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USING STABLE ISOTOPES TO ASSOCIATE MIGRATORY SHOREBIRDS WITH THEIR WINTERING LOCATIONS IN ARGENTINA

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Resumen. – Usando isótopos estables para asociar a las aves playeras migratorias con sus sitios invernales de Argentina. – Investigamos el uso de isótopos estables para identificar la ubicación de los sitios invernales de aves playeras migratorias Neotropicales en Argentina. Nuestro objetivo es asociar un individuo capturado en su área de nidificación o durante la migración con un sitio de invernada especifico, ayudando así a identificar distintas áreas utilizadas por diferentes subpoblaciones. En Enero y Febrero de 2002 y 2003, colectamos plumas remeras de aves playeras migratorias de 23 áreas invernales en siete provincias de Argentina (n = 170). Las muestras de plumas fueron preparadas y analizadas para determinar $\delta^{13}C$, $\delta^{15}N$, $\delta^{34}S$, $\delta^{18}O$ y δD con métodos de fluido continuo. Una función discriminante basada solamente en deuterio no fue precisa prediciendo el origen de aves playeras migratorias a nivel provincial, las predicciones variaron entre 8% (Santiago del Estero) a 80% (Santa Cruz) de las asignaciones correctas. Cuando se incluyeron los otros isótopos estables, la precisión de las predicciones aumentó significativamente (desde 56% en Buenos Aires a 100% en Tucumán). El aumento en la precisión se debió a C/N que separan sitios con baja concentración de deuterio en el oeste de aquellos en el sur, y la inclusión de S separó los sitios respecto a su distancia de fuentes de sulfato marino. También fue posible discriminar correctamente dos sitios geográficamente muy próximos en Tierra del Fuego. Estos resultados sugieren la posibilidad de identificar el origen de un ave playera migratoria con precisión a nivel provincial, tanto como diferenciar aves de sitios muy cercanos. Hay un alto grado de variabilidad intra y entre individuos especialmente en la región Pampeana, donde existe una amplia variedad en las condiciones del agua con respecto a la composición isotópica. De hecho, la variabilidad podría ser la "firma" característica de esta región. La inclusión futura de elementos traza a los análisis puede mejorar las predicciones basadas solamente en isótopos estables.

Abstract. – We are evaluating the use of stable isotopes to identify the wintering areas of Neotropical migratory shorebirds in Argentina. Our goal is to associate individual birds, captured on the breeding grounds or in migration with specific winter sites, thereby helping to identify distinct areas used by different subpopulations. In January and February 2002 and 2003, we collected flight feathers from shorebirds

at 23 wintering sites distributed across seven provinces in Argentina (n = 170). Feathers samples were prepared and analyzed for $\delta^{13}C$, $\delta^{15}N$, $\delta^{34}S$, $\delta^{18}O$ and δD by continuous flow methods. A discriminant function based on deuterium alone was not an accurate predictor of a shorebird's province of origin, ranging from 8% correct (Santiago del Estero) to 80 % correct (Santa Cruz). When other isotopes were included, the prediction accuracy increased substantially (from 56 % in Buenos Aires to 100% in Tucumán). The improvement in accuracy was due to C/N, which separated D-depleted sites in the Andes from those in the south, and the inclusion of S separated sites with respect to their distance from the Atlantic. We also were able to correctly discriminate shorebirds from among two closely spaced sites within the province of Tierra del Fuego. These results suggest the feasibility of identifying the origin of a shorebird at a provincial level of accuracy, as well as uniquely identifying birds from some closely spaced sites. There is a high degree of intra- and inter-bird variability, especially in the Pampas region, where there is wide variety of wetland/water conditions. In that important shorebird region, the variability itself may in fact be the "signature." Future addition of trace elements to the analyses may improve predictions based solely on stable isotopes. *Accepted 7 January 2004*.

Key words: Stable isotope, shorebird, migration, *Calidris melanotos*, Pectoral Sandpiper.

