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MORPHOLOGICAL ADAPTATION WITH NO MITOCHONDRIAL DNA DIFFERENTIATION IN THE COASTAL PLAIN SWAMP SPARROW

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ABSTRACT.—We estimated genetic differentiation between morphologically distinct tidal marsh populations of Swamp Sparrows (*Melospiza georgiana nigrescens*) and the more widespread inland populations (*M. g. georgiana* and *M. g. ericrypta*). The tidal marsh populations are consistently grayer with more extensive black markings (particularly in the crown), and their bills are larger. These differences are variously shared with other species of salt marsh birds and small mammals. We analyzed mitochondrial DNA sequences (5′ end of control region, COII/t-lys/ATPase8, and ND2) of Swamp Sparrows and found low levels of genetic variation and no evidence of geographic structure. These results suggest a rapid and recent geographic expansion of Swamp Sparrows from restricted Pleistocene populations. Morphological differentiation has occurred without long-term genetic isolation, suggesting that selection on the divergent traits is intense. The grayer and more melanistic plumage is probably cryptic coloration for foraging on tidal mud, which tends to be grayish as a result of the formation of iron sulfides, rather than iron oxides, under anaerobic conditions. *Received 14 May 1997, accepted 9 January 1998.*

VERTEBRATES SHOW CONSPICUOUS ADAPTATIONS to the distinct environment of tidal marshes. For example, diurnal terrestrial species tend toward grayish or blackish coloration (Grinnell 1913, Von Bloeker 1932, Greenberg and Droege 1990). In addition, emberizid finches often have relatively large bills compared with non-tidal populations (Greenberg and Droege 1990). Tidal marsh populations often are small and geographically restricted compared with inland populations. This leads to the question of how distinct morphological features evolve in these populations in the face of potential gene flow from larger inland populations. Few studies have addressed the degree of geographic genetic structuring in species with tidal and inland forms.

Swamp Sparrows (*Melospiza georgiana*) breed in tidal marshes of Delaware and Chesapeake bays and in inland fresh water wetlands within formerly glaciated areas and occur only sporadically south of the glacial line, inhabiting the few suitable postglacial bogs, brushy marshes, and wet meadows. Except for a few scattered populations, a hiatus extends between the inland and coastal Swamp Sparrow populations through the Piedmont of Maryland and Pennsylvania, leaving the only potential area of significant contact in northern New Jersey and southern New York State (Greenberg and Droege 1990: figure 1).

Coastal Plain Swamp Sparrows (*M. g. nigrescens*) have more extensive black coloration (e.g. almost three times the area of black in the crown), are generally grayer, and have bills that average 29% greater in overall volume than Swamp Sparrows from inland populations (Greenberg and Droege 1990). In contrast to the morphological differentiation between the estuarine and the closest freshwater populations, the interior subspecies (*M. g. ericrypta* and *M. g. georgiana*) are morphologically quite uniform across their ranges, with the two recognized subspecies exhibiting only slight differences in the degree of pale markings on the back.

Three possible scenarios can account for the evolution of the *nigrescens* subspecies: (1) tidal marsh populations have been genetically isolated from other populations for a substantial period of time; (2) selective pressures differ and are of sufficient magnitude to cause strong and rapid morphological divergence in the face

