The Auk 110(1):117–126, 1993

GENETIC DIVERGENCE AMONG POPULATIONS OF A TROPICAL PASSERINE, THE STREAKED SALTATOR (*SALTATOR ALBICOLLIS*)

GILLES SEUTIN,[1] JEFFREY BRAWN,[1,4] ROBERT E. RICKLEFS,[2] AND ELDREDGE BERMINGHAM[1,3]

[1]*Smithsonian Tropical Research Institute, Apartado 2072, Balboa, Panamá; and*
[2]*Department of Biology, University of Pennsylvania, Philadelphia, Pennsylvania 19104, USA*

ABSTRACT.—We analyzed mitochondrial-DNA (mtDNA) restriction-site variation in populations (subspecies) of the Streaked Saltator (*Saltator albicollis*) in Panama, Peru, and the Lesser Antilles. Genetic differentiation between populations (Panama vs. Peru, 0.035; Lesser Antilles vs. Panama/Peru, 0.063) greatly exceeded values reported for populations or subspecies within North American bird species (0.0028–0.0086), and was near the high end of the range reported for congeneric species of passerine birds (0.016–0.073). Nucleotide diversity within populations was similar to that reported for other species of passerines and did not differ markedly between mainland and island populations. Thus, founder effects and population bottlenecks associated with island colonization appear to have caused little, if any, loss of mtDNA nucleotide diversity. No significant mtDNA differentiation was observed between populations of named subspecies on mainland Panama (*S. a. isthmicus*) and the Pearl Islands (*speratus*), or between two subspecies in the Lesser Antilles (*albicollis* and *guadelupensis*). *Saltator albicollis* exhibits marked geographical genetic differentiation, as assayed by mtDNA polymorphism, but this bears little relationship to subspecies distinctions. Finally, the genetic data suggest that Antillean populations of Streaked Saltators should be distinguished at specific rank (*S. albicollis*), with continental forms referred to *S. striatipectus*. *Received 7 May 1992, accepted 14 August 1992.*

MITOCHONDRIAL-DNA (mtDNA) sequence divergence based on restriction-site analysis (Avise et al. 1987, Dowling et al. 1990) has provided informative measures of genetic differentiation within avian species and genera (Mack et al. 1986, Avise and Zink 1988, Ball et al. 1988, Zink and Avise 1990, Quinn et al. 1991, Zink 1991, Zink and Dittmann 1991, Zink et al. 1991a, b, Bermingham et al. 1992). With regard to North American passerine birds, for example, mtDNA divergence between species is almost an order of magnitude greater than differences among populations within species. These findings refer exclusively to temperate taxa. At present, there is little information available on mtDNA divergence among populations of tropical birds, although allozyme surveys (Capparella 1988, Peterson et al. 1992) have revealed marked genetic differentiation over short distances within several groups of Amazonian and Mexican passerines. Additionally, Escalante-Pliego (1991) has documented substantial isozyme differences among disjunct populations of yellowthroats (*Geothlypis*) in Central and South America, and Hackett and Rosenberg (1990) have demonstrated pronounced geographical subdivision and differentiation in Neotropical antwrens (Formicariidae).

Appraisals of genetic differentiation among populations of tropical birds would address questions of population structure and incipient species formation in regions of high species richness. Tropical species diversity is thought to result from high rates of species production, low rates of extinction, or great age of tropical regions. Species production presumably varies in relation to rate of evolutionary change within populations and degree of population subdivision. Estimates of mtDNA divergence directly address the issues of population subdivision and the relative genetic differentiation of taxa of specific rank. It has been suggested that the constant temperatures and diminished seasonal change in climate in the tropics have led to increased ecological specialization, increased fragmentation of species

[3] Mailing address: Smithsonian Tropical Research Institute, Unit 0948, APO, AA 34002-0948, USA.

[4] Present address: Illinois Natural History Survey, 607 East Peabody Drive, Champaign, Illinois 61820, USA.

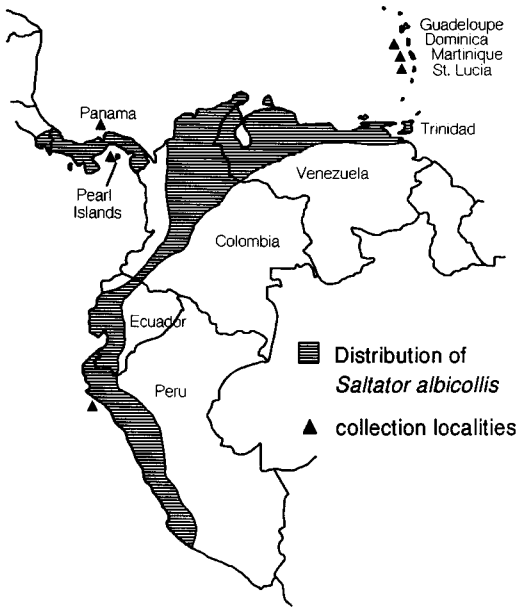

Fig. 1. Distribution of *Saltator albicollis*, with collection localities indicated.

distributions, and reduced migration of individuals between populations (Haffer 1969, 1974). Under these circumstances, one would expect to find more subpopulations per species and greater genetic differentiation among them in the tropics compared to temperate latitudes. We contribute to the analysis of genetic differentiation among tropical New World passerines by reporting mtDNA sequence divergence for populations of the Streaked Saltator (*Saltator albicollis*; Cardinalinae, Emberizidae).

