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Archipelagic genetics in a widespread Caribbean anole

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Abstract

Aim: We examine the influence of fluctuating sea levels in a land-bridge archipelago on the apportioning of intraspecific genetic diversity and divergence in the widespread Puerto Rican crested anole (*Anolis cristatellus*). We compare three alternative scenarios for genetic diversification in an archipelagic species that contrast the relative influences of periodic isolation versus island connectedness driven by fluctuating sea levels. Our approach combines information from geography and population genetics to assess the influence of island size, island isolation, island historical geography, and population genetic processes such as drift on the contemporary distribution of genetic variation within and among islands.

Location: The Puerto Rico Bank in the Caribbean focusing primarily on the Spanish, British and U.S. Virgin Islands.

Methods: We used nuclear and mitochondrial DNA sequences and microsatellite genotypes sampled from *A. cristatellus* populations to investigate: (1) the broad-scale pattern of phylogeographical divergence across Puerto Rico Bank islands and (2) diversification within the Virgin Islands archipelago. For the first component, we used sequence data to reconstruct the relationships among 542 samples from across the species range. For the second component, we examined the relative influences of island size, isolation, and population genetic processes on the distribution of genetic diversity across the Virgin Islands.

Results: In the Virgin Islands, *A. cristatellus* is represented by a monophyletic clade except on the island of Vieques, where two divergent clades coexist. We found evidence for non-equilibrium dynamics in the Virgin Islands, suggesting spatial population expansion during intraglacial periods of low sea level.

Main conclusions: We found limited evidence that periods of island isolation affected patterns of genetic diversity and differentiation. Instead, we found that the patterns of genetic diversity and divergence in *A. cristatellus* in the Virgin Islands archipelago are likely shaped by long-term persistence in the region and periods of population spatial expansion.

KEYWORDS

Anolis cristatellus, approximate Bayesian computation, Caribbean, gene flow, island biogeography, microsatellite, mtDNA, Puerto Rico, Virgin Islands

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1 | INTRODUCTION

Islands are well-suited to the investigation of evolutionary processes, as they often represent natural laboratories in which to test general evolutionary predictions in spatially discrete areas (Losos & Ricklefs, 2009). Islands closely resemble models used in population genetics, as they have distinct boundaries and harbour populations that are subject to processes such as genetic drift, gene flow, extinction/re-colonization, and other stochastic evolutionary forces (Slatkin,1987; Wade & McCauley, 1988; Whitlock & McCauley, 1990; Wright, 1977). Furthermore, variance in island age and geographical structure of these spatially discrete systems might either impede or impel divergence between island populations (Losos & Ricklefs, 2009). For example, when a species colonizes a classic oceanic island archipelago, in which the islands themselves have never been joined, a variety of evolutionary processes might serve to structure intraspecific genetic diversity in the archipelago. In such a situation, average genetic diversity might be expected to be a function of island area, as larger islands can support larger populations that experience lower rates of genetic drift, and gene flow might be expected to be higher among islands in close proximity compared to more distant islands (Johnson, Adler, & Cherry, 2000; Vellend, 2003). Thus, the structure of the islands themselves predicts an allopatric signature on intraspecific genetic divergence and diversification.

Oceanic islands have figured prominently into these predictions and empirical examinations of insular genetic divergence and diversification, yet additional insight might be gained from increased attention to other island systems (Meiri, 2017). In contrast to a classic oceanic island archipelago, a land-bridge archipelago represents a potentially complex history of island isolation and connection. Such a region might experience repeated bouts of complete connection during intraglacial periods of lower sea level, interceded by isolation into separate islands during interglacial periods of increased sea level. When islands are connected during intraglacial periods, populations of vagile terrestrial species exchange alleles across the land bridge. However, when sea levels rise and populations are spatially structured in allopatry, population genetic processes such as genetic drift and reduced gene flow might influence local and global population genetic divergence (Papadopoulou & Knowles, 2015; Slatkin, 1987; Wright, 1977). Contemporary land-bridge archipelagos present a challenge to reconstructing the evolutionary history of a species owing to a variety of potential population genetic outcomes, such as influences on gene flow or effective population sizes (Papadopoulou & Knowles, 2015). In the present interglacial Holocene period, sea levels are relatively high (Siddall et al., 2003) and some land bridges that had been exposed in the Quaternary are inundated, fragmenting these landscapes into islands often referred to as Pleistocene Aggregate Island Complexes (PAICs). We might, therefore, expect that the resulting changes in island area and degree of isolation have had an impact on population genetic processes and have thus produced a measurable pattern of genetic differentiation within such regions. Thus, "archipelagic genetics" (Table 1) takes into consideration the relative influences of island geography, population genetic processes, and island biogeographical processes on intraspecific diversification, and so lends an important perspective to an understanding of diversification in land-bridge archipelago systems (Johnson et al., 2000; Papadopoulou & Knowles, 2015; Vellend, 2003).

TABLE 1 The archipelagic genetics approach. Three hypothetical generalized scenarios describing possible influences on the distribution of contemporary genetic diversity on islands in a land-bridge archipelago. Characterizing these expectations and potential violations of the models, and then examining data relating to the expectations might yield inference regarding the evolutionary history of an island archipelagic species. IBD = isolation-by-distance, MMD = mutation–migration–drift

Scenario	Expectations
Island allopatry	(1) Positive relationship between island area and genetic diversity (2) Negative relationship between island isolation and genetic diversity (3) Positive IBD pattern (4) MMD equilibrium
Contiguous populations	(1) No relationship between island area and genetic diversity (2) No relationship between island isolation and genetic diversity (3) Positive IBD pattern (4) MMD equilibrium
Spatial expansion	(1) Positive relationship between island area and genetic diversity (2) No relationship between island isolation and genetic diversity (3) No IBD pattern (4) No MMD equilibrium

The potential historical geologic complexity of a land-bridge island archipelago might necessitate the characterization of a priori potential expectations with which to compare empirical data. For example, three main potential outcomes might be examined using this archipelagic genetics approach. First, the isolation of islands in a land-bridge archipelago could permit allopatry to play an important role in influencing genetic variation within, and divergence between, island populations. For such an allopatric divergence scenario to operate in the face of cyclical exposure and inundation, there would need to be either reduced gene flow among populations across emergent land bridges or a sufficient number of generations would need to have elapsed since island isolation to produce a measurable signal of genetic drift. As in oceanic island archipelagos, we might expect to see the following population genetic patterns (Table 1; Johnson et al., 2000; Vellend, 2003; Whitlock, 2004; Papadopoulou & Knowles, 2015): (1) a positive relationship between genetic diversity and island area, assuming effective population size is correlated with island area; (2) a negative relationship between island isolation and intra-island genetic diversity owing to reduced gene flow among distant islands; (3) a positive relationship between genetic divergence and geographical distance between island populations in accordance with an isolation-by-distance (IBD) pattern (Rousset, 1997; Wright,

1943) owing to reduced gene flow among more distant islands; and (4) no deviations from mutation–migration–drift (MMD) equilibrium in the absence of demographic bottlenecks or high gene flow (Finn, Bogan, & Lytle, 2009).

