Biological Journal of the Linnean Society, 2009, **96**, 617–634. With 3 figures

Living together but remaining apart: comparative phylogeography of *Anolis poncensis* and *A. cooki*, two lizards endemic to the aridlands of Puerto Rico

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Received 26 June 2008; accepted for publication 12 August 2008

Comparative phylogeography is a powerful method for testing hypotheses of evolutionary diversification in ecological communities. Caribbean lizards of the genus *Anolis* are a species-rich group and a well-known example of adaptive radiation. In 1983, Ernest Williams suggested that species of *Anolis* that belong to the same 'climate type' (taxa that occur sympatrically in either xeric, mesic or very wet habitats) probably evolved under similar ecological conditions, and thus have experienced a parallel evolutionary history. This hypothesis implies that the phylogeographical patterns of such species can be expected to be concordant, a prediction that has not been tested. We conducted a comparative phylogeographical and population genetic study of *Anolis poncensis* and *Anolis cooki*, two sympatric lizards restricted to the aridlands of southwestern Puerto Rico, to determine whether there are similarities in the genetic architecture of the two anoles that may have resulted from a parallel response to the same historical events, or whether each taxon displays a distinct pattern of geographical distribution of intraspecific genealogical lineages. Our dataset consisted of approximately 2120 base pairs of the ND2 and cytochrome *b* genes from specimens from the known extant populations of the two species. The average haplotype diversity in *A. poncensis* (0.36) was considerably lower than that in *A. cooki* (0.62), whereas the average nucleotide diversity in *A. cooki* was ten times higher than that in *A. poncensis*. Both anoles showed pronounced phylogeographical structure, with no shared haplotypes among populations. The gene genealogy of *A. poncensis* recovered three strongly supported clades: the westernmost population, the easternmost deme and the three intermediate populations. In *A. cooki*, the populations from the western part of the species' range formed a well-supported group, to the exclusion of the eastern demes. Pairwise F_{ST} values revealed significant genetic differentiation among all conspecific populations of both anoles. Coalescent simulations indicated that *A. poncensis* could have evolved under a scenario of simple population fragmentation during the Pleistocene, but that *A. cooki* did not. The estimate of the effective population size of *A. cooki* was an order of magnitude larger than that of *A. poncensis*. Because time to the most recent common ancestor is dependent on effective population size, this tenfold difference implies that the time to the most recent common ancestor of *A. cooki* is much longer than that of *A. poncensis*, which indicates that *A. cooki* diversified earlier than *A. poncensis*. Collectively, these findings suggest that, although *A. poncensis* and *A. cooki* are syntopic throughout much of their current distribution, intraspecific diversification in the two species has not proceeded in parallel, which does not support the hypothesis that *Anolis* lizards that occupy the same climate-type region possess spatially and temporally congruent genetic architectures. *Biological Journal of the Linnean Society*, 2009, **96**, 617–634.

ADDITIONAL KEYWORDS: Caribbean Sea – cytochrome *b* – dry forest – evolutionary diversification – mitochondrial DNA – ND2 – population genetics – West Indies.

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INTRODUCTION

Comparative phylogeography is a powerful method for assessing the roles of historical events and demographic factors in shaping genetic diversity in ecological communities. This multispecies approach enables broader conclusions to be drawn than those generated from single-species studies, and has led to the recognition that phylogeographical data can be used to depict the effects of past events on processes of population differentiation that may ultimately lead to more diverse ecological communities (Bermingham & Moritz, 1998; Templeton, 1998; Avise, 2000; Hewitt, 2000; Patton, da Silva & Malcolm, 2000; Riddle, Hafner & Alexander, 2000; Barber, Erdmann & Palumbi, 2006; Soltis *et al.*, 2006; Victoriano *et al.*, 2008). If similar forces have affected the demographies of species located in the same geographical region, we would expect these species to share major similarities in their genealogical structures. Accordingly, congruence among phylogeographical hypotheses for codistributed taxa should be considered as indirect evidence that common historical and genealogical processes have influenced regional patterns of biodiversity (Lapointe & Rissler, 2005).

Anolis lizards have radiated extensively in both mainland Central and South America (more than 250 species) and the West Indies (more than 150 species; Irschick *et al.*, 1997; Losos *et al.*, 2006). Phylogenetic and ecological studies of West Indian *Anolis* have played an important role in the testing of hypotheses and the development of theories about evolutionary diversification, as well as in the elucidation of interesting biogeographical patterns (for example, Roughgarden, 1995; Losos *et al.*, 1998; Losos & Schluter, 2000; Calsbeek & Smith, 2003; Kolbe *et al.*, 2004; Glor, Losos & Larson, 2005; Harmon *et al.*, 2005; Nicholson *et al.*, 2005; Schoener, Losos & Spiller, 2005; Butler, Sawyer & Losos, 2007; Rodríguez-Robles, Jezkova & García, 2007; Johnson *et al.*, 2008). As is typically the case in Caribbean islands, species of *Anolis* are conspicuous elements of the fauna that inhabits terrestrial ecosystems in Puerto Rico, the smallest and easternmost of the Greater Antilles. *Anolis poncensis* Stejneger and *Anolis cooki* Grant are endemic to the subtropical dry forests of Puerto Rico, aridlands that are primarily found on the southwest coast of the island, in the rain shadow of the central mountain system, and extend 3–20 km inland, from the municipality of Cabo Rojo in the west (18.09°, –67.15°) to the municipality of Guayama in the east (17.99°, –66.13°; Ewel & Whitmore, 1973; Murphy *et al.*, 1995; Helmer *et al.*, 2002). *Anolis poncensis* ('Lagartijo Jardinero del Sur', Dryland Grass Anole) is a relatively small, sexually dimorphic lizard [maximum snout-to-vent length (= body size) is 48 mm in males and 41 mm in females; Williams, 1983; M. Leal, unpubl. data]. *Anolis cooki* ('Lagartijo del Bosque Seco', Dry Forest Anole) is a medium-sized lizard (maximum snout-to-vent length is 70 mm in males and 59 mm in females; Schwartz & Henderson, 1991) that has become specialized to the light conditions (Leal & Fleishman, 2002) and thermal microhabitat (Hertz, 1992) characteristic of the localities of sparse, xeric vegetation in which it is found.

In his seminal work on the evolutionary radiation of *Anolis* in the Greater Antilles, Williams (1983) suggested that species that belong to the same 'climate type' (taxa that occur sympatrically in either xeric, mesic or very wet habitats) probably evolved under similar ecological conditions, and thus have experienced a parallel evolutionary history. This hypothesis implies that the phylogeographical patterns of such species should be concordant, a prediction that has not been tested. Herein, we present the results of a comparative phylogeographical study of *A. poncensis* and *A. cooki* to determine whether there are similarities in the genetic architecture of these two lizards that may have resulted from a parallel response to the same historical events, or whether each species displays spatially and temporally distinct phylogeographical patterns.

MATERIAL AND METHODS

TAXON SAMPLING, DNA ISOLATION AND SEQUENCING

We secured samples from the known extant populations of *A. poncensis* and *A. cooki* (except from the *A. cooki* deme from Caja de Muertos Island, located off the south-central coast of Puerto Rico; Fig. 1), and unsuccessfully looked for new localities for both species. Tissue samples were obtained from 56 *A. poncensis* (from five populations) and 52 *A. cooki* (from six populations; Table 1, Fig. 1). Because of its restricted and disjunct distribution, *A. cooki* was designated in 1991 as a threatened species by the Department of Natural and Environmental Resources of Puerto Rico (Moreno, 1991), and the dataset for this species included in the present comparative study was initially reported in an article that focused on the conservation genetics of the species (Rodríguez-Robles, Jezkova & Leal, 2008).

