1995, 1996, and 1997) and stable isotope data (November 1993, 1996, and 1997) revealed that monarchs from natal sites in Canada and the United States traveled to Cuba during November in each of the four sampled years. TLC also revealed that this movement occurred on a regular basis and was not restricted to the peak of the Wisconsinan, as Brown and Heineman (1972) had suggested. Migrants from North America outnumbered Cuban residents in November in 1993, 1996, and 1997. Only one of 14 Guanahacabibes individuals (collected in November 1993) was a Cuban resident. Of 76 butterflies analyzed from November 1996 samples, 56 were migrants; of 42 butterflies collected in November 1997 in San Antonio, 22 individuals were migrants and 18 were resident monarchs (Table 3). Information about the total numbers of migrants vs. residents collected in November 1995 was not available because we did not perform isotopic analyses on these butterflies. Our findings contrast with the single historical record of a tagged migrant reaching Cuba (Urquhart 1987) and thus reveal the benefit of using these chemical fingerprinting vs. conventional mark–recapture techniques (Hobson 1999).

TLC and the isotope data show the presence of migrant monarchs in Cuba in November, but these two techniques show the absence of migrant monarchs in March. There are three possible reasons that may explain the absence of migrants in the March sample: (1) they had died and/or we failed to collect them (because we only collected 10 individuals at one time and in one location); (2) they returned to the United States,

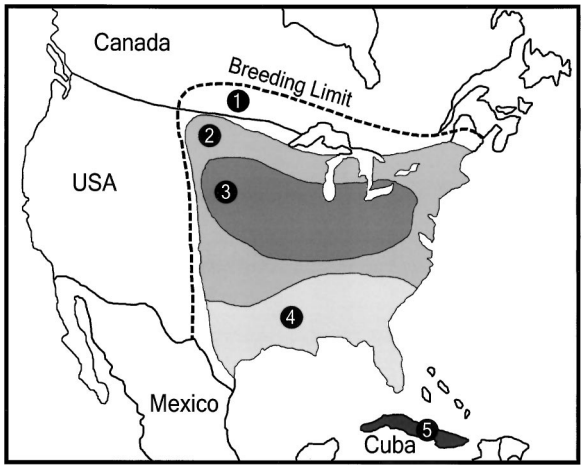

FIG. 5. Natal zones determined for monarchs collected in Cuba in November 1993, March 1995, November 1996, and November 1997 ($N = 148$ butterflies; Table 3). The dashed line represents the approximate breeding range of monarchs east of the Rockies.

most probably through the Florida peninsula; and (3) they moved to other areas of Cuba and the Caribbean. We do not have information about the first option. The research of Knight (1998) and Walker (1980) does not support the second option; their work suggests that monarch migration to Cuba (and possibly the Caribbean) is likely to be a one-way movement. Knight's (1998) two-year study in southern Florida found migrant monarchs in October and November, but not in March and April when they were expected to migrate back to the United States. Knight's findings are consistent with Walker's (1980) eight-year study of butterfly migration through the Florida peninsula. Walker found monarchs migrating southward during the fall, but none migrating back northward in the spring. However, an observation and the work of Urquhart support the third scenario. Jorje Luis Fontenla, a Cuban researcher, reported what appeared to be migrant monarchs in the most eastern portion of the island of Cuba at Punta Maisi in November. The extensive tagging program developed by Urquhart (1987) shows the arrival of North American monarchs in other areas of the insular and continental Caribbean: four in the Yucatan peninsula, two in Hispaniola, one in Jamaica, one in Puerto Rico, and two in the lesser Antilles.

Future research

The numbers of migrants generally outnumbered residents and many (~90%) of these individuals were reproductively active (Dockx 2002), suggesting gene flow into the Caribbean *D. p. megalippe* population from *D. p. plexippus* (Appendix). We suspect that this same movement from the North American continent and gene flow may also occur on other Caribbean islands and perhaps on the Yucatan peninsula. However, the impact of migrants probably becomes progressively diluted the farther the migrants are from their source in the United States and Canada. The extent to which Cuba may be a bridge for migrants to the Yucatan and to other insular Caribbean areas, and the genetic impact of this migratory influx into the Caribbean and Neotropical *D. p. megalippe* subspecies, now remains a question for further study.

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APPENDIX

A color photograph showing two monarch subspecies, *Danaus plexippus plexippus* in North America and *D. plexippus megalippe* in the Caribbean and northern South America (both collected in Cuba), is available in ESA's Electronic Data Archive: *Ecological Archives* A014-020-A1.