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RESEARCH ARTICLE

Continuous-surface geographic assignment of migratory animals using strontium isotopes: A case study with monarch butterflies

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Abstract

1. Strontium isotope ratios ($^{87}Sr/^{86}Sr$) have shown promise for tracing the geographic origin of animal tissues because they have high-resolution and show discrete spatial patterns independent and complementary to those of light isotopes. In this study, we provide a complete quantitative framework to apply $^{87}Sr/^{86}Sr$ for tracking migratory animals using the eastern North American population of monarch butterflies *Danaus plexippus* as a case study.
2. To enable continuous-surface geographic assignment using $^{87}Sr/^{86}Sr$, we recommend following five key steps: (a) assessing feasibility, (b) sample collection, (c) laboratory analysis, (d) modelling the isoscape and (e) geographic assignment. We provide a detailed outline of these steps and then focus on steps 3–5 for the case study. For monarchs, using an extensive plant $^{87}Sr/^{86}Sr$ dataset ($n = 400$), geospatial data and a machine learning approach, we first calibrate a regional, high-resolution $^{87}Sr/^{86}Sr$ isoscape (i.e. a baseline for $^{87}Sr/^{86}Sr$ assignment) over their eastern North American summer breeding range. We then use the $^{87}Sr/^{86}Sr$ isoscape to estimate the posterior probability surface of natal origin for 100 monarchs of unknown origin.
3. Our results demonstrate that $^{87}Sr/^{86}Sr$ can greatly improve the precision of isotope-based geographic assignment. Furthermore, combining δ^2H and $^{87}Sr/^{86}Sr$ into a dual assignment provides the most constrained area of natal origin.
4. We provide a framework for ecologists and palaeoecologists to apply $^{87}Sr/^{86}Sr$-based geographic assignments for animal movement studies using contemporary or archived samples. The addition of the $^{87}Sr/^{86}Sr$ assignment tool will enhance our ability to study migration and dispersal in a wide variety of animals.

KEYWORDS

Danaus plexippus, geographic assignment, hydrogen isotopes, insect migration, monarch, provenance, strontium isoscape, strontium isotope ratios

1 | INTRODUCTION

Animal migration is an understudied and threatened ecological phenomenon found in diverse taxa (Satterfield et al., 2020). Up to 18% of the ~10,000 known bird species (Sekercioglu, 2007) and 3% of the ~20,000 known butterfly species are migratory (Chowdhury et al., 2021). Despite the prevalence of this life-history strategy, the migratory behaviour of many species is not well-documented, especially in insects (Chapman et al., 2015). This lack of knowledge is problematic because many migratory populations are declining, and the causes of these declines are not always understood (Brower et al., 2012; Guo et al., 2020; Kauffman et al., 2021). In the absence of detailed knowledge of migratory connectivity and patterns, conservation efforts are likely to be ineffective (Kauffman et al., 2021; Taylor & Norris, 2010).

One of the reasons that migration is understudied is that tracking animals over long distances is often challenging. Over the last two decades, stable isotopes have become a reliable tool for tracking animal migration (Hobson & Wassenaar, 2019). Stable isotopes are particularly useful for posthumous or archived samples, such as in palaeoecological studies (Hoppe et al., 1999) and provenance studies of poached animal products (Coutu et al., 2016). Isotopes are also advantageous for insect species because they are small and short-lived, which limits the use of biologging technologies applicable to larger animals (Rutz & Hays, 2009, but see Knight et al., 2019). Isotopes vary predictably in the environment with biological and physical processes allowing the development of numerical models, called isoscapes, predicting spatial isotopic patterns across the landscape (West et al., 2009). During tissue formation, animals inherit a specific isotopic composition from their food and water that reflects the location of natal origin and is preserved in metabolically inert tissues (Hobson et al., 1999). During the migratory stage, the isotope composition measured from these inert tissues (e.g. insect wings, bird feathers, horn) can be compared to isoscapes to retrace the natal origin (i.e. continuous-surface geographic assignment; Flockhart et al., 2013; Hobson et al., 1999, 2019; Talavera et al., 2018).

Hydrogen isotope values (δ^2H) are currently the quintessential isotope to infer provenance (e.g. Flockhart et al., 2013, 2017; Hallworth et al., 2018; Hobson et al., 2019; Wassenaar & Hobson, 1998). Hydrogen isotope composition varies temporally with climate patterns on the landscape and generally produces low-resolution latitudinal gradients (Bowen et al., 2005). These latitudinal gradients are often redundant with longitude, limiting the precision of geographic assignments (i.e. the ability of the assignment to restrict the probable area of natal origin; Hobson et al., 2010).

To enhance the precision and ecological interpretations derived from δ^2H-based geographic assignment of migratory animals, other isotopes are needed. Other isotopes, such as carbon and oxygen isotopes (e.g. Borisov et al., 2020; Dockx et al., 2004; Satterfield et al., 2018), have already been used for geographic assignment in combination with δ^2H, but with limited success due, in part, to their spatial correlation with δ^2H (Hobson et al., 2019). Strontium isotope ratios (i.e. $^{87}Sr/^{86}Sr$) were proposed decades ago as a strong candidate for geographic assignment (Hobson et al., 1999), but have been limited by analytical hurdles (e.g. low strontium content of organic tissue, reviewed in Bataille et al., 2020). Using $^{87}Sr/^{86}Sr$ is advantageous because they are temporally stable, they show high-resolution patterns following geological regimes that are independent from other light isotopes (Bataille et al., 2020) and, unlike light isotopes, they do not show metabolic or trophic-level fractionation (Flockhart et al., 2015). Therefore, $^{87}Sr/^{86}Sr$ have high potential to complement δ^2H in the geographic assignment of migratory animals.

Previous studies, including some on insects (e.g. Flockhart et al., 2015; Holder et al., 2014), have analysed $^{87}Sr/^{86}Sr$ in animal tissues. However, without independent models predicting $^{87}Sr/^{86}Sr$ variations on the landscape (i.e. a $^{87}Sr/^{86}Sr$ isoscape), $^{87}Sr/^{86}Sr$ from animals can only be compared with each other (e.g. Holder et al., 2014) or with a set of pre-defined locations with known $^{87}Sr/^{86}Sr$ (e.g. Chamberlain et al., 1997; Sellick et al., 2009; Widga et al., 2010), techniques known as the nominal approach (Wunder, 2012). In contrast, continuous-surface geographic assignment compares the measured isotopic composition in animal tissue to an isoscape and produces posterior probability surfaces of origin that are less biased and facilitate visualization of origin (Wunder, 2012). Probability surfaces from multiple isotopes are easily combined within this framework to further constrain geographic origin (Flockhart et al., 2017). With the recent advances in global $^{87}Sr/^{86}Sr$ isoscapes (Bataille et al., 2020), studies have started to apply $^{87}Sr/^{86}Sr$-based, continuous-surface geographic assignment for ecological applications (e.g. Funck et al., 2021; Kruszynski et al., 2021).