This medium-sized saltator (35–45 g; Wetmore et al. 1984) is typically found in resident, sedentary populations without pronounced seasonal movements. *Saltator albicollis* is distributed from southwestern Costa Rica, through Panama, south to Peru and east through Colombia to northern Venezuela and Trinidad (AOU 1983). The species also resides on several land-bridge islands along the Pacific coast of Panama (Coiba, Taboga, Pearl Islands), and on the islands of St. Lucia, Martinique, Dominica, and Guadeloupe in the Lesser Antilles. Several authors have referred the Antillean forms and continental forms to different species (*S. albicollis* Vieillot 1817 and *S. striatipectus* Lafresnaye 1947, respectively). Paynter and Storer (1970) recognized 12 subspecies: 2 referable to the Lesser Antilles; 3 to the Pacific coastal islands of Panama; and 7 to continental Central and South America.

In this study, we characterize mtDNA restriction-site polymorphism in saltator populations from central Panama, the Pearl Islands in the Bay of Panama, Peru, and three islands in the Lesser Antilles (Fig. 1). Although our sample covers only a small part of the geographic range of the Streaked Saltator, it includes geographically distant populations and five named subspecies. Furthermore, the Streaked Saltator provides an excellent opportunity to investigate genetic diversity and differentiation of populations on islands of different age.

The Pearl Islands are land-bridge islands thought to have had a land connection to mainland Panama as recently as 9,500 to 11,000 years ago (Bartlett and Barghoorn 1973, Fairbanks 1989). In contrast, the Lesser Antilles are oceanic islands whose modern geography probably dates from the Miocene (Pregill 1981, Sykes et al. 1982, Rosen 1985, Burke 1988). The four Antillean islands inhabited by the Streaked Saltator have never been connected by land either to each other or to the mainland of South America. Saltators undoubtedly colonized these islands by long-distance dispersal and, therefore, might be expected to exhibit founder effects in the form of reduced genetic diversity. Furthermore, considering the potentially greater age of oceanic island populations, they may exhibit greater genetic divergence from mainland forms than do populations on land-bridge islands. Finally, we assess the relationship between subspecific distinctions and mtDNA differentiation in this species.

Materials and Methods

Field methods and tissue samples.—We obtained blood or pectoral muscle samples from Streaked Saltators from: three Antillean islands; Atlantic coastal and inland localities in central Panama; the Pearl Islands in the Bay of Panama; and coastal Peru (Table 1, Fig. 1). Each sample consisted of tissues collected primarily from adult birds during a single season. Judging from the presence or absence of a cloacal protuberance, both sexes were represented in most samples. In most locales our permit provisions prevented us from sacrificing birds for voucher specimens. All samples were collected and imported under appropriate permits and licenses.

We collected only blood (50 to 150 μl) from most Panamanian birds through wing-vein puncture. The samples were diluted in 1× TE buffer (10 mM Tris, 1 mM EDTA, pH 7.5) and stored frozen in liquid ni-

trogen in the field, and in an ultracold freezer in the laboratory. In representative Panamanian localities, we sacrificed a few individuals, from which we purified mtDNA from pectoral muscle to establish restriction patterns without ambiguity and to use as probe. Peruvian birds were collected by a team of the Museum of Natural Sciences, Louisiana State University; saltator specimens were stored in the field in liquid nitrogen, and later transferred to an ultracold freezer. Antillean samples were collected in July and August 1991. Birds were caught with mist nets, and 0.05 to 0.1 g of pectoral muscle was biopsied and preserved in a salt-DMSO solution (Seutin et al. 1991). The biopsy procedure followed Baker (1981), except that we excised a triangular piece of the lower part of the breast muscle; birds were released within an hour of the procedure. Pickled tissue samples were kept in the field (one to five weeks) at ambient temperature; upon arrival at the laboratory, they were transferred to 4°C. More than 98% of the birds captured were released alive whether we sampled blood or biopsied tissue. However, our experience with both blood and biopsied pectoral muscle as sources of total genomic DNA for subsequent filter hybridization with purified mtDNA strongly suggests using the biopsy technique.

DNA extraction, restriction digests, electrophoresis, and Southern blotting.—We extracted total cellular DNA from each sample. For Panamanian specimens, we digested 50 to 75 μl of whole blood with proteinase K (20 μl, 10 mg/ml), in the presence of 0.5% SDS. Samples were digested overnight at 65°C and then extracted two times with an equal volume of phenol-chloroform-isoamyl alcohol solution (PCI; 25:24:1), and once with a chloroform-isoamyl alcohol solution (CI; 24:1). The DNAs were recovered by conventional isopropanol precipitations and redissolved in 200 μl of 1× TE. One-half of the Caribbean samples were extracted by the tissue-preparation technique described by Seutin et al. (1991). The remaining Caribbean samples, and those from Peru, were first ground in 500 μl of 2× CTAB buffer (Murray and Thompson 1980) and incubated at 50 to 55°C for 1 to 16 h with constant agitation in presence of 6 to 12 units of Proteinase K. The homogenate was then extracted with an equal volume of CI solution, followed by PCI solution, and finally with CI. The DNA was recovered by cold-ethanol precipitation and redissolved in 200 μl of 1× TE, and then was dialyzed overnight against 1× TE with three changes of buffer. In addition, purified mtDNA was obtained from mainland Panamanian and Pearl Islands samples by ultracentrifugation in cesium chloride-ethidium bromide (CsCl-EtdBr) density gradients, followed by dialysis (see Lansman et al. 1981).