INTRODUCTION

Identifying linkages between the seasonal habitats of migratory birds is critical to conservation efforts because such knowledge is needed to effectively focus management efforts (Myers *et al.* 1987). The need to identify habitat linkages is especially acute for more than 30 species of Neotropical migratory shorebirds because of the hemispheric scope of their annual migrations. We cannot insure their conservation until we have an understanding of the biogeography of these shorebird populations, including: 1) the extent to which species populations are geographically subdivided on the wintering grounds, breeding grounds, and during migration, and 2) the location of specific wintering areas, breeding areas, and migratory stopovers used by each subpopulation.

Stable isotope analyses have been shown to have great potential for defining the geographic distribution of migratory bird populations (Chamberlain *et al.* 1997, Hobson & Wassenaar 1997, Kelly & Finch 1998, Marra *et al.* 1998). The success of this technique is based on two factors. First, a bird's body tissues, including feathers, carry chemical markers that reflect its diet and habitat (Mitzutani *et al.* 1990). Second, these chemical markers are known to vary spatially across the surface of the earth according to well-defined physical and chemical processes. These factors provide the ability to predict a bird's geographic origin by analyzing the chemical content of body tissues.

We are evaluating the use of stable isotopes as a means of establishing linkages between wintering grounds in Argentina and breeding grounds in the Arctic for the Pectoral Sandpiper (*Calidris melanotos*), the Baird's Sandpiper (*Calidris bairdii*), and the White-rumped Sandpiper (*Calidris fuscicollis*). The study species are widely distributed on both their breeding and wintering grounds. They breed along the Arctic coastal zone of Canada and Alaska. The Pectoral Sandpiper's breeding range also extends westward to the Taymyr Peninsula of Russia, and Baird's Sandpiper's breeding range extends eastward to northern Greenland (Hayman *et al.* 1986). On their South American wintering grounds, they occur from southern Patagonia northward into southern Uruguay, Paraguay, Bolivia and Peru (the Pectoral Sandpiper is found only as far south as Santa Cruz province). Little is know about winter site fidelity or movements during the winter in South America,

although the presumption is that individuals of *Calidris* spp. remain in the same local area during their several-month stay in Argentina.

The goal of this study is to interpret the distinct chemical markers in shorebird flight feathers in order to associate individual birds, captured on the breeding grounds or at migration stopovers, with their specific winter sites. These long distance migrants replace their flight feathers once each year, on the wintering grounds just prior to spring migration. Thus, the flight feathers carry chemical markers that are characteristic of their winter area. We must identify how these chemical signatures vary spatially across the wintering grounds to answer study questions such as: 1) can a bird's breeding location be predicted from knowledge of its winter latitude; 2) is a bird's migration path related to its winter latitude; and 3) is a bird's timing of migration related to its winter latitude.

We began a pilot study in February 2001 (Farmer *et al.* 2003) that provided evidence that there are significant differences in the isotopic signatures of feathers collected at three widely separated sites. In January 2002, we expanded the work to describe the isotopic patterns across the winter range of Argentina, including study sites in northern, central, and southern Argentina. This paper reports results from the first year's effort, both large and small-scale patterns that are evident in the data, and potential problems that must be solved to accomplish the study goal.

METHODS

Feather collection. In January and February 2002 and 2003, we collected newly grown flight feathers from White-Rumped Sandpipers (n = 77), Pectoral Sandpipers (n = 44), Baird's Sandpipers (n = 8), and 6 other shorebird species (n = 41) captured at 23 different wintering sites distributed across Argentina [Tucumán (1), Santiago del Estero (2), Córdoba (2), La Pampa (9), Buenos Aires (2), Santa Fe (4), Santa Cruz (1), and Tierra del Fuego (2)]. We collected shorebirds in mist nets and extracted two secondary feathers(secondary # 9 or the latest that had been replaced if # 9 was an old feather) were removed from each bird for analysis. One species (*Calidris canutus*) was captured with canon nets and, because of their generally larger feather mass, we extracted two primary coverts, the latest to have been replaced in the current year. Whole wings from shorebirds collected in central and northern Argentina were available for analysis.

