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CONCEPTS, REVIEWS AND SYNTHESES

Fundamental limits to the accuracy of deuterium isotopes for identifying the spatial origin of migratory animals

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Abstract Deuterium isotope analyses have revolutionized the study of migratory connectivity because global gradients of deuterium in precipitation (δD_P) are expressed on a continental scale. Several authors have constructed continental scale base maps of δD_P to provide a spatial reference for studying the movement patterns of migratory species and, although they are very useful, these maps present a static, 40-year average view of the landscape that ignores much underlying inter-annual variation. To more fully understand the consequences of this underlying variation, we analyzed the GNIP deuterium data, the source for all current δD_P maps, to estimate the minimum separation in δD_P (and latitude) necessary to conclude with a given level of confidence that distinct δD_P values represent different geographic sites. Extending analyses of δD_P successfully to deuterium in tissues of living organisms, e.g., feathers in migratory birds (δD_F), is dependent on the existence of geographic separation of δD_P, where every geographic location has a distribution of values associated with temporal variability in δD_P. Analyses were conducted for three distinct geographic regions: North America, eastern North America (east of longitude 100°W), and Argentina. At the 80% confidence level, the minimum separation values were 12, 7, and 14° of latitude (equivalent to 53, 31, and 32‰) for North America, eastern North America, and Argentina, respectively. Hence, in eastern North America, for example, one may not be able to accurately assign individual samples to sites separated by less than about 7° of latitude as the distributions of δD_P were not distinct at latitudes <7° apart. Moreover, two samples that differ by less than 31‰ cannot be confidently said to originate from different latitudes. These estimates of minimum separation for δD_P do not include other known sources of variation in feather deuterium (δD_F) and hence are a first order approximation that may be useful, in the absence of more specific information for the system of interest, for planning and interpreting the results of new stable isotope studies.

Keywords Stable isotope · Migration · Deuterium · Isotope map

Introduction

Deuterium isotope analyses have revolutionized the study of migratory connectivity (Kelly et al. 2002; Webster et al. 2002; Hobson 2005) because global gradients of deuterium in precipitation (δD_P) are expressed on a continental scale. Migration studies have been aided by the Global Network for Isotopes in Precipitation (GNIP) database, administered by and made available online by the International Atomic Energy Association (IAEA/WMO 2001). These water isotope data are comprised of monthly measurements of δD_P from many points around the world, collected over more than 40 years from about 1960 to 2004. Several authors have used these point data, with various models

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that include covariates such as temperature and altitude, and constructed continental scale base maps of δD_P (Hobson and Wassenaar 1997; Meehan et al. 2004; Bowen 2003; Bowen and Revenaugh 2003; Bowen et al. 2005).

These maps generally have several attributes in common. First, they are based on "mean annual" δD_P or the "growing season" δD_P, computed for each of the points in the GNIP dataset. Second, the mean annual or growing season δD_P is averaged over all years in the GNIP database to depict an average condition at each point. Third, these 40-year averages are extrapolated from, or interpolated between, the data points to produce a two-dimensional depiction of how δD_P is expected to vary across the face of the earth.

From the perspective of science and conservation, these deuterium base maps are a potentially powerful tool because they provide a spatial reference for identifying animal movement patterns. This is possible for two reasons. First, δD_P varies spatially according to relatively well understood atmospheric processes and, consequently, there are strong latitudinal gradients around the world, including North America and the southern cone of South America (Dansgaard 1964; Bowen et al. 2005). Second, as a consequence of its diet, an animal's body tissues reflect the isotopic signal of its local environments (Vogel et al. 1990; Lajtha and Michener 1994; Kelly and Finch 1998; Hobson 1999; McCarthy and Waldron 2000; Kelly et al. 2002; Rubenstein and Hobson 2004). Hence, as an animal moves across isotopic gradients, its various tissues reflect isotopic signals that are useful clues that can be used to reconstruct the history of its movements.

Seasonally-grown tissues such as hair, nails, and feathers are especially valuable for this purpose because they provide a chemical signal of habitats used during relatively short time periods during the annual cycle (Rubenstein and Hobson 2004). Feathers, in particular, are a useful tissue for migratory bird studies because they grow during a short period of time, after which they become inert and lock in the isotopic signature of the habitat where they were grown (Hobson and Clark 1992; Bearhop et al. 2002; Mazerolle and Hobson 2005). Migratory birds carry in their different feather tracts isotopic markers of the various seasonal habitats used throughout the year. Using knowledge about when (during which season) a particular feather tract is molted, combined with measured values of feather deuterium (δD_F) and a deuterium base map, one can, in principal, begin to identify the location of seasonal habitats used by a particular bird population.

