



Lower Colorado River Multi-Species Conservation Program

Balancing Resource Use and Conservation

Genetic Characterization of *Macrotus californicus* Populations along the Lower Colorado River

2010 Annual Report



December 2011

Lower Colorado River Multi-Species Conservation Program Steering Committee Members

Federal Participant Group

Bureau of Reclamation
U.S. Fish and Wildlife Service
National Park Service
Bureau of Land Management
Bureau of Indian Affairs
Western Area Power Administration

Arizona Participant Group

Arizona Department of Water Resources
Arizona Electric Power Cooperative, Inc.
Arizona Game and Fish Department
Arizona Power Authority
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Mohave Water Conservation District
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Other Interested Parties Participant Group

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Desert Wildlife Unlimited

California Participant Group

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Colorado River Board of California
Bard Water District
Imperial Irrigation District
Los Angeles Department of Water and Power
Palo Verde Irrigation District
San Diego County Water Authority
Southern California Edison Company
Southern California Public Power Authority
The Metropolitan Water District of Southern California

Nevada Participant Group

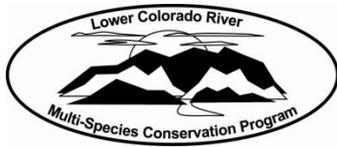
Colorado River Commission of Nevada
Nevada Department of Wildlife
Southern Nevada Water Authority
Colorado River Commission Power Users
Basic Water Company

Native American Participant Group

Hualapai Tribe
Colorado River Indian Tribes
Chemehuevi Indian Tribe

Conservation Participant Group

Ducks Unlimited
Lower Colorado River RC&D Area, Inc.
The Nature Conservancy



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ACRONYMS AND ABBREVIATIONS

LCR lower Colorado River

LCR MSCP Lower Colorado River Multi-Species Conservation Program

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ABSTRACT

This project was initiated to provide information regarding the population structuring, genetic diversity, and foraging habits of *M. californicus* along the lower Colorado River (LCR). At this time, *M. californicus* is listed as an evaluation species under the Lower Colorado River Multi-Species Conservation Program due to the paucity of information on the species in the LCR region. Genetic analysis will provide demographic estimates of *M. californicus* not easily obtained using other field techniques. The information obtained will provide data regarding where different colonies of these bats are breeding, overwintering, and foraging as well as the size of the breeding population through time. These data will also aide restoration efforts for this species by identifying nightly movement patterns from the different roosts to the various restoration sites (incorporating Work Task F-4) and possibly pinpointing foraging sites that are highly preferred if bats are flying further distances or bypassing other restoration sites to get to a particular site. Mitochondrial analysis for 37 individuals was sequenced and analyzed. Six haplotypes were present in the LCR samples, with the Baja sample nested within the northern phylogroup. The diversity of haplotypes occurring within the sample areas suggests some degree of population structuring within populations along the LCR.

INTRODUCTION

The California leaf-nosed bat (*Macrotus californicus*) is one of two bats listed as evaluation species by the Lower Colorado River Multi-Species Conservation Program (LCR MSCP). *Macrotus californicus* is a nonmigratory, colonial, cave-roosting bat. This species is not readily detected using acoustic surveys because of its low intensity calls and the similarity of their calls to other species. It also has a relatively low capture rate thus far in mist netting surveys along the lower Colorado River (LCR) (Calvert 2010); however, the species appears to be fairly common at several roost sites in the vicinity of the LCR (Brown 2006). The difficulty in remotely detecting this species using acoustic surveys, as well as the low capture rate of mist netting, precludes using classic mark-recapture or telemetry techniques to understand even basic demographics of the population using the LCR, particularly those subsets of the population using LCR MSCP restoration sites.

New molecular techniques for estimating population demographics, such as movement, current and historic effective population size, and site fidelity are opening the doors for studying the natural history of rare and elusive species (for a review, see Waits and Paetkau 2005). In particular, microsatellites are proving to be a relatively inexpensive, yet effective method for identifying population structure and gene flow in both a historic and contemporary context. Microsatellites have already been developed for *M. waterhousii* (Murray et al. 2008) and they should be applicable to the closely related *M. californicus*. Because the largest cost of sequencing using microsatellite DNA is in developing an informative set, the availability of known microsatellite regions from closely related species can significantly reduce the cost associated with producing the data.

