MOLECULAR ECOLOGY

Molecular Ecology (2010) **19**, 1860–1876 doi: 10.1111/j.1365-294X.2010.04616.x

Climatic stability and genetic divergence in the tropical insular lizard *Anolis krugi*, the Puerto Rican 'Lagartijo Jardinero de la Montaña'

JAVIER A. RODRÍGUEZ-ROBLES,* TEREZA JEZKOVA* and MANUEL LEAL†

*School of Life Sciences, University of Nevada, Las Vegas, 4505 Maryland Parkway, Las Vegas, NV 89154-4004 USA, †Department of Biology, Duke University, Durham, NC 27708-0338, USA

Abstract

Two factors that can lead to geographic structuring in conspecific populations are barriers to dispersal and climatic stability. Populations that occur in different physiographic regions may be restricted to those areas by physical and/or ecological barriers, which may facilitate the formation of phylogeographic clades. Long-term climatic stability can also promote genetic diversification, because new clades are more likely to evolve in areas that experience lesser climatic shifts. We conducted a phylogeographic study of the Puerto Rican lizard *Anolis krugi* to assess whether populations of this anole show genetic discontinuities across the species' range, and if they do, whether these breaks coincide with the boundaries of the five physiographic regions of Puerto Rico. We also assessed whether interpopulation genetic distances in *A. krugi* are positively correlated with relative climatic stability in the island. *Anolis krugi* exhibits genetic structuring, but the phylogroups do not correspond to the physiographic regions of Puerto Rico. We used climatic reconstructions of two environmental extremes of the Quaternary period, the present conditions and those during the last glacial maximum (LGM), to quantify the degree of climatic stability between sampling locations. We documented positive correlations between genetic distances and relative climatic stability, although these associations were not significant when corrected for autocorrelation. Principal component analyses indicated the existence of climatic niche differences between some phylogeographic clades of *A. krugi*. The approach that we employed to assess the relationship between climatic stability and the genetic architecture of *A. krugi* can also be used to investigate the impact of factors such as the spatial distribution of food sources, parasites, predators or competitors on the genetic landscape of a species.

Keywords: Caribbean Sea, climatic stability, cytochrome *b*, ecological niche models, evolutionary diversification, mitochondrial DNA, ND2, phylogeography, population genetics, Puerto Rico, Quaternary, West Indies

Received 7 May 2009; revision received 21 February 2010; accepted 24 February 2010

Introduction

Genetic assessments of insular species sometimes reveal little genetic differentiation among conspecific populations (demes) (e.g. Rodríguez-Robles *et al*. 2007; Browne *et al*. 2008), suggesting that the members of those species have good dispersal capabilities and that the demes have occupied ranges free of firm, long-standing barriers to gene flow, or that those populations have experienced recent demographic expansion (Avise 2000). On the contrary, some surveys uncover hidden levels of genetic diversity in island taxa, indicating that some segments of the species are demographically independent. In some cases, the genetic breaks correspond at least in part to geographic morphological differences

Correspondence: Javier A. Rodríguez-Robles, Fax: +1 702 895 3956; E-mail: javier.rodriguez@unlv.edu

among conspecific populations, and may be recognized (at the taxonomic level) to some extent already (Malhotra & Thorpe 2000; Glor *et al.* 2003; Funk & Fa 2006; Hedges & Heinicke 2007; Dehghani *et al.* 2008). However, in many cases, taxa that lack obvious spatial variation in morphology display marked genetic discontinuities (e.g. Bond & Sierwald 2002; Jackman *et al.* 2002; Brown *et al.* 2006; Jezkova *et al.* 2009b), indicating that these species consist of previously unrecognized cryptic lineages. Identifying these units is essential to accurately characterize present levels of biodiversity, and to elucidate the evolutionary processes responsible for generating such diversity.

Two factors that can lead to phylogeographic structuring in conspecific populations are barriers to dispersal and climatic stability (Jansson 2003; Noonan & Wray 2006; Araújo *et al.* 2008; Carnaval & Moritz 2008). Populations that occur in different physiographic regions may be at least partially restricted to those areas by physical and/or ecological dispersal barriers that may facilitate the formation of geographic arrays of differentiated populations. Climatic conditions can also result in the geographic structuring of genetic variance. In particular, it has been proposed that long-term climatic stability promotes genetic diversification (Cronk 1997; Graham *et al.* 2006; Araújo *et al.* 2008). Under this hypothesis, new clades are more likely to evolve and persist, with a lower risk of extinction or introgression with other clades, in areas that experience lesser climatic shifts (Jansson 2003; Graham *et al.* 2006). Evidence supporting the historic climate stability hypothesis comes from studies of terrestrial vertebrates from continental areas (Graham *et al.* 2006; Araújo *et al.* 2008; Carnaval *et al.* 2009). Paleoclimatological reconstructions typically indicate that these continental regions include large stable areas (refugia) with high levels of genetic (or species) diversity, and unstable (recently colonized) regions that experienced dramatic climatic fluctuations and that exhibit reduced genetic (or species) diversity. Smaller geographic areas (e.g. many tropical and subtropical islands) may not have undergone the drastic changes in ecological conditions that larger continental regions have, but these smaller landmasses may still have experienced detectable differences in climatic stability among neighbouring locations. Accordingly, herein we test a corollary of the historical climate stability hypothesis, namely that different degrees of climatic stability generate detectable differences in levels of genetic variation among conspecific populations. Specifically, we predicted that if paleoclimatic reconstructions suggest varying degrees of climatic stability among insular localities, then phylogeographic studies of island endemics will reveal that populations from the more stable areas exhibit higher levels of genetic variability.

Puerto Rico, the smallest and most easterly of the Greater Antilles in the eastern Caribbean Sea, is a volcanic island with a rugged topography. Puerto Rico can be divided into five physiographic regions (Picó 1974; Hedges 1999; Fig. 1): (i) the lowlands (maximum elevation: *c.* 200 m), (ii) the Cordillera Central (=Central Uplands; maximum elevation: *c.* 1340 m), a mountain

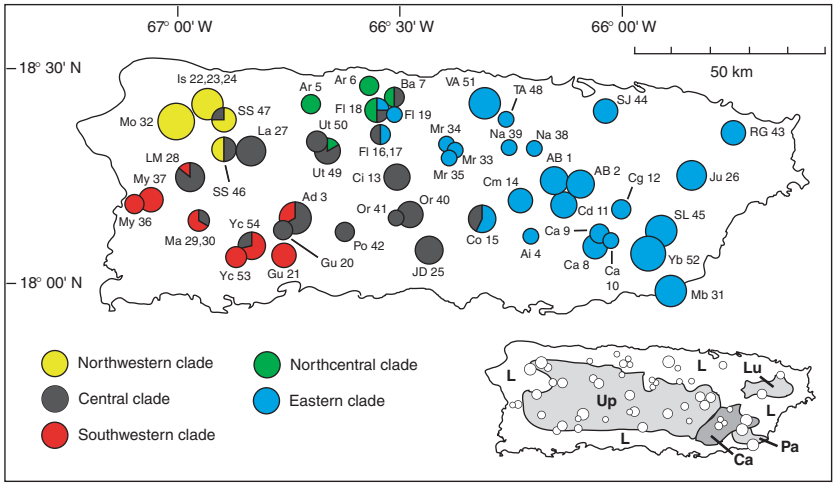

Fig. 1 Map of Puerto Rico. Circles indicate the approximate locations of the specimens of *Anolis krugi* included in this study (see Table S1, Supporting Information, for specific locality information), and the different colours represent clades identified by the maximum likelihood and Bayesian inference methods. Circle size is proportional to sample size, with the smallest circle representing one sample, and the largest one (Mo 32) representing 12 samples. Inset: Map of Puerto Rico, indicating the five physiographic regions of the island. The contour is 200 m in elevation. Abbreviations used are: Ca, Sierra de Cayey; L, Lowlands; Lu, Sierra de Luquillo; Pa, Cuchilla de Pandura; Up, Central Uplands.

system that extends in an east–west direction, (iii) the Sierra de Cayey (maximum elevation: *c.* 900 m), a southeastern extension of the Cordillera Central, (iv) the Sierra de Luquillo (maximum elevation: *c.* 1070 m), an isolated mountain range on the northeastern part of the island and (v) the Cuchilla de Pandura, a low (maximum elevation: *c.* 525 m), narrow, southeastern range consisting mostly of igneous rocks in the form of giant boulders. Because the boundaries of the five areas can constitute barriers to dispersal, species distributed across these regions may exhibit genetic breaks congruent with those boundaries.

Anolis krugi, the 'Lagartijo Jardinero de la Montaña' (Mountain Garden Lizard) is a small, sexually dimorphic lizard (maximum reported body size [=snout-to-vent length] is 45 mm in males and 36 mm in females; Schwartz & Henderson 1991). The lizard is most commonly found less than 1 m above the ground, on leaves and perches of small diameter (Rivero 1998; Henderson & Powell 2009). The species is endemic to Puerto Rico, where it widely occurs in shaded conditions in mesic habitats from near sea level up to *c.* 1300 m of elevation (personal observation). Populations of *A. krugi* are phenotypically similar, but the fact that the geographic range of the species encompasses the five physiographic regions of Puerto Rico raises the possibility that these demes may form spatially circumscribed phylogeographic clades.

We conducted a phylogeographic study of *A. krugi* to assess whether populations of this anole show genetic discontinuities across the species' range, and if they do, whether these breaks coincide with the boundaries of the five physiographic regions of Puerto Rico. We also used climatic reconstructions of two environmental extremes of the Quaternary period (*c.* 1.8 Ma–present; Bell *et al.* 2004), the present conditions and those during the LGM (*c.* 21 000 years ago; Harrison 2000), to evaluate whether historical climatic patterns in Puerto Rico have influenced the distribution and genetic structuring of *A. krugi*. We specifically tested the hypothesis that populations of *A. krugi* from more climatically stable areas exhibit greater interpopulation genetic distances than do populations from localities that have experienced greater climatic shifts.

Materials and methods

Taxon sampling and laboratory methods

We collected tissue samples from 211 individuals of *A. krugi* from 54 localities across the entire geographic range of the species (Fig. 1; Table S1, Supporting Information). Sample size varied from 1 to 12 lizards per locality, with >50% of the populations being represented by four or more individuals.