In this study, we provide a systematic framework to apply $^{87}Sr/^{86}Sr$-based, continuous-surface geographic assignment to migratory animals. We then apply this framework to monarch butterflies *Danaus plexippus*, an emblematic insect of conservation concern (Brower et al., 2012), because geographic assignments using light isotopes have been previously used to study monarch migration and provide a basis for comparing the performance of $^{87}Sr/^{86}Sr$ (e.g. Flockhart, Acorn, et al., 2018; Flockhart, Dabydeen, et al., 2018; Flockhart et al., 2013; Hobson et al., 1999; Wassenaar & Hobson, 1998). We develop an analytical protocol to rapidly and efficiently clean and analyse $^{87}Sr/^{86}Sr$ in insect wings. We then analyse the $^{87}Sr/^{86}Sr$ of 100 monarch butterflies that were previously analysed for δ^2H (Flockhart et al., 2013). To enable continuous-surface geographic assignment of $^{87}Sr/^{86}Sr$, we develop a regional plant $^{87}Sr/^{86}Sr$ isoscape for eastern North America. We use the new regional $^{87}Sr/^{86}Sr$ isoscape to estimate the natal origin of these monarchs and compare the relative performance of $^{87}Sr/^{86}Sr$ and δ^2H geographic assignments. Finally, we combine $^{87}Sr/^{86}Sr$ and δ^2H into a dual-isotope geographic assignment framework to provide the most precise estimate of natal origin with their associated ecological insights. Throughout this case study, we illustrate the key steps required to apply $^{87}Sr/^{86}Sr$ to animal mobility studies.

2 | MATERIALS AND METHODS

We detail five key steps to apply $^{87}Sr/^{86}Sr$-based, continuous-surface geographic assignment for animal mobility studies (Figure 1): (a) assessing the feasibility of $^{87}Sr/^{86}Sr$ assignment for monarch butterflies, (b) collection of known-origin plant samples and unknown-origin monarch butterfly samples, (c) laboratory $^{87}Sr/^{86}Sr$ analysis, (d) development of a regional, accurate and high-resolution $^{87}Sr/^{86}Sr$ isoscape and (e) the application of continuous-surface geographic assignment of unknown-origin monarchs using $^{87}Sr/^{86}Sr$ only, δ^2H only and dual $^{87}Sr/^{86}Sr$–δ^2H assignment. All statistical analyses were performed using R (version 3.6.3; R Core Team, 2013) and a commented R script is provided to guide ecologists through the framework.

In this study, we compare the performance of $^{87}Sr/^{86}Sr$ assignments with δ^2H assignments, the quintessential isotope used in ecological studies. The unknown-origin monarch butterflies used in this study were analysed for δ^2H and $\delta^{13}C$ as part of a previous study and assigned to their probable natal origins using these isotopes (Flockhart et al., 2013). However, we revised the δ^2H assignments generated in the study by Flockhart et al. (2013) using a recalibrated δ^2H isoscape (Hobson et al., 2019; Figure S4).

2.1 | Step 1: Assessing feasibility

There are multiple factors that need to be considered before starting any isotope-based geographic assignment project, but some considerations are $^{87}Sr/^{86}Sr$ specific (Table S1). Here, we develop $^{87}Sr/^{86}Sr$ assignment for monarch butterflies, an ideal species for $^{87}Sr/^{86}Sr$ assignment. The eastern population of monarch butterflies

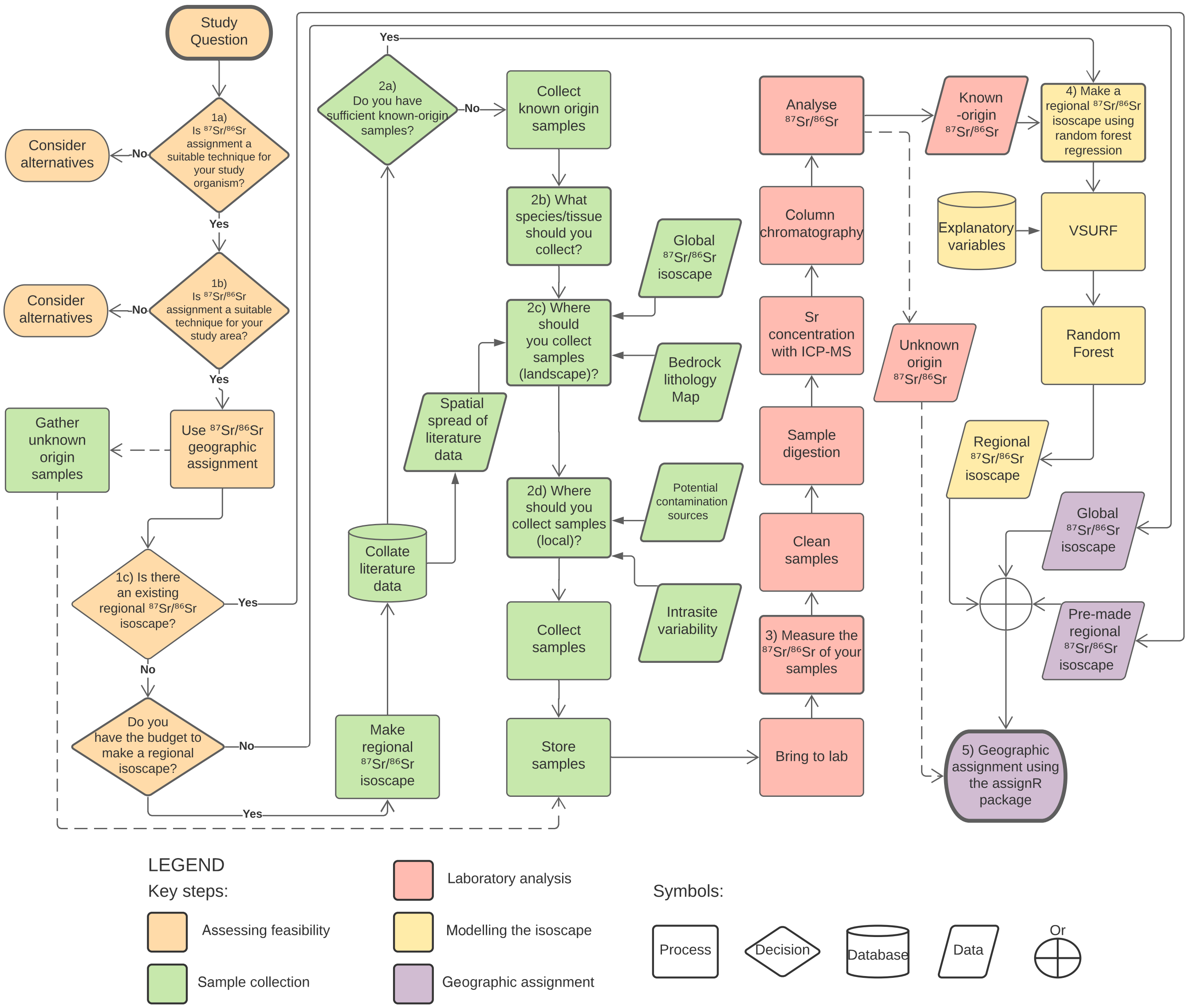

FIGURE 1 Step-by-step guide to using $^{87}Sr/^{86}Sr$-based geographic assignment. A corresponding list of important considerations and literature suggestions can be found in Table S1 to help ecologists looking to apply this technique to other systems. The hatched line shows the steps that should be taken with unknown-origin samples. Line arrows show the sequence of steps, and solid arrows indicate data that should inform a process

is famous for its annual multi-generational round-trip migration from its overwintering grounds in Central Mexico, through the United States to southern Canada, and back. Monarchs are an ideal species for $^{87}Sr/^{86}Sr$ assignment for multiple reasons. Monarch caterpillars are specialists on milkweed plants and have a very restricted larval range, therefore obtaining Sr from a specific source. The $^{87}Sr/^{86}Sr$ of monarchs is highly correlated with the $^{87}Sr/^{86}Sr$ of milkweed *Asclepias* spp. ($r^2 = 0.88$; Flockhart et al., 2015). The $^{87}Sr/^{86}Sr$ of the milkweed is fixed in inert wing tissues and is not altered by adult feeding (M.S. Reich, unpubl. data). In addition, sufficient $^{87}Sr/^{86}Sr$ variations exist across North America to allow distinct $^{87}Sr/^{86}Sr$ assignments (Bataille & Bowen, 2012).

