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INTRAFEATHER AND INTRAINDIVIDUAL VARIATION IN THE STABLE-HYDROGEN ISOTOPE (δD) CONTENT OF RAPTOR FEATHERS

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Abstract. Stable-hydrogen isotope ratios (deuterium:protium; δD) in feathers enable researchers to evaluate patterns of avian movement and to estimate the source areas of migratory birds. However, variation in feather δD remains inadequately described, thus confounding inferences of avian movement and origin. We assessed variation within a feather and among feathers within and between tracts in three species of immature raptors. Within contour feathers, measurements of δD increased from a distal section to an adjacent, proximal section; the magnitude of δD increase varied with raptor species. Furthermore, contour and flight feathers differed systematically in their δD content. Two explanations for intrafeather and intraindividual variation warrant further investigation: (1) hydrogen isotope fractionation associated with feather growth rate, and (2) the incorporation of temporal variation in environmental δD into growing feathers. We consider these explanations for raptors and passerines, which seemingly differ in the incorporation of deuterium into feathers. Additionally, corresponding sections of multiple contour feathers exhibited better measurement repeatability than multiple sections within a contour feather; the variability of multiple δD measurements within a feather tract (geometric SD: ±3.5‰) suggests that biological effects on the repeatability of δD measurements from concurrently grown feather material are difficult to distinguish from analytical effects. In most cases, intrafeather and intraindividual variation can be minimized by informed sample selection decisions, but both sources of variation must be considered when stable-hydrogen isotopes are used to infer the geographic origins of migrants, ascertain migratory connectivity, and facilitate avian conservation decisions.

Key words: *deuterium, feathers, hydrogen, passerines, raptors, stable isotopes, variation.*

Variación en una misma Pluma y entre Individuos en el Contenido del Isótopo de Hidrógeno Estable (δD) en las Plumas de Aves Rapaces

Resumen. Los cocientes de isótopos estables de hidrógeno (deuterio:protonio; δD) en las plumas permiten a los investigadores evaluar los patrones de movimiento de las aves y estimar las áreas de origen de las aves migratorias. Sin embargo, la variación de δD en las plumas no ha sido descrita de forma adecuada, lo que puede dificultar las inferencias sobre el movimiento y el origen de las aves migratorias. Determinamos la variación en una misma pluma, y entre plumas, en y entre folículos, en tres especies de aves rapaces inmaduras. En las plumas de contorno, las medidas de δD aumentaron desde una sección distal a una sección proximal adyacente de la pluma. La magnitud con la que δD aumentó varió entre las diferentes especies de aves rapaces. Además, las plumas de contorno y de vuelo difirieron sistemáticamente en sus contenidos de δD. Dos explicaciones para la variación en una misma pluma y entre las plumas de individuos diferentes merecen un análisis más profundo: (1) fraccionamiento de los isótopos de hidrógeno asociado a la tasa de crecimiento de las plumas e (2) incorporación de la variación temporal del δD ambiental en las plumas en crecimiento. Consideramos estas explicaciones para aves rapaces y paseriformes, las que aparentemente difieren en la incorporación de deuterio en las plumas. Además, la repetibilidad de las mediciones fue mejor entre las secciones correspondientes a diferentes plumas de contorno, que entre secciones diferentes en una misma pluma de contorno. La variabilidad de múltiples medidas de δD en una mismo folículo (DE geométrico: ±3.5‰) sugiere que los efectos biológicos sobre la repetibilidad de las medidas de δD en las plumas que se encuentran en fase de crecimiento son difíciles de distinguir de los efectos analíticos. En la mayoría de los casos, la variación en una misma pluma y entre plumas de individuos diferentes puede ser minimizada mediante decisiones de muestreo acertadas. Sin embargo, las dos fuentes de variación deben ser consideradas al utilizar isótopos estables de hidrógeno para inferir el origen geográfico de las aves migratorias, determinar la conectividad migratoria y facilitar las decisiones de conservación en aves.