In contrast, when populations were historically contiguous over exposed land bridges, they might have experienced relatively high levels of gene flow and would now exhibit few island, or allopatric, effects which might have been predicted given the current geography. From a population genetic perspective, these contemporary island populations could appear more similar to contiguous populations, as periodic oceanic separation might generate only temporary boundaries to gene flow. Under this scenario, we might predict that: (1) there would be no relationship between island area and genetic diversity, because contemporary emergent island areas are poorly correlated with historical effective population size; (2) there might be no relationship between island isolation and genetic diversity if drift or gene flow are not impacting allelic diversity; and (3) there might be a positive relationship between genetic divergence and geographical distance between island populations in accordance with the IBD pattern (Rousset, 1997; Wright, 1943) owing to likely incomplete panmixia; and finally, (4) island populations might not deviate from population genetic neutrality, or MMD equilibrium, owing to limited influence of drift or migration of novel alleles producing unbalanced allele frequencies.

A third possibility is that populations are not contiguous across the land bridge during intraglacial periods, instead being restricted to a regional refuge or refugia during these periods. A change in climate, environment or further reduction in sea level might then allow "recolonization" of the remainder of the land-bridge archipelago. Such a situation could obscure island-level population-genetic processes, such as a correlation between island size and genetic diversity, as well as landscape-level patterns such as IBD or MMD equilibrium. Under this spatial expansion scenario, we might predict (1) a positive relationship between island area and genetic diversity because contemporary islands support larger effective population sizes, (2) no relationship between island isolation and genetic diversity because alleles disperse from refugia with equal probability; (3) no evidence for an IBD pattern owing to little drift or local mutation and gene flow; and (4) deviation from MMD equilibrium in contemporary island populations owing to demographic fluctuations, as might be expected during spatial expansion.

The Puerto Rican crested anole, *Anolis cristatellus* Dumeril & Bibron (1837), is a small arboreal lizard species well-suited for exploring the influence of a land-bridge island archipelago on intraspecific genetic diversity and divergence. This widespread, abundant and generalist species occurs across the 350-km-long Puerto Rico Bank (PRB), which comprises an island archipelago including Puerto Rico (PR) and the Virgin Islands (Bitanja, van de Wal, & Oerlemans, 2005). The eastern extent of the PRB, or the Virgin Island (VI) Archipelago, consists of the politically independent though geographically proximate Spanish (Passage), British and U.S. Virgin Islands, excluding the island of St. Croix, which is on a separate bank. Importantly, the PRB has experienced repeated fluctuations in sea level of more than 100 m during the Quaternary (Bitanja et al., 2005). When sea levels were lowest during intraglacial periods, the PRB was fully exposed, with a maximum of 21,000 km^2 subaerial, and the VI Archipelago was a land bridge throughout much of this time period (Rohling et al., 2009). However, three relatively brief interglacial periods of inundation 190–245 ka (Dutton et al., 2009), 119–130 ka (Hearty, Hollin, Neumann, O'Leary, & McCulloch, 2007; Siddall et al., 2003), and 0–7 ka (Fairbanks, 1989; Lighty, Macintyre, & Stuckenrath, 1982) have fragmented the VI land bridge into dozens of islands of various sizes and distances one from the other.

Previous studies have shown that these island dynamics produce different population genetic patterns. For example, Barker et al. (2012) found that VI populations of the frog *Eleutherodactylus antillensis* correspond to a spatial expansion scenario (our scenario 3, above), having arisen from directional expansion from eastern PR to the VI during the most recent glaciation. Consequently, the contemporary fragmented nature of the archipelago seems to have had relatively little influence on population genetic structure. By contrast, Papadopoulou and Knowles (2015) found evidence for allopatric patterns (our scenario 1, above) in VI crickets (*Amphiacusta sanctaecrucis*). If, like *Amphiacusta sanctaecrucis*, contemporary VI populations of *A. cristatellus* are effectively allopatric (in the population genetic sense of relative isolation), we might expect that this geographical isolation would result in population genetic structure and thus drive intraspecific genetic diversification (Papadopoulou & Knowles, 2015). Otherwise, if these populations do not persist in allopatry for sufficiently long periods or are subject to other demographic dynamics such as moderate intraglacial gene flow or spatial expansion, then we might expect little allopatric signal resulting from physical separation onto islands. In these scenarios, migration or expansion effectively allows island populations to exchange alleles unhindered by periodic ocean separation, though if inundation is very recent, and gene flow has since ceased or slowed, then sufficient time may not yet have elapsed for isolation and genetic drift to have yet left their signatures on genetic divergence between islands or diversity within them. These three scenarios are not mutually exclusive; however, by contrasting their predictions we will attempt to reconstruct the relative importance of these scenarios on the present genetic diversity and divergence seen among island populations of *A. cristatellus*.

