

Wheatear molt and assignment tests: ongoing lessons in using stable isotopes to infer origins

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In an earlier paper (Rocque et al. 2006), we attempted to assign two generations of feathers in three species of intercontinental migrants to their respective breeding and wintering areas using stable isotopes. We found that while two species of *Pluvialis* plovers that breed in Alaska and winter on different sides of the Pacific Ocean (South America versus Asia and the South Pacific) showed significant differences between summer- and winter-grown feathers, we could not statistically separate feathers grown on the two continentally different wintering areas. Nor could we assign summer-grown feathers to within thousands of kilometers of their actual origin using the common approach of a North American isotope (δD) isocline map. In Alaska-breeding Northern Wheatears (*Oenanthe oenanthe*), which winter in Africa, we found no significant differences between summer- and (presumed) winter-grown feathers. Larson and Hobson (2009) helpfully point out a molt-related problem with our study, specifically with

respect to where our presumed winter-grown Northern Wheatear feathers were actually grown.

We welcome the opportunity to draw attention to wheatear molt, in which we made an error: the body plumage color changes in *O. oenanthe* between autumn and spring must be one of the most dramatic to occur in high-latitude birds due to feather wear and not to molt (Fig. 1). Measurements of 10 back feathers from five males in fresh basic plumage showed that approximately one-third (average 36.5%) of the feather length is worn off to achieve this color change. Thus, most if not all of our presumed winter-grown back feathers from adult Northern Wheatears were grown in Alaska, not Africa. This is *mea culpa* for DAR and KW. We overestimated the extent of the pre-ternate molt and appreciate the input from other colleagues and now Larson and Hobson (2009) that provides us the opportunity to correct this error. This recognition helps us understand why this group of feathers did not differ significantly from secondaries known to have grown during the previous breeding season in Alaska on these same birds. We hope this prevents others from committing a similar error, for such mistakes only add to the pitfalls associated with using stable isotope analysis to infer origins and movement patterns of animals. However, the presumed winter-grown wheatear back feathers represented only one of six datasets in our study, and we consider that the other conclusions remain robust.

In addressing our *Pluvialis* data, Larson and Hobson (2009) do not dispute the spatial location of feather growth (in the tropics on different sides of the Pacific Ocean in the respective species). Instead, they provide a series of reasons why winter-grown feathers might have high variance in δD (as we did also). They suggest that we should have excluded samples for which $\delta^{13}\text{C}$ was less negative than -20‰ because these samples represent feeding in the

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Fig. 1 A series of Northern Wheatear (*Oenanthe oenanthe*) male specimens from Alaska showing dorsally the alternate (breeding) plumage (the paler birds at left, middle, and right) and basic (wintering) plumage (the two browner birds). The majority of body color changes are achieved not by molt, but by feather wear

marine environment. Although most C_3 terrestrial ecosystems are characterized by highly depleted $\delta^{13}C$, many inland primary and secondary consumers may exhibit values more enriched than this arbitrary cutoff point. For example, aquatic snails (*Goniobasis* spp.) from Cayuga Lake had $\delta^{13}C$ lower than -20‰ , reflecting the enriched values of macrophytes in that lake (Post 2002). Similarly, mountain lions (*Puma concolor*) in northern California without access to marine food resources had more enriched $\delta^{13}C$ values (Long et al. 2005), even after accounting for discrimination associated with bone collagen. Individual red squirrels (*Tamiasciurus hudsonicus*) in southeast Alaska had values between -20 and -23‰ (Ben-David et al. 1997), which would translate into more enriched values in a specialized predator. Thus, arbitrary exclusion of data points may lead to erroneous conclusions. Regardless, marine feeding could not be implicated in the

high variation in the δD values in our *Pluvialis* samples in summer when only two of the birds had $\delta^{13}C$ values more enriched than -20‰ and near average δD values. Using these data, we were unable to assign most of the plovers to their correct area of origin. Also, it would be unreasonable to assume that overwintering *Pluvialis* plovers do not feed in C_4 foodwebs in Asia and South America. We find puzzling the suggestion by Larson and Hobson (2009) that removal of isotopic “outliers” from datasets is part of the “art and science” of stable isotope analysis. We offer that exploration of cases that deviate from our expectations may provide important insights into ecological phenomena, and arbitrary elimination of such data may lead to loss of knowledge. Is it true that in winter *Pluvialis* plovers feed in marine foodwebs? If so, why is the variance in δD four times higher for *P. fulva* compared with *P. dominica* without a similar relationship occurring in $\delta^{13}C$ or $\delta^{15}N$?

Our conclusion remains that variance in isotopic signatures among the three very widely spaced global regions from which our *Pluvialis* feathers originated precludes accurate assignment to region of origin in these species using feather stable isotopes even at the continental scale. Why such variation occurs is of secondary importance when asking whether the methodological tool can provide unequivocal answers; in the *Pluvialis* case—thus far—it could not. Many factors can introduce variance into the isotopic signatures recovered from an individual bird: marine influences are not restricted to coastlines nor to shorebirds, but rather can occur hundreds of kilometers inland (Mowat and Heard 2006); variation in soil moisture, light and temperature (Dawson et al. 2002), and nutrient inputs from animal activity (Croll et al. 2005; Craik and Ben-David 2007) can generate isotopic variation in primary producers that filter through trophic levels (Stewart et al. 2003); anthropomorphic alteration of ecosystems (e.g., the use of ground water in irrigation; S.A. Carleton, University of Wyoming, personal communication) can introduce further variation; and individual physiology, nutritional status, and age could also add to the isotopic variance (McKechnie et al. 2004; Carlton and del Rio 2005; Szép et al. 2009). In addition, our understanding of molt is incomplete and continued research is needed (e.g., Willoughby 2004; Rohwer et al. 2005); adventitious molt needs to be recognized and excluded from samples (we noted this; see errors reported by Reudink et al. 2008); and variation within the plumage coat of a bird or even a single feather can further impart unwanted variance into stable isotope datasets (Smith et al. 2008). Plucking feathers of unknown origin under field conditions, the general modus operandi in this discipline, is largely blind to all these sources of variance. Add to this the general lack of “ground truthing” to verify concordance between feather stable isotopes and isotope isocline maps—and growing

