The Auk 110(4):844–856, 1993

APPLICATION OF GENEALOGICAL-CONCORDANCE PRINCIPLES TO THE TAXONOMY AND EVOLUTIONARY HISTORY OF THE SHARP-TAILED SPARROW (*AMMODRAMUS CAUDACUTUS*)

JAMES D. RISING[1] AND JOHN C. AVISE[2]

[1]*Department of Zoology, University of Toronto, Toronto, Ontario M5S 1A1, Canada; and*
[2]*Department of Genetics, University of Georgia, Athens, Georgia 30602, USA*

ABSTRACT.—We examined geographic differentiation in mitochondrial (mt) DNA and in morphometric characters among 12 populations of the Sharp-tailed Sparrow (*Ammodramus caudacutus*) representing all recognized subspecies and geographic regions. Both data sets reveal the existence of two distinct groups of populations, a northern group from the Canadian maritime provinces and Maine, the St. Lawrence Valley, Hudson Bay lowlands, and interior prairies, and a southern group from along the Atlantic coast north to southern Maine. In one sample from southern Maine, both forms co-occur, and about 40% of the individuals there appear to be of hybrid ancestry. Recently, principles of genealogical concordance have been proposed as a conceptual basis for recognition of biological taxa. Here we provide an empirical application of these principles in the context of observed concordance between the mtDNA phylogenetic partition and the subdivisions evidenced by morphological (and behavioral) attributes in the Sharp-tailed Sparrow complex. We recommend that two subspecies of *A. caudacutus* be recognized: one (*A. c. nelsoni*) to encompass the northern populations (formerly *A. c. nelsoni, A. c. alterus,* and *A. c. subvirgatus*); and the other (*A. c. caudacutus*) to encompass the southern populations (formerly *A. c. caudacutus* and *A. c. diversus*). By taxonomically formalizing what appears to be a fundamental phylogenetic partition among Sharp-tailed Sparrow populations, study of the biogeographic history, reproductive relationships, and management of the forms will be facilitated. *Received 26 March 1992, accepted 23 November 1992.*

THE SHARP-TAILED SPARROW (*Ammodramus caudacutus*) is a widespread and polytypic species that breeds in marshes and wet grasslands. The AOU *Check-list* (1957) recognized five subspecies with breeding ranges as follows (Fig. 1): (1) *A. c. nelsoni,* freshwater prairie marshes of continental interior from eastern British Columbia and southern Mackenzie to central Manitoba and northern South Dakota (and formerly southeast to Chicago, Illinois); (2) *A. c. alterus,* freshwater marshes bordering James and Hudson bays; (3) *A. c. subvirgatus,* brackish and salt marshes on south shore of lower St. Lawrence River, the Canadian maritime provinces, and along coast to southern Maine; (4) *A. c. caudacutus,* salt marshes from southern Maine to New Jersey; and (5) *A. c. diversus,* southern New Jersey (where it intergrades into *A. c. caudacutus*) to Virginia (and doubtfully to North Carolina). With the exception of perhaps a few of the birds that breed south of central New Jersey, Sharp-tailed Sparrows are migratory, and most individuals winter in coastal marshes from South Carolina to southern Florida and (especially *A. c. nelsoni*) west to the Gulf coast of Texas, and regularly to coastal California. Although there is geographic variation in size and shape, these subspecies were described primarily on the basis of differences in plumage.

The Sharp-tailed Sparrow is of special interest to ecologists and zoogeographers because much of its current range was uninhabitable only 13,000 years ago (prior to retreat of Laurentide ice sheet; Bryson 1988), and since that time the species may have colonized and adapted to freshwater marshes in the interior of the continent. Thus, Beecher (1955) speculated that the complex originated in salt marshes along the Atlantic coast, perhaps as a sister species of the Seaside Sparrow (*A. maritimus*), and colonized the prairies as well as James Bay lowlands via a corridor provided by postglacial marine embayments south of the retreating ice front. Greenlaw (1993), however, suggested that the ancestors to the northern and southern Sharp-tailed Sparrows became isolated by glacial advance, with the pro-southern birds isolated in a coastal refugium and the pro-northern birds in an inland one. In a subsequent glacial cycle, these birds dispersed northward, with the inland Sharp-tailed Sparrows dispersing eastward into the maritimes and to the northern Atlantic

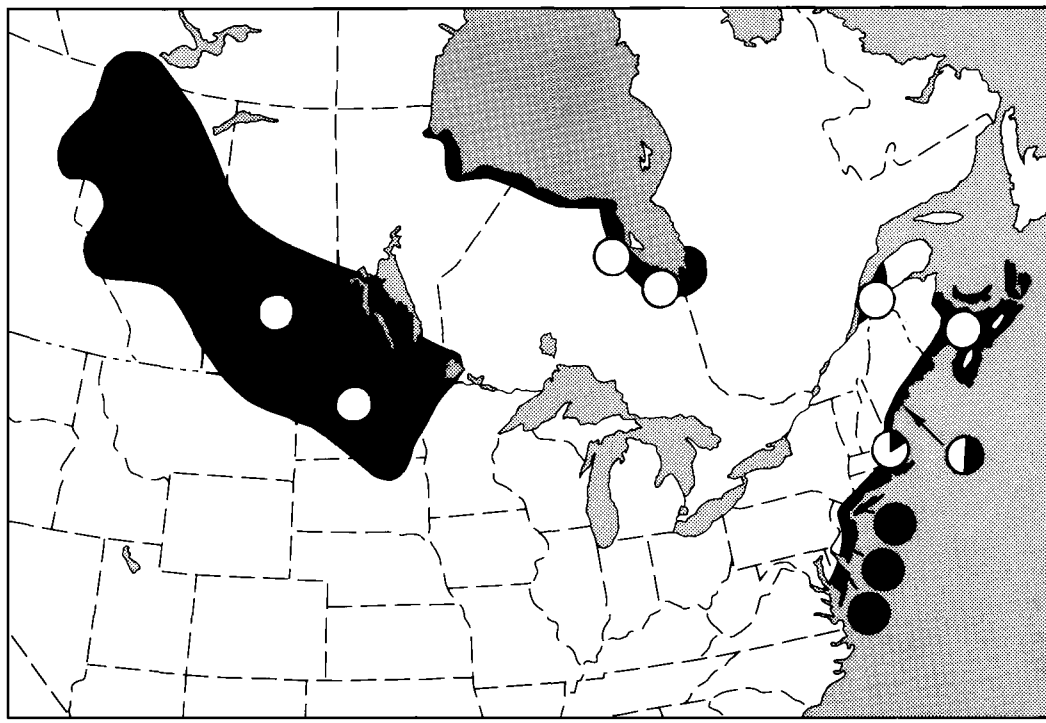

Fig. 1. Distribution of breeding populations of Sharp-tailed Sparrows. Circles represent geographic sites from which samples taken, with proportion of individuals with "southern" mtDNA indicated by proportion of circle that is black. Southern Maine local (Popham Beach), where "northern" and "southern" forms overlap, is indicated by arrow.

coast. Beecher (1955) also proposed that the Sharp-tailed and Seaside sparrows evolved from a common ancestor of the widely-distributed Savannah Sparrow (*Passerculus sandwichensis*), whereas Murray (1968) argued that LeConte's Sparrow (*A. leconteii*) is the sister taxon to *A. caudacutus*. Analyses based on skeletal measurements (Robbins and Schnell 1971) and molecular data (Zink and Avise 1990) indicate that the LeConte's Sparrow is the closest living relative of the Sharp-tailed/Seaside complex, and that the Savannah Sparrow is a distant outlier.