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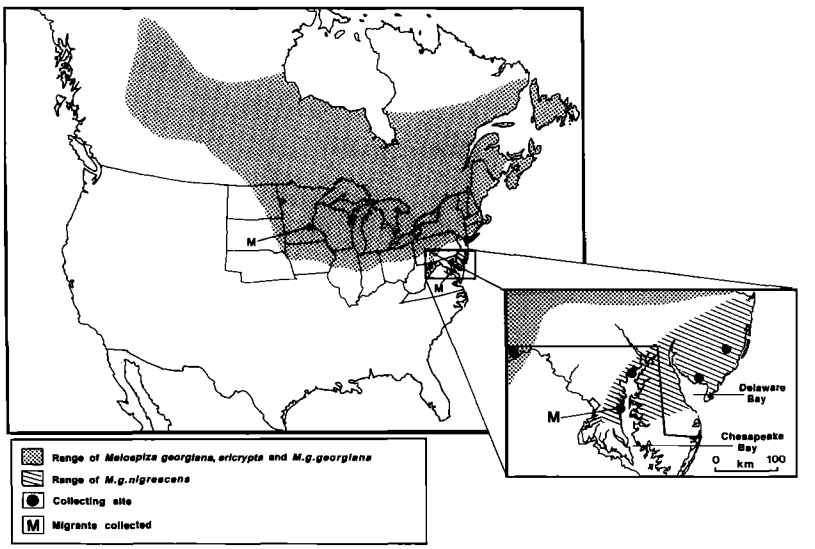

FIG. 1. Distribution of Swamp Sparrows and the *nigrescens* subspecies (AOU 1983, Greenberg and Droege 1990). M indicates sites where all specimens were non-*nigrescens* migrants. One migrant was also collected immediately north of Delaware Bay.

of continued gene flow from inland populations and/or despite recent range expansions that have not allowed sufficient time for mitochondrial DNA (mtDNA) divergence; and (3) morphological differences are environmentally induced (Von Bloeker 1932, James 1983, Price and Pavelka 1996). To examine these scenarios, we measured the degree of genetic differentiation in rapidly evolving regions of mtDNA between the estuarine (*M. g. nigrescens*) and inland populations (*M. g. ericrypta* and *M. g. georgiana*) of Swamp Sparrows.

METHODS

Field collections.—Adult and nestling *M. g. nigrescens* were collected during the breeding season (June and July) near Delaware Bay (Weeksville and Dividing Creek, Cumberland County, New Jersey, $n = 7$) and Chesapeake Bay (Black Marsh, Baltimore County, Maryland, $n = 4$; see Fig. 1). Members of inland populations were collected during the breeding season from Finzel Swamp, Garrett County, Maryland (*M. g. georgiana*, $n = 5$) and Clay County, Minnesota (*M. g. ericrypta*, $n = 2$) or during migration (Edgewater, Anne Arundel County, Maryland, $n = 7$; Dividing Creek, Cumberland County, New Jersey, $n = 1$; Minneapolis, Minnesota, $n = 3$). The subspecies identity (i.e. *nigrescens* versus non-*nigrescens*) for all migrants was verified based on the diagnostic features determined by Greenberg and Droege (1990). We could not ascertain whether migrants were *M. g. ericrypta* or *M. g. georgiana*; however, it is likely that the Maryland and Minnesota migrant samples represent birds that originated from eastern and more western parts of the breeding range, respectively. Adults were sacrificed, transported on dry ice to a freezer at −60°C, and ultimately prepared as tissue samples and study skins (see Greenberg and Droege 1990); nestlings were raised in captivity and prepared as study skins when they died.

Mitochondrial DNA analyses.—Twenty-nine specimens were analyzed: 11 *M. g. nigrescens*, 2 *M. g. ericrypta*, 5 *M. g. georgiana*, and 11 *M. g. georgiana* or *ericrypta* migrants. Sequences are available in Genbank (accession numbers AF038817 to AF038824 for control region, AF061648 to AF061651 for COII/t-lys/ATP8, and AF061652 for ND2). Tissues from ANSP were kept in DMSO buffer (Seutin et al. 1991) for a short period of transport. Tissues were digested in SET buffer (0.15M NaCl, 0.05M Tris, 1mM EDTA, pH 8) with proteinase K and SDS, extracted with phenol-chloroform, and centrifugally dialyzed (see Fleischer et al. 1994). We also purified mtDNA from two individuals (one *M. g. nigrescens* and one *M. g. georgiana*) with a sucrose step-gradient protocol (Spolsky and Uzzell 1984, Tarr and Fleischer 1993). We amplified and sequenced from the purified mtDNA to compare the sequences with those amplified from whole genomic DNA of the same birds. This procedure provided one way of identifying whether there may have been transpositions of mitochondrial genes to the nuclear genome.

