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Stable isotopes (δD and $\delta^{13}C$) are geographic indicators of natal origins of monarch butterflies in eastern North America

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Abstract Wing membranes of laboratory and field-reared monarch butterflies (*Danaus plexippus*) were analyzed for their stable-hydrogen (δD) and carbon ($\delta^{13}C$) isotope ratios to determine whether this technique could be used to identify their natal origins. We hypothesized that the hydrogen isotopic composition of monarch butterfly wing keratin would reflect the hydrogen isotope patterns of rainfall in areas of natal origin where wings were formed. Monarchs were reared in the laboratory on milkweed plants (*Asclepias* sp.) grown with water of known deuterium content, and, with the assistance of volunteers, on native milkweeds throughout eastern North America. The results show that the stable hydrogen isotopic composition of monarch butterflies is highly correlated with the isotopic composition of the milkweed host plants, which in turn corresponds closely with the long-term geographic patterns of deuterium in rainfall. Stable-carbon isotope values in milkweed host plants were similarly correlated with those values in monarch butterflies and showed a general pattern of enrichment along a southwest to northeast gradient bisecting the Great Lakes. These findings indicate that natal origins of migratory and wintering monarchs in Mexico can be inferred from the combined δD and $\delta^{13}C$ isotopic signatures in their wings. This relationship establishes that analysis of hydrogen and carbon isotopes can be used to answer questions concerning the biology of migratory monarch butterflies and provides a new approach to tracking similar migratory movements of other organisms.

Key words Monarch butterfly · Migration · Natal origins · Isotopic gradients

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Introduction

Each year, an estimated 80–120 million monarch butterflies (*Danaus plexippus*) from eastern North America migrate thousands of kilometers to overwinter in discrete colonies located in the Oyamel forests of the Transvolcanic Mountains of central Mexico (Urquhart 1976; Malcolm 1987). In recent years there has been concern that this natural phenomenon is seriously threatened. In Canada and the United States, the milkweed plant (*Asclepias* sp.), essential to monarch larval development, is considered a noxious weed and is often eliminated with pesticides. In Mexico, deforestation and thinning of the Oyamel forest in the vicinity of wintering colonies have led to concerns that these wintering roosts may eventually fail due to loss of habitat or irreversible changes in microclimate (see numerous papers in Malcolm and Zalucki 1993).

Despite more than four decades of research, numerous questions regarding monarch butterfly migration remain unanswered. Specifically, there is virtually no information on links between monarch natal origins and each of the discrete wintering colonies. It is not clear whether butterflies found at each of the wintering colonies originate from specific breeding regions in North America, or whether these sites contain a mixture of monarchs from Canada and the United States. It is also unknown whether monarchs from northern extremes of the breeding range are less well represented at winter roost sites due to the rigors of long-distance migration

compared with monarchs from southern United States breeding areas. Such basic knowledge is lacking and would be a powerful tool in the protection of the monarch butterfly, as conservation efforts could be better focused within the North American breeding range and at the Mexican wintering colonies.

Previous investigations of monarch butterfly migration and natal origin have relied largely on the use of mark and recapture methodology. For example, a tagging program was conducted from 1937 to 1994 [Urquhart 1960; Monarch Watch 1998, Season summaries (1993–1998), http://www.MonarchWatch.org] and is currently administered by Monarch Watch, based at the University of Kansas. Wintering monarch tag recovery has hitherto been critical to making links between recovery sites and natal origins (Urquhart 1976). Tagging has shown that butterflies from across of the breeding range migrate to Mexico and vectors of recoveries are being used to interpret migration patterns (Rogg et al. in press). Nevertheless, because most tagged migrants are "intercepted" en route and because numbers tagged are not coincident with monarch production per region, tagging does not yield quantitative information on the proportional origins of wintering monarchs. Clearly, if the butterflies retain an elemental signature in their cuticle in the form of stable isotopic ratios characteristic of their natal origin, it will be possible to not only establish the origins of monarchs but to determine the proportion of monarchs reaching Mexico from different breeding areas. In addition to collecting individuals, such analysis could be conducted on butterflies found dead at winter roost sites due to their significant natural over-winter mortality (Malcolm and Zalucki 1993) or possibly involve the sampling of wing membrane from live insects.

