



Initial Assessment of Genetic Diversity in Ten Bird Species of South American Cerrado

John M. Bates¹, Jose G. Tello^{1,2} and Jose Maria Cardoso da Silva^{3,4}

¹Department of Zoology, Field Museum of Natural History, Chicago, IL, USA

²Department of Biological Sciences, University of Illinois at Chicago, Chicago, IL, USA

³Conservation International do Brasil, Belém, Pará, Brazil

⁴Departament of Zoology, Federal University of Pernambuco, Recife, Pernambuco, Brazil

Abstract

We compared mitochondrial DNA sequences of portions of the cytochrome *b* (*cyt b*) and the NADH dehydrogenase subunit 2 (ND2) genes from samples of ten bird species that occur in both extremes of the Cerrado region (eastern Bolivia and Amapá, Brazil). The species include a wide sampling of taxa from several avian families: Tinamidae (1), Apodidae (1), Dendrocolaptidae (1), Furnariidae (1), Thamnophilidae (1), Tyrannidae (1), Turdidae (1), and Emberizidae (3). The taxa also exhibit a variety of distribution patterns in Neotropical open lands. Levels of genetic divergence within all taxa were low compared to comparable intraspecific values in many other widespread birds. In particular, these data suggest that there is much less genetic differentiation within these Cerrado birds than exists in birds of neighboring Amazonian forest. We suggest this implies the non-mutually exclusive possibilities that these open country birds have maintained higher levels of gene flow than forest understory birds, and that the Cerrado may have expanded to parts of its present-day distribution fairly rapidly. These data also suggest that hypothesized forest connections between Amazonia and Atlantic forest did not isolate open country bird populations from one another to a great extent.

Keywords: Cerrado, genetic diversity, birds, biogeography, Bolivia, Brazil.

Introduction

Although species diversity is the most commonly employed measure of biological diversity, it is not the only measure.

Genetic diversity, measured as the level of intraspecific genetic variation, has provided valuable information on levels of genetic variation, gene flow, population subdivision, historical patterns of population fragmentation, and the evolutionary history of populations (Moore et al., 1991; Ball & Avise, 1992; Bermingham et al., 1992; Zink & Dittmann, 1993; Zink, 1994; Gibbs, 1998; Schneider et al., 1998; Gill et al., 1999; Macey et al., 1999; Bates, 2000; Patton et al., 2000). However, genetic diversity is not generally measured because appropriate data are considered difficult and expensive to gather. In spite of these potential drawbacks, measuring genetic diversity may be especially important for conservation (Moritz & Faith, 1998). Among other things, evolutionarily significant units may be identified and these units may be more strongly supported than those used in traditional species diversity which, in ornithology, is many times based on arbitrary decision making using the Biological Species Concept (Mayr, 1963; but see Johnson et al., 1999). We present the first data on genetic diversity in birds from Cerrado, the large savanna region that occupies much of central South America south of the Amazon. Our data set is a simple one in that we have sequenced mitochondrial DNA from only a few individuals of ten biological species; however, our samples come from two regions that are 1800 km apart and separated by the lower reaches of the Amazon River. These data provide a first view of levels of genetic differentiation in birds of the Cerrado region. We have sampled a variety of taxa representing three avian orders (Tinamiformes, Apodiformes, and Passeriformes) and eight families (Table 1). The chosen taxa also occur in a range of

Received: 14 October 2001

Accepted: 4 February 2003

Correspondence: J. M. Bates, Field Museum of Natural History, 1400 S. Lake Shore Drive, Chicago, IL 60605, USA. Fax: +1 (312) 665-7754; E-mail: jrbates@fieldmuseum.org

Table 1. Voucher information for tissue samples used in this study.

