MITOCHONDRIAL-DNA VARIATION IN THE POLYTYPIC ALASKAN SONG SPARROW

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ABSTRACT.—Previous studies of mitochondrial-DNA (mtDNA) variation in the polytypic Song Sparrow (Melospiza melodia) have not found geographic structure of haplotype distributions. We used restriction-fragment analysis of mtDNA to analyze geographic variation of Song Sparrows in Alaska, where phenotypic differentiation between populations is greatest. We examined 42 individuals, representing five subspecies, from island and mainland localities. In addition, we examined two specimens each from Washington and California. An average of 55 sites per individual distinguished 12 mtDNA haplotypes, which were all closely related (d = 0.001–0.008). Geographic structure was evident in the distribution of haplotype frequencies, but not in their phylogenetic affinities. Received 21 January 1991, accepted 1 August 1991.

THE STUDY of morphological variation between allopatric populations of birds has led to inferences about microevolutionary processes based on the rarely tested assumption that the measured traits have a large additive genetic variance and, therefore, indicate at some level the genetic structure of a population (Mayr 1942, 1963). Consequently, multiple subspecies have been named for many North American bird taxa based on geographic variation in morphology (AOU 1957). Attempts to describe the genetic structure of avian populations more directly using allozymes have been stymied by the relative lack of population differentiation in birds compared with other vertebrates, presumably because of their potential for great vagility via flight (Barrowclough 1983).

In genetic terms, population differentiation will occur only if the effective population size is small relative to the level of gene flow. Analysis of mitochondrial-DNA (mtDNA) variation has proven to be a sensitive molecular assay of intraspecific genetic variation (Wilson et al. 1985, Avise et al. 1987, Shields and Wilson 1987b) in part because the effective population number for mtDNA genes is reduced (Birky et al. 1983).

Mitochondrial DNA is maternally inherited without recombination (Giles et al. 1980). Because of this uniparental inheritance, the evolutionary effective population size for mitochondrial genes is expected to be equal to the number of females under the neutral-mutation theory. Assuming a sex ratio of 1:1, the mean time to fixation or loss of mitochondrial mutations is one-half that for nuclear genes (Birky et al. 1983). The difference in effective-population number between mitochondrial and nuclear genes means that mitochondrial genes can be subdivided in a population with migration rates that result in panmixis for nuclear genes. In addition, sequence evolution in mtDNA is rapid compared to single-copy nuclear loci so that sequence differences will accumulate in mtDNA over shorter divergence times (Brown et al. 1979).

Application of mtDNA analysis to birds at the intraspecific level has revealed genetic structure with a variety of geographic patterns and degrees of divergence (Avise and Ball 1991). Two of the more extensive surveys provide a useful comparison. Red-winged Blackbirds (Agelaius phoeniceus) from across the continent possessed many closely related clones of mtDNA (the maximum genetic distance p = 0.008) with limited geographic structure (Ball et al. 1988). In contrast, the Seaside Sparrow (Ammodramus maritimus) had relatively large genetic distances (maximum p = 0.013) between Atlantic and Gulf Coast populations, but within each of those populations, p averaged only 0.002 and clonal diversity was low (Avise and Nelson 1989). This discontinuous pattern of mtDNA variation presumably results from high levels of historical contact among populations within each region (similar to that in Red-winged Blackbirds), but not between regional populations due to separation by a long-term zoogeographic barrier (Avise et al. 1987, Avise and Nelson 1989). Both species are polytypic as...
judged by the number of subspecies recognized (23 and 9, respectively; AOU 1957).

The Song Sparrow is a polytypic species that occupies a diversity of habitats across North America. Currently, 31 subspecies are recognized based on morphometric and plumage characters (AOU 1957). Song Sparrows representing several subspecies from the western United States have been examined for mtDNA variation by Ball (1990) and Zink (1991). Both studies yielded a high diversity of mtDNA haplotypes, but all types were closely related and did not show the geographic structure expected if haplotypes were taxon-specific. This is similar to the pattern of variation present in Red-winged Blackbirds (Ball et al. 1988).