In practice, however, measured values of δD_F can differ from base-mapped δD_P due to a number of factors, some of which are well understood but many of which are not (Hobson 2005). Early efforts to compare growing season δD_P and feather deuterium (δD_F) in forest passerines estimated about 30‰ enrichment in feathers (Hobson and Wassenaar 1997). A recent regression analysis of growing season δD_P and δD_F from migratory raptors (Lott and Smith 2006) suggests that there is no significant fractionation between precipitation and feathers on average at the scale of North America, but that there might be a net fractionation within some regions. Current uncertainty about the factors that decouple δD_P and δD_F suggest that it would not be advisable to apply a generic fractionation factor, and that the relationship between δD_P and δD_F needs to be investigated on a case-by-case basis (Bowen et al. 2005).

A fundamental factor contributing to observed differences between δD_P and δD_F is the inter-annual variation in δD_P, expressed in complex ways across a variety of temporal and spatial scales (Bowen 2008). Much of recent isotope ecology has been focused on the use of deuterium maps, and it is easy to forget that these maps present a static, 40-year average view of the landscape that ignores this underlying variation. The utility of current δD_P base maps therefore hinges on how closely the 40-year average δD_P values correspond to the deuterium composition of feathers (δD_F) (or other seasonally-grown tissues), which is determined by the animal's physiological state and its diet during a relatively short period of the annual cycle (Bearhop et al. 2002).

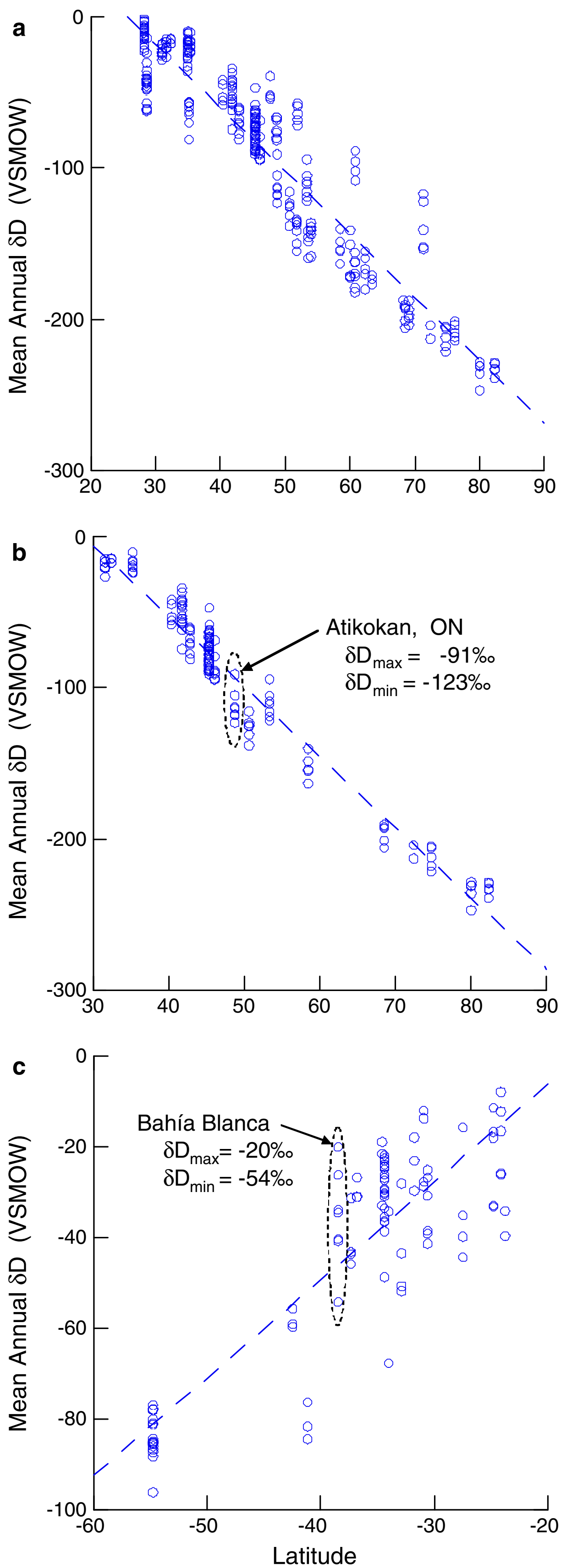
Our purpose in this paper is to explore the potential consequences of the inter-annual variation in δD_P for the accuracy of geographic assignments made with deuterium isotopes. Our personal research focus, indeed much of the ecological literature, concerns feather analyses and migratory bird movements. However, the statistical techniques that we employ and the results that we achieved should be equally useful for the study of other taxa by investigators proposing to begin new isotope studies with the goal of assigning individual animals to specific geographic origins.

Materials and methods

GNIP data

We downloaded the annual deuterium data from the GNIP website (IAEA/WMO 2001). These data are available in Microsoft Excel format for many sites and years (through about 2004) across the entire globe. We selected mean annual deuterium data for subsets of sites in North America [records for the US (USA), Canada (CA), and Mexico (MX)] and for South America [records for Argentina (AR)].

