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TRACKING DISPERSAL IN BIRDS: ASSESSING THE POTENTIAL OF ELEMENTAL MARKERS

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Abstract.—Natal dispersal in vagile species such as songbirds can shape a population's range and structure. Although effective conservation practices depend on knowledge of the scale and frequency of natal dispersal, these issues remain poorly understood because of methodological gaps. In this exploratory study, we assessed whether element signatures within natal feathers might be used to identify the geographic birth site of first-year breeders. We used two related techniques, inductively coupled plasma mass spectrometry (ICP-MS) and inductively coupled plasma with optical emission spectrometry (ICP-AES), to quantify element levels in natal feather samples from 7 species at 27 sites across the eastern United States. The techniques differed in the manner in which elements were quantified and in their detection limits. Our goal was to determine whether element analyses of feathers could discriminate (1) different species within a site and (2) different sites within a species. Additionally, because spatial autocorrelation of element levels is needed for element analysis to be an effective tool in assessing natal dispersal, we also evaluated the spatial autocorrelation of ICP-AES samples at 18 sites across the eastern United States.

Both ICP-MS and ICP-AES analyses separated species within a site with fairly high accuracy, though the discriminating elements varied with site. However, within a species, natal feather locations were not identified with high accuracy on the basis of feather elements. We were not able to determine whether there is spatial correlation among individual elements or a principal component analysis (PCA) score that described the elemental makeup of a feather. A kriging model was fit to the semivariogram of PCA scores to produce a base-map of element signatures across the eastern United States. This map was ineffective at predicting feather-element values at sample sites. Whether elemental analyses can identify natal dispersal distances requires further study. We suggest that future studies evaluate elements with ICP-MS methodologies on a single, box-nesting species that is sampled more intensively at smaller geographic scale, or on species that occur in very discrete populations. Additionally, this methodology should be evaluated in concert with stable-isotope analyses of feathers and, potentially, genetic analyses. *Received 7 June 2004, accepted 16 August 2005.*

Key words: discriminant function, dispersal, Eastern Bluebird, element analysis, *Sialia sialis*, spatial autocorrelation, *Tachycineta bicolor,* Tree Swallow.

Suivre la Dispersion chez les Oiseaux: Évaluer le Potentiel de Marqueurs Élémentaires

Résumé.—La dispersion natale chez les espèces vagiles comme les passereaux peut modeler la portée et la structure d'une population. Bien que les pratiques de conservation efficaces dépendent des connaissances acquises sur l'échelle et la

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fréquence de la dispersion natale, ces questions demeurent peu comprises en raison de lacunes méthodologiques. Dans cette étude exploratoire, nous avons cherché à savoir si la signature des éléments contenus dans les plumes natales pourrait être utilisée afin d'identifier le site géographique de naissance des reproducteurs de première année. Nous avons utilisé deux techniques semblables, l'ICP-MS (inductively coupled plasma mass spectrometry) et l'ICP-AES (inductively coupled plasma with optical emission spectrometry), pour quantifier les niveaux d'éléments dans les plumes natales issues de 7 espèces dans 27 sites à travers l'est des États-Unis. Les techniques différaient dans la manière avec laquelle les éléments étaient quantifiés et leurs limites de détection. Notre but était de déterminer si les éléments analysés dans les plumes pouvaient discriminer (1) différentes espèces à l'intérieur d'un même site (2) différents sites à l'intérieur d'une même espèce. L'autocorrélation spatiale des niveaux d'éléments est nécessaire à leur analyse, et ce dans le but de constituer un outil efficace pour l'évaluation de la dispersion natale. Par conséquent, nous avons également évalué l'autocorrélation spatiale d'échantillons ICP-AES provenant de 18 sites à travers l'est des États-Unis.

Les analyses ICP-MS et ICP-AES ont séparé les espèces à l'intérieur d'un même site avec une relativement bonne précision, bien que les éléments discriminants ont varié avec les sites. Néanmoins, pour une même espèce, la localisation des plumes natales n'a pas été identifiée avec une grande précision à partir des caractéristiques des éléments. Nous n'avons pas été capables de déterminer s'il y avait une corrélation spatiale parmi les éléments individuels ou un score d'analyse en composante principale (ACP) qui décrivaient l'assemblage des éléments dans une plume. Un modèle «kriging» a été ajusté au semi-variogramme des scores d'APC afin de produire une carte de base des signatures d'éléments à travers l'est des États-Unis. Cette carte s'est avérée inefficace pour prédire les valeurs des éléments contenus dans les plumes aux sites d'échantillonnage. D'autres études seront nécessaires pour déterminer si les analyses d'éléments permettent d'identifier les distances de dispersion natale. Nous suggérons que des études futures puissent évaluer les éléments avec la méthodologie ICP-MS pour une seule espèce qui utilise des nichoirs et qui pourrait être échantillonnée plus intensivement et à plus petite échelle, ou encore sur des espèces appartenant à des populations très distinctes. De plus, cette méthode devrait être évaluée en concert avec des analyses isotopiques stables des plumes et, potentiellement, des analyses génétiques.

