


Regional climate on the breeding grounds predicts variation in the natal origin of monarch butterflies overwintering in Mexico over 38 years

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Abstract

Addressing population declines of migratory insects requires linking populations across different portions of the annual cycle and understanding the effects of variation in weather and climate on productivity, recruitment, and patterns of long-distance movement. We used stable H and C isotopes and geospatial modeling to estimate the natal origin of monarch butterflies (*Danaus plexippus*) in eastern North America using over 1000 monarchs collected over almost four decades at Mexican overwintering colonies. Multinomial regression was used to ascertain which climate-related factors best-predicted temporal variation in natal origin across six breeding regions. The region producing the largest proportion of overwintering monarchs was the US Midwest (mean annual proportion = 0.38; 95% CI: 0.36–0.41) followed by the north-central (0.17; 0.14–0.18), northeast (0.15; 0.11–0.16), northwest (0.12; 0.12–0.16), southwest (0.11; 0.08–0.12), and southeast (0.08; 0.07–0.11) regions. There was no evidence of directional shifts in the relative contributions of different natal regions over time, which suggests these regions are comprising the same relative proportion of the overwintering population in recent years as in the mid-1970s. Instead, interannual variation in the proportion of monarchs from each region covaried with climate, as measured by the Southern Oscillation Index and regional-specific daily maximum temperature and precipitation, which together likely dictate larval development rates and food plant condition. Our results provide the first robust long-term analysis of predictors of the natal origins of monarchs overwintering in Mexico. Conservation efforts on the breeding grounds focused on the Midwest region will likely have the greatest benefit to eastern North American migratory monarchs, but the population will likely remain sensitive to regional and stochastic weather patterns.

Keywords: *Asclepias*, carbon, *Danaus plexippus*, hydrogen, migratory connectivity, provenance, seasonal migration, stable isotopes

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Introduction

Determining the geographic origin and destination of migratory animals in different periods of their annual cycle is critical for understanding population dynamics and developing effective conservation strategies (Webster *et al.*, 2002; Martin *et al.*, 2007; Iwamura *et al.*, 2014). Quantifying patterns of connectivity is challenging because most migratory species cannot carry onboard, remotely downloaded global positioning recorders, and because fitting large numbers of individuals

with tracking devices is costly (Robinson *et al.*, 2010; Bridge *et al.*, 2011). For small migratory organisms, including most insects, robust spatial patterns of key stable isotopic elements (e.g., ²H and ¹³C) in the biosphere (isoscapes) can be used to link the movement of individuals over large distances (Hobson & Wassenaar, 1996; Hobson, 1999; Rubenstein & Hobson, 2004; Hobson *et al.*, 2012). In contrast to mark–recapture efforts, the geographic origin of tissue formation inferred using stable isotopes is spatially unbiased and, therefore, can be confidently used to assess areas of natal origin (Rubenstein & Hobson, 2004; Hobson *et al.*, 2009) and to quantify population-level patterns of migratory connectivity (Webster *et al.*, 2002; Chapman *et al.*, 2015).

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Stable isotopes are intrinsic markers that can be used to link individuals between different portions of the annual cycle (Rubenstein & Hobson, 2004). Stable hydrogen (^2H) and carbon (^{13}C) isotopes are commonly applied to delineate migratory connectivity because they are predictably transferred between the food sources and the biological tissue of higher-order consumers (Hobson, 1999). The abundance of ^2H in animal tissue varies depending upon biological processes that reflect precipitation patterns (i.e., meteoric waters originating from the atmosphere), temperature, elevation, and relative humidity at continental scales (Hobson, 1999; Bowen, 2010). Terrestrial continental patterns of ^2H show depletion in more northern latitudes, at higher elevation and further from ocean (Bowen, 2010). The abundance of ^{13}C values in animal tissue reflects those of consumed plant tissues, which depends on photosynthetic pathways in C3, C4, and CAM plants (Farquhar *et al.*, 1989; Dawson *et al.*, 2002). As a result, terrestrial continental patterns of ^{13}C increase with latitude, altitude, and temperature based on environmental conditions that reflect the proportion of C3 to C4 plants and water stress (Farquhar *et al.*, 1989; Hobson *et al.*, 1999, 2003; Kelly, 2000; Dawson *et al.*, 2002). Because the stable isotopic composition of many biological tissues (hair, claw, insect wings) is fixed upon formation (Hobson, 1999; Rubenstein & Hobson, 2004), isotopic elements such as ^2H and ^{13}C can be applied to archived tissue collections to reconstruct patterns of connectivity and natal origin over time (Koch *et al.*, 1995; Hobson *et al.*, 2014), as well as understand how isotopic patterns are influenced by interannual variation in weather and other environmental factors.

Weather affects the physiology and migratory behavior of insects (Kingsolver, 1989; Bale *et al.*, 2002; Sparks *et al.*, 2005). Warmer air temperatures increase larval development rate (Taylor, 1981; Zalucki, 1982), but beyond an upper temperature threshold, larval development slows and individuals die (Dixon *et al.*, 2009). At the population level, warmer temperatures translate into additional breeding generations in multivoltine species (Kingsolver, 1989; Batalden *et al.*, 2007). Stochastic weather patterns could also cause local and interannual variation in patterns of productivity and affect seasonal migration (e.g., delayed migration due to cold, wet spring/fall). Therefore, changes in climate could shift the breeding distribution and ecology of insects (Bale *et al.*, 2002) and those of their host plants (Lemoine, 2015), in addition to affecting survival during the nonbreeding season (Anderson & Brower, 1996). However, no data have been collected to test the effect of climate on

movement and productivity across a migratory species' distribution over long timescales.

