Oecologia (2007) 152:449–457
DOI 10.1007/s00442-007-0669-3

POPULATION ECOLOGY

Hydrogen isotopic variation in migratory bird tissues of known origin: implications for geographic assignment

Kathryn M. Langin · Matthew W. Reudink · Peter P. Marra · D. Ryan Norris · T. Kurt Kyser · Laurene M. Ratcliffe

Received: 27 September 2006 / Accepted: 23 January 2007 / Published online: 17 March 2007

Abstract Continent-wide variation in hydrogen isotopic composition of precipitation is incorporated into animal diets, providing an intrinsic marker of geographic location at the time of tissue growth. Feathers from migratory birds are now frequently analyzed for stable-hydrogen isotopes (δD) to estimate the location of individuals during a preceding molt. Using known-origin birds, we tested several assumptions associated with this emerging technique. We examined hydrogen isotopic variation as a function of age, sex, feather type and the timing of molt in a marked population of American redstarts (*Setophaga ruticilla*) breeding in southeastern Ontario. We measured δD in feathers and blood from individuals that bred or hatched at our study site during the year in which those tissues were grown. Juvenile tissues from 5- to 10-day-old birds had more negative δD values than those from adults, which most likely reflected age-related differences in diet. Within adults, primary feathers had more negative δD values than contour feathers. The mean δD value in adult primary feathers was relatively consistent among years and with the value expected for our study population. However, among-individual variation in δD corresponded to an estimated latitudinal range of 6–8° (650–900 km). We conclude that feathers sampled from recently hatched juveniles may not provide a reliable estimate of expected local isotopic signatures for comparison with adult feathers of unknown origin. Furthermore, we urge researchers to use caution when using δD values in feathers to infer geographic origin, and suggest that the best approach is to assign individuals to broad geographic zones within a species' potential molting range.

Keywords American redstart · Migration · Molt · Passerine bird · Stable isotopes

Communicated by Mark Chappell.

K. M. Langin (✉) · M. W. Reudink · L. M. Ratcliffe
Department of Biology, Queen's University,
K7L 3N6 Kingston, ON, Canada
e-mail: langink@biology.queensu.ca

P. P. Marra
Smithsonian Migratory Bird Center, National Zoological Park,
Washington, DC 20008, USA

D. R. Norris
Department of Integrative Biology, University of Guelph,
N1G 2W1 Guelph, ON, Canada

T. K. Kyser
Department of Geological Sciences and Geological Engineering,
Queen's University, K7L 3N6 Kingston, ON, Canada

Introduction

Tracking migratory animals between periods of the annual cycle has been a challenging task because many species travel thousands of kilometers between their breeding and wintering areas (Greenberg and Marra 2005). Intrinsic markers, such as stable isotopes, have proven useful for tracking animals to study long-distance migration (Hobson 1999, 2005a; Rubenstein and Hobson 2004) and dispersal (Hobson 2005b). The primary advantage of using isotopes over mark–recapture techniques is that individuals only have to be captured once to estimate their geographic origin from a previous season. Stable-hydrogen isotopes (δD), in particular, are a useful intrinsic marker in regions where large-scale meteorological patterns create spatial gradients in isotopic signatures (Bowen et al. 2005). Hydrogen in precipitation is incorporated into plant tissues and then transferred to higher trophic levels (Lajtha and

Michener 1994). Values of δD in animal tissues (δD_t) can, therefore, reflect the isotopic composition of the local food web. The expected δD_t value of a locally grown tissue is commonly derived using the average δD value of growing season precipitation (δD_p), while taking into account the tissue-specific discrimination factor that describes the offset between δD_p and δD_t.

For birds, stable-hydrogen isotope values in feathers (δD_f) can serve as a geographic marker for the location of molt. Feathers are useful tissues for tracking individuals because they retain a constant isotopic signature after they are grown, unlike metabolically active tissues such as blood and claws (Mazerolle and Hobson 2005). The application of δD_f for inferring geographic origins relies upon a priori knowledge of variation in δD_p within a species' potential molting range. Isotopic base maps of growing season precipitation have been created for North America (Meehan et al. 2004; Bowen et al. 2005) and can be adjusted to correct for isotopic discrimination from precipitation to feathers. Birds of unknown origin can therefore be assigned to a region of molt (often a latitudinal band) by interpolation of the established isotopic base maps.

