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## A test of geographic assignment using isotope tracers in feathers of known origin

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**Abstract** We used feathers of known origin collected from across the breeding range of a migratory shorebird to test the use of isotope tracers for assigning breeding origins. We analyzed  $\delta\text{D}$ ,  $\delta^{13}\text{C}$ , and  $\delta^{15}\text{N}$  in feathers from 75 mountain plover (*Charadrius montanus*) chicks sampled in 2001 and from 119 chicks sampled in 2002. We estimated parameters for continuous-response inverse regression models and for discrete-response Bayesian probability models from data for each year independently. We evaluated model predictions with both the training data and by using the alternate year as an independent test dataset. Our results provide weak support for modeling latitude and isotope values as monotonic functions of one another, especially when data are pooled over known sources of variation such as sample year or location. We were unable to make even qualitative statements, such as north versus south, about the likely origin of birds using both  $\delta\text{D}$  and  $\delta^{13}\text{C}$  in inverse regression models; results were no better than random assignment. Probability models provided better results and a more natural framework for the problem. Correct assignment rates were highest when considering all three isotopes in the probability framework, but the use of even a single isotope was better than random

assignment. The method appears relatively robust to temporal effects and is most sensitive to the isotope discrimination gradients over which samples are taken. We offer that the problem of using isotope tracers to infer geographic origin is best framed as one of assignment, rather than prediction.

**Keywords** Assignment test · Feathers · Migration · Stable isotopes

### Introduction

In the study of seasonally migratory animals, strong conclusions from site-specific investigations are not possible without also considering activities over the complete annual biogeography of the species (Gill et al. 2001). For example, assuming uniform wintering histories for individuals in a study of migrant bird breeding biology overlooks a potentially important mechanistic source of variation; events during winter can influence events during the subsequent breeding season (Marra et al. 1998; Norris et al. 2004). Such an improved understanding of the links between seasonal distributions of migratory animals has obvious conservation implications. This is particularly true for rare or sparsely distributed species that are vulnerable to stochastic events that can occur at any point across their biogeography.

Traditional methods for studying continental-scale migrations of small- or medium-sized birds have been limited, expensive, and marginally helpful at best. Off-site band returns for non-game birds are notoriously scarce (Wassenaar and Hobson 2001). UHF radio telemetry is logistically impractical over the scales of most migratory routes and satellite telemetry is not yet possible for any but the largest animals. Recently, advances in the use of molecular and chemical markers to study questions of migration have been gaining momentum (Webster et al. 2002).

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Naturally occurring ratios of stable isotopes of some elements vary over ecologically convenient scales. Ratios of nitrogen have been used to trace anthropogenic inputs to birds from the environment (Hobson 1999; Hebert and Wassenaar 2001) and also to determine dietary status (Hobson et al. 1993). Carbon isotope ratios vary most strongly with the photosynthetic pathway (Smith and Epstein 1971), but also with latitude and altitude (Körner et al. 1991). Hydrogen ratios vary with precipitation and geomorphology (Meehan et al. 2004). In general, these differences in isotope ratios are transferred through the food web such that biogeographic patterns are reflected in animal tissues (see Gannes et al. 1997; Hobson et al. 1999; McKechnie et al. 2004 for important associated considerations).

A number of recent studies have exploited patterns in naturally occurring isotope ratios to link winter and summer ranges of migratory birds. Some have suggested the apparent utility of using only stable-hydrogen values ( $\delta\text{D}$ ) in feathers coupled with models interpolating long-term averages of spatially sparse precipitation values (e.g. Hobson and Wassenaar 1997; Meehan et al. 2001; Kelly et al. 2002; Smith et al. 2003), but the use of more than one element seems to offer more promise. For example, Lott et al. (2003) used sulfur isotope values ( $\delta^{34}\text{S}$ ) to censor their data such that marine effects on  $\delta\text{D}$  trends were removed from analysis. Using a combination of hydrogen and carbon isotopes has yielded promising results (Wassenaar and Hobson 2000; Hobson and Wassenaar 2001; Rubenstein et al. 2002). Chamberlain et al. (2000) suggest that a combination of carbon and nitrogen isotopes can discern geographically distinct subspecies of willow warblers (*Phylloscopus trochilus*), although few of data could be uniquely assigned to a particular area (see Fig. 2 of that study). Finally, at least three studies have shown the utility of using isotopes of three elements, but each study examined a different set of three (Chamberlain et al. 1997; Caccamise et al. 2000; Pain et al. 2004).

In general, there have been three common feather sampling scenarios, each addressing a slightly different aspect of the question of geographic links in migration. First, feathers grown on the breeding grounds in the year prior to sampling are sampled on the breeding grounds. Philopatry is often the focus in these cases (e.g. Graves et al. 2002; Hobson et al. 2004) and often only territorial males are sampled in attempts to better address the question. Second, and most commonly, feathers grown on the breeding grounds in the year of sampling are sampled away from breeding areas either during migration or on the wintering grounds. Inference from this strategy often relies on spatially interpolated maps of long-term average  $\delta\text{D}$  in precipitation, further assuming that discrimination against  $^2\text{H}$  in feathers is constant over individual birds, species, habitats, locations, and time (e.g. Hobson and Wassenaar 1997). Third, feathers grown in winter are sampled on the breeding grounds.

Studies that sample via the third scenario generally address questions of population mixing or habitat quality differences during winter, assuming that distinct wintering areas or habitats are isotopically recognizable (e.g. Evans et al. 2003). Most studies sampled feathers of unknown origin. Accordingly, the vast majority of conclusions have been based on largely untested assumptions about the degree of natural variation in molt strategy and isotopic discrimination factors among individuals, species, habitats, locations, seasons, and years. Direct tests of such key assumptions would clearly benefit not only studies of avian migration, but also the more general range of applications that interpret naturally occurring stable isotopes to infer dispersal, migration, and origins of individual animals of many varieties.

