Contrasting assignment of migratory organisms to geographic origins using long-term versus year-specific precipitation isotope maps

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Summary

1. As a result of predictable large-scale continental gradients in the isotopic composition of precipitation, stable isotopes of hydrogen (δ^2H) are useful endogenous markers for delineating long-distance movements of animals. Models to predict patterns of δ^2H in precipitation (δ^2H$_p$), and consequently determine likely geographic origin of migratory animals, have traditionally used static, amount-weighted long-term average values of δ^2H$_p$ over the growing season. However, animal tissues reflect H incorporated from food webs that integrate precipitation over a single year's growing season or portions thereof. Inter-annual variation in precipitation and other climatic variables may lead to deviations from predictions derived from long-term mean precipitation isotopic values and could therefore lead to assignment errors for specific years and locations that are atypical.

2. We examined whether using biologically relevant short-term δ^2H$_p$ isoscapes can improve estimates of geographic origin in comparison with long-term isoscapes. Using δ^2H data from known-origin tissues of two migratory organisms in North America and Europe, we compared the accuracy, precision and similarity of assigned origins using both short- and long-term δ^2H$_p$ isoscapes.

3. Relative to long-term δ^2H$_p$ isoscapes, using short-term isoscapes for assignment often resulted in dissimilar regions of likely origin but did not significantly improve accuracy or precision. This was likely due to reduced spatial coverage in the data used to generate the short-term δ^2H$_p$ isoscapes.

4. We suggest that continued efforts to collect precipitation isotope data with a large spatiotemporal range will benefit future research on incorporating temporal variation in the amount and isotopic composition of precipitation into geospatial assignment models.

Key-words: stable isotopes, wildlife forensics, IsoMAP.org, *Danaus plexippus*, *Acrocephalus scirpaceus*, animal movement

Introduction

Long-distance migration is a common feature of many species world-wide. However, fundamental information about migratory connectivity amongst geographically distinct habitats, and the effect of the quality of these habitats on population demography, is typically limited, preventing a thorough understanding of many species' basic ecology. Information on factors affecting populations throughout their annual cycle is crucial to inform appropriate conservation strategies for threatened species (Webster *et al.* 2002; Martin *et al.* 2007; Marra, Hunter & Perrault 2011).

The stable hydrogen isotopic composition (δ^2H) of inert tissues is a well-established tracer in migratory studies of terrestrial organisms owing to distinctive isotopic patterns in rainfall that are integrated into terrestrial biosphere H through water,

soil and plants (Rubenstein & Hobson 2004; Hobson & Wassenaar 2008). This approach does not require initial marking and typically involves non-invasive and non-lethal sampling regimes (Hobson & Wassenaar 2008; Szép *et al.* 2009; Rundel *et al.* 2013). The δ^2H composition of inert tissues – usually keratin of bird feathers and mammalian hair, or chitin in insect tissues – reflects the geographic region where that tissue was grown, as the organism incorporates environmental water obtained through diet and drinking water into the tissue during synthesis (Hobson & Wassenaar 1996; Hobson, Atwell & Wassenaar 1999a; Wolf, Bowen & Martínez del Rio 2011). When animals migrate away from their breeding or overwintering location, the isotopic record of that domain may be preserved in these tissues. Ascertaining the likely origin of a migrant individual requires relating measured tissue δ^2H (δ^2H$_{tissue}$) values to measured or modelled values of precipitation entering the food web and supporting tissue growth (Kelly & Finch 1998; Hobson, Wassenaar & Taylor 1999b).

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Precipitation hydrogen isotope values (δ^2H_p) vary systematically and predictably around the globe with factors including temperature, latitude, altitude and distance from the coast (Dansgaard 1964). The predictable variation of δ^2H_p values in response to these factors forms the foundation of using this isotopic marker for migratory wildlife forensics. Because precipitation isotopic data are derived from a limited numbers of observation stations sampled over the past 50 years, spatially explicit models are used to generate predictive, continuous precipitation H and O isotopic maps (isoscapes) over regional to global scales (Bowen 2010; Terzer *et al.* 2013).

Most isotope prediction models are based on data from the International Atomic Energy Agency (IAEA) Global Network of Isotopes in Precipitation (GNIP) data base (IAEA/WMO 2011), which is a spatio-temporally discontinuous data set ranging from 1961 through the present. The mean annual and growing-season (i.e. those months with a mean daily temperature >0°C) precipitation isoscapes developed by Bowen, Wassenaar & Hobson (2005) have been successfully used to determine origin of migratory species globally, and strong correlations between δ^2H_{tissue} in known-origin animals and these predicted rainfall δ^2H_p values have been demonstrated in many cases (reviewed in Hobson & Wassenaar 2008). However, inert tissues such as feather keratin and insect wing chitin primarily assimilate H from the food web during the relatively short period of tissue synthesis and reflect seasonal integrated precipitation for a limited time up to the point of synthesis (Hobson, Barnett-Johnson & Cerling 2010).

Temporal variation in δ^2H_p values at continental scales is associated with climate phases (e.g., ENSO, PNA), storm tracks and primary wind trajectories (Welker 2012; Liu *et al.* 2013). In addition, stochastic inter-annual (e.g. drought vs. wet years) and intra-annual (or seasonal) variation in precipitation and other climate variables may lead to large deviations from long-term averaged isotopic values, and particular regions may be more susceptible to higher departures from the long-term mean (Vachon *et al.* 2007; Bowen 2008; Liu, Kennedy & Bowen 2011). Therefore, using isoscapes derived from long-term mean δ^2H_p values to infer origins of tissues that presumably reflect much shorter time periods of H integration could lead to biased interpretation of model results, particularly during atypical years.

