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Migration and evolution of lesser long-nosed bats *Leptonycteris curasoae*, inferred from mitochondrial DNA

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Abstract

We used sequence variation within 297 bp of control region mitochondrial DNA (mtDNA) amplified from 53 lesser long-nosed bats, *Leptonycteris curasoae* (Phyllostomidae: Glossophaginae) captured at 13 locations in south-western United States and Mexico and one site in Venezuela to infer population structure and possible migration routes of this endangered nectar- and fruit-eating species. Phylogenetic analysis using maximum parsimony and UPGMA confirmed species and subspecies distinctions within *Leptonycteris* and revealed two clades exhibiting 3% sequence divergence within the Mexican subspecies, *L. c. yerbabuenae*. Even though many roosts contained *L. c. yerbabuenae* from both clades, weak population structure was detected both by a correlation between genetic differentiation, F_{st}, and geographical distance and by a cladistic estimate of the number of migration events required to align bat sequences with geographical location on maximum parsimony, as compared to random, trees. Three results suggest that *L. c. yerbabuenae* are more likely to migrate between sites along the Pacific coast of Mexico or along the foothills of the Sierra Madre Occidental than between these regions. (1) Seventeen of 20 bats which shared an identical sequence were captured up to 1800 km apart but within the same putative migration corridor. (2) Residuals from a regression of F_{st} on distance were greater between than within these regions. (3) Fewer migration events were required to align bats with these two groups than expected from random assignment. We recommend analysing independent genetic data and monitoring bat visitation to roost sites during migration to confirm these postulated movements.

Keywords: control region, endangered species, long-nosed bat, migration, mitochondrial DNA

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Introduction

Seasonal movements and migratory behaviour of bats have traditionally been studied by banding individuals, either in summer colonies or in winter hibernacula, and recapturing them at other sites (e.g. Tuttle 1976). Banding efforts involving species that live in large colonies and that migrate relatively long distances, however, have not been successful in locating alternate roost sites. For example, researchers banded over 430 000 *Tadarida brasiliensis*, a seasonal migrant from Mexico into Texas, New Mexico, and Arizona, but did not recover any banded bats in Mexico (Cockrum 1967; Glass 1982). An alternative to mark-recapture studies is to use genetic markers to infer migration. Although nuclear gene frequencies have been used to test for population structure in migratory bats (McCracken *et al.* 1994), mtDNA sequence variation should be more informative for assessing movement patterns (e.g. Baker *et al.* 1990; Morin *et al.* 1994; Norman *et al.* 1994) because mtDNA is inherited maternally, is not influenced by male mating patterns, and does not undergo recombination (Harrison 1989).

Although phylogenetic analysis of mtDNA sequence variation within populations has been promoted for gaining insight into processes underlying population differentiation (Avise *et al.* 1987), discriminating between alternative explanations for patterns of genetic variation is often difficult because none of the key genetic parameters – e.g. effective population size, mutation rate, or migration rate – can usually be estimated directly (Moritz 1994). For

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example, although mtDNA sequence divergence has commonly been associated with geographical isolation (Avise 1989; Avise 1994), mtDNA sequence divergence has also been linked to differences in rates of evolution (Wayne *et al.* 1990), diversifying selection (Ballard & Kreitman 1994; Nachman *et al.* 1994; Rand *et al.* 1994), and large effective population sizes (Ball *et al.* 1988). One method for dealing with this uncertainty, which we have adopted in this paper, is to test alternative explanations, such as selection and unequal mutation rates, before attempting to infer migration from phylogenetic information.

Several methods have recently been proposed for inferring genetic exchange from nucleotide sequence data. The cladistic measure estimates the minimum number of migration events, *S*, required to account for the distribution of subpopulation origins across a gene phylogeny (Slatkin & Maddison 1989). This number can then be used to estimate the product of population size and migration rate, *Nm*, from simulations assuming neutral evolution. Alternatively, *Nm* can be inferred from F_{st} estimated from pairwise comparisons of sequences within and between subpopulations (Hudson *et al.* 1992; Slatkin 1993). Both of these techniques assume an island model in which unidirectional migration events occur with equal probability between all sites. While unrealistic, this assumption offers the virtue of providing a way to identify those subpopulation pairs that are either more different or more similar than expected from this model. Differences from expectation in either method therefore can be used to infer which subpopulations among those sampled are most likely either to have had recent ancestors in common or exchanged migrants.

Here we apply these two methods to control region sequences of mtDNA to draw inferences regarding the population differentiation and migration patterns of the lesser long-nosed bat, *Leptonycteris curasoae*. This 23-g nectar-, pollen- and fruit-eating bat frequents arid regions of Mexico, the south-western United States, and Venezuela and feeds primarily at the flowers and fruit of columnar cacti and from the flowers of several *Agave* species (Arita

Fig. 1 Geographical location of the 13 sampling sites. Numbers correspond to sites listed in Table 1. Symbol type identifies sites which align genetically with either the putative coastal (circles) or inland (squares) migratory populations. Stippled area indicates the distribution of *L. c. yerbabuenae* (based on Arita 1991).