From the North America data, we produced two data files for analysis. The first file contains all the records for



◀ **Fig. 1** Mean annual deuterium (δD_P) for all available years from the GNIP yearly database versus degrees latitude (southern hemisphere latitudes are shown as negative values) for: **a** all North America sites north of 25°N latitude and between 60 and 170°W longitude; **b** "eastern" North American sites ($n = 17$) north of 25°N latitude and between 60 and 100°W longitude, and **c** all sites for Argentina that had two or more years of data ($n = 17$). *Dashed lines* are the least squares regression of δD_P on latitude. Regression summary statistics are (a) $r^2 = 0.87$, $\hat{\beta}_1 = -4.12$ (95% CI = −4.32, −3.93), $F_{1,246} = 1689$, $P \ll 0.001$; (b) $r^2 = 0.96$, $\hat{\beta}_1 = -4.67$ (95% CI = −4.83, −4.51), $F_{1,142} = 3{,}366$, $P \ll 0.001$; and (c) $r^2 = 0.75$, $\hat{\beta}_1 = 2.16$ (95% CI = 1.90, 2.41), $F_{1,95} = 285$, $P \ll 0.001$. Scatter about the regression line is mostly due to inter-annual variation within collection sites (Atikokan, ON data points for 7 years, and Bahia Blanca data points for 7 years are identified as examples)

sites north of latitude 25°N, and between 60 and 170°W longitude, to include all sites north of the Caribbean region. The latitudinal pattern of δD_P in this region is complicated by the mountain ranges of the western USA and Canada; hence, we created a second data file comprised of "eastern" sites, including those sites with longitude between 60 and 100°W. This eastern region is characterized as having a strong, linear relationship between δD_P and latitude (see δD_P maps in Bowen 2003). The δD_P for these two data files is shown in Fig. 1 and the sites comprising the eastern region are listed in Table 1. The δD_P data for Argentina (Fig. 1) are from all sites (Table 2) that had data for more than 1 year. Linear relationships with latitude appeared adequate for the three statistical models we estimated because they did not include wide spans of latitude near the equator, nor across the northern and southern hemispheres, which would have required nonlinear functions (e.g., Bowen et al. 2005).

Statistical analyses

The regression lines for the relationships between δD_P and latitude for North and South America (Fig. 1) have a precise, highly significant non-zero slope, conveying the strong latitudinal pattern of deuterium. Knowing the slope of this relationship and the proportion of variance explained (r^2), however, is not sufficient information to conclude that the distributions of δD_P are well separated at two distinct latitudes that are d units apart. To evaluate this requires incorporating information about the dispersion (e.g., the standard deviation of a normal distribution) of the probability distribution around the mean of δD_P at any latitude. We used an approach to evaluate how effectively the linear statistical relationship distinguishes among distributions of δD_P at given latitudes based on the technique of "separation of distributions" developed by Xie and Nelson (2003).

Table 1 Collection site statistics for the "eastern" North American sites ($n = 17$) north of 25°N latitude and between 60 and 100°W longitude. Deuterium (δD_P) is reported as a mean annual as well as the minimum and maximum annual values reported over the number of years of record for each site (n)

Site name	Latitude (°N)	Longitude (°W)	δD_P (‰ VSMOW)			n (years)
			Min.	Max.	Mean	
Waco, TX	31.62	97.22	−27	−15	−20	8
Hatteras, NC	35.27	75.55	−24	−10	−19	8
Coshocton, OH	40.37	81.80	−58	−42	−51	5
Chicago (Midway), IL	41.78	87.75	−74	−34	−50	16
Simcoe, ON	42.85	80.27	−81	−60	−69	8
Ottawa, ON	45.32	75.67	−91	−63	−78	31
Truro, NS	45.37	63.27	−73	−47	−63	9
Ste. Agathe, QB	46.05	74.28	−94	−69	−88	8
Atikokan, ON	48.75	91.62	−123	−91	−111	7
Gimli, MB	50.62	96.98	−138	−115	−126	6
Goose bay, NL	53.32	60.42	−122	−94	−110	7
Churchill, MB	58.45	94.00	−163	−140	−152	5
Hall beach, NT	68.47	81.15	−205	−190	−196	5
Pond inlet, NT	72.40	78.00	−212	−203	−208	2
Resolute bay, NU	74.72	94.98	−221	−204	−212	5
Eureka, NT	80.00	85.56	−247	−228	−234	5
Alert, NT	82.30	62.20	−239	−228	−232	5

For the common homogeneous variance regression model used to relate δD_P to latitude, evaluating separation of distributions of δD_P at specified latitudes requires comparing an estimated upper percentile point (τ) of the probability distributions of δD_P at a latitude x to the corresponding lower percentile point $(1 - \tau)$ of the probability distribution of δD_P at $x \pm d$ degrees separation (Fig. 2a). The separation distance (d) required to have distinguishable distributions is a function of the degree of overlap based on the selected quantile (τ), slope (β_1) of the regression model, and the dispersion of the observations around the regression relation (e.g., $\hat{\sigma}$ = standard error of the estimate). The higher the percentile (τ) chosen the less overlap [= $2 \times (1 - \tau)$] in distributions of δD_P at latitudes x and $x \pm d$ degrees and the greater confidence we can have that the distributions are well separated in the sense of representing distinctly different conditional distributions of δD_P. However, for a given slope and dispersion, decreasing the acceptable overlap (e.g., for $\tau = 0.95$ versus $\tau = 0.90$) requires greater values of separation d (compare Fig. 2a, b). Greater $\left|\hat{\beta}_1\right|$ (absolute value of estimated slope) provides separation at shorter d for a given τ and dispersion (compare Fig. 2a, c). Greater dispersion for a given slope estimate $\left(\hat{\beta}_1\right)$ and percentile τ requires greater separation distances d (compare Fig. 2a, d).