The purpose of this study was to examine whether using short-term isoscapes, in contrast to traditional long-term models, would lead to an improvement in determining geographic origin of migratory species. We used δ^2H_{tissue} values of known-origin monarch butterflies (*Danaus plexippus*) and Eurasian reed warblers (*Acrocephalus scirpaceus*) in two distinct geographic domains (Hobson, Wassenaar & Taylor 1999b; Procházka *et al.* 2013). IsoMAP (Bowen *et al.* 2014, www.isomap.org) was used to generate two types of δ^2H_p isoscapes: (i) short-term isoscapes corresponding to the period the organisms assimilate environmental H into the sampled tissue, and (ii) long-term mean annual isoscapes that reflected over 40 years of isotopic data for the study region. We used three diagnostic metrics to compare predictive accuracy, precision and geographic similarity of assignments between these models. Our hypothesis was that biologically relevant short-term models would improve the accuracy and precision of assignments and could result in significantly different source assignment surfaces in comparison to long-term annual isoscape models.

Materials and methods

CASE STUDIES

We used two previously published data sets of δ^2H_{tissue} from known-origin migratory species in eastern North America and Europe. In each case, the hydrogen isotopic data were randomly divided into (i) calibration, and (ii) validation data sets. The calibration data sets were used to correlate δ^2H_{tissue} values to amount-weighted precipitation δ^2H_p values in order to create isotopic rescaling functions. The validation points were treated as if they were from unknown locations in order to test model performance.

The first case study was a subset of δ^2H_{tissue} data from 97 monarch butterfly wing chitin samples from 27 localities within the eastern United States (Hobson, Wassenaar & Taylor 1999b). Monarch butterflies from eastern North America migrate to overwinter in central Mexico (Urquhart & Urquhart 1976). Monarch larvae were reared by volunteers in the summer (May–August) of 1996 on naturally occurring milkweed plants whose only water source was local rainwater, and wing chitin samples from the emerged adult butterflies were collected for analysis. Information about sampling design and isotopic analysis of the monarch samples is found in Hobson, Wassenaar & Taylor (1999b). Seventeen monarch sites (53 individuals) were randomly designated calibration sites, and the remaining 10 sites (44 individuals) were designated for validation (Fig. 1a, Table S1).

The second case study consisted of δ^2H_{tissue} data from 214 feather samples of Eurasian reed warblers at 26 sites within Europe and North Africa. Reed warblers are migratory passerines that breed in the wetlands of Europe and overwinter in sub-Saharan Africa (Cramp 1992). Innermost primary feathers grown in Europe were sampled between 12 June and 12 August, 2004–2006 from first-year reed warblers at sites spanning the entire latitudinal gradient of their breeding range. Information on the study design and isotopic analysis is available in Procházka *et al.* (2013). Thirteen sites (124 individuals) were randomly designated as calibration sites, and the remaining 13 sites (90 individuals) were designated for validation (Fig. 1c, Table S2).

