Range-wide patterns of migratory connectivity in the western sandpiper *Calidris mauri*

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ered from expected only among males in the Yukon-Kuskokwim (Y-K) Delta
Nome, Alaska. Males in the Y-K Delta originated overwhelmingly from western Mexico, while in Nome, there we
males from western North America and more from the Baja Peninsula than expected. An unexpectedly high p
of migrants captured at a stopover site in the interior United States originated from eastern and southern winter
while none originated from western North America. In general, we document substantial mixing between the bre
wintering populations of both sexes, which will b er the global population of western sandpipers from the e ects of loc
habitat loss on both breeding and wintering grounds.

Understanding the population dynamics of migratory animals requires knowledge about the degree of migratory connectivity of populations across the annual cycle degree – that is, the degree to which individuals in a population co-occur in di erent seasons (Webster et al. 2002, Marra et al. 2006, Norris and Marra 2007). Stable isotope analysis of inert tissues such as feathers has been widely used to identify the geographic origins of migratory animals (Hobson and Wassenaar 1997, Clegg et al. 2003, Kelly et al. 2005, Bensch et al. 2006, Jones et al. 2008, Miller et al. 2011), but only a few studies have examined the degree of connectivity across the entire range of a long-distance migratory species using this approach (Rubenstein et al. 2002, Boulet et al. 2006, Norris et al. 2006). Such range-wide information on connectivity is essential for making strong inferences about the causes of variation in population size of migratory animals (Taylor and Norris 2010) and for developing e ective conservation strategies (Martin et al. 2007). Many studies

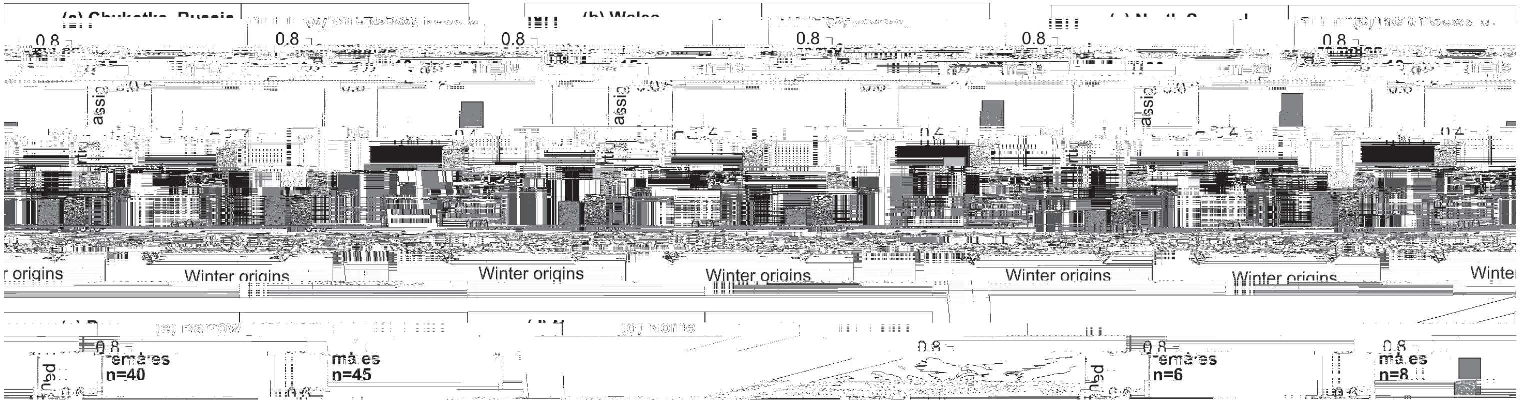

Figure 1. Map of all sites where feather samples were collected from western sandpipers (■ = winter, ▲ = migration, ● = breeding). Circled sites indicate regional groups (Supplementary material Appendix 1, Table A1). Inset graphs show the proportion of females and males from six breeding areas (a…f) and two migration sites (g, h) assigned to each winter region. An individual was assigned to the region with the greatest number of assignments out of 10 000 simulations. In each simulation, an individual was assigned to the region with the highest probability of origin. Inset graph (i) shows the expected distribution of females and males from each wintering region based on patterns of relative winter abundance (the null hypothesis). Because very few breeding and migrant birds originated from the Caribbean, we grouped these assignments with those from eastern North America. WNA = western North America, SB = Southern Baja, CB = Central Baja, GC = Gulf of California, SA = South America, ENA = eastern North America, CARIB = Caribbean.