METHODS

Sampling

Sampling was conducted twice at dusk for each cave along the LCR known to have *M. californicus*—once in the summer during breeding and once during winter. A single harp trap was positioned at the entrance prior to bats exiting the cave. Individuals were weighed, sexed, and measured, and a genetic sample was taken from the wing membrane with a 2-millimeter biopsy punch. Up to 15 samples are to be taken from each cave/season (see figure 1). Because the bats in a particular cave will likely be highly related, this number of samples will be necessary to accurately sample the variation in any given cave. Tissue from museum specimens was included as representatives of the species from outside the LCR as an out group. Wing punches collected during routine restoration site monitoring (work task F-4) are also included. Fifty-six samples were collected, with the breakdown by locality as follows:

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Locality	Samples collected	Samples sequenced
Homestake mine	2	2
Californian mine	16	9
Stone House mine	19	7
Cibola Valley Conservation Area	4	4
3C mine	15	10
Sonora, Mexico (museum specimen)	4	4
Isla Tiburon (museum specimen)	1	1
Total	56	37

SAMPLE AREA

Each point represents a sampling locality. Localities from outside the LCR region are from museum specimens.

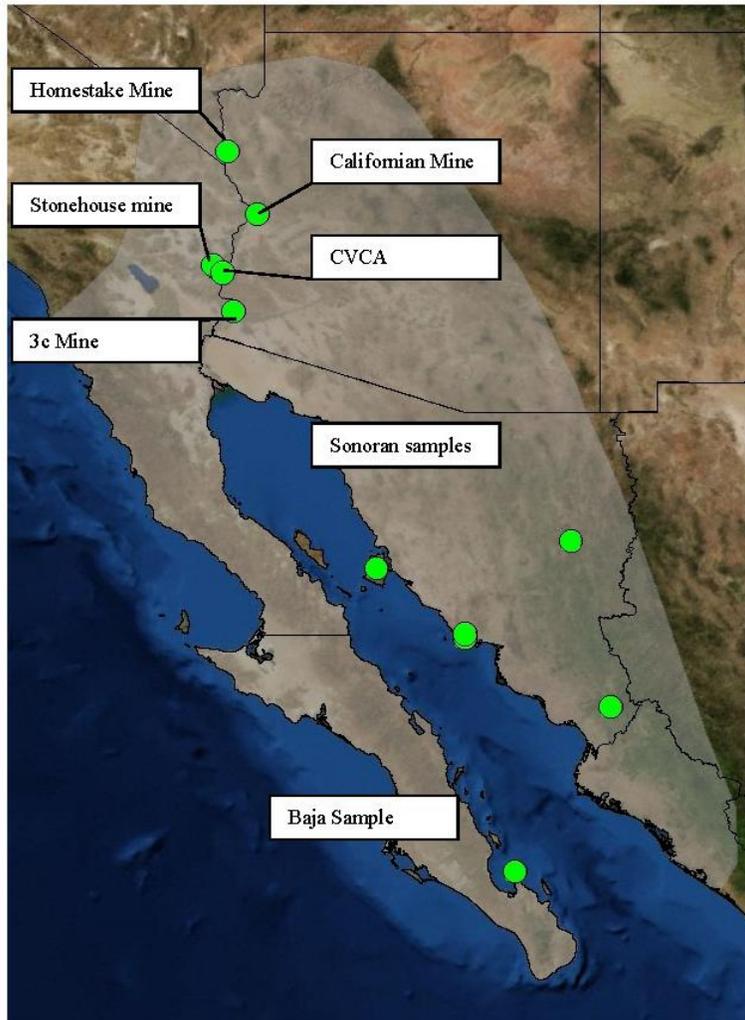


Figure 1.— Locations where tissue samples were collected.

Sequencing

Tissues were processed using a Qiagen DNEasy blood and tissue extraction kit. A polymerase chain reaction was conducted using an Applied Biosystems Geneamp 2720 thermal cycler. Up to 1,000 base pairs of the highly variable cytochrome B portion of the mitochondrial genome were sequenced for 37 individuals using an Applied Biosystems 3130 genetic analyzer.

Analysis

All sequences were aligned using Sequencher DNA sequencing software. Aligned sequences were analyzed using the BEAST phylogeographic model (figure 2) (Drummond and Rambaut 2007).