Sample preparation and laboratory analysis. Feathers were cleaned in a 2:1 chloroform:methanol solution and dried overnight. Samples were cut from the feather vane and weighed into either silver (H and O analyses) or tin capsules (C, N, and S analyses). We attempted to prepare at least two samples for each isotope for each individual bird, but there was insufficient feather mass to do this in some cases. We did a whole-wing analysis for some birds by preparing samples from the proximal and distal ends of several flight feathers (as many as 20); hence, some birds were represented by as many as 40 samples.

The samples were analyzed for $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ by continuous flow methods using Carlo Erba NC2500 elemental analyzer coupled to either a Micromass Optima, Finnigan Delta Plus XL, or a Finnigan Delta Plus XP mass spectrometer (Fry *et al.* 1992, Giesemann *et al.* 1994). Isotopic compositions of $\delta^{13}C$ and $\delta^{15}N$ were reported relative to PDB and Air, respectively, using internal laboratory standards calibrated against ANU sucrose ($\delta^{13}C$ = –10.4‰), NBS 22 ($\delta^{13}C$ = –29.6‰), USGS 25 ($\delta^{15}N$ = –30.4‰), and USGS 26 ($\delta^{15}N$ = 53.7‰). Isotopic compositions of $\delta^{34}S$ were reported relative to CDT using internal laboratory standards calibrated against NBS 127 and IAEA-S-1 ($\delta^{34}S$ =

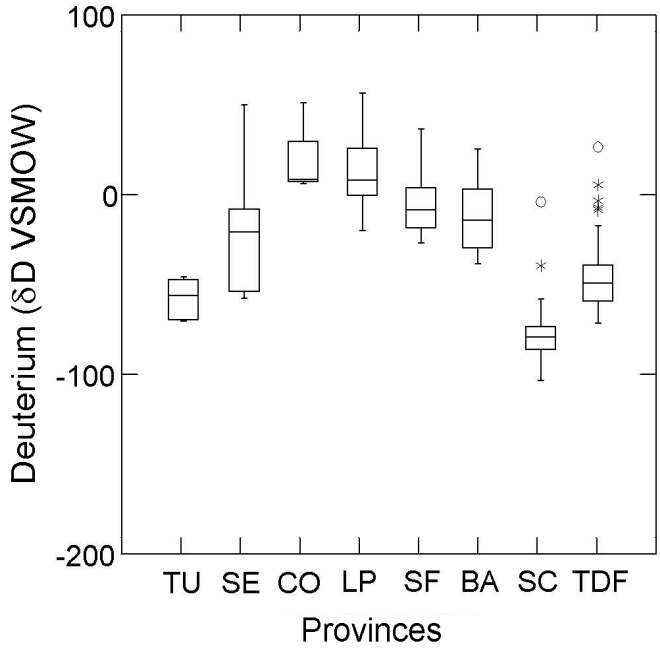

FIG. 1. Standard box plots of deuteriun ratios (referenced to the SMOW standard) by Argentine province (TU = Tucumán, SE = Santiago del Estero, CO = Córdoba; LP = La Pampa, SF = Santa Fe, BA = Buenos Aires, SC = Santa Cruz, TDF = Tierra del Fuego). Data for Pectoral, White-rumped and Baird's sandpipers..

21.1‰ and –0.3‰, respectively). Analytical error of C, N, and S analyses is ± 0.2‰. Samples were analyzed for $\delta^{18}O$ and δD using a Finnigan TC-EA coupled to a Finnigan Delta Plus XL mass spectrometer (Farquhar *et al.*1997, Wassenaar & Hobson 2003). Deuterium compositions were reported relative to Vienna Standard Mean Ocean Water (VSMOW) using internal laboratory standards calibrated against IAEA-CH-7 (δD = –100‰). Non-exchangeable hydrogen isotopic compositions are determined by comparative equilibration techniques described by Wassenaar & Hobson (2003). Analytical error of non-exchangeable hydrogen isotope values is ± 4‰. Oxygen isotopic compositions were reported relative to VSMOW using internal laboratory standards calibrated against NBS127 ($\delta^{18}O$ = 9.0‰). Analytical error of oxygen isotope values is ± 1‰.

