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Historical and contemporary demography of leaf-toed geckos

(Phyllodactylidae: Phyllodactylus) in the Mexican dry forest

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Running Head: Gene flow in Phyllodactylus geckos
Abstract

Disentangling the relative influence of historical versus contemporary processes shaping the spatial distribution of genetic variation is critical if we are to effectively mitigate key biodiversity issues. We utilize a comprehensive approach based on different molecular marker types and analytical methods to understand the demographic consequences of recent habitat fragmentation in a spatially explicit context. We focus our efforts on native leaf-toed geckos (Phyllodactylus tuberculosus saxatilis) throughout fragmented habitat in the tropical dry forest of northern Mexico. Recent evidence suggests that geographic ranges for these species may be much smaller than currently realized and no data are available regarding recent shifts in demographic trends and how these trends may correspond with recent fragmentation and introductions of non-native gecko species (Hemidactylus). Mitochondrial DNA sequences reveal substantial historical genetic divergence over a small geographic area (<40 km). We find evidence for an increase in contemporary versus historical migration rates based on 10 microsatellite loci, but evidence that many populations suffer from recent reductions in effective population sizes. Landscape genetic analyses find stronger correlations between landscape structure and contemporary versus historical migration or mtDNA divergence, suggesting that individuals have altered their dispersal routes in response to recent habitat changes. Taken together, this study suggests that long-term female philopatry, recent habitat fragmentation, and possibly introductions of non-native gecko species all contribute to the demographic patterns and the high degree of differentiation observed over fine-spatial scales in Mexican leaf-toed geckos.
Key Words: Alamos; gekko; migration; mtDNA; Sonora; tropical dry forest
Introduction

Evidence continues to accumulate showing that rates and patterns of dispersal and gene flow can be influenced by a variety of intrinsic and extrinsic factors (Ricketts 2001; Manel et al. 2003; Storfer et al. 2010). Habitat fragmentation and loss due to both natural and anthropogenic forces can represent a substantial threat to the maintenance and connectivity of populations (Sanderson et al. 2002; Ewers and Didham 2005). Maintaining genetic connectivity is essential to allow the free exchange of beneficial 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 extinction (Fahrig 2003; Crispo et al. 2011).

With the advent and continued interest in the field of landscape genetics, there has been a renewed interest in understanding the contemporary ecological processes influencing the genetic structure of natural populations (see Sork and Waits 2010; Storfer et al. 2010 for review). Although many of these studies have had a profound impact on what we now understand about the spatial distribution of genetic variation in nature, many of these studies fail to examine the influence of historical demographic processes on contemporary genetic structure (e.g. Spear et al. 2005; Vignieri 2005; Wang 2009; Goldberg and Waits 2010, but see Vandergast et al. 2007; Zellmer and Knowles 2009; Dyer et al. 2010). Historical demographic patterns can be examined through multiple mechanisms including using historical landscape layers (Vandergast et al. 2007), different molecular marker types (Wang 2011), and different analytical methods (Andersen et al. 2009; Schmidt et al. 2009; Chiucchi and Gibbs 2010).
The tropical deciduous or dry forests (TDF) of western Mexico are home to an enormous diversity of species, several of which are endemic (Robichaux and Yetman 2000; Myska 2007). Dry forests are characterized by distinct wet/dry seasons, with the latter lasting up to 10 months in some regions (Murphy and Lugo 1996; Robichaux and Yetman 2000). Prior to recent fragmentation, Mexican TDF was a predominant ecosystem in Mexico spanning most of the Pacific versant of the Sierra Madre Occidental and Sierra Madre del Sur (Robichaux and Yetman 2000; Becerra 2005; Cartron et al. 2005). Although the floral and faunal diversity within these forests is immense, conservation efforts have historically emphasized tropical rain forests and high elevation pine and pine/oak habitats, specifically throughout areas of the Mexican Volcanic Belt (Pennington et al. 2000; Robichaux and Yetman 2000; García 2006). This is problematic, as the annual deforestation rate for Mexican TDF is estimated at up to 1.4–2%, with approximately 73% of the biome altered due to agriculture and livestock (Trejo and Dirzo 2000). Indeed, some authorities have concluded that TDF is one of Earth’s most threatened ecosystems (Janzen 1988).

