

# MITOCHONDRIAL DNA HYPERDIVERSITY AND VOCAL DIALECTS IN A SUBSPECIES TRANSITION OF THE RUFIOUS-COLLARED SPARROW<sup>1</sup>

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*Abstract.* The Rufous-collared Sparrow, *Zonotrichia capensis*, is widely distributed in neotropical America and shows extensive variation in its learned song. In northwestern Argentina it exhibits song dialects which map closely onto the distribution of natural vegetation assemblages. To date, there is no evidence of a correlation between genetic (allozyme) variation and dialects. However, recent genetic structuring produced through philopatry and assortative mating by dialect is difficult to demonstrate statistically with such protein-encoding nuclear genes. Therefore, we assayed variation in more rapidly evolving mitochondrial DNA along a 50 km transect, which spans three dialect boundaries between four adjacent habitat-types (from ~1,800 m to ~3,000 m), using restriction fragment length polymorphism analysis. This revealed exceptional diversity (41 clones from 42 individuals), a level comparable with DNA-fingerprinting, and higher than reported in any passerine over such a small area to date. The degree of nucleotide divergence between the two main clusters of mtDNA haplotypes implies a separation time in excess of one million years. The mtDNA variability is not related to song dialects; rather it is interpreted as a reflection of secondary introgression between two well-differentiated subspecies whose ranges abut in this region.

*Key words:* mtDNA; hypervariability; vocal dialects; introgression; Rufous-collared Sparrow; *Zonotrichia capensis*; Argentina.

## INTRODUCTION

Among the many phenomena which may permit or enhance the genetic differentiation of local bird populations is geographic variation in sexual advertisement vocalizations, sometimes organized as vocal, or song, dialects. Though the suggestion of such a role of vocal dialects has some antiquity (Marler and Tamura 1962), its significance remains controversial (e.g., Rothstein and Fleischer 1987), and the relationships between population genetic structure and dialects seem as varied as the study species (Handford and Nottebohm 1976, Baker 1982, Zink and Barrowclough 1984, Kroodsma et al. 1985, Balaban 1988, Lougheed and Handford 1992). However, allozyme variation provides for rather low statistical power in demonstrating spatial differentiation (Lewontin 1984), and it is worth applying the increased resolution offered by DNA analyses (Quinn and White 1987) to the problem.

Here we make the first explicit application (but see Fleischer and Rothstein 1988, Fleischer et al. 1991) of restriction fragment length polymorphism (RFLP) analysis to the question of concordance between population genetic structure and vocal dialects in the Rufous-collared Sparrow, *Zonotrichia capensis*.

The vocal dialects of *Z. capensis* are well-studied in northwestern Argentina (Nottebohm 1969, 1975; Handford and Nottebohm 1976; Handford 1981, 1988; Lougheed et al. 1989; Tubaro 1990; Handford and Lougheed 1991; Lougheed and Handford 1992). The dialect areas, which are defined by quantitative variation in terminal trill rate, are usually clearly defined, at least in this region, are consistently associated with natural vegetation types, and show long-term temporal stability (Handford 1988). Though the species displays substantial variation at allozyme loci, all studies of such variation (Nottebohm and Selander 1972, Handford and Nottebohm 1976, Lougheed and Handford 1992) have failed to show "excess genetic differentiation" (Zink and Barrowclough 1984) associated with dialects, and therefore provide no support to the

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local- or genetic-adaptation hypothesis on the origin and maintenance of vocal dialects.

## MATERIALS AND METHODS

Forty-two breeding male *Z. capensis* (all with well-developed cloacal protuberances) were collected in January and February 1990 from nine sites along a 50 km transect following Ruta Provincial 308 in the Sierra de Aconquija, Tucumán Province, Argentina, centered on the village of Tafi del Valle (26°52'S, 65°41'W). This transect is that used by Nottebohm (1975; Fig. 5) and Handford and Nottebohm (1976; Fig. 1), and spans three dialect boundaries (one of which is the most abrupt known in this species) between four adjacent vegetation types between ~1,800 m and ~3,000 m: alder woodland, montane grassland, moist puna and arid prepuna scrub (Table 1; see Handford 1988 for a description of the vegetation, the song-variants, and their co-distribution). Although we did not collect singing males, we did note that *all* males' songs heard during our collecting expeditions, from any given site, were typical of the appropriate dialect region. Moreover, we have established over numerous previous field seasons in the area of the transect that it is exceptionally unusual for any male to possess a foreign song during the breeding season. We therefore assume, since all males were in breeding condition, that collected males were capable of singing the appropriate "habitat-diagnostic" dialect. The transect is situated in a well-documented morphocline between two well-differentiated subspecies (*Z. c. hypoleuca*, typical of the northern Argentine plain lying below our transect samples, and the substantially larger and more brightly-plumaged *Z. c. pulacayensis*, typical of the high-Andean plateau some 100 km to the north of our transect) whose ranges meet in this part of Argentina (Chapman 1940; Handford 1983, 1985; Lougheed and Handford 1993).