Taxon/specimen number ¹	Collector	Locality
TINAMIDAE		
<i>Crypturellus parvirostris</i>		
MPEG CH043	J. M. C. Silva	Brazil: Amapá, Tartarugalzinho, Fazenda Casemiro.
LSUMNS B13927	T. J. Davis	Bolivia: Santa Cruz: Serrania de Huanchaca, 45 km E Florida.
TROCHILIDAE		
<i>Eupetomena macroura</i>		
MPEG CH017	J. M. C. Silva	Brazil: Amapá, Porto Grande, Fazenda Teimoso.
MPEG CH018	J. M. C. Silva	Brazil: Amapá, Porto Grande, Fazenda Teimoso.
MPEG CH204	J. M. C. Silva	Brazil: Amapá: Tartarugalzinho, Lago Cujubim.
LSUMNS B14454	A. Castillo	Bolivia: Santa Cruz: Serrania de Huanchaca, 21 km SE Catarata Arco Iris.
LSUMNS B14858	A. Castillo	Bolivia: Santa Cruz: Serrania de Huanchaca, 21 km SE Catarata Arco Iris.
DENDROCOLAPTIDAE		
<i>Lepidocolaptes angustirostris</i>		
MPEG CH206	J. M. C. Silva	Brazil: Amapá: Tartarugalzinho, Lago Cujubim.
MPEG CH207	J. M. C. Silva	Brazil: Amapá: Tartarugalzinho, Lago Cujubim.
MPEG CH241	J. M. C. Silva	Brazil: Amapá: Tartarugalzinho, Lago Cujubim.
LSUMNS B15282	J. M. Bates	Bolivia: Santa Cruz: Velasco, Parque Nacional Noel Kempff Mercado, 30 km E Aserradero Moira.
LSUMNS B15292	A. Castillo	Bolivia: Santa Cruz: Velasco, Parque Nacional Noel Kempff Mercado, 30 km E Aserradero Moira.
FURNARIIDAE		
<i>Synallaxis albescens</i>		
MPEG CH062	J. M. C. Silva	Brazil: Amapá, Tartarugalzinho, Fazenda Casemiro.
LSUMNS B14438	A. P. Capparella	Bolivia: Santa Cruz: Serrania de Huanchaca, 21 km SE Catarata Arco Iris.
THAMNOPHILIDAE		
<i>Formicivora rufa</i>		
MPEG CH001	J. M. C. Silva	Amapá: Porto Grande, Fazenda Teimoso.
MPEG CH166	J. M. C. Silva	Brazil: Amapá: Amapá, Fazenda Itapoa.
MPEG CH167	J. M. C. Silva	Brazil: Amapá: Amapá, Fazenda Itapoa.
MPEG CH239	J. M. C. Silva	Brazil: Amapá: Tartarugalzinho, Lago Cujubim.
LSUMNS B14601	J. M. Bates	Bolivia: Santa Cruz: Serrania de Huanchaca, 21 km SE Catarata Arco Iris.
LSUMNS B14602	C. A. Marantz	Bolivia: Santa Cruz: Serrania de Huanchaca, 21 km SE Catarata Arco Iris.
TYRANNIDAE		
<i>Elaenia chiriquiensis</i>		
MPEG CH217	J. M. C. Silva	Brazil: Amapá: Tartarugalzinho, Lago Cujubim.
MPEG CH245	J. M. C. Silva	Brazil: Amapá: Tartarugalzinho, Lago Cujubim.
MPEG CH246	J. M. C. Silva	Brazil: Amapá: Tartarugalzinho, Lago Cujubim.
LSUMNS B15284	A. Castillo	Bolivia: Santa Cruz: Velasco, Parque Nacional Noel Kempff Mercado, 30 km E Aserradero Moira.
TURDIDAE		
<i>Turdus leucomelas</i>		
MPEG CH069	J. M. C. Silva	Amapá: Porto Grande, Fazenda Teimoso.
MPEG CH224	J. M. C. Silva	Brazil: Amapá: Tartarugalzinho, Lago Cujubim.
MPEG CH303	J. M. C. Silva	Brazil: Amapá: Tartarugalzinho, Fazenda São Bento.
LSUMNS B14694	C. A. Marantz	Bolivia: Santa Cruz: Serrania de Huanchaca, 21 km SE Catarata Arco Iris.
LSUMNS B14719	M. D. Carreño	Bolivia: Santa Cruz: Serrania de Huanchaca, 21 km SE Catarata Arco Iris.
EMBERIZIDAE		
<i>Tangara cayana</i>		
MPEG CH216	J. M. C. Silva	Brazil: Amapá: Tartarugalzinho, Lago Cujubim.
MPEG CH233	J. M. C. Silva	Brazil: Amapá: Tartarugalzinho, Lago Cujubim.
MPEG CH234	J. M. C. Silva	Brazil: Amapá: Tartarugalzinho, Lago Cujubim.
MPEG CH235	J. M. C. Silva	Brazil: Amapá: Tartarugalzinho, Lago Cujubim.
LSUMNS B14840	A. Castillo	Bolivia: Santa Cruz: Serrania de Huanchaca, 21 km SE Catarata Arco Iris.
LSUMNS B14853	A. Castillo	Bolivia: Santa Cruz: Serrania de Huanchaca, 21 km SE Catarata Arco Iris.
<i>Cypsnagra hirundinacea</i>		
MPEG CH007	J. M. C. Silva	Brazil: Amapá: Porto Grande, Fazenda Teimoso.
MPEG CH014	J. M. C. Silva	Brazil: Amapá: Porto Grande, Fazenda Teimoso.
LSUMNS B15289	J. M. Bates	Bolivia: Santa Cruz: Velasco, Parque Nacional Noel Kempff Mercado, 30 km E Aserradero Moira.
LSUMNS B15290	J. M. Bates	Bolivia: Santa Cruz: Velasco, Parque Nacional Noel Kempff Mercado, 30 km E Aserradero Moira.
<i>Sporophila plumbea</i>		
MPEG CH175	J. M. C. Silva	Brazil: Amapá: Tartarugalzinho, Fazenda Hiane.
LSUMNS B13878	T. J. Davis	Bolivia: Santa Cruz: Serrania de Huanchaca, 45 km E Florida.
LSUMNS B13986	J. M. Bates	Bolivia: Santa Cruz: Serrania de Huanchaca, 45 km E Florida.

¹ MPEG: Museu Paraense Emilio Goeldi; LSUMNS: Louisiana State University Museum of Natural Science.

Cerrado habitats from 'campo limpo,' a grassland with few or no tall woody plants to 'gallery forest,' an evergreen forest that occurs as narrow belts along rivers and streams (Silva, 1995a; Table 2). We compare our results with levels of genetic differentiation found between populations of Amazonian birds, and between pairs of lowland taxa separated by the northern Andes (Brumfield & Capparella, 1996) and the Bering Strait (Zink et al., 1995).