We amplified sections of three different gene regions of mtDNA using the polymerase chain re-

action (PCR): (1) Control region (CR; 5′ end and part of central domain); amplifications were made using primers LGL2 and H417 (Tarr 1995), which amplify what is usually the most variable segment of the passerine control region (Marshall and Baker 1997). (2) Cytochrome oxidase subunit II (COII; 3′ end only) through tRNA-lysine (t-lys) and ATPase subunit 8 (ATP8); this region was amplified using primers CO2GQL (light strand, 3′ position 8929 of the *Gallus gallus* sequence) and A6MNH (heavy strand, 3′ position 9240 of *Gallus gallus* sequence; G. Seutin and E. Bermingham unpubl. data). (3) NADH dehydrogenase subunit 2 (ND2); amplifications were made using primers MetB (light strand, 3′ position 5233 of the *Gallus gallus* sequence) and TrpC (heavy strand, 3′ position 6342 of the *Gallus gallus* sequence; G. Seutin and E. Bermingham unpubl. data).

Amplification reactions for all primer sets included the following components in a 25-μL total reaction volume: 2.5 μL of Perkin-Elmer-Cetus 10X buffer, 0.8 mM dNTPs, 1.0 mM of each primer, 1 to 2 units of *Taq* polymerase, 2 mM of $MgCl_2$, and 20 to 50 ng of template DNA. Cycle parameters were 1 min at 94°C, 1 min at 50°C, and 2 min at 72°C, for 35 cycles. Amplified products were separated in 2% low-melting point agarose gels then excised and purified from the agarose using Quiagen. Between 250 and 500 ng of purified product was used in a dye-labeled terminator reaction with cycle amplification (ABI Prism™). Unincorporated nucleotides were removed by sephadex column and the product was concentrated under a vacuum. Products were loaded onto a 4.75% denaturing polyacrylamide gel and electrophoresed in an ABI 373 automatic sequencer. Sequences were run for both strands using each end primer for the CR and COII / t-lys / ATP8 regions, and the two strands were aligned and compared for discrepancies using SeqEd (Applied Biosystems Inc. 1992).

Verified sequences were aligned among individuals using SeqEd, and any differences among sequences were confirmed by close examination of associated chromatographs. Haplotypes were designated on the basis of identity of sequence for all gene regions. The numbers of differences among haplotypes were counted, and the proportional pairwise differences among sequences were averaged to calculate the nucleotide diversity (π from Nei 1987: equation 10.6, equivalent to θ, or $4N_e\mu$ at equilibrium in a neutral model). We also calculated the mean number of segregating sites (p_n of Nei 1987), which was then used in equation 10.1a of Nei (1987) to calculate θ.

A network was constructed among haplotypes based on the minimum number of substitutions between haplotypes. Kimura two-parameter corrected distances among haplotypes were calculated with PAUP* (Swofford 1996), and these were used to construct phylograms employing a neighbor-joining algorithm with a minimum evolution criterion in PAUP* (Swofford 1996). Unrooted networks based on cladistic (character) approaches were also constructed using PAUP* for comparison, although this method may not be appropriate for population studies. We conducted analyses of molecular variance (AMOVA 1.55; Excoffier 1995) by grouping sequences into four "populations" (i.e. Minnesota *M. g. georgiana* / *M. g. ericrypta*; Garrett County *M. g. georgiana*; migrant *M. g. georgiana*; and *M. g. nigrescens*). AMOVA analogues of F_{ST} called ϕ_{ST} were calculated among all groups and tested for significant departure from zero with nonparametric permutation methods (Excoffier et al. 1992). Average long-term rates of gene flow (N_e m) were estimated from these F_{ST} analogues using Nei (1987: equation 13.79).