Although Chapman (1940) tentatively assigned the birds at the higher end of the "Tafi trail" (the location of our present transect) to *pulacayensis*, the whole region in which our transect is embedded is one showing a fairly smooth transition from one set of morphological characteristics to another (Handford 1983, 1985; Lougheed and Handford 1993) and so subspecies allocation is best described as doubtful at present. See below for discussion.

Birds were collected, eight external morphological measurements taken, using dial calipers

(±0.1 mm; see Lougheed and Handford 1992 for a description), and immediately placed on dry ice for storage and transport to the laboratory. Heart, kidney and liver tissues were removed and the samples assayed for RFLP variation in mtDNA following published protocols (Van Wagner and Baker 1990) using 14 restriction endonucleases (Table 1). The profiles for each restriction enzyme were compiled for each individual so as to give composite mtDNA haplotypes. Haplotypes were clustered using UPGMA (Sneath and Sokal 1973). A principal components analysis (SYSTAT; Wilkinson 1991) was performed on a correlation matrix of the morphological variables.

## RESULTS AND DISCUSSION

The mtDNA molecule was approximately 16.6 kilobases long, consistent with lengths estimated for other birds (Zink 1991, Zink and Dittmann 1991). The 42 individuals yielded 41 haplotypes (Table 1) and clonal diversity of the whole sample, as estimated by Nei's G-statistic (Nei 1987), was 0.999. This value is the highest reported so far in birds (but see below), slightly exceeding that found in Pied Flycatchers (*Ficedula hypoleuca*) (Tegelström et al. 1990), and rivaling the hyperdiversity found in Menhaden Fish (*Brevortia tyrannus*) and Chuckwalla Lizards (*Sauromalus obesus*) (Avisé et al. 1989). Unlike these latter two species, hypervariability in restriction fragments in *Z. capensis* is apparently due to site polymorphism since we could not detect any evidence of size variation in mtDNA among individuals. Our result is remarkable given the narrow geographic scope of the sample area: this RFLP analysis, assaying approximately 8% of the mtDNA molecule, gave a level of resolution approaching that of nuclear DNA-fingerprinting. The very high diversity reported here, and in *Ficedula hypoleuca*, is related partly to the use of tetranucleotide restriction endonucleases; earlier studies (Shields and Wilson 1987, Avisé and Nelson 1989), reporting substantially lower levels of mtDNA diversity over much larger geographical areas, primarily used hexanucleotide endonucleases. The probability of a mutation giving a particular four-base sequence is sixteen times greater than achieving a specific six-base sequence.

The average estimated nucleotide divergence between haplotypes (p) (Nei and Li 1979) was 1.40%, and ranged from 0.036% to 3.20%. The

TABLE 1. Composite RFLP haplotypes for 42 individual Rufous-collared Sparrow, *Zonotrichia capensis*, revealed by analyses using the following endonucleases (listed left to right in the table): EcoR I, Ava I, Nci I, Alu I, Cfo I, Dde I, Hinf I, Hpa II, Mbo I, Mse I, Rsa I, Sau96 I, Taq I, Tha I. Sites had the following characteristics: Site 1, distance along Provincial Route 307 measured from the town of Acheral (km) = 46, alder woodland, trill interval (TI) = 120 msec, dialect 1; Site 2, km = 52, montane grass/scrubland, TI = 50 msec, dialect 2; Site 3, km = 58, montane grass/scrubland, TI = 60 msec, dialect 2; Site 4, km = 78, montane grassland, TI = 60 msec, dialect 2; Site 5, km = 81, montane grassland/moist puna, TI = 40 msec, dialect 2/3 transition; Site 6, km = 85, moist puna, TI = 20 msec, dialect 3; Site 7, km = 88, arid prepuna scrub, TI = 300 msec, dialect 4; Site 8, km = 91, arid prepuna scrub, TI = 300 msec, dialect 4; Site 9, km = 95, arid prepuna scrub, TI = 300 msec, dialect 4.

