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Phylogeographic Inference of Insular Mule Deer (*Odocoileus Hemionus*) Divergence in North America's Desert Southwest

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PHYLOGEOGRAPHIC INFERENCE OF INSULAR MULE DEER (*ODOCOILEUS HEMIONUS*) DIVERGENCE IN NORTH AMERICA'S DESERT SOUTHWEST

by

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ABSTRACT

PHYLOGEOGRAPHIC INFERENCE OF INSULAR MULE DEER (*ODOCOILEUS HEMIONUS*) DIVERGENCE IN NORTH AMERICA'S DESERT SOUTHWEST

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Though mule deer (*Odocoileus hemionus*) persist in robust populations throughout most of their North American distribution, nearly 60% of their historic range in México has declined due to habitat loss and unregulated hunting. Two of the six subspecies inhabiting México's deserts and Baja California peninsula are of conservation concern, occurring on land bridge islands in the Pacific Ocean (*O. h. cerrosensis* on Cedros Island: threatened) and in the Sea of Cortés (*O. h. sheldoni* on Tiburón Island: endangered). Focusing on the desert southwest (n=449 deer), we obtained 1,611 bp of mtDNA sequence (control region: 583 bp; cytochrome b gene: 1,028 bp) from natural history specimens of Tiburón (n=14) and Cedros (n=15) deer from North American collections to complete the phylogeographic evaluation of the species complex. We found that both island subspecies nest phylogenetically within mainland lineages but demonstrate significantly reduced genetic variation (haplotype diversity for Cedros: $p<0.0045$; for Tiburón: $p<0.0001$) compared to their adjacent mainland counterparts. Tiburón deer form the western periphery of an unexpected geographic discontinuity for one of the six inferred mule deer mitochondrial lineages in the desert southwest which is supported as an older, basal lineage by Bayesian phylogenetic inference and relative divergence time estimates. Considering genetic signature of demographic expansions coincide with the full extent and retreat of the last glacial maximum (LGM) (estimated 7,000-26,500 years before present), we propose that the associated

climatic fluctuations and drastic turnover in biotic communities (large land herbivore extinctions and increasing aridity following recession of mesic forests to higher elevations) greatly contributed to mule deer expansion and ecological adaptation in the desert southwest. This study underscores the role natural history specimens represent for genetic studies of declining or rare populations, allowing us to provide the first phylogeographic analysis of insular mule deer for the region. Considering both Tiburón and Cedros island subspecies lack comprehensive demographic and ecological studies, our phylogeographic inference will help serve future conservation priorities of desert southwest deer for informed management.

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CHAPTER 1 – INTRODUCTION

Evaluating the role of historical biogeographic and climatic events in shaping phylogeographic patterns is critical to understanding contemporary genetic structure (Avise 2000), particularly for conservation and management needs. In this chapter (**Chapter 1**), I present an overview of mule deer (*Odocoileus hemionus*) in the desert southwest region and conservation status of the insular subspecies, *O. h. sheldoni* and *O. h. cerrosensis*. In **Chapter 2**, I build upon a phylogeographic analysis for mule deer in the desert southwest region in North America, incorporating for the first time mitochondrial DNA sequences from these island subspecies provided by natural history specimens.

Fossil record of *Odocoileus* spp. in México

Compared to other mammals with well-established histories in North America such as certain mustelids (Tedford et al. 2004), *Odocoileus* spp. (*hemionus*: mule deer and *virginianus*: white-tailed deer) represent relatively recent lineages. Following entrance to the New World via the Bering Land Bridge by a cervid ancestor (between 5-7 million years ago [mya]; Kurtén and Anderson 1980; Heffelfinger 2011), cervids radiated within North America and exchanged southwards with the Panama land bridge between North and South America during the Blancan (3.5-1.75 mya). Scant fossil evidence marred by repeated glacial-interglacial cycles (Geist 1998) has made it difficult to pinpoint the *Odocoileus* split of *O. hemionus* and *O. virginianus*. Dates spanning the early Blancan (3.7 mya) into the Late Rancholabrean (0.75 mya to present) have been offered for an *O. virginianus* and *O. hemionus* divergence (summarized in Heffelfinger 2011), though the earliest (Irvingtonian) fossil dates for *O. hemionus* were estimated in age around 1.9-0.7 mya (Jacobsen 2003).

In México, the oldest of *Odocoileus* spp. are known from the Irvingtonian (1.75-0.75mya) in northwestern Sonora, El Golfo region bordering the Colorado River Delta (Shaw et al. 2005). More recent Rancholabrean reports of confirmed *O. hemionus* exist from the Sierra

Madre Occidental, Central Plateau of México and notably, the Trans-Mexican Volcanic Belt in south-central México (Ferrusquía-Villafranca et al. 2010). Thus fossil evidence supports the idea that the geographic range of mule deer during the Rancholabrean extended further south than the current distribution today.

Mule deer distribution and desert ecology

The extant distribution of mule deer spans from the Kodiak and Alexander archipelagos in southern Alaska to the Sonoran and Chihuahuan deserts of México, including the entire Baja California peninsula (Hall 1981; Wallmo 1981). Eleven subspecies have been recognized based on morphological differences, i.e. body and skull size, pelage differences, metatarsal gland morphology (Anderson and Wallmo 1984; Cronin 1991a) as well as complex phylogeographic history (Latch et al. 2009). Mule deer occupy all climatic zones throughout their distribution with the exception of high-altitude tundra, and as generalist browsers are known for their ability to exploit a variety of different habitat types, even in anthropogenically-modified landscapes such as agricultural fields and urban areas. Bucks disperse farther distances than does, though dispersal distance and seasonal movements in different ecoregions depend heavily on habitat availability and quality (Conner and Miller 2004). In the arid desert southwest region of North America, deer are required to cover larger distances to fulfill dietary and water requirements compared to other deer in more productive (milder) habitats, leading to the occupation of home ranges which can be 78-130 square kilometers (Heffelfinger 2006) throughout the Baja California peninsula and Sonoran desert. In the extremely arid King Valley, Arizona, mule deer seasonal movements have been recorded to exceed 550 square kilometers (Rautenstrauch 1987). In the less extreme habitats of the Chihuahuan desert, mule deer home ranges are estimated to be much smaller, nearing 50 square kilometers (Heffelfinger 2006).

Desert southwest subspecies

Six subspecies are distributed throughout the desert southwest region of North America: two subspecies inhabit the Baja California peninsula, including *O. h. fuliginatus* at the northern end and *O. h. peninsulae* at the southern end. While the ranges of *O. h. eremicus* and *O. h. crooki* roughly correspond to the boundaries of the Sonoran and Chihuahuan deserts (Shreve 1942) respectively, these two subspecies have been considered to be synonymous (Heffelfinger 2000). The remaining two subspecies of mule deer in México are Baja California insular endemic subspecies of conservation concern, according to the Secretaría del Medio Ambiente Recursos Naturales y Pesca (Mexican Ministry of Environment and Natural Resources or SEMARNAT). *O. h. sheldoni* occurs on Tiburón Island, off the western coast of Sonora in the Gulf of California (Threatened). *O. h. cerrosensis* occurs on Cedros Island in the Pacific Ocean off the coast of Baja California and is considered to be Endangered (SEMARNAT 2010). The U.S. Fish and Wildlife Service also considers Cedros deer as Endangered due to low population numbers (USFWS 1975). Both islands are managed as separate Units for the Management and Sustainable Use of Wildlife (UMA from its name in Spanish) (Valdez et al. 2006). Sea level rises associated with retreat of northern glaciers in the late Pleistocene and early Holocene isolated the islands from respective mainlands approximately 12-10,000 years ago (Cody et al. 2002; Des Lauriers 2006; Rojas-Soto et al. 2010). The two islands possess distinct biogeographic histories and anthropogenic influences coupled with species introductions, but share deficiencies of comprehensive demographic or genetic studies (Colchero et al. 1999; Mellink 1993; Pérez-Gil Salcido 1981; Weber and Gonzalez 2003).

Tiburón Island mule deer

Tiburón Island is the largest of México's 230 islands and islets (Donlan et al. 2000), encompassing an area of 1,201 square kilometers. This land bridge island is located 1.7 km west of the coast of Sonora, and deer are known to swim this channel separating the island from mainland Sonora (N. Martinez-Tagüeña, personal communication). The island has a long history

of protection and conservation efforts, currently overseen by the Seri (Comcáac) tribe whose indigenous homeland encompasses Tiburón Island and the adjacent Sonoran coast (Colchero et al. 1999). For the Seri, deer have long represented the most important terrestrial source of meat, and oral tradition long supports the cultural use of deer on Tiburón Island (Felger and Moser 1985); combined with archaeological findings (White 2000), their endemicity to the island is strongly supported. An estimate of approximately 869 individual deer on the island was published in 1980 (Reyes Osario 1981), and while subsequent extrapolation from aerial surveys conducted in 1993 suggest a more recent population size of 650 individuals (R. Lee, personal communication), little information of current estimates is available. While attempts failed to introduce javelina (*Pecari tajacu*) and pronghorn (*Antilocapra americana*) to the island in 1967 (Quiñónez and Rodríguez 1979), bighorn sheep (*Ovis canadensis*) successfully established when 16 individuals were translocated to Tiburón Island to start a population in 1975 (Colchero et al. 1999). Bighorn sheep continue to be studied and managed using conservation funds raised by the sale of hunting permits. This bighorn population serves as a source population for reintroductions throughout Sonora and elsewhere (Gasca-Pineda et al. 2013). Although bighorn and mule deer generally use habitats differently, anecdotal observation suggests that deer and sheep on the island maintain overlapping niches. However, comprehensive genetic and demographic studies are lacking for endemic Tiburón deer (Colchero et al. 1999; Ezcurra et al. 2002).

Cedros Island mule deer

Cedros Island is the largest of the Southern California Channel islands (Murphy and Aguirre-León 2002), a land bridge island formed in the Pacific Ocean 24 km from Punta Eugenia in the state of Baja California del Norte, comprising an area of approximately 348 square kilometers. Deer are represented sparsely in the paleontological record predating 2,500 years ago (Des Lauriers and Des Lauriers 2006; Des Lauriers 2009); however, archaeological evidence supports deer presence on the island at least 11,500 years ago (Des Lauriers 2010). Previous

population-level studies suggest relatively low numbers of mule deer on Cedros Island (estimates range from 50-473; Pérez-Gil Salcido 1981; Povilitis and Ceballos 1986). Cedros deer appear to occupy primarily the northern two-thirds of the island (234 square km) and are noted to be much less common in the southern third of the island where anthropogenic operations (salt manufacturing plant, airport and human settlements) have been in place for decades (Pérez-Gil Salcido 1981). Extrapolations from camera-trapping data and other population size estimates suggest the current population may be as low as 15-20 individuals (de Jesus Martinez Vazquez 2012 and a governmental report referenced by Cortés-Calva et al. 2013). The large distance between Cedros Island and mainland Baja California peninsula suggests limited opportunity for gene flow with peninsular mainland deer. Assessment of phylogeographic history for Cedros deer is critical for informing future conservation and management of this subspecies, especially considering the stresses of feral dog predation (Gallo-Reynoso and García-Aguilar 2008; García-Aguilar 2012) and lack of enforcement for undocumented poaching (Pérez-Gil Salcido 1981;USFWS 1975).

Insular deer morphology

Among the most remarkable morphological effects of insular isolation is the dramatic change in body size associated with the 'island rule' (Van Valen 1973). Recent studies have shown strong correlations for dwarfism in larger mammals, including artiodactyls and carnivores (body size ratio decrease in insular relative to mainland conspecifics) (Lomolino 2005; Meiri et al. 2008). Further, higher selection coefficients associated with population size fluctuations, competition and resource limitation have been shown to impact optimal body size, where statistical models (Filin and Ziv 2004) support a faster evolution of mammal body size on smaller islands compared to larger islands (Millien 2011). Cedros deer follow the pattern larger mammals show of reduced body size, with pronounced dwarf-like size compared to mainland deer (size of skull, toothrow length and size of antlers, including the apparent lack of brow tines)

and differences in pelage coloration (Merriam 1898; Pérez-Gil Salcido 1981). Similarities in skull morphometrics between *O. h. cerrosensis*, *O. h. fuliginatus* and *O. h. peninsulae* suggested Cedros deer resemble *O. h. fuliginatus* or an intermediary between the two subspecies (Cowan 1936; Pérez-Gil Salcido 1981). Similarly, Tiburón deer were noted to exhibit pelage coloration differences, broader skulls with shorter toothrow length than other subspecies, but antler size was described as similar to mainland *O. h. eremicus* (Goldman 1939). This suggests that conditions on the island led to selective pressures and fitness benefits to favor much smaller body size in Cedros deer (consistent with smaller island size) than for Tiburón deer, which inhabit a much larger island in greater proximity to core populations and lack the pronounced decrease in body size.

Mule deer from San José Island

While pelage patterns of mule deer on San José Island may differ from other deer from the region (Heffelfinger 2006), no detailed study of San José Island deer has been conducted and these deer are not distinguished taxonomically from *O. h. peninsulae*. Thus we pool genetic analyses with Baja California peninsular mainland deer in **Chapter 2**. Lower sea levels during the Pleistocene combined with volcanic uplifting are thought to have connected San José Island to mainland Baja California del Sur, with the most recent separation as a land bridge island approximately 12,000 years ago at a distance of approximately 4.6 km (Best and Thomas 1991; Lidicker 1960). Deer on this island face anthropogenic threats, including poaching during legal hunting of feral goats, which are present in high numbers on the island (Espinoza-Gayosso and Álvarez-Castañeda 2006). Other threats include habitat loss associated with resort and tourist development, particularly in the more level western parts of the island (Lorenzo et al. 2011).

CHAPTER 2 – PHYLOGEOGRAPHIC INFERENCE OF INSULAR MULE DEER (*ODOCOILEUS HEMIONUS*) DIVERGENCE IN NORTH AMERICA'S DESERT SOUTHWEST

Introduction

Geophysical and climatic events during the Plio-Pleistocene drove biotic diversification in North America, shaping faunal diversity and distributions. For widely distributed species, vicariance in northern latitudes (e.g., glacial-interglacial cycles) triggered allopatric divergence as species experienced fragmentation and contraction into isolated refugia or displacement south of the ice sheets. This history of vicariance has provided numerous opportunities to study evolutionary processes including genetic differentiation, adaptation, speciation and extinction (Hofreiter and Stewart 2009). Phylogeographic studies of species transformed by Plio-Pleistocene flux have concentrated in northern latitudes such as Northern Europe (e.g., Taberlet et al. 1998; Tammeleht et al. 2010) and the Pacific Northwest (reviewed in Shafer et al. 2010; Soltis et al. 1997). Fewer studies have examined patterns by which geophysical events paired with climatic shifts south of the ice sheets drove divergence in species whose distributions encompass southern latitudes (Hewitt 2000). The desert southwest and Baja California peninsula harbor endemic biotic assemblages shaped by geophysical and island formation events and refined by millennia of climatic shifts and pluvial-sea level cycles (Grismer 2000). This unique region provides ample opportunity to examine vicariance in southern latitudes associated with Pleistocene biogeographic events and climatic shifts (pluvial-sea level changes leading to land bridge isolation and turnover in biotic community composition) near the time of recession of the Last Glacial Maximum (LGM: estimated 26,500-19,000 years before present; Clark et al. 2009).

Genetic patterns of mammal expansions during the LGM vary with species' dispersal ability (vagility) and other ecological factors. For relatively sessile species, limited gene flow and ecological specialization can lead to genetic isolation and local adaptation (Dieckmann et al. 1999), where vicariance associated with historical biogeographic events or island isolation leave

phylogeographic signatures that persist through time. Neutral intraspecific genealogies have commonly been used to compare patterns of divergence for co-distributed taxa observed in biogeographic regions shaped by such vicariance events (Hafner and Riddle 2005; Riddle et al. 2000a). For older taxa in the Baja California peninsula, large geophysical events such as marine transgressions in the vicinity of the Salton Trough and Son Gorgonio constriction (Imperial formation, 10-6 million years ago [mya]: Peterson 1975; Schremp 1981, Wood et al. 2008), the peninsular split from mainland México during the Pliocene (approximately 5-4 mya), and the Colorado River delta 5.5-3 mya (Bouse embayment; Lucchitta et al. 2001) are reflected through concordant genetic signatures across co-distributed taxa. More recent events in the Baja California region such as putative trans-peninsular seaways (e.g., La Paz, 3 mya; Vizcaino, 1 mya) (Riddle et al. 2000a) have also left lasting signatures in phylogeographic histories. Intraspecific phylogroups inferred from mtDNA genealogies support vicariance hypotheses through concordant divergence trends across relatively sessile (low-dispersing) peninsular species, such as *Chaetodipus* pocket mice (Riddle et al. 2000b), *Urosaurus* lizards (Lindell et al. 2008) and *Euphorbia* plants (Garrick et al. 2009), among many others. Considering more recent vicariance, land bridge formation due to sea-level rise coinciding with recession of the LGM led to insular divergence in certain taxa.

A large body of literature exists evaluating the conservation status or assessing insular divergence from mainland populations for numerous small and relatively sessile species, including rodents (reviewed in Riddle et al. 2000a, Riddle and Hafner 2006), relatively sedentary birds (*Toxostoma lecontei*, Rojas-Soto et al. 2007), reptiles (Murphy and Aguirre-León 2002; Davy et al. 2011) and more vagile bats (Frick et al. 2008). However, little attention has been paid to the role of biogeographic or island vicariance in shaping desert southwest and Baja California lineages of more vagile, generalist species, considering vagility leading to high levels of gene flow may overshadow or resist historic genetic signatures of vicariance. Thus vagility remains an underrepresented ecological factor in phylogeographic analyses (Kodandaramaiah 2009). Studies

of highly dispersive carnivores (Byun et al. 1997, Sacks et al. 2008) and ungulates (Klütsch et al. 2012; Latch et al. 2009) show unexpected genetic signatures of Plio-Pleistocene vicariance in North America, but the extent to which species' ecological requirements affect patterns of divergence suggests complex evolutionary dynamics within vagile species (Pease et al. 2009). Thus it is unknown whether the phylogeographic patterns associated with vicariance arising from biogeographic events and island isolation observed in smaller, relatively sessile taxa would hold for a larger, more vagile species such as the North American mule deer (*Odocoileus hemionus*).