Xie and Nelson (2003) estimated separation distances d by assuming the conditional probability distributions were homogenous normal distributions, i.e., standard assumptions for ordinary least squares regression and used in Fig. 2. Thus, Xie and Nelson (2003) determined the percentile point for a given τ by multiplying the τth quantile of a standard normal deviate value by the standard error of the estimate [$\hat{\sigma}$ = (mean square error)$^{0.5}$] from an ordinary least squares regression model of the conditional mean of y (δD_P) as a linear function of X (latitude). However, it is also possible to estimate the percentile points for a given τ directly with linear quantile regression without assuming any specific parametric distributional form for the errors (Cade and Noon 2003; Koenker 2005). We used this latter approach with the modification of He (1997) to provide quantile regression estimates with a common slope for τ and $1 - \tau$ quantiles as was appropriate for a homogenous regression model. The quantile regression formulation makes it very easy to see that the separation distance based on the estimated quantiles $\hat{Q}_Y(\tau|X) = \hat{\beta}_0(\tau) + \hat{\beta}_1 X$ and $\hat{Q}_Y(1-\tau|X) = \hat{\beta}_0(1-\tau) + \hat{\beta}_1 X$ in the homogeneous regression model, where $y = \delta D_P$ and X = latitude, is given by $d = [\hat{\beta}_0(\tau) - \hat{\beta}_0(1-\tau)]/|\hat{\beta}_1|$, for $\hat{\beta}_1 \neq 0$ and $\tau \geq 0.50$. We provided estimates of separation distance d with 10, 20, and 30% overlap in distributions of δD_P corresponding to $d(0.90)$, $d(0.80)$, and $d(0.70)$ by using estimates for $\tau = 0.95$, 0.90, and 0.85, respectively. Xie and Nelson (2003) proposed that 20% overlap associated with $d(0.80)$ indicated that the conditional distributions were well separated because the upper 90% of the one with the larger mean was above the lower 90% of the one with the smaller mean. We also provide the normal theory, ordinary least square regression estimates of separation distances (Xie and Nelson 2003) for comparison.

Results

Our estimates of separation distance required for distributions of δD_P to be well separated was 12° of latitude [$d(0.80)$] for the entire North American precipitation data set and was 6.8° of latitude for the eastern North American precipitation data (Table 3; Fig. 3). The Argentina precipitation data set required 13.8° of latitudinal separation for distributions of δD_P to be well separated (Table 3; Fig. 3). The 12° of latitudinal separation required for distinguishing separate δD_P distributions for North America is 22% of the latitudinal range of the precipitation data whereas the 13.8° required for Argentina is 44% of the

Table 2 Collection site statistics for Argentine sites ($n = 17$) with two or more years of data. Deuterium (δD_P) is reported as a mean annual as well as the minimum and maximum annual values reported over the number of years of record for each site (n)

Site name	Latitude (°S)	Longitude (°W)	δD_P (‰ VSMOW)			n (years)
			Min.	Max.	Mean	
Purmamarca	23.75	65.50	−39	−34	−37	2
Los Molinos	24.11	65.19	−26	−8	−17	5
Salta	24.78	65.40	−33	−12	−23	5
Corrientes	27.47	58.83	−44	−15	−33	4
La Suela	30.58	64.58	−41	−25	−33	6
Mar Chiquita	30.92	62.67	−28	−12	−21	4
Santa Fe	31.75	60.73	−30	−18	−24	3
Mendoza	32.88	68.85	−52	−28	−44	4
Nancunan	34.03	67.97	−68	−34	−51	2
Ciudad Universitaria	34.38	58.28	−48	−22	−29	21
Buenos Aires	34.58	58.48	−33	−19	−25	3
Azul	36.80	59.83	−31	−27	−30	3
Padre Buoda	37.32	64.33	−46	−31	−41	4
Bahia Blanca	38.47	62.16	−54	−20	−35	7
Bariloche	41.15	71.33	−85	−76	−81	3
Puerto Madryn	42.48	65.05	−60	−56	−58	3
Ushuaia	54.78	68.28	−96	−77	−84	18

latitudinal range of the precipitation data. Decreasing the amount of overlap to 10% [$d(0.90)$] and, thus, increasing the confidence in our predictions required greater latitudinal separation (Table 3) as expected. Estimated separation distances based on percentiles of a normal distribution were within 1–2° of latitude of the estimates based on quantile regression. The greatest discrepancies between the methods were associated with the Argentina data set which had more pronounced skewness in the lower quantiles of the δD_P distribution indicating the normal distribution was a poor approximation (Table 3).