Stable isotopes of carbon and nitrogen have been used previously to define geographically distinct populations of animals (van der Merwe et al. 1990; Vogel et al. 1990; Alisauskas and Hobson 1993). The basis of this approach is that stable isotope ratios in foodwebs can differ regionally and can thus provide naturally occurring signatures in organisms that can be related to origin (see review by Hobson 1999). Recently, Hobson and Wassenaar (1997) demonstrated that stable hydrogen isotope measurements (δD) in bird feathers can be used to evaluate broad North American origins of several species of Neotropical migratory songbirds at Central American wintering sites. Hydrogen isotope ratios in rainfall are mirrored in plants (Yapp and Epstein 1982) and are subsequently reflected in higher trophic-level consumers (Cormie et al. 1994a, 1994b). Moreover, deuterium in rainfall in North America shows a distinct continental pattern with a general depletion with latitude along a southeast to northwest gradient (Hobson and Wassenaar 1997) This pattern is the result of the differential behavior of heavy and light water molecules in response to a variety of factors including temperature gradients, altitude, season, and distance inland from the coast to point of precipitation (reviewed by Ziegler 1988). The stable-hydrogen isotope composition of migratory songbird feathers thus provided a "geographic fingerprint" that was ultimately derived from local hydrology at breeding sites where feathers were grown (Hobson and Wassenaar 1997; Chamberlain et al. 1997). The stable-hydrogen isotope technique has distinct advantages over physical tagging methods, including a broader application to small migratory species that are typically not easily tagged or recovered (e.g., insects, bats, songbirds). Further, the method provides a means of tracing numerous migratory species that move across large isotopic gradients (Hobson 1999).

We were interested in determining if the stable-hydrogen isotope technique could be used to link breeding and wintering grounds of monarch butterflies. We hypothesized that the composition of keratin in monarch butterfly wings reflects the hydrogen isotope patterns of rainfall in areas of eastern North America where wings are formed. Like feathers, butterfly wings are composed primarily of keratin, a material that is metabolically inert following synthesis and adult emergence at the natal site. After correcting for any possible exchange of hydrogen with ambient water vapor, wings should thus provide a useful isotopic signature for tracing butterfly origins (see Miller 1984; Schimmelmann et al. 1993). Similarly, we hypothesized that carbon isotope ratios might show geographic patterns in host milkweed plants due to responses to environmental gradients (e.g., Stuiver and Braziunas 1987) and could be used in tandem with hydrogen isotopes to better delineate geographic origins of butterflies (e.g., Chamberlain et al. 1997). As controls for this study, host plants and monarchs were raised in the laboratory using water of known deuterium content. To determine if monarchs retained isotopic signals characteristic of their natal origins, isotopic ratios were established for monarchs reared on milkweed subjected to local rainfall at locations across eastern North America. The goal of this project was to establish an isotopic map that can be used to trace the origins of wintering monarchs in Mexico.

Materials and methods

Our project had three components designed to test whether stable-hydrogen and carbon isotope measurements can be used to determine natal origins of migratory monarchs. First, laboratory experiments were performed to determine hydrogen and carbon isotopic fractionation among water, host plants, and monarch wing tissue. These experiments were used to confirm whether hydrogen isotopic patterns in simulated laboratory "rainfall", and stable-carbon isotope ratios in larval host plants, were correlated with the isotopic composition of monarch butterfly wings raised on these plants. The second component involved an extensive field-rearing program to establish whether geographic hydrogen and carbon isotope patterns could be found in monarchs across their eastern North American breeding range, and whether the resolution of isotopic patterns was sufficient to be able to infer monarch natal origins. The third component, published elsewhere, involved the application of δD and $\delta^{13}C$ measurements of wings of butterflies at

each of 13 overwintering roost sites in Mexico (Wassenaar and Hobson 1998). Here we report the results of the first two components of this project.

Laboratory rearing experiment

During April of 1996, milkweed plants (*Asclepias curassavica*) were grown from seed using common potting soil and tap water at the University of Kansas. Two groups of plants were watered with tapwater with added 99.9% D_2O (treatment 1:10 µl D_2O l^{-1} tap water, treatment 2:30 µl D_2O l^{-1} tap water). The control group was watered with tap water only. This gave three isotopically distinct water types used to raise the three groups of plants (Table 1), simulating a large hydrogen isotopic gradient of 286‰ in source water. At least six monarch butterflies were raised from egg to adult on each set of host plants. Newly emerged adult butterflies were frozen (Canadian Council on Animal Care 1984). Throughout the experiment, samples ($n = 4$) were saved from each carboy of water prepared for the plants. Samples of the three groups of *A. curasavica* and adult monarchs from each treatment were stored frozen and their isotopic composition later analyzed.