The intent of this study was to test the use of isotope values ( $\delta\text{D}$ ,  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ ) in feathers to make predictions about bird origin. By sampling feathers from precocial mountain plover (*Charadrius montanus*) chicks from across the breeding range in two consecutive breeding seasons, we generated two independent datasets comprised entirely of known origin samples. Using regression models, we characterized the mean variation in isotope values over the sampled latitude range. Using two different methods for inverting these conditional data, we generated models to assign specific geographic origins. We used 1 year of data to generate, or train the models. Data from the alternate year were used to test model performance. We discuss the roles of appropriate sampling and model frameworks as they relate to limits in the use of isotope ratios to predict or assign geographic origin to birds of unknown origin.

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## Materials and methods

### Mountain plovers

Mountain plover chicks forage independently for invertebrates within the first hours after hatching (Knopf 1996). At hatching, chicks are covered in natal down. Contour feathers and flight feathers in sheath begin to show by about 7 days of age (M. B. Wunder, personal observation). Because plover chicks prey on terrestrial invertebrates and because they cannot fly, isotope ratios in new feathers are likely related to those of the environment within an area of approximately 56 ha, the mean home-range size for plovers with broods (Knopf and Rupert 1996). It is possible that chicks use some tissue reserves inherited from parental birds for the development of feathers. However, because this is an energetically demanding period during which all tissues, including feathers, are growing, it is more likely that the resources necessary for such development are primarily provided by food intake (Schekkerman and Visser 2001). We therefore assume that mountain plover chicks provided feathers exclusively of known geographic origin.

## Feather sampling

We sampled body feathers from 75 mountain plover chicks caught by hand between 12 June and 18 August 2001 at six locations across 9° of latitude within the breeding range. Between 10 June and 6 August 2002, we sampled 119 chicks from eight locations across 6° of latitude within the breeding range. The three southernmost locations were also sampled in 2001 (Fig. 1). Feathers were stored in individually marked bags and frozen until analyses.

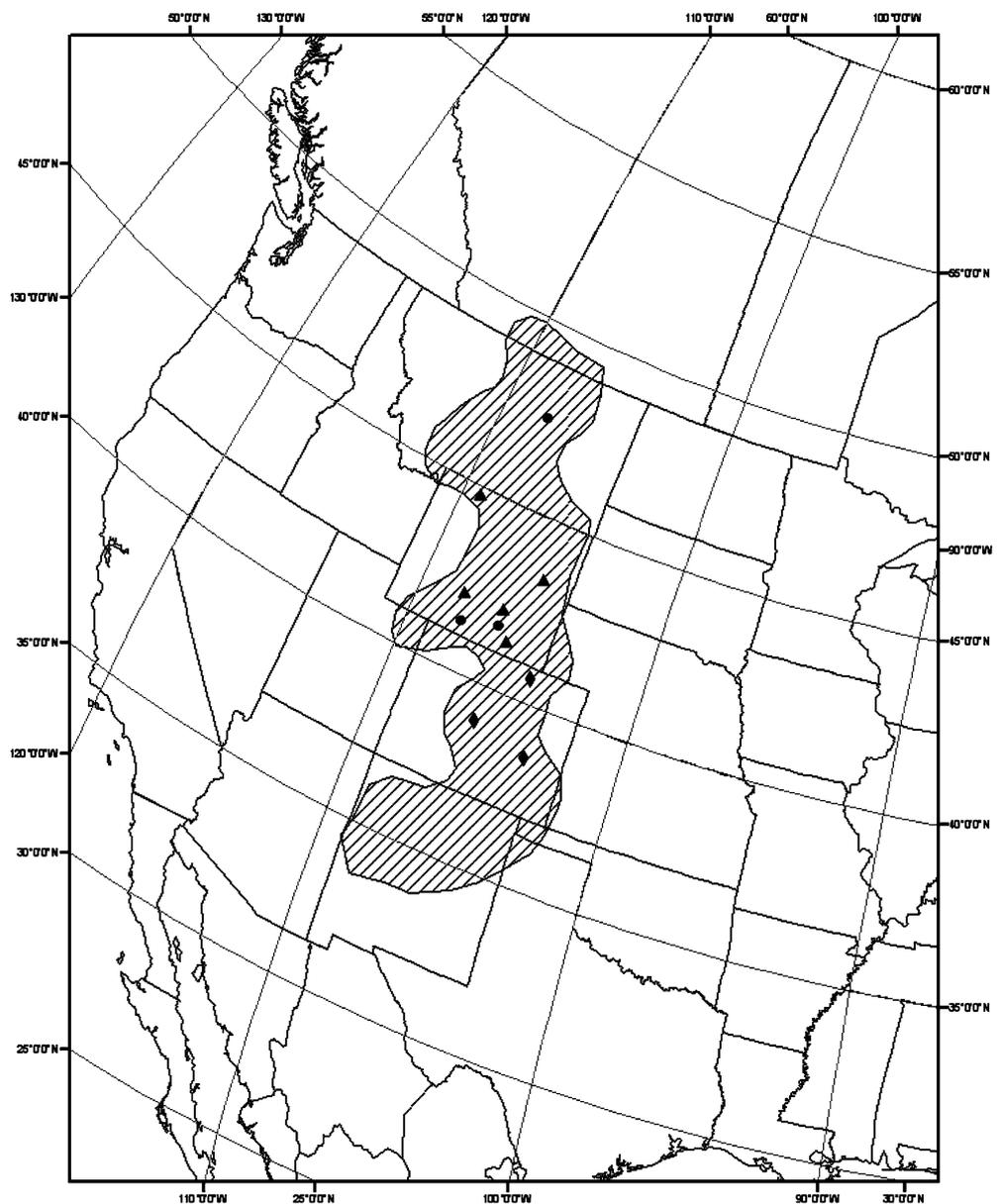
## Isotopic analysis

We report stable isotope ratios as relative values in parts per mil (‰) using the standard  $\delta$  notation:

$\delta_{\text{samp}} = (R_{\text{samp}}/R_{\text{std}} - 1) \times 1,000$  where  $\delta_{\text{samp}}$  is the reported value of isotope ratios of the sample relative to a standard, and  $R_{\text{samp}}$  and  $R_{\text{std}}$  are the isotope ratios of the sample and the standard, respectively.