A simple interpretation of our results for the entire North American precipitation pattern for a single new sample of annual δD_P from an unknown latitude is that we cannot distinguish its predicted latitudinal origination with great confidence within an interval of latitudes <12°. Thus, if we had predicted that an observed annual δD_P came from latitude 56°N, we would expect the distribution of δD_P at this latitude to be indistinguishable from δD_P distributions at latitudes between 50 and 62°N. Or, given two predicted latitudes <12° apart based on mean δD_P, we cannot really distinguish between their distributions of annual δD_P because they are not well separated. For example, a predicted distribution of annual δD_P at 56°N cannot be distinguished from the distribution at 67°N although we can distinguish it from distributions at ≥68°N. We can also turn these intervals of latitudinal separation into intervals of ‰ separation of δD_P by algebraic computations with the regression function for the mean. Expressed in terms of differences in ‰ of δD_P, the corresponding distributions of δD_P that are well separated by latitudes would have mean δD_P that differ by ≥53.5‰ (Table 3). Similar statements with different values from Table 1 could be made for eastern North America and Argentina.

Discussion

The use of stable isotopes, especially deuterium, continues to revolutionize the study of animal movement patterns. Isotope base maps, including current deuterium base maps developed from GNIP data, are an integral and necessary component of the technology driving that revolution. Like all tools, however, base maps can be misused if their limitations are not well understood. In the rapidly developing scientific field of stable isotope applications to study animal movements, it is important to strive to understand these limitations, to understand the dynamics of study systems, and to develop realistic expectations of prediction accuracy.

An analogy with remote sensing approaches may serve to put deuterium isotopes in perspective. There exists a range of remote sensing platforms and sensors, from orbiting satellite scanners to low-level airborne video. Each of these is perhaps best suited for detecting a particular type of reflectance at a particular resolution. The current deuterium base maps are analogous to satellite imagery; they provide a large-scale, but relatively low resolution, view of the landscape. They display patterns of interest at a regional or continental scale, but may not be very useful to sort out patterns at a local scale, such as movements that may occur within a single season. The limits to the

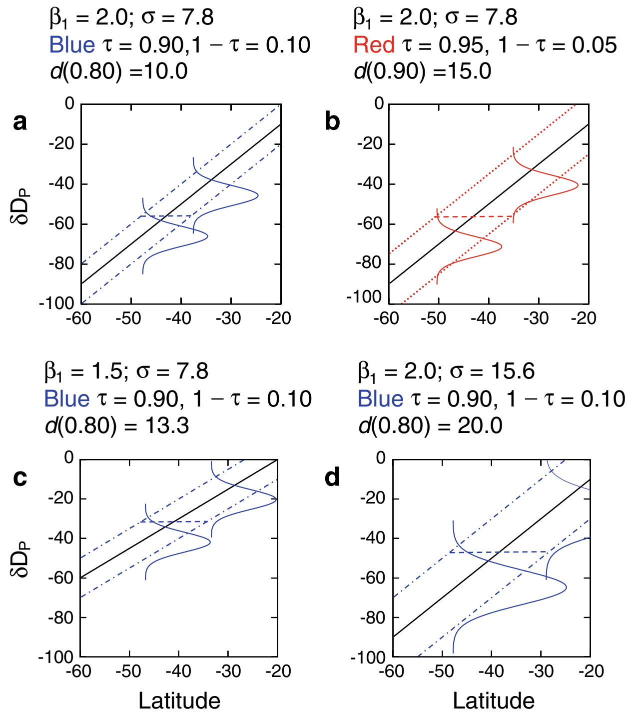


Fig. 2 Examples of separation of distributions in regression models with homogeneous, normally distributed deuterium (δD_P) as a linear function of degrees latitude. *Upper* and *lower dot-dashed lines* are the $\tau = 0.90$ and $\tau = 0.10$ conditional quantiles of the normal distributions with standard deviations given in the panels. *Upper* and *lower dotted lines* are the $\tau = 0.95$ and $\tau = 0.05$ conditional quantiles of the normal distribution with standard deviation given in the panel. *Solid lines* are the conditional means. *Horizontal dashed lines* correspond to the specified separation distance for the normal probability distributions. In **a** the intercept terms for the conditional quantiles are $\beta_0(0.90) = 40$ and $\beta_0(0.10) = 20$; **b** the intercept terms for the conditional quantiles are $\beta_0(0.95) = 45$ and $\beta_0(0.05) = 15$; **c** the intercept terms for the conditional quantiles are $\beta_0(0.90) = 40$ and $\beta_0(0.10) = 20$; and **d** the intercept terms for the conditional quantiles are $\beta_0(0.90) = 50$ and $\beta_0(0.10) = 10$