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INTRODUCTION

In 1997, two seminal papers in stable isotope ecology suggested that stable-hydrogen isotope ratios (deuterium:protium; δD) in feathers enable researchers to describe the location of feather growth and, by extension, the origins or breeding grounds of young and adult migratory birds, respectively (Chamberlain et al. 1997, Hobson and Wassenaar 1997). Since their publication, the potential of the stable isotope technique to answer previously intractable questions related to avian movements has resulted in a proliferation of studies employing this approach. Reviews of stable isotope applications to the study of avian movement have focused on how the technique has advanced our ability to study bird migration, dispersal, and migratory connectivity, or on its ability to provide insights relevant to conservation (Kelly and Finch 1998, Hobson 1999, 2005, Webster et al. 2002, Rubenstein and Hobson 2004). However, methodological issues relating to the prediction of avian origins from feather deuterium measurements have yet to be resolved, and only recently have we begun to explore variation in feather δD (Lott and Smith 2006, Norris et al. 2006, Langin et al. 2007, Wunder and Norris 2008, Smith et al. 2009).

Typically, studies of avian movement employing stable-hydrogen isotopes use a single measurement of feather δD to represent an individual bird. However, the selection of feather material to be analyzed requires decisions at several levels of avian morphology. For example, a single feather tract initially must be identified and from this a single feather selected for analysis. Depending on the feather tract, it may or may not be possible to select the equivalent feather from multiple individuals (e.g., flight feathers and contour feathers, respectively). Once a feather has been selected, a specific portion (i.e., subsample) of that feather must be chosen for isotopic analysis. Variation in deuterium composition (e.g., systematic δD differences) may occur at any level of this feather hierarchy. Furthermore, circumstances related to the storage and preparation of samples, as well as analytical error, may affect the final deuterium value obtained for a sample.

Selecting a feather grown in the geographic region of interest (e.g., the breeding area, in most studies of migratory birds) is axiomatic, but understanding plumage cycles and molt patterns in the species of interest is critical to selecting an appropriate feather, as δD can vary among generations of feathers for ecological or physiological reasons (Duxbury et al. 2003, Meehan et al. 2003, Smith and Dufty 2005, Langin et al. 2007). In certain instances, δD can vary systematically among feathers of the same generation (Kelly et al. 2002, Smith and Dufty 2005). Furthermore, subsample selection has only recently been explored in detail (Wassenaar and Hobson 2006).

The continuous-flow isotope-ratio mass spectrometry (CF-IRMS) method employed in most δD-based studies of avian movement requires only a small amount of sample material for analysis (0.25–1.00 mg; Wassenaar and Hobson 2006); that only a small fraction of all possible material from a single feather is utilized makes isotopic variation in feathers an important consideration. Here, we consider the equivalency of: (1) repeated measurements of δD within a single juvenal feather, and (2) δD measurements among multiple juvenal feathers, both within a single feather tract and between feather tracts.

METHODS

FEATHER COLLECTION AND STABLE ISOTOPE ANALYSES

We obtained feather samples from individual raptors captured during the 2003 fall migration at the Idaho Bird Observatory on Lucky Peak (43°36′N, 116°05′W, 1845 m), the southernmost peak of the Boise foothills located 12 km east of Boise, Ada County, Idaho. We collected two contour feathers from the upper breast, separated by 1–2 cm, and the greater primary covert of the second primary (right wing) from 46 individuals comprising three raptor genera and species: Merlin (*Falco columbarius*; $n = 10$), Red-tailed Hawk (*Buteo jamaicensis*; $n = 17$), and Sharp-shinned Hawk (*Accipiter striatus*; $n = 19$). We considered greater primary coverts to represent flight feathers, as raptors typically grow and replace a greater primary covert concurrently with its corresponding primary feather.