RESULTS

DNA sequences.—We obtained up to 641-bp of sequence for two regions of mtDNA for each of 29 individuals: 330 bp from the 5′ end of the control region (274 bp of region I and 56 bp of the central domain) and a segment containing 86 bp of COII, 70 bp (all) of t-lys, and 155 bp (all but 6 bp) of ATP8 (311 bp total). In addition, we amplified and obtained sequence for 450 bp of ND2 from one individual each of *M. g. georgiana* and *M. g. nigrescens*. Thus, for one individual from each of two subspecies we have assessed a total of 1,091 bp of sequence from three genes.

By sequencing three genes located at distant points along the mtDNA molecule, each amplified with separate primer sets (most of which were designed from cloned avian mtDNA sequences; Desjardins and Morais 1990), we reduced the probability that we inadvertently amplified only nuclear copies of mitochondrial genes. Nuclear copies usually evolve much more slowly than mitochondrial versions (e.g. Sorenson and Fleischer 1996); thus, one might discover them because they are much less divergent than expected. In this case, all three regions showed relatively low variation, so the test was not persuasive. However, we also found no differences between sequences amplified from purified mtDNA and those amplified from genomic DNA preparations from the same individual.

We aligned the Swamp Sparrow sequences with those from other avian species to insure that they have no unusual features indicating they are pseudogenes. We aligned CR sequences (in SeqEd and then by eye) with those of two

TABLE 1. Nine variable sites from among 330 bp of the mitochondrial control region and 311 bp of COII/t-lys/ATP8 sequences from Swamp Sparrows. Numbers at top refer to base position in sequences used for each segment. The first six sites are in the control region, site 139 is in the t-lys, and the last two are in ATP8. For reference, site 17 is 12 bp into the control regions TAS, and site 139 is 54 bp from the start of tRNA-lys. MD = Baltimore Co., Maryland; GR = Garrett Co., Maryland; MN = Clay Co. or Minneapolis (migrants only), Minnesota; NJ = Cumberland Co., New Jersey; e = *M. g. ericrypta*; g = *M. g. georgiana*; n = *M. g. nigrescens*, mig = migrant bird.

Haplotype	17	76	151	181	184	307	139	290	304
1. n-NJ (5), n-MD (1), g-GR (1), e-MN (1), mig-MD (4), mig-NJ (1)	T	T	C	G	C	G	T	G	G
2. n-MD (2), g-GR (2), e-MN (2), mig-MD (1)	·	C	·	·	·	·	·	·	·
3. g-GR (2), mig-MN (1), mig-MD (1)	·	C	·	·	·	·	·	·	A
4. n-MD (1)	·	C	·	·	·	·	·	A	A
5. mig-MN (1)	·	C	·	A	T	·	·	·	·
6. mig-MD (1)	C	C	T	·	·	·	·	·	·
7. n-NJ (1)	·	·	·	·	·	·	C	·	·
8. n-NJ (1)	·	·	·	·	·	A	·	·	·

other oscine passerines: the Chaffinch (*Fringilla coelebs*, Fringillidae; Marshall and Baker 1997) and the Apapane (*Himatione sanguinea*, Drepanidinae; Tarr 1995). Alignments of these revealed relatively high sequence conservation between Swamp Sparrow CR and that of other passerines, and we found both the putative TAS element and the F Box (Tarr 1995, Marshall and Baker 1997). The protein-coding and t-RNA regions were aligned to chicken (*Gallus gallus*) and/or passerine sequences for the COII, t-lys, ATP8, and ND2 pieces, and no inappropriate stop codons or other unusual features were found.