We used relative abundances of ^2H and ^{13}C isotopes, geospatial modeling, and multinomial regression to examine seasonal climatic factors influencing long-term patterns in the natal origin of monarch butterflies (*Danaus plexippus*), focusing on monarchs that had successfully migrated from eastern North America to overwintering colonies in central Mexico. Monarchs overwinter at up to 12 colonies annually in dense aggregations (~35 million butterflies per hectare of forest) in high-elevation conifer forests between November and March each year (Slayback & Brower, 2007; Vidal & Rendón-Salinas, 2014). In March, monarchs depart and fly north toward Texas and the Gulf Coast states where they breed and die. Butterflies that develop from these eggs continue north, laying eggs along the way, as multiple breeding generations recolonize the eastern USA and southern Canada east of the Rocky Mountains (Cockrell *et al.*, 1993; Flockhart *et al.*, 2013). Monarchs have declined by approximately 80% over the last two decades (Brower *et al.*, 2012; Vidal & Rendón-Salinas, 2014), and, as a result, the migratory phenomenon is at risk of extinction (Flockhart *et al.*, 2015; Semmens *et al.*, 2016). Many causes have been proposed to explain monarch declines, including habitat loss on the breeding grounds (Pleasants & Oberhauser, 2013; Flockhart *et al.*, 2015) and extensive overwintering habitat logging and degradation (Ramirez *et al.*, 2015; Brower *et al.*, 2016).

This paper focuses on (i) identifying the natal origins of the late-summer generation that migrates to Mexico, (ii) examining evidence for long-term trends in the relative contributions of different regions to the monarch overwintering population, and (iii) analyzing climatic predictors of interannual patterns in natal origin assignment. Future climate warming is predicted to expand the distribution and abundance of monarchs and their host plants (Batalden *et al.*, 2007; Lemoine, 2015), although northward shifts in milkweed and monarch breeding activity could translate to longer migration distances and lower migratory survival for monarchs originating from northern extremes. At the same time, other anthropogenic impacts, such as the loss of milkweed in the breeding range due to the planting of herbicide-resistant crops (Hartzler, 2010; Pleasants & Oberhauser, 2013), could decrease the relative numbers of monarchs originating from agriculturally intensive regions in North America (Flockhart *et al.*, 2015). Indeed, a previous analysis (Wassenaar & Hobson, 1998) showed that approximately half of all monarchs wintering in Mexico in one year originated from the corn belt of the United States prior to the widespread adoption of herbicide-tolerant crops. One prediction is

therefore that the relative contribution to overwintering monarchs in Mexico from this region would have decreased in recent years. Because monarch reproduction and movement is known to depend on temperature (for example, warmer summer temperatures increase monarch abundance in regions that tend to be cooler on average; Zipkin *et al.*, 2012; Saunders *et al.*, 2016), we predict that warmer temperatures in a region should increase the relative contribution of that region to the overwintering monarchs in Mexico. From an applied perspective, we expect that results from this study will inform ongoing planning for monarch habitat restoration to ensure effective conservation efforts are focussed in appropriate geographical regions.

Materials and methods

Monarch butterfly and milkweed collections

Monarchs ($n = 1058$) were collected from overwintering sites in Mexico over a 38-year period between 1976 and 2014 ($n = 20$ overwintering years; Table 1); hereafter, 'year' refers

Table 1 Monarch collections used for the analysis of natal origins over time. Year is the overwintering year that corresponds with December (e.g., December 1993 = 1993). All butterflies were collected at Sierra Chincua except for 2007 where some butterflies were also collected at Cerro Pelon ($N = 43$; Altizer *et al.*, 2015) and for 2010 where butterflies were collected in Texas, USA, in spring 2011 and assessed as overwintered based on date and wing condition (Flockhart *et al.*, 2013)

Year	Collection dates	Colony	<i>N</i>
1976	January 27/77	Sierra Chincua	26
1977	December 11/77 – March 21/78	Sierra Chincua	158
1978	March 27/79	Sierra Chincua	20
1980	November 23/80	Sierra Chincua	40
1981	November 7/81	Sierra Chincua	40
1983	December 3/83	Sierra Chincua	40
1984	January 29/85	Sierra Chincua	40
1985	February 20/86	Sierra Chincua	40
1986	March 27/87	Sierra Chincua	39
1987	January 20/88	Sierra Chincua	20
1988	March/89	Sierra Chincua or Rosario	33
1992	February 7/93	Sierra Chincua	25
1993	December 30/93	Sierra Chincua	40
1996	February/1997	Sierra Chincua	132
2003	February 22/2004	Sierra Chincua	39
2006	December 27/06	Sierra Chincua	40
2007	February/08	Sierra Chincua, Pelon	88
2010	April 13/11–May 1/11	Texas, USA	93
2012	February/13	Sierra Chincua	40
2014	November/ 14–December/14	Sierra Chincua	65

to the year in the first portion of the calendar year in a given overwintering period (i.e., overwintering period, November 1976–March 1977, is '1976'). Ninety-six percent of the samples were collected only from the Sierra Chincua overwintering colony (19°40'30"N, 110°18'15"W; Brower *et al.*, 2016; Fig. 1), but monarchs ($n = 43$) from the nearby Cerro Pelon colony were also included in 2007. Samples from 1988 did not have any colony location recorded (Table 1). One exception to butterflies being collected in Mexico was data from Flockhart *et al.* (2013) who collected butterflies in April and May 2010 in the southern USA during the spring remigration and assessed butterflies as having overwintered based on timing of capture and wing wear scores. Monarchs overwinter in dense aggregations at up to 12 colonies per year in the central highlands of Mexico (Slayback *et al.*, 2007; Vidal & Rendón-Salinas, 2014). Although most of our samples come from a single overwintering colony, previous isotope data (Wassenaar & Hobson, 1998), tag-recovery data (O.R. Taylor, per. comm.), and genetic data (Pfeiler *et al.*, 2016) indicate the butterflies in all the overwintering colonies in Mexico are panmictic. Overall, this implies that inference drawn from the Sierra Chincua colony is likely to be representative of the entire eastern North American monarch population. Patterns of plant and butterfly $\delta^{13}\text{C}$ and $\delta^2\text{H}$ values for calibration of isoscapes were taken from Hobson *et al.* (1999) and Miller *et al.* (2011), with additional milkweed sample values (see Supporting information) collected in western Canada in 2012 (Table S4) at a time when an usually large number of monarch butterflies migrated to that region (Acorn, 2012).