During the molecular-genetic survey of interspecific relationships in *Ammodramus* sparrows and relatives (Zink and Avise 1990), two rather distinct mitochondrial (mt) DNA genotypes were observed in preliminary collections of Sharp-tailed Sparrows. Here we extend the "phylogeographic" (Avise et al. 1987) survey of mtDNA in *A. caudacutus* to include samples from all recognized subspecies and from all major breeding regions of the species. In addition, we use multivariate morphometric analyses to address whether morphologic partitions in the complex are concordant with the striking genetic subdivisions observed and with the behavioral differences reported by Greenlaw (1993). Results should contribute to an understanding of the evolutionary history of Sharp-tailed Sparrow populations. In a similar analysis of the Seaside Sparrow, Avise and Nelson (1989) showed how such genetic information can also be relevant to decisions involving taxonomy and population management.

Materials and Methods

Study animals.—We collected 220 specimens of Sharp-tailed Sparrows from 12 breeding populations, as well as 20 from two migration and wintering sites (Table 1). All of the named taxa were represented by at least two samples from breeding populations. The sample from Popham Beach, Sagadahoc Co., Maine is from an area of overlap between *A. c. caudacutus* and *A. c. subvirgatus*. Study skins and partial skeletons, as well as tissue samples (heart, liver, kidney, breast muscle), were saved from specimens taken during the

TABLE 1. Samples, dates of collection, and sample sizes of Sharp-tailed Sparrows.

Locale no.	Locality	Dates collected	*n* (male, female)	*n* (mtDNA assays)[a]
1	Saskatchewan: Last Mountain Lake	5–6 July 87	12, 1	6
2	North Dakota: Upham, McHenry Co.	27–29 June 87	14, 6	6
3	New Brunswick: Sackville, Westmoreland Co.	15 June 1987	17, 3	6
4	Ont.: Attawapiskat, Kenora Dist.	30 Jn–1 Jly 1988	18, 4	5
5	Ontario: Moosonee, Kenora Dist.	5–7 July 1988	20, 3	5
6	Delaware: Prime Hook Refuge, Broadkill Beach	8–9 June 1988	19, 2	6
7	New Jersey: 4 km NE Oceanville, Atlantic Co.	14 June 1988	12, 7	6
8a	New York: Jones Island, Nassau Co.	16 June 1988	11, 6	6
8b	New York: Oak Beach Marsh, Suffolk Co.	16 June 1988	3, 1	—
8c	New York: Suffolk Co.	October 1987	—	11
9	Massachusetts: 2 km SE Newbury, Essex Co.	17–18 June 1988	16, 5	6
10	Maine: Popham Beach, 10 km S Phippsburg, Sagadahoc Co.	1 July 1989	18, 2	20
11	Quebec: 1 km N Pocatière, Kamourska Co.	29 June 1989	15, 0	15
12	Quebec: 6 km NE L'Isle Verte, Rivière-du-Loup Co.	26–27 June 1989	4, 1	—

[a] Additional specimens assayed for mtDNA were collected during migration (Long Island, New York; $n = 7$) or on wintering grounds (Cameron Parish, Louisiana; $n = 2$).

breeding season. From most birds collected in 1987 and 1988, tissues were quick frozen in liquid nitrogen; in the laboratory these were stored at −70°C. From a few birds collected in 1987, and all birds collected in 1989, part of the tissue sample was saved in MSB buffer (Lansman et al. 1981), which proved to enhance mtDNA yields.

Morphometric analyses.—J.D.R. measured the 25 variables on the cleaned skeleton of each specimen. These are essentially the same measurements used by Rising (e.g. 1987) for other studies of geographic variation in sparrows. Sample sizes of females were too small (Table 1) to test for sexual dimorphism (which exists in Sharp-tailed Sparrows; Rising pers. obs.), so we excluded females from morphometric analyses. We used analyses of variance (SPSSX/ANOVA, Release 3.1; SPSSX 1986) to test for significant interpopulational variation of the characters, and principal-components analyses (PCA; NTSYS/FACTOR, version 4, level 6, 1984; Rohlf 1985) to look for multivariate patterns of variation in males. We estimated the size of missing (broken) characters using multiple regression (BMDP/PAM; Dixon 1983), regressing the missing variable against the two non-missing ones that best predicted (within a sample) it. We omitted individuals missing more than three variables from the PCA analysis and, consequently, were able to use only 164 of the 220 birds. We extracted the first three components from a matrix of correlations among the raw measurements (Rising and Somers 1989).

We used discriminant function analysis (DFA; SPSSX/DISCRIMINANT, release 3.1; SPSSX 1986) to discriminate among the samples of males. For the DFA, samples 11 and 12 were combined, and only the variable skull length, premaxilla length (from the nostril), mandible depth, tarsometatarsus length, ulna length, hallux length, and keel length were used. These variables were selected *a priori* because they reflect different parts of the bird (bill size, leg length, wing length, toe length, etc.). Individuals missing any of the selected variables were omitted from this analysis and as a result only 179 specimens were used.

mtDNA analyses.—We prepared mtDNA from 107 birds (Table 1). Either fresh or frozen heart and liver was used in the mtDNA isolations. Fresh tissue was placed in MSB buffer (Lansman et al. 1981), shipped to the laboratory, and processed within six days of the time of collection. Frozen tissue, stored at −70°C for up to two years provided lower, but still adequate mtDNA yields. Closed-circular mtDNA was isolated using the CsCl-gradient method described in Lansman et al. (1981). Purified mtDNA was then digested with 20 restriction endonucleases under conditions recommended by the supplier (New England Biolabs). Digestion products were end-labeled with ^{35}S-nucleotides (Drouin 1980), separated by electrophoresis in 1.0 to 1.6% agarose gels, and revealed by autoradiography. Fragment sizes were determined by comparison to a 1-kilobase (kb) ladder standard. In general, no attempts were made to score fragments less than about 0.4 kb in length.

