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Historical Biogeography of the Midriff Islands in the Gulf of California, Mexico

A Dissertation submitted in partial satisfaction
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by

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ABSTRACT OF THE DISSERTATION

Historical Biogeography of the Midriff Islands in the Gulf of California, Mexico

by

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While the processes that led to the formation of modern plant communities are often cryptic, biogeographic patterns of extant species can provide clues to their origin. The Midriff Islands, an archipelago in the Gulf of California at the center of the Sonoran Desert, provide an opportunity to investigate the origins of the desert. This research uses three case studies at three different time scales to better understand the factors responsible for modern biodiversity.

Chapter 1 revisits the theory of island biogeography and incorporates the long history of humans on the Midriff Islands to determine factors controlling plant species richness. Area, habitat diversity, island type, and seabird dynamics explain 98% of the variability in species richness across this archipelago. Interestingly, human presence is not predictive, suggesting an island system with ancient human interactions that functions in a pre-Anthropocene state.

Chapter 2 investigates Holocene extinctions. In 1975, bighorn sheep (*Ovis canadensis*) were introduced as a novel element to Isla Tiburón as a conservation

measure. Fossil dung found on Isla Tiburón was ^{14}C-dated to 1476–1632 years before present and identified as *Ovis canadensis* by morphological and ancient DNA analysis. Bighorn sheep went locally extinct on the island sometime in the last ~1500 years prior to their "unintentional rewilding." This discovery questions the definition of a non-native species and extends an ecological and conservation baseline.

Disjunct long-lived plant taxa on Isla Tiburón suggest climate and vegetation change on the Midriff Islands in the Pleistocene. Chapter 3 is a phylogeographic study of the desert edge species *Canotia holacantha* (Celastraceae) that tests whether *Canotia* on Isla Tiburón is a Pleistocene relict or a recent dispersal event. Results support long isolation and divergence of *Canotia* on Tiburón but recent arrival in the core of its modern day distribution in Arizona. In contradiction to an expected temperate origin, *Canotia* seems to have tracked the northward shift of the desert's edge at the end of the last Ice Age from glacial refugia in Sonora or Chihuahua.

Collectively, this research helps illuminate the history of the desert and establishes baselines to support management decisions of the world's best-preserved archipelago.

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Clues to decipher the evolutionary and ecological patterns of present-day species diversity and communities are embedded in the landscape. Reconstruction of past climatic and ecological conditions and the natural invasions that have routinely occurred in response to long-term environmental change is fundamental to understanding contemporary patterns of biological diversity (Jackson & Overpeck 2000). In addition, patterns of genetic variation can provide a powerful tool for inferring historical routes of dispersal and vicariance barriers among populations (Hewitt 1996). The Sonoran Desert of northwestern Mexico and southwestern United States provides a promising test bed for sorting out geologic vs. climatic factors in historical biogeography.

Spanning this region and dividing the Sonoran Desert into two divergent but fundamentally united regions is the Gulf of California, one of the single greatest vicariant entities in Northwest Mexico. The Gulf began to open ca. 15 million years ago, splitting Baja California from the mainland of Mexico (Ledesma-Vázquez & Carreño 2010). The desert vegetation that is now found in the Baja California peninsula and the mainland of Sonora Mexico dates from a relatively recent origin during the Miocene (Axelrod 1958). During the Pleistocene arid-adapted species maintained a dynamic nature of range expansion and contraction due to the waxing and waning of glacial climates (Van Devender 1977). While the broad pattern of vegetation change in the Sonoran Desert during glacial times is fairly well resolved, disentangling the legacy of past geologic and climatic events in the formation of modern desert communities remains a problem. At the center of the Sonoran Desert in the Gulf of California are the Midriff Islands, an archipelago that stretches from the Baja California peninsula to the mainland of Mexico between latitudes 28º20’ and 29º40’.

A careful investigation of the flora of this desert archipelago established the baseline floristic diversity and current environmental conditions (Felger & Wilder 2012). The island floras today are shaped by aridity and maritime influences, yet contain intriguing biogeographic patterns that indicate markedly different conditions thousands of years ago. A number of plant species have distributions that suggest migration from the Baja California peninsula to the Sonoran mainland via the Midriff Islands (Cody et al. 1983). The presence of a half-dozen species with disjunct distributions at high elevations on Isla Tiburón indicate historic vegetation much different than the current suite of desert species (Wilder et al. 2008). However, many questions remain unanswered. What factors control species diversity on these islands? What was the predominant vegetation of the Midriff Islands during the Pleistocene? Do some species more likely represent relict (vicariant) populations rather than recent dispersal events?

The Midriff Islands display a continuum of adaptation of life to a harshly arid environment in an isolated setting. The vegetation and floristic makeup of each island is unique. From the cardón forests of the tiniest island, Cholludo, to the agave-dominated slopes of San Esteban, the forcing topographic, climatic, and biological forces have sculpted a set of unparalleled desert worlds (Wilder et al. 2008; Wilder & Felger 2010; Felger et al. 2011; Felger & Wilder 2012).

The Midriff Islands are the product of a complex geologic history tied to significant rifting along the San Andreas Fault line that led to the incursion of marine water and the formation of the Gulf of California. The Gulf runs N-S along the San Andreas Fault, which is marked by a series of stepped short-spreading centers connected by transform (strike-slip) faults. Together and over several million years, these forces have created an archipelago with distinct geologic origins and islands that range across a vast scale of size. The Gulf of California opened between 15 and 5.5 million years ago (mya), with most evidence indicating two stages of evolution, a proto-Gulf (mid-to late Miocene, 15–6 mya) and a modern Gulf (Late Miocene and younger, ca. 6 mya to present; Helenes et al. 2009; Ledesma-Vázquez & Carreño 2010). The Midriff Islands are a mix of land-bridge islands (part of the mainland during the Pleistocene), oceanic islands (in deep water, isolated throughout their existence), and geologically isolated islands (once part of the mainland but have existed as islands for the past several million years).

The islands, especially Tiburón and San Esteban, have long histories of human use (Bowen 2000, 2009). Tiburón and its neighboring islands, including San Esteban, are in the historical and current homeland of the Comcaac (Seri people). They and their ancestors have lived in the region for thousands of years. The Comcaac primarily lived a hunter-gatherer and seafaring existence (Felger & Moser 1985). Their knowledge of the flora and fauna of the islands is profound (e.g., Moser & Marlett 2010; Nabhan 2003), and remains strong, although known to a lesser extent by fewer people than during earlier, traditional times. The Comcaac continue to inhabit a core area of their historical lands, speak their native language, and maintain a keen interest in the plants and natural history of their region.

The Midriff Islands occur in a world of contrasts. The modern day climate is marked by severe aridity, yet the waters of the Gulf of California are one of the most productive marine environments in the world. Strong winter and spring winds from the northwest, year round tidal fluctuations, and constriction points between the Midriff Islands cause coastal trapped waves that lead to mixing and upwelling that stimulate globally high values of primary productivity (rates can exceed 4 g C/m^2/day; Álvarez-Borrego 2002; Douglas et al. 2007). The persistent cold-water upwelling in combination with the arid climate of the Sonoran Desert leads to a marked aridity on the islands. Infrequent precipitation events can be classified by a dampened bimodal rainfall distribution in the winter and summer, typical of the Central Gulf Coast subdivision of the Sonoran Desert (Shreve 1951). Infrequent yet biologically important tropical storms occur in the Fall (primarily in September and October) that deliver large pulses of moisture. From 1949 to 2008, 15 out of 52 hurricanes that struck the Baja California peninsula crossed the Midriff Islands (Wilder & Felger 2010)

Significant climatic fluctuations are seen in the Gulf of California during El Niño years. Warm El Niño waters reach the entrance to the Gulf from the eastern Pacific in about a month from origin that are propagated via coastal trapped waves (Lavín & Marinone 2003). This influx of warm water leads to positive anomalies (high temperatures) that are greatest in the Midriff Island region thermal fronts. While marine productivity and the subsequent trophic levels suffer acute collapses during El Niño years, winter precipitation tends to increase during these oceanographic anomalies, and terrestrial and insular habitats respond accordingly via a profusion of plant growth (Holmgren et al. 2001; Polis et al. 1997; Caso et al. 2007).

Climatic conditions were markedly different during the last ice age and multiple lines of inference such as global climate models (e.g., Bartlein et al. 1998; Metcalfe et al. 2000), deep sea and lake cores (Caballero et al. 2005; Ortega-Guerrero at al. 1999; Lozano-García et al. 2002), dune formation analysis (Beveridge et al., 2006), paleo-vegetation analysis via fossil packrat middens (e.g., Betancourt et al. 1990; Van Devender et al. 1994; Rhode 2002; Holmgren et al. 2007, 2011, 2014), and phylogeographic analysis of select desert plant species (Nason et al. 2002; Clark-Tapia & Molina-Freaner 2003; Fehlberg & Ranker 2009; Garrick et al. 2009) taken together reveal a fairly cohesive reconstruction of climatic conditions in the late Pleistocene and Holocene.

Increased winter rainfall and cooler summers facilitated mesic adapted plant species to occur in lowland regions throughout the area now referred to as the Sonoran Desert. While a mesic woodland dominated in central Arizona, mixed desert and mesic communities occurred on both sides of the Gulf of California and winter-rainfall adapted Baja California species were present in mainland Sonora opposite the Midriff Islands. Evidence for sites of the desert refugia consistently points to the Lower Colorado River Valley, eastern Plains of Sonora, and the Cape Region of Baja California Sur, the specific composition of these communities is not known. C_4-grasses in Pleistocene middens indicate the persistence of the North American monsoon throughout the last glacial-interglacial cycle. Continuation of the Mexican monsoon and warming temperatures in the early Holocene led to the establishment of the modern desert after a several thousand-year lag period and the distribution of mesic adapted taxa contracted to present localities in Sky Island mountains or northern latitudes.

This dissertation provides a bridge from the last glacial maximum to present day conditions. Chapter 1 focuses on the islands as they are today in the Anthropocene. Species richness of the Midriff Islands is used as a starting place to test factors of diversity that control for anomalously high or low levels of diversity as established by the species-area curve of the theory of island biogeography. This assessment of the extremely well preserved Gulf of California islands, which incorporates the longevity of human interactions, provides better understanding for why these islands have not suffered severe human driven modifications and extinctions seen in island systems around the world.

Chapter 2 represents the serendipity of science. In the process of attempting to identify the components of Pleistocene aged vegetation through the recovery and analysis of fossil packrat middens on the islands, a different form of midden was found on Isla Tiburón. Instead of Pleistocene plants it held Holocene bighorn sheep pellets that questioned the baseline of bighorn sheep as a novel introduction to Isla Tiburón. This chapter tells the story of Holocene island extinctions and raises questions of how to consider native or non-native species and future management decisions in an era of rewilding.

The third chapter addresses the phylogeography of one of the disjunct plant species of the summit of Isla Tiburón, crucifixion thorn (*Canotia holacantha*). Phylogeography – the investigation of the processes governing the geographical distribution of genealogical lineages, especially at the intraspecific level (Avise 2000) – allows the testing of hypotheses regarding the causal forces of geographic, geologic, and/or climatological phenomena that are responsible for anomalous geographic distributions on the modern landscape. Specifically, this chapter investigates whether *Canotia* represents a Pleistocene relict as opposed to a recent dispersal event and establishes the implications of the results on the location of desert species at the last glacial maximum.

The evolutionary history of Mexico's northwestern deserts is recorded in the flora of the Midriff Islands of the Gulf of California. It is written in the Pleistocene relicts of their mountainous peaks, in the plant litter accumulated in packrat middens, and in the DNA of many of their plants. This research harnesses three proxies to lift the veil of time and establish baselines for the biodiversity seen today.

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CHAPTER 1

REVISITING ISLAND BIOGEOGRAPHY IN THE GULF OF CALIFORNIA

Abstract

Insular ecosystems with functioning ecological interactions and few invasive species are rare in the modern world. This is especially true for islands with a long history of human use. There is robust archeological evidence that indigenous societies made use of the arid islands of the Gulf of California, Mexico for centuries if not millennia. This chapter revisits the theory of island biogeography and explicitly incorporates the presence of humans to identify what influences plant species richness in this natural laboratory. With comprehensive floristic data, we identify patterns in the floras of the islands using principal component analysis. The standard species-area model is used to establish which islands deviate from expected levels of plant diversity. To understand the drivers of species diversity we test six factors (human presence, area, habitat diversity, distance, island type, and seabird dynamics) with generalized linear models. The classic species-area model yielded a slope (z) of 0.3136 (±0.0102) with sets of islands both significantly above (species rich) and below (species poor) the line of expected diversity. Area accounts for 82% of the variance islands display from predicted levels of plant diversity. Likewise, habitat diversity accounts for an additional 10% of the variance, and island type (land-bridge or oceanic) ca. 5%. Seabird dynamics, while only

capturing ca. 1% of the variance, reflect the ecological interactions that connect the land and sea on small oceanic islands. Nesting seabird colonies with thousands of individuals deposit abundant amounts of guano, which both limit plant species diversity and subsidize those species that can tolerate high nutrients. Distance and human presence do not account for the variance detected in plant species diversity, which indicate that local species diversity is not limited by dispersal nor controlled by human activities. These results document an island system with ancient human interactions that largely functions in a pre-Anthropocene state.

Introduction

The word pristine is invoked to describe areas of the world yet to be altered by humans (Denevan 1992). Increasingly, the concept of humans as apart from nature no longer holds precedent, and the wilderness myth has begun to be tamed (Gómez-Pompa & Kaus 1992). However, some of the core models that continue to guide ecological thought and conservation science have yet to incorporate humans in a fundamental manner. The theory of island biogeography (MacArthur & Wilson 1967) has helped drive and propel the field of ecology. Yet, studies that compare species diversity and area via theoretical (e.g., Anderson & Wait 2001; Lomilono et al. 2001), statistical (e.g., Preston 1962a, 1962b; Rosenzweig 1995), and mechanistic (Chesson 2000) approaches by and large have omitted humans from the equation (Nabhan 2002).

The transformative role of humans in reshaping diversity and ecosystem functioning of islands across the globe is well known. Island-wide impacts have been documented in pre-historical times, such as on Easter Island (e.g., Diamond 2005) and Hawaii (Burney 2010). Megafauna extinctions across the globe coincide with the arrival of humans, especially on islands (overkill hypothesis; Martin 1984, 2005). Modification of islands has increased via limitless rates of species invasions (Wilcove et al. 1998; Mooney & Cleland 2001) and habitat modification (Pimm 1996), among others. Humanity's role as an agent of change has been constant (Burney & Flannery 2005) and multiplicative.

Beyond wide-scale habitat degradation and species extinctions, human-ecosystem interactions can just as well be regarded for their nuances (Bye & Linares 2000). Two plant-human interactions in the arid lands of North America exemplify this interwoven relationship. The "man-agave symbiosis" (Gentry 1982) represents the link between biological and cultural richness. Agaves have been renewable resources for food, drink, and artifact, and in turn have been cultivated and selected for since pre-historic times (Gentry 1982). Likewise, many columnar cacti and their delicious fruits have a long history of use by peoples of the New World (Casas et al. 1999; Casas & Babera 2002; Yetman 2007). High levels of genetic diversity in tended populations (Clark-Tapia & Molina-Freaner 2003; Casas et al. 2006), anomalous distributions (Yetman & Burquez 1996; Felger & Wilder 2012), and wide use by humans throughout the range of cacti in the New World that continues today (Felger & Moser 1985; Yetman 2007; Lira et al. 2009) reflect a close and sustainable pairing. Cultural dispersal, the movement of certain useful natural resources by local people who serve as active agents of dispersal in their regional domain (e.g., Nabhan 2002) has undoubtedly shaped landscapes around the world.

Thus, a long history of human presence, either through introduction of non-native species and ecosystem modification or cultural dispersal, is hypothesized to be a controlling factor on plant species diversity. The theory of island biogeography has served as a template to guide conservation efforts to protect the maximum combination of species and area both in wild (Diamond 1975) and urban spaces (Rosenzweig 2001; Rosenzweig 2003). It is time to revisit this classic theory, but with humans factored in at the outset. Once the role of humans in the landscape is defined in the context of species diversity, a baseline is established. Additional factors that control diversity can be clarified, and management plans can better address the controlling factors in a region.

The Midriff Islands (Figure 1.1), an isolated and arid archipelago in the middle of the Gulf of California, Mexico, is an ideal location to assess the role of humans in shaping species diversity. This set of 14 islands that span the Gulf of California from the Baja California peninsula to the Mexican mainland have been inhabited by the sea faring and hunter-gather Comcaac (Seri people) and or their ancestors for millennia (Felger & Moser 1985; Bowen 2000). Archaeological reconnaissance of these islands has revealed artifacts and evidence of pre-historic people on the majority of the islands in question (Bowen 2009). The Comcaac maintain extensive knowledge of the natural world of the Gulf of California (e.g. Felger & Moser 1985; Nabhan 2003; Moser & Marlett 2010; Marlett 2014) that reflects a close association between people and the desert and sea. The Comcaac lived on Isla Tiburón, the largest island in the Gulf of California, and it’s neighbor San Esteban, and traveled extensively throughout the Midriff region on reed *balsas* (boats made from reed grass or carrizo). Today the islands are uninhabited, a UNESCO World Heritage site, and Mexican natural protected area (DOF 2000). The Comcaac maintain control of Isla Tiburón and primarily live in two coastal villages on the Sonoran mainland opposite the island.

The Midriff Islands also factor into the history of island biogeography and its application in conservation. In 1970 Michael Soulé took the principals of McArthur and Wilson's revolutionary theory and set to apply them to the islands of the Gulf of California by investigating the genetics and population biology of island lizards. The results of Soulé's fieldwork (Soulé 1966; Soulé & Sloan 1966; Soulé & Yang 1973), as well as the efforts of many others (e.g., Case 1975; Felger 1976; Grismer 1994), combined with the sheer inspiration brought by the experience of working on the Gulf Islands, led Soulé eventually to apply his knowledge into a new discipline. The emerging science of conservation biology was launched with the publication of an article in *Bioscience* (Soulé 1985) that opened new ideas to the scientific world. Since that time the tenants and interdisciplinary approach of conservation biology has been applied worldwide.

In this paper we turn our attention to the inspirational setting of the Gulf of California to revisit the explanatory power of the theory of island biogeography in the context of human presence. We focus our attention on plants, which are readily dispersed, fairly persistent (Case & Cody 1987), and extremely responsive to short- and long-term environmental conditions. Plants reflect both the imprints of evolution in a relatively recent island system (the modern Gulf of California dates to ca. 6 Ma; Ledesma-Vázquez & Carreño 2010) and the effects of porous land-sea boundaries in one of the world's most productive oceans (Douglas et al. 2007). A recently updated and comprehensive knowledge of the flora (Felger & Wilder 2012) captures the current botanical diversity of these islands.

We first ascertain the pattern of floristic composition across the Midriff archipelago through a principal component analysis (PCA). To identify which islands vary from expected levels of diversity, we perform a species-area analysis following MacArthur and Wilson's (1967) time-honored model. We specifically test six factors of diversity (human presence, area, habitat diversity, distance, island type, and seabird dynamics) to determine which factors significantly explain the plant species richness seen today. Finally, we test whether certain plant families have anomalous levels of diversity on certain islands.

Methods

Factors of Diversity

The analyses presented here are based on the floristic work of Felger and Wilder (2012) and associated efforts (Moran 1983; Cody et al. 2002; Wilder et al. 2007; Wilder & Felger 2010; Felger et al. 2011; Velarde et al. 2014), which provide an up-to-date baseline for the flora of the 14 Midriff Islands analyzed (Supplemental Appendix 1). To understand which factors may be playing the largest role in controlling the observed plant diversity today, we compiled data on a set of six factors that are most likely to be of ecological importance (Table 1.1).

The presence of humans on the islands was incorporated into our biogeographic analysis via archaeological investigations undertaken on all islands in question (Bowen 2000, 2009). The islands, especially Ángel de la Guarda, San Esteban, and Tiburón have long histories of human use (Bowen 1976, 1983, 2009). Tiburón and its neighboring islands, including San Esteban, are in the historical homeland of the Comcaac. There is also substantial evidence within Seri oral history that cross-Gulf voyages were made to the Baja California peninsula and the adjacent islands (Bowen 2009). In traditional times the population of all Comcaac groups probably did not exceed several thousand. The Comcaac continue to inhabit a core area of their historical lands, speak their native language, and maintain a keen interest in the plants and natural world of their region (Marlett 2014).

Area and habitat diversity are correlated (Sfenthourakis & Triantis 2009), but the two quantities are not independent and each has a significant effect (Hortal et al. 2009). In Sonoran Desert ecosystems, topographical diversity is strongly related to habitat and plant diversity (Búrquez et al. 1999), and indeed, several small islands in the Gulf have a high degree of topographic complexity despite their size. Following Hortal et al. (2009) we define habitat diversity as the number of habitats on each island, which are based on their role in supporting the fourteen vegetation types found on the Midriff Islands (Felger & Lowe 1976; Felger & Wilder 2012; *see* Table 1.1 footnotes for habitat types). Isolation by distance was taken into account via an island’s proximity to the nearest mainland

(Baja California peninsula or Sonora) and in several cases neighboring island(s) that may serve as sources for immigration.

Geologic history of the island was incorporated via island type, land-bridge or oceanic (*see* Table 1.1 footnotes for geologic origin and age since last isolation). Given the importance of nesting seabirds, especially on small islands (Wait et al. 2005), we classified each island as a seabird island if there is annual presence of over ca. 5,000 breeding individuals and characteristic white-washed guano soil based on the abundant expert knowledge of seabirds in the Gulf of California (*see* Table 1.1 footnotes for island specifics). Although a continuum of seabird influence exists among islands, following Anderson and Polis (1999), islands can be divided into distinct bird and non-bird categories.

No reliable and long-term climatological or precipitation data exists for the Midriff Islands, which was not incorporated into our analyses. Bahía de los Ángeles, Baja California receives ca. 59 mm/yr (Reyes-Coca et al. 1990; Anderson & Polis 1999) and between 1991 and 2002 the mean annual precipitation was 52.67 with a deviation of 71.6 mm (Wait et al. 2005). On the eastern end of the Midriff in the vicinity of Puerto Libertad annual precipitation was recorded to be 102 mm/yr between 1925–1934 (Mallery 1936). Remarkably, data for the Sonoran side of the Gulf has not noticeably improved. Infrequent measurements in Kino Bay, Sonora show the region to receive ca. 140 mm/yr (Little 2012). Values for the central Midriff have been reported at 75–125 mm/yr (Sanchez-Piñero & Polis 2000) with yearly totals of 80.5 mm in 1995, 2.0 mm in 1996, 0 mm from January to August 1997, and 215 mm between September and December 1997

– an El Niño year (Sanchez-Piñero & Polis 2000). The higher elevations on Isla Tiburón are presumed to receive slightly higher moisture and precipitation than surrounding low-lying areas (Felger & Wilder 2012). Addition important moisture is brought to the islands in the form or fog, especially in the winter months (Wilder & Felger 2010). However, no formal recordings quantify fog drip in the Gulf of California. The region as a whole can certainly be classified as arid with a gradation from arid to extremely arid east to west (Felger & Wilder 2012). Hopefully island specific climatic data will soon become available.

Analyses

To examine patterns in the presence and absence of the 476 plant taxa on these 14 islands, a checklist of the flora of the Midriff Islands (Supplemental Appendix 1) was converted into a matrix where presence = 1 and questionable (no collections) or absent = 0. A non-standardized principal component analysis (PCA) was conducted for all 14 islands and 476 taxa. A broken-stick distribution model (Jackson 1993) was used to determine which axes were significant. To determine the forcing of the axes, the island loadings of axis 1 were plotted against species richness and the island loadings of axes 2 against the proportional distance of the island from the mainland and Baja California peninsula (Baja distance – Sonora distance / total distance). The small islands were omitted from the proportional distance correlation because they are nearly fully explained by axis 1.

