Monarch butterflies cross the Appalachians from the west to recolonize the east coast of North America

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Each spring, millions of monarch butterflies (*Danaus plexippus*) migrate from overwintering sites in Mexico to recolonize eastern North America. However, few monarchs are found along the east coast of the USA until mid-summer. Brower (Brower, L. P. 1996 *J. Exp. Biol.* 199, 93–103.) proposed that east coast recolonization is accomplished by individuals migrating from the west over the Appalachians, but to date no evidence exists to support this hypothesis. We used hydrogen (δD) and carbon (δ^{13}C) stable isotope measurements to estimate natal origins of 90 monarchs sampled from 17 sites along the eastern United States coast. We found the majority of monarchs (88%) originated in the mid-west and Great Lakes regions, providing, to our knowledge, the first direct evidence that second generation monarchs born in June complete a (*trans*-)longitudinal migration across the Appalachian mountains. The remaining individuals (12%) originated from parents that migrated directly from the Gulf coast during early spring. Our results provide evidence of a west to east longitudinal migration and provide additional rationale for conserving east coast populations by identifying breeding sources.

1. INTRODUCTION

Each year, millions of insects complete migrations in North America, with some species travelling thousands of kilometres between breeding and wintering sites [1]. However, the small size, short lifespan, and complex life histories of migratory insects make it difficult to link populations across their annual cycle [2], predict changes in population size, and develop effective conservation plans [3]. The monarch butterfly (*Danaus plexippus*) travels thousands of kilometres from breeding grounds in eastern North America to wintering sites in central Mexico [4]. During early spring, overwintering monarchs and their first generation offspring recolonize eastern North America, becoming

abundant in the mid-west and Great Lakes regions in June [5,6]. Curiously, monarchs do not become abundant in areas east of the Appalachian mountains until July [5], leading Brower [7] to hypothesize that the recolonization of the east coast is accomplished by second generation individuals born in the central and northern mid-western states in June that migrate eastwards across the Appalachians ([7]; hereafter termed the 'range expansion' strategy or RE). Alternatively, recolonization of the east coast could be accomplished by the first spring generation produced in the Gulf coast region. Under this strategy, individuals migrate northwards from the Gulf coast to areas east of the Appalachians in late May and early June, lay eggs and produce a cohort of second generation monarchs along the east coast that emerge in early July (hereafter termed the 'coastal migration' strategy or CM).

We examined both hypotheses by collecting monarchs at 17 sites in the eastern United States (figure 1) and used stable-hydrogen (δD) and -carbon (δ^{13}C) isotopes to estimate natal origins [8]. Following the RE strategy, we predicted that monarchs would have isotopic values consistent with the central-west and northwest regions of the breeding range, while those following the CM strategy would have isotopic values indicative of either the Gulf coast region (migratory adults) or the central-east and northeast regions (offspring of Gulf coast adults).

2. MATERIAL AND METHODS

(a) *Monarch and milkweed sampling*

Monarch adults were collected using standard sweep nets from 26 June to 10 July 2009 along the east coast of the USA from Virginia in the south to Maine in the north (figure 1; see the electronic supplementary material for details on sampling sites). To estimate geospatial patterns of δ^{13}C, we collected milkweed leaf tissue (monarch larval food plant; one mature leaf from five different plants per site) from 36 sites across the eastern breeding range between May and July 2009 (see the electronic supplementary material, figure A1 for details on sampling sites).