We isolated total genomic DNA from frozen tissue samples (heart, liver or tail fragments) using the DNeasy Blood & Tissue Kit (Qiagen Inc., Valencia, CA, USA). We used the primers LVT_Metf.6_AnCr (AAGCTATTGGGCCCATACC) and LVT_5617_AnCr (AAAGTGYTTGAGTTGCATTCA; Rodríguez-Robles *et al.* 2007) to amplify *c.* 1150 bp of the nicotinamide adenine dinucleotide dehydrogenase (NADH) subunit 2 ('ND2') and adjacent tRNAs ($tRNA^{Trp}$, $tRNA^{Ala}$), and we used the primers MVZ_49 (ATAARAACAATGACAATYATACGAAG; Roe *et al.* 1985) and MVZ_14 (GGTCTTCATCTYHGGYTTACAAGAC) to amplify *c.* 1100 bp of cytochrome *b* ('Cyt *b*'). We carried out PCRs in 12.5 volumes consisting of 1 μL of template DNA, 0.5 μL of each primer (10 μM), 6.25 μL of Takara Ex Taq™ Polymerase Premix (Takara Mirus Bio Inc., Madison, WI, USA), and 4.25 μL of ddH_2O. DNA was denatured initially at 95 °C for 2.5 min, and then 30–35 cycles of amplification were performed under the following conditions: denaturation at 95 °C for 1 min, annealing at 57 °C (for ND2) or 51 °C (for Cyt *b*) for 1 min, and extension at 72 °C for 1 min, followed by a final 10 min elongation at 72 °C. Two microlitres of all PCR products were electrophoresed on a 0.8% agarose gel stained with ethidium bromide to verify product band size.

We cleaned the double-stranded PCR products with ExoSap-IT® (USB Corporation, Cleveland, OH, USA). We sequenced 1044 bp of the ND2 fragment using the primers LVT_Metf.6_AnCr and LVT_L5002_AnPu (AACCAAACACARACTCGAAAAAT), and 957 bp of the Cyt *b* fragment using the primers MVZ_49 and MVZ_14. We used the Big Dye Terminator Ready Reaction Kit 3.1 (Applied Biosystems, Foster City, CA, USA) for cycle sequencing, and ran the sequences on an ABI 3130 automated sequencer.

Phylogenetic and median-joining network analyses

We used the program COLLAPSE (version 1.2; available at http://darwin.uvigo.es) to collapse the 211 sequences to 174 unique haplotypes (=mitochondrial types). An incongruence length difference (ILD) test (Farris *et al.* 1994) performed with the program PAUP* (version 4.10b; Swofford 2003) indicated that the sequences from the ND2 (1044 bp) and Cyt *b* (957 bp) genes contained congruent phylogenetic signal (100 replicates, $P = 0.74$). Accordingly, we combined the ND2 and Cyt *b* data sets for all subsequent analyses. Based on previous karyotypic, electrophoretic, and phylogenetic studies (Gorman *et al.* 1968, 1983; Brandley & de Queiroz 2004; Poe 2004; Nicholson *et al.* 2005), we used *Anolis*

gundlachi, *A. poncensis* and *A. pulchellus* as outgroup taxa. We performed all phylogenetic analyses using maximum likelihood (ML) and Bayesian inference (BI) methods.

Because many haplotypes were only separated by few mutations, analysing our large data set became time-prohibitive. Due to these computational difficulties, we used the star contraction method (Forster *et al.* 2001) in the program NETWORK (version 4.200; http://www.fluxus–technology.com) to prune the combined data set for phylogenetic analyses. This method identifies star-like clusters of haplotypes representing newly emerging mutations (hereafter referred to as 'satellite haplotypes') around a founder (ancestral) node, and reduces a cluster to the founder node (Forster *et al.* 2001). Using a contraction value of five mutational steps, we reduced the data set from 174 to 102 sequences, of which 95 are original haplotypes and seven are median vectors (Bandelt *et al.* 1999; Table S2, Supporting Information). Median vectors represent either unsampled or extinct, ancestral haplotypes necessary to connect the associated satellite haplotypes in the most parsimonious way. We used the reduced data set of 102 sequences for all ML and BI analyses.

We partitioned the data set by codon (first and second codon positions combined; third codon position), and identified the best-fitting model of nucleotide substitution for each partition using MRMODELTEST (version 2.2; Nylander 2004). Hierarchical likelihood ratio tests and Akaike Information Criteria identified GTR + I + G (for the first and second codon positions combined) and GTR + G (for the third codon position) as the most appropriate models of nucleotide substitution for the ingroup data for the ML and BI analyses.

We conducted ML analyses using the program TREEFINDER (Jobb *et al.* 2004). TREEFINDER uses a fast sampling algorithm to estimate all model parameters and construct a phylogeny. We used the 'Bootstrap Analysis' option in TREEFINDER (200 replicates, consensus level, 50) to determine nodal support. We also assessed tree topology and clade support using the program MRBAYES (version 3.1.1; Ronquist & Huelsenbeck 2003). We initiated the analyses from a random starting tree with uniform (uninformative) priors (Brandley *et al.* 2006). We produced posterior probability distributions by allowing four Monte Carlo Markov Chains with a heating value of 0.05 (to increase swapping among trees) to proceed for five million generations each, with samples taken every 100 generations, a procedure that yielded 50 000 trees. After visual evaluation (Leaché & Reeder 2002), we discarded the first 1 250 000 generations (12 500 trees) as 'burn-in' samples (trees obtained before parameter stabilization occurred), and combined the remaining samples to estimate tree topology, posterior probability values, and branch lengths. We ran the Bayesian analyses twice to ensure that the chains were not trapped on local optima. We confirmed that the contracted data set did not alter tree topology by conducting an additional run on the entire data set (174 unique haplotypes) using the same parameters as above, except that we decreased the heating value to 0.02.

We constructed a median-joining network of the 211 sequences of *A. krugi* using the program NETWORK (http://www.fluxus-technology.com; Bandelt *et al.* 1999). The median-joining method uses a maximum parsimony approach to search for all the shortest phylogenetic trees for a given data set (Bandelt *et al.* 1999). To construct the network, we weighted transversions twice as high as transitions (as recommended in the NETWORK manual). After generating the initial network, we used the MP option in NETWORK to remove excessive links and median vectors and construct the final median-joining network (Polzin & Daneshmand 2003).

Phylogeography

We explored patterns of genetic divergence among sampling localities of *A. krugi* using the program SAMOVA 1.0 (Spatial Analysis of Molecular Variance; Dupanloup *et al.* 2002; http://web.unife.it/progetti/genetica/Isabelle/samova.html). SAMOVA is a nonphylogenetic method based on *F*-statistics (also known as fixation indices; Wright 1951). In SAMOVA, information about mtDNA sequence divergence represented by pairwise distances among haplotypes is incorporated into an analysis of variance format (Excoffier *et al.* 1992). SAMOVA identifies the partitions of sampling areas that are maximally differentiated from each other by optimizing the proportion of total genetic variance due to differences among groups of populations (i.e. the F_{CT} value; Dupanloup *et al.* 2002). SAMOVA recalculates the F_{CT} values for any particular number of requested population partitions, and therefore previously identified groupings are not necessarily preserved as additional ones are requested (Dupanloup *et al.* 2002). We performed the SAMOVA analyses based on 300 simulated annealing steps for $K = 2$ through $K = n–1$ partitions, where K is the number of genetically defined units (i.e. groups), and n is the number of populations included in the analysis.

We used AMOVA (analysis of molecular variance, as implemented by SAMOVA) to explore in more detail the partitioning of genetic variance among the maximally differentiated groups identified by SAMOVA. We compared indicators of differentiation among groups (F_{CT} values) and among populations within groups (F_{SC} values) for each value of K. F_{CT} values peak at values of K that maximize differentiation among groups, whereas

F_{SC} values of zero indicate that the groups are comprised of populations with the same level of genetic variation among populations and within populations. Consequently, F_{CT} values should peak at values of K that correspond to $F_{SC} = 0$. Gene flow, recent colonization events or incomplete lineage sorting within or among groups result in lower F_{SC} and F_{CT} values.

Ecological niche modelling

Recent advances in climate modelling and paleoenvironmental reconstructions have allowed simulations of climatic conditions of past time periods for relatively small geographic areas, including tropical and subtropical islands. Specifically, a set of coupled ocean–atmosphere simulations for the climatic conditions of the LGM is now available through the second phase of the Paleoclimate Modelling Intercomparison Project (Braconnot *et al.* 2007). We relied on these simulations to evaluate whether climatic conditions in Puerto Rico during the Quaternary period may have influenced patterns of genetic diversification in *A. krugi*.

We constructed ecological niche models [ENMs; also known as species distribution models, habitat models, bioclimatic envelopes, and resource selection functions (Elith & Graham 2009)] for *A. krugi* using the current climatic conditions and those of the LGM. Our goal was to assess whether the niche of *A. krugi* has experienced noticeable changes (e.g. shifts, contractions, expansions) during these two climatic extremes of the Quaternary (Hewitt 1999). We used the software package Maxent (version 3.2.1; Phillips *et al.* 2006; Phillips & Dudík 2008) to build the ENMs. Maxent creates ENMs by combining presence-only data with ecological layers, and finds a probability distribution of maximum entropy. Maxent has been used in several recent studies that compared current and historical species distributions (Carstens & Richards 2007; Rissler & Apodaca 2007; Waltari *et al.* 2007; Peterson & Nyári 2008; Jezkova *et al.* 2009a), and is believed to outperform similar methods of niche reconstruction (Elith *et al.* 2006; Phillips 2008).