Approximately 1 μg of total genomic DNA extracted from blood (<10 μl of sample) was digested with 20 units of restriction enzyme following the manufacturer's recommendations. For samples extracted from tissue, approximately 2 μg of DNA (1–2 μl of sample) were digested with 5 to 10 units of enzyme. We used 2 enzymes recognizing a 5.3-base sequence (*Ava*I, and *Hin*cII; see Nei 1987), as well as 11 enzymes recognizing six bases (*Bam*HI, *Bcl*I, *Bgl*I, *Bgl*II, *Dra*I, *Eco*RI, *Eco*RV, *Hin*dIII, *Pvu*II, *Sac*I, and *Xba*I). Restriction fragments were separated electrophoretically in 0.9 to 1.2% agarose gels. After in-gel denaturation (see Seutin et al. 1991), the size-fractionated DNA samples were Southern blotted overnight onto ZetaBind membranes. SCP buffer (10×: 1 M NaCl, 0.3 M Na_2HPO_4, 10 mM EDTA, pH 7.0–7.5) was used for transfers and post-transfer washing of the membranes.

TABLE 1. Streaked Saltator samples used in mtDNA analysis.

Location[a]	Code	Subspecies[b]	*n*
Lesser Antilles			
Dominica	DOM	*guadelupensis*	9
Martinique	MAR	*albicollis*	8
St. Lucia	STL	*albicollis*	10
Central Panama			
Atlantic coast	PAC	*isthmicus*	10
Inland	PIN	*isthmicus*	10
Las Perlas islands	PPI	*speratus*	31
Peru			
Lambayeque	PER	*immaculatus*	3

[a] Precise locations available from authors.
[b] Subspecific designations follow Paynter and Storer (1970).

Probing.—We used a Streaked Saltator mtDNA preparation purified on CsCl-EtdBr density gradients as the probe. Because analyses of DNA extracted from blood required extremely pure probes, the samples were purified twice on density gradients and traces of nuclear DNA were removed by running *Eco*RI-digested samples in 1.0% low-melting-point agarose gels and extracting the mtDNA fragments with the GeneClean procedure (Bio101, LaJolla, California). In random priming reactions, a few nanograms of probe were radioactively labelled with [alpha-^{32}P]dCTP to very high specific activity (10^8–10^9 dpm/μg).

Transfer membranes carrying blood-extracted DNA were prehybridized for 1 to 3 h at 65°C in rotating canisters spinning at approximately 5 rpm. Three to five blots were prehybridized and hybridized at once in 25 to 40 ml of prehybridization solution (7.4% dextran sulfate, 4.4× SCP, 0.74% N-lauryl-sarcosine, 0.4 mg/ml heparin). Hybridizations (12 to 48 h) were followed by three 15-min washes of increasing stringency at 65°C (2× SCP, 1% SDS; 0.2× SCP, 0.1% SDS; 0.1× SCP, 0.05% SDS). Complete details on the techniques for analyzing blood-extracted DNA are presented by Brawn et al. (unpubl. manuscript). Membranes with tissue-extracted DNAs were prehybridized and hybridized in the conditions described above, but

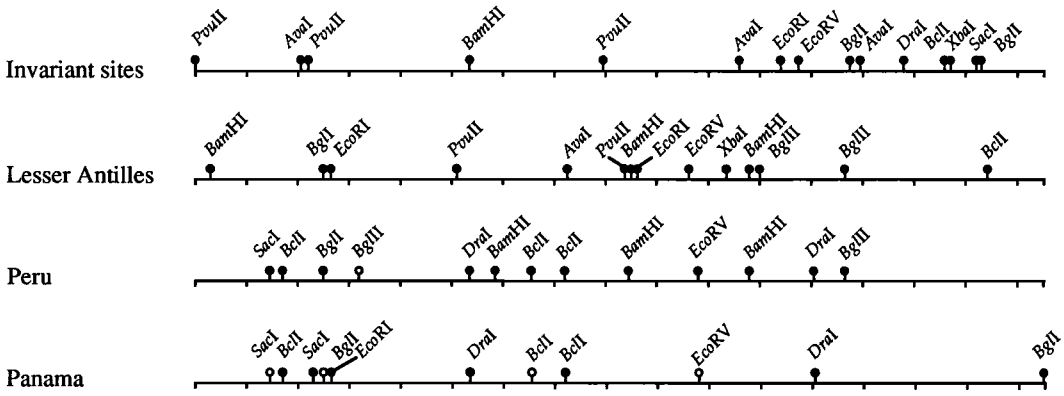

Fig. 2. mtDNA restriction-site maps for *Saltator albicollis*. Invariant-sites map shows restriction sites shared by all individuals surveyed across all three populations. *Hin*cII and *Hin*dIII not mapped because each enzyme produced large numbers of small fragments. Sites indicated as variable (O) or invariant (●) within a population. Two variable *Bam*HI sites and one variable *Bcl*I site observed in Panama samples were not mapped. Tic marks spaced at 1-kilobase intervals.

with a solution containing 10% dextran sulfate, 0.5 M NaCl and 1% SDS, and they were washed as described by Seutin et al. (1991). Scorable bands on autoradiographs were obtained in 20 to 120 h on Kodak XAR film at −70°C using one or two intensifying screens. No attempt was made to score fragments smaller than 300 base pairs (bp).

Restriction-site data analysis.—Each distinctive mtDNA fragment pattern for an enzyme was given an alphabetic label; an increase in the letter sequence indicates a site gain, and a decrease indicates a site loss. For most of the enzymes, all the fragment patterns could be related to each other by assuming the gain or loss of one or more sites. Nevertheless, to establish fragment homologies across patterns with certainty, we used double digestions to physically map the restriction sites for most of the enzymes (Fig. 2). For *Hin*cII and *Hin*dIII, we could not fully map sites delimiting many small fragments, but homology of large fragments found in representative samples from the West Indies, Panama, and Peru was verified through double digests. Once homology was established, sites were inferred for these enzymes; all fragment patterns could be related to each other without ambiguity.