Data analysis. SYSTAT v. 10 was used to conduct a series of linear discriminant analyses (complete, and stepwise/backward) as a means of 1) identifying the isotope(s) that best discriminate among groups, and 2) developing canonical classification functions that could be used to classify future feather samples from Argentina. The unit of analysis was the individual bird, hence mean isotope values were computed from the feather sample replicates within individual birds ($2 \leq n \leq 40$), and the mean values were used in the analyses.

RESULTS

Large-scale analysis. Farmer *et al.* 2003 reported

TABLE 1. Expected prediction accuracy for correctly identifying the provincial origin of a shorebird (Pectoral, White-rumped, or Baird's sandpipers) , based on a complete discriminant analysis (jackknifed classification matrix).

Provinces	Percent correct		
	H	H, O, N, C	H, O, N, C, S
Buenos Aires	31	28	56
La Pampa	71	80	79
Santa Cruz	80	80	—
Santiago del Estero	8	54	69
Tierra del Fuego	18	75	75
Tucumán	25	100	100

that deuterium ratios in precipitation varied both longitudinally and latitudinally in Argentina. These same patterns were seen in the feather analysis; generally as one moves westward or southward in Argentina, deuterium ratios decrease. The data in Figure 1 were arranged to show the longitudinal and latitudinal gradients. The longitudinal gradient was caused by both a continental effect and an orographic effect of the Andes. The latitudinal gradient reflects the worldwide equator-to-pole gradient seen in precipitation deuterium values. The most notable deviation from the large-scale pattern was for Tierra del Fuego where the δD values were larger than expected, likely because these samples came from birds feeding in estuarine areas where ocean water (δD = 0‰) has a moderating effect.

Although there was a significant large-scale pattern, deuterium alone did not provide a strong ability to predict a shorebird's winter origin. A discriminant analysis (jackknifed classification matrix) poorly predicted a shorebird's province of origin, with prediction accuracy ranging from 8% correct (Santiago del Estero) to 80 % correct (Santa Cruz)(Table 1). When C, N, and O were added to the analysis, the prediction accuracy increased substantially in the northwest (Santiago del Estero and Tucumán) and in the south (Tierra del Fuego). The multivariate function provided an improved prediction because the northwestern sites had similar δD values to Tierra del Fuego (Fig. 1), but the C and N isotope values were sufficiently different to separate these regions. The addition of S to the analysis resulted in additional prediction accuracy in Buenos Aires, likely because these sites were relatively close to the Atlantic Ocean, as compared to the other sites.

Small-scale analysis. We also evaluated this method for identifying the origin of shorebirds from among two closely spaced sites in Tierra del Fuego. These sites, Rio Grande and Bahía Lomas, were of interest because they are winter sites for Red Knots and there is an established conservation need to identify individual Red Knots from these two sites, when they are captured during spring migration at Delaware Bay and other stopovers in North and South America. A complete discriminant analysis (jackknifed classification matrix) of C, N, and D correctly predicted whether a Red Knot wintered in Rio Grande (n = 5) versus Bahía Lomas (n = 5) with 95% accuracy.

Intra-bird variability. We conducted several whole-wing analyses of δD for individual Pectoral Sandpipers from La Pampa and Buenos