Here, we examine archipelagic genetics in *A. cristatellus* from the VI using mitochondrial (mtDNA), nuclear sequence data and nuclear (microsatellite) genotype data. Because *A. cristatellus* occurs on nearly all islands of the PRB and is a habitat generalist with large census population sizes, we might expect that effective population size could be correlated with island area, as the species can inhabit nearly all habitat types on these islands. First, we consider the origins of VI populations in a phylogenetic context to confirm previous work suggesting that *A. cristatellus* from the region are monophyletic (Revell, Harmon, Langerhans, & Kolbe, 2007). Next, we investigate the influence of the island archipelago in shaping genetic diversity through an examination of the influence of island size, island isolation, and population genetic processes on the distribution of genetic diversity across the VI. Specifically, we focus on our three predicted

scenarios (Table 1) for genetic diversification in this species: (1) that island populations show the influence of allopatric isolation; or (2) that island separation has little influence on population genetic structure among these periodically connected land-bridge islands, or (3) that VI populations correspond to a spatial expansion scenario like *Eleutherodactylus* frogs. These scenarios (allopatry, persistence or expansion) represent hypothetical ends of spectra representing the relative influence of each scenario in contributing to contemporary patterns of genetic diversity and distribution.

2 | MATERIALS AND METHODS

2.1 | Sample collection and genetic data

We collected 4–59 *A. cristatellus* samples (mean = 17.1 per island) from each of 21 island populations in the VI (32 separate sampling sites; mode = 1/site, range 1–6 sites per island; mean = 8 per site) by hand-capture or noosing (Figure 1a; Table S1 in Appendix S2; also see Falk & Perkins, 2013). We extracted whole genomic DNA from all tissue samples and used PCR to amplify a fragment of the mitochondrial genome (NADH II [*ND2*]). We purified and sequenced products in both directions on an automated sequencer (ABI 3730XL) at Massachusetts General Hospital DNA Core Facility, Cambridge, MA. We assembled contigs and manually verified ambiguous base calls using GENEIOUS 7.1.2 (Biomatters, Auckland, New Zealand).

We additionally screened samples at six di-nucleotide microsatellite loci developed for *A. cristatellus* (Glor, Johnson, & Larson, 2007), as well as four tetra-nucleotide loci developed for *A. carolinensis* (*Acar8*, *9*, *23*, and *36*; Wordley, Slate, & Stapley, 2011). We modified the 5′ end of the forward primer from each primer pair with a 19-bp sequence tag (M13 method; Schuelke, 2000) to allow for the use of a third primer labelled on the 5′ end with one of four dyes (6-FAM, PET, VIC or NED; Applied Biosystems®). PCR conditions for the *Acar8* locus were as follows: denaturation at 94°C for 2 min; 30 cycles at 94°C for 30 s, 57°C for 30 s and 72°C for 45 s; and a final extension at 72°C for 10 min. All remaining loci were amplified using the following "touchdown" conditions: denaturation at 95°C for 5 min; nine cycles at 94°C for 30 s, 64–55°C for 30 s, and 72°C for 30 s (stepping down 1°C each cycle from 64 to 55°C); 20 cycles at 94°C for 30 s, 55°C for 30 s, and 72°C for 45 s; and a final extension at 72°C for 10 min. We multiplexed PCR products with different dyes and resolved genotypes using GeneScan™ 500 LIZ size standard. We called and binned alleles using the microsatellite plugin in GENEIOUS. We tested for genotyping errors by randomly selecting 29% of the samples for repeated genotyping from the PCR stage, and we used MICRO-CHECKER 2.2.3 (Van Oosterhout, Hutchinson, Wills, & Shipley, 2004) to investigate whether our genotype profiles showed evidence of allele-dropout or null alleles.

2.2 | Origins of the Virgin Islands clade

Our first objective was to investigate whether or not the VI populations of *Anolis cristatellus* consisted of a monophyletic clade with respect to the entire native range of *A. cristatellus* across the PRB, as well as to characterize the phylogenetic background for the species to contextualize the diversity and diversification in the VI archipelago. We obtained Puerto Rico *A. cristatellus* mtDNA sequences from GenBank (data from Kolbe, Larson, & Losos, 2007; Rodríguez-Robles, Jezkova, & García, 2007), which we supplemented with collection of 1–12 samples from 10 sites, yielding a total of 48 localities across PR (Figure 1a; Table S2 in Appendix S2). We then amplified six nuclear genes (Table S3 in Appendix S2) in a subset of 6–11 randomly chosen representative samples from each major *A. cristatellus* mtDNA clade (our "reduced multilocus dataset"; see clades in Figure 1b) using primers and conditions in Cádiz et al. (2013) and Reynolds et al. (2013). We purified, sequenced, and assembled products

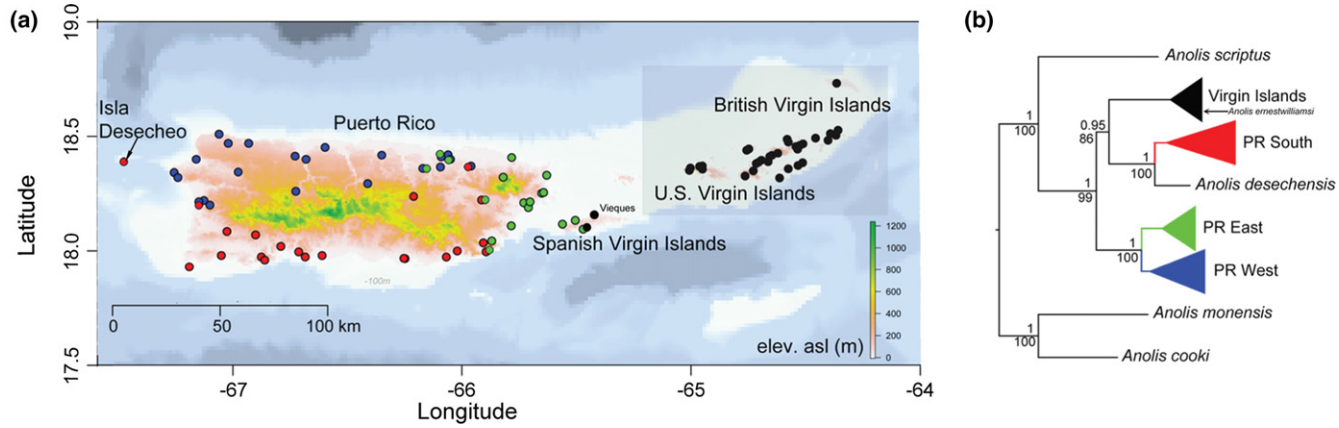

FIGURE 1 Sampling localities and main mtDNA clades of *Anolis cristatellus* on the Puerto Rico Bank. (a) Sampling locations colour-coded by mtDNA clade in panel (b). Overlapping circles are offset slightly. The approximate extent of the Puerto Rico Bank when exposed during glacial maxima is shown in white, and the inset for the Virgin Islands study area is shaded with a grey rectangle. (b) Mitochondrial DNA gene tree for all 465 haplotypes recovered in this study and the sister-species *A. scriptus* from the Turks and Caicos Islands, rooted with the outgroups *A. monensis* and *A. cooki*. Major clades are collapsed and colour-coded. Numbers at each node indicate Bayesian posterior probability (above) and maximum likelihood bootstrap support (below). Note that *A. ernestwilliamsi* is nested within the Virgin Islands clade [Colour figure can be viewed at wileyonlinelibrary.com]