Mule deer occur from southern Alaska to the deserts in northern México where they are continuously distributed throughout the Baja California peninsula, and are well known for their ability to disperse. The species complex comprises eleven currently recognized subspecies characterized by diverse morphology and complex genetic structure (Anderson and Wallmo 1984; Hall 1981; Latch et al. 2009; Wallmo 1981) (**Figure 1**). The phylogeographic histories of nine of 11 mule deer subspecies have been evaluated using mtDNA sequences and nuclear microsatellites with regards to refugial and post-glacial expansion events (Latch et al. 2009; Latch et al. submitted; Pease et al. 2009). Two of the six recognized subspecies of mule deer in México are Baja California insular endemic subspecies of conservation concern: *O. h. sheldoni* on Tiburón Island in the Gulf of California (Threatened) and *O. h. cerrosensis* on Cedros Island off the coast of Baja California in the Pacific Ocean (Endangered) (Secretaría del Medio Ambiente Recursos Naturales y Pesca or SEMARNAT 2010; USFWS 1975; **Figure 1**). Both islands are managed as separate Units for the Management and Sustainable Use of Wildlife (UMA from its name in Spanish) (Valdez et al. 2006). The two islands are estimated to have been separated from their respective mainlands with sea level rise during the late Pleistocene and early Holocene; Cedros estimated to have separated approximately 13-10,000 years before present (ybp; summarized in Des Lauriers 2006) and Tiburón approximately 11-10,000 ybp (Cody et al. 2002; Rojas-Soto et al. 2010). Each island possesses a distinct biogeographic history and suite of anthropogenic influences coupled with invasive species introductions, but they do share

deficiencies of comprehensive demographic or genetic studies (Colchero et al. 1999; Mellink 1993; Pérez-Gil Salcido 1981; Weber and Gonzalez 2003).

Separated by the western coast of Sonora by the 1.7 km-wide Infiernillo Channel, Tiburón Island is the largest of the Mexican islands (approximately 1,201 square kilometers) with a long history of protection and conservation efforts, currently overseen by the Seri (Comcáac) tribe whose indigenous homeland encompasses Tiburón Island and the adjacent Sonoran coast (Colchero et al. 1999). While bighorn sheep (*Ovis canadensis*) from Sonora were introduced to the island in 1975 are managed for hunting and as a potential source population for reintroductions throughout Sonora and elsewhere (Gasca-Pineda et al. 2013), comprehensive genetic and demographic studies are lacking for Tiburón deer (Colchero et al. 1999; Ezcurra et al. 2002). In contrast, Cedros Island (348 square kilometers) is located in the Pacific Ocean, 24 km from Punta Eugenia, Baja California del Norte. Past population-level studies suggest a decrease in deer numbers on Cedros Island from estimates of 50-473 in 1980 (Pérez-Gil Salcido 1981; Povilitis and Ceballos 1986) to more recent estimates as low as 15-20 individuals (de Jesus Martinez Vazquez 2012, and a governmental report referenced by Cortés-Calva et al. 2013). Combined with potential predation by feral dogs (Gallo-Reynoso and García-Aguilar 2008; García-Aguilar 2012), limited opportunity for gene flow with mainland deer considering distance to shore and lack of enforcement preventing undocumented poaching (USFWS 1975; Pérez-Gil Salcido 1981), genetic assessment of Cedros deer is necessary to guide management actions.

Examining phylogeographic patterns in wide-ranging species is critical to evaluating subspecies and present-day populations, particularly for management and conservation purposes in culturally and economically important game mammals (Leopold 1959; Heffelfinger 2006). No study to date has examined the phylogeographic histories of Tiburón and Cedros deer relative to their intraspecific classification or phylogeographic setting, though morphological differences, i.e., smaller size (Des Lauriers 2009) consistent with the island rule (Lomolino 2005; Meiri et al. 2008), tooth row length and pelage color imply divergence. These insular populations of *O.*

hemionus occur along the periphery of the mule deer distribution and may be isolated from their respective mainlands, and therefore may endure compounded effects of lower population sizes and reduced genetic variation associated with inbreeding, founder effect and drift. We test the hypothesis that Cedros and Tiburón deer reflect genetic signatures of respective adjacent mainland populations, though to varying degrees considering island size, distance to respective mainland and anthropogenic pressures. We thus predict that insular populations of Cedros and Tiburón deer show overall lower levels of genetic diversity (Frankham 1997) compared to adjacent mainland populations from which they separated. Given that taxa of interest in diverse or endemic 'hotspots' are often extinct, declining or rare, natural history or paleontological specimens provide genetic insight for studies of phylogeography, ecology and anthropogenic influences on species, particularly when extant samples are not attainable (Wandeler et al. 2007). Through the use of natural history specimens, this study represents the first genetic evaluation of the endangered *O. h. cerrosensis* subspecies from Cedros Island and of the threatened *O. h. sheldoni* subspecies from Tiburón Island in México's desert southwest, where the historic range of mule deer has undergone a 60% reduction (Weber and Galindo-Leal 2005).

We pursued two goals with this study. First, we sought to broadly examine the phylogeographic framework in the desert southwest and Baja California peninsula region for mule deer with regards to some of the more recent vicariance hypotheses supported for other taxa. We provide an essential complement to recognized Baja California phylogeographic patterns, and help elucidate the role of vagility in shaping evolutionary dynamics in landscapes with complex biogeographic histories. Secondly, we investigated the level of divergence of insular deer from mainland populations to evaluate genetic diversity and population demographics and further investigate signature of gene flow attributed to deer vagility between islands and respective mainland populations. In achieving these goals, we sampled mule deer from a broad desert southwest framework to assess how specific recent geophysical events, climatic shifts and island

formations shaped a broader faunal assemblage in the desert southwest and Baja California peninsula to include a generalist and vagile herbivore.

Methods

Taxonomic sampling

To complete the desert southwest regional analysis for mule deer, we focused on sampling from the two island subspecies, *O. h. sheldoni* (Tiburón Island mule deer) and *O. h. cerrosensis* (Cedros Island mule deer). Specimens from natural history collections are increasingly utilized as a source of DNA for conservation and phylogenetic studies, and are thus critical for providing phylogeographic insight when extant samples are not attainable (Wisely 2004). Owing to political constraints and geographical challenges in acquiring contemporary deer samples from Tiburón and Cedros Islands, samples were obtained from natural history specimens of Tiburón (n=14), Cedros (n=26) and San José Island (n=1) deer collected between 1896-1985 from several museums across North America (**Table S1**). We obtained samples from bones (n=9 turbinates, n=12 other bone), tissue (hide) (n=21) and antler core and tooth (dentin) core (n=10). Methodology for destructive sampling of museum specimens is provided in the **Appendix**.

To examine genetic signatures of insular isolation of Tiburón and Cedros deer in a larger desert southwest framework, we sampled n=419 contemporary deer from geographically widespread locations spanning the southwestern distribution of mule deer. Samples were obtained from the Sonoran, Chihuahuan, Mojave and Peninsular (Hafner 1992) deserts from 23 sampling locations (**Figure 1**; **Table S2**). These included representative individuals of peninsular deer *O. h. peninsulae* (n=8), *O. h. fuliginatus* (n=27), *O. h. eremicus* (n=122), *O. h. crooki* (n=188) and *O. h. hemionus* (n=56). Contemporary deer samples throughout their southwestern range in the USA and México were collected as outlined in Latch et al. (2009). Blood samples (n=8) from Tiburón Island deer were obtained during a survey for bighorn sheep on the island in fall of 2005.

Historical DNA precautions

Since DNA quantity and quality can decrease with increasing specimen age and is highly dependent on preservation method (Payne and Sorenson 2003; Rohland et al. 2004), extraction and amplification protocols require careful optimization to maximize DNA yield from specimens while minimizing potential contamination to historical DNA template. All DNA extractions from museum specimens were conducted in a dedicated laboratory designated for historical DNA work at the University of Wisconsin-Milwaukee (UWM) where no contemporary ungulate samples had ever been processed. The following actions consistent with published protocols and guidelines were followed to minimize potential contamination during the sampling, extraction and amplification process (Gilbert et al 2005; Pääbo et al. 2004; Willerslev and Cooper 2005): a) Sterilization of tools and work surfaces during specimen sampling (**Appendix**); b) single-direction transfer of extracted DNA from a low to high quality fashion; c) regular bleaching of tools, glassware and all sides of stainless-steel work surfaces; d) UV irradiation treatment of the laboratory and tools and equipment; e) use of RNAse- and DNAse-free or autoclaved reagents; f) use of sterile single-use filter tips for all pipetting; g) use of blank extraction controls for each extraction batch and negative PCR controls to monitor for potential contamination via agarose gel visualization, followed by sequencing of random negative controls and h) repeated PCR amplifications for unique haplotypes and quality control re-sequencing at two independent genomics core facilities. Purified products were first sequenced at UW-Madison's Biotechnology Center, after which unique haplotypes identified were re-amplified and re-sequenced at polymorphic regions to verify the sequence quality and consistency at a second facility (Great Lakes Genomics Center, School of Freshwater Sciences, UWM). All insular museum samples were extracted at least twice, and ten percent of working samples were re-extracted and re-amplified to verify template consistency and quality of sequence.

Extraction methods

Genomic DNA from insular museum specimens was extracted following sampling (**Table S1, Appendix**) using a modified phenol-chloroform method adapted from Smithsonian's Center for Conservation and Evolutionary Genetics for bone, tissue, teeth and antler. Approximately 5 mm^2 of dried tissue was finely chopped with a disposable scalpel blade in a plastic weigh boat, using a piece of 4 cm x 4 cm weigh paper (Fisher Scientific, USA) to cover the scalpel while chopping as a means of minimizing aerosolization associated with static cling of tissue bits. Finely chopped tissue was funneled into extraction tubes using weigh paper. Extractions were conducted in 'batches' of six, with each batch consisting of five museum samples to reduce potential for error and running a sixth as a blank extraction control. Approximately 5 mm^2 of nasal turbinates or long bone matrix bone was crushed finely by a pestle in a mortar (Fisher Scientific) in aluminum foil and funneled into an extraction tube using weigh paper where feasible to reduce static cling. For flat bone (turbinates) the blunt end of a scalpel was used to fracture the bone, followed by additional crushing using a scalpel blade in a piece of 4 cm x 4 cm weigh paper.

While other methods report using 100 mg (Kim et al. 2012) to 200 mg (Hoffman and Griebeler 2013) or more of antler material, we were able to extract reliable DNA using approximately 45-75 mg of antler material. All bone fragments and antler and dentin shavings were demineralized for 18-24 hours in a rotating 55ºC oven in 1 mL of 0.5 M EDTA. The EDTA supernatant was pipetted off after spinning down for 2 minutes at 12,000 rpm, making sure to not disturb the pellet. Macerated tissues (which were not subjected to the EDTA wash) and demineralized bone, antler or dentin shavings were then incubated in a 600 ul solution of 0.02 M TE, 0.01 M NaCl, 1% SDS, 640 ug/mL Proteinase K (5-Prime, USA) and 600 ug/mL Dithiothreitol (DTT) for 18-24 hours at 55ºC in a rotating oven. Six hundred ul of pH-adjusted phenol (to pH±7.9) was added to the digested extract where after mixing and spinning at 14,000 rpm for 2 minutes, the aqueous was transferred to a fresh tube for further protein and organic

separation via a straight phenol extraction, followed by a 1:1 phenol: chloroform and thereafter a straight chloroform wash (to remove residual phenol). The aqueous layer containing nucleic acids was then added to 1 mL of dH_20 in an Amicon Ultra-4 (ultracel 30K filters- Millipore, USA) and spun at 4,850-5,000 rpm for 9 minutes to concentrate the DNA, followed by another wash and spin with 1 mL dH_20. Approximately 65-80 ul Tris Low EDTA (TLE) was added to the concentrated nucleic acids (95-140 ul) to bring the volume up to 200ul, which was heated for 10-15 minutes at 65ºC to denature any potential DNAses. The isolated nucleic acids were aliquoted for immediate use and long-term storage. Genomic DNA was extracted from n=419 contemporary tissue samples as outlined in (Latch et al. 2009).

Markers and PCR conditions

Species divergence in a phylogeographic context has often been examined using selectively neutral markers, allowing for inference of coalescence where rates of mutation and evolution are constant and well-characterized (Avise 2008; Irwin 2012). Intraspecific divergence has been widely examined using mitochondrial (mtDNA) owing to fast yet variable mutation rates amongst different regions to provide fine-scale resolution for reconstruction of intraspecific phylogenies (Avise et al. 1987) and inference of coalescence (Avise 2008; Cronin 1992). Further, multiple copies of mtDNA per cell allow for greater chance of recovery of genetic information when samples are degraded (Keyser-Tracqui et al. 2003; Mulligan 2005).

Considering DNA extracted from natural history or ancient specimens is generally degraded due to enzymatic breakdown (resulting in shorter amplifiable fragments; Pääbo et al. 1989), several internal primer pairs were designed to capture half of the control region (or Displacement Loop, 583 base pairs [bp]) and six internal primers to capture the full sequence of a slower evolving gene (cytochrome b, approximately 1,028 bp) in overlapping fragments varying in size from 198 to 350 bp (**Table 1**). We used natural history specimens from of mule deer and white-tailed deer (*O. virginianus*) bone, tissue and antler samples from the UW-Stevens Point

(UWSP) Museum of Natural History collected between 1954-1985 to optimize the internal primer PCR conditions and extraction protocols prior to working with the insular mule deer samples. Sequence fragments from UWSP samples were assembled and compared to existing mule deer and white-tailed deer sequences to ensure accuracy and quality of sequence reads. These optimized protocols from UWSP samples were applied to the Tiburón and Cedros insular samples, followed by amplification and sequencing.

PCR amplification of DNA extracted from insular natural history specimens for control region primers were performed in 10 ul reactions using 5 pmol each forward and reverse primer (**Table 1**), 5-10 ng of template DNA, 0.2mM each dNTP (Promega, USA), 3.0 mM $MgCl_2$, 1 unit PerfectTaq DNA Polymerase (5-Prime, USA) and 1.6 mg/mL bovine serum albumin (BSA; ThermoScientific, USA). When DNA templates visualized on a 1.4% agarose gel fell below 5 ng/ul, 2.5% dimethyl sulfoxide (DMSO) was added to the mix to enhance PCR yield, reduce effects of contaminants and secondary structure in amplifying G-C rich regions (Farell and Alexandre 2012; Mamedov et al. 2008). For cytochrome b primers, the above was followed with the exception that 3 pmol of each forward and reverse primer was used (**Table 1**). PCRs were run on Eppendorf Mastercyclers for both control region and cytochrome b at conditions following Latch et al. (2008), with the exception of annealing temperature of 56ºC for all primer sets. PCR products were visualized on 2% agarose gels stained with ethidium bromide to estimate size and product concentration. Amplifications with multiple products were gel band excised and purified using the MinElute Gel Purification Kit (Qiagen, USA) following manufacturer's instructions, with the exception of a second elution in 5 ul of EB buffer. PCR products were diluted 1:10 for incorporation into 10 ul sequencing reactions following Latch et al. (2008). Fragments from insular natural history specimens were sequenced in both forward and reverse directions using 3 pmol of the same amplification primers with 0.5-0.75 ul BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, USA) following manufacturer's instructions (except cycles were increased to 50 cycles). Sequencing products were purified following a standard ethanol

precipitation method and sequenced at two independent genomics facilities (see '*Historical DNA Precautions*' above) following manufacturer's protocols for ABI 3730xl (Life Technologies, USA). Sequence chromatograms of forward and reverse fragments of insular natural history specimens were aligned using Geneious Pro 6.1.6 (Biomatters, New Zealand) and visually inspected for quality and consistency. Overlapping and replicate chromatogram fragments were aligned to referenced sequences from the nine mainland subspecies and assembled into full reads of target regions, followed naming nomenclature consistent with Latch et al. (2009), including the full natural history collection catalog/ accession number.

Molecular diversity and variation partitioning indices

Sequences were collapsed to polymorphic sites using the web-based program FaBox v1.41 (Villesen 2007) to identify individual haplotypes of insular deer in the desert southwest dataset. Representatives of each novel insular deer haplotype were deposited in GenBank. Standard estimates of nucleotide diversity and sequence polymorphism were examined using the program DnaSP v5.10.1 (Librado and Rozas 2009) for each control region and cytochrome b partition. We calculated number of haplotypes (H) and several molecular diversity and sequence polymorphism indices using ARLEQUIN v.3.5 (Excoffier and Lischer 2010) across three regional subsets of deer in the desert southwest study area to examine regional differences in overall genetic variation: a) among sampling locations (n=23); b) among seven sampled subspecies; and c) comparing Tiburón and Cedros Island deer to respective mainland populations. We also examined diversity indices among six inferred haplogroups (see Results). To account for differences in sample size within each subset, we estimated haplotype richness (H_R) by rarefaction in the program EstimateS v9.10 (Colwell 2013) using the individual-based Chao1 estimator (Chao 1984; Chao 1987; Chao and Lee 1992), which incorporates a correction factor for sample size. We minimized bias of larger sample sizes within each subset by running 200 random resampling iterations (without replacement) adjusted to n-1 of the smallest sample size of

the subset. We examined thoroughness of sampling of deer within island and inferred haplogroup populations by constructing rarefaction curves estimating randomly subsampled H_R from the total pooled sample haplotype richness. Curves demonstrating asymptotes suggested our sampling approached saturation of the potential richness and additional sampling would not yield new haplotypes. We also calculated molecular diversity indices, including haplotype diversity (*Hd*), which measures the probability that two individuals randomly chosen for comparison possess different haplotypes, as well as nucleotide diversity (π), which estimates the probability of nucleotide-site specific differences for two randomly chosen individuals (Nei and Kumar 2000). To observe overall patterns of genetic distance for the desert southwest study area, we estimated the average number of pairwise differences (Nei's D_A; Nei and Li 1979) across the 23 sampling locations.