Species numbers increase with increasing area following simple scaling laws in which species richness increases less-than-proportionally to the area that contains those species. Like many other scaling phenomena in biology, the relationship is usually described by a power function of the form $S = k\,A^z$, where S is the number of species, A is island area, z is the power exponent (usually showing values between 0.2 and 0.35), and k is a scale coefficient. Although the theory on biological scaling has dramatically expanded recently (e.g., Brown & West 2000), the basic species–area model, based on MacArthur and Wilson's (1967) "equilibrium theory" of island biogeography, has been successfully employed in research for decades and is still one of the most robust models of ecological theory (*see* Gould 1979 for a review). There are many different statistical alternatives to fit the model, ranging from log-log fit, to least squares, reduced major axes, or numerical search (Gould 1979). In our case, to estimate the power coefficient z of the area–diversity relationship for the Midriff Islands we used generalized linear modeling (GLM), a novel approach in species-area relationships. Because our dependent variable, the number of species in each island, is a count variable that is expected to have a Poisson error distribution, we used Poisson regression (also known as log-linear regression), in which the fit of the model to the data is measured as a log-likelihood function (i.e., a χ^2 distribution of the error, see McCullagh & Nelder 1989).

The derivation of our model is as follows: The species-area model for islands $S = k\,A^z$ can be rewritten in log-log form: $\log S = \log k + z \log A$. This is a linear model where $\log k$, the intercept, can be also expressed as a constant parameter $b = \log k$. Raising the exponent of both sides we get: $S = e^{(b + z \log A)}$, a log-linear function in which the count variable S is expressed as the exponent of the linear function $b + z \log A$. The GLM algorithm searches for the values of b and z that make the error of the fit minimum, and the error in turn is calculated as a log-likelihood "deviance" function: $\varepsilon = 2 \sum S_i \log (S_i / \hat{S}_i)$ that is numerically similar to the more familiar chi-squared statistic: $X^2 = \sum[(S_i - \hat{S}_i)^2 / \hat{S}_i]$ (in both equations, S_i is the number of species observed in island i, and $\hat{S}_i$ is the number of species predicted by the model for island i. In short, we fitted a Poison GLM to the species richness of the islands, using the logarithm of the area as the predictor. The slope and standard error of the fitted function gave us the value of z and its error.

In this model, each individual error term is distributed as a normal-distribution deviate that can be tested against the critical values of the Normal curve (i.e., errors are Pearson residuals, *see* Duffy 1990). Using this property, we were able to identify the islands that had significantly more or significantly fewer species than predicted by the equilibrium model of island biogeography. Once the power coefficient was estimated by regressing species richness against log-area, we identified the islands that departed significantly from the predictions of the model.

After confirming that some islands did depart significantly from the species-area model, we started testing additional predictors of species richness for the archipelago, using a step-wise process and testing for the effect of (a) presence of archeological artifacts (yes or none found) as a surrogate for prehistoric human presence, (b) habitat diversity (a number between 1–14), (c) distance to the nearest mainland, distance to the nearest island (in km), (d) island type (oceanic or land-bridge), and (e) seabird dynamics (presence of nesting seabirds, yes or no). Some of our independent predictors were quantitative variables but others were statistical factors.

At each step of the regression analysis we tried all the variables that could be added, then selected the best fit using Akaike's Information Criterion, and kept on adding variables until no variable could significantly improve the fit. For the whole modeling procedure, we used the generalized linear model (GLM) module in the R programming language (R Core Team 2014).

Finally, we determined Pearson residuals at the family level to ascertain whether plant families adhere to diversity levels expected given family proportions in the total Midriff flora. We assessed the different groups of islands as identified in the species-area analysis (species rich, species poor, expected) and compared observed and expected levels of diversity. Family classifications follow the APG III (Stevens 2012), reflecting current knowledge of evolutionary relationships.

Results

The PCA analysis had two significant axes as determined by the broken-stick distribution model (Jackson 1993; Figure 1.2). The first axis explains 41% of the variation and the second 19% (opposed to expected values due to random conditions of 23% and 16%, respectively). The PCA analysis of the floristic matrix of the Midriff Islands can be assessed either by islands as various representations of floristic communities (Figure 1.2), or species that are characterized by where they occur (Figure 1.3). When islands are assessed, axis 1 is strongly correlated with species richness (r^2=

0.9899; Figure 1.2b). The second axis represents a gradient from west (Baja California, Ángel de la Guarda) to east (Sonoran mainland, Tiburón; r^2= 0.7372; Figure 1.2c). Among the 476 plant taxa (Figure 1.3) four distinct groups of species can be recognized: rare species, shared species, Sonoran-side species, and Baja-side species. The first axis represents a gradient from rare species (endemics and single island occurrences) to species found on multiple islands. As in the assessment of islands, axis 2 separates species found only on the western side of the Midriff near Baja California from those on the eastern side, such as on Isla Tiburón adjacent to Sonora.

The species-area model (Figure 1.4) yielded a regression line with an $r^2 = 0.822$ and a slope $z = 0.3136$ (s.e. ± 0.0102). As is frequently the case in islands, the value of the estimated species-area exponent (0.3136) was significantly higher than 0.24, the "canonical" value (Preston 1962a) expected in terrestrial ecosystems ($t = 7.2$, d.f. 11, $P < 0.0001$). The analysis of the residuals identified four islands with more plant species that expected, the Tiburón archipelago: Islas Tiburón, Dátil, Alcatraz, and Cholludo. Seven islands were shown to have less plant species that expected: Ángel de la Guarda, Las Ánimas, San Pedro Mártir, Partida Norte, Patos, Salsipuedes, and Rasa. Each of these species poor islands is oceanic and can be characterized as a bird island, apart from Ángel (non-bird) and Patos (land-bridge). The other three fell within the expected range: San Esteban, San Lorenzo, and Nolasco (Table 1.2).

The analysis of variance identified four of the six factors of diversity to significantly account for the variation from expected values (Table 1.3). In decreasing order of significance the four factors found to explain the observed variation in plant species diversity on the Midriff Islands of the Gulf of California are (1) area^z (area raised to the slope of the species-area regression line, which accounted for 82% of the variance), (2) habitat diversity (10%), (3) island type (ca. 5%), and (4) seabird dynamics (ca. 1%). Together these four factors accounted for 98.4% of the observed variation, and the residuals were not significant, matching the assumptions of a random Poisson distribution. The two other factors, distance and human presence were in no way responsible for the observed variation.

Once area is accounted for, which is well accepted to be a primary driver of species diversity patterns (e.g., Preston 1962a; Rosenzweig 1995), habitat diversity accounts for 57.0% of the area model residual variation, and island type plus seabird dynamics account for 34.1%.

Pearson residual analysis of plant family diversity was determined for the three sets of islands: species rich, species poor, and equilibrium (Table 1.4). The species rich islands, or the Tiburón archipelago, are depauperate in Polygonaceae, while Ángel de la Guarda is specious in this family. The bird islands have far more species of cacti and amaranths than expected, as does San Pedro Nolasco grasses. As anticipated, the islands in equilibrium have no significant deviations in diversity from expectations.

Discussion

Humans have been a broad-scale determinant of species diversity on islands worldwide, often through habitat disruptions and species extinctions (e.g., Burney & Flannery 2005) and cultural use (e.g. Bye & Linares 2000). The consumption, utilitarian use, harvesting, and transport of plants by the Comcaac and or their ancestors is not only undeniable, but as much a part of the fabric of this region as any of the other factors we have tested (Felger & Moser 1985; Nabhan 2000; Nabhan 2002). However, our results show that humans are not a deterministic factor for plants on the Midriff Islands in the Gulf of California. What can explain this dichotomy?

Unlike Polynesians that arrived in Hawaii and peoples in the Caribbean, the Comcaac are of the Gulf of California, endemic. Their language, *Cmiique Iitom* is a language isolate (Moser & Marlett 2010) and their knowledge in varied realms of the natural world is profound (e.g., Felger & Moser 1985, Nabhan 2003, Marlett 2014). They lived in and/or visited even remote areas in the region for thousands of years, with archaeological evidence present on nearly all islands in the Midriff (Table 1.1; Bowen 2009). The environmental changes wrought by the Comcaac have been integrated with and indistinguishable from the background rate of ecological dynamics of the Midriff Islands.

Plants were harvested almost daily, with people transporting one species or another locally and sometimes regionally. The Comcaac had a cultural practice of burying the placenta from a newborn next to a large cactus such as cardón or sagüeso (*Pachycereus pringlei*), saguaro (*Carnegiea gigantea*), or sinita (*Lophocereus schottii*; Felger & Moser 1985; Moser 1970; Nabhan 2000, 2002). There is also evidence that the Comcaac planted chollas (*Cylindropuntia fulgida*), such as in association with game circles (Bowen et al. 2004). Oral histories document the Comcaac transplanting and planting certain species. *Agave subsimplex* and two prickly pears (*Opuntia engelmannii* and *O. gosseliniana*) “were said to have been planted near the base of Punta Sargento many years ago by the people of the Sargento Region” (Felger & Moser 1985:225). The dense stands of the endemic *Agave cerulata* spp. *dentiens* on Isla San Esteban were utilized as a primary food source (Felger & Moser 1985; Bowen 2000). Interactions were not limited to plants, iguanids were moved between islands (Grismer 2002; Nabhan 2002) and the role of people in the local extinction of bighorn sheep on Isla Tiburón can not be excluded (Wilder et al. 2014).

Yet, the environmental changes and the results of the active dispersal of culturally significant resources by the Comcaac have been subtle. This is principally because human population pressure was low and varied from ca. 180 to 3,500 individuals through time (Nabhan 2002), limited by the scarcity of fresh water (Felger & Moser 1985). The aridity of the region made agriculture unfeasible within their historic territory (Felger & Moser 1985) and subjugation and military pursuit difficult. A way of life evolved, forced by the aridity of the land and made possible by an intimate connection between the bounty of the desert and sea.

The plant species richness patterns from the Midriff Islands support the well-established correlation between area and species diversity and the linearity of the species-area curve (Preston 1948). The step-wise model identified that area and habitat diversity account for the majority of the deviance from expected values (92%; Table 1.3). The combination of these two factors in supporting diversity has been long recognized (e.g., Darwin 1860; Preston 1962a; Hortal et al. 2009; Panitsa et al. 2010). Similarly, the first axis of the PCA analysis of the Midriff Islands flora is significantly correlated with species richness (Figure 1.2b).

Islands in general have *z* values that start at the canonical value of 0.24 (Preston 1962a) and range up to ca. 0.33 (Rosenzweig 1995). The value 0.31 for the Midriff Islands is raised to the higher end of this range due to the species-poor small islands, where the effects of insularity are clear (Cody et al. 2002). The species-area model alone does not explain the low plant species diversity on this cluster of small islands (<3 km^2). Cody et al. (1983) in their assessment of the plant species on the Gulf islands explain the deviation in terms of the lack of habitat diversity. Based on our analysis, habitat diversity is not an appropriate explanation in itself. Seabird dynamics, which do correlate with low topography, were a significant factor in our results and the driving force in Anderson and Wait's (2001) "subsidized theory of island biogeography." The combination of seabird presence, low topography, and oceanic islands is a better explanation of the low diversity on small islands.

The six small oceanic islands in our analyses are linked to the productive waters of the Gulf of California through a long chain of ecological interactions (Figure 1.5). Due to long isolation and remote nature, there are no predatory mammals on the oceanic islands (Lawlor et al. 2002). Instead, small rodents are top-level consumers (Anderson et al. 2008). The lack of predators and shallow relief with few associated habitats set the stage for some of the largest aggregations of breeding seabirds in North America (Velarde et al. 2005).

Seabird diets consist mostly of pelagic fish that occur in abundance in the Midriff region where they feed on microscopic plankton in the upwelling zones (Velarde et al. 2013). These birds deposit copious amounts of nutrient rich guano, high in Nitrogen (N) and Phosphorus (P; Hutchinson 1950). Indeed, soil on bird islands in the Gulf of California has 7-fold more N and ca. 5-fold more P than non-bird islands (Anderson & Polis 1999; Anderson et al. 2008), which is in line with seabird islands from around the world (Ellis 2005). Guano cover is positively related to seabird nesting density on islands in the Gulf of California (Sanchez-Piñero & Polis 2000), with more than 50 such islands (Hutchinson 1950; Anderson & Polis 1999).

The presence of elevated levels of N and P on seabird islands acts as a strong filter that selects for specific plant species. Unique physiological responses and life history traits are required to tolerate these toxic conditions of nearly unlimited raw fertilizer (Anderson et al. 2008). There are two principal plant growth forms or functional categories that effectively and readily pass through this guano filter, halophytic annuals or shrubs (Amaranthaceae) and cacti (Cactaceae), which are identified in our statistical analyses of plant families on bird islands (Table 1.4). Annual or herbaceous perennial species in the amaranth family with a fast growing “ruderal” growth strategy are seen in far greater diversity on the Midriff bird islands than expected. The other functional group is xerophytic succulents, especially columnar cacti and chollas with shallow roots and capacity for sporadic rapid water absorption, storage, and fast growth (Wilder et al. 2008; Wilder & Felger 2010; Felger & Wilder 2012; Velarde et al. 2014). The percentage of succulent flora of bird islands in the Midriff region is relatively greater than that on large islands, in opposition to the standard species-area relationship (Wilder et al. 2008). Both cacti and amaranths are adapted for long distance dispersal and are facilitated in arrival to the islands by seabirds (Ellis 2005; Velarde et al. 2014)

Species that can exist on guano rich islands benefit from abundant nutrients that enrich plants (Anderson & Polis 1999) and enhance primary productivity (e.g., Heatwole 1971, Onuf et al. 1977, Ryan & Watkins 1989, Anderson & Polis 1999; Sanchez-Piñero & Polis 2000). Guano deposition has been shown to increase soil moisture in both wet and dry years (Wait et al. 2005), which is one of the most significant limiting factors for plants in arid environments (Shreve 1951). The Gulf of California regional endemic cardón cactus (*P. pringlei*) especially benefits from the lack of seed predators and abundant nutrients. This columnar cactus is seen in remarkable forests of thousands of individuals on these bird islands (Cholludo, Mártir, Partida Norte; Figure 1.1), here at the greatest density in its range (Medel-Narvaez et al. 2006; Wilder & Felger 2010).

The ecological dynamics of bird islands fluctuate, which affect species diversity in time (Figure 1.5). Seabird guano accumulates and resides in the soil year-round, but is mobilized for plant uptake only during pulsed periods of rain (Anderson and Polis 1999; Anderson et al. 2008). Accordingly, N and P are normally unavailable to plants during dry conditions (Ellis 2005). During El Niño events warm waters lower the thermocline, pushing the pelagic fish out of the reach of diving seabirds, which result in large-scale abandonment of nesting efforts (Velarde & Ezcurra 2002). At the same time, winter precipitation tends to increase during these oceanographic anomalies, cueing a profusion of plant growth that can utilize the now liberated nutrients (Polis et al., 1997; Holmgren et al., 2001; Caso et al., 2007).

In wet years, bird islands support one- to two-fold more plant cover (annuals and perennials) and ca. 10- to 12-fold more plant biomass (annuals) than non-bird islands (Polis et al. 1997; Anderson & Polis 1999). At such times herbaceous annuals such as coastal saltbush (*Atriplex barclayana*) and an annual cucurbit vine (*Vaseyanthus insularis*) blanket various islands (Wilder & Felger 2010; Velarde et al. 2014), and the cardón surges with growth. Over the five year span of 2007–2012 cardón individuals on Isla San Pedro Mártir 1 m tall and under grew ca. 7 cm, and plants 2–5 m grew ca. 25 cm (Wilder unpublished data). These fast growth rates are previously unknown in this presumed slow growing cactus (Turner et al. 1995). It is also during these wet years that establishment events most likely occur (Wilder & Felger 2010), as they do in other arid-adapted columnar cacti (Turner et al. 2003).

In these periods of heavy precipitation the flush of vegetation switches the islands from a marine based trophic web to one driven by the primary productivity of annual and biennial plants and cacti growth (Polis et al. 1997; Stapp et al. 1999). The abundance of biomass in these wet years likely exacerbates competitive exclusion, further maintaining low species diversity. Nitrogen enrichment from marine inputs that fuel the primary producers enrich secondary consumers (Caut et al. 2012). Invertebrate and rodent communities respond with significant but short-lived population increases (Polis et al. 1997; Anderson et al. 2008). The pulses of productivity produce plant detritus that is slowly released and enhances secondary productivity for years in detritavores and granivores (Sanchez-Piñero & Polis 2000).

Just as the land and sea are starkly contrasted, the islands flanking the Midriff archipelago are mirror images. Ángel de la Guarda on the western or Baja California side of the Gulf and Tiburón to the east just off Sonora represent the end members of a continuum in the factors that govern species diversity. Tiburón and its neighbors Dátil, Alcatraz, and Cholludo – the Tiburón archipelago, is a land-bridge conglomerate that was part of the Sonoran mainland ca. 6,000 years ago (Felger & Wilder 2012). Ángel, an oceanic island, disconnected from Baja California ca. 3.3–2 Ma (Aragón-Arreola & Martín-Barajas 2007). Around this period rapid strike-slip faulting started in the Ballenas Channel that separates Ángel from Baja California (Nagy & Stock 2000; Stock 2000). The Tiburón archipelago has more species than predicted and no endemics. Despite the large size of Ángel, the ruggedness of the island, and high habitat diversity, lower than expected plant species richness is encountered. Yet, four single-island endemic plants (Moran 1983), an endemic speckled rattlesnake (*Crotalus mitchellii angelensis*), and three endemic rodents (Mellink et al. 2002; Lawlor et al. 2002) are found on this island.

The differences between either side of the Midriff archipelago are also evident in the results of the PCA analysis of the floristic matrix. Axis 2 reflects the proportional distance of both islands (Figure 1.2) and species (Figure 1.3) from either the Baja California peninsula or the Sonoran mainland. Species encountered on Ángel are adapted to extreme aridity. In addition, ca. 30 plant taxa characteristic of the northern mediterranean region of the Peninsula reach their southern limit on or near Ángel (Moran

1983). The preponderance of the buckwheat family Polygonaceae on Ángel and its near absence on Tiburón (Table 1.4) highlight the gradient of both floristic affinities and climate regimes. Persistent cold-water upwelling and location of Ángel on the western edge of the North American monsoon, as well as the southeastern edge of Pacific-derived winter storms result in Ángel and the western Midriff Islands being the most arid portions of the Gulf of California (Roberts & Ezcurra 2012). The Midriff Islands have a marked xeromorphic gradient, with increasing aridity from east to west (Felger & Lowe 1976; Felger & Wilder 2012).

A final group of oceanic islands, San Esteban, San Lorenzo, and Nolasco, fall right in the middle, not just of the species-area plot (Figure 1.4), but also in the factors that are shown to control diversity. None are seabird islands, they are all of moderate size and have moderate levels of plant species diversity. San Esteban and San Lorenzo near the middle of the Gulf are dry and relatively rugged with a substantial degree of floristic overlap and no anomalous occurrence or absence of plant families. San Pedro Nolasco sits in deep water not far from the Sonoran mainland and its massive Sierra el Aguaje complex. The island is marked by a N-S ridge that is sculpted by a series of shallow canyons with abrupt topographic changes. Five unique communities segregate according to slope exposure and support a high diversity of grasses (Table 1.4) and endemic globose cacti (Felger et al. 2011). Collectively, moderate area and habitat diversity and long isolation support these islands to be in equilibrium according to the MacArthur and Wilson theory of island biogeography.

Distance, one of the widely recognized controlling factors of island diversity worldwide, has no explanatory power in the plant diversity of the Midriff Islands (Table 1.3). Here, isolation is less than in remote oceanic archipelagos. The Baja California peninsula and Sonoran mainland parallel the Gulf through its entirety, which limits the degree of isolation of any island. The importance of distance in the Gulf of California is taxon-dependent and varies with colonization and persistence rates (Case & Cody 1987; Cody 2006). There is a gradient of colonization and persistence ability. Taxa that have high colonization and low persistent rates (birds) occupy one end of the dispersal spectrum (Cody & Velarde 2002), mammals and reptiles the other (Soulé & Sloan 1966; Case 1975; Lawlor et al. 2002).

Plants have high colonization rates and relatively high persistence capability. There is a general low level of endemic plant species in the Gulf (Rebman 2002), especially on bird islands. However, cacti are actively radiating on the islands and represent 40% of the endemic plant taxa in the Gulf of California (Rebman 2002). Relict plant species occur at high elevations and present “Sky Islands” within desert islands (Moran 1983; Chapter 3). Nonetheless, in the broad picture of species diversity levels, distance is not a factor for plants on the Midriff Islands.

Further support for the diminished role of distance as a barrier to plant movement in the Midriff Islands is the large group of Sonoran Desert taxa that occur both in the Baja California peninsula and the mainland of Sonora (Figure 1.3; Cody et al. 1983). Forty-eight plant species are broadly distributed over the Peninsula, across the Midriff Islands, and narrowly onto the Sonoran coast (Dolby et al. *in press*). The role of the

Midriff Islands as stepping-stones between the Peninsula and mainland is likely and has been supported by genetic studies (Clark-Tapia & Molina-Freaner 2003; Garrick et al. 2009). Cross-Gulf and inter-island migration was especially possible during glacial periods when distance between islands was far less.

Islands with ecologic networks that function as they were hundreds of years ago are rare. Islands where humans are not a dominant and negative driver of biological diversity are scarcer yet. Understanding the underlying factors of species diversity and intact ecological chains establish baselines. This knowledge is critical in the face of widespread extinctions and ecological chains increasingly shifting to a diminished state (McCauley et al. 2012). Taken as a whole, we are left with data that supports what any visitor to the islands of the Gulf of California sees, a set of unique desert microcosms pulsing at a pace yet to be set by people. The natural wonders of the Gulf of California, including humans, inspire today just as they did a number of decades ago. The Midriff Islands offer a blueprint of hope in the Anthropocene.

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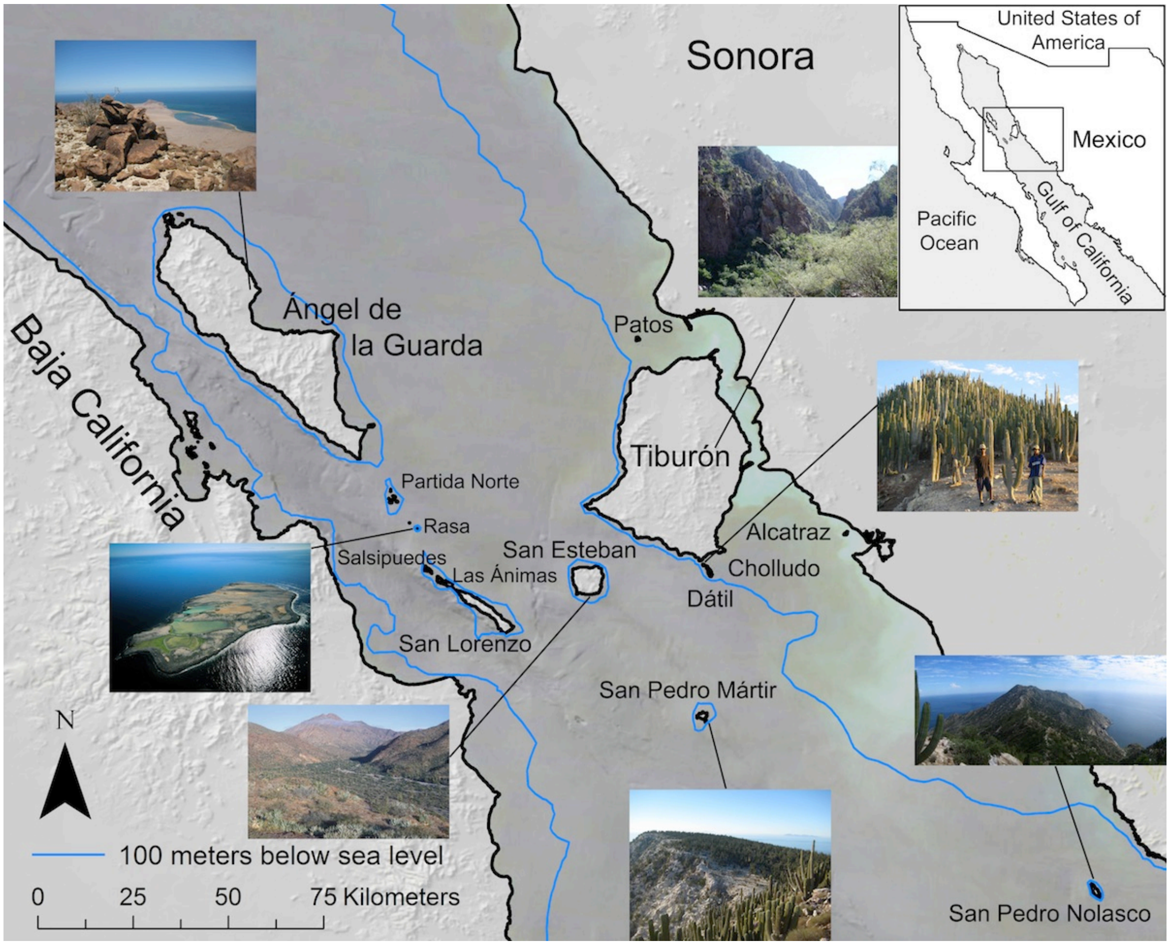

Figure 1.1. Midriff Islands of the Gulf of California, Mexico. The 100 m bathometric line indicates approximate coastline at the last glacial maximum. Photos by Wilder except Isla San Esteban by Felger and Isla Rasa by Fulvio Eccardi. Gulf of California DEM and hill shade GIS layers provided by Scott Bennett and satellite imagery courtesy of NASA. Inset regional map by Cathy Moser-Marlett.