as above. We resolved heterozygous intron sequences using PHASE v. 2.1 (Stephens & Donnelly, 2003; Stephens, Smith, & Donnelly, 2001) implemented in DNASP v5.10.1 (Librado & Rozas, 2009) using default parameters for 100 iterations with a burn-in of 100, and a cut-off of PP >0.7 for base calling. We obtained sequences for outgroup (*A. monensis* and *A. cooki*) and ingroup taxa (nominal *A. desechensis* and *A. ernestwilliamsi*) from GenBank (data from Harmon, Schulte, Larson, & Losos, 2003; Rodríguez-Robles et al., 2007). We also included a single sample of the sister-species *A. scriptus* from the Caicos Islands (RGR_Ascript004). We then aligned sequences using the MUSCLE (*ND2*; Edgar, 2004) or CLUSTALW 2.1 (all other loci; Larkin et al., 2007) algorithm implemented in GENEIOUS using reference sequences and default parameters. We deposited alignments into the online repository Dryad (https://doi.org/10.5061/dryad.4m3r4).

We used a phylogenetic approach to investigate the origin and relationships of the Puerto Rico and VI populations, analysing the complete (Puerto Rico+VI) mtDNA dataset using both maximum likelihood (ML) and Bayesian methods. We selected the best-fit model of molecular evolution for the *ND2* locus (*TrN+I+G*) using Bayesian information criterion (BIC) in JMODELTEST2 (Darriba, Taboada, Doallo, & Posada, 2012; Guindon & Gascuel, 2003). We conducted ML analysis using the RAxML algorithm (Stamatakis, 2006) implemented in the RAxML plugin-in for GENEIOUS. We used the GTRGAMMA model and the rapid bootstrapping algorithm with 1,000 bootstrap (BS) replicates followed by the thorough ML search option with 100 independent searches. We consider BS values above 70% to indicate relatively well-supported clades (Felsenstein, 2004). To estimate divergence times across the mitochondrial gene tree, we inferred a time-calibrated *ND2* coalescent tree in the program BEAST 1.8 (Drummond, Suchard, Xie, & Rambaut, 2012) using a relaxed molecular clock model and a rate of molecular evolution of 0.65% divergence per lineage, per million years. This rate has been previously used for the *ND2* locus in other lizards (Macey et al., 1998), including many studies of Caribbean anoles (e.g. Gartner, Gamble, Jaffe, Harrison, & Losos, 2013; Geneva, Hilton, Noll, & Glor, 2015). We furthermore note that we are primarily interested in the relative rather than absolute divergence times, and thus, our analyses will be largely insensitive to the specific molecular clock rate used. We ran the Markov chain Monte Carlo (MCMC) for 100 million generations using the *TrN+I+G* substitution model, a Yule speciation prior and an uncorrelated lognormal relaxed (UCLN) molecular clock model. We repeated the analyses three times with different starting numbers, sampling every 10^4 generations and discarding the first 25% of generations as burn-in. We assured adequate mixing of the chains by calculating the effective sample size values for each model parameter, with values >200 indicating adequate sampling of the posterior distribution. We assessed convergence of the independent runs by a comparison of likelihood scores and model parameter estimates in TRACER 1.5 (Rambaut, Suchard, Xie, & Drummond, 2013). We combined the results from the three analyses using LOGCOMBINER and generated a maximum clade credibility (MCC) tree using TREEANOTATOR. We then estimated genetic distances (Tamura-Nei distances) between major clades using MEGA 6.0 (Tamura, Stecher, Peterson, Filipski, & Kumar, 2013).

We estimated divergence events and times from gene histories (e.g. Degnan & Rosenberg, 2009; Edwards, Liu, & Pearl, 2007) using both mtDNA and nuclear sequences. We employed the *BEAST species-tree approach (Heled & Drummond, 2010) to analyse the reduced multilocus dataset (with corresponding mtDNA sequences) using the MCMC method implemented in BEAST 1.8. This method jointly estimates species tree topology, divergence times and effective population sizes from multiple embedded gene trees under the multispecies coalescent model, which assumes that incongruence among gene trees is owing to incomplete lineage sorting in lieu of gene flow. Because *BEAST requires a priori "species" designations, we assigned each tip to its respective mtDNA clade recovered in the previous analyses of the *ND2* gene tree, with these operational taxonomic units (OTUs) treated as "species" groupings. We constrained the recognized species *A. desechensis* and *A. ernestwilliamsi* as distinct taxa, as we had no nuclear sequence data for these groups. We partitioned sequence data by locus and assigned a locus-specific model of nucleotide substitution chosen using BIC in JMODELTEST2 (Table S3). We unlinked nucleotide substitution models, clock models and gene trees in all analyses. We employed an UCLN clock model of rate variation for the mtDNA locus and a strict clock for nuclear loci, owing to the expectation of mutations that have not yet reached fixation (Ho, Phillips, Cooper, & Drummond, 2005; Peterson & Masel, 2009), and we used a Yule process speciation prior for the branching rates. As the potential exists for interspecific molecular evolutionary rate variation (Lanfear, Welch, & Bromham, 2010), we fixed the most recent common ancestor (MRCA) of *A. cristatellus* sensu lato using a normal prior centred on the estimate obtained from the mitochondrial divergence time analysis. We ran the MCMC as above, with 100 million generations and three independent replications.

