ORIGINS OF LATE-BREEDING NOMADIC SEDGE WRENS IN NORTH AMERICA: LIMITATIONS AND POTENTIAL OF HYDROGEN-ISOTOPE ANALYSES OF SOFT TISSUE

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Abstract.   The nomadic Sedge Wren (*Cistothorus platensis*) breeds primarily in mesic grasslands in north-central North America. Following breeding in these regions from late May to early July, however, the species then "appears" en masse in the tallgrass prairie region farther south (e.g., Missouri and Kansas) and to the east to breed again from mid-July to early August (Herkert et al. 2001). The provenance of birds appearing in late summer to breed in these areas remains unknown because of problems inherent in mark–recapture surveys. Recent studies have shown how endogenous markers may be used to infer origins of individual birds. We analyzed levels of the stable hydrogen isotope ^{2}H (δD) from liver, muscle, and claws of Sedge Wrens from known northern breeding locations first to establish the relationships between δD in the wrens' tissue and mean δD in precipitation during the growing season (δD_p). From these relationships we derived expected values (mean and 95% CI) for three sites in Kansas and Missouri where late breeders colonized. The observed values of δD in these late breeders were primarily within the range expected for those locations, but more individuals than expected had δD values higher than expected. In addition, in birds apparently originating from north or south of Kansas and Missouri, the values of δD in claws were positively correlated with those in other tissues, in contrast to those with the "local" signal. This supports the idea that the isotopic outliers at these sites were more recent arrivals. For small-bodied birds like the Sedge Wren, however, the isotopic approach based on soft tissues is limited to a very narrow temporal window of inference because of rapid elemental turnover. This greatly restricts the use of this technique in inferring origins of small nomadic species.

Key words: Cistothorus platensis, *determining origins*, *deuterium*, *nomadism*, *stable isotopes.*

Origenes de la Población Nómade de *Cistothorus platensis* Crianda Tarde en América del Norte: Limitaciones y Posibilidades del Análisis de Isótopos de Hidrógeno en Tejidos Blandos

Resumen.   La especie nómade *Cistothorus platensis* cría principalmente en pastizales húmedos en el norte y centro de Norte América. Sin embargo, después de reproducirse en estas regiones entre fines de mayo y principios de julio, la especie "aparece" en masa en la región de praderas de pastos altos más hacia el sur (e.g., Missouri y Kansas) y hacia el este, en donde cría nuevamente desde mediados de julio hasta principios de agosto (Herkert et al. 2001). La proveniencia de las aves que aparecen a finales del verano para criar en estas áreas es incierta

debido a problemas inherentes a los sistemas de monitoreo de marcado y recaptura. Estudios recientes han mostrado cómo los marcadores endógenos pueden ser usados para inferir el origen de las aves individuales. Analizamos los niveles del isótopo de hidrógeno estable ^{2}H (δD) del hígado, los músculos y las garras de *C. platensis* de localidades de cría conocidas del norte para establecer inicialmente las relaciones entre δD en los tejidos de las aves y el promedio de δD en las precipitaciones durante la estación de crecimiento (δD$_p$). A partir de estas relaciones derivamos los valores esperados (media e IC del 95%) de tres sitios en Kansas y Missouri colonizados por aves reproductivas tardías. Los valores observados de δD en estas aves estuvieron principalmente dentro del rango esperado para estas localidades, pero más individuos que los esperados tuvieron valores de δD mayores que los esperados. Además, en las que se originaron aparentemente en el norte o sur de Kansas y Missouri, los valores de δD de las garras estuvieron positivamente correlacionados con los de otros tejidos, en contraste con aquellos con la "señal" local. Esto apoya la idea de que los individuos que se encontraban por fuera de los rangos en estos sitios arribaron más recientemente. Para las aves de tamaño corporal pequeño como *C. platensis*, sin embargo, el enfoque isotópico basado en tejidos blandos está limitado a una ventana temporal angosta de inferencia, debido al recambio rápido de elementos. Esto restringe enormemente el uso de esta técnica para inferir el origen de aves pequeñas nómadas.