Isotope analysis

Stable isotope preparation and analysis for determination of $\delta^2\text{H}$ and $\delta^{13}\text{C}$ values followed standard procedures (Supporting information). Nonexchangeable $\delta^2\text{H}$ values for monarch wing chitin were obtained using the Comparative Equilibrium procedure (Wassenaar & Hobson, 2003), with results normalized to the VSMOW-SLAP scales. Laboratory keratin standards and their assigned values for hydrogen isotopes were EC1 and EC2, having $\delta^2\text{H}_{\text{VSMOW}}$ values of -197‰ and -54‰ , respectively. Within-run reproducibility ($n = 5$) for $\delta^2\text{H}$ values of keratin control standards was better than $\pm 2\text{‰}$ for $\delta^2\text{H}$. Laboratory standards for $\delta^{13}\text{C}$ were BWBII and PUGEL with assigned $\delta^{13}\text{C}_{\text{VPDB}}$ values of -18.5‰ and -13.6‰ vs. the VPDB primary standard. The within-run precision of laboratory keratin control standards ($n = 5$) was better than $\pm 0.15\text{‰}$ for $\delta^{13}\text{C}$.

Assignment of natal origins

We assigned the natal origins of overwintering monarchs to one of six breeding regions (Fig. 1). These six regions were defined based on the known spatiotemporal distribution of monarch butterflies during the late-summer breeding season (Cockrell *et al.*, 1993; Wassenaar & Hobson, 1998; Flockhart *et al.*, 2013; Pleasants & Oberhauser, 2013). We used multivariate normal distribution assignment models to calculate the probability of natal origins to each of the defined regions

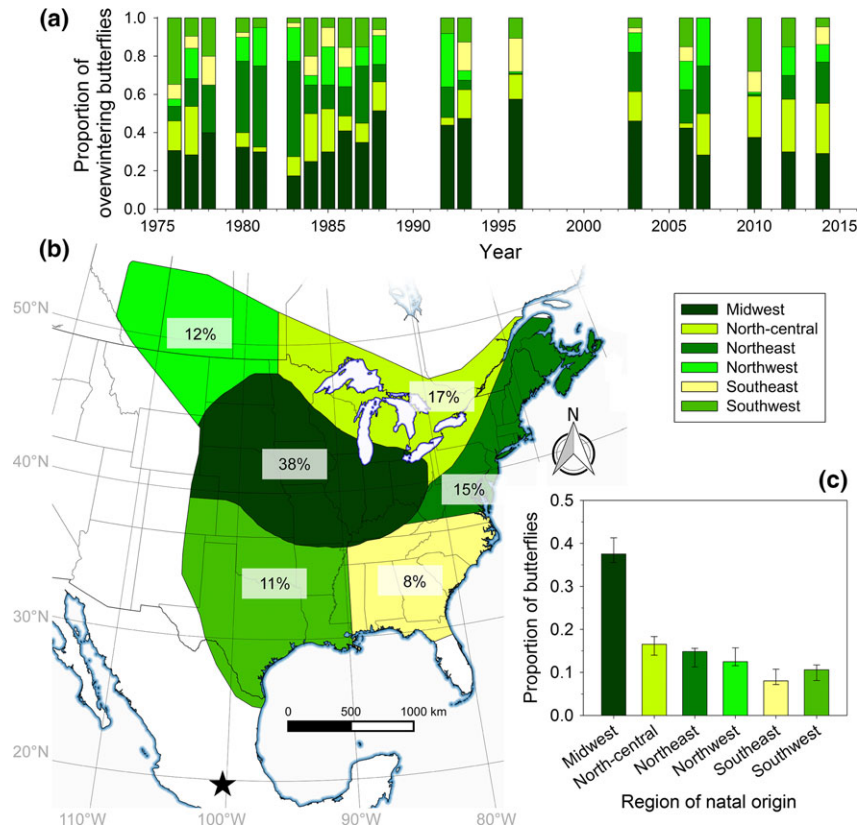


Fig. 1 (a) Raw data of the annual relative proportional assignments of monarch butterflies to different natal regions of eastern North America. Sample sizes for each year are indicated in Table 1. (b) Designated geographic regions of natal origin of monarch butterflies overwintering in Mexico. The bootstrapped mean annual percentage of monarch butterflies from the overwintering colonies (star) is indicated for each region. (c) Bootstrapped mean annual proportional assignment and 95% confidence interval from a multinomial regression that predicted relative proportion of overwintering monarch butterflies from different natal regions in eastern North America. [Colour figure can be viewed at wileyonlinelibrary.com]

(Royle & Rubenstein, 2004; Miller *et al.*, 2011). For each butterfly, the model calculated the probability of natal origin to each region based on the correspondence between its $\delta^2\text{H}$ and $\delta^{13}\text{C}$ value in wing tissue to the isoscape-predicted values of monarch $\delta^2\text{H}$ and $\delta^{13}\text{C}$ wing tissue in each region (Wunder, 2010). We assumed the underlying isoscape for $\delta^2\text{H}$ assignment did not change at the decadal scale, but we present an analysis where we develop time-dependent $\delta^{13}\text{C}$ isoscapes in the Supporting information. While we used a single long-term $\delta^2\text{H}$ isoscape, we recognize that these may change among years somewhat (Welker, 2012). Nevertheless, we consider the results presented here showing strong climate effects on origins of monarchs to be robust because spatiotemporal variation in isoscape values has a negligible effect of correctly assigning the natal origins of monarchs (Vander Zanden *et al.*, 2015).