Site	Ind	Haplotypes													
1	1	A	A	A	C	A	A	B	A	C	A	A	A	A	A
	2	A	A	A	C	A	A	B	A	H	B	A	B	A	A
	3	A	A	A	C	A	A	E	A	C	B	A	A	B	B
	4	A	A	A	C	A	A	B	A	C	A	A	B	A	A
2	1	A	A	A	C	A	A	A	A	C	E	B	B	A	A
	2	A	A	A	C	A	A	A	A	C	A	A	B	A	A
	3	A	A	A	C	A	A	A	A	C	A	A	C	A	A
	4	A	A	A	C	B	A	A	B	G	H	A	C	B	B
	5	A	A	A	C	B	B	A	B	C	D	A	C	B	A
3	1	A	A	A	D	B	B	D	B	A	C	B	C	B	B
	2	A	A	C	C	B	B	A	C	D	G	A	G	B	B
	3	A	A	A	C	A	A	A	C	A	A	A	A	A	A
	4	A	A	B	C	B	A	A	B	A	D	A	A	B	B
4	1	A	A	A	D	B	B	D	B	B	J	A	A	B	B
	2	A	A	A	C	A	A	A	B	C	A	A	A	A	A
	3	A	A	B	A	B	B	A	B	C	C	A	B	B	B
	4	A	A	A	C	A	A	A	B	C	D	A	A	B	B
5	1	A	A	A	D	B	B	A	B	B	C	B	E	B	A
	2	A	A	A	C	A	A	A	A	C	A	A	A	A	A
	3	A	A	A	C	B	A	A	B	A	D	A	F	B	B
	4	A	A	A	J	A	A	A	A	C	A	A	A	A	B
	5	A	A	A	I	A	A	E	A	A	A	A	B	B	A
6	1	A	A	B	D	B	B	D	B	B	C	B	C	A	B
	2	A	A	A	E	A	A	B	B	C	A	A	A	A	B
	3	A	A	B	D	B	B	A	B	B	C	A	C	A	B
	4	A	A	A	E	A	A	B	A	C	A	A	A	A	A
	5	A	A	A	D	A	A	A	A	C	A	A	A	A	B
7	1	A	A	B	B	A	A	A	B	A	D	A	A	B	B
	2	A	A	B	A	A	B	A	B	A	D	A	B	B	B
	3	A	A	B	A	D	B	A	B	A	D	A	B	A	B
	4	A	A	A	C	A	A	B	A	C	A	B	A	A	A
8	1	A	A	B	G	B	A	A	B	A	F	C	A	B	B
	2	A	A	B	F	B	A	E	B	D	F	D	A	A	B
	3	A	A	B	H	B	A	A	B	E	G	A	A	B	B
	4	A	A	A	F	B	A	A	A	C	A	A	B	B	B
	5	A	A	A	F	A	A	A	A	A	I	A	D	B	B
	6	A	A	A	C	B	A	B	A	C	A	A	A	A	A
	7	A	A	A	G	G	B	C	B	F	C	A	C	B	B
9	1	A	A	A	C	A	A	E	A	B	A	A	A	A	A
	2	A	A	A	C	A	A	E	A	C	A	A	A	A	A
	3	A	A	A	C	A	B	E	B	A	C	A	E	B	B
	4	A	A	A	C	A	A	E	A	C	J	A	A	A	A

UPGMA dendrogram of mtDNA haplotypes (Fig. 1) revealed that there is substantial genetic mixing among sites (i.e., individuals from the same sites do not cluster together), and that the mtDNA variation has no geographic structure

corresponding to dialect designations. This result supports findings of a lack of correlation between the patterns of variation in allozyme frequencies and dialect areas (Handford and Nottebohm 1976, Loughheed and Handford 1992).