Leaf-toed geckos, (Phyllodactylus, Phyllodactylidae; Gamble et al. 2008) encompass approximately 50 species distributed throughout arid and semi-arid forests of the New World Tropics (Dixon 1964). In Mexico, their distribution mirrors that of TDF, making them suitable models to test ecological and evolutionary hypotheses in this system. Recent work on the genus has documented the presence of substantial 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). There is also evidence that species that were once considered widespread are actually composed of several species exhibiting limited geographic ranges (Blair et al. 2009).
unpublished data). Further, recent introductions of non-native gecko species of the genus *Hemidactylus* also appear to directly compete with and displace leaf-toed geckos (pers. obs.).

These factors make leaf-toed geckos an ideal system for conservation genetics-based studies to ascertain the demographic consequences of recent habitat alteration and ecosystem functioning within TDF.

A recent landscape genetic study of *P. tuberculosus saxatilis* near Alamos, Sonora finds that patterns of gene flow are correlated with multiple landscape variables including the amount of undisturbed habitat (Blair et al. 2013). However, this study did not examine how demographic parameters such as effective population size (Ne) and migration rates vary through time. In this study we use microsatellite and mtDNA data to compare spatially explicit historical and contemporary demographic parameters for leaf-toed geckos. Because of its relatively slow rate of evolution, mtDNA is an ideal marker for examining the genetic consequences of evolutionary processes occurring over geologic time, but may not be useful to infer complex landscape-genetic relationships (Wang 2010). We specifically address the following questions: (i) How is mtDNA diversity spatially structured? (ii) How do historical demographic parameters compare to contemporary parameters? (iii) Do populations show evidence of recent bottlenecks? (iv) How does landscape structure correlate with contemporary migration, historical migration and mtDNA divergence?

**Materials and methods**

**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 a previous study (Blair et al. 2013; DRYAD). Previous analyses showed no significant deviations from Hardy-Weinberg expectations or linkage disequilibrium and an estimated genotyping error rate of <1% based on re-running 10% of all PCRs (Blair et al. 2013). We also screened 77 individuals from 11 populations for mtDNA polymorphism to assess fine-scale patterns of deep lineage divergence (Fig. 1; Supplementary Table S1). Individuals were captured by hand during chance encounters on public, private, and protected land following permit guidelines. All tissues were preserved in the field in 95% ethanol following approved animal use protocols from the Royal Ontario Museum. Vouchers were fixed in the field with 10% formalin, transferred to 70% ethanol and deposited in the Laboratorio de Herpetologia, Instituto de Biología, Universidad Nacional Autónoma de México. Fieldwork was conducted during the summers of 2007 and 2008. DNA sequences were obtained from *P. xanti* (Blair et al. 2009) and *P. homolepidurus* (this study) to root all mtDNA networks. For all demographic analyses we defined a ‘population’ based on collection locality.

**DNA extraction, amplification and sequencing**

Total genomic DNA was digested and extracted from liver or muscle tissue using standard proteinase K and phenol–chloroform protocols. We amplified and sequenced 2400 base pairs (bp) of mtDNA encompassing partial sequences of cytochrome *c* oxidase subunit I (COI), NADH dehydrogenase subunit 4 (ND4) and adjacent tRNAs, and 16S rRNA. In addition,
sequences were obtained for the entire NADH dehydrogenase subunit I (NDI) gene and flanking tRNAs. Amplified gene fragments, number of corresponding bp, primer sequences, and references were presented in Supplementary Table S2. All PCR and sequencing conditions followed Blair et al. (2009).

**mtDNA analysis**

Detailed analysis of the mtDNA data can be found in the Online Supplementary Materials. In brief, mtDNA sequences were edited and aligned in BIOEDIT 5.0.6 (Hall 1999). Genealogical relationships among individuals were assessed using both maximum parsimony (MP) and Bayesian inference (BI). DNAsP v.5.0 (Rozas et al. 2003) was used to calculate standard diversity statistics and divergence among lineages. Isolation by distance (IBD) was assessed using the Isolation By Distance Web Service (IBDWS) v.3.16 (Jensen et al. 2005).