The probability density of individual i collected in year t having region j as the natal origin is $Y_{it} \sim N(\mu_{jt}, \Sigma_j)$ where Y_{it} is a vector of observed $\delta^2\text{H}$ and $\delta^{13}\text{C}$ values, μ_{jt} is a vector of the mean predicted $\delta^2\text{H}$ and year-specific mean predicted $\delta^{13}\text{C}_t$ values for region j derived from calibrated isoscapes (Supporting information), and Σ_j is the positive-definite

variance-covariance matrix of $\delta^2\text{H}$ and $\delta^{13}\text{C}$ in region j (Royle & Rubenstein, 2004) based on monarch butterflies raised at known geographic locations (Hobson *et al.*, 1999). Here, Σ was assumed constant over time (Table S7). We applied Bayes' rule to invert the conditional probabilities of natal origin based on isotope values and probability of occurrence as follows:

$$f_{j|Y,X}(J = j|Y = y_{ijt}, X = x_{jt}) = \frac{f_{Y|X}(Y = y_{ijt}|X = x_{jt})f_j(J = j)}{\sum_j f_{Y|X}(Y = y_{ijt}|X = x_{jt})f_j(J = j)}$$

where $f_{j|Y,X}$ is the posterior probability density function for region j as the true origin of individual from year t with measured isotope value y , given the measured isotope values y_{ijt} for regions x_{jt} . The function $f_{Y|X}$ represents the conditional distribution on Y_j from above. The function f_j is the probability of occurrence for region J , as described in the Supporting information. The breeding region with the highest probability value was deemed as the region of natal origin (Royle & Rubenstein, 2004; Wunder & Norris, 2008), and this assumption was robust and consistent across moderate thresholds of probability of origin (Table S6). We used the *dmvnorm* function in the *mvtnorm* package (Genz *et al.*, 2014) in program R (R Core Team, 2014) to conduct natal origin assignments.

Weather data

We gathered global climate oscillation data (e.g., El Niño/Southern Oscillation; Ropelewski & Halpert, 1986) and regional weather data (maximum and minimum daily air temperature, daily precipitation amount) to examine year-to-year variability in the contributions of the breeding regions to the monarch butterfly population overwintering in Mexico. Global oscillations of coupled oceanic and atmospheric phenomena are well known to influence patterns of temperature and rainfall at regional scales (Ropelewski & Halpert, 1986; Shabbar *et al.*, 1997), which in turn influence food availability, growing season, and moisture availability, all of which can have effects on wildlife populations (Nott *et al.*, 2002; Sparks *et al.*, 2005). We calculated annual indices from monthly Southern Oscillation Index (SOI) data (January–December; <https://www.ncdc.noaa.gov/teleconnections/enso/indicators/soi/>).

For regional weather for each year, we considered three key variables. We calculated the mean of the daily maximum and minimum air temperature (°C) between July 20 and August 7 as well as the sum of the daily precipitation amount (cm) between January 1 and August 7 of each year. These dates were selected as they overlap with the time during the breeding period when monarch larvae that will migrate to and overwinter in Mexico are feeding on milkweeds (Nail *et al.*, 2015); thus, these temperature measurements likely provide the best estimate of the conditions experienced in regions where they developed. Precipitation amount measurements represent drought or wet conditions that can further influence larval host plant growth and condition. Details on the extraction of weather data can be found in the Supporting information; the mean and variation of the weather variables among the six natal regions are provided in Table S7 and the raw data for each monarch butterfly are provided in Table S8.

Statistical analysis

We used multinomial regression and Monte Carlo simulation bootstrapping to test the effects of year and regional weather on the assigned proportion of monarchs to each natal region. A complete description of the modeling approach is provided in the Supporting information. Multinomial models present results relative to a reference group, but in situations like ours, the reference group was arbitrary (Qian *et al.*, 2012) – we therefore selected the Midwest to be our comparative reference group.

The explanatory variables in multinomial models are of two forms: alternative-specific or individual-/cohort-specific. In our study, year and SOI variables were considered *cohort-specific* because there was no variation among regions in their measurement in a given year. From here on, we refer to these variables as *annual-specific*. In contrast, alternative-specific variables are those which vary among the different response groups. We considered the weather variables as alternative-specific because, in any given year, each region had different temperature and precipitation regimes. From here on, we refer to these variables as *region-specific*.

We used AIC to select among models in our candidate list, which we constructed in two phases. The first phase

considered annual-specific variables by comparing a linear year model, a SOI model, a model that classified years as pre- or postintroduction of genetically modified crops (GMO) in 1995 and a null model where the proportion of the natal regions would be constant across all years as single effects, while controlling for weather variables. We carried the best annual-specific variable to the second phase of model building, which considered models of maximum temperature, minimum temperature, and precipitation amount as single effects, as well as all models of the additive combinations of these region-specific variables.

We interpreted our results using the most parsimonious model in our candidate set. In linear binomial models, the coefficients can be taken directly as the marginal effects of the explanatory variable on the response variable; this is not the case for multinomial models (Croissant, 2012; Qian *et al.*, 2012). In multinomial models, the sign of the coefficients for *region-specific* variables is directly interpretable, but the magnitude of the effect is not. For *annual-specific* variables, the sign of the coefficients was not necessarily the sign of the effects. In both cases, transformation of the parameter coefficients was necessary to interpret the results of the model. Transformation of the coefficients produced marginal effects, which are the partial derivatives of the probabilities of natal origins (Croissant, 2012; Supporting information) and represent a change in the probability of a given natal region given a one-unit increase in the explanatory variable (i.e., increase in 1 °C temperature, 1 cm of precipitation, 1 year, or 1 SOI unit). Note that marginal effects are for a one-unit change in the explanatory variable (1 °C temperature or 1 cm of precipitation), but the magnitude of the effects will depend on the variability of these values within and among regions (Supporting information).