Within *A. caudacutus*, essentially all of the differences among digestion patterns could reasonably be attributed to gains or losses of particular restriction sites, so that the data could be described as a matrix of presence versus absence of sites across mtDNA clones. Thus, although sites were not mapped formally (e.g. by double-digestion procedures), the data were nonetheless interpretable in terms of restriction site differences (procedures explained in Results and in Avise et al. 1989). This matrix was then used to generate estimates of nucleotide sequence divergence

by the site method of Nei and Li (1979). Nucleotide and genotype diversities were calculated by the methods of Nei (1987:256) and Nei and Tajima (1981:formula 7), respectively. Parsimony networks were obtained from the PAUP version 3.1 program (Swofford 1993). Levels of support for network branches were estimated by bootstrapping over characters, across 200 replicates (Lanyon 1987). Estimates of nucleotide sequence divergence among mtDNA clones were used to generate a phenogram using the unweighted pair-group method with arithmetic averages (UPGMA; Sneath and Sokal 1973).

The mtDNA digestion profiles in Sharp-tailed Sparrows were also compared to those obtained for other *Ammodramus* species (Zink and Avise 1990), and were designated accordingly, using uppercase letters. In a Hennigian cladistic interpretation using outgroup criteria, mtDNA gel patterns shared with other species provisionally were assumed to be ancestral (plesiomorphic), while derived patterns were considered autapomorphic (if unique to one individual) or synapomorphic (if shared by two or more individuals in an enzyme where the ancestral state was identified). For several endonucleases, digestion profiles were obtained nowhere else in the genus and, hence, were assigned new letters. Evolutionary polarities (ancestral vs. derived) among the genotypes revealed by such enzymes cannot be established using Hennigian principles (Hennig 1966, Patton and Avise 1983), but unrooted parsimony networks could nonetheless be determined.

TABLE 2. Descriptions of mtDNA clones observed in Sharp-tailed Sparrows (n = 107). Letters (from left to right) designate multifragment gel profiles produced by digestion with *Ava*I, *Ava*II, *Bam*HI, *Bcl*I, *Bgl*I, *Bgl*II, *Cla*I, *Eco*RI, *Hin*cII, *Hin*dIII, *Msp*I, *Nde*I, *Pst*I, *Pvu*II, *Spe*I, *Sst*II, and *Xba*I. Underlined profiles also observed in other species of *Ammodramus*. Parsimony networks summarizing relationships among digestion patterns for variable endonucleases shown in Figure 2.

Clone	Genotypic description	No. birds
a	B E E D D C E D G G X C C F D C D	37
b	B E E D D C E D G G Y C C F D C D	1
c	B E E D D C E D F G X C C F D C D	1
d	B E E D D C E D H G X C C F D C D	1
e	B E E E D C E D G G Y C C F K C D	1
f	B E E D D C E D G G X C C E D C D	1
g	B E E D D C E D G G X C C G D C D	2
h	B E E D D C E D E F C D C G E C D	14
i	B E E D D C E D E G C D C G E C D	27
j	B A E D D C E D E G C D C G E C D	5
k	B E E D D C E D E G C D C G I C D	4
l	B E E D D C E D E G C C C G I C D	1
m	B F F D D C E D E G C D C G E C D	4
n	B E F D D C E D E G C D C G E C D	1
o	B E E D D C E D E H C D C G A C D	1
p	B E E D D C E D E H C D C G G C D	1
q	B E E D D C E D E H C D C G Q C D	1
r	B E B D D C E D E G C D C G E C D	2
s	B E E D B C E D E G C D C G E C D	1
t	B E E D B C E D E G C D C G I C D	1

RESULTS

Genetics.—The 17 endonucleases (listed in Table 2) produced multiple cuts in *A. caudacutus* mtDNA. These informative enzymes revealed 96 restriction sites in the study, with an average of about 82 sites (representing 450 base pairs in recognition sequence) scored per individual. Altogether, 20 distinct mtDNA genotypes were observed among the 107 assayed Sharp-tailed Sparrows (Table 2), yielding a genotypic diversity estimate (probability that any two individuals differ in genotype) of 0.835. This value is near the median of mtDNA diversities reported previously for other vertebrate species (Avise et al. 1989). Table 3 summarizes the distribution of restriction sites among the 20 genotypes.

Considered individually, most of the endonucleases produced gel profiles whose interrelationships could readily be summarized by parsimony criteria (Fig. 2). For example, three *Bam*HI patterns (E, F, and B) appeared to differ by successive site changes: F exhibited two sites, producing fragments of 12.6 and 4.2 kb; E had an additional site that cleaved the 4.2-kb fragment into fragments of lengths 3.5 and 0.7 kb; and B had an additional site from E that cleaved the 3.5-kb band into fragments of lengths 2.7 and 0.8 kb. Inferences about most other site changes were equally straightforward. Only *Msp*I and *Spe*I produced some gel profiles whose nearest relatives required an assumption of more than one restriction-site change to account for the pattern interconversions (Fig. 2).

In the UPGMA analysis, the 20 mtDNA clones grouped into two distinct clusters that joined at a level of sequence divergence p of 0.013 (Fig. 3). After statistical correction for within-region polymorphism (Wilson et al. 1985:equations 1 and 2), net sequence divergence between these clusters remained p of 0.012. In the parsimony analyses, these same two clusters were recognized in 99% of the bootstrap replicates, whereas no other groups were recognized in more than 75% of such resampling procedures (Fig. 3).

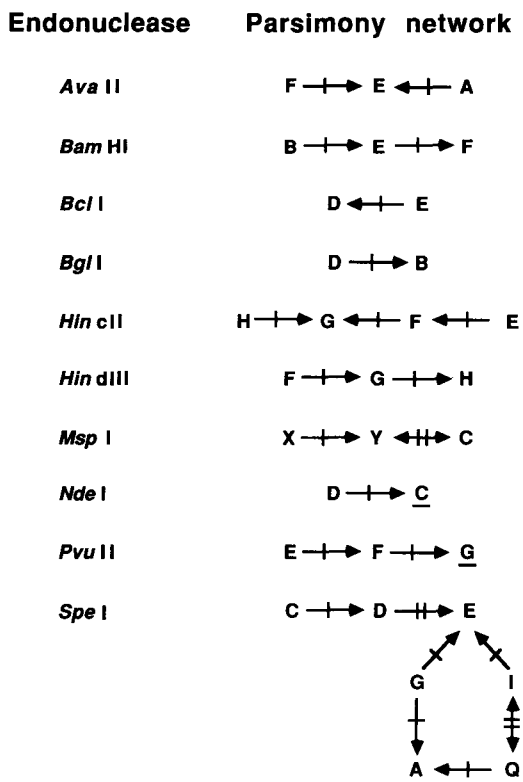

Fig. 2. Parsimony network for variable mtDNA digestion profiles produced by various endonucleases. Each arrow indicates direction of restriction-site loss, and not necessarily direction of evolutionary change. Digestion profiles observed in at least one other *Ammodramus* species are underlined, and these may represent ancestral conditions for Sharp-tailed Sparrows. All other profiles observed only in Sharp-tailed Sparrows.