To test a null hypothesis of homogeneity of recent genetic variation across the desert southwest, we conducted an analysis of molecular variance (AMOVA) using ARLEQUIN v3.5 (Excoffier and Lischer 2010) to assess how genetic variation is partitioned across the study area. This method parses out the amount of genetic variation among groups containing one or more populations (Φ_{CT}), among those populations within defined groups (Φ_{SC}) and among individuals within populations (Φ_{ST}) (Weir and Cockerham 1984; Excoffier et al. 1992). A hierarchical analysis was run using four models reflecting hypothesized groupings to assess genetic diversity among 22 sampling sites (two samples from San José Island were combined with southern peninsula BA-SM samples for n=8 samples; **Table S2**), among the six inferred haplogroups (see Results) and among the seven sampled subspecies (**Figure 1**). The fourth model included 4 biogeographic regions separated by vicariance events spanning a temporal scale of approximately <1-8 mya and supported by intraspecific phylogroups identified in several other, relatively sessile taxa (Grismer 2000; Lindell et al. 2006; Zink 2002). Considering the estimated Irvingtonian split of *Odocoileus*, we explored vicariance hypotheses with the consideration that deer experienced

allopatric divergence for the more recent splits or emigrated to geographic regions formed by such events and subsequently diverged. Region 1 was defined as Baja peninsula north of the putative Vizcaino seaway (west of the Imperial Formation), supported by divergence in San Diego pocket mice (*Chaetodipus fallax*; Rios and Álvarez-Castañeda 2010); Region 2: southern Baja Peninsula (south of Vizcaino seaway), supported by divergence in white-tailed antelope squirrels (*Ammospermophilus leucurus* – Álvarez-Castañeda 2007; Mantooth et al. 2013) and black-tailed brush lizards (*Urosaurus nigricaudus*; Lindell et al. 2008); Region 3: east of the Imperial Formation through the Sonoran Desert supported by divergence in round-tailed ground squirrels (*Xerospermophilus tereticaudus*; Bell et al. 2010) and Region 4: east of the continental divide (vicinity of the Cochise Filter Barrier; Morafka 1977) along the Sierra Madre Occidental into the Chihuahuan desert, supported by divergence in the western diamondback rattlesnake (*Crotalus atrox*; Castoe et al. 2007; **Figure 1**). We assessed significance through 10,000 permutations of pairwise differences among populations and groups.

Lineage inference with networking and phylogenetic analyses

We examined phylogeny for the study area using 140 haplotypes identified from the dataset to infer relationships to other cervid outgroups and identify the basal lineage leading to intraspecific divergence in southwest desert mule deer. We partitioned the control region separately from the cytochrome b gene sequence owing to differing rates of evolution for each region. Caribou *Rangifer tarandus* (mitochondrial genome GenBank Acc. AB245426 trimmed to control and cytochrome b regions) was selected as an outgroup, and analyses were run with white-tailed deer (*O. virginianus*, GenBank Acc. OVU12869 for control region and DQ379370 for cytochrome b region) and Sitka black-tailed deer (*O. h. sitkensis*, GenBank Acc. FJ188924 for control region and FJ188727 cytochrome b region) in phylogenetic analyses.

Use of networks for shallow divergence topologies are beneficial in parsing out intraspecific phylogeographic relationships because closely related haplotypes are best

represented in a bifurcating network rather than requiring occupation of tip positions (Posada and Crandall 2001; Templeton 2004). We examined patterns of divergence between lineages using statistical parsimony algorithms implemented in TCS v1.21 (Clement et al. 2000). The connection limit in TCS v1.21 was examined by running the default of 95% plausible connections and well as setting a manual connection limit of 50 mutational steps to ensure connection between all inferred haplogroups for the full 1,611 bp length sequence for n=449 deer. The statistical parsimony topology and connection limits were compared to the topology produced by median-joining algorithms (Bandelt et al. 1999) implemented in NETWORK v4.6.11 (Fluxus-Engineering) using epsilon=0 and unrooted settings. Both of these complementary network-building methods are highly effective at identifying intraspecific variation clade ancestry and cryptic phylogroups within a variety of mammalian taxa (e.g. Barnett et al. 2006, de Bryun et al. 2009; Ohdachi et al. 2012). For analytical purposes, we inferred deer lineages from haplogroups defined as networks of connected haplotypes separated by at least ten mutational steps from other groups of haplotypes and possessing at least five haplotypes. Identification of haplogroups followed naming nomenclature laid out in Latch et al. (2009) for consistency in reporting results.

Network visualization of haplotype divergence patterns were supported by Bayesian phylogenetic tree building algorithms. Nucleotide substitution models for each partition were assessed for 140 haplotypes using the program jModelTest v.2.4 (Posada 2008) which invokes maximum likelihood algorithms implemented in PhyML 3.0 to determine which of 88 potential substitution models best supported the observed rate of evolution, including outgroups of *R. tarandus*, *O. virginianus* and *O. h. sitkensis*. We compared the top models selected for each partition using the Bayesian Information Criterion (BIC; Schwarz 1978), which has been favored over Akaike's Information Criterion (AIC) during model selection (Luo et al. 2010). We used the top selected models for each partition as *a priori* substitution models for Bayesian phylogenetic inference (BI) for the control region and cytochrome b sequence implemented in MrBayes v3.2.2 (Ronquist and Huelsenbeck 2003; Ronquist et al. 2011). As our goal was to trace phylogenetic

structure among the inferred haplogroups, 70 haplotypes from geographically diverse locations were selected to represent haplogroups in simplifying visualization of these relationships. We set a relaxed molecular clock (independent gamma rates model –IGR) to allow lineages to explore independent variation in clock rates, and a random starting tree for a pair of two independent runs of $2.5x10^6$ MCMC generations, sampling every 500 generations with a cold chain and 3 heated chains (heating parameter=0.2) and a 10% burning (250,000 generations). We assessed convergence of the four runs through inspection of Potential Scale Reduction Factor (PSRF; Gelman and Rubin 1992) values reaching 1.0 as well as visual confirmation of trace files reaching stationarity to ensure effective sample size (ESS) values all >200 using Tracer v1.5 (Rambaut and Drummond 2007). We visualized the maximum clade credibility (consensus) tree using FigTree v1.4 (Rambaut 2008).

Demographic and lineage divergence estimations

To evaluate whether highly supported lineages of deer in the desert southwest became isolated before the LGM, we estimated relative divergence times of inferred lineages using Bayesian MCMC analyses employed in the BEAST v1.7.5 package (Drummond and Rambaut 2007). We employed a normal distribution prior for a fossil-calibrated age of the *R. tarandus* split from North and South America Odocoileini (5 mya ±1 mya; Gilbert et al. 2006; Vislobokova 1980) as well as for earliest (Irvingtonian) fossil dates for *O. hemionus* of 0.7-1.9 mya (Jacobsen 2003; Heffelfinger 2011). Partition-specific substitution models employed in BI analyses were run for the 140 haplotypes using a log-normal relaxed molecular clock model and Jeffrey's prior (Drummond and Rambaut 2007) for the coalescent tree. Statistics were sampled every 2000 MCMC generations for two independent runs of $4x10^6$ total generations (where the second run used a reduced input of 70 sequences; see above). Convergence of both MCMC runs was assessed and results visualized following those methods performed for BI. It must be noted that such relative divergence time estimates reflect bias towards maternal demographic history for the

mtDNA locus, requiring careful interpretation of results. Our interest was to infer relative timing among the inferred lineages, thus we interpret our results as such.

Considering the vast changes in vegetation in the desert southwest with the LGM and its subsequent retreat (Van Devender and Spaulding 1979), we sought to test the hypothesis that deer in the desert southwest have undergone detectable population expansions at regional scales in the last 5,000-26,500 years before present. To evaluate this, we invoked the concept of the coalescent-based mismatch distribution, which compares the distribution of observed frequencies of pairwise differences among haplotype pairs to a distribution expected under the sudden expansion model (Rogers and Harpending 1992), where departure from a stable effective population size is represented in a smooth, unimodal mismatch distribution. Mismatch distributions are commonly used to infer post-Pleistocene demographic expansion for animals with wide dispersal abilities (Thompson and Russell 2005) as the increase in mean pairwise differences suggests lack of departure from the sudden expansion model corresponding with a larger geographic range of panmixia.

We examined mismatch distributions for haplogroups possessing eight or more haplotypes using a generalized non-linear least-squares approach (Schneider and Excoffier 1999) for the 1,611 bp concatenated control region and cytochrome b sequence regions. We assessed significance through 10,000 bootstrap simulations by calculating the proportion the sum of squared deviations (SSD) of the expected values that exceeded those of the estimated values. Observed mismatch distributions that did not differ significantly ($p>0.05$) from simulated unimodal mismatch distributions meant the sudden expansion model could not be rejected. We estimated the mode of the observed mismatch distribution tau (τ) and 95% confidence intervals to estimate the date of the demographic expansion (expressed as t in years before present; $t=\tau/2\mu$) following Rogers (1995). An overall substitution rate for the control and cytochrome b regions for mule deer estimated by Latch et al. (2009) of 3.22×10^{-8} substitutions / site / year was used in calculations of t, assuming a generation time of 5 years (Robinette et al. 1977).

We further assessed haplogroups and island populations for signature of population growth for the 583 bp of the control region using Fu's *Fs* (Fu 1997) statistic in ARLEQUIN v3.5 (Excoffier and Lischer 2010), which estimates probabilistic deviations from selectively neutral sequence polymorphism and pairwise differences given the observed sequence diversity. Departure from neutral expectations inferred for expanding populations is reflected by significant negative values when an excess of rare haplotypes is detected from mutations or population expansion, while positive *Fs* values suggest fixation of older mutations from stable population sizes or potential bottleneck events (Fu 1997). We paired Fu's *Fs* statistic with Tajima's *D* statistic (Tajima 1989) to evaluate departure from a null model of pairwise nucleotide differences per segregating site for the control region only, which can be a conservative measure to changes in effective population size or varying rates of mutation across a sequence region (Thompson and Russell 2005). We adjusted for variable mutation rate across the control region using a gamma (Γ) shape (α) value of 0.561 determined from jModelTest v.2.4 using 10,000 simulations with pairwise differences.

Results

Museum samples and authenticity

We obtained full target sequence (583 bp of control region, 1,028 bp of cytochrome b gene) for a total of 29 natural history specimens (15 from Cedros and 14 from Tiburón deer), representing an overall 63% sequencing success rate for natural history specimens attempted. Bone samples yielded the most successful amplifications from extractions (76%), followed by drilled antler (70%) and tissue (33%). Only in one instance did a blank extraction control amplified by PCR yield a single, observable product visualized on a 2% agarose gel; this product was sequenced and BLASTed with medium support (75%) to *Enterobacter* sp. Combined with the fact that negative PCR controls yielded no observable amplified product on agarose gels or when amplified and sequenced, all resulting sequences from natural history specimens are

authentic and accurate. For the complete 1,611 bp sequence dataset, haplotypes observed in the museum samples (n=29) were confirmed through re-amplification and re-sequencing of polymorphic regions, leading to the confirmation of eight haplotypes in Cedros (53%) and six in Tiburón (26%) deer natural history samples. For only one Cedros bone sample (SDNHM 13938-2328) was repeated amplification and re-sequencing unable to resolve an ambiguous C-T transition at bp 241 of the control region.

Molecular diversity and variation partitioning indices

We observed a total 140 haplotypes (*H*) for the full 1,611 bp dataset (n=449). Overall desert southwest deer haplotype diversity (*Hd*) was 0.9833 (SD±0.002) with a nucleotide diversity (π) measure of 0.01066 (SD±0.00033) (**Table 2**). Across all 449 deer analyzed, the control region (583 bp) possessed 102 polymorphic sites (76 which were phylogenetically informative) with overall higher *Hd*, π, and segregating (polymorphic) sites than the cytochrome b gene (1,028 bp; 60 polymorphic sites, of which 43 were phylogenetically informative).

Patterns of molecular diversity and sequence polymorphism varied across three regional subsets examined in the desert southwest study area (subset 1: n=23 sampling locations: subset 2: n=7 subspecies, and subset 3: n=2 island-mainland pairs). When corrected for sample size for the first subset, haplotype richness (H_R) of the 23 sampling locations was highest for the Flagstaff area, Arizona (AZ_FL, H_R =25.4±13.06) and Raton area, New México (NM_RT, H_R =24.5±13.11). Among 23 sampling locations, deer from the Crockett County area in Texas showed the lowest haplotype diversity (*Hd* = 0.100) and nucleotide diversity (π =0.000062), while deer from Baja California Sur (BA_SM) showed the highest haplotype diversity (*Hd* = 0.964) and second highest nucleotide diversity (π = 0.114) (**Table S2**). We observed the greatest corrected pairwise differences (Nei's D_A; Nei and Li 1979) among the 23 sampling locations for Guadalupe Mountains, New México (NM_GM) and Tiburón deer (SO_TI) from all other sampled populations (**Figure 2**).

For the second subset examining the seven subspecies in the desert southwest region, the greatest H_R was identified for *O. h. hemionus* (H_R = 85.8±39.21) (**Table 3**). As with H_R results for sampling location, we may expect to see this considering our desert southwest study area encompassed only a small portion of the *O. h. hemionus* range (sampling locations AZ_FL and NM_RT), where H_R adjusted for sample size suggests we only marginally sampled the full haplotype diversity spectrum for this subspecies. Six of the seven subspecies examined exhibited levels of *Hd* over 0.90 (**Table 3**), with the exception of deer sampled from Tiburón Island, which possessed significantly lower *Hd* (t=9.026, df=6, p<0.0001) and slightly lower π (t=2.071, df=6, p=0.0838) than the other six subspecies. When examining the proportion of observed number of haplotypes (*H*) to the expected richness (H_R), Cedros Island deer showed the highest proportion (H/ H_R=0.8) suggesting that our sampling efforts using natural history specimens approached the asymptote of H_R for deer on Cedros Island within the last century (**Figure S1**).

Consistent with expectations for island-mainland comparisons in the third comparison subset, both Cedros and Tiburón deer showed significantly lower molecular diversity measures than adjacent mainland populations (Cedros *Hd*: t=2.957, df=58, p<0.0045) (Tiburón *Hd*: t= 54.325, df=175, p<0.0001) (**Tables 4a, 4b**). Pairwise F_{ST} measures showed that Tiburón deer significantly differed from adjacent mainland Sonoran desert deer (**Table 4b**; mean F_{ST} = 0.806, p=0.000), while Cedros deer showed overall less differentiation from mainland Baja California deer (F_{ST}= 0.348, p=0.000) (**Table 4a**).

Results of the AMOVA for the four hypothesized groupings showed that most of the genetic variation among modeled groups (Φ_{CT}) was partitioned among six inferred haplogroups (model 2: see below) equating to 69.9% of the total genetic variation explained (**Table 5**). Other models showed much lower partitioning of genetic variation among groups; model 3 (grouping of seven designated subspecies) explained 20.3% of the genetic variation among subspecies, followed by model 4 (four biogeographic regions separated by vicariance events implicated in divergence in other desert southwest taxa) with 10.3% of variation explained among groups.

While the lowest amount (7.69%) of variation was partitioned among sampling locations, it is possible that this larger number of groups (n=22) could bias the total variation explained downward (Excoffier et al. 1992; Meirmans 2006).

Lineage inference with phylogenetic analyses and networking

The networks created by TCS v1.21 and NETWORK v.4.6.11 for the full 1,611 bp region were identical in overall topology, both reflecting strong divergences for six haplogroups (MD-A, BF, I, J, N and O; inferred lineages). Substantial genetic variation was evident for the largest inferred haplogroup (MD-BF) which contained nearly half of the desert southwest samples (n=241). This inferred lineage demonstrated the highest adjusted haplotype richness (H_R=100.1±19.46) (**Table S3**) and high frequencies of clustered haplotypes separated by a few mutational steps (**Figure 3**). Using the TCS v1.21 connection limit set to 50 mutational steps, we connected a divergent haplogroup to reflect overall network topology consistent with NETWORK v4.6.11 (n=62) separated by 27 mutational steps (MD-J; Latch et al. 2009) to the remaining network (**Figure 3**). This haplogroup consisted of individuals from Tiburón Island (SO_TI; n=22), Guadalupe Mountains in New México (NM_GM; n=23) and the Alpine and Plains/ Briscoe areas of Texas (TX_AL, n=9 and TX_PL, n=7). All but one Tiburón individual fit into this MD-J haplogroup, separated by eight mutational steps to Guadalupe Mountains deer (**Figure 3**). The remaining Tiburón sample was obtained in 2003 from a carcass of a buck found on the island, and matched to haplotypes found in Altar Valley (Arizona) and other individuals from central Sonora within the MD-BF lineage. Thus we suspect this buck is a migrant who may or may not have bred on the island. Excluding this potential migrant, Tiburón Island deer (n=22) showed a 0.3-0.7% sequence divergence (eight mutational step difference) from other deer within the same lineage (MD-J) (F_{ST} = 0.520, p=0.000) and shared a unique single bp deletion with deer from the Guadalupe Mountains in the control region at bp 157.