We report on mtDNA variation in Alaskan Song Sparrows, where morphological differentiation between subspecies is most extreme (Gabrielson and Lincoln 1951, Aldrich 1984). There are at least two reasons why mtDNA haplotype distributions might corroborate subspecific distinctions based on phenotypic characters in Alaska when they have not in the western United States. We included birds from the Kenai Peninsula population that are at least partly migratory and samples from insular populations (Kodiak and the Aleutians) that are resident year round (Gabrielson and Lincoln 1951). Also, other bird species that have a subspecies boundary between the Aleutian Islands and mainland United States—Rosy Finch (Leucosticte arcata), Water Pipit (Anthus spinoloeta), Snow Bunting (Plectrophenax nivalis), Rock and Willow ptarmigan (Lagopus mutus and L. lagopus), Winter Wren (Troglodytes troglodytes), and Fox Sparrow (Passerella iliaca)—raise the possibility that vicariant events have shaped the geography of variation in these species.

**METHODS**

We collected 46 Song Sparrows, including 42 from eight localities in Alaska, and 2 each from Washington (Lewis Co.) and California (Los Angeles Co.; Fig. 1). Birds were collected during the nesting season (May through July), except for three in August from Girdwood (Turnagain Arm), and four in September from Kodiak. One Lincoln’s Sparrow (Melospiza lincolnii) was collected in Fairbanks, Alaska and used as an outgroup. Except for two birds transported alive, samples of liver, kidney, heart, gonads and breast muscle were immediately frozen or, in most cases, transported in MSB/Ca**2+/EDTA buffer on wet ice for no more than nine days (Lansman et al. 1981). Mitochondrial DNA was isolated using the methods of Shields and Wilson (1987a), except that the final purification by ultracentrifugation was done in two 10-h runs at 100,000 rpm (Carr and Griffith 1987).

Several restriction endonucleases were screened with DNA from a subsample of birds and rejected because they gave poor digestion results (i.e. partial digestion: BstNI I, Kpn I), or produced too many fragments to resolve accurately (Alu I, Hae III, Hinfl I). These latter four-base enzymes gave a general impression of homogeneity across samples, so we used one 4.0-base, two 4.6-base, and five 5.3-base enzymes in an attempt to maximize the number of fragments examined with a limited amount of DNA. The fragments were end-labeled with 32P or 35S and separated in 1.2 to 2.0% agarose, or long 3.5% polyacrylamide gels. No attempt was made to visualize fragments less than 0.4 kb except with BstU I digestions. Fragment sizes were estimated graphically from semilogarithmic standard curves using Hind III digested lambda or PM2 phage DNAs as size standards on each gel.

Each individual’s multienzyme, composite haplotype was represented by eight letters. Each letter indicated which fragment pattern was detected using a particular restriction enzyme. Because mtDNA is inherited as a single linkage group, each composite haplotype was treated as an allele to estimate *F_s* (Wright 1978) using a program written by G. Barrowclough. The maximum-likelihood method was used to calculate pairwise values for the number of substitutions for each nucleotide site and lineage from fragment presence/absence data (*d*; Nei and Tajima 1983; comparable to *p* of Upholt 1977). This was done using the program RESTSITE, which calculates values for each class of enzyme separately and weights the values by the relative number of fragments they generate (Nei and Miller 1990). Haplotype diversity (analogous to heterozygosity in a single nuclear gene with multiple alleles) was calculated as

\[(n/n-1)(1 - \Sigma f_i^2)\]

where *f_i* is the frequency of the *i*th haplotype (Nei 1987).

Phenetic clustering of composite haplotypes from a matrix of *d*-values was done using the unweighted pair-group method (UPGMA; Sheath and Sokal 1973) to produce phenograms under the assumption of a uniform evolutionary rate along all branches. Dollo- and Wagner-parsimony analyses of site and fragment data were done using PAUP 3.0 provided by D. Swoford.

**RESULTS**

The eight endonucleases produced 66 restriction fragments from the mtDNA of Song Sparrows, with an average of 55 per individual (Table 1). A subset of 11 birds (3 from islands and 8 from the Alaska mainland) that yielded a higher proportion of purified mtDNA than the
other birds was analyzed by a separate digestion with *Hinf* I and *Alu* I. The number of additional fragments produced by these enzymes was about 60, which would bring the total fragments analyzed for each of these individuals to about 126 (fragments that were partially digested prevented an exact count). The general pattern for these additional digests was homogeneity. Therefore, we are not convinced that the use of additional enzymes would substantially change the genetic-distance estimates among these Song Sparrows.

Five of the eight other enzymes produced fragment patterns in which the distribution of fragment sizes (0.5-7 kb) was conducive to a relatively precise estimate of genome size. Our estimate of 17.1 ± 0.5 kb (mean ± SE) for Song Sparrow mtDNA is within the range reported for other bird species (16.3-17.3 kb; Shields and Helm-Bychowski 1988).