Large-scale spatial movements—such as seasonal migration, natal dispersal, and breeding dispersal—are major components of the life history of many songbirds. These movements determine key aspects of songbird ecology and conservation, including links between breeding and wintering populations and the spatial scale at which breeding populations form demographically distinct management units (Webster et al. 2002). Documenting dispersal or migration patterns of individual songbirds requires tracking the movement patterns of individuals for extended periods over potentially large distances. Traditional tracking techniques, such as banding and radiotelemetry, have failed to yield substantial information on large-scale dispersal movements. Return rates of banded birds are often low, and radiotelemetry methods may not capture movements across vast areas. Because of these substantial methodological gaps, knowledge of the dispersal and migration patterns of many songbird species is extremely limited (Kelly and Finch 1998, Webster et al. 2002, Smith et al. 2003).

To fill this gap, techniques such as stable-isotope analysis (Chamberlain et al. 1997, Hobson and Wassenaar 1997), satellite telemetry (Martell et al. 2001), and DNA-based genetic "tags" (Wenink and Baker 1996, Waser and Strobeck 1998, Davies et al. 1999) have been proposed as solutions. Here, we explore the use of trace-element analyses in feathers as a method for tracking large-scale movement patterns of songbirds. Our focus is on whether trace elements can reveal information about natal dispersal distances, or the distance between a

bird's birth site and the site of its first breeding attempt (Greenwood and Harvey 1982). Such chemical profiling has been the subject of many dispersal investigations, especially in the 1970s and 1980s (Devine and Peterle 1968, Kelsall and Burton 1977, Bortolotti et al. 1989). However, technologies to quantify trace elements within feathers have rapidly advanced in the past decade, and these new technologies may provide better resolution than older methods.

The conceptual basis for hypothesizing that trace-element analyses will "source" the birthplaces of individual birds is straightforward. Elements are known to vary spatially in the environment (see the website of the National Atmospheric Deposition Program; see Acknowledgments). As young songbirds acquire new feathers in the nest, they ingest elements from food and water consumed at the time of feather growth (Mizutani et al. 1990, 1992; Hobson and Clark 1992). Hence, element levels in feathers may reflect element levels in the environment. If one or more elements vary in a predictable manner across the range of a species, and if individuals ingest different levels of given elements, a "base-map" depicting the "element signature" of locally grown feathers can potentially be developed. This base-map may consist of several elements combined (through a multivariate analysis such as principal component analysis) or may consist of several "layers," one for each element, that can be overlaid in a geographic information system (GIS).

In principle, this base-map can be used to identify natal dispersal events. When nestlings fledge and disperse to wintering areas as juveniles, and then disperse to their first breeding site the following spring, they retain the remiges, rectrices, and primary covert feathers acquired at their birthplace (Pyle et al. 1987). That is, certain feathers on first-year adults still contain the trace-element signature of their birth site. Elemental analysis of natal feathers collected from a first-year breeding adult would, theoretically, allow researchers to identify geographic areas on the element base-map or base-maps that match the sample in question, thus identifing all possible birth sites for that individual. Conceivably, the same approach could be used to identify the breeding sites of migrant individuals sampled in areas of the tropics where they overwinter (Webster et al. 2002). Additionally, depending on the molt location of adults (Szép et al. 2003), the technique may reveal breeding dispersal distances (or sites of different breeding attempts across years).

Several essential conditions must be met for element signatures to be useful in tracking natal dispersal. First, assuming that individuals within a site can be described by an element signature (the levels of various elements in down feathers), it must be determined whether element levels vary among species within a site. If signatures differ among species within a site, then elemental base-maps must be species-specific. Second, there must be systematic geographic variation in nestling element signatures, such that natal feather samples obtained from individuals from known sites should be correctly sourced back to the natal site on the basis of element composition alone. Third, there must be some level of spatial autocorrelation of element levels in feathers; samples collected in proximity to other samples should have more similar element levels than more distant samples. This condition is essential for tracking dispersal events, because it allows spatial interpolation of element levels across a large geographic range. If there is no underlying geographic pattern of feather elements collected in various sites, the method will not be useful in tracking natal dispersal unless all potential dispersal sites are sampled and analyzed, which would be prohibitive for wide-ranging species.