DNA sequence variation.—Variation among Swamp Sparrow sequences was remarkably low, especially given the high substitution rates typical for these gene regions in other taxa. In 641 bp from 29 individuals, we found only nine variable nucleotide sites and eight haplotypes (Table 1). No haplotype differed by more than two substitutions from its most similar haplotype, and the maximum difference was four substitutions. Nearly all individuals (24 of 29) had one of three common haplotypes, two of which were represented by members of all three subspecies (Fig. 2). Thus, there was no obvious geographical or subspecific structure observable in the pattern of differentiation.

Six variable sites occurred in the control region, all of which contained transitions (two G:A and four C:T changes). We found no variable sites in the 86 bp of COII, one variable site in the 70 bp of t-lys, and two variable sites in the 155 bp of ATP8 (Table 1). All three sites had transitional changes (two A:G and one C:T), thus resulting in an overall ts:tv ratio of 9:0. Oddly, in the ATP8 gene the two changes were in first and second codon positions, each resulting in amino acid replacements (alanine:threonine and serine:asparagine). Both of these positions also differ between the Swamp Sparrow and other species. Lastly, we found no variable sites in 450 bp of ND2 for the two individuals sequenced.

The average number of segregating sites (p_n of Nei 1987) for the control region is 0.0182, and for the COII/t-lys/ATP8 pieces combined is 0.0097. The p_n combined for the 641 bp is 0.0140, which predicts a θ of 0.0036 (Nei 1987). The nucleotide diversity, π, for the two regions (i.e. the average pairwise number of nucleotide differences per site, and an alternative estimate of θ; Nei 1987) is 0.0021 ± 0.0011. This estimate of θ did not differ significantly from θ estimated from p_n above (Mann-Whitney $U = 65.5$, $P > 0.25$). These small values of θ indicate a low level of variation and suggest a relatively small long-term effective population size for the species, or recent expansion from a population bottleneck. The lack of a difference between the two estimates may reflect the low power of our test, or that the populations are in drift-mutation equilibrium.

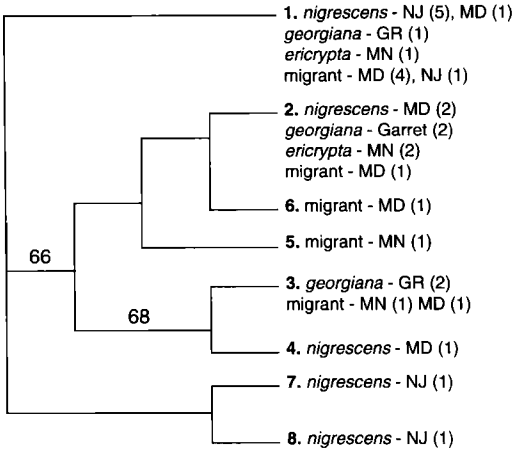

FIG. 2. Neighbor-joining tree (Saitou and Nei 1987) for the eight Swamp Sparrow haplotypes (n = 29 individuals) constructed in PAUP* from a matrix of pairwise distances among mtDNA sequences. Distances were corrected using the Kimura two-parameter method and were calculated from 641 bp of control region and COII/t-lys/ATP8. The tree was estimated in an exhaustive search with a minimum evolution criterion; a 500-replication bootstrap was applied to determine support for each node. Numbers on branches are majority-rule bootstrap percent support for the node. Analyses using cladistic-parsimony approaches yielded essentially the same tree topology, and the same two branches were supported in bootstrap consensus trees. MD = Baltimore Co., Maryland; GR = Garrett Co., MD; MN = Clay Co. or Minneapolis (migrants only), Minnesota; NJ = Cumberland Co., New Jersey; e = *M. g. ericrypta*; g = *M. g. georgiana*; n = *M. g. nigrescens*; mig = migrant bird. In these latter cases, the exact subspecies of an individual could not be determined, but it was not *M. g. nigrescens*. Note the lack of structure according to subspecies designation and geographic region.