Several assumptions underlie the ability to use δD_f as an effective geographic marker (Hobson 2005a; Norris et al. 2006a). First, the discrimination factor linking δD_p and δD_f is assumed to be constant among species (e.g., Hobson and Wassenaar 1997; Kelly 2006) and across populations (e.g., Rubenstein et al. 2002; Norris et al. 2006b). Second, birds molting at the same location are assumed to incorporate similar isotopic signatures into their feathers, regardless of age, sex or the type of feather being produced (e.g., Hobson et al. 2004; Norris et al. 2004). Lastly, inter-annual and seasonal variation in δD_f is assumed to be negligible within any given molt location (e.g., Rubenstein et al. 2002; Boulet et al. 2006). Recent studies on passerines (Powell and Hobson 2006), shorebirds (Wunder et al. 2005; Rocque et al. 2006) and raptors (Meehan et al. 2003; Smith and Dufty 2005) have called these assumptions into question. For example, Powell and Hobson (2006) reported that feathers from wood thrush (*Hylocichla mustelina*) breeding in the southern United States were more enriched in deuterium (i.e., had more positive δD_f values) than expected based on δD_p. Another study sampled locally grown contour feathers from adults of two *Pluvialis* shorebird species breeding within a narrow altitudinal range in Alaska (Rocque et al. 2006); variation in δD_f within one species (113‰ in *P. dominica*) was substantially larger than the other (20‰ in *P. fulva*). Finally, Meehan et al. (2003) found that primary feathers grown by adult Cooper's hawks (*Accipiter cooperii*) were enriched in deuterium relative to nestling feathers sampled from the same breeding territory.

Here, we examine variation in δD_f as a function of age, sex, feather type and the timing of molt using American redstarts (*Setophaga ruticilla*) of known origin. Redstarts are abundant, widely distributed songbirds that breed throughout North America and over-winter in the Caribbean, Central America and northern South America (Sherry and Holmes 1997). Like most Nearctic–Neotropical migratory songbirds, they are thought to undergo a post-breeding molt in the vicinity of their breeding or natal territory (Sherry and Holmes 1997; but see Norris et al. 2004). Using feathers of known origin from individually marked breeding adults and young, our goals were to: (1) quantify variation in δD_f within a breeding population of redstarts, (2) compare δD_f values with the expected local value based on δD_p, (3) examine variation in δD_f as a function of age, sex and feather type, and (4) test for inter-annual and seasonal variation in isotopic values.

Materials and methods

Field methods

Fieldwork was conducted during the 2003 to 2006 breeding seasons (May–August) at the Queen's University Biological Station, Ontario, Canada. Our 60-ha study area is a maple-dominated, mixed-deciduous forest located alongside a 787-ha lake, with marshes, small lakes and streams interspersed throughout (44°33′N, 76°22′W, elevation 120–140 m). Previous research on the same population suggests that some adults molt their tail feathers south of the breeding grounds (Norris et al. 2004). In light of this, we sampled the first primary (P1), which we hypothesized to be grown on or near the breeding site because adults drop and commence regrowth of their P1 feather first in the molt sequence (Pyle 1997) and have been observed doing so while feeding dependent fledglings (Sherry and Holmes 1997; K. M. L., M. W. R., D. R. N., personal observation). We therefore sampled the tip of P1 in year $x + 1$ from adults ($n = 30$ males, 12 females) known to have bred at our study site in year x (the same approach as in Norris et al. 2004). Some adults were sampled in multiple years ($n = 8$ in 2 years, $n = 1$ in 3 years). We also collected recently molted contour feathers (as indicated by the presence of a sheath) from adults during the post-breeding molt period ($n = 13$). Contour feathers were sampled from juveniles ($n = 34$, each individual from a different nest) when they were either nestlings (age 5–6 days) or fledglings (age 9–10 days). Five juveniles were sampled during the nestling and fledgling stages. We also sampled the tip of P1 and contour feathers in year $x + 1$ from second-year birds (in their first breeding season; $n = 4$, all males) known to have hatched at our study site in year x. Juveniles begin growing their primary feathers as nestlings and do not replace them until the end of the next breeding season;

they do, however, drop and re-grow contour feathers after they leave the nest (Pyle 1997). The contour feathers sampled from second-year birds (in year $x + 1$) were the re-grown feathers, so represent a distinct group from the contour feathers sampled from juveniles (in year x).

To test for age-related variation in δD within a metabolically active tissue, we sampled blood from parents ($n = 7$ females and 3 males, each from a different territory) and one of their offspring on the same day in 2004. Most offspring were sampled on the day of fledging (one was still a nestling). Blood samples (10–50 µl from the brachial vein) were collected in heparinized capillary tubes and stored in a cooler until they could be centrifuged (within 8 h) for 8 min at 14,000 r.p.m. (protocol approved by the Queen's University Animal Care Committee). The plasma component was removed using a Hamilton syringe before transferring the hematocrit to separate microcentrifuge tubes, which were then stored at –20°C until isotopic analysis.