Among the most widespread of lineages was the haplogroup MD-N (n=67) spanned between Raton (New México), Flagstaff (Arizona) and the Baja California peninsula (**Figure 4**). All fifteen sampled individuals from Cedros Island representing mtDNA genetic diversity of deer collected between 1896 and 1979 were grouped with the MD-N lineage, separated by seven mutational steps from deer from the respective mainland Baja California peninsula; no haplotypes were shared between Cedros deer and adjacent mainland deer. Of these fifteen individuals, we observed 8 nucleotide transitions (0.2% sequence divergence) and 0.4-0.9% sequence divergence (seven mutational step difference) to deer within the same lineage (MD-N) (F_{ST}= 0.506, p=0.000). The MD-A lineage (n=60) was geographically widespread like MD-N but showed the highest frequency in central Arizona (Kofa and Flagstaff areas). The MD-O lineage was the least frequent and most geographically restricted haplogroup across the desert southwest, represented by eight individuals sampled from the Portal and Phoenix areas of Arizona (**Figure 4**). Within our study area the MD-I haplogroup was represented by 11 individuals sampled from the Baja California peninsula, including two individuals from San José Island (*O. h. peninsulae* subspecies), which were genetically more similar to the MD-I lineage sampled in Northern Baja deer than deer from Southern Baja (**Figure 4**).

For BI phylogenetic analyses, the control region (583 bp) was best supported by a HKY+I+G nucleotide substitution model (BIC=7,292) with a gamma (Γ) shape α=0.561 and proportion of invariable sites (p-inv=0.520) for rate of substitution across sites. The cytochrome b region (1,028 bp) was supported with a GTR+G model of evolution (BIC=7,182). Bayesian phylogeny recovered relatively high support for the lineages inferred from network haplogroups, including posterior probability of 1.00 of the MD-J clade (all Tiburón [SO_TI], Guadalupe Mountains [NM_GM] and Texan Alpine [TX_AL] and Plains/ Briscoe [TX_PL] area deer) as a more basal lineage monophyletic to all other deer (**Figure 5**). Posterior support for the other five lineages was above 0.90 with the exception of the MD-BF lineage, which was supported as paraphyletic to lineage MD-N (posterior probability=0.82). Several unresolved relationships

within the diverse MD-BF lineage (polytomies) may be explained by unsampled intermediates prevalent throughout this central Sonoran and Chihuahuan desert region or incomplete lineage sorting. Despite extensive sampling in this particular region, lack of saturation in rarefaction curves of H_R (**Figure S2**) suggests additional haplotypes would likely be found with greater sampling from the central Sonoran and Chihuahuan desert region where the MD-BF lineage is geographically located.

Demographic and lineage divergence estimations

Relative divergence dates of desert southwest deer supported the six inferred lineages (MD-A, BF, I, J, N and O) identified by haplotype networks and BI phylogenetic tree reconstruction. The oldest split was identified for the MD-J lineage, with an estimated divergence date of approximately 67,000 ybp. Relative divergences for other desert southwest lineages were found to be slightly more recent (62,000-36,000 ybp; **Table 6**).

For each of six inferred lineages coalescent simulations of mismatch parameters failed to differ significantly ($p>0.05$) from observed distributions and the null model of the sudden expansion could not be rejected (**Table 6**). All lineages supported unimodal mismatch distributions as expected for the sudden expansion model (**Figure 6**). From the mode of the expansion (τ) and associated confidence intervals we estimated demographic expansion dates (t), all which fall within the retreat of the LGM, from expansions dated around 15,000 ybp (Clark et al. 2009) to as recent as within the last couple thousands of years (**Table 6**). The oldest demographic expansion was estimated to have occurred in the MD-J haplogroup (15,900 ybp; 95% CI: 25,100-5,300) with the youngest for the extant widespread haplogroup MD-A (6,900 ybp; 95% CI: 16,000-1,400). These demographic expansion estimates support the estimated relative divergence dates in that a pattern of timing consistency emerges when considering both sets of estimates (**Table 6**). However, the large high posterior density intervals for divergence

times emphasize the uncertainty associated in estimating shallow divergences often characteristic of intraspecific studies (**Table 6**; Brown and Yang 2010).

Calculated values of Fu's *Fs* and Tajima's *D* statistics demonstrated an overall trend of deviation from selectively neutral expectations, consistent with population expansion supported by the unimodal mismatch distributions. Both Fu's *Fs* and Tajima's *D* values were negative for all inferred haplogroups with the exception of the smallest haplogroup MD-O, which supported non-significant positive values (Fu's *Fs*= 0.491, Tajima's *D*= 1.008) likely attributable to the low sample size. Fu's *Fs* values were significantly negative for three of six haplogroups (MD-BF, I and N), with the largest haplogroup (MD-BF) demonstrating a remarkably negative statistic (-24.933, p=0.000) strongly indicative of population expansion, with estimated *t* since expansion of 13,400 ybp (95% CI: 18,100-7,200) (**Table 6**). Considering the unimodal mismatch distributions (**Figure 6**), slightly negative and non-significant values of demographic parameters for the MD-A (Fu's *Fs*=-0.898, Tajima's *D*=0.384) and MD-J (Fu's *Fs*= -1.561, Tajima's *D*= -0.0252) lineages could be due to many reasons, including perhaps fixation of older mutations and possible long-term stability in population sizes for these two lineages.

Though samples from Cedros and Tiburón deer failed to reject the sudden expansion model (**Table 6**), mismatch distributions were weakly unimodal for Tiburón deer and stronger for Cedros deer (data not shown). Neither Fu's statistic (Fu's *Fs*= -0.926, p=0.249) nor Tajima's *D* (*D*= 0.149; p=0.594) statistic for Cedros deer differed significantly from zero, making interpretation for genetic signature of population bottleneck or expansion difficult considering early Holocene island isolation and potential effects of genetic drift on rare haplotypes. However, population bottlenecks that occurred in the recent past may not always be detected by these measures. Tiburón deer considered without the putative migrant (n=22, **Table 6**) similarly showed non-significant values for both Fu's *Fs* (-1.642; p=0.0701) and Tajima's *D* (-0.814, p=0.240), but in the opposite direction, suggesting sample size may be too low to detect an effect or of a recent bottleneck or demographic expansion.

Discussion

Geophysical events, climatic shifts and island formations in the desert southwest and Baja California peninsula helped shape the diversity and divergence of faunal lineages. The study of mule deer in this region provides insight for the role these historic events played in shaping a broader faunal assemblage to include a generalist and vagile herbivore. In contrast to previous findings of many desert taxa supporting intraspecific phylogroups coinciding with vicariance hypotheses in different biogeographic regions (Grismer 2000; Lindell et al. 2006; Riddle et al. 2000a), we found limited mtDNA support across these regions, likely attributable to high deer mobility and generalist ecology. Across the Sonoran and Chihuahuan deserts, we found support for a geographically discontinuous lineage observed in Tiburón deer and deer sampled from the Guadalupe Mountains of New México and Texas localities. Observed patterns and timing of historical demography suggest that LGM retreat and associated climate-induced shifts in vegetation communities may have influenced deer expansion and contemporary mtDNA lineage patterns in the desert southwest. While the majority of insular endemic studies in the Baja California region have highlighted conservation status or divergence for smaller and less mobile taxa often vulnerable to introduced predators (Mellink et al. 2002), herbivores (Escobar et al. 2011) and invasive plants (West and Nabham 2002), more vagile taxa such as deer have been underrepresented in divergence and conservation-focused studies for the region. As SEMARNAT considers Cedros and Tiburón Islands and their deer under protected UMA Biosphere Reserve status, our study underlies the significance of using natural history specimens to assess divergence and genetic diversity for these declining subspecies in terms of their conservation status. We assessed genetic diversity and historic demographic patterns relative to other mainland desert southwest deer populations, and provide interpretations towards the taxonomic classification and conservation of these endemic deer.

Biogeographic events and subspecies divergence

Owing to mule deer generalist ecology and ability to disperse long distances, the finding of clear mtDNA genetic structure in the desert southwest is somewhat unexpected. Our findings suggest vicariance associated with biogeographic events hypotheses may influence contemporary population structure to varying degrees, and select mtDNA haplogroups show some overlap with designated subspecies boundaries. Though model 4 of the AMOVA analysis (**Table 5**) revealed marginally greater partitioning of genetic variation among groups separated by biogeographic events (10.36%; **Table 5**) over just using the sampling locations (7.69%; **Table S2**), we found support for strong regional demarcation which coincides with only one of the four proposed vicariance hypotheses separating two biogeographic regions. An abrupt transition between mtDNA lineages appears to coincide with the location of the Imperial Formation/Salton Trough area (Peterson 1975; Schremp 1981) between the San Diego and Baja California peninsula north of the putative Vizcaino seaway (Region 1: **Figure 1**) and Imperial County eastward into the Sonoran desert (Region 3) sampling areas, which roughly demarcates the current southern boundary of the Mojave and western boundary of the Sonoran deserts (Latch et al. 2009; **Figure 4**). This demarcation is further supported by nuclear microsatellite data, where Bayesian clustering methods reflect the distinct population separation (Latch et al. submitted), a pattern that also appears to support the longitudinal separation of *O. h. fuliginatus* and *O. h. eremicus* in the Imperial Valley. However, it should be noted that *Odocoileus* spp. are estimated to have diverged as the marine seaways associated with this biogeographic event were receding, thus it is more likely that the observed pattern may be due to more contemporary conditions, i.e., vegetational shifts associated with increasing aridity following LGM retreat (Betancourt 2004; van Devender and Spaulding 1979), extensive agriculture and urban development in the Salton Trough area, or a combination of these and unknown ecological factors.

Our results showed little genetic structure in support of other vicariance hypotheses and associated biogeographic regions, namely east of the Imperial Formation throughout the Sonoran

Desert (region 3; **Figure 4**) and east of the Sierra Madre Occidental inclusive of the Chihuahuan desert (region 4; **Table 5**). For the Baja California peninsula, the putative Vizcaino seaway presumed to have bisected the Baja California peninsula estimated 1 mya (**Figure 1**) is a vicariance feature implicated as a barrier leading to divergence in several smaller, more sessile species in the Baja California peninsula such as *Ammospermophilus* antelope ground squirrels (Álvarez-Castañeda 2007; Mantooth et al. 2013) and black-tailed brush lizards (*Urosaurus nigricaudus*; Lindell et al. 2008). We found limited mtDNA structure in the peninsula, though our sampling effort of deer across the peninsula was not as robust as most locations throughout the study area. Individuals belonging to the MD-A lineage were not identified or sampled south of Baja California Norte or the Vizcaino seaway (yellow lineage: **Figure 4**). Considering Cedros Island's separation from Punta Eugenia between 13-10,000 ybp having occurred north of the putative Vizcaino seaway (**Figure 1**), our finding of genetic similarity of Cedros deer to peninsular deer sampled south of the putative seaway within the MD-N lineage rather than to northern peninsular deer (where no MD-N individuals were sampled) was somewhat unexpected. Further, the MD-I lineage was only observed in both north and south ends of the Baja California peninsula in the desert southwest study region. Similarly, microsatellite data show that deer across the Baja California peninsula exhibit little population structuring (Latch et al. submitted) or latitudinal separation to support the *O. h. fuliginatus* and *O. h. peninsulae* designations. These combined results suggest that whether or not deer were present throughout the peninsula during the estimated bisection by the Vizcaino seaway (approximately 1 mya), deer dispersal ability combined with generalist ecology may have overcome the potential barrier effect of this event, leading to minimal contemporary structuring. Elsewhere in the desert southwest, we did not recover evidence of mtDNA lineage structuring coinciding with previously proposed vicariance hypotheses supported in other smaller, less mobile species such as the round-tailed ground squirrel (*Xerospermophilus tereticaudus*; Bell et al. 2010) with range restriction to east of the Imperial Formation and western Sonoran Desert (western extent of region 3) or in the western

diamondback rattlesnake (*Crotalus atrox*; Castoe et al. 2007) in the vicinity of the northern Sierra Madre Occidental (Cochise Filter Barrier; separating regions 3 and 4; **Figure 4**).

Geographic discontinuity of an older lineage

Our haplotype networks and phylogenetic analyses provide evidence for a geographically discontinuous lineage spanning the Sonoran and Chihuahuan deserts. Deer (22 of 23 animals) sampled from Tiburón Island fall within the MD-J haplogroup, demonstrating genetic similarity to deer from New México's Guadalupe Mountains and west Texas (Alpine/ Plains areas). As with several other intensively-managed game species, reintroductions and translocations of deer across regions have likely elevated observed patterns of gene flow. Such documented translocations between New México and western Texas (Heffelfinger 2006) likely explain the shared haplogroup between these states, though undocumented translocations may be contributing to some of the observed genetic patterns. Considering this, one hypothesis is that deer samples from Tiburón Island represent individuals from recently transplanted animals from the Guadalupe Mountains or Texas regions to Tiburón Island (or vice versa). We reject this hypothesis in support Tiburón deer endemicity to the island for four reasons. First, Tiburón Island is part of the homeland to the indigenous Seri (Comcáac) tribe, which has subsisted on Sonora's western coast in the Sea of Cortés for millennia. Oral tradition and written anthropological history stress the cultural significance of deer on the island for many generations (Felger and Moser 1985). To this day, Tiburón deer continue to be a significant part of the cultural history of the Seri, important for tools, basket-making, and other cultural and ceremonial use (N. Martinez-Tagüeña, personal communication). Secondly, a shell midden at the cultural site of Tecomate at the northern end of Tiburón Island (White 2000) possesses stratified layers containing an archaeological record of deer remains ranging in age from a few hundred years old near the top to 2,000 years old further down (R. White, personal communication), and future genetic analyses could track temporal genetic variation and diversity during these two millennia. Thirdly, no

translocation records are known for importing deer from New México to any Mexican island (or vice versa), and Tiburón deer were recognized as phenotypically different from the late 19th century with explorations by W. J. McGee (Fontana and Fontana 1983) until C. Sheldon collected the type specimen in 1921, followed by subspecies characterization (Goldman 1939; Sheldon 1922). Thus to consider deer as having been translocated to Tiburón Island to fit the archaeological timing of cultural deer use and remains, the translocation needed to have preceded Spanish exploration, which is extremely unlikely. Lastly, considering the 27 mutational step difference between MD-J and the remaining desert southwest lineages (**Figure 3**), it is exceptionally unlikely that potential effects of insular separation and genetic drift for Tiburón deer populations led to convergence in mtDNA sequence similarity with other members of the MD-J haplogroup (Guadalupe Mountains, Texas Plains/ Alpine deer).

Considering phylogenetic tree topology, lineage divergence dating estimates as well as consistent older demographic expansion time estimates (Latch et al. 2009; **Table 6**), we propose that the MD-J lineage represents a relict group which likely underwent extensive range modification through climatic fluctuations associated with the LGM and transition from Pleistocene to Holocene. Strong posterior probabilities support the inferred lineages identified among desert southwest mule deer, including the position of MD-J as basal to the rest of the deer sampled in the desert southwest for the Bayesian phylogram (**Figure 5**). The oldest divergence estimate for the MD-J lineage relative to other lineages coinciding with the end of the Pleistocene (approximately 62,000 ybp) supports the phylogenetic findings and reflects the oldest estimated demographic expansion (25,000-5,000ybp) for the desert southwest (**Table 6**). Evidence from packrat middens sampled from the late Wisconsin (estimated 40-30,000 ybp) to the early Holocene (approximately 11,000 ybp) suggest that middle elevation habitats in the northern Chihuahuan desert comprised a relatively stable woodland community, consisting primarily of juniper (*Juniperus* sp.) with *Quercus* and *Pinus* sp. (Van Devender 1990a). Higher elevations such as in the Guadalupe Mountains (>2,000 meters) supported more montane and sub-alpine

species (*Ponderosa* sp.) indicative of overall cooler and moister conditions (Van Devender and Spaulding 1979; Van Devender 1990a). Woodlands in middle elevations transitioned rapidly to more arid conditions with climatic shifts approximately 8,000 ybp for the Chihuahuan desert (and slightly earlier in the Sonoran region, approximately 10-9,000 ybp) driving the woodland zone to recess to higher elevations as grassland to desert scrub communities developed at lower altitudes (Van Devender and Spaulding 1979). Our demographic expansion estimates for the MD-J lineage overlap with this period of relative woodland stability (25,000 ybp, around LGM extent; Clark et al. 2009) through the shift of increasing aridity (to about 5,000 ybp). Assuming the MD-J lineage may have been more widespread, we speculate a similar pattern may have occurred with the MD-J lineage. It is possible that deer adapted to montane or sub-alpine woodland habitats in more mountainous regions spanning the Sonoran and Chihuahuan deserts may have followed the retreat of the woodlands into higher elevations (Van Devender, 1990b) as the highest elevations in a longitudinal span (from west to east) include Tiburón Island, the Sierra Madre Occidental and ranges near the Guadalupe Mountains. However, future studies considering historic distribution and fossil evidence are required to expand this idea.