The nomadic Sedge Wren (*Cistothorus platensis*) has a breeding phenology shared by no other North American bird. Breeding primarily in mesic grasslands in the northern mid-continent, it has the unique behavior of breeding in the northern sections of this region in late May to early July, then appearing en masse in the southern tallgrass prairie region and in areas to the east (Quebec, New York, Vermont) from mid-July to early August to breed again (Herkert et al. 2001). The provenance of birds appearing in late summer to breed in the south and east of the range remains unknown as a result of the difficulty in tracking this species. For decades this mystery has been intractable because this 9-gram bird is too small for radio-telemetry and the scale of banding on the May/June northern breeding grounds needed to yield a significant number of recoveries on the late July/August southern and eastern breeding grounds. With the advent of stable-isotope technology, however, we explored the possibility that this technique might shed light on the Sedge Wren's movements.

The measurement of naturally occurring deuterium (^{2}H) in the tissues of birds and other animals in North America has provided insights into latitude of origin (Hobson 2005, 2008). This is because hydrogen assimilated in terrestrial plants and animals ultimately originates in precipitation and the annual or growing-season pattern of deuterium in precipitation on this continent varies strongly by latitude (Bowen et al. 2005). Keratinous tissues such as feathers and hair have been favored for analysis since they grow during known discrete periods of the annual cycle and are metabolically inactive following the period of growth. This locks in a signal of origin where these tissues were grown, once corrections have been made for the small but important exchange of hydrogen that can take place with the atmosphere (Wassenaar and Hobson 2003).

Little research has been done on the use of deuterium measurements of metabolically active tissues to yield information on origin. In applications of other stable isotopes in animals, examination of isotopic content of tissues with varying elemental turnover rates can provide dietary or locational information based on different temporal windows (Hobson 1993, Phillips and Eldridge 2006, Hobson 2008). For deuterium the situation is more complex since metabolically active tissues can exchange a portion of their hydrogen (i.e., those involving O–H and N–H bonds) with body water, and this process is likely to interfere with interpretation of the deuterium values. Nonetheless, in a captive study using Japanese Quail (*Coturnix japonica*), Hobson et al. (1999) manipulated the deuterium content of drinking water and food and determined that strong signatures of these sources were passed on to eggs. Similarly, deuterium measurements of muscle tissue of migratory Redheads (*Aythya americana*) and their eggs varied seasonally, the change consistent with the time individuals spent in a new area or isoscape (Hobson et al. 2004). Deuterium "source" signals are maintained then in soft tissues of animals, but we currently have a poor idea of how long these signals persist in any given situation.

Our interest in determining origins of late-breeding Sedge Wrens in the southern portion of their range was complicated by the fact that these individuals apparently do not molt after their first breeding attempt before moving to more southern areas, precluding the use of feathers for deuterium analysis. We were able to use muscle and claw tissue, however, by collecting newly arrived birds. Claws resemble feathers in that they consist primarily of keratin and are metabolically inert following formation. Although the rate of growth of claws in the Sedge Wren is unknown, Bearhop et al. (2003) estimated that small passerines grow claws at a rate of ~0.04 mm per day. We examined at least 2 mm of material from the base of claws, so this tissue should provide a signal of origin during the preceding ~50 days (see also Fraser et al. 2008). We expected muscle tissue, though it exchanges hydrogen with body water, to show a deuterium signature corresponding to previous origin as well. But, on the basis of allometric relationships summarized by Hobson (2008), the species' small body size (9 g) narrows that time window to ~10 days. Liver was expected to represent only a few days of dietary integration. Our overall approach then, was to plot isotopic tissue values across a latitudinal gradient for birds of known origin during the first breeding attempts to determine the relationship between deuterium values in Sedge Wren tissue and those of the average precipitation during the growing season at those locations. This plot allowed us to see where tissue deuterium values of the southern arriving birds fell on this curve and so, all things being equal, allow insights into likely origin.