Materials and methods

A total of 42 tissue samples of ten species of Cerrado birds were studied (Table 1). These species are Small-billed Tinamou (*Crypturellus parvirostris*), Swallow-tailed Hummingbird (*Eupetomena macroura*), Narrow-billed Woodcreeper (*Lepidocolaptes angustirostris*), Pale-breasted Spinetail (*Synallaxis albescens*), Rusty-backed Antwren (*Formicivora rufa*), Lesser Elaenia (*Elaenia chiriquensis*), Pale-breasted Thrush (*Turdus leucomelas*), Burnished-buff Tanager (*Tangara cayana*), White-rumped Tanager (*Cypsnagra hirundinacea*), and Plumbeous Seedeater (*Sporophila plumbea*). The samples included one to four individuals of each taxon from each of the two studied regions. Samples were available because of general collecting and inventory research in two widely separated regions of Cerrado (*sensu* Ab'Saber, 1977; Silva, 1995a).

The Serranía de Huanchaca, Department of Santa Cruz, Bolivia, is a large raised plateau of Brazilian Shield that lies on the western edge of Cerrado, and on the eastern edge of Bolivia (Bates et al., 1992; Killeen & Schulenberg, 1998). The Bolivian portion of the plateau is protected within Parque Nacional Noel Kempff Mercado. Inventory work here was conducted by the Louisiana State University Museum of Natural Science in collaboration with the Museo de Historia Natural Noel Kempff Mercado in Santa Cruz, Bolivia.

The Amapá savannas occupy 17,000 km² on a narrow and mainly flat, north-south oriented belt composed of Tertiary sediments parallel to the Atlantic Ocean (Silva et al., 1997). These savannas are isolated from the Cerrado region and from other islands of Amazonian savannas by different types of tall, evergreen Amazonian forests and the Amazon river itself. Tissue samples were collected in buffer solution (prepared according to Seutin et al., 1991) within the general research program of the ornithology section of the Museu Paraense Emilio Goeldi, Belém, Pará.

A small (*ca.* 0.05 g wet weight) portion of tissue was used for DNA extraction using a Puregene Extraction kit (Gentra Systems, Minneapolis, Minnesota, USA) following the manufacturer's directions. The final pellet was resuspended in 100 µl of sdd H₂O. Fragments of the cytochrome *b* gene (*cyt b*), the spacer, and a small portion of the threonine t-RNA were amplified in 50 µl total volumes via the polymerase chain reaction (PCR) (Palumbi, 1996), with primers L15656 and H16065 (Helm-Bychowski & Cracraft, 1993; Lanyon & Hall, 1994). The mitochondrial NADH dehydrogenase subunit 2 (ND2) fragment was amplified via PCR

Table 2. Uncorrected pairwise percent sequence divergence (MEAN ± SE) among mtDNA sequences within and between sites in the Cerrado region.

Taxa ¹	Forest dependence ²	Subspecies ³	Cyt <i>b</i>				ND2			
			Within Amapá	Within Huanchaca	Amapá vs. Huanchaca	Within Amapá	Within Huanchaca	Amapá vs. Huanchaca	Total divergence ⁴	
<i>Elaenia chiriquensis</i> (3, 1)	1	No	0.49 ± 0.14	—	0.25 ± 0.14	0.45 ± 0.06	—	0.28 ± 0.15	—	0.27 ± 0.08
<i>Lepidocolaptes angustirostris</i> (3, 2)	1	Yes	0.00 ± 0.00	0.49 —	0.25 ± 0.11	0.00 ± 0.00	0.34 —	0.34 ± 0.08	—	0.29 ± 0.09
<i>Eupetomena macroura</i> (3, 2)	1	No	0.00 ± 0.00	0.00 —	0.25 ± 0.00	0.11 ± 0.06	0.00 —	0.40 ± 0.04	—	0.32 ± 0.02
<i>Sporophila plumbea</i> (1, 2)	1	Yes	—	0.25 —	0.13 —	—	0.00 —	0.80 —	—	0.46 —
<i>Crypturellus parvirostris</i> (1, 1)	1	No	—	—	0.50 —	—	—	0.54 —	—	0.52 —
<i>Cypsnagra hirundinacea</i> (2, 2)	1	Yes	0.00 —	0.00 —	1.47 ± 0.00	0.00 —	0.00 —	0.54 ± 0.00	—	1.01 ± 0.00
<i>Tangara cayana</i> (4, 2)	1	Yes	0.17 ± 0.05	0.25 —	0.49 ± 0.07	0.14 ± 0.06	0.00 —	1.68 ± 0.04	—	1.09 ± 0.05
<i>Formicivora rufa</i> (4, 2)	1	Yes	0.17 ± 0.05	0.49 —	1.60 ± 0.05	0.09 ± 0.04	0.34 —	1.92 ± 0.03	—	1.76 ± 0.02
<i>Turdus leucomelas</i> (3, 2)	2	Yes	0.00 ± 0.00	0.74 —	2.60 ± 0.06	0.00 ± 0.00	0.28 —	1.77 ± 0.05	—	2.18 ± 0.00
<i>Synallaxis albescens</i> (1, 1)	1	Yes	—	—	1.96 —	—	—	3.07 —	—	2.52 —

¹ Sample size per locality is in parentheses.

² Forest dependence (from Silva, 1995a). (1) Independent: species that occur in open vegetation (marshlands, campo limpo, campo sujo, campo cerrado, Cerrado *sensu stricto*, and 'campos rupestres'); and (2) semi-independent: species that occur in open vegetation and forest, including gallery forests.

³ Subspecies status of the populations at each locality (following Peters, 1931, 1951; Mayr & Paynter, 1964; Paynter, 1970; Traylor, 1979).