Total genomic DNA was extracted from frozen tissue samples (liver, muscle, tail fragments) with the DNeasy Blood & Tissue Kit (Qiagen Inc., Valencia, CA, USA), following the manufacturer's instructions. We designed two primers, LVT_Metf.6_AnCr (AAGC TATTGGGCCCATACC) and LVT_5617_AnCr (AAAG TGYTTGAGTTGCATTCA), to amplify approximately 1150 base pairs (bp) of the nicotinamide adenine

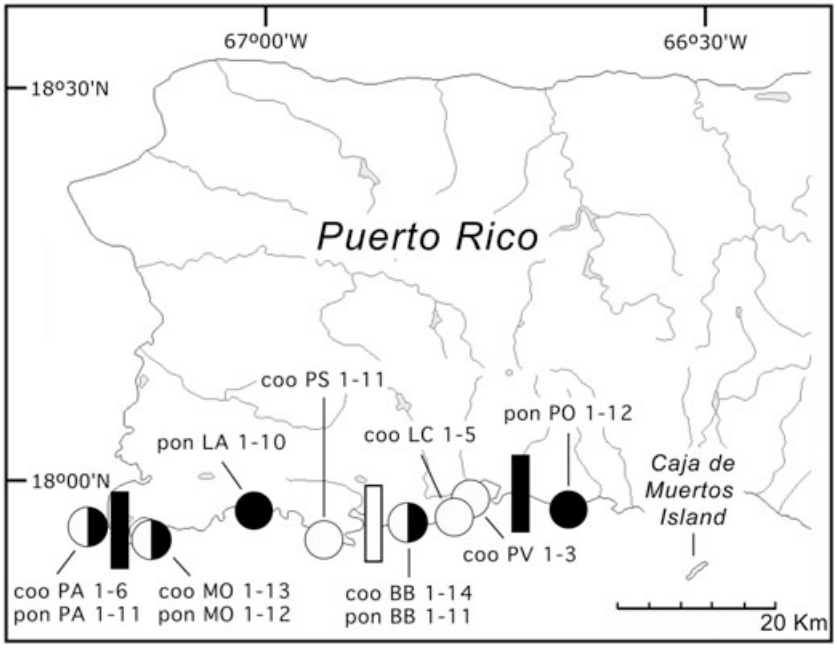


Figure 1. Map of western Puerto Rico. Circles indicate the approximate locations of the specimens of *Anolis poncensis* (filled circles) and *A. cooki* (open circles) included in this study (half-filled circles indicate locations at which both *A. poncensis* and *A. cooki* were collected; see Table 1 for specific locality information). The filled bars indicate the two basal phylogenetic breaks inferred for *A. poncensis*, and the open bar indicates the basal split within *A. cooki*. Abbreviations: coo, *Anolis cooki*; pon, *Anolis poncensis*; BB, Bahía Ballena; LA, Lajas; LC, La Cueva; MO, Morrillos; PA, Punta Águila; PO, Ponce; PS, Playa Santa; PV, Punta Verraco.

dinucleotide dehydrogenase (NADH) subunit 2 ('ND2') and adjacent tRNAs ($tRNA^{Trp}$, $tRNA^{Ala}$), and used the primers MVZ_49 (ATAARAACAATGACAAT YATACGAAG; Roe *et al.*, 1985) and MVZ_14 (GGTCT TCATCTYHGGYTTACAAGAC) to amplify approximately 1100 bp of cytochrome *b* ('Cyt b'). Polymerase chain reactions (PCRs) were carried out in 12.5 μL 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 double-distilled H_2O. DNA was denatured initially at 95 °C for 2.5 min; 30–35 cycles of amplification were then 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; this was 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 the product band size.

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

Phylogenetic and population analyses

The program COLLAPSE (version 1.2; available at http://darwin.uvigo.es) was used to collapse all sequences to unique haplotypes. An incongruence length difference test (Farris *et al.*, 1994), performed with the program PAUP* (version 4.10b; Swofford, 2003), indicated that the sequences from the ND2 (1044 bp for *A. poncensis*, 1041 bp for *A. cooki*) and Cyt b (1085 bp for *A. poncensis*, 1083 bp for *A. cooki*) genes contained a congruent phylogenetic signal (1000 replicates, $P = 1.0$) in the two anoles. Accordingly, the ND2 and Cyt b datasets were combined for all analyses conducted for each species. All subsequent phylogenetic tests were performed using maximum likelihood (ML) and Bayesian inference methods. Based on previous karyotypic, electrophoretic and phylogenetic studies (Gorman *et al.*, 1968, 1983; Brandley & de Queiroz, 2004; Poe, 2004; Nicholson *et al.*, 2005; Rodríguez-Robles *et al.*, 2007), *A. gundlachi*, and *A. pulchellus* were used as outgroup taxa for *A. poncensis*, and *A. monensis* and *A. cristatellus* as outgroup taxa for *A. cooki*.

Table 1. Taxon, sample number, voucher number, GenBank accession number, locality and coordinates of the specimens used in this study (MVZ, Museum of Vertebrate Zoology, University of California, Berkeley, CA, USA)

Taxon	Sample number	Voucher number	GenBank accession numbers for cytochrome *b* and ND2 sequences, respectively; locality	Coordinates (latitude, longitude)
Outgroup for *Anolis poncensis*				
Anolis gundlachi	1	MVZ 250939	EU095729, EU095781; Puerto Rico: Municipality of Río Grande; Rd. 903, off Km. 19.5 on Rd. 186	18.32, –65.82
	2	MVZ 252252	EU095730, EU095782; Puerto Rico: Municipality of Lares; Km. 27.0 on Rd. 129	18.30, –66.87
Anolis pulchellus	–	MVZ 251076	EU095780, EU095832; Puerto Rico: Municipality of Ponce, 9.6 km NW of intersection between Hwy. 10 and Hwy. 52, along Hwy. 10	18.14, –66.67
Anolis poncensis				
	Punta Águila 1	MVZ 257370	EU095775, EU095827; Puerto Rico: Municipality of Cabo Rojo, Km 12.0 on Rd. 301	17.96, –67.20
	Punta Águila 2	MVZ 257371	EU095776, EU095828	
	Punta Águila 3	MVZ 257372	EU095777, EU095829	
	Punta Águila 4	MVZ 257608	AB377056, AB377048	
	Punta Águila 5	MVZ 257609	AB377057, AB377049	
	Punta Águila 6	MVZ 257612	AB377060, AB377052	
	Punta Águila 7	MVZ 257614	AB377061, AB377053	
	Punta Águila 8	MVZ 257616	AB377058, AB377050	
	Punta Águila 9	MVZ 257617	AB377062, AB377054	
	Punta Águila 10	MVZ 257618	AB377059, AB377051	
	Punta Águila 11	MVZ 257619	AB377063, AB377055	
	Morrillos 1	MVZ 252288	EU095755, EU095807; Puerto Rico: Municipality of Cabo Rojo, Bosque Estatal de Boquerón, Morrillos de Cabo Rojo	17.94, –67.20
	Morrillos 2	MVZ 252289	EU095756, EU095808	
	Morrillos 3	MVZ 252290	EU095757, EU095809	
	Morrillos 4	MVZ 252291	EU095758, EU095810	
	Morrillos 5	MVZ 252292	EU095759, EU095811	
	Morrillos 6	MVZ 252293	EU095760, EU095812	
	Morrillos 7	MVZ 252294	EU095761, EU095813	
	Morrillos 8	MVZ 252295	EU095762, EU095814	
	Morrillos 9	MVZ 252296	EU095763, EU095815	
	Morrillos 10	MVZ 252297	EU095764, EU095816	
	Morrillos 11	MVZ 252298	EU095765, EU095817	
	Morrillos 12	MVZ 252299	EU095766, EU095818	
	Lajas 1	MVZ 252310	EU095753, EU095805; Puerto Rico: Municipality of Lajas, La Parguera	17.97, –67.04
	Lajas 2	MVZ 252287	EU095754, EU095806	
	Lajas 3	MVZ 257355	EU095767, EU095819	
	Lajas 4	MVZ 257356	EU095768, EU095820	
	Lajas 5	MVZ 257357	EU095769, EU095821	
	Lajas 6	MVZ 257358	EU095770, EU095822	
	Lajas 7	MVZ 257359	EU095771, EU095823	
	Lajas 8	MVZ 257360	EU095772, EU095824	
	Lajas 9	MVZ 257361	EU095773, EU095825	
	Lajas 10	MVZ 257362	EU095774, EU095826	

