

# An Isotopic Analysis of Migratory Connectivity in Ruby-throated Hummingbirds

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## ABSTRACT

Hydrogen isotope data from hatch-year (HY) Ruby-throated Hummingbirds (*Archilochus colubris*) are presented to answer questions about migratory connectivity. Baseline data from 11 sites across the eastern US show a strong relationship between feather isotope ratio and latitude. Isotope ratios from an additional seven HY feathers from Costa Rica and El Salvador were analyzed in light of these baseline data. Hummingbirds at the two sites were found to originate from different latitudes in North America, suggesting a pattern of chain migration. A second aim was to determine the relationship between the isotopic signature of HY feathers ( $D_f$ ) and estimated annual deuterium in precipitation ( $D_p$ ) for the natal sites. The calibration of  $D_p$  to  $D_f$  will enable estimation of natal latitude for HY birds sampled on the wintering grounds in Central America.

## INTRODUCTION

There is currently little information on the migration of the most widespread of North American hummingbirds, the Ruby-throated Hummingbird (*Archilochus colubris*); this includes interseasonal migration routes and patterns. Ruby-throated Hummingbirds breed in the eastern US and Canada, and spend winters in southern Mexico and Central America. There appears to be high site fidelity at breeding grounds, as indicated by the proportion of re-encounters across years at the same locations (Hutcheson 2007). It is generally believed that they migrate to wintering sites overland into and south of Mexico, but typically

cross the Gulf of Mexico on their return to northern breeding grounds (e.g., Williamson 2001). However, directional analysis of re-encounter data in the US, as well as eye-witness accounts in Mexico, suggest that a larger proportion than previously thought may return north via an overland route (Hendrix et al. 2008).

The term *migratory connectivity* refers to the extent to which individuals breeding in a given area tend to concentrate in one particular wintering area (Boulet and Norris 2006). If a given breeding population disperses and mixes together with other populations on wintering grounds, there is low migratory connectivity; conversely, connectivity is high if birds from different breeding grounds migrate to separate wintering grounds. Boulet and Norris (2006) follow Salomonsen (1955) in identifying four different patterns of migratory connectivity:

**Parallel migration** occurs when breeding populations at different longitudes migrate along parallel routes along different longitudes with, for example, the more easterly breeders taking the more easterly wintering grounds;

**Longitudinal migration** occurs when populations migrate along the same degree of longitude, presumably using similar routes as when flyways exist;

**Chain migration** is one subtype of longitudinal migration in which more northerly breeding populations take up more northerly

wintering grounds, and more southerly breeders utilize sites further south; and

*Leap-frog migration* is another subtype in which more northerly breeding populations use the more southerly wintering grounds, and the southerly breeders use the northerly wintering grounds.

Currently, there are insufficient data on Ruby-throated Hummingbird migration to apply this conceptual framework, and many questions remain unanswered. For example:

1. Do Ruby-throated Hummingbirds exhibit high migratory connectivity, or do those from one breeding area disperse and merge with others on different wintering grounds?

2. Do those that breed further north take the most northerly wintering grounds, or do they leap-frog over southerly breeders, making for a much longer migration?

3. Do their migratory routes cross or are they parallel?

Our first aim was to attempt to address the first two of the above questions. Our method—using stable isotope ratios from juvenile feathers to infer natal latitude—allows us potentially to differentiate between chain and leap-frog migration. Traditional bird banding studies suffer low recapture rates that make such a study difficult; according to the US Bird Banding Laboratory, there have been no recaptures of Ruby-throated Hummingbirds banded in the US or Canada on their wintering areas in Central America, although a recent re-encounter in Georgia of a Ruby-throated Hummingbird banded in Costa Rica was reported by Hilton (2008). However, by using analysis of deuterium (D or  $^2\text{H}$ , a stable isotope of hydrogen) in the feathers of migrating birds, it is possible to link wintering and breeding populations. Although deuterium is ubiquitous, its continental distribution is a function of temperature and rainfall; in North America, there is a predictable increasing gradient of deuterium concentration in precipitation, running approxi-

mately northwest to southeast (Bowen and West 2008). Deuterium from precipitation is taken up by plants and thus enters the food chain. As a result, biological materials will reflect the deuterium signature of the area in which the deuterium was assimilated (Wassenaar 2008).