After thawing, feathers were washed of surface oils with a 2:1 chloroform/methanol solution and allowed to air dry overnight under a fume hood. Approximately 1.7 mg of feather tissue was loaded into pressed tin capsules for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ . If a feather was still partially in sheath, only the fully developed vanes were used. Samples were analyzed for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  by continuous flow methods using an elemental analyzer coupled to either a Micromass Optima mass spectrometer or a Finnigan Delta Plus XL mass spectrometer (Fry et al. 1992). Isotopic compositions of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  are reported relative to PDB and Air, respectively, using internal laboratory standards calibrated against ANU

**Fig. 1** Map of mountain plover breeding range. *Dots* denote sample locations for 2001. *Triangles* denote sample locations for 2002. *Diamonds* denote locations sampled in both 2001 and 2002



sucrose ( $\delta^{13}\text{C} = -10.4\text{‰}$ ), NBS 22 ( $\delta^{13}\text{C} = -29.6\text{‰}$ ), USGS 25 ( $\delta^{15}\text{N} = -30.4\text{‰}$ ), and USGS 26 ( $\delta^{15}\text{N} = 53.7\text{‰}$ ). Analytical error for C and N was  $\pm 0.2\text{‰}$ .

For hydrogen isotope ( $\delta\text{D}$ ) analysis, approximately 0.5 mg of tissue was loaded into pressed silver capsules. Samples were then analyzed by continuous flow isotope ratio mass spectrometry using a Finnigan TC/EA coupled to a Finnigan Delta Plus XL mass spectrometer (Wassenaar and Hobson 2003). Hydrogen isotopic compositions are reported relative to Vienna standard mean ocean water (VSMOW), using internal laboratory standards calibrated against IAEA-CH-7 ( $\delta\text{D} = -100\text{‰}$ ). Non-exchangeable hydrogen isotopic compositions were determined by comparative equilibration techniques described by Wassenaar and Hobson (2003). Analytical error of non-exchangeable hydrogen isotope values was  $\pm 4\text{‰}$ .

## Data analysis

We observed inter-annual differences in all three isotopes from one site that was sampled in both years (95% CI for  $\delta\text{D}$  in 2001,  $-51.73:-45.55$ , and  $-32.31:-26.48$  in 2002; 95% CI for  $\delta^{13}\text{C}$  in 2001,  $-19.66:-19.13$ , and  $-18.48:-18.10$  in 2002; 95% CI for  $\delta^{15}\text{N}$  in 2001,  $9.71:10.29$  and  $10.50:11.03$  in 2002). We evaluated general latitudinal patterns in the isotopes for each year using least squares regression to model each isotope value in turn as a function of latitude. We parameterized both continuous- and discrete-response models for each year to predict the geography of origin from isotope ratios in feathers. We then used the independent datasets to evaluate model predictions. That is, we used the 2002 data to evaluate predictions from models generated with 2001 data and vice versa.

We first wrote the inverse regression model reported by Rubenstein et al. (2002). This was  $y_i = \hat{\alpha} + \hat{\beta}_1 \delta\text{D}_i + \hat{\beta}_2 \delta^{13}\text{C}_i + \varepsilon_i, i = 1, \dots, n$ , where  $y_i$  are the observed latitudes,  $\hat{\alpha}$  is the estimated constant intercept,  $\hat{\beta}_x$  is the estimated coefficient for the respective isotope ratios and  $\varepsilon_i \sim \text{N}(0, \sigma^2)$ . As with Rubenstein et al. (2002), we assumed that the data were independent random samples and estimated model parameters with least squares. However, because multiple birds were sampled at each location, the data did not represent independent random samples. Additionally, writing the model as above clearly illustrates that residual variance in the data must occur in the  $y$ -direction. In the case of these models, the overwhelming source of variance in the  $y$ -direction derived from the difference in latitudes of the sites we sampled. To address these issues, we added the sampling location as a random effect. This modified the model as follows  $y_{ij} = \hat{\alpha} + \hat{\beta}_1 \delta\text{D}_{ij} + \hat{\beta}_2 \delta^{13}\text{C}_{ij} + u_i + \varepsilon_{ij}, i = 1, \dots, g, j = 1, \dots, n$  where  $y_{ij}$  are the observed latitudes,  $g$  is the number of sample locations,  $n$  is the number of individual birds,  $\hat{\alpha}$  and  $\hat{\beta}_x$  are as in the previous model,  $u_i \sim \text{N}(0, \sigma_u^2)$  and  $\varepsilon_{ij} \sim \text{N}(0, \sigma^2)$ . This model

partitioned the variance into within- and among-location components. We estimated parameters for the mixed effects model using residual maximum likelihood (REML).

We evaluated the relative strength of evidence in the data for the fixed and mixed effects regression models by comparing AICc statistics and relative evidence ratios ( $e^{0.5\Delta\text{AICc}}$ ; Burnham and Anderson 2002). We validated these inverse regression models by examining predicted values and prediction intervals for both the model generating data and the independent datasets and by considering the associated model assumptions.