(b) *Stable isotope analysis*

Stable-hydrogen (δD) and -carbon (δ^{13}C) isotope analyses of wing chitin were conducted at the Stable Isotope Laboratory of Environment Canada, Saskatoon, Saskatchewan, Canada. Captured monarchs were killed and stored in paper envelopes. Wings were removed and placed in glass vials, solvent cleaned with a 2 : 1 chloroform : methanol solution to remove lipid residues, and air dried. For δD isotope analysis, a small section of hind wing was clipped from each monarch ($350 \pm 10 \mu g$) and loaded into 4.0×3.2 mm silver capsules. To determine the δD of non-exchangeable H_2, we used the comparative equilibration method [9–11]. Stable hydrogen isotopic ratios ($^2H/^1H$) of monarch wing tissue are reported in delta (δ) notation in parts per thousand (‰) deviation from the VSMOW–SLAP standard scale (Vienna Standard Mean Ocean Water–Standard Light Antarctic Precipitation), where $\delta D = [(\text{Ratio}_{\text{sample}}/\text{Ratio}_{\text{standard}}) - 1] \times 1000]$.

For δ^{13}C analysis, a small section of hind wing ($1000 \pm 100 \mu g$) was clipped from each monarch and loaded in 4.0×3.2 mm tin capsules. Milkweed leaf tissue was cleaned using de-ionized water and oven dried at $60°C$ overnight. Leaf tissue was then ground to a powder and also weighed into 4.0×3.2 mm tin capsules and then introduced online to a continuous flow-isotope ratio mass spectrometer. For details on equipment and standards used, see the electronic supplementary material.

(c) *Assignment test and statistical analysis*

Stable-hydrogen isotopes (δD) are reliable markers of natal origins because δD values in precipitation (δD_p) vary predictably along latitudinal gradients. Stable-carbon isotopes (δ^{13}C) in milkweed leaf tissue varies longitudinally ([8]; electronic supplementary material, figure A1). Isotopic values in milkweed are transferred up the food web to monarch wings, providing a
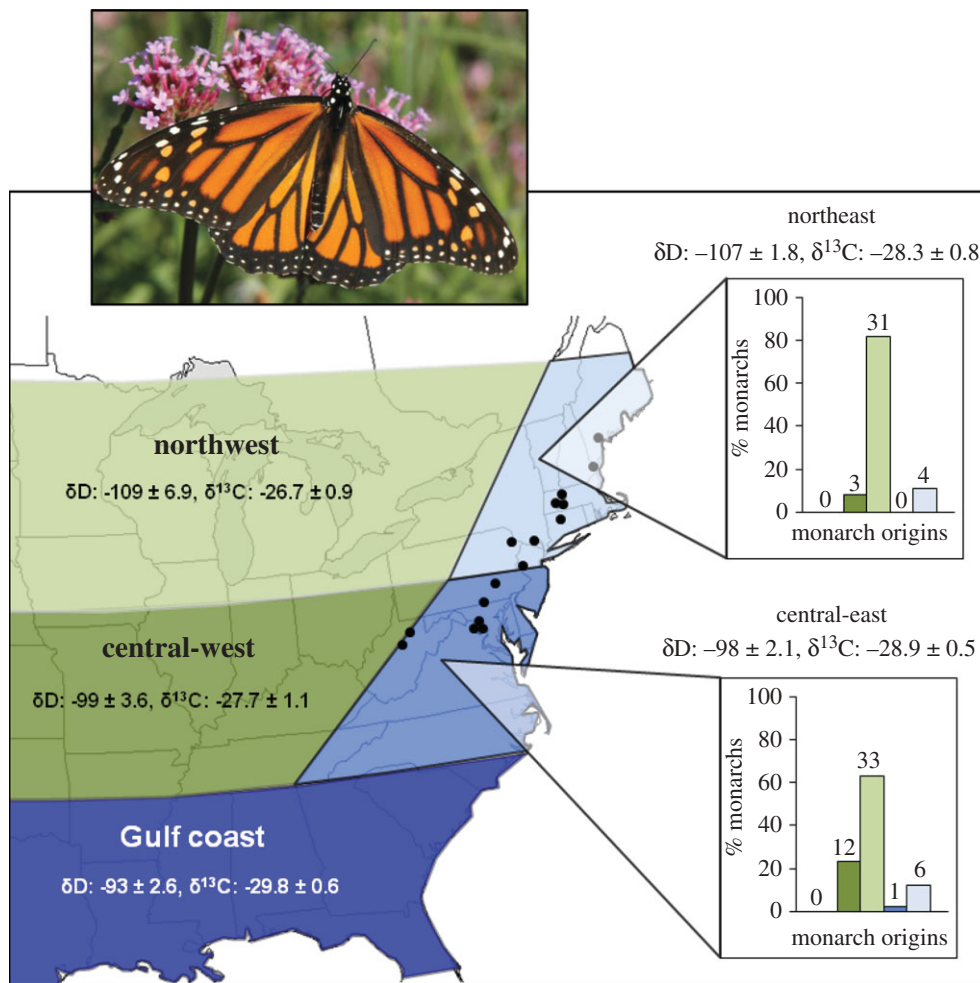
Figure 1. The natal origin of monarchs captured from 17 sites along the east coast of the USA estimated from stable isotopes. Bar graphs (number above is sample size) indicate the percentage of monarchs originating from one of five breeding regions: Gulf coast (dark blue), central-west (dark green), northwest (light green), central-east (middle blue) and northeast (light blue). Black dots indicate sampling locations. Numbers within each breeding region are the mean $\pm$ s.d. of hydrogen (δD) and ($\delta^{13}C$) isotope values calculated from precipitation and milkweed leaf tissue, respectively. Isotope values have been adjusted according to a fixed isotope discrimination factor (see §2).