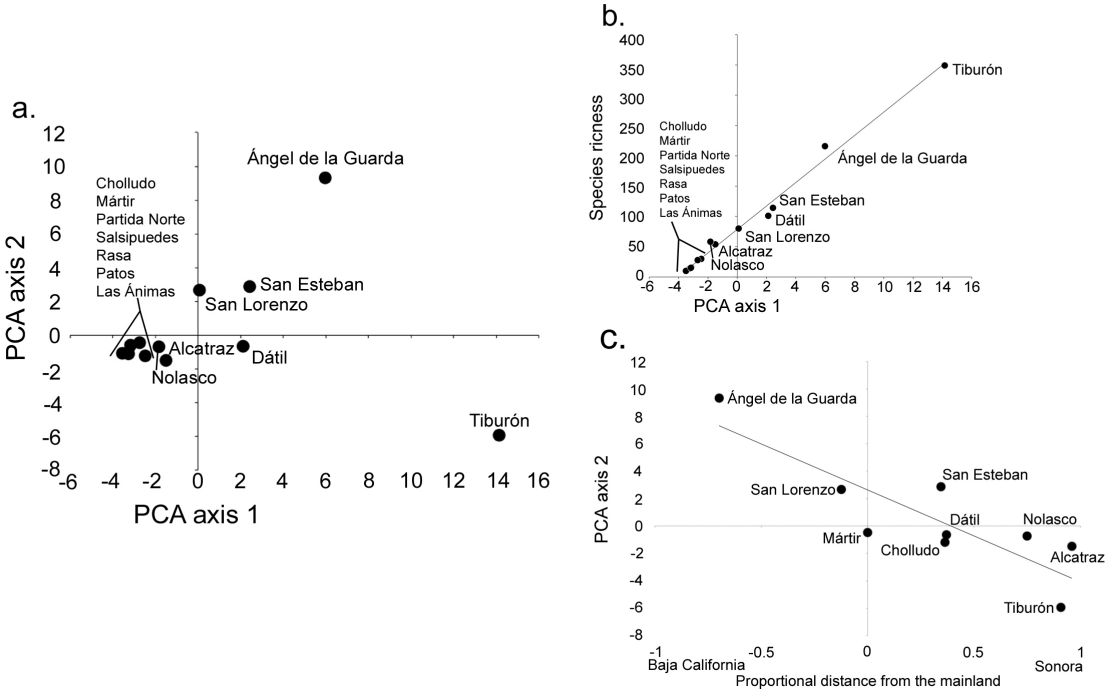

Figure 1.2. Midriff Island results of Principal Component Analysis (PCA). (a) PCA axes 1 and 2 that show the 14 islands as subsets of the floristic matrix. (b) Correlation between PCA axis 1 and species richness ($r^2 = 0.9899$). (c) Correlation between PCA axis 2 and the proportional distance from the Baja California peninsula and Sonoran mainland ($r^2 = 0.7308$). Negative x axis values reflect islands closer to Baja California, positive values are closer to Sonora.

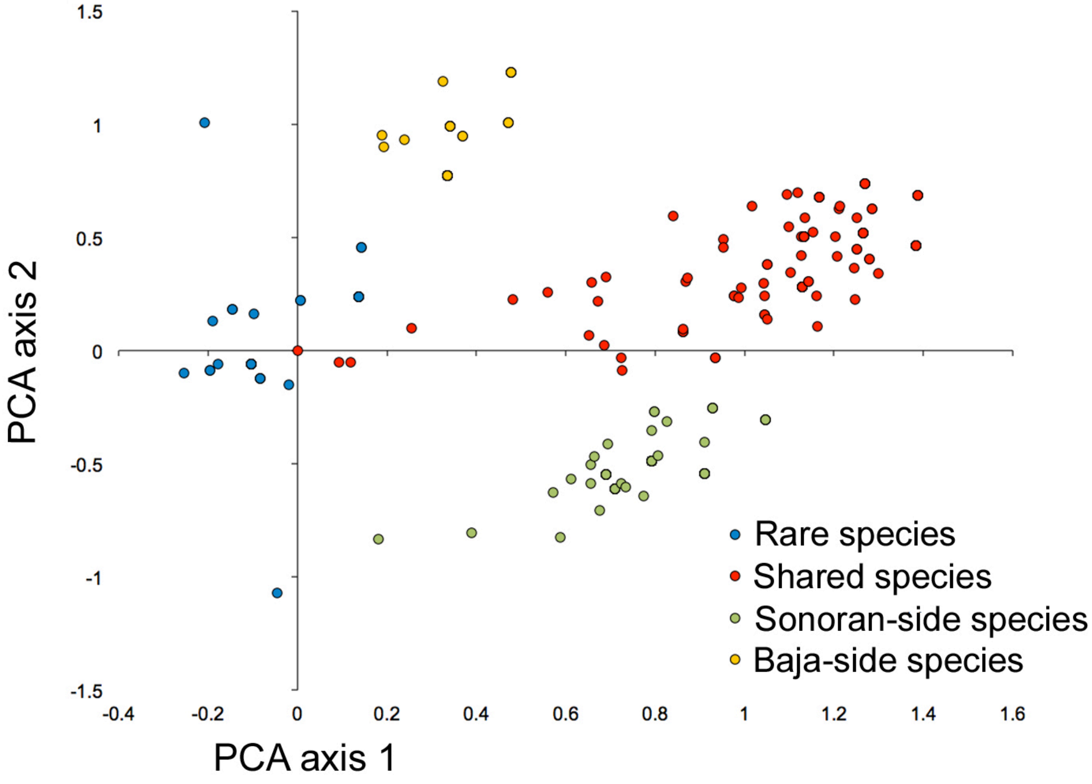

Figure 1.3. Individual species results of Principal Component Analysis (PCA). The 476 plant taxa of the Midriff Islands distributed along PCA axes 1 and 2. A species location is due to the number of islands it occurs on (axis 1; rare species only occur on one island) and presence on the Baja or Sonoran side of the Midriff (axis 2). Coding for the four species categories is based on the floristic checklist for the Midriff Islands (Supplemental Appendix 1).

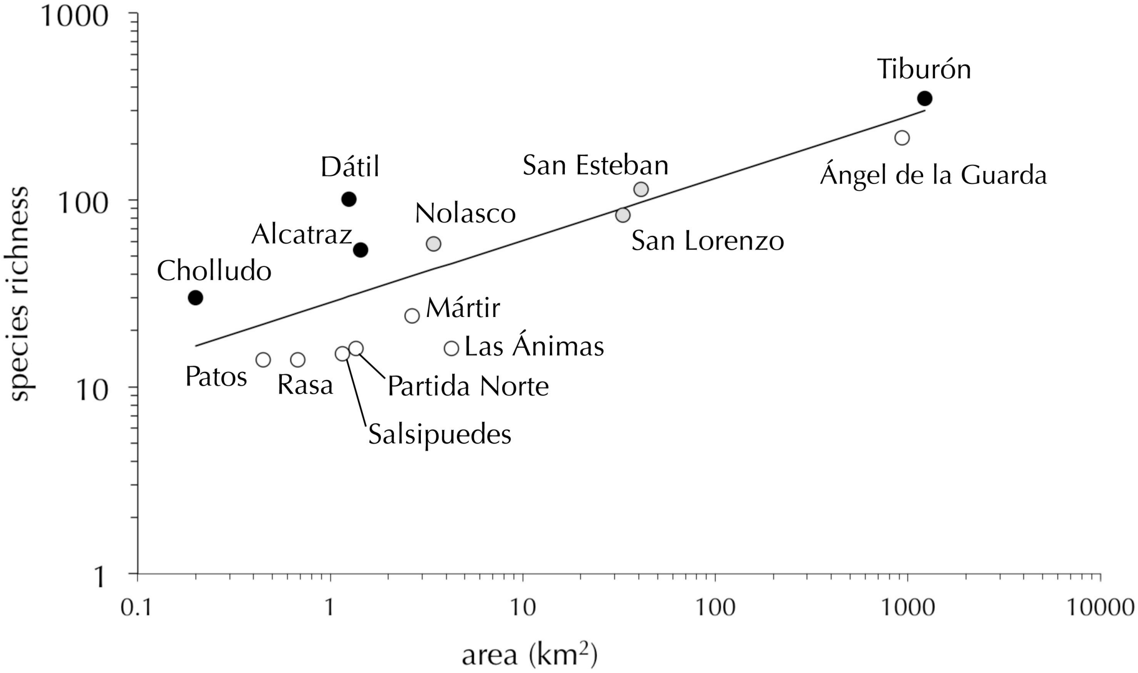

Figure 1.4. Plant species-area relationship for the Midriff Islands, Gulf of California, Mexico. Both axes are log transformed. The slope of the line (z) is 0.3136 (s.e. ±0.0102) with an $r^2 = 0.822$. Islands with significantly more species indicated by black circles, islands with significantly less species denoted by open circles, and islands with expected plant species diversity marked with grey circles.

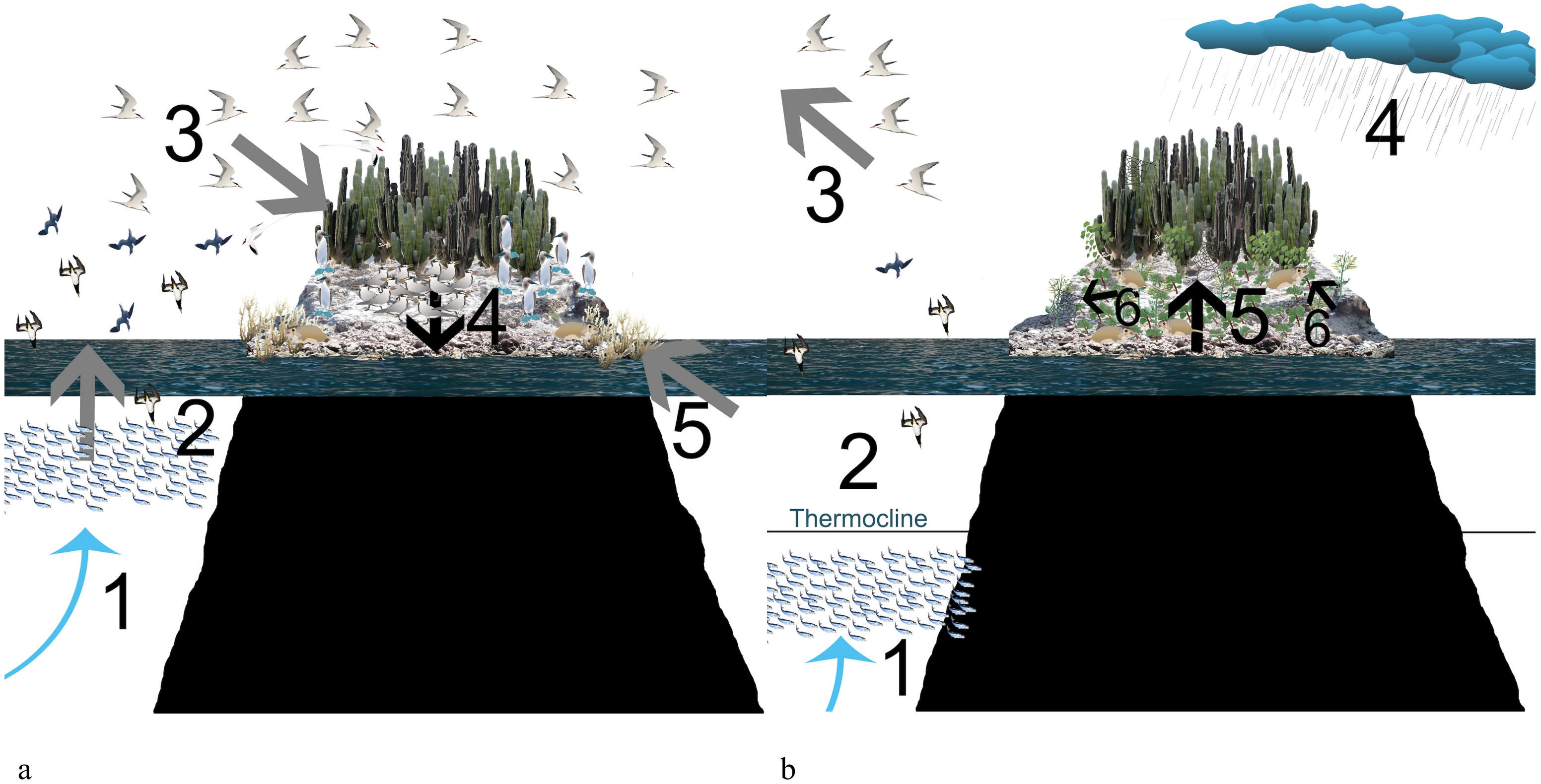

Figure 1.5. Land-sea dynamics of a representative small oceanic seabird island in the Gulf of California. (a) Normal or La Niña oceanographic conditions. Upwelling in the Midriff Island region brings nutrient rich cold-water to the surface from depth (1), which supports an abundance of pelagic fish that are fed upon by seabirds (2), that nest on predator free oceanic islands (3) where they deposit abundant marine derived nutrients in the from of guano (4), which acts as an extreme filter to plant species and limits diversity levels. Also during such times the island food web is marine based

and subsists on algal racks that arrive on the island (5). (b) El Niño conditions. Persistent upwelling continues to support the pelagic fish community (1), but is pushed down ca. 10+ m below sea level due to thermal expansion and a subsequent lowering of the thermocline that pushes fish out of the reach of diving seabirds (2) that subsequently abandon nesting efforts (3). The warm waters also produce pulses of above average precipitation events (4) that mobilize and release the N and P in the guano (5) that fuels rapid growth of annual plant species and cardón cacti, which support increases in secondary consumer populations such as small rodents and invertebrates (6), switching the island from a marine based trophic web to one driven by internal primary productivity. See text for citations for each step of this ecological interaction chain. Figures made by Wilder with select images obtained from IAN image library (ian.umces.edu/imagelibrary/).

Island	[1] Number of Plant species	[2] Area (km^2)	[3] Habitat Diversity	[4] Distance (km)	[5] Island Type	[6] Seabird island	[7] Archaeological Evidence
Tiburón	[1a] 349	1,223	14	[4a] 1.70	[5a] Land-bridge	[6a] No	Yes
Ángel de la Guarda	[1b] 215	936	10	[4b] 12.12	[5b] Oceanic	[6b] No	Yes
San Esteban	[1c] 114	41	9	[4c] 11.64, 16.85, 34.5	[5c] Oceanic	[6c] No	Yes
San Lorenzo (San Lorenzo Sur)	[1d] 83	33	4	[4d] 16.36	[5d] Oceanic	[6d] No	Yes
Las Ánimas (San Lorenzo Norte)	[1e] 16	4.26	1	[4e] 16.36	[5e] Oceanic	[6e] Yes	Yes
Nolasco	[1f] 58	3.45	5	[4f] 14.61	[5f] Oceanic	[6f] No	None known
Mártir	[1g] 28	2.67	3	[4g] 39.09, 50, 50	[5g] Oceanic	[6g] Yes	None known
Alcatraz	[1h] 54	1.44	4	[4h] 2.01	[5h] Land-bridge	[6h] Yes	Yes
Partida Norte	[1i] 16	1.36	2	[4i] 8.30, 12.18, 17.88	[5i] Oceanic	[6i] Yes	Yes
Dátil	[1j] 101	1.25	4	[4j] 1.94	[5j] Land-bridge	[6j] No	Yes
Salsipuedes	[1k] 15	1.16	1	[4k] 1.52, 19.21	[5k] Oceanic	[6k] No	Yes
Rasa	[1l] 14	0.68	1	[4l] 20.79	[5l] Oceanic	[6l] Yes	None known
Patos	[1m] 14	0.45	1	[4m] 7.45, 8.82	[5m] Land-bridge	[6m] Yes	None known
Cholludo	[1n] 30	0.2	3	[4n] 1.09	[5n] Land-bridge	[6n] Yes	None known

Table 1.1. Factors of diversity. The 14 islands considered, number of plant species and categorization or metrics for the six factors of diversity tested. Islands are listed in order of decreasing area.

[1] The flora of the Midriff Islands is presented in a checklist in Supplemental Appendix 1. Flora totals are from: [1a] Felger and Wilder (2012) with recent additions from Wilder (previously unpublished data); [1b] Moran (1983), Rebman (2010), and recent additions by Wilder and Rebman (previously unpublished data); [1c] Felger and Wilder (2012); [1d] Rebman et al. (2002); [1e] Rebman et al. (2002); [1f] Felger et al. (2011), Felger and Wilder (2012); [1g] Wilder and Felger (2010), Felger and Wilder (2012), with recent additions from Wilder (previously

unpublished data); [1h] Felger and Wilder (2012); [1i] Rebman et al. (2002); [1j] Felger and Wilder (2012); [1k] Rebman et al. (2002); [1l] Velarde et al. 2014; [1m] Felger and Wilder (2012); [1n] Felger and Wilder (2012).

[2] Island size from Murphy et al. (2002).

[3] Habitat classes that affect the fourteen vegetation types found on the Midriff Islands (Felger & Wilder 2012): ridges, arroyos, canyons, permanent springs, ephemeral tinajas, coastal bajada, foothill bajada, peaks above 500 m, coastal area and salt flats, flats, north-facing slopes, esteros, sea cliffs, valleys, dunes.

[4] Distances (Murphy et al. 2002) to nearest large land body: [4a] Sonora; [4b] Peninsula [4c] Tiburón, San Lorenzo, Peninsula; [4d] Peninsula; [4e] Peninsula; [4f] Sonora; [4g] Tiburón, Sonora, Peninsula; [4h] Sonora; [4i] Rasa, Ángel de la Guarda, Peninsula; [4j] Tiburón; [4k] Las Ánimas, Peninsula; [4l] Peninsula; [4m] Tiburón, Sonora; [4n] Tiburón.

[5] Classification of land-bridge or oceanic based on geologic origin information from Carreño and Helenes (2002; except where stated otherwise), and age since last isolation as indicated: [5a] Faulting, uplift, and erosion, ca. 6,000 ya (Wilcox 1978; Lambeck & Chappell 2001; Davis 2006; Felger & Wilder 2012); [5b] Block Faulting, 3.3–2 Ma (Aragón-Arreola & Martín-Barajas 2007; Nagy & Stock 2000; Stock 2000); [5c] Volcanic (Desonie 1992), 2.9–2.5 Ma (Desonie 1992); [5d] Block Faulting, 3.3–2 Ma (Aragón-Arreola & Martín-Barajas 2007; Nagy & Stock 2000; Stock 2000); [5e] Block Faulting, 3.3–2 Ma (Aragón-Arreola & Martín-Barajas 2007; Nagy & Stock 2000; Stock 2000); [5f] Faulting, 3–2 Ma (Felger et al. 2011); [5g] Volcanic, no age data is available but is presumably a similar age as San Esteban; [5h] Faulting, uplift, and erosion, ca. 6,000 ya (Wilcox 1978; Lambeck & Chappell 2001; Davis 2006; Felger & Wilder 2012); [5i] Volcanic, no age data is available but is presumably similar to adjacent Salsipuedes; [5j] Faulting, uplift, and erosion, ca. 6,000 ya (Wilcox 1978; Lambeck & Chappell 2001; Davis 2006; Felger & Wilder 2012); [5k] Block Faulting, 3.3–2 Ma (Aragón-Arreola & Martín-Barajas 2007; Nagy & Stock 2000; Stock 2000); [5l] Volcanic, 10,000 ya (Velarde et al. 2014); [5m] Faulting, uplift, and erosion, ca. 6,000 ya (Wilcox 1978; Lambeck & Chappell 2001; Davis 2006; Felger & Wilder 2012); [5n] Faulting, uplift, and erosion, ca. 6,000 ya (Wilcox 1978; Lambeck & Chappell 2001; Davis 2006; Felger & Wilder 2012).

[6] Seabird usage based on cumulative knowledge as identified: [6a] Cody and Velarde 2002; [6b] Cody and Velarde 2002; [6c] Cody and Velarde 2002; [6d] considered to have a large colony of seabirds (Sanchez-Piñero & Polis 2000) where nesting is confined to the northern third of island, primarily pelicans (Dan Anderson personal communication 16 Aug 2014); [6e] nesting is island wide, primarily pelicans (Dan Anderson personal communication 16 Aug 2014); [6f] not considered a seabird island (Felger et al. 2011; Dan Anderson personal communication 16 Aug

2014); [6g] a significant seabird island with 8 breeding seabirds species, especially blue footed and brown boobies (Tershy & Bresse 1997); [6h] southwestern portion of island supports 11 breeding species, especially Double-crested Cormorants (Duberstein et al. 2005); [6i] significant seabird island (Sanchez-Piñero & Polis 2000) with at least five breeding species, especially Craveri's Murrelet (Velarde et al. 2005), Least Storm-Petral (Velarde 2000), occasionally brown pelicans (D. Anderson and T. Bowen personal communication 16 Aug 2014), and the largest population of fishing bats (*Myotis vivesi*) in the Gulf of California (Maya 1968; Velarde et al. 2005); [6k] nesting over the total island, but quite spotty (D. Anderson personal communication 16 Aug 2014), we differ from Sanchez-Piñero and Polis (2000) in not designating this a seabird island; [6l] a significant seabird island (Sanchez-Piñero & Polis 2000) especially Heermann's Gull, Elegant Terns, and Royal Terns (Velarde 1989; Cody & Velarde, 2002; Velarde & Ezcurra 2002; Velarde et al. 2014); [6m] Nesting over the total island, sporadic (D. Anderson personal communication 16 Aug 2014), and perhaps not recovered from vegetation removal for guan harvesting in 1946 (Felger & Wilder 2012; Dan Anderson personal communication 16 Aug 2014); [6n] total island, but spotty within cardón forest (D. Anderson and Enriqueta Velarde personal communication 16 Aug 2014).

[7] Presence of archaeological remains is based on Bowen (2009).

Island	Area (km^2)	Number of Species (*S*)	Expected *S*	Pearson residual	*P*
Tiburón	1,223	349	292	**3.31**	**0.0005**
Ángel de la Guarda	936	215	269	**-3.29**	**0.0005**
San Esteban	41	114	101	1.31	0.0945
San Lorenzo (San Lorenzo Sur)	33	83	94	-1.15	0.1242
Las Ánimas (San Lorenzo Norte)	4.26	16	50	**-4.77**	**0.0000**
Nolasco	3.45	58	46	1.71	0.0440
Mártir	2.67	28	43	**-2.87**	**0.0020**
Alcatraz	1.44	54	35	**3.16**	**0.0008**
Partida Norte	1.36	16	35	**-3.17**	**0.0008**
Dátil	1.25	101	34	**11.58**	**0.0000**
Salsipuedes	1.16	15	33	**-3.13**	**0.0009**
Rasa	0.68	14	28	**-2.63**	**0.0043**
Patos	0.45	14	24	**-2.12**	**0.0170**
Cholludo	0.2	30	19	**2.53**	**0.0057**

Table 1.2. Pearson residual analysis for plant species-area relationship. Pearson residual scores and probabilities for islands with significantly more or fewer species than expected by chance are shown in boldface.

Source of variation	**X^2 deviance**	**d.f.**	***P***	**r^2**
Area^z	965.76	1	< 0.0001	0.822
Habitat diversity	118.59	2	< 0.0001	0.101
Island type	61.99	1	< 0.0001	0.053
Seabird dynamics	8.97	1	0.0027	0.008
Residuals	18.44.93	8	0.24	
Total	1173.75	13	< 0.0001	

Table 1.3. Analysis of variance. Results for the factors of diversity identified to significantly account for the variation from expected plant species diversity.