Because we were interested in the probability that an individual feather was from a particular geographic location given measured ratios of isotopes in the feather, we also explored a discrete-response framework. For this, we used Bayes rule to invert the conditional probabilities of all three measured isotope ratios in feathers, given the sampling location. We wrote

$$P(A_i/B) = \frac{P(B/A_i)P(A_i)}{\sum_j P(B/A_j)P(A_j)}$$
 where  $P(A_i/B)$  is the (posterior) probability distribution of a feather originating in geographic location  $i$ , given the observed  $\delta\text{D}$ ,  $\delta^{13}\text{C}$ , and  $\delta^{15}\text{N}$ .  $P(B/A_i)$  is the (sampling) probability distribution of observing the isotope values, given the geographic locations  $i$ , and  $P(A_i)$  is the (prior) probability distribution of a feather originating in geographic location  $i$ , given assumptions or knowledge prior to observing any isotope values.

We assumed our feathers were of known origin and sample data are fixed in this framework, so the distribution of sample sizes across the locations provided a reasonable a priori approximation of the prior probabilities over the locations. Therefore, we set the prior probability density proportional to the per location sample sizes. Depending on the number of isotopes considered, we assumed normal, bivariate normal, or multivariate normal distributions using the within-location variance-covariance matrices for the sampling distributions. We estimated parameters for the sampling distribution functions from the data using maximum likelihood.

If the number of sites,  $S$ , is finite, then  $P(A_i/B)$  over  $i = 1, \dots, S$  can be interpreted as a set of competing hypotheses about the geographic origin of a bird, given the measured isotope values. Thus, in all cases, individual birds were assigned to the population  $x$  for which  $P(A_x/B)$  was maximal. We applied this probability approach at two levels of spatial resolution—U.S. state (pooling data across sampling locations within a state), and individual sampling location (pooling individuals within sampling sites). We included all samples for the state-level analyses, but excluded locations with  $\leq 5$  samples from the location-level analyses. Thus, for the 2001 data, we used feathers from five locations in three states in the location-level analysis. For 2002 location-level resolution, we used feathers from four sites in two states. In all cases, we evaluated assignments with leave-one-out cross-validation and by

assigning 2001 data with posteriors from the 2002 data and vice versa.

## Results

### Latitudinal patterns of isotope ratios

Hydrogen isotope values in feathers tended to show an inverse relationship with latitude across our study area but with overlap among the latitudes (Fig. 2). The  $\delta D$  regression coefficients ranged from  $-5.3$  to  $-3.9$  (95% CI) and from  $-6.4$  to  $-3.2$  (95% CI) for 2001 and 2002, respectively. Between-location variation accounted for 80.9% of the total variance in  $\delta D$  for the 2001 data, whereas 45.7% of the total in 2002 was accounted for by between-location variation. Accordingly, the regression of  $\delta D$  on latitude explained more of the variation in the 2001 data (adjusted  $R^2=0.71$ ) than in that for 2002 (adjusted  $R^2=0.23$ ).

Carbon isotope values also tended to decrease with latitude, although less strongly (95% CI for  $\delta^{13}C$  regression coefficient, 2001 data:  $-0.33$  to  $-0.03$ , 2002 data:  $-0.81$  to  $-0.24$ ). Most of the total variation in  $\delta^{13}C$  was attributed to between-location variation in both years (78.8% in 2001, 87.1% in 2002), but the values did not vary systematically with latitude (adjusted  $R^2=0.06$  and  $0.10$  for 2001 and 2002 respectively).

Nitrogen isotope values were not associated with latitude in 2001 (95% CI for  $\delta^{15}N$  regression coefficient:  $-0.17$  to  $0.03$ , adjusted  $R^2=0.01$ ), but were in 2002 (95% CI for  $\delta^{15}N$  regression coefficient:  $-0.95$  to  $-0.57$ , adjusted  $R^2=0.35$ ). In both years, most of the total

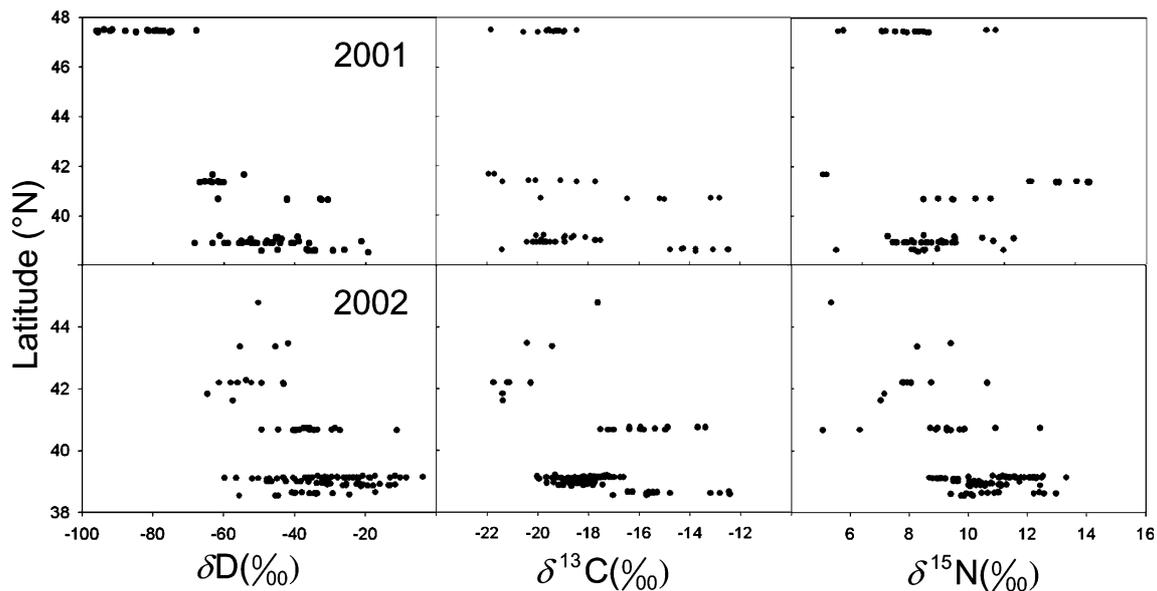
variation was between locations (83.3% in 2001, 66.4% in 2002).