To construct ENMs, we used the default parameters of Maxent (i.e. 500 maximum iterations, convergence threshold of 0.00001, 10 000 background points, regularization multiplier of 1, and autofeatures) with the following user-selected features: application of a random seed, duplicate presence records removal and logistic probabilities used for the output (Phillips & Dudík 2008). We converted the logistic probability values to presence-absence data using the 'minimum training presence' threshold, where the omission rate is set to zero. After removing all duplicate records (i.e. multiple records for a single grid cell), we obtained 45 unique occurrence records. For environmental layers of current climatic conditions, we downloaded 19 bioclimatic variables from the WorldClim data set (version 1.4) with resolutions of 30 s (*c.* 1 km) and 2.5 min (*c.* 5 km; Hijmans *et al.* 2005a). These variables are derived from monthly temperature and precipitation data layers, and represent biologically meaningful aspects of climate variation (Waltari *et al.* 2007; Peterson & Nyári 2008). Because some of the 19 bioclimatic variables are correlated (Kozak & Wiens 2006; Rissler & Apodaca 2007) we performed pairwise correlation comparisons between them. We considered two variables to be highly correlated when their correlation coefficient was ≥0.9. From pairs of variables that were highly correlated, we selected annual or quarterly averages over monthly averages, and consequently used the following eight variables to build the ENMs for *A. krugi*: mean annual temperature, isothermality, temperature seasonality, temperature annual range, annual precipitation, precipitation of wettest quarter, precipitation of driest quarter and precipitation of warmest quarter.

For environmental layers representing the climatic conditions of the LGM, we used two models: Community Climate System Model Version 3 (CCSM; Otto-Bliesner *et al.* 2006), with a resolution of 1°, and Model for Interdisciplinary Research on Climate Version 3.2 (MIROC; Hasumi & Emori 2004), with the original spatial resolution of 1.4 × 0.5° (Braconnot *et al.* 2007). These models use updated tropical cooling simulations and topographic changes (mainly caused by lowering sea levels) for the conditions of the LGM. The original variables were downloaded from the Paleoclimatic Modelling Intercomparison Project (PMIP phase II; http://pmip2.lsce.ipsl.fr/; Braconnot *et al.* 2007), and were further downscaled to a spatial resolution of 2.5 min by Robert J. Hijmans (Waltari *et al.* 2007; Peterson & Nyári 2008).

Climatic stability and the genetic landscape of A. krugi

We assessed whether the current genetic landscape of *A. krugi* can be explained by climatic stability on Puerto Rico during the Quaternary. We evaluated spatial genetic structuring in *A. krugi* by interpolating pairwise genetic distances across the landscape. We calculated genetic distances between sampling sites using the Delaunay triangulation-based connectivity network in the program Alleles in Space (Miller 2005; Miller *et al.* 2006). We used residual genetic distances (derived from the linear regression of genetic versus geographical distance) to account for the correlation between genetic and geographical distances (Manni *et al.* 2004; Miller *et al.* 2006). The genetic distances were imported into ArcGIS 9.2 (ESRI, Redlands, CA, USA) and interpolated across uniformly spaced 2.5 min (for statistical analyses)

and 30-s grids (for illustrative purposes) using the inverse distance weighted interpolation procedure (Watson & Philip 1985; Watson 1992) in the Spatial Analyst extension of ArcGIS 9.2. We used the default interpolation parameters in ArcGIS 9.2 to derive the final rasters.

From the 19 bioclimatic variables downloaded from the WorldClim data set we selected the 11 variables (seven temperature and four precipitation variables) that best described the habitat model of *A. krugi* (i.e. annual mean temperature, temperature seasonality, minimum temperature of the coldest month, mean temperature of the wettest quarter, mean temperature of the driest quarter, mean temperature of the warmest quarter, mean temperature of the coldest quarter, annual precipitation, precipitation of the wettest month, precipitation of the wettest quarter, precipitation of the warmest quarter). We selected these variables based on their relative contributions to the Maxent species distribution model and on their scores on the 'Jacknife of Regularized Training Gain' procedure (Phillips & Dudík 2008). We defined climatic stability as the difference between the current and the LGM values of a particular bioclimatic variable. We calculated this difference separately for the 11 variables, using both the CCSM and MIROC estimates of each LGM variable. We defined the environmental variables and genetic patterns as spatial rasters, and tested for correlation between them. We used the program SAM (Rangel *et al.* 2006) to calculate Spearman's correlation coefficients (r) and their corresponding P-values (uncorrected and corrected for spatial autocorrelation) to evaluate the statistical association between the genetic landscape of *A. krugi* and climatic stability.

Climatic niche comparisons among phylogeographic clades of A. krugi

We performed climatic niche comparisons among phylogeographic clades of *A. krugi* to determine whether these genetic groupings are ecologically differentiated. For occurrence records we used our sampling localities, and for climatic factors representing the environmental conditions at those localities we used the 19 bioclimatic variables downloaded from the WorldClim data set. We used the DIVA-GIS computer program (Hijmans *et al.* 2005b) to extract values for each of the 19 bioclimatic variables for all sampling sites. We compared the similarity of the climatic niches of phylogeographic clades by conducting a principal component analysis (PCA), following the methodology described in Kozak & Wiens (2006) and Rissler & Apodaca (2007). In systat (version 12; Hilbe 2008) we performed PCA to reduce the 19 bioclimatic variables to a smaller number of principal components that account for most of the variance in the observed variables. Only variables that had an eigenvalue ≥1 were retained for additional analysis. We then used an unbalanced anova, with axis scores from PCA as dependent variables and phylogeographic clade as the fixed factor, to assess whether the separation in the ecological niche was statistically significant. If there was an overall statistical significance, we employed a Tukey HSD post hoc test to determine which pairwise comparisons were significantly different.

Results

Phylogenetic and median-joining network analyses

The ML and Bayesian phylogenetic analyses recovered virtually identical topologies, and revealed a high degree of geographic structuring in *A. krugi*. The haplotypes of this anole are divided into five strongly supported phylogeographic clades (phylogroups): the 'Eastern', 'Central', 'Northwestern', 'Northcentral' and 'Southwestern' clades (Fig. 2). Uncorrected, pairwise sequence divergence among the five phylogroups varies from 0.015 to 0.033 (Table 1). The Eastern clade contains the greatest number of haplotypes (n = 78), followed by the Central (n = 49), Southwestern (n = 20), Northwestern (n = 19), and Northcentral clades (n = 8; Table 2). Haplotype diversity is very high (≥0.97) in each of the five phylogroups. The Eastern clade has four well supported subclades, but there is little phylogenetic structure in the remaining phylogroups. The Eastern clade has the largest geographic distribution, followed by the Central clade, whereas the Northwestern and Northcentral phylogroups are the most spatially restricted (Fig. 1). Maximum likelihood and Bayesian methods suggest that the Eastern and Central clades probably are each other's closest relative, but their relationship with the remaining three clades (i.e. Northwestern, Northcentral and Southwestern clades) is unresolved.

The Eastern clade consists of haplotypes from the five physiographic areas of the island (i.e. lowlands, Central Uplands, Sierra de Luquillo, Sierra de Cayey, Cuchilla de Pandura). The mitochondrial types of the Central clade predominantly occur in the western part of the Central Uplands, but are also present in the lowlands. The Northcentral clade is primarily composed of haplotypes from the lowlands, although it encompasses mitochondrial types from the Central Uplands as well. On the contrary, the Northwestern clade is mainly composed of haplotypes from the Central Uplands, but it also includes mitochondrial types from the lowlands. The Southwestern clade consists of mitochondrial types from the lowlands and the Central Uplands.

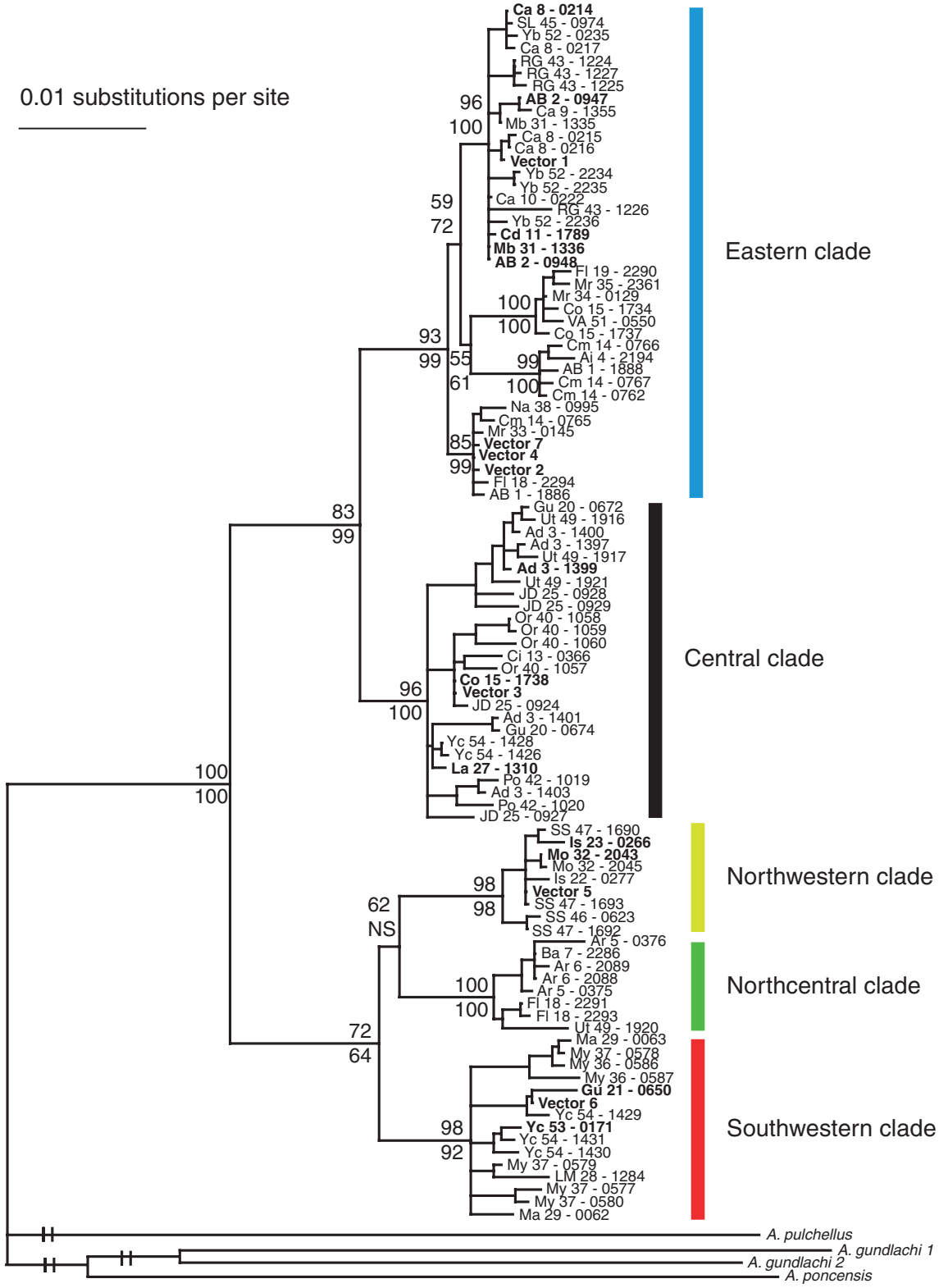

Fig. 2 Maximum likelihood tree for 95 unique mtDNA haplotypes and seven median vectors of *Anolis krugi*. *Anolis gundlachi*, *A. poncensis* and *A. pulchellus* were used as outgroup taxa. (Trees inferred with maximum likelihood and Bayesian methods recovered virtually identical topologies.) Nodal support was assessed with nonparametric bootstrap values for ML analyses (numbers above) and with Bayesian posterior probabilities (numbers below). See section 'Materials and methods' for details of phylogenetic analyses.