natal geospatial fingerprint that is fixed for the duration of the individual's lifespan [8].

To estimate latitudinal natal origins, we derived a mean and s.d. of δD_p values for each of five pre-defined breeding regions (Gulf coast (GC), central-west (CW), central-east (CE), northwest (NW) and northeast (NE); figure 1), using the Online Isotopes in Precipitation Calculator [12,13]. δD_p values were constrained to the growing season dates of each pre-defined breeding region. Growing season dates were calculated by obtaining mean milkweed emergence time for 2009 using the Journey North [14] citizen science programme [14]. To estimate longitudinal natal origins, we used carbon isotope values from milkweed leaf tissue ($\delta^{13}C_L$), sampled widely across the monarch breeding range to derive a mean and s.d. values for each of the above breeding regions.

Field- and laboratory-rearing studies have shown that both δD_p and $\delta^{13}C_L$ are directly reflected in the isotopic composition of wing tissue of monarch larvae that feed on milkweed and in emergent adults [8]. δD_p is correlated with δD values in monarch wing chitin ($r^2 = 0.69$) according to the linear function: $\delta D_m = 0.62\delta D_p - 79‰$ and isotope discrimination between $\delta^{13}C_L$ and monarch wing chitin is minimal, with $\delta^{13}C$ values 0.5‰ lower in wings compared with milkweed [8]. We used these discrimination factors to adjust the δD_p and $\delta^{13}C_L$ values for each breeding region to equivalent values in monarch wing tissue. We then used a bivariate normal-likelihood assignment test to estimate the probability of originating from each breeding region, with P_b* being the region with the highest probability (see the electronic supplementary material). We also incorporated a resampling approach to account for analytical error (see the electronic supplementary material). An individual was considered to have originated from the breeding region that had the

greatest number of P_b* designations from the 100 resamples (see the electronic supplementary material, table SA1). We used a χ^2-test to compare the frequency distribution of monarchs at both the CE and NE breeding regions against the statistical null hypothesis that individuals have an equal chance of originating from east (CM strategy) and west (RE strategy) of the Appalachian mountains.

