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SPECIES LIMITS AND RECENT POPULATION HISTORY IN THE CURVE-BILLED THRASHER[1]

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Abstract. We surveyed 1,115 base pairs of mitochondrial DNA sequence from three gene regions in 66 Curve-billed Thrashers (*Toxostoma curvirostre*) taken from throughout their range. We found that populations sorted unambiguously into three groups. Two of these groups, the Curvirostre and Palmeri groups, have been known for many years because of their distinctive phenotypic characteristics, and their status was recently confirmed with an intensive morphometric analysis. The third (southern) group consists of populations in Puebla and Oaxaca, Mexico. The strong morphological and mtDNA distinctiveness of the Curvirostre and Palmeri groups suggests that they warrant species status. We recommend that more specimens of the southern group be obtained prior to formal taxonomic recognition of this form. Coalescence analyses suggest that the Curvirostre group has undergone a recent population increase, whereas the Palmeri group seems to have been more stable in its Sonoran Desert range.

Key words: coalescence theory, Curve-billed Thrasher, mismatch distribution, mitochondrial DNA, phylogeography, subspecies, Toxostoma curvirostre.

INTRODUCTION

The Curve-billed Thrasher (*Toxostoma curvirostre*) is a widely distributed and relatively common inhabitant of desert and semi-desert regions of the southwestern United States and Mexico (Tweit 1996). Taxonomically it is divided into seven subspecies (AOU 1957), which vary in range from the highly localized *T. c. insularis*, which breeds on Tiburon Island, to *T. c. celsum*, found in much of central Mexico; disagreement exists as to the precise distribution and distinctiveness of the subspecies (Phillips 1986, Tweit 1996). The subspecies are typically classified into two subspecies groups, an eastern "Curvirostre group" (including *T. c. curvirostre, T. c. celsum, T. c. oberholseri*) and a western or Sonoran Desert "Palmeri group" (including *T. c. palmeri, T. c. maculatum, T. c. occidentale,* and *T. c. insularis*). The subspecies groups differ in degree of ventral coloration (Curvirostre group is lighter with more contrasting spots) and size of white tail spots (smaller in Palmeri group). The western group occurs primarily in the Sonoran Desert, whereas the eastern group ranges throughout the Chihuahuan Desert southwards to the Mexican state of Chiapas. After intensive multivariate study of morphometric and colorimetric characters, Rojas-Soto (1998) concluded that subspecies were not supported, and he proposed that only the two subspecies groups merited taxonomic recognition.

To clarify taxonomic limits, we sequenced parts of three mitochondrial genes, cytochrome *b* (Cyt *b*), ND2, and the control region. We used phylogenetic methods to discover patterns of genetic differentiation, and methods derived from coalescence theory to reconstruct past population history.

METHODS

Specimens were collected at 13 localities (Fig. 1), where tissues were removed and either frozen in liquid nitrogen or placed in a room-temperature buffer (Seutin et al. 1991). Voucher specimens are deposited in the Bell Museum (University of Minnesota), American Museum of Natural History, Museum of Natural Science (Louisiana State University), and the Museo de Zoología (Universidad Autonoma de México). Locality information is given with the GenBank accessions (see below). DNA was extracted following chelex (Ellegren 1992) and phenol/chloroform methods. Polymerase chain reaction (PCR) followed standard techniques. Primers ND6E (Edwards 1993) and HCR4 (Tarr 1995) were used to amplify and sequence a 367-bp portion of the Control Region I (CRI; see Baker and Marshall 1997 for discussion and characterization of this region of the mitochondrial ge-

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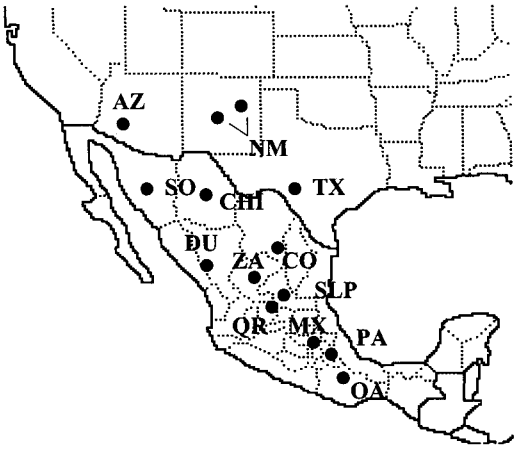

FIGURE 1. Distribution of sampling sites of specimens of Curve-billed Thrasher. Abbreviations listed in Table 1.

nome). A 433-bp portion of Cyt *b* was amplified and sequenced with primers L14841 (Kocher et al. 1989) and H15299 (Hackett 1996). Primers L5215 and H5578 (Edwards 1993) amplified a 315-bp portion of ND2. PCR products were purified with a Qiaquick PCR purification kit (Qiagen, Valencia, California). Dye-terminator sequencing was performed on an ABI Prism 310 automated sequencer. ND2 and Cyt *b* PCR products were sequenced on both light and heavy strands. CRI was sequenced only on the heavy strand because the light primer failed to function in sequencing. Sequences were aligned visually and with the use of Sequencher 3.0 (Gene Codes Corporation 1999).