Sample preparation and stable isotope analysis occurred during 21–22 September 2005 at the Stable Isotope Hydrology and Ecology Laboratory at the National Water Research Institute (Environment Canada, Saskatoon, Saskatchewan). Feathers were cleaned of surface oils and debris using a 2:1 chloroform to methanol solution and allowed to air dry for at least 48 hr. After cleaning, vane material (0.35 ± 0.01 mg) was clipped from an area perpendicular to the rachis at the distal tip of each feather (location A in Fig. 1), packaged into silver capsules, and stored in plastic culture trays. When a second measurement of the same feather was required (for contour feathers), a second sample was taken from a location immediately proximal to the initial sample (location B in Fig. 1). The deuterium composition of the nonexchangeable component of a feather sample was measured using the online pyrolysis and CF-IRMS techniques detailed by Wassenaar and Hobson (2003, 2006). Feather δD results are reported in parts per thousand (‰) deviation from the VSMOW-SLAP standard scale. We randomized samples within the laboratory session to eliminate potential bias from drift in feather δD values (i.e., a systematic shift in δD related to the analysis order of samples). Hydrogen isotope reference material (IAEA-CH-7; –100‰ VSMOW) exhibits a measurement repeatability of better than ±2.0‰; calibrated keratin standards used for comparative equilibration exhibit measurement repeatabilities of ±3.2‰. However, isotopic measurements of unhomogenized feather samples as used in most studies may be more variable than those of homogenized feather standards (Wassenaar and Hobson 2006, Smith et al. 2009).

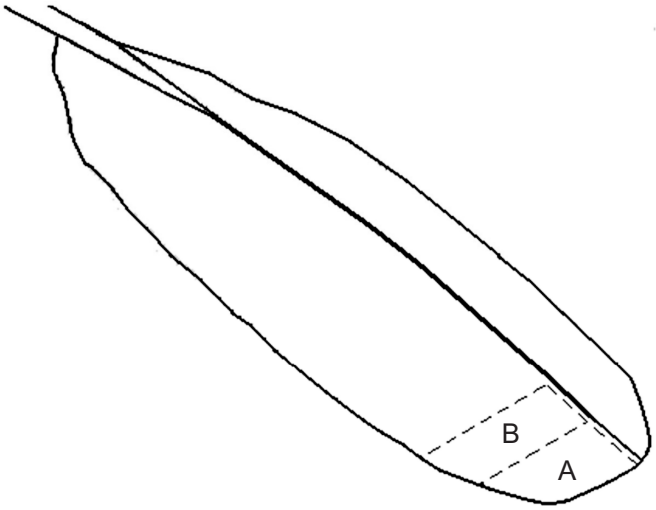

FIGURE 1. Schematic of a generic feather showing the locations of feather vane subsamples used to explore intrafeather and intraindividual variation in raptor feather stable-hydrogen isotope ratios (δD). For feather δD comparisons within a feather, measurements from a distal subsample (location A) were compared to similar measurements from an adjacent, proximal subsample (location B). Comparisons of feather δD among feathers were based on measurements from a distal subsample (A) from each feather.

TABLE 1. Model selection results for competing variance structures in the analysis of deuterium variation within a contour feather in three raptor species (Merlin, $n = 10$; Red-tailed Hawk, $n = 17$; Sharp-shinned Hawk, $n = 19$). K is the number of variance parameters estimated; –2RLL is the –2(restricted log-likelihood) for a given model; ΔAIC_c is the difference in second-order Akaike's information criterion (AIC_c) of a given model relative to the smallest AIC_c in the model set; and w_i is the Akaike weight, interpreted as the probability that model i is the Kullback-Leibler model best approximating reality (Burnham and Anderson 2002).

Covariance structure	–2RLL	K	ΔAIC_c	w_i
Heterogeneous compound symmetry[a]	673.3	3	0.0	0.82
Compound symmetry	678.4	2	3.1	0.18
Heterogeneous variance	778.5	3	105.3	0.00
Variance components	790.8	1	113.3	0.00

[a]The AIC_c of the best model was 679.5.

