Mammalian Biology 92 (2018) 78–85

Contents lists available at ScienceDirect

Mammalian Biology

journal homepage: www.elsevier.com/locate/mambio

Original investigation

Genetic diversity distribution among seasonal colonies of a nectar-feeding bat (*Leptonycteris yerbabuenae*) in the Baja California Peninsula

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ARTICLE INFO

Article history:
Received 8 July 2017
Accepted 18 April 2018
Available online 22 April 2018

Handled by Paul Grobler

Keywords:
Cytochrome *b*
ND1 region
Genetic differentiation
Historical demography
Microsatellites

ABSTRACT

Gene flow and historical demography influence the level and distribution of population genetic variation. The nectar-feeding bat *Leptonycteris yerbabuenae* is a colonial and migratory species in tropical and subtropical regions of North America. We examined the distribution of genetic diversity among colonies of this species and assess whether a population in Baja California Peninsula shows signature of historical demographic change. We expected low genetic differentiation, because individuals are highly mobile and share mating sites. We also predicted a demographic signature consistent with past climatic fluctuations. During the spring maternity season, we sampled 120 individuals of six colonies along a 450 km transect in the Baja California Peninsula, Mexico. Individuals were genotyped with eight nuclear microsatellite loci and 1739 bp of two mitochondrial markers. We record weak but significant levels of nuclear structure and no mitochondrial differentiation among these colonies suggesting a high level of gene flow mediated by females. Genetic diversity estimation per colony and in the region was moderate, and consistent with previous studies. The mitochondrial data indicate that the population in the Baja California Peninsula experienced a demographic expansion during or after the late Pleistocene, probably related to the expansion of food resources. This is the first detailed genetic population study of *L. yerbabuenae* on the spatially disjunct part of its geographical range and it is the first record of a demographic expansion in a migratory nectar-feeding bat species from North America. Our results contribute to understanding the past demography and the natural history of this species in the Baja California Peninsula.

Introduction

The level and distribution of population genetic variation are known to be shaped by historical and ecological factors as well as the life-history traits of a species. In particular, many migratory bats exhibit high mobility and share mating roosts (reviewed by Moussy et al., 2013), which could result in high genetic mixing and in a near panmictic population structure (Bryja et al., 2009; McCracken et al., 1994). Migrations often occur in response to seasonal changes in resource availability and weather (Moussy et al., 2013). For example, a few nectar-feeding bats migrate from tropical and subtropical regions of Mexico to form maternal colonies in the Sonoran Desert in spring and summer. Females give birth and raise young during peak periods of availability of nectar and fruit from columnar cacti and agaves (Ceballos et al., 1997; Cockrum, 1991; Cole and Wilson, 2006). Some of these nectar-feeding bats (*Leptonycteris* spp.) are considered endangered or threatened in some or all parts of their range due to concerns about foraging habitat degradation and disturbance of maternal roosts (Medellín, 2016a,b). Therefore, assessing the distribution of genetic diversity among seasonal colonies can help to infer the level of population

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https://doi.org/10.1016/j.mambio.2018.04.008
1616-5047/

differentiation in wide ranging migratory species, which can in turn inform their conservation planning.

Historical factors such as past climatic fluctuations caused changes in the demography and geographical range of species, in turn influencing the genetic diversity of populations (Hewitt, 2004a). Signals detected in mitochondrial markers indicate post-glacial expansions in several animals and plants. These expansions have been related to the appearance of more suitable climatic conditions, and also with greater food and shelter resources availability (reviewed by Hewitt, 2004b), as it is expected that if the availability of these resources increase, the size of populations that can be sustained in the ecosystems also increase. Plants used as food by migratory nectar-feeding bats in North America experienced demographic expansion during the last glacial maximum (Scheinvar et al., 2017) and more recently (Clark-Tapia and Molina-Freaner, 2003). This suggests that bat populations likely also experienced demographic fluctuations during this period. Estimating genetic connectivity of populations and range expansions from past climatic shifts can help predict species vulnerability and how species can respond to climate change.

The nectar-feeding bat *Leptonycteris yerbabuenae* is distributed in tropical and subtropical regions of North America (Arita, 1991; Simmons, 2005). In central and southern Mexico, some populations are resident through the year, while others migrate, depending on seasonal food availability (Ceballos et al., 1997; Stoner et al., 2003; Valiente-Banuet et al., 1996). A spatially disjunct part of its range is located from the middle to the southern end of the Baja California (BC) Peninsula (Medellín et al., 2008), and it is separated from the rest of its geographical range by the Gulf of California (Baker and Cockrum, 1966) (Fig. 1). The BC Peninsula is the fourth longest peninsula in the world, covering 10° of latitude. It has a dynamic geological and climatic history that has influenced the phylogeographic and diversity genetic patterns of species inhabiting the region (reviewed by Dolby et al., 2015). Due to its history and broad latitudinal range, the BC Peninsula harbors great biodiversity (Grismer, 2000). However, this biodiversity is threatened because of rapid tourism development, growth of mining activity, and increasing growth of the human population. If foraging habitats of *L. yerbabuenae* become degraded or if roost sites experience increased disturbance through renewed mining or unregulated cave tourism (Medellín et al., 2017), regional population of this species could decline and its migration movement to this area can decrease.