# PERCENT SEQUENCE DIVERGENCE

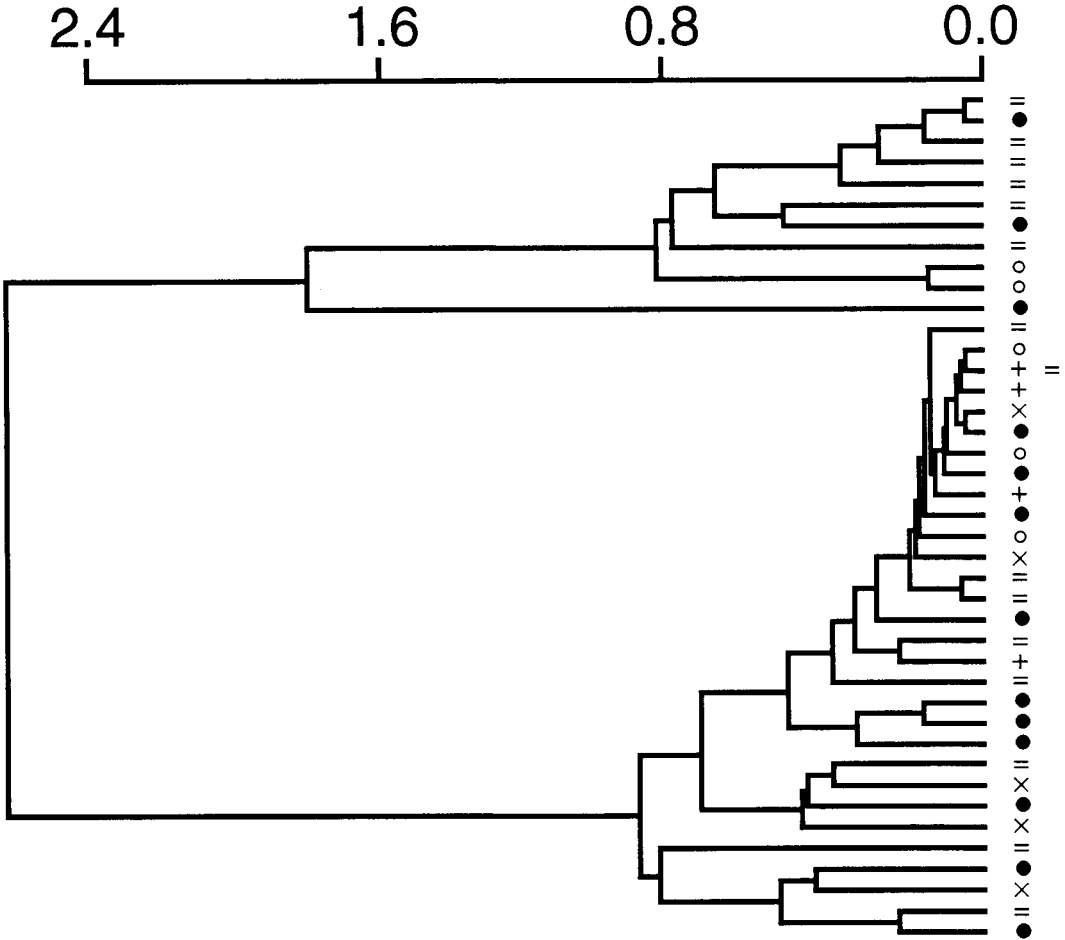


FIGURE 1. UPGMA dendrogram of 42 male Rufous-collared Sparrows, *Zonotrichia capensis*, clustered according to their composite mtDNA profiles. Individuals are coded by their dialect group (see Table 1).

Songs in *Z. capensis* are understood to be learned (Egli 1971, Tubaro 1990), and therefore both the present DNA results, and the allozyme data (Lougheed and Handford 1992), strongly imply that cultural evolution resulting in dialects does not affect dispersal or mating patterns, and thus does not promote genetic differentiation. This has the corollary that strong cultural selection is operating such that birds dispersing among dialects (as they evidently do) will switch their song types, or that naive (first-breeding) birds learn the song of the dialect area in which they settle, rather than persist with a song which they may have acquired the previous year on their

natal area. These inferences are all consistent with some findings in *Z. leucophrys* (Baptista and Morton 1981, 1982; Baptista and Petrinovich 1986) and, thereby, with the honest convergence hypothesis (Rothstein and Fleischer 1987) about the origin and maintenance of vocal dialects in birds.

Having concluded that vocal dialects have no impact on mtDNA variation in this case, in what follows, we investigate other possible factors involved in the spatial patterning of this variation. The dendrogram in Figure 1 shows two strongly defined clusters (approximately 2.7% sequence divergence). Only one of a large number of trees

generated is shown; however, all of the trees show this deep branching-point, and with only minor rearrangements in branch tips within each major cluster. Application of the molecular clock, estimated for other avian species (e.g., Shields and Wilson 1987), suggests an overall divergence time between these two major groups in excess of 1.0 million years before present, with most clones within the two groups diverging less than 0.5 million years before present. This depth of separation is one of the largest reported within any bird species.