Table 1 Uncorrected pairwise sequence divergence among the five phylogroups of *Anolis krugi*

Phylogroup	Central	Northwestern	Northcentral	Southwestern
Eastern	0.015	0.032	0.033	0.033
Central	–	0.03	0.028	0.029
Northwestern	–	–	0.017	0.018
Northcentral	–	–	–	0.018

Table 2 Measures of haplotype and nucleotide diversity for the five phylogroups of *Anolis krugi* (SD, standard deviation)

Phylogroup	No. of gene copies (no. of haplotypes)	Haplotype diversity (±SD)	Nucleotide diversity (±SD)	Mean no. of pairwise differences (±SD)
Eastern	93 (78)	0.99 (±0.01)	0.00679 (±0.00341)	13.6 (±6.2)
Central	61 (49)	0.98 (±0.01)	0.00688 (±0.00347)	13.8 (±6.3)
Northwestern	25 (19)	0.97 (±0.02)	0.00271 (±0.0015)	5.4 (±2.7)
Northcentral	8 (8)	1.0 (±0.06)	0.00537 (±0.00312)	10.8 (±5.5)
Southwestern	24 (20)	0.98 (±0.02)	0.00957 (±0.00489)	19.1 (±8.8)
Total	211 (174)			

Eleven populations included haplotypes belonging to more than one phylogroup, indicating the existence of population-level admixture of mtDNA haplotypes. All these 'mixed' populations are geographically intermediate between or among different geographic clades. Ten populations (Adjuntas [Ad] 3, Barceloneta [Ba] 7, Coamo [Co] 15, Florida [Fl] 16, 17, Las Marías [LM] 28, Maricao [Ma] 30, San Sebastián [SS] 46, San Sebastián [SS] 47, Utuado [Ut] 49, Yauco [Yc] 54 [abbreviations are those used in Fig. 1 and Table S1, Supporting Information]) shared mitochondrial types belonging to two adjacent clades, whereas only one deme (Florida [Fl] 18) included haplotypes belonging to three adjacent phylogroups (Eastern, Central, Northcentral).

The haplotype network (Fig. 3) clearly illustrates the separation between the phylogroups of *A. krugi*, as well as the haplotype diversity within each phylogroup. Most haplotypes in the Northwestern, Central, and Eastern clades are separated by only one mutational step, but the mitochondrial types in the Northcentral and Southwestern clades are more divergent. The Eastern and Central phylogroups contain three mitochondrial types (E1, C1, C2) with multiple satellite haplotypes (Fig. 3). Similarly, the Northwestern and Southwestern phylogroups exhibit a smaller cluster of satellite haplotypes, although in each case the ancestral mitochondrial type is either extinct or was not sampled, and is represented by a median vector (Mv1, Mv2, respectively; Fig. 3).

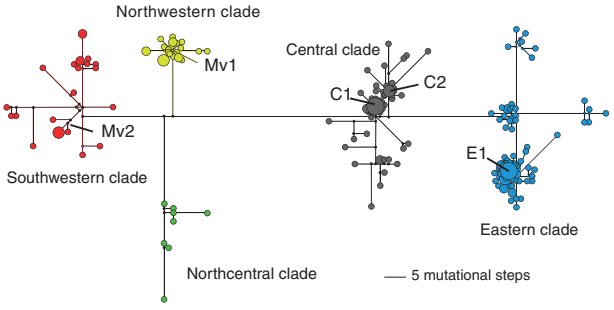

Fig. 3 Median-joining network representing the relationships among haplotypes of the Southwestern (24 sequences, 20 unique haplotypes), Northwestern (25 sequences, 19 unique haplotypes), Northcentral (eight sequences, eight unique haplotypes), Central (61 sequences, 49 unique haplotypes) and Eastern (93 sequences, 78 unique haplotypes) phylogeographic clades of *Anolis krugi*. The Southwestern clade is separated from the Northwestern, Northcentral, Central and Eastern phylogroups by 42, 46, 73 and 106 mutations, respectively. The Northwestern clade is separated from the Northcentral, Central, and Eastern phylogroups by 36, 59 and 92 mutations, respectively. The Northcentral clade is separated from the Central and Eastern phylogroups by 67 and 100 mutations, respectively, whereas the Central and Eastern clades are separated by 33 mutations. The smallest, black circles indicate median vectors (Bandelt *et al*. 1999). Circle size is proportional to haplotype frequencies, with the smallest circle representing one sample and the largest circle representing nine samples; branch length is proportional to number of mutations separating the haplotypes. The designations E1, C1, C2 and Mv1 and Mv2 indicate haplotypes or median vectors referred to in section 'Results', respectively.

Phylogeography

We discuss the results of the SAMOVA analyses conducted with populations with a minimum sample size of five individuals, for this threshold allowed us to include in the analyses as many populations as possible, without compromising accuracy (Fig. S1, Supporting Informa-

tion). As a consequence of only using populations represented by at least five individuals, the SAMOVA analyses were performed using a subset of the samples included in the phylogenetic analyses. We evaluated this data set for $K = 2$ through $K = 8$ partitions, because the latter yielded the last positive F_{SC} value, which indicates that the number of maximally differentiated groups was identified (Fig. S1, Supporting Information). Setting the number of partitions (K) to two groups ($K = 2$) separated the Northwestern phylogroup (represented by the populations Isabela [Is] 22, Isabela [Is] 23, Isabela [Is] 24, and Moca [Mo] 32) from the remainder *A. krugi* demes (Fig. 4). Setting the partitions to $K = 3$ further isolated the Eastern phylogroup, with the exception of the Coamo [Co] 15 population, a mixed deme that includes four haplotypes from the Eastern clade and three from the Central clade. When $K = 4$, the Southwestern clade, represented by the mixed population Yauco [Yc] 54, was isolated. However, Adjuntas [Ad] 3, which is also a mixed population that includes mitochondrial types belonging to the Southwestern phylogroup, was not included in this partition. The likely explanation for this result is that 8 of 10 individuals from the Yauco 54 deme have haplotypes belonging to the Southwestern clade,

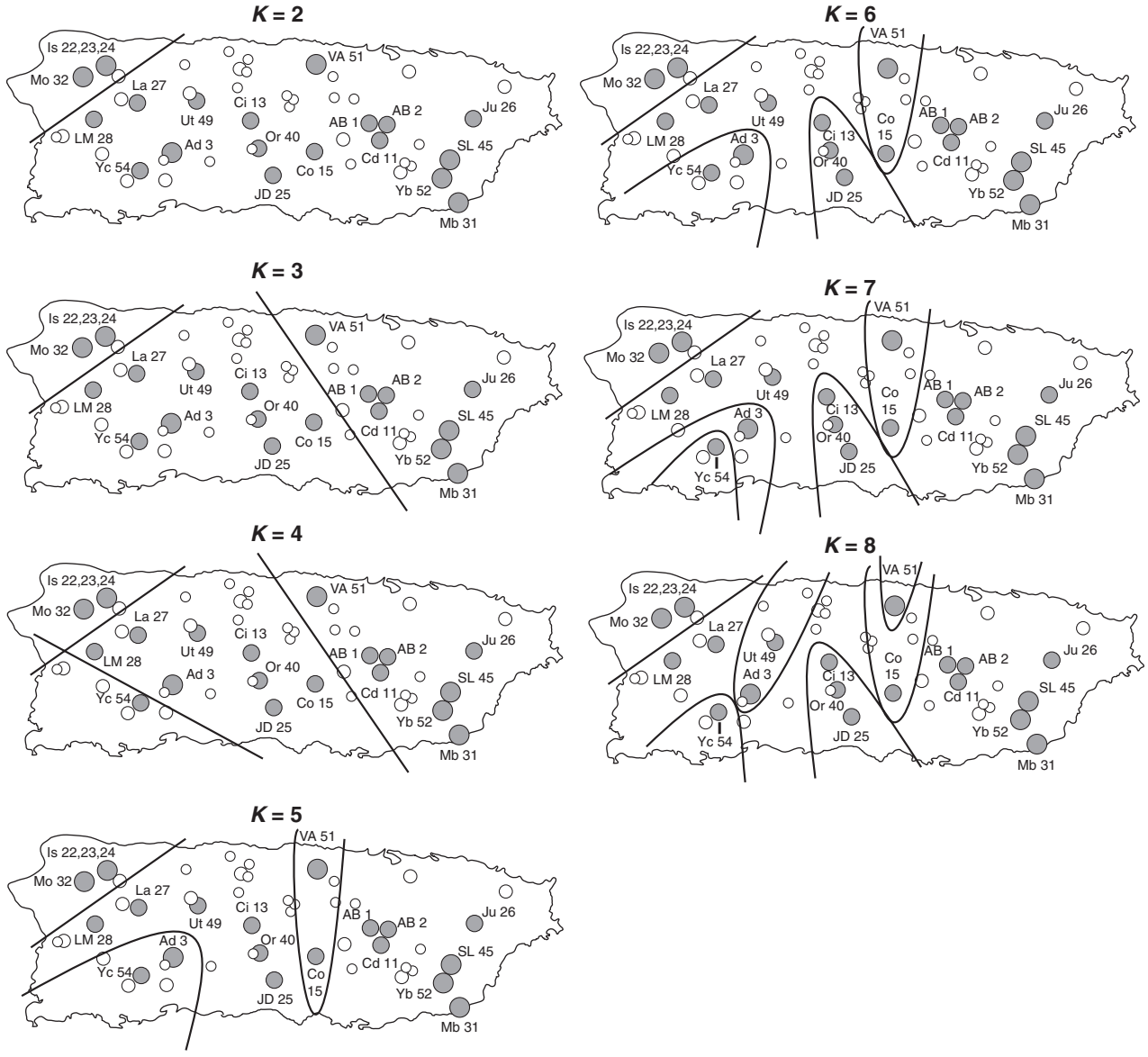

Fig. 4 Genetic partitions of populations of *Anolis krugi* identified by the SAMOVA analyses for $K = 2$ through $K = 8$, where K is the number of predefined groups. Circles indicate the approximate location of sampling localities of *A. krugi*. Dark grey circles indicate populations included in the analyses; white circles indicate populations excluded from the analyses (see text for details).