To estimate phylogenetic relationships of haplotypes, we used PAUP* (Swofford 1999) to construct maximum parsimony trees; base positions were equally weighted. Given typical coalescence times, we believe that this gene tree captures major divisions in the organismal tree. Outgroup taxa included *Toxostoma rufum, T. bendirei,* and *T. crissale.* The data were bootstrapped 10,000 times using the fast bootstrap method in PAUP*.

To estimate aspects of recent population history, we used the program Arlequin (Schneider et al. 1996) to compute nucleotide diversity (π), haplotype diversity, F_{st} (pairwise among population samples and across all populations), Nm, and a mismatch distribution (for pooled samples). Nucleotide diversity measures the degree of genetic variability, F_{st} the degree of population subdivision, and Nm the average rate of interpopulational gene flow. The mismatch distribution is simply the frequency distribution of pairwise (individual) sequence differences, which is compared with a chi-squared test to a distribution for a population recently experiencing exponential growth (Rogers 1995). Fu and Li's (1993) test for departure from neutral expectation was computed with the program DnaSP 3.0 (Rozas and Rozas 1999).

RESULTS

We resolved 1,115 base pairs for 66 individual Curve-billed Thrashers and one of each of the three outgroups; sequence data have been deposited in GenBank (U75574, AF287160–228, AF287493–629). The two singleton gaps in the control region presented no alignment difficulties. Forty-eight of the 66 (73%) individuals represented distinct haplotypes; the high percentage of haplotypes is evidence that our sequences are mitochondrial, because nuclear copies should be much less variable (Zhang and Hewitt 1996). Considering only the sequences from the Curve-billed Thrasher, there were 67 variable positions of which 40 were phylogenetically informative. Maximum parsimony analyses revealed three clades of haplotypes (Fig. 2). Clade one included the six identical individuals from southern Mexico (Oaxaca and Puebla), the second clade included individuals from the Sonoran Desert (Palmeri group), and the third clade included individuals from the eastern Curvirostre group. The phylogenetic analyses revealed no geographic structure within the Palmeri or Curvirostre clades.

Levels of sequence variation were typical of those found in other birds (Baker and Marshall 1997). For all individuals, haplotype (gene) diversity was 0.97 and π was 0.011. There was considerable geographic variation. F_{st} was 0.72, and Nm values (not shown) among population samples averaged above 2 within the two main groups, except for the Texas sample, in which values were ca. 1.0. The most widespread haplotype was CBTH63QR, which was also found in CHI, CO, DU, and SLP; its frequency was 9.1%. The mismatch distribution (Fig. 3) for the Curvirostre clade is significantly different (P = 0.036) from the expectation for an exponentially expanding population. However, the observed distribution is very similar in shape to the expected one, which we take as evidence of a