The present exploratory study examines all these issues. We collected natal feathers from seven songbird species throughout eastern North America to determine (1) variation in signatures among species within a site, and the error rates in classifying natal feathers to their correct species; (2) variation in natal feather elements among different geographic sites, and the error rates in classifying natal feathers to their correct site; and (3) spatial autocorrelation of various natal feather elements across eastern North America. The first two objectives assessed element levels in natal feathers with two different methodologies, whereas the third objective used a single methodology.

METHODS

Feather collection.—Four to five down feathers were collected from hatchlings of seven species in 27 sites in 1996–1998 (Fig. 1). The species analyzed included Tree Swallow (*Tachycineta*

FIG. 1. Map of study locations in which down feathers were collected from nestlings of various species and analyzed using either ICP-MS (squares) or ICP-AES (diamonds) technique. Circles indicate locations where both techniques were evaluated.

bicolor), Eastern Bluebird (*Sialia sialis*), Wood Thrush (*Hylocichla mustelina*), Prairie Warbler (*Dendroica discolor*), Pine Warbler (*Dendroica pinus*), Ovenbird (*Seiurus aurocapilla*), and Eastern Towhee (*Pipilo erythrophthalmus*). A site was defined generically as a point on a map (see Fig. 1); all feather samples within a given site were collected within ~10 km of this point. Sampling sites were generally separated by ≥50 km, with the exception of three sites: IL, ILL, and ILLI were separated by 10 km and analyzed separately, because the feather collections were clustered within very small geographic areas (<1 km radius). A sample consisted of down feathers collected from a single individual within a single nest; thus, each sample came from a distinct geographic position within a sampling site. The number of species and number of individuals within a species varied widely among sites. Therefore, sample sizes varied depending on the research objective (see below). All collections were done in accordance with U.S. Fish and Wildlife Service scientific collecting protocols, and collected feathers were stored in sealed plastic bags until they were analyzed in the lab.

Element analysis.—Essential to trace-element analysis is the ability to rapidly and accurately measure levels of trace elements in feathers. We used two methods in our assessments (Montaster 1997): inductively coupled plasma mass spectrometry (ICP-MS) and inductively coupled plasma with optical emission spectrometry (ICP-AES). Both techniques disperse a liquid sample into a stream of hot plasma, which vaporizes the samples. The techniques differ, however, in the manner in which elements are quantified and in their detection limits.

For ICP-MS, samples are vaporized and products are then carried to the ICP unit. The high thermal energy and electron-rich environment of the ICP unit converts the atoms into ions. The

ions are then separated according to mass with a quadrupole analyzer and detected, multiplied, and counted on the basis of their mass:charge ratio. The detection limit for most elements is in the sub-parts per billion (ppb) range. Thus, ICP-MS is a precise technique that produces element composition of feather samples at a very fine level of resolution. The ICP-MS analyses were performed in the Geology Department at McMaster University, Hamilton, Ontario, on a Perkin Elmer (PE; Wellesley, Massachusetts) Sciex Elan 250 ICP-MS equipped with a PE AS90 autosampler.

A multi-element analysis technique, ICP-AES dissociates a sample into its constituent atoms and ions and causes them to emit light at a characteristic wavelength by exciting them to a higher energy level. Light from the different elements is separated (grated) into different wavelengths and is captured by light-sensitive detectors, one for each element being analyzed. This information is used to calculate the concentration of that particular element in the sample. In terms of sensitivity, ICP-AES detection limits are typically at the microgram-per-liter level in aqueous solutions, and thus ICP-AES is less sensitive than ICP-MS. The ICP-AES analyses were performed on a PE Optima DV in the Plant and Soils Department at the University of Vermont.

For the ICP-MS analysis, feather samples from 120 individuals from 10 sites (Fig. 1) were washed in distilled water and then oven-dried. Approximately 2 mg of down feathers from a single individual, weighed to the nearest 0.01 mg, were placed into a 15-mL polyethylene test tube. One milliliter of concentrated, purified nitric acid was added, and tubes were immersed in boiling water for 6 min. After cooling, distilled deionized water was added to the 10-mL mark. Samples were loaded into the ICP-MS and were analyzed with the PE TotalQuant II analytical method, which is used for the rapid analysis of unknown solutions. We used standard solutions to calibrate the instrument and to improve analytical accuracy of elements in the standard solution mix. Other, nonstandardized elements were estimated on the basis of average instrumental response and, therefore, were considered semiquantitative. Concentrations of these other elements were determined by calibrating the response factors of adjacent elements present in the standard solutions. In total, 62 elements were analyzed by ICP-MS methods: Ag*, Al*, As*, Au, Ba*, Be, Bi, Ca, Cd*, Ce*, Co, Cr*, Cs, Cu*, Dy, Er, Eu, Fe*, Ga, Gd, Ge, Hf, Hg*, Ho, In, Ir*, La, Li, Lu, Mg*, Mn*, Mo*, Na*, Nb, Nd*, Ni*, Os*, Pb*, Pr, Pt, Rb*, Re, Rh, Sb*, Sc, Si*, Sm, Sn*, Sr, Ta, Tb, Te, Th*, Ti*, Tl, U, V*, W*,Y, Yb*, Zn*, and Zr (elements with asterisks showed enough sample variation to be retained for analysis; see below).