STATISTICAL ANALYSES

Intrafeather variation in feather δD. We employed repeated-measures two-way analysis of variance (ANOVA) to determine whether feather δD differed between a distal section of a contour feather vane and an adjacent, proximal section, and to determine if this difference varied among species; we anticipated differences in feather δD averages among species, but because we had no particular interest in interspecific differences, we do not discuss them further. Prior to repeated-measures ANOVA, we assessed within-subject covariance structures using restricted maximum likelihood, and selected among competing covariance structures using the second order Akaike's information criterion (AIC_c; Burnham and Anderson 2002; but see Kincaid 2005); a heterogeneous compound symmetric covariance structure best fit the data (Table 1). With the appropriate covariance structure identified, we evaluated fixed effects using maximum likelihood. We used Levene's test to determine whether variability in the δD difference between outer and inner contour feather varied among species.

Intraindividual (within- and between-tract) variation in feather δD. We used separate univariate ANOVAs to determine whether the δD difference between equivalent subsamples from: (1) two contour feathers in the same tract, and (2) contour and flight feathers varied among species. For the within-tract analysis, we used the absolute δD difference between two contour feathers from an individual, because it was impossible to select equivalent contour feathers from each individual and thus assess δD differences between specific contour feathers; we log-transformed this difference to accommodate ANOVA assumptions. Our inability to select specific contour feathers did not affect the assessment of the δD difference between tracts; for each individual, we used the difference between the average δD of the two contour feathers and primary covert δD. In each comparison, we used Levene's test to determine whether variation in δD differences varied among species. We conducted all statistical analyses using SAS version 8.2 (SAS Institute 1999).

RESULTS

INTRAFEATHER VARIATION IN FEATHER δD

Feather δD measurements differed between two adjacent locations of the same contour feather (ANOVA: $F_{1,43} = 74.5$, $P < 0.001$). Specifically, distal contour feather material contained consistently less deuterium than proximal contour feather material (Fig. 2A). However, the magnitude of deuterium enrichment in proximal contour feather samples relative to distal contour feather samples varied among species (ANOVA: $F_{2,43} = 7.5$, $P = 0.002$; Fig. 2A); the difference was least pronounced in Red-tailed Hawks (least squares mean ± SE: –3.0‰ ± 1.4‰; Fig. 2A) and more distinct in Merlins (least squares mean ± SE: –10.9‰ ± 1.8‰; Fig. 2A) and Sharp-shinned Hawks (least squares mean ± SE: –9.0‰ ± 1.3‰; Fig. 2A). Variability in the δD difference between adjacent locations of a feather was similar among species (Levene's test: $F_{2,43} = 1.1$, $P = 0.36$; Fig. 2A), as was also suggested by the inadequacy of a heterogeneous variance covariance structure (Table 1).

INTRAINDIVIDUAL (WITHIN- AND BETWEEN-TRACT) VARIATION IN FEATHER δD

Contour feathers from the same tract exhibited small but significant absolute differences in feather δD (geometric mean ± SD, 95% CI: 1.9‰ ± 3.5‰, 0.4‰–3.3‰; Fig. 2B) but the absolute difference did not vary among species (ANOVA: $F_{2,42} = 0.0$,