Island Group	Family with significant deviation	Observed	Expected	Pearson Residual
Species rich islands				
Tiburón and satellites	Polygonaceae	1	6.69	-2.20
Species poor islands				
Ángel de la Guarda	Polygonaceae	9	4	2.43
Bird Islands	Amaranthaceae	6	1.71	3.29
	Cactaceae	11	3.41	4.11
Equilibrium islands				
San Esteban or San Lorenzo	None			
San Pedro Nolasco	Poaceae	14	5.24	3.83

Table 1.4. Pearson residual analysis for plant family diversity. Pearson residual scores for plant families identified to be significantly abundant (positive values) or depauperate (negative) for the three island groups identified by the species-area model. Ángel de la Guarda and bird islands, as well as Islas San Esteban/San Lorenzo and San Pedro Nolasco, are analyzed separately due to known biogeographic differences (Felger & Wilder 2012).

Supplemental Appendix (see online supplemental information).

Appendix 1. Checklist for the flora of the Midriff Islands. Islands are presented in order of decreasing size. Sources are as follows, with recent additions included with voucher information as footnotes: Tiburón, Felger and Wilder (2012); Ángel de la Guarda, Moran (1983), Rebman (2010); San Esteban, Felger and Wilder (2012); San Lorenzo, Rebman et al. (2002); Las Ánimas, Rebman et al. (2002); Nolasco, Felger et al. (2011), Felger and Wilder (2012); Mártir, Wilder and Felger (2010), Felger and Wilder (2012); Alcatraz, Felger and Wilder (2012); Partida Norte, Rebman et al. (2002); Dátil, Felger and Wilder (2012); Salsipuedes, Rebman et al. (2002); Rasa, Velarde et al. 2014; Patos, Felger and Wilder (2012); Cholludo, Felger and Wilder (2012).

CHAPTER 2

LOCAL EXTINCTION AND UNINTENTIONAL REWILDING OF BIGHORN SHEEP (*OVIS CANADENSIS*) ON A DESERT ISLAND [1]

Abstract

Bighorn sheep (*Ovis canadensis*) were not known to live on Isla Tiburón, the largest island in the Gulf of California and Mexico, prior to the surprisingly successful introduction of 20 individuals as a conservation measure in 1975. Today, a stable island population of ~500 sheep supports limited big game hunting and restocking of depleted areas on the Mexican mainland. We discovered fossil dung morphologically similar to that of bighorn sheep in a dung mat deposit from Mojet Cave, in the mountains of Isla Tiburón. To determine the origin of this cave deposit we compared pellet shape to fecal pellets of other large mammals, and extracted DNA to sequence mitochondrial DNA fragments at the 12S ribosomal RNA and control regions. The fossil dung was ^{14}C-dated to 1476–1632 calendar years before present and was confirmed as bighorn sheep by morphological and ancient DNA (*a*DNA) analysis. 12S sequences closely or exactly matched known bighorn sheep sequences; control region sequences exactly matched a haplotype described in desert bighorn sheep populations in southwest Arizona and

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[1] Chapter two was published as presented here in 2014 in *PLoS ONE* 9(3): e91358. doi:10.1371/journal.pone.0091358, written by B.T. Wilder, J.L. Betancourt, C. Epps, R. Crowhurst, J.I. Mead, & E. Ezcurra, and reproduced in accordance with the permissions and conditions of the journal *PLoS ONE*.

southern California and showed subtle differentiation from the extant Tiburón population. Native desert bighorn sheep previously colonized this land-bridge island, most likely during the Pleistocene, when lower sea levels connected Tiburón to the mainland. They were extirpated sometime in the last ~1500 years, probably due to inherent dynamics of isolated populations, prolonged drought, and (or) human overkill. The reintroduced population is vulnerable to similar extinction risks. The discovery presented here refutes conventional wisdom that bighorn sheep are not native to Isla Tiburón, and establishes its recent introduction as an example of unintentional rewilding, defined here as the introduction of a species without knowledge that it was once native and has since gone locally extinct.

Introduction

As recorded in *Cmiique Iitom* —the language of the Seri people, an indigenous community of the coast of Sonora, Mexico and nearby Isla Tiburón— Orion's belt, *Hapj*, consists of three stars. The middle star represents the mule deer, *hap*, and the two flanking stars are bighorn sheep, *mojet*, and pronghorn antelope, *haamoja*. When the great hunter of the sky, *Azoj Cmiique* (Scorpius), fired his arrow, it struck *hap* but missed the others. After dripping onto Isla Tiburón, the mule deer's blood remained in the sky as the red star *Azoj haait* (Alpha or Betelgeuse). For the Seri, this myth explains why mule deer, but not bighorn sheep or pronghorn antelope, historically inhabited the island (Montaño Herrera 2010).

The events that have led to the formation of modern ecosystems, especially extinctions, are often cryptic in occurrence and causation. The anomalous absence of species in either the fossil record or on modern landscapes raises several questions. Did particular species once occur that are now lost? If so, what caused their extinctions, and are they reversible? How do we establish biological baselines to determine conservation priorities and strategies in the absence of historical data?

For example, controversial rewilding efforts to restore and even resurrect lost megafauna (Donlan et al 2005, 2006; Sandler 2013; Sherkow & Greely 2013) at the very least demand accurate baselines. In this paper, we coin the term "unintentional rewilding" to mean the introduction of a species, deliberate or otherwise, without knowledge that it was once native and has since gone locally extinct. Here are a couple of examples. In North America, European horses were introduced during the Spanish conquest, subsequently went feral, and unwittingly replaced native horses that were genetically the same but became extinct at the end of the Pleistocene (Hofreiter et al. 2001; Jansen et al. 2002). European domesticated horses gone wild are regarded by federal land management agencies in the USA as an exotic species that is harmful to native wildlife habitat and thus should be eradicated or reduced in numbers. Accepting these feral horses as native would challenge current management mandates within the federal government.

A second example involves bison. Extreme drought at the start of the twenty-first century drove bison from the adjacent Kaibab Plateau, where they were introduced and bred with cattle during the 1930s into Grand Canyon National Park, where bison are now trampling riparian areas and archeological sites. Holocene evidence for bison is scant in the Grand Canyon, and the modern herd may include bison-cattle hybrids, bolstering the National Park Service's case for removal. But what if the modern herd contains no hybrids and future paleontological evidence shows that bison occupied the Grand Canyon intermittently throughout the Holocene? Awareness of cases such as the North American horses and Grand Canyon bison will surely increase with expanded paleoecological studies and advances in genomics (Lorenzen et al. 2011; Murray et al. 2012), and will continue to raise fundamental questions about conservation targets and measures.

Here we report on the unintentional rewilding of bighorn sheep (*Ovis canadensis*) on Isla Tiburón in the Gulf of California, a few kilometers off the west coast of the State of Sonora and the largest island in Mexico (1,218 km^2; Fig. 2.1; Fig. 2.2). The Canal del Infiernillo —a narrow (~2–10 km wide) and shallow (~5.5 m deep) channel —separates the island from the mainland and was submerged by rising sea level only 6,000 – 4,700 cal yrs B.P. (Richman et al. 1988; Lambeck & Chappell 2001; Graham et al. 2003).

Mountain ranges on the mainland (the Sierra Seri) and on the island (the Sierra Kunkaak) are very similar in area and suitable habitat. Mule deer (*Odocoileus hemionus*) and desert bighorn sheep (*Ovis canadensis nelsoni*, see end of methods for a discussion on bighorn sheep taxonomy) are found on the coastal mainland today, and pronghorn (*Antilocapra americana sonoriensis*) occurred along the coastal plains in the past (Medellín et al. 2005). Given that the island was part of the Sonoran mainland as recently as ca. 6,000 years ago, we expect the past occurrence of these species on the island. However, mule deer (*Odocoilus hemionus sheldoni)*, an endemic subspecies, is the only non-introduced ungulate on Isla Tiburón, and all previous accounts of bighorn sheep and pronghorn antelope correspond to modern attempts to introduce them (Ezcurra et al. 2002).

The island has a diverse mammal fauna relative to other Gulf islands, but lacks top predators such as bobcat (*Lynx rufus*) and mountain lion (*Puma concolor*) that are common on the mainland. The reports of early explorers in the region confirm the historic absence of bighorn sheep on Tiburón. Charles Sheldon, an early 20th century naturalist and avid sportsman, hunted bighorn sheep on the mainland ranges of coastal Sonora and mule deer on Isla Tiburón in 1921, in each case accompanied by Seri guides. Sheldon's detailed field notes record the absence of bighorn sheep on Tiburón, as indicated in this passage: "The chief, Buro Alesan, tells me there are no wild sheep on Isla Tiburón, but a few are in the Sierra Seri" (Carmony & Brown 1983; Felger & Moser 1985).

In 1975, sixteen female and four male desert bighorn sheep were introduced from the Sonoran mainland adjacent to Isla Tiburón as a conservation measure (Montoya & Gates 1975; Hernández-Alvídrez & Campoy-Favela 1989; Medellín et al. 2005). The population grew rapidly to ~500 animals, where it seems to have reached its carrying capacity (DeForge & Valdez 1984; Lee & López-Saavedra 1994; Pallares 1999; Medellín et al. 2005). Low levels of hunting and other human disturbance, lack of mountain lion, absence of domesticated sheep and their contagious diseases, expansiveness of suitable habitat, and wetter conditions from 1976 to 1995 all probably contributed to introduction success. In agreement with early reports that bighorn sheep were not native to the island (Carmony & Brown 1983), all published studies about the transplanted population have treated the operation as an introduction of an alien species into a previously unoccupied ecosystem (Mellink 2002; Medellín et al. 2005).

For wildlife biologists, Isla Tiburón has become both a long-term field experiment and an object lesson in conservation. The introduction of bighorn sheep was not only successful in establishing a viable population but, through translocations back to Sonora, Tiburón animals also have contributed significantly to recovery efforts on the mainland. In 1995, a coalition of institutions initiated an innovative program to fund bighorn sheep research and conservation while providing needed income for the Seri through international auctioning of exclusive hunting tags on the island. Starting in 1999, hunting permits garnered six-figure auction bids (Navarro 1999); most recent prices range from US $80,000–90,000 a tag (Paulson 2009; Wild Sheep Foundation 2012). Revenue from these auctions offers the Seri incentive to maintain Isla Tiburón in an undisturbed state (Ezcurra et al. 2002; Medellín et al. 2005; Felger & Wilder 2012). To date, this conservation story has been regarded as controversial due to the non-native status of bighorn sheep on the island. The impact of unchecked bighorn sheep herbivory on the island's Sonoran Desert flora, which includes several regional endemic species (Felger & Wilder 2012), was not considered prior to the introduction.

During a recent survey for fossil woodrat (*Neotoma*) middens on Isla Tiburón, we discovered large pieces of an apparent sheep dung mat in Mojet Cave, a small rock shelter in the eastern foothills of the Sierra Kunkaak. Pellets from the recovered dung mat were ^{14}C-dated to 1476–1632 calendar years before present (cal yr B.P.). We used morphological and ancient DNA (*a*DNA) analyses to determine the identity of the species that deposited the pellets. The 'molecular caving' (Hofreiter et al. 2003) approach taken here adds a new dimension to the study of paleoenvironments in aridlands, and an opportunity to link theory and observations that address long-standing questions of lost populations and future conservation strategies.

Results

Morphological Identification

The Mojet Cave dung deposit contained both isolated complete pellets and those incorporated into an amorphous mat of crushed pellets; all were consolidated with crystallized urine. The pellets analyzed were small (averaging 15.5 x 10.1 mm; n=3), showing the characteristically blunt proximal ends and pointed distal ends that attribute them to bighorn sheep (Fig. 2.3). This morphology indicated that the pellets represented a dry-season diet, typical of desert vegetation throughout most of the year. Desert bighorn sheep diets focus on diverse array of desertscrub species, especially succulents (Tarango et al. 2002; Felger & Wilder 2012). Pellets of wapiti (*Cervus*; Cervidae), the extinct shrub-ox (*Euceratherium*; Ovibovinae), and the extinct Harrington's mountain goat (*Oreamnos harringtoni*; Rupicaprinae), all known to have inhabited mountains in the now arid Sonoran Desert during the late Pleistocene (Mead et al. 2005), are significantly larger in size, heavier, and have a conspicuously more robust pellet form (Mead et al. 1986; Kropf et al. 2007). Pellets of extant deer and pronghorn (including the extinct *Stockoceros*, which is known to have frequented shallow rock shelters) are similar in size with those of *Ovis*, yet they are characteristically and typically longer in form (Fig. 2.3).

Dung pellets of the living mountain goat (*Oreamnos americanus*) are distinctly smaller than those of *Ovis*. Thus, the morphology of the pellets from Mojet Cave suggests that bighorn sheep produced them.

Ancient DNA

From exterior scrapings of several ancient pellets within dung mat sample B (see methods for sample descriptions), we sequenced one 90 base pair (bp) region of the 12S ribosomal RNA subunit of the mitochondrial genome (GenBank accession number KF769974) from DNA amplified in 12 replicate PCRs (7 forward, 5 reverse), and the three control region fragments from 2–4 replicate PCRs (78–117 bp each; KF769975 [3 forward, 4 reverse], KF769976 [3 forward, 3 reverse], KF769977 [3 forward, 2 reverse]). Neither the exterior scrapings of pellets from dung mat sample A nor the interior pellet material from either mat amplified successfully. Neither of the two extraction controls amplified at any locus, and none of the blank PCR controls amplified at the 12S or control region fragments. Despite the relatively short length of the target fragments (12S and control region), none of the PCR amplicons were successfully sequenced in their entirety in only one direction, but bi-directional reads could be aligned with each other. We saw no evidence of competing sequences (indicating contamination by multiple templates), and sequences were consistent over multiple PCR replicates, except for variation at one location in numbers of repeated base pairs (3 or 4) identified by different people reviewing the sequences.

From a BLAST search, the consensus sequence for the 12S region of the midden sample clearly matched known bighorn sheep sequences, with 1–2 differences from published *O. c. canadensis* (Rocky Mountain bighorn sheep) sequences, and 0–1 differences from published *O. c. nelsoni* (desert bighorn sheep) sequences (Fig. 2.3). The ancient pellet 12S sequence differed from reference sequences for *Antilocapra americana* by 8 bp, from *Odocoileus hemionus* by 6 bp, from *Odocoileus virginianus* (white-tailed deer) by 7 bp, from *Oreamnos americanus* by 4 bp, from *Bos taurus* (domestic cattle) by 7 bp, and from *Ovis aries* (domestic sheep) by 3 bp (Fig. 2.4).

Phylogenetic analysis of the 46 bp alignment of ancient and reference 12S sequences (after trimming primer sites) resulted in 9 equally parsimonious trees each with 24 steps, of which the majority rule consensus appears in Fig. 2.5. The ancient DNA sequence is identical to one of the modern bighorn samples, and clusters within a clade that includes all desert bighorn sheep samples as well as the Rocky Mountain bighorn, mountain goat, and domestic sheep individuals. The short length of the sequence alignment made it impossible to resolve relationships within this clade unambiguously, but the exact match of the ancient DNA to one of the modern desert bighorn sheep leaves no doubt about the identity of that sample.

In total, we sequenced 280 bp of three control region fragments from the ancient sample (Fig. 2.6). The ancient sequence exactly matched two published desert bighorn sheep haplotypes (*O. c. nelsoni* haplotypes B and V, GenBank accession numbers AY903995, AY904015). Haplotypes B and V were differentiated by only 4 sites outside the three fragments that we sequenced for this project, and originated from southern

Mojave (B) (Epps et al. 2010) and the Sierra Pinta mountains of southern Arizona (V). The ancient sequence varied from each of the two known modern Tiburón bighorn sheep control region haplotypes (by 5 and 2 bp), and by one bp from a published sequence from Baja California identified as *O. c. weemsi* (Weem's desert bighorn sheep from Baja California). The ancient sequence varied from a published *O. c. canadensis* sequence originating in the Canadian Rocky Mountains (Hiendleder et al. 2002) at 11 sites. Lastly, there were 53–57 differences between the ancient DNA sequence and published haplotypes for *Ovis aries* (Fig. 2.5).

Discussion

The accumulation of plant and animal remains in aridland caves and rock shelters, sometimes imbedded in crystallized mammalian urine, offers unique opportunities for *a*DNA preservation and analysis. Although DNA molecules rapidly disintegrate after death and fossil DNA is highly fragmented (Marota & Rollo 2002), both the desiccation and protection within crystallized urine substantially slow DNA degradation (Chelomina 2006). Modern genetic techniques and diverse arrays of molecular primers for a multitude of taxa make it possible to analyze tiny amounts of DNA from dung, plant, and other material preserved in fossil middens (Murray et al. 2012). In the case of Mojet Cave, the bighorn sheep pellets in the urine-hardened mat yielded low-quality DNA that could still be amplified via primers designed from extant material. However, extreme temperatures and temperature fluctuations, UV-radiation, and humidity in the area may have compromised the preservation of these samples, leading to fragmentation of DNA strands and the truncated sequences we observed.

We conducted extractions and PCR setup for ancient DNA samples in a laboratory with no history of mammalian genetic research, used new reagents, and observed standard *a*DNA practices (Pääbo et al. 2004). We are confident that our *a*DNA sequences do not result from contamination. Sequence analysis of the *a*DNA recovered confirms that desert bighorn sheep deposited the pellets recovered from the Mojet Cave dung mat. Moreover, it is noteworthy that the *a*DNA control region haplotype does not match either of the two haplotypes recovered from 63 modern Tiburón sheep captured in 2007 (Gasca-Pineda et al. 2013) and previously reported in an unpublished study (GENBANK AY116622.1, AY116621.1).

We cannot specify whether bighorn sheep persisted or repeatedly colonized Isla Tiburón during the Holocene, or pinpoint when or why they became locally extinct during the past 1500 years. Population model simulations under two future climate change scenarios show that extinction risk for the Tiburón bighorn sheep population increases more with mean drought severity than with drought variability (Colchero et al. 2009). According to the tree-ring record, mean drought severity and variability have varied substantially during the past two millennia. Gridded reconstructions of the July Palmer Drought Severity Index for the two grid points nearest Tiburón (Cook et al. 2004) (centered at 27.5N, 110W and 30N, 112.6W) show increased mean drought severity from AD 400 to 1400, encompassing the Medieval Climate Anomaly (MCA). The tree-ring record is not long enough to evaluate if this dry period was unprecedented in the

Holocene, causing a unique extinction on Tiburón. Oceanic conditions can induce periods of extreme drought in the coastal deserts of the Gulf of California (Caso et al. 2007), which can greatly increase risk of population extinction of bighorn sheep in desert environments (Epps et al. 2004, 2006).

Bighorn sheep have a highly fragmented distribution through much of their current range, resembling a metapopulation in many regions, including the deserts of North America (Bleich et al. 1996). High rates of local population extinction in desert environments (Epps et al. 2004) and strong genetic drift (Epps et al. 2006) are counterbalanced to varying degrees by gene flow and recolonization, depending on population isolation and local climatic conditions (Epps et al. 2005, 2006, 2010). In the absence of gene flow, the small population size characteristic of desert bighorn sheep populations results in very rapid genetic drift and likely inbreeding (Epps et al. 2005). This situation is further exacerbated by the stochastic nature of precipitation and forage availability in Sonoran Desert systems, and potential hunting by indigenous peoples. The Seri lived off the seasonal bounty of the desert and sea (Felger & Moser 1985; Nabhan 2000), and it can be safely assumed that they occasionally hunted bighorn sheep. Bighorn sheep remains figure prominently in the archeological record of the Southwest, ranging from cremated remains in the Pinacate Mountains in northwestern Mexico (Hayden 1985) to widespread evidence of bighorn as a food resource in southern Arizona to the Colorado Plateau (Grant 1980; Nabhan 1993).

We hypothesize that isolation of the prehistoric Tiburón bighorn sheep population resulting from sea level rise, combined with subsequent drivers that act on small populations, including inbreeding, overharvesting by hunters, and megadroughts typical of Northern Mexico and the Southwestern U.S.A., figured in their local extinction. For similar reasons, the re-introduced population on Isla Tiburón likewise is vulnerable to extinction. Genetic diversity of the island population is demonstrably low, attributed mainly to genetic drift and lack of gene flow with other populations (Hendrick et al. 2001; Gasca-Pineda et al. 2013); low genetic diversity has been correlated with lower fitness in bighorn sheep (Johnson et al. 2011). The Tiburón population also was modeled to be susceptible to stochastic effects via increased aridity and warmer temperatures associated with climate variability and change (Seager et al. 2007), as well as continued extraction of animals for repopulation efforts on the mainland (Colchero et al. 2009).

Confirmation of the prior presence and local extinction of bighorn sheep on Isla Tiburón refutes their status as a non-native species. This extended baseline anchors bighorn sheep within the changing ecology of Isla Tiburón, furthers their importance as a focal point for conservation and management, and presents a cautionary tale. The introduction of bighorn sheep on Tiburón in 1975 is more justifiable than realized at the time. It raises other questions, however. Now that we know that bighorn sheep were native to the island, does this necessarily quell concern about the impacts of reintroduction? Did mountain lion, their main predator on the mainland, also occur on the island but suffer extinction at the same time as bighorn sheep? Does the absence of predators matter, or is it mimicked by current levels of trophy hunting that focuses on the healthiest animals, and not on the weak?

As evidenced by local extinction on Tiburón and throughout their range (Epps et al. 2005), bighorn sheep, as other large ungulate species, face increased extinction rates from future droughts exacerbated by warming (Colchero 2009; Duncan et al. 2012). The Tiburón bighorn sheep population is of critical significance as a source population for mainland introductions, great economic and cultural importance for the Seri community, and an indicator of environmental conditions. Careful management of trophy hunting and introductions of new animals from the mainland to broaden genetic diversity could help avoid another local extinction.

So how should we regard the reintroduction of bighorn sheep now that we know how recently it occurred naturally on the island? Is it a restoration or a biological invasion? This question should apply to most cases of rewilding and de-extinction efforts. Native plant communities on Isla Tiburón likely co-evolved with bighorn sheep and, given the short time since local extinction, should still be resilient to the perturbations caused by renewed herbivory. It is unlikely that local plants lost their defenses since the sheep's extinction, which happened sometime between 1500 and 100 years ago. Yet, the degree of herbivory on the island's flora by the modern bighorn sheep population may be greater than in the past. Mountain lion may or may not have also occurred on the island, and predator pressure on the introduced population now may be greatly diminished.

In other cases of rewilding, exactly how long is required for the remaining native species to evolve and lose their defenses and resilience? Native horses have been gone from North America for more than 10,000 years, and so have most of their predators. This could have had evolutionary consequences in plant and animal communities that eroded their potential resilience to rewilding. Even if bighorn "belong" on Tiburón, as well as horses in North America, does this necessarily absolve wildlife managers and conservationists from considering any and all unintended impacts of rewilding?

Finally, we recognize that our discovery of the bighorn sheep deposit in a remote cave on Isla Tiburon also was unintentional. We did not set out to test whether or not bighorn sheep were native to the island; we were actually looking for something else. We now envision strategic and purposeful 'molecular caving' to pinpoint the timing and circumstances for other Holocene extinctions, and thus inform conservation efforts, in the Gulf of California and other arid regions worldwide.

Methods

To better understand past plant communities and the origin of the modern Sonoran Desert, fossil packrat (*Neotoma* spp.) middens were collected on Isla Tiburón in March 2012. Among the middens we found a different type of urine-hardened deposit containing ungulate dung in a rock shelter at 235m elevation on Hast Coopol, a low-lying volcanic peak on the eastern foothills of the Sierra Kunkaak (Fig. 2.1).

Thick crusts of urine-hardened sheep dung commonly line the floors of caves and rock shelters occupied by both wild and domestic sheep worldwide. Domestic sheep (*Ovis aries*) are not known to have inhabited the island. Bighorn sheep (*O. canadensis*) are the only late Pleistocene wild sheep (*Ovis* sp.) known to inhabit the Intermountain West and south into Mexico (Valdez & Krausman 1999; Mead et al. 2005; Groves & Grubb 2011). Unlike other extant Sonoran Desert artiodactyls (e.g., pronghorn, and mule deer), bighorn sheep commonly use caves and rock overhangs to bed and escape the midday heat in summer (Cain et al. 2008) and their kidneys concentrate urine to conserve water (Horst 1971). As it evaporates, the viscous urine can crystallize and cement both sediment and dung on the cave floor, much like the process that forms packrat and other rodent middens (Mead et al. 2003).

