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MITOCHONDRIAL DNA VARIATION AND THE TAXONOMIC STATUS OF THE LARGE-BILLED SAVANNAH SPARROW[1]

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The Savannah Sparrow (*Passerculus sandwichensis*) is widely distributed in North America (AOU 1983). The species includes several phenotypically distinct and geographically restricted forms, some of which were once considered species, as well as a more "typical" or familiar form that is found throughout much of the continent (van Rossem 1947, AOU 1957). We assessed mitochondrial DNA (mtDNA) differentiation between one of the divergent forms, the large-billed Savannah Sparrow (*P. s. rostratus*), and representatives of more "typical" forms of the species. Although van Rossem (1947) proposed that Savannah Sparrow morphology changed gradually from typical (interior North American forms) to large-billed forms (coastal Mexican forms), we suggest that the large-billed Savannah Sparrow is a distinct taxon and not merely the end-point of a gradual cline. The mtDNA of *rostratus* differed considerably from that of "typical" Savannah Sparrows. Although we do not plan an extensive survey of mtDNA variation in the Savannah Sparrow at this time, we suggest that further study will support our hypothesis of species status for *rostratus*.

The large-billed Savannah Sparrow, once considered a species (AOU 1931), differs (Bent 1968) from more typical Savannah Sparrows in having: a thicker and longer bill with a more decurved culmen; reduced dorsal streaking and an absence or near absence of yellow lores and supercilium (contrary to some field guides); darker tarsi and toes; different rectrix shape; proportionately shorter wings; and, larger body size. The bill differences are particularly striking, especially considering that bill size and shape vary little among other Savannah Sparrow populations (Rising 1987). Furthermore, after breeding in salt marshes along the northern and eastern coasts of the Gulf of California many adults and immatures migrate north to winter in saline marshes in southwestern California, unlike other Savannah Sparrows, which migrate south or are sedentary. Migratory behavior likely has a genetic basis, which suggests genetic differences in addition to those controlling morphological variation.

MtDNA analysis is being used with increasing frequency to document genetic variation within avian species (Ball et al. 1988, Avise and Nelson 1989, Shields 1990, Fleischer et al. 1991, Moore et al. 1991, Zink et al. 1991b). In contrast to most allozymic analyses, mtDNA surveys sometimes reveal considerable differentiation among closely spaced North American bird populations (Avise and Nelson 1989, Fleischer et al.

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1991, Zink 1991). We examined *P. s. rostratus* to discern its level of mtDNA distinctness and to contribute to the mtDNA data base on the geography of mtDNA variation in birds.

SPECIMENS AND MOLECULAR METHODS

Three *P. s. rostratus* were collected from the south edge of the Salton Sea, Imperial County, California, where they were wintering with "typical" Savannah Sparrows. By "typical" we mean that the specimens were not referable to the distinct forms such as those from the Aleutian Islands (*sandwichensis*), Labrador (*labradorius*), coastal southern California and northern Baja California (*beldingi*), Sable Island off Nova Scotia (*princeps*), other coastal Mexican taxa (*anulus, guttatus, magdalenae, atratus*), or San Benito Island (*sanctorum*) (AOU 1957, van Rossem 1947). That is, "typical" refers to the main continental populations of Savannah Sparrows in which geographic variation is limited (Rising 1987). Our "typical" specimens were collected on their wintering grounds in California (specimens 4, 5, and 6 in Table 1) and Louisiana (specimens 7–11); these appeared to include prairie and western forms of the weakly differentiated "typical" group (Rising, pers. obs.). In addition, data on mtDNA variation for four additional "typical" wintering Savannah Sparrows taken in Louisiana were added from a study by Zink and Avise (1990). Methods for preservation of tissues, isolation and recovery of mtDNA, restriction endonuclease analysis of mtDNA, and agarose gel electrophoresis follow Lansman et al. (1981) and Zink (1991). An example of a restriction enzyme fragment profile is given in Figure 1. We determined the size of each mtDNA fragment by comparison with a molecular size standard (the 1 "kb" ladder purchased from Bethesda Research Laboratories). From the pattern of fragments we inferred the distribution of restriction sites within and between individuals. Each individual was scored for the presence and absence of restriction fragments and restriction sites, the latter of which were used to infer the percentage nucleotide divergence (p) between mtDNA haplotypes (Nei and Li