whereas only three of nine samples in Adjuntas 3 have Southwestern mitochondrial types. Additional partitioning of the populations ($K = 5$) did group Adjuntas 3 and Yauco 54, and created a new cluster formed by Coamo 15 and Vega Alta [VA] 51. The latter population belongs to the Eastern clade, and as previously stated, Coamo 15 is a mixed deme that includes four Eastern and three Central haplotypes. Although the Coamo 15-Vega Alta 51 grouping may seem unintuitive, the two populations nest in a strongly supported subclade of the geographically structured Eastern phylogroup (Fig. 2). Setting $K = 6$ further isolated populations Ciales [Ci] 13, Juana Díaz [JD] 25, and Orocovis [Or] 40 (all belonging to the Central clade), whereas $K = 7$ split populations Adjuntas 3 and Yauco 54. Finally, $K = 8$ joined populations Adjuntas 3 and Utuado [Ut] 49, two mixed demes mainly composed of Central mitochondrial types (Adjuntas 3 has six Central and three Southwestern haplotypes, and Utuado 49 has five Central and one Northwestern haplotypes).

Ecological niche modelling

The Puerto Rican Bank is composed of Puerto Rico (8768 km^2), its outlying islands (of which the largest are Vieques and Culebra), the US (St. Thomas, St. John) and British Virgin Islands (Jost Van Dyke, Tortola, Virgin Gorda, Anegada), and more than 180 associated islets and cays (Fig. S2, Supporting Information). With the Pleistocene lowering of the sea level, the Puerto Rican Bank constituted a single elongate island (*c.* 21 000 km^2) that extended from Puerto Rico on the west to Anegada on the east (Heatwole & MacKenzie 1967; Thomas 1999).

The two resolutions (30 s and 2.5 min) used to reconstruct the current ecological niche of *A. krugi* produced very similar models, and we only present the finer one (30 s; Fig. S2 A, Supporting Information). The ecological niche model for the current climatic conditions of the Puerto Rican Bank shows continuous, suitable habitat for *A. krugi* throughout Puerto Rico, with the exception of the southern coast, the arid most region of the island (Murphy *et al.* 1995; Helmer *et al.* 2002). The projections of the current habitat model for *A. krugi* onto the palaeoclimatic reconstructions indicate an overall retention of suitable habitat throughout the island during the LGM. However, the two reconstructions depict somewhat different predictions regarding the distribution of *A. krugi*, with the CCSM (Fig. S2B, Supporting Information) model predicting a more restricted range than the MIROC model (Fig. S2C, Supporting Information). For both current and LGM habitat models, climatic conditions on the islands off the east coast of Puerto Rico (Vieques, Culebra, the US and British Virgin Islands, and associated islets and cays) are inferred to be unsuitable for *A. krugi*, which is consistent with the fact that this anole is not known to occur or have ever occurred on any of those islands.

Climatic stability and the genetic landscape of A. krugi

The genetic landscape of *A. krugi* (Fig. 5) is largely congruent with the geographic distribution of the five phylogeographic clades and the genetically defined groups identified by SAMOVA. The largest genetic distances between clades occur in northwestern (separating the Northwestern and the Central clades) and southwestern (separating the Southwestern and the Central clades) Puerto Rico. The genetic distance between the Central and Eastern clades is smaller than the separation between any other two clades. The genetic landscape revealed a previously undetected break within the Eastern clade. This discontinuity separates the seven populations in southeastern Puerto Rico (Caguas [Cg] 12, Cayey [Ca] 8, Cayey [Ca] 9, Cayey [Ca] 10, Maunabo [Mb] 31, San Lorenzo [SL] 45, Yabucoa [Yb] 52) from the remainder demes in the Eastern clade.

When we evaluated climatic stability in Puerto Rico using the CCSM model, six of the seven temperature variables (annual mean temperature, minimum temper-

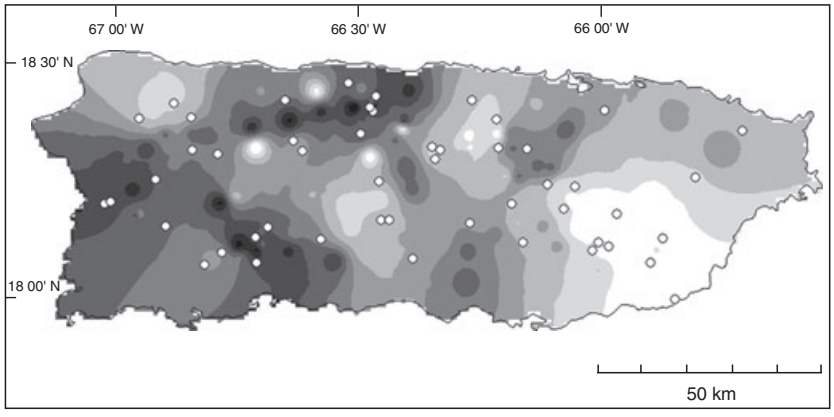

Fig. 5 Interpolated pairwise genetic distances within *Anolis krugi* across Puerto Rico using a 30 s (*c.* 1 km) grid size. The white circles indicate the approximate location of sampling localities of *A. krugi*. The shading gradation represents genetic distances ranging from small (white) to large (black).

ature of the coldest month, mean temperature of the wettest quarter, mean temperature of the driest quarter, mean temperature of the warmest quarter, mean temperature of the coldest quarter, $P < 0.001$) and one of the four precipitation variables (precipitation of the wettest month, $P = 0.003$) were positively correlated with the genetic landscape of *A. krugi*. When we assessed climatic stability using the MIROC model, only two variables (precipitation of the wettest month, precipitation of the wettest quarter) were significantly correlated with the genetic landscape of *A. krugi* ($P < 0.001$ and $P = 0.019$, respectively). However, none of these comparisons was significant when we adjusted the probability values for spatial autocorrelation (for the nine comparisons, $0.16 < P < 0.54$).

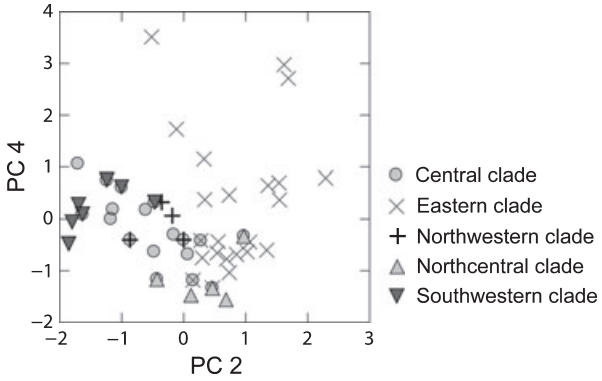

Fig. 6 Principal component analysis of climatic niche differences among the five phylogeographic clades of *Anolis krugi*. The *x* axis (PC2) explains 29% of the variation, and the *y* axis (PC4) explains 6% of the variation.

Climatic niche comparisons among phylogeographic clades of A. krugi

The PCA reduced the 19 bioclimatic variables to four principal components (PCs) with an eigenvalue ≥1. PC1, PC2, PC3, and PC4 explained 50%, 29%, 11% and 6% of the total variance in the observed variables, respectively. PC1 is positively loaded with annual mean temperature, extreme monthly temperatures, and average quarter temperatures. PC2 is positively loaded with extreme values of seasonal precipitation and temperature seasonality, and negatively loaded with precipitation seasonality, isothermality, mean temperature diurnal range and temperature annual range. PC3 is positively loaded with annual precipitation and precipitation during the warmest and coldest quarters of the year. The highest value of PC4 corresponds to precipitation during the warmest quarter of the year, whereas the lowest value corresponds to temperature annual range. The ANOVA analyses indicated that PC2 and PC4 statistically differentiated phylogroups of *A. krugi* ($P < 0.001$; Fig. 6), but that PC1 and PC3 did not ($P > 0.05$). Tukey's HSD post hoc tests revealed that PC2 discriminated between the Eastern–Central, Eastern–Northwestern, Eastern–Southwestern, Central–Southwestern, Northwestern–Southwestern and Northcentral–Southwestern phylogeographic clades ($P \leq 0.01$), whereas PC4 differentiated the Eastern from the Northcentral phylogroup ($P = 0.02$).

Discussion

Phylogenetic and phylogeographic analyses

Our genetic assessment of *A. krugi* revealed unsuspected high levels of diversity within this species. Because of the widespread, largely continuous distribution of *A. krugi* throughout most of Puerto Rico, populations of this lizard could have exhibited relatively high levels of gene flow among demes, and therefore low levels of interpopulation genetic differentiation. On the contrary, our findings revealed the existence of five strongly supported intraspecific phylogroups in *A. krugi*. Further, each of these clades includes numerous divergent haplotypes. This result is consistent with recent genetic analyses that showed that conspecific populations of other species of Puerto Rican *Anolis* (*A. cristatellus*, *A. cooki*, *A. poncensis*; Kolbe *et al.* 2007; Rodríguez-Robles *et al.* 2007; Jezkova *et al.* 2009b) also are phylogeographically structured, even though some of these populations are separated by relatively small geographic distances. Collectively, these results demonstrate that, despite several evolutionary studies involving Puerto Rican *Anolis* (e.g. Gorman *et al.* 1983; Losos *et al.* 1998; Brandley & de Queiroz 2004; Poe 2004), an important aspect of the diversity of these lizards, namely intraspecific genetic differentiation, has been overlooked.