The rock shelter on Isla Tiburón named here Mojet Cave, (mojet ['mox ɛ t], the Seri name for bighorn sheep; Moser & Marlett 2010) is about 8 m deep and 15 m wide. Two blocks or mats of artiodactyl dung (sample A consisted of two small pieces both ~20×10×8 cm, and sample B one large mat ~40×30×10 cm) were collected amid roof fall on the cave floor, following the same protocols used for sampling packrat middens (Spaulding et al. 1990). Five fecal pellets taken from both the top and bottom layers of dung mat sample B were sent to the UC, Irvine W. M. Keck Carbon Cycle Accelerator Mass Spectrometry Laboratory for radiocarbon dating. All pellets dated fell within the age range of 1595–1725 ± 20 ^{14}C yr B.P., which was calibrated to a mean age of 1476–1632 calendar years before present (cal yr B.P.) using the Intcal13 calibration (Reimer et al. 2013). A pellet from the bottom of the midden dated to 1530 ± 15 ^{14}C yr B.P. and one from the top as 1625 ± 20 ^{14}C yr B.P. That the dates are stratigraphically reversed indicates the deposit likely formed at once. Two independent methods, morphological identification and ancient DNA analysis were used to identify the fossil pellets. The same pellets used for *a*DNA extraction were dated to 1720 ± 20 and 1725 ± 20 ^{14}C yr B.P. Those used for morphological identification were not dated to preserve the intact nature of the pellet and are deposited in the East Tennessee Vertebrate Paleontology Laboratory

(Eastern Tennessee State University) under collection number ETVP 4999. All necessary permits were obtained for the described study, which complied with all relevant regulations. The material for this study was collected under collector's permit FAUT–0265 granted to Exequiel Ezcurra by the Secretaría de Medio Ambiente y Recursos Naturales (SEMARNAT; authorization document SGPA/DGVS/02213-13).

Morphological Identification

We compared identically sized and shaped pellets as the one dated with an extensive dung collection of both modern and extinct herbivores housed in the East Tennessee Vertebrate Paleontology Laboratory. Isolate dung pellets of adult *Cervus* and the extinct *Euceratherium*, *Symbos*, and *Oreamnos harringtoni* typically have a ratio of the width:length (measurements in mm) versus the weight (g) distinctly greater than that produced by *Ovis*, *Antilocapra*, and the living *Oreamnos americanus*; weights greater than 0.5 g readily distinguish these larger ungulates from *Ovis* and *Antilocapra* (Kropf et al. 2007). Isolated pellets of living adult *Ovis* spp., *Oreamnos*, *Antilocapra*, and the extinct *Stockoceros* can overlap in weight (all typically weigh less than 0.3 g in weight). When considering size/shape, pronghorns will more often have a longer pellet, with width:length ratios greater than 1:1, while most often *Ovis* spp. produce a pellet ratio between 0.9 to 0.5, creating their more cuboid appearance (Mead et al. 1986). This can vary with a more boreal and green vegetation diet. The classification of the pellets from Mojet Cave as *Ovis* is based on gross morphology and should be viewed as a 'best fit' identification.

Ancient DNA

To substantiate our identification from pellet morphology we also sequenced DNA from the fossilized pellets. We observed basic tenets of ancient DNA handling (Pääbo 1990; Pääbo et al. 2004; Willserslev & Cooper 2005) by 1) restricting all handling of material prior to PCR amplification to a laboratory at Oregon State University that had never been used for genetic research or mammalian research of any kind; 2) restricting laboratory equipment used in all pre-PCR operations to equipment that had never been used with mammalian genetic samples; 3) only allowing personnel to enter the ancient DNA laboratory if they had not previously entered the modern DNA laboratory that day. The modern DNA laboratory is on a different floor within the same building. We used only newly-purchased reagents, bleached surfaces between extractions, and autoclaved supplies. We attempted to extract and amplify DNA from pellets from two different dung deposits within the larger dung mat (sample B). We scraped the exterior of pellets (where epithelial cells are concentrated; Wehausen et al. 2004) with a bleached and flamed razor blade, but also collected dust from the interior of the pellets for a second extraction, in the event that the exterior surface had been contaminated or degraded. Due to the small size of the pellets, we used two pellets for each extraction of surface material; inner material for the second extraction was taken from a single pellet. We used the Aquagenomics/AquaPrecipi DNA extraction kit (Multitarget Pharmaceuticals, Utah), to extract DNA from 0.03 g of scraped dust from each replicate. We added 15 mAU of proteinase K (Qiagen, California) and approximately 0.2mL of 1.0mm zirconia beads

(Biospec, Oklahoma) to the dust, vortexed briefly to lyse the cells, and incubated the samples for one hour at 60°C. Using the same protocol, we conducted two blank extractions to detect contamination of reagents or supplies. Samples were rehydrated with 100uL of 1x TE buffer (pH 8.0) and stored at 4°C.

We attempted to amplify fragments of varying lengths to evaluate the *a*DNA principle that amplification success will increase with decreasing fragment size (Pääbo et al. 2004). As expected, efforts to amplify a 350bp fragment of the 16S ribosomal RNA gene (Table 2.1) that has previously been used for species identification of ungulate pellets were unsuccessful, with the exception of one contaminated PCR negative that was shown by BLAST-search to have amplified human DNA. Instead, we used Primer3 (Koressaar & Remm 2007; Untergrasser et al. 2012) to design primer pairs to amplify a ~90bp fragment of the 12S ribosomal RNA gene on the mitochondrial genome (Table 2.1). Using sequences published on GenBank (see Table 2.2 for accession numbers), we designed primers for locations that we identified as largely conserved but with variation between priming sites across mule and white tailed deer (*Odocoileus hemionus* and *Odocoileus virginianus*, respectively), mountain goats (*Oreamnos americanus*), pronghorn antelope (*Antilocapra americana*), domestic sheep (*Ovis aries*), domestic cattle (*Bos taurus*), and bighorn sheep (*Ovis canadensis*). We used the polymerase chain reaction (PCR) to amplify this gene fragment in reactions consisting of 5x Qiagen Multiplex PCR master mix, 5 μg bovine serum albumin, 0.2 μM of each primer, and 0.5 μL of DNA extract and brought the reaction to a 10 μL reaction volume with nuclease-free molecular grade water. Thermalcycling conditions were as follows: 15

minutes at 95°C, followed by 35 cycles of [95°C for 30s, 54°C for 90s, 72°C for 60s] and a final elongation of 30 minutes at 60°C. We included a negative control (molecular grade water) in each PCR run to monitor for contamination, and also attempted to amplify the two negative extraction controls. We verified amplification using an agarose gel stained with GelRed (Biotium, California) and used 2 μ L shrimp alkaline phosphatase (ExoSAP-IT, Affymetrix, Santa Clara, CA) to prepare 4 μ L of DNA from each amplicon for sequencing. PCR products were submitted to the Molecular Research Core Facility at Idaho State University for bidirectional sequencing on an ABI 3130XL DNA analyzer. We also sequenced the same section of the12S gene in one bighorn sheep sample from each of: Chocolate Mountains (CA), Marble Mountains (CA), Orocopia Mountains (CA), and the Sierra Pintas (AZ). These samples were chosen to assess potential variation in the 12S gene in desert bighorn sheep across a broad geographical area, and because this subspecies (*O. c. nelsoni*) was not represented in GenBank at this 12S gene.

To compare variation between the fossil haplotypes and those of modern bighorn sheep we also designed primers for three short sections of the mitochondrial DNA control region (~80–120 bp each; Table 2.1; Fig. 2.6) within a 515 bp region previously sequenced for numerous modern bighorn sheep populations (Hiendleder et al. 2002). Because of the highly degraded nature of ancient DNA, we did not attempt to sequence this entire region, but chose three sections that 1) showed variability in published sequences, and 2) supported reliable primers in conserved regions. We designed primers using sequences published on GenBank for bighorn sheep described as *O. c. nelsoni*, *O. c.*

weemsi, and *O. c. mexicana* to capture variation across a wide range of desert bighorn sheep (Table 2.2), and included two published haplotypes from bighorn sheep descended from individuals translocated to Isla Tiburón during the 1970s (accession numbers AY116621 and AY116622, Table 2.S2). Amplification of the control region fragments followed the same PCR recipe and cycling conditions as 12S.

Because of the degraded nature of most ancient DNA and the possibility that base modifications over time could alter the sequences, for the ancient pellets, we attempted to amplify and sequence each targeted region in a minimum of three separate PCR reactions. Raw chromatogram traces were trimmed to the length at which all bases could be unambiguously identified. We verified base calls, searched each sequence on NCBI BLAST, and aligned all replicate sequences in Geneious (v.6.1.2; Biomatters, available from http://www.geneious.com/) and had two other experienced researchers independently do the same. In case of a discrepancy between calls, we reviewed sequences again with a fourth experienced observer and corrected obvious errors, after which we selected the call made by the majority of observers. Discrepancies, when they arose, typically resulted from difficulty in interpreting strings of single repeated base pairs (e.g., AAAA). We generated consensus sequences, resolving discrepancies based on majority of calls and re-evaluation of the original sequences, and compared them with sequences published on Genbank for other candidate species (Table 2.2). In that comparison, we included 4 published sequences from domestic sheep (*O. aries,* GenBank AF010406, EF490455, NC001941, and HE577848) and two from domestic cattle (*Bos taurus*, GenBank AB074968 and AF492351) to rule out contamination from domestic animals in our lab reagents. Published sequences were selected from different studies to capture intraspecific diversity. However, as we observed no intraspecific diversity in these two species for this 12S fragment, we included only one example for each species in further analyses.

To confirm the species identity of the ancient DNA sample and rule out the possibility of contamination from other mammals, we conducted a phylogenetic analysis on the ancient 12S sequence and reference sequences for other wild and domestic artiodactyls that we downloaded from GenBank (a 46 bp region after trimming primers). While molecular data are most frequently analyzed with model-based likelihood or Bayesian methods, those methods treat alignment gaps due to insertions and deletions as missing data, and require moderately long sequence alignments in order to infer an appropriate model of nucleotide substitution. Our very short ribosomal alignment includes several indels that clearly demonstrate phylogenetic signal; treating these as missing data in a pilot likelihood analysis resulted in a loss of almost all phylogenetic resolution in the resulting consensus. To capture the signal in the indels, we inferred a phylogeny under parsimony in PAUP* 4.0 beta (Swofford 2003), while treating gaps as a fifth character state, using *Antilocapra americana* as the outgroup and conducting 1000 heuristic searches with random addition sequences.

The taxonomy of bighorn sheep subspecies remains somewhat confused. Cowan originally described seven subspecies of bighorn sheep (Cowan 1940). However, recent genetic and morphometric evaluations (Ramey 1993, 1995, 1999; Wehausen & Ramey 1993, 2000; Wehausenet al. 2005) demonstrated that there are likely only three valid subspecies: *O. c. canadensis* (including bighorn sheep formerly described as *O. c. canadensis, O. c. auduboni* and *O. c. californiana* excepting populations in the Sierra Nevada mountains of California), *O. c. nelsoni* (desert bighorn sheep, including bighorn sheep formerly described as *O. c. nelsoni*, *O. c. mexicana*, *O. c. cremnobates*, and likely *O. c. weemsi* although insufficient specimens were available to confirm the validity of *O. c. weemsi*), and *O. c. sierrae* (populations in the Sierra Nevada Mountains of California, previously described as *O. c. californiana*).

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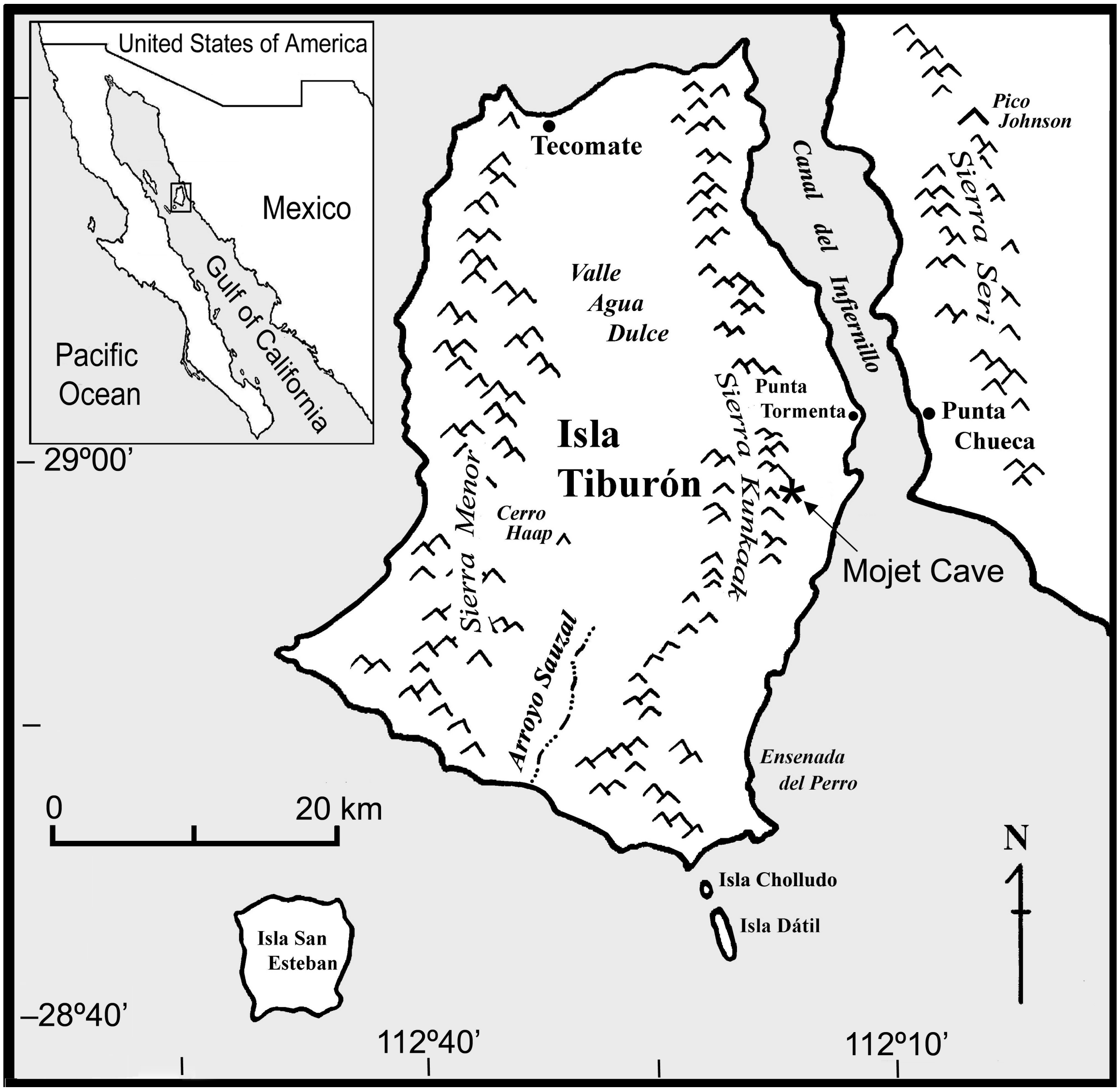

Fig. 2.1. Isla Tiburón in Gulf of California. By Cathy Moser Marlett.

Figure 2.2. Bighorn sheep (*Ovis canadensis*) skull on Isla Tiburón. Skull from bighorn sheep population introduced on Isla Tiburón, Gulf of California, in 1975. Radiocarbon dating and ancient DNA analysis from a local cave now show that this introduction unintentionally re-established bighorn sheep that had gone extinct on the island. (Photo by B.T. Wilder).

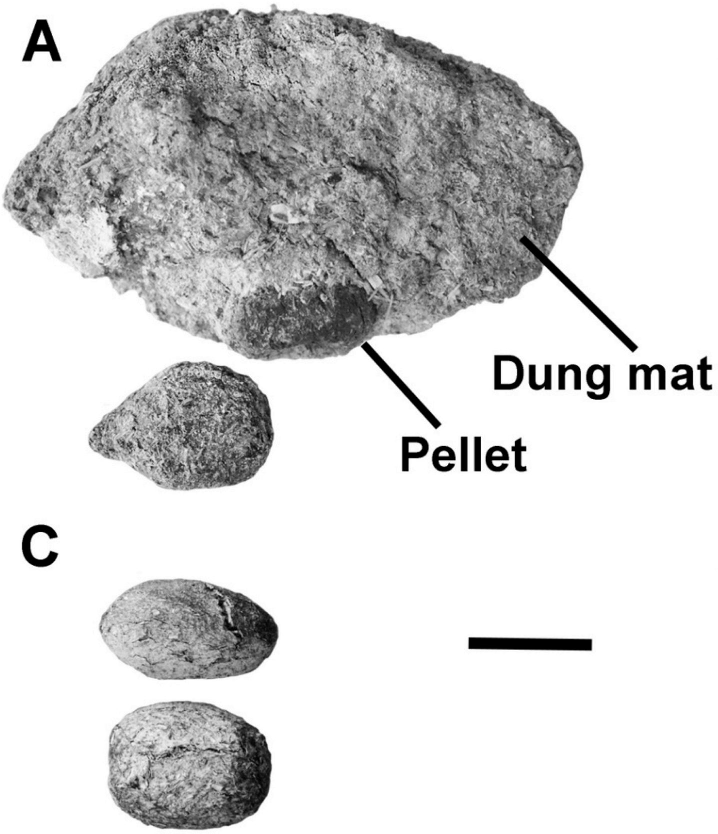

Fig. 2.3. Dung from Mojet Cave and recent potential dung producers. A) dung pellet and mat (ETVP 4999) from Mojet Cave; identified as bighorn sheep, *Ovis canadensis* (see text). Note pellet in dung mat is featured below after removal. Opposite side of dung mat has a thin layer of bat guano adhering to the surface. Recent dung: B) bighorn sheep, *Ovis canadensis* (ETVP 6083); C) pronghorn antelope, *Antilocapra americana* (ETVP 6028); C) mule deer, *Odocoileus hemionus* (ETVP 6017). Scale bar equals 10 mm.

Accession Number	Species	680										690										700									
NC015889	*Ovis canadensis canadensis*	A	T	A	C	C	G	C	C	A	T	C	T	T	C	A	G	C	A	A	A	C	C	C	T	A	A	A	A	A	A
JN181255	*Ovis canadensis canadensis*	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
OCU86983	*Ovis canadensis canadensis*	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	.	.	.	.	.
AF010406.1	*Ovis aries*	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
JN632597	*Antilocapra americana*	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
AF091706	*Antilocapra americana*	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
FJ207535	*Oreamnos americanus*	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	.	.	.	.	.
JN632670	*Odocoileus hemionus*	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	.	.	.	.	.
JN632672	*Odocoileus virginianus*	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	.	.	.	.	.
KF769971	*Ovis canadensis nelsoni*, Sierra Pintas, AZ	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	.	.	.	.	.
KF769972	*Ovis canadensis nelsoni*, Chocolate Mountains, CA	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	.	.	.	.	.
KF769973	*Ovis canadensis nelsoni*, Marble Mountains, CA	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	.	.	.	.	.
KF769974	Isla Tiburon aDNA	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	.	.	.	.	.

		710										720											730								
	Ovis canadensis canadensis	G	G	A	A	C	A	A	A	A	G	T	A	A	G	C	T	C	A	A	T	-	A	A	T	A	A	T	A	C	A
	Ovis canadensis canadensis	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	.	.	.	.	.	.	.	.	.
	Ovis canadensis canadensis	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	.	.	.	.	.	.	.	.	.
	Ovis aries	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	.	.	.	.	.	C	.	.	.
	Antilocapra americana	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	A	T	.	.	.	-	.	.	.	.	G	C	.	.	.
	Antilocapra americana	.	.	.	.	.	.	.	G	.	.	.	N	.	.	.	A	T	.	.	.	-	.	.	.	.	G	C	.	.	.
	Oreamnos americanus	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	-	.	G	.	C	.	.	.
	Odocoileus hemionus	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	C	.	C	.	.	-	.	.	.	.
	Odocoileus virginianus	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	C	.	C	.	.	-	.	.	.	.
	Ovis canadensis nelsoni, Sierra Pintas, AZ	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	.	-	.	.	.	.	.	.	.	.	.
	Ovis canadensis nelsoni, Chocolate Mountains, CA	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	.	.	.	.	.	.	.	.	.
	Ovis canadensis nelsoni, Marble Mountains, CA	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.
	Isla Tiburon aDNA	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	.	-	.	.	.	.	.	.	.	.	.

			740										750										760								
	Ovis canadensis canadensis	T	A	A	A	G	A	C	G	T	T	A	G	G	T	C	A	A	G	G	T	G	T	A	A	C	C	T	A	T	G
	Ovis canadensis canadensis	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
	Ovis canadensis canadensis	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
	Ovis aries	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
	Antilocapra americana	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
	Antilocapra americana	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
	Oreamnos americanus	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
	Odocoileus hemionus	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
	Odocoileus virginianus	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
	Ovis canadensis nelsoni, Sierra Pintas, AZ	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
	Ovis canadensis nelsoni, Chocolate Mountains, CA	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
	Ovis canadensis nelsoni, Marble Mountains, CA	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
	Isla Tiburon aDNA	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.

Fig. 2.4. Isla Tiburón *a*DNA and Sonoran Desert ungulate sequences. Partial sequences of the 12S ribosomal RNA subunit of the mitochondrial genome for the Isla Tiburón *a*DNA sample and published sequences from all ungulate species thought to have existed in the area since the start of the Holocene, with GenBank accession numbers. Open box denotes primer region, dot indicates identical bases between sequences, N indicates unknown base. Sequence position numbers are derived from *O. c. canadensis* haplotype (GenBank Accession NC015889).

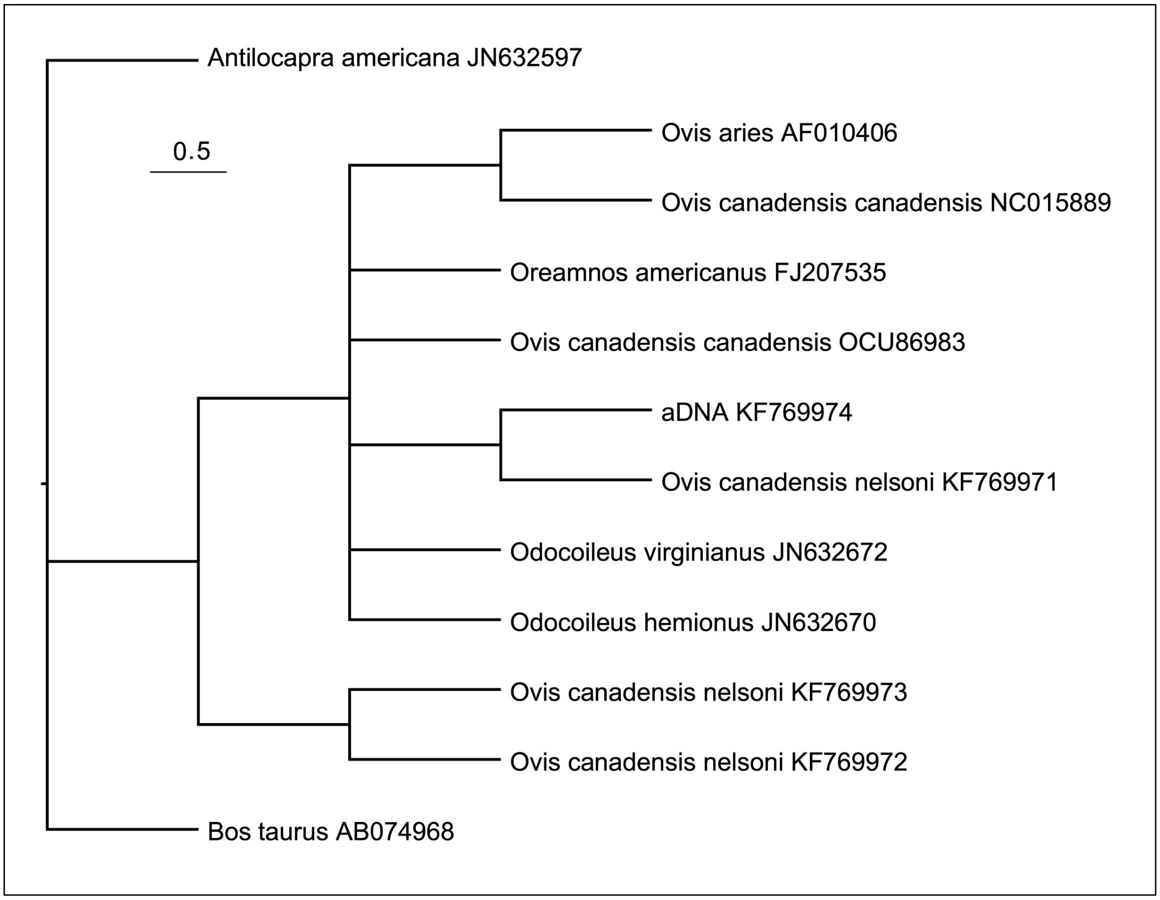

Fig. 2.5. Phylogenetic tree for Isla Tiburón *a*DNA and ungulate sequences. Majority rule consensus tree from phylogenetic analysis of a 46 bp region of the 12S ribosomal RNA subunit of the mitochondrial genome, including the Isla Tiburón *a*DNA sample and published sequences from all ungulate species thought to have existed in the area since the start of the Holocene, with GenBank accession numbers. The phylogeny was inferred using parsimony and treating indels as a 5th character state.

a)

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Accession Number  Species                                    10                  20                  30                  40
AY903995          O. c. nelsoni, haplotype B          A G C G G G T T G T T G G T T T C A C G C G G C A T G G T A G A T A A
AY904015          O. c. nelsoni, haplotype V          . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
AY903996          O. c. nelsoni, haplotype C          . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
AY904011          O. c. nelsoni, haplotype R          . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
AY904013          O. c. nelsoni, haplotype T          . . . . . . . . . . . . . . . . . . . . . . . . . . . . . G . . . . .
AY116623          O. c. weemsi                        . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
AY116621          O. c. mexicana, Tiburon-haplotype1  . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . C . .
AY116622          O. c. mexicana, Tiburon-haplotype2  . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
AF010407          Ovis aries                          . . . . . . . . . . . . . . . . . . . . . . . . . . . . . G A . C . .
AY091499          Ovis aries                          . . . . . . . . . . . . . . . . . . . . . . . . . . . . . G A . C . .
KF769975          Isla Tiburon aDNA                   . . . . . . . . . . . . . . . N . . . . . . . . . . . . . . . . . . .
AY091486          O. c. canadensis                    . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .

                                                                       50                  60                  70
                  O. c. nelsoni, haplotype B          G C T C G T G A T C T A G T G G A C A G G A T A C G C A T G T T G A C
                  O. c. nelsoni, haplotype V          . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
                  O. c. nelsoni, haplotype C          . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
                  O. c. nelsoni, haplotype R          . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
                  O. c. nelsoni, haplotype T          . . . . . . . . . . . . . . . . . . G . . . . . . . . . . . . . . . .
                  O. c. weemsi                        . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
                  O. c. mexicana, Tiburon-haplotype1  . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
                  O. c. mexicana, Tiburon-haplotype2  . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
                  Ovis aries                          . . . . . . . . . . . . . . . . . . G . . . . . T . . . . . . . . . .
                  Ovis aries                          . . . . . . . . . . . . A . . . . . . . . . . . . . . . . . . . . . .
                  Isla Tiburon aDNA                   . N N N N N N . . . . . . . . . . . . . . . . . . . . . . . . . . . .
                  O. c. canadensis                    . . . . . C . . . . . . . . . . G . . . . . . . . . . . . . . . . . .

                                                             80
                  O. c. nelsoni, haplotype B          T A G A A C G G
                  O. c. nelsoni, haplotype V          . . . . . . . .
                  O. c. nelsoni, haplotype C          . . . . . . . .
                  O. c. nelsoni, haplotype R          . . . . . . . .
                  O. c. nelsoni, haplotype T          . . . . . . . .
                  O. c. weemsi                        . . . . . . . .
                  O. c. mexicana, Tiburon-haplotype1  . . . . . . . .
                  O. c. mexicana, Tiburon-haplotype2  . . . . . . . .
                  Ovis aries                          . . . G . . . .
                  Ovis aries                          . . . A . T . .
                  Isla Tiburon aDNA                   . . . . . . . .
                  O. c. canadensis                    . G . G . . . .
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Figure 2.6a

b)

Accession Number	Species						70										80										90									
AY903995	*O. c. nelsoni,* haplotype B	C	G	C	A	T	G	T	T	G	A	C	T	A	G	A	A	C	G	G	A	T	T	T	G	A	C	T	T	T	A	T	G	T	G	C
AY904015	*O. c. nelsoni,* haplotype V	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
AY903996	*O. c. nelsoni,* haplotype C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
AY904011	*O. c. nelsoni,* haplotype R	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
AY904013	*O. c. nelsoni,* haplotype T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
AY116623	*O. c. weemsi*	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
AY116621	*O. c. mexicana,* Tiburon-haplotype1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
AY116622	*O. c. mexicana,* Tiburon-haplotype2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
AF010407	*O. aries*	T	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	A	.
AY091499	*O. aries*	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	T	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	A	.
KF769976	Isla Tiburon aDNA	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
AY091486	*O. c. canadensis*	.	.	.	.	.	.	.	.	.	.	.	.	G	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.

	100										110										120										130				
O. c. nelsoni, haplotype B	C	A	T	G	T	A	C	G	A	A	C	A	A	G	C	A	G	T	T	G	A	A	T	G	T	C	C	C	A	T	G	T	A	C	T
O. c. nelsoni, haplotype V	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
O. c. nelsoni, haplotype C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
O. c. nelsoni, haplotype R	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
O. c. nelsoni, haplotype T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
O. c. weemsi	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
O. c. mexicana, Tiburon-haplotype1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
O. c. mexicana, Tiburon-haplotype2	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
O. aries	T	.	.	.	.	.	.	.	G	T	.	.	.	.	.	.	.	.	.	C	.	.	.	.	C	.	.	T	.	C	A	.	G	.	.
O. aries	T	.	.	.	.	.	.	.	G	T	.	.	.	.	.	.	.	.	.	T	.	.	.	A	C	.	.	T	.	C	A	.	G	.	.
Isla Tiburon aDNA	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	N	.	.	.	.	.	.	.	.	.	.	.	.	.	.
O. c. canadensis	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.

						140										150										160								
O. c. nelsoni, haplotype B	T	T	A	A	T	G	A	A	G	T	T	A	A	T	G	T	G	C	T	T	G	C	T	T	A	T	A	T	G	C	A	T	G	G
O. c. nelsoni, haplotype V	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
O. c. nelsoni, haplotype C	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
O. c. nelsoni, haplotype R	.	.	G	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
O. c. nelsoni, haplotype T	.	.	.	G	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
O. c. weemsi	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
O. c. mexicana, Tiburon-haplotype1	.	.	G	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
O. c. mexicana, Tiburon-haplotype2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
O. aries	.	C	.	G	.	.	.	.	.	.	G	.	.	.	.	.	A	.	A	.	.	.	C	.	.	.	.	C	.	.	.	.	.	.
O. aries	.	C	.	G	.	.	.	.	A	C	A	.	.	.	.	.	A	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Isla Tiburon aDNA	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
O. c. canadensis	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A