⁴ Total percent sequence divergence between localities was calculated from combined gene sequences.

using one of two sets of primers; L5215 (LMet)-H5802, or L5204-H5578 (first set designed by S. Hackett; second set from Hackett, 1996). These two primer sets amplified fragments that included portions of the methionine t-RNA gene directly 5' to the ND2 gene. Numbers refer to the 3' base of the primer referenced to the complete mtDNA sequence of *Gallus gallus* (Desjardins & Morais, 1990). 'H' and 'L' refer to the primers located in the heavy and light strands of the mitochondrial genome, respectively. PCR profiles were: initial cycle of 2 min at 94°C, followed by five cycles of 20 s at 94°C, 15 s at 48°C, and 45 s at 72°C, and then 30 cycles of 20 s at 94°C, 15 s at 50°C, and 45 s at 72°C, with a final extension of 2 min at 72°C. For certain taxa it was necessary to lower the annealing temperature to facilitate the PCR amplification. PCR products were purified with GeneClean Kits (Bio 101, La Jolla, California, USA) and both strands were directly sequenced with the respective primers using the D-rhodamine (Perkin Elmer, Foster City, California, USA) cycle sequencing kits for dye-terminator chemistry following the manufacturer's instructions. Products of sequencing reactions were separated on a 5% polyacrylamide/6M urea gel by fluorescent electrophoresis on an ABI 377 automated DNA sequencer. The resulting sequences were aligned to the cytochrome *b* and ND2 sequences of the chicken (Desjardins & Morais, 1990) using Sequencher (version 3.1) (Genecodes, Ann Arbor, Michigan, USA). Uncorrected pairwise percent sequence divergence among samples were determined using PAUP* (Swofford, 1998).

Subspecies status was examined from the literature and when possible assigned to the samples. In order to assess diagnosability of subspecies, JMB and JGT compared subspecific characteristics from original descriptions using specimen series from The Field Museum collection.

Results

Sequences of the studied individuals are deposited in GenBank (Accession Nos. AY115390–AY115473). The gene regions (numbering follows the chicken [*Gallus gallus*] Desjardins & Morais, 1990) we compared in this study are positions 5205–5577 (*Crypturellus parvirostris*, *Turdus leucomelas*, *Sporophila plumbea*, *Cypsnagra hirundinacea*, and *Tangara cayana*) and 5216–5801 (*Eupetomena macroura*, *Lepidocolaptes angustirostris*, *Synallaxis albescens*, *Formicivora rufa*, and *Elaenia chiriquensis*) in the methionine t-RNA-ND2 fragment, and 15657–16035 in cyt *b*, 16036–16038 representing the spacer between cyt *b* and the threonine t-RNA, and 16039–16064 in the threonine t-RNA. Thus, the data set contained 586 or 373 bps for ND2, 379 bps for cyt *b*, 0 to 4 bps for the spacer, and 25 to 26 bps for the spacer-t-RNA piece. Cytochrome *b* sequences of one of the species, *Crypturellus parvirostris*, lacked the last two bases of the terminal (stop) codon and the spacer preceding the threonine t-RNA. Presumably, this incomplete stop codon is completed via polyadenylation as has been proposed for mammals (Quinn, 1997).

Levels of sequence divergence between samples from the same site were low for all comparisons for both genes (Table 2). Levels of uncorrected divergence (both gene regions combined) between the Huanchaca and Amapá samples vary from 0.3% or less in *Eupetomena macroura*, *Lepidocolaptes angustirostris*, and *Elaenia chiriquensis*, to values of above 2.0% for *Synallaxis albescens* and *Turdus leucomelas*. Levels of divergence for the two genes separately exhibit patterns that largely are consistent with combined levels of divergence. The ND2 sequences exhibited slightly higher levels of divergence in all but two species. As has been commonly observed in avian mtDNA sequencing studies at the population level, the majority of the changes represented third-position transitions (Edwards, 1997).

Discussion

In straight-line distance, the two regions we studied are separated by *ca.* 1800 km of mostly Amazonian forest. Based on the current paleoecological and biogeographic data, an historical connection between these two savanna sites must have been indirect, through the northern border of the Cerrado region to the Atlantic Coast and across the Amazon estuary, rather than directly through Central Amazonia (Silva et al., 1997, Silva & Bates, 2002). Thus, the actual biogeographic distance between these two sites is likely much more than the straight-line distance separating them (>2300 km, see Fig. 1). In spite of this substantial separation, the levels of genetic divergence between samples from the two sites are low compared to values between populations of Amazonian forest understory birds (Aleixo, 2002; Bates, 2002; Marks et al., 2002). For the same gene regions, understory birds from southern Amazonian forests can exhibit differences of up to 6% across rivers 200–400 m wide (Bates, unpub. data). Bates et al. (1999) found 6% sequence divergence between populations of Warbling Antbird, *Hypocnemis cantator*, separated by 300 km of Amazonian forest (*ca.* 600 km north of Huanchaca) with no obvious barriers to gene flow. Comparisons of protein electrophoretic data from bird populations separated by the Andes found genetic distances (Nei, 1978) to average 0.056 (Brumfield & Capparella, 1996); reviews of the avian literature find mean values between avian *species* to average 0.044 (Barrowclough, 1980); thus these trans-Andean values are high. Zink et al. (1995) used restriction fragment length polymorphisms to look at genetic structure of several avian taxa separated by the Bering Sea. They found up to 6% of genetic differentiation between taxa occurring on opposite sides of Beringia. Levels of divergence in the ten taxa we examined did not exceed 2.6% in spite of the substantial geographic distances separating the sampling localities (including a substantial water barrier).

