Spatial and Temporal Patterns of Diversification in Leaf-toed Geckos (Phyllodactylidae: *Phylodactylus*) throughout the Mexican Dry Forest

by

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A thesis submitted in conformity with the requirements for the degree of Doctor of Philosophy

Department of Ecology and Evolutionary Biology
University of Toronto

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Abstract

Understanding the ecological and evolutionary processes responsible for shaping patterns of genetic variation in natural populations is a long-standing goal in molecular ecology. Although an extensive number of recent studies focus on patterns and processes throughout tropical rain forest ecosystems, substantially less effort has been placed on tropical dry forests (TDFs); a habitat known to harbour a large percentage of Earth’s diversity. In this thesis I use leaf-toed geckos of the genus *Phylloactylus* to understand both the historical and contemporary processes influencing diversification throughout Mexico’s TDFs. In Chapter 2 I isolate and characterize microsatellite markers for the gecko *P. tuberculosus*. Chapter 3 uses these loci to conduct a landscape genetic analysis of the species near Alamos, Sonora. I find that the inclusion of landscape variables explains more genetic variance versus Euclidean distance alone. Chapter 4 examines the evolutionary history of the *P. tuberculosus* group throughout western Mexico. Results suggest that habitat and climate shifts during the Miocene and Pleistocene were important divers of diversification. Chapter 5 uses microsatellite and mtDNA markers to compare historical and contemporary demographic
parameters in *P. tuberculosus*. I find evidence for low historical gene flow and high female philopatry, recent reductions in population sizes, and higher correlations between landscape and contemporary gene flow versus historical gene flow and mtDNA divergence. In Chapter 6 I examine the biogeographic and taxonomic consequences of the dynamic history of Baja California. My phylogenetic results provide evidence for a trans-peninsular seaway in the Isthmus of La Paz region and suggests that *P. xanti nocticolus* warrants species status. My results also suggest the possibility of a seaway near the Loreto area that needs to be evaluated further. By combining multiple molecular marker-types and analytical methods, this thesis adds to our understanding of diversification processes throughout the threatened Mexican TDF.
Acknowledgments

Taking on a graduate degree, especially a Ph.D. in a foreign country with limiting funding opportunities is no easy task. Throughout our graduate programs we must learn to troubleshoot potential research issues and partition the limited time we have in an effective manner. To this end, I feel that graduate school adds a second dimension of education beyond a researcher’s focus on a specific discipline. With that said, I would first like to thank my supervisor, Prof. Bob Murphy, for all his advice and guidance over the years. Bob accepted me into his lab knowing that I did not have my own research funding, in part, because I was an international student. However, he welcomed me into his lab with open arms and gave me the freedom to pursue my own research interests and was always available for advice if needed. I also thank the remainder of my thesis committee, Prof. Allan Baker and Prof. Marie-Josée-Fortin for their insightful discussions regarding methods of statistical analysis.

Special thanks also go to my fellow lab-mates Christina Davy, Andre Ngo, and Pedro Bernardo for all the help and advice they have given me. Graduate school, like life, is a continuous learning process and these individuals taught me much more than I ever thought possible. I would also like to thank graduate students from other labs at the Royal Ontario Museum (ROM) and the University of Toronto for their thoughtful discussions about research methods and philosophy. In particular, I thank Ida Conflitti, Patrick Schaefer, Ruben Cordero, Julio Rivera, Damon Dewsbury, Santiago Sanchez, Yessica Rico, Pasan Samarasin, Ilona Naujokaitis-Lewis, Oliver Haddrath, and Erika Tavares for all their help over the years.

As those of us in the field know, molecular genetic laboratory work can be a stressful, time-consuming process that results in many days without the generation of data. However, throughout my time at the ROM I was fortunate to have the help from a variety of talented individuals including Amy Lathrop, Kristen Choffe, and Oliver Haddrath. These knowledgeable researchers were always close-by to lend a helping hand when needed. I also thank Christopher Law and Pedro Bernardo for all their help in the laboratory and Christopher Wilson and Christopher Kyle for their assistance with 454 sequencing.
During my time as a Ph.D. student at the University of Toronto I had the opportunity to travel for my research. Because of Bob, I was fortunate to have the chance to conduct research on geckos throughout western Mexico. Conducting field work throughout the country over the course of three years enabled me to form new collaborations that I hope will last a lifetime. Working in Mexico also allowed me to grow as an individual and understand the lives and cultures of people from different backgrounds. I first thank my collaborator and friend Fausto Méndez de la Cruz for all of his help with logistics in Mexico including securing permits. Without Fausto, this thesis would not have been possible. Special thanks also go to his students Victor Jiménez-Arcos and Anibal Diaz de la Vega for all their help. Not only are these guys excellent herpers, but they are very knowledgeable about Mexico’s diverse ecosystems and about where and when to sample. I also thank Stephanie Meyer and Martin Gabriel Figueroa Martinez for their help with all of the Alamos work. Finally, I wish to thank Marie-Josée-Fortin and Helene Wagner for the opportunity to attend the landscape genetics conference in Santa Barbara. This was a tremendous opportunity and I hope to continue to collaborate on projects with the people I met.

As research is not possible without adequate funding, I must thank the appropriate funding sources for this research. The majority of this research was funded by a Natural Sciences and Engineering Research Council of Canada (NSERC) grant (A3148) issued to Bob. Funding for lab work was also provided by the Schad Foundation at the ROM. Field work in Mexico was partly funded through a Theodore Roosevelt Memorial Grant issued by the American Museum of Natural History.

Finally, and most importantly, I would like to thank my parents, Burt and Debbie Blair, and my brother, Michael Blair, for all their support over the years. Words cannot express how supportive they have been to me during my time as a struggling student. Throughout my time as a college student (over 12 years) my parents gave me the freedom to pursue my interests in life even at times when it was difficult financially. For this I will always be in your debt. Pursuing advanced degrees also takes us away from the people we care about, and although this is difficult on families, my parents understood that life is short and we must follow our hearts. You raised me to be a polite, caring, hard-working man and I always wanted to make you proud of me. I hope this thesis and degree adds to that endeavor.
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**Appendix I.** Mitochondrial and nuclear loci sequenced for *Phylodactylus* and their corresponding primer sequences. Different combinations of primers were used to obtain full reads of regions.
1. Background

Evolutionary and molecular ecological research often strives to understand spatial and temporal patterns of biological diversification. Elucidating how and when species and populations evolved allows us to draw robust conclusions regarding the ecological and historical processes governing diversification. Recent advances in both DNA sequencing technologies and analytical methods are providing empiricists with a toolbox of new methods to test hypotheses of general interest to molecular ecology and evolution (Edwards 2008; Brito & Edwards 2009; Heled & Drummond 2008,2010; Storfer et al. 2010; Blair & Murphy 2011). However, both careful selection of molecular markers and analytical methods are required to identify accurately pattern-process relationships (Wang 2010, 2011).

The field of phylogeography seeks to identify the historical processes that shape the spatial distribution of genetic lineages. Early animal studies relied on mitochondrial DNA (mtDNA) sequences to test historical hypotheses including the effects of both major geologic changes and climatic shifts on biological diversification (Hewitt 2000, 2004). Now, with the continual reduction in costs of DNA sequencing, these studies are beginning to incorporate variable nuclear DNA (nDNA) loci to provide a multilocus perspective regarding historical processes shaping the evolutionary history of species and populations (Zhang & Hewitt 2003; Brito & Edwards 2009). Analysis of multiple unlinked genetic loci is often beneficial as sole
reliance on mtDNA for evolutionary inference is fraught with potential issues including its maternal inheritance, which makes it generally unsuitable as a tool to measure gene flow (Edwards & Bensch 2009). Further, phylogenetic analysis of a single gene provides but a single glimpse into history. Indeed, both theory and empirical data show that gene divergence will predate population and species divergence, producing a biased perspective regarding historical relationships and tempo of divergence (Edwards & Beerli 2000; McCormack et al. 2011). Because of stochasticity in the coalescent process (Kingman 1982), analysis of unlinked loci will often result in different histories and conclusions regarding the influence of historical processes on evolutionary patterns (Moore 1995; Maddison 1997; Degnan & Rosenberg 2006, 2009). This is particularly the case in recently diverged groups separated by relatively short branch-lengths, where deep gene-coalescence is common (Fig. 1). The debate regarding the utility of mtDNA versus nDNA-based phylogeography and phylogenetics continues (Zink & Barrowclough 2008; Edwards & Bensch 2009) and has spawned a new era in molecular evolution and systematics that focuses efforts away from concatenation and gene-tree approaches (Edwards 2008; Knowles 2009). These so-called “species-tree” methods use multispecies coalescence to estimate phylogenetic relationships by explicitly modeling the stochastic nature of the coalescent process (Liu et al. 2008; Kubatko et al. 2009; Heled & Drummond 2010). Unlike concatenation, these species-tree methods statistically account for gene-tree/species-tree discordance in phylogenetic studies, most of which attribute the discordance to the incomplete sorting of ancestral alleles (e.g. Liu 2008). New multilocus Bayesian methods are also being developed to reconstruct the demographic history of populations (Heled & Drummond 2008) and estimate historical migration rates and effective population sizes (Beerli & Felsenstein 2001; Beerli 2006).
Although the analysis of nDNA loci provides a more comprehensive perspective regarding patterns and processes of evolution, care must be taken when working with these data (Hare 2001; Zhang & Hewitt 2003). For example, unlike mtDNA that is inherited directly through the maternal line in most animals, nDNA is bi-parentally inherited and sequences can show signs of genetic recombination that can affect downstream phylogenetic and demographic inference (Arenas & Posada 2010; Martin et al. 2011). Determining haplotypic phase is also an issue when working with nDNA sequences that is traditionally overlooked in higher-level phylogenetic studies. Although molecular cloning procedures can be used to determine allelic phase, Bayesian methods have been developed to phase haplotypes in a statistical framework (Stephens et al. 2001). Subsequent analysis with phased nuclear data is still in its infancy and new models continue to be developed that incorporate genetic information from both alleles at a locus (Heled & Drummond 2008, 2010).

Regardless, recent evidence suggests that the arbitrary selection of one allele from each gene and concatenating it with mtDNA can lead to erroneous conclusions (Weisrock et al. 2012). Thus, although phylogenetic and phylogeographic studies continue to adopt multilocus approaches, testing the performance of new and existing analytical methods is required if we are to select the appropriate methods to test our hypotheses.

Complementing the inferences made possible by molecular phylogenetics and phylogeography, the nascent field of landscape genetics seeks to understand the contemporary landscape and ecological factors influencing microevolutionary processes (Manel et al. 2003; Holderegger & Wagner 2006, 2008). A key distinction between landscape genetics and phylogeography is the former’s focus on understanding contemporary ecological and evolutionary processes whereas the latter focuses on historical processes.
(Manel et al. 2003; Storfer et al. 2010; Wang 2010). The focus of landscape genetics on contemporary versus historical processes usually requires a certain class of molecular marker with enough variability to capture processes operating over the recent timescales of interest (e.g. microsatellites). Thus, recent discussions have highlighted the importance of careful molecular marker selection in both phylogeography and landscape genetics (Storfer et al. 2010; Wang 2010, 2011). For example, sequence-based markers such as mtDNA and nDNA evolve too slowly to capture ecological processes operating over recent timescales (see Vandergast et al. 2007 for counter-argument). Conversely, microsatellites evolve too rapidly to be useful for inferring historical processes (Zink 2010). Common questions addressed by adopting a landscape genetics approach include the influence of environmental and landscape variables on rates and patterns of gene flow, the influence of environmental and landscape variables on the spatial distribution of adaptive genetic variation, and the influence of environmental and landscape variables on certain ecological processes (Spear & Storfer 2008; Manel et al. 2010; Storfer et al. 2010; Balkenhol & Landguth 2011). Landscape genetics can also be used for effective corridor design to maintain adequate rates of functional connectivity between populations (Epps et al. 2007; Braunisch et al. 2010). The enormous interest in landscape genetics comes from a combination of its interdisciplinary nature—combining population genetics, landscape ecology, and spatial statistics—along with the continual ease in which molecular genetic data can be gathered. This interest has spawned numerous recent simulation studies that test the relative power of different analytical methods for inferring landscape-genetic relationships (e.g. Balkenhol et al. 2009; Landguth et al. 2010; Blair et al. 2012). Additional simulation and empirical studies are vital
if we are to gain an in-depth understanding of the processes governing the spatial distribution of genetic variation in natural populations.

2. Tropical Dry Forest

The tropical dry or deciduous forests (TDF) of western Mexico harbor an enormous diversity of species (Ceballos & García 1995; Gillespie et al. 2000; García 2006). In Mexico, these forests form a nearly continuous belt throughout much of the Pacific coast from southern Sonora to Chiapas with isolated stands scattered throughout the south and east as well as in Baja California Sur (Becerra 2005). Unlike tropical rainforests, the TDF is characterized by a distinct seasonality with up to 8 months of dry, arid-like conditions separated by 4 months of deluge (Murphy & Lugo 1986). These forests typically grow at low to moderate elevations where they gradually transition to pine-oak and pine forests (Robichaux & Yetman 2000). Molecular phylogenetic evidence suggests that these forests originated approximately 30 million years ago in response to major geologic changes associated with the Sierra Madre Occidental and the Mexican Volcanic Belt (Becerra 2005). Although the herpetofaunal diversity in these forests is substantial, effective conservation strategies throughout this threatened ecosystem have been lacking (García 2006) and we still know little about the historical processes important in shaping the evolutionary and biogeographic histories of organisms inhabiting this ecosystem (Becerra & Venable 2008). The few phylogenetic and phylogeographic studies focusing on genetic patterns of species in this region have found evidence for several cryptic mtDNA lineages that correspond to several traditional biogeographic barriers (e.g. Devitt 2006; Zarza et al. 2008). However, additional data from
diverse species are required to understand better the potential suite of historical processes influencing cladogenesis throughout the TDF.

To date, no landscape genetic study has focused explicitly on TDF habitat. Indeed, at present there is a general lack of landscape genetic studies focused on tropical ecosystems in general (Storfer et al. 2010). This is problematic, as the majority of Earth’s species reside in these environments (Myers et al. 2000). Further, dry forests, particularly in Mexico, have been experiencing elevated rates of recent fragmentation due to continual anthropogenic influence (Trejo & Dirzo 2000). Thus, it remains unknown how species are responding to fragmentation. The adoption of a landscape genetic approach can help us understand the importance of landscape and environmental variables on functional connectivity and how populations are responding to recent habitat fragmentation. Thus, by combining multilocus phylogenetic analyses along with a landscape genetics we can begin to understand both the evolutionary history of species inhabiting TDF and if and how they are responding to recent changes in the landscape. The combination of molecular markers and analytical methods suitable for testing hypotheses operating over different temporal scales serves as a powerful approach to understand the evolutionary ecology of TDF dependent species.

3. Leaf-toed Geckos

Leaf-toed geckos, genus *Phyllodactylus*, encompass approximately 50 species distributed throughout the TDF of the Neotropics from northwestern Mexico and extending to southern California and the peninsula of Baja California southward to north-central South America and the West Indies (Dixon 1962). *Phyllodactylus* are primarily nocturnal commonly found in a diverse array of microhabitat types depending on geographic region (Dixon 1962). In
Mexico, for example, most species are strictly saxicolous (found on rocky outcroppings), although some species can also be found on trees or inside cacti (Dixon & Huey 1970). Although these lizards are often quite common throughout their range, the phylogenetic relationships, biogeographic history, population genetic structure, and taxonomy of this diverse group of lizards remains uncertain (Dixon 1960, 1964). However, recent investigations utilizing genetic data have documented cryptic diversity throughout the Mexican dry forests (Blair et al. 2009). Further, their apparent reliance on TDF habitat makes them an ideal choice to examine both historical and contemporary processes governing genetic diversification throughout this ecosystem.

4. Objectives of Thesis

The goals of this thesis are to test multiple spatial and temporal hypotheses affecting genetic diversification in leaf-toed geckos throughout the Mexican TDF. All fieldwork was in collaboration with Prof. Robert W. Murphy (supervisor; University of Toronto), Prof. Fausto Roberto Méndez de la Cruz (National Autonomous University of Mexico), and Victor Hugo Jimenez Arcos (National Autonomous University of Mexico), with additional field assistance provided by numerous other individuals (see Acknowledgments). In Chapter 2, I use a combination of standard molecular cloning along with next-generation 454 DNA sequencing to isolate 12 highly polymorphic microsatellite loci for the Mexican yellow-bellied gecko *Phyllodactylus tuberculosus saxatilis*. In Chapter 3, I use these microsatellite loci to perform a landscape genetic analysis of *P. tuberculosus* in the region surrounding Alamos, Sonora. Specifically, I seek to determine if forest structure, stream connectivity, slope, anthropogenic disturbance, and minimum temperature influence rates and patterns of gene flow between
populations. In Chapter 4, I conduct a multilocus phylogenetic study of the *P. tuberculatus* species group (*P. tuberculatus*, *P. lanei*, and *P. muralis*) throughout the TDF of western Mexico. Using new, powerful coalescent methods, I test hypotheses regarding the relative roles of climate change, geography, and geological formations on rates and patterns of cladogenesis in these geckos. Chapter 5 explores the use of the microsatellite data gathered in Chapter 3 along with *de novo* mtDNA sequences by comparing and contrasting historical and contemporary demographic processes in *P. tuberculatus* throughout the region surrounding Alamos. More specifically, I test for a significant difference in historical versus contemporary migration rates and effective population sizes, and how these metrics correlate with contemporary landscape structure. I then test the ability of mtDNA to capture contemporary landscape processes influencing genetic connectivity. Finally, in Chapter 6, I examine the taxonomic consequences of historical biogeographic processes operating throughout the peninsula of Baja California. This chapter is now published in the journal *Zootaxa* (Blair et al. 2009). Chapter 7 briefly summarizes the main findings of the thesis. All chapters are written in collaboration with Robert W. Murphy. References follow the style of the journal *Molecular Ecology*. 
References


Vandergast AG, Bohonak AJ, Weissman DB, Fisher RN (2007) Understanding the genetic effects of recent habitat fragmentation in the context of evolutionary history:


Figure Legends

**Fig. 1.** Example of a gene tree embedded within a species tree illustrating the influence of deep coalescence (black dots) on species tree inference. The figure shows that deep coalescence can result from both incomplete lineage sorting in recently diverged species (A and B) as well as from older speciation events shaped by short internal branches (Species E versus Species C and D). All allelic copies in Species C, D, and E share a most-recent common ancestor indicating that enough time has passed to reach reciprocal monophyly. Different colour shades represent different ancestral species. Figure taken from Knowles (2009).
CHAPTER 2

Using next-generation DNA sequencing for rapid microsatellite discovery in Mexican leaf-toed geckos (*Phylodactylus tuberculatus*).

Abstract

I use a combination of 454 shotgun pyrosequencing and traditional molecular cloning to isolate microsatellite loci for the Mexican yellow-bellied leaf-toed gecko (*Phylodactylus tuberculatus*). A pyrosequencing run on a 454 GS Junior yields 62 Mbp of data composed of 150k fragments with an average length of 412 bp. Among the fragments, 18,144 (12%) contain a suitable microsatellite. Dinucleotides and tetranucleotides are most frequently encountered. I then genotype 30 individuals of *P. tuberculatus* using 12 polymorphic loci. I find moderate allelic diversity and heterozygosity. No loci are in linkage disequilibrium and only two loci show deviations from Hardy-Weinberg expectations. These results further highlight the utility of next-generation sequencing for discovering molecular markers in non-model taxa and suggest that sequencing on a 454 GS Junior system is a rapid and cost-effective approach for microsatellite characterization.
Leaf-toed geckos (*Phyllodactylus*) occur in arid to semi-arid regions of the Neotropics (Dixon 1964). The yellow-bellied gecko, *P. tuberculosus*, ranges from southern Sonora, Mexico to northern Costa Rica. In Mexico, species are commonly encountered throughout tropical dry forests (TDF) habitat. TDF is considered one of Earth’s biodiversity hotspots (Myers *et al.* 2000; García 2006) that are experiencing rapid rates of deforestation (Trejo & Dirzo 2000). However, little is known about how fragmentation will influence population connectivity for species inhabiting this ecosystem. Further, species within this genus pose a conservation concern as local people actively kill species of the genus as they are assumed to be venomous (pers. obs.). At present, the species has yet to be assessed by the IUCN. I combine molecular cloning with NGS to isolate and characterize polymorphic microsatellite loci for *P. tuberculosus*. The microsatellite loci obtained will be used for subsequent studies that examine fine-scale population genetic structure of geckos within Mexico’s TDF.

In 2008 I sampled 30 individuals of *P. tuberculosus* from a single population adjacent to the Río Cuchujaqui near Alamos, Sonora. Tail tips were collected for genetic material and preserved in the field with 95% ethanol. DNA was extracted using standard phenol-chloroform procedures. Extracts were precipitated and resuspended in distilled water.

I first used standard molecular cloning methods to isolate microsatellite loci (Hamilton *et al.* 1999). Genomic DNA was digested with the restriction enzymes NheI RsaI and XmnI. Following digestion, SNX linkers were ligated to the digested fragments for amplification via PCR. Following amplification, I enriched for the following microsatellite motifs: (CGG)$_4$, (AAAC)$_6$, (AAT)$_{12}$, (AAAT)$_8$. Fragments containing the microsatellite motifs were captured by streptavidin beads. The linker-ligated enriched DNA was then amplified, inserted into a plasmid vector, and cloned into *E. coli* cells. Positive clones were then amplified using M13
forward and reverse primers and directly sequenced using the BigDye Terminator Cycle Sequencing Kit (Applied Biosystems). Sequences were then read on an ABI 3730 DNA sequencer (Applied Biosystems) and edited using BioEDIT version 7.0.9 (Hall 1999). A single individual was also sent to Trent University's Natural Resources DNA Profiling and Forensics Centre for 454 shotgun sequencing using a full run on a 454 GS Junior. For genomic sequencing, I extracted DNA using a DNeasy Tissue Kit (QIAGEN) to obtain a cleaner product. This extract was then sent to Trent for library construction, emulsion PCR, and 454 sequencing.

MSATCOMMANDER (Faircloth 2008) was used to search for 454 fragments (~150k) containing microsatellites. When suitable clones and 454 fragments were found, I used PRIMER3 (Rozen & Skaletsky 2000) to develop primers. The total data set consisted of 12 loci (5 from cloning and 7 from 454 sequencing). I chose loci from the 454 run containing tetranucleotide repeats to minimize potential scoring errors due to stutter.

PCR amplification was performed using a 12.5 µL reaction volume containing 1.25 µL 10X PCR buffer containing 1.5 mM MgCl₂, 0.1 µL BSA, 1 µL 10 mM of each primer, 0.4 µL 10 mM dNTPs and 0.1 µL taq DNA polymerase (New England Biolabs). Water and MgCl₂ concentrations were adjusted to obtain optimal PCR products (Table 1). Each forward primer was fluorescently labelled with a 6-FAM M13 tag following Schuelke (2000). All PCRs were performed on an Eppendorf AG 5345 thermal cycler using the following conditions: initial denaturation at 95 °C for 2 min, followed by 35 cycles of 95 °C for 30 s, annealing at the optimal temperature for 45 s (Table 1), and extension at 72 °C for 45 s, with a final extension temperature of 72 °C for 6 min. Genotyping was performed by mixing 2 µL of each PCR product with a mixture of Hi-Di and GENESCAN 500 LIZ size standard (Applied
Biosystems). Samples were read on an ABI 3730 DNA sequencer and alleles were scored using the software GENEMARKER version 1.9.1 (SoftGenetics).

I used MICROSEATELLITE ANALYSER version 4.05 (Dieringer & Schlötterer 2003) to calculate the number of alleles per locus, allelic range, and observed and expected heterozygosities ($H_O$ and $H_E$, respectively). Hardy-Weinberg and linkage equilibrium (log likelihood ratio statistic) were tested using GENEPOP version 4.0.10 (Rousset 2008) using default Markov chain parameters. I used MICRO-CHECKER version 2.2.3 (van Oosterhout et al. 2004) to check for scoring errors due to stuttering, long allele dropout, or null alleles.

I was able to obtain reliable genotypes for 12 loci (three trinucleotide and nine tetranucleotide). Diversity was moderate, with the number of alleles per locus ranging between three and 14. Observed heterozygosity ranged between 0.6207 to 0.9000 and expected heterozygosity ranged from 0.5668 to 0.9136 (Table 1). No loci showed signs of linkage disequilibrium after Bonferroni correction ($P > 0.05$). Loci G2_37 and Pt1 showed deviations from Hardy-Weinberg expectations with an excess of homozygotes ($P < 0.01$ and $P = 0.0224$, respectively). No loci showed evidence of stutter or long allele dropout. However, G2_37 showed evidence of null alleles (0.0835). This study adds to a small body of literature that uses NGS to isolate polymorphic microsatellite markers in reptiles (e.g. Castoe et al. 2009; Metzger et al. 2011; Smith et al. 2011) and suggests that the 454 GS Junior is a rapid and cost-effective means to isolate microsatellite loci.

Acknowledgments

I kindly thank the generation sequencing facilities available at Trent University's Natural Resources DNA Profiling and Forensics Centre for performing the 454 sequencing for this
study. I thank Martin Gabriel Figueroa for help in the field. Stephanie Meyer provided logistic support for potential field sites. For laboratory assistance I thank Oliver Haddrath, Amy Lathrop, and Kristen Choffe at the Royal Ontario Museum. I thank Brant Faircloth for assistance with MSATCOMMANDER. This work was supported by the Natural Sciences and Engineering Research Council (NSERC) Discovery Grant A3148 (RWM) and the Theodore Roosevelt Memorial Grant through the American Museum of Natural History (CB).
References


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throughput sequencing: when innovation meets conservation. *Conservation Genetics Resources*, 3, 589-592


Table 1 Characterization of twelve microsatellite loci for the leaf-toed gecko Phyllodactylus tuberculosus (n = 30) based on molecular cloning and 454 shotgun sequencing.

<table>
<thead>
<tr>
<th>Locus</th>
<th>Method</th>
<th>Repeat Unit</th>
<th>Primer Sequence (5'–3')</th>
<th>Label</th>
<th>Ta (°C)</th>
<th>MgCl₂ (mM)</th>
<th>Alleles</th>
<th>Range (bp)</th>
<th>H₀</th>
<th>Hₑ</th>
<th>GenBank Accession</th>
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<td>60</td>
<td>1.50</td>
<td>14</td>
<td>231–282</td>
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<td>0.9136</td>
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<td>FAM</td>
<td>60</td>
<td>2.00</td>
<td>3</td>
<td>259–265</td>
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<td>0.5668</td>
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<tr>
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<td>231–267</td>
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<td>60</td>
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All forward primers were labelled with an M13 tail and amplified using the three primer method of Schuelke (2000). Ta, annealing temperature; MgCl₂, concentration of MgCl₂ in each reaction; H₀, observed heterozygosity; Hₑ, expected heterozygosity.
CHAPTER 3
Landscape genetics of leaf-toed geckos in the sub-tropical dry forests of northern Mexico

Abstract

Habitat fragmentation due to both natural and anthropogenic forces continues to threaten the evolution and maintenance of biological diversity. This is of particular concern in tropical regions that are experiencing elevated rates of habitat loss. Although less well-studied than tropical rain forests, tropical dry forests (TDF) contain an enormous diversity of species and continue to be threatened by anthropogenic activities including grazing and agriculture. However, little is known about the processes that shape genetic connectivity in species inhabiting TDF ecosystems. I adopt a landscape genetic approach to understanding functional connectivity for leaf-toed geckos (*Phyllodactylus tuberculatus*) at multiple sites near the northernmost limit of this ecosystem at Alamos, Sonora, Mexico. Traditional population genetic analyses are combined with univariate and multivariate landscape analyses to test hypotheses on the potential drivers of spatial genetic variation. Moderate levels of within-population diversity and substantial levels of population differentiation are revealed by $F_{ST}$ and $D_{est}$. Analyses using STRUCTURE suggest the occurrence of from three to five genetic clusters. Landscape genetic analysis suggests that forest cover, stream connectivity, undisturbed habitat, slope, and minimum temperature of the coldest period explain more genetic variation than do simple Euclidean distances. Additional landscape genetic studies
CHAPTER 3—Landscape Genetics of *P. tuberculosus* 26

throughout TDF habitat are required to understand species-specific responses to landscape and climate change and to identify common drivers. I also urge researchers interested in using multivariate distance methods to test for, and report, significant correlations among predictor matrices which can impact results, particularly when adopting least-cost path approaches.

1 Introduction

Dispersal is a fundamental process that can greatly influence ecological and demographic trajectories within and between subpopulations (Clobert *et al.* 2001). For example, dispersal often leads to gene flow, the transfer of genetic information from one population to another (Slatkin 1987; Bohonak 1999). Maintaining adequate rates of gene flow is often beneficial because populations experiencing low gene flow are susceptible to a loss of genetic diversity due to inbreeding and drift (Slatkin 1987; Frankham *et al.* 2002; Vilá *et al.* 2002). A lack of genetic diversity may also make it difficult for populations to adapt to changing environmental conditions and eventually lead to local extinction (Lande 1993; Crispo *et al.* 2011).

Dispersal rates can result from a suite of different factors including species-specific philopatry, intra- and interspecific interactions, predation pressures, physiological tolerances, and simple geographic (Euclidean) distance (Spear *et al.* 2010). Landscape heterogeneity often plays a substantial role in an organism’s choice to disperse or not (With *et al.* 1997; Ricketts 2001; Bowne & Bowers 2004) and both natural and anthropogenic habitat-fragmentation can detrimentally affect the connectivity and persistence of populations
CHAPTER 3—LANDSCAPE GENETICS OF P. TUBERCULOSUS

(Fahrig 1997, 1998, 2003, 2007). Landscape genetics seeks to explicitly quantify the influence of landscape and environmental variables on microevolutionary processes such as gene flow and natural selection (Manel et al. 2003; Holderegger & Wagner 2008). The approach extends traditional population genetic studies by explaining the spatial distribution of genetic variation by using components of the landscape. This particularly powerful approach to studying fine-scale population structure and its application is meeting with much success as Storfer et al. (2010) review. By combining rapidly evolving molecular markers such as microsatellites with novel approaches to statistical analysis, landscape genetics identifies a suite of environmental variables likely to influence population genetic structure (Spear et al. 2005; Vignieri 2005; Wang et al. 2009). Identifying the landscape components facilitating or constraining gene flow can aid in delimiting areas for conservation (Neel 2008), for example, by designing corridors that maximize functional connectivity (Epps et al. 2007; Braunisch et al. 2010). Today, there is a dearth of landscape genetic studies focusing on tropical localities (Storfer et al. 2010), which harbour the majority of species (Myers et al. 2000). Thus, compared to temperate localities, relatively little is known about the processes influencing functional connectivity in species inhabiting this mega-diverse region.

Tropical dry forests (TDF), which also occur in the Neotropics, are a major biodiversity hotspot (Myers et al. 2000). They occur in a semi-continuous belt throughout the New World from northern Mexico southward into northern South America. Both structural and functional differences differentiate these forests from tropical rainforests (Murphy & Lugo 1986). The amount of annual rainfall is a primary distinction between these forests, with TDF experiencing up to eight months of arid-like conditions followed by four months of deluge. Further, canopy height is far lower in TDF than in rainforests and many plant species
in these forests possess thorny spines throughout their shoots. Although evidence suggests that these forests may be far more diverse than currently realized (Robichaux & Yetman 2000), habitat fragmentation due to both natural and anthropogenic factors is threatening the evolutionary potential of inhabiting species (Trejo & Dirzo 2000; Sigala-Rodríguez & Greene 2009). Habitat fragmentation is of particular concern in rapidly developing countries such as Mexico, where dense continuous forest is being cleared for both livestock and agriculture (Trejo & Dirzo 2000). In Mexico, TDF reaches its northern limit near Alamos, Sonora, whereas forest density is highest in the southwest states of Jalisco, Colima, Michoacán, and Guerrero (Becerra 2005). Fragmentation of these forests is documented to have occurred for decades and continues to increase, yet we know relatively little about how fragmentation effects species and populations distributed throughout these ecosystems.