Table 1. *Continued*

Taxon	Sample number	Voucher number	GenBank accession numbers for cytochrome *b* and ND2 sequences, respectively; locality	Coordinates (latitude, longitude)
	Bahía Ballena 1	MVZ 252301	EU095744, EU095796; Puerto Rico: Municipality of Guánica, Bahía Ballena	17.96, –66.86
	Bahía Ballena 2	MVZ 252302	EU095745, EU095797	
	Bahía Ballena 3	MVZ 252303	EU095746, EU095798	
	Bahía Ballena 4	MVZ 252304	EU095747, EU095799	
	Bahía Ballena 5	MVZ 252305	EU095748, EU095800	
	Bahía Ballena 6	MVZ 252306	EU095749, EU095801	
	Bahía Ballena 7	MVZ 252307	EU095750, EU095802	
	Bahía Ballena 8	MVZ 252308	EU095751, EU095803	
	Bahía Ballena 9	MVZ 252309	EU095752, EU095804	
	Bahía Ballena 10	MVZ 257379	EU095778, EU095830	
	Bahía Ballena 11	MVZ 257380	EU095779, EU095831	
	Ponce 1	MVZ 226163	EU095732, EU095784; Puerto Rico: Municipality of Ponce, Km 221.2 on Hwy. 2	17.98, –66.67
	Ponce 2	MVZ 226164	EU095733, EU095785	
	Ponce 3	MVZ 226165	EU095734, EU095786	
	Ponce 4	MVZ 252312	EU095735, EU095787; Puerto Rico: Municipality of Ponce, El Tuque, Km 219.6 on Hwy. 2	17.97, –66.67
	Ponce 5	MVZ 252313	EU095736, EU095788	
	Ponce 6	MVZ 252314	EU095737, EU095789	
	Ponce 7	MVZ 252315	EU095738, EU095790	
	Ponce 8	MVZ 252316	EU095739, EU095791	
	Ponce 9	MVZ 252317	EU095740, EU095792	
	Ponce 10	MVZ 252318	EU095741, EU095793	
	Ponce 11	MVZ 252319	EU095742, EU095794	
	Ponce 12	MVZ 252320	EU095743, EU095795	
Outgroup for *Anolis cooki*				
Anolis cristatellus	–	MVZ 242846	EF553539, EF184065; Puerto Rico: Municipality of Lajas, Km 3.3 on Rd. 304	17.98, –67.05
Anolis monensis	–	MVZ 235440	EF553612, EF184138; Mona Island: vicinity of Playa Sardinera	18.09, –67.94
	–	MVZ 235454	EF553622, EF184148; Monito Island	18.16, –67.95
Anolis cooki				
	Punta Águila 1	MVZ 257364	EU095684, EU095723; Puerto Rico: Municipality of Cabo Rojo, Punta Águila	17.95, –67.21
	Punta Águila 2	MVZ 257365	EU095685, EU095724	
	Punta Águila 3	MVZ 257366	EU095686, EU095725	
	Punta Águila 4	MVZ 257367	EU095687, EU095726	
	Punta Águila 5	MVZ 257368	EU095688, EU095727	
	Punta Águila 6	MVZ 257369	EU095689, EU095728	
	Morrillos 1	MVZ 235170	EU119666, EF184066; Puerto Rico: Municipality of Cabo Rojo, Bosque Estatal de Boquerón, Morrillos de Cabo Rojo	17.94, –67.20
	Morrillos 2	MVZ 235172	EU119667, EF184067	
	Morrillos 3	MVZ 252194	EU119675, EF184068	
	Morrillos 4	MVZ 252195	EU119676, EF184069	
	Morrillos 5	MVZ 252196	EU119677, EF184070	
	Morrillos 6	MVZ 252197	EU119678, EF184071	

Table 1. *Continued*

Taxon	Sample number	Voucher number	GenBank accession numbers for cytochrome *b* and ND2 sequences, respectively; locality	Coordinates (latitude, longitude)
	Morrillos 7	MVZ 252198	EU095667, EU095706	
	Morrillos 8	MVZ 252199	EU095668, EU095707	
	Morrillos 9	MVZ 252200	EU095669, EU095708	
	Morrillos 10	MVZ 252201	EU095670, EU095709	
	Morrillos 11	MVZ 252202	EU095671, EU095710	
	Morrillos 12	MVZ 252203	EU095672, EU095711	
	Morrillos 13	MVZ 252204	EU095673, EU095712	
	Playa Santa 1	MVZ 252211	EU095665, EU095704; Puerto Rico: Municipality of Guánica, Balneario Playa Santa	17.94, –66.96
	Playa Santa 2	MVZ 252212	EU095666, EU095705	
	Playa Santa 3	MVZ 257334	EU095675, EU095714	
	Playa Santa 4	MVZ 257335	EU095676, EU095715	
	Playa Santa 5	MVZ 257336	EU095677, EU095716	
	Playa Santa 6	MVZ 257337	EU095678, EU095717	
	Playa Santa 7	MVZ 257338	EU095679, EU095718	
	Playa Santa 8	MVZ 257339	EU095680, EU095719	
	Playa Santa 9	MVZ 257340	EU095681, EU095720	
	Playa Santa 10	MVZ 257341	EU095682, EU095721	
	Playa Santa 11	MVZ 257342	EU095683, EU095722	
	Bahía Ballena 1	MVZ 250896	EU119668, EF184072; Puerto Rico: Municipality of Guánica, Bahía Ballena	17.96, –66.86
	Bahía Ballena 2	MVZ 250897	EU119669, EF184073	
	Bahía Ballena 3	MVZ 250898	EU119670, EF184074	
	Bahía Ballena 4	MVZ 250899	EU119671, EF184075	
	Bahía Ballena 5	MVZ 250900	EU119672, EF184076	
	Bahía Ballena 6	MVZ 250901	EU119673, EF184077	
	Bahía Ballena 7	MVZ 250902	EU119674, EF184078	
	Bahía Ballena 8	MVZ 252206	EU095660, EU095699	
	Bahía Ballena 9	MVZ 252207	EU095661, EU095700	
	Bahía Ballena 10	MVZ 252208	EU095662, EU095701	
	Bahía Ballena 11	MVZ 252209	EU095663, EU095702	
	Bahía Ballena 12	MVZ 252210	EU095664, EU095703	
	Bahía Ballena 13	MVZ 251147	EU095651, EU095690	
	Bahía Ballena 14	MVZ 251148	EU095652, EU095691	
	La Cueva 1	MVZ 252213	EU095653, EU095692; Puerto Rico: Municipality of Guayanilla, La Cueva	17.97, –66.79
	La Cueva 2	MVZ 252214	EU095654, EU095693	
	La Cueva 3	MVZ 252215	EU095655, EU095694	
	La Cueva 4	MVZ 252216	EU095656, EU095695	
	La Cueva 5	MVZ 252219	EU095674, EU095713	
	Punta Verraco 1	MVZ 252205	EU095659, EU095698; Puerto Rico: Municipality of Guayanilla, Punta Verraco	17.98, –66.78
	Punta Verraco 2	MVZ 252217	EU095657, EU095696	
	Punta Verraco 3	MVZ 252218	EU095658, EU095697	

The software MRMODELTEST (version 2.2; Nylander, 2004) was used to select the best-fit models of nucleotide substitution for the data for the ML and Bayesian analyses. Hierarchical likelihood ratio tests and Akaike Information Criteria (AIC) identified HKY + I (for the first plus second codon positions) and GTR + G (for the third codon position) as the most appropriate models for *A. poncensis*, and HKY + G + I (for the first plus second codon positions) and GTR + G (for the third codon position) for *A. cooki*. ML analyses were conducted using TREEFINDER (Jobb, von Haeseler & Strimmer, 2004). TREEFINDER uses a fast sampling algorithm to estimate all model parameters and to construct a phylogeny. The accuracy of the program with regard to the correct inference of tree topologies and estimation of branch lengths is similar to that of other likelihood programs, such as FASTDNAML (Olsen *et al.*, 1994) and PAUP* (Jobb *et al.*, 2004). The 'Bootstrap Analysis' option in TREEFINDER (1000 replicates; consensus level, 50) was used to assess nodal support on the ML tree.

Tree topology and clade support were also estimated using Bayesian inference methods, as implemented in MRBAYES (version 3.1.1; Huelsenbeck & Ronquist, 2001; Ronquist & Huelsenbeck, 2003). Using the models of sequence evolution selected by MRMODELTEST, analyses were initiated from a random starting tree with uniform (uninformative) priors (Brandley *et al.*, 2006). Posterior probability distributions were produced by allowing four Monte Carlo Markov chains (using default heating values) to proceed for ten million generations each, with samples taken every 100 generations, a procedure that yielded 100 000 trees. Parameter stabilization was assessed by examining plots of log-likelihood scores versus number of generations (Leaché & Reeder, 2002). The first 2 500 000 generations (25 000 trees) were discarded as 'burn-in' samples (that is, trees obtained before parameter stabilization occurred), and the remaining samples were combined to estimate tree topology, posterior probability values and branch lengths. Bayesian analyses were run three times to ensure that they were not trapped on local optima.