Statistical analyses were based on the presence and absence of restriction sites. Data were summarized using the programs REAP (McElroy et al. 1992) and DXY (H. Lessios unpubl. manuscript) to calculate indices of nucleotide diversity (π) and divergence (D_{xy}) described by Nei and coworkers (Nei and Tajima 1983, Nei 1987, and Nei and Miller 1990). UPGMA clustering of haplotypes was performed using the NTSYS package (Rohlf 1990), and phylogenetic networks and a consensus tree of haplotypes were produced using PAUP (Swofford 1990) and MacClade (Maddison and Maddison 1987).

RESULTS

Restriction-site analysis.—We used 13 restriction enzymes to analyze the mitochondrial genomes of 81 Streaked Saltators collected from populations in the Lesser Antilles, mainland Panama (and some nearshore Pacific islands), and coastal Peru (Fig. 1). The most common mtDNA haplotype recovered from each general region (Lesser Antilles, Panama, and Peru) was mapped (Fig. 2); according to these maps, which were based on all enzymes used except *Hin*dIII and *Hin*cII, the size of the saltator mtDNA is approximately 16.5 kilobase pairs (kbp). Including sites inferred from the restriction-fragment data for *Hin*dIII and *Hin*cII, an average of 42 restriction sites were assayed for each bird. Of the 62 restriction sites analyzed in total, 25 were shared by all 81 saltators.

Cluster analysis (UPGMA; Fig. 3) of the 14 unique mtDNA haplotypes, based on the genetic distance matrix presented in Table 2, revealed three genotypic groupings that corresponded to the geographic sources of the samples (Fig. 4). One of the clusters contained only the five *S. albicollis* mtDNA haplotypes (I–V) identified from the islands of St. Lucia, Dominica, and Martinique in the Lesser Antilles. We found that 78% of the Antillean birds carried mtDNA haplotype I, and this haplotype predominated on the three islands. Three of the four rarer haplotypes (II, III, V) were obtained only once; the fourth mtDNA haplotype (IV) occurred only on Martinique, where it was rel-

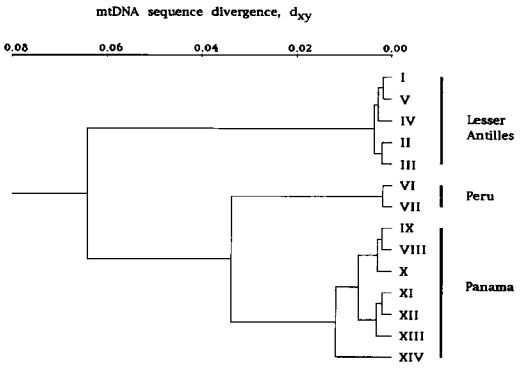

Fig. 3. UPGMA cluster analysis of *Saltator albicollis* mtDNA haplotypes based on genetic distance matrix in Table 2.

atively common (37%). Thus, the measure of mtDNA nucleotide diversity among the Lesser Antillean birds surveyed on each island was low to moderate (π = 0.0000, 0.0008, 0.0016). The greatest mtDNA sequence divergence (D_{xy}) between any pair of Antillean saltators was 0.0060 (Table 2). This level of intrapopulational mtDNA sequence divergence strongly contrasts with the mean D_{xy}-value of 0.063 (range 0.054–0.076) observed between the Antillean saltators and the clade that includes all Panama and Peru birds.

Saltator mtDNAs from Panama and Peru, although less differentiated than Antillean and continental haplotypes, fall into geographically congruent clusters separated by a genetic distance (mean D_{xy}) of 0.035 (range 0.030–0.040). Only three birds from Peru were analyzed and, thus, the nucleotide diversity for this population is not informative. Our analysis of 51 saltators from mainland Panama and nearshore Pacific islands in the Pearl Archipelago revealed a relatively high level of intrapopulation variability (π = 0.0018, *S. a. isthmicus*; 0.0024, *S. a. speratus*). The two most-common Panamanian saltator mtDNA haplotypes (VIII and XI) are widely distributed across mainland and island locales. Among Panamanian samples, the maximum D_{xy}-value (0.013) separated two unique mtDNA haplotypes recovered from individuals collected in the Pearl Islands. The level of mtDNA sequence divergence between Panamanian mainland and Pearl Island populations of saltators (D_{xy} = 0.0034) was similar to the levels observed within each of those two populations.

Wagner-parsimony analysis revealed 52 rival, minimum-length trees of 46 steps and a consistency index of 0.80. All trees support the distinctiveness of the three geographic groupings of mtDNA haplotypes discussed above; this finding was further supported by bootstrap analysis (Fig. 4). These trees identify Panamanian and Peruvian saltators as sister mtDNA taxa with the Antillean saltators as the outgroup. A minimum of 10 restriction-site changes distinguish the two Peruvian mtDNA haplotypes from the seven haplotypes observed in Panamanian saltators; a minimum of 20 site changes differentiate the five Lesser Antillean mtDNA haplotypes from all others. There is not strong bootstrap support for mtDNA haplotype relationships within the geographic locales of the Lesser Antilles, Panama, and Peru (Fig. 4).

TABLE 2. Genetic distances (lower left) and their standard errors (SE; upper right) between mtDNA haplotypes of *Saltator albicollis*.