The phylogenetic analyses do not support the hypothesis that populations of *A. krugi* are partitioned into genetic sets that correspond to the five physiographic regions of Puerto Rico. This observation implies that the boundaries between these regions do not represent a dispersal barrier for the anoles. As its Spanish vernacular name suggests, 'Lagartijo Jardinero de la Montaña' (Mountain Garden Lizard), *A. krugi* mainly occurs at higher elevation, mesic areas (the Central Uplands, Sierra de Cayey, Sierra de Luquillo and Cuchilla de Pandura). However, none of the genetic discontinuities within the species defines a phylogroup restricted to any of these four regions. For example, the Sierra de Luquillo is an isolated mountain chain on northeastern Puerto Rico that has the highest species diversity of amphibians on the island (14 species; Hedges 1999).

The Sierra has an endemic species, the eleutherodactylid frog *Eleutherodactylus unicolor* (Joglar 1998) and genetically differentiated populations of the widespread *E. antillensis* (Brittany S. Barker, University of New Mexico, Albuquerque; unpublished), suggesting that other species associated with mesic habitats may also have genetically distinctive populations on the Sierra de Luquillo. However, the haplotypes of the Sierra de Luquillo populations of *A. krugi* (Juncos [Ju] 26 and Río Grande [RG] 43) do not form a clade and are part of the widespread Eastern phylogroup, which includes mitochondrial types from the other four physiographic regions of Puerto Rico. It could be argued that the current phylogeographic structure of *A. krugi* may reflect *past* barriers to gene flow. Although we cannot refute this scenario, we are not aware of any evidence that indicates the existence of past physical or ecological barriers in Puerto Rico that even broadly coincide with the location of the boundaries between the intraspecific clades of *A. krugi*.

Our findings are based on the analysis of mitochondrial sequence data. Gene trees are a useful way of describing phylogeographic diversity, but we acknowledge that nuclear markers make an important contribution to the quantification of genetic processes, and that using mitochondrial and nuclear genes has more resolving power than does mtDNA alone (Barrowclough & Zink 2009; Edwards & Bensch 2009). Yet, studies of evolutionary diversification in *Anolis* that have employed both mitochondrial and nuclear genes found general congruence in results from the two markers (Glor *et al.* 2005; Nicholson *et al.* 2005), and several more extensive projects currently underway indicate that relationships inferred from nuclear DNA data give the same general result as published studies based on mtDNA (Losos 2009; Richard E. Glor, University of Rochester, New York, unpublished). Accordingly, we believe that our study represents an informative characterization of the pattern of genetic diversification in *A. krugi*.

Genetic variation in *A. krugi* is not uniformly distributed across the range of the species. Instead, the interpolated pairwise genetic distances indicate the existence of various regions characterized by relatively small or relatively large genetic differentiation between populations. The areas with the more pronounced levels of distinctiveness are those where the Southwestern and Northcentral clades occur, followed (in order) by the northwestern and central regions of Puerto Rico. The Eastern clade contains by far the largest number of haplotypes ($n = 78$) and, contrary to the other phylogroups, has internal phylogenetic structure, being composed of four well supported subclades. Nevertheless, the Eastern phylogroup also exhibits the lowest genetic distances across the landscape, despite the fact that it is the most geographically widespread *A. krugi* clade. This paradoxical result can be explained at least in part by two related factors: (i) the Eastern clade is mainly composed of a large cluster of satellite (newly evolved) haplotypes closely related to an ancestral mitochondrial type (E1; Fig. 3), and (ii) this ancestral haplotype occurs in six populations (Aguas Buenas [AB] 2, Cayey [Ca] 9, Juncos [Ju] 26, Maunabo [Mb] 31, San Lorenzo [SL] 45, Yabucoa [Yb] 52) on the southeastern region of Puerto Rico. Occurrence of multiple satellite, private haplotypes and retention of ancestral mitochondrial types in multiple populations usually indicate a relatively recent and rapid colonization event or re-colonization of a particular area, followed by demographic expansion (Slatkin & Hudson 1991). The very small genetic distances in southeastern Puerto Rico (indicated by the white area on Fig. 5) correspond to the distribution of the widespread haplotype E1 and also suggest an episode of range expansion into this area. If this pattern is the result of population expansion, then the high number of accumulated, satellite haplotypes in the Eastern and Central clades suggests that the expansion possibly did not occur very recently. Collectively, these observations suggest that southeastern Puerto Rico (including the Sierra de Cayey and the Cuchilla de Pandura) may be the area of the island most recently colonized by *A. krugi*.

Phylogenetic analyses recover patterns of ancestor–descendant relationships among taxa (or the analysed genetic material that they house) without considering the spatial location of the samples included in the study. In contrast, SAMOVA analyses assess the level of genetic similarity among neighbouring populations, irrespective of whether the alleles or haplotypes present in those demes are ancestral or derived. Because phylogenetic and SAMOVA analyses are complementary, groupings identified by both methods are likely to represent accurate estimates of the genetic structure of a species. Indeed, the phylogroups of *A. krugi* inferred by the phylogenetic analyses are largely congruent with the groups identified by SAMOVA. The first three breaks (Fig. 4, $K = 2$, $K = 3$, $K = 4$) define groups that correspond to the Northwestern, Eastern and Southwestern clades. (The minimum sample size threshold used [$n = 5$] for the SAMOVA analyses precluded the recovery of the Northcentral clade.) A major break within the Eastern clade is also captured when $K = 5$ (Fig. 4). The remaining breaks ($K = 6$, $K = 7$, $K = 8$) partition the Central, Southwestern, and Eastern clades, but these breaks are unstable and more driven by the admixture of haplotypes from different genetic partitions than by divergence between areas.

Climatic stability and the genetic landscape of A. krugi

Can historical climatic conditions in Puerto Rico during the Quaternary account for the observed pattern of genetic structuring in *A. krugi*? Tropical and subtropical regions did not experience the dramatic climatic extremes that temperate areas did during the Pleistocene (1.8 Ma to 10 000 years ago; Ballantyne *et al.* 2005; Braconnot *et al.* 2007). Still, improved LGM reconstructions suggest that even tropical regions underwent a large-scale cooling, both over the ocean and over land, with surface air temperatures *c.* 5 °C lower than those today (Ballantyne *et al.* 2005). These cooler temperatures may have resulted in significant shifts and/or fragmentation of the distribution of certain species. In fact, recent studies have demonstrated that changes in climatic patterns can greatly impact the distribution of a species, in particular that of tropical lizards (Huey *et al.* 2009, and references therein). Therefore, we assessed the stability of the climatic niche of *A. krugi* since the LGM by projecting the species' current climatic niche across this past time period using the CCSM and MIROC models. Because the LGM and the present interglacial period represent two climatic extremes of the Quaternary, the comparison is indicative of the largest climate-driven changes in *A. krugi*'s distribution that could have occurred during this period.

The CCSM and MIROC models produced slightly different reconstructions of the climatic conditions in Puerto Rico during the LGM. CCSM estimated less precipitation (i.e. a drier climate) throughout the island, whereas MIROC estimated an annual mean temperature *c.* 1 °C lower than CCSM did. Because *A. krugi* is a mesic species, its distribution should be more affected by a decrease in precipitation, rather than by lower temperatures (cf. Heatwole *et al.* 1969). Compared with the MIROC reconstruction, the CCSM model indeed estimated a slightly more restricted climatic niche available to *A. krugi*. Nevertheless, overall the two models indicated that despite the general cooling and drying that occurred in the tropics during the Quaternary glacial cycles, the climatic niche of *A. krugi* probably remained fairly stable. Species distribution models are only as informative as the parameters and resolution of those parameters that are incorporated into the niche model, and thus it is possible that our habitat models for *A. krugi* only inferred minor changes between the LGM and the present because key environmental parameters were not considered. For example, microhabitat variation was not incorporated in the distribution model, yet anoles can be sensitive to microclimatic conditions (Losos 2009), which may differ regionally. In the absence of microclimatic information, available evidence suggests that the ecological niche of *A. krugi* has been stable in the Quaternary, and therefore there is no expectation of higher genetic diversity in some parts of the distribution of the species as opposed to others based on the historic climate stability hypothesis.

Given the inferred stability of the climatic niche of *A. krugi* since the LGM, we tested the hypothesis that different degrees of climatic stability generate detectable differences in levels of genetic variation among populations of *A. krugi*. Specifically, we predicted that demes from the more stable areas exhibit higher levels of genetic variability than populations from regions that have experienced greater climatic shifts. The CCSM and MIROC habitat models indicated that seven of 11 and two of 11 bioclimatic variables, respectively, showed a positive association between climatic stability and the magnitude of genetic divergence among populations of *A. krugi*. However, these comparisons became statistically nonsignificant when revised for spatial autocorrelation, probably because due to the small spatial scale used, the estimated levels of climatic stability across the geographic range of *A. krugi* are similar, and the small differences may not allow for a statistically significant relationship between climatic stability and genetic differentiation.

Climatic niche comparisons among phylogeographic clades of A. krugi

In general, the most pronounced climatic and ecological habitat differences in Puerto Rico occur between the subtropical dry forest, the vast majority of which is located on the southcentral and southwestern coast, and the rest of the island, which predominantly consists of mesic habitat (Lugo 2005). Because *A. krugi* is absent from xeric habitats, its natural distribution precludes us from evaluating the potential effects of these environmental differences on patterns of genetic divergence in the species. Instead, we assessed whether the five phylogroups of *A. krugi* are climatically differentiated. Principal component analysis revealed some climatic niche differences among the five phylogeographic clades of *A. krugi*, particularly regarding temperature and precipitation seasonality and temperature fluctuation. As previously indicated, the Eastern clade contains the highest number of haplotypes and also has the largest geographic distribution, and thus it is not surprising that the populations that comprise this phylogroup collectively experience the broadest range of climatic conditions. The two principal components (PC2 and PC4) that significantly differentiated between some phylogroups suggest a slight niche structuring of *A. krugi* in an east–west and north–south direction (Fig. 7), but it is unclear whether these climatic differences could have

caused and/or maintain the observed levels of genetic structuring in this anole.