Tree-building methods tend to resolve intraspecific gene genealogies poorly when levels of genetic divergence are low, multifurcations occur and ancestral haplotypes are still present in the populations (Crandall & Templeton, 1996). Accordingly, NETWORK 4.200 (http://www.fluxus-technology.com) was used to construct median-joining networks (Bandelt *et al.*, 1999) to visualize better the evolutionary paths among the haplotypes of *A. poncensis* and *A. cooki.* Mean, pairwise, uncorrected sequence divergences were estimated between populations of both anoles with MEGA (version 3.1; Kumar, Tamura & Nei, 2004).

For population genetic analyses, haplotype (h) and nucleotide diversity (π) were calculated, and tests of selective neutrality (Tajima's D, Fu's F_S) were conducted using ARLEQUIN (version 3.1; Excoffier, Laval & Schneider, 2005). Genetic differentiation among the sampling localities was assessed using Sewall Wright's fixation index F_{ST} (10 000 permutations).

COALESCENT SIMULATIONS

Coalescent simulations were used to evaluate whether the present genetic structures of *A. poncensis* and *A. cooki* could have been influenced by the same historical processes, and whether any incongruence that may exist between the gene trees for the two species could have been caused by different demographic and/or stochastic processes. The generation time T was estimated using the equation $T = \alpha + [s/(1 - s)]$ (Lande, Engen & Sæther, 2003), where α (age of sexual maturity) was 9 months (based on an estimate for *Anolis gundlachi*; Turner & Gist, 1970; Andrews, 1976) and s (the annual adult survival rate) was 0.13% (estimated from the monthly loss rates of male and female *A. gundlachi*; Lister, 1981). Solving this equation produced an estimated generation time of 1 year, a value that was used for all coalescent simulations.

Because the performance of coalescent simulations requires an estimate of total effective population size, the program MIGRATE (version 2.1.3; Beerli & Felsenstein, 1999, 2001; Beerli, 2006; available at http://popgen.csit.fsu.edu/) was used to calculate theta (θ) values (the effective population size scaled to the neutral mutation rate), and these values were employed to estimate the effective population size of females ($N_{e(f)}$) for each population of *A. poncensis* and *A. cooki* using the equation $\theta = 2N_{e(f)}\mu$, assuming $\mu = 0.65 \times 10^{-8}$ (cf. Macey *et al.*, 1998). The following search parameters in MIGRATE were used to estimate $N_{e(f)}$: ten short chains (5000 steps each) followed by two long chains (50 000 steps each), and transition/transversion ratios (calculated in PAUP*) of 4.26 (for *A. poncensis*) and 5.46 (for *A. cooki*). Each chain had a burn-in period of 10 000 steps. The default values of MIGRATE were used for all other parameters. The program was run twice with different random seed numbers to ensure that the estimates were robust. The program produced similar estimates in each run, and herein we report the values obtained on the first run. The total effective population size was calculated as the sum of the effective population sizes for each population because, when gene flow among populations is limited or nonexistent, the combination of all haplotypes into a single population may lead to a substantial overestimate of the effective population size as a result of the disproportionate effect of inter-

population coalescences on the calculations (P. Beerli, Florida State University, Tallahassee, pers. comm.).

Coalescent simulations were performed with the program MESQUITE (version 1.12; Maddison & Maddison, 2006) to test *a priori* hypotheses of population histories. The *S statistic* (Slatkin & Maddison, 1989) was used to evaluate the concordance, or lack thereof, between the observed gene trees and the subdivision of *A. poncensis* and *A. cooki* into populations under three temporal scenarios (see below). A simple model of population fragmentation was tested, because such a model may be appropriate for the elucidation of patterns of diversification in *Anolis* (cf. Lazell, 1996; Jackman *et al.*, 2002). The model was represented by a multifurcating tree (i.e. a polytomy), where each tip comprised one of the sampled populations. For each population, the effective female population size ($N_{e(f)}$) was held constant through time (see 'Discussion'). On the basis of our divergence estimates for *A. poncensis* and *A. cooki*, based on pairwise sequence differences (see 'Results'), we tested whether the fragmentation of each species into populations could have occurred during one of three major episodes of the Pleistocene: the Pliocene–Pleistocene boundary (1.8 million years ago; Hypothesis 1, or H_1); the middle Pleistocene interglacial (800 000 years ago; H_2) or the late Pleistocene (100 000 years ago; H_3) (Gradstein, Ogg & Smith, 2004).

For *A. poncensis* and *A. cooki*, in MESQUITE, the neutral coalescence of gene sequences was simulated within each of the three temporal hypotheses (H_1, H_2, H_3) using the model of DNA evolution selected by MRMODELTEST. One hundred gene matrices were simulated for each hypothesis, and trees were reconstructed in PAUP* from these matrices using maximum parsimony. The distribution of *S* values for the simulated trees was then compared (in MESQUITE) with the *S* value of the actual gene tree. The *S* statistic was considered to be significant if it was found to be less than 5% of the values generated at random (Haenel, 2007).

RESULTS

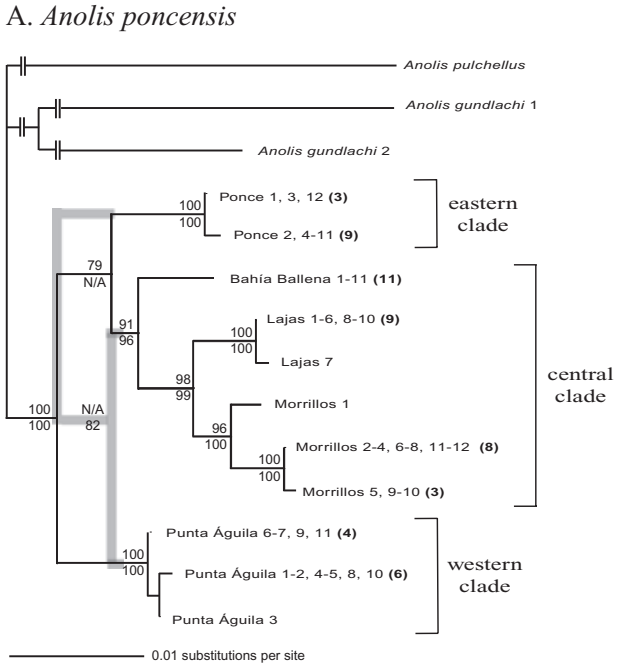
PHYLOGENETIC ANALYSES

For *A. poncensis*, ML and Bayesian methods inferred trees with the same three main clades (Fig. 2A). One clade contained individuals from the westernmost population of the species (Punta Águila), and another grouping was composed of specimens from the easternmost deme (Ponce). The geographically intermediate populations of *A. poncensis* from Morrillos, Lajas and Bahía Ballena constituted the third clade. The haplotypes from each locality formed strongly supported monophyletic groups. The two phylogenetic methods recovered weakly supported, incongruent relationships among the three clades: ML suggested that the eastern and central clades were sister taxa, whereas Bayesian analyses indicated that the central and western clades were each other's closest relative.

In *A. cooki*, the specimens from the three westernmost demes (Punta Águila, Morrillos and Playa Santa) formed a group, to the exclusion of individuals from the three easternmost populations (Bahía Ballena, La Cueva and Punta Verraco) of the species (Fig. 2B). Within the western clade, the specimens from Punta Águila and Morrillos nested together, except for one individual (Punta Águila 6) that grouped with a clade from Playa Santa. Specimens from the Playa Santa deme formed two different lineages. With regard to the three easternmost populations of *A. cooki*, the haplotypes from Bahía Ballena formed two different groups, whereas those from La Cueva and Punta Verraco nested together. However, the relationships among the mitochondrial types (haplotypes) from the last three populations were not well supported.