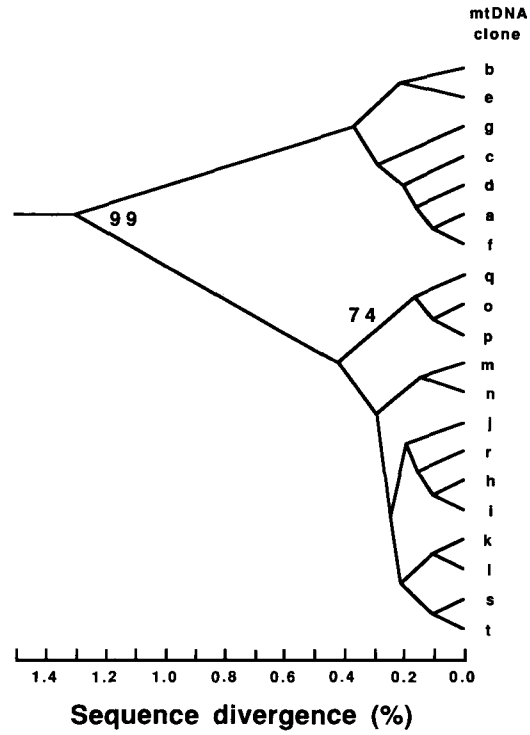

Fig. 3. UPGMA phenogram based on matrix of *P*-values derived from observed patterns of mtDNA restriction sites in Sharp-tailed Sparrows.

The validity of these two major clusters received some degree of additional support from the Hennigian cladistic perspective. The species of *Ammodramus* assayed previously (Zink and Avise 1990) exhibited large mtDNA genetic distances and, hence, were scored only with respect to fragment profiles (rather than restriction sites). Most of the enzymes employed in the current study produced fragment profiles in *A. caudacutus* that were either: (a) monomorphic or polymorphic in Sharp-tailed Sparrows but unique to this species; or (b) monomorphic in Sharp-tailed Sparrows and shared with other *Ammodramus* (Table 2). However, two putative synapomorphies within *A. caudacutus* could be defined, and these identified clades that essentially corresponded to the two major groupings already noted in the UPGMA and parsimony analyses. Thus, the derived *Nde*I pattern D united mtDNA clones *h–t* (with the exception of clone *l*), and the derived patterns *Pvu*II E and F united clones *a–f* (Table 2).

Therefore, the major feature of these molecular data is the fundamental phylogenetic break between two mtDNA clonal arrays (*a–g* versus *h–t*). The first haplotype array characterized all (except one) sampled breeding individuals from Massachusetts, New York, New Jersey, and Delaware (conventionally, *A. c. caudacutus* and *A. c. diversus*), whereas the latter array characterized all assayed breeding specimens from the continental interior, James Bay, the St. Lawrence River, New Brunswick, and Quebec (conventionally *A. c. nelsoni, A. c. alterus,* and *A. c. subvirgatus*). Hereafter, we refer to these as the "southern" and "northern" clades, respectively. In one geographically intermediate locale (10, Popham Beach, Sagadahoc Co., Maine), the northern and southern mtDNA types occurred together and were represented in equal frequency among 20 specimens. Also, one of five

TABLE 3. Matrix of presence (1) versus absence (0) of 96 restriction sites in 20 mtDNA clones observed in Sharp-tailed Sparrows.

Clone	Restriction sites
a	11111111111001011111101111111111111111111100001111111111111111111011101111111010101111111111011111
b	11111111111001011111101111111111111111111100001111111111111111111001111011111110101011111111111011111
c	11111111111001011111101111111111111111111100011111111111111111111011101111111010101111111111011111
d	11111111111001011111101111111111111111111101001111111111111111111011101111111010101111111111011111
e	11111111111001011111111111111111111111111100001111111111111111111001111011111110101011111111111111
f	11111111111001011111101111111111111111111100001111111111111111111011101111111110101111111111011111
g	11111111111001011111101111111111111111111100001111111111111111111011111111111001010111111111011111
h	11111111111001011111101111111111111111111101111111101111111111110111111111111001010111110011011111
i	11111111111001011111101111111111111111111101011111101111111111110111111111111001010111110011011111
j	11111111111011011111101111111111111111111101011111101111111111110111111111111001010111110011011111
k	11111111111001011111101111111111111111111101011111101111111111110111111111111001110111110011011111
l	11111111111001011111101111111111111111111101011111101111111111110111111111111001101111110011011111
m	11111111111010011111011111111111111111111101011111101111111111110111111111111001010111110011011111
n	11111111111001001111011111111111111111111101011111101111111111110111111111111001010111110011011111
o	11111111111001011111101111111111111111111101001111101111111111110111111111111001011111110010101111
p	11111111111001011111101111111111111111111101001111101111111111110111111111111001011111110011011111
q	11111111111001011111101111111111111111111101001111101111111111110111111111111001111111110010101111
r	11111111111001111111101111111111111111111101011111101111111111110111111111111001010111110011011111
s	11111111111001011111101111011111111111111101011111101111111111110111111111111001010111110011011111
t	11111111111001011111101111011111111111111101011111101111111111110111111111111001110111110011011111

TABLE 4. Correlations (>0.3) between variables and principal-component loadings from a PCA of correlation matrix of raw measurements of 164 male Sharp-tailed Sparrows.

Variable	PC1	PC2	PC3
Skull length	0.92		
Skull width	0.76		
Premaxilla length (nostril)	0.73	−0.31	
Premaxilla length (skull)	0.53		
Premaxilla depth			−0.58
Narial width	0.42		−0.57
Premaxilla width	0.61		
Interorbital width	0.53		−0.34
Mandible length	0.90		
Gonys length	0.66	−0.37	
Mandible depth			−0.71
Coracoid length	0.88		
Scapula length	0.82		
Femur length	0.93		
Femur width	0.65		
Tibiotarsus length	0.84		
Tarsometatarsus length	0.55	0.56	
Humerus length	0.93		
Ulna length	0.94		
Carpometacarpus length	0.80	0.33	
Hallux length	0.50	0.46	
Sternum length	0.78	0.34	
Sternum depth	0.40	0.66	
Keel length	0.42	0.73	
Synsacrum width	0.85		
Eigenvalue	12.4	2.6	1.5
Percent variance explained	49.7	10.4	5.8

assayed birds from the Massachusetts site (locale 9, Table 1) carried a northern mtDNA haplotype.

Under a conventional mammalian mtDNA clock calibration of 2% sequence divergence between a pair of lineages per million years (Brown et al. 1979), the separation of northern from southern Sharp-tailed Sparrow mtDNA clades may have occurred about 600,000 years ago. However, given uncertainties about mtDNA "clocks" in birds or other taxa, this estimate should be interpreted with considerable caution (but see also Shields and Wilson [1987], who argued that the mammalian calibration does also apply to birds).

In addition to the fundamental genetic split distinguishing northern from southern *A. caudacutus*, the mtDNA data also gave preliminary indications of within-region substructuring. For example, five of six assayed specimens (83%) from the New Brunswick site (locale 3, Table 1) exhibited mtDNA haplotype *h*, a genotype observed elsewhere only in 9 of 98 specimens (9%) from scattered breeding locales. Similarly, genotype *j* was observed in 5 of 15 of the birds from Pocatière, Kamourska Co., Quebec (locale 11), but nowhere else in the survey. On the other hand, the most common genotypes *a* and *i* (Table 2) were observed in all locales within their respective southern and northern regions.

