Stable isotopes (δD and δ13C) 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 (δ13C) 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 δ13C 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

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 roosts 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 Brazuni 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 isotopes in larval host plants, were correlated with the isotopic composition of monarch butterfly wings raised on those 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 δ13C measurements of wings of butterflies at