2.3 | Archipelagic genetics in the Virgin Islands

Our archipelagic genetics approach to distinguish among alternative scenarios (Table 1) potentially influencing contemporary genetic diversity and distribution in the VI relies upon the relative influences of historical geological and demographic processes operating in the archipelago. Specifically, we hypothesized that island characteristics (island area/proximity), IBD and MMD equilibrium might influence the distribution of genetic variation within and among *A. cristatellus* populations in the VI. We, thus, used population genetic and spatial genetic approaches to examine intraspecific diversity in this archipelago using mtDNA sequence data and microsatellite genotypes (nuclear sequences lack resolution for these population genetic-level analyses).

2.3.1 | Population structure

We used R 3.3.1 (R Core Team 2016) for subsequent analyses. We pruned the mtDNA dataset to include only VI samples, also excluding the aforementioned PR East haplotypes from Vieques Island, for analyses in the VI archipelago. We first tested for within-island

substructure on the three islands (St. Thomas, St. John, Tortola) for which we had more than one sampling site using a discriminant analysis of principal components (DAPC; Jombart, Devillard, & Balloux, 2010) implemented in the R package "Adegenet" (Jombart, 2008) to identify genotypic clusters in the datasets. This method attempts to maximize genetic differentiation between groups and minimize variation within groups by clustering individual genotypes using a principal components transformation of the genetic data prior to discriminant analysis. We used a BIC approach to obtain the predicted number of clusters between $K = 1$ and $K = 6$ after retaining all PCs. To perform the DAPC, we selected the optimal number of PCs using optim.a.score() in "Adegenet" with 1,000 replications, resulting in the retention of the first three PCs and first eigenvalue in the analysis. We found a single cluster in separate analyses for St. Thomas and St. John. We had six sampling sites on Tortola and found two clusters on this island, though neither cluster corresponds to our sampling sites and each cluster consisted of individuals from across sampling sites. We, therefore, consider sampling location to have no influence on allelic composition of individual samples.

To investigate partitioning of genetic variation by island across the VI, we calculated Φ_{ST} and F_{ST} statistics for the mtDNA and microsatellite datasets, respectively, in an analysis of molecular variance (AMOVA) framework (Excoffier, Smouse, & Quattro, 1992). To examine relationships among mtDNA haplotypes, we used the neighbour-net algorithm in SplitsTree 4.13.1 (Huson & Bryant, 2006) to visualize a phylogenetic network for island populations in the VI (e.g. Volkmann, Martyn, Moulton, Spillner, & Mooers, 2014). We assessed support among major groups using 1000 nonparametric bootstrap replicates.

2.3.2 | Population genetic summary statistics

Finding no evidence of within-island spatial substructure in our mtDNA (Figs. S1, S2 in Appendix S1) or microsatellite data (Fig. S3 in Appendix S1), we pooled samples by island to calculate an island average for population genetic summary statistics. We estimated genetic variation within and across islands as nucleotide (π) and haplotype (h) diversity using Arlequin 3.5.1.3 (Excoffier & Lischer, 2010). We also used Arlequin to calculate population pairwise Φ_{ST} (using haplotype frequencies) as well as Tamura-Nei distance to capture both haplotype and nucleotide divergence, respectively.

For the microsatellite data, we calculated the number of alleles (N_A), effective number of alleles (N_E), observed heterozygosity (H_O) and expected heterozygosity (H_E) using GenAlEx 6.4 (Peakall & Smouse, 2006). We calculated allelic richness (A_R) and tested for departures from Hardy–Weinberg equilibrium (HWE) in the package "diveRsity" (Keenan, McGinnity, Cross, Crozier, & Prodöhl, 2013) implemented in R. We estimated the fixation index within islands (F_{IS}) in GenePop 4.0 (Raymond & Rousset, 1995) using exact tests with 10,000 dememorizations, 2,500 batches and 20,000 iterations per batch. We calculated pairwise divergence using the F_{ST} analogue in Arlequin. We feel that using such an allele-identity-based measure is appropriate to our dataset, as stepwise mutation and the associated measurement of allele size variation (e.g. R_{ST}) better capture divergence when focusing on the interspecific level (Goldstein & Pollock, 1997; Hardy, Charbonnel, Fréville, & Heuertz, 2003). We determined significance of Φ_{ST} and F_{ST} values via 9×10^3 permutations in Arlequin. To ensure that our results were not biased by variable sample sizes, we examined the effect of sample size on our calculated dependent genetic summary statistics in a regression framework (Fig. S4 in Appendix S1).

2.3.3 | Tests for the influence of island characteristics

If population divergence is driven primarily by current or historical allopatry, then island characteristics such as size and isolation may shape contemporary patterns of genetic diversity in an archipelago. We tested for relationships between the dependent population genetic summary statistics h (haplotype diversity), π (nucleotide diversity), H_O (observed heterozygosity), and A_R (allelic richness) versus the explanatory variables island area (log km^2, from Mayer, 2012) and island isolation (*PX*, see below) in a multiple regression framework. In a simple island-mainland or stepping-stone scenario, isolation is a sufficient description of the distance between populations. Alternatively, an archipelago is a non-monotonic arrangement of isolation distances; hence, a proximity index might better characterize actual isolation. We calculated a metric of island isolation as the proximity index patch metric (*PX*; Gustafson & Parker, 1992):

$$PX = \sum_{s=1}^{n} \frac{a_{js}}{h_{ijs}}$$

where a_{js} is the area (km^2) of patch js in the neighbourhood of patch i, and h_{ijs} is the distance (km) between patch i and patch js. We calculated *PX* from the stepping-stone distance between islands (see below). Here, a high index value indicates closer proximity to neighbouring patches weighted by neighbouring patch area.

2.3.4 | Tests for isolation by distance

A signal of IBD in the archipelago might indicate either limited dispersal (reflecting island isolation) and/or population substructure during intraglacial periods (Meirmans, 2012; Wright, 1943). To test for patterns of IBD, we calculated genetic distances as Rousset's (1997) distance measure ($\Phi_{ST}/(1–\Phi_{ST})$) for the mtDNA data and as ($F_{ST}/(1–F_{ST})$) for the microsatellite data. In a stepping-stone population at equilibrium, there is a nearly linear relationship expected between genetic and geographical distance between pairs of populations (Rousset, 1997). We calculated geographical distance matrices using two approaches. In the first approach, we estimated island centroids in Google Earth® and then converted spatial coordinates to Euclidean distance measures using the Geographic Distance Matrix Generator 1.2.3 (Ersts, 2014). In the second approach, we generated a matrix of stepping-stone distances from the island centroids, which we defined as the sum of centroid distances among nearest-neighbour islands. We then regressed genetic distances against the log-

transformed geographical distance matrices. We evaluated correlations using Mantel test (Mantel, 1967; Wright, 1943) using 1,000 permutations implemented in the R package "Adegenet" (Jombart, 2008).