**Historical and contemporary migration rates**

We used the program MIGRATE 3.2.16 (Beerli and Felsenstein 2001; Beerli 2006) to estimate historical \( N_e \) and asymmetric migration rates between populations based on the microsatellite data. MIGRATE uses the coalescent in a Bayesian or maximum likelihood framework to calculate two parameters from the data, \( \Theta \) and \( M \), where \( \Theta \) represents effective population size (\( 4N_e\mu \) for nuclear DNA) and \( M \) represents the mutation-scaled immigration rate (\( m/\mu \)). This coalescent-based approach is most suitable for detecting migration rates over thousands of years or approximately \( 4N_e \) generations in the past (Beerli 2008). The data were assumed to follow a Brownian motion mutation model. We used the \( F_{ST} \) calculation method to generate staring values for both \( \Theta \) and \( M \). We specified uniform priors for both parameters with a minimum of 0, mean
We then implemented the Bayesian method to infer $\Theta$ and $M$, specifying two independent runs, static heating with four chains (temperatures: 1, 1.5, 3.0, 10,000.0), 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. We also ran the program multiple times to make sure the algorithm was reaching the same posterior distribution of parameter estimates. We also used MIGRATE to estimate $\Theta$ and $M$ based on the mtDNA data. Two independent Bayesian searches were performed with the same number of chains and heating scheme as the microsatellite analysis. Default priors were used for $\Theta$ and a uniform prior (minimum = 0, mean = 1000, maximum = 4000, delta = 400) was used for $M$. Each independent run used 5,000 steps and a sampling increment of 500.

We used the Bayesian method implemented in BAYESASS EDITION 3.0 (Wilson and Rannala 2003) to estimate contemporary migration rates between populations. Unlike MIGRATE, BAYESASS does not assume genetic equilibrium and is therefore more suitable for inferring contemporary (over the past few generations) processes. The model in BAYESASS assumes linkage equilibrium between loci, but allows for deviations in Hardy-Weinberg proportions by introducing an additional inbreeding (F) parameter. We ran BAYESASS for 30 million generations with a burn-in of 3 million and a sampling interval of 300. We modified the MCMC mixing parameters to obtain the target values recommended by the program authors. We ran the program multiple times starting from a different random number seed in order to make sure that the chain was converging adequately. We also examined the raw trace files in the program TRACER v1.6.1 (Rambaut and Drummond 2007) to assess convergence.

Migration estimates obtained from MIGRATE were based on the mutation-scaled immigration rate $M (m/\mu)$ whereas BAYESASS returned the immigration rate $m$. Thus, to obtain
estimates of $m$ from MIGRATE we multiplied all $M$-values by an estimated mutation rate of $5 \times 10^{-4}$ (Garza and Williamson 2001; Waples and Do 2010). To statistically quantify the relationship between historical and contemporary migration rates we implemented a Mantel test (Mantel 1967) in the R package ECODIST (Goslee and Urban 2008) using 10,000 permutations.

**Historical and contemporary effective population sizes**

Historical $N_e$ was estimated in MIGRATE. To estimate contemporary $N_e$ we used the linkage disequilibrium method (Hill 1981) in the program LDNE v. 1.3.1, which implemented separate bias correction factors for sample sizes greater than or less than 30 individuals (Waples 2006; Waples and Do 2008). We 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 and Do 2008).