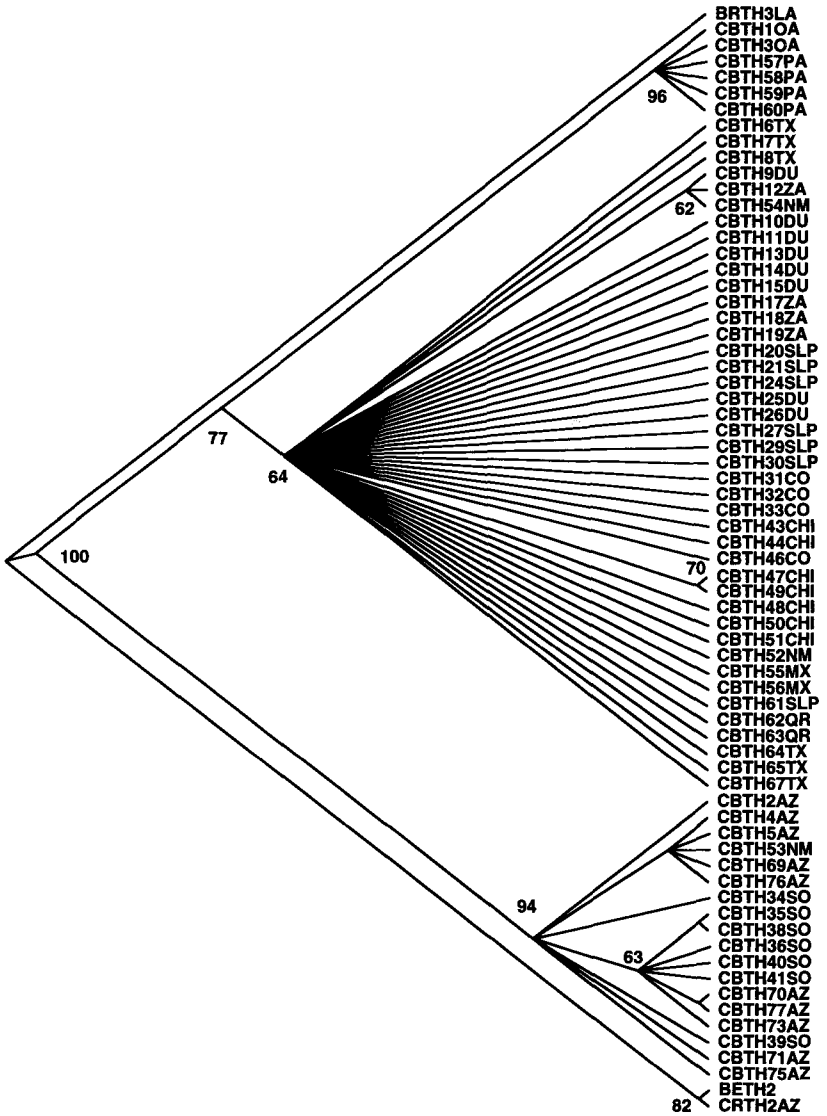

FIGURE 2. Bootstrap consensus tree of haplotypes showing three main clades. CBTH = Curve-billed Thrasher followed by lab numbers, and letter abbreviations which refer to sampling sites shown in Figure 1. Numbers at nodes are bootstrap percentages for main clades only.

growing population. The mismatch distribution (Fig. 3) for the Sonoran sample does differ ($P < 0.05$) from the expectation of the sudden expansion model; however, it is not close in shape to the expected distribution (not shown) for a constant population size. Fu and Li's (1993) test was significant ($P < 0.05$) for the eastern group, and insignificant for the Sonoran sample. The pattern of nucleotide diversity among sites (Table 1) does not suggest directionality of population expansion because all values are similar. Nucleotide diversity values for the pooled eastern and Sonoran samples were 0.0039 and 0.0044, respectively.

DISCUSSION

SPECIES LIMITS

The high F_{st} value and the phylogenetic reconstruction of haplotype evolution show that the Palmeri group and the Curvirostre group de-

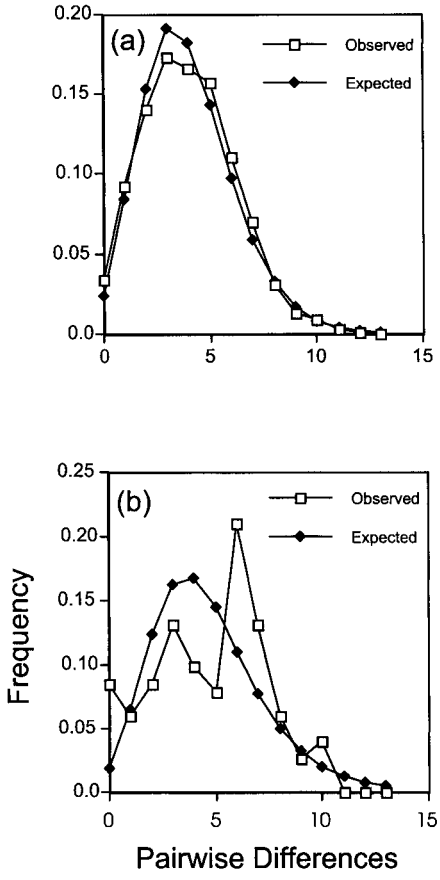

FIGURE 3. Observed mismatch distributions for the combined eastern (a) and western (b) samples. Black boxes with connecting lines show the expected distribution for a sudden population expansion (Rogers 1995).

serve recognition as evolutionarily significant units (Moritz 1994), as well as phylogenetic species (Cracraft 1989). Each group is reciprocally monophyletic and separated from each other by approximately 2% sequence divergence. Furthermore, the individuals from Oaxaca and Puebla, representing the southern part of *T. c. curvirostre,* are distinct and likely also merit species status. The geographic distance between the samples from MX (Fig. 1), representing the southernmost part of the mtDNA-defined Curvirostre group, and the sample from southern Puebla (PA; Fig. 1), is approximately 250 km. Nonetheless, more sampling is warranted before altering species status of the southern group, which differs by ca. 1% from the Curvirostre group. We emphasize that it is the pattern of reciprocal monophyly shown in the haplotype tree, and not the degree of sequence divergence, that indicates phylogenetic species status for the Palmeri and Curvirostre groups. Furthermore, morphological support exists for the Palmeri and Curvirostre groups (Tweit 1996, Rojas-Soto 1998), although none was apparent for the southern group (Oaxaca and Puebla) detected here. Reciprocally monophyletic groups, especially those with corroborating evidence from morphology, deserve species status owing to their evolutionary independence.