1991; Cockrum 1991). Although it has long been known that some North American populations of *L. curasoae* migrate because maternity sites in northern Mexico and southern Arizona are occupied only during the spring and summer (Cockrum 1991), little evidence is available to indicate migration routes or the extent to which populations in the southern part of the range in Mexico make seasonal movements. Such information is needed as this species is currently listed as endangered (Schull 1988). Based on an analysis of the carbon stable isotope composition of the muscle tissue of *L. c. yerbabuenae*, Fleming *et al.* (1993) postulated that some populations of *L. curasoae* migrate north from central Mexico along a 'nectar corridor' of blooming columnar cacti in the spring and then move south following blooming paniculate agaves in the fall. A more complex migration pattern is suggested, however, by the arrival of bats at north-western maternity sites in May and at some north-eastern non-maternity sites in July or August (Cockrum 1991). In addition, pregnant and lactating females have been observed or captured in southern roosts during the winter months (Quiroz *et al.* 1986; Cockrum 1991; this study). Whether all or only some females migrate and give birth biannually is currently unknown.

We selected the 5′ end of the mtDNA control region for sequence analysis because recent work in bats (Wilkinson & Chapman 1991; Worthington Wilmer *et al.* 1994), primates (Morin *et al.* 1994), mice (Nachman *et al.* 1994), and cetaceans (Hoelzel *et al.* 1991; Arnason *et al.* 1993), has revealed high substitution rates in this region. In several species of vespertilionid bats, this region often contains extensive variation in length and heteroplasmy is commonly observed (Wilkinson & Chapman 1991; Petrie *et al.* 1995; F. Mayer, personal communication). Both length variation and heteroplasmy could complicate analyses based on sequence similarity. Fortunately, we detected no evidence of length or sequence heteroplasmy in any of the species sequenced for this study.

In this paper we first report on tests of selection and substitution rate equality and then examine evidence for evolution, population subdivision and migration of *Leptonycteris* bats sampled throughout their range in the United States and Mexico (*L. c. yerbabuenae*) and Venezuela (*L. c. curasoae*). To test for selection in the control region of *L. curasoae*, we compare the number of segregating sites to nucleotide diversity (Tajima 1989). To test for equal rates of evolution, we compare sequences obtained from both subspecies of *L. curasoae* with its sole congener, *L. nivalis*, and a species from the same subfamily, *Glossophaga soricina*, using phylogenetic tests (Tajima 1993). *L. nivalis* is similar to *L. curasoae* in diet, morphology and habits, but is larger and typically occurs at higher elevations in central Mexico (Arita 1991). By using substitution rates for the same mtDNA region in mice (Nachman *et al.* 1994), we infer separation times among these taxa. We test for unrestricted gene flow by comparing levels of genetic differentiation (Hudson *et al.* 1992; Slatkin 1993) to geographical distance and by applying the cladistic measure of genetic exchange (Slatkin & Maddison 1989) between sampling sites. Finally, we evaluate whether our data are more consistent with one or two migratory populations. Because some *L. c. yerbabuenae* arrive at north-eastern roost sites later than at north-western sites, we consider two migration routes: either along the western coast of Mexico and Baja California extending north into south-western Arizona or along the foothills of the Sierra Madre Occidental connecting with roosts in south-eastern Arizona (Fig. 1).

Methods

Sampling locations

Between April 1992 and May 1993, one of us (THF) estimated colony size and captured *L. c. yerbabuenae* at 13 day roosts in Arizona and Mexico (Table 1, Fig. 1). We refer to seven of these sites as northern summer colonies because *L. curasoae* usually is not found in these caves or mines outside of the spring and summer months (Table 1). The remaining six sites – Santa Domingo Mine (Aduana), Isla San Andres Cave (Chamela), Cueva La Mina (Ajiijic), Gruta Juxtlahuaca, Cueva La Capilla (Baja), and Cueva Rancho Tempisque (Chiapas) – contain bats throughout the year and will be termed southern permanent sites even though the number of individuals in most of these roosts varies seasonally. To determine if different populations of bats utilized the Aduana site, we sampled this cave on two occasions when the resident population differed in size (Table 1).

In addition, THF captured *L. c. curasaoae* at Cueva Piedra Honda on the Paraguana Peninsula in northern Venezuela in December 1992 and Rodrigo Medellin captured several *L. nivalis* at Cueva del Diablo, Morelos, south of Mexico City in June 1993. GSW captured an individual *Glossophaga soricina* in a mine near the town of Los Angeles, Guanacaste Province, Costa Rica, in July 1994.

At most sites 10 bats were captured in mist nets while exiting the roost or with hand nets inside the roost. After sexing and weighing each bat, a small piece of patagial membrane, ≈ 10 mm^2, was excised with biopsy scissors and stored in a concentrated salt solution (Seutin *et al.* 1991). Membrane samples were shipped without refrigeration to GSW's laboratory in Maryland but then kept at 4 °C until extraction.