For the ICP-AES analysis, feather samples from 148 individuals from 18 sites (Fig. 1 and Table 1) were washed in distilled water and oven-dried. With the exception of a single site (WI-CNF), these sites were different from the sites in the ICP-MS analysis, and focused on two box-nesting species, Eastern Bluebird and Tree Swallow. These species were selected because an extensive nest-box system exists throughout North America, and volunteers from different sites assisted in sample collection, thereby maximizing the number of spatial sites for geographic comparisons. The same protocol for digesting feathers in the ICP-MS analysis was used for the ICP-AES analysis. A blank (quality control, or QC) was prepared in the same manner after every 11 unknowns. Samples were analyzed in the axial mode, which allowed for greater analytical sensitivity. Standards and QC samples were prepared from NIST (National Institute of Standards and Technology) traceable solutions. In total, 27 elements were analyzed: Al*, As, B, Ca*, Cd, Co, Cr, Cu, Fe*, K*, Mg*, Mn, Mo, Na*, Ni, P*, Pb, S*, Sb, Se, Sm, Sr, V, W, Y, Zn*, and Zr (elements with asterisks showed enough sample variation to be retained for analysis).

Data reduction.—We investigated only those sites in which element signatures from at least four different individuals from different nests of the same species were sampled. As a first approach for reducing the number of potential ICP-MS elements analyzed, we used a discriminant-function procedure (PROC DISCRIM, SAS; SAS Institute, Chicago, Illinois) to identify those elements that best discriminated among sites. This analysis was done separately for each species in which samples were collected at two or more sites. Of the 62 potential ICP-MS elements, we dropped any element in which the univariate *F*-statistic from the disciminant-function output suggested that the element was not a good discriminator of sites for any of the species analyzed ($P > 0.05$). This procedure reduced the number of

TABLE 1. Results of discriminant-function analysis to separate species within a study site (see Fig. 1 for site locations). Sample size (*n*) is the number of individuals sampled per site per species. Entry order gives the order of variable entry in the stepwise discriminant-function analysis.

Technique	Site	Species	*n*	Entry order	Element	*F*	Error rate
ICP-MS	WI-CNF	Eastern Bluebird	13	1	Cr	9.0	0.23
		Tree Swallow	5	2	Cd	9.9	0
ICP-MS	SC-SRS	Eastern Bluebird	4	1	Fe	127.6	0
		Pine Warbler	26	2	Sn	22.0	0
		Prairie Warbler	8				0.75
		Eastern Towhee	6				0.75
ICP-MS	VT-GMNF	Ovenbird	4	1	Na	518.5	0
		Tree Swallow	4	2	Mg	4.7	0
ICP-AES	IA	Eastern Bluebird	5	1	S	34.6	0
		Tree Swallow	5	2	Na	14.6	0
	IL	Eastern Bluebird	5	1	Mg	9.9	0
		Tree Swallow	5	2	Ca	15.0	0
	MN	Eastern Bluebird	5	1	Mg	12.2	0
		Tree Swallow	5	2	P	9.5	0
	NEB	Eastern Bluebird	5	1	Mg	30.0	0
		Tree Swallow	5	2	Na	13.2	0
	WI-CNF	Eastern Bluebird	6	1	P	5.2	0
		Tree Swallow	5	2	Mg	14.5	0
	OH	Eastern Bluebird	5	1	Na	23.7	0
		Tree Swallow	3	2	Mg	20.3	0

candidate elements from 62 to 29 elements (previously identified with an asterisk) for element signature analysis.

For the AES data, means and standard deviations (SD) of element levels were compared with the QC mean and SD levels. We dropped those elements in which the mean of the QC was greater than the mean of the observed samples. (QC data were blanks that were analyzed with the feather samples; yet in some cases, some measure of elements was detected.) We also dropped elements where variability in the QC data was comparable with that in the sample data. Such data were interpreted to be noise resulting from machinery error, rather than true variation across sites. For AES, this reduced the number of element signatures from 27 to 9: Al, Ca, Fe, K, Mg, Na, P, S, and Zn.