Twelve composite haplotypes were found among the 46 Song Sparrows (Table 2). Individuals with the same composite haplotype were considered to be members of the same mtDNA clone. Unscorable results were obtained from the *BstU* I digestion of one Seldovia specimen and the *Hinc* II digestion of one specimen each from Washington and California. Because of analytical requirements, the most common fragment pattern found in other individuals from the same sample was substituted for the missing data.

Within Alaska, a nonuniform distribution of clones is apparent (Fig. 1) in which C is the only clone found in both the mainland (Kenai Peninsula) and island populations. Excluding Kenai postbreeders, we computed an *F*ₚₚ of 0.50 (corrected for sampling error) among all Alaskan localities except Amak (*n* = 1). This value is significantly different from zero (*X²* = 114, *df* = 36, *P* < 0.001; Workman and Niswander 1970), indicating that one-half of the mtDNA clonal diversity in Alaska is distributed among the seven localities. In contrast to the geographic structure found within Alaska, one of the two clones from Washington (A) was also the predominant clone in the Aleutian Islands, and the other (J) was shared with a postbreeding bird from the Kenai Peninsula sample.

Haplotype diversity was 0.77 in Alaskan Song Sparrows (Kenai postbreeders excluded), and 0.82 in the entire sample. All of the island samples combined (*n* = 24) had a haplotype diversity of 0.52 compared to 0.81 estimated from combined mainland samples (*n* = 15) with postbreeders excluded. Genetic-distance estimates among Song Sparrow clones were all low, ranging from 0.001 to 0.008 (*d* = 0.004 ± 0.002).
TABLE 1. Fragment sizes (in kilobases) generated using eight restriction enzymes on 46 Song Sparrows and one Lincoln Sparrow. Letters refer to haplotype designations given in Table 2, except L, which refers to Lincoln Sparrow.

<table>
<thead>
<tr>
<th>Restriction enzyme</th>
<th>Fragment sizes</th>
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<tbody>
<tr>
<td>Ava</td>
<td>A&amp;L: 1.20, 2.25, 1.7, 1.26, 0.6, 0.5. B: 3.5, 3.1, 2.75, 2.0, 1.7, 1.5, 1.3, 1.26, 0.6, 0.5. C: 3.5, 3.1, 2.75, 2.0, 1.7, 1.5, 1.3, 1.26, 0.6, 0.5. D: 3.5, 3.1, 2.75, 2.0, 1.7, 1.3, 1.26, 0.6, 0.5. E: 3.5, 3.1, 2.75, 2.0, 1.3, 1.26, 1.22, 0.6, 0.55, 0.5. F: 4.9, 3.1, 2.75, 2.0, 1.26, 1.22, 0.6, 0.55, 0.5. L: 6.3, 3.1, 1.8, 1.26, 1.22, 0.91, 0.72, 0.61, 0.55, 0.54, 0.49.</td>
</tr>
<tr>
<td>Ban</td>
<td>A: 3.2, 3.0, 2.8, 2.5, 1.95, 1.85, 1.2, B: 4.5, 3.2, 3.0, 2.8, 1.85, 1.2. L: 4.5, 3.2, 2.1, 1.55, 1.2, 0.95, 0.8, 0.64.</td>
</tr>
<tr>
<td>BstU I</td>
<td>A&amp;L: 5.9, 4.85, 2.58, 1.43, 9.3, 8.4, 6.2, 2.9, 2.4, 1.9. B: 5.9, 4.85, 2.58, 1.43, 9.3, 8.4, 6.2, 3.5, 2.9, 1.9.</td>
</tr>
<tr>
<td>BstY I</td>
<td>A: 10.0, 3.3, 1.7, 1.6, 0.8. B: 7.0, 3.3, 2.9, 1.7, 1.6, 0.8. C: 10.0, 3.65, 1.7, 1.6, 0.8. L: 10.0, 5.0, 1.85, 0.8.</td>
</tr>
<tr>
<td>Hinc II</td>
<td>A: 10.0, 3.1, 2.78, 1.48, 0.61, 0.56. L: 10.0, 3.1, 2.78, 1.75, 0.9, 0.61.</td>
</tr>
<tr>
<td>Nci I</td>
<td>A: 5.5, 2.05, 1.65, 1.43. L: 7.8, 3.8.</td>
</tr>
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</table>

Between Song Sparrow clones and the Lincoln Sparrow, $d$ ranged from 0.045 to 0.076 ($\bar{x} = 0.058 \pm 0.010$). Cluster analysis indicated that the geographic structuring of clone frequencies has little phylogenetic basis (Fig. 2). Closely related clones are not necessarily proximal to each other geographically as with Aleutian clone A and California clone L.