Levels of divergence do not necessarily provide an indication of taxonomic rank. Zink et al. (1995) stressed that mtDNA data could constitute a gene tree embedded in the organismal phylogeny, thus other characters (and more

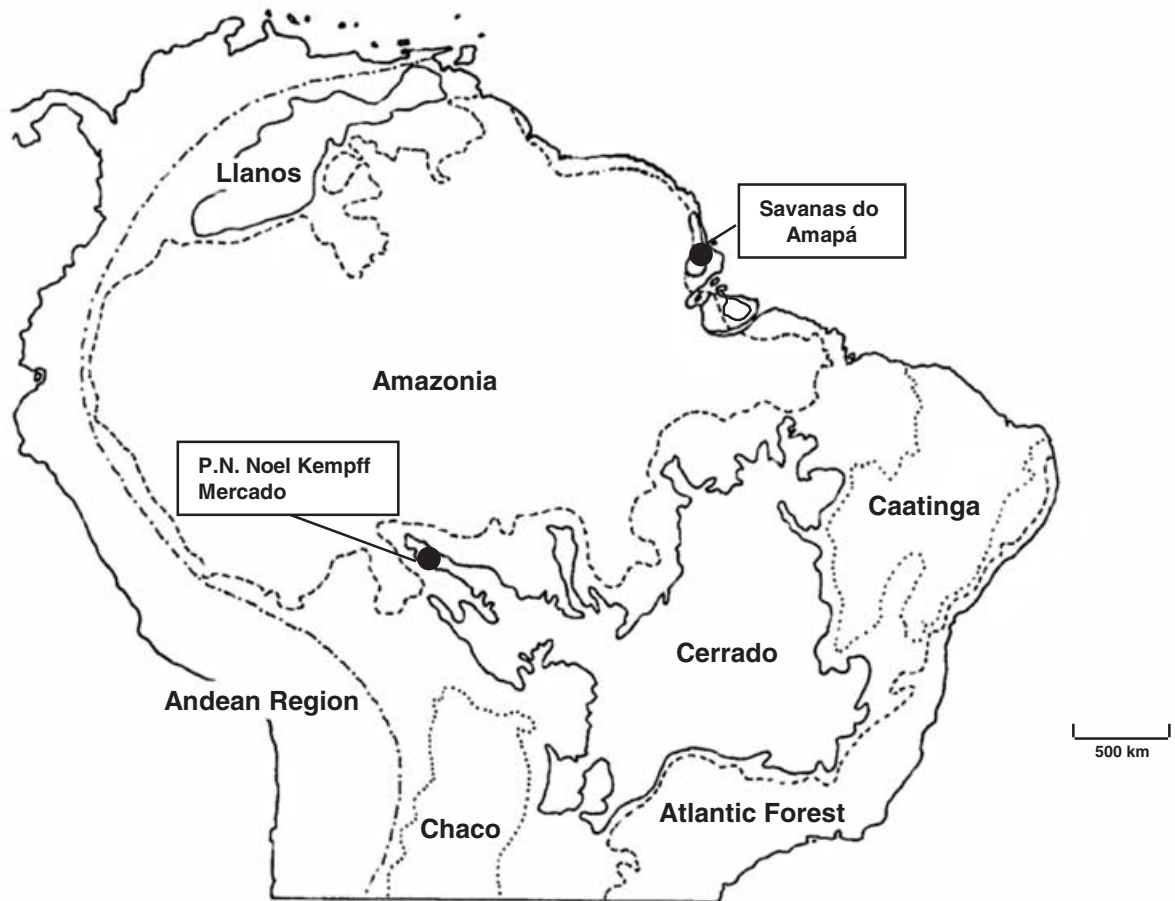


Fig. 1. Location of the study sites in the Cerrado region relative to the distribution of other major South American morphoclimatic domains (following Ab'Saber, 1977).

complete geographic sampling) should also be considered before making taxonomic decisions (Davis & Nixon, 1992). Among other things, our data do not rule out the possibility of genetic breaks between the regions we sampled even within species with little differentiation, and additional sampling of intervening regions is needed. Recognizing the limits of our data, we make no taxonomic recommendations based on the sequences we present.

Although most of the species we studied are typical of the Cerrado region (Silva, 1995a), their complete distributions and habitat requirements represent a variety of patterns that do not clearly correlate with levels of genetic differentiation (Table 2). One species, *Turdus leucomelas*, inhabits several different types of habitats ranging from borders of evergreen forests to deciduous woodlands and gallery forest and has a wide range encompassing northern South America, eastern Amazonia and tropical regions south Amazon (Table 2, Ridgely & Tudor, 1989). Two species, *Synallaxis albescens* and *Elaenia chiriquensis*, are widespread and occupy different types of open habitats (Silva, 1995a), but only the later species is known to make long-distance migrations (Marini & Cavalcanti, 1990). *Tangara cayana* inhabits several types of savannas and forest borders, presenting a 'circum-

Amazonian' distribution pattern (Remsen et al., 1991). Six other species possess a 'peri-Atlantic' pattern (Silva, 1995b), which means they occur throughout the Cerrado and north of the Amazon in northern and/or eastern South America. *Crypturellus parvirostris*, is known from isolated savannas north (only Amapá) and south (Santarém) of the Amazon. *Lepidocolaptes angustirostris* also is found in isolated Amazonian savannas north (Amapá, Monte Alegre and Sipaliwini) and south of the Amazon (Santarém). *Cypsnagra hirundinacea* is found within Amazonia only in patches of savanna located north (Amapá and Sipaliwini) of the Amazon (Silva, 1995b). In the east, *Formicivora rufa* is found in several Amazonian savanna patches south (Santarém) and north (Amapá, Monte Alegre and Sipaliwini) of the Amazon, but there also is an isolated population west of Amazonia in the Marañon Valley in Perú. *Eupetomena macroura* and *Sporophila plumbea* have populations above and below Amazonia and some populations in isolated savannas within Amazonia (Silva, 1995b).