DISCUSSION

These early results show genetic diversity within populations along the LCR, with several haplotypes being widespread and found at multiple sampling sites (figure 3). There is divergence between the Northern samples and Sonoran samples, suggesting the two have been reproductively isolated for some time. The Baja sample is nested within the Northern haplotypes, which suggests gene flow between the two areas. Microsatellite analysis will provide a more fine-scale view of the genetic makeup of the LCR populations. If sufficient microsatellite variation exists within LCR populations, we may be able to assign individuals to roosts with distinct genetic haplotypes. Current and historic population size will also be estimated when the full data set is available. These analyses will be a useful tool in characterization of the Northern populations of *M. californicus*, of which little is currently known. For 2011, sampling will continue in Arizona and California, and mitochondrial/microsatellite analysis will also continue.

Genetic Characterization of *Macrotus californicus* Populations along the Lower Colorado River

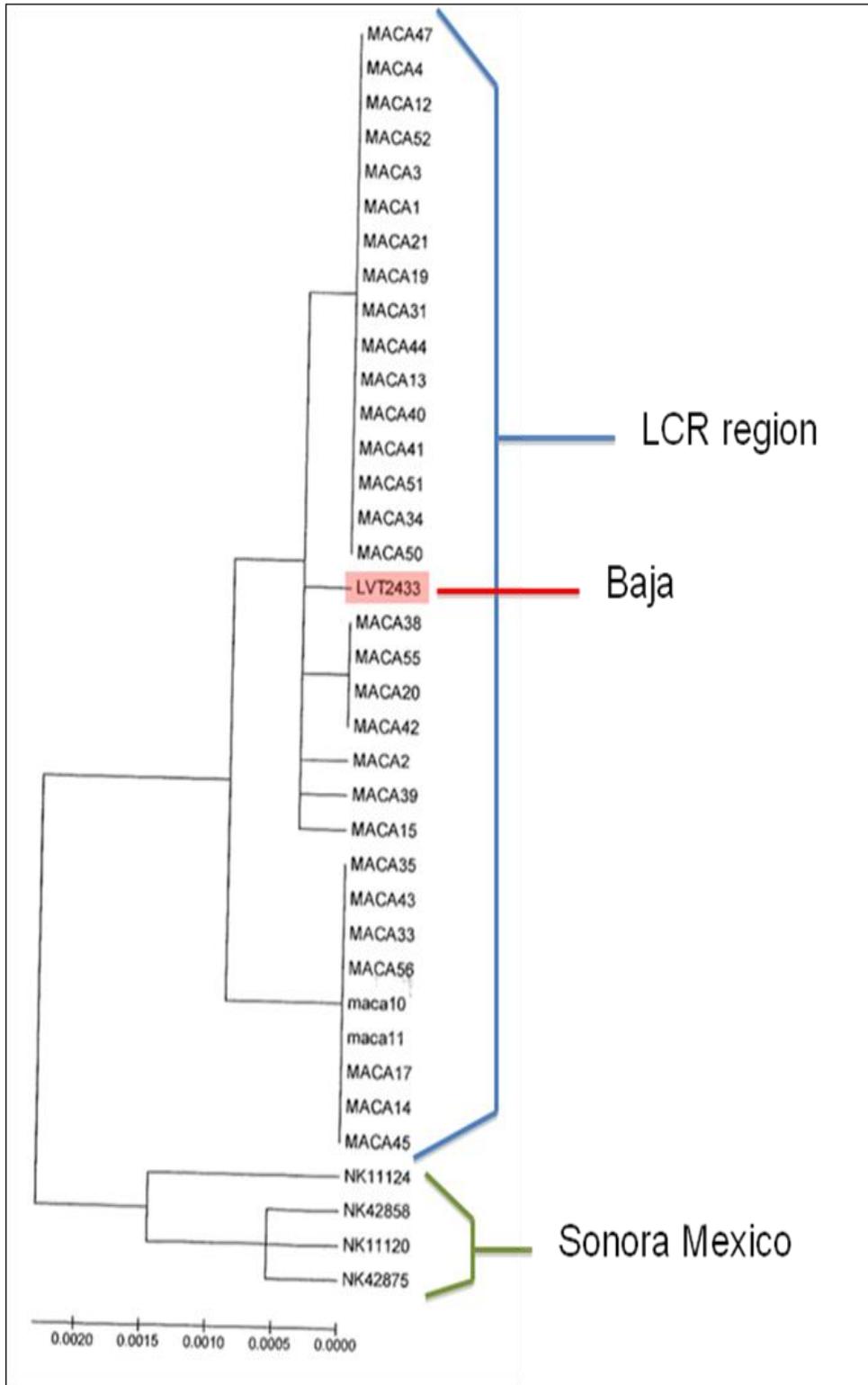


Figure 2.—Relatedness tree constructed using the BEAST phylogeographic model.

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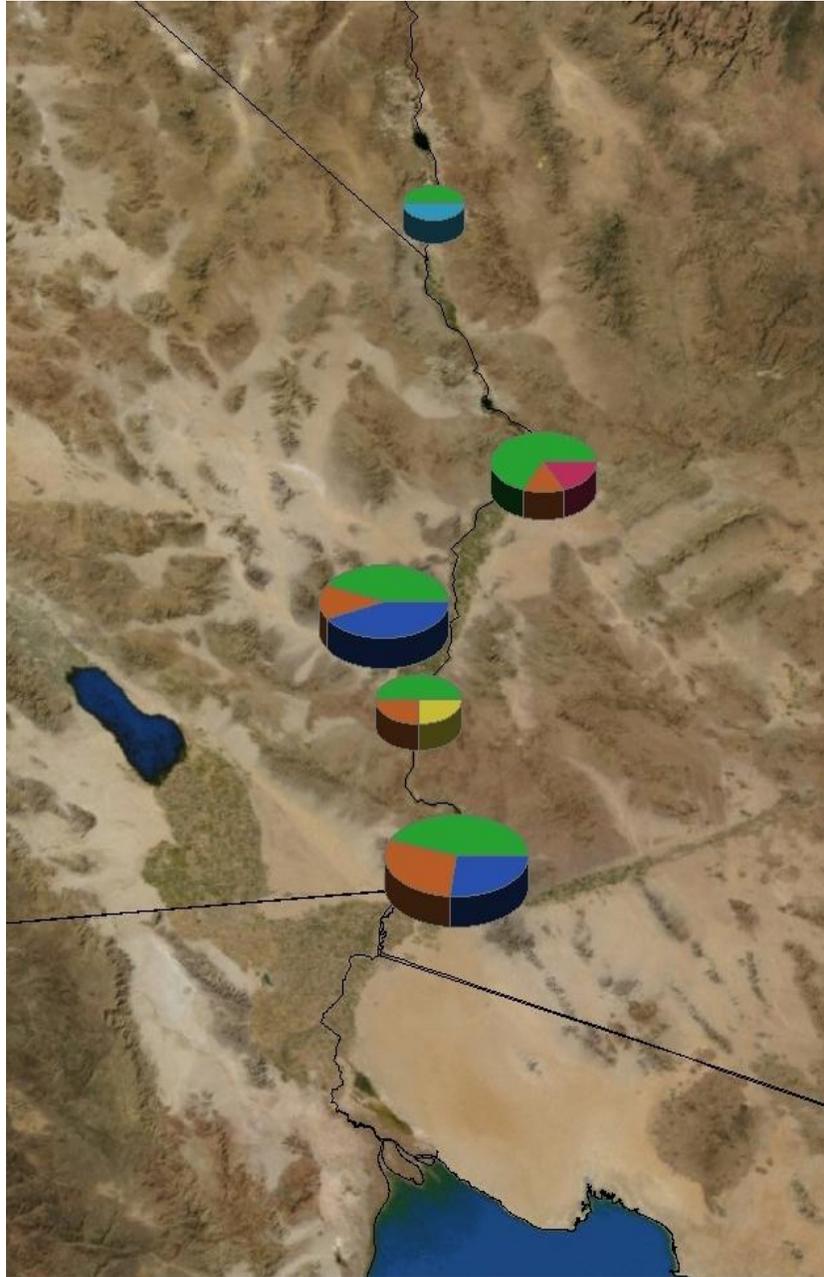


Figure 3.—Pie charts showing number of haplotypes per locality. Chart size relative to number of individuals per site.

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