DNA extraction, amplification and sequencing

DNA was extracted from a tiny portion of each wing membrane sample using either Chelex (Walsh *et al.* 1991)

Table 1 Location, date, reproductive state of captured females, number of sequences obtained from and estimated size of *Leptonycteris curasoae* roosts sampled in Arizona and Mexico (cf. Fig. 1)

Location	Date	No. of sequences	Repro. state*	No. of bats	Method of estimation†
Northern summer sites					
1. Bluebird	14 May 1992	4	PG	2000 adults	VC
2. Copper Mtn	1 May 1992	4	PG	10 400 adults	EC
3. Pinacate	16 May 1992	3	PG	80 000 adults	EC
4. Patagonia	29 August 1992	3	PL	19 800 adults and pups	VC
5. Hilltop	15 May 1993	3	N	400 adults	VC
6. Carbo	2 May 1992	3	PG	1000 adults	VC
7. Kino Bay	27 April 1992	4	PG	2600 adults	EC
Southern permanent sites					
8. Aduana	20 October 1992	4	N	1000 adults	VC
	13 February 1993	4	PG	20 000 adults	VC
9. Baja	12 April 1993	4	L	20 000 adults and pups	VC
10. Chamela	29 October 1992	4	N	50 000 adults	VC
11. Ajiijic	2 November 1992	3	N	20 000 adults	EC
12. Juxtlahuaca	14 November 1992	4	PG	2000 adults	VC
13. Chiapas	20 January 1993	2	L	10 000 adults	VC

*PG, pregnant; L, lactating; PL, post-lactating; N, neither pregnant nor lactating.
†EC, exit count; VC, visual census inside roost.

or a Qiagen DNA extraction kit following the manufacturer's protocol. A 372 bp fragment from the 5′ end of control region mtDNA was amplified using two 22-bp primers, P and CSB-F (Wilkinson & Chapman 1991). The P primer begins at position 15975 in the human proline tRNA gene (Anderson *et al.* 1981) while the CSB-F primer ends at position 16425 in a conserved sequence block found in the middle of the control region (Southern *et al.* 1988). Double-stranded amplifications using the polymerase chain reaction (PCR) were performed as described in Wilkinson & Chapman (1991) using 40 cycles of 95 °C for 1 min, 55 °C for 1.5 min, and 72 °C for 2 min in a Peltier thermal cycler. Amplification products were purified and concentrated using either ethanol precipitation or a Qiagen PCR-prep kit following the manufacturer's instructions. Double-stranded DNA was sequenced by the dideoxy chain termination method using an ABI automated sequencer at the Molecular Genetics Instrumentation Facility at the University of Georgia.

Control region sequence was obtained in both directions from 56 bats by using either the P or F primer to initiate the sequencing reaction. We restricted sequence comparisons within the amplified fragment to a 297-bp region for which there was complete overlap of sequence from both reactions. If the complementary sequences in this region exhibited any ambiguous nucleotide sites after pairwise alignment using the program Gene Jockey, then a new fragment was amplified, cleaned, and sequenced. The number of sequences obtained for *L. curasoae* from each sampling location is given in Table 1.

Phylogenetic and statistical techniques

A critical assumption of all phylogenetic methods is that the characters being used for reconstructing the phylogeny are not under selection. We used Tajima's D statistic (Tajima 1989) to test that assumption by comparing nucleotide diversity, π, to the number of segregating sites, θ, for both subspecies of *Leptonycteris curasoae*. If mutations are neutral, then these two statistics should be equal (Nei 1987).

Because maximum parsimony, which is used for the cladistic measure of genetic exchange (Slatkin & Maddison 1989), can fail to resolve evolutionary relationships correctly when the rate of evolution along different branches of a phylogenetic tree are not equal (Felsenstein 1988), we employed Tajima's 1D and 2D methods (Tajima 1993) to test if branch lengths change at a constant rate over the phylogeny. This method tests whether the number of substitutions inferred for each branch of a dichotomous tree are equal using a χ^2 test for goodness-of-fit. We used the 1D test, with 1 degree of freedom, for comparisons within subspecies where insufficient sample sizes were available to partition substitutions into transitions and transversions and the 2D test, with 2 degrees of freedom, for branch length comparisons at higher nodes where transitions and transversions could be counted separately.

We reconstructed phylogenetic relationships among taxa and sampling sites with PHYLIP (Felsenstein 1993) using UPGMA (Unweighted Pair Group Method with Arithmetic Mean) on Kimura's two-parameter distances

(Kimura 1980) assuming a transition/transversion ratio of 2.5:1. We resampled the data 100 times to obtain bootstrap values (Felsenstein 1985) for each node on the tree. We also used the heuristic search method in PAUP (Swofford 1993) to identify all minimal trees from informative sites by maximum parsimony. After comparing over 16 million trees a strict consensus tree was created from 7600 equally parsimonious trees. The average transition/transversion ratio across a sample of 100 equally parsimonious trees was 2.565 with a minimum of 2.527 and a maximum of 2.567.

To estimate *S*, the minimum number of migration events (Slatkin & Maddison 1989), we created a new character in the nucleotide data matrix which contained 13 states to represent the collection site for each of the 49 samples of *L. c. yerbabuenae*. Then, using MacClade (Maddison & Maddison 1992), we computed *S*, the minimum number of steps, i.e. migration events, necessary for all samples to share a common geographical origin. To determine if there is evidence of restricted movements between sites we then compared the distribution of *S* obtained from 1000 equally parsimonious trees to that obtained from 1000 randomly formed trees with 49 tips. The proportion of *S*-values obtained using random trees which were lower than observed *S*-values indicated the probability of obtaining as many *S*-values in a panmictic population. We performed a similar test to evaluate an hypothesis of two migratory populations by computing the minimum number of steps required to cluster each sample with a putative migratory population again for either 1000 equally parsimonious trees or 1000 random trees.