METHODS

FIELD COLLECTIONS

From 21 to 25 June 2005 Robbins collected seven breeding Sedge Wrens per site at six localities along a latitudinal transect from central Iowa to central Manitoba (see below; Fig. 1). Field surveys by Robbins and several observers (see acknowledgments) from late May to mid-June 2005 in western Missouri and northeastern Kansas revealed no breeding populations in that area, thus we chose DeSoto National Wildlife Refuge on the Missouri River near Blair, Nebraska, as the southernmost point of the transect. The central Manitoba site is near the northern terminus of this species' breeding range (Godfrey 1986). On the basis of the anticipated latitudinal resolution deuterium permits, we spaced sampling sites a minimum of 150 km apart. Collecting localities: Iowa/Nebraska: (1) DeSoto National Wildlife Refuge, Harrison/Washington counties (41° 30.6′ N, 95° 59.8′ W). Minnesota: (2) Nobles County, south of Worthington (43° 33.4′ N, 95° 35.6′ W); (3) Traverse County, east Brown's Valley (45° 36.1′ N, 96° 45.6′ W); (4) Polk County, southeast Crookston (47° 36.6′ N, 96° 23.2′ W). Manitoba: (5) southwest Lake Francis (50° 14.1′ N, 97° 56.6′ W); (6) west-northwest of St. Martin Junction (51° 44.4′ N, 98° 50.1′ W).
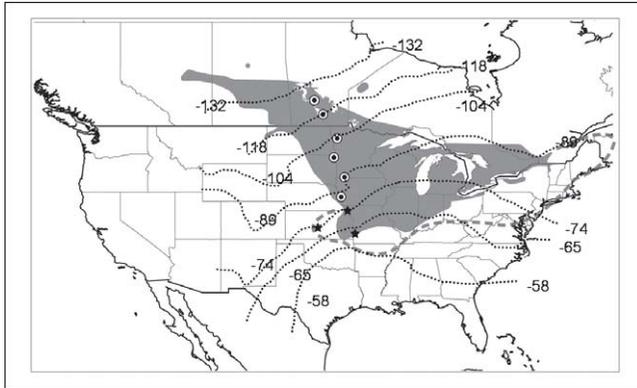
FIGURE 1.   Breeding range of the Sedge Wren in North America. Circles enclosing dots, sites of sampling in early summer along a latitudinal transect; stars, sites of sampling in late summer, representing the influx in July and August; dashed line, areas of sporadic breeding. Superimposed, for reference only, are contours of expected values of δD for feathers, based on Hobson and Wassenaar (1997).

Birds arriving to breed in July–August were collected at the following localities and dates: Missouri: Holt County, Bob Brown Conservation Area (39° 58.8′ N, 95° 14.3′ N; 22 July–11 August 2005; $n = 23$); Missouri: Vernon County, Bushwacker Conservation Area (37° 39.0′ N, 94° 26.9′ N; 28 July 2005; $n = 5$); Missouri: Barton County, Prairie State Park (37° 30.5′ N, 94° 31.6′ N; 28 July 2005; $n = 7$), given the close proximity of the latter two localities they are treated as a single site in the text and in Fig. 1; Kansas: Stafford County, Quivira National Wildlife Refuge (38° 11.8′ N, 98° 29.4′ N; 5 August 2005; $n = 4$).

Upon collection, each specimen was immediately frozen on dry ice. Liver and muscle were preserved from each specimen when voucher specimens were prepared at KUMNH (catalog numbers 96631–96672). After deuterium results were obtained from liver and muscle, toe nails were clipped from voucher specimens.