2.2 | Step 2: Sample collection

The monarchs used in this study (n = 100) were collected in the United States between 13 April and 8 May 2011 and were assumed to be individuals returning from the overwintering grounds in Mexico because they were caught early during the northward migration and had high wing wear scores, a proxy for butterfly age (Flockhart et al., 2013; Figure S1). We targeted the overwintering generation because they are known to be a mix of individuals originating from across the summer breeding grounds (i.e. east of the Rocky Mountains in the United States and southern Canada; Flockhart et al., 2017; Wassenaar & Hobson, 1998) and could thus provide a good test case to compare $^{87}Sr/^{86}Sr$ and δ^2H geographic assignments.

2.3 | Step 3: $^{87}Sr/^{86}Sr$ analysis of unknown-origin monarch butterflies

We developed a new protocol to prepare and analyse $^{87}Sr/^{86}Sr$ in insect wings. Unlike plants and other organic tissues (e.g. bones and teeth), for which a large amount of material and relatively high Sr concentration are available, monarch butterfly wings are small (9.3 ± 1.3 mg, n = 99) and have low Sr concentration (3.1 ± 1.8 mg/kg, n = 99). Consequently, the total mass of Sr extracted from a single wing is small for monarchs and insects in general. Such small Sr mass makes the analysis challenging and the sample sensitive to contamination from exogenous sources (e.g. dust particles). To prevent potential contamination from solvent-based cleaning methods, we used a dry-cleaning protocol (Font et al., 2007; Holder, 2012) to remove all particles stuck to the butterfly wings by blasting pressurized nitrogen gas (~10 psi) for 2 min. We validated the efficiency of this dry-cleaning protocol for monarch butterflies by comparing butterfly wings before and after cleaning under scanning electron microscopy (Figure S7).

Once the cleaning protocol was established, we analysed the $^{87}Sr/^{86}Sr$ of the very small Sr mass extracted from the single-wing digestion (28.4 ± 16.4 ng, n = 99). The traditional analytical set-up of MC-ICP-MS instrument uses a self-aspiration method which requires large volumes and masses of sample to analyse $^{87}Sr/^{86}Sr$ precisely. However, only a small mass of Sr is available for analysis after insect wing digestion. We used a novel instrument, the micro-*FAST* MC (Elemental Scientific), to minimize the required volume for $^{87}Sr/^{86}Sr$ analysis and maximize analytical precision. Specifically, the Sr extracted from wings was dissolved in 2% v/v HNO_3 (200 µl, ~100 ppb Sr) and injected at 30 µl/min into the MC-ICP-MS using the micro*FAST* MC. Procedural blanks were negligible (signal to noise ratio >5,000) with excellent reproducibility of $^{87}Sr/^{86}Sr$ for SRM® 987 and an in-house Sr standard across five analytical runs (mean ± *SD*): 0.71024 (±0.00002, n = 5) and 0.70815 (±0.00020, n = 25) respectively. Using this analytical set-up, even smaller masses of Sr could be analysed (as low as a few ng) which would allow $^{87}Sr/^{86}Sr$ to be applied for provenance of most migratory insect species and other animals.

2.4 | Step 4: Modelling the regional plant $^{87}Sr/^{86}Sr$ isoscape

We applied the general random forest regression model proposed by Bataille et al. (2018) to calibrate a regional and substrate-specific $^{87}Sr/^{86}Sr$ isoscape for the breeding range of the eastern North American population of monarch butterflies. Strontium isotope ratios are driven largely by bedrock age and lithology and are therefore expected to change quickly at the discrete boundaries of bedrock units. Random forest regression is a machine learning algorithm that outperforms other traditional spatial statistical approaches (e.g. ordinary kriging) when modelling $^{87}Sr/^{86}Sr$ (Bataille et al., 2018).

Unlike δ^2H, which shows strong isotopic fractionation between insects, host plants, and precipitation and therefore requires an additional calibration (Figure S4), the $^{87}Sr/^{86}Sr$ of monarchs and milkweed display a nearly 1:1 relationship (Flockhart et al., 2015). Consequently, we used the $^{87}Sr/^{86}Sr$ of known-origin herbaceous plants as a basis to predict $^{87}Sr/^{86}Sr$ of monarchs. In this study, we analysed 67 plants from an existing collection (Miller et al., 2011) and 77 newly collected herbaceous plants (Supporting Information). After sample collection, the leaves of the 144 new plant samples were used for $^{87}Sr/^{86}Sr$ analysis. To augment this dataset, we searched the literature to compile 256 plant $^{87}Sr/^{86}Sr$ from across eastern North America (Esker et al., 2019; Hoppe et al., 1999; Widga et al., 2010, 2017). This literature search resulted in a calibration dataset of 400 herbaceous plant $^{87}Sr/^{86}Sr$ ratios (144 new plant $^{87}Sr/^{86}Sr$ ratios plus 256 from the literature) for training the random forest regression model (Figure 2).

Once the plant $^{87}Sr/^{86}Sr$ calibration dataset was compiled, we collected 29 geospatial data products that represent potential factors influencing $^{87}Sr/^{86}Sr$ variation on the landscape (Bataille et al., 2018, 2020) and used random forest regression to create the regional $^{87}Sr/^{86}Sr$ isoscape. The geospatial data products used here included bedrock geology, tectonostratigraphic terrane age (i.e. a fault-bounded area with a distinctive stratigraphy, structure and geological history), surficial geology, soil properties, aerosol