The diversity of Mexico’s herpetofauna is substantial, with approximately 1 000 described species and many more awaiting formal description (Robichaux & Yetman 2000; Wilson et al. 2010). Flanking the Pacific coast, Mexico’s TDF also appears to be a centre of endemism for a variety of amphibian and reptilian species (García 2006). A variety of species of amphibians and reptiles are restricted to the TDF of western Mexico, including many species of leaf-toed geckos of the genus *Phyllodactylus* (Dixon 1964) These lizards inhabit arid to semi-arid areas from southern California southward through Middle America into northern South America and into the West Indies. Like many geckos, they are commonly found on vertical surfaces including bridges and buildings.

Herein I use the Mexican yellow-bellied gecko, *Phyllodactylus tuberculosis*, to test a variety of landscape genetic hypotheses operating in Mexico’s TDF. This species is an ideal choice to examine relationships between landscape and genetics for two reasons. First, the
gecko’s geographic distribution mirrors the distribution of TDF in Mexico. Second, this species, along with others in the genus, are at risk of local and area-wide extinctions due to habitat fragmentation and recent introductions of non-native, all female species, such as geckos of the genus *Hemidactylus*, which appears to be displacing leaf-toed geckos (pers. obs.). The study site lays in the northernmost limit of TDF near Alamos, Sonora. I test the following null hypotheses: 1) Genetic diversity not spatially structured, other than isolation-by-distance; 2) forest fragmentation does not influence functional connectivity and gene flow; and 3) univariate and multivariate landscape and environmental data do not correlate with the spatial distribution of genetic differentiation between populations. Rejection of these null hypotheses and drawing robust conclusions favouring alternative hypotheses requires principles and practices drawn from diverse research disciplines (Manel *et al.* 2003).

Therefore, I use recent advances in landscape genetic techniques (Storfer *et al.* 2007, 2010; Balkenhol *et al.* 2009; Dyer *et al.* 2010) to test the null hypothesis (Cushman *et al.* 2006) and identify which landscape variables influence functional connectivity of leaf-toed geckos in this threatened ecosystem.

## 2 Materials and Methods

### 2.1 Sampling

My study area was the northern limits of TDF (Fig. 1), an area that today has a relatively high degree of forest cover compared to other locations throughout western Mexico due, in part, to the federal protection of land (Sierra de Alamos/Río Cuchujaqui Reserve). The
landscape at lower elevations (generally under 500 m) consisted of tropical dry thornscrub that gradually transitioned into TDF closer to the Sierra de Alamos with increasing elevation. From 2008 to 2010, I sampled 336 leaf-toed geckos from 12 different localities (mean = 28 individuals per locality) throughout the landscape surrounding the Alamos region. Sampling localities were chosen based on landscape characteristics to allow for testing hypotheses. I sampled on opposite banks of two relatively large rivers or arroyos (Cuchujaqui and Tabelo) to determine if these features served as barriers to gene flow. When possible, I obtained a minimum of 30 individuals per population. Following protocols approved by the Royal Ontario Museum Animal Care Committee, tail tips were taken in the field and immediately preserved in 95% ethanol for subsequent genetic analysis.

2.2 DNA extraction and genotyping

DNA was extracted using standard phenol-chloroform procedures. I used the polymerase chain reaction (PCR) to amplify 12 polymorphic microsatellite loci developed specifically for *Phyllodactylus tuberculosus* (see Blair et al. in review for description of loci and PCR conditions). Both negative and positive controls were run on each PCR plate. PCR products were visualized on an ABI 3730 automated sequencer (Applied Biosystems Inc.) at the Royal Ontario Museum. Genotyping was performed using the software GeneMarker v. 1.95 (SoftGenetics). I re-ran PCRs for approximately 10% of the samples to quantify any potential errors in genotyping.

2.3 Genetic diversity
I calculated diversity statistics for each site including number of alleles and allelic richness using MIcroSatellite Analyser v. 4.05 (Dieringer & Schlötterer 2003). Observed \( (H_0) \) and expected \( (H_E) \) heterozygosites were calculated in TFPGA v. 1.3 (Miller 1997). I tested for site-specific deviations from Hardy-Weinberg (exact test) and linkage equilibrium using GENEPop on the web v. 4.0.10 (Raymond & Rousset 1995; Rousset 2008). Significance for tests was assessed using the Markov chain method using 100 batches with 1 000 iterations per batch. I used the false-discovery rate method to control for multiple comparisons (Benjamini & Hochberg 1995).

### 2.4 Genetic differentiation

There has been considerable recent debate as to the best \( F_{ST} \)-like analogue for assessing genetic differentiation between populations (Heller & Siegismund 2009; Jost 2009; Ryman & Leimar 2009; Gerlach et al. 2010). The traditional \( F_{ST} \) metric (\( G_{ST} \) for multiple loci and alleles) has been shown to be highly sensitive to within-population heterozygosity, making it difficult to compare values between studies and markers (Hedrick 2005; Jost 2008). Further, because \( F_{ST} \) is dependent on the number of alleles and heterozygosity, two populations have shown low \( F_{ST} \) values despite sharing no alleles. For example, Gerlach et al. (2010) showed that in two hypothetical populations with a total of 16 alleles, \( F_{ST} \) was constrained to less than 0.1 even when the two populations shared no alleles. New metrics such as \( G'_{ST} \) (Hedrick 2005) and \( D \) (Jost 2008) have been developed that circumvent some of the more common issues found with \( F_{ST} \) (reviewed by Meirmans & Hedrick 2011). Although these new metrics have been criticised (Whitlock 2011), information about differentiation can be maximized by comparing multiple statistics (Meirmans & Hedrick 2011). Thus, I calculated both traditional
$F_{ST}$ and Jost’s $D$ between populations. Whereas $F_{ST}$ dealt with fixed biallelic markers, $D$ measured the degree of allelic differentiation between populations and was particularly useful for highly polymorphic markers such as microsatellites (Jost 2008). MICROSATELLITE ANALYSER was used to calculate both global and pairwise multilocus $F_{ST}$ following Weir & Cockerham (1984). Significance of comparisons was assessed using 10,000 permutations while implementing a Bonferroni correction for multiple tests. I used SMOGD v. 1.2.5 (Crawford 2010) to calculate pairwise $D_{est}$ based on harmonic means estimated over all 10 loci. I used the unbiased estimates of all metrics to account for artefacts of sample size (Jost 2008, 2009).

### 2.5 Population structure

I tested for the presence of genetic clusters using STRUCTURE v. 2.3.3 (Pritchard et al. 2000; Falush et al. 2003) to classify population structure. STRUCTURE, a Bayesian program, used Hardy-Weinberg and linkage disequilibrium to cluster individuals into populations ($K$) based on data from multi-locus genotypes. I employed the admixture model with correlated allele frequencies while specifying a range of $K$-values (1–12). For simplicity, I created a batch run specifying the range of $K$-values to be evaluated and running 10 independent runs per $K$. Each run was composed of a burnin of 30,000 followed by 100,000 iterations, which was sufficient to reach convergence. I evaluated the most likely number of clusters using both the $Pr (X/K)$ method as described by the program’s authors and the $\Delta K$ method of Evanno et al. (2005). STRUCTURE HARVESTER (Earl & vonHoldt 2011) was used to visualize outputs and calculate $K$ based on both methods. To deal with the multimodality of utilizing multiple independent runs, I used CLUMPP v. 1.1.2 (Jakobsson & Rosenberg 2007) to permute the
admixtures for the runs with the chosen $K$-value using the “Greedy” algorithm with 1,000 random input orders. DISTRUCT v.1.1 (Rosenberg 2004) was then used to visualize the output from CLUMPP.

Geographic (spatial) distributions have provided valuable insights in population genetic analyses (Guillot 2009). Thus, I compared the non-spatial STRUCTURE results to those that incorporated information about sampling locations as prior information (Hubisz et al. 2009). I introduced an additional parameter (LOCPRIOR) into the clustering analysis by specifying a different integer for each sampling location. Often this approach has increased the ability of the algorithm to detect relatively weak population structure that may not be found using the aspatial model (Hubisz et al. 2009). I did not perform fully spatial models using programs such as TESS (Chen et al. 2007; Durand et al. 2009) and GENELAND (Guillot et al. 2005a,b) because I had GPS data for populations and not individuals. Although GENELAND can potentially accommodate this type of sampling by the introduced error term, some subjectivity remained as to which values to use for this parameter.

### 2.6 Landscape genetic analysis

I calculated effective distances between populations using least-cost path modeling (Michels et al. 2001; Adriaensen et al. 2003; Spear et al. 2005; Wang et al. 2009). This assessed the influence of different landscape and environmental variables on population genetic structure (Spear et al. 2010). Unlike using Euclidean distances (IBD) to correlate geographic and genetic distances, effective distances between individuals or populations allowed for the direct incorporation of landscape variables into the model (Cushman et al. 2006). These effective distances were created in a GIS environment by parameterizing different resistance
surfaces that represented the hypothesized relationships between a specific habitat feature and gene flow (Spear et al. 2010). For example, if hypothesizing that urban development versus undisturbed forested habitat constrained the movement of individuals, I assigned a higher cost value to cells representing urban habitat. The difficulty in using resistance surfaces to model functional connectivity lied in the assignment of relative cost values for different landscape features (Rayfield et al. 2010). Although parameterization based on radio telemetry, mark-recapture studies or experimental approaches may be the most quantitative way to assess relative costs, often for many species these methods have not been feasible due to financial or logistical reasons (Spear et al. 2010). Parameterization based on expert opinion of assigning relative cost values provided an alternative approach to model gene flow and the values were tested in a model-selection framework.

I tested for the relative influence of several landscape variables on genetic differentiation based on pairwise $D_{est}$. Landscape variables were selected based on expert knowledge of which habitat characteristics were most likely important in shaping patterns of gene flow in the species (e.g. Spear et al. 2010). My first model was based on the traditional isolation-by-distance (IBD) model (Wright 1943) that assumed genetic differentiation was a by-product of simple Euclidean distance without regard to the landscape. Next, I tested a variety of landscape genetic hypotheses that explicitly consider the intervening matrix (Ricketts 2001). First, I tested for the influence of land cover-type (TDF vegetation) on genetic connectivity by downloading a raster data set from the North American Land Change Monitoring System (NALCMS). Nineteen different land cover-types were classified at a 250 m spatial resolution. I created resistance surfaces by re-classifying the data to assign higher cost values to non-forested versus forested habitat. I tested several different cost ratios (1:2,
CHAPTER 3—LANDSCAPE GENETICS OF P. TUBERCULOSUS

1:10, 1:100, 1:1000) to determine how parameterization might have influenced the results. I used the Mantel test function in the R package ECODIST (R Development Core Team 2008; Goslee & Urban 2008) to test for both the presence of IBD and to select among the four relative cost values chosen for parameterization. Optimal values were selected based on the Mantel $r$ correlation statistic using 10 000 randomizations.

Because P. tuberculosus occurred in lowland tropical environments only, my second set of analyses developed least-cost paths based on slope. These data were derived from a GTOPO30 digital elevation model (DEM) with a 1 km$^2$ spatial resolution produced from Natural Resources Canada and the U.S. Geological Survey. This layer consisted of seven elevation classes that were reclassified into slope using the Spatial Analyst extension of ARCMAP 10 (Environmental Systems Research Institute) to test the prediction that gene flow occurred primarily throughout lowland habitats. The study area encompassed an elevation range between 100 to 500 m above sea level. For computational efficiency and because I assumed a linear relationship between elevation and gene flow (e.g. Spear & Storfer 2008), I reclassified the data into values ranging between 1 and 9, with 9 representing the highest slope values in the study.

Because I often captured geckos adjacent to streams and arroyos, I tested if dispersal occurs primarily through stream corridors. I first obtained a polyline file representing all of Mexico’s streams and tributaries from the GISDataDepot, a site that compiled multiple data layers based on ESRI’s Digital Chart of the World (DCW). I then converted this data-file into a raster file with a cell size of 100 m$^2$ and assigned different cost values to cells encompassing streams versus those that did not. I tested the same relative cost values as out
land cover analysis (1:2, 1:10, 1:100, 1:1000) and selected the best values based on Mantel correlations.

I tested for effects of minimum temperature of the coldest period of the year because these lizards are predominantly found in warm tropical lowland habitats. A significant correlation between gene flow and minimum temperatures was predicted. Temperature data were obtained from the WorldClim database at a resolution of 1 km$^2$. Because these data were continuous variables, I reclassified values to represent natural breaks in temperature. As with slope, I assumed a linear relationship between temperature and gene flow and assigned cost values ranging from 1 to 9, with 9 representing the highest cost to gene flow representing cells with the lowest temperature.

Finally, I utilized a multivariate resistance surface representing the combined effects of anthropogenic land-use on gene flow. These data were obtained from the Wildlife Conservation Society (WCS) and the Center for International Earth Science Information Network (CIESIN) and represented the combined effects of population density, built-up areas, roads, railroads, navigable rivers, coastlines, land-use, and nighttime lights. The data were categorized based on the Human Influence Index with values ranging between 0 and 64, with 0 representing no human influence and 64 representing maximum influence. To create least-cost paths, I recategorized the continuous data into discrete classes and assigned cost values ranging from 1 to 9, with 9 representing the highest cost to gene flow representing cells with the highest anthropogenic influence.

For all least-cost path-analyses, I used the LANDSCAPE GENETICS TOOLBOX 1.2.3 (Etherington 2011) implemented in ARCMAP 10 to calculate effective distances between sampling localities. This calculated both the cost-distance and the length of the least-cost
path between any two sampling points. Because both distances could have been sensitive to relative cost values (Rayfield et al. 2010), I tested several different relative values for categorical variables as described above. For all least-cost path-analyses, I used the cumulative cost-distance because this metric minimized the degree of multicollinearity in the predictors. For all least-cost path calculations, rasters were standardized to a cell size of 100 m$^2$.

Functional connectivity and gene flow were modeled using circuit theory (McRae 2006; McRae & Beier 2007; McRae et al. 2008). Unlike least-cost path approaches to modeling functional connectivity, a circuit theoretic approach was particularly powerful in that it modeled all possible pathways linking populations and accounted for wider habitat swaths. This so-called isolation-by-resistance (IBR) approach has been shown to be powerful in modeling gene flow and functional connectivity in both simulated and empirical data sets (McRae 2006; McRae & Beier 2007). I calculated resistance distances between populations using CIRCUITSCAPE 3.5.7 (Shah & McRae 2008). I first used the EXPORT TO CIRCUITSCAPE tool in ARCMAP to modify the input rasters for use with CIRCUITSCAPE. I then calculated resistance distances between each population for each landscape variable in CIRCUITSCAPE. Each calculation used focal points in pairwise mode and a four-neighbors connection scheme. All calculations were based on values of per-cell resistance.

Multiple regression analyses on distance matrices (MRM; Legendre et al. 1994; Lichstein 2007) were used to evaluate landscape genetic relationships. Although a potentially powerful method to elucidate landscape genetic relationships (Balkenhol et al. 2009), few studies have incorporated MRM analyses (Dyer et al. 2010). Similar to the commonly used partial Mantel test (Smouse et al. 1986), MRM was developed to test for significant
relationships between a dependent distance matrix (e.g. linearized $D_{est}$) and any number of indicator matrices and identify the contribution of each explanatory variable to the overall fit of the model. Further, MRM can model polynomial and nonlinear relationships (Lichstein 2007). Each distance matrix was unfolded into vectors representing pairwise distances. The response vector was then regressed against each indicator vector and the significance of the model was assessed by permuting the objects of the response matrix. Because several of the landscape variables were highly correlated (forest cover, minimum temperature, slope, anthropogenic disturbance), I created multivariate resistance surfaces using the Weighted Sum function under the Spatial Analyst tool in ARCMAP. I created four different surfaces representing a composite of the four correlated variables. To understand which of the four variables were influencing genetic structure, I assigned a weight of 10 to a different input raster for each of the four multivariate surfaces. For example, to test whether forest structure was the primary feature influencing functional connectivity or not, I first multiplied cost values in the forest raster by 10 before summing the values with the remaining three rasters. I then re-calculated least-cost paths and resistances based on the new multivariate resistance surfaces.

I performed all MRM using ECODIST. To select among competing models, I used a stepwise regression approach, although using a backwards elimination approach gave similar results. First, I tested the predictive power of each distance metric to determine the variable with the largest coefficient of determination. This variable was then added to the model and subsequent variables were either added or removed depending whether their corresponding $p$-value was lower or higher than the predetermined cut-off of $p = 0.05$, respectively. Included variables were required to be statistically significant.
Multivariate models using all variables were not created for the least-cost analyses due to a relatively high degree of multicollinearity among the predictors, which could result in coefficients with large variances, leading to erroneous conclusions regarding the direction and magnitude of slope. Kutner et al. (2004) suggested calculating Variance Inflation Factors (VIF) for each predictor in a model to ascertain if collinearity might be a problem in parameter estimation. Like previous studies, I used VIF values > 10 as evidence for substantial multicollinearity (Dyer et al. 2010). Thus, I ran four separate models for regressions based on least-cost paths to minimize the potential error in estimated regression coefficients. All models contained both Euclidean distance and least-cost paths based on stream connectivity. Each of the four models contained one of the remaining variables (forest cover, slope, temperature, or anthropogenic disturbance). Because multicollinearity was not a problem in the resistance distances calculated from CIRCUITSCAPE I included all variables in the stepwise regression.

To validate the stepwise procedure for selecting a model, I calculated values of AIC—measures the amount of information lost in a given model \( i \) relative to the true model \( j \) (Kullback & Leibler 1951)—for competing models. The best model minimized the amount of information lost as represented by the combination of variables with the lowest AIC value. Corrected values of AIC accounted for smaller sample sizes. Different combinations of variables were compared to the null model of IBD to determine if the incorporation of landscape variables explained more of the variation in \( D_{est} \). I followed Burnham & Anderson (2002) in assuming that models with \( \Delta \text{AIC} < 2 \) were strongly supported, values between 3 and 7 represented models with considerably less support, and values above 10 were highly unlikely. I used MUMIN (Barton 2009) to calculate AIC weights for each model.
3 Results

3.1 Genetic diversity

The genotyping and scoring of microsatellite alleles had an error rate of less than 1%, and, thus, high reproducibility. After controlling for false discovery rates, some loci showed significant deviations from Hardy-Weinberg expectations within sites. For example, locus G2_96 showed heterozygote deficits at six of the 12 sites, locus P7 at eight of 12, and locus G2_59 at four of 12. However, only three alleles were present at locus G2_59 and, thus, there was a high probability that random chance resulted in significance. Because loci G2_96 and P7 showed a significant heterozygote deficit at multiple sites, I ran preliminary analyses with and without these loci to see how results changed. Although results did not differ substantially, I adopted a conservative approach and chose to report results from subsequent analyses excluding these two loci.

After controlling for false discovery rates, a few loci showed signs of linkage disequilibrium. For example, locus G2_22 showed linkage to loci G2_96, P2, P7, P12, and P19. Locus G2_85 showed signs of linkage with locus P7, and locus P2 with P15. However, linkage was suggested at only two of the 12 sites (Arroyo Tabelo B and Mocuzari). If linked, I expected to find significant linkage across multiple sites. Therefore, I concluded that the loci were independent, unlinked markers.

Exploratory results of genetic diversity were shown in Table 1. In general, within-site diversity was moderate as shown by both expected heterozygosity and allelic richness. Expected heterozygosity ranged from 0.7025 to 0.7812 and allelic richness from 54.2341 to 65.6058. Allelic richness was calculated based on the minimum sample size per locality (10...
individuals) over all 10 loci. Allelic diversity within loci over all populations ranged from 3 alleles at locus G2_59 to 26 alleles at locus G2_37 (mean number of alleles per locus = 13.1). In general, diversity estimates were fairly similar among sites. There was a significantly positive relationship between elevation and genetic diversity ($R^2 = 0.5836; p = 0.004$; Fig. 2).

### 3.2 Genetic differentiation

Global genetic differentiation was reasonably moderate based on both $F_{ST}$ ($F_{ST} = 0.0869; p = 0.0001$) and $D$ ($D_{est} = 0.248$). Pairwise measures of differentiation revealed moderate to high levels of population divergences (Table 2). Pairwise $F_{ST}$ values ranged from zero between Aduana and the road to Navojoa to 0.201 between La Sierrita and El Quintero. Further, the majority of $F_{ST}$ values were significant based on 10 000 random permutations of alleles after a Bonferroni correction. Similarly, values of $D_{est}$ were moderate to high, showing similar relative values between populations. However, estimated values of differentiation were generally much higher based on $D_{est}$ versus $F_{ST}$. El Quintero and Choquincahui showed the greatest differentiation among all comparisons based on both statistics. I also detected a significantly positive correlation between Euclidean and genetic distances (Mantel $r = 0.4897, p < 0.001$).

### 3.3 Population structure

I used two methods to assess the degree of genetic structure and to assign individuals to populations. I first used the traditional aspatial model in STRUCTURE that did not incorporate sampling locality information into the clustering. Results from the batch run suggested a $K$-
value of 5 using the Pr(Χ|Κ) method (Supplementary Fig. S1a). Conversely, inference based on the second order rate of change in likelihood values (ΔΚ method; Evanno et al. 2005) suggested a Κ = 3 (ΔΚ = 138.332; Fig. S1b). With a Κ of 3, the following localities were grouped together into clusters with varying degrees of admixture: Cluster 1 = Road to Navojoa, House/Alamos, Arroyo Aduana, Rio Cuchujaqui A and B, La Sierrita Nature Reserve, and San Antonio; Cluster 2 = Arroyo Tabelo A and B and Mocuzari; Cluster 3 = El Quintero and Choquincahui (Fig. 2A). Similar clustering was found with Κ = 5. However, both sites Mocuzari and Cuchujaqui A,B formed an additional cluster with varying degrees of admixture from other clusters (Fig. 2B). Neither clustering analysis suggested that individuals on opposite banks of Rio Cuchujaqui or Arroyo Tabelo formed a distinct cluster. However, individual-based Mantel tests found a significant barrier effect for both Rio Cuchujaqui (Mantel r=0.1148; p <0.0001) and Arroyo Tabelo (Mantel r=0.1126; p=0.0006) after 10,000 permutations.

A second set of analyses utilized the model that explicitly incorporated prior information for sampling localities to aid in clustering. These results differed somewhat from the clustering results that did not utilize sampling localities. For example, the plot of Κ versus Ln Pr(Χ) never reached a single peak (Fig. S2a) whereas the ΔΚ method suggested a Κ of 2 (ΔΚ = 80.915; Fig. S2b). However, the plot of Κ versus Ln Pr(Χ) showed that likelihood values began to stabilize at about Κ = 3, which was a value similar to that chosen using the ΔΚ method in the aspatial analysis. At Κ = 3, cluster memberships and admixture coefficients were very similar to those of the aspatial analysis.
3.4 Landscape genetics

Because the parameterization of resistance surfaces in landscape genetic studies of gene flow can be difficult, especially for categorical landscape variables such as forest cover, I first used Mantel $r$ values to select among relative cost values. For forest cover, I tested four relative cost values (1:2, 1:10, 1:100, 1:1000), in each case assigning a cost value of 1 to TDF and a higher cost to all other types of land-cover. The same relative cost values were tested on the stream data, with cells containing streams assigned a value of 1 and all other cells a higher cost value. For forest cover, Mantel $r$ values were highest with a cost ratio of 1:2 (Mantel $r = 0.4344$, $p = 0.002$) whereas for the stream data the best cost surface was based on a 1:10 ratio (Mantel $r = 0.4901$, $p < 0.001$).

The MRM analysis based on least-cost path distances suggested that the incorporation of landscape variables explained significantly more variance in genetic differentiation than a simple IBD model (Table 3). Whereas Euclidean distance was able to explain approximately 44% of the variation in $D_{est}$ values, the incorporation of landscape variables increased this value to approximately 57%. Because of substantial multicollinearity among some of the predictors, I ran four separate MRM analyses to test the influence of different variables. The most statistically supported model (using AIC weights) was based on a combination of Euclidean distance and the degree of anthropogenic disturbance ($R^2 = 0.5725; w_i = 0.974$). The least-cost paths based on temperature and slope explained slightly more variation in $D_{est}$ than Euclidean distance (0.49 and 0.45 versus 0.44, respectively). A model containing Euclidean distance and forest was also supported through stepwise regression. However, these two distances were highly correlated (Mantel $r = 0.995; p<0.001$) leading to very large VIF values. After accounting for Euclidean distance in models, regression coefficients for
stream connectivity, forest, and undisturbed habitat (anthro model) were negative and, thus, associated with a lower $D$ and higher gene flow.

Results of MRM based on resistance distances calculated in CIRCUITSCAPE were similar to those based on least-cost path distances (Table 4). However, slope appeared to be an important variable influencing gene flow under a circuit-theoretic approach. The stepwise MRM analysis suggested a model containing Euclidean distance, slope, and stream connectivity to best explain patterns of differentiation, with the latter variable showing a negative relationship with $D_{est}$. This model explained approximately 20% more variance in $D_{est}$ than a pure IBD model (0.6192 vs. 0.4464). The most supported model based on AIC weights included Euclidean distance, slope, stream connectivity, and temperature ($w_i = 0.521$), with stream being the only variable with a significantly negative coefficient. Visual examination of resistance distances based on a composite map of stream networks and slope was highly congruent with the genetic clusters inferred from the STRUCTURE analysis (Fig. 4). In most cases AIC weights were higher for models incorporating landscape variables versus a model of simple IBD. VIF values were less than 10 for all models suggesting that multicollinearity among predictors was not likely to be a problem.

4 Discussion

This study highlights the power of adopting a landscape genetics approach to understanding genetic structure and functional connectivity for tropical organisms. Few landscape genetic studies focus explicitly on tropical taxa (Storfer et al. 2010). This is problematic, because tropical regions contain the majority of Earth’s biodiversity (Myers et al. 2000). To exacerbate this issue, rates of deforestation and habitat fragmentation are increasing
exponentially throughout these regions and we still know relatively little about how organisms will respond to these continuing threats (Cascante et al. 2002; Burgos & Maass 2004; Miles et al. 2006; Becerra & Venable 2008). My results suggests that if we are to fully comprehend how habitat fragmentation influences functional connectivity in tropical organisms, additional landscape genetic studies are required to infer species-specific responses to continued habitat change.

4.1 Genetic diversity and population structure

Results suggest a moderate to high level of genetic diversity within populations of \textit{P. tuberculosus} surrounding the Alamos region. Diversity is not substantially higher in the population near Alamos versus the other areas, as might be predicted given the tendency for many gecko species to aggregate in human settlements. Further, a statistically significant relationship occurs between elevation and allelic richness. These results corroborate field observations that these lizards are predominantly encountered in TDF habitat. For example, elevation of the sites ranges from 100m to 500m. This range in elevation spans two distinct habitat types: tropical thornscrub and TDF (Robichaux & Yetman 2000). An abrupt change in tropical vegetation occurs at approximately 400m as well as an apparent change in abundance of geckos; more individuals are encountered per unit of time at El Quintero and Choquincahui than the other sites.

Significant population structure occurs based on both pairwise differentiation statistics and \textsc{structure} runs. Most pairwise $F_{st}$ values are high and statistically significant, with the localities El Quintero and Choquincahui being most divergent. Similar results are obtained based on values of $D_{est}$. Thus, leaf-toed geckos appear to exhibit substantial
population differentiation over relatively fine spatial scales. Blair et al. (unpublished data) examine the molecular phylogenetics of the *P. tuberculosus* species group; they report substantial genetic differentiation among populations and suggest that these lizards evolved in concert with Mexico’s TDF. However, few studies examine the population and/or landscape genetic structure of other lineages of geckos. Examining two species of gecko, Hoehn et al. (2007) report significant differences in genetic diversity and structure. Their result suggests different dispersal abilities in sympatric species, with one species exhibiting a maximum dispersal distance of only 500 m. The results of my landscape genetic analysis of *P. tuberculosus* also suggest that many gecko species may have limited dispersal abilities.

The STRUCTURE analyses reveal cryptic population structure. The number of genetic clusters, *K*, are inferred using two different methods both with and without incorporating prior information on sampling localities. Under the aspatial model, I detect three clusters using the \( \Delta K \) method and five using Pr(\( \mathcal{X} | K \)). Incorporating geographic locality information as a prior changes the inference of *K*. The \( \Delta K \) method suggests a *K* of 2, whereas the plot of *K* versus Ln Pr(\( \mathcal{X} \)) never reaches a peak. Populations at El Quintero and Choquincahuí always group into a single cluster. I never detect structure on opposite banks of the Río Cuchujaqui or Arroyo Tabelo based on Bayesian clustering (i.e. individuals on opposite banks were placed in the same cluster). However, Mantel tests did suggest a significant barrier effect for these features. Although Bayesian clustering methods, and STRUCTURE in particular, can be a powerful tool for inferring recent linear barriers to gene flow (Safner et al. 2011; Blair et al. in press), their high type-I error rates and difficulty in interpretation require caution when using these methods to test barrier hypotheses.
Genetic clustering is a difficult task and many authors have sought to develop more sophisticated algorithms to achieve this endeavour. Although early algorithms are entirely aspatial in nature (e.g. Pritchard et al. 2000), recent algorithms and programs incorporate prior locality information into the clustering analysis (Guillot et al. 2005a; Chen et al. 2007; Durand et al. 2009). These powerful spatial genetic models utilize GPS data and free or constrained Voronoi tessellation to cluster individuals into discrete units. In contrast, the new spatial method implemented in STRUCTURE incorporates geographic information by assigning different codes to different populations (Hubisz et al. 2009). Although relatively underutilized, this method is appealing in cases like this where I have geographic information only by population and not by individual. As different results are often encountered when using different Bayesian clustering programs (Safner et al. 2011; Blair et al. in press), additional empirical and simulation studies are required to test the power of the spatial approach in STRUCTURE in comparison to the fully spatial models implemented in other software packages.

4.2 Landscape genetics

At present, few studies focus on understanding landscape-genetic relationships in Neotropical vertebrates (Sork & Waits 2010; Storfer et al. 2010). My landscape genetic analysis identifies several landscape variables important in shaping the genetic connectivity of leaf-toed geckos. This suggests that the incorporation of landscape variables can explain significantly more variation in genetic differentiation than a simple IBD model. These conclusions make intuitive sense when the general biology and life-history of the study organism is considered. For example, Blair et al. (unpublished data) report that the evolution
of Mexican leaf-toed geckos is tied to the formation of the TDF throughout western Mexico. Combining this information with qualitative observations of relative abundance, it seems reasonable to conclude that forest fragmentation will have detrimental effects on functional connectivity. Several landscape genetic studies also report a negative relationship between forest fragmentation and genetic connectivity in small vertebrate species (e.g. Spear & Storfer 2008; Goldberg & Waits 2010), but again, most such studies focus on temperate systems. After controlling for Euclidean distance in my models, the least-cost path results show a negative relationship between forest connectivity and genetic differentiation, suggesting that gene flow is higher through forest patches (Table 3). However, I view these result with caution for two reasons. First, VIF values are exceptionally high for this model and this may be causing large variances in regression coefficients. Second, the study area contains a relatively large amount of undisturbed forest compared to localities in southern Mexico. Thus, Euclidean distances and least-cost path distances based on forest are nearly identical. I suggest that additional landscape genetic studies are required in areas experiencing rapid loss of TDF in order to understand the effect of forest patch dynamics on functional connectivity.