The savannas of Amapá are located on Tertiary sediments and therefore are possibly more recent with respect to other Cerrado areas including the Serrania de Huanchaca, which are mostly on the old crystalline and sedimentary rocks of

the Brazilian Shield (Cole, 1986). For birds, a mixture of Cerrado species with taxa typical of northern South American savannas (e.g., Crested Bobwhite [*Colinus cristatus*] and Eastern Meadowlark [*Sturnella magna*]) demonstrates that the Amapá avifauna has been formed by contributions from both regions, although the Cerrado element is numerically dominant (Silva et al., 1997). Peri-Atlantic species probably arrived in Amapá from the south by crossing the lower reaches of the Amazon (Silva, 1995b; Silva et al., 1997). Although dispersal is possible, so is passive transport by 'rafting' across on an island in the ever-changing mouth of the river. No matter what the ultimate mechanism, for largely terrestrial taxa such as the tinamous *Crypturellus parvirostris*, this is an impressive feat. Two study species, *Formicivora rufa* and *Cypsnagra hirundinacea*, have not been recorded from the islands in the mouth of the Amazon (Pinto Henriques & Oren, 1997), but neither are obvious candidates for long-distance dispersal.

The degree of sequence divergence between populations is largely consistent with the presence or absence of described geographic variation (subspecies) for the study taxa (Table 2). Thus, the five species with levels of genetic divergence above 1% have subspecific differences recognized between the regions we sampled, and we suspect that these different taxa are Phylogenetic Species (following the definition of Zink & McKittrick, 1995). Three of the five species with less than 1% divergence have no recognized geographic variation (*Crypturellus parvirostris*, *Eupetomena macroura*, and *Elaenia chiriquensis*). *Lepidocolaptes angustirostris* and *Sporophila plumbea* have described geographic variation, but little or no divergence (only a single *Sporophila plumbea* was available from each region).

The geographic boundaries between subspecies of differentiated taxa vary, suggesting that there is not a simple history of vicariance. In *Formicivora rufa*, populations inhabiting the Amazonian savanna isolates are recognized as a separate subspecies (*chapmani*), although known specimens from Maranhão and Piauí (Silva et al., 1997) are intermediate between *chapmani* and the nominate subspecies, which is widespread south of Amazonia. *Synallaxis albescens* has one subspecies that is widespread south of Amazonia (*albescens*) and four described subspecies within Amazonia (Pinto, 1979). No intermediate specimens between Amazonian and extra-Amazonian subspecies are known. *Synallaxis albescens* is found in the savannas of the Amazon estuary whereas *Formicivora rufa* is not. The other differentiated taxa have forms in the east and west that may intergrade where they meet in the central Cerrado. In *Turdus leucomelas*, our examination of specimens indicates more population differences than those accounted for in the currently accepted subspecies. *Turdus leucomelas* from central Bolivia (including the Serranía de Huanchaca) are distinct in plumage from other Cerrado populations south of the Amazon, and these are distinct from populations north of the Amazon including Amapá; however, only two subspecies have been described across this distribution.

Our data provide insight into the effects of long term expansion and contraction of forest and open country habitats across the landscape of central South America (Silva, 1996). In examining the biogeography of South American forest birds, various authors (Haffer, 1985; Willis, 1992), have postulated that over the past 7–10 million years, various connections between Amazonia and Atlantic forest must have existed to permit exchange of forest species between regions that are isolated from one another today by Cerrado and other open country habitats. The timing and degree of connection are largely speculative. The low levels of genetic differentiation in the Cerrado taxa suggest that the paleoecological conditions which must have permitted the exchange of forest species between Amazonia and Atlantic forest did not isolate populations of the Cerrado birds we studied. This could be because the forest corridors were never particularly wide or completely continuous, but another possibility is that the distribution of Cerrado was historically much more limited (perhaps confined to the central portion of its current range) making gene flow possible. In this sense, savanna birds inhabiting the northern Cerrados (Amapá) may be the result of a more recent expansion from the 'core area' of Cerrado. Connections may have been possible through the savannas located along the Atlantic coast (Silva, 1995b, Silva et al., 1997, Silva and Bates, 2002). This is supported by the distribution of four of the study taxa (*Lepidocolaptes*, *Eupetomena*, *Crypturellus*, and *Cypsnagra*) in which most of their ranges are found within the Cerrados southeast from Amapá.

These data provide an initial view of levels of genetic diversity in Cerrado birds. We find relatively low levels of genetic divergence even within taxa that would seem to be poor dispersers (e.g., a tinamou), especially when compared to levels of genetic divergence in birds of the Amazonian forest understory. These low levels do not rule out the possibility that genetic structure exists across the Cerrado, and additional data from intervening regions need to be incorporated. However, for the taxa we studied it appears that any genetic structure that may exist evolved recently, and it would appear unlikely that many Cerrado bird species would have levels of population subdivision that approach those seen in many Neotropical forest birds.