Lineages in the desert southwest

Combined with the timely extinction of several genera of land herbivores with the onset of the Holocene, other *Odocoileus* deer were probably able to take advantage of the newly available scrub and shrub communities of middle elevations in the Sonoran and Chihuahuan deserts (Heffelfinger 2011). This is the core area for the large MD-BF lineage, which spanned the largest geographic portion of our study area and was represented by nearly half of the individuals examined, though it is also widespread throughout more northern populations of *O. h. hemionus* (Latch et al. 2009). Inferred (missing) haplotypes reflected in the median joining network for MD-BF emphasize the polymorphism of this particular lineage, which may be explained by incomplete sampling as geographic range extends beyond our sampling area or incomplete

lineage sorting (shown by the Bayesian phylogram, **Figure 5**) separated by high frequencies of substitutions. This widespread mtDNA lineage supports demographic expansion (significantly large *Fs* value) approximately 18-7,000 ybp, which coincides with the retreat of the LGM and which may reflect colonization or large expansion into previously unoccupied areas (e.g., lower elevation woodlands transitioning to desert scrub communities). Considering pattern of maternal inheritance and quarter the effective population size inferred by nuclear markers, demographic estimates and genetic patterns inferred from mtDNA in a species such as deer where male-biased dispersal (i.e., female tendency towards philopatry - Cronin 1991b; Lansman et al. 1981) likely represent conservative estimates of population structure. Particularly for the Sonoran desert, a combination of sparse resources (reliable forage and watering corridors) in arid environments leads to occupation of larger annual and seasonal home ranges (Heffelfinger 2006) required to satisfy ecological needs, which may result in high gene flow between deer populations across the arid deserts. Population clusters spanning large geographic areas inferred from nuclear microsatellite data (Latch et al. submitted) support the idea that high levels of contemporary gene flow occur in the Sonora as well as within the Chihuahuan deserts.

While similarly broad in geographic distribution as the Sonoran and Chihuahuan MD-BF lineage, deer representing the MD-N lineage were observed in the Baja California peninsula and eastward north of the deserts (**Figure 4**). Demographic expansion for this lineage was estimated at 15,600 ybp, coinciding with LGM retreat and vegetation shifts. Our patterns of mtDNA spatial structure observed for mule deer in the western Sonoran desert and San Diego area (belonging to lineage MD-N; **Figure 4**) are further supported by demographic expansion date estimates calculated for mule deer populations across California (Pease et al. 2009). Recent population splits between a large area encompassing a coastal, southern latitudinal stretch of California and deer sampled in the San Diego and eastern Sonoran region provided demographic expansion dates of 13,000 ybp, an estimate which falls within the 20,800-8,800 ybp confidence interval we identified for demographic expansion of the MD-N lineage (**Table 6**). Further north of our study

area, demographic expansions were observed in northern parts of California that coincide with the full extent of the LGM (mountainous eastern and coastal western latitudinal stretches of California, estimated 16-26,500 ybp) (Pease et al. 2009). These findings congruent with ours lend support to the role of climatic fluctuations associated with the LGM in shaping contemporary mule deer genetic structure, while considering how ecological requirements (i.e., dispersal ability) allow for adaption to changing vegetation regimes (Klütsch et al. 2012, Sacks et al. 2008).

Insular deer genetic diversity and demography

Dispersal ability greatly influences connectivity of peripheral island and core mainland populations. Mule deer are strong swimmers capable of crossing several kilometers of open water (Reimchen et al. 2003; Robinette 1966). The finding of a mainland haplotype on Tiburón Island from an approximately six year-old buck with identical mtDNA haplotype to deer from Central Sonora and Altar Valley (AZ) further supports observations made by Seri tribal members that deer are fully capable of swimming the approximately 1.7 km-wide Infiernillo Channel (N. Martinez-Tagüeña, personal communication). We saw no additional signature of maternal gene flow from adjacent mainland lineages in the Tiburón island samples. Despite Cedros deer forming a monophyletic group separated by seven mutational steps from mainland deer (lineage MD-N) and thus exhibiting a stronger genetic signature of land bridge isolation to mainland Baja peninsula than Tiburón deer to adjacent mainland Sonora, our data did not show sign of maternal gene flow of Cedros deer to mainland Baja or vice versa. As the ocean distance between Cedros Island and Punta Eugenia at the Vizcaino peninsula is approximately 24 kilometers, this distance combined with strong ocean currents likely makes deer movement between Cedros Island and the mainland very infrequent.

Small colonizing populations represent only a proportion of genetic variation found within source populations; therefore drift, inbreeding, and mortality can negatively affect population viability and fitness (Bouzat 2010; Stuessy et al. 2012). Gene flow may have

continued initially following sea level rise separating the founding populations of deer on each island and their respective mainland approximately 13-10,000 ybp but with time and a greater expanse of open water to cross (equating to a barrier effect), we would expect to see greater effects of genetic drift and inbreeding for a founder event. While larger islands closer to the mainland are predicted to have a higher potential for gene flow than more distant islands with smaller populations (Habel and Zachos 2012; MacArthur and Wilson 1967; Stuessy et al. 2012), island-specific conditions combined with effects of drift and inbreeding make it difficult to parse out a single underlying mechanism explaining genetic diversity. The more distant island (Cedros) demonstrated overall higher number of polymorphic sites, haplotype diversity, nucleotide diversity (**Table 4a**) than deer from Tiburón, the larger and more proximal (i.e., less isolated) island (**Table 4b**), a pattern likely due more to unknown historical demography than effects of insular biogeographic isolation. Yet, the eight haplotypes private to Cedros deer separated by seven mutational steps (0.4-0.9% sequence divergence) nested within the older MD-N lineage. Such relatively low level of genetic divergence has been found for other Cedros Island endemics also demonstrating notable morphological differences from their respective mainland counterparts, including woodrats (*Neotoma bryanti bryanti*; Patton et al. 2008), pocket mice (*Chaetodipus fallax anthonyi*; Rios and Álvarez-Castañeda 2010) and rattlesnakes (*Crotalus ruber exsul*; Douglas et al. 2006). It is likely that the population size of Cedros deer was never very large, and while the positive Tajima's *D* value 0.149 (p=0.594) for Cedros deer hints at a bottleneck event, the lack of significance suggests the timing of such an event combined with low sample size might not have been robust enough to detect an actual effect. However, a known recent population bottleneck of a dramatic decrease from several hundred deer in the 1980s (Pérez-Gil Salcido 1981) to population sizes as low as 15-20 individuals (de Jesus Martinez Vazquez 2012 and a governmental report referenced by Cortés-Calva et al. 2013) is documented following the temporal period of genetic variation captured by the natural history specimens.

Thus it is evident that the Cedros deer have undergone a considerable demographic decline which will likely be associated with a reduction in genetic variation in contemporary deer samples.

While recent population numbers for Tiburón deer are thought to be higher than those for Cedros deer (approximately 650 individuals in 1993: R. Lee, personal communication), we observed overall lower mtDNA genetic diversity than for Cedros deer. Tiburón deer (n=22) are separated by eight mutational steps from other deer within the MD-J lineage (0.3-0.7% sequence divergence), while the putative buck migrant matches genetically with central Sonoran deer. The 14 sequences obtained from natural history specimens of deer collected 1911-1985 from Tiburón Island share haplotypes with the eight contemporary deer sampled in 2005. Four of the five nucleotide transitions (0.2% sequence divergence) were private to natural history specimens, and were not sampled in contemporary populations. While Tiburón deer are genetically very different from adjacent Sonoran mainland deer, other species on the island show low genetic divergence from mainland Sonora, including the curve-billed thrasher (*Toxostoma curvirostre*; Rojas-Soto et al. 2007) and Sonoran desert tortoise (*Gopherus morafkai*; Murphy et al. 2011). As with our molecular polymorphism indices, nuclear microsatellite analyses for the eight (high quality) contemporary deer samples obtained from Tiburón Island (Latch et al. submitted) show significantly lower allelic richness and observed heterozygosity than the average values for individuals sampled across the species' distribution ($t= 7.747$, $df=64$, $p<0.0001$), possibly due to a small effective population size combined with effects of genetic drift relative to mainland deer. Insular isolation during the Holocene and morphological differentiation noted for this subspecies, combined with mtDNA membership in a geographically discontinuous and relict lineage, with no or low levels of gene flow between the mainland and the island (observed through both maternal mtDNA or biparentally inherited microsatellites) implies that Tiburón deer represent a unique management unit.

Conservation implications

North America during the Plio-Pleistocene was home to a much broader array of artiodactyl species than are known today (Frick 1937). Throughout what is now the desert southwest, many genera of large herbivores (e.g., *Bison*, *Mammuthus* and *Camelops* sp.) occupied extensive geographic distributions (Kurtén and Anderson 1980) and exploited a variety of biotic communities (Betancourt 2004). With dramatic turnover in vegetation communities associated with climatic oscillations during the late Quaternary, combined with prehistoric hunting, artiodactyls experienced the highest loss in species numbers of all land mammals at the end of the Pleistocene (approximately 24 known species; Ceballos et al. 2010). However, as extinctions during turnovers in dominant land herbivores shifted species richness and population numbers, it is very likely that *O. hemionus* was able to radiate into the array of habitats and vegetation communities observed today (Heffelfinger 2006). While the majority of deer and other artiodactyls in México are not considered to be of sensitive status and can be exploited with restriction (Gallina and Mandujano 2009), the two insular subspecies of mule deer are considered to be of conservation concern by SEMARNAT. Further, both island populations demonstrate nested reciprocal monophyly within more broadly distributed lineages, suggesting these subspecies should be upheld as Evolutionary Significant Units (ESUs) (Kizirian and Donnelly 2004).

Cedros (*O. h. cerrosensis*) – Our results show that Cedros deer form a monophyletic group which nests within a lineage of mule deer found within the Baja California peninsula and northern extents of the desert southwest. Though a nuclear genetic analysis is necessary to fully support taxonomic status, we believe that the endemicity of Cedros deer to the island and associated morphological distinction combined with our mitochondrial derived pattern of isolation from mainland deer defend the current subspecies classification of the *O. h. cerrosensis* subspecies. Moreover, while we caution management recommendations should not be based solely on genetic patterns observed from a single locus, it is imperative to note that our mtDNA

haplotype and molecular diversity estimates are baseline and likely 'best case' or near maximal estimates of individuals representing the island population from 34-117 years ago when population size probably exceeded the current one. Compounding this issue are intensified effects of inbreeding and genetic drift, which pose a major threat for future maintenance of genetic diversity of this island population, considering recent low population estimates. Combined with anthropogenic threats (among them, predation by feral dogs and poaching) and a dramatic population bottleneck presumed from at least the last 30 years, we stress that genetic variation loss is real for this subspecies. An urgent effort should be undertaken to examine nuclear markers from contemporary or non-invasive samples (e.g., fecal pellets, hair snares) collected during field surveys on the island to help identify the severity of potential bottleneck, genetic loss, inbreeding and to assess levels of heterozygosity for management considerations (i.e., determine genetic similarity to mainland peninsular populations should genetic rescue be needed in the future). Intensive field-based surveys to augment recent remote camera station, foot and aerial survey population size estimates (de Jesus Martinez Vazquez 2012; Cortés-Calva et al. 2013) and assessment of overall deer health, sex ratios and age class will strengthen management considerations. Efforts to monitor and eradicate the feral dog packs on the island and enforcement of restricted access to inland parts of the island are recommended consistent with the protected UMA status of the island. If such future work verifies current estimates of population numbers, a potential management strategy could include captive management and propagation of Cedros deer for later reintroduction efforts to preserve the diverged genetic composition that these deer represent.

Tiburón (*O. h. sheldoni*) – Twenty-two of the 23 deer sampled from Tiburón Island form a monophyletic clade nesting within an older, relict lineage of mule deer exhibiting geographic discontinuity in the desert southwest. Considering the founding event presumably included mainland ancestors of the MD-J lineage potentially leading to the divergence of deer on

Tiburón Island, future sampling of deer in the Sierra Madre Occidental may uncover persistence of this lineage. Cultural, paleontological and biogeographic indicators point to the endemicity of Tiburón deer to the island, and morphological differentiation supports their isolation from mainland Sonora. On a population level, nuclear microsatellite patterns point to a unique cluster consisting uniquely of Tiburón deer (Latch et al. submitted), and multi-locus inference with mtDNA (this study) show genetic variation is significantly lower in Tiburón deer than observed in deer populations on the adjacent mainland. These reasons, combined with the fact that these deer were isolated and subsequently diverged from adjacent mainland deer and the MD-J lineage, support the assertion that subspecific classification of Tiburón deer as *O. h. sheldoni* should be upheld for only deer on the island. We stress the need for available recent demographic information for these deer towards assessing population bottleneck events and level of inbreeding. While numerous studies have focused on founder effect and carrying capacity of bighorn sheep that were introduced on the island in 1975 (Gasca-Pineda et al. 2013; Hedrick et al. 2001; Hedrick 2013), such population level-studies, including habitat and resource use by both ungulates would benefit management considerations for deer and sheep on the island. As with Cedros deer, estimating population sizes and sex ratios are necessary for Tiburón deer and evaluating level of interaction and ecosystem balance with bighorn sheep on Tiburón island should be understood prior to translocations of sheep to the island (Gasca-Pineda et al. 2013). Further, details of Tiburón deer evolutionary history could be revealed with study of the island's archaeological remains (White 2000) at Tecomate's shell midden, allowing for examination of historical genetic diversity towards inferring ancestral population genetic patterns and numbers.

Conclusions

In this study, we examined patterns of mitochondrial genetic variation, desert deer ecology and geographic distribution of a vagile North American mammal in the desert southwest. Through inference of mule deer lineages across our study area we were able to reconstruct

phylogenetic relationships and relative dates of divergence and demographic expansions that coincide with climatic fluctuations associated with the LGM. We highlight the use of museum specimens to complete the phylogeographic analysis for the mule deer species complex and provide baseline genetic diversity information towards identifying priorities for conservation and management of insular endemic subspecies of Cedros and Tiburón mule deer. We strongly recommend that future studies of mule deer in the Sonoran desert region maximize the formation of international research teams from interdisciplinary fields towards open communication and collaborative efforts in building a comprehensive management strategy for this culturally and economically important mammal.

Figure 1. Map showing distribution of a) 11 subspecies of mule deer (modified from Anderson and Wallmo 1984; Heffelfinger 2000), b) inset for desert southwest study area zoomed in to seven subspecies and n=23 sampling localities (shaded circles) for desert mule deer, as well as locations of vicariance hypotheses and boundaries of 4 biogeographic regions examined in the AMOVA analysis and c) the Salton Trough (Imperial Formation) vicariance event separating biogeographic regions 1 and 3. Desert outlines modified from Shreve (1942).

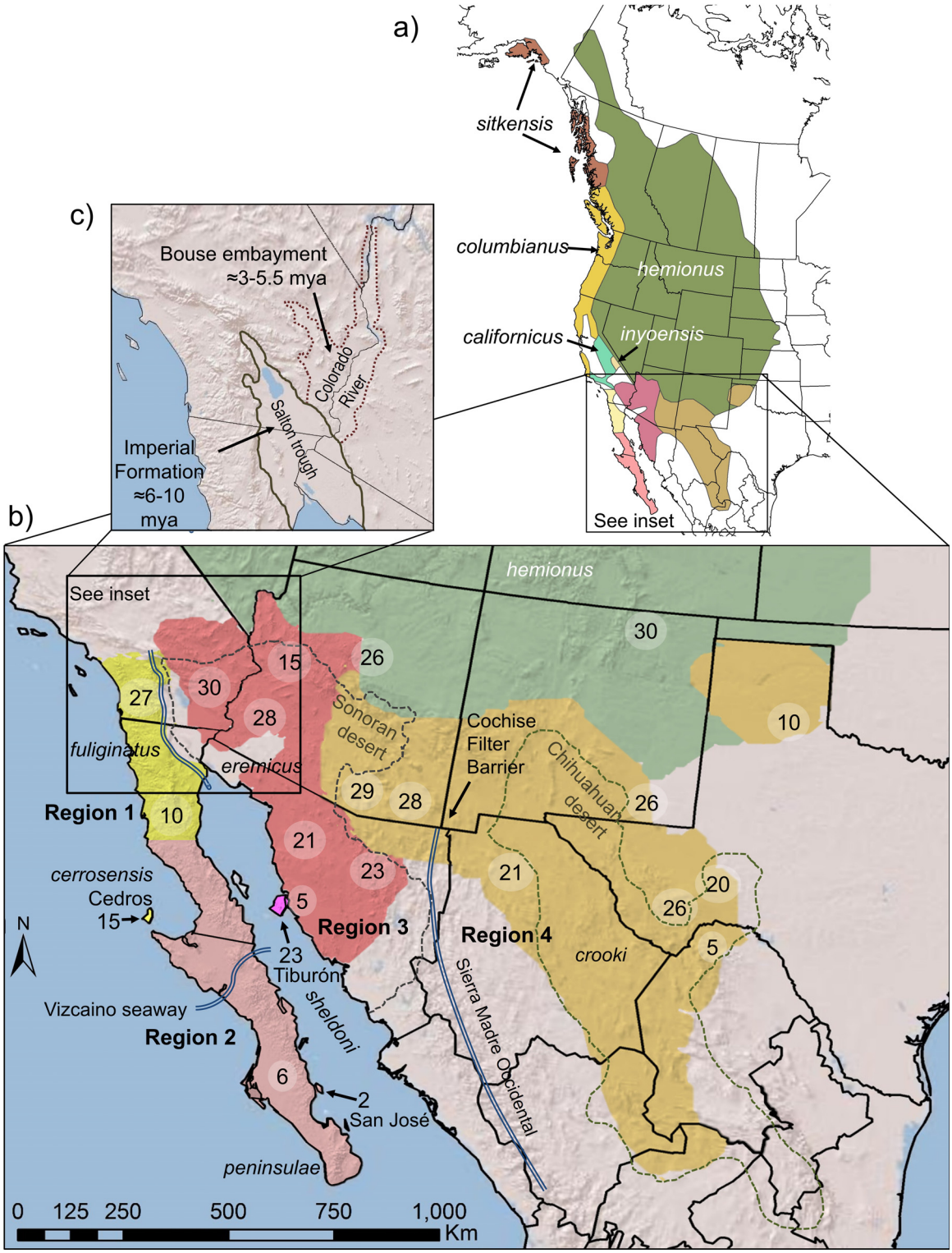

Figure 2. Heat-map distance matrix of genetic (pairwise) distances for n=23 sampling locations of mule deer. Orange diagonal represents pairwise difference observed within sampling locations; diagonals in green represent pairwise differences between locations; and diagonals in blue signify Nei's D_A distance (corrected average pairwise difference). BA_CE represents n=15 deer sampled from Cedros Island: BA_SJ represents n=2 deer sampled from San José Island and SO_TI represents n=22 deer sampled from Tiburón Island.

