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Using stable hydrogen and oxygen isotope measurements of feathers to infer geographical origins of migrating European birds

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Abstract Successful application of stable-hydrogen isotope measurements (δD_f) of feathers to track origins of migratory birds and other wildlife requires a fundamental understanding of the correlation between δD_f and deuterium patterns in rainfall (δD_p) over continental scales. A strong correlation between δD_p and δD_f has been confirmed for birds and insects in North America, but not yet for other continents. Here, we compare δD_f data from resident European birds to new δD_p basemaps for Europe. Three maps, representing growing-season and mean annual δD_p estimates from an elevation-explicit, detrended interpolation model and growing-season δD_p estimates from simple Kriging, all indicate that strong isotope gradients occur across Europe with a general depletion occurring in a northeast direction. The feather data, representing 141 individuals of 25 avian species from 38 sites, ranged from −131 to −38‰. Regression analysis showed that strong correlations existed between both mean annual and growing-season δD_p estimated by detrended interpolation and δD_f of non-aquatic and non-corvid birds (r^2=0.66 and 0.65, respectively). We also examined mean annual and growing-season $\delta^{18}O_p$ vs. $\delta^{18}O_f$ for our samples. Both oxygen regressions were similar (r^2=0.56 and 0.57, respectively) but poorer than for deuterium. Our study reveals that δD measurements of feathers from migratory birds in Europe may be used to track their origin and movements, and so provide a powerful investigative tool for avian migration research in Europe.

Keywords Deuterium · Feathers · GNIP · Growing season · Migration · Oxygen · Precipitation · Stable isotopes

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Introduction

There is increasing scientific evidence that environmental conditions and habitat quality at overwintering and summer locales used by migratory species may profoundly influence the fitness and survival of individuals (Webster et al. 2001; Norris et al. 2003; Møller and Hobson 2003). This appears to be particularly true for migratory birds that may overwinter thousands of kilometers from their summer breeding areas. Recently, it has been shown that habitat and dietary quality directly impacts body condition, the timing of departure, and survival (Marra et al. 1998). Only by making scientifically sound linkages between breeding, wintering, and stopover sites used by individuals can we hope to better understand and unravel the factors that determine the overall demography and health of migratory bird populations.

Unfortunately, establishing geographical life cycle connectivity has proven extremely elusive for most migratory birds since the traditional mark-recapture (banding) technique rarely provides sufficient or statistically valid recoveries, especially for small-bodied organisms (Hobson 2003). Conventional mark-recapture results are always geographically biased, and are frequently proportional to the banding efforts made at the specific marking locations. Furthermore, mark-recapture provides no quantitative information on the larger population, and provides no data on geographically remote areas where no banding activities occur (Wassenaar and Hobson 2001). Newer technologies like satellite tracking are prohibitively expensive, and are generally useful only for large bodied or high profile species.

The recent development of stable isotope analyses of feathers of migratory birds as a means of inferring geographical origin has provided a new and powerful

Table 1 Summary data for individual species δD_f and $\delta^{18}O_f$ values used to investigate the relationship between feather deuterium and various estimates of δD_p

Site	Species	*n*	Age	δD_f (‰)	$\delta^{18}O_f$ (‰)
1	Blue Tit (*Parus caeruleus*)	6	J	−73±8.2	17.1±0.3
2	House Sparrow (*Passer domesticus*)	4	J	−65±10.6	19.0±0.3
3	Pheasant (*Phasianus colchicus*)	7	A	−79±5.7	17.7±0.5
3	Red Grouse (*Lagopus lagopus*)	1		−88	
4	Black Grouse (*Tetrao tetrix*)	1	J	−84	
5	Black Grouse	1	J	−98	
6	Black Grouse	1	J	−98	
7	Pheasant	2	A	−76, −86	
8	Red Grouse	4	A	−99±4.1	16.7±0.6
9	Black Woodpecker (*Dryocopus maritus*)	1		−62	
9	Warbler sp.	2		−64, −67	
10	Warbler sp.	1	J	−55	
10	Tit sp.	3	J	−54±8.7	19.0±0.2
10	European Serin (*Serin serin*)	2	J	−67, −70	
11	Lapwing (*Vanellus vanellus*)	3		−112±5.5	16.8±0.4
11	Carrion Crow (*Corvus corone*) (C)	1		−93	
13	Eurasian Blackbird (*Turdus merula*)	3	J	−78±1	17.3±0.6
13	Thrush sp.	3	J	−72±1.9	17.9±0.5
14	Carrion Crow (C)	3	J	−101±5.1	
14	Jackdaw (*Corvus monedula*)	3	J	−94±8.7	
15	Common Wood Pigeon (*Columba palumbus*)	5	J	−74±3.3	18.8±1.0
16	Common Wood Pigeon	3	J	−77±3.7	16.6±1.0
17	Common Wood Pigeon	1	J	−80	17.4
18	Common Wood Pigeon	5	J	−87±5.3	17.9±0.5
19	Common Wood Pigeon	5	J	−92±5.8	17.6±0.8
20	Hazel Grouse (*Tetrastes bonasia*)	5		−116±5.8	16.7±0.4
21	Hazel Grouse	5		−116±12.5	16.3±1.2
22	Partridge (*Perdix perdix*)	5		−96±3.2	18.3±0.6
23	Mallard (*Anas platyrhyncos*) (A)	4		−82±10.9	14.3±0.4
24	Common Wood Pigeon	1		−80	
25	Carrion Crow (C)	1		−38	
26	Canada Goose (*Branta canadensis*) (A)	1		−111	
27	Rock Pigeon (*Columba livia*)	1		−71	
28	Eurasian Curlew (*Numenius arguata*)	4	J	−89±5.4	16.5±0.4
29	Fieldfare (*Turdus pilaris*)	1	J	−93	
30	Redwing (*Turdus iliacus*)	5	J	−91±4	13.4±0.2
31	Willow Grouse (*Lagopus lagopus*)	5	J	−127±6	14.4±0.7
32	Black Grouse	5	J	−97±5.1	15.0±0.5
33	Willow Grouse	5	J	−122±8.7	13.3±0.8
34	Black Grouse	6	J	−112±5.3	13.3±1.3
35	Grey Partridge	2	A	−58, −77	17.6, 17.9
35	House Sparrow	2	J	−65, −66	18.2
36	Black Grouse	6	J	−99±3.7	14.9±0.7
37	Eurasian Blackbird	2	J	−64, −73	
	Song Thrush (*Turdus philomelos*)	2	J	−55, −48	
	Chaffinch (*Fringilla coelebs*)	1	J	−53	
38	Mallard (A)	1	J	−94	
	Grey Partridge	1	A	−73	