furnace (Finnigan TC/EA) at 1450°C and introduced on-line to an isotope ratio mass spectrometer (Delta^{Plus} XP). Samples for $\delta^{13}C$ and $\delta^{15}N$ analysis (0.2…0.4 mg) were loaded into tin capsules, crushed, converted to gas in an oxidation/reduction furnace (Costech ECS 4010 elemental analyzer), and introduced on-line to an isotope ratio mass spectrometer (Delta^{Plus} XP). Isotope analyses were conducted between October and December 2007, between October 2009 and January 2010, in August 2010, and in February 2011. Stable isotope ratios are reported in delta (δ) notation in per mil (‰) units, where $\delta X = ((R_{sample}/R_{standard}) - 1) \times 1000$. For hydrogen ($\delta D$), $R = {}^2H/{}^1H$ and $R_{standard}$ is Vienna standard mean ocean water; for carbon ($\delta^{13}C$), $R = {}^{13}C/$

used linear mixed effects models to examine the relationship of each stable isotope (δD, $\delta^{13}C$, and $\delta^{15}N$) with latitude and with longitude in wintering adult sandpiper feathers. We included site as a random effect to account for isotopic differences between sampling sites, and centered latitude and longitude around their respective means to reduce collinearity between linear and non-linear terms within models. We used an information-theoretic approach to evaluate the relative support for a null (intercept only), linear, or non-linear (quadratic and/or exponential) relationship of each isotope with latitude and longitude (Burnham and Anderson 2002, Table 1 for candidate model set). Non-linear relationships were included in the candidate model set after graphically visualizing the data. Site was included as a random intercept in all models. Models were ranked according to their ΔAIC_c score, calculated as the difference between a model's AIC_c value and that of the best-supported model in the candidate set. The support for each model given by the data was evaluated using Akaike weight ($wAIC_c$), which represents the probability of the model given the data in relation to all other models in the candidate set. Parameter likelihoods and weighted parameter estimates for each explanatory variable were calculated to assess an individual variable's relative importance within the candidate model set (Supplementary material Appendix 1, Table A3). All analyses were conducted in R ver. 2.14.0 (R Development Core Team). We used the package nlme for linear mixed effects models (Pinheiro et al. 2011). We specified no within-group correlations and used the maximum likelihood method to compare different fixed effect model structures. The package AICcmodavg was used to calculate AIC_c values, produce model selection results and calculate model averaged predicted response values (Mazerolle 2011).

Delineating winter regions

We divided the wintering grounds into seven regions (Fig. 1, Supplementary material Appendix 1, Table A1) based on prior knowledge of western sandpiper winter distribution patterns and what we believe to be geographically and biologically relevant regions, as well as the degree to which certain areas of the wintering range were isotopically distinct (e.g. Southern Baja, Central Baja). Assignments of known-origin birds to individual sampling sites was not possible using a maximum likelihood approach because some sites had samples sizes that were too small ($n < 4$). However, for individual sampling sites where sample size was large enough, we used a preliminary assignment test to examine how often known-origin wintering individuals were assigned to another sampling site in the same geographic area. We then grouped sites into regions where this was a frequent occurrence. Grouping sites regionally by pooling values across several sites increased sample size without substantially increasing variance (Supplementary material Appendix 1, Table A2).

Probability assignment tests

We used a maximum likelihood assignment approach to assign individuals to their most probable region of winter origin (Royle and Rubenstein 2004). To determine the likelihood that an individual originated from any one region based on its feather isotope values, we used a multivariate (δD, $\delta^{13}C$, $\delta^{15}N$) normal probability density function.

Table 1. The candidate linear mixed effects models used in the evaluation of the relationship between each stable isotope (δD, $\delta^{13}C$ and $\delta^{15}N$) and latitude and longitude of wintering adult western sandpiper feathers. All models include a random intercept of site. Sampled feathers are from 17 sites during the winter of 2008–2009. The number of parameters (K) includes a parameter for the intercept, the random effect of site, and the residual variance. Deviance is equal to $-2\times$ log-likelihood and was used to calculate AIC_c (Akaike's information criterion corrected for small sample size). Competing models were ranked according to ΔAIC_c and Akaike weight. Sample size $n = 109$ for all models.