The next supported model based on least-cost paths includes Euclidean distance and anthropogenic disturbance. Because I parameterize the multivariate anthropogenic resistance surface by assigning higher costs to disturbed areas, the significantly negative regression coefficient suggests that undisturbed habitat is associated with a lower $D_{est}$ or higher gene flow. These results are concordant with other recent studies that show a negative relationship between anthropogenic disturbance and rates of gene flow (e.g. Wofford et al. 2005; Leclerc et al. 2008; Raeymaekers et al. 2008). This concordance suggests that although geckos are
frequently encountered in close proximity to human settlements, these areas have a detrimental impact on genetic connectivity. Although geckos are common on abandoned houses in TDF habitat, individuals of *P. tuberculosus* are only present in and around houses in absence of introduced geckos of the genus *Hemidactylus*. Very few individuals of *P. tuberculosus* can be found syntopically with *Hemidactylus* and on one occasion I witnessed the head of a *P. tuberculosus* in the jaws of *H. frenatus*. Thus, in heavily anthropogenically influenced areas, it appears that the introduced *Hemidactylus* are directly competing against native species of *Phyllodactylus*. To exacerbate this issue, people often kill leaf-toed geckos on site as they believe the darker colour of these geckos indicates that they are venomous. Conservation efforts should focus on educating local people on differences between native and non-native flora and fauna to aid in the maintenance and protection of the native species. This is especially important for species commonly found close to human settlements.

The next most-significant variable in the least-cost models is the minimum temperature of the coldest period, which explains slightly more of the variance in $D_{est}$ than Euclidean distance. Neotropical dry forest is a seasonal forest with approximately eight months of warm, wet conditions and four months of dry, cooler conditions (Murphy & Lugo 1986). Because these lizards are commonly encountered in hot tropical lowland environments, I predict that localities experiencing colder temperatures will impede gene flow. To parameterize the temperature resistance surface I assign higher cost values to cells experiencing colder temperatures. The MRM results suggest that these geckos are avoiding areas experiencing colder temperatures. Seasonality and climate are important variables shaping connectivity for other species (Geffen *et al.* 2004; Hirao & Kudo 2004, 2008; Banks *et al.* 2007; Dionne *et al.* 2008; Garroway *et al.* 2008).
My sampling includes geckos from near bridges and on rocky outcroppings adjacent to streams so I test the hypothesis that stream connectivity is an important component shaping patterns of gene flow. None of the results from MRM based on least-cost distances suggest that gene flow occurs primarily through riparian corridors. Indeed stream networks are often the first variable removed through the stepwise procedure except in the anthropogenic models. However, when stream connectivity is included in a highly supported model, its coefficient is negative; geckos may be following stream corridors. Riparian networks may or may not shape functional connectivity in small terrestrial vertebrates. For example, streams facilitate gene flow among populations of blotched tiger salamander (*Ambystoma tigrinum melanosticum*) (Spear *et al.* 2005) as they do in Rocky Mountain tailed frogs (*Ascaphus montanus*; Spear & Storfer 2010) and the Pacific jumping mouse (*Zapus trinotatus*; Vignieri 2005). Conversely, gene flow occurs terrestrially in coastal tailed frogs (*Ascaphus truei*) and does not follow riparian corridors (Spear & Storfer 2008). Combined, these results illustrate the utility of a GIS-based landscape genetic approach to understanding the influence of stream networks on genetic connectivity of small terrestrial vertebrates and reaffirm the necessity for examining species-specific processes in landscape genetic studies (Storfer *et al.* 2010).

My results based on resistance-distances derived from a circuit theoretic approach are similar to those based on least-cost paths. However, slope becomes a more important predictor of genetic variation in the former models, where populations are separated by higher slopes experiencing lower rates of gene flow (Table 4). These results are similar to numerous other studies that show a direct relationship between topological relief, elevation, and slope on rates and patterns of gene flow in terrestrial vertebrates (e.g. Funk *et al.* 2005;
Spear et al. 2005; Pérez-Espona et al. 2008; Murphy et al. 2010). Temperature, stream connectivity, and forest appear as important components based on resistance-distances. Unlike MRM, resistance-distances show a strong effect of stream connectivity on gene flow. Circuit theoretic approaches complement least-cost path modeling and often explain more of the variance in genetic differentiation than more traditional methods (McRae & Beier 2007; McRae et al. 2008). The model with the largest AIC weight based on resistance distances explains more variation in $D_{est}$ than the least-cost models (0.6462 vs. 0.6161). However, unlike the least-cost analysis, I am able to combine all resistance distances into the model due to the lack of multicollinearity among predictors. My results corroborate previous findings in suggest that a combination of circuit theoretic and least-cost models provides a powerful tool for investigating functional connectivity in dynamic landscapes.

4.3 Statistical methods in landscape genetics

Landscape genetics is still a relatively new discipline (Manel et al. 2003) and a large number of recent studies focus on testing the power of various analytical techniques for understanding the influence of landscape variables on microevolutionary processes (Cushman et al. 2006; Balkenhol et al. 2009; Cushman & Landguth 2010; Landguth et al. 2010; Safner et al. 2011; Blair et al. in press). Although the Mantel and partial Mantel tests continue to be the most widely used methods to link landscape and genetic data (Storfer et al. 2010), recent research suggests that these methods suffer from low power and high type-1 errors (Balkenhol et al. 2009; Legendre & Fortin 2010). Recognizing these limitations, Cushman et al. (2006) and Cushman & Landguth (2010) conclude that partial Mantel tests implemented in a causal modeling framework can be a powerful tool. However, landscape-
genetic relationships are often multivariate and are best represented in models that simultaneously consider multiple landscape and environmental variables (Spear et al. 2010). Thus, a MRM approach can serve as a powerful method to understanding the complex suite of factors important in shaping the spatial distribution of genetic variation (Balkenhol et al. 2009). My study highlights the value of applying MRM analyses to both least-cost and resistance distances. Surprisingly, few studies use this analytical method (Dyer et al. 2010).

Although potentially powerful, MRM approaches have limitations that need to be addressed (Legendre et al. 1994; Lichstein 2007). Multicollinearity often occurs among independent variables because they are in the form of distances and it will often manifest itself in least-cost analyses, with the severity depending on landscape-structure and the distance metric chosen. Although multicollinearity will not affect predictions of the variance of the dependent variable, it may have a consequence on individual regression coefficients because standard errors will be higher. Regression coefficients for predictors can change drastically depending on what other predictors are included in the model. Researchers interested in using MRM should examine the influence of multicollinearity on model results and how regression coefficients change with different models. Adopting rigorous model-selection criteria such as AIC or stepwise regression methods can serve as a means to understanding the best combination of explanatory variables. Although multicollinearity occurs in my data, coefficients change little with different explanatory models (Table 3,4). Thus, I am confident in my conclusions regarding landscape-genetic relationships. In cases where coefficients change drastically between models, examination of VIF values may be necessary to understand the degree of correlation among the predictors. I recommend that future landscape genetic studies using methods such as MRM and Mantel approaches report
VIF for each model examined. The application of ridge regression techniques to landscape genetic studies is also an avenue that could be explored.

4.4 Conservation implications for Mexican TDF

Habitat fragmentation and extirpation continue to threaten tropical ecosystems throughout the globe (Vitousek et al. 1997; Miles et al. 2006). Fragmentation of Mexico’s TDF is of particular concern as these forests are the country’s predominant vegetation-type and are known to be a biodiversity hotspot (Ceballos 1995; Robichaux & Yetman 2000; Becerra 2005). Trejo & Dirzo (2000) perform a time-series analysis of Mexico’s TDF and report that by 1990 only 27% of intact forest remained due to continual anthropogenic conversion for agriculture and pastureland. At present, the TDF near the Alamos region represents the country’s most undisturbed tract of continuous forest due, in part, to federally protected reserves (Robichaux & Yetman 2000). However, even in areas of relatively high forest cover, slight fragmentation may have detrimental impacts to functional connectivity. Thus, this study highlights the need for additional landscape genetic studies focusing on TDF ecosystems to better understand how habitat fragmentation and climate change will influence ecological and evolutionary processes. To this end, researchers should focus on developing GIS data sets at finer spatial resolutions that will provide a more comprehensive examination of the effect of landscape-level processes on the spatial distribution of genetic variation. The combination of the high resolution landscape layers with highly polymorphic genetic markers and sophisticated analytical techniques is required if we are to design movement corridors to fully maximize functional connectivity for species inhabiting this threatened ecosystem.
Acknowledgments

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CHAPTER 3—LANDSCAPE GENETICS OF *P. TUBERCULOSUS*


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(*Canis lupus*) population by a single immigrant. *Proceedings of the Royal Society of

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Wang JJ, Savage WK, Shaffer BH (2009) Landscape genetics and least-cost path analysis
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californiense*). *Molecular Ecology*, 18, 1365–1374.


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watershed genetic variation of coastal cutthroat trout. *Ecological Applications*, 15, 628–
637.

Table 1. Characterization of genetic diversity of *Phylodactylus tuberculatus* at each sampling site included in this study based on data from 10 microsatellite loci.

<table>
<thead>
<tr>
<th>Population</th>
<th>Elevation (m)</th>
<th>n</th>
<th>n(msat)</th>
<th>H₀</th>
<th>Hₑ</th>
<th># alleles</th>
<th>allelic richness</th>
</tr>
</thead>
<tbody>
<tr>
<td>Road to Navojoa</td>
<td>455</td>
<td>10</td>
<td>10</td>
<td>0.717</td>
<td>0.7547</td>
<td>65</td>
<td>65.0000</td>
</tr>
<tr>
<td>Alamos</td>
<td>375</td>
<td>36</td>
<td>35.2</td>
<td>0.7615</td>
<td>0.7725</td>
<td>88</td>
<td>64.8873</td>
</tr>
<tr>
<td>Tabelo A</td>
<td>167</td>
<td>27</td>
<td>26.3</td>
<td>0.7007</td>
<td>0.7143</td>
<td>70</td>
<td>56.5553</td>
</tr>
<tr>
<td>Tabelo B</td>
<td>199</td>
<td>30</td>
<td>29</td>
<td>0.7213</td>
<td>0.734</td>
<td>69</td>
<td>54.2341</td>
</tr>
<tr>
<td>Aduana</td>
<td>497</td>
<td>17</td>
<td>16.5</td>
<td>0.7562</td>
<td>0.7799</td>
<td>75</td>
<td>65.6058</td>
</tr>
<tr>
<td>Rio Cuchujaqui A</td>
<td>358</td>
<td>30</td>
<td>28.9</td>
<td>0.7487</td>
<td>0.7619</td>
<td>86</td>
<td>65.4514</td>
</tr>
<tr>
<td>Rio Cuchujaqui B</td>
<td>261</td>
<td>42</td>
<td>41.3</td>
<td>0.7717</td>
<td>0.7812</td>
<td>83</td>
<td>64.5302</td>
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<tr>
<td>Sierrita</td>
<td>483</td>
<td>38</td>
<td>36.4</td>
<td>0.6976</td>
<td>0.7073</td>
<td>89</td>
<td>63.8813</td>
</tr>
<tr>
<td>Mocuzari</td>
<td>124</td>
<td>30</td>
<td>29.6</td>
<td>0.7005</td>
<td>0.7125</td>
<td>70</td>
<td>55.2195</td>
</tr>
<tr>
<td>El Quintero</td>
<td>361</td>
<td>30</td>
<td>29</td>
<td>0.6903</td>
<td>0.7025</td>
<td>83</td>
<td>61.7606</td>
</tr>
<tr>
<td>Choquincahui (El Cobre)</td>
<td>433</td>
<td>31</td>
<td>29.2</td>
<td>0.7089</td>
<td>0.7214</td>
<td>81</td>
<td>59.3397</td>
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<tr>
<td>San Antonio</td>
<td>388</td>
<td>15</td>
<td>13.6</td>
<td>0.7242</td>
<td>0.7518</td>
<td>69</td>
<td>62.7309</td>
</tr>
</tbody>
</table>

n= number of individuals, n(msat) = number of individuals accounting for missing data, H₀ = observed heterozygosity, Hₑ = expected heterozygosity; allelic richness calculated based on population with smallest sample size (n = 10).
Table 2 Pairwise genetic differentiation between populations estimated from 10 microsatellite loci. Values above diagonal represent $D_{est}$ and values below diagonal $F_{ST}$. Bold values of $F_{ST}$ indicate significance ($P < 0.05$) after Bonferroni correction.

<table>
<thead>
<tr>
<th></th>
<th>Road to Navojoa</th>
<th>Alamos</th>
<th>Tabelo A</th>
<th>Tabelo B</th>
<th>Aduana</th>
<th>Cuch A</th>
<th>Cuch B</th>
<th>La Sierrita</th>
<th>Mocuzari</th>
<th>El Quintero</th>
<th>Choquincahui</th>
<th>San Antonio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rd to Navojoa</td>
<td>--</td>
<td>0.106</td>
<td>0.181</td>
<td>0.174</td>
<td>0.000</td>
<td>0.119</td>
<td>0.075</td>
<td>0.048</td>
<td>0.094</td>
<td>0.325</td>
<td>0.311</td>
<td>0.113</td>
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<tr>
<td>Alamos</td>
<td>0.032</td>
<td>--</td>
<td>0.197</td>
<td>0.166</td>
<td>0.072</td>
<td>0.086</td>
<td>0.095</td>
<td>0.139</td>
<td>0.151</td>
<td>0.217</td>
<td>0.309</td>
<td>0.032</td>
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<td>Tabelo A</td>
<td>0.080</td>
<td>0.080</td>
<td>--</td>
<td>0.050</td>
<td>0.125</td>
<td>0.196</td>
<td>0.223</td>
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<td>0.133</td>
<td>0.260</td>
<td>0.309</td>
<td>0.248</td>
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<tr>
<td>Tabelo B</td>
<td>0.065</td>
<td>0.059</td>
<td>0.026</td>
<td>--</td>
<td>0.143</td>
<td>0.224</td>
<td>0.232</td>
<td>0.252</td>
<td>0.100</td>
<td>0.293</td>
<td>0.377</td>
<td>0.197</td>
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<tr>
<td>Aduana</td>
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<td>0.021</td>
<td>0.058</td>
<td>0.059</td>
<td>--</td>
<td>0.074</td>
<td>0.084</td>
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<tr>
<td>Cuch A</td>
<td>0.039</td>
<td>0.028</td>
<td>0.082</td>
<td>0.076</td>
<td>0.024</td>
<td>--</td>
<td>0.059</td>
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<td>0.195</td>
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<td>Cuch B</td>
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<td>0.029</td>
<td>0.087</td>
<td>0.075</td>
<td>0.031</td>
<td>0.021</td>
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<td>0.197</td>
<td>0.236</td>
<td>0.319</td>
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<tr>
<td>La Sierrita</td>
<td>0.042</td>
<td>0.056</td>
<td>0.154</td>
<td>0.123</td>
<td>0.047</td>
<td>0.078</td>
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<td>0.176</td>
<td>0.345</td>
<td>0.378</td>
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<tr>
<td>Mocuzari</td>
<td>0.045</td>
<td>0.063</td>
<td>0.068</td>
<td>0.049</td>
<td>0.056</td>
<td>0.055</td>
<td>0.070</td>
<td>0.097</td>
<td>--</td>
<td>0.267</td>
<td>0.318</td>
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<td>El Quintero</td>
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<td>0.147</td>
<td>0.151</td>
<td>0.128</td>
<td>0.096</td>
<td>0.111</td>
<td>0.201</td>
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<td>San Antonio</td>
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<td>0.019</td>
<td>0.099</td>
<td>0.078</td>
<td>0.027</td>
<td>0.040</td>
<td>0.042</td>
<td>0.069</td>
<td>0.083</td>
<td>0.135</td>
<td>0.144</td>
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</table>
Table 3 Multiple regression on distance matrices (MRM) results showing the relationship between pairwise genetic distance (linearized Dest) and least-cost path distances incorporating landscape heterogeneity. Four different MRM analyses were performed (models B–D) using a stepwise approach with a $p$-to-enter value <0.05. Models in italics are those selected with only significant regression coefficients. Optimal cost values used to parameterize resistance surfaces prior to calculating each least-cost path were selected based on Mantel $r$ correlation coefficients. VIF=Variance Inflation Factor with values above 10 in boldface.

<table>
<thead>
<tr>
<th>Model</th>
<th>Variables</th>
<th>$\beta$</th>
<th>P</th>
<th>Model R$^2$</th>
<th>P</th>
<th>VIF</th>
<th>Model AICc</th>
<th>$\Delta$AIC</th>
<th>Akaike Weight (w)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>Euclidean</td>
<td>8.49E-06</td>
<td>0.0001</td>
<td>0.444</td>
<td>0.0001</td>
<td>-97.46</td>
<td>20.34</td>
<td>0.0000453</td>
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<tr>
<td>A2</td>
<td>Stream</td>
<td>1.08E-06</td>
<td>0.01</td>
<td>0.2397</td>
<td>0.01</td>
<td>-76.8</td>
<td>40.48</td>
<td>1.48E-09</td>
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<tr>
<td>B1</td>
<td>Anthro</td>
<td>1.92E-07</td>
<td>0.0037</td>
<td>0.2631</td>
<td>0.0037</td>
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<tr>
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<tr>
<td>B3</td>
<td>Euclidean</td>
<td>2.17E-05</td>
<td>0.0017</td>
<td>0.5725</td>
<td>0.0002</td>
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<td></td>
<td>Anthro</td>
<td>-4.12E-07</td>
<td>0.0422</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B4</td>
<td>Euclidean</td>
<td>2.87E-05</td>
<td>0.0005</td>
<td>0.6161</td>
<td>0.0001</td>
<td><strong>16.35</strong></td>
<td>-117.28</td>
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<td>0.913</td>
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<tr>
<td></td>
<td>Anthro</td>
<td>-5.18E-07</td>
<td>0.0055</td>
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<td>-103.01</td>
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Table 4 Multiple regression on distance matrices (MRM) results showing the relationship between pairwise genetic distance (linearized \(D_{est}\)) and resistance distances incorporating landscape heterogeneity. Optimal cost values used to parameterize resistance surfaces prior to calculating resistance distances were selected based on Mantel \(r\) correlation coefficients. For clarity, only models with significant support are shown. VIF=Variance Inflation Factor.

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Figure Legends

Fig. 1. Sampling sites for all individuals and populations of *Phyllodactylus tuberculosus* included in this study. Darker shades of gray represent tropical dry forest. Dark lines represent streams and arroyos throughout the study area.

Fig. 2. Relationship between elevation and genetic diversity (allelic richness) for all populations of *Phyllodactylus tuberculosus* included in this study.

Fig. 3. *STRUCTURE* results excluding prior information on sampling locality for *Phyllodactylus tuberculosus* included in this study. A) Results for $K=3$ selected using the $\Delta K$ method. B) Results for $K=5$ selected using $\text{Pr}(X|K)$

Fig. 4. Results from *CIRCUITSCAPE* depicting cumulative currents between populations of *Phyllodactylus tuberculosus* based on the combined effect of slope and riparian connectivity. ‘High’ in figure legend represents high levels of current whereas ‘Low’ represents low levels of current.
Fig. 1
Fig. 2

![Graph showing the relationship between Allelic Richness and Elevation (m). The graph includes a linear regression line with the equation $y = 0.0255x + 52.884$ and an $R^2 = 0.5836$. The x-axis represents Elevation (m) ranging from 0 to 600, and the y-axis represents Allelic Richness ranging from 45,000 to 70,000. The data points are plotted along the line, indicating a positive correlation.](image-url)
Figure 3

A

K=3 ΔK

B

K=5 Pr(X|K)
CHAPTER 3—LANDSCAPE GENETICS OF *P. TUBERCULOSUS*

Fig. 4
Supplementary Material

**Fig. S1.** A) STRUCTURE results illustrating changes in Pr(\(X|K\)) under the aspatial model. B) STRUCTURE results based on the second order rate of change (\(\Delta K\) method) under the aspatial model. For each \(K\), 10 independent simulations were performed.

**Fig. S2.** A) STRUCTURE results illustrating changes in Pr(\(X|K\)) under the spatial model. B) STRUCTURE results based on the second order rate of change (\(\Delta K\) method) under the spatial model. For each \(K\), 10 independent simulations were performed.
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Fig. S2a

L(K) (mean ± SD)

![Graph showing L(K) for different values of K.](image)

Fig. S2b

$\Delta K = \text{mean}(L''(K)) / \text{sd}(L(K))$

![Graph showing Delta K for different values of K.](image)
CHAPTER 4
Genes, geography, climate and cryptic diversity in leaf-toed geckos (Phyllodactylidae: *Phyllodactylus*) illuminate evolutionary processes throughout the Mexican dry forest

Abstract

Neotropical dry forests (TDF) are among the planet’s most diverse ecosystems. However, in comparison to moist tropical forests, little is known about the mode and tempo of biotic evolution throughout this threatened biome. Mitochondrial and nuclear DNA sequence data are used to investigate the drivers of diversification in leaf-toed geckos (*Phyllodactylus*) of Mexico’s dry forests. Speciation and substantial, cryptic molecular diversity seems to have originated as the forest was forming due to orogenesis of the Sierra Madre Occidental and Mexican Volcanic Belt in western Mexico. Multilocus species-tree and diversification analyses suggest that lineage accumulation was low and consistent until ~2.6 million years ago when diversification rates increased at an exponential rate. Key vicariant events play important roles in driving the biogeographic history of these lineages and southwestern Mexico is suggested to be an area for future diversification. Estimates of historical migration rates suggest that this extraordinary diversity may also be driven by limited dispersal abilities. Multilocus tests of demographic expansion indicate weak evidence for increases in population size since the Last Glacial Maximum. These results suggest that the formation of
TDF vegetation in the Miocene combined with Pleistocene climate shifts affected the evolution of leaf-toed geckos throughout western Mexico.

1 Introduction

The emergence of next generation DNA sequencing techniques and novel statistical methods are revolutionizing molecular phylogenetics and genealogical inference (Brito & Edwards 2009; Edwards 2009; Blair & Murphy 2011). New methods of phylogenetic inference account for gene-tree discordance (Liu 2008, Kubatko et al. 2009; Heled & Drummond 2010) and use more realistic models to estimate the timing of lineage divergence (Drummond et al. 2006). Multilocus algorithms have also been recently developed to estimate demographic history (Heled & Drummond 2008), reconstruct ancestral distributions (Yu et al. 2010), and estimate effective population sizes and asymmetric migration rates (Beerli & Felsenstein 2001; Beerli 2006). These new statistical methods formulate and test hypotheses pertaining to the historical forces shaping the evolutionary histories of species and populations.

The tropical dry forests (TDF) of the Neotropics are home to an extraordinary diversity of species (Ceballos & García 1995; Gillespie et al. 2000; García 2006). Unlike tropical rainforests, the TDF is characterized by a distinct seasonality with up to 8 months of dry, arid-like conditions separated by 4 months of deluge (Murphy & Lugo 1986). Because of this seasonality, many plants lose their leaves in a similar fashion to the vegetation of temperate deciduous forests. It is hypothesized that the TDF of Mexico originated sometime between 30–20 million years ago (Ma) due to the further orogenesis of two of México’s major mountain systems: the Sierra Madre Occidental and the Mexican Volcanic Belt (MVB;
Becerra 2005). These mountain chains effectively block colder northern temperatures allowing the TDF to expand throughout much of western Mexico and Middle America.

Although Neotropical dry forests are a diversity hotspot (Myers et al. 2000), relatively little is known about the historical forces that drive and shape patterns of genetic diversity throughout this ecosystem (Werneck et al. 2011). Recent empirical analyses conflict as to the role Pleistocene climatic shifts played in driving lineage divergence in lowland Neotropical species (Klicka & Zink 1997; Weir & Schluter 2004; Zink et al. 2004; Weir 2006; Zarza et al. 2008). Some discrepancy may be due to the different statistical methods employed, yet these results also suggest that additional data are required to test for the influence of palaeoclimatic shifts on rates of cladogenesis. Alternatively, the regions dynamic orogenesis and diverse landscape also may have played a vital role in speciation and lineage divergence (Devitt 2006; Mulcahy et al. 2008; Zarza et al. 2008).

How TDFs responded to the climatic fluctuations of the Pleistocene remains uncertain. Early studies suggest that during the Pleistocene TDF encompassed a much broader distribution that was subsequently fragmented as temperature and precipitation levels increased during the Pleistocene-Holocene transition (Pennington et al. 2000). This hypothesis partly explains the disjunct distributions of several plant and animal taxa restricted to these forests. Conversely, recent evidence from palaeodistribution modeling suggests that the distribution of TDF was much more disjunct during the Last Glacial Maximum than present-day (Werneck et al. 2011). Regardless of the temporal dynamics of TDF vegetation during the Pleistocene, it is likely that genetic signatures dating to this time period are present in species largely restricted to this ecosystem.
Recent molecular phylogenetic and phylogeographic studies in Mexico focus on patterns and processes of species inhabiting highland pine and pine-oak habitats and suggest a dual effect of Neogene vicariance and Pleistocene climate change (Bryson et al. 2011a,b,c,d). The few empirical studies that focus on genetic patterns of species throughout the lowlands of western Mexico report several cryptic lineages (Hasbún et al. 2005; Devitt 2006; Zarza et al. 2008), many of which correspond to major biogeographical provinces within Mexico. Additional studies are warranted to adequately assess regional patterns of biodiversity, especially at a time when the TDF is severely threatened due to burnings, agriculture, urbanization, and selective logging (Trejo & Dirzo 2000; Burgos & Maass 2004).

Analyses of spatial distribution of cryptic diversity and hypothesis testing can illuminate palaeoclimatic, geologic, and geographic histories within Mexico’s TDF (sensu Che et al. 2010).

Mitochondrial DNA (mtDNA) continues to be the marker of choice for examining spatial patterns of lineage divergence in closely related groups. However, the validity of the approach for extrapolating population history is questioned (Barrowclough & Zink 2009; Edwards & Bensch 2009). Some evidence suggests that a relatively large number of nuclear loci are required to obtain the same level of signal found with mtDNA (Lee & Edwards 2008). Further, analyses based on mtDNA resolve matrilineal histories only, whereas nuclear DNA (nDNA) provides evidence of gene flow including paternal contributions to evolutionary history. It is also well-known that the slower rate of evolution and larger effective population sizes of nuclear versus mitochondrial markers result in longer coalescence times with the former (Degnan & Rosenberg 2006, 2009). This realization has spawned a rapid growth of theory and computational methods aimed at estimating a species-
or population-tree versus a gene-tree (Edwards & Beerli 2000; Edwards 2009; Knowles 2009). Although these methods are particularly powerful at estimating phylogenetic relationships among recently diverged groups, few studies have utilized these techniques in a hypothesis-testing framework in Neotropical lowland taxa.

Leaf-toed geckos, genus *Phyllodactylus*, have a largely Neotropical distribution, ranging from southern California, the peninsula of Baja California, and the Sea of Cortes, to western lowlands of Mexico, Middle and South America, northeastern South America, and the West Indies (Dixon 1964). They occur in arid and semi-arid regions and are commonly found in tropical thornscrub and TDF; they avoid rainforests. The species are ideal for testing hypotheses pertaining to historical diversification within the Mexican TDF. Like many other geckos, *Phyllodactylus* appear to be microhabitat specialists, and are commonly found on rocky outcroppings adjacent to drainages. Little molecular work exists for these lizards (Blair *et al.* 2009). Thus, we still know relatively little about the evolutionary history of this widespread group.

Recent research demonstrates the utility of employing molecular phylogenetic analyses to understand historical processes and biotic evolution in highly diverse regions (e.g. Che *et al.* 2010; Fujita *et al.* 2010). Thus, my primary goal is to use multilocus sequence data to elucidate the drivers of cladogenesis in leaf-toed geckos distributed throughout the Mexican TDF. I test hypotheses pertaining to the influence of palaeoclimate, geology, geography, and landscape configuration on rates and patterns of cladogenesis for the *P. tuberculosus* species group using phylogenetic, biogeographic, demographic, molecular dating, and multilocus coalescent techniques. I am particularly interested in understanding
the relative roles of Pleistocene climate change and the origin of the Mexican TDF on rates
and patterns of lineage diversification.

2 Materials and Methods

2.1 Study area and sampling

From 2008 to 2010 I sampled approximately 150 individuals of the *P. tuberculosus* group (*P.
tuberculosus, P. lanei, P. muralis*) from distant sites covering most of the Mexican ranges for
each species (Fig. 1). When possible, I obtained sample sizes large enough to capture
intrapopulation diversity. Tissue samples (tail tips) were collected in the field and directly
preserved in 95% ethanol. Detailed sampling locality information was provided in
Supplementary Appendix I. I used *Phylodactylus xanti* and *Coleonyx variegatus* as outgroup
taxa.

2.2 Laboratory methods

Total genomic DNA was extracted using standard phenol-chloroform procedures following
initial digestion with proteinase K. I then amplified two mitochondrial genes (NADH
dehydrogenase subunit 4 (*ND4*) and cytochrome *b* (*cyt b*) following the polymerase chain
reaction (PCR) protocols described previously (Blair et al. 2009). Because I was also
interested in employing new multilocus analytical methods, I amplified and sequenced two
rapidly evolving nuclear introns (alpha-enolase and lamin-A) previously used for other
temperatures used for each of the four loci were summarized in Table 1. Following amplification, each PCR product was separated on a 1% agarose gel stained with ethidium bromide. Bands were then excised and directly sequenced in both directions using the BigDye Terminator Cycle Sequencing Kit (Applied Biosystems). Sequences were then read on an ABI 3730 automated sequencer (Applied Biosystems) and protocols followed the manufacturer’s recommendations.

2.3 Sequence alignment and haplotype estimation

Sequences were edited and assembled using BIOEDIT v7.05 (Hall 1999). Sequence identity was confirmed using a BLASTn search. Mitochondrial genes were translated into their corresponding amino acid sequence to check for premature stop codons which would suggest a nuclear pseudogene. Next, all sequences were aligned manually in BIOEDIT. Haplotypes from heterozygous individuals in the intron data were identified using PHASE V2.1 (Stephens et al. 2001; Stephens & Donnelly 2003) with the default parameters and a threshold value of 0.90. Input files for PHASE were generated using SEQPHASE (Flot 2010). All unique sequences were deposited in GenBank (accession numbers here upon acceptance).

2.4 Recombination

Although the inclusion of nDNA sequences provided a more comprehensive analysis of evolutionary history, recombinant sequences have been shown to be a serious concern for subsequent phylogenetic and demographic analyses (Posada 2001, 2002; Arenas & Posada 2010). I used RDP3 (Martin et al. 2010) to test for the presence of recombination in the two
nuclear intron data sets. Because the data set was relatively large, I only implemented the RDP (Martin & Rybicki 2000), GENECONV (Padidam et al. 1999), and MAXCHI (Maynard Smith 1992) methods as per recommendation of the program’s authors using the default parameters.

2.5 Gene-tree reconstruction

I used RAxML v7.2.6 (Stamatakis 2006) run through RAxMLGUI 0.93 to estimate genealogical (microevolutionary) and phylogenetic (macroevolutionary) relationships for the species group under a maximum likelihood (ML) framework. I performed several different analyses on different data sets—mtDNA only, alpha-enolase only, lamin-A only, and concatenated data—specifying an independent GTRGAMMA model for each data set. For ease of visualization I only included unique sequences from the phased intron data sets. A single phased haplotype from each individual of the intron data was concatenated with the corresponding mtDNA sequence. Each analysis started from a maximum parsimony tree and implemented a full ML search with 10 000 bootstrap replicates (BS) using the rapid bootstrap algorithm (Stamatakis et al. 2008). Phylloactylus xanti was used as the outgroup to root each topology. I accepted BS values greater than 70 as strong support for a given node (Hillis and Bull 1993).