resolution of deuterium maps arise because the level of deuterium in tissues is decoupled from the mapped values of δD_P by factors at three levels. First, animal tissues such as feathers do not precisely reflect the isotope signatures of an animal's diet because fractionation can occur along the biochemical chain as materials are assimilated into tissue. Second, the diet may not reflect the isotopic values of precipitation because the δD of local surface and ground water can be modified by local biogeochemical processes, for example evaporation. And third, the δD_P for any single year can be quite different from the 40-year mean value. Even if the first two factors were insignificant, feather deuterium can be quite different from the mapped δD_P because these two measures of deuterium represent a fundamentally different time frame. Bird feathers grow within a period of days or weeks, capturing the isotopic signature of food and water ingested during and immediately prior to that period of time. These short-term conditions may differ substantially from the mean annual value of deuterium in the system. Furthermore, the mean annual (and the growing season) value of δD_P can vary at a given site by as much as 30‰ between years (see Fig. 1). Hence, bird feathers collected over a number of years from a given site may falsely appear to have originated from widely separated sites.

Our task in this paper was to estimate a statistical parameter (minimum separation) that could be used to identify distinct differences in distributions of deuterium in precipitation that might prove useful for identifying geographic origins of organisms. Although we based our estimates (Table 3) on an analysis of mean annual precipitation, a similar analysis of "growing season" values or any other seasonally constrained values would produce comparable measures of separation. Of the three factors mentioned above that potentially contribute to decoupling of tissue and mapped δD_P values, inter-annual variation in δD_P is a fundamental source of uncertainty in all systems and for all stable isotope applications that make geographic assignment of individual samples. In a future with much enhanced computer technology and more rapid dissemination of information it might be possible to produce deuterium maps for individual years, months, or weeks,

Table 3 Summary statistics for separation distance expressed in both mean parts per mil (‰) and degrees of latitude based on quantile regression (QR) and ordinary least squares (OLS) regression with an assumed normal distribution

Region	SEP(d)	QR separation (mean ‰)	QR separation (°latitude)	OLS separation (mean ‰)	OLS separation (°latitude)
North America	0.9	76.110	17.087	72.509	17.586
	0.8	53.501	12.011	56.494	13.702
	0.7	42.244	9.484	45.688	11.081
E. North America	0.9	42.276	9.124	41.342	8.854
	0.8	31.498	6.798	32.210	6.899
	0.7	26.632	5.748	26.050	5.579
Argentina	0.9	39.696	16.704	39.575	18.335
	0.8	32.713	13.766	30.834	14.285
	0.7	25.930	10.912	24.936	11.553