### Continuous-response prediction of latitudinal origin

Fixed-effects inverse regression models for latitude using hydrogen and carbon isotope values as explanatory variables appeared to describe the data reasonably well in 2001 (adjusted  $R^2=0.76$ ,  $P<0.0001$ ,  $\sqrt{MSE}=1.75$ ), and less so in 2002 over a reduced latitudinal range (adjusted  $R^2=0.24$ ,  $P<0.0001$ ,  $\sqrt{MSE}=1.08$ ). However, the models produced poor predictions for both the generating and the independent datasets (Fig. 3). Prediction intervals were similar in magnitude to the entire sampling ranges; the intervals for individual predictions of latitude ranged  $7^\circ$  and  $4.3^\circ$  for the 2001 and 2002 data, respectively.

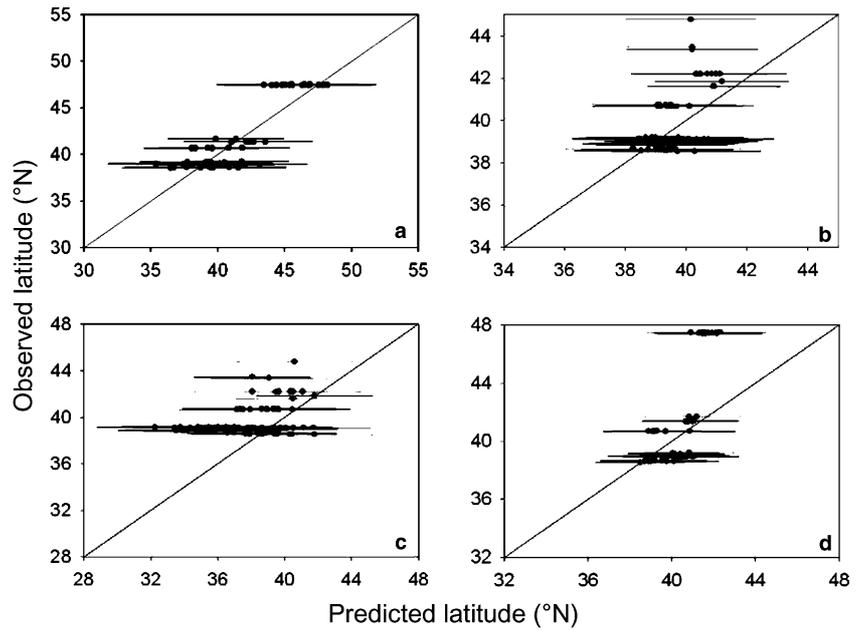
The fixed-effects inverse regression models for both years yielded substantial non-random structure in the residuals violating the assumption  $\epsilon_i \sim N(0, \sigma^2)$  (Fig. 4a). Most of this structure was attributed to residuals associated with  $\delta D$  (Fig. 4b). This is an artifact of the sampling structure that was likely amplified for  $\delta D$  because the within-location variance of  $\delta D$  was substantial, yet most of the overall variation was still among locations. Partitioning the variance into within- and among-location components removed the structure in the residuals, satisfied the assumption, and resulted in much smaller AICc scores (Fig. 4b, Table 1).

The most parsimonious model for latitude in both years included only a fixed intercept and location as a random effect. Evidence ratios were overwhelmingly in favor of this intercept-only mixed model over models