The lack of geographic structure was further substantiated by the dendrogram based on the neighbor-joining algorithm with the Kimura two-parameter distances (Fig. 2). Results of the AMOVA (Excoffier et al. 1992, Excoffier 1995) revealed that nearly all of the variation in the Swamp Sparrow mtDNA haplotypes was distributed within, rather than among, populations (ϕ_{ST} = 0.057, P = 0.208 for permutation test of whether ϕ_{ST} differs significantly from zero). Assuming this value is equal to F_{ST} and using equation 13.79 of Nei (1987), we estimated the long-term mean N_em for the study populations to be 6.20 effective migrants per generation, a value consistent with little or no differentiation expected among populations. This value is estimated under the assumptions of neutrality of markers, a negligible mutation rate, and genetic equilibrium. The last of these may be violated if the Swamp Sparrow populations resulted from a relatively recent expansion and are not yet in equilibrium. Such a violation would imply that some part of the 6.2 migrants per generation was due to expansion and not to current levels of gene flow.

DISCUSSION

Lack of genetic variation and structure.—Our results support the findings of Balaban (1988) that there is little geographic variation among Swamp Sparrow populations in the southern portion of the species' range, including those living in tidal marshes. The low genetic variation (mean divergence of 0.21% in 641 bp of sequence) suggests that populations were very small prior to a rapid post-Pleistocene expansion. A minimum rate calibration for ATP8 sequences from drepanidines based on ages of Hawaiian islands is about 2.5% uncorrected sequence divergence per million years (R. Fleischer et al. unpubl. data, Tarr and Fleischer 1993). Applying this calibration to 0.1% mean divergence over just the ATP8 sequence implies the most recent common mitochondrial ancestor in Swamp Sparrows was only 40,000 years ago. Regardless of the potential errors of this rate, and of our estimate of ATP8 divergence in Swamp Sparrows, the coalescent point of Swamp Sparrow mtDNA haplotypes appears to be very recent.

The lack of geographic structure is to be expected for a migratory songbird whose range was almost entirely covered by ice during the Pleistocene, thus requiring a rapid expansion in the past 10 to 15,000 years. Even the tidal marshes of Delaware and Chesapeake bays are postglacial features resulting from the flooding of river mouths (Flint 1947). During the ice ages, one or a few scattered populations of Swamp Sparrows may have bred south of the glaciers. This is not surprising considering the generally xeric conditions of most of eastern North America during this period (Webb et al. 1993). Similar post-Pleistocene expansion scenarios have been suggested to explain low levels of mtDNA variation in other North Ameri-

can birds (Ball et al. 1988, Zink and Dittmann 1993, Fleischer et al. 1995, Zink 1996).

Origins of morphological differentiation of tidal marsh Swamp Sparrows.—The lack of genetic differentiation between the tidal marsh population and other populations and the existence of strong morphological differences could result if: (1) selection is relatively strong in these traits (plumage color and bill size) and sufficient heritable variation exists in these presumably polygenic traits, or (2) the differences are environmentally induced (Beebe 1907, Von Bloeker 1932, James 1983, Zink and Remsen 1986). Regarding sufficient heritable variation, mutation rates are effectively high in polygenic traits (10^{-3} or higher; Lande and Barrowclough 1987, Lynch 1988, Keightley et al. 1993), and variability would be recovered more rapidly for these traits than for mtDNA (effectively a single locus) following a population bottleneck.

These two hypotheses represent adaptive and nonadaptive explanations, respectively. Although we are unable to reject completely the environmental-induction hypothesis, we note that the color pattern and bill morphology of *M. g. nigrescens* reared from 3- to 4-day-old nestlings indoors under very different conditions (e.g. low humidity) and with very different food than would be found in a salt marsh were statistically indistinguishable from those collected in the wild (Greenberg and Droege 1990) but significantly different from *M. g. georgiana.* This includes plumage characteristics (i.e. proportion of black in the crown of males) acquired during the prealternate molt following nine months of captivity.