Statistical analysis: Element signatures between species within a site.—We compared the element signatures of feather samples collected from various species within a site to determine whether signatures are species-specific. For the ICP-MS, three sites were analyzed and species within those sites were compared: WI-CNF (13 Eastern Bluebirds vs. 5 Tree Swallows), SC-SRS (4 Eastern Bluebirds vs. 26 Pine Warblers vs. 8 Prairie Warblers vs. 6 Eastern Towhees), and VT-GMNF (4 Ovenbirds vs. 4 Tree Swallows). For the ICP-AES, we compared signatures of Eastern Bluebirds and Tree Swallows within six sites: WI-CNF, IA, IL, MN, NEB, and OH.

We constructed a linear discriminant function to determine whether species within a site could be identified solely on the basis of their feather element composition. We limited the number of predictor variables to two elements to avoid overfitting and used a stepwise procedure to choose the two-element discriminant function. The proportion of correct classifications for species within a site (classification hit-rate) was estimated using the leave-one-out method, also referred to as either "jack-knife" or "cross-validation" (Huberty 1994). Equal prior probabilities for assigning feathers to species were used in estimating hit-rates.

Statistical analysis: Element signatures within species among sites.—For objective 2 (variation in natal feather elements among different geographic sites), we compared the element signatures within a species across different geographic sites to determine whether individuals

of a particular species could be classified to their birth site. We used a stepwise discriminant-function procedure (in SAS, version 8.2) to identify the best-discriminating elements, and the leave-one-out method with equal priors to assess error rates as described for objective 1 (variation in signatures among species within a site). We limited the number of predictor variables to two elements to avoid overfitting the discriminant model. Analyses were conducted for Eastern Bluebird (2 sites for ICP-MS and 18 sites for AES analyses), Ovenbird (5 sites; ICP-MS samples only), Tree Swallow (2 sites for ICP-MS and 6 sites for AES analyses), and Wood Thrush (3 sites; ICP-MS samples only).

Statistical analysis: Spatial autocorrelation.—We investigated spatial autocorrelation in ICP-AES Eastern Bluebird samples only. Analyses were limited to Eastern Bluebird because it was the only species for which we had sufficient samples at several sites across a large spatial gradient (n = 18 sites). Sampling sites were mapped onto an ARCGIS layer (ESRI, Redlands, California) (Fig. 1), and mean element levels were computed for Eastern Bluebird in each site. We also ran a principal component analysis (PROC PRINCOMP, SAS) on the mean element values within a site to generate a single PCA score for each site. The score comprises a linear combination of elements.

Spatial correlation is necessary if element analyses are to be useful in tracking natal dispersal, because it allows natal feathers collected from a first-year adult to be sourced back to the location where the feathers were grown; these locations can be inferred even if the sites were unsampled. A semivariogram is a measure of spatial correlation (i.e. whether data points close in space are more similar in value than data points that are distant from each other). Spatial autocorrelations for the first PCA score and for individual elements were examined by generating an empirical semivariogram for the 18 sites (18 sites results in 153 pairs of points; PROC VARIOGRAM, SAS).

We grouped points into one of seven bins, where points within a bin had similar distances among pairs. We selected a bin size of 166 km, indicating that points within a bin were within 166 km of other points. The bin size and bin number were selected in an attempt to include enough points in each bin to get a reasonably precise estimate of spatial correlation while following the general rule that the bin size multiplied by the number of bins is approximately half the largest distance among all sampling sites (ARCGIS; SAS helpfiles). We then computed the average of the squared differences in element values and the PCA scores among points within each bin. Semivariances (0.5× the variance) of paired points within each bin were plotted as a function of distance (i.e. empirical semivariogram). Empirical semivariograms were inspected to assess the level of spatial autocorrelation.

For the first PCA score only, a spherical model was fitted to the semivariogram data. The spherical model assumes a progressive decrease of spatial autocorrelation until some distance, beyond which autocorrelation is assumed to be 0. Ordinary kriging was used with the spherical model to obtain a map (the elemental base-map) of predicted PCA scores.

To assess the kriging model's prediction ability, we used the cross-validation procedure in ARCGIS. This procedure omits a data point, fits a kriging model, and then uses the new model to predict the value of the omitted point. This type of cross-validation procedure was selected because of the small number of sites evaluated (n = 18). We evaluated the sensitivity of our results to model assumptions by altering the bin number, bin size, and model structure (e.g. anisotropy) to determine whether errors could be decreased.

RESULTS

Element signatures between species within a site.—In all analyses, ICP-MS provided sufficient resolution to separate species within a site on the basis of one or two most-discriminating elements (Table 1). Assignment errors occurred between Eastern Towhee and Prairie Warbler (6 of 8 Prairie Warblers classified as Eastern Towhees, and 6 of 6 Eastern Towhees classified as Prairie Warblers) in SC-SRS site. The ICP-AES was able to separate Eastern Bluebirds from Tree Swallows in all sites evaluated. These results suggest that, in general, natal-elemental base-maps should be species-specific to be of use in tracking natal dispersal.

