

## NOTE / NOTE

# Trace element profiles as unique identifiers of western sandpiper (*Calidris mauri*) populations

D.R. Norris, D.B. Lank, J. Pither, D. Chipley, R.C. Ydenberg, and T.K. Kyser

**Abstract:** Understanding the ecology and evolution of migratory animals requires information on how populations are geographically linked between periods of the annual cycle. To examine whether trace elements could be used to track migratory birds, we analyzed concentrations of 42 trace elements in feathers of western sandpipers (*Calidris mauri* (Cabanis, 1857)) that were grown at five different wintering sites ranging from San Francisco Bay (USA) to the Bay of Panama. Linear discriminant analysis of 15 elements correctly classified all 26 individuals to their wintering sites, including two sites that were separated by less than 3 km. A randomization procedure confirmed the robustness of these findings. Our analysis suggests that trace elements can be used to assign individuals to specific sites of origin. Although we did not sample feathers from all wintering areas, the regions our sites represented comprised a significant percentage of the global population. However, since trace element profiles appear to be highly specific to geographic sites, we suggest that this technique is best suited for cases where samples can be obtained from the majority of populations throughout a species range. Thus, under certain circumstances, trace element profiles may provide the potential to identify populations with a high degree of spatial accuracy.

**Résumé :** Afin de comprendre l'écologie et l'évolution des animaux migrateurs, il est nécessaire de savoir comment les populations sont reliées géographiquement d'une période à l'autre de leur cycle annuel. Nous avons déterminé les concentrations de 42 éléments en traces dans les plumes de bécasseaux d'Alaska (*Calidris mauri* (Cabanis, 1857)) élevés dans cinq sites différents d'hivernage répartis de la baie de San Francisco (É.-U.) à la baie de Panama afin de vérifier si ces éléments peuvent servir à suivre les oiseaux migrateurs. Une analyse discriminante linéaire de 15 éléments permet de classer correctement les 26 individus d'après leur site d'hivernage, y compris dans deux emplacements situés à moins de 3 km l'un de l'autre. Une procédure de randomisation confirme la robustesse de ces résultats. Notre analyse indique que les éléments en traces peuvent servir à relier les individus à des sites d'origine spécifiques. Bien que n'ayons pas échantillonné le plumage dans tous les sites d'hivernage, les régions où nos sites se trouvent représentent un pourcentage élevé de la population globale. Cependant, parce que les profils d'éléments en trace semblent hautement spécifiques aux sites géographiques, nous croyons que cette technique peut servir de manière optimale dans les cas où il est possible d'échantillonner la majorité des populations dans l'aire de répartition d'une espèce. Ainsi, dans certaines circonstances, les profils d'éléments en traces peuvent être utilisés pour identifier des populations avec un fort degré de précision spatiale.

[Traduit par la Rédaction]

## Introduction

One of the greatest challenges for understanding the ecology and evolution of migratory animals is determining how populations are geographically linked between different periods of the annual cycle (Webster et al. 2002). Knowledge of migratory connectivity is essential for understanding the factors that limit populations (Webster and Marra 2005) and for developing models that successfully predict declines in

the future (Marra et al. 2006). Intrinsic markers such as stable isotopes and genes have dramatically improved our ability to link breeding and non-breeding populations (Rubenstein and Hobson 2004). Elemental and genetic information is incorporated into tissues during one period of the year, and animals can then be sampled in subsequent periods to estimate their location in the previous period. However, owing in part to the high ratio of within- to among-site variability of these markers, the resolution to assign individuals

Received 30 November 2006. Accepted 6 March 2007. Published on the NRC Research Press Web site at [cjz.nrc.ca](http://cjz.nrc.ca) on 16 May 2007.

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to specific areas has thus far been limited to coarse regional scales (e.g., Hobson and Wassenaar 1997; Rubenstein et al. 2002; Norris et al. 2006). A pioneering study based on elemental analysis assigned migrant and wintering *Anser* geese species to breeding/moult grounds (Hanson and Jones 1976), but further use and development of the technique was limited. Advances in the use of high-resolution inductively coupled plasma mass spectrometers (HR-ICP-MS) provide a unique opportunity to analyze multiple elements at low concentrations from a small tissue sample (e.g., Szep et al. 2003; Donovan et al. 2006). Simultaneous analysis of multiple markers should increase the probability of correctly assigning individuals to specific sites (Webster et al. 2002).