Because the hydrogen in feathers and blood originates from both dietary and water sources (Hobson et al. 1999), we sampled redstart prey (insects), rainwater and standing water in 2005. Insects were sampled using Malaise traps (Diptera) and taken directly from vegetation (Lepidopteran larvae) in mid June, then frozen at –20°C until isotopic analysis. Precipitation and standing water were sampled from 14 June to 18 August. For precipitation, open jars ($n = 9$) were located throughout the study site and a thin layer of mineral oil was placed in them to prevent subsequent evaporation. The water was collected using a syringe on three separate occasions, each sample representing precipitation over the previous 3 weeks. Standing water was sampled 4 times at seven sites along marshes, lakes and streams. Mean δD values at each site were used for analysis.

Isotopic analysis

Stable-isotope analyses were conducted at the Queen's Facility for Isotope Research in Kingston, Ontario. Feathers were washed of surface oils and debris using a 2:1 chloroform:methanol solution and air dried under a fume hood for 72 h, allowing them to equilibrate with the local atmosphere. Blood samples and insect specimens were freeze-dried and powdered prior to analysis (except for Diptera, which were freeze-dried and analyzed whole). Organic samples were loaded (0.10–0.15 mg) into silver capsules and placed in an oven at 100°C for either 24 h (feathers) or 1 h (blood and insects) to remove any surface water. The capsules were then crushed, loaded into a reduction furnace (Finnigan TC/EA) at 1,450°C and introduced online into an isotope ratio mass spectrometer (Finnigan MAT Delta Plus XL). For water samples, 1 µl was injected into an H/Device coupled to an isotope ratio mass spectrometer (Finnigan MAT 252). One in-house standard was run for every five unknowns. Stable-hydrogen isotope values are reported in per mil notation (‰) relative to Vienna standard mean ocean water according to the formula:

$$\delta D = \left[\left({}^{2}\mathrm{H}/{}^{1}\mathrm{H}_{\mathrm{sample}}/{}^{2}\mathrm{H}/{}^{1}\mathrm{H}_{\mathrm{standard}}\right) - 1\right] \times 1{,}000$$

Measurements on the same feather were repeatable to within 2 ± 2‰ (mean ± SD, $n = 12$). Similarly, SDs for repeated measurements of mineral standards were between 2 and 3‰ (kaolinite $n = 16$; brucite $n = 13$).

The hydrogen isotope values reported here include a combination of exchangeable and non-exchangeable hydrogen. To control for potential seasonal variation in δD in the ambient water vapor of the laboratory—and hence in the exchangeable hydrogen portion of the feathers—we analyzed the majority of samples ($n = 95$) within a span of 2 months in 2006. Excluding samples analyzed outside of this period ($n = 17$) did not change the results of most analyses, with the exception of sex-related variation in δD_f. There was no isotopic difference between the sexes when all of the feathers were included in the analysis (see results); however, with the reduced sample size primary feathers from males had more positive δD_f values than those from females (*t*-test, $t = 3.6$, $P = 0.001$, $df = 27$). Further sampling is needed to determine if there is sex-related variation in δD_f in American redstarts.

Data analysis

The interpolated growing season mean δD_p value at our study site is –58‰, based on isotopic data from the International Atomic Energy Agency (IAEA) and the interpolation method described in Bowen et al. (2005). Confidence intervals are not available for interpolations of growing season precipitation (Bowen et al. 2005) but are for annual precipitation (Bowen and Revenaugh 2003). At our study site the 95% confidence interval for the interpolated *annual* mean δD_p value is relatively low (4‰) due to the close proximity of the nearest IAEA sampling site (within 100 km). Two different discrimination factors are frequently used to estimate the origin of passerine birds in North America, one based on measures of non-exchangeable hydrogen in feathers from central portions of the continent (–25‰; Wassenaar and Hobson 2001), and the other based on measures of exchangeable and non-exchangeable hydrogen in feathers from across the continent (–19‰; Bowen et al. 2005). According to these estimates, the predicted δD_f value for our study population is between –83 and –77‰.