ISOSCAPE MAPPING

The δ^2H_p isoscapes were created using IsoMAP, which is an online resource that allows users to create region- and time-specific maps for geographic assignment purposes (Bowen *et al.* 2014, www.isomap.org). Short-term δ^2H_p isoscapes were created for years specific to our two case studies: 1996 for the monarchs and three individual years from 2004 to 2006 for the reed warblers (Table 1). Long-term δ^2H_p isoscapes used all available data and represented 40 years or more of GNIP collections: 1960–2000 for the monarchs and 1960–2006 for the reed warblers (Table 1). Only the months of April–August were included for the monarchs in order to encompass the period of growth of milkweed associated with the summer larval period. We included all months of the year for the reed warbler analysis, as they are wetland birds using resources that likely integrate precipitation over a longer time period (Procházka *et al.* 2013). Additional information about the modelling
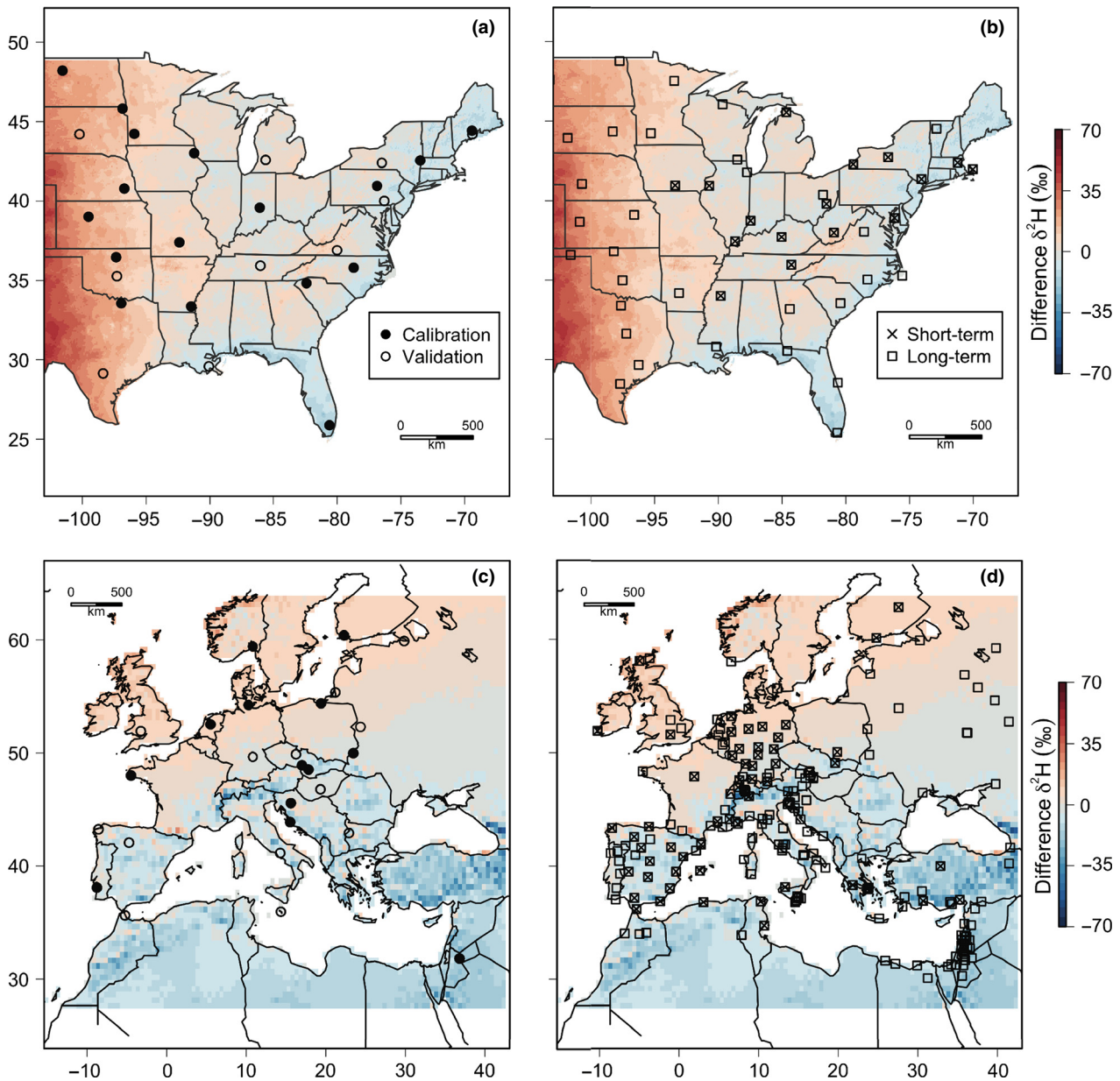
Fig. 1. Difference in δ^2H_p values between short- and long-term precipitation models in (a, b) monarch butterfly and (c, d) reed warbler sampling ranges. Location of calibration and validation tissue sampling sites (a, c) as well as the short- and long-term precipitation sampling stations used to develop the δ^2H_p isoscapes (b, d). A complete list of tissue sampling sites is available in Tables S1 and S2. The overall mean difference (short-term minus long term) was more positive for eastern North America (8·9 ‰), while it was negative in Europe (−3·8 ‰). Years represented in the monarch butterfly isoscapes were 1996 for the short-term and 1960–2000 for the long-term isoscape; and for the reed warbler isoscapes were 2004–2006 for the short term and 1960–2006 for the long-term isoscape.

process to generate the isoscapes in this study is available in Appendix S1.

MEAN RESCALING FUNCTIONS

Calibration individuals were used to create a universal rescaling, or conversion, function to convert δ^2H_p values to animal δ^2H_{tissue} values. This step is critical in the assignment to origin process, as the precipitation-tissue relationship can be specific to species, age class and isoscape used (Hobson *et al.* 2012; Van Dijk, Meissner & Klaassen 2014). Ordinary least-squares regression was used to determine a mean slope

and intercept of 1000 simulated regression lines between δ^2H_p and animal δ^2H_{tissue} values for each species and time period, and these mean regression equations were then used to perform precipitation to tissue isoscape conversions (see Appendix S1).

VARIANCE ESTIMATION

We considered three sources of variance, each of which was assumed to be independent and normally distributed (see Appendix S1 for additional detail). This assumption allowed pooling using the equation:

Table 1. Number of sampling sites and individuals represented in the case study data sets for monarch butterflies and reed warblers. The years and number of data stations used to generate precipitation isoscapes for this study are also included. Precipitation maps can be accessed with the job key numbers at http://isomap.org (Bowen *et al.* 2013)

	Monarch butterflies	Eurasian reed warblers
Tissue sampling		
Number of collection sites	27	26
Calibration sites (individuals)	17 (53)	13 (124)
Validation sites (individuals)	10 (44)	13 (90)
Short-term precipitation models		
Time period	1996	2004, 2005, 2006
Number of stations	16	37–55
Stations per 10^6 km^2	3·1	7·2–10·7
IsoMAP job key(s)	27010	28972–74
Long-term precipitation models		
Time period	1960–2000	1960–2006
Number of stations	49	202
Stations per 10^6 km^2	3·9	16·0
IsoMAP job key	27016	28928

$$\sigma_{pooled} = \sqrt{\sigma^2_{rescale} + \sigma^2_{precip} + \sigma^2_{individ}} \qquad \text{eqn 1}$$

where $\sigma^2_{rescale}$ was the variance of the rescaled precipitation to tissue δ^2H isoscapes using the 1000 regressions (this varied across the raster, depending on the cell value); σ^2_{precip} was the variance of the original δ^2H$_p$ isoscape generated in IsoMAP (this also varied across the raster, depending on uncertainty in the isoscape model parameters); $\sigma^2_{individ}$ was the mean of the variances in δ^2H$_{tissue}$ values of the individuals at the calibration sites. Both $\sigma^2_{rescale}$ and σ^2_{precip} were represented by raster maps (Figs S4 and S5), whereas $\sigma^2_{individ}$ was a single constant value specific to the case study organism (monarchs: $\sigma^2_{individ} = \pm$ 14·4 ‰, warblers: $\sigma^2_{individ} = \pm$ 41·0 ‰). While other sources of error may exist, we expect those in equation 1 to be the major contributing sources.