Wild-rearing experiment

During the summer of 1996, volunteers were solicited from throughout eastern North America to raise monarch butterflies from eggs on naturally occurring milkweed whose only source of moisture was local rainwater. Based on location and experience 99 volunteers were selected from the eastern United States and Canada. Volunteers were instructed not to use milkweed from gardens, irrigated fields, drainage ditches, inner city lots, and other locations in which the water may have had inputs other than that of rainwater. Each volunteer was sent a monarch rearing kit containing fertilized eggs, instructions, and data sheets. Following rearing, volunteers returned at least six adult male and female butterflies as well as an air-dried sample of local milkweed plant. Most volunteers successfully reared 4–12 butterflies, and 86 samples of milkweed (*Asclepias* sp.) and over 600 wild-reared monarchs from throughout the eastern monarch breeding range were returned. All samples were stored at −40°C until further processing. For practical purposes, a geographically representative sub-sample of 33 sites was selected for stable isotopic analyses.

Stable isotope analyses

Wings were separated from the thorax and placed in glass scintillation vials, cleaned of surface oils by rinsing three times with a solution of 2:1 chloroform:methanol (Dobush et al. 1985), air-dried, and stored until analysis. Samples of milkweed used in the laboratory-rearing experiment were oven-dried at 80°C, ground, and stored in glass vials.

All samples were prepared for δD analysis by eliminating uncontrolled hydrogen isotope exchange with laboratory air moisture (see Chamberlain et al. 1997) using the steam equilibration method of Schimmelmann (1991) as modified by L.I. Wassenaar and K.A. Hobson (unpublished work). Briefly, 7.5 mg of monarch wing material (~4% by weight total H) were weighed into 9-mm Vycor break-seal tubes along with 1 g of cupric oxide and 2 cm of silver wire. Each sample was evacuated for 1 h at 130°C to drive off adsorbed water. Samples were then equilibrated at 130 ± 0.2°C for 2 h individually with 300 µl of injected water (steam) of known isotopic composition (−132‰ Vienna Standard Mean Ocean Water standard, VSMOW). Samples were then re-evacuated for 1 h to remove all water vapor (or longer until $< 1^{-4}$ torr vacuum was attained). The equilibration water-hydrogen to membrane exchangeable hydrogen ratio was greater than 99:1, ensuring that available exchangeable hydrogen (~18% of total H at 130°C) was replaced with hydrogen of fixed isotopic composition. This procedure eliminated effects of uncontrolled hydrogen isotope exchange with ambient moisture through a calibration process and effectively allowed interpretation of the results as a proxy for non-exchangeable hydrogen (Schimmelmann 1991; L.I. Wassenaar and K.A. Hobson, unpublished work). The Vycor breakseal tubes were then sealed under vacuum and combusted at 850°C for 2 h using the classical Dumas combustion. Water of combustion was cryogenically transferred to 6 mm Pyrex break seal vessels containing 120 mg of zinc alloy (Biogeochemical Laboratories, Indiana University). The 6-mm vessels were then sealed and placed in a heating block and reacted at 510°C for 30 min to reduce H_2O to H_2. The CO_2 of combustion was cryogenically separated from N_2 and transferred to 6 mm Pyrex vessels for subsequent ^{13}C analysis. Water samples were prepared for δD measurement using standard techniques. Stable hydrogen and carbon isotope measurements for the wild-reared samples were performed using dual inlet isotope-ratio mass spectrometry on a Micromass Optima at the National Hydrology Research Centre, Saskatoon. Results for δD are expressed in per mil notation relative to the VSMOW standard, and normalised to the VSMOW/SLAP scale. Results for $\delta^{13}C$ are expressed relative to the PDB standard. Replicates of samples and intercomparison material (IAEA-CH-7) yielded an external reproducibility of better than ±2‰ for δD and ±0.1‰ for $\delta^{13}C$ measurements.