Two specimens collected on wintering grounds in Louisiana carried mtDNA genotypes characteristic of the northern clade, as did seven birds collected during migration on Long Island. The latter specimens have been identified as representatives of northern subspecies (*A. c. alterus*, *A. c. subvirgatus*, or *A. c. nelsoni*) on the basis of plumage characteristics (Jon Greenlaw pers. comm.).

Morphometrics.—The ANOVAs show that there is highly significant ($P < 0.001$) interpopulational variation in all 25 variables. The pattern of variation that emerges from these ANOVAs is that the prairie birds are smallest in size, followed by James Bay, New Brunswick and Quebec, and Atlantic coast specimens, in that order.

The first three components extracted from the PCA explain 49.7, 10.4, and 5.8% of the total variation among the 164 male specimens in the 25-character space (Table 4). Components 4, 5, and 6 have nearly equal and small eigenvalues; these are difficult to interpret (and have little explanatory value). The correlations of 23 characters with PC 1 are positive and high (>0.4; Table 4), such that sparrows with high PC 1 values (to right in Fig. 4) are large individuals. PC 2 is highly positively correlated with tarsometatarsus length, hallux length, and keel and sternum size, and is negatively correlated with bill length (premaxilla and gonys; Table 4). Thus, individuals with high PC 2 scores (near top in Fig. 4) have relatively short bills, long legs and toes, and large keels. PC 3 is negatively correlated with the two measures of bill depth and with narial and interorbital widths (Table 4).

In the plot of PC 1 and PC 2 (Fig. 4), the relatively large Sharp-tailed Sparrows from Massachusetts, New York, New Jersey, and Delaware (the southern mtDNA types) are grouped together (to lower right of dashed line in Fig. 4), as are the relatively small birds (to upper left of dashed line) from the prairies, James Bay, St. Lawrence River, and New Brunswick (the northern mtDNA types). On the PC 1 axis there is considerable overlap between the sparrows from Quebec and New Brunswick, and those from the south Atlantic coast (New York, New

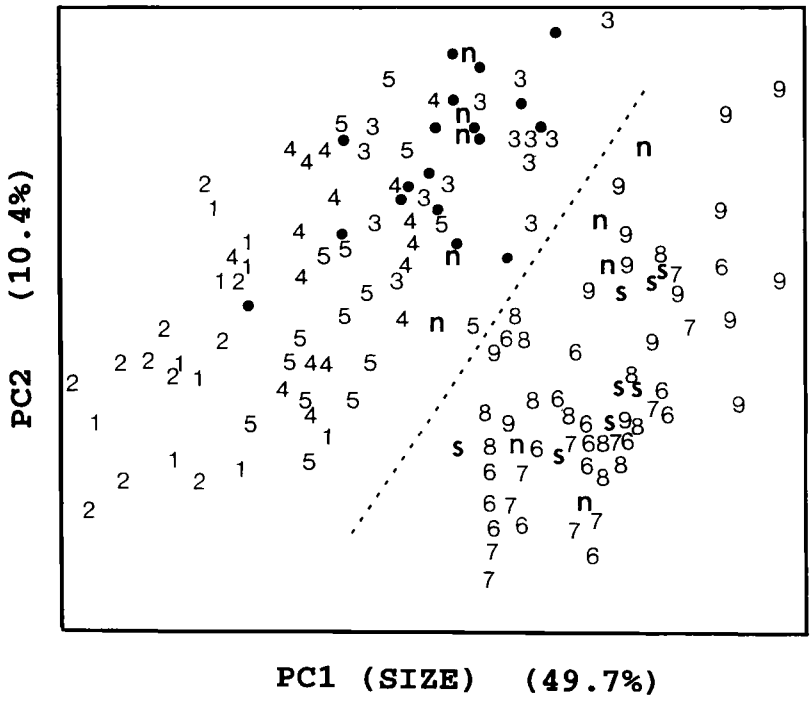

Fig. 4. Position of 164 male Sharp-tailed Sparrows in space defined by PC 1 and PC 2 from analysis based on matrix of correlations among 25 skeletal measures. PC 1 and PC 2 explain 60.1% of variance in 25-dimensional space. Position of each bird indicated by a number for locale from which specimen taken (Table 1). Localities 11 and 12 pooled, and indicated by single dots. Letters "n" and "s" indicate birds from Popham Beach, Maine (locale 10), where both northern and southern mtDNA genotypes occur.

Jersey, and Delaware), but virtually no overlap between these groups on the PC 2 axis. The DFA also demonstrates the phenetic distinctiveness of the northern and southern mtDNA clones, with all specimens correctly identified to these two assemblages (Table 5).

In the sample from southern Maine where both mtDNA clades co-occur in high frequency, 5 of 10 males with northern mtDNA cluster with maritime (Quebec, New Brunswick) birds, but the remainder cluster morphometrically with the southern birds (three of five with sparrows from Massachusetts) in both the PC and DF analyses. However, those with southern mtDNA all cluster with the southern birds, perhaps especially with those from the southern Atlantic populations (Fig. 4, Table 5). The bird from Massachusetts with the northern mtDNA type was a female and, therefore, could not be identified by mensural criteria. Its plumage phenotype is southern.

Table 6 summarizes the identity of the 20 birds from the southern Maine site based on their mtDNA clade membership, PCA (for males) of the 25 morphometric measures, and general plumage pattern (*A. c. caudacutus* [southern] vs. *A. c. subvirgatus* [northern], as assessed by J.D.R.). Eleven of the 18 males (61%) were identified as being in the same group by all three criteria, and another was identified concordantly as southern by mtDNA and PCA, but was intermediate in plumage. Of the five individuals identified differently by the mtDNA and PCA criteria, two were northern in plumage, two intermediate, and one southern. In 2 × 2 tests of independence using the *G*-test and Yate's correction for small sample size (Sokal and Rohlf 1981), associations between mtDNA and plumage in the southern Maine sample were significant ($G = 8.2$, $P < 0.01$), whereas associations between plumage and PCA (excluding intermediate conditions), and between mtDNA and PCA, were marginally nonsignificant with our sample sizes ($G = 2.8$ and 4.5, respectively; $P > 0.05$). With the possible exception of specimen 110090, all of the birds that appear to be