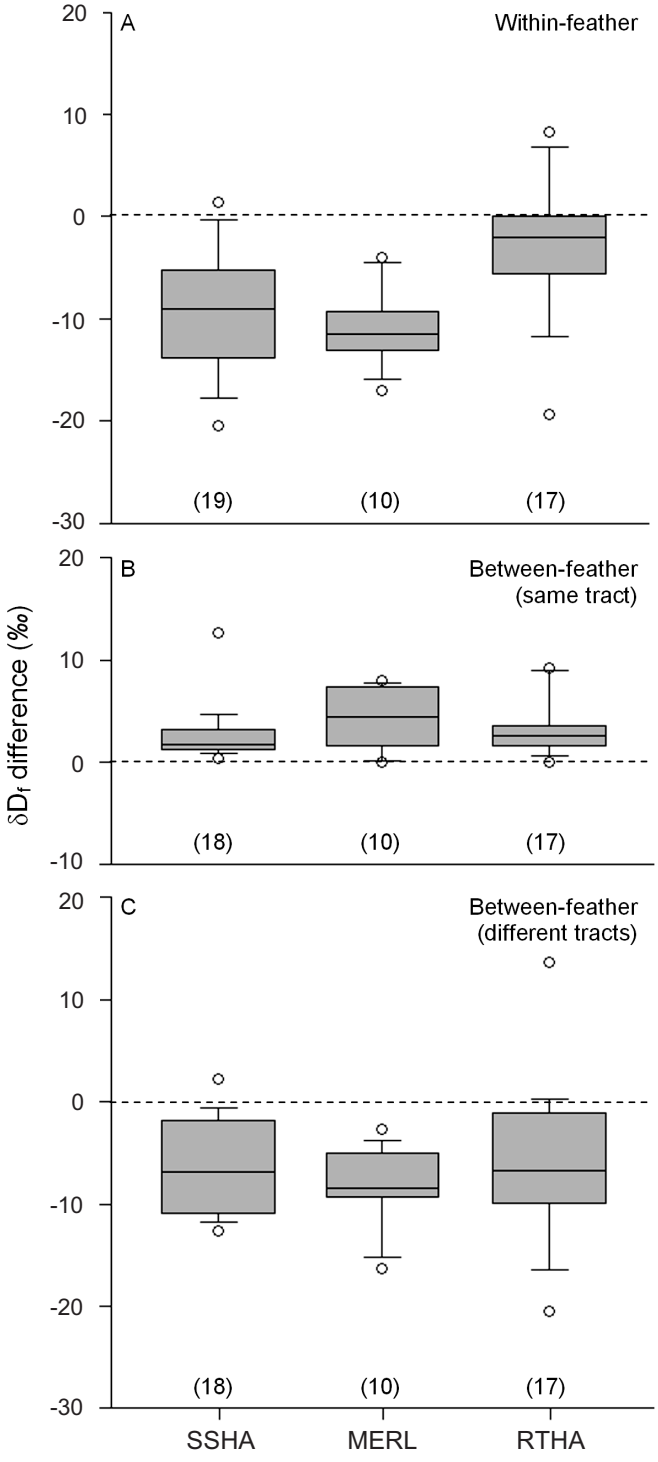

FIGURE 2. Within a single contour feather, distal feather material possessed consistently lower ratios of stable-hydrogen isotopes (δD) than proximal feather material (A). However, the magnitude of deuterium enrichment in proximal feather samples relative to distal feather samples varied among species; the difference was least pronounced in Red-tailed Hawks (RTHA) and more distinct in Merlins (MERL) and Sharp-shinned Hawks (SSHA). Contour feathers from the same tract exhibited small but significant absolute differences in feather δD (B), although the difference is tenuous due to our inability to select equivalent contour feathers from each individual. The absolute difference did not vary among species. Flight feathers possessed substantially lower δD values than contour feathers (C), but again this difference did not vary among species. Ordinate values represent feather δD differences between (A) adjacent subsamples from the same contour feather (distal sample-proximal sample); (B) two contour feathers from the same individual (absolute difference between contour feathers); and (C) a primary covert and the average of two contour feathers of the same individual (primary covert-contour feather average). Species are given in order of increasing body size and contour feather width, based on measurements from museum specimens. Box plots indicate the median and the 25th and 75th percentiles of the difference; whiskers indicate the 10th and 90th percentiles. Circles indicate outliers. The broken horizontal line at 0‰ indicates no isotopic difference between feather δD measurements; 0‰ is the lower bound in (B). Sample sizes are indicated in parentheses.