Because we had no a priori information on how trace elements in tissues vary over large spatial scales, we compared elemental profiles between sites within the same period of the annual cycle. We analyzed 42 trace elements in flight feathers of western sandpipers (*Calidris mauri* (Cabanis, 1857)) that were grown at five different wintering sites ranging from San Francisco (USA) to the Bay of Panama. Feathers are metabolically inert after growth and should therefore provide an elemental signature from the location where they were grown (Hobson and Wassenaar 1997; Szep et al. 2003; but see Bortolotti and Barlow 1988; Bortolotti et al. 1988). If trace element profiles in feathers are distinguishable among wintering sites, this would provide a unique opportunity to sample individuals in other periods of the year to estimate their winter origin with a high degree of spatial accuracy.

## Methods

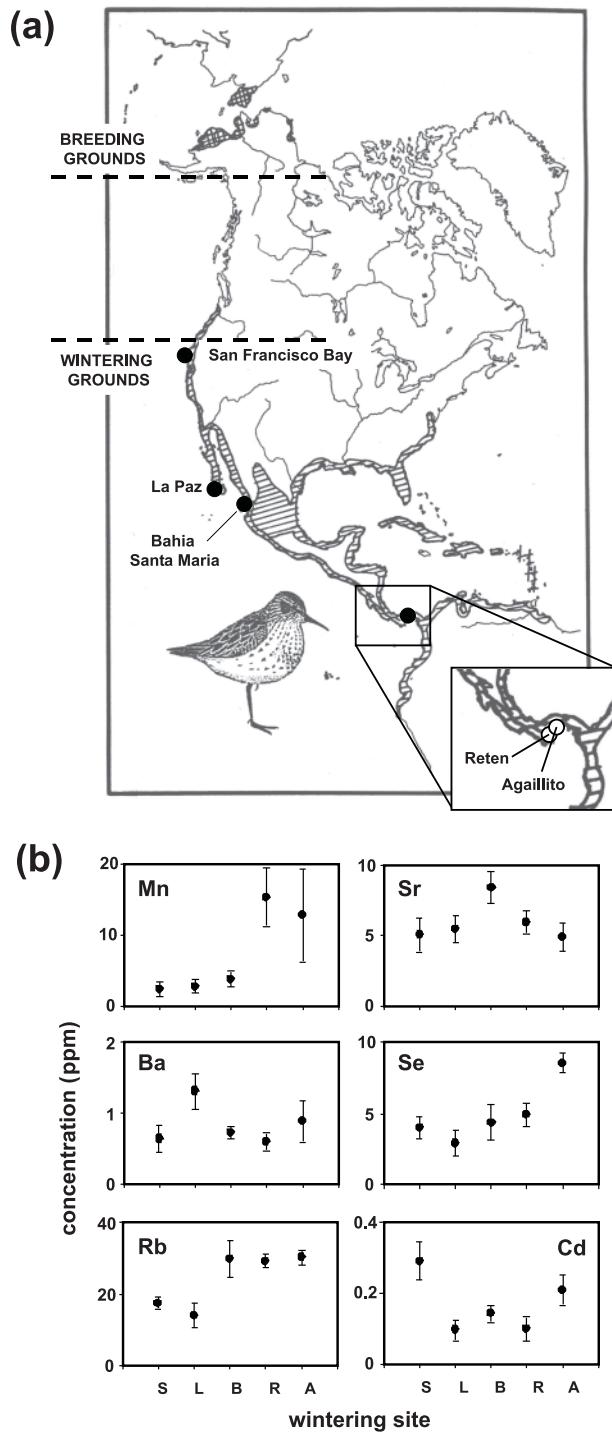
### Study species and sampling sites

Western sandpipers breed in the high Arctic of western Alaska and far eastern Siberia and spend the boreal winter in coastal estuaries on the Pacific coast from California to Ecuador and, in smaller numbers, along the Atlantic coast and in the Caribbean (Fig. 1a). Adults moult their flight feathers on the wintering grounds. Between 1998 and 2001, we sampled primary flight feathers from after-hatch-year (second wintering season or more) western sandpipers at each of five wintering sites: San Francisco Bay (USA;  $n = 5$ ;  $37.5^\circ\text{N}$ ,  $122.3^\circ\text{W}$ ), Bahía Santa María (Mexico;  $n = 6$ ;  $25^\circ 02'\text{N}$ ,  $108^\circ 18'\text{W}$ ), La Páz (Mexico;  $n = 5$ ;  $24^\circ 06'\text{N}$ ,  $110^\circ 22'\text{W}$ ), Reten (Panama;  $n = 6$ ;  $08^\circ 00'\text{N}$ ,  $80^\circ 50'\text{W}$ ), and Agaillito (Panama;  $n = 6$ ;  $08^\circ 00'\text{N}$ ,  $80^\circ 50'\text{W}$ ).