Over the last decade or so, deuterium analysis has been employed to investigate migration of passerines (Hobson and Wassenaar 1997; Hobson et al. 2004 a; Hobson et al. 2004b; Hobson et al. 2004c; Mazerolle and Hobson 2005; Mazerolle et al. 2005; Greenberg et al. 2007; Langin et al. 2009) as well as hummingbirds (Hobson et al. 2003) and other non-passerines (Hebert and Wassenaar 2005; Mehl et al. 2005; Hobson et al. 2006; Rocque et al. 2006), and even migratory invertebrates such as monarch butterflies in which chitin, rather than keratin, incorporates the isotopic signature (Hobson et al. 1999). Hutcheson et al. (2007) demonstrated that stable isotope analysis could distinguish between locally hatched Ruby-throated Hummingbirds and migrants.

In the present study, we first obtained feather samples from banders across the breeding range of Ruby-throated Hummingbirds to create a base sample for comparison with feathers obtained from banding stations in the winter range of Ruby-throated Hummingbirds. We were able to obtain seven feather samples from El Salvador and Costa Rica, and we attempted to obtain samples from other banding stations throughout Central America. However, Ruby-throated Hummingbirds are encountered rarely at banding stations, and we had the further restriction of using hatch-year (HY) bird feathers, since their feathers are grown in the nest, thereby containing the isotopic signature of their natal area.

Our second aim was to determine the relationship between the deuterium signature of HY Ruby-throated Hummingbird feathers ( $D_f$ ) and the estimated annual deuterium in precipitation ( $D_p$ ) for the site at which each feather was grown (i.e., the natal site). The calibration of  $D_p$  to  $D_f$  will enable  $D_f$  values of rectrices collected from HY birds

overwintering in Central America to be assigned an estimated geographical area of natal origin by direct comparison with published North American deuterium isoscapes.

## METHODS

**Feather Collection.** From July through August 2006, the 4th rectrix (R4) was removed from the first 10 HY Ruby-throated Hummingbirds banded at 11 stations across the species' breeding range with the assumption that these first few birds were from the local breeding population. These 11 stations represent a fairly complete sampling of the Ruby-throated Hummingbird range excepting Canada, and include one station in Louisiana (latitude  $\sim 30^\circ$  N), Alabama ( $\sim 32^\circ$  N), North Carolina ( $\sim 35^\circ$  N), Oklahoma ( $\sim 36^\circ$  N), Illinois ( $\sim 37^\circ$  N), Pennsylvania ( $\sim 40^\circ$  N), Michigan ( $\sim 42^\circ$  N), New York (three stations ranging from  $\sim 42^\circ$  to  $43^\circ$  N), Maine ( $\sim 44^\circ$  N) and Minnesota ( $\sim 47^\circ$  N). These samples provided a range-wide variation of isotopic values to compare feathers obtained from banding operations in El Salvador and Costa Rica. The Central American feather samples ( $n = 7$ ) were obtained from Ruby-throated Hummingbird wintering areas and were collected from HY birds prior to their first molt; as a result, these feathers contained the isotopic signature of the natal sites.

**Stable Isotope Analysis.** Analyses were carried out following the procedure of Hutcheson et al. (2007). Briefly, the feathers were cleaned in a 2:1 chloroform/methanol solution, then analyzed for deuterium at the National Water Research Institute in Saskatoon, SK, using continuous-flow isotope-ratio mass spectrometry (CF-IRMS). The results are expressed in delta ( $\delta$ ) notation, per mil (‰), referenced to the Vienna Standard Mean Ocean Water-Standard Light Antarctic Precipitation (VSMOW-SLA) standard. Laboratory error was estimated at  $\pm 3$  ‰.