2.3.5 | Tests for MMD equilibrium

Deviation from MMD equilibrium might indicate support for our third scenario—that island populations are influenced by spatial expansion. We implemented three tests for MMD equilibrium in *A. cristatellus* using the mtDNA dataset. For the first two tests, we calculated Tajima's *D* and Fu's F_S in ARLEQUIN. Negative values for these two test statistics indicate an excess of recent mutations, which is evidence for a number of potential processes including demographic expansion, which might be expected to coincide with a spatial expansion scenario.

Third, we investigated potential demographic fluctuation by conducting mismatch analyses for each island population in ARLEQUIN to estimate goodness-of-fit between our observed data and the expectations under a sudden expansion model. In this case, a unimodal mismatch distribution is expected to signify either evidence of past demographic expansion (Rogers & Harpending, 1992; Slatkin & Hudson, 1991) or a signature of migration between structured subpopulations (i.e. Excoffier, 2004); though it is worth noting that levels of migration or time since expansion might influence our ability to reconstruct these demographic fluctuations (Excoffier, Foll, & Petit 2009). We used both the sum of squared deviation (SSD) and the Harpending's raggedness index to assess demographic stability via 9×10^3 permutations in ARLEQUIN.

2.3.6 | Tests for migration and spatial expansion

If island populations do not retain some degree of population genetic cohesiveness during periods of land-bridge connection, then relatively higher levels of migration across the land bridge might also contribute to a signal of non-equilibrium. Concomitantly, if lizards are capable of dispersing between islands either naturally or via human-mediated transport (Perry, Powell, & Watson, 2006), then island populations might show signatures of recent migration. Hence, we directly tested for rates of migration among larger islands in the archipelago for which we had >20 samples (eight islands; Table S1). As we found no evidence for population genetic substructuring within islands (Fig. S3 in Appendix S1), we pooled individuals by island and estimated recent directional rates of migration using the program BAYESASS 3.0 (Wilson & Rannala, 2003). BAYESASS does not assume HWE, a situation that might not be expected in the presence of non-random mating and/or genetic drift (Hedrick, 2009; Loew, Williams, Ralls, Pilgrim, & Fleischer, 2005). We calculated a measure for directional migration among a priori populations (islands), where $m[i_n][j_n]$ are measures of the per cent of individuals in the i^{th} population that are migrants from the j^{th} population per generation (Wilson & Rannala, 2003). This method generally requires a relatively high F_{ST} (≥0.05) among populations being compared (Faubet, Waples, & Gaggiotti, 2007; Meirmans, 2014). As some of our VI populations do not conform to this expectation (Table S4), we consider migration rates to be relative and not absolute (Samarasin, Shuter, Wright, & Rodd, 2016). We conducted 10 independent runs, each with 2.1×10^7 iterations sampling every 2,000 generations, with 2×10^6 generations of burn-in. We varied initial starting values for each run and calculated Bayesian deviances for each run to select the most appropriate analysis given the data (Faubet et al., 2007; Meirmans, 2014).

We used coalescent Bayesian skyline plots (BSP; Drummond, Rambaut, Shapiro, & Pybus, 2005) implemented in BEAST 1.8 (Drummond et al., 2012) to ascertain whether (female) effective population size (N_ef) has fluctuated through time—a potential signal of demographic expansion or contraction. The BSP method uses an MCMC sampling algorithm to generate a posterior distribution of effective population size through time. We used a piecewise-constant Bayesian skyline tree prior, ran the MCMC for 100 million generations, and checked convergence statistics as above.

We explicitly modelled changes in effective population size through time in the VI using summary statistics calculated from Approximate Bayesian Computation (ABC) implemented in DIYABC 2.0 (Cornuet et al., 2014). For this analysis, we used the microsatellite genotypes and treated the VI as a single population, to compare to results from the BSP above. We established three basic demographic scenarios corresponding to fluctuations in effective population size (N_e) over the course of the last 10^5 years to capture cycles of potential demographic expansion, assuming an anole generation time of 1 year. Model 1 is a single discrete increase in effective population size following demographic expansion of 1–2 orders of magnitude (uniform prior distribution with $10^5 < N_e < 10^6$); model 2 is stable N_e through time (uniform prior distribution with $10^2 < N_e < 10^5$), and model 3 is a reduction in N_e (uniform prior distribution with $10 < N_e < 10^4$). The time of these events is a series of priors drawn from a uniform distribution including a range of event times ($10 < t < 10^4$), and we used a mean microsatellite mutation rate uniformly distributed between 1.00×10^{-4} and 1.00×10^{-3}. We calculated summary statistics including the mean number of alleles, mean genic diversity and mean size variance for 2×10^5 simulated datasets to compare to the empirical dataset, and we confirmed that our empirical data lie within the parameter space of the simulated datasets (goodness-of-fit) using principal components analysis in DIYABC (Cornuet et al., 2014).

3 | RESULTS

3.1 | Sample collection and genetic data

We aligned a maximum of 1,104 bp of mitochondrial *ND2* sequence data (full coding sequence plus 3′ *tRNA-TRP*) from 542 individuals of *A. cristatellus* (80 sampling locations across the PRB), three ingroup (but nominally distinct) taxa, and two outgroup taxa. For the reduced multilocus dataset, we aligned a maximum of 4,435 bp across six nuclear loci and the ND2 locus. Of the 10 microsatellite loci we

screened, six were repeatable and amplified consistently across all samples, yielding between 13 and 22 alleles per locus (Table S5 in Appendix S2). We resolved 363 genotypes at these loci, with between 3 and 62 genotypes per island for each of the 21 islands (Table 2). We failed to amplify or resolve only 5.9% of the 4,356 possible allelic states. We found an allele-scoring error rate of only 6.5% based on independent replications from the PCR stage. MICRO-CHECKER did not identify allelic dropout, but suggested some evidence of null alleles across the dataset. Null alleles are expected in populations with large effective population size, though they do not appear to bias genetic distance measures when populations are minimally diverged (Chapuis & Estoup, 2007).