GEOGRAPHIC ASSIGNMENT METHODS

Geographic assignments were conducted within a Bayesian framework, whereby we determined the probability that an individual sample was from a particular geographic location, given the measured δ^2H$_{tissue}$ value of the sample (cf. Wunder 2010). The precipitation isoscape corresponding to the year of sample collection (1996 for monarch butterflies; 2004, 2005, or 2006 for reed warblers) was rescaled to the expected values for δ^2H$_{tissue}$ to find assignment probabilities for each pixel in the grid. Posterior probability surfaces were normalized such that all pixel probabilities summed to 1 across each spatial domain. These surfaces were then rescaled by the largest observed density value to range between values of 0 and 1 for ease of visualization and comparison among individuals. Assignments and statistical analyses were conducted in R (R Core Team 2013), but the geographic assignment algorithm used in this study was identical to that in Wunder (2010) and equivalent to the assignment process when conducted in IsoMAP (Bowen *et al.* 2014).

EVALUATION OF ASSIGNMENT EFFICACY

Assignment efficacy was evaluated over a range of thresholds using a suite of three metrics: accuracy, precision and similarity. Each metric was evaluated across a sequence of relative probability density values from 0·01 (low probability) to 0·99 (high probability) in 0·01 increments, creating 99 intervals for analysis. Accuracy and precision were evaluated at the population and individual levels, whereas the similarity index could only be evaluated at the individual level. The R scripts used to conduct assignments and calculate accuracy, precision and similarity are available in Appendix S2.

Population-level assignment accuracy was evaluated as the proportion of the validation locations contained within the assignment region for a given probability interval. This metric evaluates the ability of the assignment model to correctly circumscribe a region of origin that includes the true location. Individual-level accuracy was evaluated as the change in relative probability (short-term minus long term) between assignments at the known-origin site and reflects whether the relative likelihood of origin at the true location increased or decreased with adoption of the short-term isoscape.

Population-level precision was evaluated as the median proportion of the total surface area covered by the assignment region for each probability interval. Smaller areas (or smaller proportions of the spatial domain) indicate higher precision in the assignment surface. Individual-level precision was evaluated as the change in area (short-term minus long term) of the posterior probability surface at a relative probability density value equal to that at the known origin. This metric demonstrates whether precision for a single individual increased (as indicated by smaller areas) or decreased in the short-term assignment. Two-sided paired t-tests were used to determine if mean individual-level accuracy and precision values differed from 0.

A similarity index was adopted from the Expected fraction of Shared Presences (ESP) metric proposed by Godsoe (2014) (described in detail in Appendix S1). Briefly, this index calculates the ratio of the number of shared cells between two assignment rasters to the total number of possible cells. A function of relative probability versus similarity was created for each individual, which was then integrated to calculate a value between 0 and 1 representing the area under the curve (AUC). The AUC equals 1 when two assignments match exactly and equals 0 when no part of the two assignments match. The similarity index can be informally considered as the percentage overlap between the two assignments over the entire range of relative probabilities.

Results

The range in δ^2H$_{tissue}$ values among all sampling sites was more than twice as large for reed warblers (112·7‰) as for monarch butterflies (53·9‰), and ranges within individual sites were also generally larger for reed warbler sampling sites (Fig. 2, Tables S1 and S2). This may in part reflect the larger range in the δ^2H$_p$ values for Europe compared to the restricted portion of North America analysed here (Fig. S1).

The short-term and long-term precipitation isoscapes developed for the two study regions differed between the time periods investigated. Both positive and negative differences in δ^2H$_p$ (short-term minus long term) existed in each of the study regions (Fig. 1). In the eastern United States, there was an east to west trend such that negative δ^2H$_p$ differences were dominant in the east with positive differences in the west (Fig. 1a). Whereas reed warbler assignments were made to isoscapes representing a single year between 2004 and 2006, we combined all years in the short-term versus long-term δ^2H$_p$ comparison to examine overall patterns between the time periods (Fig. 1c).
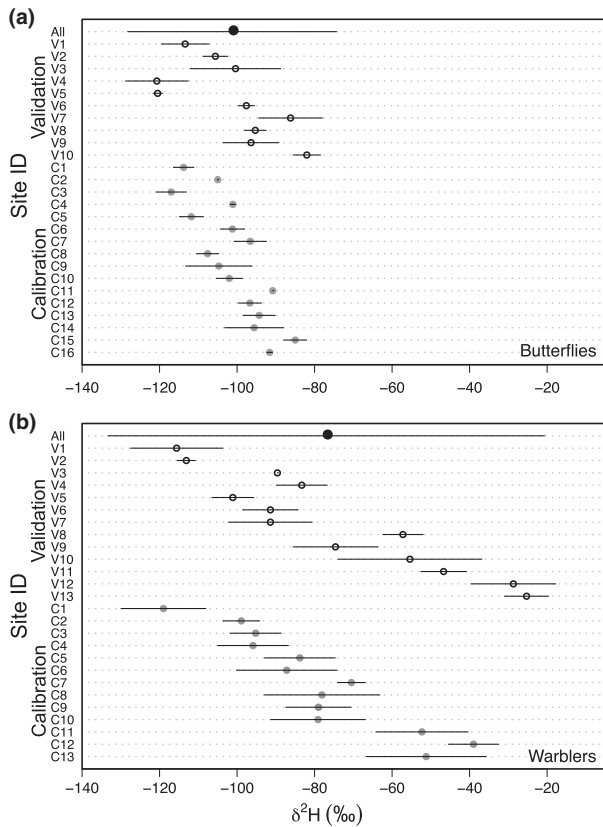
Fig. 2. Mean and range in δ^2H_{tissue} values for all sampling sites (top line) as well as each individual validation and calibration sampling site for monarch butterflies (a) and reed warblers (b). A complete list of sampling sites is available in Tables S1 and S2. The overall range in reed warbler δ^2H_{tissue} values is nearly twice as large as that for the monarch butterflies.