Figure 2.6b

c)

```
Accession Number  Species                                             320                 330                 340
AY903995          O. c. nelsoni, haplotype B          A C A T T C A A G G T G G A C A T A G G G T A T A T T - G A T T T G T G
AY904015          O. c. nelsoni, haplotype V          . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
AY903996          O. c. nelsoni, haplotype C          . . . . . . . . . . . . . . . . . . . . . . . C . . . . . . . . . . . .
AY904011          O. c. nelsoni, haplotype R          . . . . . . . . . . . . . . . . . . . . . . . C . . . . . . . . . . . .
AY904013          O. c. nelsoni, haplotype T          . . . . . . . . . . . . . . . . . . . . . . . C . . . . . . . . . . . .
AY116623          O. c. weemsi                        . . . . . . . . . . . . . . . . . . . . . . . C . . . . . . . . . . . .
AY116621          O. c. mexicana, Tiburon-haplotype1  . . . . . . . . . . . . . . . . . . . . . . . C . . . . . . . . . . . .
AY116622          O. c. mexicana, Tiburon-haplotype2  . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
AF010407          O. aries                            . . . . . . . . . A C A . . . . . . A . A . . . . . . G . G . . . A . .
AY091499          O. aries                            . T G . . T . . . A C A . . . . . . A . A . . . G . . G . G . . . A . .
KF769977          Isla Tiburon aDNA                   . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
AY091486          O. c. canadensis                    . . . . . . . . . . . . . . . . . . . . . C . C . . . . . . . . . . . .

                                                                        350                 360                   370
                  O. c. nelsoni, haplotype B          - - - - - - G A T G T C C G T G G G T G G A T G G - T G T G C T T T T C
                  O. c. nelsoni, haplotype V          . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
                  O. c. nelsoni, haplotype C          . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
                  O. c. nelsoni, haplotype R          . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
                  O. c. nelsoni, haplotype T          . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
                  O. c. weemsi                        . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
                  O. c. mexicana, Tiburon-haplotype1  . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
                  O. c. mexicana, Tiburon-haplotype2  . . . . . . . . . . . . . . . . . . . . . G . . . . . . . . . . . . . .
                  O. aries                            A A C G C T C G . . . . . . . . . . . . . . . . . T . . . . . . . . . .
                  O. aries                            A A C G C T C . . . . . . . . . . . . . . G . . . T . . . . . . . . . .
                  Isla Tiburon aDNA                   . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
                  O. c. canadensis                    . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .

                                                              380                 390                 400                 410
                  O. c. nelsoni, haplotype B          T T G T A T G G G T A T G G G T T G T T G T T A T G T G A G C T T G T G
                  O. c. nelsoni, haplotype V          . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
                  O. c. nelsoni, haplotype C          . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
                  O. c. nelsoni, haplotype R          . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
                  O. c. nelsoni, haplotype T          . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
                  O. c. weemsi                        . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
                  O. c. mexicana, Tiburon-haplotype1  . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
                  O. c. mexicana, Tiburon-haplotype2  . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
                  O. aries                            . . . . . . . . . - - - - - - - - - . . . . . . . . . . G . T . . . . .
                  O. aries                            . . . . . . . . . - - - - - - - - - . . . . . . . . . . G . . . . . . .
                  Isla Tiburon aDNA                   . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .
                  O. c. canadensis                    . . . . . . . . . . G . . . . . . . . . . . . . . . . . . . . . . . . .

                                                                      420
                  O. c. nelsoni, haplotype B          G A G T G G A A A G T C C G T G T
                  O. c. nelsoni, haplotype V          . . . . . . . . . . . . . . . . .
                  O. c. nelsoni, haplotype C          . . . . . . . . . . . . . . . . .
                  O. c. nelsoni, haplotype R          . . . . . . . . . . . . . . . . .
                  O. c. nelsoni, haplotype T          . . . . . . . . . . . . . . . . .
                  O. c. weemsi                        . . . . . . . . . . . . . . . . .
                  O. c. mexicana, Tiburon-haplotype1  . . . . . . . . . . . . . . . . .
                  O. c. mexicana, Tiburon-haplotype2  . . . . . . . . . . . . . . . . .
                  O. aries                            . . . . . . G . . . . . . . . . .
                  O. aries                            . . . . . . G . . . . . . . . . .
                  Isla Tiburon aDNA                   . . . . . . . . . . . . . . . . .
                  O. c. canadensis                    . . . . . . . . . . . . . . . . .
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Figure 2.6c.

Fig. 2.6. Control regions of Isla Tiburón *a*DNA and select bighorn sheep populations. Partial control region sequences of Isla Tiburón *a*DNA sample and published bighorn sheep samples, sequenced as three fragments (a-c). Open boxes denote primer region, dot indicates identical bases between sequences, dash indicates deletion, N indicates unknown base. Sequence position numbers are derived from the reverse complement of *O. c. nelsoni* haplotype B (Epps et al. 2005; GenBank Accession AY903995).

Gene	Primer sequence	Fragment size (bp)
16S ribosomal RNA	F: AAG ACG AGA AGA CCC TAT GGA R: CCG GTC TGA ACT CAG ATC ACG T	~350
12S ribosomal RNA	F: ATA CCG CCA TCT TCA GCA AA R: CAT AGG TTA CAC CTT GAC CTA ACG	89
Control region, fragment 1	F: AGC GGG TTG TTG GTT TCA C R: CCG TTC TAG TCA ACA TGC GTA	78
Control region, fragment 2	F: CGC ATG TTG ACT AGA ACG GAT T R: CCA TGC ATA TAA GCA AGC ACA	104
Control region, fragment 3	F: ACA TTC AAG GTG GAC ATA GGG R: ACA CGG ACT TTC CAC TCC AC	117

Table 2.1. Primer sequences and fragment size. The genes sequenced or attempted from ancient fecal pellets from Isla Tiburón, Mexico.

Accession number	Species	Gene
JN632597.1	*Antilocapra americana*	mitochondrion, complete genome
JN632670.1	*Odocoileus hemionus*	mitochondrion, complete genome
JN632672.1	*Odocoileus virginianus*	mitochondrion, complete genome
FJ207535.1	*Oreamnos americanus*	mitochondrion, complete genome
JN181255.1	*Ovis canadensis canadensis*	mitochondrion, complete genome
NC_015889.1	*Ovis canadensis canadensis*	mitochondrion, complete genome
AF091706.1	*Antilocapra americana*	12S ribosomal RNA gene, partial sequence
OCU86983	*Ovis canadensis canadensis*	12S ribosomal RNA gene, partial sequence
AY091486	*Ovis canadensis canadensis*	control region, complete sequence
AY116621	[1]*Ovis canadensis mexicana*	control region, partial sequence, Tiburón-hap1
AY116622	[1]*Ovis canadensis mexicana*	control region, partial sequence, Tiburón-hap2
AY903995	*Ovis canadensis nelsoni*	control region, partial sequence, haplotype B
AY903996	*Ovis canadensis nelsoni*	control region, partial sequence, haplotype C
AY904011	*Ovis canadensis nelsoni*	control region, partial sequence, haplotype R
AY904013	*Ovis canadensis nelsoni*	control region, partial sequence, haplotype T
AY904015	*Ovis canadensis nelsoni*	control region, partial sequence, haplotype V
AY116623	[1]*Ovis canadensis weemsi*	control region, partial sequence

[1] Subspecies designations not warranted (*O. c. mexicana*) or probably not warranted (*O. c. weemsi*); should be considered *O. c. nelsoni*.

Table 2.2. Accession numbers of sequences used. The sequences used for primer development and sequence alignment for reference sequences and ancient fecal DNA of unknown origin from Isla Tiburón, Mexico.

CHAPTER 3

TRACKING THE DESERT'S EDGE WITH A PLEISTOCENE RELICT

Abstract

The southern extent of Pleistocene woodlands and location of desert refugia in North America remain unknown. A series of 900–1,200 m desert peaks surrounded by arid lowlands occur throughout the southwestern U.S. and northwestern Mexico where temperate affiliated species occur at highest elevations. The presence of disjunct long-lived plant taxa on under-explored summits, especially Isla Tiburón at 29º latitude in the Gulf of California, suggests a more southerly extent of Ice Age woodlands than previously understood. The phylogeography of the desert edge species *Canotia holacantha* (Celastraceae) was investigated to test the hypothesis that this insular desert peak population represents remnants of Pleistocene woodlands rather than a recent dispersal event. Sequences of four chloroplast DNA regions totaling 2,778 bp were amplified from 59 individuals of 14 populations across the entire range of *C. holacantha* as well as the other two species in its clade. Five haplotypes were recovered; three in *C. holacantha* (Arizona populations, disjunct Sonora populations, and Isla Tiburón) and one for each outgroup. Greater genetic variation in Sonora and no detectable variation in Arizona indicate a broader distribution in southern reaches of mainland Sonora during the Pleistocene. The Chihuahuan Desert microendemic *C. wendtii* is shown to be sister to the Tiburón population, with this group basal to all other *Canotia* populations. These results

suggest that the occurrence of *Canotia* in Sonora is not due to recent long distance dispersal. Instead and contrary to the expectation of a temperate origin, it seems *Canotia* has been present in Sonora since at least the last glacial maximum. *Canotia* has likely tracked the desert's shifting edge since the end of the last Ice Age from refugia in Sonora or Chihuahua, and is a relatively recent arrival in Arizona.

Introduction

The story of the Pleistocene is written on the high elevations of mountains across North America. When the cooler and wetter environments of the last glacial maximum (LGM; ca. 21 kya) began to transition to warmer and drier conditions, species once widespread in the lowlands followed favorable habitat and moved up in elevation. Novel communities established in their place.

A considerable amount of work has revealed profound changes to the landscapes of the world from the LGM to present, especially in the northern hemisphere. These include a fundamental shift from a mesic to more arid climate (e.g., Bartlein et al. 1998; Cheshire et al. 2005; Metcalfe et al. 1997), a ca. 120 m rise in global sea level (Clark & Mix 2002; Peltier 2002), a full scale reorganization of species and communities (e.g., Van Devender 1977; Graham & Grimm 1990; Huntley 1991; Webb & Bartlein 1992), and the loss of 90 of the world's megafauna genera (Martin 2005; Koch & Barnosky 2006) that has had underappreciated ecological ramifications (e.g., Janzen 1986; Gill et al. 2009). However, the history and origin of a substantial portion of modern biodiversity remains unknown, especially in arid regions. By looking up in elevation and into the genetic and biogeographic patterns of disjunct taxa, we can further understand and appreciate the imprint of the past on the diversity we see today.

Analysis of fossil packrat middens has reconstructed the vegetation of the northern portion of what is now the Sonoran Desert in North America (Betancourt et al. 1990). Results provide data on species composition and confirm the presence of a relatively mesic woodland vegetation and flora in lowland regions (<300 m) prior to 11,000 ya (Van Devender 1990). Pleistocene woodlands were dominated by Single-leaf pinyon, Utah juniper, shrub live oak, and Joshua tree. The early Holocene from 11,000 to about 9,000 ya was a transitional period, and modern communities were attained ca. 6,000 ya (Van Devender 1977). Yet, the majority of fossil packrat midden studies have been in the northern portion of the Sonoran Desert with few in-depth paleo records in the vicinity of the Gulf of California (*but see* Van Devender et al. 1994).

Recent comprehensive fossil packrat midden series in the rainshadow of the eastern escarpment of the Sierra San Pedro Mártir show the presence of woodland elements at low elevations co-occurring with Sonoran Desert taxa during the Pleistocene (Holmgren et al. 2011; Holmgren et al. 2014). This finding argues against a desert refugium in one of the most stable arid regions of North America. Where was the desert during the Ice Age? The extent of Ice Age woodlands into southern latitudes, areas along the Gulf of California, and the core of what is now the Sonoran Desert remain unknown.

Beyond the iconic and well-studied sky island mountains of the southwestern U.S. and northwestern Mexico (e.g., Merriam 1890; Shreve 1915; Whittaker & Niering 1965; Brusca et al. 2013), many lower but perhaps equally informative desert peaks ca. 900–

1,200 m in elevation occur across the region. While lacking the full suite of community transitions from desert at the base to oak woodlands and pine forests at the summits, these desert peaks are mountains in the midst of arid lowlands. The highest elevations often harbor species characteristic of Pleistocene woodlands. Floristic inventories of a select few of these mountain peaks, many of which remain unexplored especially in Sonora, have identified mixed grasslands, chaparral, and long-lived mesic-adapted perennial species on peaks throughout Arizona (Brown 1978; Felger et al. 2013a, 2013b), the length of the Baja California peninsula (Moran 1983; Bullock et al. 2008), and parts of Sonora (Felger 2000; Wilder et al. 2008).

Isla Tiburón, the largest in Mexico (1,218 km^2; Figure 3.1), is a land-bridge island in the Gulf of California a few kilometers off the west coast of the state of Sonora. Botanical studies revealed the presence of a half-dozen species confined to higher elevations on the island (> 500 m) that are isolated by more than 200 km of intervening Sonoran Desert from their nearest conspecific populations (Wilder et al. 2007; Felger & Wilder 2012). The occurrence of relatively mesic-adapted perennial species on insular mountains can be likened to a “Sky Island” within a desert island. These disjunct taxa have present day distributions in the Sky Island Mountains at the northern fringe of the Sierra Madre Occidental and northwards in temperate regions. The presence of these species on Tiburón may be relictual: they may have arrived with the extension of Ice Age woodlands to southern latitudes in the Pleistocene (Turner et al. 1995; Wilder et al. 2008). However, that conjecture has remained untested. This paper is the first phylogeographic study of a disjunct species found on desert peaks.

The pattern of genetic variation within *Canotia holacantha* was investigated to better understand the disjunct occurrence of temperate affiliated species on desert peaks at southern latitudes. One of the evolutionary convergent taxa of thorny shrubs known as crucifixion thorns, *C. holacantha* is characteristic of the northern edge of the Sonoran Desert, and intriguingly occurs on the highest ridge of Isla Tiburón (Wilder et al. 2008). We test the hypotheses that (1) the insular population of *Canotia holacantha* is relictual of once widespread and near contiguous populations, rather than recent dispersal events and (2) the Tiburón and N. Sonora populations originated via glacial expansion from central Arizona to the south, as suggested by present day distribution. Results that would argue against recent dispersal would show the Isla Tiburón *Canotia* population to have a unique haplotype and significant genetic differentiation relative to northern mainland populations. Southward expansion in the Pleistocene and post-glacial retreat would be supported by multiple haplotypes in Arizona and reduced or fixed haplotypes across Sonora and Isla Tiburón, which would also be nested within Arizona populations.

Methods

Study species

Canotia (Celastraceae) is a genus of two species, *C. holacantha* Torrey (Torrey 1856) and *C. wendtii* M.C. Johnston (Johnston 1975). This genus has been shown to form a well supported clade with the monotypic southern Mexican endemic *Acanthothamnus aphyllus* (Schlechtendal) Standley via morphology (Johnston 1975) and genetics (Simmons et al. 2001, 2012). Outside of this three-taxon clade, relationships become harder to reconcile. *Canotia* and *Acanthothamnus* are supported, although with moderate significance, as sister to *Euonymus* (Coughenour et al. 2010; Simmons et al. 2012), a genus of evergreen shrubs and small trees mostly native to East Asia but also present in North America, Europe, Australasia, and Madagascar. In turn, that clade is sister to *Wimmeria*, the largely New World tropical genus that includes *W. mexicana*, which occurs in the tropical dry forests of southern Sonora (Martin et al. 1998). *Mortonia*, the other arid-adapted genus in Celastraceae occurs on the periphery of the Sonoran Desert where it is common on limestone in the Chihuahuan Desert and is shown to be an early evolving member of the Celastraceae and distantly related to *Canotia* (Simmons et al. 2012). The *Canotia – Acanthothamnus* clade is on a long branch at the tip of the Celastraceae phylogeny.

Canotia is a hardwood shrub or small tree, essentially leafless with scale-like leaves that are quickly drought deciduous. *Canotia holacantha* is one of a small group of unrelated desert shrubs with photosynthetic stems termed "crucifixion thorns". The crucifixion-thorn growth form occurs in four families in the deserts of North America: Koeberliniaceae (*Koeberlinia*), Rhamnaceae (*Ziziphus obtusifolia*), Simaroubaceae (*Castela emoryi* and *C. polyandra*), and Celastraceae. These plants share the holacanthoid (Muller 1941; meaning wholly [hola] thorny [cantha], Gray 1854) or the retamoid syndrome (Shmida 1981; Zohary 1962; named after the Old World legume *Retama* with spiny photosynthetic stems). These spinescent shrubs and trees are a fascinating adaptation to arid environments (Muller 1941), a growth form and syndrome more common and perhaps derived in the arid lands of South America (Johnston 1940).

Canotia holacantha is a widespread and occasional dominant in central Arizona where it is characteristic of northern and upper elevations of the Sonoran Desert in desert grassland and juniper-chaparral communities. The plants are thick-trunked shrubs or small trees 3–6 (occasionally to 10) m tall (Turner et al. 1995; Brasher 1998). The disjunct populations in the southern portion of its range in southern Arizona and northern Sonora mostly host plants that are 0.5–1.3 m tall on exposed ridges, favorable niches sometimes support small trees ca. 3 m tall (Felger et al. 2001; Wilder et al. 2008; Felger & Wilder 2012). The five-merous white flowers are minute with no clear pollination syndrome. The fruits are persistent woody capsules 1.5–2 cm long, with 5 carpels that split apically into awned valves containing seeds 7–8 mm long with a thin, aril-derived basal wing (Coughenour et al. 2010). The seeds are likely gradually or tardily wind-dispersed relatively short distances. The role of animals, especially ants, in dispersal is not known. The plants are long-lived (Turner et al. 1995).

Canotia wendtii is a micro-endemic in the Chihuahuan Desert in the vicinity of Coyame, Chihuahua and the Chihuahua/Texas border near Big Bend National Park. It is reminiscent of a dwarf version of *C. holacantha*, 0.7–1.2 m tall, lacking a winged seed, and found on dry "badland" hillsides of lime-cemented rounded alluvial gravels in typical Chihuahuan Desert vegetation (Johnston 1975). *Acanthothamnus aphyllus* is likewise a small spiny shrub 1.0–1.5 m tall with photosynthetic stems. This species is restricted to the southern extent of North American deserts in the Mexican states of Hidalgo, Puebla, Querétero, San Luis Potosí, and Tamaulipas (Clevinger & Clevinger 2010). Johnston (1975:119) states, "The thorny shrubs of *Acanthothamnus*… are essentially holacanthoid, though they have a few, small, scattered leaves. In the minutest details of epidermis, bracts, calyx, petals, stamens, gynophore, ovaries, and ovules, *Acanthothamnus* is a diminutive replica of *Canotia*."

Sample collections

A total of 107 individuals of *Canotia holacantha* were sampled from 14 populations throughout the range of the species in the field (Figures 3.1 and 3.2; Table 3.1), with 1–15 and an average of 8 individuals per population. A disjunct population in northern Sonora in the Saric-Tubutama region (red dot southwest of the Waterman population, Figure 3.1) was not sampled due to narco-related violence in the region. Ten field-sampled individuals of *Canotia wendtii* from Coyame, Chihuahua and one *Acanthothamnus aphyllus* from a herbarium specimen were included as outgroups (Table 3.1). Because *Canotia* usually lacks leaves, collections were made of the photosynthetic stem tissue, which was obtained by scrapping away the thick waxy cuticle with a knife and then making thin strips of the green stem tissue. The strips were placed in separate coin envelopes for each individual and kept in silica gel until DNA extraction.

DNA extraction, sequencing, and alignment

DNA was extracted following the standard CTAB based extraction protocol of Doyle and Doyle (1987) using a bead beater to pulverize the liquid nitrogen-frozen plant tissue. Preliminary work identified four regions of the chloroplast genome (cpDNA) to be reliably amplified, sequenced, and variable, which were used in this study: three intergenic spacers (atpB-rbcL, trnL-F, rpl20-rps12), and one intron (trnL; Table 3.2). The chloroplast genome is circular, non-recombinant, and maternally inherited in angiosperms, and continues to be one of the most widely used markers in plant phylogenetics since early successes (Gielly & Taberlet 1994). A number of studies have shown the chloroplast genome to evolve at a rate that retains intraspecific patterns and processes, allowing biogeographic inference within species (Palmer 1987; Comes & Kadereit 1998; Fehlberg & Ranker 2009; Duran et al. 2012).

Each of the four cpDNA fragments was amplified by polymerase chain reaction (PCR). All amplifications had a total volume of 25 μL using: 1.5 μL MgCl2, 0.2 μL deoxynucleotides (dNTPs), 2.5 μL of each primer at 10μM concentration, 0.2 μL Taq polymerase, 2.5 μL Promega 10 reaction buffer, 1.0 μL Bovine Serum Albumin (BSA), 2.0 μL genomic DNA, and 12.6 μL of autoclaved DI water treated in a UV microwave. Thermocycling conditions for the trn L, trnL-F, and atpB-rbcL markers were: 94°C for 2min; 35 cycles of 94°C for 15sec, 48°C for 30sec, 72°C for 90sec; and 72°C for 3min. The following PCR profile was used for the rpl20-rps12 marker: 80°C for 5min; 35 cycles of 94°C for 30sec, 52°C for 30sec, 72°C for 2min; and 72°C for 5min. PCR products were sent for Sanger sequencing to the High Throughput Genomics Unit at the University of Washington in Seattle. Sequences were assembled and edited in Geneious v. 7.0 and aligned using MUSCLE v. 3.8.31.

Genetic Analysis

The four cpDNA data sets were combined for phylogenetic analysis. The concatenated nexus file was assembled using Sequence Matrix v. 1.7.8 and included 831 bp (atpB-rbcL), 592 bp (trnL intron), 475 bp (trnL-F spacer), and 880 bp (rpl20-rps12). Between 88–90 individuals were sequenced for each cpDNA fragment, but only 59 individuals have all four of the target cpDNA regions (Table 3.1). This reduced data set was used for all analyses: 55 individuals of *C. holacantha*, 3 of *C. wendtii*, and 1 of *Acanthothamnus aphyllus*

The number of haplotypes, haplotype network, and population clustering among haplotypes were identified using the program Popart v. 1.5 (Leigh & Bryant 2014). In order to determine the pattern of genetic variation among populations, a statistical parsimony (Templeton 1998) TCS network analysis (as implemented in the program TCS v. 1.21; Clement et al., 2000) was undertaken in Popart. A traits matrix block was used to identify the number of haplotypes and the individuals that belong to specific haplotypes.

Population genetic diversity analyses were performed in the program DNAsp (Librado & Rozas 2009). Locations of high and low genetic diversity were determined through calculation of the amount of haplotype (h; Nei, 1987) and nucleotide diversity (Π_n; mean number of pairwise differences per site; Nei, 1987) within individual populations, populations that correspond to the different haplotypes, and between haplotypes of plants from Arizona and Sonora. Demographic history and evidence of range expansion were investigated by calculating Tajima’s D (Tajima 1989) and Fu’s Fs (Fu 1997). These statistics indicate the degree of departure of a DNA sequence from neutrality (random evolution), which may be due to population expansion or purifying selection (negative values of D and Fs) or a population bottleneck or overdominant selection (positive values).

Relationships among the 14 sampled populations and the two outgroup taxa were resolved by constructing a phylogenetic tree. A maximum parsimony analysis using a heuristic search was undertaken in PAUP* 4.0 (Swofford 2002). All characters were unordered and weighted equally. Bootstrap values were generated with 200 replicates using a heuristic search.

Results

The concatenated data set of four cpDNA fragments for 59 individuals across 14 populations of *Canotia holacantha* and one population of both *C. wendtii* and *Acanthothamnus aphyllus* consisted of a total aligned length of 2,778 bp. Among all sequences there were 22 variable sites.

Five unique haplotypes were identified, three within *Canotia holacantha*, with no populations having more than one haplotype (Figures 3.3; 3.4a). The Tiburón population has a unique haplotype (Haplotype 3), three substitutions removed from the populations in Sonora, which form a separate haplotype. The Sonoran haplotype (Haplotype 2) consists of the Sierra Seri, Magdalena, and Imuris populations as well as the Waterman plants from southern Arizona. All other Arizona populations share a single haplotype, from here on referred to as the Arizona haplotype or Haplotype 1, that apparently characterizes most of the present day distribution of *C. holacantha*. This haplotype differs by a single substitution from the Sonoran haplotype. Both *C. wendtii* and *A. aphyllus* have unique haplotypes (Haplotypes 4 and 5 respectively). Congeneric *C. wendtii* is most similar to the Isla Tiburón *C. holacantha* haplotype from which it differs by three substitutions, whereas 16 substitutions distinguish the southern Mexico aridland *A. aphyllus* from *Canotia*.

When sequences of all populations of these three species are combined, Tajima's D value is significantly negative that suggests recent population expansion, although the stricter Fu's Fs value is positive and non-significant. There was no genetic variation within any of the individual populations of the three taxa or within individual haplotypes (Table 3.2). When the Sonoran and Tiburón populations (Haplotypes 2 and 3) were analyzed as a unit (based on their disjunct occurrence at the southern edge of the range of *C. holacantha*), the highest levels of haplotype and nucleotide diversity were observed (Table 3.2). Fu's Fs and Tajima's D both had positive values for the combined Sonoran haplotypes ($P<0.05$ and $0.10 > P >0.05$ respectively), which suggests a population bottleneck. In contrast, all Arizona populations are fixed for Haplotype 1 and accordingly have zero haplotype and nucleotide diversity, consistent with expectations of populations that have undergone recent expansion.

The maximum parsimony phylogeny (Figure 3.4b) provides resolution of the relationship between the three taxa and context for the association between *C. wendtii* and *C. holacantha*. It shows a strongly supported nested structure with the Isla Tiburón *C. holacantha* population sister to *C. wendtii*. These two populations, which are the most geographically isolated, are basal to all other *C. holacantha* populations. The populations that form the Arizona haplotype are strongly supported as their own clade, which is sister to the populations of the Sonoran haplotype or clade.

Discussion

The disjunct occurrence of the desert edge plant species *Canotia holacantha* in Sonora and Isla Tiburón suggests previously unidentified vegetation patterns in the core of the Sonoran Desert. With chloroplast DNA sequences from across the range of this species and the other two taxa in this clade, we attempted to determine whether the insular occurrence of *Canotia holacantha* is indeed relictual of once widespread populations instead of a recent long-distance dispersal event. The cpDNA sequence data supports Tiburón as genetically unique, its presence on the island is unlikely due to a recent dispersal event. Yet, the results conflict with the expected pattern of glacial expansion of *C. holacantha* from central Arizona to Sonora. Instead, they support post-glacial expansion from glacial refugia for arid-adapted taxa in Sonora or Chihuahua during the LGM.

On the present day landscape *Canotia holacantha* occurs along a broad transition form the highest to mid-elevations of the Sonoran Desert, with disjunct populations on isolated desert peaks. The sister species *C. wendtii* is a narrow Chihuahuan Desert endemic, several hundred kilometers east of *C. holacantha* in Arizona. The biogeographic patterning of the genetic data of *Canotia* populations (Figure 3.4) highlights the imprints of time on this arid-adapted desert-edge taxa. Results do not support *C. holacantha* populations at the southern edge of its range to be remnants of a temperate tongue of vegetation that descended and retracted in elevation and latitude. Instead, the genetic evidence points to a recent arrival of *C. holacantha* in the core of its present day distribution.