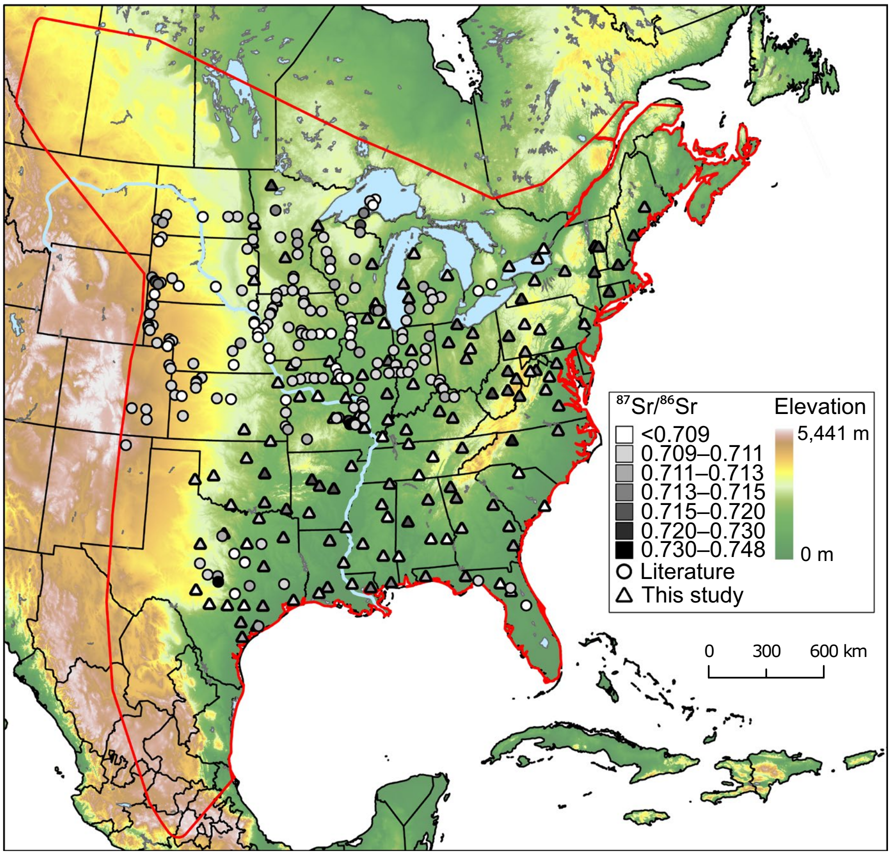

FIGURE 2 Location of plant samples with $^{87}Sr/^{86}Sr$ analysed in this study or compiled from the literature, and used as training data in the random forest regression. Of these plant $^{87}Sr/^{86}Sr$, 256 are from pre-existing studies (circles), and 144 are presented in this paper (triangles). The base map is a digital elevation model (Commission for Environmental Cooperation, 2007), and the red line represents the breeding distribution of monarch butterflies in eastern North America (Flockhart et al., 2017)

deposition, topography, climate and agricultural activity (Table S2). We used the sample locations of the georeferenced plant calibration dataset to extract the local pixel values of each of these geospatial data products.

Next, we used the VSURF package (Variable Selection Using Random Forest) to select the geospatial variables that were the best able to predict the $^{87}Sr/^{86}Sr$ variation (Genuer et al., 2015). We then used random forest regression to integrate the calibration dataset and geospatial data products into a modelling framework. To optimize and assess the performance of the model, a repeated 10-fold cross-validation was performed using the root mean squared error (RMSE) and coefficient of determination (R^2) as metrics through the CARET package (Max, 2008). Using the selected geospatial predictors and the optimized random forest model, we mapped the mean predicted plant $^{87}Sr/^{86}Sr$ across eastern North America, clipping the predictions to the breeding range of monarch butterflies (Flockhart et al., 2017).

In addition to the mean $^{87}Sr/^{86}Sr$ predictions, $^{87}Sr/^{86}Sr$-based geographic assignment requires a spatially explicit uncertainty layer (Ma et al., 2020). Quantile random forest regression has been proposed to overcome the absence of built-in approaches to assess spatial uncertainty in random forest regression (Fox et al., 2020). However, this approach can overestimate uncertainty, particularly when data have skewed distributions and outliers, as is sometimes seen with $^{87}Sr/^{86}Sr$ (Fox et al., 2020). We calculated spatially explicit uncertainty values by using the logarithmic relationship between absolute residual values and predicted $^{87}Sr/^{86}Sr$ as in the study by Bataille et al. (2020). Although we did not take this step, collecting a known-origin dataset could help further validate the tissue-specific $^{87}Sr/^{86}Sr$ isoscape by using tools available in the ASSIGNR package (Ma et al., 2020).

2.5 | Step 5: Geographic assignment

We calculated the continuous-surface geographic assignments of natal origin based on $^{87}Sr/^{86}Sr$ only, δ^2H only and dual $^{87}Sr/^{86}Sr$–δ^2H. The geographic assignment method depicts the most likely natal origin of each unknown-origin individual as a probability (i.e. a heat map of probable natal origin). Using the newly developed $^{87}Sr/^{86}Sr$ isoscape and the analysed $^{87}Sr/^{86}Sr$ of the monarchs, we used the ASSIGNR package (Ma et al., 2020) to calculate the posterior probability surface (i.e. assignment map) for each individual. The same procedure was followed for δ^2H assignment using the updated δ^2H isoscape (Supporting Information) and previously analysed δ^2H (Flockhart et al., 2013). The ASSIGNR package does not currently support dual-isotope assignment, so a function developed in a previous study (Wunder, 2010) and applying multivariate normal probability density was used for the dual $^{87}Sr/^{86}Sr$–δ^2H assignment (Supporting Information).

Once assignment maps of monarch natal origin were obtained, we produced a histogram of probability from the assignment map (0%–100%) in 5% increments. Using this histogram, we calculated the cumulative area represented within each incremental probability bin by dividing the calculated geographic area for each bin by the entire area of the study region. We plotted these cumulative areas for each assignment method to compare the single- and dual-isotope assignments. The precision of geographic assignment is often tested using a known-origin dataset by verifying that probabilities are scaled with the proportion of sites properly assigned (e.g. QA function in ASSIGNR). In the absence of a monarch known-origin dataset, we defined precision following Rushing et al. (2017) as the ability of the model to restrict the area of natal origin represented by the zone with high probability of natal origin within the study area. We defined high probability using a 2:1 odds ratio (e.g. Flockhart et al., 2013). The 2:1 odds ratio was used to create binary surfaces representing the most probable 33.3% of the probability distribution. These binary surfaces were then used to calculate the area of highly probable natal origin and to assess if the individuals had a local isotopic composition (Supporting Information).

To summarize the assignment maps, we applied a *k*-means cluster analysis to separate individuals into groups of similar natal origin. We also assessed the average probability of origin by summing the probability of all individuals and dividing by the number of individuals; this procedure was also repeated for each cluster. Additionally, to compare summarization techniques with the averaged probability surfaces, the binary surfaces were summed both by cluster and by all individuals, and a nominal assignment was performed (Figures S9 and S10).

3 | RESULTS

3.1 | Unknown-origin monarch butterflies

All monarch $^{87}Sr/^{86}Sr$ fell within the $^{87}Sr/^{86}Sr$ range of the plant samples that were used to construct the plant $^{87}Sr/^{86}Sr$ isoscape. No association between capture date or capture longitude with log-transformed $^{87}Sr/^{86}Sr$ was detected, but associations with capture latitude ($F_{1,93} = 59.8$, $p < 0.001$), sex ($F_{1,93} = 15.7$, $p < 0.001$) and wing wear score ($F_{1,93} = 7.2$, $p < 0.01$) were found. $^{87}Sr/^{86}Sr$ tended to increase with capture latitude ($\beta = 0.00029 \pm 0.00009$, $t = 3.12$, $p = 0.002$), and males tended to have lower $^{87}Sr/^{86}Sr$ than females ($\beta = -0.00058 \pm 0.00016$, $t = -3.56$, $p < 0.001$) These five variables explained 46% of the variance in logged $^{87}Sr/^{86}Sr$ ratios ($F_{6,93} = 15.03$, $p < 0.001$). There was also evidence that sex ($F_{1,93} = 4.6$, $p = 0.03$) influenced δ^2H, but no evidence that capture location, capture date or wing wear score had an effect on δ^2H. Males tended to have lower δ^2H than females ($\beta = -5.4 \pm 2.6$, $t = -2.07$, $p = 0.04$).