In Europe, the trend extended north to south such that positive δ^2H_p differences characterized the north with negative δ^2H_p differences in the south (Fig. 1c). The overall mean difference was 8·9 ‰ in eastern North America and −3·8 ‰ in Europe (Fig. 1). The spatial patterns are of more interest than the mean difference, however, and indicate that continental sites in North America are more affected by seasonal variability than coastal sites, and high latitude sites are more affected in Europe than lower latitude sites.

The rescaling functions differed depending on the organism and time period used (Fig. S2). The butterfly rescaling equations were similar between short- ($\delta^2H_{tissue} = 0.40 \times \delta^2H_p - 90.8$, $r^2 = 0.54$) and long-term isoscapes ($\delta^2H_{tissue} = 0.34 \times \delta^2H_p - 90.2$, $r^2 = 0.41$), whereas the warbler rescaling equations were more different between short- ($\delta^2H_{tissue} = 1.23 \times \delta^2H_p - 18.6$, $r^2 = 0.65$) and long-term ($\delta^2H_{tissue} = 1.06 \times \delta^2H_p - 26.5$, $r^2 = 0.85$) isoscapes. Assignments were made using the rescaled tissue-specific isoscapes as the mean or expected values (Fig. S3).

Population-level assignment accuracy increased as the relative probability interval for assignment was increased, such that all (or nearly all) of the validation locations were included in the assignment surface by using the widest assignment interval. On the other hand, restricting the assignment surface to
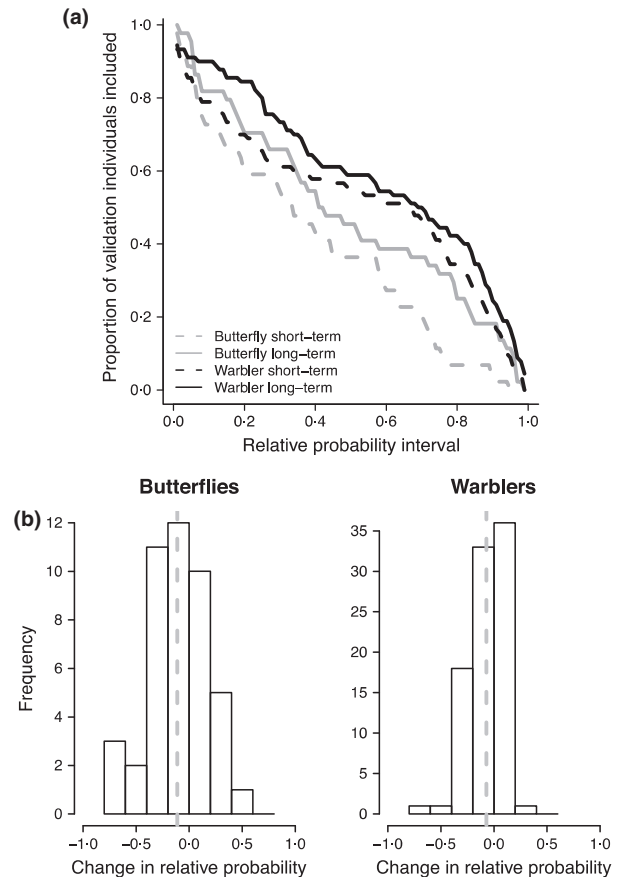
Fig. 3. (a) Population-level accuracy was measured as the proportion of validation individuals in which the known origin was contained within the relative probability interval. Accuracy increased with decreasing relative probability and was lower for the short-term models than the long-term models in both species. (b) Individual-level accuracy was measured as the change in relative probability (short-term minus long term) between assignments at the known origin. The mean change (denoted by the dotted grey line) was significantly less than 0 for both species (see results), indicating individual accuracy decreased in the short-term assignments.

very high relative probabilities resulted in very low accuracy, and the change in accuracy across the range of relative probability intervals was nearly linear (Fig. 3a). Population-level accuracy was generally higher when using long-term precipitation models compared to short-term models and was also higher for reed warblers than monarch butterflies. Individual-level assignment accuracy was significantly lower for assignments to short-term isoscapes for both species, meaning that the relative probability at the known origin was lower, on average (monarch butterflies: mean = −0·11, $t = -2.71$, d.f. = 43, $P = 0.001$; reed warblers: mean = −0·07, $t = -4.07$, d.f. = 89, $P < 0.001$) (Fig. 3b).