Relationships between stable isotope values involving captive and field-reared monarch wings or milkweed were analysed using Spearman correlation analysis (Zar 1984). Isotopic contours for δD patterns in field-reared monarch butterfly wings were interpolated by kriging δD data at arbitrary 10 per mil contours using Surfer (Golden Software) and imported into a basemap using Mapviewer (Golden Software).

Results

Laboratory rearing experiments

δD values of monarch butterfly wings were strongly correlated with the δD of water used to raise host plants (Fig. 1a) and with the host plants themselves (Fig. 1b). For both carbon and hydrogen, the isotopic compositions of milkweed host plants were also similar to those measured for the butterfly wing material grown on those plants, regardless of the isotopic composition of the treatment water (Table 1). However, for hydrogen, a large isotopic fractionation occurred between water and milkweed and depended on the δD value of the water used (range −43 to −174‰, Table 1).

Field rearing experiments

Average δD values for monarch wings followed an expected gradient of more enriched values in the south to more depleted values in the north and ranged from −82‰ in southern Texas to −137‰ in Manitoba (Fig. 2). Following the methodology of Hobson and Wassenaar (1997), we compared wing δD values from each location with the growing season average δD values in precipitation expected for that location and found a positive correlation ($r = 0.83$, Fig. 3). In a similar manner to our approach for hydrogen, we investigated $\delta^{13}C$ patterns in monarch butterflies across eastern North America. Carbon isotope measurements ranged from about −25.9 to −30.5‰ (Table 2) and described a

Table 1 Carbon and hydrogen isotope results (mean ± SD, ‰) for laboratory rearing experiments using three water treatments including average fractionations between water, milkweed, and monarch butterfly wing keratin. Sample sizes given in *parentheses*

Control	Treatment 1	Treatment 2	
Water			
δD	−33 ± 9 (4)	+70 ± 8 (4)	+253 ± 8 (4)
Milkweed			
δD	−76 ± 2 (2)	−10.3 ± 4 (4)	+79 ± 2 (4)
$\delta^{13}C$	−30.6 ± 0.1 (4)	−30.7 ± 0.1 (4)	−30.9 ± 0.2 (4)
Monarch			
δD	−73 ± 11 (13)	−11 ± 4 (7)	+81 ± 6 (7)
$\delta^{13}C$	−31.2 ± 0.5 (12)	−30.8 ± 0.4 (9)	−31.4 ± 0.3 (6)
Tissue-diet fractionation			
δD	+3	−1	+2
$\delta^{13}C$	−0.6	−0.1	−0.5

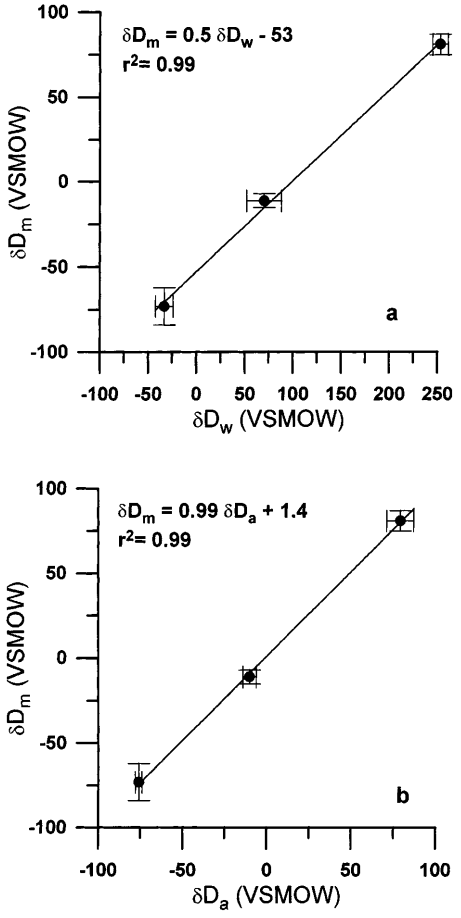

Fig. 1 Relationship between δD values of wings of laboratory-reared monarchs (δD_m) and those of **a** growth water (δD_w) used to raise the larval host plant (*A. curassavica*), and **b** the larval host plant (δD_a). Data from Table 1

gradient of more depleted values in the southwest to more enriched values in the northeast along a gradient bisecting the Great Lakes (Fig. 4). We found a poorer correlation between $\delta^{13}C$ values in monarchs and local milkweed ($r = 0.65$). This was not unexpected as the milkweed measurements from each site were conducted on single leaves that did not necessarily correspond to the host plants of the monarch individuals returned.