3.2 | Regional plant $^{87}Sr/^{86}Sr$ isoscape

The *VSURF* variable selection procedure identified five geospatial data products to be dominant predictors of $^{87}Sr/^{86}Sr$ in plants across eastern North America, including bedrock model products (Bataille et al., 2018), geological age of rock units (Hartmann & Moosdorf, 2012) and soil pH in H_2O solution (×10; Hengl et al., 2017; Figure 3a; Figures S4 and S5). The resulting model predicted $^{87}Sr/^{86}Sr$ in plants across eastern North America (Figure 4c) with an RMSE of 0.0017 ($R^2 = 0.44$; Figure 3b), which represented ~8% of the observed plant $^{87}Sr/^{86}Sr$ range across the dataset (i.e. the normalized RMSE). The random forest regression model produced a high-resolution $^{87}Sr/^{86}Sr$ isoscape that displayed discrete spatial patterns associated with specific geological features, but also variation associated with local environmental conditions (e.g. alkaline soil in the Interior Plains; Figure S6). Strontium isotope ratios between 0.709 and 0.711 were predominant in the American Midwest and across the western extremes of the monarch range (Figure 4c). Higher $^{87}Sr/^{86}Sr$ (0.711–0.715) were found in the Gulf Coast and north through the Appalachian Mountains. Strontium isotope ratios >0.713 dominated across the north-eastern portions of the United States and southern Canada east of Manitoba (Figure 4c).

3.3 | Geographic assignment

We compared the precision of single- and dual-isotope geographic assignments. When averaging across the 100 individuals, $^{87}Sr/^{86}Sr$ assignments showed higher accuracy and precision (Figure S8) compared to δ^2H assignment. Dual $^{87}Sr/^{86}Sr$–δ^2H assignment showed higher precision than single-isotope assignment. At high probability (i.e. most probable 33.3%), δ^2H assignment removed on average 89% (±3) of the study area from the predicted natal origin and $^{87}Sr/^{86}Sr$ assignment removed 93% (±5) of the area. Dual $^{87}Sr/^{86}Sr$–δ^2H assignment removed 97% (±2), a nearly fourfold decrease in the area of estimated origin compared to δ^2H assignment. However, this increase in precision seemed to come with some accuracy trade-offs: single isotope assignments tended to have higher accuracy than dual $^{87}Sr/^{86}Sr$–δ^2H assignments, as evidenced by the overall higher probability distribution for δ^2H and $^{87}Sr/^{86}Sr$ assignments (Figure S8).

To facilitate discussion and visualization, we selected two individuals, MOTF009 and MOTF003, that had distinct and different assignment patterns (Figure 5). At the individual level, geographic assignment using δ^2H displayed broad regions of potential origin. Like most of the individuals in this study, both MOTF009 and MOTF003 showed similar δ^2H assignment precision with ~13% of the study area estimated at high probability (Figure 5). Conversely, $^{87}Sr/^{86}Sr$ geographic assignment showed more variation in precision between individuals (Figure 5c). For 77% of individuals, such as MOTF009, the $^{87}Sr/^{86}Sr$ assignments were restricted to very specific zones and disqualified more of the study area than δ^2H assignment (mean of 5% (±3.3) of the area estimated; Figure 5b). For 23% of individuals, like MOTF003, the area of potential natal origin estimated by $^{87}Sr/^{86}Sr$ assignment exceeded the δ^2H assignment area (mean of 14% (±3.0) of the area estimated; Figure 5a). The potential area of origin was more restricted by dual $^{87}Sr/^{86}Sr$–δ^2H assignment than by single-isotope assignment in all cases (Figure 5).

The averaged dual $^{87}Sr/^{86}Sr$–δ^2H assignment suggested that the highest probability of origin is an area encompassing Texas and

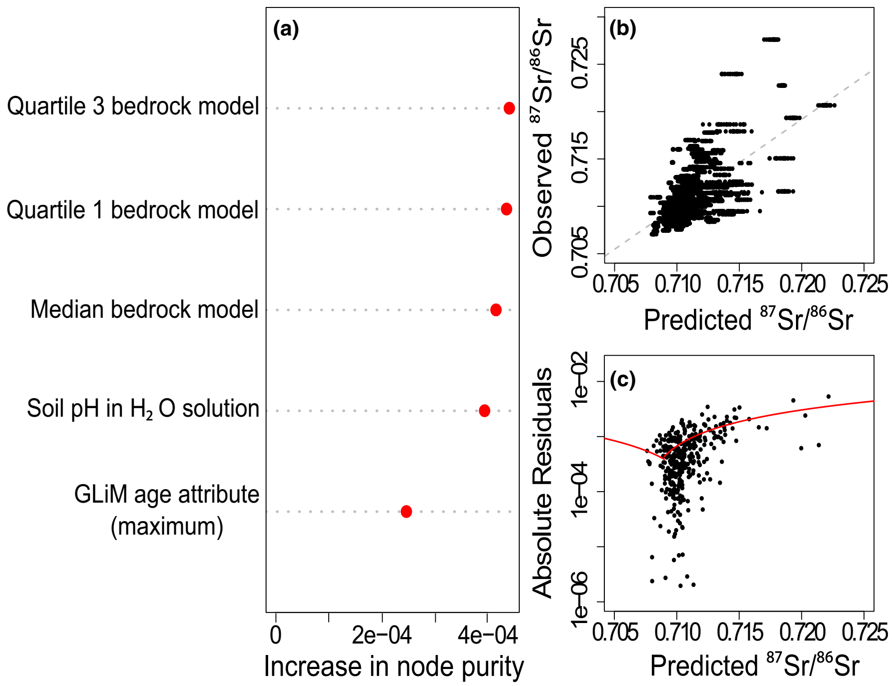

FIGURE 3 Performance of the random forest regression model used to develop the regional $^{87}Sr/^{86}Sr$ isoscape. (a) Variable importance plots for the variables selected using VSURF. Explanatory variables are listed by the mean increase in node purity, an index of importance to the model. Explanatory variable descriptions can be found in the Supporting Information, Table S2; (b) Observed versus predicted $^{87}Sr/^{86}Sr$ from the 10-fold cross-validation (RMSE 0.0017, $R^2 = 0.44$, $p < 0.001$). The 1:1 line is marked in grey; (c) Absolute residuals at predicted $^{87}Sr/^{86}Sr$. Red lines show the global equation used to calculate the uncertainty raster (Bataille et al., 2020): $x < 0.709$, $y = \log(1.08–0.11x)$, if $x > 0.709$, $y = \log(0.83 + 0.24x)$

northern Mexico (Figure 6a). The *k*-means cluster analysis found three isotopic clusters, predominately driven by differences in δ^2H. When considering the dual $^{87}Sr/^{86}Sr$-δ^2H assignment maps, monarchs in Cluster 1 ($n = 45$) displayed natal origin in the southern part of the breeding range in Texas and northern Mexico (Figure 6b), Cluster 2 monarchs ($n = 32$) showed natal origin in a few patches in northern Mexico and the American Midwest (Figure 6c) and Cluster 3 monarchs ($n = 23$) showed natal origin along the Gulf of Mexico in Texas and Florida (Figure 6d). These clusters did not differ in their capture location, capture date, proportion of males to females, or wing wear score.