When individuals were sampled on multiple occasions, we only included the first feather in our analyses. Contour

feathers sampled from nestlings [–92 ± 8‰ (mean ± SD), n = 20] and fledglings (–90 ± 5‰, n = 14) did not differ significantly in isotopic composition (test, t = 0.74, P = 0.47, df = 32), so these groups were pooled for all analyses and are hereafter referred to as ''juvenile contour feathers''. Multiple comparison procedures were used to test for variation in δD_f as a function of sex (t-test), feather type (one-way ANOVA) and growth year (two-way ANOVA, with growth year and feather type as model factors). Raw data were converted to ranks before performing both ANOVA procedures because of unequal variance among groups. For the two-way ANOVA, there was no interaction between growth year and feather type (P = 0.08). We tested for seasonal variation in δD_f using hatch date as a proxy for the start of feather growth in juveniles and offspring fledge date as a proxy for the start of molt in adults. For the latter analysis, we only included adults that raised a brood to fledging during the year of feather growth (n = 15). Data were analyzed using JMP (SAS Institute 2006).

Results

Based on the δD_p value for local growing season precipitation, the predicted isotopic composition of feathers grown at our study site was between –83 and –77‰. On average, δD_f values in American redstart feathers were slightly more negative than predicted [–85 ± 8‰ (mean ± SD), n = 97] and ranged from –115 to –70‰. Age accounted for much of this variation. Hydrogen isotope values in adult primary feathers corresponded more closely with the predicted values and were not as variable (–82 ± 4‰, n = 42; ranged from –92 to –70‰). Half of those values were within ±2‰ of the mean and 80% were within ±6‰. Based on the isotopic base map proposed by Meehan et al. (2004) for growing season precipitation, the total variation in δD_f we measured among adult primary feathers corresponds to an estimated latitudinal range of 6–8° (650–900 km) around our study site (see Fig. 1).

Primary feathers from males (–81 ± 4‰, n = 30) and females (–84 ± 5‰, n = 12) had similar δD_f values (t-test, t = 1.8, P = 0.08, df = 40), so adults were pooled for all subsequent analyses. Juvenile contour feathers were more depleted in deuterium than both adult feather types [ANOVA on ranked data, $F_{2,88}$ = 51.6, P < 0.0001, Tukey's honestly significant difference (HSD) test; Fig. 2a]. Within adults, contour feathers had more positive δD_f values than primary feathers (Tukey's HSD test). Only a small fraction of birds banded as nestlings returned the following year (n = 4), so they could not be included in the above analysis. Nevertheless, values of δD_f in feathers from second-year birds (nestlings returning in year x + 1) appear more similar to those of adult feathers than to those of juvenile contour feathers (Fig. 2a).

Temporal variation in δD_f was examined using adult primary feathers and juvenile contour feathers, the groups with the most samples. Values of δD_f did not vary among years when controlling for feather type (two-way ANOVA on ranked data, $F_{2,75}$ = 1.8, P = 0.18; Fig. 2b). However, when juveniles were analyzed separately feathers grown in 2005 had more negative δD_f values than those grown in 2004 (ANOVA, $F_{2,33}$ = 6.9, P = 0.003, Tukey's HSD test). We did not detect seasonal variation in the isotopic composition of juvenile contour feathers (ordinary least squares r^2 = 0.01, P = 0.54, n = 34) or adult primary feathers (r^2 = 0.04, P = 0.49, n = 15; Fig. 2c).

For birds that were sampled on multiple occasions, within-individual variation in δD_f was highly variable (see Fig. 3). Hydrogen isotope values were more variable within juveniles sampled twice in 4–5 days than within adults sampled in consecutive years. The SD for any given individual was also largest when its mean δD_f value was furthest removed from the population mean (controlling for age). Thus, individuals that formed a feather with a δD_f value more positive (or negative) than expected did not consistently do so.