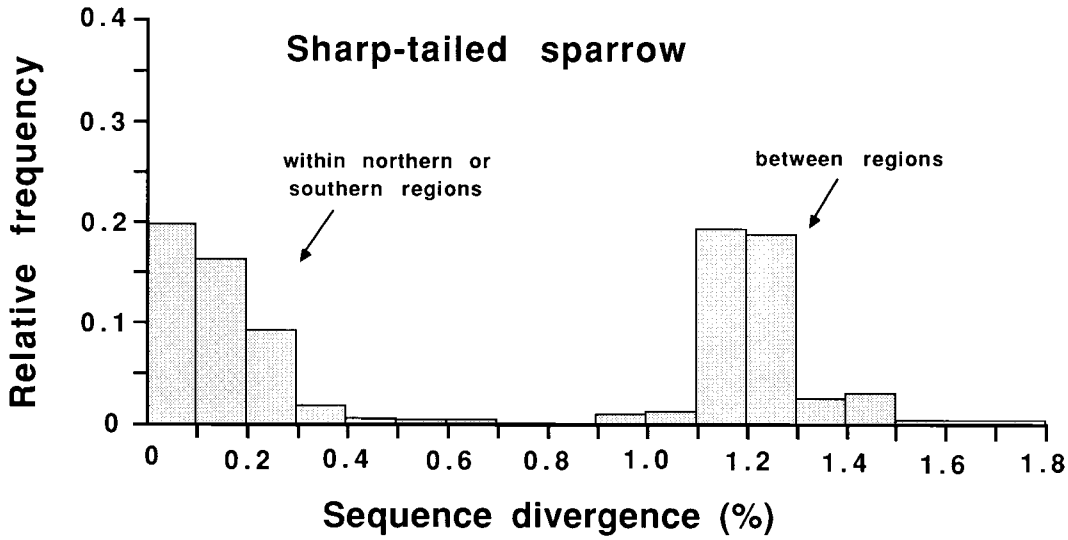

Fig. 5. Frequency histogram of 5,671 pairwise mtDNA distance estimates among 107 Sharp-tailed Sparrows assayed.

mixed have northern mtDNA. Inasmuch as the mtDNA comes from the female parent without recombination, this implies that female southern Sharp-tailed Sparrows tend to mate selectively with southern males, whereas (at Popham Beach) northern females mate nonselectively. Because Sharp-tailed Sparrows practice scramble-competition polygyny (Post and Greenlaw 1982), this suggests that the larger southern males are more successful in mating competition than the smaller northern ones, or perhaps that the females tend to select southern rather than northern males.

In addition to the general distinctions between the northern and southern populations, the morphometric analyses allowed some finer resolutions within regions (Fig. 4, Table 5). For example, the two prairie samples (locales 1 and 2) overlap extensively in the PCA plot, but are consistently among the smallest of all Sharp-tailed Sparrows (Fig. 4). Within the northern array, the New Brunswick specimens (locale 3) are consistently among the largest; in the southern array, Massachusetts birds (locale 9) typically score among the highest along both PC 1 and PC 2 (Fig. 4).

DISCUSSION

Distinctions between northern and southern forms.—Figure 5 is a frequency histogram for the 5,671 pairwise distance comparisons among the 107 Sharp-tailed Sparrows assayed for mtDNA. As expected, given the clade structure, the distribution is strongly bimodal, with the two frequency peaks reflecting within-region and between-region (northern vs. southern) comparisons, respectively. Thus, *A. caudacutus* provides a classic example of "phylogeographic category I," in which mtDNA clades characterized by sharp phylogenetic discontinuities display a clear geographic orientation (Avise et al. 1987). This distinction strongly suggests a historical population subdivision of *A. caudacutus* into two major phylogenetic lines.

The fundamental phylogeographic subdivision of Sharp-tailed Sparrow mtDNA may be interpreted as concordant with a multivariate partition of morphometric characters (Fig. 4). However, other boundary lines might justifiably be drawn through Figure 4 emphasizing other regional subdivisions (such as the clustering of locales 1 and 2 in lower left corner of PC 1/PC 2 space). Thus, while a morphological partition can be identified that is concordant with the mtDNA subdivision, it is not obvious that the separation into northern and southern Sharp-tailed Sparrows (as defined here) is the most fundamental morphological separation, nor that a historical population separation led to the "northern" versus "southern" distinction. In contrast, the partition in the mtDNA tree evidences not only fixed and known genetic differences between regions, but also cu-

mulative mutational divergence that goes well beyond the level of mtDNA sequence diversity reflected as polymorphisms within either region (Fig. 3).

The argument for a northern-versus-southern distinction in Sharp-tailed Sparrows is strengthened further by other differences previously noted by other researchers. Montagna (1942) was the first to write about behavioral distinctions, noting that Maine birds (*A. c. subvirgatus*) sang more frequently than did birds from New York or New Jersey (*A. c. caudacutus*), and that the flight display of the southern birds was less spectacular than that of the northern birds. The flight of both the prairie and James Bay birds is quite spectacular: the male ascends to about 10 m, sings, drops about 3 m, sings again, and then glides back to the marsh, landing perhaps 50 m from where he initiated the display (Rising pers. obs.). The songs of the northern and southern birds also appear to differ substantially (Greenlaw 1993).

The dichotomy we find between the prairie, James Bay, and maritime birds versus the southern Atlantic coastal birds can help us select between Beecher's (1955) and Greenlaw's (1993) biogeographic hypotheses. Beecher postulated that, following the last cycle of Pleistocene glaciation, ancestral Sharp-tailed Sparrows from salt marshes along the Atlantic coast moved north and west to the present-day Great Lakes. Prior to the isostatic rebounding of the Hudson Bay lowlands, there may have been a nearly continuous corridor of marshy habitat between the St. Lawrence Valley and the then much larger James Bay, and from there into the Great Plains, providing an avenue of dispersal for Sharp-tailed Sparrows from the Atlantic seaboard to the prairies. If Beecher's scenario is correct, our mtDNA data indicate that the ancestors of present-day northern forms came from pro-*subvirgatus* stock and, presumably, would have dispersed west to the shores of Glacial Lake Agassiz (and later to James Bay) only after the last glacial recession, less than 11,000 years ago. However, if the date of the separation of these clades is as old as 600,000 years, as we have estimated on the basis of the mtDNA data, Beecher's scenario is not supported. Rather, the older date supports Greenlaw's hypothesis that the pro-northern and pro-southern clades were separated during an earlier glacial advance, and that the ancestor to *A. c. subvirgatus* has secondarily colonized the Canadian maritimes and the

TABLE 5. Actual versus predicted group membership (numbers with percentages in parentheses) of male Sharp-tailed Sparrows from 11 samples.