Most individuals showed a probability of origin that was incompatible with their capture site, indicating that they did not originate locally. When looking at δ^2H assignments, only 7% of the individuals had a high probability of natal origin at their capture location (i.e. in the most probable 33.3%). When looking at $^{87}Sr/^{86}Sr$ assignments, 24% of the individual were compatible with local origin. Only 14% of individuals had dual $^{87}Sr/^{86}Sr$–δ^2H isotopic compositions compatible with their location of capture.

4 | DISCUSSION

4.1 | Isoscapes

The $^{87}Sr/^{86}Sr$ isoscape showed discrete patterns following the changes in geological units combined with high-resolution intra-unit variations associated with soil and atmospheric deposition processes. These patterns were complementary to those of δ^2H which showed broad latitudinal gradients (Figure 4). Strontium isotope ratios can therefore constrain area of natal origin within a latitudinal band defined by δ^2H, particularly when the geology in this latitudinal band is heterogeneous.

The plant $^{87}Sr/^{86}Sr$ isoscape presented here performed better than other recent, less regional or substrate-specific, $^{87}Sr/^{86}Sr$ isoscapes when comparing model metrics (Bataille et al., 2018, 2020). The overall RMSE of the model was reduced by half in comparison with Bataille et al. (2018, 2020) models. In these previous studies, the $^{87}Sr/^{86}Sr$ isoscape was trained using a dataset mixing samples from different substrates, different sampling strategies, and over geologically unrelated regions. In this study, we only used one specific substrate (i.e. herbaceous plants), we carefully selected our sampling sites and we trained a model over a region with a common geological history (i.e. eastern North America). All these factors likely contributed to reducing the overall uncertainty of the $^{87}Sr/^{86}Sr$ isoscape.

4.2 | Performance of the geographic assignments

The potential of $^{87}Sr/^{86}Sr$ assignment to constrain the natal origin of individuals was more variable than for δ^2H assignment (Figure 5c). While δ^2H almost always ruled out 89% of the study area, $^{87}Sr/^{86}Sr$ showed either lower or higher influence on the precision of geographic assignment. For some individuals, $^{87}Sr/^{86}Sr$ were not very diagnostic and geographic assignments were similar, or even less precise, than the δ^2H assignment (Figure 5a). These individuals tended to have $^{87}Sr/^{86}Sr$ in the range of 0.7085–0.711. For other individuals, $^{87}Sr/^{86}Sr$ were much more diagnostic, for example, two individuals with low $^{87}Sr/^{86}Sr$ (i.e. <0.708) had assignment maps pointing to very restricted areas for natal origin in Texas. This observation supports the findings of Bataille et al. (2020) showing that

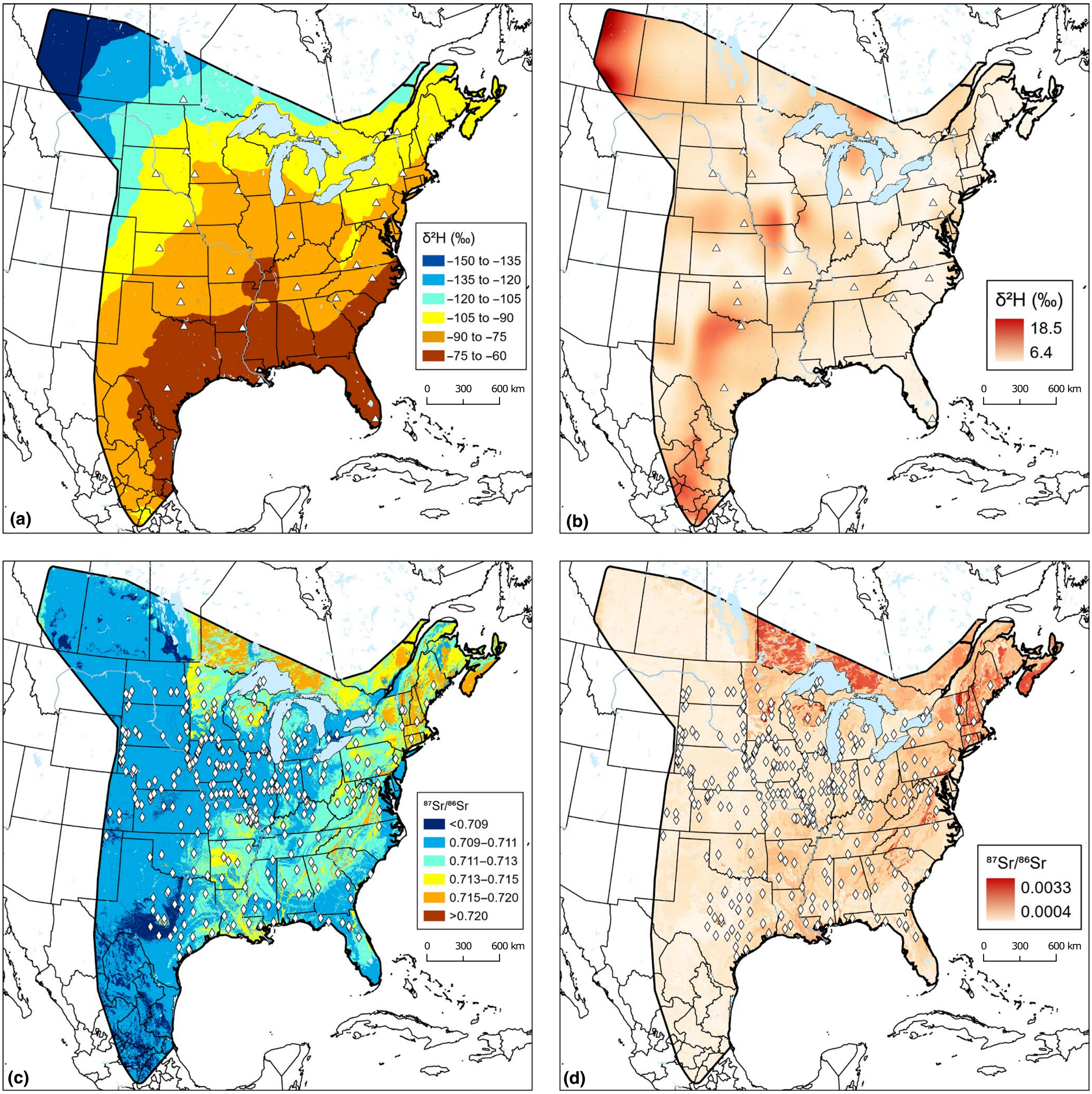

FIGURE 4 Isoscapes and their associated uncertainty. (a) Monarch δ^2H isoscape with locations (white triangles) of the known-origin monarch butterflies (Hobson et al., 2019) used to calibrate the isoscape in *assignR* using the growing-season precipitation δ^2H isoscape (Bowen, 2018; Bowen et al., 2005; IAEA/WMO, 2018), (b) uncertainty map (±1 *SD*) for the monarch δ^2H isoscape; dark red areas represent higher uncertainty, (c) plant ^{87}Sr/^{86}Sr isoscape with locations (white diamonds) of the plant ^{87}Sr/^{86}Sr used to train the random forest regression model, (d) uncertainty map for the plant ^{87}Sr/^{86}Sr isoscape; dark red areas represent higher uncertainty

about 50% of bioavailable ^{87}Sr/^{86}Sr of the world fall within a tight range of 0.7085–0.711. When individual ^{87}Sr/^{86}Sr fell outside this tight range, the potential of ^{87}Sr/^{86}Sr assignment to constrain natal origin became increasingly high. Areas that fell outside this range include geological areas with old or young rock units, including young volcanic regions (e.g. Central Mexico) and older igneous and metamorphic regions (e.g. Appalachian Mountains and their associated sedimentary basins).