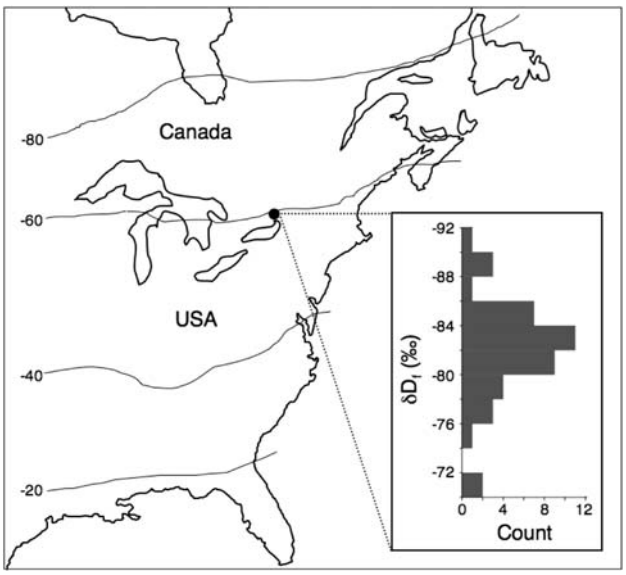

Fig. 1 *Inset* Histogram of stable-hydrogen isotope values in primary feathers (δD_f) (n = 42) sampled in year x + 1 from adult American redstarts (*Setophaga ruticilla*) known to have bred at the study site (Queen's University Biological Station, Ontario, Canada; *dark filled circle*) in year x. Feathers were grown during the post-breeding molt period in 2003, 2004 and 2005. *Contour lines* represent mean δD_p values for growing season precipitation across eastern North America (approximated from Meehan et al. 2004). Feathers grown in these locations are assumed to reflect the local δD_p value with an offset in the order of –19 (Bowen et al. 2005) to –25‰ (Wassenaar and Hobson 2001)

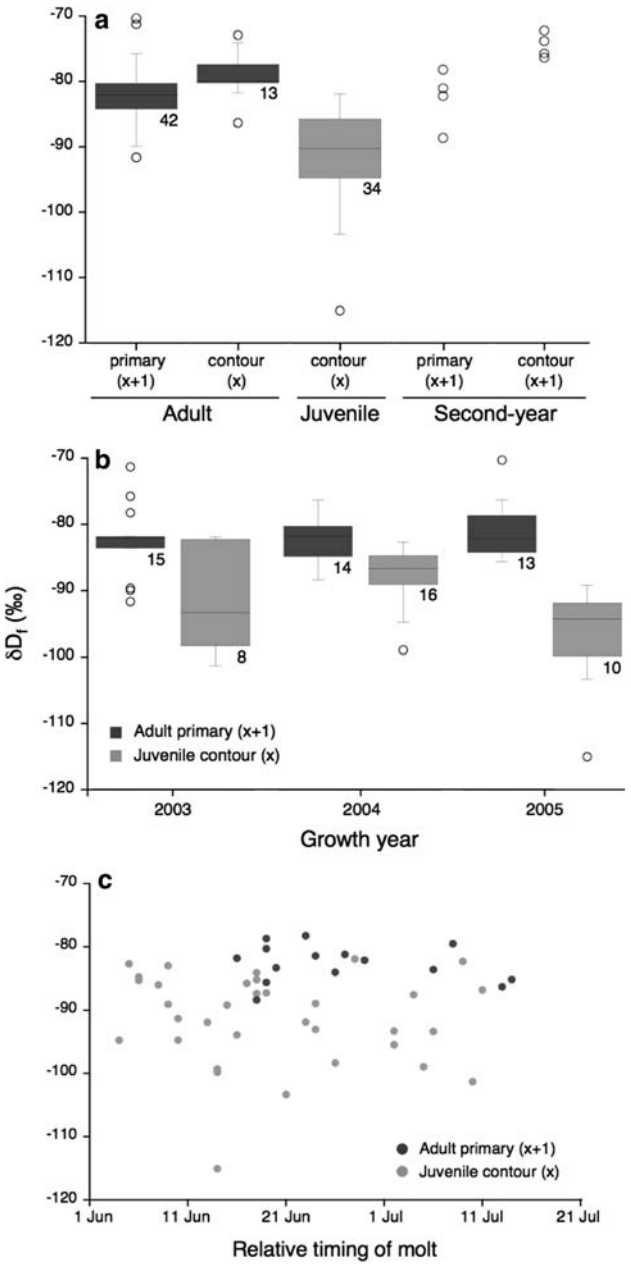

Fig. 2 δD_f in American redstart feathers grown in southeastern Ontario as a function of **a** bird age (adult, juvenile, second-year) and feather type (primary, contour), **b** growth year (2003, 2004, 2005) and **c** relative timing of molt. Age designations are used to describe the birds' age at the time of feather sampling. Feathers collected in the same year they were grown are labelled as (x) and those collected in the following year are labelled as ($x + 1$). **a** and **b** Box plots show 10, 25, 50, 75 and 90th percentiles as *horizontal lines* and outlying values as *circles*. Sample sizes are noted below each box. The data for second-year birds are represented as *individual circles*, rather than as boxes, because of low sample size ($n = 4$). **c** The date an adult's offspring left the nest in year x was used as a proxy for the timing of molt ($n = 15$), while for juveniles hatch date was used as a proxy ($n = 34$)

Age-related variation in δD was also detected in blood, a metabolically active tissue. Controlling for location within the study site (adults and offspring were from the same territory) and time of season, hematocrit sampled from parental redstarts was more enriched in deuterium than hematocrit sampled from their offspring on the same day (paired t-test, $t = 2.8$, $P = 0.02$, $df = 9$; Table 1).