3.2 | Origins of the Virgin Islands clade

The 542 mitochondrial sequences obtained from individuals across the PRB consisted of 465 unique haplotypes, which we used for subsequent analyses. Both Bayesian and ML analyses recovered similar topologies and nodal support for the *A. cristatellus ND2* gene tree (Figure 1b). *Anolis cristatellus* is rendered paraphyletic in the mtDNA gene tree by the populations referred to as *A. desechensis* (Isla Desecheo) and *A. ernestwilliamsi* (Carrot Rock, British Virgin Islands); henceforth, we refer to the inclusive (*A. cristatellus*+*A. desechensis*+*A. ernestwilliamsi*) lineage as *A. cristatellus* sensu lato. Within *A. cristatellus*, we identified four well-supported (PP ≥ 0.95; BS ≥ 70%) mitochondrial clades (Figure 1b), with 3.5%–7.5% respective Tamura-Nei corrected sequence divergence. Two of these clades ("PR South" and "PR West") are restricted to the main island of Puerto Rico, while a third clade ("PR East") is represented in both eastern Puerto Rico and the western part of Vieques Island. The VI clade (Figure 1b; Fig. S1 in Appendix S1) is restricted to the VI (including eastern Vieques) and is sister to *A. cristatellus* from the southern half of Puerto Rico (including *A. desechensis*) with an estimated mtDNA coalescent time of 5.4 Ma (95% HPD 4.1–6.8 Ma; Table S6 in Appendix S2). For the multilocus sequence dataset, we find that the VI clade is sister to *A. cristatellus* from eastern/western Puerto Rico with an estimated divergence time of 1.3 Ma (95% HPD 0.4–2.3 Ma; Fig. S5 in Appendix S1; Table S6 in Appendix S2). *Anolis ernestwilliamsi*, presently restricted to Carrot Rock in the VI, is nested within the VI clade of *A. cristatellus* and is the sister taxon of a specimen from nearby Peter Island (PP = 0.98; Fig. S2 in Appendix S1).

3.3 | Archipelagic genetics in the Virgin Islands

Our ML, Bayesian, and Splitstree analyses demonstrate that most of the VI populations exhibited little phylogenetic structure (Figs. S1,

TABLE 2 Microsatellite DNA summary statistics for populations of *Anolis cristatellus* sampled in the Virgin Islands. N = number of genotypes, N_A = number of alleles, A_R = allelic richness, N_E = effective number of alleles, H_O = observed heterozygosity, H_E = expected heterozygosity, F_{IS} = fixation index, *HWE* = p value for Hardy–Weinberg equilibrium. Standard error is reported alongside mean values

Island	N	N_A	A_R	N_E	H_O	H_E	F_{IS}	*HWE*
Anegada	40	10.2 ± 3.3	3.18	4.87 ± 0.99	0.54 ± 0.07	0.73 ± 0.2	0.22 ± 0.08	<.001*
Beef	6	4.3 ± 1.0	2.84	3.37 ± 0.34	0.47 ± 0.07	0.74 ± 0.1	0.29 ± 0.11	.080
Cooper	7	4.5 ± 1.0	2.91	3.43 ± 0.44	0.52 ± 0.06	0.73 ± 0.1	0.21 ± 0.09	.03*
George Dog	24	4.8 ± 1.7	2.42	2.88 ± 0.50	0.33 ± 0.07	0.59 ± 0.2	0.42 ± 0.08	<.001*
Guana	33	8.8 ± 2.9	3.17	5.35 ± 1.24	0.57 ± 0.10	0.76 ± 0.2	0.24 ± 0.09	<.001*
Jost van Dyke	31	9.3 ± 2.2	3.23	5.38 ± 0.78	0.55 ± 0.08	0.78 ± 0.2	0.25 ± 0.12	<.001*
Little Camanoe	9	4.5 ± 1.4	2.65	3.16 ± 0.37	0.44 ± 0.10	0.70 ± 0.1	0.36 ± 0.13	.001*
Little Thatch	5	5.0 ± 1.4	3.21	3.96 ± 0.49	0.57 ± 0.08	0.80 ± 0.1	0.22 ± 0.09	.117
Marina Cay	11	5.3 ± 1.9	2.99	3.92 ± 0.70	0.53 ± 0.09	0.72 ± 0.2	0.25 ± 0.11	.045*
Moskito	7	3.5 ± 1.8	2.27	2.53 ± 0.72	0.43 ± 0.14	0.49 ± 0.3	0.06 ± 0.17	.072
Necker	7	4.0 ± 1.1	2.25	3.20 ± 0.50	0.26 ± 0.07	0.71 ± 0.1	0.61 ± 0.09	<.001*
Norman	12	6.0 ± 2.2	3.00	4.18 ± 0.80	0.63 ± 0.06	0.74 ± 0.2	0.08 ± 0.09	.428
Peter	5	4.0 ± 0.9	2.63	2.94 ± 0.27	0.43 ± 0.10	0.72 ± 0.1	0.33 ± 0.15	.137
Prickly Pear	15	4.8 ± 1.6	2.64	3.42 ± 0.60	0.41 ± 0.11	0.65 ± 0.3	0.45 ± 0.15	.002*
Salt	7	4.8 ± 1.7	2.86	3.68 ± 0.69	0.54 ± 0.13	0.73 ± 0.2	0.19 ± 0.20	.235
Scrub	3	2.8 ± 1.0	2.36	2.29 ± 0.29	0.61 ± 0.16	0.60 ± 0.3	0.22 ± 0.18	.515
St. John	22	9.7 ± 2.7	3.25	5.75 ± 0.89	0.54 ± 0.07	0.82 ± 0.1	0.32 ± 0.09	<.001*
St. Thomas	29	9.8 ± 3.6	3.07	5.70 ± 1.03	0.43 ± 0.08	0.80 ± 0.1	0.46 ± 0.09	<.001*
Tortola	62	12.0 ± 3.0	3.49	6.51 ± 0.89	0.57 ± 0.09	0.83 ± 0.1	0.30 ± 0.10	<.001*
Vieques	23	8.5 ± 3.0	3.33	5.30 ± 0.84	0.57 ± 0.10	0.77 ± 0.2	0.23 ± 0.11	<.001*
Virgin Gorda	5	5.7 ± 1.0	3.22	4.39 ± 0.389	0.58 ± 0.09	0.87 ± 0.0	0.23 ± 0.13	<.001*
Mean	17.3	6.3 ± 1.9	–	4.11 ± 0.17	0.50 ± 0.02	0.73 ± 0.2	0.27 ± 0.03	–

*Significant at $p \leq .05$.