STABLE-ISOTOPE ANALYSIS

Prior to analysis, soft tissues were first freeze-dried, then lipid-extracted by means of a 2:1 chloroform:methanol solvent rinse, and then air dried for several days in a fume hood. Claws were similarly cleaned of surface oils with this solvent rinse. Tissues were then ground and prepared for analysis of stable hydrogen isotopes at the Environment Canada stable-isotope laboratory in Saskatoon, Saskatchewan. Analyses followed the comparative-equilibration method described in detail by Wassenaar and Hobson (2003) and used isotope-reference materials calibrated for keratin. No equivalent standards are available for soft tissues and so were run against the keratin standards. Stable hydrogen isotopes were measured by continuous-flow isotope-ratio mass spectrometry in H_2 derived from high-temperature flash pyrolysis of feathers and nails. All deuterium results are expressed in the typical delta notation with "D" representing deuterium (δD), in parts per thousand (‰) and normalized on the Vienna Standard Mean Ocean Water–Standard Light Antarctic Precipitation (VSMOW-SLAP) standard scale. Repeated analyses of hydrogen isotope inter-comparison material IAEA-CH-7 (−100 ‰) and keratin references yielded an external repeatability of better than ±2 ‰ based on the distribution of residuals within autoruns for three keratin references.

STATISTICAL ANALYSES

Deuterium values for growing-season average precipitation ($δD_p$) were taken for each collection site from the online tool http://www.waterisotopes.org/. Regressions of tissue δD values against $δD_p$ were performed using SPSS (version 15). We used Sigmaplot (version 10) to portray these regressions with 95% confidence intervals. We used this approach to examine how those δD values for Sedge Wrens at the two southern collection sites differed from values expected for that location.

RESULTS

As expected, in birds of known origin tissue δD values were most depleted at the northern end of the range and most enriched at the southern end. That is, the relationship between tissue δD values and estimated $δD_p$ values was positive (Fig. 2). The relationship for muscle tissue, however, was stronger ($r^2 = 0.62$) than that for liver ($r^2 = 0.28$) and claw ($r^2 = 0.32$). We then plotted the distribution of tissue δD values for the southern sites of newly arriving birds in Kansas and Missouri. For all tissues, there was a slight tendency for outliers to be more enriched in their δD values than expected for those sites. We apportioned the values for muscle and claw δD values from the Missouri sites into three groups (top third, middle third, and lowest third). For the claw values, we found a significant positive relationship for the groups most enriched and most depleted in δD (enriched: $r^2 = 0.25$, $P < 0.01$; depleted $r^2 = 0.27$, $P < 0.01$) but no relationship for the central group.

DISCUSSION

The broad overlap of muscle and claw δD values of Sedge Wrens arriving later in the breeding season in Kansas and Missouri with values expected for the latitude of those locations is consistent with the notion that those tissues were formed primarily at those locations or at other sites with food webs of similar isotope composition. In liver δD values more individuals had more enriched values than expected from the extrapolation of the regression of known-source birds. This was surprising since we anticipated these southern breeders to have arrived primarily from the north, where their tissues would have equilibrated with food webs more depleted in δD. Liver samples in particular were expected to represent very recent locations (i.e., their southern collection sites) in comparison to muscle and claw samples. Within eastern North America, Sedge Wrens are not expected to breed farther south than the locations sampled in Missouri and Kansas.