Potential sources of hydrogen for redstarts in this population—insects and drinking water—differed markedly in isotopic composition. Hydrogen isotope values in insects ranged from –170 to –97‰, with Diptera samples having more negative δD values than Lepidopteran larvae (Table 1). Relative to insects, all potential sources of drinking water were enriched in deuterium (δD ranged from –71 to –38‰). Hydrogen isotope values were similar in precipitation and standing water (Table 1).

Discussion

Stable-hydrogen isotopes are increasingly used to address fundamental questions about the ecology and life history of mobile organisms. Yet, few researchers have adequately tested critical assumptions of this method by analyzing tissues of known origin. Most such studies on passerine birds (Hobson and Wassenaar 1997; Kelly et al. 2002; Hobson et al. 2004) have used feathers collected from

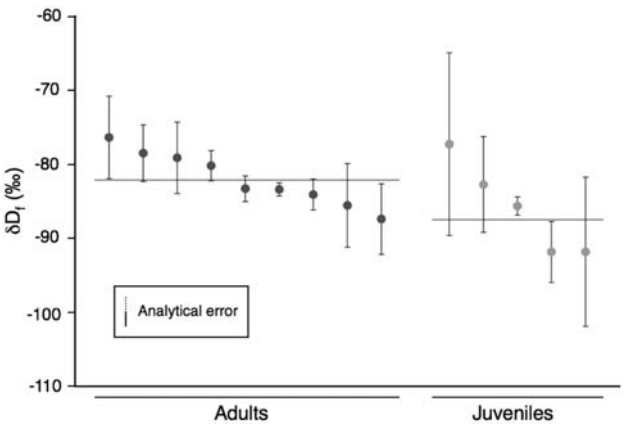

Fig. 3 Within-individual variation in δD_f for American redstarts sampled on multiple occasions. Adults ($n = 9$; *dark filled circle*) were sampled for primary feathers in 2 consecutive years (one in all 3 years), while juveniles ($n = 5$; *light filled circle*) were sampled for contour feathers twice within the first 10 days of hatching in 2004 (mean ± SD for each individual). *Horizontal lines* denote the mean δD_f value for adult primary feathers in all years and for juvenile contour feathers in 2004. The analytical error includes the mean difference between repeated δD_f measurements of the same feather (*solid line*) and the SD of those measurements (*dotted line;* $n = 12$)

Table 1 Stable-hydrogen isotope values in American redstart (*Setophaga ruticilla*) feathers and blood hematocrit, as well as in potential sources of dietary hydrogen (insects) and drinking water

	Mean ± SD (*n*)
Feathers[a]	
Adult (primary)	–82 ± 4 (42)
Juvenile (contour)	–91 ± 7 (34)
Blood[b]	
Adult	–84 ± 9 (10)
Juvenile	–92 ± 9 (10)
Insects[c]	
Diptera	–143 ± 27 (5)
Lepidoptera	–116 ± 22 (5)
Drinking water[d]	
Precipitation	–54 ± 3 (9)
Standing water	–57 ± 13 (7)

[a] Grown in 2003, 2004 and 2005

[b] Sampled in June and July 2004

[c] Sampled in June 2005

[d] Sampled in June, July and August 2005

breeding areas and have assumed that those individuals bred and molted in the same location the previous year (i.e., no dispersal or molt migration; but see Powell and Hobson 2006). Our study analyzed isotopic signatures of individually marked birds over multiple years and could therefore limit feather collection to birds known to have hatched or bred at our study site during the year in which those feathers were grown. Furthermore, by sampling the first primary feather (sampling tail feathers is a more common approach in passerines; Smith et al. 2003) we increased the likelihood that adults completed feather growth prior to their departure for fall migration. It does, however, remain possible that some primary feathers were replaced south of the study site, as we could not track the post-breeding movements of adults. Had that been the case, we would have expected the distribution to be skewed, with the tail pointing towards more positive δD_f values (more enriched in deuterium than expected). Instead, outliers were dispersed evenly around the mean (i.e., both positive and negative; see Fig. 1).