Differences between Song Sparrow fragment patterns generated by each enzyme could be explained by gains or losses of one to three sites (e.g. Fig. 3), which allowed analysis of a site presence/absence matrix. Parsimony methods did not resolve evolutionary relationships between clones. Bootstrap confidence intervals in Dollo and Wagner trees from site data were all below 50%, and the consensus of fragment-based Wagner trees lacked any phylogenetic information. Convergent or parallel evolution of restriction sites was indicated by a network linking Ava II restriction patterns based on site-change relationships (Fig. 4). The closed loop in the network means that a particular restriction pattern could evolve independently from two adjacent patterns regardless of the direction of evolution through the network (Lansman et al. 1983). Because 6 of the 12 clones differ only by Ava II restriction sites (Table 1), the ability of parsimony analyses to unambiguously relate the 12 clones seems to have been limited by homoplasy.

### DISCUSSION

Haplotype diversity of Song Sparrows in Alaska is comparable to that found in the northwestern United States (0.73, $n = 11$; Ball 1990) and southwestern United States (0.87, $n = 27$; Zink 1991). The Adak Island sample, which was the largest for island localities ($n = 14$), had a haplotype diversity of 0.27. Samples from the other island localities were too small to accurately represent haplotype diversity, but all were monomorphic, leading to a low combined haplotype diversity for islands relative to the mainland samples. The most likely explanation for this difference is that a bottleneck in population size occurred during colonization of the islands. Because it is haploid and transmitted maternally, mtDNA is sensitive to founder effects. A similar pattern and level of haplotype diversity
was observed in deer mice (*Peromyscus maniculatus*) from the Channel Islands of California (Ashley and Wills 1987).

There does not appear to be geographic segregation of mtDNA lineages in Alaskan Song Sparrows, which would be expected if currently recognized subspecies had been separated by long-term historical barriers to gene flow. This conclusion is provisional because we analyzed only 1.8% of the mtDNA genome (300 nucleotides), and because the mtDNA phylogeny represents the genealogy of a single linkage group (gene) within an organismal phylogeny composed of many gene genealogies. Nevertheless, there is clearly no large genetic distance between any of the Song Sparrow clones, despite examination of subspecies with very divergent phenotypes (Gabrielson and Lincoln 1951). Our result corroborates studies of this species in the contiguous United States involving phenotypically less divergent subspecies (Ball 1990, Zink 1991).

When clonal frequencies are analyzed irre-
Fig. 4. Network connecting six fragment patterns generated with Ava II. Arrows indicate direction of a single-site gain and not necessarily direction of evolution.

respective of their genetic distance, geographic structure becomes apparent. Our $F_{st}$ of 0.5 contrasts with 0.04 for mtDNA in Song Sparrows of the western United States (Zink 1991). It is mostly the intermediate distribution of clone C between the mainland and island localities that prevents $F_{st}$ from approaching 1.0 when calculated for Alaskan birds (Fig. 1). Clone frequencies are imprecisely estimated for localities with small sample sizes, but the order-of-magnitude difference in estimates of $F_{st}$ for Song Sparrows suggests that there has been limited historical gene flow between the mainland and island populations relative to that between mainland populations in the contiguous United States (Zink 1991).

It is not clear from our results whether: (1) isolation by distance limits gene flow as suggested by the distribution of clone C; or (2) differences in migratory behavior between the island and mainland populations, or patchiness of habitat, contribute to population differentiation. If Washington shares clones with Alaska because of gene flow, then isolation by distance is unlikely in Song Sparrows. Dispersal distances are generally small in Song Sparrows (Arcese 1989), however, and the two Washington clones (A and J) are more easily explained by convergence, since each differs from the other clones only at Ava II sites (Table 1).

There appear to be barriers to gene flow between island and mainland populations of Alaskan Song Sparrows that are more severe than barriers of some populations in the contiguous United States, but not effective enough, or ancient enough, to have produced evolutionary deep branches in the intraspecific mtDNA phylogeny. Shallow mtDNA branch lengths suggest that phenotypic differences used to define the subspecies have evolved fairly rapidly, or that they are environmentally induced. The events or conditions that have produced phenotypic differences and differentiation of mtDNA clone frequencies among Alaskan Song Sparrow populations may be responsible for a similar pattern of intraspecific variation in other bird species in Alaska. Comparison of these species would help clarify the processes responsible for differentiation of island and mainland populations in Alaska.

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