A aquatic species, *C* corvids
Age categories include adult (A) and juvenile (J)

approach to establish migratory connectivity (Hobson 1999; Wassenaar and Hobson 1998). For most avian species in North America, predictable continental-scale hydrologic patterns in the stable hydrogen isotope deuterium (δD) in rainfall strongly correlate, through local diet, with δD in locally grown feathers, thereby providing an independent and unbiased means of tracing the molting origins of birds and other wildlife (see Hobson and Wassenaar 1997; Wassenaar and Hobson 2001; Meehan et al. 2001; Kelly et al. 2001; Rubenstein et al. 2002). The North American pattern of δD in rainfall shows a strong negative trend from the southeast to northwest, which by translation into plants and dietary assimilation through the food web into birds establishes an isotopic marker of the geographical location of feather molt (Hobson and Wassenaar 1997).

The well-known correlation between δD and δ^{18}O in meteoric waters (Craig 1961; Taylor 1974) suggests that δ^{18} O values of feathers might similarly provide information of molting location, although such a relationship and the overall partitioning of ^{18}O from water and diet into animal tissues has not yet been investigated. One perceived advantage of using δ^{18}O values of feathers is that organic oxygen, unlike hydrogen, does not exchange with ambient water vapor (see Methods), although this idea has not yet been tested. A potential disadvantage is

Table 2 Summary of feather collection site information with three measures of average δD_p and two measures of $\delta^{18}O_p$: growing-season detrended average (GSD), growing season kriged average (GSK), and mean annual detrended (MAD)

Site	Country	Town	Latitude (°)	Longitude (°)	Elevation (m)	GSD δD (‰)	GSK δD (‰)	MAD δD (‰)	MAD δ^{18}O (‰)	GSD δ^{18}O (‰)
1	Ireland	Bray	53.2	−6.1	0	−53	−41	−53	−7.7	−7.7
2	Spain	El Saler	39.4	−0.3	3	−26	−32	−26	−4.4	−4.4
3	England	Hillborough	54.6	−0.7	41	−61	−49	−61	−8.7	−8.7
4	England	Rothershope	55.3	−2.2	413	−67	−49	−67	−9.6	−9.6
5	England	Raby	54.7	−2.3	113	−61	−48	−61	−8.8	−8.8
6	England	Harthope	54.2	−2.2	454	−64	−48	−64	−9.3	−9.3
7	England	Lodington	52.6	0.8	100	−56	−49	−56	−8.1	−8.1
8	Scotland	Drumochter	56.9	−4.2	450	−71	−49	−71	−10.2	−10.2
9	Romania	Greci	45.2	28.2	28	−51	−58	−54	−7.8	−7.5
10	Italy	Castelfidardo	44.0	13.1	81	−46	−48	−46	−6.9	−6.9
11	Bielorussia	Turov	52.1	27.7	125	−64	−62	−73	−10.2	−9.1
13	Poland	Czempin	52.1	16.8	69	−60	−63	−67	−9.3	−8.5
14	Estonia	Jogeva	58.5	26.5	60	−76	−70	−85	−11.5	−10.5
15	France	NA	46.4	−0.3	70	−43	−41	−43	−6.6	−6.6
16	France	NA	48.6	−4.0	76	−43	−33	−43	−6.5	−6.5
17	France	NA	47.5	−2.6	1	−42	−37	−42	−6.4	−6.4
18	France	NA	48.3	1.4	157	−53	−46	−50	−7.4	−7.4
19	England	Guernsey	49.8	3.7	142	−84	−50	−53	−7.8	−7.8
20	Russia	NA	62.0	42.8	42	−69	−78	−101	−13.6	−11.5
21	Russia	Perm	57.3	55.8	175	−58	−81	−104	−14.0	−11.7
22	Russia	NA	55.0	38.8	119	−59	−71	−85	−11.5	−9.5
23	Slovakia	NA	48.6	19.1	308	−63	−64	−66	−9.3	−8.2
24	England	NA	53.8	−1.8	150	−59	−48	−59	−8.6	−8.6
25	England	NA	55.0	−2.4	220	−63	−48	−63	−9.1	−9.1
26	England	NA	54.3	−3.0	60	−59	−47	−59	−8.5	−8.5
27	France	NA	42.4	1.9	162	−49	−36	−49	−7.5	−7.5
28	Finland	Alaraumo	65.8	24.3	10	−85	−88	−100	−13.6	−11.6
29	Russia	Rappolovo	59.0	30.9	60	−74	−70	−89	−12.0	−10.3
30	Russia	Poddubye	60.2	30.4	60	−77	−72	−92	−12.4	−10.7
31	Finland	Utsjöki	69.5	27.3	106	−89	−87	−109	−14.8	−12.0
32	Finland	Viitasaari	63.2	25.8	127	−84	−80	−98	−13.3	−11.6
33	Finland	Kemijärvi	66.7	27.4	161	−87	−85	−103	−14.0	−11.9
34	Finland	Suomussalmi	64.9	29.2	191	−85	−81	−102	−13.8	−11.7
35	Czech Republic	Putim	49.3	14.2	386	−59	−63	−66	−9.3	−8.4
36	Finland	Partano	62.1	23.0	106	−81	−78	−95	−12.8	−11.3
37	Netherland	Klarenbeek	52.2	6.1	9	−55	−50	−55	−8.0	−8.0
38	Czech Republic	Prostredni fishpond	49.3	14.1	350	−68	−63	−65	−9.2	−8.3

NA not applicable
Precipitation deuterium and oxygen isotope values based on the long term GNIP dataset

the fact that the range of $\delta^{18}O$ in nature is quite small compared to δD, and the analytical error is comparatively greater, and so the process of resolving populations geographically using $\delta^{18}O$ might be compromised.