To provide a second measure of genetic interchange we calculated the product-moment correlation between F_{st} and geographical distance between all pairs of the 13 sites where *L. c. yerbabuenae* were collected. For this analysis $F_{st} = 1 - H_w/H_b$ where H_w is the mean number of nucleotide differences between samples from a single site and H_b is the mean number of nucleotide differences between samples taken from two different sites (Hudson *et al.* 1992). Because we used Kimura two-parameter distances to correct for multiple hits when calculating H_w and H_b, our F_{st} is comparable to N_{st} (Lynch & Crease 1990; Hudson *et al.* 1992). We did not compare estimates of the neutral theory parameters, *Nm*, against distance as proposed by Hudson *et al.* (1992) because in many cases H_b was less than H_w which caused *Nm* to be negative or undefined. H_b can be less than H_w when two individuals from each of two clades are present in the same population. For example, if the sequence divergence between clades is 3%, then the average pairwise difference within both locations would be 3%. In contrast, the between-site pairwise difference might only be 1.5% if the sequences from each clade were identical.

We assessed the significance of the correlation between F_{st} and geographical distance using a Mantel test (Mantel & Valand 1970; Dietz 1983). This test compares two matrices by randomizing without replacement the columns and then the rows of one of the matrices using the same random order for both columns and rows. After each randomization, we calculated a pairwise element product-moment correlation. We then estimated the probability of obtaining the observed correlation by chance from the proportion of 1000 ranked correlations that exceeded the observed correlation.

In addition to testing for a relationship between genetic differentiation and geographical distance we also evaluated an hypothesis of two different migratory populations by comparing the residuals from the regression of F_{st} on geographical distance. For this test we calculated the difference between the average residual for pairs of sites postulated to be from the same migratory population to the average residual for pairs of sites from different populations. The null expectation is that the average residuals will not differ. We determined the probability associated with the observed difference by comparing it to a distribution of differences obtained from the 1000 randomizations used in the Mantel test described above. During each randomization, we recomputed the regression of F_{st} on geographical distance, the residuals for each pair of sites, and the difference between the average residuals. Thus, this test controls for geographical distance to determine if sites within a putative population exhibited less genetic differentiation than those between populations.

Results

Selection, mutation rate and evolution

Comparison of nucleotide polymorphisms with substitutions did not provide any evidence for selection on this part of the mtDNA control region. Nucleotide diversity, π, was approximately equal to the number of segregating sites, θ, for both *L. curasoae yerbabuenae* and *L. c. curasoae* as expected if mutations were neutral. For *L. c. yerbabuenae* there were 38 variable sites giving $\theta = 0.02870$ while $\pi = 0.01599$. Tajima's test statistic $D = -0.23$ was not significantly different from zero. The four *L. c. curasoae* individuals exhibited three variable sites resulting in $\theta = 0.00485$, $\pi = 0.00561$ and $D = 0.04$, which also did not differ significantly from zero.

Phylogenetic analysis using either UPGMA (Fig. 2) or maximum parsimony provided strong support for the taxonomic distinctions made at the species and subspecies levels (Arita & Humphrey 1988) with bootstrap values of 100% at each of these nodes. Each species and subspecies also produced a uniquely sized fragment. In comparison to the control region sequence amplified for *L. c. curasoae*, *G. soricina* had five deletions, *L. nivalis* exhibited three

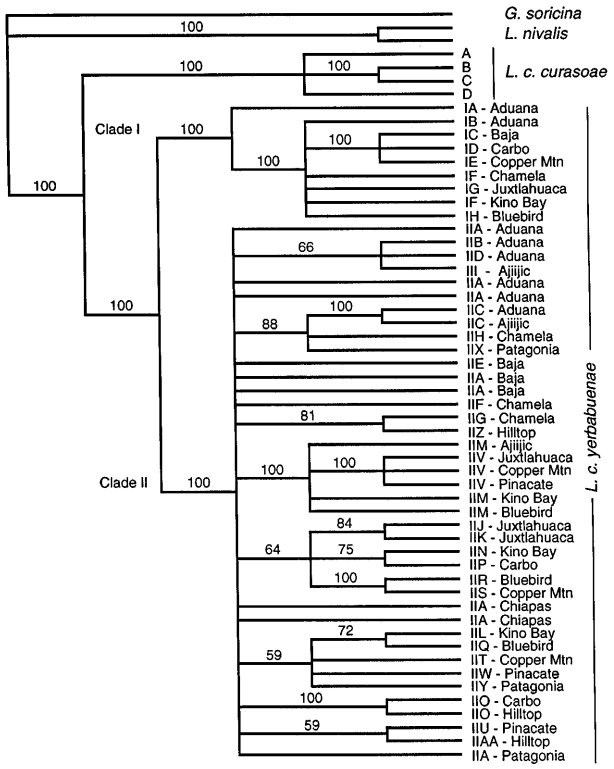

Fig. 2 UPGMA tree for all 56 samples based on two-parameter Kimura distances (Kimura 1980) with transition:transversion ratio set to 2.5. Number of bootstrap replicates supporting each node is above branch. Sequences for each *L. c. yerbabuenae* haplotype are given in Table 3.