We used the program BOTTLENECK v 1.2.02 (Piry et al. 1999) to compare estimates of observed heterozygosity to number of alleles at each locus. Following a population bottleneck, the number of alleles is reduced faster than heterozygosity, resulting in an observed heterozygosity larger than would be expected if the locus were at mutation-drift equilibrium (Cornuet and Luikart 1996). This test is useful for tracking bottlenecks occurring over the last 0.2–4.0$N_e$ generations (Cornuet and Luikart 1996). Heterozygosity excess was tested using the infinite allele mutation model (IAM) with 1,000 iterations. Although microsatellites were traditionally assumed to follow an approximate SMM model (Di Rienzo et al. 1994, Piry et al. 1999), more recent evidence suggests that recent bottlenecks may be better detected under an IAM (Cristescu et al. 2010). Significance was assessed using the Wilcoxon signed rank test.
(Cornuet and Luikart 1996). Second, we examined allele frequency distributions for each locus and population. Populations in mutation-drift equilibrium tend 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 tend 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 is most suitable for detecting bottlenecks occurring relatively recently (i.e. a few generations ago; Luikart et al. 1998).

Landscape genetics

We adopted a landscape genetic approach to understand the relationship between landscape structure, historical migration, contemporary migration and mtDNA divergence. We first created resistance surfaces in ArcMap 10 (ESRI) based on landscape variables shown to be important in shaping functional connectivity for this species (see Blair et al., 2013 for details). Next, we used Circuitscape 3.5.7 (Shah and McRae 2008) to calculate pairwise resistance distances between populations based on each resistance surface. We then used a matrix regression approach (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, we averaged both values to obtain a single rate between populations. Although averaging rates potentially discarded valuable information regarding source-sink dynamics, it was a necessary step to correlate these values with resistance distance between populations. We assumed that if recent fragmentation was altering dispersal routes then we would observe a
higher correlation between contemporary migration and landscape versus historical migration and landscape. All regression analyses were conducted in ECODIST. As a second measure of historical migration we correlated resistance distances with pairwise mtDNA sequence divergence ($D_{xy}$). We did not perform regressions using migration rate estimates based on the mtDNA data because of difficulty in parameter estimation (see Results).

Results

mtDNA diversity and phylogenetic analysis

We recovered 2400 bp of mtDNA sequence data for 77 individuals from 11 populations distributed throughout the immediate area surrounding Alamos. The total number of bp per partition, the proportion of potentially phylogenetically informative characters, and selected models of sequence evolution were shown in Supplementary Table S3. Bayes Factors suggested the most heavily partitioned dataset (by gene and codon position) to be the most efficient (Supplementary Table S4). Thus, all Bayesian analyses were based on this partitioning strategy. MP analysis resulted in eight most parsimonious trees (MPTs) of 723 steps. Both MP and BI resulted in a highly supported genealogical hypothesis comprised of four mitochondrial lineages differing up to 6% (uncorrected $p$-distances; Fig. 2). Lineage A was the most divergent and was comprised solely of individuals from Choquincahui. This population was sister to the remaining populations with strong statistical support from both MP bootstrap proportions (BSP = 99) and Bayesian posterior probabilities (BPP = 1.0). The remaining three lineages were also supported by moderate to high statistical support from both inference methods (Lineage B = 53/0.80;
Lineage C = 96/1.0; Lineage D = 100/1.0) and showed east-to-west geographic structuring.

Populations central to our study area served as a secondary contact zone for multiple divergent mitochondrial lineages, whereas no haplotypes were shared among eastern and western populations (Fig. 2). Sequence divergence between Lineages B, C, and D approached 3% uncorrected p-distances.

Population genetic analysis of mtDNA

Standard nucleotide diversity statistics for nine populations sampled for mtDNA polymorphism were presented in Supplementary Table S5. As expected, genetic diversity was greater for populations composed of haplotypes from multiple lineages (e.g. \( \pi = 0.0147 \) vs. 0.0032). Haplotype diversity was high in all populations, with a value of 1.0 recovered for several populations. Significant population differentiation was present throughout the region (Supplementary Table S5). 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 versus populations east of Alamos. Rio Cuchujaqui differed from western populations by approximately 29.059 changes or 0.0239 substitutions per site. The results of the Mantel and reduced major axis regression (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.

Historical and contemporary migration rates
Multiple runs of BAYESASS and MIGRATE for the microsatellite data gave consistent results within each program, indicating that the MCMC chains were mixing well and adequately sampling from the posterior distribution. Contemporary migration rates estimated from BAYESASS (i.e. proportion of migrants per generation) were generally low to moderate and for most population pairs 95% confidence intervals included zero (Supplementary Table S7).