Median-joining networks provided additional information on the intraspecific relationships among the populations of *A. poncensis* and *A. cooki*. The mitochondrial types of *A. poncensis* could be assigned to five groups that corresponded almost precisely to each of the five sampling localities, namely Morrillos (2–12), Lajas and Morrillos (1), Punta Águila, Bahía Ballena and Ponce (Fig. 3A). The mitochondrial types of each of the five sampling localities of *A. poncensis* were separated by one or two mutations only. The exception was one haplotype from the Morrillos population (Morrillos 1) that differed from its closest mitochondrial type from the same locality by 13 mutational steps. The haplotypes from the westernmost deme of *A. poncensis* (Punta Águila) were the most divergent, being separated from their closest relative (from Bahía Ballena) by 33 mutations.

The haplotype network of *A. cooki* showed three main groups (Fig. 3B). One cluster was composed of specimens from Punta Águila and Morrillos. These two nearby populations did not share haplotypes, but five unique mitochondrial types from the two localities were separated by only one to three mutational steps. The exception was a unique haplotype from Punta Águila (Punta Águila 6) that was most closely related to one of the two mitochondrial groups from Playa Santa. In contrast, in the second cluster, the five haplotypes from the Playa Santa population formed two subgroups that differed by up to 35 mutations. The third cluster comprised populations from Bahía Ballena, La Cueva and Punta Verraco. These demes, particularly that from Bahía Ballena, exhibited higher haplotype diversity than those from Punta Águila, Morrillos and Playa Santa. The mitochondrial types from Bahía Ballena were separated by 1–20



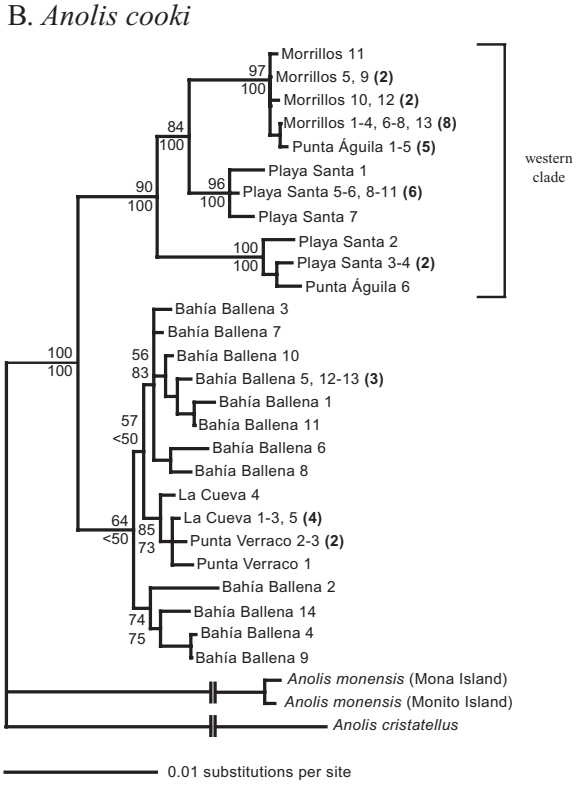


Figure 2. A, Maximum likelihood (ML) tree for 11 unique mitochondrial DNA haplotypes of *Anolis poncensis*. *Anolis gundlachi* and *A. pulchellus* were used as outgroup taxa. Trees inferred with ML and Bayesian methods recovered different relationships among the three main clades of *A. poncensis* (Ponce, Morrillos/Lajas/Bahía Ballena, Punta Águila), and the shading indicates the internodes recovered by Bayesian analyses. B, Maximum likelihood tree for 27 unique mitochondrial DNA haplotypes of *A. cooki*. *Anolis cristatellus* and *A. monensis* were used as outgroup taxa. For both species, nodal support was assessed using nonparametric bootstrap values for ML analyses (numbers above) and Bayesian posterior probabilities (numbers below). See 'Material and methods' for details of phylogenetic analyses.

mutational steps. The haplotypes from La Cueva and Punta Verraco clustered together and nested with mitochondrial types from Bahía Ballena.

We could not calibrate a precise molecular clock for *A. poncensis* or *A. cooki* because of the absence of fossils for either species. However, the average rate of evolution of the fragment of the ND2 gene used in this study is 0.65% (range, 0.61–0.7%) per lineage per million years (Macey *et al.*, 1998), a rate that has been used to estimate divergences in previous investigations of Caribbean *Anolis* (for example, Creer *et al.*, 2001; Jackman *et al.*, 2002; Glor *et al.*, 2003; Rodríguez-Robles *et al.*, 2007). Applying this rate to the mean, pairwise, uncorrected sequences, the divergences obtained in this study yielded a maximum age of *c.* 8.7 million years (range, 8.1–9.3 million years; 11.3% divergence) for the split between *A. poncensis* and *A. gundlachi*; *c.* 1.6 million years (range, 1.5–1.7 million years; 2.1% divergence) for the divergence between the Punta Águila and Morrillos populations of *A. poncensis*; *c.* 770 000 years (range, 710 000–820 000 years; 1% divergence) for the separation between the demes from Morrillos and Lajas; and *c.* one million years (range, 930 000–1.1 million years; 1.3% divergence) and *c.* 1.1 million years (range, 1.0–1.15 million years; 1.4% divergence) for the split between the populations from Lajas and Bahía Ballena, and Bahía Ballena and Ponce, respectively. For *A. cooki*, we estimated a maximum age of *c.* 690 000 years (range, 640 000–740 000 years; 0.9%) for the divergence between the three westernmost and the three easternmost assemblages of the species; *c.* 310 000 years (range, 290 000–330 000 years; 0.4% divergence) for the separation of the two populations from Cabo Rojo (Punta Águila and Morrillos) and that from Playa Santa; and *c.* 150 000 years (range, 140 000–160 000 years; 0.2% divergence) for the split between Bahía Ballena and the two Guayanilla demes (La Cueva and Punta Verraco).

Population analyses

Haplotype and nucleotide diversity varied greatly between the two species (Table 2). We identified 11 (from 56 individuals) and 27 (from 52 individuals) unique haplotypes in *A. poncensis* and *A. cooki*,