Element signatures within species between sites: ICP-MS results.—For Eastern Bluebird, two sites had sufficient samples to be analyzed: SC-SRS and WI-CNF. All feather samples were correctly placed into the site in which they were collected

(Table 2). For Ovenbird, five sites had sufficient samples to be analyzed, and most classification errors occurred between two sites: WI-BENA and WI-CART (both in Wisconsin). For Tree Swallow, no classification errors occurred (n = 2 sites). For Wood Thrush, error rates ranged from 0.2 to 0.6 (n = 3 sites; Table 2).

ICP-AES results.—Tree Swallows sampled at six sites (IA, IL, MN, NEB, OH, and WI-CNF) were analyzed with ICP-AES. Sodium and Mg were selected as the two best discriminators among sites, and error rates in classification of a natal feather to the site in which it was collected ranged from 0 to 80% (Table 2). Eastern Bluebirds were sampled at 18 sites and analyzed with ICP-AES: TX, OKL, NEB, MN, NC, IA, OH, OHI, CNF, FL, IL, ILL, ILLI, MO, CT, OK, AL, VI. Sodium and P were the first two discriminators selected by the stepwise procedure (Table 2). Error rates in classifying natal Eastern Bluebird feathers to the site in which they were collected were generally very high.

Table 2. Results of discriminant-function analysis to separate sites within a species (see Fig. 1 for site locations). Sample size (n) is the number of individuals sampled per site per species. Predictors are listed in the order of entry in the stepwise discriminant-function analysis.

Technique	Species	Sites	n	Predictors	F	Error rate
ICP-MS	Eastern Bluebird	SC-SRS	13	Sn	18.42	0.00
		WI-CNF	4	Rb	20.71	0.00
ICP-MS	Ovenbird	MN-BENA	13	Cu	7.23	0.23
		WI-CART	6	Nd	6.05	1.00
		VT-GMNF	4			0.25
		TN-ORNL	4			0.25
		MN-STCR	8			0.13
ICP-MS	Tree Swallow	WI-CNF	5	Ce	28.00	0.00
		VT-GMNF	4	Th	9.52	0.00
ICP-MS	Wood Thrush	WV-RAND	5	Na	23.71	0.60
		DE-UDEL	10	Rb	2.87	0.20
		OH-VINT	4			0.50
ICP-AES	Tree Swallow	IA	5	Na	6.85	0.00
		IL	5	Mg	14.91	0.60
		MN	5			0.80
		NEB	5			0.40
		OH	3			0.00
		IL	5			0.20
ICP-AES	Eastern Bluebird	AL	5	Na	5.34	1.00
		WI-CNF	6	P	5.71	0.00
		CT	10			0.47
		FL	6			1.00
		IA	5			0.60
		IL	5			0.40
		ILL	10			1.00
		ILLI	5			1.00
		MN	5			1.00
		MO	5			0.80
		NC	5			1.00
		NEB	5			1.00
		OH	5			0.60
		OHI	5			0.60
		OK	5			0.40
		OKL	5			0.80
		TX	5			1.00
		VI	21			0.67

In summary, error rates for classifying the site in which natal feathers were collected were statistically significantly lower than if classification had been done by random assignment (one-sample binomial test: all exact *P*-values < 0.01). This is true for both the ICP-MS and ICP-AES methodologies. Although error rates for ICP-MS tended to be lower than those for ICP-AES, it is not possible to conclude that ICP-MS has greater ability to discriminate, because the location and number of sites differed between methodologies. Additionally, the elements that discriminated sites varied between species.

Spatial autocorrelation.—The empirical semivariograms of each element did not exhibit clear spatial autocorrelation (Fig. 2). With regards to PCA of element levels within Eastern Bluebird among 18 sites, eigenvalues indicated that two or three components provided a good summary of the data. The first component accounted for 50% of total variance in Eastern Bluebird elements, two components accounted for 68% of total variance, and three components explained 82%. The first principal component (PC1) loaded heavily on Mg (PCA coefficient = 0.44), S (0.44), K (0.43), Na (0.37), and P (0.37). Aluminum and Fe had negative loadings (–0.29 and –0.22, respectively), and Ca did not contribute much to PC1 (loading = 0.07).