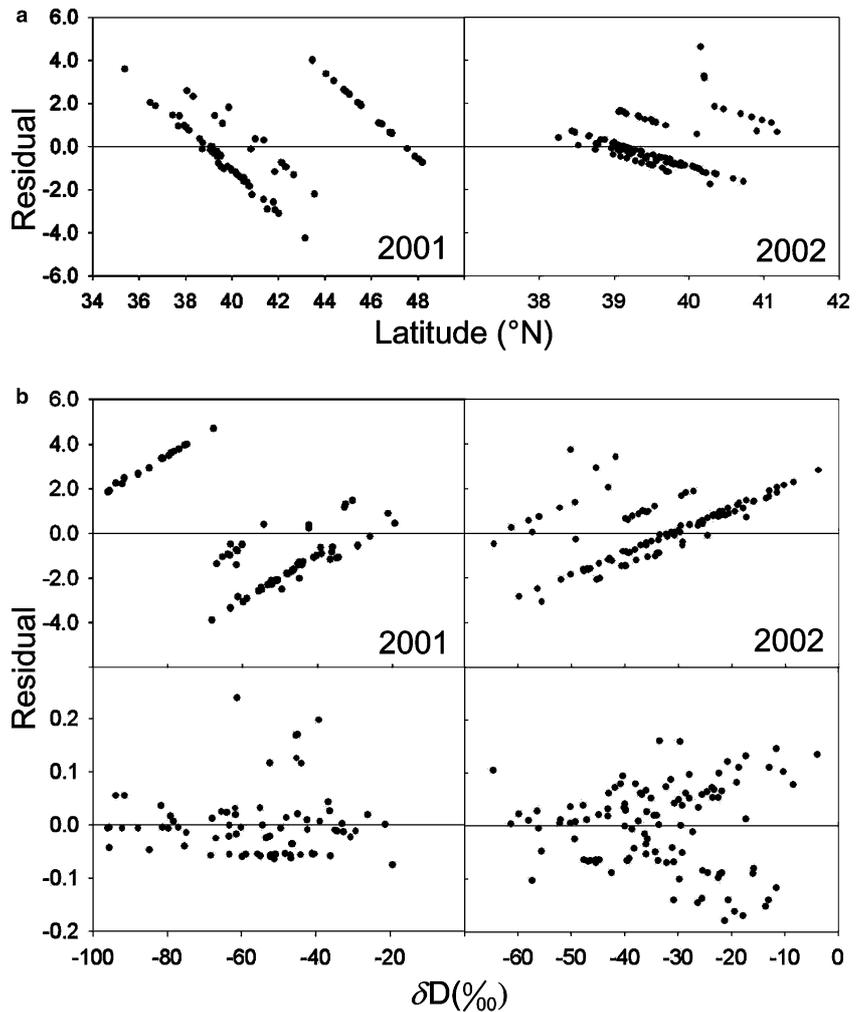


**Fig. 2** Data for  $\delta D$ ,  $\delta^{13}C$ , and  $\delta^{15}N$  from mountain plover chick feathers collected in 2001 (top row) and 2002 (bottom row).  $\delta D$  is for non-exchangeable hydrogen only and is reported relative to VSMOW.  $\delta^{13}C$  is reported relative to PDB.  $\delta^{15}N$  is reported relative to Air. Note the between-year difference in ranges of latitude sampled

**Fig. 3** Observed versus predicted latitudes of origin using inverse regression model framework.  $\delta D$  and  $\delta^{13}C$  in mountain plover chick feathers were used as independent variables to model latitude of origin.  $\delta^{13}C$  is reported relative to PDB, and  $\delta D$  is reported relative to VSMOW. Error bars extend to the 95% prediction limits. Diagonal lines show 1:1 observed:predicted ratio. **a** 2001 predictions for model-generating data. **b** 2002 predictions for model-generating data. **c** model generated from 2001 data used to predict 2002 observations. **d** model generated from 2002 data used to predict 2001 observations



**Fig. 4** Residuals from inverse regression model of latitude using  $\delta D$  and  $\delta^{13}C$  as explanatory variables.  $\delta^{13}C$  is reported relative to PDB, and  $\delta D$  is reported relative to VSMOW. Horizontal bars at zero are for reference and do not indicate mean of the residuals. Left column is 2001 data, right column is 2002 data. **a** Residuals of predicted latitude for model-generating data. **b** residuals of  $\delta D$  from model with  $\delta D$  and  $\delta^{13}C$  as explanatory variables (top row) and with location added as a random effect (bottom row)



**Table 1** Differences in AICc scores and evidence ratios (ER) for inverse regression models of latitude. Mixed models included sample location as a random effect in addition to the fixed effects of either just an intercept or an intercept plus hydrogen (H) and carbon (C) isotope values

Model	2001 Data		2002 Data	
	$\Delta$ AICc	ER	$\Delta$ AICc	ER
Intercept only, mixed	0 <sup>a</sup>	1	0 <sup>a</sup>	1
H + C, mixed	20.0	22,026	26.6	597,195
H + C, fixed	442.8	1.4×10 <sup>96</sup>	526.4	2.0×10 <sup>114</sup>

<sup>a</sup>AICc score for the top model was −136.1 and −184.5 for 2001 and 2002, respectively

that included isotope ratios as predictors (Table 1). Removing the intercept-only model from the candidate set did not reduce the disparity between the fixed- and mixed-effects models. Evidence ratios favor the model that includes location as a random effect and isotope values as fixed effects by  $6.5 \times 10^{91}$  to 1 and  $3.4 \times 10^{108}$  to 1 over the model that includes only the isotopes for 2001 and 2002, respectively.

#### Discrete-response prediction of geographic origin

The probability models correctly assigned 73%–93% of the observations in a leave-one-out cross-validation of the 2001 data. Correct assignment rates increased with the number of isotopes used. Cross-validation of the 2002 data was similar (Table 2). For comparison, random assignment rates for tests based on 2001 data were 33% to the correct state and 20% to the correct location. Random assignment rates for 2002-based tests were 50% to state and 25% to location.

State-level assignment rates of 2002 data using posteriors estimated from 2001 data were similar to those from cross-validation and were an improvement from random rates. State-level assignment rates of 2001 data using posteriors from 2002 data were also improvements from random for all isotope combinations considered, but only the use of all three isotopes provided better than a marginal improvement (Table 2). Recalling that only two states were sampled in 2002 and three in 2001,

23% of the 2001 data were necessarily misclassified. When considering only the remaining 77% of the data that were from the two states also sampled in 2002, correct assignment rates were 73, 66, and 84% for posteriors from hydrogen, hydrogen + carbon, and hydrogen + carbon + nitrogen, respectively.

Location-level assignment using only hydrogen from 2001 to assign 2002 data yielded a slight improvement over random assignment. When considering only those locations that were sampled in both years, the rate increased slightly to 29%. Correct assignment rate more than doubled when using all three isotopes (Table 2). That rate increased to 69% when considering only locations sampled in both years. Similarly, tests based on only hydrogen values in 2002 assigned 26% of the 2001 data to the correct location. That compared with a 25% random assignment rate. This improved to 38% if we considered data only from locations that were sampled in both years. The assignment rate again doubled when using all three isotopes from 2002 to assign 2001, and increased to 81% if considering only locations sampled in both years.

## Discussion

### Latitudinal patterns of variation in isotope values

Isotope values in feathers of mountain plover chicks varied with geography, but not always systematically so. As expected, mean hydrogen values varied systematically to different degrees over ranges of both 6° and 9° of latitude. This supports most other North American studies that have used hydrogen values in feathers to describe geographic origins of birds (Chamberlain et al. 1997; Hobson and Wassenaar 1997; Meehan et al. 2001; Kelly et al. 2002).