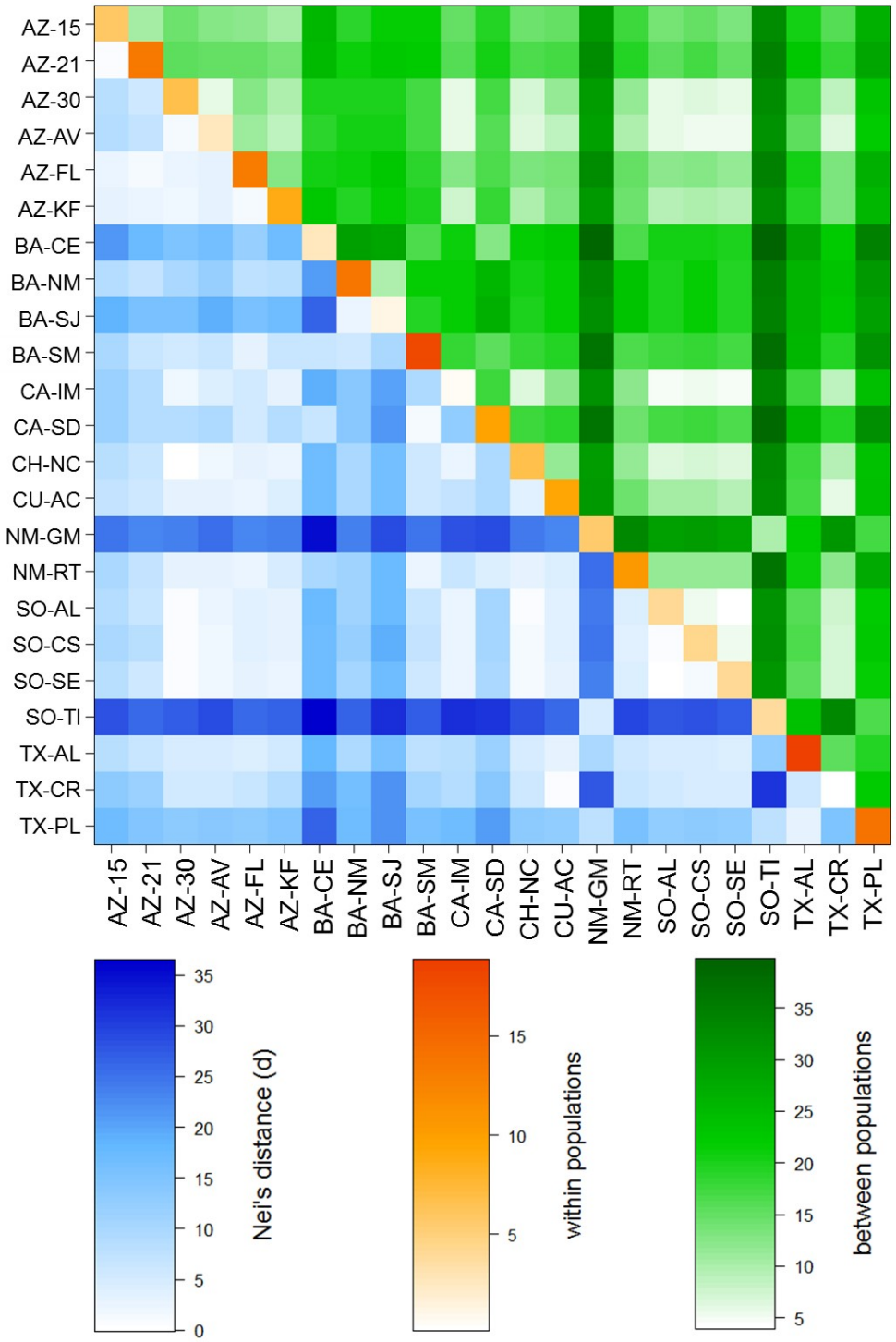

Figure 3. Median-joining network created in NETWORK v4.6.11 and supported in topology by TCS v1.21 for 1,611 bp of control region and cytochrome b gene sequences. Vector length approximately proportional to number of mutational step differences. Smaller red circles (median nodes) connect vectors representing inferred unsampled or ancestral mutational steps. Inferred haplogroups were characterized based on at least 10 mutational steps; color coding of haplogroups is followed throughout the results and discussion. Haplotype position of island deer depicted by dashed circles (pink= Tiburón Island deer; purple = Cedros Island deer), with the exception of one probable migrant carrying an MD-BF haplotype. Tiburón deer are separated by eight mutational steps from the remainder of MD-J; Cedros deer are separated by seven mutational steps from the remainder of MD-N.

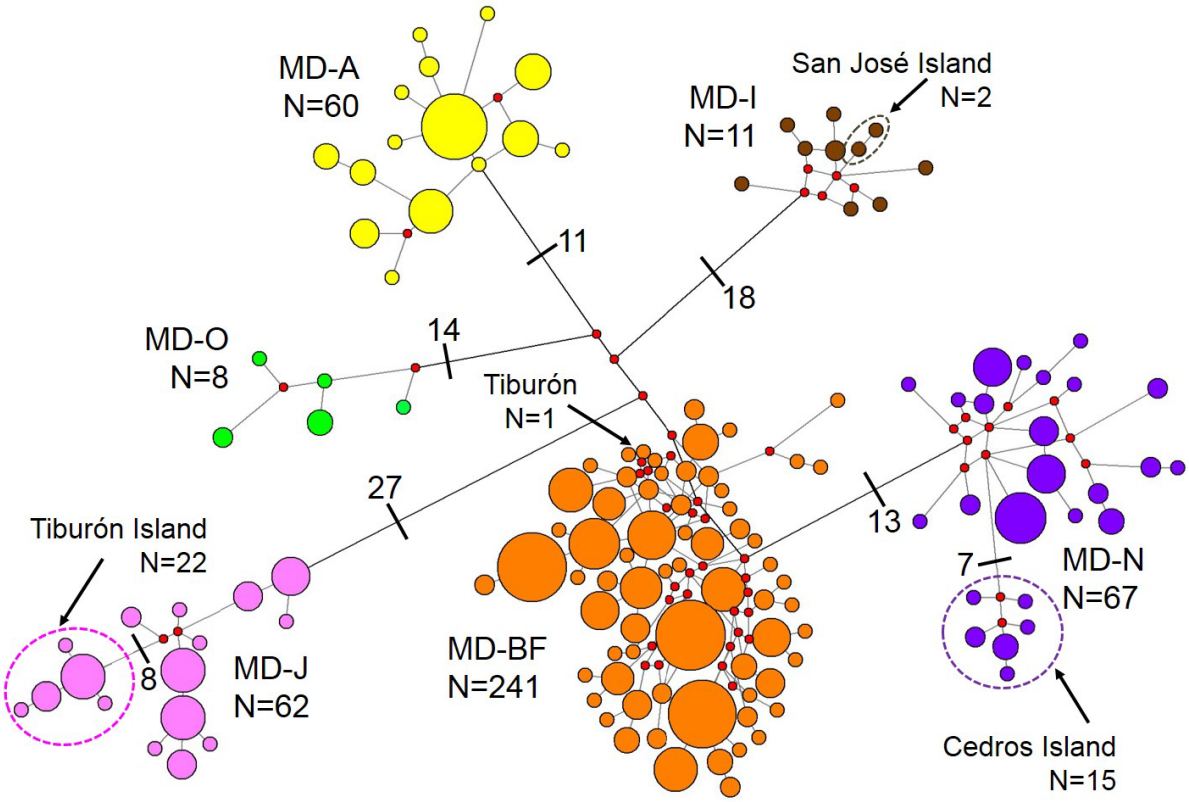

Figure 4. Haplogroup (n=6) membership overlaid across sampling locations of for n=449 mule deer in the desert southwest study area. Size of circle corresponds to frequency of samples collected at a particular location, with the largest sample size of n=27, while pies show proportion of haplogroup membership at a particular sampling location. Colors correspond to inferred haplogroups: purple=MD-N; orange=MD-BF; yellow=MD-A; pink=MD-J; green=MD-O, brown=MD-I. Four major biogeographic regions examined in this study are also shown.

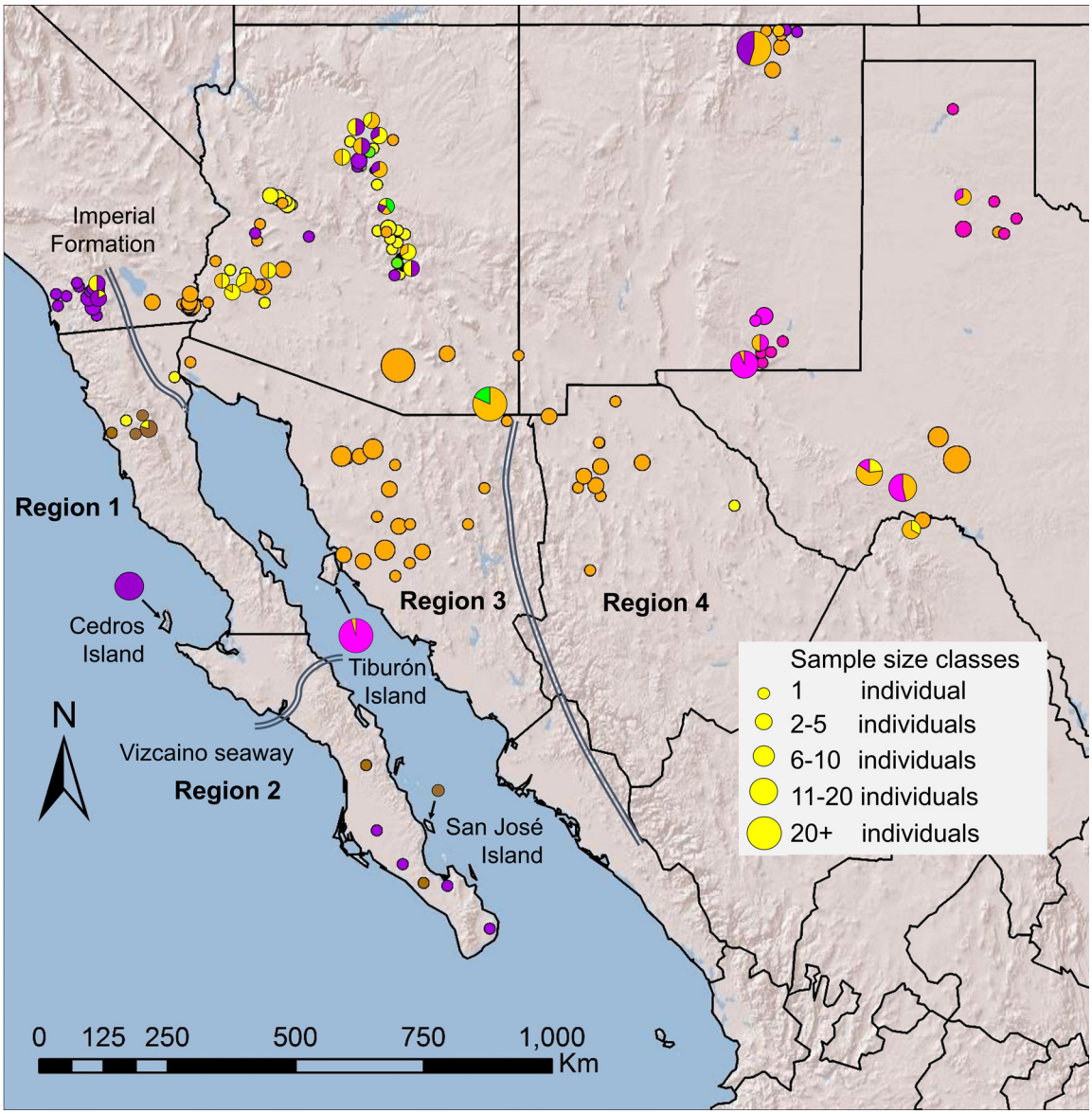

Figure 5. Bayesian maximum clade credibility tree (phylogram) of 70 representative haplotypes for n=449 desert southwest deer. Support for major lineages shown by posterior probabilities and for other nodes by heat bar. *Rangifer tarandus*, *Odocoileus hemionus sitkensis* and *O. virginianus* are outgroups. Phylogenetic position of island deer depicted by dashed lines (purple= Cedros, BA_CE; pink=Tiburón, SO_TI).

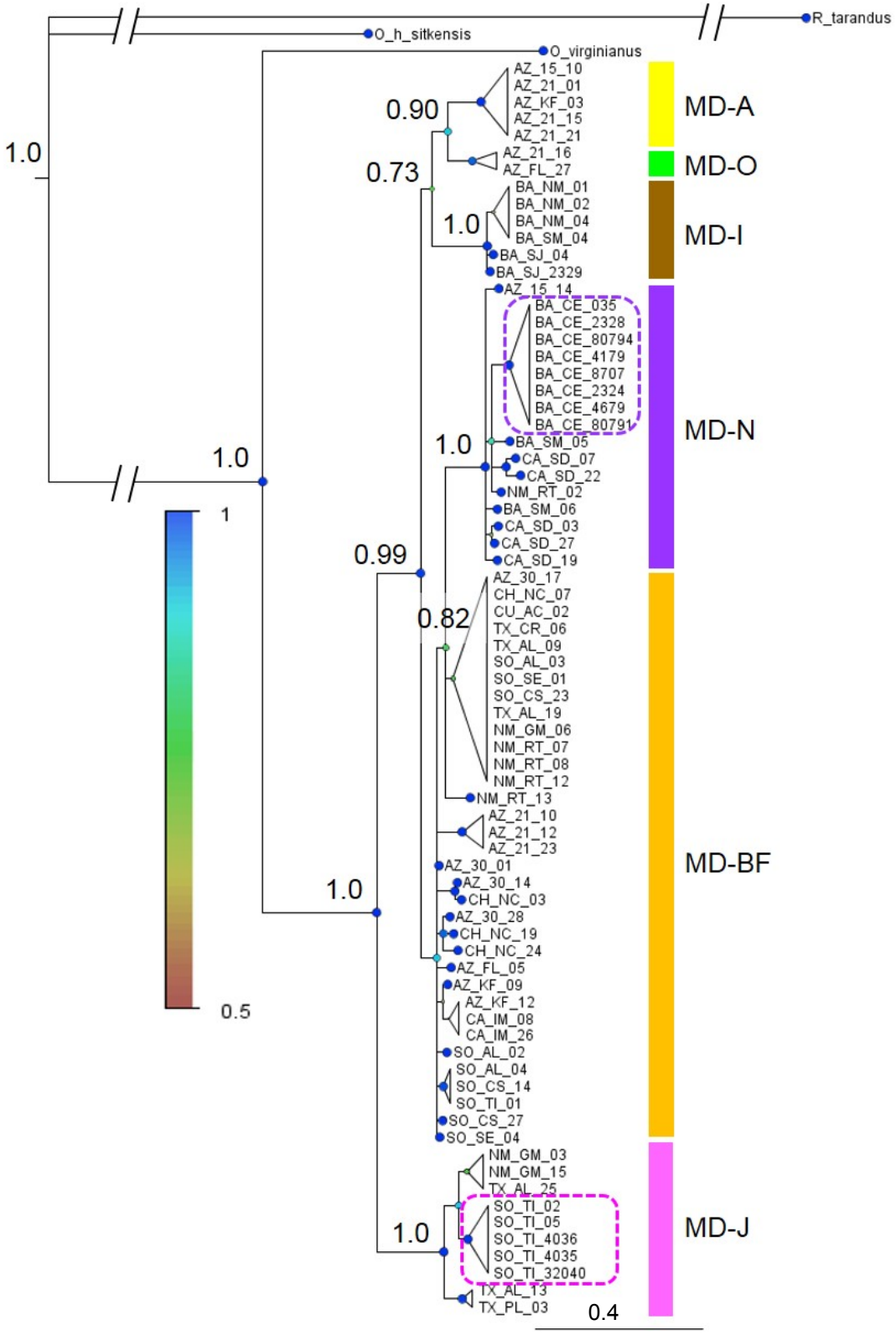

Figure 6. Mismatch distributions for each of n=6 inferred haplogroups. The y-axis shows the observed and expected number of comparison pairs, while the x-axis shows the number of pairwise differences across 10,000 simulated replicates. Solid black line denotes observed pairs and fine dotted black line shows expected pairs. Coarser grey dotted lines indicate upper and lower 95% confidence intervals (CIs), and lightest lines indicate 90 and 99% CIs.