### Trace element analysis

We measured 41 trace elements (Mn, Hg, Bi, Mg, Al, Ca, Sc, Sr, Ba, Fe, As, Se, Na, Pb, U, Li, Ti, V, Cr, Ni, Cu, Zn, Rb, Mo, Cd, Th, Y, La, Ce, Pr, Nd, Sm, Eu, Gd, Dy, Ho, Er, Tm, Yb, Lu, and Hf) in feathers using a Finnigan MAT ELEMENT HR-ICP-MS (Finnigan MAT, Bremen, Germany). The ELEMENT is a single-collector, double-focusing sector-field mass spectrometer with reverse Nier-Johnson geometry (Gießmann and Greb 1994). To prepare samples, whole feathers (20–30 mg) were cut into small pieces (approx. 5 mm), placed in glass vials with deionized water ( $>18\text{ M}\Omega$ ), and then washed in an ultrasonic bath for 10 min. Water was decanted and samples were dried on a hot plate at  $70^\circ\text{C}$ , cooled, and weighed into screw-top Tef-

**Fig. 1.** (a) Breeding and wintering grounds of western sandpipers (*Calidris mauri*) (sampling sites indicated by dots). Although wintering distribution is depicted as continuous, sandpipers are patchily concentrated in major estuaries, primarily along the Pacific coast. (b) Mean ( $\pm\text{SE}$ ) concentrations of six elements, out of 42 analyzed, in feathers of western sandpipers that were grown at the five wintering sites shown above (S, San Francisco Bay [US]; R, Reten [Panama]; A, Agaillito [Panama]; L, La Páz [Mexico]; B, Bahía Santa María [Mexico]). Examples were chosen to illustrate how elements varied differently across sites, thereby increasing the ability to discriminate between populations.



Ion<sup>TM</sup> beakers. After 2 mL of concentrated nitric acid ( $\text{HNO}_3$ ) had been added, capped beakers were placed on a 70 °C hot plate for digestion of samples. Once the resulting liquid had cooled, 0.25 mL of hydrogen peroxide was added to digest dissolved organic compounds; samples were again dried on a 70 °C hot plate. After cooling, another 1 g of 2%  $\text{HNO}_3$  was added to dissolve residue. Samples were transferred to a clean, tared high-density polyethylene bottle. Each Teflon beaker was rinsed twice with 2%  $\text{HNO}_3$  and the rinse solution was added to the bottle. Samples were diluted with 2%  $\text{HNO}_3$  to a total mass of 5–6 g. To monitor contamination, procedure blanks were prepared in the same fashion. One ppb of indium was mixed with all  $\text{HNO}_3$  solutions. Indium is an internal standard used to monitor and correct for instrument sensitivity drift, sample density, and viscosity variations.

Samples were introduced to the HR-ICP-MS via a 100  $\mu\text{L}/\text{min}$ , self-aspirating Teflon nebulizer (Elemental Scientific, Inc., Omaha, Nebraska) and a Scott double-pass spray chamber. Signal intensities were maximized by adjusting plasma gas flows and source lens potentials at low resolution ( $\text{M}/\Delta\text{M} = 300$  at 5% peak height). Tuning lenses at medium ( $\text{M}/\Delta\text{M} = 3\,000$  to 5 000 at 5% peak height) and high ( $\text{M}/\Delta\text{M} = 7\,500$  to 10 000 at 5% peak height) resolutions achieved narrow peaks while maintaining a symmetrical, triangular peak shape such that interfering isotopes could be separated from the analyte peaks. Isotopes of the elements were measured at resolutions appropriate to separate isobaric inferences.