2.6 Species-tree reconstruction and divergence times

Divergence dating in geckos can be difficult due to both the poor fossil record and the taxonomic uncertainty surrounding the fossils that are present (Grimaldi 1995; Kluge 1995; Fujita & Papenfuss 2011). However, I was interested in gaining a broad understanding of the
tempo of lineage divergence in leaf-toed geckos and thus, I relied on previous estimates of mtDNA substitution rate in geckos (see below). Calibrating phylogenetic trees based on independently-derived mutation rates can be robust, particularly if a parametric distribution prior is applied to the rate (Ho & Phillips 2009). Because incomplete lineage sorting can be ubiquitous at and below the species level, I performed a species-tree analysis for the *P. tuberculosus* group using the coalescent model in the program *BEAST V.1.7.1* (Heled & Drummond 2010; Drummond *et al.* 2012). Of the rapidly growing number of species-tree methods available, *BEAST* is one of the few algorithms that can simultaneously estimate individual gene-trees, the species-tree, population sizes, and divergence times under a Bayesian framework. For the analysis, I defined a ‘species’ based on previous species and subspecies designations, sampling locality, and results from the calculated mtDNA gene-tree topology; an approach similar to Fujita *et al.* (2010). For computational efficiency I pruned data for each individual gene to 3 individuals per species which has been shown to be adequate for estimating species-trees (Heled & Drummond 2010; McCormack *et al.* 2011). I created a text file defining traits for each species and used BEAUTI v.1.7.1 to create the input file for *BEAST*. I used BEAUTI to define two species sets; one constraining the monophyly of *Phyllodactylus* and the other based on the root. I specified a GTR+I+G model for the mtDNA and an HKY model for both nDNA loci. Both phased nuclear haplotypes were used for the analysis. I used an uncorrelated lognormal relaxed clock, Yule tree prior, and a random starting tree. I specified a substitution rate for the mtDNA gene-tree of 0.0057 substitutions per site per million years as estimated from previous studies on geckos and other small vertebrates (Macey *et al.* 1999; Fujita & Papenfuss 2011). To incorporate uncertainty in this value (Ho & Phillips 2009), I implemented a normal prior for the mtDNA rate with a mean
and initial value of 0.0057 and a standard deviation of 0.001. Further, because recent research suggested the utility of calibrating species-trees versus gene-trees in molecular dating analyses (McCormack et al. 2011), I placed a prior on the root node of the species-tree separating *Phyllodactylus* from *Coleonyx*. I specified a normal distribution with a mean of 140 and standard deviation of 20 (Gamble et al. 2008). Although using so-called “secondary calibrations” for molecular dating can be less than ideal, results are often robust, particularly if parametric prior distributions are used and subsequent conclusions based on 95% confidence intervals versus simple mean estimates (Sauquet et al. 2012). I ran five separate runs of 200 million generations each, sampling every 20,000 generations. Runs were combined in LOGCOMBINER and trees annotated in TREEANNOTATOR. Adequate sampling for all parameters was assessed with ESS values of at least 200.

### 2.7 Diversification rates

I tested hypotheses on whether or not formation of the Mexican TDF as well as Pleistocene climatic shifts substantially influenced rates of cladogenesis in leaf-toed geckos. Rejection of the null hypotheses—no influence—would be concluded if diversification rates were correlated with the timing of these events. I used LASER v. 2.3 (Rabosky 2006a,b) to test for significant departures from diversification-rate constancy over evolutionary history. LASER marked an improvement over previous methods in testing for temporal increases in diversification rate versus decreases as measured by the gamma statistic ($\gamma$) based on a constant rate test (Pybus & Harvey 2000). I calculated the test statistic $\Delta\text{AIC}_{RC}$ by comparing the likelihood of multiple rate-constant and rate-variables models. Model 1 assumed a pure birth or Yule model of diversification; Model 2 assumed a constant birth-death model;
Models 3 and 4 assumed density-dependent diversification rates under an exponential (DDX) or logistic distribution (DDL); Model 5 assumed a single change in diversification rate over time (yule2rate). A significantly positive value of the $\Delta \text{AIC}_{RC}$ statistic signified that a rate-variable model was a better fit to the data than a rate-constant model using the equation

$$\Delta \text{AIC}_{RC} = \text{AIC}_{RC} - \text{AIC}_{RV}$$

To visualize trends in lineage accumulation through time, I generated Lineage Through Time (LTT) plots in LASER. Finally, I calculated $\gamma$ to test for a decrease in diversification through time as expected if the number of available niches was being depleted. Significantly negative values of $\gamma$ would indicate a decrease in diversification through time with a large number of internal nodes distributed towards the root of the phylogeny (Pybus & Harvey 2000). All LASER analyses were based on both the dated mtDNA gene-tree topology and the dated species-tree calculated from the *BEAST analysis.

### 2.8 Biogeography

I used RASP v2.0 (Yu et al. 2010, 2011) to estimate ancestral distributions and elucidate the biogeographic history of the *P. tuberculosus* species group throughout western Mexico.

RASP, the most recent version of S-DIVA (Statistical Dispersal-Vicariance Analysis), incorporated both phylogenetic uncertainty and multiple equally optimal ancestral states into consideration when calculating ranges at ancestral nodes given a single or a set of phylogenetic trees (Nylander et al. 2008; Harris & Xiang 2009). I implemented the Bayesian Binary MCMC analysis using the default settings (2 runs, 50,000 cycles, 10 chains, sampling frequency of 100) under an F81+$\Gamma$ model. I estimated ancestral ranges based on the dated mtDNA gene-tree obtained in *BEAST versus the species-tree or concatenated topology due
to the higher support values in the former. To estimate ancestral ranges I first combined tree
files for each of the five independent runs of *BEAST implementing a burnin of 10%. I then
performed a Bayesian Binary analysis using the parameters above specifying the mtDNA
gene-trees file as input to RASP. Distribution was classified based on the Mexican state where
the animal was captured and by the mtDNA gene-tree topology calculated from RAxML.

2.9 Population genetic and historical demographic analysis

I used DNASP v5 (Librado & Rozas 2009) to calculate nucleotide diversity statistics for each
major lineage recovered in the phylogenetic analysis of mtDNA sequences. These statistics
were not calculated for the intron data because of the relatively low variation within
populations. I calculated the number of haplotypes ($H$), haplotype diversity ($h$), average
number of nucleotide differences assuming no recombination ($k$), number of segregating sites
($S$), and nucleotide diversity ($\pi$). Sequence divergence between mitochondrial lineages
(uncorrected p-distances and Tamura-Nei corrected distances) was calculated in MEGA 5
(Tamura et al. 2011).

To investigate signs of demographic expansion with the mtDNA data I calculated the
Ramos-Onsins and Rozas’ $R_2$ statistic (Ramos-Onsins & Rozas 2002), Tajima’s $D$ (Tajima
1989) and Fu’s $F_s$ (Fu 1997). Results from simulated data have suggested that Fu’s $F_s$ test is
particularly powerful when testing for expansion in large data sets, whereas the Ramos-
Onsins and Rozas’ $R_2$ is more sensitive for smaller populations (Ramos-Onsins & Rozas
2002). Significance of $D$ followed the critical values of Tajima (1989). Significance of both
$F_s$ and $R_2$ was determined from 1000 coalescent simulations assuming no recombination.
Statistics were calculated for populations/lineages with at least four individuals.
I also applied a multilocus Bayesian approach to infer historical changes in effective population sizes through time (Ho & Shapiro 2011). I used an Extended Bayesian Skyline model (Heled & Drummond 2008) implemented using BEAST to infer demographic history, as simulation evidence suggested that doubling the number of independent loci in demographic analyses reduces the error rate by a factor of $\sqrt{2}$ leading to more robust parameter estimates (Heled & Drummond 2008). Extended Bayesian Skyline Plots were obtained from each lineage recovered in the mtDNA gene-tree analysis. I used JMODELTEST to calculate best-fit models for each gene within each lineage using BIC; model parameters were estimated separately for each gene using BEAST. Bayesian skyline models required either a known substitution rate or internal calibrations to date times of demographic changes. I specified the same mtDNA substitution rate and priors as for the species-tree analysis. I used starting rates of 0.005 substitutions per site per million years for the nDNA data with a uniform prior between 0 and 100. All phased haplotypes were included. I then specified an Extended Bayesian Skyline tree prior under a strict clock model and designated the loci as either mitochondrial or autosomal. Operators were adjusted according to the recommendations of the program’s authors. Each analysis was run for a 30 million generations, logging parameters every 3 000 generations to obtain a final sample of 10 000 states. Adequate sampling for all parameters was assessed with ESS values of at least 200.

2.10 Migration rates and effective population sizes

I used MIGRATE-N v3.2.16 (Beerli & Felsenstein 2001; Beerli 2006) to estimate demographic parameters including effective population sizes and asymmetric migration rates between lineages recovered in the phylogenetic analysis. MIGRATE-N used the coalescent in a
Bayesian or maximum likelihood framework to calculate two parameters from the data, \( \Theta \) and \( M \), where \( \Theta \) represented the mutation-scaled effective population size \((4N_e \mu \text{ for a nuclear locus, } N_e \mu \text{ for mtDNA})\) and \( M \) represented the mutation-scaled immigration rate \((m/\mu)\). The effective number of immigrants per generation was estimated by multiplying \( \Theta \) and \( M \). I performed separate analyses on lineages of \( P. \) *tuberculosis/muralis* and \( P. \) *lanei* while specifying collecting locality and major mitochondrial lineages as separate populations. Only populations/lineages with at least five individuals were included to increase computation efficiency. I used default values for all starting parameters. To infer \( \Theta \) and \( M \), I specified two independent runs, static heating with four chains using recommended temperatures, a sampling increment of 100, 100,000 recorded steps, and a burnin of 10,000. To account for the difference in effective population sizes of the loci (mtDNA vs. nDNA), I introduced inheritance scalars \((4 1 1)\) for the mtDNA, alpha-enolase, and lamin-A data, respectively. All phased nuclear haplotypes were included in the analysis. Convergence was assessed by examination of ESS values with a target of at least 1,000.

### 2.11 Isolation-by-distance

I used IBDWS v3.2.1 (Jensen *et al.* 2005) to test for significant correlations between geographic (Euclidean) and genetic distance for both \( P. \) *tuberculosis* and \( P. \) *lanei*. As an estimate of genetic distance, I used Rousset’s measure of differentiation based on sequence data \((\Phi_{ST}/(1 – \Phi_{ST}))\). Significance was assessed by Mantel tests (Mantel 1967) using 30,000 randomizations. Because I tested the hypothesis that dispersal was not influenced by drainage configuration, I created a third binary matrix specifying a value of 0 for populations adjacent to the same river body and a value of 1 for different systems. I then used partial
Mantel tests (Smouse et al. 1986) to test for a significant correlation between genetic distance and river configuration; a significant correlation was used to reject the null hypothesis. Partial Mantel tests controlled for the influence of Euclidean distance when estimating a riverine effect. Data for Mexico’s major rivers and tributaries were obtained from the Instituto Nacional de Estadistica y Geografia.

3 Results

3.1 Sequence characteristics

The concatenated mitochondrial and nuclear data set contained a total of 2334 characters. The combined mtDNA data contained 1301 characters, 776 of which were variable and 670 potentially parsimony-informative. The phased alpha-enolase intron alignment including \( P. \) \( xanti \) contained a total of 441 characters, 134 of which were variable and 116 potentially parsimony-informative. The phased lamin-A alignment contained 592 characters, 113 of which were variable and 105 potentially parsimony-informative. No premature stop codons were found for either mitochondrial gene suggesting that authentic mitochondrial sequences were obtained. No recombinant breakpoints were detected for the nuclear loci with any of the three methods used. \textit{JMODELTEST} selected the GTR+\( I + \Gamma \) model for both the concatenated mtDNA and alpha-enolase partitions and the HKY+\( \Gamma \) model for lamin-A. Within the mtDNA data the GTR+\( I + \Gamma \) model was selected for both \( ND4 \) and \( cytb \), and HKY+\( I + \Gamma \) for the tRNA. No indels were present in the alpha-enolase alignment. However, the lamin-A data set contained a total of nine indels ranging from 1–20 bp in length.
3.2 Gene-tree analysis

ML analysis of the mtDNA data resulted in a well-supported topology illustrating highly divergent maternal lineages (Fig. 2). Two deep divergences largely corresponded to species (*P. tuberculosus* and *P. lanei*). However, *P. tuberculosus magnus*, *P. muralis muralis*, and *P. muralis isthmus* were not monophyletic. Two highly divergent and sympatric lineages (A1 and B1) occurred at El Charco, Jalisco. Bootstrap support for each lineage varied from moderate to high. Genetic structure was much less pronounced for both intron data sets versus the mtDNA and showed evidence for substantial incomplete lineage sorting. ML analysis of the phased alpha-enolase data resulted in three lineages that largely corresponded to species and sub-species (Supplementary Fig. S1). Conversely, ML analysis of the phased lamin-A data showed less structure, with several haplotypes of some species nested within haplotypes of other species (Supplementary Fig. S2). The concatenated ML analysis was largely congruent with the mtDNA gene-tree (Supplementary Fig. S3). However, lineage A1 from the mtDNA analysis shifted from the *P. tuberculosus/muralis* lineage to *P. lanei*. Further, haplotypes from Cosalá, Sinaloa clustered more closely with haplotypes from Alamos, Sonora versus haplotypes from Villa Unión, Sinaloa. Lastly, *P. muralis isthmus* was monophyletic in the concatenated tree and haplotypes for *P. lanei rupinus* from Michoacán were removed from lineage B2.

3.3 Species-tree analysis and divergence times

The five independent *BEAST* runs all showed adequate mixing and convergence as estimated by ESS values greater than 200. The 95% highest posterior density (HPD) for *ucld.stdev* and
coefficient of variation did not include zero, indicating that the data did not conform to a strict clock model. The mtDNA gene-tree indicated that the most recent common ancestor of *Phyllophactylus* was present during the Cretaceous ~75 Ma (HPD: 40–127; Fig. 3). Cladogenesis progressed relatively slowly until about 2.67 Ma when the number of lineages increased rapidly up to the present. The *BEAST* mtDNA gene-tree was highly congruent with the ML topology, with most of the maternal lineages sharing a most recent common ancestor in the Early Pleistocene. There was evidence for an increase in evolutionary rate for many lineages approximately 25 Ma; a time when speciation was occurring throughout the species group.

The species-tree topology was generally less well-supported than the mtDNA gene-tree and suggested a slightly different topology (Fig. 4). However, unlike the mtDNA tree, the species-tree analysis recovered strong evidence for a monophyletic *P. lanei rupinus*, *P. lanei lanei*, and *P. tuberculosus magnus*. In contrast, *P. tuberculosus saxatlis*, *P. muralis isthmus*, and *P. muralis muralis* were not monophyletic. Divergence times were more recent than the mtDNA tree, with speciation and population divergence occurring in the Miocene.

### 3.4 Diversification rates

I used the maximum likelihood model in LASER to statistically quantify shifts in rate of cladogenesis throughout the tree and, thus, test for significant departures from a rate-constancy model of diversification. No decrease in diversification rate was detected through time over the evolutionary history of these geckos based on either the mtDNA gene-tree ($\gamma = 6.364; P = 1$) or the species-tree topology ($\gamma = 1.485; P = 0.9312$). The simultaneous comparison of multiple rate-constant and rate-variable models favoured the rate-variable
yule2rate model for the mtDNA tree \( \text{AIC} = 22.85 \) indicating that rates of diversification increased through time \( \Delta \text{AIC}_{RC} = 16.0045 \). Cladogenesis progressed at a rate of 0.0242/million years until 2.67 Ma when the rate shifted to 0.3261/million years. Significance of the calculated \( \Delta \text{AIC}_{RC} \) statistic based on 5000 simulated trees under a Yule process (constant birth) indicated a significant difference \( (P = 0.0002) \). Diversification through time progressed gradually up until the Early Pleistocene when cladogenesis followed a more exponential distribution versus linear and never reached an asymptote (Fig. 3). Diversification analysis based on the dated species-tree also suggested that a rate-variable model explained the data better than a rate-constant model \( \text{yule2rate AIC} = 81.91; \text{bd AIC} = 83.17; \Delta \text{AIC}_{RC} = 1.2512 \). An increased in diversification rate from 0.0100 to 0.0406 occurred approximately 28 Ma (Fig. 4). However, this shift in diversification was not significantly different from a constant birth model \( (P = 0.2006) \).

### 3.5 Biogeography

The distance between independent runs of the Bayesian MCMC analysis was less than 0.01, indicating congruence in ancestral range estimates. Different biogeographic histories were recovered depending on which tree or sets of trees were used an input (results not shown). However, all three data sets (mtDNA, concatenated, species-tree) suggested an origin of *Phyllodactylus* in the state of Jalisco. I focus additional discussion on the mtDNA topology because of the relatively high support for relationships (Fig. 5). Biogeographic histories were presented for both the *P. tuberculosus/muralis* and *P. lanei* lineages. For *P. tuberculosus*, the results suggested an ancestral distribution originating in Jalisco, with subsequent dispersal and divergence to the north (*P. tuberculosus saxatlis*) and to the south (*P. tuberculosus*
magnus, P. muralis isthmus, P. muralis muralis). Three distinct vicariant events were hypothesized for the tuberculosus lineage. Ancestral distributions for P. lanei were also concentrated in the Jalisco region with a single vicariant event separating P. lanei lanei from P. lanei rupinus.

3.6 Population genetics and demographics

Sequence divergence between major matrilines was exceptionally high, with uncorrected p-distances and Tamura-Nei distances approaching 32% (Table 2). Genetic diversity within lineages was low-to-moderate as revealed by nucleotide and haplotype diversities (Table 3). None of the neutrality tests indicated signs of recent demographic expansion. However, significantly positive values of Fu’s Fs test indicated recent population subdivision for lineages A2 and B2. The extended Bayesian skyline plots also showed weak evidence for recent demographic expansion (Fig. 6). ESS values for all parameters were >100 and most were >200 indicating adequate sampling from the posterior distribution. I was unable to get adequate sampling from the posterior distribution for lineage B1. Although the demographic.population.size-changes parameter often suggested one, two, and three changes, the 95% confidence intervals were large for many lineages and no plot showed an obvious trend.

3.7 Migration rates and effective population sizes

Results from the MIGRATE-N analyses suggested that population sizes and migration rates between populations were low. For the P. tuberculosus/muralis lineage, the number of
migrants per generation ($4N_m$) ranged from 0.0057 between lineages A3 and A10 to 6.7195 from lineage A5 to A9 (Supplementary Table 1). For *P. lanei*, the number of migrants per generation ($4N_m$) was one or less for all comparisons of populations (Supplementary Table 2). Estimated effective population sizes ($4N_eu$) for *P. tuberculosus* were smallest for lineages A7 (0.0008) and A10 (0.00003). Effective population size for *P. lanei* at Chamela, Jalisco (0.0006) was much smaller than sizes for the remaining populations.

### 3.8 Isolation-by-distance

The Mantel test for *P. tuberculosus* found a significant correlation between log geographic distance and genetic distance ($r = 0.2892$, $P = 0.0101$). Partial Mantel tests showed no significant correlation between drainage structure and genetic distance with ($r = -0.0620$, $P = 0.2596$) and without ($r = 0.1502$, $P = 0.2596$) controlling for the effects of Euclidean distance. There was no significant correlation between log geographic distance and genetic distance for *P. lanei* ($r = -0.2625$, $P = 0.8128$)

### 4 Discussion

Neotropical dry forests are one of Earth’s most diverse ecosystems based on both species richness and number of endemics (Myers *et al.* 2000). However, these forests occur primarily in developing countries where the threat of extirpation is drastically increasing due to a suite of anthropogenic factors (Trejo & Dirzo 2000). To focus conservation efforts in this highly threatened system in Mexico, recent efforts seek to determine both specific areas of high richness and endemism as well as source-sink areas for future diversification (García 2006;
Becerra & Venable 2008). However, comprehensive analyses from diverse scientific disciplines are required to fully understand the mode and tempo of biotic evolution in this ecosystem. By combining phylogenetic, biogeographic, molecular clock and demographic analyses from multiple loci, I seek to determine the patterns and the drivers of diversification in western Mexico’s TDF. Results suggest that these forests may be much more diverse than previously realized, and point to several potential mechanisms of diversification including Pleistocene climatic cycling.

### 4.1 Phylogenetic analysis

My phylogenetic analysis suggests that the degree of cryptic molecular diversity for leaf-toed geckos throughout western Mexico is substantial, with sequence divergence values approaching 32%. *Phyllodactylus tuberculatus* and *P. muralis* are composed of at least 11 highly differentiated lineages whereas *P. lanei* contains five cryptic lineages. Phylogenetic analysis of the nuclear haplotypes also reveals genetic structure, albeit less pronounced than the mtDNA analysis. Analysis of the phased alpha-enolase haplotypes suggests that *P. tuberculatus saxatilis* is not exchanging genetic information with other populations throughout southern Mexico. These patterns are not resolved in analyses of the lamin-A.

The discrepancy between gene-trees from independent loci at and below the species level often suggests gene flow or incomplete lineage sorting as causal factors (Degnan & Rosenberg 2006, 2009). Although sex-biased dispersal can explain these patterns, no data to suggest this occurs. Interspecific hybridization could also be a factor. However, my results suggest that haplotypes are shared between individuals over relatively broad spatial scales. This would seem counter to a hypothesis of limited dispersal abilities in geckos. More likely,
the differences between the mtDNA and nDNA gene trees is due to the incomplete sorting of ancestral haplotypes due to the larger effective population sizes of the nuclear markers. One potentially powerful approach to deal with rampant incomplete lineage sorting is to employ powerful species-tree methods that incorporate the stochasticity of the coalescent process into genealogic and phylogenetic inference (Liu et al. 2008, 2009; Heled & Drummond 2010). Indeed the multilocus *BEAST analysis resulted in a different hypothesis versus both the mtDNA and concatenated analyses, albeit with lower statistical support. However, several species and subspecies that were not monophyletic in the gene-tree analysis (e.g. *P. tuberculosus magnus*) were monophyletic in the species-tree analysis. These results add to the growing body of literature and suggest that combining novel species-tree methods with traditional gene-tree based approaches can be a powerful means to elucidate historical relationships at and below the species level.

These results highlight the utility of incorporating both haplotypes from heterozygous individuals in genealogical and phylogenetic inference. Many researchers choose to construct a genealogy choosing one of the two haplotypes from heterozygous individuals. The arbitrary (or statistical) choice of including one haplotype over the other can result in biased conclusions regarding genetic structure and evolutionary relationships (Weisrock et al. 2012). This is of particular concern in studies at and below the species level where incomplete lineage sorting is likely to be common. As multilocus studies are now commonplace, I urge researchers to present genealogies and perform analyses with both nuclear haplotypes so correct genetic structure can be visualized. The continual development of more sophisticated phylogenetic and demographic algorithms (e.g. Heled & Drummond 2008, 2010) is now making this a feasible endeavour.
A growing body of literature focuses on phylogenetic patterns and processes throughout the diverse ecosystems of Mexico. Some of these studies test climatic and geologic hypotheses influencing the evolution of montane species (Bryson et al. 2011a,b,c), whereas others focus on lowland tropical taxa (Mulcahy & Mendleson 2000; Mulcahy et al. 2006). My study adds to the limited number of vertebrate studies focusing on the TDF of western Mexico that find extensive cryptic molecular diversity throughout the region (Hasbún et al. 2005; Devitt 2006; Zarza et al. 2008). However, to my knowledge this study is only the second to incorporate variable nuclear markers to infer historical processes. Although not as variable as the mtDNA, the intron data allowed for a multilocus perspective of processes governing the evolutionary history of the P. tuberculosus group. I urge researchers to continue to scan the genome for variable nuclear markers to test historical processes effecting organisms distributed throughout the TDF of western Mexico. The combination of markers with both different mutation rates and inheritance modes can provide a much more comprehensive investigation into patterns and processes of molecular evolution.

### 4.2 Divergence times and diversification rates

The relaxed molecular clock analysis of the mtDNA gene-tree dates the most recent common ancestor of Phyllodactylus to the Cretaceous approximately 75 Ma, corroborating other recent molecular evidence for an ancient origin of geckos (Gamble et al. 2008). The diversification analysis in LASER suggests that cladogenesis progressed gradually up until about 2.67 Ma when the rate of lineage accumulation increased up to the present. I also find evidence for increased rates of evolution and speciation approximately 25 Ma. Interestingly,
these results are highly congruent to a time-calibrated phylogeny and diversification analysis of *Bursera*, a conspicuous member of the Mexican TDF (Becerra 2005). For example, it is hypothesized that *Bursera* also has an origin dating back to the Cretaceous approximately 70–80 Ma. Further, the tree genus is hypothesized to have experienced rapid diversification at around 20–30 Ma, a time when extensive tectonic activity was altering Mexico’s Sierra Madre Occidental and MVB, and creating the TDF. Most of the species identified in my mtDNA analysis coalesce within this time frame. Taken together, this suggests that *Phyllodactylus* evolved in concert with this genus of trees, and that the formation of the Mexican TDF may be responsible for driving cladogenesis in these geckos. The dated species-tree also suggests an influence of climate change during the Miocene as a driver of cladogenesis in these geckos. Although many divergence dates in the species-tree are younger than those of the mtDNA as theory predicts, my diversification analysis did suggest a moderate shift in diversification rate approximately 28 Ma.

The importance of Pleistocene climatic shifts in shaping the evolutionary history of Neotropical taxa is a hotly debated topic (e.g. Klicka & Zink 1997; Hewitt 2000). My results suggest a substantial influence of both Miocene/Pliocene and Pleistocene effects in shaping the evolutionary history of these geckos. For example, most of the mtDNA lineages date back to the Early Pleistocene approximately 2.5 Ma, making the shifts in TDF habitat during the Quaternary (Pennington *et al.* 2000; Werneck *et al.* 2011) a likely model to explain patterns of intraspecific divergence in these geckos. My results are also concordant with previous studies that suggest an older timeframe of speciation in Neotropical lowland taxa (Weir & Schluter 2004; Weir 2006) as many of the species and populations diverged during the Miocene. However, my results cannot refute the hypothesis of Pleistocene effects on
diversification patterns within the TDF of western Mexico. This generally agrees with other studies that find evidence for both Pleistocene and older climatic and geologic events important in shaping the evolution of TDF biota (Pennington et al. 2004).

The continual reduction of next-generation sequencing costs is resulting in studies that incorporate information from multiple unlinked genetic loci to test evolutionary hypotheses. These multilocus data sets have, in turn, spawned the development of several new phylogenetic algorithms that explicitly model the stochastic nature of gene coalescence (Heled & Drummond 2008; 2010; Liu 2008). The species-tree method implemented in *BEAST is particularly powerful given its flexibility regarding model specification and priors for a variety of parameters. An additional attractive feature of *BEAST is its ability to simultaneously estimate gene- and species-trees. Previously, molecular clock analyses focused on obtaining divergence dates for a given gene-tree topology. However, in analyses such as these it can be difficult to select the appropriate tree prior for the data (Ho 2005). For example, a Yule prior is generally appropriate for data at and above the species level whereas coalescent priors are suitable for intrapopulation sequences. However, many data sets, such as this, consist of sequences where neither a Yule nor a coalescent prior would be appropriate to estimate a gene-tree. In this case, a species-tree model would be an appropriate alternative as ‘species’ and ‘populations’ better fit the Yule species-tree prior (A. Drummond, pers. comm.). Further, although these species-tree methods were explicitly designed to deal with multilocus sequence data, the can also be applied to single locus data to provide a rough estimation of the species- or population-tree (Heled & Drummond 2010). As inappropriate selection of tree priors coupled with incorrect fossil information can severely bias divergence dating analyses (Ho 2005, 2007; Ho & Phillips 2009), employing a *BEAST model may be
more accurate for particular sampling schemes. My study also adds to a recent study in demonstrating the utility of calibrating species-trees versus gene-trees (McCormack et al. 2011). I predict that this will become more common in the near future due to both the increase in multilocus data sets and the ease in which this can now be performed (Drummond et al. 2012).

4.3 Biogeography

My biogeographic analysis suggests several key vicariant events shaping the history of both the *P. tuberculosus/P. muralis* lineage and the *P. lanei* lineage. Interestingly, the proposed events correspond to several previously hypothesized biogeographic barriers. First, my analysis suggests the clade has its origin in the state of Jalisco, southwestern Mexico with subsequent dispersal to the north and south. Previous studies also suggest that this region may explain both current patterns of richness and endemicity as well as potential source areas for future diversification (García 2006; Becerra & Venable 2008). Becerra and Venable (2008) suggest that the high levels of species accumulation in the area are due to the diverse set of environmental conditions generated by the confluence of several of Mexico’s major mountain chains. Becerra (2005) postulates an ancestral distribution of the tree genus *Bursera* in the area. Thus, it appears that Jalisco’s TDF is an important area for species accumulation and diversification across broad taxonomic groups.

My biogeographic analysis for the *P. tuberculosus/P. muralis* lineage suggests three vicariant events that coincide with previously hypothesized biogeographic boundaries. Event number one partitions individuals into groups north and south of the MVB. Numerous studies suggest that this mountain chain serves as a biogeographic barrier between Nearctic and
Neotropical biotas (Marshall & Liebherr 2000; Mulcahy & Mendleson 2000; Mateos et al. 2002; Hulsey et al. 2004; Mateos 2005; Devitt 2006; Huidobro et al. 2006; Morrone 2006). Many lowland species are, thus, composed of highly differentiated groups occurring on either side of the MVB. Recent studies also suggest that orogenesis of the MVB drove rates of cladogenesis for montane species (Bryson & Riddle 2011; Bryson et al. 2011a,b,c,d). The MVB is known to have formed in a west-east progression beginning at around 23 Ma and ending approximately 2.5 Ma (Moran-Zenteno 1994). The most extensive ridges of this belt are found in western regions throughout Jalisco, with the number and prominence of volcanoes dissipating towards Veracruz. However, phylogenetic evidence suggests that even the smaller ridges to the east can serve as major biogeographic barriers to lowland taxa (Mulcahy & Mendleson 2000; Mulcahy et al. 2006). My molecular clock and biogeographic analysis cannot refute the hypothesis of vicariance due to the early orogenesis of the MVB in Jalisco.

Event number two separates populations of *P. tuberculosus saxatilis* occurring in Sonora and Sinaloa. Devitt (2006) provides a mtDNA study of the western lyresnake (*Trimorphodon biscutatus*) and also reports a strong mtDNA discordance in the same general area. My study confirms these findings, suggesting a common biogeographic barrier between the states of Sonora and Sinaloa that may be a result of vicariance due to the Río Fuerte or the Sierra de Barabampo (Hafner & Riddle 2005).

Vicariant event three separates populations of *P. tuberculosus magnus* in Guerrero from populations in Oaxaca and Chiapas. These results are concordant with a phylogeographic study of black spiny-tailed iguanas that found a mtDNA break in the same general vicinity (Zarza et al. 2008). The biogeographic feature responsible for these vicariant
events is presently unknown. However, the Sierra Madre del Sur occurs in a belt throughout southern Mexico and is composed of numerous small mountain chains which may have served as a historical biogeographic barrier between lineages. Additional phylogenetic and phylogeographic studies are required to test the generality of a Guerrero/Oaxaca break.

An additional vicariant event previously hypothesized in the literature, but not corroborated by my biogeographic analysis separates populations of *P. tuberculosus magnus* east and west of the Isthmus of Tehuantepec. Unlike the MVB, there is still substantial controversy over whether there was a historical barrier across the isthmus in southern Oaxaca or not (Mulcahy *et al.* 2006). Some researchers suggest that a Pliocene seaway fragmented the isthmus into eastern and western components, in turn, isolating populations on either side (Sullivan *et al.* 2000). Although my biogeographic analysis does not suggest a vicariant event, the phylogenetic analysis does suggest a possible historical barrier across the isthmus.

My biogeographic analysis for *P. lanei* suggests a single key vicariant event, separating populations in Guerrero from those in Jalisco and Michoacan. This division corresponds to the current taxonomy of *P. lanei* (Dixon 1964), with *P. l. lanei* being restricted to Guerrero and *P. l. rupinus* occurring in Jalisco and Michoacan. These subspecies are distributed on opposite sides of the Balsas Basin, a highly speciose region that contains many endemic taxa (Rzedowski 1993; Zaldivar-Riverón *et al.* 2004; Becerra & Venable 2008). Vicariance is most likely due to the Rio Balsas, a large river that flows southwestwardly through the states of Mexico, Puebla, Morelos, and Guerrero. Alternatively, the Sierra de Taxco, which divides Balsas Basin into eastern and western units, may be responsible for the split within *P. lanei* (Becerra & Venable 1999). Conversely, my biogeographic analysis suggests an ancestral range in Jalisco with an associated vicariant
event dating to the formation of the MVB. Thus, it remains uncertain as to the relative
importance of orogenesis of the MVB versus alternative hypotheses for the split between *P.
lanei lanei* and *P. lanei rupinus*.