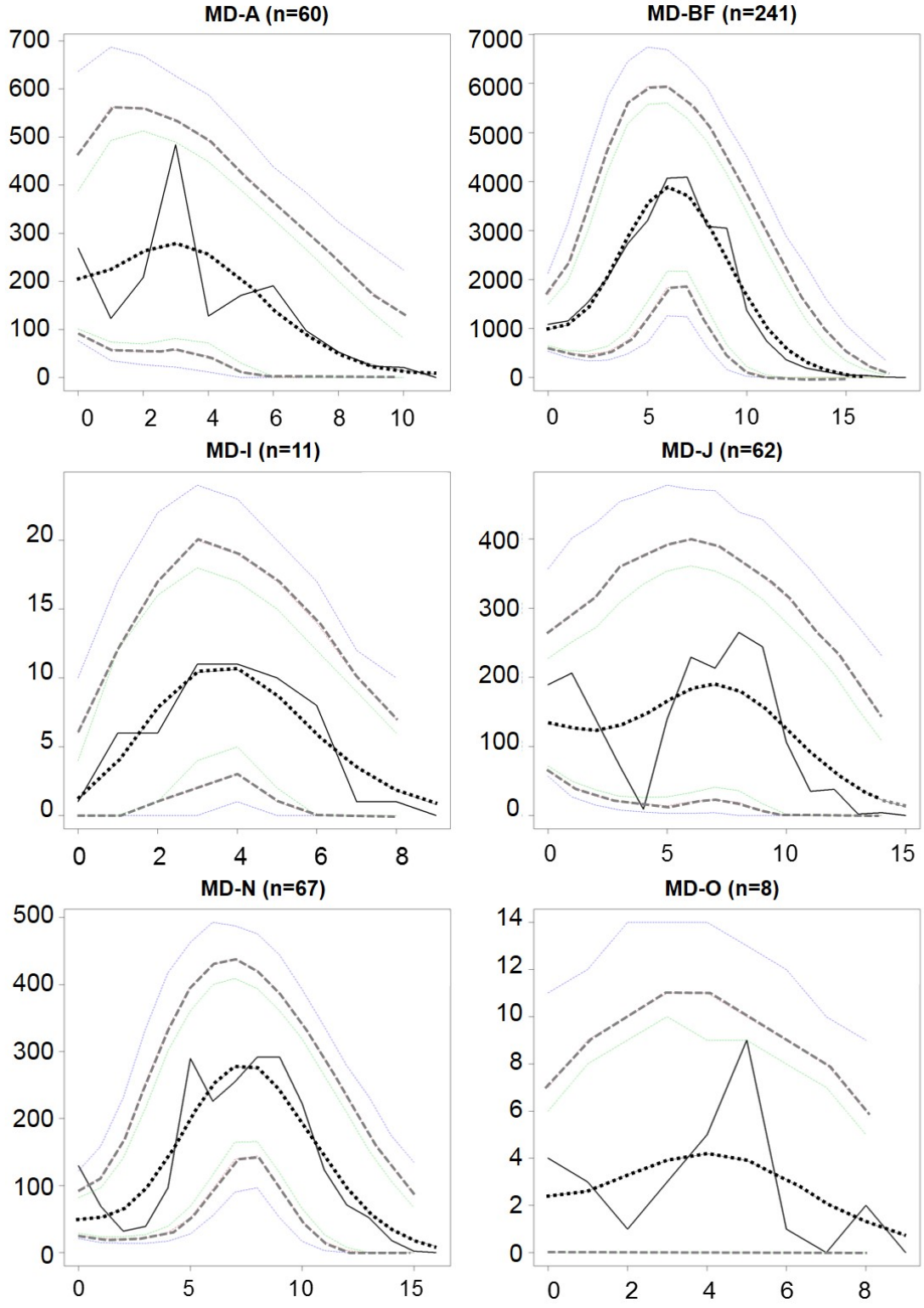

Figure S1. Rarefaction curves of haplotype richness (H_R) plotted with increasing numbers of individuals sampled for two island subspecies. Diamonds represent mean Chao1 estimator of haplotype richness (Chao 1987), and solid lines above and below the mean represent the upper and lower 95% confidence intervals for the Chao1 estimator (respectively). Dotted lines represent the observed haplotype richness controlled for sample size.

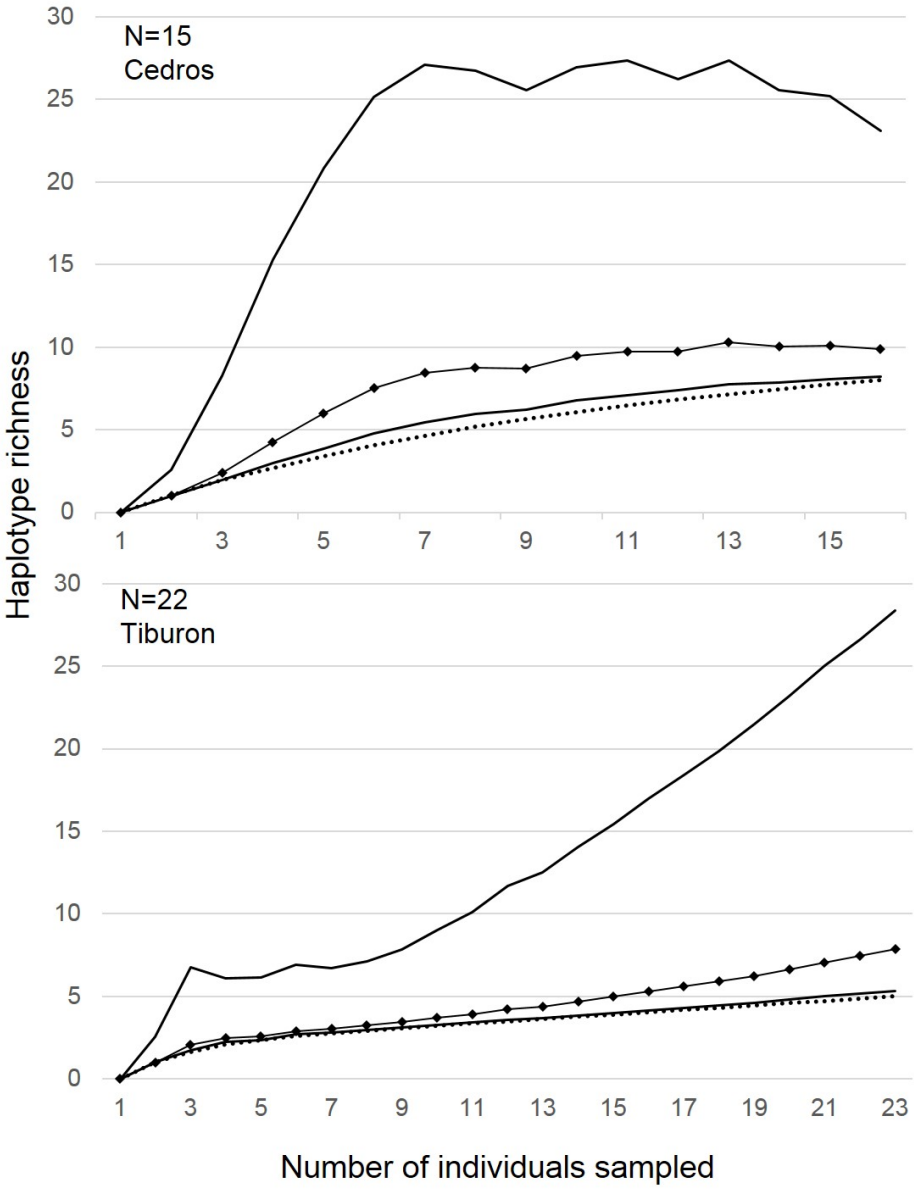

Figure S2. Rarefaction curves of haplotype richness (H_R) plotted with increasing numbers of individuals sampled for n=6 inferred haplogroups. Diamonds represent mean Chao1 estimator of haplotype richness (Chao 1987), and solid lines above and below the mean represent the upper and lower 95% confidence intervals for the Chao1 estimator (respectively). Dotted lines represent the observed haplotype richness controlled for sample size.

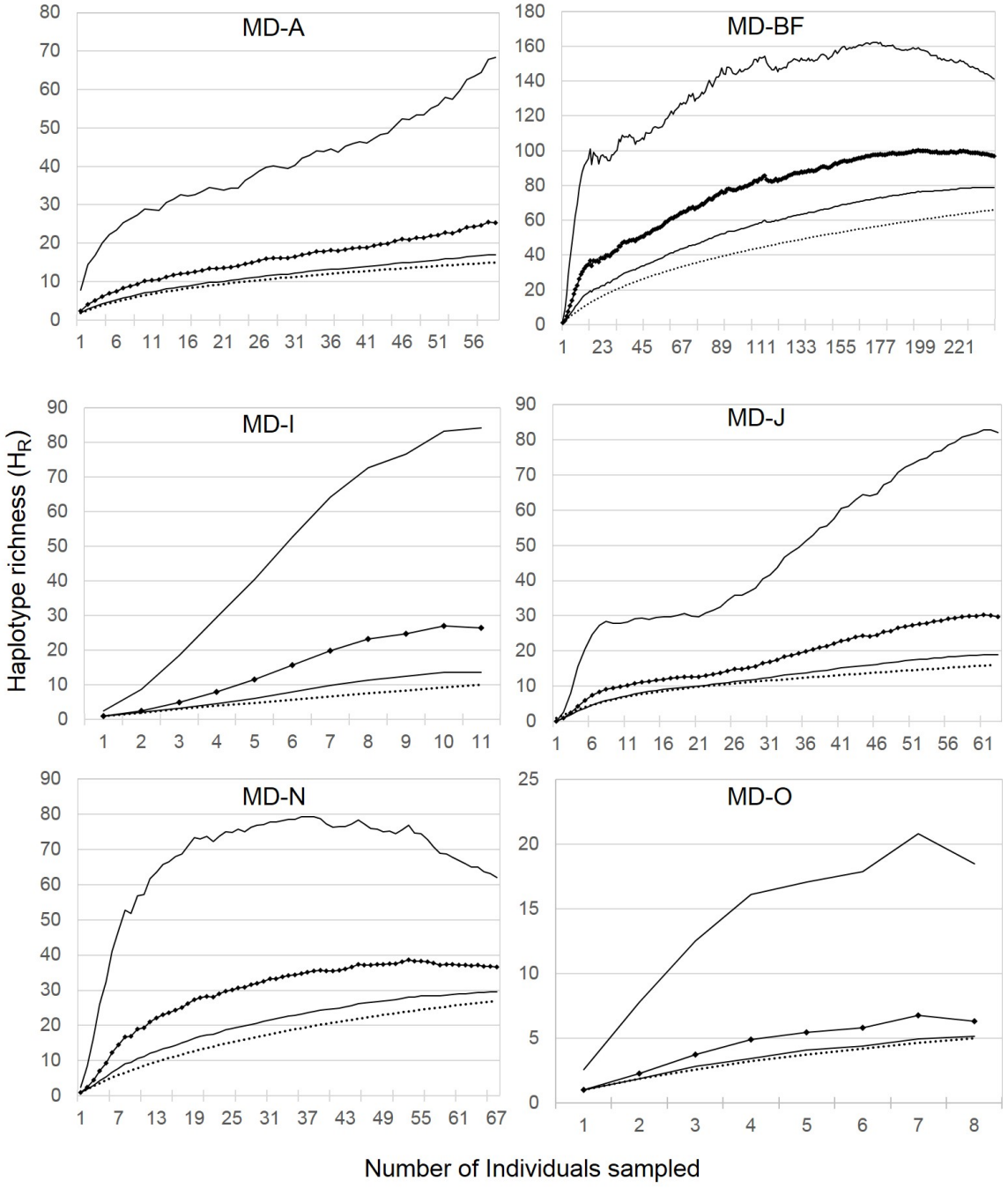

Table 1. Internal primers developed for this study for PCR amplification and sequencing of overlapping mtDNA control region and cytochrome b fragments of insular natural history samples. All annealing temperatures were optimized at 56ºC.

Primer name and pair	Primer sequence (5'→3')	GC content (%)	Target fragment length (bp)
Control region (583 bp total)			
Odh_dloopF [1]	GAGCAACCAATCTCCCTGAG	55	227
Odh_dL_int1R	CGTTATTGTACAGTTTGTGTAGGTAATG	36	
Odh_dL_int2aF	GCTCCATAAAATCCAAGAG	42	235
Odh_dL_int2aR	ACTTGCTTATAAGTATGGGG	40	
Odh_dL_int2F	CAGTTTTGCACTCAATAGCCATA	39	321
Odh_dL_2bR	AGGGTAGATTTGACTGAATGTG	41	
Odh_dL_int2b.1F	TTTATGCGGGTATAGTACATAA	32	239
Odh_dL_2bR	--	--	
Odh_dL_int2bF	CCTACACAAACTGTACAATAACG	39	306
Odh_dL_int2R	ATCTAAGGGACGGGATACGC	55	
Odh_dL_int2cF	AGCAAGTCCATATAACCACTTTA	35	211[2]
Odh_dL_int2cR	TTCATTAAATAGCTACCCCCAC	41	
Odh_dL_int3F	CGTCCATAGCACATTAAGTCAAA	39	275
Odh_dLoopR [1]	GTGTGAGCATGGGCTGATTA	50	
Odh_dL_int3F	--	--	288
Odh_dL_int3aR	ACACCACAGTTATGTGTGAGCA	45	
Cytochrome b region (1,028 bp total)			
Odh_cytb_14153F [1]	TCAATGACCAACATCCGAAA	40	221
Odh_cytb_1R	ACGTCTCGGCAGATGTGAGT	55	
Odh_cytb_int2F	CATCCGACACAATAACAGCA	45	265
Odh_cytb_int2R	GTTGCCCCTCAGAATGACAT	50	
Odh_cytb_int3F	TCTCCTATTCACAGTTATAGCCACA	40	228
Odh_cytb_int3R	ATAGCAAGTGCTGCGATGAT	45	
Odh_cytb_int4F	ACCTTAACCCGATTCTTCGC	50	244
Odh_cytb_int4R	TGTCTGGGTCTCCGAGTAGG	60	
Odh_ctyb_int5F	CAGACCTACTCGGAGACCCA	60	282
Odh_cytb_int5R	GGCCTCCAATTCATGTGAGT	50	
Odh_cytb_int6F	CATTCAGCCAATGCCTATTC	45	290
Odh_cytbR_15399 [1]	TGGGTGTTGATAGTGGGGTA	50	

[1] denotes primers used and referenced from Latch et al. (2008)

[2] denotes primer pair only amplifiable for Tiburón deer

--denotes duplicate primer used in pair to amplify an additional fragment

Table 2. Molecular diversity and sequence polymorphism indices for control region and cytochrome b for 449 deer calculated by DnaSP v5.10.1 (Librado and Rozas 2009). *H* = observed number of haplotypes, *S* = Segregating (polymorphic) sites, *Hd* = haplotype diversity and π = nucleotide diversity.

Diversity Index	**Control region (583 bp)**	**Cytochrome b (1,028 bp)**	**Overall (1,611 bp)**
H	121	50	140
S (parsimony informative)	102 (76)	60 (43)	165 (119)
Hd (±SD)	0.980 (0.002)	0.816 (0.017)	0.9833 (0.002)
π (±SD)	0.02282 (0.00068)	0.00383 (0.00015)	0.01066 (0.00033)

Table 3. Molecular diversity and sequence polymorphism indices for seven *O. hemionus* subspecies for 1,611 bp of mtDNA. Values for Tiburón deer (n=23) shown with[1] and without[2] single probable migrant whose haplotype matches with deer from Altar Valley, AZ and Central Sonora. N=number individuals sampled, *H*=observed number of haplotypes, H_R=rarefacted haplotype richness, *Hd* =haplotype diversity, π=nucleotide diversity, PD=mean number of pairwise differences and *S*= number of segregating (polymorphic) sites.

***O. hemionus* subspecies**	***N***	***H***	***H_R* (±SD)**	***Hd* (±SD)**	**π (±SD)**	**PD (±SD)**	***S***
cerrosensis	15	8	10.0 (3.32)	0.943 (0.0403)	0.00161 (0.00102)	2.590 (1.469)	8
fuliginatus	37	20	30.2 (12.09)	0.947 (0.0213)	0.0102 (0.00518)	16.480 (7.509)	59
peninsulae	8	7	13.7 (7.48)	0.964 (0.0772)	0.0112 (0.00632)	17.964 (8.942)	39
[1]*sheldoni*	23	6	11.7 (6.75)	0.672 (0.0701)	0.00235 (0.00137)	3.779 (1.976)	36
[2]*sheldoni*	22	5	7.9 (4.20)	0.641 (0.0702)	0.000540 (0.000441)	0.870 (0.636)	5
eremicus	122	36	54.1 (16.55)	0.937 (0.0109)	0.00487 (0.00253)	7.841 (3.674)	48
crooki	188	51	76.4 (14.72)	0.955 (0.00610)	0.0103 (0.00510)	16.604 (7.425)	101
hemionus	56	21	85.8 (39.21)	0.919 (0.0204)	0.00819 (0.00415)	13.198 (6.028)	62
Totals	449	140	--	0.9833 (0.002)	0.01066 (0.00033)	9.913 (4.707)	165

Table 4. Molecular indices for a subset of deer comparing island populations to respective mainland populations for Cedros Island (**a**) and Tiburón Island (**b**). Values for Tiburón (n=22) shown with[1] and without[2] single probable migrant whose haplotype matches with deer from Altar Valley, AZ and Central Sonora. *H*=observed number of haplotypes, H_R=rarefacted haplotype richness (200 randomized iterations) to account for differing sample sizes based on the Chao1 estimator of haplotype richness, *S*=number of segregating (polymorphic) sites, *Hd* =haplotype diversity, π=nucleotide diversity and PD=mean number of pairwise differences.

(**a**)

Index	**Cedros (n=15)**	**Mainland Baja (n=45)**
H	8	27
H_R (±SD)	10.0 (3.32)	46.4 (14.18)
Hd (±SD)	0.943 (0.0403)	0.964 (0.0152)
S	8	70
π (±SD)	0.00161 (0.00102)	0.0106 (0.00533)
PD (±SD)	2.590 (1.469)	17.063 (7.7314)
Pairwise F_{ST}	0.348 (p=0.000)	

(**b**)

Index	**Tiburón (n=23)** [1]	**Tiburón (n=22)** [2]	**Mainland Sonora (n=155)**
H	6	5	45
H_R (±SD)	11.7 (6.75)	7.9 (4.20)	59.4 (6.75)
Hd (±SD)	0.672 (0.0701)	0.641 (0.0702)	0.954 (0.0074)
S	36	5	63
π (±SD)	0.00235 (0.00137)	0.000540 (0.000441)	0.00435 (0.00227)
PD (±SD)	3.779 (1.976)	0.870 (0.636)	7.007 (3.310)
Pairwise F_{ST}	0.797 [2] – 0.820 [1] (p=0.000)		

Table 5. Results of AMOVA for partitioning of genetic variation for 4 hypothesized scenarios for mtDNA control region and cytochrome b genetic variation (1,611 bp). Model 4 biogeographic regions are depicted in **Figure 1**.