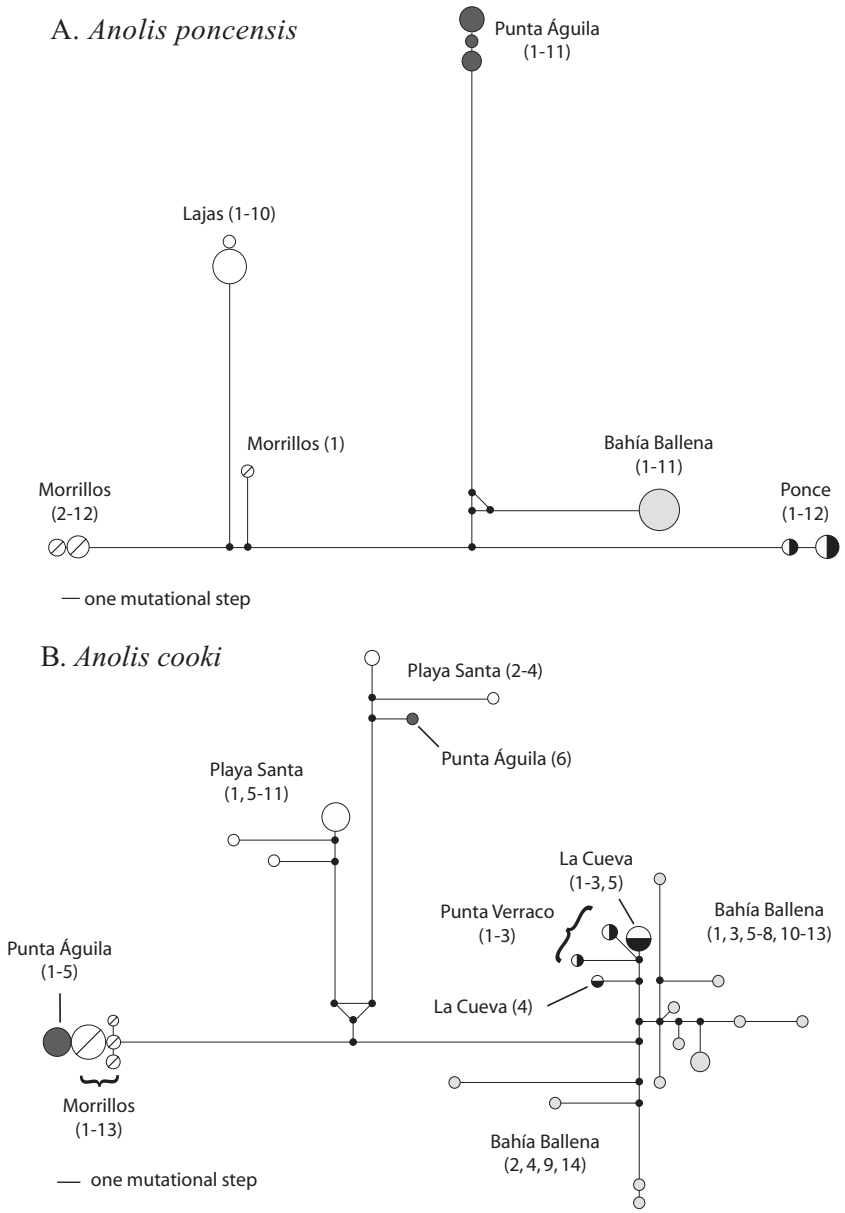


Figure 3. A, Median-joining network representing the relationships among haplotypes of *Anolis poncensis* from the Morrillos (divided open circles, $N = 12$), Lajas (open circles, $N = 10$), Punta Águila (dark grey circles, $N = 11$), Bahía Ballena (light grey circles, $N = 11$) and Ponce (half-filled circles; $N = 12$) populations. B, Median-joining network representing the relationships among haplotypes of *A. cooki* from the Punta Águila (dark grey circles, $N = 6$), Morrillos (divided open circles, $N = 13$), Playa Santa (open circles, $N = 11$), Bahía Ballena (light grey circles, $N = 14$), La Cueva (half-filled circles, horizontal pattern, $N = 5$) and Punta Verraco (half-filled circles, vertical pattern, $N = 3$) populations. In both networks, the smallest filled circles indicate median vectors (Bandelt *et al.* 1999). Circle size is proportional to haplotype frequency, with the smallest circle representing one sample and the largest circle representing 11 (*A. poncensis*) or eight (*A. cooki*) samples; branch length is proportional to the number of mutations separating the haplotypes.

respectively. There were no shared haplotypes between any of the populations of either species (Fig. 3); that is, each of the five populations of *A. poncensis* and six populations of *A. cooki* only had private mitochondrial types. Overall, the haplotype diversity tended to decrease from west to east in *A. poncensis*, and from east to west in *A. cooki*. The haplotype diversity in *A. poncensis* ranged from zero (Bahía Ballena) to 0.62 (Punta Águila); the nucleotide diversity was low in all populations, and ranged from zero (Bahía Ballena) to 0.00121 (Morrillos). In *A. cooki*, the haplotype diversity ranged from 0.33

Table 2. Measures of haplotype and nucleotide diversity and tests of selective neutrality for populations of *Anolis poncensis* (values in bold) and *A. cooki*

Population	No. of samples (no. of haplotypes)	Haplotype diversity (±SD)	Nucleotide diversity (±SD)	Tajima's *D*	Fu's F_S
Punta Águila	**11 (3)**	**0.62 (±0.11)**	**0.0005 (±0.0004)**	**1.665**	**0.694**
				***P* = 0.97**	***P* = 0.64**
Punta Águila	6 (2)	0.33 (±0.22)	0.00471 (±0.00290)	–1.513	8.007
				P = 0.001	*P* = 0.997
Morrillos	**12 (3)**	**0.53 (±0.14)**	**0.00121 (±0.00079)**	**–1.896**	**3.057**
				***P* = 0.02**	***P* = 0.91**
Morrillos	13 (4)	0.62 (±0.14)	0.00045 (±0.00037)	–0.059	–0.628
				P = 0.46	*P* = 0.21
Lajas	**10 (2)**	**0.20 (±0.15)**	**0.00009 (±0.00014)**	**–1.112**	**–0.339**
				***P* = 0.20**	***P* = 0.14**
Playa Santa	11 (5)	0.71 (±0.14)	0.00640 (±0.00352)	0.226	5.490
				P = 0.61	*P* = 0.98
Bahía Ballena	**11 (1)**	**0.0 (±0.0)**	**0.0 (±0.0)**	**0.0**	–
				***P* = 1.0**	–
Bahía Ballena	14 (12)	0.97 (±0.04)	0.00477 (±0.00260)	–1.169	–2.596
				P = 0.12	*P* = 0.09
Ponce	**12 (2)**	**0.41 (±0.13)**	**0.00038 (±0.00033)**	**0.688**	**1.961**
				***P* = 0.77**	***P* = 0.81**
La Cueva	5 (2)	0.40 (±0.24)	0.00075 (±0.00062)	–1.094	2.202
				P = 0.11	*P* = 0.85
Punta Verraco	3 (2)	0.67 (±0.31)	0.00157 (±0.00136)	0.0	2.357
				P = 0.80	*P* = 0.79

Table 3. Pairwise F_{ST} values for populations of *Anolis poncensis*

	Punta Águila	Morrillos	Lajas	Bahía Ballena	Ponce
Punta Águila	–	0.96	0.984	0.985	0.975
		P < 0.0001	*P* < 0.0001	*P* < 0.0001	*P* < 0.0001
Morrillos	–	–	0.935	0.956	0.957
			P < 0.0001	*P* < 0.0001	*P* < 0.0001
Lajas	–	–	–	0.997	0.985
				P < 0.0001	*P* < 0.0001
Bahía Ballena	–	–	–	–	0.986
					P < 0.0001

(Punta Águila) to 0.97 (Bahía Ballena), whereas the average nucleotide diversity was approximately ten times higher than that in *A. poncensis*, and ranged from 0.00045 (Morrillos) to 0.0064 (Playa Santa).

Pairwise F_{ST} values revealed significant genetic differentiation among all populations of *A. poncensis* and *A. cooki* (Tables 3 and 4). Tajima's *D* differed significantly from the expectation under neutrality in only one population of each species, whereas Fu's F_S was not significant for any deme. These results suggest that, for nine of the 11 populations included in this study, evolution has been relatively independent of selection and/or major population perturbations during the coalescent history of the analysed genes (Templeton, 2006; Hartl & Clark, 2007). Tajima's *D* was significantly negative for the *A. poncensis* deme from Morrillos and for the *A. cooki* population from Punta Águila. Tajima's *D* is based on the relationship between the average number of pairwise differences ($\bar{\pi}$) and the number of segregating sites (*S*); the statistic becomes negative when there is an excess of low-frequency alleles (haplotypes). This may happen when the genes under investigation are under natural selection (or linked to a gene that is under selection),

Table 4. Pairwise F_{ST} values for populations of *Anolis cooki*

	Punta Águila	Morrillos	Playa Santa	Bahía Ballena	La Cueva	Punta Verraco
Punta Águila	–	0.235 $P = 0.001$	0.454 $P < 0.0001$	0.710 $P < 0.0001$	0.816 $P = 0.003$	0.773 $P = 0.012$
Morrillos	–	–	0.682 $P < 0.0001$	0.827 $P < 0.0001$	0.965 $P < 0.0001$	0.961 $P < 0.0001$
Playa Santa	–	–	–	0.647 $P < 0.0001$	0.699 $P = 0.001$	0.662 $P = 0.004$
Bahía Ballena	–	–	–	–	0.321 $P < 0.0001$	0.299 $P = 0.002$
La Cueva	–	–	–	–	–	0.392 $P = 0.032$

Table 5. Mean theta (θ) values (with associated lower and upper 95% confidence limits) for populations of *Anolis poncensis* and *A. cooki*. We combined the *A. cooki* populations from La Cueva and Punta Verraco because of an insufficient number of samples from each deme and the genetic closeness of the populations

	Anolis poncensis			*Anolis cooki*		
	Lower 95% Confidence limit	Mean	Upper 95% Confidence limit	Lower 95% Confidence limit	Mean	Upper 95% Confidence limit
Punta Águila	0.000043	0.000494	0.00127	0.00000002	0.00000076	0.000004
Morrillos	0.000738	0.00152	0.0039	0.000088	0.000519	0.0016
Lajas	0.000009	0.000061	0.000443	–	–	–
Playa Santa	–	–	–	0.00252	0.00606	0.0137
Bahía Ballena	0.000001	0.000006	0.000052	0.0057	0.0115	0.0285
Ponce	0.000065	0.000119	0.000979	–	–	–
La Cueva and Punta Verraco	–	–	–	0.000472	0.0025	0.00662

or when populations have experienced demographic or geographical expansion (Tajima, 1989; Johnson, Dunn & Bouzat, 2007). The two populations with significantly negative Tajima's *D* values had two nearly identical mitochondrial types (*A. poncensis*; Fig. 3A) or one common haplotype (*A. cooki*; Fig. 3B), plus a single, more distantly related haplotype that increased the number of segregating sites and caused the statistic to become significant [when these haplotypes were removed, Tajima's *D* became nonsignificant for each population ($D = 0.67$, $P = 0.83$ for *A. poncensis*; $D = 0.0$, $P = 1.0$ for *A. cooki*)].