	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV
I		.0027	.0039	.0027	.0026	.0120	.0121	.0102	.0104	.0104	.0103	.0103	.0102	.0125
II	.0020		.0028	.0039	.0038	.0119	.0120	.0104	.0106	.0106	.0105	.0105	.0104	.0129
III	.0041	.0020		.0028	.0048	.0123	.0124	.0106	.0107	.0108	.0106	.0107	.0106	.0132
IV	.0019	.0041	.0020		.0038	.0124	.0125	.0104	.0106	.0106	.0105	.0105	.0104	.0129
V	.0019	.0040	.0061	.0040		.0117	.0118	.0100	.0102	.0103	.0101	.0102	.0100	.0122
VI	.0596	.0574	.0614	.0636	.0557		.0026	.0069	.0071	.0069	.0069	.0070	.0071	.0094
VII	.0616	.0596	.0636	.0657	.0578	.0020		.0070	.0072	.0070	.0070	.0071	.0072	.0096
VIII	.0607	.0649	.0693	.0649	.0576	.0311	.0332		.0028	.0039	.0052	.0060	.0062	.0054
IX	.0649	.0693	.0741	.0693	.0607	.0341	.0363	.0020		.0028	.0042	.0052	.0054	.0050
X	.0671	.0716	.0764	.0716	.0628	.0311	.0332	.0041	.0020		.0052	.0060	.0062	.0054
XI	.0605	.0648	.0695	.0648	.0563	.0299	.0320	.0064	.0043	.0064		.0030	.0031	.0051
XII	.0627	.0671	.0718	.0671	.0585	.0320	.0341	.0085	.0064	.0085	.0021		.0044	.0055
XIII	.0582	.0625	.0671	.0625	.0541	.0330	.0351	.0087	.0066	.0087	.0022	.0044		.0047
XIV	.0634	.0679	.0726	.0679	.0593	.0378	.0400	.0131	.0110	.0131	.0113	.0135	.0091	

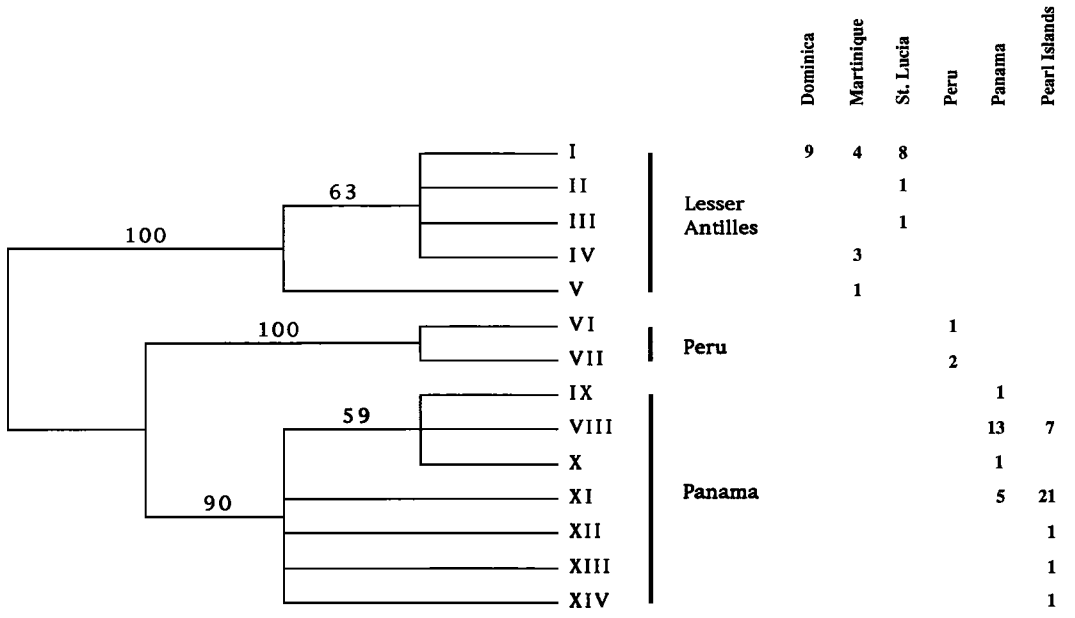

Fig. 4. Consensus of unrooted minimum-length Wagner-parsimony trees (length = 44+; CI = 0.84) based on mtDNA restriction sites. Numbers along branches indicate percentage of times that clade was distinguished in 500 bootstrapped trees (only percentages greater than 50 shown). Distribution of haplotypes among sample localities shown to right.

Discussion

The major feature of the mtDNA analyses is the distinctiveness of Lesser Antillean, Panamanian, and coastal Peruvian Streaked Saltators. No mtDNA haplotypes are shared among these localities, and the mtDNA clades observed are distinguished from one another by multiple mutational steps. The average genetic distance between the Antillean *albicollis* and the subspecies representing Panama and Peru (0.063) exceeds all but a few of the published estimates of mtDNA differences between congeneric species of passerine birds, and all of those between populations within species (Table 3). The average genetic distance between the Peruvian *immaculatus* and the two Panamanian subspecies (0.035) also exceeds other intraspecific comparisons and is consistent with levels of differentiation between congeneric species.