The European avifauna is comprised of both short-distance and long-distance birds that summer in northern Europe and winter primarily in southern Europe and sub-Saharan Africa, respectively. Hobson (2003) recently suggested that there may be sufficient δD latitudinal structure to allow the measurement of deuterium in feathers of European migrant birds to infer latitudinal molting origins, however, that preliminary analysis used a simple Kriging interpolation method (cf. Hobson and Wassenaar 1997) which did not take into account the European topographical relief that is known to strongly influence precipitation deuterium values (Craig 1961; Dansgaard 1964; Poage and Chamberlain 2001).

The relationship between δD and $\delta^{18}O$ values in rainfall and that of feathers grown in Europe has not yet been systematically examined, nor is it known if there is sufficient isotopic structure to allow these isotopes to be used as a tool in avian migration research. The objective of this paper is twofold: First, we present new growing-season and annual rainfall δD and $\delta^{18}O$ contour maps for Europe that account for orographic isotope effects. This interpolated data is required for subsequent correlations with feather isotope data, as feather samples can not be associated 1:1 with sites where water isotopes in precipitation have been measured directly. Second, we compare the results of several precipitation model outputs with measured feather δD and $\delta^{18}O$ values (δD_f, $\delta^{18}O_f$) collected from birds of known geographical molt origin at 38 sites across Europe. Our goal was to determine the degree of confidence with which H and O isotopes could be used to track the geographical origins of migrant birds from Europe and, in the case of deuterium, compare our findings with those which have proven successful in North America.

Methods

Feather stable isotopic analyses

Feathers were obtained from 141 individuals of 25 avian species at 38 sampling sites across Europe. We concentrated our sample collection efforts almost exclusively on hatch-year birds of resident, mostly insectivorous and granivorous, species from natal sites across Europe (Tables 1 and 2). The feathers obtained were cleaned with a chloroform:methanol solvent mixture to remove surface contaminants and oils. Cleaned feather vanes were then sub-sampled for hydrogen and oxygen isotope analysis.

Stable-hydrogen isotope analyses of feathers are significantly affected by uncontrolled isotopic exchange between a fraction of available hydrogen in the feather matrix with ambient water vapor in the laboratory (Wassenaar and Hobson 2000). The routine use of co-analyzed and calibrated keratin standards as a means of correcting for this effect is described elsewhere (Wassenaar and Hobson 2003), and the values reported here are for non-exchangeable feather hydrogen.

Briefly, the isotopic analysis process involved the measurement of unknown feather samples in daily batches along with replicates of three different keratin references whose non-exchangeable δD values were known and spanned the range of expected feather values. Algorithms generated from each sample and reference batch that related δD values of unknowns to their expected non-exchangeable values were constructed to correct for drift due to seasonal and temporal changes in the δD of the lab air moisture, otherwise referred to as comparative equilibration (Wassenaar and Hobson 2003).

Stable hydrogen isotope measurements on feathers and keratin standards were performed on H_2 derived from high-temperature (1,275°C) flash pyrolysis of feathers and continuous-flow isotope-ratio mass-spectrometry. A minor analytical modification to the pyrolysis reactor described in Wassenaar and Hobson (2003) was the removal of the inner glassy carbon reactor tube and a helium flow rate of 120 ml/min. A ceramic pyrolysis reactor was filled with glassy carbon chips (Eurovector, Italy) to the middle of the hot zone of the reactor. This minor modification eliminated rapid plugging of the reactor column with silver that occurs when an inner glassy carbon tube is used (60–120 samples) and allowed for analysis of over 500 consecutive samples.

Pure H_2 was used as the sample analysis gas and the isotopic reference gas. A Eurovector 3000 high temperature elemental analyzer (EA) fitted with a Costech Zero-Blank autosampler was used to automatically pyrolyse 99 feather and reference sample batches to single pulses of H_2 gas. The resolved H_2 sample pulses were then introduced to the isotope-ratio mass spectrometer (VG Intruments Isoprime with electrostatic analyser) via an open split capillary. All δD results are expressed in the typical delta notation, in units of per mil (‰), and normalized on the VSMOW-VSLAP standard scale. Repeated analyses of hydrogen isotope inter-comparison material IAEA-CH-7 (−100‰), routinely included as a check, yielded an external repeatability of better than ±1.0‰.

Similar to hydrogen, the online analysis process for $\delta^{18}O$ involved the measurement of unknown feather samples in batches along with replicates of three certified organic isotope reference materials whose known $\delta^{18}O$ values that spanned the range of expected feather values. Oxygen isotope reference materials were IAEA 601 and IAEA 602 and IAEA C3. Algorithms generated from these references were used to normalize and correct the $\delta^{18}O$ values of unknown samples. Stable oxygen isotope measurements on feathers and references were performed on CO derived from high-temperature flash pyrolysis (1,200°C) of 200–350 ug aliquots of feather and reference materials using continuous-flow isotope-ratio mass-spectrometry. The online pyrolysis reactor was set up using the modifications described above for hydrogen, but the GC oven temperature was reduced to 65°C in order to chromatographically separate N_2 from CO. Similarly, this setup allowed for analysis of over 500 consecutive $\delta^{18}O$ samples.

Pure CO was used as the sample analysis gas and the isotopic reference gas. A Eurovector 3000 high temperature EA fitted with a Costech Zero-Blank autosampler was used to automatically pyrolyse batches of 99 feather and reference sample batches to single pulses of CO gas. The resolved CO sample pulses were then introduced to the isotope-ratio mass spectrometer (VG Instruments Isoprime) via an open split capillary. All $\delta^{18}O$ results are expressed in the typical delta notation, in units of per mil, and normalized on the VSMOW-VSLAP standard scale. Repeated analyses of oxygen isotope reference materials, routinely included as a check, yielded an external repeatability of ±0.4 ‰.

Isotopic base map and data analysis

Data on the deuterium and oxygen isotope composition of precipitation were obtained from the global network of isotopes in precipitation (GNIP) database administered by the international atomic energy association and world meteorological organization (IAEA 2001).