In summary, our findings concerning the patterns of genetic differentiation in *A. krugi* are intriguing, and suggest that the relationship between climatic stability and spatial genetic patterns should be explored in more detail in other taxa. Further, the approach that we employed to assess how environmental conditions may shape the genetic architecture of a species can also be used to investigate the impact of factors such as soil or vegetation type, or spatial distribution of food sources, parasites, predators or competitors, on a taxon's genetic landscape.

Acknowledgements

We thank Carmen D. Ortiz, Michael J. Quiñones, Yahirí Rodríguez, Miguel A. García and Kimberly Franco for assistance in the field and the laboratory; Carla Cicero and Jimmy A. McGuire (Museum of Vertebrate Zoology, University of California, Berkeley) for loaning of tissue samples; Eric Waltari for providing the downscaled palaeo-climatic layers; the CCSM and MIROC modelling groups for providing data for analyses; the Laboratoire des Sciences du Climat et de l'Environnement for collecting and archiving the model data; Jonathan B. Losos, Christopher J. Conroy, and Carol L. Spencer for valuable information; and the Department of Natural and Environmental Resources of Puerto Rico for conceding the necessary collecting permits to conduct this investigation. This study was partly funded by grants from the National Science Foundation (DBI-0001975, DEB-0327415, IOS-0413791) and the American Museum of Natural History to JAR-R and M.L., and by a Major Research Instrumentation grant (DBI-0421519) to the University of Nevada, Las Vegas.

The DRYAD identifier for the sequence data generated for this study is: http://hdl.handle.net/10255/dryad.1257.

References

Araújo MB, Nogués-Bravo D, Diniz-Filho JAF, Haywood AM, Valdes PJ, Rahbek C (2008) Quaternary climate changes explain diversity among reptiles and amphibians. *Ecography*, **31**, 8–15.

Avise JC (2000) *Phylogeography: The History and Formation of Species*. Harvard University Press, Cambridge, Massachusetts.

Ballantyne AP, Lavine M, Crowley TJ, Liu J, Baker PB (2005) Meta-analysis of tropical surface temperatures during the Last Glacial Maximum. *Geophysical Research Letters*, **32**, L05712.

Bandelt HJ, Forster P, Röhl A (1999) Median-joining networks for inferring intraspecific phylogenies. *Molecular Biology and Evolution*, **16**, 37–48.

Barrowclough GF, Zink RM (2009) Funds enough, and time: mtDNA, nuDNA and the discovery of divergence. *Molecular Ecology*, **18**, 2934–2936.

Bell CJ, Lundelius EL Jr, Barnosky AD *et al.* (2004) The Blancan, Irvingtonian, and Rancholabrean mammal ages. In: *Late Cretaceous and Cenozoic Mammals of North America: Biostratigraphy and Geochronology* (ed. Woodburne MO), pp. 232–314. Columbia University Press, New York.

Bond JE, Sierwald P (2002) Cryptic speciation in the *Anadenobolus excisus* millipede species complex on the island of Jamaica. *Evolution*, **56**, 1123–1135.

Braconnot P, Otto-Bliesner B, Harrison S *et al.* (2007) Results of PMIP2 coupled simulations of the Mid-Holocene and Last Glacial Maximum—Part 1: experiments and large-scale features. *Climate of the Past*, **3**, 261–277.

Brandley MC, de Queiroz K (2004) Phylogeny, ecomorphological evolution, and historical biogeography of the *Anolis cristatellus* series. *Herpetological Monographs*, **18**, 90–126.

Brandley MC, Leaché AD, Warren DL, McGuire JA (2006) Are unequal clade priors problematic for Bayesian phylogenetics? *Systematic Biology*, **55**, 138–146.

Brown RP, Hoskisson PA, Welton J-H, Báez M (2006) Geological history and within-island diversity: a debris avalanche and the Tenerife lizard *Gallotia galloti*. *Molecular Ecology*, **15**, 3631–3640.

Browne RA, Collins E, Anderson DJ (2008) Genetic structure of Galápagos populations of the yellow warbler. *Condor*, **110**, 549–553.

Carnaval AC, Moritz C (2008) Historical climate modelling predicts patterns of current biodiversity in the Brazilian Atlantic forest. *Journal of Biogeography*, **35**, 1187–1201.

Carnaval AC, Hickerson MJ, Haddad CFB, Rodrigues MT, Moritz C (2009) Stability predicts genetic diversity in the Brazilian Atlantic forest hotspot. *Science*, **323**, 785–789.

Carstens BC, Richards CL (2007) Integrating coalescent and ecological niche modeling in comparative phylogeography. *Evolution*, **61**, 1439–1454.

Cronk QCB (1997) Islands: stability, diversity, conservation. *Biodiversity and Conservation*, **6**, 477–493.

Dehghani R, Wanntorp L, Pagani P, Källersjö M, Werdelin L, Veron G (2008) Phylogeography of the white-tailed mongoose (Herpestidae, Carnivora, Mammalia) based on partial sequences of the mtDNA control region. *Journal of Zoology (London)*, **276**, 385–393.

Dupanloup I, Schneider S, Excoffier L (2002) A simulated annealing approach to define the genetic structure of populations. *Molecular Ecology*, **11**, 2571–2581.

Edwards S, Bensch S (2009) Looking forwards or looking backwards in avian phylogeography? A comment on Zink and Barrowclough 2008. *Molecular Ecology*, **18**, 2930–2933.

Elith J, Graham CH (2009) Do they? How do they? Why do they differ? On finding reasons for differing performances of species distribution models. *Ecography*, **32**, 66–77.

Elith J, Graham CH, Anderson RP *et al.* (2006) Novel methods improve prediction of species' distributions from occurrence data. *Ecography*, **29**, 129–151.

Excoffier L, Smouse PE, Quattro JM (1992) Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics*, **131**, 479–491.

Farris JS, Källersjö M, Kluge AG, Bult C (1994) Testing significance of incongruence. *Cladistics*, **10**, 315–319.

Forster P, Torroni A, Renfrew C, Röhl A (2001) Phylogenetic star contraction applied to Asian and Papuan mtDNA evolution. *Molecular Biology and Evolution*, **18**, 1864–1881.

Funk SM, Fa JE (2006) Phylogeography of the endemic St. Lucia whiptail lizard *Cnemidophorus vanzoi*: conservation

genetics at the species boundary. *Conservation Genetics*, **7**, 651–663.

Glor RE, Kolbe JJ, Powell R, Larson A, Losos JB (2003) Phylogenetic analysis of ecological and morphological diversification in Hispaniolan trunk-ground anoles (*Anolis cybotes* group). *Evolution*, **57**, 2383–2397.

Glor RE, Losos JB, Larson A (2005) Out of Cuba: overwater dispersal and speciation among lizards in the *Anolis carolinensis* subgroup. *Molecular Ecology*, **14**, 2419–2432.

Gorman GC, Thomas R, Atkins L (1968) Intra- and interspecific chromosome variation in the lizard *Anolis cristatellus* and its closest relatives. *Breviora*, **293**, 1–13.

Gorman GC, Buth D, Soulé M, Yang SY (1983) The relationships of the Puerto Rican *Anolis*: electrophoretic and karyotypic studies. In: *Advances in Herpetology and Evolutionary Biology: Essays in Honor of Ernest E. Williams* (eds Rhodin AGJ, Miyata K), pp. 626–642. Museum of Comparative Zoology, Cambridge, Massachusetts.

Graham CH, Moritz C, Williams SE (2006) Habitat history improves prediction of biodiversity in rainforest fauna. *Proceedings of the National Academy of Sciences, USA*, **103**, 632–636.

Harrison SP (2000) Palaeoenvironmental data sets and model evaluation in PMIP. In: *Paleoclimate Modelling Intercomparison Project* (ed. Braconnot P), pp. 25–42. Proceedings of the Third PMIP Workshop. World Climate Research Programme–111, ICPO Publications Series No. 34. La Huardière, Canada.

Hasumi H, Emori S (eds) (2004) *K-1 Coupled GCM (MIROC) Description*. K-1 Technical Report No. 1. Center for Climate System Research, University of Tokyo, Japan.

Heatwole H, MacKenzie F (1967) Herpetogeography of Puerto Rico. IV. Paleogeography, faunal similarity and endemism. *Evolution*, **21**, 429–438.

Heatwole H, Lin T-H, Villalón E, Muñiz A, Matta A (1969) Some aspects of the thermal ecology of Puerto Rican anoline lizards. *Journal of Herpetology*, **3**, 65–77.

Hedges SB (1999) Distribution patterns of amphibians in the West Indies. In: *Patterns of Distribution of Amphibians: A Global Perspective* (ed. Duellman WE), pp. 211–254. Johns Hopkins University Press, Baltimore.

Hedges SB, Heinicke MP (2007) Molecular phylogeny and biogeography of West Indian frogs of the genus *Leptodactylus* (Anura, Leptodactylidae). *Molecular Phylogenetics and Evolution*, **44**, 308–314.

Helmer EH, Ramos O, López T del M, Quiñones M, Díaz W (2002) Mapping the forest type and land cover of Puerto Rico, a component of the Caribbean biodiversity hotspot. *Caribbean Journal of Science*, **38**, 165–183.

Henderson RW, Powell R (2009) *Natural History of West Indian Reptiles and Amphibians*. University of Florida Press, Gainesville.

Hewitt GM (1999) Post-glacial re-colonization of European biota. *Biological Journal of the Linnean Society*, **68**, 87–112.

Hijmans RJ, Cameron SE, Parra JL, Jones PG, Jarvis A (2005a) Very high resolution interpolated climate surfaces for global land areas. *International Journal of Climatology*, **25**, 1965–1978.

Hijmans RJ, Guarino L, Jarvis A *et al.* (2005b) DIVA-GIS (Version 5.2). http://www.diva-gis.org/.

Hilbe JM (2008) Systat 12.2: an overview. *American Statistician*, **62**, 177–178.

Huey RB, Deutsch CA, Tewksbury JJ *et al.* (2009) Why tropical forest lizards are vulnerable to climate warming. *Proceedings of the Royal Society, B*, **276**, 1939–1948.