$P = 1.00$). However, flight feathers (i.e., greater primary covert) possessed substantially less deuterium than contour feathers (mean ± SD, 95% CI of difference: 6.7‰ ± 5.9‰, 5.0‰–8.4‰; Fig. 2C); again, this difference did not vary among species (ANOVA: $F_{2,44} = 0.7$, $P = 0.52$; Fig. 2C). Variability in multiple δD measurements was similar among species within a tract (Levene's test: $F_{2,42} = 2.6$, $P = 0.08$; Fig. 2B) and between tracts (Levene's test : $F_{2,44} = 1.9$, $P = 0.16$; Fig. 2C).

DISCUSSION

In our study, changes in geographic location, diet, or metabolism (Wassenaar and Hobson 2006), the contribution of water-derived hydrogen (Langin et al. 2007), or trophic level (Birchall et al. 2005) during feather growth seem inadequate to account for the systematic difference in δD observed within a contour feather and between contour and flight feathers, as the growth of feather material occurred entirely in the nest. Thus, while we have no definitive explanation for these systematic differences in feather δD, we discuss two explanations that warrant further investigation: (1) hydrogen isotope fractionation associated with feather growth rate, and (2) the incorporation of temporal variation in the δD of North American precipitation (δD_p) into feather keratin.

HYDROGEN ISOTOPE FRACTIONATION

The large relative difference in mass between deuterium and protium makes hydrogen highly susceptible to fractionation (i.e., the partitioning of heavy and light isotopes; Hoefs 2004) during tissue formation. Heavy isotopes form stronger bonds and react more slowly, suggesting that the δD content of feather keratin may depend to a large extent on the production rate of feather material (i.e., growth rate). Specifically, we might expect feather material grown more quickly to be relatively depleted in deuterium (i.e., have lower δD values). To our knowledge, however, no study has examined

hydrogen isotope fractionation as a function of feather growth rate. Feather growth rates vary depending on the feather type; flight feathers generally grow more quickly than contour feathers (Widelitz et al. 2003). Furthermore, feather growth rate typically is not constant throughout the period of feather growth (Redfern 1989). Our results for intraindividual, though not intrafeather, variation support this hypothesis.

Within a contour feather, we observed a systematic enrichment of deuterium from a distal feather vane subsample to an adjacent, proximal subsample. Contour feather growth rates are unknown for the three species that we studied, but the growth rate of Budgerigar (*Melopsittacus undulates*) contour feathers changed very little throughout the period of growth (Widelitz et al. 2003:fig. 5). If the same is true for raptors, hydrogen isotope fractionation fails to explain deuterium enrichment within a contour feather; growth rates for raptor contour feathers are needed. However, among species, the absolute growth rate of feathers tends to decrease with increasing body size (Redfern 1989). Consequently, hydrogen isotope fractionation resulting from faster feather growth rates in Merlins and Sharp-shinned Hawks, which are similar in size but much smaller than Red-tailed Hawks, might have produced the increased deuterium enrichment observed in the contour feathers of these species. Patterns of deuterium enrichment similar to those observed in raptor contour feathers have been observed in juvenal primary feathers of a Bald Eagle (*Haliaeetus leucocephalus*; Wassenaar and Hobson 2006) and Pectoral Sandpipers (*Calidris melanotos*; Farmer et al. 2004).

Within an individual, flight feathers grow more quickly than contour feathers (Widelitz et al. 2003). Thus, our observation of flight feathers containing consistently less deuterium than contour feathers supports the possibility of increased fractionation of hydrogen isotopes in flight feathers relative to contour feathers. An independent comparison of flight and contour feathers in nine immature Merlins revealed no δD difference between flight feathers but distinctly lower δD values in flight feathers relative to contour feathers (ADS, unpubl. data), corroborating the findings of this study. That the magnitude of feather δD differences was similar among species suggests similar relative growth rates of flight and contour feathers for the three species.