Two approaches for deriving point estimates and maps from the station data were investigated. A growing-season δD_p map without elevation correction was created using simple Kriging, as described in Hobson and Wassenaar (1997). Precipitation amount-weighted, growing season δD_p values were calculated for 94 stations in and around Europe using the monthly δD_p, precipitation amount, and temperature data in the GNIP database, where the growing season was defined as all months with mean monthly temperature >0°C (Hobson and Wassenaar 1997). These values were interpolated onto a 5′×5′ grid covering the European continent by simple Kriging with a linear semivariogram model in the software package Surfer (Golden Software; Golden, Colorado). Elevation-explicit growing season and mean annual δD_p and $\delta^{18}O_p$ maps were created using a detrended interpolation algorithm (Bowen and Revenaugh 2003). The algorithm was first used to create grids of monthly water isotope values, precipitation amount, and temperature using the GNIP isotope data and monthly mean temperature and precipitation amount data from the global historical climatology network (Peterson and Vose 1997) spanning the same years as the GNIP data (1960–2000). Data from the monthly grids was then combined to derive precipitation amount-weighted mean annual and growing season δD_p and $\delta^{18}O_p$.

The underlying premise for using detrended interpolation to create maps of water isotope values is that δD_p and $\delta^{18}O_p$ estimates can be improved if empirical relations between geographic variables and water isotopes in precipitation are used to inform the interpolation model (Bowen and Wilkinson 2002; Bowen and Revenaugh 2003). These relations, which reflect the distillation of the heavy isotope from air masses as they proceed along the trajectories of atmospheric circulation and over orographic barriers, are used to express a background isotope field. Superimposed upon this field is regional variation in δD_p and $\delta^{18}O_p$ attributable to the unique isotopic properties of individual air mass trajectories. This regional variation is represented by spatial interpolation of station measurements following subtraction of the background field (detrending). The algorithm used here is a modified implementation of that described by Bowen and Revenaugh (2003) for interpolation of mean annual water isotope data, and is fully described and further explored by Bowen et al. (in press). Briefly, the detrending model:

$$p_x = a\left(elat^2\right) + b(elat) + c(alt), \tag{1}$$

is used to describe the large-scale variance in the isotope data set. In this equation, p_x is the model estimate at location x, *elat* is the latitude at site x scaled to account for the meridional asymmetry of atmospheric circulation, and *alt* is the site elevation. The interpolated estimate of δD_p or $\delta^{18}O_p$ at gridpoint x is given by:

$$\hat{\delta}_x = p_x + \frac{\sum_{i=1}^{n}\left((\delta_i - p_i)\mathrm{e}^{(-D_{xi}/\beta)}\right)}{\sum_{i=1}^{n}\mathrm{e}^{(-D_{xi}/\beta)}} \tag{2}$$

where the first term is the detrending model estimate at the gridpoint and the second term is the average of the model residuals at all data stations, weighted according to each station's distance from the gridpoint (D_{xi}). Parameters a–c in Eq. 1 and β in Eq. 2 were fit simultaneously using the iterative adjustment method described by Bowen and Revenaugh (2003). Calculations were carried out on a 5′×5′ global grid, using elevations derived from the ETOPO-5 digital elevation model (USNGDC 1998). The detrended interpolation model was also used to generate grids of mean monthly temperature, which exhibits large-scale spatial variation similar to that of δD_p and $\delta^{18}O_p$. Precipitation amount does not vary in a simple and systematic way with latitude, longitude, or elevation, and precipitation amount grids were calculated using an inverse distance method with a linear weighting function. Weighting parameters were estimated from the empirical semivariogram for precipitation amount values and used to create 5′×5′ monthly precipitation amount grids.

Comparison of feather and precipitation values

Comparisons of feather hydrogen and oxygen isotope data were made against all three δD_p grids and both grids of $\delta^{18}O_p$. We present data for all feather samples analyzed, but for our regression analysis we have excluded corvids (omnivorous species often using anthropogenically produced foods) and taxa that feed within aquatic food webs. We separate these samples because their feeding behavior is likely to affect the isotopic relationship between their body tissues and local precipitation in a different way than for the other birds sampled. The relationship between feather and water isotope values was determined using reduced major axis regression. This regression is more appropriate than simple *y* on *x* least squares because both variables have similar levels of variance. Finally, we provide estimates of the error associated with reconstructing precipitation δD or δ^{18}O from single feather measurements based on the feather-water regressions. These are given as the standard deviation for back-predicted water isotope values (σ_p) according to the formula:

$$\sigma_p = \sqrt{\mathrm{MSE}_p\left(1 + \frac{1}{n} + \frac{\left(\delta_f - \bar{\delta}_f\right)^2}{\mathrm{VAR}_f}\right)} \tag{3}$$

(Rao 1998), where MSE_p is the mean square error of the regression, n is the number of regression pairs, δ_f is the