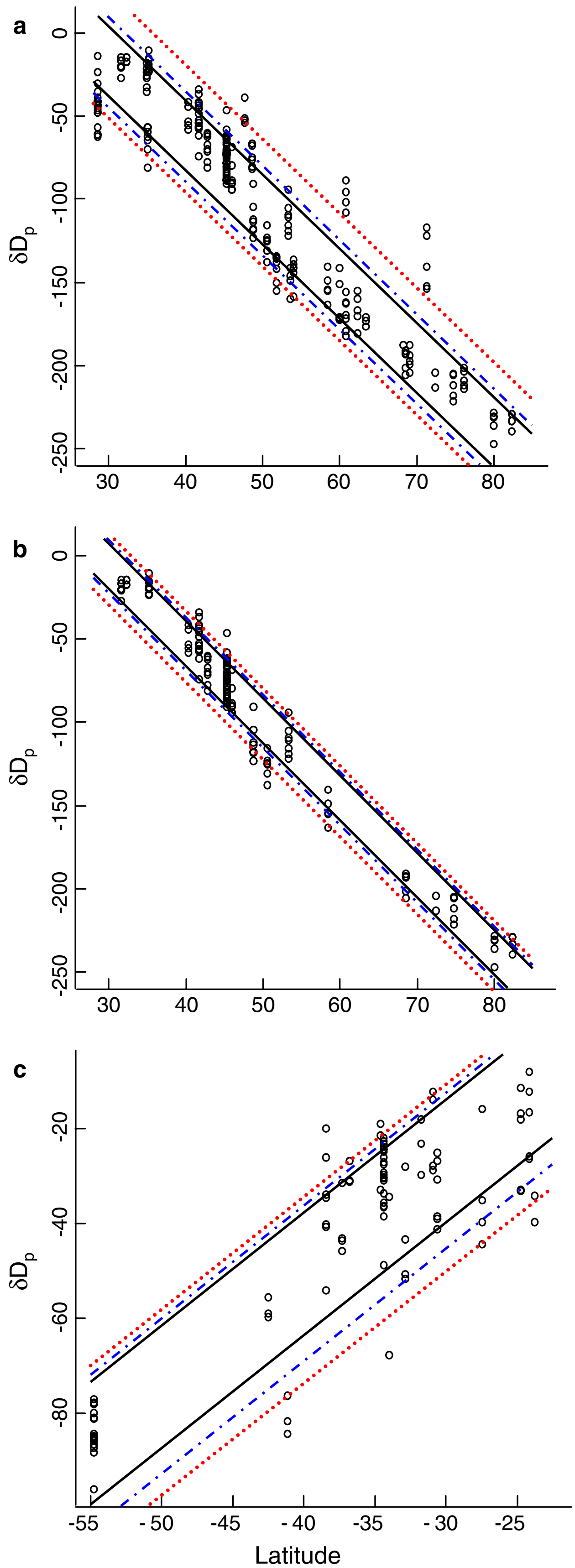


Fig. 3 Mean annual deuterium (δD_P) for all available years from the GNIP yearly database versus degrees latitude (southern hemisphere latitudes are shown as negative values) for: **a** all North America sites north of 25°N latitude and between 60 and 170°W longitude; **b** "eastern" North American sites ($n = 17$) north of 25°N latitude and between 60 and 100°W longitude, and **c** all sites for Argentina that had two or more years of data ($n = 17$). *Dotted lines* are the $\tau = 0.95$ (*upper*) and $\tau = 0.05$ (*lower*) homogeneous quantile regressions used to estimate $d(0.90)$; *dot-dashed lines* are the $\tau = 0.90$ (*upper*) and $\tau = 0.10$ (*lower*) homogeneous quantile regressions used to estimate $d(0.80)$; and *solid lines* are the $\tau = 0.85$ (*upper*) and $\tau = 0.15$ (*lower*) homogeneous quantile regressions used to estimate $d(0.70)$. Slope and intercept estimates are (a) $\hat{\beta}_1 = -4.45$, $\hat{\beta}_0(0.95) = 158.2$, $\hat{\beta}_0(0.90) = 142.4$, $\hat{\beta}_0(0.85) = 137.8$, $\hat{\beta}_0(0.15) = 95.5$, $\hat{\beta}_0(0.10) = 88.9$, and $\hat{\beta}_0(0.05) = 82.1$; (b) $\hat{\beta}_1 = -4.63$, $\hat{\beta}_0(0.95) = 151.9$, $\hat{\beta}_0(0.90) = 148.0$, $\hat{\beta}_0(0.85) = 145.9$, $\hat{\beta}_0(0.15) = 119.3$, $\hat{\beta}_0(0.10) = 116.5$, and $\hat{\beta}_0(0.05) = 109.6$; and (c) $\hat{\beta}_1 = 2.38$, $\hat{\beta}_0(0.95) = 60.6$, $\hat{\beta}_0(0.90) = 58.6$, $\hat{\beta}_0(0.85) = 57.2$, $\hat{\beta}_0(0.15) = 31.3$, $\hat{\beta}_0(0.10) = 25.9$, and $\hat{\beta}_0(0.05) = 20.9$

and therefore reduce this uncertainty. But there will always be uncertainty in geographic assignment, and we must learn to deal with this uncertainty in stable isotope studies. How might our estimates of minimum separation be interpreted and utilized for that purpose?

Some studies have aimed to discriminate between origins at a small spatial scale, with a geographic span less than minimum separation. For example, Szymanski et al. (2007) applied deuterium analysis to discriminate Mallards origins across a latitude range of 5° in northern central USA (North and South Dakota, Minnesota and Wisconsin). Wunder et al. (2005) analyzed the utility of deuterium to determine Mountain Plover origin across 9 degrees of latitude in the western United States. Hobson et al. (2001) studied the usefulness of deuterium to discriminate between Bicknell's Thrushes origin spanning only 5° of latitude. Finally, in our own work on Neartic shorebirds (Torres-Dowdall et al., unpublished manuscript) we tried to apply deuterium analysis to discriminate between White-rumped Sandpiper wintering sites covering a latitudinal range of 7° in northern Argentina. All of these studies found that deuterium fails to discriminate between origins, and combining deuterium with other markers (e.g., $\delta^{13}C$, $\delta^{15}N$) increased the assignment rate. Working in Mexico, Central, and South America (a region with deuterium gradients less distinct than in most of North America), Kelly et al. (2008) found that they could only distinguish two large winter regions: Central Mexico and Ecuador. These studies do not suggest that deuterium is an inappropriate marker; rather that the scale of application was simply too small given the necessary minimum separation for the study region.

The inter-annual variation in δD_P has other implications for ecological studies. Some studies have attempted to infer site fidelity using deuterium analysis (Hobson et al. 2004a, b; Yohannes et al. 2007). In this type of study, stability of deuterium values among years is the test statistic; if freshly molted feathers and feathers molted in the previous year are similar in an individual, then it is inferred that this individual used the same site, otherwise it is inferred that the individual used different sites in successive years. Such an analysis could be misleading, however, if inter-annual variability results in different δD_F values across years, or if site fidelity is inferred from a comparison between δD_F and expected values from a deuterium map. Our results suggest that successive years must differ by the minimum separation (e.g., 31‰ in eastern North America) (Table 3) to be 80% confident in rejecting a hypothesis that an individual used the same site in successive years.