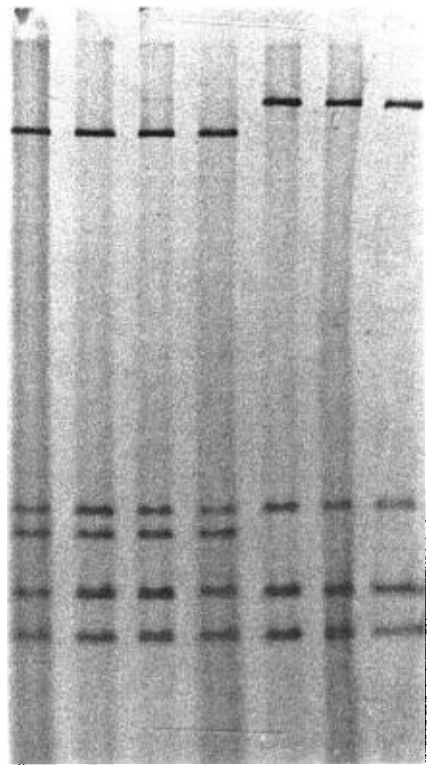

FIGURE 1. Autoradiograph of mtDNA digested with the restriction endonuclease *Ava* I, which reveals one restriction site difference between *rostratus* and "typical" Savannah Sparrows. Lanes 1 (left) to 4 depict "typical" Savannah Sparrows and 5 to 7 depict the three *rostratus*. Sizes of fragments in "typical" Savannah Sparrows are: 8,800 base pairs (top band), 2,400, 2,200, 1,900, and 1,600.

TABLE 1. Summary of restriction fragment profiles for Savannah Sparrows. Each letter refers to a distinct fragment profile for the following endonucleases: *Ava* I, *Nde* I, *Ava* II, *Nci* I, *Ban* II, *Bgl* I, *Bcl* I, *Bgl* II, *Cfo* I, *Kpn* I, *Eco*R I, *Hin*d III, *Pst* I, *Bam*H I, *Pvu* II, *Stu* I, *Sal* I, *Xba* I, *Hinf* I, and *Sst* II. Dashes in specimens 8–11, examined by Zink and Avise (1990), refer to endonucleases not examined. Dots indicate the "A" pattern.

Specimen	Haplotype																					Clone
Large-billed Savannah Sparrows																						
1	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	1
2, 3	.	.	.	.	.	.	B	.	.	.	.	.	.	.	.	.	.	.	.	.	.	2
"Typical" Savannah Sparrows																						
4, 7	B	.	B	B	B	B	.	B	B	.	.	.	.	B	.	B	.	.	B	.		3
5	B	.	C	B	B	B	.	B	B	.	.	.	.	B	.	B	.	.	B	.		4
6	B	.	D	C	C	C	B	.	C	.	.	.	.	B	.	C	.	.	B	.		5
8	B	.	B	–	–	B	.	B	–	–	.	.	.	B	.	B	–	.	–	.		6
9	B	.	E	–	–	B	.	B	–	–	.	.	.	B	.	B	–	.	–	.		7
10	B	.	D	–	–	B	B	B	–	–	.	.	.	B	.	B	–	.	–	.		8
11	B	.	F	–	–	C	B	B	–	–	.	.	.	B	.	C	–	.	–	.		9

1979). Each distinct restriction fragment profile is assigned a letter, and each individual is characterized by a composite (haplotype) of its patterns for each restriction endonuclease. The computer program HENNIG86, written by J. S. Farris, was used to infer a phylogenetic (unrooted) network among clones, based on the presence/absence matrix of restriction sites, according to the principle of maximum parsimony.