Discussion

Laboratory rearing experiments

We found a strong relationship between the δD value of water and the δD value of milkweed host plants, a finding consistent with numerous plant hydrogen isotopic studies (reviewed by White 1988; Ziegler 1988). The δD value of butterfly wings from monarchs reared in the laboratory were strongly correlated with the δD values of the milkweed host plants. These findings provided a strong empirical basis for further investigations of the use of isotopic techniques to examine origins of wild monarch butterflies.

For the laboratory-raised monarchs, the non-unity slope of 0.5 for the regression between water δD values and monarch butterfly wing δD was in contrast to an almost 1:1 relationship between monarch wings and milkweed. This suggests that under similar environmental conditions of temperature and humidity (although not strictly controlled in this experiment), the large deuterium gradient in growth water was not proportionally passed on to milkweed plant tissue. However, little isotopic change then took place between plant and butterfly. In nature, the δD of local source water and ambient water vapor are both likely to reflect the δD of local precipitation. In contrast, the leaf water of all three groups of plants grown in the laboratory exchanged with a single batch of ambient water vapor. The reduced slope of the relationship of δD of butterfly wings and δD of source water was likely due to exchange between leaf water and ambient water vapor in the laboratory. Alternatively, differential hydrogen isotopic discrimination between water and plant could simply be a characteristic of milkweed plants (cf. White 1988). Nevertheless, the strong linear relationship observed between water and both milkweed and butterflies indicates that the milkweed water source (e.g., soil water, precipitation) controls the hydrogen isotopic composition of both the milkweed and ultimately the monarch butterfly larvae that feeds upon it.

Hydrogen isotopic fractionation between adult butterflies and their larval host plants is negligible, averaging around +1.5‰ (Table 1), and is within the error of the hydrogen isotope measurement. Similar findings were obtained by Miller (1984) who observed little δD fractionation between dietary hydrogen and chitin for insects raised in captivity on known isotopic diets. These experiments demonstrate that the hydrogen isotopic composition of monarch wing keratin, which is metabolically inert following adult emergence from the larval pupae, should reflect the local isotope hydrology of the natal site. As a result, the observed continental hydrogen isotopic gradient in precipitation (Hobson and Was-