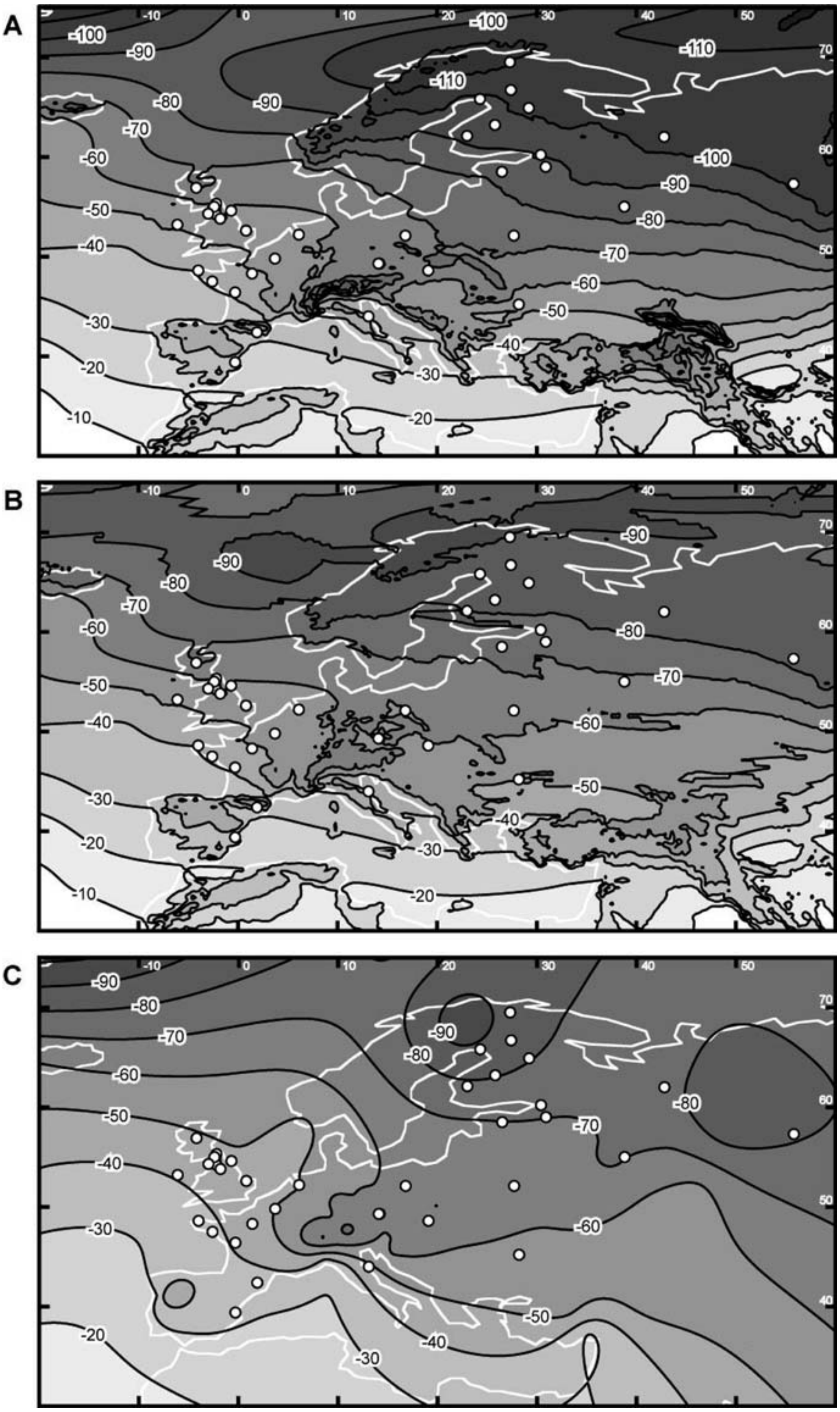

Fig. 1a–c Contour maps of δD_p estimated by three methods, with bird feather sampling sites shown (*circles*). Maps represent mean annual (**a**) and growing-season (**b**) δD_p interpolated by the detrended, elevation-explicit model, and growing-season δD_p estimates made by simple Kriging (**c**)

measured feather isotope value, δ(*bar*)$_f$ is the average feather isotope value for the regression set, and VAR$_f$ is the variance of the feather isotope values in the regression set.

Results

Precipitation isotope base maps for Europe

The three contour basemaps for European δD$_p$ demonstrate considerable structure and variability across the continent. All three maps reveal similar patterns of variation at the continental scale, but careful comparison of the maps reveals significant differences associated with the choice of interpolation method employed.

A general δD$_p$ gradient from the southwest to northeast across the continent is reproduced by all three methods. The range for mean annual δD$_p$ across the mapped area (−124.6 to +2.3‰, Fig. 1a) is considerably greater than the range for growing-season δD$_p$ estimated by either interpolation method (detrended interpolation: −99.0 to +2.3‰, Fig. 1b; Kriging: −101.7 to −16.0‰, Fig. 1c). As a result, the southwest to northeast gradient across the continent is somewhat steeper for mean annual δD$_p$. Both maps created using detrended interpolation depict more complex fields of spatial variation than the Kriged growing-season δD$_p$ map, much of the additional complexity being due to topography within the mapped region. The detrended interpolation maps also suggest somewhat stronger and more consistent south to north δD$_p$ gradients across the region. Mean annual δ^{18}O$_p$ values determined by detrended interpolation range from −17.9 to −0.7‰ and growing-season δ^{18}O$_p$ from −14.2 to −0.7‰ for the mapped region. The patterns of variation and differences between mean annual and growing-season fields are similar to those described for δD$_p$.

Relationship between feather and precipitation isotopes

Our feather samples showed a broad range of δD values (range −131 to −38‰) across the European sampling sites. Feather δD was correlated with collection site δD$_p$ as estimated by each of the three interpolation methods (Fig. 2). The strongest correlations were found between δD$_f$ and mean annual or growing-season δD$_p$ from the detrended interpolation model (r^2=0.66 or 0.65, respectively). In other words, about 66% of the hydrogen isotopic variance in feathers is a function of the precipitation isotopic composition at molting sites. A somewhat weaker correlation was found between δD$_f$ and Kriged growing-season δD$_p$ (r^2=0.54).

For each regression, many samples from corvid and aquatic-feeding taxa fall near the regression line, but our small set of samples from this group does not demonstrate a strong relationship between site δD$_p$ and measured δD$_f$.