However, some pairs show signs of exchanging a relatively high number of migrants per generation. For example, migration rates from Aduana to the Road to Navojoa population were 0.155, and rates from El Quintero to Choquincahui were 0.256. Conversely, 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 (Supplementary Table S7). We 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. Mean historical estimates of $m$ were approximately one-tenth the size of contemporary $m$ (Fig. 3) with no significant correlation detected ($\text{Mantel} \ r = 0.2592, \ p = 0.055$). We could not obtain reliable parameter estimates using the mtDNA data in MIGRATE (results not shown). This was the case even after altering priors, increasing chain length and decreasing the number of populations. Therefore, these analyses were not pursued further.

Historical and contemporary effective population sizes
For estimates of contemporary $N_e$, we focused our results based on a $P_{\text{crit}}$ value of 0.02 as this value was shown to be an adequate choice to minimize bias and maximize precision in most situations (Waples and Do 2010). Estimates for Road to Navojoa 1, Arroyo Tabelo, and Mocuzari showed relatively small sizes in comparison to all remaining populations (Table 1). Point estimates from Aduana and El Quintero were negative. Although 95% confidence intervals were broad, estimates of historical $N_e$ were generally greater than contemporary estimates (Table 1). Tests for recent population bottlenecks using the excess heterozygosity method showed recent reductions in $N_e$ for all populations except La Sierrita (Table 2). Tests for mode-shifts in allele frequency distribution showed an L-shaped curve for all populations except for the Road to Navojoa 1 population, which showed a shifted distribution.

Landscape genetics

Results from the regression analyses indicated significant correlations between landscape resistance and contemporary migration rates in comparison to historical rates and mtDNA divergence (Table 3). There was no significant correlation 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), whereas correlations in contemporary models ranged from 0.0214 for slope to 0.1994 for Euclidean distance (mean= 0.1268). Euclidean distance was the only variable significantly correlated with mtDNA divergence.

Discussion
Disentangling the relative influence of historical and contemporary processes in shaping the spatial distribution of genetic variation is currently a major research focus of landscape genetics (Vandergast et al. 2007; Spear and Storfer 2008; Anderson et al. 2010; Burbrink 2010; Dyer et al. 2010; Wang 2010). We expand upon previous studies (e.g. Chiucchi and Gibbs 2010) utilizing a spatially explicit approach to understand the temporal dynamics of demographic processes. We demonstrate high levels of lineage divergence over small spatial scales, consistent with high female philopatry and low levels of historical gene flow. In contrast to our expectations, we find an increase in contemporary versus historical migration rates, but find evidence that landscape heterogeneity better correlates with contemporary gene flow versus historical estimates. We also find evidence for recent reductions in $N_e$ for many populations.

Below we extrapolate on these major findings and discuss the utility of adopting similar multi-tiered approaches to understand the demographic consequences of recent habitat modification.

Sequence divergence and mitochondrial genealogy

We find substantial lineage divergence among populations of leaf-toed geckos with divergence values approaching 6%. If we assume the widely used vertebrate mtDNA divergence rate of 2% per million years, divergence between lineages occurred at least 3 million years ago illustrating the influence of historical processes. As no major biogeographic barrier separates these deep lineages, these results suggest a high degree of female philopatry through evolutionary history. Substantial geographic structure and significant IBD are detected with an apparent east-west pattern (Fig. 2). Further, several populations central to the study area show signs of sympatric lineages. It is possible that these sites are composed of hybrids of eastern and western populations. For example, Blair et al. (2013) find evidence of substantial admixture within
geckos from many of these areas. Additional taxonomic work is needed to clarify the specific status of these individuals. However, combining our mtDNA results (>6% sequence divergence) with previous nuclear DNA evidence (Blair et al. 2013) suggests that geckos from Choquincahui and El Quintero are not exchanging genetic information with other populations and should be recognized as a distinct species. In addition, the fact that no haplotypes are shared among the most eastern and western populations suggests the notion that home range sizes of these lizards are small and dispersal capabilities are limited to <20 km (Fig. 2). These results are consistent with previous studies and provide further evidence that many gecko species possess small home-range sizes (Gübitz et al. 2000; Kaspidis et al. 2005; Rato et al. 2011).