### Statistical analyses

We used linear discriminant analysis (LDA) to examine whether trace element concentrations in feathers (predictor variables) could be used to distinguish individuals originating from different wintering sites (groups). LDA produces linear combinations of predictor variables (discriminant functions) and maximizes the among-group variation relative to the within-group variation, with the constraint that successive discriminant functions are not correlated with one another. Since we had more elements ( $n = 42$ ) than the total number of feathers ( $n = 28$ ), we reduced the number of elements that were included in the LDA based on two criteria: elements were removed if more than 25% of the individuals sampled had zero concentrations (below detectable limits) or if there was no suggestive difference in the concentration of an element between winter sites (one-way ANOVA with  $P > 0.15$ ). This reduced the total number of elements used in the LDA to 15: Mn, Mg, Sr, Ba, Fe, Se, Na, Pb, V, Ni, Cu, Mo, Cd, Y, and Ce.

Because the sample size within each group was small, the assumption of normality in the LDA was not met. Therefore, we used a randomization procedure for LDA described by Manly (1998, p. 275). First, we conducted a standard LDA using the untransformed concentrations of elements as predictor variables and the five wintering sites as the groups to be discriminated among. This analysis generated four discriminant functions (equal to the number of groups – 1) with corresponding eigenvalues. For the randomization, the same procedure was repeated 999 times, except that wintering sites were allocated randomly among the coherent groups of trace element values (i.e., the rows containing the

trace elements were kept fixed). Each iteration produced a new set of four eigenvalues. The four original (observed) eigenvalues were then compared with the “null” distribution of randomized eigenvalues. If 50 or fewer of the 999 null eigenvalues were larger than the observed eigenvalue, then the given discriminant function was considered significant (95% confidence level).

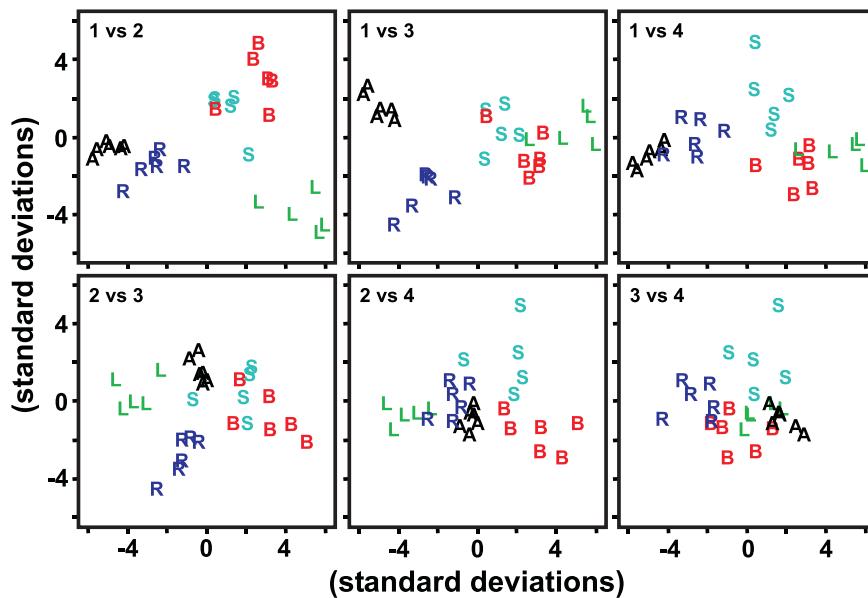
### Results and discussion

We found that many elements varied differently across wintering sites, such that concentrations of some elements were similar between sites “A and B” while concentrations of other elements were similar between sites “A, B, and C” or between sites “A and C” (Fig. 1b). As a result, the discriminant functions calculated from 15 trace element concentrations correctly classified all individuals to the wintering sites where they were sampled. The randomization procedure strongly suggested that this was not a fortuitous result: the magnitude of each of the four observed eigenvalues (9.4, 6.05, 4.13, and 3.40) was highly significant when judged against its corresponding distribution of “null” eigenvalues (first eigenvalue:  $P = 0.013$ ; remaining eigenvalues:  $P < 0.001$ ). Pairwise scatterplots illustrate how the individuals from each of the five wintering sites are aligned across the discriminant axes and show the significant contribution of each discriminant function to the classification of individuals (Fig. 2). An assessment of analogous bivariate plots using randomized data (i.e., when locations are randomly assigned to individuals; cf. Manly 1998) emphasized the robustness of these findings (not shown).