### 4.4 Population genetics and demographics

My population genetic results suggest low to moderate diversity within populations and
substantial differentiation between populations. Analyses suggest that almost every sampling
locality constitutes a highly-differentiated group, with sequence divergence values
approaching 32%. Summary statistics-based tests for demographic expansion fail to find
signs of expansion for any of the major mitochondrial lineages. Fu’s $F_s$ test shows
significantly positive values for lineages A2 and B2 which may be a result of recent
fragmentation. However, both of these lineages include individuals from different areas that
are grouped together because of sample sizes. Thus, these significantly positive values are
likely the result of combining divergent lineages into a single unit for analysis.

My multilocus Bayesian estimates of demographic changes show weak signs of
increases in effective population sizes through time. The Extended Bayesian Skyline Plots
are a recent extension of the more-traditional single-locus skyline plots used to track
demographic changes through time (Heled & Drummond 2008). Although some of the
lineages show relatively wide 95% confidence intervals, results suggest that populations of
these geckos did not expand during or following Pleistocene climatic shifts. Conversely,
some of the lineages (e.g. A2, B2) showed trends of decreasing population size. However, I
feel this is due to combining genetically diverse individuals into a single unit for these
analyses (Ho & Shapiro 2011) and not an actual demographic phenomenon. To maximize
genetic information for these tests I included all phased nuclear haplotypes. Evidence suggests that increasing the number of independent loci, versus increasing the number of individuals and lengths of sequences, has a drastic influence on parameter estimates (Heled & Drummond 2008). In contrast to between-population comparisons, sequence diversity within populations is low to moderate for both nuclear loci, resulting in relatively little information to estimate historical demographic parameters. The collection of additional highly variable loci will likely decrease error estimates and provide a more comprehensive estimate of demographic trends through time for these geckos. Notwithstanding artefacts of sampling bias, these results indicate that the Pleistocene had little impact on demographic trends in leaf-toed geckos.

4.5 Migration

My MIGRATE-N analyses suggest both small effective population sizes and migrates rates between populations and lineages. For *P. lanei* the number of migrants per generation (*4Nm*) is less than one for most comparisons. Migration rates for *P. tuberculatus/muralis* appear higher, with some populations exchanging approximately six migrants per generation. However, these results should be taken with caution. Unlike the analysis for *P. lanei*, confidence intervals for several parameters are relatively large for *P. tuberculatus/muralis*. For example, the effective population size estimate for lineage A9 is 0.0797 with a 97.5% confidence interval between 0.0518 and 0.1000. Because I multiply $\Theta$ and $M$ to calculate the number of migrants per generation, these relatively large confidence intervals can affect parameter estimates substantially. Further, confidence intervals for several $M$ parameters are high. Although this could be an artefact of analysis parameters, I specify multiple runs with
different heating parameters to better sample parameter space. ESS values are also large for all parameters suggesting that the chain was run long enough. I suggest that the large confidence intervals are due to the signal in the data and that the collection of additional independent loci will improve parameter estimates (Beerli & Felsenstein 2001; Beerli 2006). Nevertheless, these results agree with my other analyses and suggest that these geckos exhibit limited dispersal abilities. These results are also concordant with previous genealogical studies of geckos that suggest high levels of differentiation over small spatial scales (Gübitz et al. 2000; Kasapidis et al. 2005; Thorpe et al. 2008).

4.6 IBD and drainage systems

Results suggest a mixed influence of Euclidean distance on genetic differentiation, providing additional evidence for cryptic species. Contrary to expectations, partial Mantel tests fail to find a significant correlation between drainage configuration and genetic divergence between populations. Although visualizing my sampling localities on a map of Mexico’s drainage systems may suggest a riverine effect on dispersal patterns, these results suggest that it is more likely that these geckos simply encompass very small home range sizes. These conclusions are also corroborated by the phylogenetic and migration rate analyses. Unfortunately, I cannot perform partial Mantel tests for P. lanei due to the relatively small number of populations. In addition, one of my populations (El Charco) is not adjacent to a river-body, yet it is highly diverged from the nearby population of Chamela. Taken together, these results suggest a limited influence of drainage configuration on genetic structure in these geckos.
4.7 Gecko diversity and taxonomic implications

Few studies examine geographic patterns of genetic differentiation in geckos, particularly throughout the New World. Most studies focusing of historical processes at and below the species level are restricted to North African, European, or Australian biotas (e.g. Gübitz et al. 2000; Kaspidis et al. 2005; Fujita et al. 2010; Rato et al. 2011). However, a general trend among these studies and mine is that geckos often harbour substantial cryptic diversity and that dispersal distances are presumably quite small. To my knowledge, this is the first study to statistically quantify migration rates between populations of geckos. My multilocus analysis of migration rates, coupled with the high levels of mtDNA sequence divergence (32%) suggests that the taxonomy of the genus may be in need of substantial revision.

Because both the mtDNA and alpha-enolase data suggest that *P. tuberculosus saxatilis* is not exchanging genetic information with other populations, I elevate this subspecies to species level as *Phyllodactylus saxatilis* new combination. Further, my mtDNA and species-tree analysis combined with previous karyotypic (Castiglia et al. 2009) and morphological data (Dixon 1964) suggests that *P. lanei rupinus* should be elevated to species status and I consider it to be *Phyllodactylus rupinus* new combination. Additional data from variable nuclear markers, morphology, and ecology are needed to determine if additional maternal lineages warrant species status (e.g. mtDNA lineage A1). Regardless, these results suggest that cryptic molecular diversity in taxa distributed throughout the TDF of western Mexico is drastically underestimated. If we are to conserve this highly biodiverse ecoregion, additional studies are needed from diverse organismal groups to elucidate genetic patterns and the evolutionary processes generating them.
4.8 Conclusions

The results of this study suggest that both the formation of TDF throughout western Mexico in the Miocene and Pleistocene climatic shifts had a substantial influence on biotic evolution throughout the region. Dry forests are one of Earth’s most diverse yet threatened ecosystems (Myers et al. 2000) yet, compared to moist tropical forests, we still know relatively little about the tempo and mechanisms of diversification (Werneck et al. 2011). By employing sophisticated multilocus phylogenetic, molecular clock, demographic, and biogeographic analyses, my results point to diversification processes operating prior-to, and including, Pleistocene climate change. This study adds to a limited literature that illustrates to utility of these approaches to understanding the biological history of species-rich areas (e.g. Che et al. 2010). If we are to adequately conserve Earth’s biodiversity at a time when natural and anthropogenic change threaten future diversification, similar multi-tiered approaches are required to elucidate the diverse processes contributing to the evolution of our planet’s rich biota.

Acknowledgments

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Foundation at the Royal Ontario Museum. All research was conducted using approved Animal Use Protocols. Collection (FLOR0030, SGPA/DGFS/047097/09, SGPA/DGVS/02320/10, SGPA/DGVS/02372/11) and export permits (028, 059) were obtained from SEMARNAT through the Universidad Nacional Autónoma de México (UNAM).
References


**Table 1.** Primer information for loci used in this study.

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<th>Sequence</th>
<th>Reference</th>
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</thead>
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<td>Friesen et al. (1997)</td>
</tr>
<tr>
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<td></td>
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<td></td>
</tr>
<tr>
<td>EnolH 912</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LamL724</td>
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<td>Friesen et al. (1997)</td>
</tr>
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<td></td>
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</tr>
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</tr>
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<td></td>
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<tr>
<td>ND413824H</td>
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<td></td>
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<td>ND4phyL</td>
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<td></td>
</tr>
<tr>
<td>CytbB1L</td>
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</tr>
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Table 2. Mitochondrial DNA sequence divergence between major lineages of leaf-toed geckos (*Phyllodactylus*) recovered in the phylogenetic analysis. Values below diagonal represent uncorrected p-distances and values above diagonal Tamura-Nei corrected distances.

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<tr>
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<td>0.199</td>
<td>0.107</td>
<td>—</td>
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**Table 3.** Diversity statistics and tests for demographic changes for each major mitochondrial lineage of leaf-toed geckos (*Phyllodactylus*) recovered in the phylogenetic analysis.

<table>
<thead>
<tr>
<th>Species</th>
<th>Lineage</th>
<th>n</th>
<th>H</th>
<th>h</th>
<th>S</th>
<th>K</th>
<th>π</th>
<th>Tajima's $D$</th>
<th>Fu's $Fs$</th>
<th>$R_2$</th>
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<td>6</td>
<td>5</td>
<td>0.933</td>
<td>10</td>
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<td>0.942</td>
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<td>0.689</td>
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<td>N/A</td>
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* signifies that statistics were calculated including sites with gaps or missing data. Bold values indicate significance at $P < 0.05$. 
Figure Legends

Fig. 1. Map of collecting localities for all species and subspecies used in this study. Shaded landscape represents the present distribution of Mexican dry forest. Solid lines represent previously documented biogeographic barriers.

Fig. 2. Genealogical relationships among representatives of the *P. tuberculosus* species group based on maximum likelihood analysis of mtDNA. Values adjacent to nodes represent bootstrap proportions estimated from 10000 replicates using the fast bootstrapping algorithm in RAxML.

Fig. 3. Relaxed molecular clock analysis for the *P. tuberculosus* group based on mtDNA sequences. Horizontal lines at nodes represent the 95% highest posterior density for inferred nodal ages. Vertical line represents the hypothesized origin of the Mexican dry forest (Becerra 2005). Horizontal scale is in million of years ago. Branch colors represent rate of evolution (blue=slow; red=fast). Lineage Through Time plot illustrates the accumulation of evolutionary lineages as a function of time.

Fig. 4. Species-tree analysis of the combined mitochondrial and nuclear data. Horizontal lines at nodes represent the 95% highest posterior density for inferred nodal ages. Horizontal scale is in million of years ago. Lineage Through Time plot illustrates the accumulation of evolutionary lineages as a function of time.

Fig. 5. Historical biogeography of the *P. tuberculosus* species group based on the dated mtDNA gene-tree. Colors represent the Mexican state where the animal was captured. Letters at nodes represent ancestral areas with the highest likelihood from Bayesian reconstructions. Green circles surrounding nodes represent postulated vicariant events.
Dotted lines represent hypothesized biogeographic barriers. I=Sierra Madre Occidental; II=Mexican Volcanic Belt; III=Isthmus of Tehuantepec.

**Fig. 6.** Extended Bayesian skyline plots showing demographic trends through time for each major mtDNA lineage recovered in the phylogenetic analysis. Dashed line represents the median population size, while solid lines denote 95% confidence intervals. Scale on the $x$-axis represents time in millions of years before present, while the $y$-axis represents log population size ($N_e$) x generation time ($\tau$).
CHAPTER 4—MOLECULAR PHYLOGENETICS OF *P. TUBERCULOSUS* GROUP

Fig. 1

Species
- lanei
- lanei/tuberculosis
- muralis
- t. magnus
- t. saxatilis

![Map of Mexico with specific locations marked]
CHAPTER 4—MOLECULAR PHYLOGENETICS OF *P. TUBERCULOSUS* GROUP

---

**Fig. 3**

Log-Lineages Through Time

- **P. lanei rupinus**
- **P. lanei lanei**
- **P. sp.**
- **P. tuberculosis saxatilis**
- **P. tuberculosis magnus**
- **P. muralis isthmus**
- **P. muralis muralis**

Log Lineages

Million Years Before Present

st = 2.67 Ma
Fig. 4

Log-Lineages Through Time

Million Years Before Present

Log Lineages

Cretaceous
Paleocene
Eocene
Oligocene
Miocene
LEGEND
A. Sonora  
B. Sinaloa  
C. Jalisco  
D. Guerrero  
E. Oaxaca  
EF. Oaxaca/Chiapas

Fig. 5

CHAPTER 4—MOLECULAR PHYLOGENETICS OF *P. TUBERCULOSUS* GROUP

Million Years Before Present

- Coleonyx variegatus
- *P. lanii lanii*
- *P. lanii rupinus*
- *P. muralis isthmus*
- *P. muralis muralis*
- *P. muralis saxatilis*
- *P. tuberculosus magnus*
- *P. tuberculosus saxatilis*
- *P. tuberculosus magnus*
- *P. tuberculosus magnus*
- *P. tuberculosus magnus*
- *P. tuberculosus magnus*
- *P. tuberculosis*
Fig. 6
## Supplementary Material

**Appendix I.** Locality information and GenBank accession numbers for all species and populations used in this study.

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- CB0584
- Jalisco
- El Charco house
- El Charco

### P. lanei rupinus
- ROM 35439
- Michoacan
- Lombardia, 10 km N (by road, Hwy 37)
- Lombardia

### P. lanei rupinus
- ROM 35440
- Michoacan
- Lombardia, 10 km N (by road, Hwy 37)
- Lombardia

### P. muralis isthmus
- CB0280
- 42837
- Oaxaca
- MX 200; Puente Nizaburra
- PN

### P. muralis isthmus
- CB0281
- 42838
- Oaxaca
- MX 200; Puente Nizaburra
- PN

### P. muralis isthmus
- CB0282
- 42839
- Oaxaca
- MX 200; Puente Nizaburra
- PN

### P. muralis isthmus
- CB0283
- 42840
- Oaxaca
- MX 200; Puente Nizaburra
- PN

### P. muralis isthmus
- CB0284
- 42841
- Oaxaca
- MX 200; Puente Nizaburra
- PN

### P. muralis isthmus
- CB0286
- 42843
- Oaxaca
- MX 200; Puente Merro II
- PMII

### P. muralis isthmus
- CB0287
- 42844
- Oaxaca
- MX 200; Puente Merro II
- PMII

### P. muralis isthmus
- CB0288
- 42845
- Oaxaca
- MX 200; Puente Merro II
- PMII

### P. muralis isthmus
- CB0291
- 42848
- Oaxaca
- MX 200; Puente Chipuehau
- Pchip

### P. muralis isthmus
- CB0292
- 42849
- Oaxaca
- MX 200; Puente Chipuehau
- Pchip

### P. muralis isthmus
- CB0293
- 42850
- Oaxaca
- MX 200; Puente Chipuehau
- Pchip

### P. muralis isthmus
- CB0294
- 42851
- Oaxaca
- MX 200; Puente Chipuehau
- Pchip

### P. muralis isthmus
- CB0295
- 42852
- Oaxaca
- MX 200; Puente Chipuehau
- Pchip

### P. muralis muralis
- ROM 35268
- Oaxaca
- San Pedro Totolapan, 23.8 km E of town (by road)
- SPT

### P. muralis muralis
- ROM 35269
- Oaxaca
- San Pedro Totolapan, 23.8 km E of town (by road)
- SPT

### P. muralis muralis
- ROM 35270
- Oaxaca
- San Pedro Totolapan, 23.8 km E of town (by road)
- SPT

### P. muralis muralis
- ROM 35271
- 45088
- Oaxaca
- San Pedro Totolapan, 23.8 km E of town (by road)
- SPT

### P. muralis muralis
- ROM 35272
- Oaxaca
- San Pedro Totolapan, 23.8 km E of town (by road)
- SPT

### P. muralis isthmus
- CB0278
- Oaxaca
- Tehuantepec, ~16 km W

### P. sp.
- CB0575
- Jalisco
- El Charco house
- El Charco

### P. sp.
- CB0580
- Jalisco
- El Charco house
- El Charco

### P. sp.
- CB0582
- Jalisco
- El Charco house
- El Charco

### P. sp.
- CB0587
- Jalisco
- El Charco house
- El Charco

### P. sp.
- CB0597
- Jalisco
- El Charco house
- El Charco

### P. tuberculosis magnus
- CB0238
- 42800
- Guerrero
- MX 200; km 50; 9 km S San Marcos; Puente La Estancia I
- SM

### P. tuberculosis magnus
- CB0239
- 42801
- Guerrero
- MX 200; km 50; 9 km S San Marcos; Puente La Estancia II
- SM

### P. tuberculosis magnus
- CB0240
- 42802
- Guerrero
- MX 200; 3 km S San Juan Grande
- SJG

### P. tuberculosis magnus
- CB0241
- 42803
- Guerrero
- MX 200; 3 km S San Juan Grande
- SJG

### P. tuberculosis magnus
- CB0259
- 42816
- Oaxaca
- MX 200; km 225 at bridge
- km225
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**Supplementary Table 1.** **MIGRATE-N** estimates of mutation-scaled effective population sizes and migration rates for *Phyllodactylus tuberculosus* and *P. muralis* along with confidence intervals. Locus ‘All’ signifies that all three loci were used to calculate values. $4N_m = \text{number of migrants per generation} (\Theta M)$.

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**Supplementary Table 2.** MIGRATE-N estimates of mutation-scaled effective population sizes and migration rates for *Phyllodactylus lanei* along with confidence intervals. Locus 'All' signifies that all three loci were used to calculate values. $4N_m$ = number of migrants per generation ($\Theta M$).

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Supplementary Figure Legends

Fig. S1. Relationships among representatives of the *P. tuberculosus* species group based on maximum likelihood analysis of phased alpha-enolase haplotypes. For ease of visualization only unique haplotypes are presented. Values adjacent to nodes represent bootstrap proportions estimated from 10000 replicates using the fast bootstrapping algorithm in RAxML. I=Sierra Madre Occidental; II=Mexican Volcanic Belt; III= Isthmus of Tehuantepec.

Fig. S2. Relationships among representatives of the *P. tuberculosus* species group based on maximum likelihood analysis of phased lamin-A haplotypes. For ease of visualization only unique haplotypes are presented. Values adjacent to nodes represent bootstrap proportions estimated from 10000 replicates using the fast bootstrapping algorithm in RAxML. I=Sierra Madre Occidental; II=Mexican Volcanic Belt; III= Isthmus of Tehuantepec.

Fig. S3. Relationships among representatives of the *P. tuberculosus* species group based on maximum likelihood analysis of the concatenated data. For concatenation with mtDNA a single phased nuclear haplotype was selected from both intron alignments for each individual. Values adjacent to nodes represent bootstrap proportions estimated from 10000 replicates using the fast bootstrapping algorithm in RAxML.
Fig. S1

Chap. 4—Molecular Phylogenetics of *P. tuberculosus* Group
CHAPTER 4—MOLECULAR PHYLOGENETICS OF \textit{P. tuberculosus} GROUP

Fig. S2

P. tuberculosus saxatilis (Sonora, Sinaloa, Jalisco)
P. tuberculosus magnus (Oaxaca)
P. muralis muralis (Oaxaca)
P. muralis isthmus (Oaxaca)

P. lanei lanei (Guerrero)
P. lanei rupinus (Jalisco, Michoacan)
P. muralis isthmus (Oaxaca)

P. tuberculosus magnus (Guerrero, Oaxaca, Chiapas)
P. lanei lanei (Guerrero)
Fig. S3

- **P. tuberculosis saxatilis**
- **P. tuberculosis magnus**
- **P. muralis muralis**
- **P. muralis isthmus**
- **P. sp.**
- **P. lanei rupinus**
- **P. lanei lanei**
CHAPTER 5
Consideration of historical demographic processes in landscape genetic studies of gene flow: a case study with Mexican leaf-toed geckos

Abstract

It is critical to disentangle the relative influence of historical versus contemporary processes that shape the spatial distribution of genetic variation. I employ different molecular marker types and analytical methods in a landscape genetic context to understand the genetic consequences of recent habitat fragmentation using leaf-toed geckos (Phyllodactylus) from northern Mexico. Mitochondrial DNA sequences obtained from 77 individuals are synthesized with microsatellite DNA data from 336 geckos to reveal substantial genetic differentiation over a relatively small geographic area (<40 km). MtDNA sequence divergences of up to 6% are resolved. I find a 10-fold increase in contemporary versus historical migration rates based on microsatellite data, and evidence that many populations suffer from reduced effective population sizes. Landscape genetic analyses find a stronger correlation between landscape structure and contemporary versus historical migration, suggesting that individuals are altering their dispersal routes in response to recent habitat changes. Finally, my analyses suggest that mtDNA is a poor metric for inferring contemporary landscape genetic relationships and that the utility of Bayesian methods needs to be evaluated further. This study suggests that both long-term female philopatry and recent
fragmentation contribute to the high degree of differentiation observed over fine-spatial scales in leaf-toed geckos.

1 Introduction

Rates and patterns of dispersal, dispersion, and gene flow can be influenced by a variety of intrinsic and extrinsic factors including species-specific philopatry, predation, competition, physiological tolerances, and landscape characteristics (Ricketts 2001; Manel et al. 2003; Storfer et al. 2010). Habitat fragmentation and loss due to both natural and anthropogenic forces can be a substantial threat to the maintenance and connectivity of populations (Sanderson et al. 2002; Ewers & Didham 2005). Maintaining genetic connectivity is essential to allow the free exchange of neutral and adaptive alleles between demes. Processes that disrupt or prevent dispersal and gene flow could lead to inbreeding depression, the accumulation of deleterious alleles, reduced adaptive potential, and local extirpation (Fahrig 2003; Crispo et al. 2011).

The field of landscape genetics (Manel et al. 2003) is renewing interest in contemporary ecological processes that drive the genetic structure of natural populations (Sork & Waits 2010; Storfer et al. 2010). Although many studies profoundly enhance our understanding of the spatial distribution of genetic variation, most studies do not examine the influence of historical demographic processes on contemporary genetic structure (e.g. Spear et al. 2005; Vignieri 2005; Wang 2009; Goldberg & Waits 2010). I note that by historical I refer to processes operating over geologic time and not within the past few dozen generations. Temporal components of landscape genetics studies that drive pattern-process
relationships need to be identified (Anderson et al. 2010; Wang 2010). For example, based on simulations Landguth et al. (2010) find that historical processes may confound contemporary landscape-genetic inference in species with small dispersal abilities. Thus, recent efforts have sought to disentangle the relative influence of historical and contemporary landscape processes on contemporary population genetic structure (Vandergast et al. 2007; Zellmer & Knowles 2009; Dyer et al. 2010). One particularly valuable approach utilizes powerful statistical analyses to detecting migration rates operating over different timescales (Beerli & Felsenstein 2001; Wilson & Rannala 2003; Beerli 2006), although this approach is not often applied in many contemporary studies. Combining estimates of historical and contemporary population genetic parameters provides a powerful means to determine if recent habitat fragmentation has had detrimental impacts on population size and connectivity (e.g. Chiucchi & Gibbs 2010). Although the comparative demographics approach is promising, most studies directly comparing historical versus contemporary demographic parameters focus on temperate systems (Anderson et al. 2009; Schmidt et al. 2009; Chiucchi & Gibbs 2010). Few studies center explicitly on tropical habitats yet the majority of Earth’s biodiversity resides in these environments (Myers et al. 2000). Further, most studies have sought to compare historical and contemporary parameters in an aspatial context, disregarding the effects of landscape configuration on functional connectivity.

Although generally not as diverse as tropical rain forests, the tropical deciduous forests (TDF) of western Mexico are home to an enormous biodiversity including many endemic species (Robichaux & Yetman 2000; Myska 2007). In contrast to tropical moist or
rain forests, TDFs are characterized by distinct wet/dry seasons, with the latter lasting up to 10 months in some regions (Murphy & Lugo 1996; Robichaux & Yetman 2000). Functional and structural differences between these forest types include differences in plant phenology, canopy height, ground cover, soil chemistry, net primary productivity, and the proportion of thorny plant species (Murphy & Lugo 1996; Pennington et al. 2000). The geographic range of Mexican TDF spans most of the Pacific versant of the Sierra Madre Occidental and Sierra Madre del Sur, with isolated stands scattered throughout eastern Mexico including the Yucatán Peninsula (Robichaux & Yetman 2000; Becerra 2005; Cartron et al. 2005). These forests originated sometime during the Oligocene due to the uplift of the western sierras; forest maintenance relies heavily on these mountain ranges blocking cold fronts from the north (Becerra 2005). TDF is commonly encountered at elevations less than 2 000 m, where they gradually transition at higher elevations to pine/oak and pine forests.

The floral and faunal diversity within these forests is immense. Unfortunately, conservation efforts emphasize tropical rain forests and high elevation pine and pine-oak habitats, specifically throughout areas of the Mexican Volcanic Belt (Pennington et al. 2000; Robichaux & Yetman 2000; García 2006). Whereas rain forest biodiversity is substantial, only a small portion of Mexico can be classified as tropical rain forest while TDF is a predominant habitat-type (Becerra 2005). In order to conserve Mexico’s TDF, both broad and fine-scale genetic studies are required to assess spatial patterns of diversity and the processes generating them. Molecular genetic data can be used to detect local and widespread patterns that can be used to inform conservation priorities, including distinguishing between
source and sink areas (Becerra & Venable 2008).

Leaf-toed geckos, genus *Phyllodactylus* (Phyllodactylidae), comprise approximately 50 species distributed throughout the New World Tropics, with most Mexican species concentrated in coastal Pacific states south of the Trans-Mexican Volcanic Belt (TMVB) (Dixon 1964). These lizards are common on vertical surfaces throughout TDF habitat. Recent molecular, karyological and morphological work on the genus documents the presence of cryptic diversity, diagnosable only with a careful selection of molecular and morphological characters (Blair et al. 2009; Castiglia et al. 2009; Murphy et al. 2009; Blair et al. unpublished). Molecular evidence also suggests that the evolution of these lizards is tied to the formation of Mexican TDF during the Oligocene (Blair et al. unpublished).

*Phyllodactylus tuberculatus* Wiegmann 1835 (yellow-bellied gecko) currently exhibits the broadest geographic range of any leaf-toed gecko. The species is distributed throughout the tropical thornscrub and dry forests of the Neotropics from northwestern Mexico to Costa Rica (Dixon 1964). Although Mosauer (1936) reports little morphological variation, Dixon (1964) documents substantial variation in several meristic characters, leading to the possibility of potential cryptic species. In *P. tuberculatus* from near Alamos, Sonora rates of gene flow are correlated with forest structure, stream connectivity, undisturbed habitat, slope, and temperature (Blair et al. unpublished). A multilocus phylogenetic analysis of the *P. tuberculatus* species group (*P. tuberculatus, P. lanei, P. muralis*) throughout western Mexico suggests that species richness is far greater than currently recognized (Blair et al. unpublished).
unpublished). Based on data from mtDNA, nDNA, and previous morphological characters, these authors elevate the subspecies *P. tuberculosus saxatilis* to species.

Herein I use microsatellite data and *de novo* mtDNA sequence data to test for confounding effects of genealogical history on landscape-genetic inference. Subsequently, I compare and contrast historical and contemporary demographic parameters for multiple populations of *P. saxatilis* throughout Alamos to test hypotheses related to the following: (i) cryptic mtDNA structure resulting from historical demographic processes; (ii) historical vs. contemporary migration rates and effective population sizes; (iii) bottlenecks; (iv) landscape structure correlates with contemporary vs. historical migration; and (v) response metric influences on landscape genetic relationships. This multi-tiered approach utilizing different molecular markers and analytical methods allows for robust conclusions to be drawn regarding the demographic consequences of recent habitat fragmentation.

2 Materials and methods

2.1 Study site and tissue sampling

All sampling localities were mapped in Figure 1. Microsatellite data (10 loci) for 336 individuals (mean=28 individuals per locality) were obtained from Blair *et al.* (unpublished). I screened 77 individuals for mtDNA polymorphism to test for confounding historical processes influencing population demography (Fig. 1; Supplementary Appendix I). Individuals screened for mtDNA were chosen based on location and previous microsatellite
results. All tissues were preserved in the field in 95% ethanol. Vouchers were fixed in the field with 10% formalin and eventually transferred to 70% ethanol and deposited either in the Laboratorio de Herpetologia, Instituto de Biologia, Universidad Nacional Autónoma de México, or the Royal Ontario Museum. Fieldwork was conducted during the summers of 2007 and 2008. Sequences were also obtained from *P. xanti* (Blair et al. 2009) and de novo for *P. homolepidurus* to root all mtDNA networks.

2.2 DNA extraction, amplification and sequencing

Total genomic DNA was extracted from liver or muscle tissue using standard Proteinase K and phenol-chloroform protocols (Sambrook *et al.* 1989; Hillis *et al.* 1996). I amplified and sequenced 2400 base pairs (bp) of the mitochondrial genome encompassing partial sequences of cytochrome *c* oxidase subunit I (COI), NADH dehydrogenase subunit 4 (ND4), and 16S rRNA. Sequences were also obtained for the entire NADH dehydrogenase subunit I (NDI) gene and the flanking tRNAs leucine, isoleucine, and glutamine. Amplified gene fragments, number of corresponding bp, primer sequences, and references were presented in Supplementary Table 1. PCR reactions (25 µl) were composed of the following reaction mix: 18.55 µl ddH₂O, 1 µl 10 mM primer (forward and reverse), 2.5 µl 10x buffer with 1.5 mM MgCl₂, 0.8 µl 10mM dNTPs, 0.15 µl 5 U Taq DNA Polymerase (Boehringer Mannheim), and 1 µl template DNA. Amplification was performed on a GeneAmp PCR System 9700 or Eppendorf AG 5345 thermal cycler using 38 cycles with the following conditions: initial denaturation of 94°C (2 min) followed by 94°C (30 s), 50–55°C (45 s), 72°C (45 s), with a
final extension temperature of 72°C (7 min). Amplified DNA products were separated on a 1% agarose gel stained with ethidium bromide and visualized under ultraviolet (UV) light. Visible bands were excised and filtered through spin columns for 10 min via centrifugation. The resulting solution was used as the template for sequencing reactions.

Sequencing reactions (10 µl) were performed on an Eppendorf AG 5345 thermal cycler using the BigDye Terminator v 3.0 Cycle Sequencing Kit (Applied Biosystems) with the following reaction mix: 1 µl BigDye, 2 µl 5x BigDye Terminator Buffer, 2 µl ddH₂O, 1 µl of 10 mM primer solution (same as PCR), and 4 µl PCR product. Sequencing conditions were run with the following conditions: initial denaturation of 96°C (1 min), followed by 25 cycles of 96°C (10 s), 50°C (5 s), 60°C (4 min), and 4°C indefinitely. All fragments were sequenced in both directions. Following sequencing reactions, samples were cleaned, ethanol precipitated, and visualized on an ABI 3100 Automated Sequencer (Applied Biosystems).

2.3 Matrilineal genealogy

Mitochondrial DNA sequences were initially imported and edited in BIOEDIT 5.0.6 (Hall 1999). Forward and reverse gene fragments were manually combined and multiple sequence alignments were performed manually in BIOEDIT. Individual datasets were exported into MACCLADE 4.0 (Maddison & Maddison 2005) to identify and define codon positions for each protein-coding gene. Because the amplified ND1 fragment encompassed several genes (Leaché & McGuire 2006), I used the complete mitochondrial genome of *Gekko gecko* (NC 007627) to determine gene position. The MACCLADE nexus file was used for genealogical
I reconstructed genealogical relationships using both maximum parsimony (MP) and Bayesian inference (BI). MP was based on the concatenated mtDNA dataset implemented using TNT 1.1 (Goloboff et al. 2008). I employed the parsimony ratchet (Nixon 1999) and tree drifting with subsequent rounds of tree fusing (Goloboff 1999; Goloboff et al. 2008) using default parameters. All characters were treated as nonadditive and equally weighted, and gaps were treated as missing data. Support for nodes was assessed from 10,000 nonparametric bootstrap replicates using a “fast” stepwise heuristic search (Felsenstein 1985) implemented in PAUP* v.4.0b10 (Swofford 2002), retaining groups with >50% nodal support. I assumed a bootstrap value >70 indicated reliability (Hillis & Bull 1993).

Bayesian inference (BI; Huelsenbeck & Ronquist 2001; Huelsenbeck et al. 2002; Nylander et al. 2004) was used to reconstruct genealogical relationships. I implemented a partitioned analysis (Nylander et al. 2004; Brandley et al. 2005) with three strategies: the entire dataset, by gene, and by gene and codon position for protein-coding loci. Bayes Factors (BF; Kass & Raftery 1995), obtained by using the sump command in MRBAYES 3.1.2 (Huelsenbeck & Ronquist 2001), were used to select the best partitioning scheme with a BF_{12} >10 indicating a preference one partition over another (Brandley et al. 2005). Because rate variation among partitions significantly influence parameter estimation in posterior distributions (Marshall et al. 2006) with regard to branch length estimates, I allowed rates to vary by using the command prset applyto=(all) ratepr=variable in MRBAYES.