**Historical and contemporary demography**

To date, only a small handful of studies have simultaneously tested for similarities in historical and contemporary demographic parameters with the majority focusing on temperate species (e.g. Ross et al. 2007; Andersen et al. 2009; Schmidt et al. 2009; Muscarella et al. 2011). This is problematic, as tropical regions are generally considered to be global hotspots of biodiversity (Myers et al. 2000). In addition, tropical ecosystems (and TDF in particular) continue to suffer from high rates of habitat fragmentation (Janzen 1988; Trejo and Dirzo 2000). Thus, from a conservation perspective, additional studies are needed to quantify temporal dynamics in key demographic parameters in species distributed throughout these ecosystems.

We find weak evidence for a reduction in contemporary versus historical migration rates. Conversely, our results suggest that contemporary migration rates may be an order of magnitude higher in many cases. Further, our Mantel test shows no significant correlation between these two estimates. This suggests that recent habitat fragmentation is not impacting the ability of
individuals to disperse. These conclusions are based on an estimated microsatellite mutation rate of $5 \times 10^{-4}$ recommended by previous studies (e.g. Garza and Williamson 2001; Waples and Do 2010). Although a range of microsatellite mutation rates have been estimated for humans (Ellegren 2004), an unrealistically high value of 0.01 would need to be assumed to yield comparable historical and contemporary migration rates in these geckos. Thus, these results provide strong evidence for recent increases in pairwise migration rates.

Although a direct explanation for higher contemporary migration rates is presently untenable, it is possible that these results are a due to anthropogenic influence. It is well-known that gecko species are commonly encountered in and around human settlements. Thus, it is possible that the relatively recent colonization of humans in the area has increased migration rates via hitchhiking. Additional studies conducted on gecko species will be useful to determine if similar patterns occur in other taxa distributed in areas with varying anthropogenic influence.

Acknowledging the large confidence intervals recovered in our analyses, historical $N_e$ estimates for several populations are generally larger than contemporary estimates, suggesting that recent habitat fragmentation and/or introduction of competing species ($Hemidactylus$) has reduced $N_e$ in these areas irrespective of an increase in overall migration rate. Further, the majority of populations show signs of a recent bottleneck. A finding of recent bottlenecks and reduced contemporary $N_e$ in a region of relatively intact Mexican TDF (Fig. 2) suggests that similar studies are needed for other less-common TDF taxa inhabiting even more fragmented forest patches through western Mexico. Quantifying the demographic consequences of habitat fragmentation in diverse taxa is imperative if adequate conservation measures are to be implemented throughout Mexican TDF.
There has been considerable recent debate as to 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 and Vandergast 2011). To date, most landscape genetic studies correlate different effective distances with genetic distance using an $F_{ST}$-like metric based on microsatellite loci. Here, we use different molecular markers and analytical methods to test if correlations between the landscape and population connectivity are larger when using estimates of contemporary population genetic metrics (e.g. contemporary migration). Our results suggest that the landscape is significantly more correlated with contemporary versus historical migration rates, as would be expected if populations are altering dispersal routes in response to recent habitat changes.

Correlations between landscape structure and mtDNA divergence contrast sharply with those based on contemporary migration rate. 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). We assume that the choice of mtDNA as a marker for many of these studies is partly a factor of cost and ease of data collection. However, sole reliance on mtDNA comes with issues that must be acknowledged including the stochasticity of the lineage sorting process, relatively slow mutation rates and the fact that mtDNA is maternally inherited (Wang 2010). The fact that we obtain drastically different correlations when using mtDNA versus microsatellites raises 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 and require the use of more sophisticated spatial analysis and molecular markers powerful enough to give adequate resolution at the spatial and temporal scale under investigation (Cushman et al. 2006). Our results suggest that though mtDNA may be an acceptable marker to test hypotheses in deep time, it may not be the ideal marker for modeling complex landscape-genetic relationships. We agree with Wang (2010) that researchers should be explicit about their hypotheses and the timeframe under consideration as to avoid erroneous conclusions regarding landscape influences on genetic divergence.