indications that such concordance may not be very tight (e.g., Wunder et al. 2005; Rocque et al. 2006; Langin et al. 2007)—and accurate geographic assignment using these methods seems increasingly unlikely despite some successes (e.g., Bensch et al. 2006; Yohannes et al. 2008).

In their criticism, Larson and Hobson (2009) focused on only one aspect of our paper: where presumed winter-grown feathers originated, continent- and ecosystem-wise. As they noted, how and where these feathers are grown will affect datasets and the results of hypothesis testing. In reviewing the literature, it seems that uncertainties about, and the realities of, biotic systems presently blunt the utility of using feather stable isotopes to infer origins. Indeed, even if feather δD did accurately reflect δD in rainfall, Farmer et al. (2008) have determined that assignments to origin cannot be accurately made within 7–14° of latitude, depending on where in North or South America one is working. Given the nature of this marker (δD), in which the steepest geographic gradient is latitudinal, we can expect substantially greater levels of error across longitudinal space, which has a shallower gradient of change. Thus, even ignoring the lack of concordance between a biotic and an abiotic marker system (which is not possible given results such as ours and Langin et al. 2007), the variation inherent in the underlying abiotic marker can be sufficiently high to preclude accurate within-continent assignment. And this is without considering the third and final source of variation: measurement error (Wunder et al. 2005). In at least one case, measurement error of δD has been shown to be so profound that Smith et al. (2009) “caution against continued use” of this methodology until improved reproducibility is demonstrated (though see Yohannes et al. 2008 for evidence of between-year repeatability within individual Great Reed Warblers, *Acrocephalus arundinaceus*). To our knowledge, no study has yet measured and included all three sources of error in a statistically rigorous analysis. As such studies develop, we should be able to better assess the utility of this methodological tool.

Two other aspects of Rocque et al. (2006) deserve reiteration. First, we used improved rigor in the statistics used to calculate the probability of correctly assigning individuals to regions of origin, an important and needed step forward for this discipline (see Wunder and Norris 2008 for a brief review of this developing front). When determining the probabilities that an individual originated from one of a heterogeneous series of groups, one (or a restricted subset) of those groups will nearly always have a higher probabilistic value. It is an error to assume that the highest probability obtained indicates the actual group of origin; one must then determine the likelihood of that group containing such an individual. When we implemented this test in our study of the two plover species

(using a rather lax 80% probability of membership for an exclusion threshold), we found strikingly low abilities to assign individual birds to known groups of origin. Indeed, in *Pluvialis* plovers, correct assignment among three groups (pooled summer-grown and two groups of winter-grown feathers) was worse than random (41%), and not a single winter-grown feather could be confidently assigned to the group from which it originated. Sometimes biology meets statistical rigor in undesired ways; this is one of those cases. Nonetheless, it is reassuring that the statistical methods of assigning feathers to isoscapes are improving (e.g., Wunder and Norris 2008; Farmer et al. 2008), and we hope that future work will elucidate the strengths and caveats of using stable isotopes to infer origins.

Second, our ability to geographically assign Alaska-breeding plovers was poor. In assessing summer-grown feathers of known origin (we collected birds on their breeding grounds), we found that the nearest rainfall-based δD isoscapes that provided a quantitative match were thousands of kilometers away. Thus, simply matching feather stable isotope signatures to δD isocline maps is a demonstrably insufficient method to assign unknown samples to a geographic region. Larson and Hobson (2009) referred to a “feather δD isocline map,” a mistake we believe too many researchers have made. The more detailed maps available today are rainfall δD isocline maps—averaging values over many years—many of which have yet to be “ground truthed” with respect to δD in feathers grown across the same geographic space. While rainfall δD isoclines provide us with an a priori expectation for the δD signature in feathers grown in a specific area, our results show that for *Pluvialis* plovers in Alaska the fit between these biotic (plover body feathers) measures and abiotic (rainfall δD) maps is not good. Nor does it seem to be without error elsewhere (e.g., Meehan et al. 2003; Langin et al. 2007). Clearly, using stable isotopes to assign migratory birds to the areas where their feathers were grown is a new field in which we can anticipate continued improvements. Larson and Hobson (2009) suggested that “researchers investigate more carefully the nature of isotopic variation in tissues of target organisms under controlled conditions and only attempt to infer assignment to origins under highly constrained scenarios.” We fully agree with this statement, though it is perhaps disheartening to observe that it places some remarkably narrow boundaries on a discipline whose promise was widely hailed just a short time ago. We hope that new advances in isotope analyses (such as compound-specific assays), development of additional markers (such as trace elements, Hanson and Jones 1976; Szép et al. 2009), and development of more accurate feather δD isocline maps will render this tool more broadly applicable.

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