deletions, while all *L. c. yerbabuenae* had a single deletion at position 248 (Table 2). Within the subspecies *L. c. yerbabuenae*, two distinct clades were evident from both the UPGMA and parsimony analyses. Although further resolution within the two *L. c. yerbabuenae* clades was suggested by UPGMA (Fig. 2), only those nodes with 100% bootstrap values were completely concordant with the strict consensus maximum parsimony tree. Examination of the collecting locations represented in each clade revealed no geographical concordance between the two *L. c. yerbabuenae* clades and roosting location. Every site represented in the smaller clade (henceforth clade I), was also represented by an individual aligned with the larger clade (clade II).

Comparison of nucleotide substitutions along the branches involving any three clades in the phylogeny provided no evidence for unequal mutation rates. Comparison of clade I and clade II substitutions, using *L. c. curasoae* as the outgroup, yielded a Tajima 1D $\chi^2 = 0.67$ ($P > 0.5$), *L. c. yerbabuenae* compared to *L. c. curasoae* with *L. nivalis* as the outgroup gave Tajima 2D $\chi^2 = 1.4$ ($P > 0.4$), and *L. nivalis* compared with *L. curasoae* using *G. soricina* as the outgroup resulted in a Tajima 2D $\chi^2 = 5.1$ ($P > 0.05$).

Average pairwise distances, using the 2-parameter Kimura metric between species, subspecies and the two clades within *L. c. yerbabuenae* are presented in Table 2 along with estimates of divergence times between each taxonomic unit based on corrected genetic distances (Nei 1987). Divergence times were estimated assuming a divergence rate of 10%/MY as estimated for the control region among mice in the genus *Mus* (Nachman *et al.* 1994). Under this assumption, the two clades of *L. c. yerbabuenae* diverged about 200 000 years ago.

Table 2 Average uncorrected pairwise genetic distances on and above diagonal and estimated divergence times (MYBP) from corrected genetic distances (Nei 1987) below the diagonal assuming 10% divergence/MY

				L.c.y.	
	G.s.	*L.n.*	*L.c.c.*	c.I	c.II
G. soricina	–	0.269	0.217	0.245	0.232
L. nivalis	2.624	0.014	0.108	0.113	0.103
L. c. curasoae	2.140	0.984	0.006	0.062	0.060
L. c. yerbabuenae, clade I	2.412	1.016	0.550	0.008	0.030
L. c. yerbabuenae, clade II	2.262	0.912	0.519	0.203	0.011

Population subdivision

Comparison of genetic differentiation with geographical distance between sampling locales for *L. c. yerbabuenae* indicated that movement between populations was partially restricted over the range of the subspecies (Fig. 3). The correlation between F_{st} and geographical distance was 0.376 ($P = 0.015$, Mantel test). The impact of the phylogenetic structure on the pairwise sequence differences is clearly seen in Fig. 4. The distribution of all possible pairwise sequence differences among *L. c. yerbabuenae* sites was bimodal, not Poisson. A Poisson distribution would be expected if each sample experienced mutations independently, as might occur in an exponentially growing population or a true 'star' phylogeny (Slatkin & Hudson 1991).

Weak population subdivision was also supported by the cladistic analysis of migration. The median minimum number of migration events, $S = 31$, inferred from 1000 equally parsimonious trees for the 49 samples of *L. c. yerbabuenae* was lower than the median minimum number of migration events, $S = 37$, inferred from 1000 random trees. Because 8 random trees required 32 or fewer steps (Fig. 5a), $P = 0.008$ that assignment of sequence to geographical location occurred by chance.

Migration

Here we consider two alternative hypotheses regarding possible migration patterns of *L. c. yerbabuenae*. (1) A single

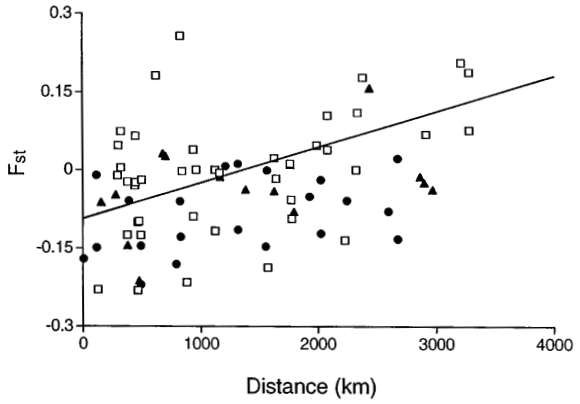

Fig. 3 Least squares regression plot of genetic differentiation (F_{st}) on geographical distance between all pairs of sampling locations for *L. c. yerbabuenae*. Comparisons among inland pairs of sites – Chiapas, Ajiijic, Aduana, Carbo, Patagonia and Hilltop – are indicated by triangles, while those among the remaining coastal sites are indicated by circles and boxes denote comparisons between inland and coastal sites. Solid symbols indicate comparisons within inland or coastal corridors and open symbols indicate comparisons between inland and coastal corridors.

panmictic migratory population in which all southern sampling sites contribute migrants with equal probability to all northern summer sites. (2) Two populations of bats migrating either along the coast or along the foothills of the Sierra Madre Occidental. The putative coastal corridor contained the Juxtlahuaca, Chamela, Baja, Kino, Pinacate, Bluebird and Copper Mountain sampling sites while the inland corridor consisted of the Chiapas, Ajiijic, Aduana, Carbo, Patagonia and Hilltop locations (Fig. 1). Three lines of evidence supported the second hypothesis.