Studies conducted with deuterium samples collected across years and that span large regions are likely to be more robust than small-scale, single-year analyses. For example, we believe that the conclusions drawn by Norris et al. 2004 seem to be well within the resolving power of deuterium, because they do not try to assign to specific sites and their study region spans more than 20° in eastern North America. In another large-scale, multi-year study, Mazerolle et al. (2005) attempted to use variance in δD_F to infer the span of area used by a population. Their logic was that a high variance (as compared to control sites) would indicate that individuals came from multiple sites. However, their results do not make a convincing case for multiple sites because their δD_F data did not span more than 31‰, the minimum separation to make such a decision in eastern North America. Moreover, Bowen (2008) has recently shown that there is a spatial component to variation in δD_P, and some sites are inherently more variable than others.

Some estimates of resolving power of deuterium differ substantially from our minimum separation values. Hobson (2005), citing Meehan et al.'s (2001) data on Cooper's Hawks, reported that assignment of individuals can be accurate to within 1.5° of latitude (equivalent to 3‰). This was an improper interpretation of Meehan et al.'s (2001) data, however, because the 3‰ was the confidence interval on the population mean. The better indication of accuracy for assignment of individuals would have been their prediction interval, which was about ±16‰ (or an interval of 32‰), much closer to our estimates of minimum separation for eastern North America. Moreover, their prediction interval was derived for a regression between δD_F and δD_P but was based on data from only one year. If multiple years were included, their prediction interval would no doubt increase in magnitude to more than 32‰.

Bowen et al. (2005) also analyzed variation in feather deuterium from North American warblers (Hobson and Wassenaar 1997). Using selected δD_F values of −150, −100, and −50‰ they derived 95% prediction regions that are similarly sized (~10° latitude) to our separation distances for North America. Their approach includes other sources of variation in addition to inter-annual variation in δD_P, but their data were for North American forest birds, and hence their confidence regions may not apply for other bird groups that use other habitats. The prediction intervals for a single new observation used by Bowen et al. (2005) are conceptually related to the separation of distributions calculated based on an assumed normal distribution (Xie and Nelson 2003). Estimates of separation of distributions based on the normal distribution do not include the smaller component of variation associated with the sampling variation of the estimated mean (a function of $\hat{\sigma}n^{-0.5}$) as do prediction intervals for a new observation. However, hypothesis tests and confidence intervals for estimates of separation of distributions that account for sampling variation are discussed in Xie and Nelson (2003) although we did not perform those calculations here.

Minimum separation (or related prediction intervals) for a single new observation of deuterium in precipitation allows investigators to evaluate the feasibility of testing specific hypotheses about bird distribution or movement patterns in the absence of prior information on feather isotope data. The annual variability in isotopes in precipitation can be considered a base amount of variability of isotopic signatures likely to be found in bird feathers until additional information is obtained to refine estimates. Estimating separation of distributions can also be applied to isotopes in feathers (e.g., δD_F) as a function of isotopes in precipitation (e.g., δD_P). Although we analyzed only latitudinal gradients, one could easily extend the technique to the multivariate case (e.g., latitude and longitude) to explore more complex geographical patterns. Nonlinear, quadratic functions of latitude are required for statistical models that include latitudes near the equator, or across northern and southern hemispheres (Bowen et al. 2005). Separation of distributions can be estimated for quadratic polynomial functions of latitude although the algebraic computations are more complicated that those used for the linear function.

Our estimates of minimum separation are only meaningful, however, if bird diets reflect the same magnitude of inter-annual variation as seen for δD_P. This may or may not be the case, depending on local biogeochemical processes in the system being studied. Some systems, for example the larger lakes and wetlands in our shorebird studies, buffer the inter-annual variation in δD_P. They have a relatively slow turnover rate in their water budget; hence, the aquatic food chains on which shorebirds depend are based on a heterogeneous mixture of water derived from precipitation events across many months and perhaps years. The use of deuterium to identify bird origins has been most successful for forest birds in North America (Hobson and Wassenaar

1997; Marra et al. 1998; Hobson et al. 2004a, b; Langin et al. 2007), perhaps because the food chains in these systems are based on sub-surface water that integrates precipitation events over a relatively long period of time. This is not necessary true for all forest systems. For example, some forest systems in the Chaco region of Argentina are based on the current year's precipitation given that the phreatic level is below 70 m, inaccessible to plants (Villagra et al. 2005), and one might expect, *Ceteris paribus*, that δD_F of birds feeding in these systems to more closely track the annual values of δD_P. Birds that are more closely dependent on annual plants might also be expected to reflect more of the inter-annual variation in δD_P. Moreover, feathers from shorebirds that use ephemeral wetlands during the feather molt could reflect the deuterium signature of a single precipitation event during the year, and hence display even more inter-annual variation than the mean annual (or mean growing season) value would suggest.

Finally, our estimates do not include other known sources of variation in feather deuterium (see Wunder and Norris 2008 for an analysis of other potential sources) and hence are a first order approximation that may be useful in the absence of more specific information for the system of interest, for planning new stable isotope studies. Above all, we recommend that if at all possible one undertake to do obtain preliminary source samples from potential study areas to make informed decisions about variability and possible inferences that can be made in ecosystems and for species of interest.

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