The top model was bootstrapped with 10 000 simulations to estimate the uncertainty in the predicted proportion of butterflies assigned to each natal region as well as the marginal effects with respect to the explanatory variables. Results were plotted as the mean and 95% confidence interval natal region assignment probabilities and marginal effects to capture the within- and among-year variability (Qian *et al.*, 2012).

Results

The predicted origins of overwintering monarch butterflies varied annually for each of the natal regions (Fig. 1a), but showed no long-term directional shifts. Considering *annual-specific* variables first, the best model to explain probability of natal origin region included only the SOI variable, which suggested monarch natal origins vary over time based on global weather oscillations (Table 2). Models that considered the introduction of GMO crops, year, or the null model were not supported (Table 2), indicating that the data do not indicate any long-term shift in the natal origins over almost four decades nor a shift in the geographic representation of monarchs wintering in Mexico that correlates with the conversion to GMO. Carrying SOI

Table 2 Ranking of multinomial regression models for cohort-specific (annual-specific) independent variables of annual effects to predict area of natal origin. Probability of natal origin of each region was considered to be constant (Null) over time, or vary in a linear time-dependent model (Year) or covariate model of the annual measurement of Southern Oscillation Index (SOI) or years classified as being pre- or postintroduction of genetically modified crops (GMO) crops in 1995 (GMO). For each model, the table presents the Akaike information criterion (AIC), the difference between the AIC and the AIC of the best-supported model (Δ AIC), the model weight (w_i), model likelihood (l_i), and the number of parameters in the model (K). The null model was Origin \sim effect|1, where 'effect' was the variables in the table

Model	AIC	Δ AIC	w_i	l_i	K	Log-likelihood
SOI	3460.1	0.00	1.000	1.00	10	-1720.1
GMO	3492.7	32.60	0.000	0.00	10	-1736.4
Year	3505.7	45.61	0.000	0.00	10	-1742.9
Null	3506.5	46.37	0.000	0.00	5	-1748.2

through, the best model that considered *region-specific* variables included mean maximum temperature and precipitation (Table 3). A more complex model that also included mean minimum temperature had a higher AIC score (Δ AIC = 0.55), but as the more complex model had six more parameters and the variables in the top model were a subset of those in the more complex model, we drew inference from the top-ranked model (Arnold, 2010). The coefficients of the top model are presented in Table S5.

Bootstrapping the top model showed that the region of highest probability for natal origins of monarchs in Mexico was the US Midwest (mean = 38%; 95% CI: 36–41), followed by the north-central (17%; 14–18), northeast (15%; 11–16), northwest (12%; 12–16), southwest (11%; 8–12), and southeast (8%; 7–11; Fig. 1c). Although the US Midwest produced the greatest percentage of monarchs relative to the other regions, there were only two years (1988 and 1996; Fig. 1a) for which the fraction of monarchs originating from the Midwest exceeded 50%. Surprisingly, for some years (e.g., 1980, 1981, and 1983), the majority of overwintering monarch butterflies originated from the northeast breeding region and, in one year (1976), the southwest region (Fig. 1a). Overall, fewer butterflies originated from the eastern portion of the breeding range (northeast and southeast regions; mean annual percentage = 25%; range 12–53%) compared to the western portion (Midwest, north-central, northwest, southwest regions) of the breeding range (mean annual percentage = 75%; range 48–88%).

The marginal effects of SOI showed that the probability that an overwintering butterfly was born in the Midwest, southeast, and southwest increased with the SOI,

Table 3 Ranking of multinomial regression models for alternative-specific (region-specific) weather variables to predict area of natal origin. Probability of natal origin of each region was compared among additive models of mean daily maximum temperature (Max temp) or mean daily minimum temperature (Min temp) in each region between July 20 and August 7. Precipitation (Precip) was the average sum of precipitation amount between January 1 and August 7 in each region. For each model, the table presents the Akaike information criterion (AIC), the difference between the model AIC and the AIC of the best-supported model (Δ AIC), the model weight (w_i), model likelihood (l_i), and the number of parameters in the model (K). The null model was Origin \sim Year|weather, where 'Year' is the top model from Table 2 and 'weather' was the variable in the table.

Model	AIC	Δ AIC	w_i	l_i	K	Log-likelihood
Max temp + Precip	3414.2	0.00	0.528	1.00	22	-1685.1
Max temp + Min temp + Precip	3414.8	0.55	0.402	0.76	28	-1679.4
Max temp	3419.7	5.42	0.035	0.07	16	-1693.8
Max temp + Min temp	3419.7	5.48	0.034	0.06	22	-1687.9
Min temp + Precip	3436.7	22.47	0.000	0.00	22	-1696.4
Min temp	3445.0	30.78	0.000	0.00	16	-1706.5
Precip	3455.9	41.67	0.000	0.00	16	-1712.0
Null	3460.1	45.87	0.000	0.00	10	-1720.1

whereas the probability that an overwintering butterfly was born in the northeast and northwest region declined (Fig. 2a). Even after accounting for the effects of SOI, *regional-specific* temperature and precipitation also influenced the distribution of natal origins (Fig. 2b, c). In the northeast, north-central, northwest, and southwest regions, an increase in daily maximum temperature resulted in an increase in the probability of monarchs being born in that region and decreased the probability of monarchs being born in the other two regions (Fig. 2b). By contrast, an increase in daily maximum temperature in the southeast decreased the probability of monarchs being born in the southeast and increased the probability of monarchs being born in other regions (Fig. 2b). Changes in temperature within the US Midwest had little influence on the probability of origin from other regions (Fig. 2b). For most regions, an increase in precipitation had similar effects as increasing temperature. For instance, an increase in precipitation in the north-central and northeast regions increased the probability of natal origins from those regions and decreased the probability of natal origins from all other regions (Fig. 2c). In contrast, an increase