Dual ^{87}Sr/^{86}Sr–δ^2H assignment was able to constrain the area of highly probable natal origin nearly four times better than δ^2H assignment, and twice as well as ^{87}Sr/^{86}Sr assignment (Figure S8). As the patterns of ^{87}Sr/^{86}Sr and δ^2H are independent on the landscape, the precision improvement in dual-isotope assignments is dependent on the fortuitous complementarity of their isotope patterns. In some cases, ^{87}Sr/^{86}Sr and δ^2H assignments are highly complementary with their probability surfaces only intersecting on very small areas

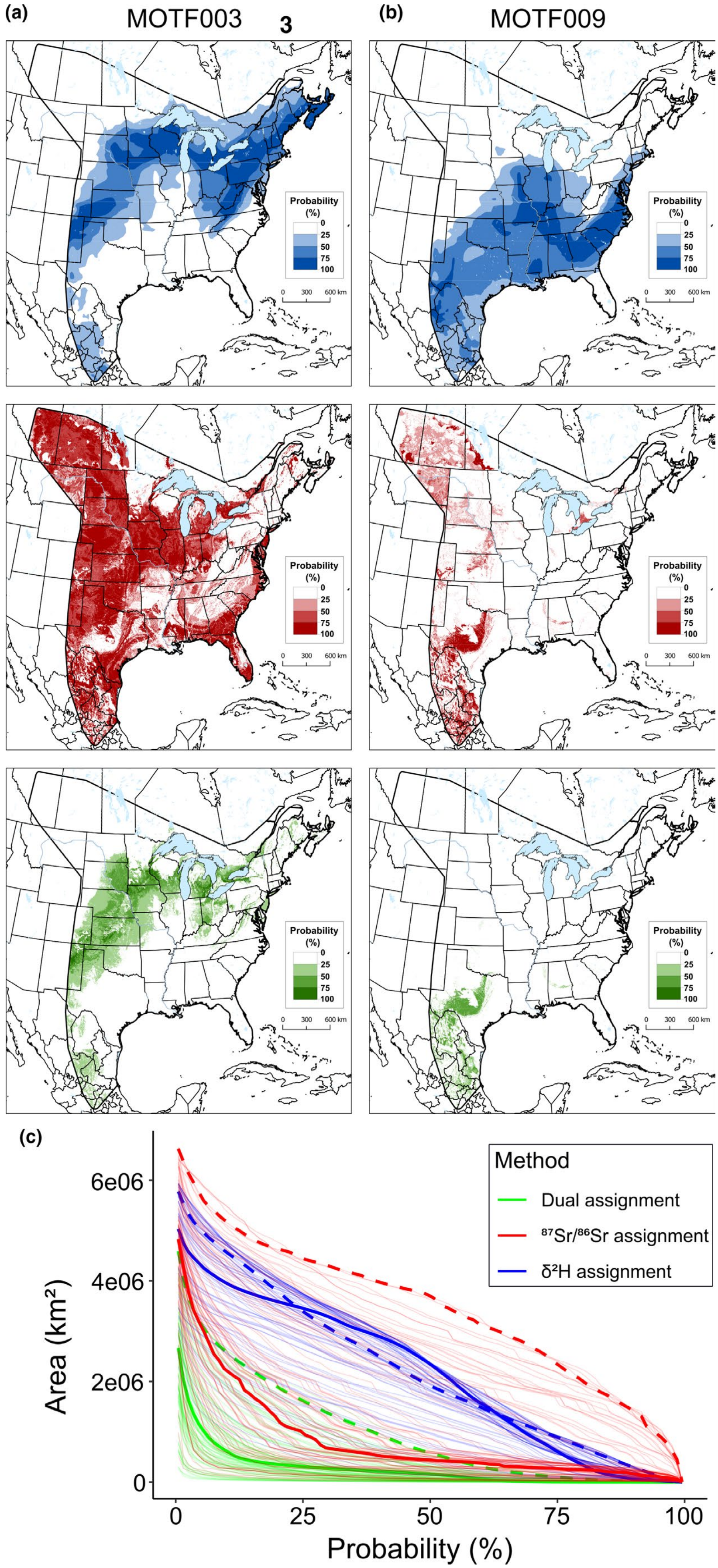

FIGURE 5 Comparison of single- and dual-isotope geographic assignment at the individual level illustrated using two individuals. (a) Individual MOTF009 and (b) individual MOTF003. For both, the top panel corresponds to the δ^2H-only assignment, middle panel corresponds to the ^{87}Sr/^{86}Sr-only assignment and the bottom panel corresponds to the dual ^{87}Sr/^{86}Sr–δ^2H assignment. Dual ^{87}Sr/^{86}Sr-δ^2H assignment performs better in both cases; (c) Comparison of individual assignment precision performance for the 100 individuals. The graph shows the amount of area with an assignment probability greater than or equal to a given assignment probability. Individual MOTF009 is highlighted by a bold solid line and individual MOTF003 by a bold dashed line