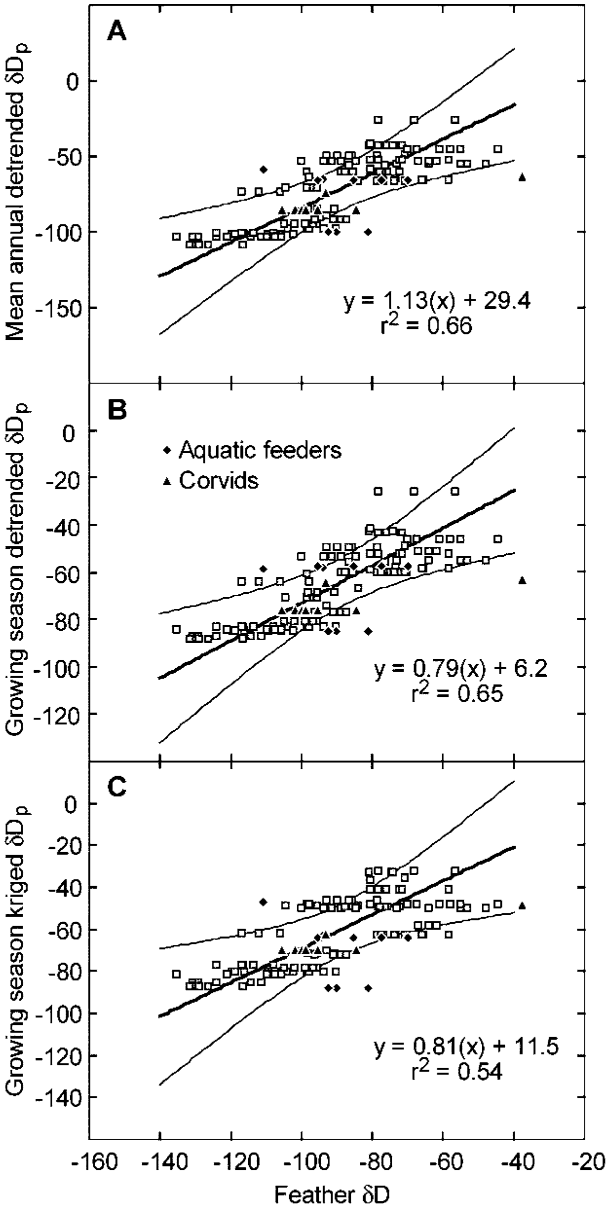

Fig. 2a–c Regressions between feather sample δD values and δD$_p$ estimates for the collections sites. Water isotope estimates are **a** mean annual detrended, **b** growing-season detrended, and **c** growing-season Kriged values corresponding to those shown in the three panels of Fig. 1. Best-fit lines were determined by reduced major axis regression, and thin lines indicate ±1 standard deviation for δD$_p$ predictions made from single feather measurements using the regression equation. Samples from birds that feed or live in terrestrial aquatic environments and corvids (see Table 1) were excluded from the regressions

To investigate the effect of site elevation on our feather/water correlations, we tested for a significant relationship between the average residual of the δD$_f$/Kriged δD$_p$ regression for each collection site and elevation. We found that there was no significant correlation ($F_{1,36}$=0.394, p=0.534).

The correlation between δ^{18}O$_f$ and δ^{18} O$_p$ is weaker than the δD regressions for both growing-season and mean annual precipitation values (r^2=0.57 and 0.56, respectively). Despite the moderate r^2 values for these regression, the $\delta^{18}O_f/\delta^{18}O_p$ relation in the European data set appears to be strongly non-linear and has very little predictive power (Fig. 3).

Discussion

Hydrogen isotopes

Our altitude-corrected, precipitation amount-weighted, growing-season and mean annual average δD precipitation maps for Europe show significant spatial structure in deuterium patterns, with a general decrease in δD_p along a southwest to northeast geographical gradient This structure provides the basis for using hydrogen and oxygen isotopes in animal migration research. The European precipitation isotope patterns are analogous to that found in North America, where there is a general decrease in δD_p toward more northerly latitudes, but also reflect rainout along the strong zonal circulation pathways dominant over Europe. This leads to a monotonic decline in δD values from west to east along longitudinal bands, particularly at high latitudes. As expected, considerable complexity was captured in all mountainous areas, reflecting the well documented response of precipitation deuterium with altitude. Nonetheless, for much of the European continent sufficient spatial δD_p patterns exist that can potentially be employed in tracking migratory wildlife moving between isotopically distinct regions.

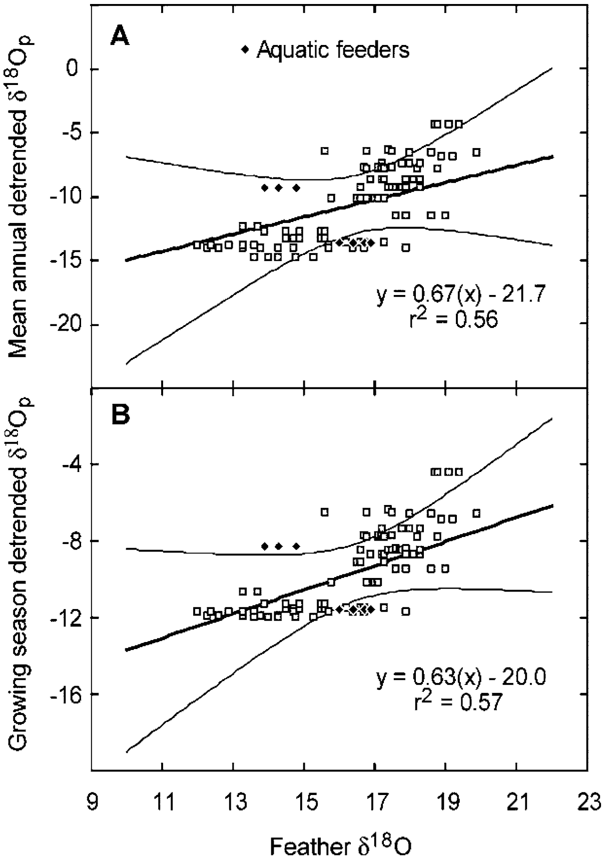

Fig. 3a, b Regressions between feather sample $\delta^{18}O$ and mean annual detrended $\delta^{18}O_p$ (**a**) and growing-season detrended $\delta^{18}O_p$ (**b**). Methods and symbols as in Fig. 2

Prior to regression analysis of precipitation and feathers, we binned our samples into three general feeding groups, allowing us to examine the effect of generalized feeding strategy on the $\delta D_f/\delta D_p$ relationship. Corvids were treated independently because their diets tend to be broad and can include anthropogenic inputs, in the form of carrion or refuse, whose δD values may not be reflective of local precipitation and foodweb δD values. Birds feeding in aquatic-based foodwebs may also have diets that are similarly disconnected from local δD_p values as the result of hydrological processes that filter the primary rainwater isotopic signal. Based on our small samples of birds from these two categories, it is difficult to say with certainty whether these concerns prevent the use of δD_f analysis to reconstruct the habitats of corvid and aquatic-feeding birds. We can say that the samples from these groups, which are derived from collecting sites with δD_p values spanning a range of several tens of per mil, demonstrate no clear correlation with δD_p, and include some of the most extreme outliers of the regressions determined for other taxa.