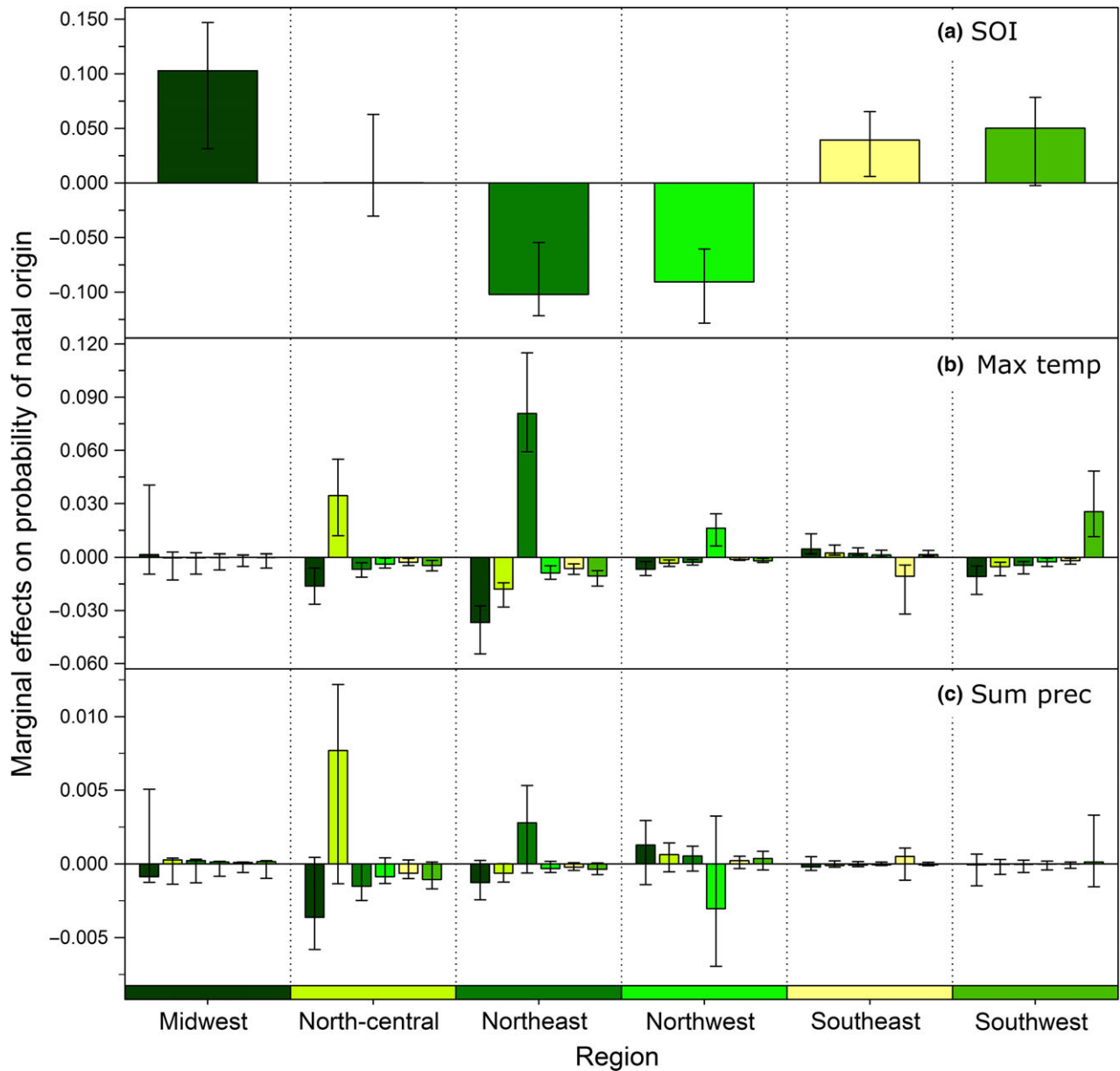


Fig. 2 Marginal effects (mean and 95% CI from 10 000 bootstrap simulations) of (a) Southern Oscillation Index (SOI), (b) mean daily maximum temperature, and (c) sum of daily precipitation on the relative change in the proportion of overwintering butterflies born in each breeding region. The relative change in the region of natal origin for a one-unit increase in one variable taken at the mean values of the other variables across the data set. For example, at the mean SOI and mean sum of precipitation of the study, a 1 °C increase of the mean daily maximum temperature (panel b) in the northeast region (*x*-axis) is predicted to decrease the proportionate assignment from the Midwest by 3.7%, the north-central by 1.8%, the northwest by 0.9%, southeast by 0.6%, and southwest by 1.1% and increase the proportionate assignment from northeast by 8.1% (bars). The figure presents a one-unit change in the explanatory variable, but region-specific changes in temperature or precipitation could be by any amount so the effect size would, for example, be doubled by a 2 °C increase in temperature or a 20-mm increase in precipitation. Note the change in scale among the panels. [Colour figure can be viewed at wileyonlinelibrary.com]

in precipitation in the northwest and Midwest decreased the probability of monarchs being born in these regions and increased the probability of monarchs being born in all other regions (Fig. 2c).

Discussion

Our results affirmed, from data spanning almost four decades, that migratory monarchs overwintering in

Mexico originated from a wide geographic distribution, and that the proportional contributions of different regions vary among years. Perhaps the most significant finding is the absence of any directional shift in the proportions of butterflies originating from the six areas over almost four decades. A relatively high proportion of overwintering butterflies were born in the US Midwest but, in all but two years (1988 and 1996), constituted less than half of all overwintering butterflies. Previous findings of natal origin based on stable isotope (Wassenaar & Hobson, 1998; Flockhart *et al.*, 2013) and cardenolide (Seiber *et al.*, 1986) analyses emphasized that the majority of overwintering butterflies originated from the US Midwest. Our results show that the proportion of butterflies with natal origins in the Midwest regions depends on the year that overwintering colonies were sampled, such that the relative variations in breeding-ground productivity would be difficult to identify without conducting long-term annual sampling of a larger number of overwintering individuals.