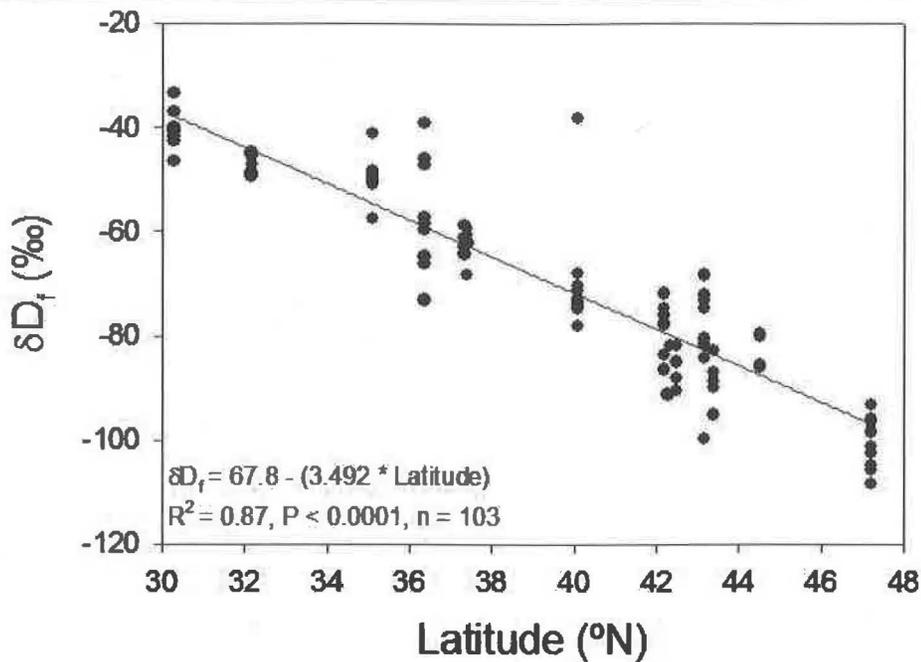
**Data Analyses.** Data were analyzed with the Statistical Package for the Social Sciences (SPSS; SPSS Inc., Chicago, IL). Hand calculations were used to construct origin latitude estimates for the

Central American specimens using formula from Steel and Torrie (1960) and Sokal and Rohlf (1969). Estimates for annual deuterium in precipitation ( $D_p$ ) were obtained for each collection site using the Online Isotopes in Precipitation Calculator (OIPC v.2.2, Bowen, 2008, – see Bowen and Revenaugh, 2003, for background). We followed Hobson et al. (2009) in using Reduced Major Axis regression (RMA) for calibration of  $D_p$  to  $D_f$ . Regression graphs were plotted using SigmaPlot v. 8.02 (SPSS Inc., Chicago, IL).

## RESULTS AND DISCUSSION

For the feathers collected in the US, regressing feather isotope ratio ( $D_f$ ) on latitude provides baseline data. This analysis shows a strong relationship between  $D_f$  and latitude (see Fig. 1). Natal latitude accounts for approximately 87% of the variation in  $D_f$  in the feathers collected in the US, with a slope of  $-3.49$ . This strong relationship helps to counteract the problems of statistically estimating the latitudes of origin for the Central American samples. Two extreme outliers occurred at  $40.10^\circ$  N and  $43.16^\circ$  N (Fig. 1). Both are far from their expected values, at 3.6 and 5.5 standard deviations, respectively. Ordinarily, outliers such as these two would be excluded from the analysis with the assumption that some unknown error occurred. Such an error could come from a bird moving some distance before its feather was collected, from a diet rich in cane sugar-water (grown in more southern locations) at feeders, or other sources. Since we use  $D_f$  to estimate the unknown natal latitudes for the feathers collected in Central America, it is uncertain whether to include or to delete these two cases. We present parallel analyses with these outliers included and excluded.