At each site, variation in tissue δD values for known breeding birds was considerable. Thus the correlations between claw and liver δD values and $δD_p$ values across our north–south transect were weak. In contrast, a moderate ($r^2 = 0.62$) corresponding relationship was found for muscle δD values. This result suggests that muscle tissue was a more faithful indicator of provenance than liver or claws. Claws from early breeding individuals may have included an isotopic signal from the wintering grounds or migratory stopovers. Claws are also difficult to sample consistently from individual to individual, especially in small birds like wrens. Liver has such a fast turnover rate that it provides only a contemporary signal of local dietary variation in D, and because of Sedge Wren's small size, muscle extends the signal only a few additional days. Although muscle provided a better match to precipitation data, it still was a poor indicator of where later breeding birds in Kansas and Missouri originated. Ours is the first latitudinal transect of soft-tissue δD for birds, so we cannot currently compare the results of our regressions of tissue δD and $δD_p$ with other published material.
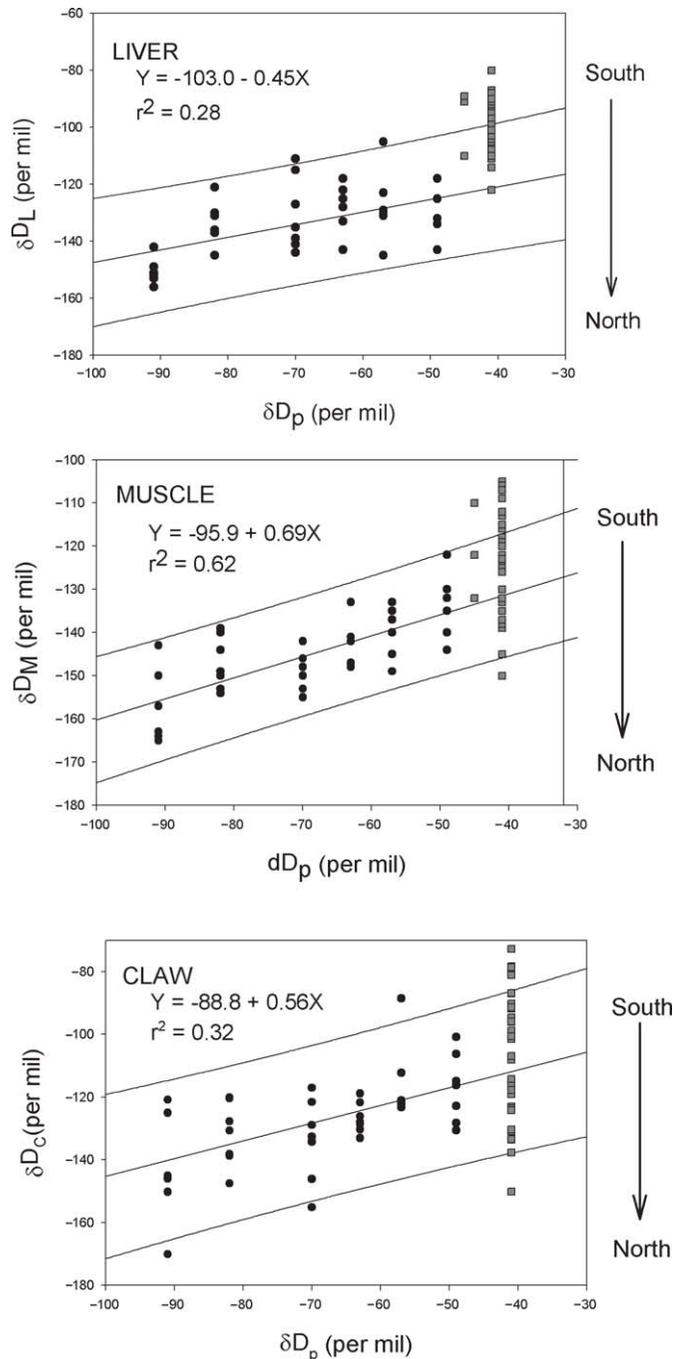
FIGURE 2. Relationship between δD values for Sedge Wren tissue and the mean in precipitation during the growing season (δD_p), calculated for collection sites from data in Bowen et al. (2005). Shown are the regression and prediction 95% confidence intervals for the regression without the Kansas and Missouri birds later sampled (shown as squares).

An alternative hypothesis for the origin of late-summer breeders at our Missouri sample sites is that these individuals arrived from more southern wintering locations or locations of food webs with more enriched δD values such as brackish marshes (with inputs of hydrogen of marine origin). The winter range of the Sedge Wren includes the Gulf states, where it is typically found in coastal grass marshes and wet meadows (Herkert et al. 2001). One line of evidence supporting this hypothesis is that birds with claws with more enriched or depleted δD values than expected for the southern site of Missouri showed stronger correlations between claw and muscle δD values than those with intermediate values, indicating longer-term presence at the southern sites. That result is consistent with the expectation that more recent arrivals will show stronger correlations among tissues than birds that have resided at the site for periods exceeding the replacement period for the tissue with fastest turnover (i.e., muscle vs. claws). Within the southern late-summer breeding range (such as Missouri and Kansas), Sedge Wrens arrive a few days or a week earlier farther north than they do farther south (MBR, unpubl. data). Because of this schedule, coupled with the species' well-documented breeding distribution (Herbert et al. 2001), we consider the hypothesis of southern origin highly improbable.