The proportion of butterflies originating in the Midwest fluctuated annually between 18% and 58% and correlated with global climate oscillations (Ropelewski & Halpert, 1986) but did not show any consistent directional trend in relative proportion over time. In other words, the US Midwest is producing, on average, a similar proportion of the overwintering population in recent years as it did in the mid-1970s. This is an unexpected result because of the documented extreme declines in milkweed host plant abundance in this region (Hartzler, 2010; Brower *et al.*, 2012; Pleasants & Oberhauser, 2013; Pleasants, 2016). This decline in the monarch's milkweed food plants coincides with the increasing use of glyphosate herbicide on genetically engineered soybeans and corn that is having an impact on the monarch butterfly population (Flockhart *et al.*, 2015). Given that the overall fall population in Mexico has declined over the past two decades (Brower *et al.*, 2012; Vidal & Rendón-Salinas, 2014; Semmens *et al.*, 2016), our results suggest that *all regions* in eastern North America, including the Midwest, produced fewer monarch butterflies over time, but our analysis does not distinguish among the potential causes of decline at the population level.

The mechanisms by which each natal region produces butterflies that successfully reach Mexico will likely differ based on regional colonization patterns, the number of realized breeding generations, and predator/parasite communities across space and time. For instance, butterflies reaching Mexico that originated from the northwest probably depend on late-spring, early-summer monarchs from the southern United States that recolonize this distal portion of the breeding range (Sparks *et al.*, 2005; Acorn, 2012; Chapman *et al.*,

2015), particularly in years with abnormally warm spring weather patterns in the southern portion of the breeding range which promote long-distance movement of butterflies (Cockrell *et al.*, 1993; Sparks *et al.*, 2005; Zipkin *et al.*, 2012; Flockhart *et al.*, 2013). Regional weather conditions have also been shown to predict the productivity of monarchs both within and across successive breeding generations (Saunders *et al.*, 2016). In contrast, the probability of natal origin for the southern portions of the breeding range likely depends upon changes in the distribution and condition of milkweed host plants (Calvert, 1999; Baum & Sharber, 2012) or the relative prevalence of disease (Satterfield *et al.*, 2015). Migratory monarchs breed during the autumn in the southwest and southeast regions with eggs being laid on newly emergent native host plants (Calvert, 1999; Flockhart *et al.*, 2013) that may increase productivity in years with stochastic regional disturbances (e.g., fires) during the summer (Baum & Sharber, 2012). At the same time, monarchs may breed throughout the winter on introduced host plants now grown year-round near the Gulf of Mexico (Satterfield *et al.*, 2015). Year-round breeding has been hypothesized to lead to an increase of parasites (Satterfield *et al.*, 2016), shed as spores by adults on food plants, which are subsequently ingested by larvae causing infection. Heavily infected monarchs are less likely to survive during fall migration (Bartel *et al.*, 2011), which suggests butterflies born in the southwest and southeast regions suffer higher mortality during the migration to Mexico in years with higher regional parasite levels (Satterfield *et al.*, 2015). Overall, monarchs overwintering in Mexico came from a wide geographic distribution across eastern North America, but whether a region produces more or less butterflies that overwinter in Mexico in any given year depends on several successive, and stochastic, events across the annual cycle.

Butterflies with natal origins in the eastern portion of the breeding distribution made up only 25% of the overwintering population in Mexico. Landscape changes over the past two centuries have increased the abundance of common milkweed (*Asclepias syriaca*) in the eastern portion of the breeding distribution, and monarchs now breed there regularly (Brower, 1995). However, tagging studies show that few monarchs migrating along the eastern seaboard successfully arrive in Mexico compared to inland areas (Urquhart & Urquhart, 1978; Brindza *et al.*, 2008). Instead, many butterflies born in this portion of the breeding distribution could migrate through Florida (Knight & Brower, 2009) and possibly on to Cuba (Dockx *et al.*, 2004) and thereby may not contribute to the population that overwinters in Mexico.

Weather on the breeding grounds had a significant effect on the probability of natal origin of monarch

butterflies overwintering in Mexico. The effects of global weather oscillations on weather patterns in North America are well documented and show contrasting patterns between positive and negative phases of El Niño/SOI. During positive phases of the oscillations typical of La Niña conditions (i.e., positive SOI values), below-average temperatures and above-average precipitation occur in western Canada and west-central United States (Shabbar *et al.*, 1997). Negative phases of the oscillations typical of El Niño conditions (i.e., negative SOI values) result in above-average precipitation in the Gulf Coast and northern Mexico (Ropelewski & Halpert, 1986), and below-average precipitation (Shabbar *et al.*, 1997) and above-average temperatures in western Canada and west-central United States (Ropelewski & Halpert, 1986; Shabbar *et al.*, 1997). Our results suggest that above-average precipitation in the Gulf Coast translated into a lower relative proportion of monarchs with natal origins from the southeast and southwest regions. In contrast, lower precipitation and higher maximum temperatures increased the relative proportion of the northwest and northeast, which implies that successful reproduction may be dependent on temperatures that promote faster growth and development. For the northwest region, this may dictate where breeding is successful, whereas in the northeast region these conditions may dictate whether an additional breeding generation is produced (Batalden *et al.*, 2007). The Midwest had a higher probability of natal origins in years with positive SOI, which is in contrast to the negative probability of natal origin in the northeast and northwest under these same climate conditions. These contrasting effects of SOI indicate that under certain weather conditions recolonizing butterflies can move beyond the US Midwest to areas to the east or west and that region-specific weather variation within and among regions controls regional productivity.