Conclusions

Our results suggest a complex demographic history in leaf-toed geckos and that studies that simultaneously examine historical and contemporary demographic parameters can serve as a powerful tool to understand the population genetic consequences or recent habitat fragmentation. We find high levels of mtDNA divergence over small spatial scales and evidence for recent reductions in $N_e$ for several populations. Although our results suggest relatively high levels of contemporary migration, landscape genetic analyses suggest that geckos are altering dispersal routes in response to habitat changes. Finally, our results suggest that mtDNA is poor marker for inferring contemporary landscape genetic relationships, presumably due to its slow mutation rate and stochasticity associated with the coalescent process. As TDF contains an extraordinary diversity of species (Robichaux and Yetman 2000), yet remains a conservation concern (Trejo and Dirzo 2000), additional molecular genetic studies are needed to assess recent demographic changes in the native flora and fauna. Only then will conservation initiatives be most effective.
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Blair et al. 23

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Table 1. Estimates of contemporary and historical effective population sizes ($N_e$). Contemporary $N_e$ was estimated in the program LDNe using a $P_{\text{crit}}$ value of 0.02 (Waples and Do 2010). Historical $N_e$ was calculated from theta estimated in MIGRATE and assuming a microsatellite mutation rate of $5 \times 10^{-4}$ (Garza and Williamson 2001; Waples and Do 2010). Values in parentheses represent 95% confidence intervals.

<table>
<thead>
<tr>
<th>Locality</th>
<th>n</th>
<th>Contemporary $N_e$</th>
<th>Historical $N_e$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Road to Navojoa 1</td>
<td>10</td>
<td>25.4 (15.5–56.0)</td>
<td>483.355 (0–1333)</td>
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<td>36</td>
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<td>749.5 (0–1566)</td>
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<td>703.34 (0–1500)</td>
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<td>Aduana</td>
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<td>-331 (74.1–infinity)</td>
<td>501.685 (0–1333)</td>
</tr>
<tr>
<td>Rio Cuchujaqui</td>
<td>72</td>
<td>636.7 (225.3–infinity)</td>
<td>1456.435 (466–2400)</td>
</tr>
<tr>
<td>Sierrita</td>
<td>38</td>
<td>142.9 (65.2–infinity)</td>
<td>668.235 (0–1500)</td>
</tr>
<tr>
<td>Mocuzari</td>
<td>30</td>
<td>29.2 (21.0–43.9)</td>
<td>272.6 (0–1066)</td>
</tr>
<tr>
<td>El Quintero</td>
<td>30</td>
<td>-865.0 (110.9–infinity)</td>
<td>432.8 (0–1266)</td>
</tr>
<tr>
<td>Choquincahui</td>
<td>31</td>
<td>121.3 (43.9–infinity)</td>
<td>296.295 (0–1100)</td>
</tr>
<tr>
<td>San Antonio</td>
<td>15</td>
<td>224.4 (45.2–infinity)</td>
<td>323.865 (0–1133)</td>
</tr>
</tbody>
</table>
Table 2. Results of tests for population bottlenecks performed using the software Bottleneck. Wilcoxon IAM represents P-values based on the Infinite Allele 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 IAM</th>
<th>Allelic Distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Road to Navojoa 1</td>
<td>10</td>
<td>0.042</td>
<td>Shifted</td>
</tr>
<tr>
<td>Alamos</td>
<td>36</td>
<td>0.001</td>
<td>Normal</td>
</tr>
<tr>
<td>Arroyo Tabelo</td>
<td>57</td>
<td>0.001</td>
<td>Normal</td>
</tr>
<tr>
<td>Aduana</td>
<td>17</td>
<td>0.001</td>
<td>Normal</td>
</tr>
<tr>
<td>Rio Cuchujaqui</td>
<td>72</td>
<td>0.001</td>
<td>Normal</td>
</tr>
<tr>
<td>La Sierrita</td>
<td>38</td>
<td>0.065</td>
<td>Normal</td>
</tr>
<tr>
<td>Mocuzari</td>
<td>30</td>
<td>0.001</td>
<td>Normal</td>
</tr>
<tr>
<td>El Quintero</td>
<td>30</td>
<td>0.005</td>
<td>Normal</td>
</tr>
<tr>
<td>Choquincahuí</td>
<td>31</td>
<td>0.001</td>
<td>Normal</td>
</tr>
<tr>
<td>San Antonio</td>
<td>15</td>
<td>0.002</td>
<td>Normal</td>
</tr>
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</table>
Table 3. Regression on distance matrices results showing the relationships between landscape resistance and historical migration, contemporary migration, and mtDNA sequence divergence ($D_{xy}$). Model M = MIGRATE; Model B = BAYESSASS; Model MtDNA = mtDNA divergence. Models in italics significant at an alpha = 0.05.