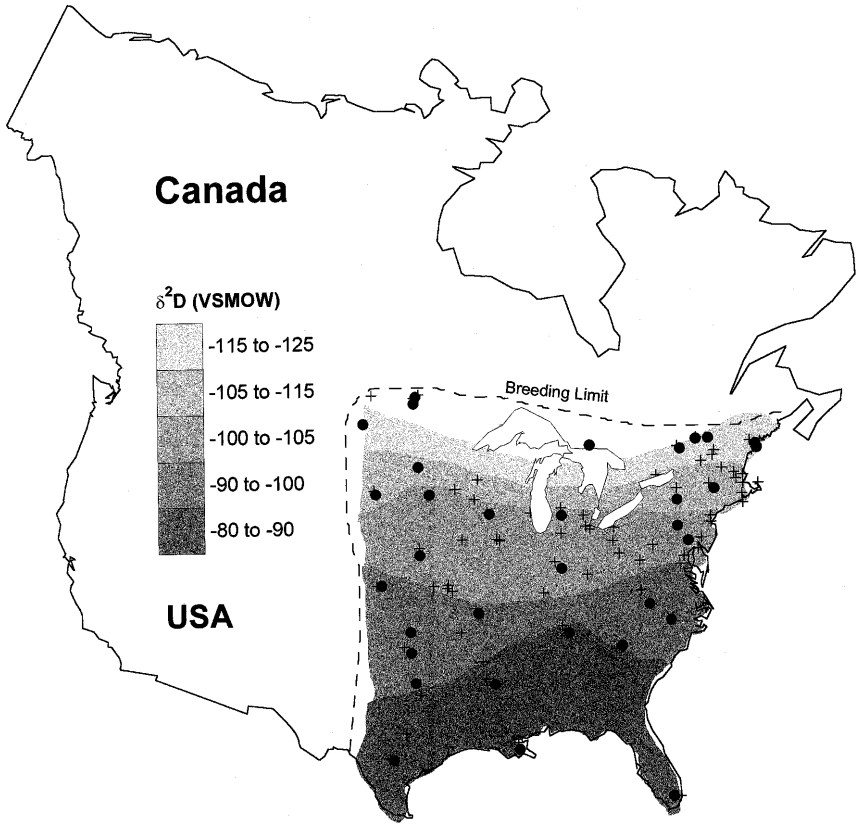

Fig. 2 Geographic patterns of δD values (‰) of wings from field-raised monarch butterflies in eastern North America. All *symbols* indicate wild rearing sites, *circles* indicate sub-sites selected for isotopic analyses (see Table 2). *Dashed line* indicates the approximate breeding limit of eastern North American monarch butterfly

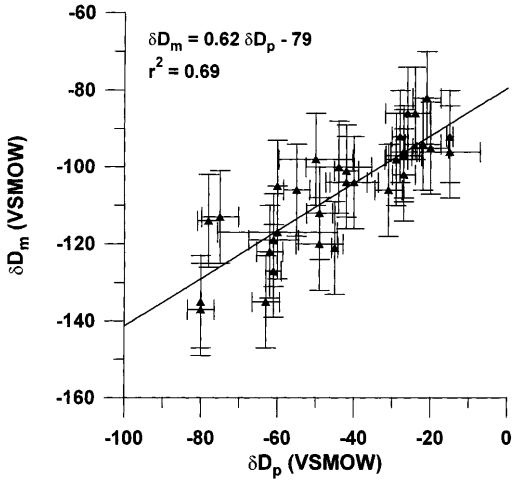

Fig. 3 Relationship between δD measurements of wings of field-raised monarchs and δD of weighted mean growing season precipitation at the natal site (δD_p, see Table 2). δD of precipitation was interpolated from an update of the database compiled by Hobson and Wassenaar (1997)

senaar 1997) should be reflected in monarchs whose natal origins lie across that gradient.

Stable-carbon isotope fractionation between milkweed and butterfly wings was of the order of −0.5‰ and is similar to values of −0.1 to −1.3‰ observed previously by Ostrom et al. (1997) for ladybird beetles (*Hippodamia variegata*) and aphids raised on controlled diets. Thus, potential geographic carbon isotopic gradients in milkweed host plant resulting from changes in climatic conditions might also be used along with δD to infer natal origins.

Field-rearing experiments

We found a positive relationship between δD values of field-reared monarch wings and our interpolated δD values for average growing season precipitation at those sites. Similarly, Schimmelmann et al. (1993) showed a strong positive relationship between δD values of beetle chitin and δD values of local meteoric rainfall (see also Schimmelmann and DeNiro 1986). Cormie et al. (1994a) also found a strong relationship between δD in deer collagen and δD of rainfall, a result also found in feathers of forest songbirds from across North America (Hobson and Wassenaar 1997; Chamberlain et al. 1997). Thus, the strong relationship observed in our laboratory- and field-rearing studies was consistent with previous evidence that continental patterns in rainfall δD values can be used to infer geographic origins of organisms. However, the correlation ($r = 0.83$) between δD values in field-reared monarch wings and inferred average precipitation δD values (δD_p) for monarchs, was not as strong as that observed in our controlled captive experiment or for songbirds ($r = 0.91$) sampled across North America (Hobson and Wassenaar 1997). This may be related to the fact that the monarch butterfly breeding range lies across a lesser isotopic gradient

Table 2 Location, coordinates, and mean δD (±SD‰) and $\delta^{13}C$ (±SD‰) of wild reared monarch butterflies from eastern North America. δD_p indicates hydrogen isotopic composition for weighted average precipitation during the growing season interpolated from the database compiled by Hobson and Wassenaar (1997)