Assigning breeding origin of migratory birds using geographic patterns in δD_p depends on an accurate estimate for the discrimination factor linking δD_p and δD_f. Across North America, a strong relationship exists between δD_p and δD_f (Hobson and Wassenaar 1997; Lott and Smith 2006); however, the discrimination factor linking them has been found to vary among taxonomic groups (songbirds –25 to –19‰, Wassenaar and Hobson 2001; Bowen et al. 2005; raptors –5.6‰, Lott and Smith 2006) and, in the case of raptors, at a regional level (Lott and Smith 2006). The accurate assignment of migratory birds is further complicated by potential inconsistency in the measurement of δD_f values. As mentioned previously, a small portion of the hydrogen in feathers can exchange with the hydrogen in ambient water vapor, the isotopic signature of which may vary seasonally or among laboratories. Despite the potential influence of exchangeable hydrogen, the mean δD_f value we measured in adult primary feathers (–82‰) corresponded closely with the predicted value (–83‰) based on the discrimination factor for non-exchangeable hydrogen (Wassenaar and Hobson 2001). The isotopic signature of adult feathers was also remarkably consistent across all 3 years, with the mean varying by less than 2‰.

Recent studies on shorebirds have reported substantial within-population variation in δD_f, questioning the precision of this intrinsic geographic marker. Wunder et al. (2005) sampled contour feathers from mountain plover (*Charadrius montanus*) chicks at multiple breeding sites ranging from Colorado to Montana and found that, within a given site, the isotopic signature of feathers grown in the same year varied by upwards of 60‰. The use of juvenile contour feathers—which, in redstarts at least, are more isotopically variable than adult feathers—may have contributed to the large within-site variation. However, Rocque et al. (2006) reported an even larger isotopic range for adult American golden plovers (*Pluvialis dominica*) breeding in Alaska, with δD_f ranging from –175 to –62‰. For American redstarts in our study population, in contrast, the range of δD_f values among adult primary feathers in a given year was as high as 20‰ in 2003 and as low as 12‰ in 2004. Furthermore, half of the values were within ±2‰ of the mean and 80% were within ±6‰. These results suggest that δD_f may serve as a more precise estimate of geographic origin in passerines than in shorebirds.

We also found that adult tissues were more enriched in deuterium than juvenile tissues from 5- to 10-day-old birds. Similar results have been previously reported for feathers of Cooper's hawks (Meehan et al. 2003) and northern goshawks (*Accipiter gentilis*; Smith and Dufty 2005). Adults of those species, however, begin molt during the incubation phase, so the authors presumed their findings would not apply for passerines that undergo a post-breeding molt. In redstarts, age-related variation in δD appears to be limited to recently hatched juveniles, as feathers from second-year birds (nestlings returning in year $x + 1$) had isotopic signatures that were consistent with adult feathers of the same type (Fig. 2a). Primary feathers begin growing when juveniles are in the nest, but they do not become inert until the feather barbs emerge and the follicles reabsorb the blood vessels (Gill 1989). The isotopic signature of flight feathers could therefore be altered later during the fledgling phase. In the case of contour feathers, they are re-molted after the juveniles fledge (Pyle 1997), so a distinct isotopic signature may be incorporated into the re-grown feathers

compared to those grown in the nest. We suggest that during the early stages of growth, juvenile tissues are depleted in deuterium relative to adults and even relative to juveniles in the later stages of growth.

In raptors, evaporative water loss was proposed as a possible explanation for elevated δD_f values in adults relative to nestlings (Meehan et al. 2003; Smith and Dufty 2005). Birds release heat through the evaporation of water along cutaneous and respiratory surfaces (Wolf and Walsberg 1996). Water containing protium is preferentially evaporated during this process, leaving the body water pool enriched in the heavier hydrogen isotope (McKechnie et al. 2004). Raptors combine molt with reproductive activities; thus it was suggested that they may rely on evaporation during the period of feather growth more so than in passerines. We detected an isotopic difference between adults and nestlings that was in the same direction, but of a lower magnitude, than that reported in raptors (difference of 30–60‰, on average). It is possible that adults have higher rates of evaporative water loss than nestlings, regardless of the timing of molt. However, there is some evidence that the reverse may actually be the case in temperate-breeding species (Kirkley and Gessaman 1990; Marder et al. 2003).