Acknowledgments

We thank the Louisiana State University Museum of Natural Science and Museu Paraense Emílio Goeldi for access to specimens and/or tissues in their care. We also thank the Centro de Desarrollo Florestal in Bolivia and IBAMA in Brazil for granting permits. For help with Bolivian fieldwork, we thank staff of the Parque Nacional Noel Kempff Mercado, the Museo de Historia Natural 'Noel Kempff Mercado' and the people of the town of Florida. For work in Amapá, we thank the support from Chamflora, David C. Oren and Dionisio Pimentel. We gratefully acknowledge the field

efforts of colleagues who helped collect this material during the course of biotic surveys in Bolivia and Brazil. The molecular research was carried out in the Pritzker Laboratory for Molecular Systematics and Evolution operated with support from the Pritzker Foundation. We thank Lee Weigt, Jeffrey Hunt, and researchers in the Field Museum's Pritzker Lab for their assistance. Support for this project came from the office of Academic Affairs and the Ellen Thorne Smith Fund of the Field Museum of Natural History. José Maria C. da Silva received support from the Conselho Nacional de Desenvolvimento Científico e Tecnológico (Grant number 302464/88-3). The manuscript was improved by the comments of Shannon Hackett, Karl Schuchmann, and Jon Fjeldså.

References

- Ab'Saber AN (1977): Os domínios morfoclimáticos da América do Sul. Primeira aproximação. *Geomorfologia* 52: 1–21.
- Aleixo A (2002): Molecular systematics and the role of the “varzea”–“terra-firme” ecotone in the diversification of *Xiphorhynchus* woodcreepers (Aves: Dendrocolaptidae). *Auk* 119: 621–640.
- Ball RM Jr., Avise JC (1992): MtDNA phylogeographic differentiation among avian populations and the evolutionary significance of subspecies. *Auk* 109: 626–636.
- Barrowclough GF (1980): Genetic and phenotypic differentiation in a wood warbler (genus *Dendroica*) hybrid zone. *Auk* 97: 655–668.
- Bates JM (2000): Allozymic genetic structure and natural habitat fragmentation: data for five species of Amazonian forest birds. *Condor* 102: 770–783.
- Bates JM (2002): The genetic effects of forest fragmentation on five species of Amazonian birds. *J Avian Biol* 33: 276–294.
- Bates JM, Hackett SJ, Goerck J (1999): High levels of mitochondrial DNA differentiation in two lineages of antbirds (*Drymophila* and *Hypocnemis*). *Auk* 116: 1093–1106.
- Bates JM, Parker III TA, Capparella AP, Davis TJ (1992): Observations on the *campo*, *Cerrado* and forest avifaunas of eastern Dpto. Santa Cruz, Bolivia, including 21 species new to the country. *Bull Br Ornithol Club* 112: 86–98.
- Bermingham E, Rohwer S, Freeman S, Wood S (1992): Vicariance biogeography in the Pleistocene and speciation in North American wood warblers: a test of Mengel's model. *Proc Natl Acad Sci* 89: 6624–6628.
- Brumfield RT, Capparella AP (1996): Historical diversification of birds in northwestern South America: A molecular perspective on the role of vicariant events. *Evolution* 50: 1607–1624.
- Cole MM (1986): *The Savannas: Biogeography and Geobotany*. London, Academic Press.
- Davis JI, Nixon KC (1992): Populations, genetic variation and the delimitation of the phylogenetic species. *Syst Biol* 41:421–435.
- Desjardins P, Morais R (1990): Sequence and gene organization of the chicken mitochondrial genome. *J Mol Biol* 212: 599–634.
- Edwards SV (1997): Relevance of microevolutionary processes to higher level molecular systematics. In: Mindell DP, ed., *Avian Molecular Evolution and Systematics*. London, Academic Press, pp. 251–278.
- Gibbs JP (1998): Genetic structure of redback salamander *Plethodon cinereus* populations in continuous and fragmented forests. *Biol Conserv* 86: 77–81.
- Gill FG, Slikas B, Agro D (1999): Speciation in North American Chickadees: II. Geography of mtDNA haplotypes in *Poecile carolinensis*. *Auk* 116: 274–277.
- Hackett SJ (1996): Molecular phylogenetics and biogeography of tanagers in the genus *Ramphocelus* (Aves). *Mol Phyl Evol* 5: 368–382.
- Haffer J (1985): Avian zoogeography of the Neotropical lowlands. *Ornithol Monogr* 36: 113–146.
- Helm-Bychowski K, Cracraft J (1993): Recovering phylogenetic signal from DNA sequences: relationships within the corvine assemblage (class Aves) as inferred from complete sequences of the mitochondrial cytochrome-*b* gene. *Mol Biol Evol* 10: 1196–1214.
- Johnson NK, Remsen JV, Cicero C (1999): Resolution of the debate over species concepts in ornithology: a new comprehensive biologic species concept. In: Adams NJ, Slotow RH, eds., *Proceedings of the 22nd International Ornithological Congress*. Durban, Birdlife South Africa, pp. 1470–1482.
- Killeen TJ, Schulenberg TS, eds. (1998): *A Biological Assessment of Parque Nacional Noel Kempff Mercado, Bolivia*. RAP Working Papers 10. Washington DC, Conservation International.
- Lanyon SM, Hall JG (1994): Reexamination of barbet monophyly using mitochondrial-DNA sequence data. *Auk* 111: 398–397.
- Macey JR, Wang Y, Ananjeva N, Larson A, Papenfuss T (1999): Vicariant patterns of fragmentation among gekkonid lizards of the genus *Teratoscincus* produced by the Indian collision: a molecular phylogenetic perspective and an area cladogram for central Asia. *Mol Phyl Evol* 12: 320–332.
- Marini MA, Cavalcanti RB (1990): Migrações de *Elaenia albiceps chilensis* e *Elaenia chiriquensis albivertex* (Aves: Tyrannidae). *Bol Mus Para Emilio Goeldi (Zoologia)* 6: 59–67.
- Marks BD, Hackett SJ, Capparella AP (2002): Historical relationships among Neotropical lowland forest areas of endemism as determined by mitochondrial DNA sequence variation within the Wedge-billed Woodcreeper (Aves: Dendrocolaptidae: *Glyphorhynchus spirurus*). *Mol Phyl Evol* 24: 153–167.
- Mayr E (1963): *Animal Species and Evolution*. Cambridge, Harvard University Press.
- Mayr E, Paynter RA Jr. (1964): *Checklist of Birds of the World*. Vol. X. Cambridge, Massachusetts, Museum of Comparative Zoology.
- Moore WS, Graham JH, Price JT (1991): Mitochondrial DNA variation in the Northern Flicker (*Colaptes auratus*, Aves). *Mol Biol Evol* 8: 327–344.