Recently, Meehan et al. (2003) noted that raptors may represent an ecological group that differs in their δD_f vs. δD_p relationship from insectivorous songbirds. Should this extend to occasionally carnivorous or carrion feeding birds like corvids, it may contribute the observed deviation of some of our corvid samples from the regression lines determined for non-corvid, non-aquatic taxa. Previous studies, although few, have not shown trophic-level effects of isotopic fractionation between diet and feathers (Hobson et al. 1999; Schimmelman et al. 1993), but more work is still needed on this topic.

Previous studies in North America have found excellent correlations between site δD_f and the weighted growing-season mean δD_p (Hobson and Wassenaar 1997; Meehan et al. 2001; Kelly et al. 2001). The relationship between δD_f and mean annual δD_p has not been examined, however. The choice of growing-season values in previous work reflects the findings that rainfall occurring during the growing months provides the primary isotopic signal taken up by plants and incorporated in foodwebs (cf. Cormie et al. 1994), but this may not be valid in all cases.

For the European sample set examined here, we found almost identical correlation coefficients for δD_f vs. mean annual detrended (MAD) δD_p and δD_f vs. growing season detrended (GSD) δD_p regressions, and so the choice of which precipitation δD values represent the appropriate input signal is somewhat ambiguous. Indeed, the range and variance of the δD_f data set exceed those of the growing-season δD_p data for the feather sampling sites, and are somewhat closer to those for the mean annual δD_p estimates. This contrasts sharply with the results of Hobson and Wassenaar (1997) for North America, where the δD_f variance was substantially lower than that of GSD δD_p. A variety of post-rainfall processes, including hydrological storage and mixing will smooth out the rainwater isotope signal and influence the $\delta D_f/\delta D_p$ relationship. Understanding the propagation of rainwater isotope signals through hydrological and biological systems constitutes a major task for future studies, and we note that the "correct" representation of input signals will not be the same for all δD tracer studies.

Incorporation of elevation and spatial detrending of the growing season δD_p data during interpolation improved the correlation between δD_p and δD_f values. The importance of altitude effects on δD_f values was not clearly manifested in our re-analysis of the residuals from the non altitude-explicit (Kriged) regression, but this is not surprising given that the bird sampling sites used in this study span only a relatively small ~400 m elevation range. Given the average altitude-dependence of δD_p shown in our detrended interpolation analysis of monthly data (−14‰/km), this corresponds to an altitude-induced δD_p difference of only 5.6‰ between our lowest and highest-elevation sampling stations. By contrast, the root mean square error of the Kriged growing season $\delta D_f/\delta D_p$ regression was 12.2‰, meaning that any elevation effects over such a small elevation range were likely to be obscured by the noise inherent in this regression. In general, we suggest that incorporation of elevation-driven δD_p patterns in basemaps for animal migration studies will help elucidate situations in which elevation-driven δD_p variation might lead to erroneous conclusions of the location of origin. This potential elevation effect must be evaluated on a case-by-case basis, taking into account the relative magnitude of spatial and altitudinal δD_p variation within the region of study as well as the known geographic and topographic ranges of the taxa of interest.

The *y*-intercept of the regression between δD_f and δD_p gives the isotopic discrimination factor associated with the process of feather formation from the elemental form of hydrogen available as water at the base of the foodweb. Most of this isotopic discrimination takes place with the incorporation of hydrogen from water into plant material with little further trophic discrimination occurring after that (White 1988; Hobson and Wassenaar 1997). Hobson and Wassenaar (1997) reported an intercept of −31‰ for the relationship in North America, with a value of −34‰ subsequently determined by Meehan et al. (2001) for North American Cooper's Hawks (*Accipiter cooperi*). Based on controlled captive experiments using Red-winged Blackbirds (*Agelaius phoeniceus*), Wassenaar and Hobson (1997) estimated the discrimination factor to be −27.4 ‰. For the current European data set and our three δD_p grids, we obtain fractionation factor estimates ranging from −26‰ (mean annual detrended δD_p) to −8‰ (growing-season δD_p). These estimate range from values similar to the previous estimates for North America to much higher values. Interpretation of the estimated fractionation factors is complicated by ambiguity about which δD_p values reflect the "correct" input signal. The estimate based on the European avifauna collections and mean annual detrended δD_p is not statistically different than North American estimates (the upper 95% confidence limit for the intercept of Wassenaar and Hobson 1997, regression is −24.1‰). The most parsimonious interpretation of this data, therefore, might be that the discrimination factor for European birds in this study is near the lower end of the range derived here and similar that for North American birds, and that the δD signal input to European ecosystems is most closely approximated in our study by the mean annual detrended δD_p grid.

Oxygen isotopes

We found a relatively poorer correlation between $\delta^{18}O_p$ and $\delta^{18}O_f$ (r^2=0.57). In our regression analysis this is revealed by very high variance for the regression coefficients, giving a regression without power to constrain the $\delta^{18}O$ of precipitation at the site of sample collection. This may reflect a number of co-related and unknown factors. First, the source and routing of oxygen incorporated into specific biological tissues may be more complex than hydrogen. There are comparatively more oxygen sources (water, diet, air) and sinks (urine, exhaled CO_2) than hydrogen sources and sinks, and tissue specific isotope fractionation associated with oxygen isotopes remains unknown. Second, the analytical error for online $\delta^{18}O$ assays of organic samples is quite poor relative to δD. For example, the range in mean annual $\delta^{18}O_p$ for Europe is ~17.2‰ and for δD_p is ~126‰. Expressed as a ratio of annual range to analytical measurement error (±0.4 and ±1 respectively, see Methods) this gives ratios of 43 and 126. In other words, δD has the sensitivity to resolve geospatial origins that is three times better than $\delta^{18}O$. As a result, we suggest that δD measurements in animal tissues will likely prove to be the most useful indicator of geographical origin of animals over continental scales. However, $\delta^{18}O$ measurements of animal tissues may prove to be useful in situations where populations of animals with similar tissue δD values (e.g., animals whose origins lie generally on similar lines of latitude in North America or Europe) nonetheless differ in their $\delta^{18}O$ values. Clearly, far more research on $\delta^{18}O$ of animal tissues is still required.