Coalescent simulations

For populations of *A. poncensis*, the θ values varied from 0.000006 for the Bahía Ballena population to 0.00152 for the Morrillos deme (Table 5). For *A. cooki* populations, the θ values were, on average, ten times higher than those for *A. poncensis*, and ranged from 0.00000076 for the Punta Águila deme to 0.011458 for the Bahía Ballena population (Table 5). We estimated a total effective population size of 169 308 and 1 579 751 females of *A. poncensis* and *A. cooki*, respectively. These high estimates may be biologically unrealistic, particularly for *A. cooki*, a threatened island endemic lizard. The large estimate of the total effective population size of *A. cooki* females is mainly caused by the large θ values calculated for the Playa Santa and Bahía Ballena populations, values that partly result from the large haplotype and nucleotide diversities in these two populations. If either population is further substructured or comprises previously isolated populations that have recently come into secondary contact (as may be the case for the Bahía Ballena deme; Rodríguez-Robles *et al.*, 2008), the assumption of random mating would be violated, which would result in overestimates of θ, and consequently an overestimate of the effective population size of *A. cooki* (cf. Edwards & Beerli, 2000). Further, if the mutation rate of $\mu = 0.65 \times 10^{-8}$ for the ND2 gene (Macey *et al.*, 1998) is an undervalue, estimates of the effective population size based on this mutation rate will be high. However,

Table 6. Average values of the *S* statistic calculated for the simulated matrices used to evaluate the concordance, or lack thereof, between the observed gene trees of *Anolis poncensis* and *A. cooki* and a simple model of population fragmentation during the Pliocene–Pleistocene boundary (1.8 million years ago; Hypothesis 1), during the middle Pleistocene interglacial (800 000 years ago; Hypothesis 2) or during the late Pleistocene (100 000 years ago; Hypothesis 3)

	Anolis poncensis		*Anolis cooki*	
	Average *S* statistic	*P* value	Average *S* statistic	*P* value
Hypothesis 1				
Lower 95% $N_{e(f)}$ confidence limit	4.64	0.48	13.56	< 0.001
Mean $N_{e(f)}$	5.86	0.07	14.02	< 0.001
Upper 95% $N_{e(f)}$ confidence limit	6.73	< 0.001	14.01	< 0.001
10% of $N_{e(f)}$	4.26	0.76	13.33	< 0.001
Hypothesis 2				
Lower 95% $N_{e(f)}$ confidence limit	4.76	0.38	13.77	< 0.001
Mean $N_{e(f)}$	6.07	0.04	14.02	< 0.001
Upper 95% $N_{e(f)}$ confidence limit	6.79	< 0.001	13.94	< 0.001
10% of $N_{e(f)}$	4.4	0.65	13.54	< 0.001
Hypothesis 3				
Lower 95% $N_{e(f)}$ confidence limit	5.82	0.06	13.63	< 0.001
Mean $N_{e(f)}$	6.65	0.01	13.95	< 0.001
Upper 95% $N_{e(f)}$ confidence limit	6.87	< 0.001	13.83	< 0.001
10% of $N_{e(f)}$	5.45	0.10	13.48	< 0.001

using Cyt b sequences and four geological calibration points, Thorpe, Leadbeater & Pook (2005) calculated a mutation rate for *A. extremus* and *A. roquet* (from the Caribbean islands of Barbados and Martinique, respectively) similar to that of Macey *et al.* (1998). [Nevertheless, calibrations based on geological dates can only approximate the earliest date of a colonization event (Thorpe *et al.*, 2005), and if there was a time lag between the emergence of an island and its colonization, the actual mutation rate could be higher.] These considerations indicate that our estimates of the total effective population size of *A. cooki* should be interpreted with caution.

When there is no gene flow among populations, as is the case in *A. poncensis* and *A. cooki*, the *S* statistic measures the degree of lineage sorting, and can be used to test different spatial and temporal hypotheses of population histories (for example, Carstens *et al.*, 2005; Spellman & Klicka, 2006; Steele & Storfer, 2006). The *S* statistic for the empirical gene genealogy of *A. poncensis* was $S = 4$, and that for *A. cooki* was $S = 5$. Table 6 reports the average *S* statistic for the simulated genealogies for each of the three temporal hypotheses of subdivision into populations of the two anoles. Based on the estimated $N_{e(f)}$ values for these different scenarios, we could reject the hypotheses that populations of *A. poncensis* underwent fragmentation during the middle Pleistocene interglacial or late Pleistocene, but could not refute that this fragmentation may have taken place during the Pliocene–Pleistocene boundary. None of the three hypotheses, however, could be rejected when we used the lower 95% confidence intervals for $N_{e(f)}$, nor when we used an admittedly subjective, although probably more biologically realistic, estimate of effective female population size of 10% of $N_{e(f)}$. In contrast, we could decisively reject the hypotheses that the subdivision of *A. cooki* into populations occurred during any of the three tested time intervals (Table 6). These results suggest that *A. poncensis* could have evolved under a scenario of simple population fragmentation during the Pleistocene, but that *A. cooki* did not.

DISCUSSION

Phylogenetic analyses and coalescent simulations

The intraspecific evolutionary patterns inferred for *A. poncensis* and *A. cooki* were only partially congruent. The gene genealogy of *A. poncensis* recovered three strongly supported clades: the westernmost population (Punta Águila), the easternmost deme (Ponce) and the three intermediate populations (Morrillos, Lajas, Bahía Ballena). The relationships among these three clades were unresolved, but there was a strong phylogenetic signal in the central clade. In contrast, in *A. cooki*, the three westernmost populations (Punta Águila, Morrillos, Playa Santa) formed a well-

supported group to the exclusion of the three easternmost demes (Bahía Ballena, La Cueva, Punta Verraco), among which there was little phylogenetic structure.

The populations of *A. poncensis* and *A. cooki* were phylogeographically structured. Conspecific populations of both species exhibited relatively deep interpopulation mitochondrial DNA divergences. The maximum uncorrected sequence divergence was > 2% in *A. poncensis* and > 1% in *A. cooki*, despite the small geographical range of the two species. [The most distant populations of *A. poncensis* and *A. cooki* were only *c*. 60 km and 50 km (airline distance) apart, respectively.] Pronounced intraspecific divergences have been documented in other Caribbean *Anolis* (Malhotra & Thorpe 2000; Jackman *et al.*, 2002; Kolbe *et al.*, 2004, 2007; Thorpe *et al.*, 2005), including some species that occur on islands less than 50 km in diameter and without any obvious geographical barriers (Malhotra & Thorpe 2000). Therefore, insular species of *Anolis* often are markedly geographically structured, with most genetic variation occurring among, rather than within, populations. This repeated pattern suggests that the initial stages of differentiation and probably diversification in *Anolis* lizards may result from the geographical isolation of conspecific populations (cf. Losos *et al.*, 2006).

Estimates of the timing of intraspecific diversification within *A. poncensis* and *A. cooki* were contradictory. Estimates based on mean sequence divergences suggested that *A. poncensis* diversified earlier than *A. cooki*, but estimates of effective population size indicated that *A. cooki* diversified before *A. poncensis*. Molecular dating based strictly on pairwise sequence divergences is a simplistic approach that assumes that the population sizes of the two species are equal, that demes have not changed in size over time, that there is no gene flow among populations, and that the rate of evolution is constant (Milot, Gibbs & Hobson, 2000). The estimate of the effective population size of *A. cooki* is an order of magnitude larger than that of *A. poncensis*. Because time to the most recent common ancestor is dependent on effective population size, this tenfold difference implies that the time to the most recent common ancestor of *A. cooki* is much longer than that of *A. poncensis*, which indicates that *A. cooki* diversified prior to *A. poncensis*. In summary, our analyses indicate that intraspecific diversification in *A. poncensis* and *A. cooki* was not caused by the same historical events, and therefore that these two anoles do not have parallel evolutionary histories.