Jackman TR, Irschick DJ, de Queiroz K, Losos JB, Larson A (2002) Molecular phylogenetic perspective on evolution of lizards of the *Anolis grahami* series. *Journal of Experimental Zoology*, **294**, 1–16.

Jansson R (2003) Global patterns in endemism explained by past climatic change. *Proceedings of the Royal Society B: Biological Sciences*, **270**, 583–590.

Jezkova T, Jaeger JR, Marshall ZL, Riddle BR (2009a) Pleistocene impacts on the phylogeography of the desert pocket mouse (*Chaetodipus penicillatus*). *Journal of Mammalogy*, **90**, 306–320.

Jezkova T, Leal M, Rodríguez-Robles JA (2009b) Living together but remaining apart: comparative phylogeography of *Anolis poncensis* and *A. cooki*, two lizards endemic to the aridlands of Puerto Rico. *Biological Journal of the Linnean Society*, **96**, 617–634.

Jobb G, von Haeseler A, Strimmer K (2004) TREEFINDER: a powerful graphical analysis environment for molecular phylogenetics. *BMC Evolutionary Biology*, **4**, 18.

Joglar RL (1998) *Los Coquíes de Puerto Rico: Su Historia Natural y Conservación*. Editorial de la Universidad de Puerto Rico, San Juan, Puerto Rico.

Kolbe JJ, Glor RE, Rodríguez Schettino L, Chamizo Lara A, Larson A, Losos JB (2007) Multiple sources, admixture, and genetic variation in introduced *Anolis* lizard populations. *Conservation Biology*, **21**, 1612–1625.

Kozak KH, Wiens JJ (2006) Does niche conservatism promote speciation? A case study in North American salamanders *Evolution*, **60**, 2604–2621.

Leaché AD, Reeder TW (2002) Molecular systematics of the eastern fence lizard (*Sceloporus undulatus*): a comparison of parsimony, likelihood, and Bayesian approaches. *Systematic Biology*, **51**, 44–68.

Losos JB (2009) *Lizards in an Evolutionary Tree: Ecology and Adaptive Radiation of Anoles*. University of California Press, Berkeley.

Losos JB, Jackman TR, Larson A, de Queiroz K, Rodríguez-Schettino L (1998) Contingency and determinism in replicated adaptive radiations of island lizards. *Science*, **279**, 2115–2118.

Lugo AE (2005) Los Bosques. In: *Biodiversidad de Puerto Rico: Vertebrados Terrestres y Ecosistemas. Serie de Historia Natural* (ed. Joglar RL), pp. 397–548. Editorial del Instituto de Cultura Puertorriqueña, San Juan, Puerto Rico.

Malhotra A, Thorpe RS (2000) The dynamics of natural selection and vicariance in the Dominican anole: patterns of within-island molecular and morphological divergence. *Evolution*, **54**, 245–258.

Manni F, Guérard E, Heyer E (2004) Geographic patterns of (genetic, morphologic, linguistic) variation: how barriers can be detected by using Monmonier's algorithm. *Human Biology*, **76**, 173–190.

Miller MP (2005) Alleles In Space (AIS): computer software for the joint analysis of interindividual spatial and genetic information. *Journal of Heredity*, **96**, 722–724.

Miller MP, Bellinger MR, Forsman ED, Haig SM (2006) Effects of historical climate change, habitat connectivity, and vicariance on genetic structure and diversity across the

range of the red tree vole (*Phenacomys longicaudus*) in the Pacific Northwestern United States. *Molecular Ecology*, **15**, 145–159.

Murphy PG, Lugo AE, Murphy AJ, Nepstad DC (1995) The dry forests of Puerto Rico's south coast. In: *Tropical Forests: Management and Ecology* (eds Lugo AE, Lowe C), pp. 178–209. Springer-Verlag, New York.

Nicholson KE, Glor RE, Kolbe JJ, Larson A, Hedges SB, Losos JB (2005) Mainland colonization by island lizards. *Journal of Biogeography*, **32**, 929–938.

Noonan BP, Wray KP (2006) Neotropical diversification: the effects of a complex history on diversity within the poison frog genus *Dendrobates*. *Journal of Biogeography*, **33**, 1007–1020.

Nylander JAA (2004) MrModeltest v.2. Program distributed by the author. Evolutionary Biology Centre, Uppsala University, Sweden.

Otto-Bliesner BL, Brady EC, Clauzet G, Tomas R, Levis S, Kothavala Z (2006) Last Glacial Maximum and Holocene climate in CCSM3. *Journal of Climate*, **19**, 2526–2544.

Peterson AT, Nyári AS (2008) Ecological niche conservatism and Pleistocene refugia in the thrush-like mourner, *Schiffornis* sp., in the neotropics. *Evolution*, **62**, 173–183.

Phillips SJ (2008) Transferability, sample selection bias and background data in presence-only modelling: a response to Peterson *et al.* (2007). *Ecography*, **31**, 272–278.

Phillips SJ, Dudík M (2008) Modeling of species distributions with Maxent: new extensions and a comprehensive evaluation. *Ecography*, **31**, 161–175.

Phillips SJ, Anderson RP, Schapire RE (2006) Maximum entropy modeling of species geographic distributions. *Ecological Modelling*, **190**, 231–259.

Picó R (1974) *The Geography of Puerto Rico*. Aldine Publishing Company, Chicago.

Poe S (2004) Phylogeny of anoles. *Herpetological Monographs*, **18**, 37–89.

Polzin T, Daneshmand SV (2003) On Steiner trees and minimum spanning trees in hypergraphs. *Operations Research Letters*, **31**, 12–20.

Rangel TFLVB, Diniz-Filho JAF, Bini LM (2006) Towards an integrated computational tool for spatial analysis in macroecology and biogeography. *Global Ecology and Biogeography*, **15**, 321–327.

Rissler LJ, Apodaca JJ (2007) Adding more ecology into species delimitation: ecological niche models and phylogeography help define cryptic species in the black salamander (*Aneides flavipunctatus*). *Systematic Biology*, **56**, 924–942.

Rivero JA (1998) *The Amphibians and Reptiles of Puerto Rico*, 2nd rev. edn. Editorial de la Universidad de Puerto Rico, San Juan, Puerto Rico.

Rodríguez-Robles JA, Jezkova T, García MA (2007) Evolutionary relationships and historical biogeography of *Anolis desechensis* and *A. monensis*, two lizards endemic to small islands in the eastern Caribbean Sea. *Journal of Biogeography*, **34**, 1546–1558.

Roe BA, Ma D-P, Wilson RK, Wong JF-H (1985) The complete nucleotide sequence of the *Xenopus laevis* mitochondrial genome. *Journal of Biological Chemistry*, **260**, 9759–9774.

Ronquist F, Huelsenbeck JP (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics*, **19**, 1572–1574.

Schwartz A, Henderson RW (1991) *Amphibians and Reptiles of the West Indies: Descriptions, Distributions, and Natural History*. University of Florida Press, Gainesville.

Slatkin M, Hudson RR (1991) Pairwise comparisons of mitochondrial DNA sequences in stable and exponentially growing populations. *Genetics*, **129**, 555–562.

Swofford DL (2003) *PAUP*: Phylogenetic Analysis Using Parsimony (and Other Methods)*, Version 4.10b. Sinauer Associates, Sunderland, Massachusetts.

Thomas R (1999) The Puerto Rico area. In: *Caribbean Amphibians and Reptiles* (ed. Crother BI), pp. 169–179. Academic Press, San Diego.

Waltari E, Hijmans RJ, Peterson AT, Nyári AS, Perkins SL, Guralnick RP (2007) Locating Pleistocene refugia: comparing phylogeographic and ecological niche model predictions. *PLoS ONE*, **2**, e563.

Watson DF (1992) *Contouring: A Guide to the Analysis and Display of Spatial Data*. Pergamon Press, New York.

Watson DF, Philip GM (1985) A refinement of inverse distance weighted interpolation. *Geo-Processing*, **2**, 315–327.

Wright S (1951) The genetical structure of populations. *Annals of Eugenics*, **1**, 323–354.

J.A.R.-R. is an Associate Professor and Associate Director of the School of Life Sciences of the University of Nevada, Las Vegas. He is interested in the systematics, biogeography, and evolutionary and behavioural ecology of squamate reptiles and anurans from the Puerto Rican Bank. T.J. is a doctoral student in the laboratory of Brett R. Riddle at the School of Life Sciences, University of Nevada, Las Vegas. Her current research efforts focus on integrating molecular genetics, ecological niche modelling, and GIS-based landscape analyses to understand how extant species responded to past climatic changes. M.L. is an Assistant Professor at the Department of Biology, Duke University. His research interests include animal communication and behavioural and evolutionary ecology of lizards.

Supporting Information

Additional supporting information may be found in the online version of this article.

Table S1 Locality abbreviation, sample number, voucher number, GenBank accession numbers, specific locality, and coordinates of the specimens used in this study. Abbreviation: MVZ, Museum of Vertebrate Zoology, University of California, Berkeley

Table S2 Redundant and contracted haplotypes of *Anolis krugi*. The first column indicates all the haplotypes that were redundant with other mitochondrial types (second column) and/or that represent an ancestral haplotype to which one or more other haplotypes were contracted using the star contraction method (see Materials and Methods). Haplotypes in bold appear on the maximum likelihood tree (Fig. 2). The third column indicates the satellite haplotypes that were contracted to the ancestral mitochondrial type listed on the first column

Fig. S1 Effect of sample size (i.e. minimum number of individuals per population) on (A) F_{CT} values (indicators of the proportion of total genetic variance due to differences among groups of populations), and on (B) F_{SC} values (indicators of differentiation among populations within groups).

Fig. S2 Ecological niche models for *Anolis krugi* for the (A) current climatic conditions (30 sec resolution) in Puerto Rico, and for the climatic conditions during the last glacial maximum (2.5 min resolution) estimated using the (B) CCSM and (C) MIROC models. The current shoreline of Puerto Rico and adjacent islands is depicted in light grey, whereas the approximate shoreline of the Puerto Rican Bank during the last glacial maximum (adjusted to the resolution of 2.5 min) is shown in black. The shading represents habitat modeled as suitable for *A. krugi*.

Please note: Wiley-Blackwell are not responsible for the content or functionality of any supporting information supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.