3. RESULTS AND DISCUSSION

The confidence in assigning individuals to specific regions based on the likelihood tests was high (see the electronic supplementary material). From these assignments ($n = 90$), we estimated that 15 monarchs originated from the CW region, 64 from the NW region, one from the CE region and 10 from the NE region. None originated from the GC region. Hence, consistent with the RE hypothesis, 88 per cent of monarchs ($n = 79$) originated from west of the Appalachians. Only 12 per cent of monarchs were estimated to have been born at or near the capture locations in the CE and NE regions, indicating that these individuals are most probably the second generation offspring of monarchs originating from the Gulf coast. Frequency distributions of monarchs in the NE and CE regions differed significantly compared

with expectations when individuals are evenly distributed across all potential natal regions (NE: $\chi^2 = 23.7$, $p = <0.0001$, d.f. = 1; CE: $\chi^2 = 27.8$, $p = <0.0001$, d.f. = 1).

Our results provide, to our knowledge, the first direct support of Brower's [7] range expansion hypothesis that re-colonization of the east coast is by monarchs born in the mid-west and Great Lakes, which then migrate eastwards over the Appalachians by mid-summer [7]. To explain the geographical patterns and timing of monarch abundance, Brower [7] suggested that monarch orientation shifts by $1° d^{-1}$ in a clockwise fashion [7]. Under this scenario, the second generation born in late spring and early summer in CE and NE regions would adopt an eastern migratory orientation. It is also possible, however, that eastern monarchs originating from the west could have resulted from a multi-directional migration strategy, which would predict that east coast individuals would not be the only monarchs that originate from the mid-west during this time period.

Nevertheless, the adaptive explanation of such a lengthy eastward movement remains unclear. Longitudinal migrations in some bird species have been attributed to seasonal variation in habitat quality (e.g. hummingbirds; [15]). Although the northward spring migration arrival of monarchs seems to coincide with milkweed emergence [16], milkweed is equally abundant and emerges at the same time east of the Appalachians. Thus, it is unlikely that longitudinal movement of monarchs is related to milkweed phenology [14].

Alternatively, there is some evidence showing that monarchs avoid laying eggs on plants, which already have eggs deposited on their leaves, suggesting that there are density-dependence mechanisms operating during egg-laying [17]. If monarch larvae experience decreased fitness in the presence of other caterpillars, then the benefits of migrating to areas of low density such as the east coast of the USA may be quite high. Future work should focus on estimating the strength of density-dependence and understanding of the fitness benefits of longitudinal migration.

We also found that some individuals (12%) had isotope values indicating that they were born east of the Appalachians. Knight *et al.* [18] found that the wave of monarchs arriving from Mexico on the Gulf coast peaks in mid-April and it takes their eggs approximately one month to develop to adults [18]. These adults will reach the north by 1st June, and their adult offspring will appear from late June to July, hence, monarchs with isotope signatures from the CE and NE are most probably second generation monarchs. We reached this conclusion because the timing of migration is such that there is insufficient time for second generation individuals born in the mid-west to cross the Appalachians and produce offspring within our sampling window (N. G. Miller & D. R. Norris 2009, unpublished data). It is also unlikely that second generation monarchs born in the mid-west completed a migration eastward during late May and early June since during this time of the year monarchs should be oriented north-eastwards, arriving in the Great Lakes (NW) region during June [11]. Hence, monarchs with isotopic values indicative of

the east coast are most probably the offspring of the first spring generation born on the Gulf coast, providing evidence that a small proportion of these individuals adopt a coastal migration strategy.

Because the monarch recolonization of eastern North America requires multiple generations from geographically discrete breeding regions, developing conservation plans for this species is challenging compared with species with distinct wintering and breeding sites. Based on estimates of natal origin from individuals sampled in the Great Lakes and in Mexico, previous work suggests that the mid-west is the most productive breeding region [11,19]. Our results suggest that the most productive breeding region for monarchs found along the east coast is the northwest region, which produced nearly 90 per cent of the monarchs. Thus, conservation planning will require targeting multiple regions to maintain all life-history strategies, emphasizing the importance of targeting the Great Lakes (NW) region for conservation efforts of the east coast monarch population.

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