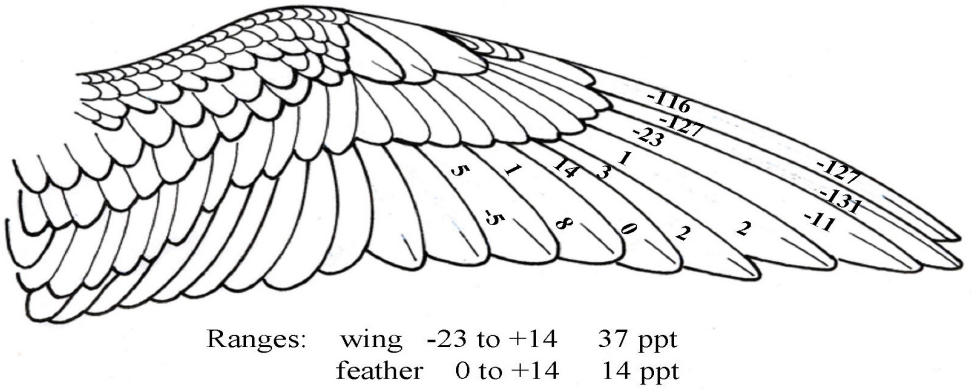

FIG. 2. Deuterium ratios at the base and tip for primaries 3–10 from a Pectroal Sandpiper collected in the Pampas region of Argentina. Primaries 3–8 were new and had deuterium values from Argentina. Primaries 9 and 10 had not been replaced and had Arctic signatures.

Aires provinces. One of these wings is shown in Figure 2. This bird had recently molted primaries 1–8. Primaries 9 and 10 were old and reflected Arctic δD values, indicating the bird was a juvenile. The range of values within feathers was high (as much as 14‰), as was the variability among feathers (as much as 37 ‰ within a single shorebird). This range is a significant portion of the variability seen across all of Argentina.

DISCUSSION

These first-year results are promising because they provide support for our initial goal of predicting the origin of a shorebird at a provincial level of accuracy. To fully accomplish our goal, however, we must expand the geographic scope of feather collection on the wintering grounds by adding additional sites in northeastern Argentina and in Patagonia. Eventually, we must also expand sampling beyond Argentina across the entire wintering range including portions of Chile, Paraguay, Uruguay, and Bolivia.

Ultimately, the accuracy with which one can predict the geographic winter location of a shorebird will be limited by the degree of variability seen within and among feathers from individual birds. Farmer *et al.* 2003 reported values of intra-bird variability for $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$. In the current study, we conducted several whole-wing analyses of δD for individual Pectoral Sandpipers from La Pampa and Buenos Aires provinces. The range of values within individual birds was high (37‰), which is a significant portion of the variability seen across all of Argentina. This variability can be viewed in the traditional way, by employing a power analysis to estimate the sample size necessary to detect some minimum difference between populations, at some specified level of probability. We believe that this would be an advisable thing to do in any case. However, one might go beyond this basic statistical treatment, and attempt to understand the cause of the variation. Several possible mechanisms that might introduce variability were identified by Farmer *et al.* 2003. In the present case, the high degree of variability in δD from shorebirds in the center of Argentina might indicate the wide variety of surface water δD values due to fractionation associated with evaporation. In the central Pampas region of Argentina, there are many evaporated lakes in

close proximity to smaller wetlands containing relatively new rainwater. Such variety introduces strong local variations in δD that complicate the large-scale gradients evident from the precipitation data. A shorebird that spends the winter in a relatively small area of the Pampas could consume food from a wide variety of waters during a brief period when flight feathers are being grown. Hence, the degree of variability in δD itself might be a diagnostic marker of the Pampas region, and we would be ill advised to attempt to ignore it or mask it with statistics. This seems to be a fertile area for new research that might yield valuable ecological insights.

For achieving higher prediction accuracy, the relatively high variability of δD may be partially overcome by incorporating other chemical analyses. In the next year we plan to incorporate radiogenic isotopes (207Pb/206Pb and 87Sr/86Sr) using thermal ionization mass spectrometers, as well as trace element analyses (Parrish *et al.* 1983) utilizing a relatively new technique, laser ablation ICPMS (Ridley 1998, Ridley 2000) in our exploratory analyses. Although deuterium has been very important for bird migration studies in North America, utilizing a suite of isotopes and trace elements may substantially enhance our ability to identify a shorebird's specific winter region in South America.

We began this study with the initial expectation that we might be able to achieve provincial-level accuracy. It now appears that this is an achievable goal. However, the analyses that we have conducted to date are based on provincial boundaries drawn with human political systems in mind. There are ecological/geological criteria that might be used to characterize discrete spatial units within Argentina in a manner that would improve prediction accuracy within approximately "provincial" sized units. Additionally, there are other statistical approaches to data analysis that would treat isotopic gradients as spatially continuous rather than discrete. These alternative analyses might provide additional insights in this expanding field of using stable isotopes to identify movement patterns of organisms.

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