First, the geographical distribution of identical haplotypes from *L. c. yerbabuenae* suggested that extensive north–south movements of bats occurs between sites, largely as predicted by the postulated coastal and inland migration routes. Four of 35 unique haplotypes were detected both at northern summer sites and at southern permanent sites, some of which were over 1800 km apart (Table 3). Despite the presence of lactating females in winter (Table 1), the Chiapas and Juxtlahuaca sites shared unique haplotypes with two northern summer sites, Patagonia and Pinacate, respectively. Such shared unique haplotypes would not be expected if there were two genetically distinct populations of *L. c. yerbabuenae*, one of which did and the other did not migrate. The distribution of shared unique haplotypes was also not consistent with independent exchange of migrants between all sites. In contrast, 17 of 20 sequences shared by more than one bat supported the hypothesis of two migration routes (Table 3, Fig. 1). The probability of incorrectly assigning no more than 3 of 20 binomial events was only 0.004. The three exceptions included haplotype IIA, which occurred in two Baja bats and in six other bats from three inland sites, and haplotype IIM, which was found in an Ajiijic inland bat and in two other IIM bats from coastal sites (Table 3). The sequences obtained for bats captured at the Aduana site in October did not differ consistently from those taken in February and included representatives from both clades (Table 3), as expected if these bats were sampled from a common population.

Secondly, comparison of residual F_{st} values within and between postulated corridors from a regression of F_{st} on distance (Fig. 3) was more compatible with two, rather than one, migratory populations. Of 42 coastal-inland corridor comparisons, 28 fell above the least squares regression of F_{st} on distance while 24 of 36 within-corridor comparisons fell below the regression line (Fig. 3). The difference between the mean residuals for the between migration corridor populations and the within corridor populations was 0.0622. A difference that large or larger had a probability of 0.041 (1000 randomizations) of occurring by chance.

Third, the cladistic measure of migration supported two migratory routes. When sites were aligned with either a coastal or an inland migration route as described above, median $S = 9$ using 1000 equally parsimonious trees for the 49 samples of *L. c. yerbabuenae*. In contrast, median $S = 16$ when migration events were estimated between two populations using 1000 random trees. Because 7 random trees required 11 or fewer steps (Fig. 5b), $P = 0.007$ that the observed sequences were distributed randomly among the two proposed migration corridors.

Discussion

The control region mtDNA sequence variation presented in this paper has implications for the evolution, population structure, and migration within Mexico of the lesser long-nosed bat, *Leptonycteris curasoae*. Interpretation of this genetic information does not appear to be confounded by selection or by unequal mutation rates along different branches of the *Leptonycteris* phylogenetic tree. Sequence comparisons among species suggest that *Glossophaga*, which is a primitive member of the subfamily, and *Leptonycteris*, which is a relatively derived member of the subfamily (Koopman 1981), last shared a common ancestor about 2.4 million years ago. The two species of *Leptonycteris* last had a common ancestor about 1 million years ago, the two subspecies of *L. curasoae* separated about 0.54 million years ago, and the two clades of *L. c. yerbabuenae* diverged about 200 000 years ago.

If these dates accurately reflect evolutionary history, they suggest that these taxa are the products of climatic

events occurring during the late Pliocene and Pleistocene. Prior to the uplift of the Mexican plateau and its flanking Sierra Madres in the late Pliocene about 2 million years ago (Axelrod 1979), a single arid-adapted species of *Leptonycteris* is likely to have occurred in Mexico. After the uplift, this species must have split into the current two species – an upland *L. nivalis* and a lowland *L. curasoae* (Arita 1991). The presence of *L. curasoae* in Mexico and northern South America suggests that an arid or semi-arid corridor connected these regions during at least one Pleistocene glacial advance. Remnants of that corridor, as represented by night-blooming columnar cacti, are evident as far south as north-western Costa Rica today (Janzen & Liesner 1980). During mid-Pleistocene this corridor may have broken, producing the two subspecies of *L. curasoae*.

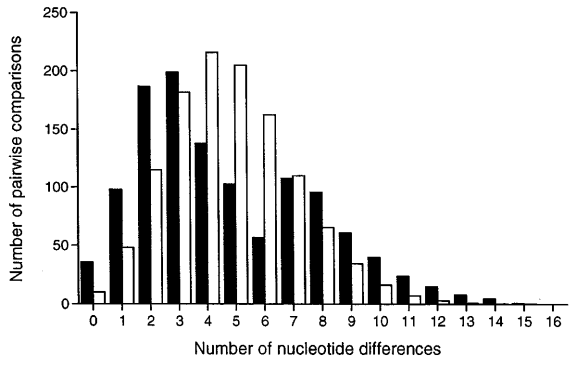

Fig. 4 Frequency histograms of number of sample pairs against number of nucleotide differences for the 297-bp control region product from 49 *L. c. yerbabuenae* (solid bars) compared to Poisson expectation (open bars).