MRMODELTEST 2.2 (Nylander 2004) was used to select the best evolutionary model.
for each data partition using the Akaike Information Criterion (AIC; Akaike 1974, 1979). BI was performed using MRBAYES. Two simultaneous runs of six chains were implemented for 1 x 10^6–3 x 10^6 generations, depending on partitioning strategy, while sampling every 100 generations. Stationarity was assessed when log–likelihood scores versus generation reached a stable equilibrium using the *sump* command in MRBAYES. I monitored convergence using Tracer 1.5 (Rambaut & Drummond 2007) with target ESS values of at least 200. A burn-in value of (25%) was used to discard topologies with sub-optimal likelihood scores prior to generating a 50% majority rule consensus tree. Nodal support within the consensus tree was assessed from the posterior distribution of topologies (Erixon *et al.* 2003). Although Bayesian posterior probabilities (BPP) have often provided relatively high support for incorrect clades (Suzuki *et al.* 2002), a BPP value >95 with a bootstrap value >70 was assumed to indicate a real biological entity.

2.4 Population genetic analysis of mtDNA

DNASP 5.0 (Rozas *et al.* 2003) was used to estimate a suite of population genetic diversity indices from mtDNA data for each population including the number of haplotypes, haplotype diversity and its variance (Nei 1987), nucleotide diversity (π; Nei 1987), the average number of nucleotide differences k (Tajima 1983) and total variance (stochastic + sampling) assuming no recombination (Tajima 1993), and the number of segregating sites S (Tajima 1989). Fu’s Fs (Fu 1997) tests were used to test for the presence of demographic expansion. Fu’s Fs was assumed to be the more powerful test for detecting population growth (Fu 1997)
than the raggedness index (Harpending 1994). Significantly negative values of Fu’s $F_s$ (compared to neutral expectations under the coalescent) indicated an excess number of rare alleles compared to $\theta$ (estimated from $\pi$) and suggested a recent population expansion (Fu 1997). DNASP was used to estimate the degree of genetic divergence between populations using both the average number of nucleotide differences between populations and the average number of nucleotide substitutions per site between populations (Nei 1987).

A Mantel test and Reduced Major Axis (RMA) regression analysis were implemented to detect the presence of significant isolation-by-distance (IBD) using the IBD Web Service 3.16 (Jensen et al. 2005). The analysis was run for 30,000 randomizations using PhiST/(1-PhiST) as a measure of genetic distance, with PhiST estimated from uncorrected $p$-distances (Rousset 1997).

### 2.5 Historical and contemporary migration rates

A multi-tiered analytical approach was used to compare contemporary demographic parameters and historical patterns. Migrate 3.2.16 (Beerli & Felsenstein 2001; Beerli 2006) was used to estimate historical $N_e$ and asymmetric migration rates between populations based on the microsatellite data. Migrate employed coalescence in either a Bayesian or maximum likelihood framework to calculate $\Theta$ and $M$, where $\Theta$ represented the mutation-scaled effective population size ($4N_e\mu$ for a nuclear locus, $N_e\mu$ for mtDNA) and $M$ represented the mutation-scaled immigration rate ($m/\mu$). I estimated the effective number of immigrants per generation over thousands of years or approximately $4N_e$ generations in the past (Beerli 2008) by multiplying $\Theta$ and $M$. The $F_{ST}$ calculation method was used to generate starring
values for both $\Theta$ and $M$ while specifying uniform priors for both parameters with a minimum of zero, mean of 50, maximum of 100, and a delta of 10. The Bayesian method was used to infer $\Theta$ and $M$, specifying two independent runs, static heating with four chains using recommended temperatures, a sampling increment of 200, 5,000 recorded steps, and a burn-in of 100,000. Convergence was assessed by examination of ESS values with a target of at least 1,000. The analysis was run multiple times to confirm reaching the same posterior distribution of parameter estimates.

**BAYESASS 3.0** (Wilson & Rannala 2003) was used to estimate contemporary migration rates between populations. The method assumed linkage equilibrium between loci, but allowed for deviations in Hardy-Weinberg proportions by introducing an additional inbreeding ($F$) parameter. **FORMATOMATIC** (Manoukis 2007) was used to generate a **BAYESASS** input file from a **GENEPOL** file. **BAYESASS** was run for $3 \times 10^6$ generations with a burn-in of $3 \times 10^6$ and a sampling interval of 300. I modified the MCMC mixing parameters to obtain target values recommended by the program’s authors. Adequate convergence was assessed by running the program multiple times starting with different random number seeds and by employing **TRACER**.

Estimated migration rate estimates from **MIGRATE** and **BAYESASS** were compared by adapting an approach similar to that of Chiucchi & Gibbs (2010). Estimates of $m$ from **MIGRATE** were obtained by multiplying all $M$-values by an estimated mutation rate of $5 \times 10^{-4}$ (Garza & Williamson 2001). To statistically quantify the relationship between historical and contemporary migration rates I implemented a Mantel test (Mantel 1967) in the R package **ECODIST** (Goslee & Urban 2008) using 10,000 permutations.
2.6 Historical and contemporary effective population sizes

I tested the null hypothesis that historical and contemporary effective population sizes were the same. The hypothesis would be rejected, $N_e$ changed through time, assuming that such indicated continual habitat fragmentation and anthropogenic affects. First, I estimated historical effective population sizes in a Bayesian framework using the coalescent model implemented in MIGRATE. To estimate contemporary $N_e$, I used the linkage disequilibrium method (Hill 1981) in LDNE 1.3.1 (Waples 2006; Waples & Do 2008). Whereas the temporal method required samples from multiple points in time (Waples 1989), the linkage disequilibrium method accommodated samples from a single time frame and corrected for potential bias in sample sizes (Waples 2006). I specified a random mating model with default critical values for allele frequencies. Jackknife 95% confidence intervals were used as a measure of variance and negative values were interpreted as infinity (Waples & Do 2008).

To test for reductions in $N_e$ operating over different timescales, I used BOTTLENECK 1.2.02 (Piry et al. 1999) to compare estimates of observed heterozygosity to number of alleles at each locus. The null hypothesis was rejected, i.e. bottlenecking was indicated, if heterozygosity excess was observed (Cornuet & Luikart 1996) over the last 0.2–4.0 $N_e$ generations. Heterozygosity excess was tested using all three mutation models for comparison, each with 1 000 iterations. For the two-phase mutation model (TPM), I specified a variance of 12 and set the proportion of stepwise mutation events to 95% (Piry et al. 1999). Significance was assessed using the Wilcoxon signed rank test (Cornuet & Luikart 1996).

Second, I examined allele frequency distributions for each locus and population. Populations
in mutation-drift equilibrium were assumed to show L-shaped distributions representing a large proportion of alleles at low frequency (<0.1; Luikart et al. 1998). In contrast, recently bottlenecked populations were assumed to show a mode-shift in allele frequency distribution because low frequency alleles are lost more quickly versus alleles present in high frequency. This test was most suitable for detecting bottlenecks occurring a few generations ago (Luikart et al. 1998).

2.7 Landscape genetics

Landscape genetics was employed to understand the relationship between physiographic structure, historical and contemporary migration rates, and mtDNA divergence. I tested the hypothesis that landscape-level processes did not act over historical timescales. First, I created resistance surfaces in ArcMap 10 (ESRI) based on landscape variables shown to be important in shaping functional connectivity for this species (Blair et al., unpublished). Next, I used Circuitscape 3.5.7 (Shah & McRae 2008) to calculate pairwise resistance distances between populations based on each resistance surface and then used multiple regression on distance matrices (MRM; Legendre et al. 1994; Lichstein 2007) to correlate resistance distance with both historical and contemporary migration rates. Because MIGRATE and BAYESASS produced asymmetrical pairwise rates, I averaged both values to obtain a single rate between populations. If recent fragmentation was altering dispersal routes, then I expected to observe a higher correlation between contemporary vs. historical migration and landscape. Conversely, a stronger correlation between landscape and historical migration was
interpreted to indicate the importance of landscape-level processes acting over older timescales. All MRM analyses were conducted in ECODIST. As a second measure of historical migration I correlated resistance distances with pairwise mtDNA sequence divergence ($D_{xy}$). The incorporation of clonally inherited mtDNA allowed me to examine signs of long-term sex-biased dispersal. Finally, I tested the sensitivity of choice-of-response in landscape genetic inference by utilizing a stepwise MRM procedure to correlate contemporary migration rates with different combinations of landscape variables. I used a p-to-enter value of < 0.05 and a p-to-remove of > 0.05 in all models. Significance was assessed using 10 000 permutations of the response matrix. I also calculated AIC values for each model to assess relative importance. Results were then compared to a previous study that used pairwise $D_{est}$ as the response variable (Blair et al., unpublished).

3 Results

3.1 Genetic diversity and phylogenetic model selection

I obtained microsatellite data for 10 loci from 336 individuals in 10 different populations from a previous study (Blair et al. unpublished). All 10 loci were independent and in Hardy-Weinberg equilibrium for each population. Within-site diversity was moderate as shown by both expected heterozygosity and allelic richness. Expected heterozygosity over all loci ranged from 0.7025 to 0.7812 and allelic richness from 54.2341 to 65.6058.
I recovered 2400 bp of mtDNA sequences for 77 *P. saxatilis* from 11 populations distributed throughout the immediate area surrounding Alamos, Sonora. The total number of bp per partition, the proportion of potentially phylogenetically informative characters, and the selected model of sequence evolution were shown in Supplementary Table 2. No premature stop codons were found in the protein-coding genes. Variation was more pronounced at third positions in codons versus first and second, as was expected for protein-coding genes. Variation in the three tRNAs and 16S was moderate as compared to the protein-coding loci.

### 3.2 Matrilineral genealogy

Bayesian analyses using three alternate partitioning strategies were summarized in Supplementary Table 3. The calculation of Bayes Factors suggested that the most heavily partitioned dataset (by gene and codon position) was the most efficient approach based on improved marginal likelihood values. Thus, analyses were based on this partitioning strategy.

Maximum parsimony analysis of the concatenated dataset resulted in eight most parsimonious trees (MPTs) of 723 steps. The MP strict consensus tree with both bootstrap proportions and posterior probabilities was shown in Fig. 2A. To illustrate branch lengths, I also presented the Bayesian majority rule consensus tree (Fig. 2B). Both methods resulted in a highly supported genealogical hypothesis comprised of four maternal lineages differing up to 6% (uncorrected *p*-distances). Lineage A, comprised solely of individuals from Choquincahui, was the most distinct and sister to the remaining populations with strong
statistical (BSP = 99; BPP = 1.0). The remaining three lineages received moderate to high statistical support (Lineage B = 53/0.80; Lineage C = 96/1.0; Lineage D = 100/1.0) and showed east-to-west geographic structuring. Lineage B was composed of individuals from Arroyo Tabelo, Rio Cuchujaqui, Los Camotes, El Palmarito, and a population 55 km northeast of the Alamos cemetery. Lineage C contained haplotypes from La Sierrita Nature Reserve, central Alamos, and Rio Cuchujaqui. Lineage D included haplotypes from western areas including Aduana, road to Navojoa (both populations), Arroyo Tabelo, Alamos, and La Sierrita Reserve. Thus, populations central to the study sites served as a secondary contact zone for multiple divergent mitochondrial lineages (Fig. 3). Uncorrected $p$-distances between lineages B, C, and D approached 3%.

### 3.3 Population genetic analysis of mtDNA

Standard nucleotide diversity statistics for nine populations sampled for mtDNA polymorphism were presented in Table 1. Genetic diversity was greater for populations composed of haplotypes from multiple lineages. For example, nucleotide diversity estimates ($\pi$) for populations at Alamos and La Sierrita are almost two-fold higher than values from other populations (0.0147 vs. 0.0032). Haplotype diversity was high in all populations, with a value of 1.0 recovered for Alamos, Road to Navojoa 1, Choquincahui, and Los Camotes. Fu’s $F_s$ test showed no significant deviations from neutral expectations in each population, indicating no demographic expansion.

As indicated by the genealogy, significant population differentiation was present throughout the region (Table 2). Choquincahui, on average, differed from all other
populations by 106.0525 substitutions or 0.0588 substitutions per site. Individuals from the Alamos population were more similar genetically to the populations west of Alamos (Aduana, Road to Navojoa) versus populations east of Alamos (Rio Cuchujaqui).

Conversely, individuals from La Sierrita Nature Reserve were generally just as related to western populations as they were to the eastern populations. Rio Cuchujaqui differed from western populations (Aduana, Road to Navojoa) by approximately 29.059 changes or 0.0239 substitutions per site.

The results of the Mantel and RMA tests showed a significantly positive relationship between geographic distance (km) and mtDNA genetic distance ($Z = 5314.0176; r = 0.5007; P = 0.0002$). Model estimates for the RMA analysis included an intercept of –6.435, a slope of 0.4518, and an $R^2$ value of 0.251 (Fig. 4).

### 3.4 Historical and contemporary migration rates

Multiple runs of BAYESASS and MIGRATE gave consistent results, indicating that the MCMC chain was mixing well and adequately sampling from the posterior distribution.

Contemporary migration rates estimated from BAYESASS were generally low to moderate and for most population pairs 95% confidence intervals included zero (Table 3). However, some pairs showed signs of exchanging a relatively high number of migrants per generation. For example, migration rates from Aduana to the Road to Navojoa were 0.155, and rates from El Quintero to Choquincahui were 0.256. Interestingly, rates from Choquincahui to El Quintero and from the Road to Navojoa to Aduana were much lower (0.008 and 0.012, respectively). The fraction of non-migrants per generation ranged from 0.675 in Choquincahui to 0.940 in Rio Cuchujaqui.
Estimates of $M$ obtained from MIGRATE also suggested little migration between populations with 95% confidence intervals including zero for most pairs (Supplementary Table 4). I used $M$ to calculate the number of migrants per generation ($N_m$) using the formula $N_m = (M\Theta)/4$. Many of these values were less than one indicating little historical migration between populations. To compare historical rates to contemporary rates I multiplied $M$ by a mutation rate of $5 \times 10^{-4}$ to obtain an estimate of historical $m$; historical estimates of $m$ were approximately one-tenth the size of contemporary $m$ (Fig. 5). Because I found evidence for relatively high contemporary migration from El Quintero to Choquincahui compared to historical rates (mean 0.256 vs 0.000935) I performed Mantel tests both with and without this population. When including El Quintero there was a near-significant correlation between historical and contemporary migration rates based on 10 000 permutations (Mantel $r = 0.2592$, $p = 0.055$). Excluding this population increased the $p$-value, indicating no significant correlation (Mantel $r = 0.2773$, $p = 0.070$).

3.5 Historical and contemporary effective population sizes

I used MIGRATE to estimate historical $N_c$ and LD$N_c$ to estimate contemporary $N_c$.

Contemporary $N_c$ was large for most populations with 95% confidence intervals approaching infinity (Table 4). However, three populations (Road to Navojoa 1, Arroyo Tabelo, and Mocuzari) showed relatively small sizes in comparison. Averaging over each allele frequency models, estimated $N_c$ at these three sites was 25.4, 35.6, and 32.1, respectively. Some populations exhibited considerable variation in estimates of $N_c$ depending on which
allele frequency model was used (0.05, 0.02, or 0.01). Estimates of historical and contemporary \( N_e \) were similar. However, mean estimates for historical \( N_e \) were generally larger for most populations, but 95% confidence intervals were quite large (Table 4).

Tests for recent reductions in \( N_e \) using the excess heterozygosity method in BOTTLENECK showed no evidence for a decrease in size for any population under the TPM or SMM model (Table 5). However, under IAM, all populations showed recent reductions. Tests for mode-shifts in allele frequency distribution showed an L-shaped curve for all populations except for Road to Navojoa 1, which showed a shifted distribution.

### 3.6 Landscape genetics

Results from the MRM analyses indicated a higher correlation between landscape resistance and contemporary migration rates versus landscape resistance and both historical rates and mtDNA divergence (Table 6). No significant correlation occurred between Euclidean distance and historical migration rates. Slope was the only variable to show a significant correlation with historical rates. Coefficient of determination \((R^2)\) values in the historical models ranged from 0.0073 to 0.1145 (mean=0.0551). Correlations in the contemporary models ranged from 0.0214 for slope to 0.1994 for Euclidean distance (mean=0.1268). Further, four of the six landscape variables were significantly correlated with contemporary migration rates. Euclidean distance was the only variable significantly correlated with mtDNA divergence. The stepwise MRM analysis suggested that Euclidean distance and anthropogenic disturbance were the best predictors of contemporary gene flow as estimated with BAYESASS (AIC weight=0.1604; Supplementary Table 5).
4 Discussion

Population and landscape genetics seek to disentangle the relative roles historical and contemporary processes play in shaping the spatial distribution of genetic variation (Vandergast et al. 2007; Anderson et al. 2010; Burbrink 2010; Dyer et al. 2010; Wang 2010). I use landscape genetics to investigate the temporal dynamics of demographic processes. High levels of mtDNA divergence between lineages occur over small spatial scales, in the absence of obvious geographic barriers, which is consistent with female philopatry and low levels of historical gene flow. A 10-fold increase in contemporary versus historical migration rates occurs yet contemporary versus historical migration appears to be better explained by present-day landscape-level processes. I show that the choice of response-variable (equilibrium vs. non-equilibrium metrics, microsatellites vs. mtDNA) can influence subsequent landscape-genetic inference. Below I expand on these major findings and discuss the advantages of adopting similar multi-tiered approaches when investigating the demographic consequences of recent habitat fragmentation in a spatial context.

4.1 Sequence divergence and mitochondrial genealogy

Substantial lineage divergence occurs among populations of leaf-toed geckos distributed near Alamos, Sonora, with samples from Choquincahui showing the highest divergence values (approximately 6%). Pairwise comparisons between several other populations show divergence estimates (uncorrected \(p\)-distances) ranging from 1% to 4%. The MP and BI analyses yield highly compatible topologies and several nodes are clearly resolved (Fig. 2A,
B). Geographic structure and significant isolation-by-distance is detected. Whereas lineage D (blue lineage) occurs predominantly west of Alamos the remaining lineages are distributed between Choquincahui and the western populations (green and yellow). Lineages B, C, and D sometimes occur sympatrically (Arroyo Tabelo, Alamos, La Sierrita, and Rio Cuchujaqui). The two sympatric mitochondrial lineages at each of these sites may result from contemporary female dispersal. If asymmetric female dispersal drives these patterns, then females from Rio Cuchujaqui must be dispersing to Alamos and females from Arroyo Aduana and Road to Navojoa are dispersing to both Alamos and La Sierrita. Dispersal from western to eastern populations is non-existent. Thus, the home ranges must be quite small and dispersal capabilities limited to approximately 20 km. Indeed I find moderate, but significant isolation-by-distance with the mtDNA data (Fig. 4). This explanation also requires asymmetrical female dispersal via migration from both eastern and western populations into Alamos, but not vice versa. Asymmetrical migration rates appear to occur in these lizards (Table 3). Another explanation involves incomplete lineage sorting at sites containing sympatric lineages but, again, this requires asymmetrical female dispersal. Finally, these sites might be composed of hybrids between eastern and western populations. Blair et al. (unpublished) find evidence of substantial admixture within populations of these geckos. Taken together, this suggests a high degree of female philopatry and male-biased dispersal in these lizards.

The co-occurrence of both mitochondrial lineages throughout Alamos proper might suggest an ecological component is involved. Geckos often occur among human settlements, which provide additional microhabitat in the form of houses and other man-made structures. These animals tend to prefer dark, moist areas on vertical substrate, a microhabitat that is
quite abundant in residential areas. However, Blair et al. (unpublished) find that leaf-toed geckos prefer to disperse via undisturbed habitat.

The discovery of significant cryptic lineage divergence is consistent with previous studies and provides further evidence that geckos possess extremely small home-range sizes (Gübitz et al. 2000; Kaspidis et al. 2005; Rato et al. 2011). Whether these cryptic lineages warrant species status will require evidence from additional sources. Notwithstanding, the mtDNA and microsatellite evidence suggests that the population from Choquincahui should be recognized as a species.

4.2 Historical and contemporary migration rates and effective population sizes

Contrary to expectations, I find no reduction in contemporary versus historical migration rates but rather a 10-fold increase in contemporary migration rates. The Mantel test shows no significant correlation between these two metrics. These observations suggest that recent habitat fragmentation is not impacting the ability of individuals to disperse. Only a handful of studies have tested for similarities in historical and contemporary migration rates (Ross et al. 2007; Andersen et al. 2009; Schmidt et al. 2009; Muscarella et al. 2011) and few have pursued a hypothesis-testing framework (Chiucchi & Gibbs 2010). Many of these studies inappropriately compare $m$ estimated from BAYESASS with $N_m$ as estimated with MIGRATE. BAYESASS uses MCMC to estimate contemporary asymmetric migration rates ($m$) among populations, whereas MIGRATE uses MCMC in a coalescent framework to estimate both historical effective population sizes ($4N_e\mu$ for an autosomal locus) and the mutation-scaled migration rate $M$ ($m/\mu$). Migration rate and the number of migrants per generation ($N_m$) are not equivalent. The accurate comparison of results from these two methods requires
calculating $m$ from MIGRATE by multiplying $M$ times the mutation rate $\mu$. Thus, many previous studies fail to elucidate temporal differences in migration rates and how these correspond to recent landscape or environmental dynamics. The only previous study to use these methods in a strict hypothesis-testing framework has found no significant difference between historical and contemporary rates, suggesting that populations have lived in small isolated populations for a long period of time (Chiucchi & Gibbs 2010). Conversely, in leaf-toed geckos contemporary gene flow is higher than historical gene flow. A non-anthropogenic explanation for these patterns is wanting. Geckos commonly occur in and around human settlements. Relatively recent colonization of humans appears to have increased migration rates via hitchhiking. Similar studies conducted on geckos will determine if similar patterns occur.

Lower contemporary versus historical population sizes are predicted when habitat fragmentation is having negative consequences on populations. Estimates of contemporary effective population sizes are high for most populations, with 95% confidence intervals approaching infinity. However, Road to Navojoa, Arroyo Tabelo, and Mocuzari show signs of small effective population sizes irrespective of the employed allele frequency model. MIGRATE estimates large historical effective population sizes and historical population sizes for these populations are substantially higher than contemporary estimates. In these cases, recent fragmentation appears to have reduced population sizes. No evidence of recent bottlenecks is detected using the models TPM and SMM but most populations show signs of a bottleneck when assuming IAM. Although microsatellites are traditionally assumed to follow an approximate SMM model (Di Rienzo et al. 1994, Piry et al. 1999), new evidence suggests that recent bottlenecks may be better detected assuming an IAM when working with
microsatellites (Cristescu et al. 2010). Thus, all of the populations except La Sierrita have experienced a recent bottleneck. Tests for a mode-shift in allele frequency distribution yield a shift for Road to Navojoa.

4.3 Landscape genetics

Considerable recent debate has surrounded the utility of different molecular marker types and analytical methods for landscape genetic inference (Manel et al. 2003; Vandergast et al. 2007; Storfer et al. 2010; Wang 2010, 2011; Bohonak & Vandergast 2011). Most landscape genetic studies correlate effective and genetic distances, the later usually using $F_{ST}$-like metrics based on microsatellites. I probe the power of the ability of different analytical methods (MIGRATE vs. BAYESASS) and genetic marker-types (mtDNA vs. microsatellites) to determine if and how the choice of method or marker influences landscape genetic inference. MRM analysis more significantly correlate landscape with contemporary versus historical migration rates, as expected when populations respond to recent habitat fragmentation. These results would have been missed if I based my inference solely on differential historical and contemporary migration rates in an aspatial context.

Correlations between landscape and mtDNA divergence contrast sharply with an analysis based on microsatellites (Blair et al. unpublished). The stepwise MRM analysis indicates that Euclidean distance is the only significant predictor of mtDNA divergence. Although landscape genetic studies continue to rely predominantly on microsatellites (Storfer et al. 2010), a handful of studies have relied solely on mtDNA to infer contemporary ecological and evolutionary processes (e.g. Vandergast et al. 2007; Koscinski et al. 2009). The prior employment of mtDNA may owe to cost and relative ease of data collection.
However, this approach comes with costs that must be acknowledged by researchers (Wang 2010). Major issues include the stochasticity of the lineage sorting process, relatively slow mutation rates, and, most importantly, mtDNA is maternally inherited and does not document bi-parental gene flow. I obtain drastically different landscape-genetic relationships when using mtDNA versus microsatellites (Blair et al. unpublished). Similar results are found when comparing landscape-genetic relationships using different markers for wood frog populations (Lee-Yaw et al. 2009). Taken together, this begs the question as to the utility of mtDNA for inferring contemporary ecological processes. Although mtDNA may be adequate for testing simple barrier hypotheses using binary matrices (e.g. Vandergast et al. 2007), landscape-genetic relationships are often more complex (e.g. Cushman et al. 2006) and require the use of more sophisticated GIS-based modeling approaches. My analysis suggests that mtDNA is a poor choice for modeling landscape resistance based on a circuit theoretic approach. To avoid reaching erroneous conclusions regarding the influence of recent habitat fragmentation on genetic divergence, researchers should test explicit hypotheses and define the timeframe under consideration (Wang 2010).

My tests of landscape genetic inference based on different response metrics determine that contemporary migration rates differ substantially from those based on equilibrium approaches (Blair et al. unpublished). Most landscape genetic studies test for correlations between landscape variables and gene flow (Manel et al. 2003; Storfer et al. 2010), yet most have not used migration rates estimated with BAYESASS as the dependent variable. Perhaps this owes to the difficulty of Bayesian MCMC algorithms used to estimate the posterior distribution of demographic parameters (Wilson & Rannala 2003). The 95% confidence intervals are quite large and this may confound subsequent inference. Further, the number of
populations, individuals, and loci can greatly impact the inference of contemporary migration rates (Wilson & Rannala 2003). As simulation studies continue to define and direct empirical landscape genetic research (e.g. Epperson et al. 2010; Landguth et al. 2010; Blair et al. 2012), future studies should test the power of migration rates estimated with BAYESASS versus the more traditional equilibrium-based statistics such as $F_{ST}$. Additional Bayesian methods that statistically correlate recent migration with environmental variables (e.g. BIMr; Foll & Gaggiotti 2006) also show promise (Balkenhol et al. 2009).

I envision three different approaches to help disentangle the relative influences of historical and contemporary processes that shape contemporary genetic structure. First, methods that correlate genetic data with both contemporary and historical landscape and environmental data can aid in determining if and when shifts in landscape structure influenced genetic divergence (Vandergast et al. 2007; Zellmer & Knowles 2009). Second, it is possible to partition out the effects of deep genealogical history prior to conducting landscape genetic inference by incorporating residuals from regression analysis (Vandergast et al. 2007; Dyer et al. 2010). However, the use of residuals for subsequent statistical analysis is questioned due to biased parameter estimates as compared to multiple regression approaches (Freckleton 2002). Third, multiple markers and analytical methods can be used in a landscape genetic context to probe the population genetic consequences of recent habitat fragmentation. This method is particularly attractive for areas generally lacking in fine-scale geospatial data for multiple temporal periods. This approach is also useful for species that show signs of strong genealogical structure at small spatial scales that cannot be attributed to common ecological or biogeographic barriers.
4.4 Implications for Mexican biodiversity

Mexico is hypothesized to house some of Earth’s richest biodiversity (particularly reptiles) due to its geographic location intersecting the Nearctic and Neotropical realms (Devitt 2006; García 2006). Further, this diversity can be attributed to the dynamic geological and climatic histories of Mexico, which has resulted in numerous mountain ridges, river systems, and plateaus, contributing to a diverse array of habitats and ecosystems ranging from desert habitats in the north to tropical rain forest in the south and east (Cartron et al. 2005). This varied landscape, in turn, serves as an ideal region to address questions pertaining to population differentiation, landscape resistance, and barriers to gene flow. Species diversity in southern Sonora, for example, may be partly explained by drastic shifts in habitat characteristics, with the arid Sonoran Desert coming into contact with tropical thornscrub, deciduous, and pine-oak forest in the south (Robichaux & Yetman 2000; Cartron et al. 2005).

Further, orogenesis of the Sierra Madre Occidental during the Eocene/Oligocene transition has had a major impact on not only the geology and climate of western Mexico, but also on biogeographic patterns in several widespread groups (Cartron et al. 2005).

A multi-tiered analytical approach can help clarify both the historical and contemporary processes contributing to diversification throughout this ecosystem. Research is addressing phylogenetic, population genetic, and landscape genetic questions throughout Mexico and Middle America utilizing a diverse array of taxa including lizards (Blair et al., unpublished), snakes (Mulcahy 2008), birds (Zink et al. 2001; Rojas-Soto et al. 2007), frogs (Mulcahy & Mendelson 2000; Pfeiler & Markow 2008), fish (Hulsey et al. 2004; Huidobro...
et al. 2006) and mammals (Harris et al. 2000; Ortega et al. 2009). Most of these studies attribute historical patterns to the orogenesis of Mexico’s major mountain systems including the TMVB or climatic fluctuations during the Pleistocene. However, most studies have not focused specifically on patterns of genetic diversity throughout the TDF of western Mexico (but see Devitt 2006; McCormack et al. 2008; Zarza et al. 2008; Blair et al. unpublished) and, thus, we still know relatively little about the spatial and temporal processes contributing to diversification of species inhabiting this ecosystem.

4.5 Conclusions

I present a comprehensive method to understanding the population genetic consequences of recent habitat fragmentation for leaf-toed geckos inhabiting Mexican TDF. My results suggest that migration rates have increased in the recent past, but that geckos are altering their dispersal routes in response to recent landscape change. I find evidence for recent reductions in effective population sizes, likely due to continual anthropogenic infringement, and a deep genealogical history over small spatial scales, which reflects low historical gene flow and strong female philopatry. My analyses suggest that mtDNA is a poor marker for inferring contemporary landscape-genetic relationships due to its low mutation rate, error associated with the coalescent process, and matrilineal inheritance. Additional simulation studies are needed to test the power of different measures of genetic connectivity ($F_{ST}$ vs BAYESASS) when inferring landscape genetic relationships.

Interestingly, a high level of mtDNA variation occurs on a very small geographic scale. Generally, DNA sequences lack the variation necessary to make demographic inferences on
such scales. Markers such as microsatellites or AFLPs are usually used in order to document such genetic patterning (Storfer et al. 2010). However, my analysis suggests that the examination of mtDNA sequence data provides insight into deeper historical divergences at the population genetic level, in contrast to markers such as microsatellites that are more suitable for detecting more recent genetic patterns. Thus, studies should use mtDNA data in addition to, rather in lieu of, other markers such as microsatellites and SNPs to understand the spatial and temporal dynamics of population connectivity. This may be of particular relevance to species likely to possess limited dispersal abilities.
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**Table 1.** Nucleotide diversity statistics and their variances for all populations sampled for mtDNA.

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<td>16.762</td>
<td>72.519</td>
<td>34</td>
<td>2.782</td>
</tr>
<tr>
<td>Los Camotes</td>
<td>4</td>
<td>4</td>
<td>1</td>
<td>0.0313</td>
<td>0.0081</td>
<td>17.667</td>
<td>100.111</td>
<td>35</td>
<td>0.978</td>
</tr>
<tr>
<td>Road to Navojoa 2</td>
<td>10</td>
<td>7</td>
<td>0.911</td>
<td>0.006</td>
<td>0.0032</td>
<td>5.533</td>
<td>8.442</td>
<td>15</td>
<td>-0.496</td>
</tr>
<tr>
<td>Arroyo Tabelo</td>
<td>14</td>
<td>7</td>
<td>0.879</td>
<td>0.0033</td>
<td>0.0075</td>
<td>11.209</td>
<td>29.352</td>
<td>41</td>
<td>3.106</td>
</tr>
<tr>
<td>Aduana</td>
<td>4</td>
<td>3</td>
<td>0.833</td>
<td>0.0495</td>
<td>0.0034</td>
<td>4.833</td>
<td>8.861</td>
<td>9</td>
<td>1.453</td>
</tr>
</tbody>
</table>

n = number of individuals; H = number of haplotypes; h = haplotype diversity; V(h) = variance in haplotype diversity; π = nucleotide diversity; k = average number of nucleotide differences; V(k) = variance in average number of nucleotide differences; S = number of segregating sites. Fu's Fs calculated from the total number of mutations. All Fu's Fs non-significant with an alpha=0.05.
Table 2. DNA divergence between populations. Numbers above diagonal are the average number of nucleotide differences and numbers below correspond to the average number of substitutions per site ($D_{xy}$).