Empirical semivariograms for PC1 and each element did not exhibit clear spatial autocorrelation for any of the bin groupings evaluated (Fig. 2). A spherical model that was fit to the PC1 semivariogram showed weak autocorrelation up to 458 km (major range = 458,050 m); beyond this distance, samples were uncorrelated. The ordinary kriging model produced a map of predicted PCA scores across the extent of the study area (Fig. 3). However, this model proved to be a very poor predictor of PCA scores in the cross-validation analysis, given that the regression line between observed and predicted PCA scores was nowhere close to the 1:1 expected relationship. Changes in bin number, bin size, and model structure (e.g. anisotropy) did not improve these results.

Discussion

The topic of natal dispersal has been of interest to avian biologists for many years. Devine and Peterle (1968), Bortolotti et al. (1989), and Parrish et al. (1983) used neutron activation analyses to differentiate sources of North American waterfowl, Spruce Grouse (*Falcipennis canadensis*), and Peregrine Falcons (*Falco peregrinus*), respectively. This method involves irradiating feathers or bones across locations and comparing the gamma-ray spectra. Neutron-

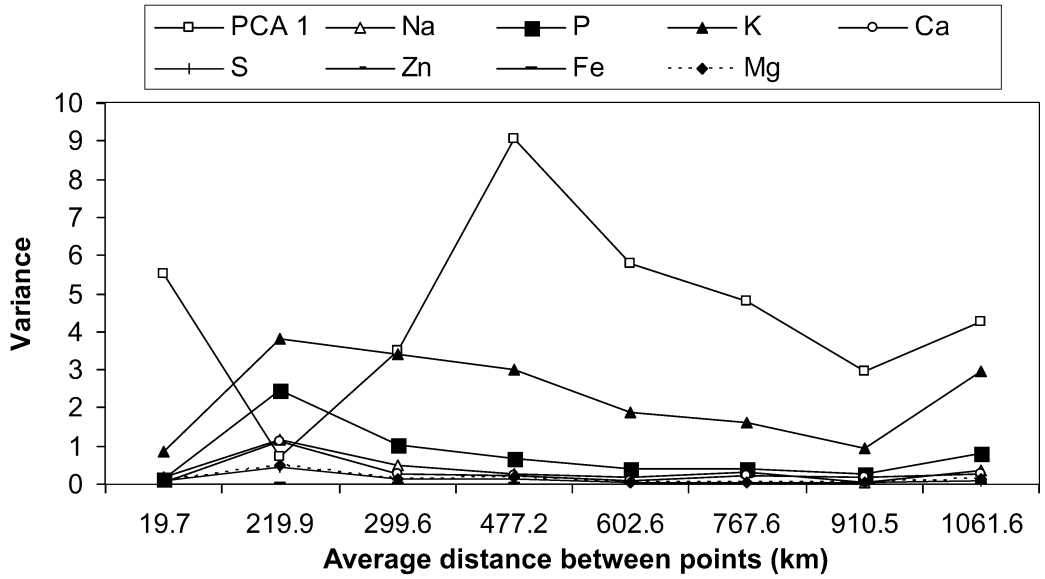

FIG. 2. Empirical semivariograms of element scores and the first principal component score, where pairs of points were placed into one of seven bins, where bin size = 166 km.