Actual group	Predicted group (with mtDNA clone[a] below)										
	1 N	2 N	3 N	4 N	5 N	6 S	7 S	8 S	9 S	10 M	11 N
1 Saskatchewan (12)	7 (58.3)	2 (16.7)	0 (0)	1 (8.3)	2 (16.7)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
2 North Dakota (14)	2 (14.3)	10 (71.4)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	2 (14.3)
3 New Brunswick (17)	0 (0)	0 (0)	5 (29.4)	2 (11.8)	1 (5.9)	0 (0)	0 (0)	0 (0)	0 (0)	3 (17.6)	6 (35.3)
4 Attawapiskat (18)	1 (5.6)	0 (0)	0 (0)	8 (44.4)	4 (22.2)	0 (0)	0 (0)	0 (0)	0 (0)	1 (5.6)	4 (22.2)
5 Moosonee (20)	1 (5.0)	1 (5.0)	2 (10.0)	4 (20.0)	5 (25.0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (5.0)	6 (30.0)
6 Delaware (19)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	6 (31.6)	3 (15.8)	1 (5.3)	4 (21.1)	5 (26.3)	0 (0)
7 New Jersey (12)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	4 (33.3)	4 (33.3)	0 (0)	1 (8.3)	3 (25.0)	0 (0)
8 New York (14)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	6 (42.9)	1 (7.1)	4 (28.6)	2 (14.3)	1 (7.1)	0 (0)
9 Massachusetts (16)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	2 (12.5)	1 (6.3)	0 (0)	12 (75.0)	1 (6.3)	0 (0)
10 Maine (18)	0 (0)	0 (0)	3 (16.7)	0 (0)	0 (0)	2 (11.1)	1 (5.6)	0 (0)	5 (27.8)	7 (38.9)	0 (0)
11 Quebec (19)	0 (0)	0 (0)	2 (10.5)	2 (10.5)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (5.3)	14 (73.7)

[a] N = northern; S = southern; M = mixed.

TABLE 6. Identity of 20 Sharp-tailed Sparrows (*Ammodramus caudacutus*) from Popham Beach, Maine, based on mtDNA clade affiliation, principal-components analysis of 25 skeletal measures, and general plumage coloration.

Specimen	mtDNA type[a]	Plumage	PCA
11088	N	S	S
11089	N	N	N
11093	N	S > N	S
11094	N	N	S
11095	N	S	N
11098	N	N	N
11099	N	S > N	S
11101	N	N	N
11102	N	N	N
11104	N	N	S
11087	S	S	F[b]
11090	S	S > N	S
11091	S	S	S
11092	S	S	S
11096	S	S > N	S
11097	S	S	S
11100	S	S	F
11103	S	S	S
11105	S	S	S
11106	S	S	S

[a] N = "northern" (*A. c. subvirgatus*); S = "southern" (*A. c. caudacutus*).
[b] Female (thus, not analyzed).

northern Atlantic coast, perhaps following the last glacial recession. If this is correct, they now seem to be hybridizing in secondary contact with *A. c. caudacutus* in southern Maine, with evidence of introgression south into northern Massachusetts. Possibly the hybridization is asymmetrical, with southern females generally mating with southern males, but northern females apparently mating more randomly.

Taxonomic recommendations.—In terms of mtDNA phylogeny, the northern and southern forms of the Sharp-tailed Sparrow appear to be more closely related to one another than to any other species (i.e. they are sister groups; Zink and Avise 1990). Their closest living relative is either the Seaside Sparrow or LeConte's Sparrow (Zink and Avise 1990, Murray 1968). The magnitude of mtDNA sequence divergence between the two Sharp-tailed Sparrow clades is nearly identical to that between two distinct forms of the Seaside Sparrow (those that inhabit salt marshes of the Atlantic coast vs. those of the Gulf of Mexico; Avise and Nelson 1989), and is considerably lower than any mtDNA distances observed between conventionally recognized species of *Ammodramus* (Zink and Avise 1990).

Avise and Ball (1990) suggested that, in principle, historical population subdivisions worthy of formal taxonomic recognition should be evidenced by concordant partitions in the genealogies of multiple, independent (unlinked and nonepistatic) loci. Since mtDNA is uniparentally transmitted, it provides only one such genealogical tracing. Because detailed phylogenies for particular nuclear loci are currently unavailable for most species (including the Sharp-tailed Sparrow), searches for concordant patterns of divergence across loci will (for the time being) normally necessitate use of assayable "surrogates" for such complete genealogies. Such partial surrogates can include morphological or other traits, provided that they have a genetic basis and are not influenced solely by environmental conditions.

Northern and southern forms of Sharp-tailed Sparrows clearly differ in mtDNA phylogeny, coloration, size, and behavior. According to Avise and Ball (1990), such concordant differences in presumably independent genetically based attributes are likely to appear only when populations have existed as separate gene pools for reasonably long periods of time. Furthermore, they suggest that such arrays should be recognized formally as species if the gene-pool separations would be maintained in sympatry by intrinsic (genetically based) reproductive barries, whereas subspecies status is indicated if the gene-pool differences stem solely from historical geographic separations without attendant evolution of reproductive barriers. Thus, subspecies will typically be allopatric (unless secondary sympatry has been achieved very recently, or over a restricted portion of a species' range), whereas species may be either sympatric or allopatric.

In the current situation, the northern-versus-southern forms of Sharp-tailed Sparrow are largely allopatric. Taxonomic recognition is clearly indicated because of concordant differences, but whether the forms should be considered distinct subspecies or species depends on the degree to which intrinsic reproductive barriers are present. The only information currently available on this matter involves the population in southern Maine, where the ranges of the northern and southern forms overlap. About 60% of the assayed specimens from this locale

exhibit concordant assignment by morphology and mtDNA clade (Table 6) and, hence, appear not to be hybrids between northern and southern birds (well-defined genetic markers from the nuclear genome would be required to confirm this suspicion). However, other birds from this locale exhibit mixed assortments of mtDNA and morphological attributes (Table 6), strongly suggestive of some genetic exchange through hybridization. Furthermore, the appearance of one specimen from Massachusetts with northern mtDNA type (out of only six assessed), and of a second specimen with a northern plumage phenotype, raise the likelihood that some introgression has occurred beyond the current contact zone.

On balance, and in the absence of additional genetic information from nuclear loci, we conservatively recommend that the northern and southern forms of the Sharp-tailed Sparrow be considered subspecies, to be designated *A. c. nelsoni* and *A. c. caudacutus*, respectively. The former would encompass the previously recognized forms *A. c. nelsoni*, *A. c. alterus*, and *A. c. subvirgatus*, and the latter would encompass *A. c. caudacutus* and *A. c. diversus*. If further study provides stronger evidence for intrinsic reproductive barriers between these forms, we would then urge elevation to full species status.

We acknowledge one potential objection to this recommendation: populations defined by finer distinctions within either region (including some currently recognized subspecies) will go unnamed. Some ornithologists have argued that all distinguishable populations, even if identifiable by a single trait, warrant taxonomic recognition (Cracraft 1983, McKitrick and Zink 1988). However, within any species showing limited dispersal relative to range size, sufficiently detailed genetic inspection likely would reveal that virtually all populations would be "distinguishable" by at least some recently arisen mutations. Similarly, given sufficiently large sample sizes, statistically significant differences in size or coloration usually could be found between samples. For these and other reasons, Avise and Ball (1990) argued that concordant support from genetically independent traits would provide a meaningful guide to the more significant phylogenetic subdivisions within species. Such concordant support for finer within-region distinctions in the Sharp-tailed Sparrow is currently lacking (e.g. mtDNA and morphological data suggest very recent genetic contact between the previously recognized *A. c. diversus* and *A. c. caudacutus*).