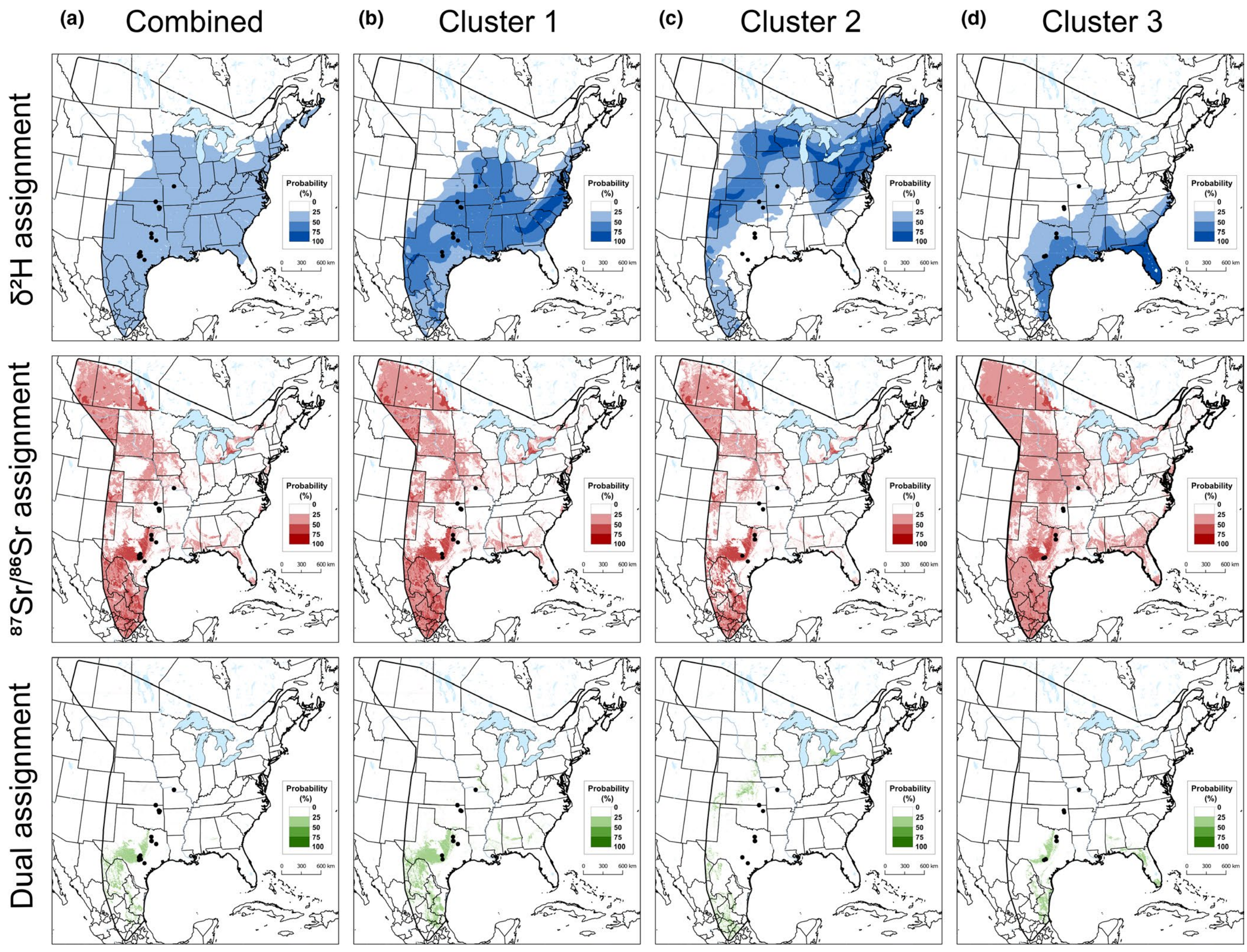

FIGURE 6 Geographic assignment maps of monarch butterflies summarized by group. (a) Averaged assignment maps for all monarchs ($n = 100$), (b) averaged assignment map for Cluster 1 ($n = 45$), (c) averaged assignment map for Cluster 2 ($n = 32$) and (d) averaged assignment map for Cluster 3 ($n = 23$). The black points mark the collection sites of the monarchs

leading to highly precise dual-isotope assignments (e.g. Figure 5b). In other cases, $^{87}Sr/^{86}Sr$ and δ^2H assignments cover similar regions on the landscape, leading to only small improvements in precision.

4.3 | Ecological implications

While our sample size was too small to make inferences at the population scale, the geographic assignment results were generally aligned with previous studies (Flockhart et al., 2013, 2017; Wassenaar & Hobson, 1998). The American Midwest is often considered to be the most important region contributing to the monarch overwintering population numbers (Dockx et al., 2004; Flockhart et al., 2017; Wassenaar & Hobson, 1998), and the population decline over the last two decades has been associated with a decrease in host-plant availability in this area (Pleasants & Oberhauser, 2013). In our dataset, we identified a cluster of individuals likely to have originated from a few areas in the American Midwest (Figure 6c).

Our analysis also demonstrated that many monarchs had probable natal origins in Texas and northern Mexico (Figure 6; Figures S9 and S10). However, it is unclear whether these were individuals hatched in the late summer of the previous year (i.e. a 'fifth generation') that overwintered in Mexico or if they were first-generation individuals hatched in the year they were sampled. Relatively, high wing wear scores from this sample suggest that they had flown substantial distances and, therefore, may have been the previous year's fifth generation (Calvert, 1999; Flockhart et al., 2013; Figure S1). However, other proxies of monarch age, such as wing shape and size (Altizer & Davis, 2010) and wing pigmentation (Satterfield & Davis, 2014), could confirm that the samples are from the fifth generation. Recent studies have shown that monarchs in Texas can represent a non-negligible proportion (~5%) of the pre-migratory population (Momeni-Dehaghi et al., 2021), and that up to 18% of migratory monarchs sampled in Texas from October to December are reproductive (i.e. not in diapause, as would be expected if they were migratory; Satterfield et al., 2018). However, the South Central

United States is generally not considered a dominant contributing region to the overwintering monarch population, and Texas is well known as the primary spring breeding area for the first generation (Miller et al., 2012). Furthermore, the spring migration had already progressed further north than the sampling locations during the sampling period (i.e. 13 April to 8 May 2011; Figure S2). Thus, we cannot exclude the possibility that some or all of these monarchs might be the first-generation offspring of overwintering monarchs.

A third possible explanation for the prevalence of assignments in Texas is that the $^{87}Sr/^{86}Sr$ of monarchs have experienced exogenous contamination from dust ingestion or through adsorption of Sr from water during the overwintering stage. Caterpillars could ingest dust as they feed on dust-coated leaves. While Sr adsorption from water exists for human hair (Hu et al., 2020), it seems unlikely for monarchs because they behaviourally avoid submergence in water and have superhydrophobic wings (Pass, 2018). These contamination sources might superimpose on the natal origin signal obtained from the milkweed (Flockhart et al., 2015). These hypotheses call for more experimentation to test the role of aerosols from dust and rain in modifying $^{87}Sr/^{86}Sr$ in insects.

4.4 | Implications and future improvements

We encourage researchers interested in studying animal mobility to consider applying the framework described in this study (Figure 1) to combine δ^2H and $^{87}Sr/^{86}Sr$ and enhance the precision of geographic assignment. Geographic assignments derived from isotopes can be combined with other types of ecological data including community science data (Flockhart et al., 2013), trace element analysis (Holder et al., 2014), wind-trajectory modelling (Talavera et al., 2018), genetic data (Ruegg et al., 2017) and ecological niche modelling (Ruegg et al., 2017). Combining these approaches will considerably enhance our ability to refine the geographic origins and migration pathways of animals. For example, in this study, we illustrate how dual-isotope geographic assignments could help understand the productivity of monarchs across the migration cycle over time (Flockhart et al., 2017). These spatial data are critical to constrain the factors contributing to the monarch population decline and focus habitat restoration efforts.

5 | CONCLUSIONS

We provided and tested a novel framework to use $^{87}Sr/^{86}Sr$ for continuous-surface geographic assignment of migratory animals. High-resolution, accurate, regionally calibrated $^{87}Sr/^{86}Sr$ isoscapes can be generated using carefully selected samples, geospatial data and machine learning regression. Using appropriate cleaning and analytical procedures, $^{87}Sr/^{86}Sr$ can be precisely analysed even in tissues with low Sr abundances. We demonstrated that, depending on the underlying geology, $^{87}Sr/^{86}Sr$ can complement δ^2H and substantially constrain geographic origin. The framework, isoscapes and dataset generated in this study provide a basis to apply dual $^{87}Sr/^{86}Sr$–δ^2H assignment to contemporary or ancient animals expanding our ability to study the migration phenomenon.

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AUTHORS' CONTRIBUTIONS

C.B., D.T.T.F. and D.R.N. conceived the ideas and designed the sampling methodology; M.S.R. and D.T.T.F. collected the samples; M.S.R., L.H. and C.B. analysed the data; M.S.R. and C.B. led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

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DATA AVAILABILITY STATEMENT

Data and all R code are archived with the Open Science Foundation (https://osf.io/8wzam/; https://doi.org/10.17605/OSF.IO/8WZAM).

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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