Applications to wildlife studies in Europe

Although additional research will be required to further refine our understanding of the distribution of hydrogen isotopes in precipitation and meteoric waters across Europe, and the relationship between these parameters and δD_f values from migrant European avifauna, our findings presented here are encouraging enough to suggest a number of current and future isotope applications to tracking migratory bird populations. Indeed, our initial interest in pursuing this research was motivated by a desire to determine the breeding origins of several gamebird species such as Wood Pigeon (*Columba palumbus*) and Eurasian Woodcock (*Scolopax rusticola*) that are present throughout France and other mid-continental areas where they are hunted as year-round residents, in migration, or as wintering birds (Ferrand and Gossman 2001). The number of migrating woodpigeons and Woodcocks crossing the French Pyrenean mountains to winter in Spain and Portugal has steadily decreased since 1960 (Jean and Razin 1993) while populations of sedentary birds are increasing everywhere in Europe (Saari 1997; Ferrand and Gossman 2001). Effective conservation of these types of short-distance migrants in Europe requires that we can scientifically discriminate among and quantify locally produced birds from those produced elsewhere, or identify the primary production areas of strictly migratory populations.

For future research investigations we recommend that avian populations of interest (e.g. resident or local vs. distant) be generally classified isotopically, so that a good estimate of isotopic variance associated with these populations can be established. Then, statistical inference based on tests such as discriminant function analyses (Alisauskas and Hobson 1992; Mehl et al. 2004) can provide probability of assignment of individuals (see also Royle and Rubenstein in press; Hebert and Wassenaar 2004). Overall, the utility of using deuterium assays of birds in Europe based on the GSD δD_p pattern is not quite as encouraging for North America, which has so far shown much less variance associated with the $\delta D_f/\delta D_p$ relation (e.g., Hobson and Wassenaar 1997; Kelly et al. 2001; but see Chamberlain et al. 1997; Hobson et al. 2001).

Nonetheless, there are three clear regions of the European continent that show a good deal of promise as areas of isotopic assignment for birds and other wildlife. These are: (1) Southern Europe, including Mediterranean regions where average δD_p is of the order of −30‰, (2) mid-Europe west and east of the Alps where δD_p ranges from −60 to −40‰, and (3) the northern and northeastern regions where δD_p ranges below −70‰. Furthermore, within the northern regions, there are areas with relatively steep gradients in δD_p such as in eastern Europe and southern Scandinavia where information on origins of birds will be possible over relatively shorter geographical distances. Already, subspecific delineation of birds such as the Scandinavian Willow Warbler (*Phylloscopus trochilus*) are known to occur zonally, and measurements of δD_f can potentially allow further information on population segregations (see also Chamberlain et al. 2000). We also note that the proportion of breeding species that are migratory varies linearly with latitude in Europe (Newton 2003), and so northern birds with negative δD_f values migrate to wintering locations further south in Europe or beyond the continent where they should be readily distinguished from more southern populations.

Although the GSD pattern of precipitation in Europe manifests higher variance than that in North America, the MAD δD pattern shows some encouraging results with a much more uniform gradient of greater magnitude. Our results suggest a better fit between the MAD δD pattern and δD_f suggesting that some of this annual precipitation contributes to foodweb δD. If this is the case, the application of the deuterium isotope technique to tracking European migrants may approach the power seen in North America and so further tests of this hypothesis are now needed.

Unlike most migratory birds in North America that molt *prior* to migration, the situation is generally reverse among long-distant migrants in Europe that winter in sub-Saharian Africa. Those European species tend to molt on the wintering grounds and so the isotopic measurement of their feathers will reflect more “positive” hydrogen isotopic values at sites in Africa. Pilot research to date suggests that deuterium measurements of feathers grown in Africa may not be particularly useful as geographic indicators largely due to the highly dynamic nature of precipitation on that continent (Møller and Hobson 2004). However, $\delta^{13}C$ and $\delta^{15}N$ measurements of feathers show much more promise since they reflect long-term foodweb biogeoclimatic effects (Chamberlain et al. 2000; Møller and Hobson 2004). However, hatch-year birds produced in Europe and sampled en route to Africa or on their wintering grounds there will retain deuterium isotopic information in their feathers reflecting their European origins.

Future research

Our research has provided critical information on how the measurement of deuterium in feathers and other tissues of birds and other migratory species from Europe may be used to assign broad areas of origin. The additional measurement of other stable isotopes such as ^{13}C, ^{15}N, ^{18}O, and ^{34}S in feathers might also be useful in some circumstances (e.g., Hebert and Wassenaar 2004). Recently, Szép et al. (2003) showed that the analysis of trace elements in feathers of Sand Martins (*Riparia riparia*) in Europe can also provide information on origins of individuals. Clearly, the combination of several independent techniques show the greatest promise in tracking migration on this and other continents (e.g., Dockx et al. 2004). Even in cases where the breeding distribution of a species and the broad range of potential origins based on stable isotope profiles results in very imprecise geographical information on origins of individuals, the ability to discriminate between local individuals and “others” can represent a powerful tool in ecological and conservation research. This approach was recently described by Hobson et al. (2004) using stable-hydrogen and carbon isotope analyses of feathers for populations of Ovenbirds (*Seiurus aurocapillus*) and American Redstarts (*Setophaga ruticilla*) in North America as a means of quantifying dispersal of individuals into local breeding populations (see also Hobson et al. 2001; Graves et al. 2002; Szép et al. 2003). Møller and Hobson (2004) also recently used isotopic analyses of feathers of a single population of European Barn Swallows (*Hirundo rustica*) to describe population heterogeneity based on different wintering areas in Africa.

Finally, we note that our approach here used a broad range of species from numerous sites across Europe. Hence, this sample set likely represents a “worst-case scenario" in terms of the anticipated isotopic variance. For most single species studies (excluding potentially problematic omnivorous species such as corvids) we would expect the range of isotopic variation to be significantly reduced and the relationship between δD_f and δD_p to improve. Future studies investigating isotopic relationships for single species of interest are therefore encouraged (e.g., Wassenaar and Hobson 1998; Meehan et al. 2001; Kelly et al. 2001; Rubenstein et al. 2002).

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