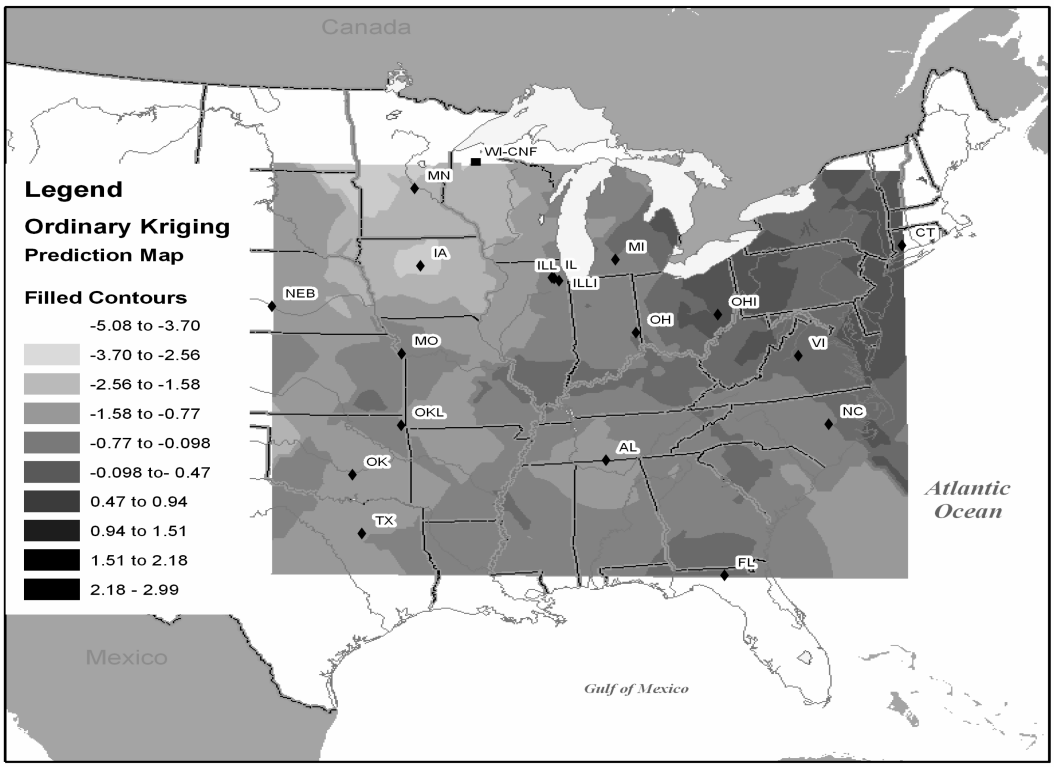

FIG. 3. Results of kriging analysis on the PCA scores obtained from each site for Eastern Bluebird, based on a spherical model fit to the empirical semivariogram. The spherical model assumes that values decrease as a function of distance and is used to predict PCA score of unsampled locations on the basis of the following information: sill = 2.2082, nugget = 2.5643, major range = 458.050 km.

activation analysis was able to source Peregrine Falcons to one of three locations (Parrish et al. 1983), but generally it was not found to be effective at sourcing birds to their correct location in waterfowl species (Devine and Peterle 1968) or in Spruce Grouse (Bortolotti et al. 1989). One reason for the poor performance is that feather chemistry varied substantially among feathers collected from the same individual and among portions within the same feather (Bortolotti and Barlow 1985). Additionally, feather chemistry varied among age and sex classes within the same geographic location (Bortolotti et al. 1989), and ambient deposition may also contribute to high variation within a site. These same issues may have caused high within-site variation in our analyses as well. Other methods evaluated for tracking dispersal include X-ray spectrometric of flight feathers (Kelsall and Burton 1977). This method was also of limited utility in tracking dispersal, because feathers can show significant within-year changes in feather chemistry (Kelsall et al. 1975).

Here, we sought to determine whether ICP-MS and ICP-AES methodologies can be used to track natal dispersal in songbirds. We acknowledge that our sampling scheme was not ideal; samples were collected by willing volunteers, and the number of actual feathers that could be analyzed varied greatly across sampling sites, limiting comparisons and inferences. Still, two basic findings resulted from our analyses. First, within a site, species differed in element signatures for both ICP-MS and ICP-AES methodologies. This result suggests that if elemental base maps can be interpolated across a large geographic extent to track dispersal, the maps will likely need to be species-specific. And second, the elements that discriminated the sites were species-specific, such that researchers will need to carefully consider their study species, as well as which elements can be evaluated meaningfully.

We explored spatial autocorrelation of the feather elements and PCA scores at 18 sites for Eastern Bluebird. Such autocorrelation is necessary because models fit to spatially autocorrelated data can be "kriged" over space to show predicted natal-element levels across large spatial extents (the base-map). This predictability, in theory, would allow researchers to identify zones or areas on the base-map that match a sample of feathers collected from a first-year adult, thus identifying possible birth locations of that adult. The empirical semivariogram of ICP-AES samples did not show clear spatial autocorrelation for any of the elements or PCA scores. This result has many possible causes; one obvious explanation is that we sampled too few sites to adequately test spatial autocorrelation. The kriging map, based on a spherical model fit to the PCA scores, showed the type of gradient that would be required for an elemental base-map to be useful in tracking dispersal, with highest PCA scores in the northeastern United States, lowest PCA scores in the Midwest, and average PCA scores in the South (Fig. 3). However, cross-validation of this kriging model performed very poorly at predicting element levels at sampled sites. This result may be attributable to the small sample of sites (n = 18) as well as the large extent to which those sites were separated. Other potential explanations for lack of spatial autocorrelation include (1) high variation among individuals within a site and (2) changes in feather composition related to ambient conditions. Thus, whether elemental analyses can identify the natal dispersal distances requires further study. We believe that future studies should focus sampling efforts within a much smaller geographic region than we sampled and sample more sites so that spatial autocorrelation can be explored more fully.

In summary, the present study suggests that future research should focus on a single species across much smaller geographic extents, with larger numbers of sampling sites as well as more geographically restricted sampling within sites. One possibility is that elemental analysis may be most productively combined with other more "coarse-grained" methods for sourcing the natal origin of songbirds in a hierarchical assignment framework: "coarse-gained" methods such as genetic markers or stable-isotope ratios could be used to assign birds to relatively large geographic regions, with elemental analyses like those described here used to source individuals within such regions.

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