Whether the Curvirostre and Palmeri groups would function as biological species is unclear. Our small sample from New Mexico includes haplotypes from each of the two main groups, which might indicate hybridization. However, the two individuals (CBTH52NM, CBTH54NM)

TABLE 1. Population samples and basic sequence statistics for population samples of the Curve-billed Thrasher. See Figure 1 for geographical locations of samples.

Location	No. birds	No. haplotypes	π
Chihuahua (CHI)	7	7	0.0038
Coahuila (CO)	4	4	0.0027
Durango (DU)	8	8	0.0033
Mexico (MX)	2	2	0.0027
New Mexico (NM)	2	2	0.0063
Queretaro (QR)	2	2	0.0027
San Luis Potosi (SLP)	7	6	0.0027
Texas (TX)	6	5	0.0032
Zacatecas (ZA)	4	4	0.0064
Arizona (AZ)	11	8	0.0042
Sonora (SO)	7	6	0.0042
Puebla (PA)	4	1	0.0000
Oaxaca (OA)	2	1	0.0000

with the Curvirostre-type sequences are from eastern New Mexico (Quay and Harding counties), whereas the specimen (CBTH53NM) that has the Palmeri-type sequence was taken just south of Albuquerque (Socorro County), New Mexico, nearer the main part of the range of that form. Hence the division between Curvirostre and Palmeri groups might reside in central New Mexico. Although there are specimens of probable hybrid status between the Palmer and Curvirostre groups (O. Rojas-Soto, pers. comm.), it is noteworthy that these two groups are not sister groups (Fig. 2), adding to the growing list of such examples in birds (Freeman and Zink 1995).

Lack of support for the named subspecies of *T. curvirostre* is unsurprising in light of recent general surveys of mtDNA data (Ball and Avise 1992, Zink 1997, Klicka and Zink 1999). Many avian subspecies do not receive corroborating support from mtDNA data because they are often based on divisions of single character clines (Zink et al., in press). When characters are considered simultaneously in multivariate analyses, these clines tend to cancel each other out. That is, each character is likely responding to a different environmental gradient, which themselves overlap in a non-hierarchical fashion. Thus, if taxonomists were to emphasize different characters, the resulting (subspecies) classifications would differ. Such was the case in Rojas-Soto's (1998) multivariate analysis of *T. curvirostre,* which showed that although there is considerable geographic variation within the Curve-billed Thrasher (revealed by the prior description of seven subspecies), only two main groups have attained evolutionary independence worthy of taxonomic recognition. Resolution of the systematic status of the populations in southern México await study of additional specimens.

POPULATION HISTORY

Coalescence theory (Slatkin and Hudson 1991) provides methods for detecting events in past population history, such as exponential increases in size. The mismatch distribution for the eastern group of Curve-billed Thrashers is consistent with a major population increase, which might be expected given that the areal extent of deserts has been increasing (Axelrod 1983). Population increases are known to cause departures from neutral expectations, which might explain the significant value of Fu and Li's (1993) test statistic for the Curvirostre group. Recent population expansion and concomitant gene flow would also explain why there is no geographic structure in the haplotype tree over this large area. The mismatch distribution of the Sonoran group is not consistent with exponential population growth, suggesting that the population in this region might have been historically more stable. Interestingly, nucleotide diversity was similar in each region, suggesting that each has had similar long-term effective population sizes. Although we only obtained six individuals from Puebla and Oaxaca, the lack of variability suggests a population bottleneck in this region.

Although molecular clocks are controversial (Klicka and Zink 1997), dating of cladogenic events by calibrated DNA sequence distances provides general times of divergence. Although there are no independent fossil- or biogeography-based calibrations for the combination of genes used here, the Cyt *b* data give similar distance values. Using the rate estimated by Fleischer et al. (1998) for Cyt *b,* the coalescent point for the Curvirostre and Palmeri groups occurred approximately one million years before present. Accounting for ancestral haplotype diversity (Avise and Walker 1998, Klicka and Zink 1999), the time of lineage splitting (as opposed to the coalescence point of haplotypes) occurred at least 500,000 years before present. This date is older than that predicted for some pairs of passerine bird species (Klicka and Zink 1999). Even though these groups of thrashers currently are only recognized taxonomically at the subspecies level, mtDNA calibrations suggest that these divergences were also prior to the global climate changes precipitated by the last two cycles of Pleistocene glaciations (Klicka and Zink 1997).

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