RESULTS

Twenty restriction endonucleases were used to assess mtDNA variation for the three *rostratus* and four "typical" Savannah Sparrows (Table 1). A total of 108 fragments and 95 sites was scored; for some endonucleases that have 4-base recognition sequences site variation was not fully resolvable and we scored the minimum number of site differences. The average percentage of the mtDNA genome surveyed per individual was 3.4%. The size of the mtDNA genome is 16.9 kb ± 0.18 (SD), typical for birds (Shields and Helm-Bychowski 1988), and was estimated from six endonucleases that produced fragments between 500 and 9,000 base pairs. Five clones were observed among the seven individuals examined with all 20 enzymes (Table 1), and each individual surveyed by Zink and Avise (1990) for 14 of the 20 restriction endonucleases used in this study had a unique haplotype. The sample of *rostratus* differs from the other Savannah Sparrows at nine of the 20 endonucleases (see Fig. 1), and shares a polymorphism at *Bcl* I. Savannah Sparrow "#6" (a wintering California individual, one of two collected on the Mojave Desert) also exhibits several unique mtDNA fragment profiles (Table 1), although this individual is phenotypically "typical" (Rising, pers. obs.). The remaining "typical" individuals differed little from each other in mtDNA patterns. The average nucleotide divergence (p) among the clones is 1.18%, the samples of *rostratus* differ from the other Savannah Sparrows by 1.7%, and values among the "typical" Savannah Sparrows surveyed for all 20 endonucleases averaged 0.46%. Individual #6 and *rostratus* exhibited the maximum p-value observed, 1.9%. Inspection of the data in Table 1 reveals that the four "typical" specimens from Louisiana used by Zink and Avise (1990) are very similar to those collected in California for this study. The single most parsimonious phylogenetic network (not shown; length = 19, consistency index = 1.00) among the clones depicts *rostratus* as a distinct group.

DISCUSSION

Our data indicate that the large-billed Savannah Sparrow exhibits considerable mtDNA differentiation from more "typical" Savannah Sparrows. Although the mtDNA of individual #6 exhibits fewer site differences from "typical" specimens than does *rostratus,* there could be substantial geographic differentiation within "typical" Savannah Sparrows; we suggest, however, that its magnitude is less than that between *rostratus* and "typical" forms. The level of divergence of *rostratus,* 1.7%, is at the low end of values documented for other avian congeners, but it exceeds most intraspecific comparisons (Avise and Zink 1988). Avise and Zink (1988) listed mtDNA p-values for six species pairs that are lower than or equal to the value for *rostratus* versus other Savannah Sparrows. As noted previously (e.g., Zink and Dittmann 1991), level of genetic differentiation (irrespective of genetic marker) cannot be used as an absolute taxonomic yardstick. For example, the Golden-crowned Sparrow (*Zonotrichia atricapilla*) and White-crowned Sparrow (*Z. leucophrys*) differ at only 1 of 80 restriction sites, a sequence divergence of only 0.11% (Zink et al. 1991a), but their species status is not in dispute. We suggest that morphological evidence and the level and pattern of mtDNA variation support species status for *rostratus* (irrespective of the species concept invoked; McKitrick and Zink [1988]). Although larger sample sizes would reveal more haplotypes among all taxa surveyed, we suggest that the existence of such haplotypes would not reduce the mtDNA distinctiveness of *rostratus.* We also note that mtDNA comparisons should be used cautiously for taxonomic purposes. Because of a maternal mode of inheritance, mtDNA patterns might be paraphyletic with respect to the nuclear genome (Avise et al. 1987, Neigel and Avise 1986). In the case of *rostratus,* both morphological (obviously encoded by nuclear genes) and mtDNA patterns support a common theme of evolutionary independence of *rostratus.* This hypothesis should be tested with additional data sets, including allozymes and song characters, and extended to include phenotypically intermediate as well as distinct forms. Such studies would clarify evolutionary patterns (i.e., species limits) within the group and identify the sister taxon of *rostratus.*

The specialized breeding habitat and restricted breeding range along the Gulf of California seem to have allowed for allopatric differentiation. Other avian species restricted to narrow coastal habitats exhibit differentiation, such as the Seaside Sparrow (*Ammodramus maritimus*; Avise and Nelson 1989). Applying a commonly used but poorly tested calibration of mtDNA divergence, namely 2% sequence divergence accrues per million years (Shields and Wilson 1987), *rostratus* has been isolated for ca. 750,000 years. California Towhee (*Pipilo crissalis*) samples from California and Baja California do not exhibit the degree of mtDNA differentiation observed (Zink and Dittmann 1991) in our sample of Savannah Sparrows whose breeding ranges span the same or greater geographic areas (depending on where our wintering specimens bred). Allozymic comparisons of populations of California Quail (*Callipepla californica*) also revealed little differentiation between northern California and southern Baja California (Zink et al. 1987). The differences in apparent level of genetic differentiation among these species suggest that historical events in this region did not affect these currently co-distributed species in the same way. Ecological isolation seems likely to have effected the evolution of *rostratus,* rather than a vicariant event, which might have fragmented the entire ancestral biota of the region.

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