Experimental evidence suggests that geographic variation in bill shape has an environmental component (James 1983). However, with respect to differences in plumage, the distribution of rusty (i.e. phaeomelanic) versus gray to black (i.e. eumelanic) coloration appears to be under strong genetic control in birds (Smythe 1990). At this time, the adaptive explanation, at least for plumage variation, appears most parsimonious. For a diurnal, terrestrially foraging bird, selection on substrate color-matching should be intense, although we have no direct evidence on this point. Sharp differences in substrate color exist between tidal and freshwater marshes resulting from fundamental differences in chemical processes. Brackish or salt water has low levels of oxygen compared with fresh water, which causes soil iron to be reduced through anaerobic processes and forms grayish or blackish iron sulfides rather than rusty-colored iron oxides (T. Jordan pers. comm.). Terrestrial vertebrates exhibit coloration that camouflages them on gray and black exposed soils of tidal ponds and sloughs. Given that inland and coastal Swamp Sparrows are active on these strikingly divergent substrates, rapid selection for color pattern should occur despite an apparent absence of genetic structuring of the population. Although other salt marsh taxa exhibit much higher levels of sequence divergence, and hence apparently have been isolated in this habitat for longer periods (e.g. Large-billed Savanna Sparrow [*Passerculus sandwichensis rostratus*], Zink et al. 1991; Seaside Sparrow [*Ammodramus maritimus*], Avise and Nelson 1989; Saltmarsh Sharp-tailed Sparrow [*A. caudacutus*], Rising and Avise 1993; Clapper Rail [*Rallus longirostris*], R. Fleischer unpubl. data), the Swamp Sparrow estimates show that morphological adaptation to this ecologically distinctive habitat can be rapid, can occur in the face of substantial gene flow, or both.

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Literature Cited

American Ornithologists' Union. 1983. Checklist of North American birds, 6th ed. American Ornithologists' Union, Washington, D.C.

Applied Biosystems Incorporated. 1992. SeqEd, version 1.03. Foster City, California.

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

Balaban, E. 1988. Cultural and genetic variation in the Swamp Sparrow. Behaviour 195:250–289.

Ball, R. M. Jr., S. Freeman, F. C. James, E. Bermingham, and J. C. Avise. 1988. Phylogeographic population structure of Red-winged Blackbirds assessed by mitochondrial DNA. Proceedings of the National Academy of Sciences USA 85:1558–1562.

Beebe, W. 1907. Geographic variation in birds with

special reference to the effects of humidity. Zoologica 1:9–41.

DESJARDINS, P., AND R. MORAIS. 1990. Sequence and gene organization of the chicken mitochondrial genome: A novel gene order in higher vertebrates. Journal Molecular Biology 212:599–634.

EXCOFFIER, L. 1995. AMOVA 1.55 (Analysis of Molecular Variance). Computer program. University of Geneva, Geneva, Switzerland.

EXCOFFIER, L., P. SMOUSE, AND J. M. QUATTRO. 1992. Analysis of molecular variance inferred from metric differences among DNA haplotypes: Application to human mitochondria DNA restriction data. Genetics 131:479–491.

FLEISCHER, R. C., C. L. TARR, AND T. K. PRATT. 1994. Genetic structure and mating system in the Palila, an endangered Hawaiian honeycreeper, as assessed by DNA fingerprinting. Molecular Ecology 3:383–392.

FLINT, R. F. 1947. Glacial geology and the Pleistocene epoch. John Wiley and Sons, New York.

GREENBERG, R., AND S. DROEGE. 1990. Adaptations to tidal marshes in breeding populations of the Swamp Sparrow. Condor 92:393–404.

GRINNELL, J. 1913. The species of the mammalian genus *Sorex* of west-central California with a note on the vertebrate palustrine fauna of the region. University of California Publications in Zoology 20:179–205.

JAMES, F. C. 1983. Environmental component of morphological differentiation in birds. Science 221: 184–186.