Model	Hypothesized grouping	N groups	% variation among groups	Φ_{CT}	Φ_{SC}	Φ_{ST}
1	Sampling location (see **Table S2**)	22[1]	7.69%	0.0769	0.552	0.586
2	Inferred haplogroups MD-A, B, I, J, N, O	6	69.93%	0.699	0.428	0.828
3	*O. hemionus* subspecies *cerrosensis, sheldoni, hemionus, eremicus, crooki, fuliginatus, peninsulae*	7	20.82%	0.208	0.503	0.606
4	Vicariance – biogeographic **regions** **1.** N. Vizcaino + W Imperial Formation **2.** S. Vizcaino (peninsula) **3.** E Imperial Formation + Sonoran desert **4.** Sierra Madre Occidental + Chihuahuan Desert	4	10.36%	0.104	0.558	0.603

[1]due to low sample size, San José Island (n=2) samples were grouped together with BA-SM samples for n=8 samples.

Table 6. Demographic expansion indices for each haplogroup. Fu's *Fs* and Tajima's *D* values calculated using gamma shape parameter α=0.56 for 583 bp of control region only. Estimates of tau (τ) and 95% confidence intervals calculated for 1,611 bp of mtDNA for each haplogroup. All exhibited unimodal mismatch distributions and shown is the sum of squared deviations (SSD) and P (sim SSD>=Obs SSD). Estimated time since expansion (*t*) was calculated from t= τ /2μ and rounded to the nearest 10^2. Estimated divergence time (DT) for each lineage calculated in BEAST v1.7.5 shown with 95% high posterior density intervals (analogous to 95% CIs).

Demographic signature (583 bp control region)			**Sudden expansion model** (1,611 bp total)			
Lineage, (N), color	Fu's *Fs* (p-value)	Tajima's *D* (p-value)	SSD (p-value)	τ (95% CI)	*t* (ybp) (95% CI)	DT (ybp) (95% HPD)
A (60), yellow	-0.898 (0.384)	-0.428 (0.375)	0.0256 (0.141)	3.609 (0.748-8.287)	7,000 (1,400-16,000)	35,000 (170,000-8,000)
BF (241), orange	-24.933 (0.000)	-1.164 (0.0965)	0.00118 (0.748)	6.961 (3.739-9.406)	13,400 (7,200-18,100)	62,000 (90,000-present)
I (11), brown	-7.031 (0.000)	-1.298 (0.109)	0.00679 (0.703)	4.082 (1.604-6.055)	7,900 (3,100-11,700)	36,000 (220,000-7,000)
J (62), pink	-1.561 (0.313)	-0.0252 (0.550)	0.0155 (0.278)	8.27 (2.754-13.023)	15,900 (5,300-25,100)	67,000 (360,000-18,000)
N (67), purple	-7.020 (0.0243)	-0.693 (0.272)	0.00556 (0.201)	8.098 (4.588-10.477)	15,600 (8,800-20,200)	52,000 (105,000-4,000)
O (8), green	0.491 (0.582)	1.008 (0.850)	0.0575 (0.279)	4.98 (1.125-8.682)	9,600 (2,200-16,700)	44,000 (160,000-5,000)
Islands						
BA_CE (15)	-0.926 (0.249)	0.149 (0.594)				
SO_TI (22)	-1.642 (0.0701)	-0.814 (0.240)				

Table S1. Natural history specimens of insular *O. h. cerrosensis* and *O. h. sheldoni* used in this study (deposited in GenBank). Locality information for *O. h. sheldoni*: MÉXICO: Sonora, Tiburón Island. Locality information for Cedros is MÉXICO: Baja California Norte, Cedros Island. Sample types extracted include: B=long bone or other marrow, B(T)= turbinate bone, B(S) = bone shavings, T=tissue, D=dentin, A=antler core. MVZ=Museum of Vertebrate Zoology, University of California, Berkeley; AMNH=American Museum of Natural History; NMNH=Smithsonian Institution National Museum of Natural History; LACM=Los Angeles County Museum; SDNHM=San Diego Natural History Museum; HCZ=Harvard Museum of Comparative Zoology; CAS=California Academy of Sciences; CNMA=Colección Nacional de Mamíferos, Universidad Nacional Autónoma de México.

Catalog No.	Museum	Island locality (subspecies)	Date collected	Sample type	Control region (dL) haplotype	Cyt b (cb) haplotype
M-31988	AMNH	Tiburón (*sheldoni*)	4/13/1911	B(T)	SO_TIdL-2	SO_TIcb-2
M-32014	AMNH	Tiburón (*sheldoni*)	4/17/1911	B	SO_TIdL-2	SO_TIcb-2
M-32036	AMNH	Cedros (*cerrosensis*)	3/2/1911	T	BA_CEdL-4	SO_TIcb-2
M-32040	AMNH	Tiburón (*sheldoni*)	4/12/1911	A	SO_TIdL-4	SO_TIcb-1
M-32044	AMNH	Tiburón (*sheldoni*)	4/12/1911	A	SO_TIdL-2	SO_TIcb-2
4179	CAS	Cedros (*cerrosensis*)	8/13/1922	B(T)	BA_CEdL-4	BA_CEcb-1
4679	CAS	Cedros (*cerrosensis*)	none	B	BA_CEdL-5	BA_CEcb-1
4680	CAS	Cedros (*cerrosensis*)	none	B (S)	BA_CEdL-4	BA_CEcb-1
035	LACM	Cedros (*cerrosensis*)	3/26/1905	B	BA_CEdl-1	BA_CEcb-1
8707	MCZ	Cedros (*cerrosensis*)	4/18/1906	B	BA_CEdL-4	BA_CEcb-2
97796	MVZ	Tiburón (*sheldoni*)	11/9/1941	B(T)	SO_TIdL-2	SO_TIcb_2
97797	MVZ	Tiburón (*sheldoni*)	11/11/1941	B(T)	SO_TIdl-1	SO_TIcb-2
97799	MVZ	Tiburón (*sheldoni*)	1/11/1942	T	SO_TIdl-1	SO_TIcb-2
97800	MVZ	Tiburón (*sheldoni*)	1/9/1942	T	SO_TIdl-1	SO_TIcb-2
80784	NMNH	Cedros (*cerrosensis*)	8/14/1896	T	BA_CEdl-1	BA_CEcb-1
80791	NMNH	Cedros (*cerrosensis*)	1896	T	BA_CEdL-6	BA_CEcb-1
80794	NMNH	Cedros (*cerrosensis*)	1896	T	BA_CEdL-7	BA_CEcb-1
82801	NMNH	Cedros (*cerrosensis*)	1896	A	BA_CEdL-2	BA_CEcb-1
514035	NMNH	Tiburón (*sheldoni*)	1976	B(T)	SO_TIdL-5	SO_TIcb-2
514036	NMNH	Tiburón (*sheldoni*)	1976	B(S)	SO_TIdL-3	SO_TIcb-2
530150	NMNH	Cedros (*cerrosensis*)	1979	A	BA_CEdL-5	BA_CEcb-1
530151	NMNH	Cedros (*cerrosensis*)	1979	B(S)	BA_CEdL-1	BA_CEcb-1
1206	SDNHM	Cedros (*cerrosensis*)	8/7/1922	B	BA_CEdL-2	BA_CEcb-1
13937	SDNHM	Cedros (*cerrosensis*)	7/16/1939	B	BA_CEdL-1	BA_CEcb-1
13938	SDNHM	Cedros (*cerrosensis*)	7/16/1939	B	BA_CEdL-3	BA_CEcb-1
19159	SDNHM	Tiburón (*sheldoni*)	3/19/1952	B	SO_TIdL-1	SO_TIcb-2
19160	SDNHM	MX: Baja California Sur, San José Island (*peninsulae*)	4/12/1962	B(T)	BA_SJdL-1	BA_SJcb-1
4172	CNMA	Tiburón (*sheldoni*)	11/19/1985	A	SO_TIdL-2	SO_TIcb-2
26391	CNMA	Tiburón (*sheldoni*)	5/30/1978	B(T)	SO_TIdL-1	SO_TIcb-2
32546	CNMA	Tiburón (*sheldoni*)	11/13/1978	T	SO_TIdL-2	SO_TIcb-2

Table S2. Sample location (abbreviation), size, and diversity indices for a subset of deer at n=23 locations (1611 bp concatenated mtDNA dataset) in the U.S. and México (MX). *H*=observed number of haplotypes, H_R=rarefacted haplotype richness (200 randomized iterations) to account for differing sample sizes based for the Chao1 estimator of haplotype richness, *S* = segregating (polymorphic) sites, *Hd* =haplotype diversity, π= nucleotide diversity and PD= mean pairwise differences. GMU signifies Game Management Unit. [1]due to low sample size, San José Island (n=2) samples grouped together with BA-SM samples for n=8 samples.

Abbrev.	Sampling locality	*N*	Lat; Long (WGS 84 datum)	*H*	H_R (±SD)	*S*	*Hd* (±SD)	PD (±SD)	Π (±SD)
AZ-15	Arizona: GMUs 15B/16A/18AB	15	34.456; -113.190	6	11.6 (6.59)	28	0.7619 (0.813)	5.7333 (2.909)	0.003559 (0.002024)
AZ-21	Arizona: GMUs 21/22/23	23	33.938; -111.301	11	15.8 (5.32)	50	0.8972 (0.0404)	13.3360 (6.2252)	0.008278 (0.004309)
AZ-30	Arizona: GMU 30A	28	31.471; -109.590	8	9.2 (2.65)	24	0.8280 (0.0448)	6.6984 (3.2567)	0.004158 (0.002251)
AZ-AV	Arizona: Altar Valley	29	32.075; -111.164	3	3.0 (0.48)	44	0.8769 (0.0451)	13.2677 (6.1674)	0.008236 (0.004265)
AZ-FL	Arizona: Flagstaff area	26	35.212; -111.733	11	25.4 (13.06)	7	0.3941 (0.0937)	2.5764 (1.4222)	0.001599 (0.000983)
AZ-KF	Arizona: Kofa area	28	33.431; -113.756	8	8.6 (1.64)	29	0.8439 (0.0422)	8.7037 (4.1418)	0.005403 (0.002862)
BA-CE	MX: Baja California Norte, Cedros Island	15	28.144; -115.230	8	10.2 (3.51)	8	0.9429 (0.0403)	2.5908 (1.4689)	0.001608 (0.001022)
BA-NM	MX: Baja California Norte, mainland Norte	10	31.151; -115.547	7	11.7 (5.69)	31	0.9111 (0.0773)	13.8000 (6.7779)	0.008566 (0.04758)
BA-SM/ SJ	MX:Baja California Sur, Mainland Sur/ San José Island[1]	6 2	24.654; -110.834 24.971; -110.629	7	13.6 (7.22)	9	0.9643 (0.0772)	17.9643 (8.9415)	0.01115 (0.006322)
CA-IM	California: Imperial County	30	32.941; -114.859	4	4.0 (0.24)	3	0.5402 (0.0798)	0.6299 (0.5075)	0.000391 (0.000351)
CA-SD	California: San Diego County	27	33.101; -116.580	13	17.5 (6.23)	43	0.9117 (0.0365)	9.7778 (4.6201)	0.006069 (0.003194)
CH-NC	MX: Chihuahua: Northcentral	21	30.561; -107.611	13	13.8 (4.16)	32	0.9524 (0.0256)	6.7047 (3.2938)	0.004162 (0.002282)
CU-AC	MX: Coahuila: Acuna area	5	29.694; -102.040	4	5.5 (2.74)	22	0.9000 (0.01610)	9.2000 (5.1063)	0.005711 (0.003705)

Table S2 (continued)

Abbrev.	Sampling locality	*N*	Lat; Long (WGS 84 datum)	*H*	H_R (±SD)	*S*	*Hd* (±SD)	PD (±SD)	II (±SD)
NM-GM	New México: Guadalupe Mountains	26	32.380; -104.784	7	12.8 (6.81)	38	0.7600 (0.0522)	5.5261 (2.7448)	0.003432 (0.001899)
NM-RT	New México: Raton area	30	36.851; -104.447	10	24.5 (13.11)	35	0.8046 (0.0578)	10.6046 (4.9693)	0.006583 (0.003433)
SO-AL	MX: Sonora, Altar area	21	30.737; -111.885	10	12.9 (5.41)	15	0.9143 (0.0364)	4.0952 (2.1252)	0.002542 (0.001473)
SO-CS	MX: Sonora, Central Sonora	23	29.497; -111.023	10	13.7 (5.0)	15	0.8893 (0.0370)	4.4585 (2.2802)	0.002768 (0.001578)
SO-SE	MX: Sonora, Seri tribal land	5	29.176; -111.980	4	5.3 (2.64)	10	0.9000 (0.0161)	4.000 (2.3992)	0.002483 (0.001741)
SO-TI	MX: Sonora, Tiburón Island	23	29.000; -112.417	6	11.7 (6.75)	36	0.6719 (0.0701)	3.7787 (1.9756)	0.002347 (0.001368)
TX-AL	Texas: Alpine, Stockton, Sanderson Counties	26	30.326; -102.685	13	18.4 (5.98)	52	0.9169 (0.0331)	18.4061 (8.4343)	0.01143 (0.005836)
TX-CR	Texas: Crockett County	20	30.819; -101.512	2	2.0 (0.48)	1	0.1000 (0.0880)	0.1000 (0.1775)	0.000062 (0.000123)
TX-PL	Texas: Plains area (Briscoe, Cottle, Floyd, Hall, Hutchinson, Motley Counties)	10	34.441; -101.001	3	3.0 (0.5)	30	0.6000 (0.1305)	13.4667 (6.6220)	0.008364 (0.004652)

Table S3. Molecular diversity and sequence polymorphism indices for a subset of deer by inferred haplogroup**.** Values rounded to the nearest 0.001. *H*=observed number of haplotypes, H_R= rarefacted haplotype richness (200 randomized iterations) to account for differing sample sizes based on the Chao1 estimator of haplotype richness, *Hd* =haplotype diversity, π =nucleotide diversity, PD=mean pairwise differences and *S*=segregating (polymorphic) sites.

Haplogroup	***N***	***H***	H_R **(±SD)**	***Hd*** **(±SD)**	**π (±SD)**	**PD (±SD)**	**S**
A	60	15	24.3 (10.36)	0.848 (0.033)	0.00212 (0.00122)	3.412 (1.770)	21
BF	241	67	100.1 (19.46)	0.962 (0.0044)	0.00374 (0.00198)	6.025 (2.881)	76
I	11	10	27.0 (14.76)	0.982 (0.0463)	0.00235 (0.00144)	3.782 (2.062)	15
J	62	16	18.9 (13.21)	0.900 (0.0152)	0.00348 (0.00188)	5.606 (2.727)	26
N	67	29	38.7 (10.70)	0.942 (0.0154)	0.00438 (0.00231)	7.049 (3.351)	47
O	8	5	6.7 (2.92)	0.857 (0.108)	0.00224 (0.00144)	3.607 (2.043)	10

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APPENDIX

Destructive sampling protocols observed for specimens sampled at the American Museum of Natural History, California Academy of Sciences and CNMA - Colección Nacional de Mamíferos, Universidad Nacional Autónoma de México.

All destructive sampling from natural history specimens was conducted in a manner to minimize damage or harm to the specimens. Locations on a specimen were chosen for destructive sampling in accordance with collections' destructive sampling policies. All work surfaces were sterilized with 30% bleach solution before and between sampling from individual specimens. Tools (scissors, scalpels, forceps, etc.) were sterilized in between sampling of specimens using a 30% bleach solution followed by a rinse with deionized H_20 and flame sterilization, paired with a change of gloves in between. Samples were labeled with the catalogue number, sampling date and name of person sampling and placed in a sterile 2 mL sampling tube with silica gel desiccant beads. Samples were frozen upon arrival at UWM.

Tissues

Tissue samples consisted of ± 1 cm^2 from either incision of the ventral side of *untanned* study skin (when known), epithelial ear tissue, palate/ interorbit or overhanging tissue or "crusties" (in the case of tissue present on skulls or post-cranial material).

Bone

We sampled turbinate bones with careful dislodging using sterilized forceps and placement of approximately 20-100 mg of bone fragments into sterile tubes. We also sampled similar quantities of marrow from long bones.

Antler/ bone/ dentin

Antler and dentin core were obtained with a Dremel® hand drill using a 5/64" or 3/32" drill bit flame sterilized with 30% bleach solution followed by a rinse with deionized H_20 and wipe with 70% isopropyl alcohol in between each specimen sampled. The first 1-2 mm of surface powder from drilling was discarded and drilling continued to target the hard (cortical) bone (Hoffman and Griebeler 2013). Drilling targeted the antler core (closest to skull) spongy bone to maximize DNA yield. Prior to starting a new specimen, the surface of the hard tissue was wiped with 70% isopropyl and the drill bit was flame-sterilized. Considering the 10,000 rpm at which the drill operates, sampling involved quick bursts of drilling to minimize heat to the surface of the hard cortical bone or dentin core and thus to the DNA. Drilling was performed over a sterile weigh boat or weigh paper to catch dentin or antler core powder, followed by transfer to a sterile 2 mL sampling tube.