Annual variation in the pattern of natal origins of overwintering butterflies was also explained by variation in regional weather, specifically the daily maximum during late July and early August and sum of precipitation amount during January to early August of each year. Temperatures are well known to have a large influence on insect behavior and physiology and can give rise to population-level responses (Kingsolver & Wiernasz, 1991). At the individual level, warmer temperatures can cause darker wing coloration of monarchs (Davis *et al.*, 2005; Hanley *et al.*, 2013), which influences flight performance and successful migration to Mexico (Davis *et al.*, 2012). Warmer temperatures are expected to decrease longevity of monarch butterflies and reduce the egg-laying potential (Oberhauser, 1997), while, at the same time, increase development rate (Zalucki, 1982) making larvae less likely to be exposed

to predators (Benrey & Denno, 1997). Variation in temperatures among regions could result in dynamic population-level responses as regional temperature patterns can have contrasting effects on productivity among regions. For instance, theoretical models suggest that warmer temperatures can lead to additional breeding generations of monarchs (Batalden *et al.*, 2007), which implies variation in temperatures among regions could give rise to variation in the relative abundance of butterflies from each region at the overwintering grounds. At the same time, regional patterns of precipitation influenced the probability of natal origin but the mechanism is likely to be different from that of temperature. For instance, variation in precipitation is likely to influence food plant abundance and quality, which, in turn, could influence adult egg-laying behavior, immature survival, and development (Zalucki & Kitching, 1982). If global climate prediction models prove to be correct, there may be faster warming in the northern regions of the monarch range compared to the south (Wang *et al.*, 2016), which could shift the relative contribution of each breeding region to the overwintering colonies over this century.

To our knowledge, our analysis is one of few spatiotemporal studies of provenance to test for changes in areas of natal origin over time (Koch *et al.*, 1995). Our work highlights the critical importance of appropriate systematic and long-term collections of samples that provide scientific data to develop efficacious conservation strategies (Hobson *et al.*, 2014). For example, using two years (1996 and 2010) of sampling to estimate the natal origin of overwintering monarchs (e.g., Wasseenaar & Hobson, 1998; Flockhart *et al.*, 2013), we would erroneously conclude that the US Midwest contributes *significantly more* to the overwintering population than any of the other regions (Fig. 1a) and that known habitat changes have likely contributed to proportionately fewer monarchs coming from this region over time (Pleasants & Oberhauser, 2013). Instead, our findings suggest although the relative proportion of butterflies coming from the US Midwest is indeed the largest, the proportion of butterflies from this region did not vary systematically over the past four decades and that, for most years, over 50% of monarchs arose from regions other than the US Midwest. The implication is that monarch conservation efforts focused solely on the Midwest region are likely to benefit the largest number of individuals arriving in Mexico, but cannot ensure the entire eastern North American population is sufficiently resilient to stochastic weather patterns related to global climate change.

Effective conservation of migratory animals relies on making informed optimal investment strategies across the annual cycle to enhance population viability

(Martin *et al.*, 2007; Sheehy *et al.*, 2010; Iwamura *et al.*, 2014). Our findings indicate that the US Midwest region has produced the highest proportion of the monarchs collected in Mexico over the past four decades, and a simplistic implication might be that restoration efforts should focus primarily on this particular region. However, this disregards variation caused by land-use changes across North America and annual weather patterns and climate (Wang *et al.*, 2016) that could change the distribution and abundance of both monarchs and their host plants (Batalden *et al.*, 2007; Lemoine, 2015) in the next century. Buffering population responses to the dynamics of environmental conditions, therefore, suggests hedge betting conservation efforts to reduce the risk of extinction by maintaining vigilance across the entire breeding range rather than limiting efforts to only one particular region.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. The six natal regions considered in the study and the location of known-origin monarchs (Hobson *et al.*, 1999) and milkweed (Miller *et al.*, 2011, this study) used to formulate isoscapes.

Figure S2. The bounding box depicted in red for the weather data from 1980-present overlaid with the natal regions of monarch butterflies used in the study.

Figure S3. Measured $\delta^{13}\text{C}_m$ and geographically weighted model-estimated $\delta^{13}\text{C}_m$ values. The line is the 1 : 1 line.

Figure S4. Year-specific $\delta^{13}\text{C}_m$ isoscape for monarch butterfly wing tissues based on kriging results of a geographically weighted regression at the start (1976; A) and end (2016; B) of the study.

Table S1. Ranking of geographic-weighted regression models to explain stable-carbon isotope values of monarch butterfly wing tissue ($\delta^{13}\text{C}_m$) across eastern North America.

Table S2. Results of OLS Regression of the most parsimonious model ($\delta^{13}\text{C}_m \sim \text{Latitude} + \text{Year} + \text{Altitude} + \text{Annual Temperature}$) to explain $\delta^{13}\text{C}_m$ in eastern North America. The $R^2 = 0.4367$.

Table S3. Coefficient estimates of Geographically Weighed Regression of the most parsimonious model ($\delta^{13}\text{C}_m \sim \text{Latitude} + \text{Year} + \text{Altitude} + \text{Annual Temperature}$) to explain spatial variation in $\delta^{13}\text{C}_m$ in eastern North America ($R^2 = 0.52$).

Table S4. Measured mean \pm SD (sample size) $\delta^{13}\text{C}_m$ values and geographically-weighted model-estimated $\delta^{13}\text{C}_m$ values of monarch butterfly wing tissue using the geographically-weighted regression model $\delta^{13}\text{C}_m = \text{Latitude} + \text{Altitude} + \text{Mean Annual Temperature} + \text{Year}$.

Table S5. Parameter estimates for the most parsimonious model to explain the region of natal origin of monarch butterflies overwintering in Mexico.

Table S6. Counts (proportion of butterflies assigned to a region) of monarch butterflies assigned to natal regions given increasing normalized probability of assignment.

Table S7. Summary of region-specific covariates used in the multinomial regressions and the expected isotope values and variance-covariance matrices used in the natal origin assignments.

Table S8. Raw data used in the analysis.