Where along the regression line in Fig. 1 do the Central American birds fit? Point and interval estimates for latitude can be hand-calculated from the isotope ratios from the feathers collected in Central America, but the calculations are cumbersome and the intervals are not symmetrical around the latitude point estimate for each (Sokal and Rohlf 1969: 446-448). We opted instead for a simpler



**Fig. 1. Relationship of Hydrogen Isotope Ratio ( $D_f$ ) to Latitude for HY Ruby-throated Hummingbirds.**

“backwards regression” analysis, by switching the independent and dependent variables. Using latitude as the dependent and isotope ratio as independent, SPSS can provide both point estimates and confidence intervals for origin latitude for the individual feathers collected in Central America. Additional hand-calculations yield confidence intervals for origin latitude for the mean isotope ratios of the feathers separately for Costa Rica and El Salvador.

Feathers collected from Central America in this trial include two from one site in Costa Rica and five from two sites in El Salvador. The two Salvadoran sites are separated by less than half a degree of both latitude and longitude. The collection sites in El Salvador are four degrees farther north and are 4.00° and 4.28° further east than those in Costa Rica (Table 1).

**Table 1. Individual feather isotope ratios ( $D_f$ ) and expected natal latitudes with 95% confidence intervals for Ruby-throated Hummingbird feathers collected in Central America.**

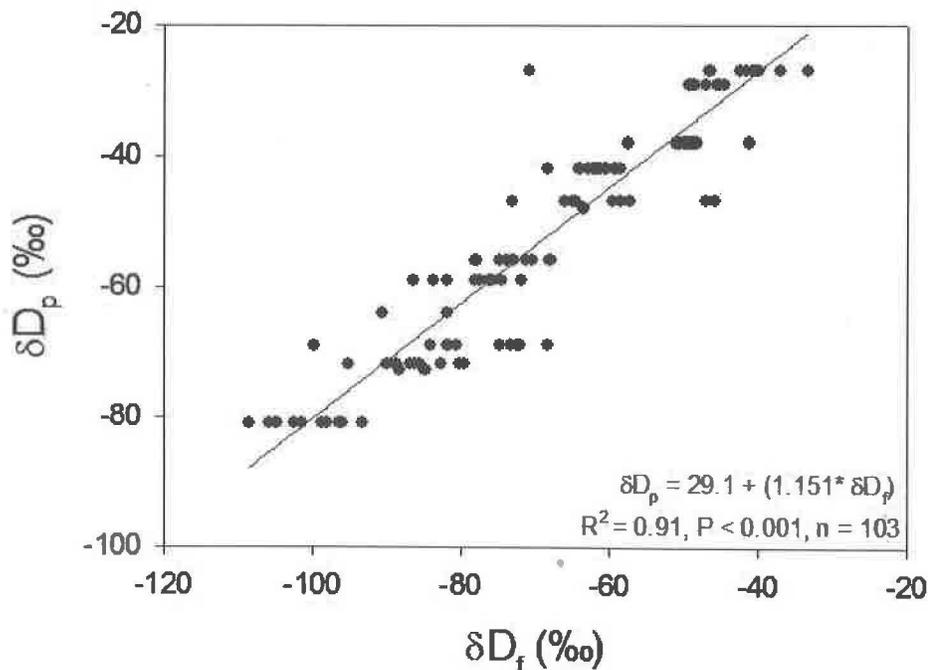
Feather Sample	Collection Coordinates	Country	Observed $D_f$	Predicted Natal Latitude	95% CI	Predicted Natal Latitude (outliers deleted)	95% CI (outliers deleted)
1	10.37N, 85.37W	Costa Rica	-55.69	36.12	31.60 to 40.63	35.69	32.32 to 39.06
2	10.37N, 85.37W	Costa Rica	-56.67	36.33	31.82 to 40.84	35.91	32.54 to 39.29
Summary: 1 and 2			-56.13	36.21	33.00 to 39.42	35.82	33.44 to 38.20
3	14.39N, 89.37W	El Salvador	-85.96	43.27	38.75 to 47.79	43.40	40.02 to 46.08
4	13.82N, 89.65W	El Salvador	-54.70	35.88	31.37 to 40.40	35.44	32.06 to 38.81
5	13.82N, 89.65W	El Salvador	-72.98	40.20	35.70 to 44.71	40.09	36.73 to 43.46
6	13.82N, 89.65W	El Salvador	-65.01	38.32	33.81 to 42.82	38.06	34.70 to 41.43
7	13.82N, 89.65W	El Salvador	-88.88	43.96	39.43 to 48.49	44.14	40.76 to 47.53
Summary: 3 thru 7			-73.51	40.31	38.26 to 42.36	40.25	38.75 to 41.76