Carbon values were not well predicted by latitude in either year. In contrast to many studies of migrating birds using stable isotopes, both C3 and C4 plants occur in habitats used by plover chicks. For example, in Colorado plovers use a combination of habitats including native prairie dominated by C4 grasses and dryland crops of C3 plants, especially winter wheat (Knopf and Rupert 1999). Regional trends in carbon values that

**Table 2** Assignment rates from Bayes Rule inversions. Leftmost column lists isotopes used to generate probability density functions. Second and third columns from the left show results from leave-one-out cross-validation. Rightmost two columns show assignment results for independent datasets

Isotopes	2001 CV		2002 CV		2001–2002 <sup>a</sup>		2002–2001 <sup>b</sup>	
	State	Loc	State	Loc	State	Loc	State	Loc
HCN	0.93	0.86	0.95	0.90	0.94	0.64	0.63	0.55
HC	0.88	0.77	0.94	0.86	0.91	0.52	0.51	0.56
H	0.91	0.73	0.92	0.65	0.88	0.25	0.57	0.26

<sup>a</sup>Assignment of 2002 data with probability densities estimated from 2001 data

<sup>b</sup>Assignment of 2001 data with probability densities estimated from 2002 data

have been reported by others (Körner et al. 1991; Rubenstein et al. 2002) are likely swamped by local effects from C3–C4 mixtures at similar latitudes across the western Great Plains of North America (Fig. 2). Wassenaar and Hobson (2000) reported similar results for central North America in red-winged blackbirds (*Agelaius phoeniceus*) that used both C3 and C4 habitats. Graves et al. (2002) demonstrated that local geographic and demographic effects are stronger sources of variation in  $\delta^{13}\text{C}$  than more regional or continental effects. Our data support the assertion that variation of  $\delta^{13}\text{C}$  in bird feathers from across a continental geography derives predominantly from local sources, rather than regional phenomena.

Surprisingly, nitrogen values appeared to vary systematically with latitude in 2002 (Fig. 2). In fact, the adjusted  $R^2$  for the nitrogen model was greater than that for hydrogen in the same year, improbably suggesting that latitude explained more variation in nitrogen than in hydrogen. We do not believe the pattern has any phenomenological merit and offer instead that this finding was spurious; it exposes how ad hoc regression approaches to modeling data that are not independent random samples can be misleading. The 2001 data for nitrogen were not remotely related to latitude, as expected.

#### Predicting latitudes with continuous models

Predictions from the inverse regression models were not useful for assigning latitudinal origins. The model based on the 2001 data generated large location-specific prediction intervals. For example, when predicting the model-generating data, known Colorado birds from the two southernmost sites (latitude 38.5°N–39.2°N) were predicted as having originated somewhere between 32°N and 47°N (Fig. 3a). This range includes the full extent of the breeding range for mountain plover (Fig. 1). When using the same 2001-based model to predict the independent data from 2002 for those same two southernmost sites, the predicted range was even larger, spanning from Mexico (28°N) north to Montana (45°N). The predictions from the 2002-based model were similarly vague, failing to distinguish northern birds from southern birds in either the model-generating data or in the independent 2001 data (Fig. 3b, d). This precludes even qualitative statements about the likelihood of a bird originating from any particular portion of the overall breeding range.

Kelly et al. (2002) reported that an inverse regression model using only  $\delta\text{D}$  for range-wide Wilson's warbler (*Wilsonia pusilla*) samples yielded similarly poor results. For most values of  $\delta\text{D}$ , prediction intervals that incorporated model uncertainty included the entire breeding range of the warblers, despite the fact that the data in that study more closely represented independent random samples. Rubenstein et al. (2002) used inverse regression with both  $\delta\text{D}$  and  $\delta^{13}\text{C}$  to predict latitude and longitude

of origin for samples of black-throated blue warblers (*Dendroica caerulescens*) and reported better precision in their predictions. However, that study reported but did not consider or propagate any uncertainty in the model predictions. Reported values of  $\sqrt{\text{MSE}} = 3.18$  for latitude and  $\sqrt{\text{MSE}} = 6.40$  for longitude yield prediction intervals for an individual bird that span  $\sim 13^\circ$  of latitude and  $\sim 26^\circ$  of longitude, or slightly larger than the geographic range over which birds were sampled. If variance among individuals within each of the ten locations is also considered, the overall uncertainty range of isotope values would widen even further, effectively precluding assignments of origin. Such results align better with those of Kelly et al. (2002) and results reported here.

Both Kelly et al. (2002) and Rubenstein et al. (2002) pooled samples over many years, used feathers of uncertain origin, and measured isotope values for combined exchangeable and non-exchangeable hydrogen. This study attempted to control for those three sources of potential variation, yet inverse regressions, albeit over a more restricted range of latitudes, yielded similarly poor results. Thus, the inverse regression framework is inappropriate for the problem of assigning geographic origins. The problems with regression are most easily seen in the comparisons between the fixed- and mixed-effects models.

The difference in AICc scores between the fixed- and mixed-effects models (Table 1) demonstrates the importance of incorporating the spatially hierarchical structure of the data. Pooling data across years revealed a substantial cost associated with ignoring the temporal structure in the data as well. We fit the temporally pooled data to the same models for latitude as for each year separately. The difference in AICc between the fixed- and mixed-effects models for the temporally pooled data ( $\Delta\text{AICc} = 1,034$ , evidence ratio =  $4.1 \times 10^{224}$ ) was about twice as large as that for the year-specific comparisons. This disparity relative to that for the year-specific models represents the compounded costs of ignoring both temporal and spatial structure in the data. It suggests that the cost of pooling over annual fluctuations in isotope values is on the same order as that for ignoring spatial dependencies, and both are large. This finding further implies potential challenges associated with prediction methods that rely on the use of spatially smoothed models of temporally averaged (pooled)  $\delta\text{D}$  in precipitation.