TEMPORAL PATTERNS OF δD IN PRECIPITATION

In North America, long-term average δD values in precipitation increase markedly from March to June and then change relatively little through July and August (Smith and Dufty 2005:fig. 4). We confirmed this general pattern in δD_p at 10 inland stations reporting precipitation isotope data from 2000 to 2002 in North America (Birks et al. 2003, International Atomic Energy Agency 2008); at the time of publication, data were not yet available for 2003. Consequently, feather material or feather tracts grown earlier in this period might be expected to contain less deuterium than feather material or feather tracts grown later. Our results in raptor feathers support this hypothesis consistently.

Within a contour feather, distal portions become metabolically inert prior to proximal portions, so lower δD values in distal feather vane samples relative to proximal vane samples correspond with seasonal patterns of δD_p. Assuming a feather growth rate of approximately 1.0–1.8 mm day^{-1} (i.e., body feather growth rate estimated from Widelitz et al. 2003: fig. 5), the distal and proximal contour feather material in our comparison represented growth occurring, on average, 5–10 days apart. Feather material grown 5–10 days apart could record significantly different environmental δD signatures, but such an explanation requires an as yet untested assumption: that the consumption of materials (e.g., food, water) characterized by disparate and discrete δD content can produce a continuous, longitudinal gradient of δD in growing feathers on the order of 1‰–2‰ day^{-1}. Notably, a similar gradient (~2‰ day^{-1}) observed in human hair was attributed to seasonal variation in δD_p (Sharp et al. 2003).

Within the context of temporal δD_p patterns, differences in deuterium enrichment among raptor species may relate to differences in feather size. That is, wide feathers require shorter lengths of feather material to obtain subsamples adequate for isotopic analysis. Contour feathers (breast feathers, measured 1 cm from the distal feather tip) from Red-tailed Hawks (mean ± SD: 22.6 ± 0.5 mm; $n = 4$) were distinctly wider than contour feathers from Merlins (mean ± SD: 16.9 ± 0.6 mm; $n = 6$) and Sharp-shinned Hawks (mean ± SD: 16.1 ± 1.1 mm; $n = 4$), which possess similarly sized contour feathers. Consequently, on the wider Red-tailed Hawk feathers, subsamples from adjacent locations may represent feather material grown over a shorter period of time (decreasing the window during which environmental δD is incorporated and decreasing the magnitude of deuterium enrichment), although differences in feather growth rates related to body size might negate some of the differences due to feather size.

Within an individual, different feather tracts erupt asynchronously during prejuvenal and prebasic molts. In the three raptor species we compared, primary feathers and greater coverts erupt 5–10 days prior to ventral contour feathers (Preston and Beane 1993, Sodhi et al. 1993, Bildstein and Meyer 2000). As mentioned previously, feather material grown 5–10 days apart may incorporate significantly different δD values based on temporal patterns in δD_p, whether that material is derived from adjacent locations on a single feather or, in this case, from feathers in different tracts grown at different stages of the prejuvenal molt.

APPLICABILITY TO PASSERINES

Isotopic work to date suggests that the incorporation of deuterium into feathers may be more complicated in raptors than passerines (Meehan et al. 2003, Smith and Dufty 2005, Lott and Smith 2006, Smith et al. 2009). For example, Mazerolle

et al. (2005) found no difference in δD between the tip and the base of tail feather vane in White-throated Sparrows (*Zonotrichia albicollis*), and Wassenaar and Hobson (2006) did not find deuterium enrichment in flight feather vane in Swainson's Thrushes (*Catharus ustulatus*). Depending on the explanation invoked, the absence of deuterium enrichment in these cases suggests either a consistent growth rate throughout the period of feather growth (hydrogen isotope fractionation), or samples comprised primarily of after-hatching-year (hereafter, adult) individuals (temporal patterns of δD_p). Adult passerines customarily replace flight feathers subsequent to breeding and prior to migration during a period of relative stability in environmental δD, so we might expect deuterium enrichment due to temporal patterns of δD_p to be less prominent. Conversely, in hatching-year (hereafter, immature) individuals, we would expect patterns comparable to those observed in immature raptors, in which case the absence of deuterium enrichment is problematic for this hypothesis. Unfortunately, age information is not available for these studies (K. Hobson, Environment Canada, pers. comm.; D. Mazerolle, Parks Canada, pers. comm.).