The lack of sizable differentiation in mtDNA between populations in the Pearl Islands and various locations in central Panama is not surprising in that the Pearl Islands are land-bridge islands with connections to the mainland as recently as 9,500 to 11,000 years ago (Bartlett and Barghoorn 1973, Fairbanks 1989). The presence of a few rare haplotypes in the Pearl Islands not observed in central Panama raises the possibility that Pearl Island saltators were derived from a different region of the mainland. Wetmore et al. (1984) suggested that the endemic subspecies from the Pearl Islands, *S. a. speratus*, was more closely related to *S. a. striatipectus* of eastern Panama and Colombia than to the central Panamanian *S. a. isthmicus*. Alternatively, the rare Pearl Island haplotypes may have arisen *in situ* either after the land bridge was submerged, or earlier but without dispersing from the area. The probability of origination within the last 10,000 years seems relatively remote given the average rate of mutation in mtDNA. Finally, we may have failed to sample the rare haplotypes on the mainland simply because they are present there at low frequency. Our data neither support nor exclude any of these possibilities. They do, however, emphasize the observation that subspecific distinctions bear little relationship to degree of mtDNA divergence among populations.

The genetic similarity among the three Antillean populations sampled suggests a recent expansion of the taxon within the Lesser Antilles. All the mtDNA polymorphism within the Lesser Antilles was revealed by *Hin*dIII and rep-

TABLE 3. mtDNA divergence between populations and congeneric species of passerine birds.[a]

Taxa	Sample sizes: *n*	Localities	Enzymes	Haplotypes	π	D_{xy}	Reference
Intraspecific comparisons							
Quiscalus quiscula	35	8	20	29	—	0.0028	Zink et al. (1991b)
Parus bicolor	8	2	19	4	—	0.0040	Avise and Zink (1988)
Dendroica nigrescens	11	2	14	6	0.0024	0.0077	Bermingham et al. (1992)
Passerella iliaca	46	9	20	5	0.0008	0.0086	Zink (1991)
Ammodramus maritimus	40	2	18	11	—	0.0098	Avise and Nelson (1989)
Agelaius phoeniceus	127	19	18	34	0.0020	—	Ball et al. (1988)
Melospiza melodia	27	7	20	15	0.0027	—	Zink (1991)
Intrageneric comparisons							
Quiscalus	14	2	19	3	—	0.0160	Avise and Zink (1988)
Melospiza	9	3	18	3	—	0.0290	Zink (1991)
Quiscalus	10	3	20	4	—	0.0295	Zink et al. (1991b)
Zonotrichia	22	5	19	10	—	0.0320	Zink et al. (1991a)
Sturnella	—	2	—	2	—	0.0340	Freeman (1991)
Icterus	—	2	—	2	—	0.0370	Freeman (1991)
Dendroica	11	5	17	5	—	0.0440	Kessler and Avise (1985)
Dendroica	35	8	14	18	—	0.0523	Bermingham et al. (1992)
Pipilo	26	4	16	11	—	0.0640	Zink and Dittman (1991)
Parus	10	2	18	3	—	0.0700	Mack et al. (1986)
Ammodramus	58	8	16	19	0.0060	0.0730	Zink and Avise (1990)
This study							
Saltator albicollis	81	7	13	14	—	0.0630	

[a] π = genotypic diversity within populations; D_{xy} = genetic differentiation between populations.

resents a series of four single-substitution differences. Haplotype I occurs on the three islands sampled. The Martinique haplotypes represent a series (V-I-IV) with the gain of a single restriction site between I and V and the loss of a site between I and IV. The St. Lucia haplotypes similarly represent a series (I-II-III) with the loss of a single restriction site at each step. Haplotype III also can be obtained by a single site loss from IV of Martinique, but geographical considerations make the sequence II-III more likely.

We exclude the possibility that the populations of Streaked Saltators on the individual Antillean islands have had as long a history of isolation from each other as they have had from continental populations; in such a case, substantial levels of mtDNA sequence divergence would have been observed among our Antillean samples. We also believe it unlikely that gene flow among the islands has persisted throughout their history in the Lesser Antilles given that the species occurs on neither St. Vincent nor Grenada, which lie between St. Lucia and the South American continent. Presumably, dispersal abilities sufficient to maintain genetic uniformity among the islands would also be sufficient for colonization of neighboring islands. The presence of a common genotype (37%) on Martinique that was absent from our samples from Dominica to the north and St. Lucia to the south reinforces the idea that movement of females among islands is limited. The probability of missing a haplotype that common in our samples from Dominica ($n = 9$) and St. Lucia ($n = 10$) by chance alone is about 0.01. The data raise two possibilities to explain the geographic pattern. Either Martinique was the source population for the recent expansion and haplotype IV was lost in the founding events, or birds carrying the common haplotype colonized Martinique from another island following a period of isolation and genetic differentiation.

The genetic diversity on the Pearl Islands (π = 0.0009 on Pacheca Island and 0.0028 on Rey Island) is similar to that in central Panama (0.0017 in an Atlantic coastal population and 0.0018 in a central population), indicating that some of the Pearl Islands suffered neither a founder effect during colonization nor loss of genetic diversity since isolation from the mainland. These values are similar to those observed in North American passerines (Table 3). The

genetic diversities on Martinique (0.0016) and St. Lucia (0.0008) also provide no compelling evidence of founder effects or of bottlenecks on the Antillean islands. The Antillean populations are old enough to obscure founder effects and presumably large enough to prevent loss of genetic diversity through drift. The absence of genetic diversity in the Dominican sample suggests, however, the possibility of recent colonization from Martinique by a small number of individuals.