On the positive side, our recommended taxonomic revision would have several desirable effects. First, it would provide formal recognition for a previously unappreciated, but major phylogenetic disjunction within the species. This recognition is fundamentally important in several arenas, including the reconstruction of the biogeographic history of the marshland sparrows, and formation of management plans should either taxon become threatened or endangered. Second, it would acknowledge the possibility of hybridization and introgression between these forms, and the desirability of further scientific study of these phenomena, particularly where the subspecies currently meet along the northeastern coast. Third, it would leave open the possibility for elevation to full species status if subsequent studies find that strong intrinsic reproductive barriers do indeed exist between the forms where they overlap.

Acknowledgments

We thank William S. Nelson for excellent assistance in the laboratory. We are indebted to Jon Greenlaw for providing specimens as well as useful observations on the biology of Sharp-tailed Sparrows; Joel Cracraft, Alvaro Jaramillo, Richard Johnston, Trudy Rising, Bob Zink, and Jon Greenlaw also made valuable comments on the manuscript. Work was supported by NSF grants to J.C.A., and NSERC grants to J.D.R.

Literature Cited

American Ornithologists' Union. 1957. Check-list of North American birds, 5th ed. Am. Ornithol. Union, Washington, D.C.

Avise, J. C., and W. S. Nelson. 1989. Molecular genetic relationships of the extinct Dusky Seaside Sparrow. Science 243:646–648.

Avise, J. C., J. Arnold, R. M. Ball, Jr., E. Bermingham, T. Lamb, J. E. Neigel, C. A. Reeb, and N. C. Saunders. 1987. Intraspecific phylogeography: The mitochondrial DNA bridge between population genetics and systematics. Annu. Rev. Ecol. Syst. 18:489–522.

Avise, J. C., and R. M. Ball. 1990. Principles of genealogical concordance in species concepts and biological taxonomy. Oxford Surv. Evol. Biol. 7:45–67.

AVISE, J. C., B. W. BOWEN, AND T. LAMB. 1989. DNA fingerprints from hypervariable mitochondrial DNA genotypes. Mol. Biol. Evol. 6:258–269.

BEECHER, W. J. 1955. Late-Pleistocene isolation in salt-marsh sparrows. Ecology 36:23–28.

BROWN, W. M., M. GEORGE, JR., AND A. C. WILSON. 1979. Rapid evolution of animal mitochondrial DNA. Proc. Natl. Acad. Sci. USA 76:1967–1971.

BRYSON, R. A. 1988. What the climatic past tells us about the environmental future. Pages 230–247 *in* Earth '88: Changing geographical perspectives. National Geographic Society, Washington, D.C.

CRACRAFT, J. 1983. Species concepts and speciation. Curr. Ornithol. 1:159–187.

DIXON, W. J. (ED.). 1983. BMDP statistical software. Univ. California Press, Berkeley.

DROUIN, J. 1980. Cloning of human mitochondrial DNA in *Escherichia coli*. J. Mol. Biol. 140:15–34.

HENNIG, W. 1966. Phylogenetic systematics. Univ. Illinois Press, Champaign.

GREENLAW, J. S. 1993. Behavioral and morphological diversification in Sharp-tailed Sparrows (*Ammodramus caudacutus*) of the Atlantic coast. Auk 110: 286–303.

LANSMAN, R. A., R. O. SHADE, J. F. SHAPIRA, AND J. C. AVISE. 1981. The use of restriction endonucleases to measure mitochondrial DNA sequence relatedness in natural populations. III. Techniques and potential applications. J. Mol. Evol. 17:214–226.

LANYON, S. M. 1987. Jackknifing and bootstrapping: Important "new" statistical techniques for ornithologists. Auk 104:144–146.

MCKITRICK, M., AND R. M. ZINK. 1988. Species concepts in ornithology. Condor 90:1–14.

MONTAGNA, W. 1942. The Sharp-tailed Sparrows of the Atlantic coast. Wilson Bull. 54:107–120.

MURRAY, B. G. 1968. The relationships of sparrows in the genera *Ammodramus, Passerherbulus,* and *Ammospiza* with a description of a hybrid between LeConte's × Sharp-tailed Sparrow. Auk 85:586–593.

NEI, M. 1987. Molecular evolutionary genetics. Columbia Univ. Press, New York.

NEI, M., AND W.-H. LI. 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases. Proc. Natl. Acad. Sci. USA 76:5269–5273.

NEI, M., AND F. TAJIMA. 1981. DNA polymorphism detectable by restriction endonucleases. Genetics 97:145–163.

PATTON, J. C., AND J. C. AVISE. 1983. An empirical evaluation of qualitative Hennigian analyses of protein electrophoretic data. J. Mol. Evol. 19:244–254.

POST, W., AND J. S. GREENLAW. 1982. Comparative costs of promiscuity and monogamy: A test of reproductive effort theory. Behav. Ecol. Sociobiol. 10:101–107.

RISING, J. D. 1987. Geographic variation in sexual dimorphism in size of Savannah Sparrows (*Passerculus sandwichensis*): A test of hypotheses. Evolution 41:514–524.

RISING, J., AND K. M. SOMERS. 1989. The measurement of overall body size in birds. Auk 106:666–674.

ROBINS, J. D., AND G. D. SCHNELL. 1971. Skeletal analysis of the *Ammodramus-Ammospiza* grassland sparrow complex: A numerical taxonomic study. Auk 88:567–590.

ROHLF, F. J. 1985. NT-SYS. Numerical taxonomy system of multivariate statistical programs. Tech. Rep. State Univ. New York, Stony Brook.

SHIELDS, G. F., AND A. C. WILSON. 1987. Calibration of mitochondrial DNA evolution in geese. J. Mol. Evol. 24:212–217.

SNEATH, P. H. A., AND R. R. SOKAL. 1973. Numerical taxonomy. W. H. Freeman, San Francisco.

SOKAL, R. R., AND F. J. ROHLF. 1981. Biometry, 2nd ed. W. H. Freeman, San Francisco.

SPSSX. 1986. SPSSX guide, 2nd ed. McGraw-Hill, New York.

SWOFFORD, D. L. 1993. PAUP: Phylogenetic analysis using parsimony, version 3.1. Computer program distributed by the Illinois Nat. Hist. Survey, Champaign.

WILSON, A. C., R. L. CANN, S. M. CARR, M. GEORGE, U. B. GYLLENSTEN, K. M. HELM-BYCHOWSKI, R. G. HIGUCHI, S. R. PALUMBI, E. M. PRAGER, R. D. SAGE, AND M. STONEKING. 1985. Mitochondrial DNA and two perspectives on evolutionary genetics. Biol. J. Linn. Soc. 26:375–400.

ZINK, R. M., AND J. C. AVISE. 1990. Patterns of mitochondrial DNA and allozyme evolution in the avian genus *Ammodramus*. Syst. Zool. 39:148–161.