Previous genetic assessments of *L. yerbabuenae* have focused on broad latitudinal sampling and recorded moderate levels of diversity in mitochondrial control region sequences and nuclear microsatellites (Ramirez, 2011; Wilkinson and Fleming, 1996), as well as with RAPDS (Morales-Garza et al., 2007). These studies focused mainly on colonies from the north and center of the range of *L. yerbabuenae* in mainland Mexico, and only included marginal sampling from the BC Peninsula. A detailed description of the genetic diversity of seasonal colonies from the BC Peninsula is still lacking but necessary to understand the connectivity and genetic historical context on this spatially disjunct part of the species range. In this study we used both nuclear and mitochondrial markers to analyze the level and distribution of genetic variation of *L. yerbabuenae* colonies that occupy seasonal roosts on the BC Peninsula, Mexico. Specifically, we examine the extent of genetic structure among six seasonal colonies on the BC Peninsula. We hypothesize a low genetic differentiation among colonies, because individuals of this species are highly mobile and share mating sites (Cole and Wilson, 2006; Horner et al., 1998). We further assess diversity on maternal inherited markers in the context of past demographic processes, and predict that we will find a signature such as bottleneck or expansion consistent with glacial or interglacial cycles respectively. Our results highlight moderate variation and low genetic structure, and suggest a higher level of gene flow mediated by females than by males. Additionally, we detected that this population experienced a historical demographic expansion during or after the late-Pleistocene.

Material and methods

Amplification and genotyping of nucDNA and mtDNA markers

In the spring of 2014, we collected tissue samples from 120 *Leptonycteris yerbabuenae* from six roost sites (n = 20 bats per site) along a 450 km transect in the Baja California (BC) Peninsula and one nearshore gulf island. The six roosts included five maternity colonies and one male colony (Fig. 1; Table 1). During the nights, we captured the bats at cave entrances, using hand nets and mist nets. A sterile 3 mm biopsy punch was used to take a tissue sample from the wing of each bat, and tissue samples were desiccated and frozen at −20 °C. All animal handling methods were in accordance to the guideline of the American Society of Mammalogist (Sikes and Gannon, 2011).

Total genomic DNA was extracted using a DNeasy Kit (Qiagen) and stored at 4 °C. Individuals were genotyped with eight nuclear (nucDNA) microsatellite loci previously reported for this species (*Leye3a*, *LeyeGT2*, *Leye11*, *Leye27*, *Leye17*, *LeyeCT2*, *LeyeL71*, *Leye23*: Ramirez et al., 2011). The PCR reaction was in a final volume of 25-μl containing 1X buffer with 0.4 μM of each primer, 0.16 mM of dNTP, 1–3 mM of $MgCl_2$ and 1 U of *Taq* DNA polymerase. The thermal profile for amplification consisted of an initial denaturation at 94 °C for 5 min, followed by 30 cycles of 30 s at 94 °C, 30 s at 53–60 °C and 30 s at 72 °C, with a final extension of 5 min at 72 °C (Ramirez et al., 2011). PCR products were run on an ABI PRISM genetic analyzer system (Applied Biosystems, Foster City, CA) and sized with an internal lane standard using GENEMAPPER 4.0.

For mtDNA analysis, we sequenced 1739 bp with the primers L14725 and H15915 (Steppan et al., 1999) for cytochrome *b* gene (1127 bp; cyt *b*), and ER65 and ER66 for a ND1 region (612 bp) from the NADH mitochondrial gene (Mayer and von Helversen, 2001). For both markers, 1 U of *Taq* polymerase was added per 25 μl of reaction volume. The final 1X buffer had 0.4 μM of each primer, 0.4 mM of dNTP and 1.7 mM or 1.5 nM of $MgCl_2$, for cyt *b* and ND1 respectively. The thermal profile for amplification of cyt *b* consisted of an initial denaturation cycle at 95 °C for 3 min, followed by 35 cycles at 96 °C for 30 s, 52 °C for 1 min and 72 °C for 2 min, and a final extension at 72 °C for 7 min. For amplification of a segment of ND1, thermal profile consisted of an initial denaturation cycle at 95 °C for 5 min, followed by 30 cycles at 94 °C for 30 s, 50 °C for 30 s and 72 °C for 90 s, and a final extension at 72 °C for 7 min. All amplifications were performed in a Perkin–Elmer GeneAmp PCR system 9600 (Applied Biosystems, Foster City, CA). Sequencing was done in both directions by the Sanger method using forward and reverse primers. Because we used complete sequences for each marker for analyses, there are differences in sample sizes between nucDNA and mtDNA data set (see Results).

Genetic diversity and structure in nuclear microsatellites (nucDNA)

For nuclear microsatellite data, we estimated standard population genetic statistics such as the allelic richness (AR), the inbreeding coefficient (F_{is}), and the observed (H_o) and expected (H_e) heterozygosity for each colony and the total sample, using GenALEx 6.5 (Peakall and Smouse, 2006) and FSTAT (Goudet, 2001). We determined the presence of null alleles in MICRO-CHECKER (Van Oosterhout et al., 2004), and we tested Linkage Equilibrium between pairs of loci within each colony and Hardy Weinberg dis-