Our results suggest that trace element profiles in feathers can be used to accurately distinguish among moulting (wintering) areas used by western sandpipers. Importantly, elemental profiles correctly classified birds overwintering at two coastal sites in the Bay of Panama (Reten and Agaillito) that were separated by less than 3 km, one up-current and the other down-current from a river mouth, and two sites in Mexico (La Páz and Bahía Santa María) that were separated by less than 100 km of water. We also show that trace element profiles in feathers are relatively consistent within sites. This confirms that adults show high within-season fidelity to wintering sites and suggests that most individuals do not move between different habitats, at least not while they are moulting.

We suggest that this technique will probably be useful only when there are well-defined boundaries between populations and the majority of populations throughout the range of a species can be sampled (Donovan et al. 2006). Clearly, the use of trace element profiles will be most feasible for migratory species that are patchily distributed, for example, coastal waders that winter in major estuaries, insects that aggregate during the non-breeding season, anadromous fish that spawn in discrete river systems, or ungulates that use specific sites for calving. In other cases, habitats that generate specific elemental profiles may be dispersed throughout a species’ range. For example, Bortolotti et al. (1989) found that profiles of five minerals in known-origin spruce grouse (*Dendragapus canadensis* (L., 1758)) were highly specific to different forest types but because these forest types were distributed throughout the species’ range, the successful as-

**Fig. 2.** Pairwise scatterplots of the scores from the four discriminant functions (labeled in upper left corners of panels) generated by the linear discriminant analysis of 15 trace elements in feathers of western sandpipers that were grown at five different wintering sites (letters correspond with those in Fig. 1b). Overall, excellent discrimination was achieved among wintering sites.



assignment of individuals of unknown origin to particular geographic regions appeared unlikely.

In our study, we sampled only five wintering sites, but two of these sites were in the Bay of Panama, where 35% of the global population is estimated to overwinter (Morrison et al. 2001). It is estimated that an additional 10% of western sandpipers overwinter in Bahía Santa María. Samples from three or four more key sites in the Bay of Panama plus another three or four sites in Mexico would increase the global representation to over 75%. Based on the number of additional sampling sites needed and our current discriminatory ability, we suggest that trace element profiles could be used to accurately separate all major western sandpiper wintering sites. However, because elemental profiles appear to be highly site-specific, trace elements will likely not be useful for species with a more continuous distribution, where only a small fraction of the populations can be sampled (Donovan et al. 2006).

The key advantage of this technique is that 40 or more markers can be analyzed simultaneously from a single tissue sample (Szep et al. 2003), thereby increasing the chances that a subsample of elements will vary independently between sites. The number of elements than can be measured simultaneously by HR-ICP-MS analysis may balance out the relatively high cost (stable isotope analysis is approximately one-tenth to one-half the price). Other issues associated with these markers are likely more important to consider. Analysis of multiple stable isotopes is also possible, but these markers often show high spatial autocorrelation (e.g.,  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ,  $\delta^{18}\text{O}$  and  $\delta\text{D}$ ), reducing the likelihood that the geographic resolution of assignments will improve with the addition of only one or two more isotopes. The combination of stable isotopes with genetic markers has proven useful (e.g., Kelly et al. 2005; Boulet et al. 2006) and will un-

doubtedly improve as more genes can be screened. However, genetic markers are constrained to assigning individuals to breeding areas where genetic structuring occurs and cannot account for adult dispersal within a season because they can only link individuals with their place of birth.

We emphasize that trace elemental markers, like other intrinsic markers, should ideally be verified using known-origin birds (Hanson and Jones 1976; Bortolotti et al. 1988). In addition, there is some evidence that the chemical composition of feathers can change after growth (Hanson and Jones 1976; Bortolotti and Barlow 1988; Bortolotti et al. 1988), implying that assigning individuals of unknown origin to geographic areas in previous periods of the annual cycle could be problematic. Further experimental tests are required to determine whether chemical changes after feathers are fully grown are specific to certain mineral types, habitats, and (or) species. However, our results provide evidence that trace elements can be used to identify shorebird populations with high geographic resolution and provide the potential for tracking individuals between breeding and non-breeding areas.

## Acknowledgements

We thank G.J. Fernández, S. Nebel, D. Galindo-Espinoza, and P. O'Hara for help collecting feathers. D.R.N. and J.P. were supported by Natural Sciences and Engineering Research Council of Canada (NSERC) Postdoctoral Fellowships. Funding was provided by NSERC (D.B.L., R.C.Y., T.K.K.), the Centre for Wildlife Ecology, Environment Canada (D.B.L., R.C.Y.), the Ontario Innovation Trust (T.K.K.), and the Canadian Foundation for Innovation (T.K.K.).

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