We report precision as an area-standardized value because the surface area of the precipitation isoscape was more than twice as large for the reed warblers (12·6 × 10^6 km^2) than for the monarch butterflies (5·1 × 10^6 km^2). The assignment areas were generally smaller for short-term precipitation models than for the long-term models, meaning that the precision of

(a)

(b)
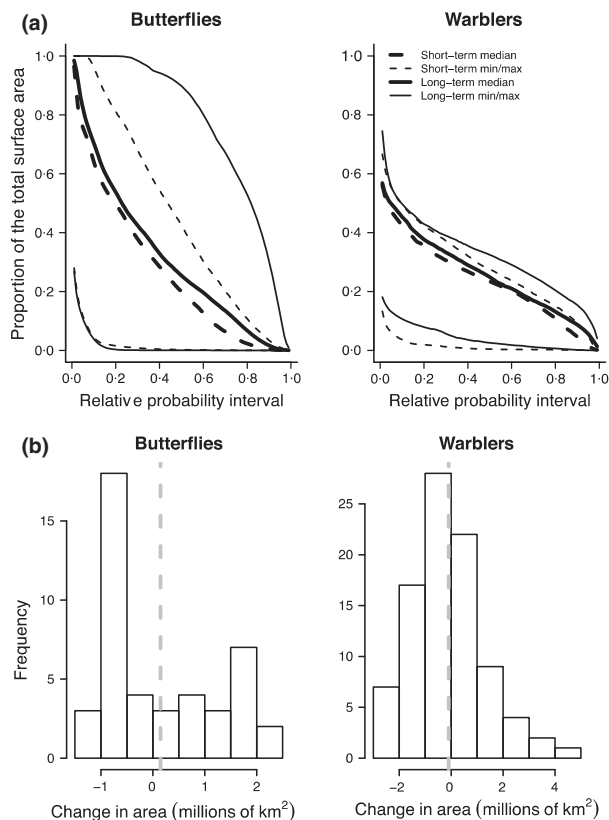
Fig. 4. (a) Population-level precision was measured as the median proportion of the total surface area covered by the posterior probability surface at a given relative probability interval. Minimum and maximum values are also provided for each interval. Precision was generally improved (smaller surface areas) with the use of short-term models for both species. (b) Individual-level precision was measured as the change in area (short-term minus long-term) of the posterior probability surface at a value equal to the relative probability at the known origin. The mean change (denoted by the dotted grey line) was not significantly different from 0 for either species (see results).

the short-term models was higher, but the relationship was nonlinear (Fig. 4a). The relative assignment areas were also generally larger for monarch butterflies than for reed warblers (Fig. 4a). This may be a result of the smaller range in δ^2H_{tissue} values of the monarch wing isoscapes (Fig. 2), such that the overall power to distinguish between locations within the isoscape was lower. Along with a lower precision in the monarch butterfly assignments, the values were also much more variable, as indicated by the range in precision values for a given relative probability interval (Fig. 4a). This is likely due to variation in individual monarch butterfly δ^2H_{tissue} values that represented a larger portion of the total isotopic range in the species (Fig. 2). Individual-level precision did not change significantly for either species between time periods (monarch butterflies: mean $= 0.15 \times 10^6$ km^2, $t = 0.89$, d.f. $= 43$, $P = 0.38$, reed warblers: mean $= -0.10 \times 10^6$ km^2, $t = -0.67$, d.f. $= 89$, $P = 0.50$) (Fig. 4b).

The similarity index was lower for monarch butterflies (0.45) than for reed warblers (0.54), indicating there was generally less similarity between short- and long-term assignments for the monarch butterflies than for the reed warblers (Fig. 5).
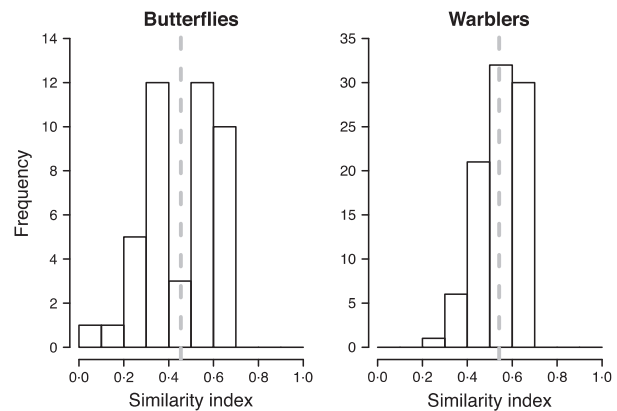
Fig. 5. The similarity index indicates the degree of overlap between assignments using both short-term and long-term precipitation models for each individual. The mean similarity (indicated by the grey dotted line) between assignments was lower for monarch butterflies (0·45) than for reed warblers (0·54), indicating less similarity was observed in monarch assignments between time periods.

The maximum similarity for either species was 0·68, and the minimum was 0·07, indicating that the overlap between assignments was never extremely high, and in some cases, was quite low.

Discussion

SELECTING A BIOLOGICALLY RELEVANT TIME FRAME

We found that posterior probability assignment surfaces for monarch butterflies and reed warblers differed between those based on short- and long-term δ^2H_p models (as indicated by the similarity index). This result indicates that short-term variation in spatial patterns of precipitation isotope values might be expected to influence assignment accuracy and precision. Contrary to our expectations, the targeted use of short-term isoscapes did not improve the efficacy of predicting geographic origin of validation individuals over the use of long-term isoscapes for the species investigated. Short-term isoscapes resulted in a small albeit significant reduction in the individual-level accuracy, but there was little difference in the individual-level precision.

Our analysis incorporates assumptions about the time period over which H fell as precipitation and was propagated into the food web and incorporated in the study organism. The assumptions are reflected in the time ranges selected for the short-term models, which may be incorrect and negatively affect the short-term assignment (i.e., the 'water memory' of the system may be shorter or longer than assumed). In the case of monarchs, wing chitin is formed from milkweed consumed during the larval period (Hobson, Wassenaar & Taylor 1999b). Because rain was the sole source of water to the milkweed in this study, the growing season for milkweed is the maximum period of water memory for this system. However, this season is not uniform, beginning earlier in the south than the north, for example, and if plant growth or larval feeding were unevenly distributed throughout the season, then the true

period of water memory could be different from that assumed here. In the case of reed warblers, δ^2H_{tissue} values of North American insectivorous bird feathers have previously been shown to be well-correlated to amount-weighted mean growing season δ^2H_p values (Bowen, Wassenaar & Hobson 2005; Hobson *et al.* 2012), indicating that the primary months of plant growth are a good proxy for the period of H incorporation into feathers for this group. Although the reed warbler is also an insectivorous bird, we used the mean annual δ^2H_p values rather than growing-season averages, due to its association with wetlands that likely incorporate rainfall throughout the year (Procházka *et al.* 2013).