Response	Model	K	Deviance	AIC_c	ΔAIC_c	AIC_c w
δD	latitude + latitude^2	5	904.4	915.0	0.0	0.57
	latitude	4	908.0	916.4	1.4	0.28
	NULL	3	911.4	917.6	2.7	0.15
$\delta^{13}C$	NULL	3	520.8	527.0	0.0	0.67
	latitude	4	520.6	529.0	2.0	0.25
	latitude + latitude^2	5	520.6	531.2	4.2	0.08
$\delta^{15}N$	latitude + latitude^2	5	508.9	519.5	0.0	0.89
	latitude	3	518.3	524.5	5.0	0.07
	NULL	4	517.3	525.6	6.1	0.04
δD	longitude	4	906.3	914.7	0.0	0.64
	longitude + longitude^2	5	906.3	916.9	2.2	0.21
	NULL	3	911.4	917.6	3.0	0.14
$\delta^{13}C$	longitude	4	516.3	524.7	0.0	0.59
	longitude + longitude^2	5	516.0	526.6	1.9	0.23
	NULL	3	520.8	527.0	2.4	0.18
$\delta^{15}N$	$e^{-\text{longitude}/5}$	4	508.1	516.5	0.0	0.61
	$e^{-\text{longitude}/10}$	4	509.5	517.9	1.4	0.30
	longitude + longitude^2	5	511.7	522.3	5.8	0.03
	$e^{-\text{longitude}}$	4	514.7	523.1	6.5	0.02
	longitude	4	515.1	523.5	7.0	0.02
	NULL	3	518.3	524.5	8.0	0.01

Assignment tests were conducted in R using the mvnmle and mvtnorm packages (Genz et al. 2011, R Development Core Team). To account for analytical error in isotope measurements, we used a resampling simulation approach for the cross-validation of known-origin individuals and assignment of unknown-origin individuals (Wunder and Norris 2008, Miller et al. 2011). For each stable isotope observation per individual, we first randomly sampled 100 values from a normal distribution with a mean equal to the isotope value for that feather, and a standard deviation equal to the mean standard deviation of the lab standards for that isotope. This resampling procedure produced 100 new datasets of isotope values for all individuals. Secondly, we randomly chose one of these 100 datasets to define the parameters (mean, variance-covariance) of the multivariate probability density functions for the regions of winter origin. For each individual in every dataset, we determined its probability of being assigned to each region of winter origin, producing 100 assignment outcomes for each individual. Thirdly, we repeated step two a total of 100 times, each time using a new dataset from step one to define the parameters of the regional probability density functions. This produced 10 000 (100 × 100) assignment outcomes for each individual (Supplementary material Appendix 3, Table A5–7). Finally, we considered an individual to have originated from the region with the greatest number of assignments out of 10 000 simulations. We assessed confidence in the assignment by the number of times an individual was assigned to the region out of the total number of simulations.

To cross-validate our isotopic basemap of the wintering grounds, we evaluated the ability of the isotope data to correctly assign known-origin individuals back to their region of origin using the resampling simulation approach described above, but with an exclusion criterion. Specifically, if the dataset being assigned in step two was the same as the dataset chosen to define the regional probability density functions, we re-calculated the parameters (mean, variance-covariance) of the probability density functions with the individual to be assigned omitted from the dataset. We then determined that individual's likelihood of coming from each of the candidate regions. We repeated this probability assignment for the remaining 99 datasets, but without using an exclusion criterion; in these remaining 99 cases, the dataset being assigned was different than the dataset chosen to define the regional probability density functions.

Age class identification

Previous work suggests that some individuals identified as adult by plumage characteristics have feathers with Arctic-type δD isotope values, indicating that these individuals may in fact be young birds (Franks et al. 2009). Because we were interested in the winter origins of adult birds, we needed to identify 'true' adults and remove young birds from all datasets, which we did using probability assignment of individuals to two possible regions of origin, Arctic or non-Arctic (Supplementary material Appendix 2). After removing juveniles and outliers from the datasets, 109, 243, and 137 'true' adults remained in the winter, breeding, and migration datasets, respectively.

Estimating migratory connectivity of breeding and migrant birds to wintering regions

Estimating the most likely origin of an individual based solely on stable isotope values assumes an equal prior probability of origin among all regions, which is unlikely since population density varies across space. To determine the most probable region of winter origin for breeding and migrant birds, we used a Bayesian approach that considers the likelihood of an individual's origin based on isotope data as well as prior previous year. Low trophic level brine shrimp comprise a large proportion of available food resources in salt ponds (Franks unpubl.), while intertidal mudflats may encompass a wider spectrum of resources. This may potentially explain the lower ^{15}N values observed at Guerrero Negro in 2008…2009. Because of the difference in the between-year sampling locations at Guerrero Negro and the fact that individuals show relatively high fidelity to specific wintering sites (Fernández et al. 2004), the comparison of interannual variation at Guerrero Negro may not be valid. Since D, ^{13}C and ^{15}N values were not significantly different between years at any other site, all years of data were pooled.