Instead of a physiological mechanism, we suggest that variation in diet is a more parsimonious explanation for the age-related variation in δD. Hydrogen incorporated into feathers and blood can be derived from food items and drinking water (Hobson et al. 1999), sources that may differ in isotopic composition. Plants discriminate against the heavier hydrogen isotope during the synthesis of organic compounds, producing tissues with more negative δD values than local waters (Smith and Epstein 1970; Epstein et al. 1976). Isotopic fractionation of hydrogen is thought to be minimal during the formation of animal tissues (Estep and Dabrowski 1980; Hobson and Wassenaar 1997). Even so, a recent study found that animals at higher trophic levels tend to have more positive δD values (Birchall et al. 2005), a pattern that may reflect the incorporation of hydrogen derived from drinking water. We sampled a variety of local waters in 2005, all of which had more positive δD values than those in insects (Diptera and Lepidopteran larvae) sampled at the same time (Table 1). Adults in our study population have access to both of these sources of hydrogen whereas nestlings only have access to food items (assuming precipitation plays a negligible role). All else being equal, if adults incorporate more water-derived hydrogen into their tissues they would have more positive δD values than nestlings, which is consistent with our observations. Once the nestlings fledge their access to drinking water may increase, thus altering the isotopic composition of their tissues. Given the wide range in δD values of insects, the exclusive use of food items by nestlings may have also contributed to the substantial variance in δD_f among juvenile contour feathers.

Regardless of the underlying mechanism, deuterium enrichment in adults relative to nestlings appears to be present in multiple tissue types (feathers, blood) and across diverse avian taxa (raptors, passerines). Based on these findings, we caution against using feathers sampled during the nestling phase or shortly thereafter to estimate expected local isotopic signatures for comparison with adult feathers of unknown origin (e.g., Hobson and Wassenaar 2001; Norris et al. 2004). Feathers from fully grown hatch-year or second-year birds, however, appear to reflect the same local isotopic signature as adults. A similar discrimination factor should therefore apply when estimating their geographic origin.

Implications for future research

Feathers from migratory birds are more and more frequently being analyzed for stable-hydrogen isotopes to link the location of molt (as inferred using δD_f) with the location of feather collection. One of the most useful applications of this technique is to document patterns of migratory connectivity, which is the degree to which breeding, wintering and migratory stopover areas are geographically linked (Webster et al. 2002; Marra et al. 2006). Hydrogen isotope values in feathers have also been used to study long-distance dispersal, an application that applies to both resident and migratory species (Hobson 2005b). The ability to infer the mean δD_p value in the source region based on the mean δD_f value for a group of birds has been reported to be as precise as ±3‰ (Meehan et al. 2001). Our findings are consistent with that level of resolution. Furthermore, at the individual level more than half of the adults sampled for primaries had δD_f values within ±3‰ of the mean. Yet, a few individuals known to be local breeders had isotopic signatures that were indicative of much more northerly or southerly origins (see Fig. 1). Our results therefore suggest that the origin of individual redstarts, and possibly other songbirds, cannot be accurately estimated within a narrow band of latitude using δD_f values alone.

When used cautiously, we believe stable-hydrogen isotopes remain a valuable tool with which to study movement ecology. Instead of treating δD_f values as pinpoint estimates of molting latitude, we recommend that individuals should be assigned to a broad geographic zone within a species' potential molting range (e.g., Norris et al. 2006b). The recent development of likelihood-based assignment tests has provided more rigorous statistical techniques for assigning birds to geographic zones of origin (Royle and Rubenstein 2004; Wunder et al. 2005; Marra et al. 2006). Multivariate approaches, which combine δD_f with the use of additional markers such as trace elements (e.g., iron,

mercury), other stable isotopes (e.g., $\delta^{13}C$, $\delta^{15}N$) and genetic markers, can also be used to provide more precise estimates of geographic origin, particularly when the additional markers vary with longitude (Kelly et al. 2005; Boulet et al. 2006).

Acknowledgements Funding was provided by the Natural Sciences and Engineering Research Council of Canada (L. M. R., T. K. K., K. M. L., D. R. N.), the National Science Foundation (0089565; P. P. M.), the Canadian Foundation for Innovation (T. K. K., L. M. R.), the Wilson Ornithological Society (D. R. N.), Sigma Xi (M. W. R.), the American Ornithologists' Union (M. W. R., D. R. N.), the Society of Canadian Ornithologists (M. W. R., D. R. N.), the Ontario Government (K. M. L.), Queen's University (M. W. R., D. R. N.) and the Smithsonian Institution (P. P. M.). Thanks to C. Studds, S. McWilliams, J. Kelly and one anonymous reviewer for helpful comments, to F. Phelan and F. Connor for logistical support at the Queen's University Biological Station, to K. Klassen and A. Vuletich for assistance with isotope analysis, to K. Shorter and R. Zavitz for collecting and analyzing water samples, to C. Hasler for GIS help, and to our many excellent field crews for their tireless efforts.

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