In the present case studies, the animal tissues demonstrated strong correlation with long-term δ^2H_p values (Hobson, Wassenaar & Taylor 1999b; Procházka *et al.* 2013). In organisms for which the long-term δ^2H_p values have far less explanatory power, short-term δ^2H_p isoscapes may provide a greater improvement in assignment efficacy. This may be the case, for example, in arid regions (unlike the sites studied here) and regions where more annual variation in the isotopic composition of precipitation occurs, such as the south-western United States (Vachon *et al.* 2007).

ACCURACY AND PRECISION

We have demonstrated that in two case studies the use of temporally refined δ^2H_p isoscapes, assumed to reflect the period of animal tissue growth and assimilation of environmental H, did not improve the accuracy of geographic assignments to origin. The observed reduction in accuracy may be related to the data density upon which the short-term δ^2H_p isoscapes were created, specific both to the time period and region. More stations were available over the same surface area in Europe in comparison to the eastern United States (Table 1). In addition, there were three times fewer stations available from which to generate the short-term (vs. long-term) isoscapes in each region (Fig. 1b,d; Table 1), which highlights the gaps in the GNIP data and the need for data collection that is spatially and temporally continuous. The lack of spatial coverage has been improved here by including data from non-GNIP sources, such as the United States Network for Isotopes in Precipitation (USNIP), but these supplementary data may only be available for a limited number of years (Welker 2000).

The reduction in data available for isoscape model parameter estimation might have decreased the predictive power of the short-term isoscapes and those in the United States compared to those in Europe. Furthermore, in contrast to the long-term models, neither short-term model exhibited significant spatial autocorrelation of the model residual values, suggesting the distribution of the short-term precipitation data was insufficient to resolve some of the smaller-scale features reflected in the long-term data set (Bowen 2010). The estimates of uncertainty on the model parameter values, which are based on the correlation structure of the relatively sparse observational data set, may not adequately reflect this small-scale variability for the short-term models, leading to an optimistic estimation of prediction uncertainty. The lower uncertainty values in the short-term precipitation models likely contributed to the decreased accuracy for these assignments.

Short-term assignments exhibited higher population-level precision (as indicated by smaller relative surface areas), but individual-level precision did not change significantly between approaches. The higher population-precision coupled with the lower population-level accuracy in the short-term models suggests an accuracy-precision trade-off. Precision in the assignments was also related to the ratio of the variance and total range in the δ^2H_{tissue} isoscape. For example, population-level precision was lower and more variable for the monarch butterflies such that a larger fraction of the total surface area was selected at any relative probability interval (Fig. 4). This may result from both a smaller range in the δ^2H_{tissue} isoscape in North America and a mean variance term that was nearly double that of the reed warblers, as a proportion of the total isoscape range. Therefore, these differences in the two regions likely contributed to the incongruity in population-level precision patterns.

SOURCES OF VARIATION

There are few migratory animal studies that have examined the effect of temporal variability in δ^2H_p values in making assignments of geographic origin largely due to the fact that the disciplines of biology, isotope hydrology and meteorology traditionally had little overlap. Temporal variation in the isotopic composition of precipitation can be affected by stochastic inter-annual differences in weather (hot spells, drought, cold wet summers, etc.) as well as by fluctuations in storm tracks, dominant climatic oscillations and large-scale climate modes (Bowen 2008; Sjostrom & Welker 2009; Field 2010; Liu *et al.* 2013). Increased variance in the amount of precipitation falling may result in larger deviations from the long-term predictions of δ^2H_p values (Van Wilgenburg *et al.* 2012).

Farmer, Cade & Torres-Dowdall (2008) incorporated temporal variability in determining a minimum separation distance required to distinguish two origins as distinct; however, they calculated separation distance as a monotonic function of latitude, which does not adequately characterize spatial patterns in δ^2H_p. In a second study, Van Wilgenburg *et al.* (2012) suggested that the creation of year-specific δ^2H isoscapes might improve the assignment of individuals origin where models documenting the regional effects of climate variation had been established but lacked synchronous biological and precipitation H isotope data sets to test this hypothesis.

We focused on the temporal variations in δ^2H_p values that ultimately drive δ^2H patterns in food webs, but other types of variation may also affect the ability to correctly predict geographic origins. First, the degree of heterogeneity in the underlying isoscapes can affect the posterior probability distributions, and this issue needs to be explored. Secondly, the uncertainty associated with the δ^2H_p isoscape (σ^2_{precip}) was much higher in the short-term isoscapes, and higher uncertainty occurred on the edges of the spatial domain, likely due to the more limited data density in these time periods (Fig. S5). In general, this component of variance was greater than the

others considered in the current study (σ_{precip} means ranged from 6·7 to 8·0 ‰) and strongly affected the assignments.