Site	Latitude	Longitude	δD	*n*	$\delta^{13}C$	*n*	δD_p
San Antonio, Tex.	29.14	98.37	−82 (3.7)	3	−28.4 (0.1)	3	−21
Monticello, Ariz.	33.37	91.48	−86 (4.0)	6	−29.7 (0.1)	3	−24
Madison Tenn.	35.93	86.01	−86 (5.8)	6	−28.8 (0.2)	6	−26
Miami, Fla.	25.88	80.6	−92 (0.8)	3	−30.0 (0.1)	3	−15
Red Rock, Okla.	36.46	97.35	−92 (2.1)	2	−27.2 (0.4)	3	−28
Greenville, S.C.	34.83	82.4	−94 (4.7)	3	−28.3 (0.3)	3	−22
Cedar Hill, Tex.	33.56	96.95	−95 (4.7)	9	−27.6 (0.3)	5	−20
Metairie, La.	29.58	90.09	−96 (7.9)	3	−30.5 (0.1)	3	−15
Oklahoma City	35.27	97.29	−96 (3.4)	5	−28.3 (0.3)	5	−27
Raleigh, N.C.	35.8	78.7	−97 (2.1)	6	−27.8 (0.3)	6	−27
Lincoln, Neb.	40.78	96.71	−97 (4.7)	3	−28.1 (0.1)	3	−27
Ferrum, VA	36.9	80	−98 (3.0)	2	−28.3 (0.3)	3	−29
Omaha, Neb.	44.23	95.93	−98 (9.7)	3	−26.4 (0.2)	3	−50
Grand Rapids, Mich.	42.58	85.6	−100 (8.6)	6	−26.1 (0.5)	7	−44
Lewisburg, Pa.	40.95	76.88	−101 (3.2)	3	−29.3 (0.8)	3	−42
Lebanon, Mo.	37.4	92.4	−102 (3.0)	4	−26.6 (0.3)	6	−27
Wakeeney, Kan.	39.01	99.51	−104 (6.3)	6	−28.9 (0.1)	5	−40
Monona, Iowa	43.01	91.24	−104 (5.4)	3	−25.9 (1.1)	3	−42
Fargo, N.D.	45.83	96.85	−105	1	−27.1	1	−60
Carmel, Ind.	39.58	86.06	−106 (3.6)	7	−26.5 (0.1)	3	−31
Jefferson, Me.	44.2	69.4	−106 (3.4)	3	−26.9 (0.1)	3	−55
Round Lake, N.Y.	42.55	73.48	−112 (3.4)	3	−30 (0.1)	3	−49
Fort Pierre, S.D.	44.21	100.2	−113 (4.8)	8	−27.3 (0.4)	7	−75
Des Lacs, N.D.	48.21	101.6	−114 (2.9)	3	−28.4 (0.2)	3	−78
Madison, Me.	44.44	69.43	−117 (15.7)	7	−25.9 (0.1)	7	−60
Ottawa, Ont.	45.23	75.42	−119 (6.5)	7	−28.9 (0.3)	7	−61
Ithaca, N.Y.	42.4	76.5	−120 (6.2)	6	−26.7 (0.2)	7	−49
Lancaster, Pa.	40	76.3	−121 (0.8)	6	−26.3 (0.8)	6	−45
St. Aug.-des-Maures, P.Q.	45.58	74	−122 (3.4)	3	−27.1 (0.4)	3	−62
Elliot Lake, Ont.	46.37	82.65	−127 (2.0)	3	−26.2 (0.4)	3	−61
Chambly, P.Q.	45.45	73	−135 (3.6)	5	−26.3 (0.3)	5	−63
Winnipeg, Man.	49.86	97.13	−135 (0.1)	3	−27.1 (0.2)	3	−80
Springstein, Man.	49.46	97.29	−137 (3.5)	3	−27.3 (0.1)	6	−80