<table>
<thead>
<tr>
<th>Population</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
<th>G</th>
<th>H</th>
<th>I</th>
</tr>
</thead>
<tbody>
<tr>
<td>B. Rio Cuchujaqui</td>
<td>0.0214</td>
<td>-</td>
<td>31.5000</td>
<td>72.3700</td>
<td>7.6570</td>
<td>22.5750</td>
<td>27.5400</td>
<td>26.2710</td>
<td>30.9250</td>
</tr>
<tr>
<td>C. Road to Navojoa 1</td>
<td>0.0061</td>
<td>0.0244</td>
<td>-</td>
<td>135.1000</td>
<td>11.7140</td>
<td>51.2500</td>
<td>5.4500</td>
<td>9.0000</td>
<td>4.0000</td>
</tr>
<tr>
<td>D. Choquincahui</td>
<td>0.0594</td>
<td>0.0560</td>
<td>0.0587</td>
<td>-</td>
<td>70.5430</td>
<td>125.3000</td>
<td>106.5000</td>
<td>83.1570</td>
<td>119.1500</td>
</tr>
<tr>
<td>E. La Sierrita</td>
<td>0.0122</td>
<td>0.0145</td>
<td>0.0103</td>
<td>0.0619</td>
<td>-</td>
<td>20.7500</td>
<td>11.5140</td>
<td>8.6940</td>
<td>12.1430</td>
</tr>
<tr>
<td>F. Los Camotes</td>
<td>0.0230</td>
<td>0.0184</td>
<td>0.0235</td>
<td>0.0573</td>
<td>0.0182</td>
<td>-</td>
<td>41.7000</td>
<td>34.3210</td>
<td>44.7500</td>
</tr>
<tr>
<td>G. Road to Navojoa 2</td>
<td>0.0070</td>
<td>0.0245</td>
<td>0.0031</td>
<td>0.0614</td>
<td>0.0106</td>
<td>0.0240</td>
<td>-</td>
<td>7.5290</td>
<td>4.7500</td>
</tr>
<tr>
<td>H. Arroyo Tabelo</td>
<td>0.0096</td>
<td>0.0229</td>
<td>0.0060</td>
<td>0.0556</td>
<td>0.0122</td>
<td>0.0247</td>
<td>0.0058</td>
<td>-</td>
<td>8.3570</td>
</tr>
<tr>
<td>I. Aduana</td>
<td>0.0063</td>
<td>0.0239</td>
<td>0.0002</td>
<td>0.0602</td>
<td>0.0107</td>
<td>0.0242</td>
<td>0.0027</td>
<td>0.0056</td>
<td>-</td>
</tr>
</tbody>
</table>
Table 3. Estimates of contemporary migration rates between populations. Values in parentheses represent 95% confidence intervals. Bold values on diagonal represent the proportion of individuals derived from the source population each generation.

<table>
<thead>
<tr>
<th>Locality</th>
<th>1 Road to Navojoa</th>
<th>2 Alamos</th>
<th>3 Tabelo</th>
<th>4 Aduana</th>
<th>5 Cuchujaqui</th>
<th>6 Sierrita</th>
<th>7 Mocuzari</th>
<th>8 El Quintero</th>
<th>9 Choquincahui</th>
<th>10 San Antonio</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Road to Navojoa</td>
<td>0.685</td>
<td>0.018</td>
<td>0.020</td>
<td>0.155</td>
<td>0.031</td>
<td>0.021</td>
<td>0.018</td>
<td>0.018</td>
<td>0.017</td>
<td>0.017</td>
</tr>
<tr>
<td></td>
<td>(0.651–0.719)</td>
<td>(-0.015–0.051)</td>
<td>(-0.016–0.056)</td>
<td>(0.080–0.229)</td>
<td>(-0.021–0.083)</td>
<td>(-0.017–0.059)</td>
<td>(-0.015–0.052)</td>
<td>(-0.015–0.050)</td>
<td>(-0.015–0.049)</td>
<td>(-0.014–0.049)</td>
</tr>
<tr>
<td>2 Alamos</td>
<td>0.007</td>
<td>0.834</td>
<td>0.030</td>
<td>0.011</td>
<td>0.059</td>
<td>0.018</td>
<td>0.017</td>
<td>0.010</td>
<td>0.007</td>
<td>0.007</td>
</tr>
<tr>
<td></td>
<td>(-0.007–0.021)</td>
<td>(-0.766–0.902)</td>
<td>(-0.001–0.061)</td>
<td>(0.001–0.117)</td>
<td>(-0.013–0.048)</td>
<td>(-0.011–0.044)</td>
<td>(-0.008–0.028)</td>
<td>(-0.007–0.021)</td>
<td>(-0.007–0.022)</td>
<td></td>
</tr>
<tr>
<td>3 Tabelo</td>
<td>0.005</td>
<td>0.007</td>
<td>0.937</td>
<td>0.006</td>
<td>0.011</td>
<td>0.005</td>
<td>0.014</td>
<td>0.005</td>
<td>0.005</td>
<td>0.005</td>
</tr>
<tr>
<td></td>
<td>(-0.005–0.015)</td>
<td>(-0.006–0.020)</td>
<td>(0.900–0.974)</td>
<td>(-0.005–0.017)</td>
<td>(-0.005–0.027)</td>
<td>(-0.005–0.015)</td>
<td>(-0.011–0.039)</td>
<td>(-0.005–0.015)</td>
<td>(-0.005–0.015)</td>
<td></td>
</tr>
<tr>
<td>4 Aduana</td>
<td>0.012</td>
<td>0.028</td>
<td>0.020</td>
<td>0.824</td>
<td>0.022</td>
<td>0.031</td>
<td>0.026</td>
<td>0.013</td>
<td>0.012</td>
<td>0.012</td>
</tr>
<tr>
<td></td>
<td>(-0.011–0.035)</td>
<td>(-0.020–0.077)</td>
<td>(-0.016–0.056)</td>
<td>(0.731–0.916)</td>
<td>(-0.018–0.061)</td>
<td>(-0.020–0.082)</td>
<td>(-0.014–0.065)</td>
<td>(-0.012–0.038)</td>
<td>(-0.011–0.035)</td>
<td>(-0.011–0.035)</td>
</tr>
<tr>
<td>5 Cuchujaqui</td>
<td>0.004</td>
<td>0.007</td>
<td>0.006</td>
<td>0.009</td>
<td>0.940</td>
<td>0.007</td>
<td>0.006</td>
<td>0.012</td>
<td>0.004</td>
<td>0.004</td>
</tr>
<tr>
<td></td>
<td>(-0.004–0.012)</td>
<td>(-0.006–0.021)</td>
<td>(-0.005–0.017)</td>
<td>(0.907–0.973)</td>
<td>(-0.006–0.020)</td>
<td>(-0.005–0.016)</td>
<td>(-0.003–0.027)</td>
<td>(-0.004–0.012)</td>
<td>(-0.004–0.013)</td>
<td></td>
</tr>
<tr>
<td>6 Sierrita</td>
<td>0.007</td>
<td>0.010</td>
<td>0.010</td>
<td>0.021</td>
<td>0.023</td>
<td>0.891</td>
<td>0.018</td>
<td>0.007</td>
<td>0.007</td>
<td>0.007</td>
</tr>
<tr>
<td></td>
<td>(-0.006–0.020)</td>
<td>(-0.009–0.029)</td>
<td>(-0.009–0.029)</td>
<td>(0.813–0.854)</td>
<td>(-0.010–0.055)</td>
<td>(0.841–0.941)</td>
<td>(-0.009–0.046)</td>
<td>(-0.006–0.020)</td>
<td>(-0.006–0.021)</td>
<td></td>
</tr>
<tr>
<td>7 Mocuzari</td>
<td>0.008</td>
<td>0.013</td>
<td>0.021</td>
<td>0.010</td>
<td>0.011</td>
<td>0.011</td>
<td>0.898</td>
<td>0.012</td>
<td>0.008</td>
<td>0.008</td>
</tr>
<tr>
<td></td>
<td>(-0.007–0.023)</td>
<td>(-0.010–0.035)</td>
<td>(-0.011–0.054)</td>
<td>(-0.009–0.030)</td>
<td>(-0.009–0.031)</td>
<td>(-0.009–0.031)</td>
<td>(0.848–0.949)</td>
<td>(-0.009–0.032)</td>
<td>(-0.007–0.023)</td>
<td>(-0.007–0.023)</td>
</tr>
<tr>
<td>8 El Quintero</td>
<td>0.008</td>
<td>0.009</td>
<td>0.008</td>
<td>0.009</td>
<td>0.014</td>
<td>0.009</td>
<td>0.008</td>
<td>0.917</td>
<td>0.008</td>
<td>0.008</td>
</tr>
<tr>
<td></td>
<td>(-0.008–0.024)</td>
<td>(-0.008–0.027)</td>
<td>(-0.008–0.024)</td>
<td>(-0.008–0.027)</td>
<td>(-0.010–0.037)</td>
<td>(-0.008–0.026)</td>
<td>(-0.008–0.025)</td>
<td>(-0.873–0.961)</td>
<td>(-0.007–0.024)</td>
<td>(-0.008–0.024)</td>
</tr>
<tr>
<td>9 Choquincahui</td>
<td>0.008</td>
<td>0.008</td>
<td>0.010</td>
<td>0.008</td>
<td>0.009</td>
<td>0.009</td>
<td>0.008</td>
<td>0.675</td>
<td>0.009</td>
<td>0.009</td>
</tr>
<tr>
<td></td>
<td>(-0.008–0.024)</td>
<td>(-0.008–0.025)</td>
<td>(-0.009–0.029)</td>
<td>(-0.008–0.024)</td>
<td>(-0.008–0.025)</td>
<td>(-0.008–0.025)</td>
<td>(-0.008–0.024)</td>
<td>(-0.208–0.303)</td>
<td>(-0.659–0.692)</td>
<td>(-0.008–0.026)</td>
</tr>
<tr>
<td>10 San Antonio</td>
<td>0.014</td>
<td>0.095</td>
<td>0.034</td>
<td>0.061</td>
<td>0.043</td>
<td>0.049</td>
<td>0.014</td>
<td>0.014</td>
<td>0.684</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(-0.012–0.039)</td>
<td>(-0.013–0.177)</td>
<td>(-0.012–0.039)</td>
<td>(-0.033–0.155)</td>
<td>(-0.012–0.098)</td>
<td>(-0.009–0.106)</td>
<td>(-0.012–0.040)</td>
<td>(-0.012–0.040)</td>
<td>(0.649–0.718)</td>
<td>(-0.012–0.039)</td>
</tr>
</tbody>
</table>
Table 4. Estimates of contemporary and historical effective population sizes ($N_e$) and 95% confidence intervals. Results in first three columns represent $N_e$ estimates based on the linkage disequilibrium method employed in the software LDNe. Results are reported based on lowest allele frequency values used (0.05, 0.02, 0.01). Estimates in last column represent historical $N_e$ estimates using the coalescent model in MIGRATE and calculated assuming a mutation rate of $5 \times 10^{-4}$ (Garza & Williamson 2001).

<table>
<thead>
<tr>
<th>Locality</th>
<th>$n$</th>
<th>0.05</th>
<th>0.02</th>
<th>0.01</th>
<th>MIGRATE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Road to Navojoa 1</td>
<td>10</td>
<td>25.4 (15.5–56.0)</td>
<td>25.4 (15.5–56.0)</td>
<td>25.4 (15.5–56.0)</td>
<td>483.355 (0–1333)</td>
</tr>
<tr>
<td>Alamos</td>
<td>36</td>
<td>172.4 (65.2–infinity)</td>
<td>464.1 (104.8–infinity)</td>
<td>362.9 (106.5–infinity)</td>
<td>749.5 (0–1566)</td>
</tr>
<tr>
<td>Arroyo Tabelo</td>
<td>57</td>
<td>26.8 (20.7–35.5)</td>
<td>36.4 (28.1–48.7)</td>
<td>43.7 (33.5–59.6)</td>
<td>703.34 (0–1500)</td>
</tr>
<tr>
<td>Aduana</td>
<td>17</td>
<td>132.5 (33.6–infinity)</td>
<td>-331 (74.1–infinity)</td>
<td>-331 (74.1–infinity)</td>
<td>501.685 (0–1333)</td>
</tr>
<tr>
<td>Rio Cuchujaqui</td>
<td>72</td>
<td>-3903.1 (308.0–infinity)</td>
<td>636.7 (225.3–infinity)</td>
<td>654.9 (226.4–infinity)</td>
<td>1456.435 (466–2400)</td>
</tr>
<tr>
<td>Sierrita</td>
<td>38</td>
<td>148.2 (57.1–infinity)</td>
<td>142.9 (65.2–infinity)</td>
<td>239.7 (91.6–infinity)</td>
<td>668.235 (0–1500)</td>
</tr>
<tr>
<td>Mocuzari</td>
<td>30</td>
<td>28.8 (19.6–47.3)</td>
<td>29.2 (21.0–43.9)</td>
<td>38.4 (26.7–62.1)</td>
<td>272.6 (0–1066)</td>
</tr>
<tr>
<td>El Quintero</td>
<td>30</td>
<td>442.8 (62.5–infinity)</td>
<td>-865.0 (110.9–infinity)</td>
<td>584.8 (90.6–infinity)</td>
<td>432.8 (0–1266)</td>
</tr>
<tr>
<td>Choquincahui</td>
<td>31</td>
<td>1093.3 (57.4–infinity)</td>
<td>121.3 (43.9–infinity)</td>
<td>95.6 (41.7–infinity)</td>
<td>296.295 (0–1100)</td>
</tr>
<tr>
<td>San Antonio</td>
<td>15</td>
<td>38.8 (15.3–infinity)</td>
<td>224.4 (45.2–infinity)</td>
<td>224.4 (45.2–infinity)</td>
<td>323.865 (0–1133)</td>
</tr>
</tbody>
</table>
Table 5. Results of tests for population bottlenecks performed using the software BOTTLENECK. Numbers represent P-values based on the corresponding mutation model. Allelic distribution represents the test for a shift in the relative abundance of alleles at different frequencies.

<table>
<thead>
<tr>
<th>Locality</th>
<th>n</th>
<th>Wilcoxon TPM</th>
<th>Wilcoxon SMM</th>
<th>Wilcoxon IAM</th>
<th>Allelic Distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Road to Navojoa 1</td>
<td>10</td>
<td>0.539</td>
<td>0.652</td>
<td>0.042</td>
<td>Shifted</td>
</tr>
<tr>
<td>Alamos</td>
<td>36</td>
<td>0.216</td>
<td>0.348</td>
<td>0.001</td>
<td>Normal</td>
</tr>
<tr>
<td>Arroyo Tabelo</td>
<td>57</td>
<td>0.385</td>
<td>0.722</td>
<td>0.001</td>
<td>Normal</td>
</tr>
<tr>
<td>Aduana</td>
<td>17</td>
<td>0.385</td>
<td>0.577</td>
<td>0.001</td>
<td>Normal</td>
</tr>
<tr>
<td>Rio Cuchujaqui</td>
<td>72</td>
<td>0.116</td>
<td>0.216</td>
<td>0.001</td>
<td>Normal</td>
</tr>
<tr>
<td>La Sierrita</td>
<td>38</td>
<td>0.935</td>
<td>0.998</td>
<td>0.065</td>
<td>Normal</td>
</tr>
<tr>
<td>Mocuzari</td>
<td>30</td>
<td>0.688</td>
<td>0.884</td>
<td>0.001</td>
<td>Normal</td>
</tr>
<tr>
<td>El Quintero</td>
<td>30</td>
<td>0.813</td>
<td>0.935</td>
<td>0.005</td>
<td>Normal</td>
</tr>
<tr>
<td>Choquincahui</td>
<td>31</td>
<td>0.991</td>
<td>0.998</td>
<td>0.001</td>
<td>Normal</td>
</tr>
<tr>
<td>San Antonio</td>
<td>15</td>
<td>0.652</td>
<td>0.784</td>
<td>0.002</td>
<td>Normal</td>
</tr>
</tbody>
</table>

TPM = two-phase model; SMM = stepwise mutation model; IAM = infinite allele model
Table 6. Regression on distance matrix results showing the relationships between landscape resistance and historical migration, contemporary migration, and mtDNA sequence divergence ($D_{xy}$). Model M = MIGRATE; Model B = BAYESASS; Model MtDNA = mtDNA divergence. Models in italics significant at an alpha = 0.05.

<table>
<thead>
<tr>
<th>Model</th>
<th>Variables</th>
<th>β</th>
<th>Model $R^2$</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>M1</td>
<td>Euclidean</td>
<td>-8.2E-09</td>
<td>0.0337</td>
<td>0.231</td>
</tr>
<tr>
<td>M2</td>
<td>Temp</td>
<td>-1.3E-05</td>
<td>0.0742</td>
<td>0.0795</td>
</tr>
<tr>
<td>M3</td>
<td>Forest</td>
<td>-2.3E-05</td>
<td>0.0514</td>
<td>0.1447</td>
</tr>
<tr>
<td>M4</td>
<td>Stream</td>
<td>-4E-05</td>
<td>0.0497</td>
<td>0.1578</td>
</tr>
<tr>
<td>M5</td>
<td>Anthro</td>
<td>-3.1E-06</td>
<td>0.0073</td>
<td>0.6023</td>
</tr>
<tr>
<td>M6</td>
<td>Slope</td>
<td>-1.3E-05</td>
<td>0.1145</td>
<td>0.0217</td>
</tr>
<tr>
<td>B1</td>
<td>Euclidean</td>
<td>-8.1E-07</td>
<td>0.1994</td>
<td>0.0016</td>
</tr>
<tr>
<td>B2</td>
<td>Temp</td>
<td>-0.0005</td>
<td>0.0587</td>
<td>0.0656</td>
</tr>
<tr>
<td>B3</td>
<td>Forest</td>
<td>-0.0017</td>
<td>0.1768</td>
<td>0.0077</td>
</tr>
<tr>
<td>B4</td>
<td>Stream</td>
<td>-0.0027</td>
<td>0.1342</td>
<td>0.008</td>
</tr>
<tr>
<td>B5</td>
<td>Anthro</td>
<td>-0.0006</td>
<td>0.1703</td>
<td>0.002</td>
</tr>
<tr>
<td>B6</td>
<td>Slope</td>
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Figure Legends

**Fig. 1.** Map of the Alamos region of Sonora showing sampling sites for leaf-toed geckos, *Phyllodactylus*, included in the study. Site names in italics represent sites where mtDNA sequences were obtained.

**Fig. 2.** A) Maximum parsimony (MP) strict consensus tree of eight most parsimonious trees based on the concatenated mtDNA data for leaf-toed geckos, *Phyllodactylus*. Numbers above nodes represent 10,000 nonparametric bootstrap proportions. Numbers below nodes represent Bayesian posterior probabilities sampled from the posterior distribution of trees. B) Bayesian majority rule phylogram resulting from the optimal partitioning strategy of gene + codon position.

**Fig. 3.** Geographic distribution of major mitochondrial lineages for leaf-toed geckos, *Phyllodactylus*, from throughout the Alamos region. Areas of sympatry are represented by localities encircled by more than one color.

**Fig. 4.** Scatter plot with Reduced Major Axis (RMA) regression line illustrating isolation by distance for leaf-toed geckos, *Phyllodactylus*, resulting from a Mantel test of 30 000 randomizations. PhiST/(1-PhiST) values used to calculate test statistic were based on the uncorrected *p*-distances between haplotypes. Geographic distances shown in kilometers.
Fig. 5. A) Frequency histogram of historical migration rates for leaf-toed geckos, *Phylodactylus*, estimated from MIGRATE. B) Frequency histogram of contemporary migration rates estimated from BAYESASS.
Fig. 3

Lineage A

Lineage B

Lineage C

Lineage D

0.6

[Map showing various locations and lineages]

[206]
Fig. 4
**Fig. 5**

**Histogram of Migrate**

A

**Histogram of BayesAss**

B
Appendix 1 Population and outgroup sampling with corresponding GenBank accession numbers for all *Phyllodactylus* sequenced for mtDNA. Population ID for each population follows that presented in all population genetic analyses. N/A = Not applicable.

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<td>27°02'10.0&quot;N 109°00'46.1&quot;W</td>
<td>ROM 41120</td>
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<td>27°01'57.6&quot;N 108°47'04.9&quot;W</td>
<td>ROM 41135</td>
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<td>Mexico Sonora ca. 55 km NE Alamos Cemetery by lower road to Míjillas, large boulders near stream</td>
<td>27°14'44.1&quot;N 108°45'26.3&quot;W</td>
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<td>Mexico Sonora ca. 55 km NE Alamos Cemetery by lower road to Míjillas, large boulders near stream</td>
<td>27°14'44.1&quot;N 108°45'26.3&quot;W</td>
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<td>27°58'05&quot;N 111°29'04&quot;W</td>
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<td>23°44'09&quot;N 109°51'12&quot;W</td>
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**Supplementary Table 1.** Gene, number of sequenced bases, primer name and sequence, and reference for all mtDNA loci used.

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<th>Base Pairs</th>
<th>Primer</th>
<th>Primer Sequence</th>
<th>Source</th>
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<td>400</td>
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<td>5’–TGA TTC TTC GGT CAC CCA GAA GTG TA–3’</td>
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<td></td>
<td></td>
<td>COI_m73H</td>
<td>5’– CCT ATT GAT AGG ACG TAG TGG AAG TG–3’</td>
<td>Austin et al. 2004</td>
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<tr>
<td>ND4</td>
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<td>5’–CCT ATC CCC CAC AAC CCA AAC–3’</td>
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<tr>
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<td>ND4_13824H</td>
<td>5’–CAT TAC TTT TAC TTG GAT TTG CAC CA–3’</td>
<td>Arevalo et al. 1994</td>
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<td>ND1, 16S,</td>
<td>1300</td>
<td>ND1_16dR</td>
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<td>ND1_tMet</td>
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<td>ND1_phyR</td>
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**Supplementary Table 2.** Total and number of potentially phylogenetically informative characters for each data partition along with the chosen model of nucleotide substitution based on AIC.

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<th>Characters</th>
<th>Phylogenetically Informative</th>
<th>Model</th>
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<td>SYM+Γ</td>
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<td>6</td>
<td>HKY+I</td>
</tr>
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**Supplementary Table 3.** Alternate partitioning strategies tested using Bayes Factors.

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<th>Harmonic Mean</th>
<th>2ln Bayes factor</th>
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<tr>
<td>P3</td>
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<td>-7084.85</td>
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Bayes factors were calculated by comparing the harmonic means of different partitioning strategies. P3 served as the null hypothesis.
### Supplementary Table 4.

*MIGRATE* estimates of historical asymmetric migration rates between populations. Locus 'All' signifies that all 10 microsatellite loci were used for calculations. $\Theta = 4N_e \mu$, $M = m/\mu$, $N_m =$ number of migrants per generation ($\Theta M/4$). 1 = Road to Navojoa, 2 = Alamos, 3 = Arroyo Tabelo, 4 = Aduana, 5 = Rio Cuchujaqui, 6 = La Sierrita, 7 = Mocuzari, 8 = El Quintero, 9 = Choquincahui, 10 = San Antonio.

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<th>Parameter</th>
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<th>25.00%</th>
<th>mode</th>
<th>75%</th>
<th>97.50%</th>
<th>median</th>
<th>mean</th>
<th>$4N_m$</th>
<th>$N_m$</th>
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**Supplementary Table 5.** Multiple regression on distance matrices (MRM) results showing the relationship between contemporary migration rates (estimates using BAYESASS) and resistance distances incorporating landscape heterogeneity. Optimal cost values used to parameterize resistance surfaces prior to calculating resistance distances were selected based on Mantel $r$ correlation coefficients. VIF = Variance Inflation Factor.

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CHAPTER 6

Molecular phylogenetics and taxonomy of leaf-toed geckos
(Phyllodactylidae: *Phyllodactylus*) inhabiting the peninsula of
Baja California

Abstract

Herein I assess the phylogenetic relationships and taxonomy of geckos of the genus
*Phyllodactylus* inhabiting the peninsula of Baja California, Mexico using five mitochondrial
and two nuclear genes. Phylogenetic analysis using maximum parsimony (MP) and Bayesian
inference (BI) recovered three distinct peninsular clades with high statistical support.
Sequence divergence estimates between peninsular taxa approached 13%. Two of the
species, *P. unctus* and *P. xanti* are Cape Region endemics, whereas *P. nocticolus* is
widespread throughout much of the peninsula and extreme southern California. Monophyly
of the peninsular taxa was strongly supported. In the MP analysis, *P. unctus* rooted at the
base of the peninsular clade, resolving *P. xanti* and *P. nocticolus* as sister taxa. Conversely,
BI placed *P. nocticolus* and *P. unctus* as sister taxa. These data provide further evidence for a
trans-peninsular seaway near the Isthmus of La Paz, severing the Cape Region from the rest
of the peninsula. The analysis also supports the validity of *P. nocticolus* as a distinct species
and suggests a single invasion to the peninsula from mainland Mexico, presumably during
tectonic activity during the Miocene.
1 Introduction

The complex geological history of Baja California and the Gulf of California has contributed significantly to the evolution of their regional biota. One hypothesis on the formation of the peninsula and the origin of its flora and fauna suggests a trans-gulfian vicariance model. This posits that tectonic activity during the Miocene led to rifting partitions of western Mexico off the North American Plate (Murphy 1983a,b; Hausback 1984; Lonsdale 1989; Riddle et al. 2000). This biogeographic model, in part, explains the patterns of evolutionary relationships observed between several taxa distributed on opposite sides of the Gulf of California (Murphy 1983b).

The dynamic paleogeographic history of the peninsula has, in turn, led to numerous taxonomic and phylogenetic studies conducted throughout Baja California, islands in the Gulf of California, and mainland northwestern Mexico. Several of these studies evaluated genetic breaks in mitochondrial DNA (mtDNA) lineages that coincide with trans-peninsular seaways, one mid-way on the peninsula and one across the Isthmus of La Paz, just north of the Cape Region (Upton & Murphy 1997; Riddle et al. 2000; Lindell et al. 2005, 2006, 2008).

The entire peninsula of Baja California is home to three endemic species of leaf-toed geckos: *Phylodactylus unctus*, *P. xanti* and *P. nocticola*. The latter species has been considered by some authorities to be a subspecies of *P. xanti* based on morphological data (Dixon 1964, 1966; Grismer 2002). Analyses, based on morphology and allozymes suggested that *P. nocticola* warrants full species status (Murphy 1983b). Disjunct populations of these and other species of *Phylodactylus* also occur on islands of the Gulf of California (Dixon 1966; Murphy & Aguirre León 2002). These populations and species are not reported from
mainland Mexico and, thus, it is suggested that island colonization resulted from over-water dispersal from the peninsula of Baja California (Savage 1960, Murphy 1983a,b), translocation by Seri Indians (Murphy & Aguirre León 2002) and/or paleotectonic activity (Murphy 1983a; Lonsdale 1989; Carreño & Helenes 2002).

The taxonomy of *Phyllodactylus* remains controversial, mainly due to significant morphological variation within species (Dixon 1964). Several subspecies of *Phyllodactylus* were erected to assist in the classification of intraspecific morphological variability (Dixon 1964, 1966). However, many insular subspecies distributed throughout the Gulf of California were synonymized with their mainland counterparts due to the lack of diagnosable characters differentiating them from the latter (Grismer 1999). Molecular data for *Phyllodactylus* are also generally lacking, making phylogenetic relationships and taxonomic designations difficult to ascertain.

There are two main objectives of the present study. First, I provide further molecular data indicating the validity of *P. nocticolus* and second, I determine the phylogenetic relationships of peninsular and representative mainland forms and compare the results with previously published data to infer and discuss concordant biogeographic patterns throughout the peninsula of Baja California.

2 Materials and Methods

2.1 Tissue collection

Specimens and tissues were collected from populations of each species throughout the peninsula of Baja California (Table 1; Fig. 1). Taxon identification follows Murphy (1983b).
Fieldwork was conducted in 1978, 1985, and 2000. Geographic coordinates were obtained for the latter year only, although additional geographic coordinates were obtained using Google Earth. Voucher specimens were deposited in collections housed at the National Autonomous University of Mexico (UNAM), California Academy of Sciences (CAS), Los Angeles County Museum (LACM) and the Royal Ontario Museum (ROM). Additional sequences were obtained from *P. lanei, P. paucituberculatus, P. duellmani, and P. davisi* from mainland Mexico as primary outgroup taxa. GenBank sequences were also obtained for *Gekko gecko* (AY282753) to root all networks.

2.2 DNA extraction, amplification, and sequencing

Total genomic DNA was digested and extracted from liver or muscle tissue using standard phenol–chloroform protocols (Sambrook *et al.* 1989; Hillis *et al.* 1996; Palumbi 1996). Tissue samples were first mixed with Proteinase K and Laird’s buffer and digested overnight prior to extraction with phenol–chloroform. Following extraction, samples were re-suspended in ddH$_2$O prior to amplification.

Approximately 4300 bp encompassing two nuclear genes and five mitochondrial genes were amplified using the polymerase chain reaction (PCR; Saiki *et al.* 1988). Nuclear genes amplified included brain-derived neurotrophic factor (BDNF, 670 bp) and proto-oncogene C-mos (380 bp). Mitochondrial loci included cytochrome c oxidase subunit I (COI, 420 bp), and NADH dehydrogenase subunit 4 (ND4, 690 bp). In addition, two ribosomal RNA (rRNA) fragments and the intervening transfer RNA tValine (tRNA$^{Val}$) were amplified, encompassing 1600 bp of 16S rRNA and 900 bp of 12S rRNA. Primers used for
amplification and sequencing, their corresponding sequences and references were presented in Appendix I.

PCR reactions (25 μl) were composed of the following reaction mix: 18.55 μl ddH₂O, 1 μl 10 mM each primer, 2.5 μl 1.5 mM MgCl₂ buffer, 0.8 μl 10mM dNTPs, 0.15 μl 5 U Taq DNA Polymerase (Boehringer Mannheim), and 1 μl template DNA. Amplification was performed on a Perkin Elmer GeneAmp 9700 (Applied Biosystems) or a PT200 DNA Engine (MJ Research) thermal cycler. Amplification conditions were as follows: initial denaturation of 94 °C (2 min) followed by 39 cycles of 94 °C (30 sec), 49-50 °C (45 sec), 72 °C (45 sec), with a final extension temperature of 72 °C for 6 min (Lindell et al. 2005). Following amplification, DNA products were separated on a 1% agarose gel stained with ethidium bromide under ultraviolet (UV) light. Visible bands were then excised from the gel and centrifuged through a filter pipette tip for 10 min. The resulting solution was used as the template for sequencing reactions.