Despite the small number of feathers collected, our analysis shows that the two sets appear to have distinct, but overlapping areas of origin, depending upon whether the regression outliers are included or deleted. The summary rows of Table 1 show the mean  $D_f$  for each group, the expected latitude predicted by that mean (i.e., where  $D_f$  falls on the regression line), and a 95% confidence interval around that particular expected latitude. The average of the isotope ratios for the feathers from El Salvador places them on the regression line about  $4^\circ$  farther north than those from Costa Rica. This also is about the same latitudinal distance as that between the two Central American feather collection sites. With regression outliers included in the analysis, there is a slight overlap of  $1.2^\circ$  in the 95% confidence intervals for the natal latitudes of the two sets of feathers. When outliers are removed the confidence intervals become narrower, and this small overlap disappears. Assuming that the outliers do stem from measurement errors, we can be 95% confident that the two sets of feathers come from different latitudes. The Costa Rican birds apparently came from about the same latitude as our

baseline sites in Oklahoma or southern Illinois. The Salvadoran birds on the other hand likely were hatched near the latitudes of our sites in Pennsylvania or Michigan.

The  $D_f$  values—and hence the origin latitudes and their confidence intervals—of the two feathers from Costa Rica are almost identical, those from El Salvador are more varied. In fact, one feather from El Salvador has the highest  $D_f$  value of all, higher even than the two from Costa Rica.

A confidence interval for the natal latitude of an individual case will always be wider than a confidence interval for the mean of a set of cases. Hence, the findings with regression outliers in the baseline sample included show overlap of all the confidence intervals on natal latitudes of the feathers from the two countries. Each confidence interval contains about  $9^\circ$  of latitude. Only two confidence intervals for the five Salvadoran feathers (cases 3 and 7) come close to being higher than those for the Costa Rican feathers. With regression outliers deleted from the baseline



**Fig. 2. Relationship of hydrogen isotope ratio in precipitation ( $D_p$ ) to that in HY Ruby-throated Hummingbird feathers ( $D_f$ ).**

sample, confidence intervals hover around a span of 6.7° latitude (Table 1, far right columns). Again, the confidence intervals are narrowed by omitting the two outliers. Hence, the confidence intervals of cases 3 and 7 that only slightly overlap with the confidence intervals of cases 1 and 2 from Costa Rica now indicate a distinctly more northern origin. While the two feathers from Costa Rica are inferred with 95% confidence to have originated between 32° and 39° N, cases 3 and 7 originated at 40° N or greater.

We found a strong linear relationship between  $D_p$  and  $D_f$  using RMA regression:  $D_p = 29.1 + 1.151 * D_f$  ( $R^2 = 0.91$ ,  $P < 0.001$ , as shown in Fig. 2.) This relationship is somewhat stronger than that of feather isotope ratios to latitude. This is to be expected since the precipitation isotope ratio intervenes between, or causally links, the isotope ratio in precipitation to that in feathers.

## CONCLUSIONS

The Ruby-throated Hummingbird feathers collected within the two Central American countries appear statistically to have originated on average in different latitudes. Except for one case, there is an obvious latitudinal separation of Ruby-throated Hummingbirds between the two countries, which suggests high connectivity and chain migration (Boulet and Norris 2006). Although the small sample size makes this conclusion tentative, the findings are encouraging. Furthermore, this project samples only two sites from a large geographic area. Research using larger samples from more banding stations in Mesoamerica is needed for more thorough documentation. Data from two wintering ground sites are hardly adequate for a strong inference of chain migration throughout the breeding and wintering ranges of Ruby-throated Hummingbirds. However, the data are consistent with such a pattern. Future research will also benefit from the  $D_p$  to  $D_f$  calibration equation, which will allow  $D_f$  values from HY birds wintering in Central America to be assigned an estimated  $D_p$  value for direct mapping onto North American deuterium isoscapes.

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