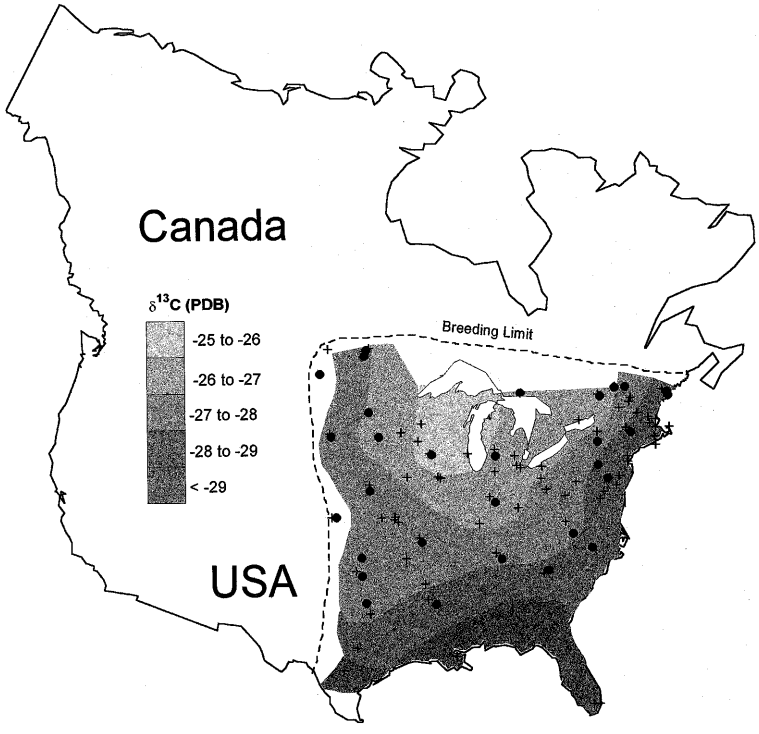

Fig. 4 Geographic patterns of $\delta^{13}C$ values (‰) of wings of field-raised monarch butterflies in eastern North America. *Symbols* as in Fig. 2

compared to both the continental scale of the songbird study and the large δD gradient in water used in our laboratory-rearing experiment. Another factor may be that, for many sites, δD_p was sometimes interpolated in areas where few precipitation isotopic data actually exist (Hobson and Wassenaar 1997). Thus local isotope hydrology and climatic effects were unknown factors. This underscores the need for an improved database and understanding of the distribution of stable-hydrogen isotope ratios in rainfall and how these relate to local food webs that concern migratory species.

Cormie et al. (1994a) found that relative humidity (RH) accounted for significant variation in the relationship between deer bone collagen δD values and average growing season precipitation δD throughout much of North America. We examined the effect of RH in our dataset by similarly including average growing season RH in a trivariate regression model (see Cormie et al. 1994a). However, we found that including RH in our model did not increase the slope of the precipitation δD and monarch δD toward 1.0. In contrast to the study of Cormie et al. (1994a), growing season RH did not differ substantially across our study area.

Stable-carbon isotope signatures in milkweed and butterfly wings across eastern North America showed a distinct pattern of enrichment along a southwest to northeast gradient bisecting the Great Lakes. Unlike patterns in deuterium, these isotopic patterns for carbon are more difficult to explain because there are few studies of carbon isotope variations in a single species of plants over broad regional scales. Several studies have reported that $\delta^{13}C$ values of C_3 plants decrease with increasing latitude, altitude and relative humidity (e.g., Körner et al. 1991; Bird et al. 1994; Stuiver and Braziunas 1987), the result of combined temperature and pressure effects. Chamberlain et al. (1997) also found that $\delta^{13}C$ values of songbird feathers along the eastern USA seaboard decreased with increasing latitude, although the effects of potential shifts in the relative proportion of C_3- and C_4-based dietary inputs could not be resolved. Clearly, the effect we observed of increasing $\delta^{13}C$ values in milkweed with latitude requires further investigation that might include measurement of relative humidity, temperature and soil nitrogen content (Körner et al. 1991). Nevertheless, when combined with hydrogen isotopic data, the carbon isotopic patterns observed in our data allow further resolution in the determination of monarch butterfly natal origins. Whereas deuterium provided a good indicator of latitude, $\delta^{13}C$ values provided further resolution according to regions of occurrence along these bands of latitude. Further resolution will likely occur when other isotopes such as ^{87}Sr, ^{34}S, ^{15}N are also considered (see Hobson 1999).

Summary

We have shown that the stable hydrogen and carbon isotopic compositions of adult monarch butterflies closely resemble those of their natal (i.e., larval) diets. The isotopic composition of the larval host plant (*Asclepias* sp.) is in turn controlled by the local isotope hydrology (δD), and climatic factors (δD and $\delta^{13}C$), which show definitive patterns across the monarch butterfly breeding range. The broad regional scale isotopic patterns reflected in the monarch and its food web in eastern North America can therefore be used as geographic indicators of natal origins of this species. This relationship will be critical in gaining a better understanding of the origins of wintering monarchs in Mexico.

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