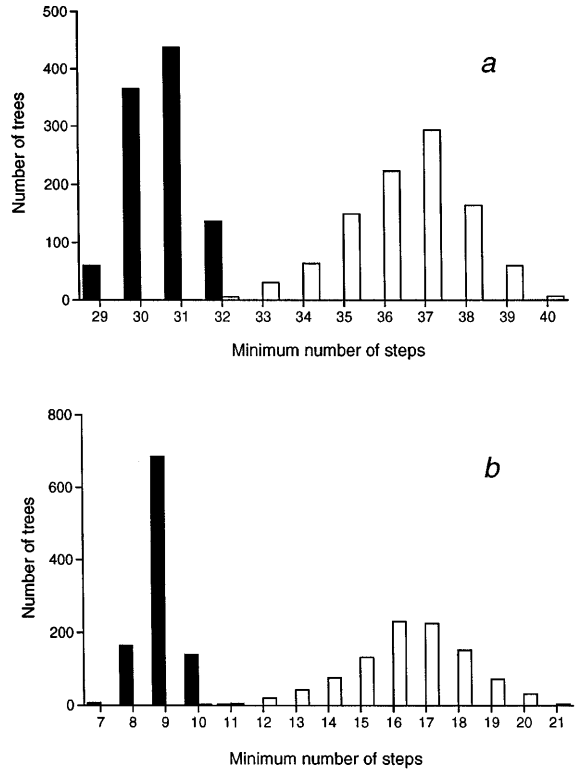

Fig. 5 Minimum number of steps required to align all *L. c. yerbabuenae* alleles with (a) geographical locations or (b) either of two putative migration corridors (see text) using 1000 equally parsimonious (■) or random trees (□).

The presence of two clades with 3% sequence divergence at both northern and southern *L. c. yerbabuenae* roost sites is unusual among mobile animals. In most cases studied to date, levels of mtDNA sequence divergence among populations of highly mobile animals are typically low (i.e. < 1%) in the absence of geographical isolation (Avise 1989). Often lineage sorting within populations leads to the presence of a single common haplotype. Sequence divergence on the order of several percentage or greater has, in most cases, only been documented among geographically separate or isolated populations (Avise 1989, 1994). For example, the ghost bat, *Macroderma gigas*, exhibits 4.6% sequence divergence among 330 bp of control region mtDNA, but different alleles associate geographically with disjunct Australian populations (Worthington Wilmer *et al.* 1994). Our results suggest, in contrast, that movements between roost sites has maintained considerable mtDNA haplotype diversity among *L. c. yerbabuenae*. If we assume that mtDNA alleles become monophyletic between 2N and 4N generations after population isolation (Neigel & Avise 1986), then N, the effective population size, of *L. c. yerbabuenae* must have recently been between 50 000 and 100 000 individuals, assuming one generation per year.

Our most compelling evidence for migration is the presence of shared unique haplotypes between distant sites. These results, in conjunction with a relatively high number of putative migration events required to enable the sequences to align parsimoniously with the sampling sites and a significant, but low, correlation between F_{st} and geographical distance between sampling sites, strongly support extensive movements among sampling locations by individual *L. c. yerbabuenae*.

While genetic drift within a single large panmictic population could explain the sequence divergence observed between the two clades, this hypothesis is not sufficient to explain the pattern of shared unique haplotypes, the relationship between F_{st} and geographical distance, or the estimated number of migration events required to align the phylogeny with sampling locations. Instead, our results suggest that *L. c. yerbabuenae* seasonally move more often

Table 3 Nucleotide sequences for all unique *Leptonycteris curasoae* haplotypes sampled at each site. Numbers in parentheses indicate number of individuals with identical haplotypes. Letters refer to month of capture for bats sampled at the Aduana site