The high degree of genetic differentiation observed in *S. albicollis* suggests that a broad survey of tropical species may reveal more pronounced geographical structure than observed in temperate species (Hackett and Rosenberg 1990). Characterizing such differences in detail may elucidate processes of species formation and the generation of diversity in tropical avifaunas. The genus *Saltator*, for example, is moderately diverse, containing 12 named species that include 52 subspecies between Mexico and tropical South America (Paynter and Storer 1970). As we have seen, continental populations of *S. albicollis* (Panama and Peru) have been isolated for a sufficient period that they share no haplotypes. Even though gene flow between Panama and Peru would be possible owing to the availability of suitable habitats, the two populations effectively are evolutionarily independent. A similar relationship may be found for other *saltator* species.

The degree of genetic differentiation between named subspecies of *S. albicollis* ranges from none or very little (*isthmicus* vs. *speratus*, *guadelupensis* vs. *albicollis*) to levels characteristic of congeneric species (*guadelupensis*/*albicollis*, *immaculatus*, and *isthmicus*/*speratus*). Thus, taxonomic differentiation must be used with caution in historical biogeographic analyses (Bermingham et al. 1992). For example, Ricklefs and Cox (1972) inferred age of species within the Antilles partly on the basis of taxonomic differentiation at the subspecies/species level. While taxonomy may provide a general indication of age, estimates of genetic differentiation based on methods such as DNA restriction-site analysis and sequencing will be necessary for detailed work.

Applying the conventional mtDNA molecular clock, which in primates ticks at a rate of roughly 2% divergence per million years (Brown et al. 1979), to saltators suggests that populations in the Lesser Antilles became separated from lines that produced Panamanian and Peruvian populations about three million years ago. Streaked Saltators almost certainly colonized the Antilles from the coast of Venezuela, but we have not collected material from that area. Thus, we cannot date the colonization of the islands themselves, but can conclude that differentiation within the species dates from before the Pleistocene. If the Lesser Antilles were colonized by *S. albicollis* before the Pleistocene, the ages of other bird taxa within the Antilles, many of which are endemic at specific and generic rank, must be much older than usually thought. Thus, much of the biogeographic structure of both Antillean and continental Central and South American populations may have been established considerably before the onset of Pleistocene climate cycles, as argued in Capparella (1991) for Amazona. Alternatively, the mtDNA clock for these passerines may be ticking considerably faster than the primate mtDNA clock (concerning rate differences in mtDNA sequence evolution, see Martin et al. 1992). In any event, our results support Hackett and Rosenberg (1990), who found levels of genetic differentiation among Neotropical congeneric species that greatly exceed those reported from North America.

Taxonomic conclusions.—Our genetic data reinforce the conclusion of Ridgway (1901) that Antillean populations on Guadeloupe, Dominica, Martinique, and St. Lucia should be treated as a distinct species, *Saltator albicollis* Vieillot 1817, with continental populations referable to *Saltator striatipectus* Lafresnaye 1847. The genetic difference between Panamanian and Peruvian populations (0.035) also exceeds differences between conspecific populations reported in other studies, and lies well within the levels of differentiation observed between congeneric species. Because we have no genetic information on populations occupying the region between Panama and Peru, we cannot comment further on their relationships to one another.

ACKNOWLEDGMENTS

G.S. is supported by a Canadian NSERC Postdoctoral Fellowship and J.B. was supported by a Smithsonian Institution postdoctoral fellowship. R.E.R. was supported at the Smithsonian Tropical Research Institute by a Smithsonian Regents' Fellowship. Field work was funded by the National Geographic Society, and laboratory work was supported by the Smithsonian Institution's Scholarly Studies Program and Ab-

bott Fund. For assistance in the field, we thank Katie Sieving, David McLain, William A. Schew, Victor Apanius, Doug Wechsler, and Georges Tayalay. Permits to collect birds in Panama were provided by INRENARE. Robert M. Zink (Museum of Natural Sciences, Louisiana State University) provided tissue samples from Peru. We thank Monica Medina, Tim Collins, and Marivi Walker for assistance in the laboratory, and Angelo P. Capparella and two anonymous reviewers for comments that improved the quality of the manuscript.

Literature Cited

American Ornithologists' Union. 1983. Check-list of North American birds, 6th ed. Am. Ornithol. Union, Washington, D.C.

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

Avise, J. C., and R. M. Zink. 1988. Molecular genetic divergence between avian sibling species: King and Clapper rails, Long-billed and Short-billed dowitchers, Boat-tailed and Great-tailed grackles, and Tufted and Black-crested titmice. Auk 105: 516–528.

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

Baker, M. C. 1981. A muscle biopsy procedure for use in electrophoretic studies of birds. Auk 98: 392–393.

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. Proc. Natl. Acad. Sci. USA 85:1558–1562.

Bartlett, A. S., and E. S. Barghoorn. 1973. Phytogeographic history of the Isthmus of Panama during the past 12,000 years (a history of vegetation, climate and sea-level change). Pages 203–299 *in* Vegetation and vegetational history of northern Latin America (A. Graham, Ed.). Elsevier, New York.

Bermingham, E., S. Rohwer, S. Freeman, and C. Wood. 1992. Vicariance biogeography in the Pleistocene and speciation in North American wood warblers: A test of Mengel's model. Proc. Natl. Acad. Sci. USA 89:6624–6628.

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.

Burke, K. 1988. Tectonic evolution of the Caribbean. Annu. Rev. Earth Planet. Sci. 16:201–230.

Capparella, A. P. 1988. Genetic variation in Neotropical birds: Implications for the speciation process. Pages 1658–1664 *in* Acta XIX Congressus Internationalis Ornithologici (H. Ouellet, Ed.). Ottawa, Ontario, Canada, 1986. Univ. Ottawa Press, Ottawa.