Hydrogen isotope fractionation related to differences in feather growth rates among tracts tends to provide a more parsimonious explanation for differences in δD values among tracts in passerines. For example, consistently lower δD values in primary feathers relative to body feathers in Wilson's Warblers (*Wilsonia pusilla*) did not relate to age ($t_6 = 0.57$, $P = 0.59$, $n = 8$; Kelly et al. 2002), a relationship which might be expected if temporal δD_p patterns are considered with the differences in molt chronology between immature and adult individuals in this species (Ammon and Gilbert 1999). Langin et al. (2007) found a similar pattern of feather δD in adult American Redstarts (*Setophaga ruticilla*), although primary and contour feathers represented different individuals. Mazerolle et al. (2005) found no difference in feather δD values between tail and tertial feathers in White-throated Sparrows. Distinguishing between the two hypotheses in this case is more difficult, however, as tail feather replacement often coincides with tertial replacement in White-throated Sparrows (Falls and Kopachena 1994; ADS, pers. obs.) and tail and tertial feathers likely grow at similar rates.

INTRAINDIVIDUAL (WITHIN-TRACT) VARIATION IN FEATHER δD

We observed only a marginal δD difference between contour feathers, and that difference is tenuous, as we quantified only absolute δD differences between feathers due to our inability to select equivalent contour feathers from each individual. Thus, we cannot conclude with certainty that systematic deviations in feather δD content exist within a tract, although feather tracts grown in a prolonged sequence (e.g., primary feathers in most prebasic molts) may exhibit such deviations (Meehan et al. 2003).

The variability of paired measurements within a feather tract (geometric SD: ±3.5‰) approached the proposed "best-case" repeatability for feather δD measurements due strictly to metabolic processes and analytical error (±2‰–3‰; Wassenaar and Hobson 2006, Paxton et al. 2007), suggesting that biological effects on the repeatability of δD measurements from feather material grown concurrently within an individual are difficult to distinguish from analytical effects. Similarly to the results of this study, Paxton et al. (2007) observed slightly less variability in δD measurements from separate Wilson's Warbler feathers within a tract than in repeated measurements of the same feather.

CONSIDERATIONS FOR FUTURE STUDIES

We documented variation in immature raptor feathers in the form of systematic δD differences between equivalent subsamples from feathers in different tracts and between adjacent subsamples in a single feather. The consistency and reduced variability in δD measurements between feathers within a tract relative to similar measurements within a feather or between tracts underscores the need for forethought in the selection of feather material. As with immature raptors in this study, deriving feather δD measurements from equivalent subsamples of equivalent feathers eliminates most risk of introducing unnecessary natural variation, including any variation associated with hydrogen isotope fractionation. However, considering temporal patterns of δD in precipitation, the choice of feather material for comparisons of migratory origins among individuals with different molt chronologies (e.g., immature versus adult passerines, multiple broods) would benefit greatly from an understanding of species-specific molt patterns. Beyond the selection of suitable feather material in such comparisons, other sources of variation warrant further consideration (Langin et al. 2007, Smith et al. 2009). Moreover, some potentially significant sources of deuterium variation will remain out of the control of researchers (e.g., intra- and interspecific variation in breeding phenology). Finally, we encourage controlled investigations of the generality of hydrogen isotope fractionation related to feather growth rate and the incorporation of temporal variation in δD_p as explanations for systematic differences in δD within feathers and among feather tracts in both raptors and passerines.

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