<table>
<thead>
<tr>
<th>Model</th>
<th>Variables</th>
<th>$\beta$</th>
<th>Model $R^2$</th>
<th>$P$</th>
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<tr>
<td>M1</td>
<td>Euclidean</td>
<td>-8.2E-09</td>
<td>0.0337</td>
<td>0.231</td>
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<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>Anthropogenic</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></td>
<td></td>
<td></td>
<td></td>
<td></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>
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<td>0.0587</td>
<td>0.0656</td>
</tr>
<tr>
<td>B3</td>
<td>Forest</td>
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<td>0.1768</td>
<td>0.0077</td>
</tr>
<tr>
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<td>Stream</td>
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<td>0.008</td>
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<tr>
<td>B6</td>
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<td>0.0214</td>
<td>0.2446</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mt1</td>
<td>Euclidean</td>
<td>1.78E-06</td>
<td>0.6235</td>
<td>0.0193</td>
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<tr>
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<td>Temp</td>
<td>0.002</td>
<td>0.558</td>
<td>0.1135</td>
</tr>
<tr>
<td>Mt3</td>
<td>Forest</td>
<td>0.003</td>
<td>0.3978</td>
<td>0.0724</td>
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<tr>
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<td>Stream</td>
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<td>Anthropogenic</td>
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<td>0.0029</td>
<td>0.8827</td>
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<tr>
<td>Mt6</td>
<td>Slope</td>
<td>0.001</td>
<td>0.5219</td>
<td>0.1012</td>
</tr>
</tbody>
</table>
Figure Legends

Fig. 1. Map of the Alamos region of Sonora showing sampling locality information for all *Phyllodactylus tuberculosis saxatilis* included in this study. Gray shading represents the current distribution of tropical dry forest throughout the landscape. Lines represent stream, rivers and arroyos throughout the study area. Triangles = sites that were assayed for mtDNA polymorphism; circles = sites assayed for microsatellite DNA polymorphism (Blair et al. 2013); squares = sites assayed for both mtDNA and microsatellite polymorphism. Populations targeted for mtDNA sequencing were based on genetic results from previous studies (Blair et al. 2013). Values in parentheses adjacent to site names represent sample sizes (mtDNA, microsatellite DNA). Inset shows the general area of Alamos, Sonora within Mexico.

Fig. 2. Bayesian majority rule phylogram resulting from the optimal partitioning strategy of gene + codon position. Numbers above nodes represent 10,000 nonparametric bootstrap proportions using maximum parsimony. Numbers below nodes represent Bayesian posterior probabilities sampled from the posterior distribution of trees. Gray shading represents the current distribution of tropical dry forest throughout the landscape. Lines represent stream, rivers and arroyos throughout the study area. Colored pies represent the mtDNA lineages represented at specific sites.

Fig. 3. A) Kernel density plot of historical migration rates (*m*) estimated from MIGRATE. B) Kernel density plot of contemporary migration rates (*m*) estimated from BAYESASS.