Capparella, A. P. 1991. Neotropical avian diversity and riverine barriers. Pages 307–316 *in* Acta XX Congressus Internationalis Ornithologici. Christchurch, New Zealand, 1990. New Zealand Ornithol. Congr. Trust Board, Wellington.

Dowling, T. E., C. Moritz, and J. D. Palmer. 1990. Nucleic acids II: Restriction site analysis. Pages 250–317 *in* Molecular systematics (D. M. Hillis and C. Moritz, Eds.). Sinauer, Sunderland, Massachusetts.

Escalante-Pliego, B. P. 1991. Genetic differentiation in yellowthroats (Parulinae: *Geothlypis*). Pages 333–343 *in* Acta XX Congressus Internationalis Ornithologici. Christchurch, New Zealand, 1990. New Zealand Ornithol. Congr. Trust Board, Wellington.

Fairbanks, R. G. 1989. A 17,000-year glacio-eustatic sea level record: Influences of glacial melting rates on the Younger Dryas event and deep-ocean circulation. Nature 342:637–642.

Freeman, S. 1991. Molecular systematics and morphological evolution in the blackbirds. Ph.D. dissertation, Univ. Washington, Seattle.

Hackett, S. J., and K. V. Rosenberg. 1990. Comparison of phenotypic and genetic differentiation in South American antwrens (Formicariidae). Auk 107:473–489.

Haffer, J. 1969. Speciation in Amazonian forest birds. Science 165:131–137.

Haffer, J. 1974. Avian speciation in tropical South America. Publ. Nuttall Ornithol. Club No. 14.

Kessler, L. G., and J. C. Avise. 1985. A comparative description of mitochondrial DNA differentiation in selected avian and other vertebrate genera. Mol Biol. Evol. 2:109–125.

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.

Mack, A. L., F. B. Gill, R. Colburn, and C. Spolsky. 1986. Mitochondrial DNA: A source of genetic markers for studies of similar passerine bird species. Auk 103:676–681.

Maddison, W., and D. Maddison. 1987. MacClade. Univ. California, Berkeley.

Martin, A. P., G. J. P. Naylor, and S. R. Palumbi. 1992. Rates of mitochondrial DNA evolution in sharks are slow compared with mammals. Nature 357:153–155.

McElroy, D., P. Moran, E. Bermingham, and I. Kornfield. 1992. REAP: The restriction enzyme analysis package. J. Hered. 83:157–158.

Murray, M. G., and W. F. Thompson. 1980. Rapid

isolation of high molecular weight plant DNA. Nucleic Acids Res. 8:4321–4325.

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

NEI, M., AND J. C. MILLER. 1990. A simple method for estimating average number of nucleotide substitutions within and between populations from restriction data. Genetics 125:873–879.

NEI, M., AND F. TAJIMA. 1983. Maximum likelihood estimation of the number of nucleotide substitutions from restriction sites data. Genetics 105: 207–217.

PAYNTER, R. A., JR., AND R. W. STORER. 1970. Checklist of birds of the world, vol. XIII. Museum of Comparative Zoology, Cambridge, Massachusetts.

PETERSON, A. T., P. ESCALANTE P., AND A. NAVARRO S. 1992. Genetic variation and differentiation in Mexican populations of Common Bush-Tanagers and Chestnut-capped Brush-Finches. Condor 94: 244–253.

PREGILL, G. K. 1981. An appraisal of the vicariance hypothesis of Caribbean biogeography and its application to West Indian terrestrial vertebrates. Syst. Zool. 30:147–155.

QUINN, T. W., G. F. SHIELDS, AND A. C. WILSON. 1991. Affinities of the Hawaiian Goose based on two types of mitochondrial DNA data. Auk 108:585–593.

RICKLEFS, R. E., AND G. W. COX. 1972. Taxon cycles in the West Indian avifauna. Am. Nat. 106:195–219.

RIDGWAY, R. 1901. The birds of North and Middle America. Part 1, Family Fringillidae—The finches. Government Printing Office, Washington, D.C.

ROHLF, F. J. 1990. NTSYS-pc: Numerical taxonomy and multivariate analysis system. Exeter Software, Applied Biostatistics, Setauket, New York.

ROSEN, D. E. 1985. Geological hierarchies and biogeographic congruence in the Caribbean. Ann. Mo. Bot. Gard. 72:636–659.

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

SWOFFORD, D. L. 1990. PAUP: Phylogenetic analysis using parsimony. Ill. Nat. Hist. Survey, Champaign.

SYKES, L. R., W. R. MCCANN, AND A. L. KAFKA. 1982. Motion of the Caribbean plate during the last 7 million years and implications for earlier Cenozoic movements. J. Geophys. Res. 87:10656–10676.

WETMORE, A., R. F. PASQUIER, AND S. L. OLSON. 1984. The birds of the Republic of Panamá, Part 4.—Passeriformes: Hirundinidae (swallows) to Fringillidae (finches). Smithsonian Institution Press, Washington, D.C.

ZINK, R. M. 1991. The geography of mitochondrial DNA variation in two sympatric sparrows. Evolution 45:329–339.

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.

ZINK, R. M., AND D. L. DITTMANN. 1991. Evolution of Brown Towhees: Mitochondrial DNA evidence. Condor 93:98–105.

ZINK, R. M., D. L. DITTMANN, AND W. L. ROOTES. 1991a. Mitochondrial DNA variation and the phylogeny of *Zonotrichia*. Auk 108:578–584.

ZINK, R. M., W. L. ROOTES, AND D. L. DITTMANN. 1991b. Mitochondrial DNA variation, population structure, and evolution of the Common Grackle (*Quiscalus quiscula*). Condor 93:318–329.