Sequencing reactions (10 μl) were performed on an Eppendorf AG 5345 thermal cycler with the BigDye Terminator v 3.1 Cycle Sequencing Kit (Applied Biosystems) using the following recipe: 1 μl BigDye, 2 μl 5x BigDye Terminator Buffer, 2 μl ddH₂O, 1 μl of 10 mM primer solution, and 4 μl PCR product. Reactions were run for 25 cycles with the following conditions: initial denaturation at 96 °C (1 min), 96 °C (10 sec), 50 °C (5 sec), 60 °C (4 min), and 4 °C indefinitely. All gene regions were sequenced in both forward and reverse directions. Following sequencing reactions, samples were cleaned and precipitated with sodium acetate and ethanol and visualized on an ABI 3100 Automated Sequencer (Applied Biosystems).
2.3 Sequence alignment and phylogenetic analysis

Forward and reverse sequences were initially imported and edited in BioEdit v.5.0.6 (Hall 1999) and subsequently combined for manual alignment. Aligned sequences were then imported into MacClade v.4.08 (Maddison & Maddison 2005) and checked by eye. The final MacClade alignment was then exported into PAUP* v.4.0b10 (Swofford 2002) for phylogenetic inference.

Maximum parsimony analysis (MP) was performed on the concatenated dataset using PAUP* v.4.0b10 (Swofford 2002). Uninformative characters (3373) were excluded from the analysis. All characters were treated as unordered with equal weighting. Gaps were treated as missing data. Because of the relatively small dataset (24 specimens), I used a branch-and-bound search strategy with furthest addition sequences. Branches were collapsed if the maximum branch length equaled zero. Support for nodes was assessed via 10,000 nonparametric bootstrap replicates using “fast” stepwise additions and retaining groups with >50% support (Felsenstein, 1985).

Bayesian inference (BI) was also used to infer the phylogenetic relationships among Baja Californian Phyllodactylus. To better account for heterogeneous rate variation across sites both within and between regions, I employed a partitioned analysis for each gene (Ronquist & Huelsenbeck 2003; Nylander et al. 2004; Brandley et al. 2005). Theoretical and empirical evidence suggests several benefits to employing a partitioned Bayesian analysis to explain different subsets of data (gene, codon position, etc.) including higher –lnL scores and clade credibility values (i.e. posterior probabilities). Combining different genes and/or codon positions into one evolutionary model ignores the fact that different partitions are presumably
evolving at very different rates, which will in turn increase the systematic error involved in model fitting (Brandley et al. 2005).

MrModeltest v.2.2 (Nylander 2004) was used to obtain evolutionary models for each gene partition using the Akaike Information Criterion (AIC; Akaike 1974, 1979). The AIC was chosen over hierarchical likelihood ratio tests (hLRTs) for model selection because the former tends to penalize more over-parameterized models more strongly than the latter and, thus, minimizes the degree of variance involved in model fitting (Akaike 1974, 1979). Bayesian inference was performed using MrBayes v.3.1 (Huelsenbeck & Ronquist 2001). Two simultaneous runs of six chains were run for $3 \times 10^6$ generations, sampling every 100 generations. Stationarity was assessed when likelihood scores reached a stable equilibrium. A burn-in value of 7500 was then implemented to discard topologies with low likelihood scores prior to generating a 50% majority rule consensus tree. Node support within the Bayesian consensus tree was assessed from the posterior distribution of topologies (Erixon et al. 2003).

In order to assess potentially conflicting phylogenetic signals from nuclear and mtDNA, I performed MP and BI on each dataset independently. For both MP analyses, a heuristic search was run with 100 replicate additions and tree bisection-reconnection (TBR) branch swapping. Nodal support for each MP analysis was also assessed by nonparametric bootstrapping with 10,000 “fast stepwise addition replicates (Felsenstein 1985). BI was performed on each dataset independently under the same conditions as the concatenated dataset described above. For all nuclear data, Gekko gecko, L12, and L103 were removed prior to phylogenetic analysis due to missing data. Trees were thus rooted using P. lanei.
3 Results

3.1 Genetic diversity

For the concatenated dataset, 915 characters were potentially phylogenetically informative. Variability within nDNA was low with only eight characters potentially phylogenetically informative. There was a significant difference in average base frequencies ($\chi^2 = 164.80; df = 69; P < 0.001$) with an adenine/cytosine bias. Percent sequence divergence based on uncorrected $p$-distances revealed significant differentiation between major groups (Table 2). Further, divergence estimates between $P$. nocticolus and $P$. xanti approached 10%, clearly indicating the mtDNA distinctiveness of both species. MrModeltest selected the GTR+I+G model of nucleotide evolution for the COI, ND4, 12S and 16S partitions, the HKY model for C-mos and BDNF and GTR+G for tRNA$^{val}$.

3.2 Phylogenetic analysis of concatenated data set

The branch-and-bound search resulted in 2 most parsimonious trees (MPTs) of 2596 steps (CI = 0.5458; RI = 0.6837; RC = 0.3732). The only difference between the two trees involved the relationships among samples L4, L15, and L33 of $P$. nocticolus. In order to illustrate branch lengths I chose one of the MPTs (Fig. 2).

BI and MP analyses of the concatenated dataset resulted in highly compatible topologies with high support from both Bayesian posterior probabilities (BPP) and MP bootstrap proportions (BSP) (Figs. 2,3). Both analyses resulted in three major well-differentiated peninsular clades corresponding to species. Phylodactylus lanei rooted as the
sister group of all ingroup taxa and *P. paucituberculatus* was sister to a clade containing *P. davisi* and *P. duellmani*. Both analyses supported the monophyly of peninsular species from mainland Mexican taxa (BSP = 92; BPP = 1.00). *Phyllodactylus unctus* rooted the base of the peninsular clade in the MP analysis, resolving *P. xanti* as sister to a clade containing *P. nocticola* from across the peninsula. However, support for the sister relationship of *P. xanti* and *P. nocticola* was relatively weak (BSP < 50%). Conversely, BI placed *P. nocticola* and *P. unctus* as sister taxa with moderate support (0.94). Monophyly of *P. nocticola* was strongly supported with both methods (BSP = 99; BPP = 1.00).

### 3.3 Phylogenetic analysis of nDNA

MP analysis of the two nuclear loci resulted in 42 MPTs of 28 steps (CI = 0.9643; RI = 0.9500; RC = 0.9161). Highly compatible topologies were recovered with both MP and BI (Fig. 4). *Phyllodactylus duellmani* and *P. davisi* were again resolved as sister taxa. However, unlike the total evidence analysis and mtDNA analysis (presented below), the relationship of *P. paucituberculatus* to its congeners was unresolved. The phylogenetic relationships of all peninsular species were also ambiguous. However, both MP and BI found strong support for the monophyly of both *P. xanti* and *P. nocticola*.

### 3.4 Phylogenetic analysis of mtDNA

MP analysis of the mitochondrial genes resulted in 2 MPTs of 3192 steps (CI = 0.6313; RI = 0.6826; RC = 0.4309). The strict consensus mtDNA tree was identical to the total evidence tree with strong support for three monophyletic peninsular clades (Figs. 2,5). As with the
total evidence topology, MP of mtDNA placed *P. xanti* and *P. nocticolus* as sister taxa, although support was relatively low (<50%). Monophyly of the three peninsular species was also strongly supported in the Bayesian topology (BPP = 1), although the phylogenetic relationships of these taxa were ambiguous (*P. nocticolus + P. unctus* = 0.84). Monophyly of *P. nocticolus* and *P. xanti* was strongly supported.

## 4 Discussion

### 4.1 Taxonomy of *Phyllodactylus*

The phylogenetic analyses presented herein provide further genetic evidence for the validity of *P. nocticolus* as a distinct species, separate from *P. xanti*. The MP analysis suggests that *P. nocticolus* is sister of *P. xanti*, which together form a clade sister to *P. unctus*. In contrast, the more strongly supported BI analysis resolved *P. nocticolus* and *P. unctus* as sister taxa. These results are of interest because, historically, *P. nocticolus* was considered a subspecies of *P. xanti* (Dixon 1964; Grismer 1999). Both *P. nocticolus* and *P. xanti* are easily distinguished from *P. unctus* because the latter lacks enlarged dorsal tubercles.

Estimates of pairwise mtDNA divergence approached 13% between peninsular species of *Phyllodactylus*. This is in concordance with previous genetic work based on allozyme data, which showed significant genetic differences between Cape Region geckos and those north of La Paz (Murphy 1983b). Although previous researchers documented morphological variation between *P. xanti* and *P. nocticolus* including color pattern, absence of thigh tubercles in *P. nocticolus*, and scutellation differences, authors felt that it was not significant enough to warrant full species status (Dixon 1964; Grismer 1994, 2002). Refer to
Dixon (1964) for a full list of morphological characters used to differentiate *P. nocticolus* from *P. xanti* and other congeners.

The taxonomy and delimitation of species ranges in *Phyllodactylus* has been ambiguous due to high intraspecific variability in several morphological characters (Dixon 1964). This variability, in conjunction with discordance between molecular and morphological characters, has hindered the elucidation of accurate phylogenetic hypotheses for the genus (Dixon 1964; Murphy 1983b). However, my molecular data, when taken in conjunction with previous allozyme and morphological investigations, provides comprehensive evidence for the validity of *P. nocticolus* as a distinct species.

*Phyllodactylus xanti* occurs both in the Cape Region of the peninsula as well as on several islands in the Gulf of California (Dixon 1964, 1966; Murphy & Ottley 1984; Grismer 1999, 2002). Historically, the range of *P. xanti* encompassed most of the peninsula of Baja California into extreme southern California (Dixon 1964). Populations residing in areas north of La Paz were designated as *P. xanti nocticolus*, whereas populations south of La Paz were classified as *P. xanti xanti* (Dixon 1964) with potential intergradation zones around Loreto and Comondu (Dixon 1964; Stebbins 2003). My genetic analysis combined with previous allozyme and morphological comparisons suggests that peninsular populations north of La Paz be elevated to full species status. Thus, I recognize these populations as *P. nocticolus*. Further, the lack of suitable habitat surrounding the La Paz region (Fig. 1) suggests that populations of *P. xanti* and *P. nocticolus* are likely not in contact. Additional molecular studies are needed to ascertain the taxonomic status of insular forms north of La Paz.

*Phyllodactylus unctus* has long been considered a monotypic species, even though insular populations exhibiting slight morphological variation occur on islands associated with
the Cape Region (Banks & Farmer 1962; Grismer 1999). On the peninsular mainland, the species is restricted to the Cape Region, where it occurs sympatrically and syntopically with *P. xanti* (Dixon 1964). The high statistical support for the monophyly of the *P. unctus* clade is in concordance with previous investigations (Dixon 1964) and suggests that it constitutes a single species.

### 4.2 Biogeography

Several paleobiogeographic hypotheses have been put forth to explain patterns of diversification in *Phyllodactylus* and other Baja Californian taxa (Murphy 1983a,b; Murphy & Aguirre León 2002). Biogeographic and geological evidence suggests that the peninsula of Baja California was continually fragmented into smaller landmasses and islands throughout periods of the Miocene through the Pleistocene (Murphy 1983b; Smith 1991; Aguirre León *et al.* 1999; Riddle *et al.* 2000; Carreño & Helenes 2002; Murphy & Aguirre León 2002). This fragmentation resulted in a trans-peninsular seaway during the late Miocene, which temporarily isolated the Cape Region from the remainder of the peninsula (Lindell *et al.* 2008). Furthermore, somewhat later during the late Miocene, a temporary mid-peninsular seaway separated northern and southern peninsular biotas (Upton & Murphy 1997; Murphy & Aguirre León 2002; Lindell *et al.* 2005, 2006, 2008). My genetic data support the hypothesis of a trans-peninsular seaway that isolated the Cape Region from the rest of the peninsula (Schwennicke *et al.* 1996; Carreño & Helenes 2002]. This is shown in the high mtDNA sequence divergence estimates between the Cape Region’s endemic species (*P. xanti* and *P. unctus*) and more widespread species north of La Paz (*P. nocticolus*).
Several empirical studies have documented genetic breaks in maternal lineages that coincide with a seaway across the mid-peninsular region (Upton & Murphy 1997; Riddle et al. 2000; Murphy & Aguirre León 2002; Lindell et al. 2005, 2006, 2008; Lindell & Murphy 2008). In contrast, my genetic data for the widespread *P. nocticola* shows no such break (Figs. 2–5); there was no significant geographic structure throughout the peninsula for this species congruent with a mid-peninsular seaway. However, two reciprocally monophyletic lineages were detected, with a break between San Evaristo and Juncalito (Figs. 2–5). It is possible that this break in maternal lineages corresponds to the intergradation zone described in Dixon (1964).

Few studies have examined intraspecific trans-peninsular variation at nuclear loci. However, Murphy (1983b) found substantial allozyme evidence for the validity of *P. nocticola* north of the Isthmus of La Paz. Conversely, Adest (1987) found no significant genetic differentiation in the zebra tailed lizard, *Callisaurus draconoides* throughout seven peninsular populations. These data contrast significantly to those utilizing mtDNA as molecular markers. Lindell et al. (2005), for example, showed highly divergent maternal lineages for *C. draconoides* distributed across the peninsula of Baja California. A similar discordance between mitochondrial and allozyme data is evident in *Urosaurus nigricaudus* (Aguirre León et al. 1999; Lindell et al. 2008). This mitochondrial-nuclear discordance clearly illustrates the need to incorporate bi-parentally inherited nuclear loci such as nuclear sequences and/or microsatellite DNA when examining biogeographic patterns of genetic differentiation throughout the peninsula of Baja California. Unfortunately, my nuclear data provided only eight potentially phylogenetically informative characters to the MP analysis.
The peninsular clade was resolved as monophyletic with high support from both MP and BI (Figs. 2,3). These results suggest a single invasion to the peninsula from mainland Mexico, presumably resulting from the rifting of western Mexico off the North American Plate during the increased tectonic activity associated with the Miocene (Murphy 1983b; Lonsdale 1989; Carreño & Helenes 2002; Murphy & Aguirre León 2002; Lindell et al. 2005). This rifting isolated peninsular populations from their mainland counterparts through the formation and northward expansion of the Gulf of California.

This study is the first to incorporate molecular data to infer evolutionary relationships among species of Phyllodactylus. In my phylogenetic analysis P. lanei rooted the base of the ingroup taxa. Phyllodactylus duellmani was resolved as sister to P. davisi, which together formed a clade sister to P. paucituberculatus (Figs. 2,3). However, only seven of the approximately 20 Mexican species were included in the analysis and until a more comprehensive sampling is undertaken, phylogenetic relationships among Phyllodactylus inhabiting mainland Mexico should remain tentative. Regardless, the inclusion of these taxa supports a single origin for Baja Californian species with high support from both BPP and BSP in the total evidence and mitochondrial analyses (P. paucituberculatus was unresolved with nDNA). Further, these results contrast to those of Dixon (1964), Grismer (1994), and Murphy & Papenfuss (1980) who suggested multiple independent origins to the peninsula.

Researchers have postulated alternative vicariant hypotheses to explain the evolution of Baja Californian biota, suggesting that climatic oscillations during the Quaternary contributed significantly to speciation in the flora and fauna of the region (Hafner & Riddle 1997). However, Lindell et al. (2006) suggested that paleogeographic and geologic forces have played a more significant role since the age of the concordant patterns are older than the
Quaternary climatic fluctuations. Thus, it is highly probable that the biogeographic patterns observed in *Phylodactylus* and other taxa distributed throughout the peninsula of Baja California are primarily due to the geological history of the region and not Quaternary climatic phenomena.
Acknowledgments

I am grateful to a number of individuals and organizations. Norberto Martínez, Felipe Rodriguez and Francisco Soto provided assistance with fieldwork. Oliver Haddrath, Ross MacCulloch, Pat Ross and Tanya Trépanier helped with laboratory work. All permits were granted to RWM by the Dirección General de la Fauna Silvestre and Secretaría de Medio Ambiente y Recursos Naturales, Mexico [65-78-866; 1-78-832; 122.4.3/128-33.26; 12140]. This work was funded by the Natural Sciences and Engineering Research Council (NSERC) Discovery Grant A3148 to Robert W. Murphy, the ROM Foundation to Robert W. Murphy, the Theodore Roosevelt Foundation of the American Museum of Natural History to Johan Lindell, the Royal Swedish Academy of Sciences to Johan Lindell, and Sigma Xi’s Grants-in-Aid programme to Johan Lindell.
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Lindell J, Méndez-de la Cruz FR, Murphy RW (2008) Discordance between deep genealogical history and population differentiation in the black-tailed brush lizard (Urosaurus nigricaudus) of Baja California. Biological Journal of the Linnean Society, 94, 89–104.


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Table 2. Percent sequence divergence (uncorrected p-distances from mtDNA) for Baja Californian and mainland Mexican Phyllodactylus compared to Gekko gecko. N/A = undefined distance between sequences.

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Figure Legends

**Fig. 1.** Map of Baja California, the Gulf of California, southwestern USA, and northwestern Mexico illustrating the geographic location of samples of *Phyllodactylus* included in this study. Sample numbers correspond to those presented in Table 1. The break in geographic ranges in the Isthmus of La Paz region is due to unsuitable natural habitat (lack of rocky outcrops). Gray area represents the geographic range of *P. nocticolus*, black area represents the geographic range of *P. xanti*, striped area represents the range of *P. unctus*.

**Fig. 2.** Phylogenetic relationships of Mexican *Phyllodactylus* based on a branch-and-bound maximum parsimony (MP) analysis of the concatenated dataset. Numbers above nodes represent MP bootstrap proportions (BSP) resulting from 10,000 pseudoreplicates.

**Fig. 3.** Phylogenetic relationships of Mexican *Phyllodactylus* based on Bayesian inference (BI) of the concatenated dataset. Numbers above and adjacent to nodes represent Bayesian posterior probabilities (BPP; * = 1.0) sampled from the posterior distribution of trees. *Gekko gecko* was removed from the tree due to its long branch length.
Fig. 4. Phylogenetic relationships of Mexican *Phyllodactylus* based on maximum parsimony (MP; A) and Bayesian Inference (BI; B) of nuclear DNA (nDNA). Numbers above nodes on MP tree represent nonparametric bootstrap proportions (BSP). Numbers above nodes on the Bayesian topology represent Bayesian posterior probabilities (BPP; * = 1.0). Refer to text for a description of loci and total base pairs (bp) sequenced.

Fig. 5. Phylogenetic relationships of Mexican *Phyllodactylus* based on maximum parsimony (MP; A) and Bayesian Inference (BI; B) of mitochondrial DNA (mtDNA). Numbers above nodes on MP tree represent nonparametric bootstrap proportions (BSP). Numbers above nodes on the Bayesian topology represent Bayesian posterior probabilities (BPP; * = 1.0). *Gekko gecko* was removed from the BI tree due to its long branch length. Refer to text for a description of loci and total base pairs (bp) sequenced.
Fig. 1
CHAPTER 6—PHYLOGENETICS OF BAJA CALIFORNIAN *PHYLODACTYLUS*

Fig. 2

Mainland Mexico

Cape Region

North of La Paz
CHAPTER 6—PHYLOGENETICS OF BAJA CALIFORNIAN *Phyllodactylus*

Mainland Mexico

Cape Region

North of La Paz
CHAPTER 6—PHYLOGENETICS OF BAJA CALIFORNIAN *PHYLODACTYLUS*

Fig. 5

A

B
### Supplementary Material

Appendix I. Mitochondrial and nuclear loci sequenced for Phyllodactylus and their corresponding primer sequences. Different combinations of primers were used to obtain full reads of regions.

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* Modified after Palumbi, 1996
CHAPTER 7
Conclusions and future directions

1 General Background

The continual reduction in DNA sequencing costs is revolutionizing molecular ecology and evolution and enabling us to ask more sophisticated questions regarding spatial and temporal patterns of biological diversification in non-model taxa. Accompanying these advances in DNA sequencing technologies are new, powerful multilocus statistical methods that are able to provide a more comprehensive analysis of the processes influencing the evolutionary history of populations, species, and higher-level taxa (Blair & Murphy 2011). Conversely, the nascent field of landscape genetics seeks to determine the relative importance of contemporary ecological variables in shaping microevolutionary processes (Manel et al. 2003; Holderegger & Wagner 2006, 2008). Landscape genetic analysis can be seen as a complement to phylogenetic and phylogeographic inference by providing a temporal component regarding drivers of contemporary genetic structure (Manel et al. 2003; Storfer et al. 2010; Wang 2010). In this thesis, I utilize multiple types of molecular markers and statistical analyses to test hypothesis of interest to both molecular phylogenetics and landscape genetics. In the following sections I extrapolate on the main findings of the thesis, provide a historical background about why this research is both timely and necessary, and discuss future research directions.

2 Mexican Tropical Dry Forest
Over the past few decades, tropical rain forests have garnered a substantial amount of attention from molecular ecologists and evolutionary biologists. This is due, in part, to the fact that these ecosystems contain the vast majority of Earth’s species and are disappearing at an alarming rate (Myers et al. 2000). Many studies seek to determine the relative importance of Neogene vicariance versus Quaternary climate change as engines for speciation and genetic diversification in taxa distributed though rain forest habitat (Klicka & Zink 1997; Moritz et al. 2000; Weir 2006). Conversely, tropical dry forests (TDFs) have received little attention from both an evolutionary and conservation perspective (Trejo & Dirzo 2000). Although they are both tropical forests, a variety of structural and functional differences separate these habitat-types. For example, TDFs are seasonal with up to eight months of drought per year. The vegetation generally consists of short deciduous or semi-deciduous trees (~8–12 m in height) and thorny vegetation along with columnar cacti (Murphy & Lugo 1986). A primary distinction between TDF and tropical thornscrub lies with the height of the canopy in relation to cactus-height (i.e. canopy-height is higher than cactus-height in TDF; Robichaux & Yetman 2000). Further, unlike rain forests, the soil in TDFs is generally nutrient rich with a moderate to high pH (Murphy & Lugo 1986).

Although the biological diversity in TDF is substantial (Ceballos 1995; Ceballos & García 1995; Gillespie et al. 2000; García 2006), both natural and anthropogenic disturbance threaten the evolutionary potential for species inhabiting this ecosystem (Janzen 1988; Miles et al. 2006). At a global level, Neotropical dry forests suffered from a deforestation rate of 12% between 1980 and 2000 (Miles et al. 2006). Mexican TDF occurs in a semi-continuous belt on the west coast from southern Sonora to the Guatemalan border. It is hypothesized that the Mexican TDF originated between 30–20 million years ago in response to the orogenesis
of the Sierra Madre Occidental and Neovolcanic Axis (Becerra 2005). Trejo & Dirzo (2000) perform a time-series analysis and find that as of 1990 only 27% of Mexico’s TDF had remained intact. They estimate an annual deforestation rate of 1.4%. The majority of fragmentation is due to anthropogenic influences including slash-and-burn practices and the conversion of forest to pasturelands and agriculture fields (Burgos & Maass 2004).

Although we are now beginning to realize the biological importance of these tropical forests, few molecular genetic studies have focused explicitly on testing diversification hypotheses for species inhabiting this ecosystem. For example, most molecular phylogenetic and phylogeographic studies in Mexico focus on evolutionary processes throughout the peninsula of Baja California (e.g. Lindell et al. 2005, 2008; Blair et al. 2009), the Sonoran Desert (e.g. Hafner & Riddle 2005), and the Mexican Highlands (e.g. Bryson et al. 2011a,b,c). The few studies focusing on genetic patterns throughout the TDF of western Mexico find evidence for cryptic lineages, many of which correspond to previously hypothesized biogeographic barriers (e.g. Hasbún et al. 2005; Devitt 2006; Zarza et al. 2008). At present, there is a lack of landscape genetic studies focused on tropical systems (Storfer et al. 2010) and to my knowledge there has been no published study that examines functional connectivity in species inhabiting TDF vegetation.

3 Leaf-toed Geckos

Leaf-toed geckos, genus *Phyllodactylus*, represent approximately 50 species that encompass a Neotropical distribution ranging from southern California to southwestern South America and the West Indies (Dixon 1964). Species are commonly found in arid and semi-arid environments throughout their ranges. In Mexico, the majority of species are confined to the
TDFs of the west coast and southern Baja California Sur. The genus is characterized by leaf-like extensions at the terminal ends of the digits. To date, little molecular work has been done with the genus with most phylogenetic studies utilizing a few representative species in order to understand higher-level gekkonid relationships (e.g. Gamble et al. 2008, 2011). Dixon (1964) provides a comprehensive morphological investigation into the systematics of the genus throughout North and Middle America and suggests that diversity within the genus may be substantially underestimated. The fact that these lizards are primarily restricted to TDF habitat makes them ideal to test both historical and contemporary ecological and evolutionary hypotheses operating throughout the ecosystem.

4 General Results of Thesis

By combining different molecular marker-types useful for testing ecological and evolutionary hypotheses over multiple spatial and temporal scales (Wang 2010), I elucidate the historical and contemporary processes influencing the spatial distribution of genetic variation in leaf-toed geckos. In Chapter 2, I use a combination of molecular cloning and next-generation 454 pyrosequencing to isolate and characterize 12 microsatellite loci for the Mexican yellow-bellied gecko, *P. tuberculosus*. In Chapter 3, I use these loci to conduct a landscape genetic study of *P. tuberculosus* throughout the landscape surrounding Alamos, Sonora. Results suggest highly differentiated populations and that dispersal is influenced by variables including forest structure, stream networks, slope, temperature, and the degree of human perturbation.

Chapter 4 utilizes data from two mitochondrial genes and two intron markers in order to understand the historical processes influencing rates and patterns of diversification of
geckos in the *P. tuberculosus* species group (*P. t. saxatilis, P. t. magnus, P. muralis muralis, P. m. isthmus, P. lanei lanei, P. l. rupinus*). My results suggest that both the origin of Mexican TDF and Pleistocene climate change were important drivers of cladogenesis for these geckos, although demographic expansion did not occur during or following the Pleistocene. I also find low effective population sizes and migration rates between contemporary mtDNA lineages. Further, unlike my landscape genetic analysis, I find no significant correlation between riverine networks and genetic differentiation. These results are concordant with a recent analysis of the wood frog (*Lithobates sylvaticus*) and suggest that processes operating over a landscape genetic scale may not translate to processes over a phylogeographic scale (Lee-Yaw *et al.* 2009). The discrepancy in my results may be partly explained by inherent differences in molecular marker-types (mtDNA versus microsatellites), a result of specific ecological characteristics at field sites, or simply the fact that other ecological processes are more important over relatively broad spatial scales. For example, most of the streams and arroyos sampled for the landscape genetic study had extensive adjacent rocky outcroppings. Conversely, many of the larger rivers included in the phylogeographic analysis of geckos lacked extensive rocky habitat. As geckos are commonly encountered on rocky vertical substrate, the discordance may be partly explained by these microhabitat differences. Additional studies are required to test the ability of landscape genetic inference to predict similar phylogeographic patterns and processes.

In Chapter 5, I use the microsatellite data generated for Chapter 3 along with *de novo* mtDNA sequence data to compare and contrast historical and contemporary demographic parameters in geckos surrounding the region surrounding Alamos, Sonora. I find evidence for substantial cryptic lineage divergence over a small spatial scale (~40 km$^2$), a 10-fold increase
in contemporary versus historical migration rates, and evidence for recent bottlenecks and smaller contemporary effective population sizes for many populations. Because the utility of mtDNA for testing landscape genetic hypotheses is still hotly debated (e.g. Vandergast et al. 2007; Wang et al. 2010, 2011; Bohonak & Vandergast 2011), I created resistance surfaces based on variables previously shown to be important predictors of connectivity for the species (Chapter 3). I then use circuit theory to correlate different measures of landscape connectivity with mtDNA divergence, historical migration rate, and contemporary migration rate. My results show that landscape-genetic relationships based on mtDNA data contrast with those based on microsatellites. Higher correlations are also found between landscape structure and contemporary versus historical migration rate. Taken together, this suggests that although migration rates have increased since humans began modifying the landscape, geckos are altering their dispersal routes in response to recent habitat fragmentation. Thus, contemporary genetic structure is likely a product of low historical migration and strong female philopatry along with recent anthropogenic fragmentation to the landscape.

In Chapter 6, I examine the biogeographic and taxonomic consequences of the dynamic paleogeographic history of the peninsula of Baja California. Previous molecular genetic studies suggest that the peninsula was fragmented by trans-peninsular seaways at multiple locations throughout the Miocene–Pleistocene (e.g. Upton & Murphy 1997; Lindell et al. 2005, 2008). Evidence suggests that these seaways resulted in a vicariant event that separated populations on either side. Traditionally, the peninsula was home to two species of leaf-toed gecko, *P. unctus* and *P. xanti*, the latter being composed of multiple subspecies both on the peninsula and on islands within the Gulf of California (Dixon 1964). *Phyllodactylus unctus* and *P. x. xanti* are restricted to the TDFs of the southern Cape Region of the peninsula.
whereas \textit{P. x. nocticolus} has a more desert-like distribution ranging from just north of the La Paz region all the way to extreme southern California. Using data from five mitochondrial and two nuclear genes, I find substantial genetic divergence both inter- and intraspecifically. This provides further evidence that \textit{P. x. nocticolus} deserves species-status and, thus, I elevate the taxon to be a species. Phylogenetic analyses also provide further evidence for a trans-peninsular seaway near the Isthmus of La Paz region that led to the separation and speciation of \textit{P. nocticolus} from its Cape Region ancestor. Unfortunately, my analysis is unable to determine the sister group to \textit{P. nocticolus} (due to low nodal support). Further, unlike other studies (e.g. Upton & Murphy 1997; Lindell \textit{et al.} 2005, 2008) I find no evidence for a mid-peninsular seaway during the Miocene. However, my analysis recovers two reciprocally monophyletic lineages of \textit{P. nocticolus} that may correspond to the previously hypothesized break near the Loreto region (Lindell \textit{et al.} 2008). Unlike the biogeographic breaks commonly observed at the mid-peninsular and La Paz areas, few studies find deep genealogical breaks near Loreto (Lindell \textit{et al.} 2005, 2008). However, the presence of marine sediments of Pliocene origin suggests a trans-peninsular seaway between 3.4–2.0 Ma (McLean 1989; Umhoefer \textit{et al.} 1994). Additional data are required to test the generality of a trans-peninsular seaway near Loreto.

5 Future Directions

Although challenging, writing a multidisciplinary Ph.D. thesis does have its benefits. The effort has taught me to think critically and always ask questions regarding methods of analysis, philosophy, and assumptions. I am interested in applying the skills I have learned during my Ph.D. to future projects in the diverse fields of molecular phylogenomics,
landscape genetics, and ecological genomics. Methods of phylogenetic inference have progressed rapidly over the past few years to handle the vast quantities of multilocus sequence data being generated (Edwards 2009; Blair & Murphy 2011) and I am interested in adopting a phylogenomic approach to better understand the evolutionary history of different vertebrate lineages. Applying these methods in a hypothesis-testing framework can illuminate the historical processes responsible for shaping Earth’s rich biota.

On a finer spatial and temporal scale, I am interested in continuing my work in landscape and conservation genetics. The field of landscape genetics is progressing rapidly since its inception almost 10 years ago (Manel et al. 2003; Storfer et al. 2010). I plan on pursuing additional empirical studies with small vertebrate species inhabiting fragmented tropical habitats to better understand how recent disturbance may be influencing functional connectivity. I am presently working on collaborations with Brazilian researchers to understand the genetic consequences of fragmentation throughout the Brazilian Atlantic forest. Simulation studies also continue to play a vital role in the development of the field of landscape genetics and I hope to continue my research in this area (Landguth et al. 2010; Safner et al. 2011; Blair et al. 2012).

The continual reduction in costs for next-generation DNA sequencing is making ecological genomic studies in non-model taxa now within reach for many researchers. Many of these studies use genome-scans (microsatellites, AFLPs, or SNPs) to determine if and how populations may be adapting to specific environmental conditions (Holderegger et al. 2006; Joost et al. 2007; Manel et al. 2010). Early methods utilized outlier approaches to test for signs of loci exhibiting differentiation higher than expected under neutrality, which may suggest that certain markers are linked to loci under selection (Luikart et al. 2003). Newer
methods continue to be developed that take the analysis one step further and statistically correlate specific loci or alleles with certain environmental features (e.g. Joost et al. 2007); a so-called ‘landscape genomics approach.’ I am interested in conducting a variety of landscape genomic studies in small vertebrates throughout the Mexican TDF to test for selection and adaptation at the molecular level. This will enable me to determine how species are responding to continual habitat change from both natural and anthropogenic sources.
References