- Moritz C, Faith DP (1998): Comparative phylogeography and the identification of genetically divergent areas for conservation. *Mol Ecol* 7: 419–429.
- Nei M (1978): Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics* 89: 583–590.
- Palumbi SR (1996): Nucleic acids II: the polymerase chain reaction. In: Hillis DM, Moritz C, Mable BK, eds., *Molecular Systematics, 2nd edition*. Massachusetts, Sinauer Associates, pp. 205–247.
- Patton JL, da Silva MNF, Malcolm JR (2000): Mammals of the Rio Jurúa and the evolutionary and ecological diversification in Amazonia. *B Am Mus Nat Hist* 244: 1–306.
- Paynter RA Jr. (1970): *Checklist of Birds of the World*. Vol. XIII. Cambridge, Massachusetts, Museum of Comparative Zoology.
- Peters JL (1931): *Checklist of Birds of the World*. Vol. I. Cambridge, Massachusetts, Museum of Comparative Zoology.
- Peters JL (1951): *Checklist of Birds of the World*. Vol. VII. Cambridge, Massachusetts, Museum of Comparative Zoology.
- Pinto OMO (1979): *Novo catálogo das Aves do Brasil. Primeira Parte*. São Paulo, Editora Gráfica da Revista dos Tribunais.
- Pinto Henriques LM, Oren DC (1997): The avifauna of Marajó, Caviana, and Mexiana islands, Amazon river estuary, Brazil. *Rev Bras Biol* 57: 357–382.
- Quinn TW (1997): Molecular sequences and evolutionary history in birds. In: Mindell DP, ed., *Avian Molecular Evolution and Systematics*. London, Academic Press, pp. 3–28.
- Remsen JV, Rocha O, Schmitt CG, Schmitt DC (1991): Zoogeography and geographic variation of *Platyrinchus mystaceus* in Bolivia, Peru, and the circum Amazonian distribution pattern. *Ornitol Neotrop* 2: 77–84.
- Ridgely RS, Tudor G (1989): *The Birds of South America. Part I: The Oscine Passerines*. Austin, University of Texas Press.
- Schneider CJ, Cunningham M, Moritz C (1998): Comparative phylogeography of Hawaiian terrestrial arthropods. *Mol Ecol* 7: 519–531.
- Seutin G, White BN, Boag PT (1991): Preservation of avian blood and tissue samples for DNA analyses. *Can J Zool* 69: 82–90.
- Silva JMC (1995a): Birds of the Cerrado Region, South America. *Steenstrupia* 21: 69–92.
- Silva JMC (1995b): Biogeographic analysis of the South American Cerrado avifauna. *Steenstrupia* 21: 49–67.
- Silva JMC (1996): Distribution of Amazonian and Atlantic birds in gallery forests of the Cerrado region, South America. *Ornitol Neotrop* 7: 1–18.
- Silva JMC, Bates JM (2002): Biogeographic patterns and conservation in the South American Cerrado: a tropical savanna hotspot. *BioScience* 52: 225–233.
- Silva JMC, Oren DC, Roma JC, Pinto Henriques LM (1997): Composition and distribution patterns of the avifauna of an Amazonian upland savanna, Amapá, Brazil. *Ornithol Monogr* 48: 743–762.
- Swofford DL (1998): PAUP*: Phylogenetic Analysis Using Parsimony (* and other methods), v. 4.0 Beta. Sunderland, Massachusetts, Sinauer Associates Inc.
- Traylor MA Jr. (1979): *Checklist of Birds of the World*. Vol VIII. Cambridge, Massachusetts, Museum of Comparative Zoology.
- Willis EO (1992): Zoogeographical origins of eastern Brazilian birds. *Ornitol. Neotrop.* 3: 1–16.
- Zink RM (1994): The geography of mitochondrial DNA variation, population structure, hybridization, and species limits in the Fox Sparrow (*Passerella iliaca*). *Evolution* 48: 96–111.
- Zink RM, Dittmann DL (1993): Gene flow, refugia, and evolution of geographic variation in Song Sparrow (*Melospiza melodia*). *Evolution* 47: 717–729.
- Zink RM, McKittrick MC (1995): The debate over species concepts and its implications for ornithology. *Auk* 112: 701–719.
- Zink R, Rowher MS, Andreev AV, Dittmann DL (1995): Trans-Beringia comparisons of mitochondrial DNA differentiation in birds. *Condor* 97: 639–649.