The disparity between the fixed- and mixed-effects models for all datasets was substantial, but not surprising. Birds from a given sample location shared the same (or nearly so) latitude such that the values of the response variable for all data were clustered into a small set of discrete groups (Fig. 2). The mixed-effects model used some of the degrees of freedom to adjust the intercept for each location-specific group of data, whereas the fixed-effect model was forced to interpolate between the clusters. This means that the fixed-effect model predicted the pattern in general, but not in par-

ticular, whereas the reverse was true for the mixed-effects model. This likely stems from the mismatch between sample and model structures that resulted in assumption violations. Unfortunately, the mixed-effects model that satisfies the assumption for the residuals requires a priori information about sample origin, precisely the information we hope to learn from the model.

### The discrete-response framework

The use of discrete-response models is not new to assignment problems using stable isotopes. Rocque (2003) used a Bayesian approach that jointly incorporated both continuous (isotopes and skeletal measurements) and discrete (microsatellite) marker types. Royle and Rubenstein (2004) used a likelihood approach that they extended to a Bayesian approach incorporating estimated relative abundance. Caccamise et al. (2000) and Wassenaar and Hobson (2000) each used linear discriminant analysis, which is similar to the likelihood approach described in Royle and Rubenstein (2004).

In general, the probability approach in this study did better than random assignment, and therefore also better than the inverse regressions. The probability approach performed better with increasing number of isotope markers. In all but one case, using posteriors generated from all three isotopes resulted in the highest proportion of correct assignments (Table 2). The approach provided promising results from cross-validation with the training data, regardless of the spatial resolution of assignment. The tests with independent data, however, were much more sensitive to the relative geographic representations of the datasets. Specifically, the geographic range sampled in 2002 was more restricted than that of 2001. Consequently, assignment rates were similar for independent and training data only in the case where the range of training data was greater than that of the independent data.

Our results were most similar to those of Caccamise et al. (2000), a study that also used three isotope values from newly growing (known origin) feathers collected in a single year. The sample groupings were stratified across clear discrimination gradients in the isotopes considered (carbon, nitrogen, and sulfur) and the authors reported a 92% correct assignment rate. Wassenaar and Hobson (2000) correctly classified 80% of their data among 11 locations that spanned discrimination gradients in both isotope values considered (carbon and hydrogen), despite the facts that the feathers were collected and pooled over a 17-year period and were of ultimately uncertain origin. Royle and Rubenstein (2004) pooled feathers of uncertain origin collected over 10 years into three vast geographic categories, each represented by three collection sites. Using the same two isotopes (carbon and hydrogen), 62% of the observations were assigned to the correct region. The relative results from the latter two studies perhaps seem counter-intuitive, given the need for greater discrimination

among 11 categories as compared with three. The primary difference between the two studies is that the latter did not span strong discrimination gradients in the isotopes, and it may have also suffered from geographic misrepresentation in the samples, given that many birds sampled from each of a few sites were used to represent much wider geographies.

Probability models do not make assumptions about structural (mechanistic) relationships between isotope values and sample locations. It is a simple matter to directly estimate and apply posteriors empirically from isotope data. This is simultaneously beneficial and limiting. On the one hand, we do not require mechanistic assumptions about the relationship between isotope values and locations. However, for precisely the same reason, the models are difficult to “train”. In particular, the precision of these assignment methods is limited by the extent of geographic sampling. Sample design can have a tremendous effect on the resulting inferences, as evidenced by the test results with the independent datasets in this study.

In parametric applications such as in this study, the representation and stratification of locations sampled as well as the absolute number and relative proportions of birds sampled from each location are clear sources of bias in the probability estimations. Take for example the variability in the per-location sample sizes and associated ranges of observed values of  $\delta D$  presented here. All feathers in this study were grown locally where collected, yet  $\delta D$  values for a single location in a single year ranged from 7‰ to nearly 60‰ (Fig. 2), suggesting vastly different precisions in the origin assignment probabilities. Interestingly, no obvious outliers were responsible for the widest single-location distributions of  $\delta D$  (Fig. 2), which forces us to question the notion of a characteristic range of  $\delta D$  values for any single location. Although it was not the focus of this paper, this finding has clearly significant implications about the utility of predictive contour maps of  $\delta D$  in feathers or precipitation. Indeed, had we used precipitation-based maps to assign origin based on feathers spanning 60‰ in  $\delta D$  values, we would have predicted that the feathers derived from most of North America, not a single discrete location as was the actual case. We will address this finding more thoroughly elsewhere.

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## Conclusions

That every animal is “isotopically marked” and therefore intrinsically provides biogeographic information remains a compelling notion. And indeed, many questions concerning migration or dispersal via isotopes are presently approachable with appropriate study designs. This is particularly true for those that are less concerned with geography than with habitat-specific differences over which distinct discrimination patterns occur. There is a swiftly growing collection of case-specific evidence suggesting general isotopic patterns in feathers across

vast geographies. However, very few of these studies have tested the idea with tissue of known origin. Thus, the question of how to appropriately structure the spatial interpolation remains largely unanswered. Increasing knowledge of particular physiological mechanisms in stable isotope biogeochemistry will likely continue to guide efforts to address that question. In the meantime, the ad hoc use of assumptive regression models for inferring geographic origin will only continue to mislead. Probability-based models provide a more flexible framework that can be structurally expanded to incorporate mechanistic models as appropriate or available. As pointed out by Webster et al. (2002), the refinement of assignment tests is an active area of research in population genetics. Assignment problems using isotopes are directly analogous to those using molecular markers. Continuing to adapt and expand modeling approaches developed for other disciplines to problems using isotopes seems another obvious direction for future effort and collaboration.

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