Geographic patterns in stable isotope values on the wintering grounds

We found some support for a non-linear relationship between D and latitude and a positive linear relationship between D and longitude (Table 1, Fig. 2a, b). We found little evidence that ^{13}C varied with latitude, as the null model received the most support (Table 1, Fig. 2c). However, we did find some evidence that ^{13}C decreased linearly from west to east (Table 1, Fig. 2d). The data provided strong relative support for a quadratic relationship of ^{15}N with latitude and some evidence of a negative exponential relationship of ^{15}N with longitude (Table 1, Fig. 2e, f).

Cross-validation of an isotopic basemap of the wintering grounds

The wintering range was divided into seven regions: western North America, Central Baja, Southern Baja, the Gulf of California, eastern North America, the Caribbean, and South America. Sixty percent (65/109) of winter individuals were correctly assigned back to their region of origin. Of these, 82% (53/65) were assigned with greater than 70% confidence, while 97% (63/65) were assigned with greater than 50% confidence. The highest rates of correct assignment occurred on the Baja Peninsula (Fig. 3a, b), followed by western North America (Fig. 3c), South America (Fig. 3d), and eastern North America (Fig. 3e); only 39% of birds from the Gulf of California region of Mexico were assigned back to their site of origin (Fig. 3f), while just under 50% of birds from the Caribbean were assigned back to their site of origin (Fig. 3g).

Estimating migratory connectivity of breeding and migrant birds to wintering regions