Thirdly, organisms may be susceptible to a suite of secondary processes in assimilating water from the diet or drinking water that complicate the relationship between isotopic values in precipitation and those in tissue. For instance, monarchs that are forming wings in the early stages of development, while in the pupa stage assimilate hydrogen from leaf tissue that may reflect appreciable ^2H enrichment associated with plant transpiration (Yakir & Sternberg 2000). For laboratory-reared monarchs, isotopic discrimination between butterflies and their host milkweed plants was substantially smaller than the discrimination that occurred between water and milkweed (Hobson, Wassenaar & Taylor 1999b). In the case of the reed warblers, there is an additional trophic discrimination because they are secondary consumers. Moreover, the routing of food versus meteoric water in migrants may vary among species and further complicate the rescaling function of precipitation water into tissue. The fractional contribution of drinking water to bird feather δ^2H_{tissue} values ranged between 18% and 32% (Hobson, Atwell & Wassenaar 1999a; Wolf, Bowen & Martínez del Rio 2011), though additional feeding and drinking trials may help improve assignments of origin by reducing the uncertainty in the processes that affect the integration of precipitation water H into inert tissue.

Finally, inter-individual variation in δ^2H_{tissue} values contributes to dissimilar isotopic values in tissues grown at the same geographic location (Wunder, Jehl & Stricker 2012). Substantial individual-level isotopic variation exists in both of the organisms used in this study, with differences in δ^2H values of up to 23·3‰ in monarch butterflies and 31·0‰ in reed warblers sampled at the same site. (The use of different isotopic analytical methods in the two studies may affect this comparison.) This variation may be a result of dietary differences or microhabitats in the foraging areas used (Powell & Hobson 2006; Fraser *et al.* 2011; Hobson *et al.* 2012), or it could be inherent variation, such that differences in individual physiologies result in isotopic differences even when diet and environmental conditions are equal (Vander Zanden *et al.* 2012). Wunder (2010) demonstrated that individual isotopic heterogeneity has a much larger effect than typical measurement error on the geographic precision of assignments. Targeting the mechanisms behind the individual isotopic variation may contribute to improvements in the accuracy and precision of geographic assignments (Kelly *et al.* 2008), though investigating these mechanisms was beyond the scope of this study. On the other hand, determining the correlation between precipitation and tissue δ^2H values may be difficult in complex food webs where both plant and animal physiology alter the δ^2H_p signal, and thus, there may be more benefit in developing species-specific isotope basemaps when extensive calibration samples across the species range permit doing so.

ASSESSING GEOGRAPHIC ASSIGNMENT EFFICACY

We used three distinct metrics to evaluate and compare the geographic assignment products. We expect that these metrics will be useful in future studies to determine acceptable study- or organism-specific cutoffs, using validation data sets, to apply in assignments of unknown-origin individuals. Investigators need to choose an acceptable level of risk that balances the accuracy-precision trade-off but that is also relevant to their research or management questions. The similarity index proposed here may have additional use for comparing posterior probability distribution from paired individuals. Although we used the similarity index to compare the assignments of the same individual using two models, it could be used to determine whether two individuals shared a likely origin or to quantify the degree of overlap in regions of likely origin.

Conclusions

We created short-term precipitation isoscapes that we believed represented the time period over which $\delta\ ^2H_p$ was routed through food webs and into tissues sampled in two case studies of migratory animals. Despite the temporal heterogeneity in δ^2H_p values, the use of seasonally targeted, short-term isoscapes did not improve assignment accuracy for these case studies. It is probable that assignment efficacy could be improved for other organisms or regions, particularly if equal spatial coverage of precipitation collecting stations was available in the short-term periods. Additionally, in regions where inter-annual isotopic variation in rainfall is substantial, the usefulness of developing time-specific isoscapes may be enhanced. We suggest that efforts to incorporate other sources of δ^2H_p measurements and collect future precipitation data that are both spatially and temporally continuous will increase our understanding of the importance of variation in δ^2H_p isoscapes for ecological applications. We recommend continued investigation into how variability in precipitation δ^2H values affects both inter-individual variations in δ^2H_{tissue} values and geographic assignment ability.

Acknowledgements

Data accessibility

R scripts: uploaded as online supporting information.

Butterfly and warbler data sets: these data originate from previously published articles (doi: 10.1111/j.1600-048X.2013.00185.x and 10.1007/s004420050872), and data from the sites used in this study are included in the online supporting information.

Precipitation isoscapes: area available at http://isomap.org with the job key numbers provided in Table 1.

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Supporting Information

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

Appendix S1. Additional description of methods plus tissue and precipitation isoscapes and associated error.

Table S1. Monarch butterfly sampling site latitude, longitude, elevation, as well as mean, range, SD of δ^2H_{tissue} values and number of individuals sampled (divided by calibration and validation sites).

Table S2. Reed warbler sampling site latitude, longitude, elevation, as well as mean, range, SD of δ^2H_{tissue} values and number of individuals sampled (divided by calibration and validation sites).

Fig. S1. Values of δ^2H in precipitation across North America and Europe representing short- (a, c, d, e) and long-term (b, f) time periods. (a) 1996, (b) 1960–2000, (c) 2004, (d) 2005, (e) 2006, (f) 1960–2006.

Fig. S2. Rescaling functions used to convert precipitation isoscapes to tissue-specific isoscapes with ordinary least squares regression.

Fig. S3. Values of δ^2H_{tissue} in monarch butterfly (North America) and reed warbler (Europe) tissues representing short- (a, c, d, e) and long-term (b, f) time periods. (a) 1996, (b) 1960–2000, (c) 2004, (d) 2005, (e) 2006, (f) 1960–2006.

Fig. S4. Values of $\sigma_{rescale}$ across North America and Europe representing short- (a, c, d, e) and long-term (b, f) time periods.

Fig. S5. Values of σ_{precip} across North America and Europe representing short- (a, c, d, e) and long-term (b, f) time periods.

Appendix S2. R codes used to perform and evaluate assignments.