Population analyses

The populations of *A. poncensis* and *A. cooki* were demographically independent. There were no shared haplotypes between any demes of either species, suggesting that current gene flow between the populations is limited or non-existent. Because the aridlands of southwestern Puerto Rico are not continuous (because of natural fragmentation as well as recent anthropogenic disturbance), we predicted the existence of a certain level of genetic distinctiveness among intraspecific populations of the two anoles. However, the degree of isolation uncovered was more pronounced than expected. For example, in *A. poncensis*, the greatest divergence occurred between the two populations from Cabo Rojo (Punta Águila and Morrillos), despite the close proximity of these two localities (*c*. 2 km) and the continuity of the habitat in this area. In *A. cooki*, the divergences between the two closest localities (Punta Águila and Morrillos, and La Cueva and Punta Verraco) were shallower, but, as stated, the lack of shared haplotypes suggests that gene flow between the two pairs of demes is limited. The maternal mode of inheritance of mitochondrial DNA does not allow us to determine whether gene flow is limited in both sexes, or whether our findings reflect female philopatry. However, in the Caribbean island of Dominica (in the Lesser Antilles), male and female *A. oculatus* exhibit abrupt changes in morphology over short geographical distances, and these changes are congruent with mitochondrial DNA differentiation, suggesting that, in at least some species of *Anolis*, gene flow is restricted in both sexes (Malhotra & Thorpe 2000).

Anolis poncensis and *A. cooki* exhibited contrasting patterns of genetic diversity. First, overall nucleotide diversity in *A. cooki* was ten times larger than that in *A. poncensis*. Second, populations of *A. poncensis* had very low haplotype variation, with only one or two closely related (only one to two mutations apart) mitochondrial types per deme (Fig. 3A), whereas populations of *A. cooki* had several unique, more distantly related haplotypes (Fig. 3B). Indeed, the average haplotype diversity in *A. cooki* (0.62) was considerably higher than that in *A. poncensis* (0.36). The variation in mitochondrial types in the Playa Santa and Bahía Ballena demes of *A. cooki* was particularly noticeable. Playa Santa haplotypes formed two divergent clades, whereas the individuals from Bahía Ballena exhibited very little haplotype redundancy (12 specimens yielded ten unique haplotypes). We did not anticipate these findings, given the relatively small areas from which the samples were collected. The animals from Playa Santa were taken from two *c*. 5 m^2 localities along a *c*. 0.5 km transect, whereas all the specimens from Bahía Ballena came from a *c*. 550 m × 70 m area (Rodríguez-Robles *et al.*, 2008).

The larger *A. cooki* was less abundant than *A. poncensis*, and its habitat preferences were more

restrictive, as *A. cooki* was typically found only in exposed, coastal areas (Leal & Fleishman, 2002), whereas *A. poncensis* occurred in both open and denser xeric forest (M. Leal & J. A. Rodríguez-Robles, pers. observ.). We thus predicted that *A. cooki* would display lower levels of genetic diversity compared with *A. poncensis*; however, contrary to our expectations, *A. cooki* exhibited higher haplotype and nucleotide diversity than *A. poncensis*. The larger population size may have contributed in part to the high genetic diversity in the *A. cooki* deme from Bahía Ballena, which occupies one of the most extensive remaining patches of dry forest within the species' range. In addition, the Bahía Ballena population may have been formed recently by the admixture of two demes that had been isolated previously for a period of time, an event that would partly explain the presence of two haplotype subgroups in this population (Rodríguez-Robles *et al.*, 2008). In contrast, at Playa Santa, *A. cooki* was relatively rare, and the population seemed to be restricted to a very small area (see above). High genetic diversity can also persist in populations that have experienced a reduction in size only recently (Ellis *et al.*, 2006), especially when the effective population size prior to the contraction was large (Harpending *et al.*, 1998). Currently, only about 4% (5000 ha) of the original xeric forest believed to have existed in Puerto Rico still persists. The rest of the forest has been destroyed or modified mainly because of agriculture, urbanization and industrial development (Murphy *et al.*, 1995), activities that probably have altered the distribution of *A. cooki*. Consequently, this anole could still preserve the genetic footprints of a species that was once more widespread. Although we did not infer a recent bottleneck in any of the populations of *A. cooki* (an event that can be revealed, for example, by significantly positive Tajima's *D* values; Tajima, 1989; Johnson *et al.*, 2007), factors such as strength and length of the demographic event (i.e. the bottleneck) or the timing of sampling relative to the demographic event limit the power of tests such as Tajima's *D* (Simonsen, Churchill & Aquadro, 1995; Depaulis, Mousset & Veuille, 2003; Johnson *et al.*, 2007). Instead of wondering why genetic variation is so high in *A. cooki* compared with *A. poncensis*, we could ask the opposite question, namely why is genetic variation so low in *A. poncensis* compared with *A. cooki*. As stated above, *A. poncensis* has less restrictive habitat preferences, and thus may be more locally vagile than *A. cooki*, which may have resulted in the higher degree of genetic homogeneity among populations of *A. poncensis*.

The geographical distribution of genetic diversity in *A. poncensis* and *A. cooki* was incongruent. In *A. poncensis*, there was higher variability in both haplotype and nucleotide diversity in the westernmost populations (Punta Águila, Morrillos, Lajas) than in the two easternmost populations (Bahía Ballena, Ponce). Conversely, in *A. cooki*, haplotype and nucleotide diversity were much higher in the easternmost populations (Playa Santa, Bahía Ballena, La Cueva, Punta Verraco) than in the westernmost demes (Punta Águila and, especially, Morrillos). These patterns can result from differences in effective population sizes among demes of the two species. Alternatively, the populations with higher diversity may represent ancestral populations, which would imply that *A. poncensis* originated in the west and extended its range to the east, and that *A. cooki* originated in the east and spread west.

The coalescent simulations used to test the hypotheses of population histories suggested that intraspecific diversification in *A. poncensis*, but not in *A. cooki*, could be consistent with a simple hypothesis of population fragmentation in the Pleistocene, despite the fact that estimates of the timing of diversification varied for different estimates of effective population size. However, we could not include information about ancestral population size (and, consequently, possible population growth or decline over time in either species) in these simulations because the calculation of ancestral θ is problematic once populations become reciprocally monophyletic (Edwards & Beerli, 2000). Changes in effective population size through time greatly affect the coalescent time. For example, if *A. poncensis* experienced a recent bottleneck throughout most of its range (that is, if the ancestral population size was much larger than at the present), the time to the most recent common ancestor of *A. poncensis* would be severely underestimated if such information was not included in the analyses. One solution to this problem is to use multiple loci and/or loci that have not completed lineage sorting (which can only be determined empirically), because such loci provide more accurate estimates of ancestral θ (Edwards & Beerli, 2000).

In conclusion, our findings suggest that *A. poncensis* and *A. cooki*, two lizards endemic to the aridlands of Puerto Rico, have undergone historical patterns of genetic diversification that differ in both space and time. Consequently, the hypothesis that anole species that belong to the same climate type have experienced a parallel evolutionary history is not supported by our data. Two other anoline lizards, *A. evermanni* (Puerto Rican Green Anole) and *A. gundlachi* (Puerto Rican Yellow-shinned Anole), are restricted to the moist, montane forests of Puerto Rico (Schwartz & Henderson, 1991; Rivero, 1998). These two species are syntopic throughout much of their distribution, and have much broader ranges than *A. poncensis* and *A.*

cooki. It will also be informative to conduct a comparative phylogeographical study of *A. evermanni* and *A. gundlachi* to determine whether these two species are also examples of codistributed anoline lizards with distinct evolutionary histories and patterns of genetic variation, or whether they possess spatially and temporally congruent genetic architectures.

ACKNOWLEDGEMENTS

We thank Carmen D. Ortiz, 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, CA, USA) for loaning of the tissue samples; Peter Beerli and Christopher J. Conroy 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, NV, USA.

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