Confidence in assignment of breeding birds to winter regions was

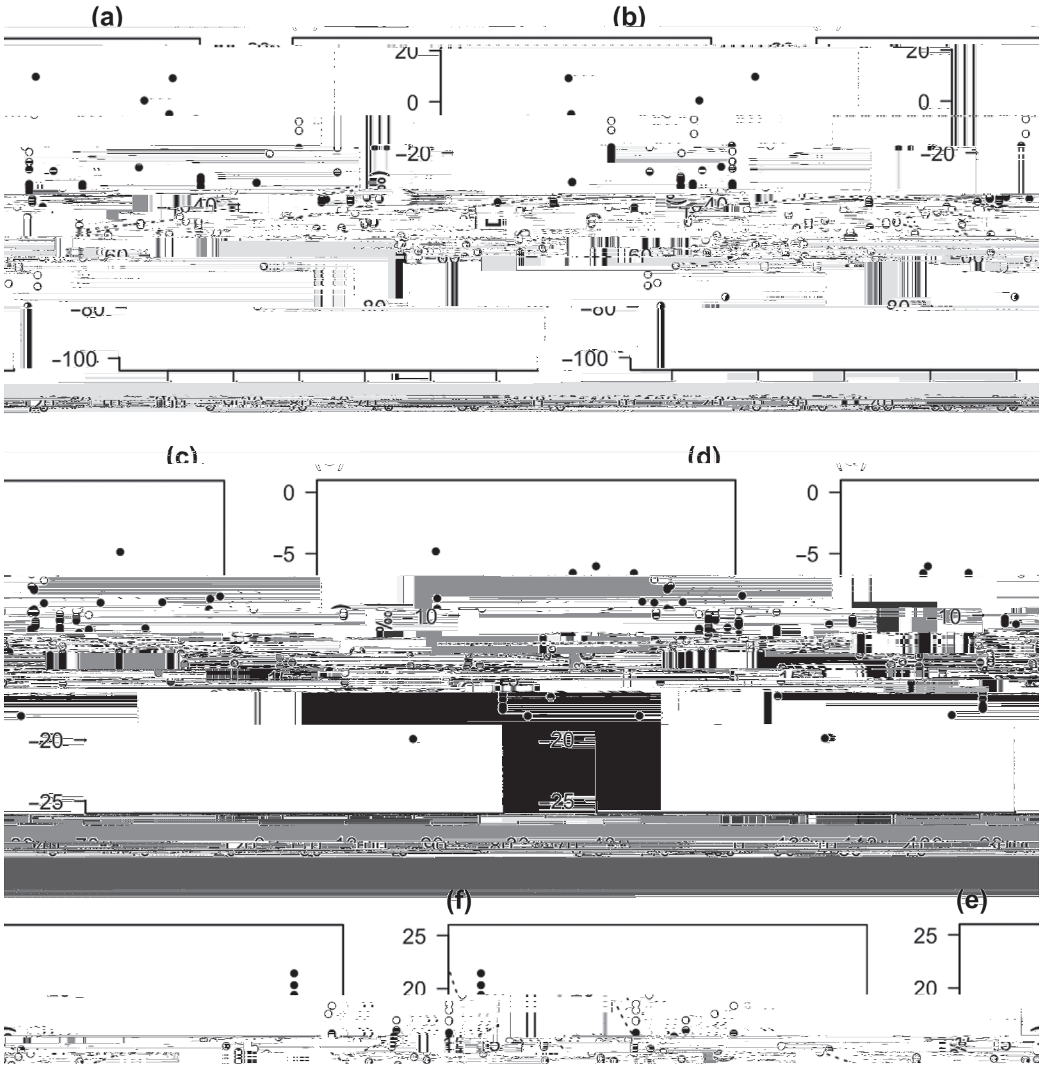

Figure 2. The relationship of each stable isotope (δD, δ^{13}C and δ^{15}N) with latitude (a, c, e) and longitude (b, d, f) for western sandpiper feathers sampled on the wintering grounds. Data are adults only ($n = 109$). Dashed lines represent the model-averaged predicted response from the candidate set of linear mixed effects models (Table 1).

that was marginally significantly different from that expected based on patterns of relative abundance. At this site, relatively more birds originated from the Gulf of California than expected (Table 2). The distribution of female winter origins did not differ from expected at any breeding site. The distribution of male winter origins in Nome differed from that expected, with relatively fewer birds originating from western North America and more from Central Baja than expected (Table 2, Fig. 1d). In the Y-K Delta, males originated overwhelmingly from the Gulf of California region of Mexico, with relatively few from other locations on the wintering grounds (Table 2, Fig. 1f).

Confidence in assignment of migrant birds to winter regions was > 70% for 79% (108/137) of individuals and was > 50% for 99% (136/137) of individuals. The distribution of migrants in Kansas differed significantly from that expected, with relatively more individuals originating from eastern and southern wintering sites, as well as the Baja Peninsula (Table 2, Fig. 1g). No individuals in Kansas originated from western North America. The distribution of male migrant origins in the Fraser Delta also differed from that expected, with fewer birds originating from western and eastern North America and relatively more originating from western Mexico (Table 2, Fig. 1h).

Figure 3. e proportion of winter adults assigned to each region from the cross-validation resampling simulation. An individual was assigned to the region with the greatest number of assignments out of 10 000 simulations. In each simulation, an individual was assigned to the region with the highest probability of origin. (a) SB = Southern Baja; (b) CB = Central Baja; (c) WNA = western North America; (d) SA = South America; (e) ENA = eastern North America; (f) GC = Gulf of California; (g) CARIB = Caribbean.

Table 2. Fisher's exact tests of the observed distribution of winter origins at each site compared to the expected distribution of winter origins based on patterns of relative abundance for pooled sexes, females, and males. Expected frequencies for pooled sexes were weighted by the sex ratio of the sample (i.e. we calculated an average expected frequency from the expected frequencies of males and females and their respective proportion at each site). Exact p values in bold with asterisks indicate sites where the observed distribution of winter origins is significantly different at a level of < 0.05 from that expected based on patterns of winter relative abundance, while p values in bold indicate marginally significant differences. Dashes (–) indicate where sample sizes were too small to conduct a ^{2} goodness of fit test.

	Pooled sexes			Females			Males		
	^{2}	exact p	n	^{2}	exact p	n	^{2}	exact p	n
Barrow	2.47	0.715	14	Š	Š	6	Š	Š	8
Nome	7.43	0.189	89	0.91	0.948	42	15.88	**0.017***	44
North Seward	5.37	0.365	34	3.91	0.432	14	4.59	0.416	20
Russia	1.43	0.887	38	1.37	0.895	18	5.56	0.311	17
Wales	3.14	0.616	26	3.93	0.428	10	4.94	0.355	15
YK Delta	10.74	0.060	42	5.67	0.258	19	16.26	**0.014***	23
Fraser Delta	9.14	0.101	107	6.08	0.271	37	18.72	**0.008***	68
Kansas	13.55	**0.027***	30	9.33	0.088	14	9.95	0.113	14

is is contribution number 5 from the Arctic Shorebird Demographics Network.

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Supplementary material (Appendix J5573 at <www.oikoso ce.lu.se/appendix>). Appendix 1…3.