Haplotype	Site	Sequence* at variable sites†
L. c. yerbabuenae		
IA	Aduana (O)	ATTGGTTCACAAGGACAAGTTTCATTAGTCATTCGCTAATTCG
IB	Aduana (F)	.....C..A.....
IC	Baja	.....A...G.....A.....
ID	Carbo	.....G.....A.....G....
IE	Copper Mtn	.....G.....G.....G...A.....C.....
IF	Chamela Kino Bay	.....A.....
IG	Juxtlahuaca	.....T.....A.....
IH	Bluebird	.....GA.....
IIA	Aduana (2,O) Aduana (F) Patagonia Baja (2) Chiapas (2)	.....GT...A.....C..T.....G....
IIB	Aduana (O)	.....GT...A.....C....C.....G....
IIC	Aduana (F) Ajiijic	...A....GT...A.....CC.T.....G....
IID	Aduana (F)	.....TGT...A.....C..T.C.....G....
IIE	Baja	.....GT...A.....C..T.....G..G....
IIF	Chamela	.....GT...A.....C..T.....T..G....
IIG	Chamela	.....GT...A.....C..T.....T.A..G....
IIH	Chamela	..A....TGT...A.....CC.T.....G....
III	Ajiijic	.....GT...A.....C..T.C.....G....
IIJ	Juxtlahuaca	.....GT...A.....C.....C.....C.G....
IIK	Juxtlahuaca	.....TGT...A..G..C.....T..C.G....
IIL	Kino Bay	.....GT.....C..T.....CG....
IIM	Kino Bay Ajiijic Bluebird	.C.....GT...A.....C..T.....GG...
IIN	Kino Bay	.....A..GT...A.....C..T.C.....C.G....
IIO	Carbo Hilltop	.....GT...A.....C.CT.....G....
IIP	Carbo	.....TGT...A.....C..T.C.....C.G....
IIQ	Bluebird	.....GT.....C..T.....GG...
IIR	Bluebird	.....GT...AG....C..T.....C.GGCT.
IIS	Copper Mtn	.....GT...AG....C..T.....C.GG...
IIT	Copper Mtn	.....TGT.....C..T.....G....
IIU	Pinacate	.C.....GT...A.....C.....G....
IIV	Pinacate Copper Mtn Juxtlahuaca	.....TGT...A.....C.....G....
IIW	Pinacate	.....GT.....C..T.....G....
IIX	Patagonia	.....GT...A.....CC.T.....G....
IIY	Patagonia	.....GT.....T...C..T.....A...G....
IIZ	Hilltop	G.....GT.....C..T.....T....G....
IIAA	Hilltop	.....TGT...A.....C..T.....G.....G....
L. c. curasoae		
A	Piedra Honda	.AC.T.....GAA.T.....T.....ATAC...C.G...A
B	Piedra Honda	.AC.T..T...GAA.T.....T.....ATAC...C.G...A
C	Piedra Honda	.AC.T..T...GAA.T.....T.....ATAC..TC.G...A
D	Piedra Honda	.AC.T.A....GAA.T.....T.....ATAC...C.G...A

*IA complete sequence:

CTGGCTATGTTATTCGTGCATAATTCCCATTACCACATATTAATGAATGACATATATGTATAATAGTACATTATATTATGTACCCCATGAATAT-
TAAGCAAGTAATTTAATTCAATGTATGGATGACATAATATTATATATCAACAGTTAAGATTAATAC-
CAATATGGATAATTCATTAATGATTATATATGATTAATCAGCTGTAGGACATAATATTTATTAATCGTGCATAGACCATTCCATTTTC_ATT-
TAATCCTTGTCAATACGCCTATCCCCTACCAAAGGATGTCTCTTG

†Variable sites: 23, 24, 30, 45, 49, 72, 77, 85, 110, 113, 114, 119, 121, 122, 123, 127, 130, 146, 147, 149, 155, 158, 160, 162, 177, 178, 183, 201, 240, 241, 243, 244, 245, 248, 269, 270, 279, 284, 288, 289, 291, 292, 297.

within than between two migratory corridors: (1) along the Pacific coast ranging from at least Juxtlahuaca in Guerrero to south-western Arizona and (2) inland along the Sierra Madre possibly ranging from as far south as Chiapas to south-eastern Arizona. These putative migration routes are consistent with the bats using topographic relief, i.e. the coastline or the foothills of the Sierra Madre Occidental, to navigate at night. Whether bats from Chiapas also make seasonal movements along the north-eastern edge of the Sierra Madre Oriental, as might be expected from the species' range (Fig. 1), requires additional sampling and sequencing.

Available phenological information appears to be consistent with coastal migrants following a cactus corridor in the spring (Fleming *et al.* 1993). Many columnar cacti, especially those in Sonora, are located in the Pacific coastal lowlands. The peak blooming time of columnar cacti species located south of the Rio Balsas (18°N) is March while that of species north of the Rio Balsas is May (A. Valiente-Banuet, personal communication). By early May, most of the coastal bats have arrived at their northern maternity roosts, such as Pinacate, Bluebird and Copper Mountain (cf. Table 1). In contrast, most inland bats do not arrive at roosts in south-eastern Arizona until July or August (Cockrum 1991). These bats may migrate north along an inland nectar corridor of paniculate *Agave* flowers. Agaves are more common in the foothills and uplands of the Sierra Madre Occidental than in the coastal lowlands and peak flowering activity occurs in July and August (Gentry 1982). Southward migration in both groups of bats could follow *Agave* corridors (Fleming *et al.* 1993).

Because we have relatively few mtDNA sequences from each site, have not systematically sampled all known roosts of *L. c. yerbabuenae* in Mexico at different times of the year, and acknowledge that several bats exhibited haplotypes that did not conform to our proposed movements, at present we consider these two putative migratory corridors an hypothesis deserving of further study. Independent information needs to be collected to test this idea before any management decisions are made. For example, additional genetic information from unlinked, i.e. nuclear, loci would permit independent estimates of gene flow and geographical structure (Slatkin 1987). In addition, careful monitoring of bats visiting roosts in the spring simultaneously at sites in western and eastern Arizona would reveal when most bats move through these areas.

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