Allozyme variation in the area immediately surrounding this transect (estimated from other samples) is slight (Handford and Nottebohm 1976, Loughheed and Handford 1992), as is commonly the case in birds (Barrowclough 1983). However, allozyme variation assayed in a sample (474 males; 24 sites; 20 loci) of *Z. capensis* derived from a large region (22° to 27°S; 64° to 67°W) surrounding the present area (Loughheed and Handford 1992) shows very high levels of divergence (overall  $F_{ST} = 0.118$ ). This high  $F_{ST}$  is primarily driven by heterogeneity at the phosphoglucumutase-1 locus (E.C. 5.4.2.2) among the four most northerly, high altitude, sites (>3,000 m.a.s.l.). Those four samples are the only ones in that study unambiguously attributable to the subspecies *Z. c. pulacayensis*, whose range is centered on the Bolivian high plateau (Chapman 1940, Handford 1985); the remainder belong either to subspecies *Z. c. hypoleuca* of the Argentine plains, or to populations transitional between the two. Chapman (1940) located the intergrade between *Z. c. hypoleuca* and the substantially larger *Z. c. pulacayensis* in the very same locality as our present transect (his "Tafi trail").

These observations suggest that members of each of the mtDNA clusters might be identifiable with these two subspecies. However, ANOVA shows that the two main mtDNA clusters do not differ significantly on either PC1 or PC2 scores from a principal components analysis ( $P = 0.153$  and  $0.197$  respectively). Nevertheless, a UPGMA cluster analysis based on site means of PC1 and PC2 scores (Fig. 2) shows that birds from sites 7, 8 and 9 are strongly separated from all others by their larger size, as expected from other findings (Handford 1983, 1985; Loughheed and Handford 1992). If we inspect Figure 1 we see that the less numerous haplotype-group is absent from the two lowest-altitude sites (1 and 2), and

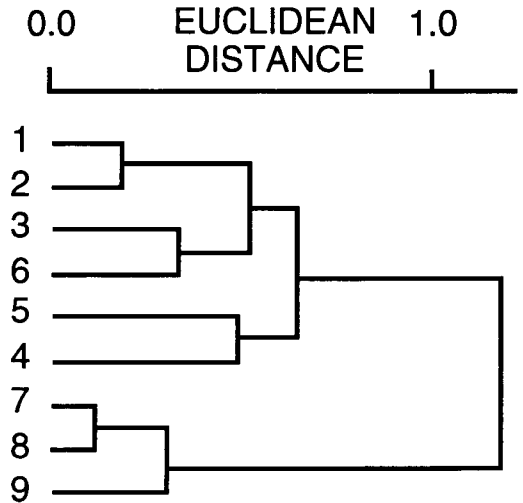


FIGURE 2. UPGMA dendrogram based on the mean site scores on principal components 1 and 2, using eight external morphological characters from 42 male Rufous-collared Sparrows, *Zonotrichia capensis*. Sites are as coded in Table 1.

from site 5, so it is possible to identify this smaller cluster provisionally as *Z. c. pulacayensis*, and the larger cluster as *Z. c. hypoleuca*. The estimated separation time of these two mtDNA clusters (see above) is consistent with the 0.8 million year estimate derived from the allozyme samples (Loughheed and Handford 1992), assuming 28.3 million years per unit Nei's (1978) corrected genetic distance (Zink and Avise 1990).

Combining these observations, we would provisionally infer that the deep cleft in Figure 1 corresponds to a subdivision between *Z. c. hypoleuca* and *Z. c. pulacayensis*. This is consistent with the scenario of Chapman (1940) for the Pleistocene colonization of South America by this species from the presumed North American homeland of the genus (but see Zink et al. 1991). He envisaged one direct route to the south, following open country at higher elevations along the Andean chain, and another more indirect route around the coastal margins of the Amazonian lowlands, moving among temporary regions of open habitat as they became available during dry phases (Prance 1982), eventually reaching the extensive and more permanently-open habitats of the Matto Grosso and the Paraguay/Argentine plain; the two long-sundered groups would enter secondary contact in our study region. The estimated time of divergence (ap-

proximately one million years) is of the order required to accommodate Chapman's scheme. If his reconstruction is accurate, it would suggest that there is a zone of secondary contact situated in the front ranges of the Andes of northwestern Argentina, and our allozyme and mtDNA results suggest that this zone is at least 300 km deep. We are at present evaluating this matter with further samples which together span most of the species' range in Argentina, from Bolivia to Tierra del Fuego, and from the Andean foothills to the Atlantic coast.

In summary, hyperdiverse mtDNA and allozyme characters together provide preliminary evidence of large-scale patterns corresponding to subspecies divisions generated by Pleistocene biogeographic events; these patterns are overlain by, and unrelated to, a recent mosaic of a cultural character, that of learned song dialects.

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