Sites of putative glacial refugia are expected to have higher levels of genetic variation, while post-glacially colonized regions are expected to have reduced genetic variation and large geographic areas fixed for a single haplotype (Hewitt 2000). The present-day center of distribution for *C. holacantha* in central and northern Arizona is genetically uniform. Both widely spaced (e.g., Kingman and Globe) and topographically isolated (Kofa and Table Top) populations are fixed for Haplotype 1. Contrastingly, the disjunct Sonora and Tiburón populations support significantly greater genetic variation than those found in Arizona (Table 3.2). The absence of genetic diversity in Arizona, two haplotypes in Sonora, and one in Chihuahua suggest a recent arrival in the core of its modern day distribution from postglacial expansion south to north either from Sonora or Chihuahua. The negative Tajima's D value (Table 3.2) indicates a population that has undergone post-glacial expansion.

The Isla Tiburón population of *C. holacantha* is strongly supported as sister to the Chihuahuan Desert microendemic *C. wendtii*, which substantiates the south to north directionality of post-glacial expansion (Figure 3.4b). The two haplotypes differ by three substitutions (Figure 3.3). *Canotia wenditii* is only known from one small region within the Chihuahuan Desert, over 720 km nearly due east of Isla Tiburón, and on the opposite side of one of the greatest vicariant barriers in North America, the Sierra Madre Occidental (Bye 1994; Marshall & Liebherr 2000). The uplift of the Sierra Madre

Occidental and Rocky Mountains, and the uplift of the Continental Divide on the Deming Plain in southern New Mexico date to the Miocene (Ferrari et al. 1999). The great distance and intervening Sierra Madre Occidental suggest that the presence of *C. wendtii* in E. Chihuahua is not likely due to a recent long-distance dispersal directly from Isla Tiburón. A recent dispersal origin is further questioned by the apparent absence of a long-distance dispersal syndrome for *Canotia* and morphological differences between the two species (Johnston 1975) that were observed to be maintained in the field.

What can explain this result? Indeed, there are similar disjunct Sonoran-Chihuahuan plant taxa such as the Turk's head cactus (*Echinocactus horizonthalonius*), which is widespread in the Chihuahuan Desert and on the unusual limestone outcrops in the Waterman Mountains both in the LGM (Anderson & Van Devender 1991) and at present. This rare cactus is also known from the isolated limestone Sierra del Viejo mountain range near Caborca, Sonora (Yatskievych & Fischer 1983; Paredes-Aguilar et al. 2000). Additional Sonora-Chihuahua disjunct taxa include *Parkinsonia florida-P. texana, Senna wislizeni* (Fabaceae), *Purshia* (Rosaceae), and *Lantana macropoda* (Verbenaceae; Van Devender personal communication Dec 2014).

Results of recent genetic studies of mule deer (*Odocoileus hemionus*) across North America surprisingly reveal the same disjunct Chihuahua-Tiburón connection as seen in *Canotia* (Latch et al. 2009; Alminas 2013). An endemic subspecies of mule deer, *O. hemionus sheldonii* is present on Isla Tiburón (Goldman 1939; Felger & Moser 1985; Wilder et al. 2014) and, like *Canotia* on the island, was shown to genetically vary from mule deer on the adjacent mainland (Alminas 2013). Twenty-two of the 23 deer sampled from Isla Tiburón form a monophyletic clade that nests within a distinct haplogroup of mule deer restricted to the Guadalupe Mountains of western Texas in the Chihuahuan Desert (Alminas 2013).

The Guadalupe Mountains are the exposed portion of a Permian aged limestone reef, ca. 775 km to the northeast of Tiburón and ca. 260 km due N of *C. wendtii*. Latch et al. (2009) attribute the distinctiveness of the Guadalupe Mountains mule deer haplogroup to its likely persistence through the LGM. Their molecular data suggests this population to be older than those in the surrounding areas (15,900 ybp; 95% CI: 25,100–5300; Alminas 2013). This conclusion is supported by the presence of deer remains in ca. 13,000-year-old cave deposits from the Guadalupe Mountains (Harris & Hearst 2012). Similarly, molecular and fossil midden results support the Guadalupe Mountains as a glacial refugium for piñon pine (*Pinus edulis*; Duran et al. 2012). Fossil packrat midden studies indicate that plant communities in the Guadalupe Mountains changed from a relatively mesic woodland at the late Wisconsin to present day xeric Chihuahuan Desert vegetation (Van Devender et al. 1979). Records also indicate a delayed but abrupt expansion of desert shrubs elsewhere in the northern Chihuahuan Desert not until ca. 5,000–4,000 yr BP (Holmgren et al. 2007).

North of the Sierra Madre Occidental, the Chihuahuan and Sonoran Deserts are separated by a low-elevation gap in the Continental Divide in the vicinity of the Deming Plains of southern New Mexico, termed the Cochise filter-barrier (Morafka 1977). During glacial periods the closed basins of this area filled with pluvial lakes and C_4-dominated grasslands were present due to the likely persistence of the North American monsoon throughout the last glacial-interglacial cycle (Holmgren et al. 2007). Relatively mesic vegetation predominated, effectively closing the pass to arid elements (Hafner & Riddle 2011). During interglacials, the lakes became playas and the filter barrier was open to arid-adapted taxa. At such times throughout the Pleistocene the Cochise filter-barrier has likely permitted the repeated dispersal of Sonoran elements east and Chihuahuan elements north and west (Hafner & Riddle 2011).

The directionality and timing of migration of *Canotia* and mule deer is uncertain. The presence of *Canotia holacantha* and the endemic subspecies of mule deer on Tiburón could be viewed as Sonoran elements that expanded from the Plains of Sonora north, or Chihuahuan Desert elements that expanded from Chihuahua and west likely through the Cochise filter-barrier. In both cases it is unlikely that the connection between Tiburón and Chihuahua dates to the LGM, especially in the seemingly dispersal limited *Canotia*. Migrations related to a prior glacial, ca. 120 kya seems more likely. With an origin from either Sonora or Chihuahua at the LGM or prior, a scenario can be envisioned in which both taxa were far more widespread across the landscape, and as climates changed their ranges retracted. Both species became isolated on the insular refugium of Isla Tiburón, and thousands of kilometers away, mule deer in the Guadalupe Mountain Sky Island and *Canotia* on the limestone badlands of the Chihuahuan Desert.

The Waterman Mountain population, which is part of the Sonoran haplotype, offers some insight into the possible postglacial expansion of *Canotia*. The Waterman Mountains are an isolated block of uplifted and fractured limestone just to the northwest of Tucson, AZ. A fossil packrat midden series from the Waterman Mountains (Anderson

& Van Devender 1991), as in much of the northern Sonoran Desert, shows that most woodland plants (*Juniperus*, *Berberis*, *Quercus turbinella*) disappeared by 8,910 yr B.P. Modern Sonoran desertscrub elements including creosote bush (*Larrea divaricata*) arrived by 6,200 yr B.P.

Interestingly, creosote bush, which exhibits three ploidy races with geographic patterns through space and time in the North American deserts, was determined to be diploid in the 6,200 year-old Waterman Mountain fossil midden (Hunter et al. 2001). This is one of the first occurrences of diploid creosote bush individuals east of the Lower Colorado River Valley in the Holocene, which expanded widely in the eastern range of this species in the Chihuahuan Desert ca. 5,000 years ago and is the dominant ploidy race in the Chihuahuan Desert today (Hunter et al. 2001; Laport et al. 2012; Holmgren et al. 2014). Diploid individuals in the Waterman Mountains at ca. 6,200 y B.P. are thought to represent the leading edge of diploid expansion from the Lower Colorado River Valley east into areas of central and southern Arizona then dominated by tetraploid populations (Hunter et al. 2001). A similar situation may apply to *C. holacantha*. The preference of *Canotia* for limestone likely explains its past and continued presence in this isolated granitic range. The Waterman Mountains population may represent a similar founder event en route to subsequent expansion, just as the diploid creosote bush in its march to the east.

Another intriguing population of *Canotia* is found on the Sierra Seri, an isolated 1,000 m desert peak on the Sonoran mainland just opposite Isla Tiburón. Surprisingly, the Sierra Seri population is associated with the Sonoran populations rather than that of adjacent Isla Tiburón. It is separated from the island (ca. 15 km) by the narrow and shallow Canal del Infiernillo, a water filled Basin and Range valley that connected Tiburón to the mainland as recently as ca. 6 ka (Wilcox 1978; Felger & Wilder 2012). *Canotia holacantha* was not known to occur on the Sierra Seri prior to this study, but based on its occurrence on the summit of Isla Tiburón and following expectations of the desert peak disjunct pattern, it was indeed found to be present (Felger & Wilder 2012:478). Given the proximity and isolation of these two peaks and the land-bride connection of Tiburón to the mainland during glacial periods, they would be expected to have either the same haplotype or be similarly differentiated from the other populations. The unique haplotype of *C. holacantha* on Tiburón may represent a past wave of dispersal or contraction on the landscape. It also supports the Sierra Kunkaak of Tiburón being a "Sky Island" within a desert island that contradicts the concept of Isla Tiburón being "hardly distinguishable from a chunk of mainland" (Cody et al. 1983).

The only fossil record for *Canotia holacantha* is 11,100 yr B.P. twig fragments in a fossil packrat midden from Picacho Peak, Arizona (Van Devender et al. 1991), just north of the Waterman Mountains. Despite thousands of Pleistocene-aged fossil packrat middens throughout the northern Sonoran Desert, the presence of *Canotia* was only observed in this one midden. If *Canotia* were a common member of the Pleistocene woodlands in what is now the northern Sonoran Desert, more fossil records would be expected. Further fossil evidence from the Gulf of California that could help determine the duration of this species in this region is elusive. A predominantly volcanic geology of the Midriff Islands (Bennett & Oskin 2014) and the absence of granitic outcrops necessary for a Pleistocene aged midden-series limit the depth of paleo-packrat midden studies. All fossil packrat middens discovered on Isla Tiburón were of Holocene age (Wilder et al. 2014; Wilder unpublished data).

Another line of inference that can provide information on past distribution patterns is comparative phylogeography – the investigation of genetic data from multiple co-distributed taxa (Bermingham & Moritz 1998; Hickerson et al. 2010). Areas with overlapping genetically distinct populations or evolutionary significant units (ESU's) are considered possible sites for glacial refugia (Hewitt 2000). A meta-analysis overlaid the geographic distributions of 85 terrestrial taxa from the Baja California peninsula for which molecular studies identified ESUs (Dolby et al. *in press*). This analysis revealed a high level of genetic diversity along the spine of mountains in the Baja California peninsula and a similar overlap of phylogroups in the foothills of the Sierra Madre in central Sonora (also called the Plains of Sonora, Shreve 1951). A similar examination of the ESUs of 31 arid-adapted rodents of North American deserts (Hafner & Riddle 2011) indicate a high concentration of species density both in the Plains of Sonora and Chihuahuan Desert. For the Plains of Sonora, this pattern of higher levels of genetic variation has been specifically documented in multiple molecular studies of Sonoran taxa (Hunter et al. 2001; Nason et al. 2002; Jaeger et al. 2005; Smith & Farrell, 2005; Castoe et al. 2007; Fehlberg & Ranker 2009). Collectively they identify reduced genetic variation and areas with a single or fewer haplotypes to the north in Arizona and higher genetic diversity in the Plains of Sonora, which supports the concept of central Sonora as a glacial refugium.

The collective results of fossil packrat midden studies and community ecology consistently demonstrate the independent nature of species in their response to climate change (Gleason 1926; Whittaker 1953; Van Devender 1977; Huntley 1991; Holmgren et al. 2014). Accordingly, the concept of a desert glacial refugium as a static entity roughly equivalent to the modern Sonoran Desert is likely erroneous (Van Devender 1990; Holmgren et al. 2014). Instead, in many locations, such as the rain shadow of the Sierra San Pedro Mártir (Holmgren et al. 2014), arid-adapted taxa were likely able to exist in dry microsites from where they expanded, rather than from a distinct refugium (Axelrod 1979). Yet, there may be localities, such as the Plains of Sonora or southern Baja California, where multiple arid-adapted taxa clustered on the landscape from where they expanded in an individualistic manner at the onset of warmer climates in the Holocene.

The results for *C. holacantha* may not apply to the other disjunct taxa on the high elevations of Isla Tiburón. These additional temperate affiliated species may indeed have origins that correspond to the southern extension of woodland vegetation. The presence of cool season-adapted species now restricted to the Baja California peninsula on the coast of Sonora ca. 9,000 years ago (Van Devender et al. 1994) suggest a cooler climate at the Pleistocene-Holocene transition in the region. It is possible Tiburón (then connected to the mainland) was analogous to various transition zones seen on the northern border of the Sonoran Desert today. Baja California, woodland, and arid-adapted taxa like *Canotia* may have coexisted on Tiburón and in the vicinity of the Gulf of California in Sonora at the LGM.

Conclusion

The genetic signatures of diverse taxa provide an indication of how desert environments and arid-adapted taxa have shifted in response to post-glacial climatic change throughout the Pleistocene. Analysis of chloroplast regions in *Canotia holacantha* suggests that this arid-adapted hardwood perennial has tracked the northern or temperate boundary of the Sonoran Desert through time. Collectively, the results presented here support that *Canotia* is relatively newly established in the core of its present day range and the Tiburón and Sonoran populations likely did not originate from recent long distance dispersal or from temperate woodlands that may have descended to southern latitudes and retracted north. Instead, these disjunct southern populations likely represent past centers of distribution for *C. holacantha* and the sources for post-glacial expansion. It is not clear if migration was from Sonora to Chihuahua or vice versa. The fascinating connections of Isla Tiburón to the northern Chihuahuan Desert as seen in *Canotia wendtii* and the mule deer in the Guadalupe Mountains might reflect a migration through the low pass of the Continental Divide at the Cochise filter-barrier of the Deming Plains. Likewise, the Sonora-Chihuahua connection may not be a post LGM result, and could be the legacy of more ancient dispersal and contraction events. These results stress the danger in assuming directionality of biogeographic patterns based on modern distributions. *Canotia*, other Sonoran-Chihuahuan disjunct species and species pairs, and desert peak relicts are exemplar taxa to understand the boundaries of the desert today, in the past, and in the future.

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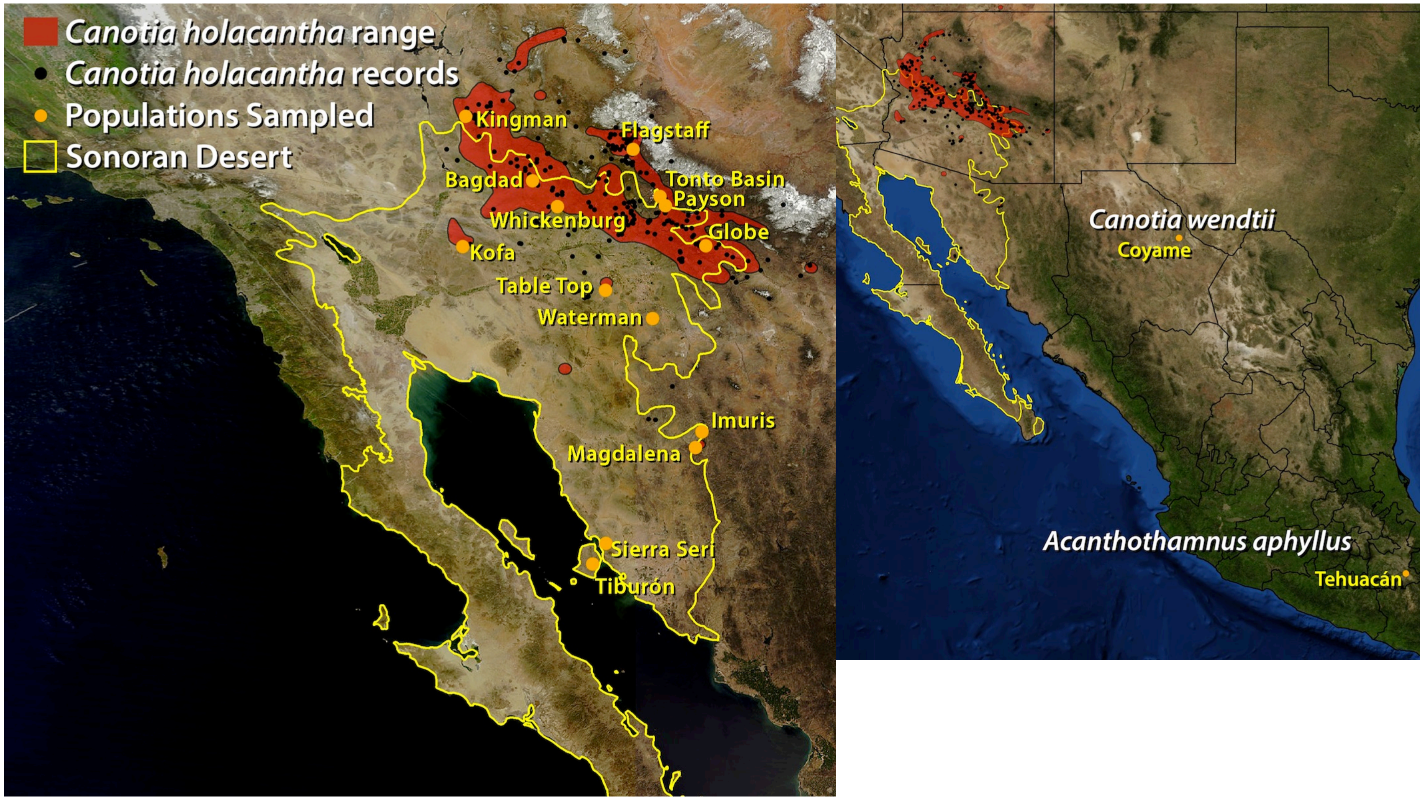

Figure 3.1. Range and populations sampled of *Canotia holacantha* and outgroups *C. wendtii* and *Acanthothamnus aphyllus*. (Left) *Canotia holacantha* distribution, the 14 sampled populations are marked by orange dots and population name (Table 3.1). (Right) Location of the outgroup populations *Canotia wendtii* and *Acanthothamnus aphyllus* in relation to the range of *C. holacantha*. Black dots are herbarium records of *C. holacantha* (SEINet 2014) and the red polygon is

the present range of *C. holacantha* (Little 1976). Golden outline is boundary of the Sonoran Desert (Shreve 1951; Felger & Lowe 1976) courtesy of Charlotte González Abraham. Background image courtesy of NASA LANCE Rapid Response MODIS imagery.

Figure 3.2. *Canotia holacantha* throughout its range. Images are featured from north (top) to south (bottom). Top left, Flagstaff, AZ, *C. holacantha* to left of alligator juniper (*Juniperus deppeana*). Top right, Kingman, AZ. Middle left, near Bagdad, AZ within a Joshua tree forest (*Yucca brevifolia*). Middle right, summit of Table

Top Mountain, AZ. Bottom left, Isla Tiburón, upper ridge of island with Jose Ramon Torres (left) and Brad Boyle (right), note Canal del Infiernillo and Sierra Seri on mainland Sonora in background. Bottom right, Sierra Seri, Son, N-slope of mountain looking N with Gulf of California on left. All photos by B. Wilder.

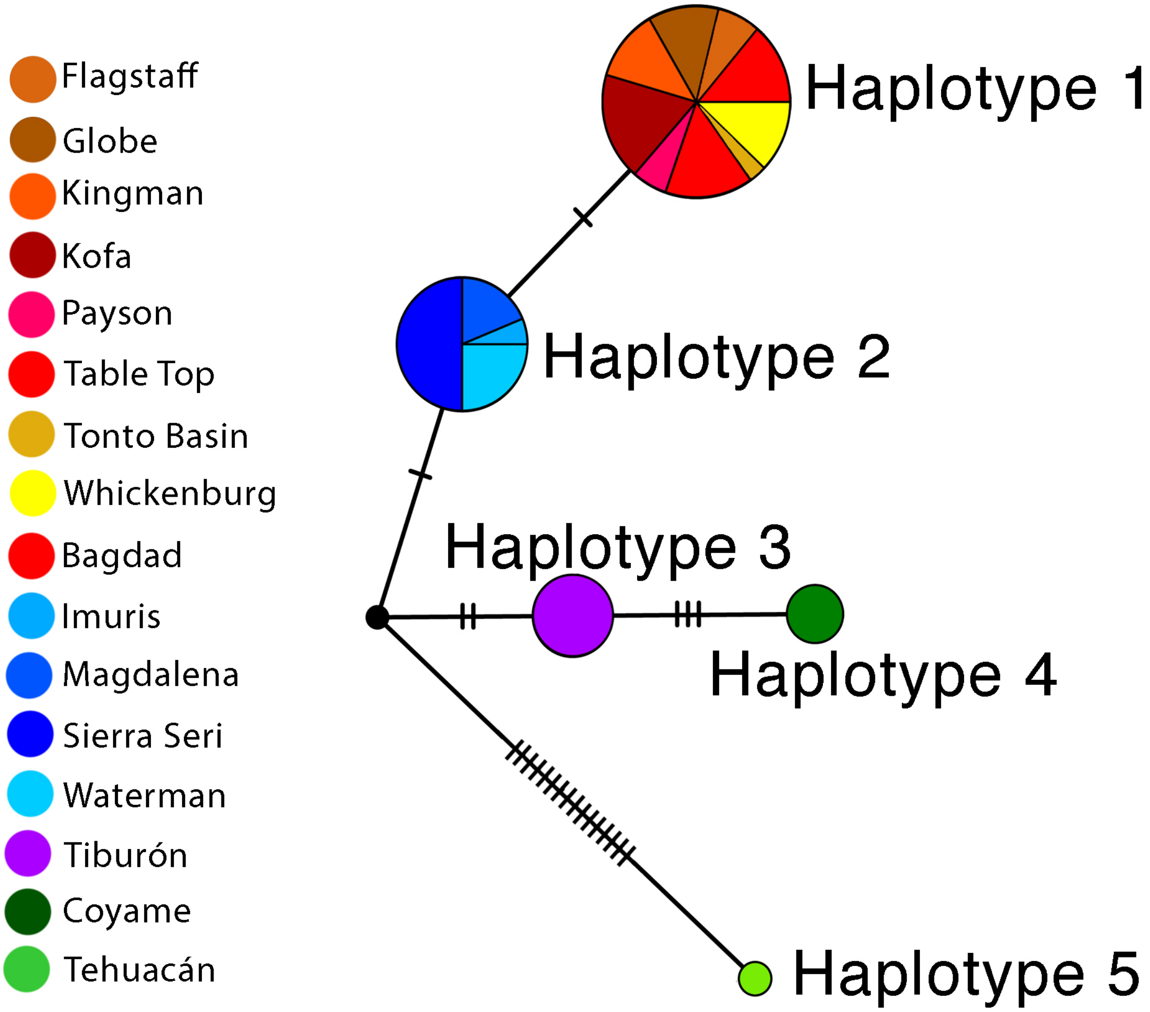

Figure 3.3. Haplotype network and associated populations. Coyame is *Canotia wendtii*, Tehuácan is *Acanthothamnus aphyllus*, and all others are *C. holacantha*. Tick marks between population clusters represent number of substitutions between haplotypes.

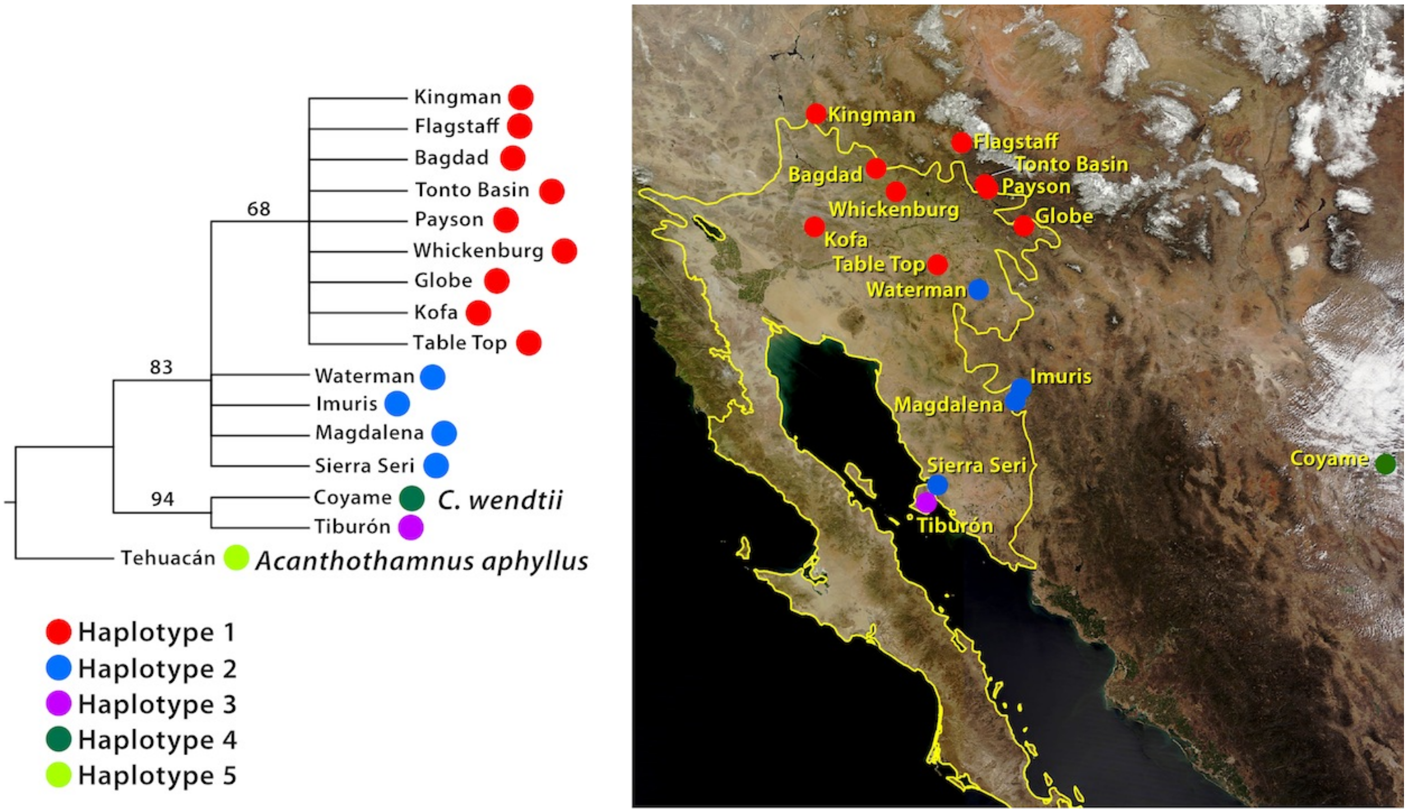

Figure 3.4a and b. Genetic structure of cpDNA results from *Canotia holacantha* and two outgroups. (a, right) Geographic distribution of the five haplotypes. Note Haplotype 5, *Acanthothamnus aphyllus*, occurs far to the south of the projected area. Golden outline is boundary of the Sonoran Desert as in Figure 1. (b, left) Consensus parsimony tree. Bootstrap values higher than 50 are shown. Cardinal directions apply in both figures, with north at top and east at right.

Species	Locality	State	Code	# of Indivds.[1]	Latitude	Longitude	Elevation (m)	Herbarium Voucher[2]
Canotia holacantha	Imuris	Son, MX	Ch1	1/1	30.76241	-110.78475	1040	*Wilder 10-541*
Canotia holacantha	Magdalena	Son, MX	Ch2	10/3	30.56362	-110.87667	829	*Wilder 10-494*
Canotia holacantha	Sierra Seri	Son, MX	Ch3	9/8	29.21498	-112.1274	610	*Wilder 11-238*
Canotia holacantha	Table Top	AZ, USA	Ch4	11/5	32.74898	-112.1285	1332	*Wilder 11-260*
Canotia holacantha	Waterman	AZ, USA	Ch5	8/4	32.35134	-111.46812	983	*Wilder 11-268*
Canotia holacantha	Kingman	AZ, USA	Ch6	8/4	35.17215	-114.07588	1025	*Wilder 12-240*
Canotia holacantha	Flagstaff	AZ, USA	Ch7	12/0	34.70864	-111.74116	1263	*Wilder 12-247*
Canotia holacantha	Payson	AZ, USA	Ch8	5/2	34.03617	-111.36568	992	NA
Canotia holacantha	Tonto Basin	AZ, USA	Ch9	2/1	33.96975	-111.32192	779	NA
Canotia holacantha	Globe	AZ, USA	Ch10	7/5	33.37453	-110.74232	1110	*Wilder 12-253*
Canotia holacantha	Tiburón	Son, MX	Ch11	15/6	28.92912	-112.31796	600	*Wilder 12-107*
Canotia holacantha	Whickenburg	AZ, USA	Ch12	5/4	33.92119	-112.79398	768	*Wilder 14-3*
Canotia holacantha	Bagdad	AZ, USA	Ch13	7/7	34.29412	-113.11359	802	*Wilder 14-4*
Canotia holacantha	Kofa	AZ, USA	Ch14	7/6	33.36066	-114.10135	680	*Wilder 14-7*
Canotia wendtii	Coyame	Ch, MX	Cw1	10/3	29.54956	-104.92725	910	*Wilder 11-193*
Acanthothamnus aphylllus	Tehuácan	Pueb, MX	Aa1	1/1	18.47222	-97.436389	1730	*Chiang F-2271*

[1] Number of individuals sequenced for each populations: Number of individuals with sequences / number of individuals with sequences for all 4 cpDNA gene regions.

[2] All herbarium specimens are at the University of Arizona herbarium (ARIZ).

Table 3.1. Locality information for population samples.

cpDNA region	Primer sequence (5’–3’)	Source
trn L	F: CGAAATCGGTAGACGCTACG R: GGGGATAGAGGGACTTGAAC	Taberlet et al. 1991
trn L-F	F: GGTTCAAGTCCCTCTATCCC R: ATTTGAACTGGTGACACGAG	Taberlet et al. 1991
atpB-rbcL	F: ACATCKARTACKGGACCAATAA R: AACACCAGCTTTRAATCCAA	Weising & Gardner 1999
rpl20-rps12	F: CGYYAYCGAGCTATATATCC R: ATTAGAAANRCAAGACAGCCAAT	Weising & Gardner 1999

Table 3.2. Primers used for amplification of *Canotia holacantha* chloroplast DNA regions

Haplotype	# of individuals	Haplotype Diversity ± SD	Nucleotide Diversity ± SD	Tajima’s D	P	Fu’s Fs
All	59	0.616 ± 0.50	0.00087 ± 0.00027	-1.93677	< 0.05	2.169
Haplotype 1	33	0	0	0	0	0
Haplotype 2	16	0	0	0	0	0
Haplotype 3	6	0	0	0	0	0
Haplotypes 2-3	22	0.416 ± 0.090	0.00813 ± 0.00039	1.75608	0.10 > P >0.05	9.713
Haplotype 4	3	0	0	0	0	0
Haplotype 5	1	NA	NA	NA	NA	NA

Table 3.3. General genetic diversity results of recovered haplotypes.

CONCLUSION

Historical biogeography addresses how and why species' distributions change through time. The investigation of island floras, desert bighorn sheep, and the desert edge plant *Canotia holacantha* provide insights into various patterns and histories that have led to modern day communities. These case studies span the Anthropocene, Holocene, and Pleistocene epochs. They encompass a diverse range of the geologic, climatic, and ecological conditions that acted on the biota of the islands and surrounding region of the Gulf of California. Taken together, the results can help answer fundamental questions:

Why do we see these plants and animals on the landscape?

Present day diversity levels of the Midriff Islands are in large part governed by the geologic legacy of the opening of the Gulf of California (Dolby et al. *in press*). Island size and topographical diversity lay the foundations for the number of both plant species and habitats that can exist. As is true for most species-area relationships (e.g., Preston 1962), larger islands with greater topographic complexity support higher levels of diversity. The proximity of an island from the mainland determined whether it was connected to a larger landmass when global sea levels were 120 m lower during glacial periods in the Pleistocene (Graham et al. 2003). The results of Chapter 1 show that oceanic islands that have been surrounded by water since the opening of the Gulf of California have depressed levels of diversity and higher endemism, especially in reptiles and mammals (Cody 2006), and cacti have developed numerous unique forms in arid isolation.

Climatic change since the last glacial maximum and possible vegetation transitions on the islands are harder to distinguish. The islands have a primarily volcanic substrate (Bennett & Oskin 2014) that does not provide the conditions needed for Pleistocene-aged fossil packrat middens, which are predominantly found in protected nooks in granitic batholiths (Betancourt et al. 1990). Inferences into past vegetation patterns are restricted to other proxies. The local extinction of bighorn sheep on Isla Tiburón may have been facilitated by intense drought during the Holocene. However, multiple other factors were likely at play, possibly including humans.

Genetic investigations of geographically disjunct species can provide insights into historic vegetation. The results from the genetic study of the desert-edge plant *Canotia holacantha* on Isla Tiburón is the first such study of plants isolated on desert peaks hundreds of kilometers south of their core distribution in the Sonoran Desert. The unique genetic signatures in the chloroplast DNA of the Tiburón plants support the long persistence of this species on the island. This result in combination with the other half-dozen disjunct long-lived plants at the high elevations of Isla Tiburón, Ángel de la Guarda, the Sierra Seri, and other regional desert peaks at ca. 29º suggest the occurrence of a mixed assembly of mesic and arid-adapted taxa along the Gulf of California during the Pleistocene.

Oceanographic conditions of the Gulf of California, which may have been relatively stable for thousands of years, are a significant driver of modern diversity levels. A trophic cascade is fed by marine derived nutrients brought to the surface from depth by persistent cold-water upwelling. The marine nutrients are transported to small oceanic islands via nesting seabirds that have consumed tons of pelagic fish and deposit large quantities of guano on the islands. High concentrations of N and P in the guano soils of these bird islands filter and limit the plant species that can establish. The flora of these islands, dominated by halophytes and succulents (cacti and amaranths) is determined by ecological dynamics dictated by oceanographic conditions.

What is the baseline for modern biodiversity?

Bighorn sheep are now known to have been present on Isla Tiburón prior to their re-introduction in 1975. This finding extends the baseline of bighorn sheep on the island to at least 1500 years before present, prior to their local extinction on the island. It alleviates certain concerns regarding the conservation action to bring them to the island in the 1970s and warns of the same potential fate. However, many questions remain unanswered and subsequent management actions need to be carefully researched and considered. The past population size of bighorn sheep on the island is not known. Likewise, there is still not an adequate baseline from which to assess the impact the modern population is having on the island's flora, especially in the Sierra Kunkaak. Actions to extract additional individuals for repopulation efforts, continued hunting efforts, and especially future efforts to introduce new individuals to increase genetic diversity need to carefully consider unintended consequences to both the island and this politically, economically, and culturally important species (Gore et al. 2011; Räikkönen et al. 2013).

The long chain of ecological interactions that link the land and sea, which control plant species diversity on bird islands in the Gulf of California reflect a robust ecosystem. However, this system is dynamic and prone to change and the shifting baseline syndrome. Both climatic (e.g., El Niño and La Niña oceanic anomalies, Caso et al. 2007) and human actions (e.g., seabird egg harvesting, Ezcurra et al. 2002; guano mining, Bowen 2000, 2009; introduction of non-native rodents, Velarde et al. 2014) are known to have shifted the system. Research to date has documented the various states of these bird islands in the past century (e.g., Tershy et al. 1997; Wilder & Felger 2010; Felger & Wilder 2012; Velarde et al. 2014). Continued investigations can track the effect of potential agents of change such as overfishing, drought, non-native species, and unforeseen events.

Efforts to understand the origin of the desert, especially migrations and contractions of arid-adapted species ranges during glacial periods of the Pleistocene are still developing. The genetic data of *Canotia holacantha* thus far suggests this species' recent arrival in the northern edge of the modern Sonoran Desert where it is common today. The Tiburón and Chihuahuan populations of *Canotia* are the most divergent and appear to be basal to the rest of the populations sampled. The results indicate post glacial expansion from the south to the north that correspond to comparable phylogeographic studies of North American desert taxa (e.g., Fehlberg & Ranker 2009; Hafner & Riddle 2011; *see* Chapter 3 for additional studies). Growing evidence indicates the low-lying area at the foothills of the Sierra Madre in the Plains of Sonora to have supported the presence of arid-adapted taxa that expanded in the Holocene and now compose the modern Sonoran Desert. Additional studies of disjunct taxa both in Baja California, Chihuahua, and the desert peaks of Sonora and Arizona will provide much needed data to refine directionality and timing of migrations.

What are the implications of this baseline for conservation?

The islands of the Gulf of California are in a remarkable state of conservation. The results of this research demonstrate that humans were not and are not determinants of plant species diversity on the Midriff Islands. The diversity, healthy terrestrial ecosystems, and minimal involvement of humans on the islands of the Gulf of California are in stark contrast to global trends. There are low levels of non-native plants, which represent only 2.7% of the flora of the Midriff Islands. In comparison, 28% of the flora of the California Channel Islands is non-native (Ratay et al. 2014). People do not inhabit any of the Midriff Islands today outside of a single Mexican naval marine outpost on Isla Tiburón just opposite the Sonoran mainland.

The uniqueness of these islands has been recognized. Conservation actions began in the 1950s in response to the full-scale desecration of seabird populations on Isla Rasa due to excessive egg collecting. An international collaboration led to the declaration of the island as a natural wildlife refuge in 1964, paving the way for multiple conservation legislations to come (Ezcurra et al. 2002). A network of natural protected areas now encompasses all islands in the Gulf of California (DOF 2000). The marine and coastal portions of the Gulf of California were named a United Nations World Heritage site in 2005, honoring their outstanding universal value.

The terrestrial and marine ecosystems of the Gulf of California have proven to be robust and resilient. Breeding seabird populations have returned to islands overtaken for guano harvesting operations in the late 1800s (Bowen 2000). Likewise, following the eradication of non-native rodents, seabird populations have soared on Isla Rasa (Velarde et al. 2014). The establishment of marine protected areas in the Gulf of California supported by enforcement has resulted in recovery (e.g., Aburto-Oropeza et al. 2011) and maintenance (e.g., Basurto 2005) of diversity and abundance.

The islands of the Gulf of California are the world's best-preserved archipelago. Beyond healthy ecosystems, low rates of non-native species, and multiple conservation mandates, the Gulf of California has human capital. The majority of local communities throughout the region base their livelihoods on the natural resources of the sea and to a lesser extent the islands. There are multiple innovative and locally lead actions in the Gulf of California that strive to maintain or restore ecological resilience. Overt collaborative efforts between researchers, Mexican and foreign conservation organizations, the Mexican government, and local communities are necessary to further the wise use of the Gulf of California's natural heritage. The conservation of the islands of the Gulf of California is a globally unique but transient opportunity. Humanity's negative impact on island ecosystems is evident across the world. Recognition of both the challenges and potential in this region, followed by science-guided action can help maintain the Gulf of California and its islands for generations to come.

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