Other factors may explain the enriched δD values in these southern breeders. In small wetlands or wet grasslands δD values of water can become enriched seasonally as a result of evapotranspiration: isotopically lighter water evaporates more readily, leaving behind more enriched water. Thus, it is possible that a shift to more enriched tissue δD values seen in the southern later breeders may have simply reflected this tendency in small wetlands, an effect that would not be accounted for in the long-term data on the ^2H content of precipitation. This hypothesis is difficult to test because no Sedge Wrens were available for isotopic testing at the southern sites until they arrived in July, confounding location and time of occupancy. The isotopic analysis of wren tissues known to be in equilibrium with the local food web at these southern sites would be useful to test for any late-season enrichment in deuterium. However, the four individuals collected at the Kansas site were sampled later than desired (5 August) and without a prior check on their presence in June or July and thus constituted a sample that had likely equilibrated to the local signal, at least for liver and muscle (claws not included for this subgroup). Two individuals were positive outliers for liver and one for muscle (Fig. 2). These presumably locally equilibrated individuals provide weak anecdotal evidence that that Kansas sites were more enriched than expected, undermining the possibility that birds were moving in from more southern locations. Another important factor is the nature of the isotopic contours expected across the Sedge Wren's range (Fig. 1). Inspection shows that while there is a strong north–south isotopic gradient over much of the range, birds originating from the east and northeast of our Kansas and Missouri sites can have similar or even more positive tissue δD values. Currently, we have no idea if birds move to these southern locations from the east.

Grasslands and wetlands may also be more susceptible to pulsed δD signals related to short-term variation in precipitation. In their study of the Swamp Sparrow (*Melospiza georgiana*), Greenberg et al. (2007) provided indirect evidence that δD values in winter-grown feathers of coastal plain populations of this species follow winter precipitation δD_p. Other studies, however, have shown good agreement between feather δD values and δD_p for wetland or wetland-associated species (Wassenaar and Hobson 2000, Clark et al. 2006, but see Szymanski et al. 2006). We examined the monthly average δD values for precipitation in Barton and Holt counties in Missouri and found that the July averages were more depleted than those for May and June by about 8‰. July rainfall tended to be more depleted than the mean annual growing-season average of −41‰. So, if later seasonal rainfall were more important at these sites, the trend is in the direction

opposite of that expected from our observation of birds with more enriched claw δD values. Thus we have no strong isotopic evidence that seasonal (i.e., May–June) departures in tissue δD values from δD_p could be responsible for the claw δD values more enriched than expected in birds from more northern origins.

Our study reveals the utility of deuterium analyses of soft tissues in addition to those of keratinous tissues such as feathers and claws in investigating the origins of migratory birds and other animals. This approach has some precedence (Hobson et al. 1999, 2004), but more controlled laboratory studies are now needed to work out the influence of body water on soft tissues and the temporal window over which δD values for soft tissue represent origins. In our case, the small body size of the Sedge Wren presents, in some respects, a worst-case scenario. All soft tissues in this species will represent a fairly brief interval during which origins can be inferred. These problems are expected to be alleviated with larger-bodied species. Our study also demonstrates an approach that we believe is appropriate in general. Researchers interested in inferring origins of individuals or populations need to attempt, wherever possible, to establish the relationship between tissue δD values and expected δD_p values or latitude. Such relationships allow researchers to then investigate how origins of birds can be inferred on a species-specific basis. In our case there was no previous information relating claw or muscle tissue to long-term estimates of δD_p for sites in North America or elsewhere. This necessitated the verification of a relationship for birds of known origin that could then be used in testing birds of unknown provenance.

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