KEIGHTLEY, P. D., T. F. C. MACKAY, AND A. CABALLERO. 1993. Accounting for bias in estimates of the rate of polygenic mutation. Proceedings of the Royal Society of London Series B 253:291–296.

LANDE, R., AND G. F. BARROWCLOUGH. 1987. Effective population size, genetic variation and their use in population management. Pages 87–123 *in* Viable populations for conservation (M. Soulé, Ed.). Cambridge University Press, New York.

LYNCH, M. 1988. The rate of polygenic mutation. Genetic Research 51:137–148.

MARSHALL, H. D., AND A. J. BAKER. 1997. Structural conservation and variation in the mitochondrial control region of fringilline finches (*Fringilla* spp.) and the Greenfinch (*Carduelis chloris*). Molecular Biology and Evolution 14:173–184.

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

PRICE, T., AND M. PAVELKA. 1996. Evolution of a colour pattern: History, development and selection. Journal of Evolutionary Biology 9:452–470.

RISING, J. D., AND J. C. AVISE. 1993. Application of genealogical-concordance principles to the taxonomy and evolutionary history of the Sharp-tailed Sparrow (*Ammodramus caudacutus*). Auk 110:844–857.

SAITOU, N., AND M. NEI. 1987. The neighbor-joining method: A new method for reconstructing phylogenetic trees. Molecular Biology and Evolution 4:1406–1425.

SEUTIN, G., B. N. WHITE, AND P. T. BOAG. 1991. Preservation of avian blood and tissue samples for DNA analyses. Canadian Journal of Zoology 69: 82–90.

SMYTHE, J. R. 1990. Genetics of plumage, skin and eye pigmentations in chickens. Pages 109–160 *in* Poultry breeding and genetics (R. D. Crawford, Ed.). Elsevier Science, Amsterdam, The Netherlands.

SORENSON, M. D., AND R. C. FLEISCHER. 1996. Multiple independent transpositions of mitochondrial DNA control region sequences to the nucleus. Proceedings of the National Academy of Sciences USA 93:1539–1543.

SPOLSKY, C., AND T. UZZELL. 1984. Natural interspecies transfer of mitochondrial DNA in amphibians. Proceedings of the National Academy of Sciences USA 81:5802–5805.

SWOFFORD, D. 1996. PAUP*. Sinauer Associates, Sunderland, Massachusetts.

TARR, C. L. 1995. Amplification and determination of mitochondrial control region sequences in oscine passerines. Molecular Ecology 4:527–529.

TARR, C. L., AND R. C. FLEISCHER. 1993. Mitochondrial-DNA variation and evolutionary relationships in the Amakihi complex. Auk 110:825–831.

VON BLOEKER, J.C., JR. 1932. Three new mammal species from salt marsh areas in southern California. Proceedings of the Biological Society of Washington 45:131–138.

WEBB T. III, P. J. BARTLEIN, S. P. HARRISON, AND K. J. ANDERSON. 1993. Vegetation, lake levels and climate in eastern North America for the past 18,000 years. Pages 415–467 *in* Global climates since the last glacial maximum (H. E. Wright, Ed.). University of Minnesota Press, Minneapolis.

ZINK, R. M. 1996. Comparative phylogeography in North American birds. Evolution 50:308–317.

ZINK, R. M., AND D. L. DITTMANN. 1993. Gene flow, refugia, and evolution of geographic variation in the Song Sparrow (*Melospiza melodia*). Evolution 47:717–729.

ZINK, R. M., D. L. DITTMANN, AND S. W. CARDIFF. 1991. Mitochondrial DNA variation and the taxonomic status of the Large-billed Savannah Sparrow. Condor 93:1016–1019.

ZINK, R. M., AND J. V. REMSEN, JR. 1986. Evolutionary processes and patterns of geographic variation in birds. Current Ornithology 4:1–69.

Associate Editor: R. M. Zink