S2 in Appendix S1). Two exceptions are Anegada Island, one of the largest (38.7 km^2) and most isolated (PX = 4.7) islands in the archipelago, and Norman Island, which is neither large nor isolated (2.5 km^2; PX = 11.7) (Fig. S1, S2 in Appendix S1). All other major clades consist of haplotypes from multiple islands, and no other island is monophyletic (Figs. S1, S2 in Appendix S1). Nevertheless, individuals with identical ND2 haplotypes are nearly invariably found on the same island, despite in some cases being sister to haplotypes from other islands. Only one individual, from Prickly Pear Island, shared an identical haplotype with individuals from Little Camanoe Island, located ~18 km to its southwest.

3.3.1 | Population genetic summary statistics

Summary statistics for the microsatellite and mtDNA datasets, referenced below, are shown in Tables 2 and 3, respectively. We found that most VI populations of *A. cristatellus* shared haplotypes with other islands across the archipelago (Figure 2b; Figs. S1, S2 in Appendix S1) and that intra-island divergence was generally low (Figure 3). AMOVA for the mtDNA dataset revealed that variation within islands accounts for 53.8% of total variation, whereas variation among islands accounts for the remaining 46.2% (Φ_{ST} = 0.5) (Table 4). For the microsatellite data, AMOVA analyses revealed that the vast majority (92.7%) of genetic variance is contained within islands (F_{ST} = 0.1) (Table 4).

3.3.2 | Tests for influence of island characteristics

Multiple regression of population genetic summary statistics (h, π, H_O, A_R) on island area and island proximity (isolation metric "PX") showed a strong positive relationship between genetic diversity and island area, but genetic diversity exhibited no relationship with island proximity (Table 5; Table S1 in Appendix S2).

3.3.3 | Tests for isolation by distance

Isolation-by-distance regression analyses revealed a non-significant relationship between Rousset's-transformed genetic distance (Φ_{ST}) for mtDNA (Mantel test, R^2 = −.31; p = .99) and F_{ST} for microsatellite loci (R^2 = −.23; p = .97) against Euclidean log-transformed geographical distance between islands (Figure 4a). Regressions of IBD were similarly non-significant for stepping-stone distances (Φ_{ST} R^2 = −.32; p = .99; F_{ST} R^2 = −.22; p = .97; Figure 4a).

3.3.4 | Tests for MMD equilibrium

For the island of St. Thomas, both Tajima's D and Fu's F_S statistical tests for population genetic neutrality rejected the null hypothesis of mutation–drift equilibrium. Fu's F_S test alone also rejected the null hypothesis for eight additional islands. Results from mismatch analysis indicated significant evidence for demographic instability

TABLE 3 Mitochondrial DNA summary statistics for populations of *Anolis cristatellus* sampled in the Virgin Islands. N = number of individuals, n = number of haplotypes, S = number of segregating sites, h = haplotype diversity ± SD, π = nucleotide diversity ± SD, D = Tajima's D, F_S = Fu's F_S, SSD = sum of squared deviation, RI = Harpending's raggedness index

Island	N	n	S	h	Π	D	F_S	SSD	RI
Anegada	35	31	44	0.99 ± 0.01	0.006 ± 0.003	−1.43	−24.94*	0.00	0.01
Beef	6	6	13	1.00 ± 0.09	0.005 ± 0.003	0.04	−1.90	0.01	0.03
Cooper	7	5	15	0.90 ± 0.10	0.005 ± 0.003	−0.37	0.55	0.08	0.27
George Dog	23	8	9	0.82 ± 0.06	0.002 ± 0.001	−0.66	−1.94	0.12*	0.08
Guana	33	18	26	0.91 ± 0.03	0.004 ± 0.002	−1.13	−7.01*	0.01	0.02
Jost van Dyke	28	25	53	0.99 ± 0.01	0.009 ± 0.005	−0.88	−12.52*	0.00	0.01
Little Camanoe	8	4	10	0.75 ± 0.14	0.004 ± 0.003	1.01	2.01	0.09	0.11
Little Thatch	4	2	1	0.67 ± 0.20	0.001 ± 0.001	1.63	0.54	0.09	0.56
Marina Cay	11	5	9	0.71 ± 0.14	0.002 ± 0.001	−0.47	0.33	0.10	0.19
Moskito	7	4	7	0.86 ± 0.10	0.002 ± 0.001	−0.52	0.35	0.10	0.18
Necker	7	6	13	0.95 ± 0.09	0.004 ± 0.003	−0.86	−1.35	0.04	0.06
Norman	12	8	10	0.91 ± 0.06	0.002 ± 0.001	−0.80	−2.96*	0.02	0.09
Peter	5	5	27	1.00 ± 0.13	0.010 ± 0.007	−0.44	−0.08	0.10	0.12
Prickly Pear	14	6	20	0.74 ± 0.11	0.005 ± 0.003	−0.25	2.05	0.09	0.18
Salt	7	4	6	0.81 ± 0.13	0.002 ± 0.001	0.06	0.28	0.08	0.20
Scrub	4	4	12	1.00 ± 0.18	0.006 ± 0.004	0.44	−0.12	0.19	0.50
St. John	20	20	66	1.00 ± 0.02	0.013 ± 0.007	−0.88	−9.43*	0.01	0.01
St. Thomas	29	27	47	0.99 ± 0.01	0.005 ± 0.003	−1.92*	−24.21*	0.00	0.02
Tortola	59	52	87	0.99 ± 0.00	0.012 ± 0.006	−1.07	−24.36*	0.00	0.00
Vieques	10	10	55	1.00 ± 0.04	0.014 ± 0.008	−0.95	−2.33	0.02	0.03
Virgin Gorda	18	18	50	1.00 ± 0.02	0.009 ± 0.005	−1.14	−9.96*	0.00	0.01

*Significant at $p \leq .05$.

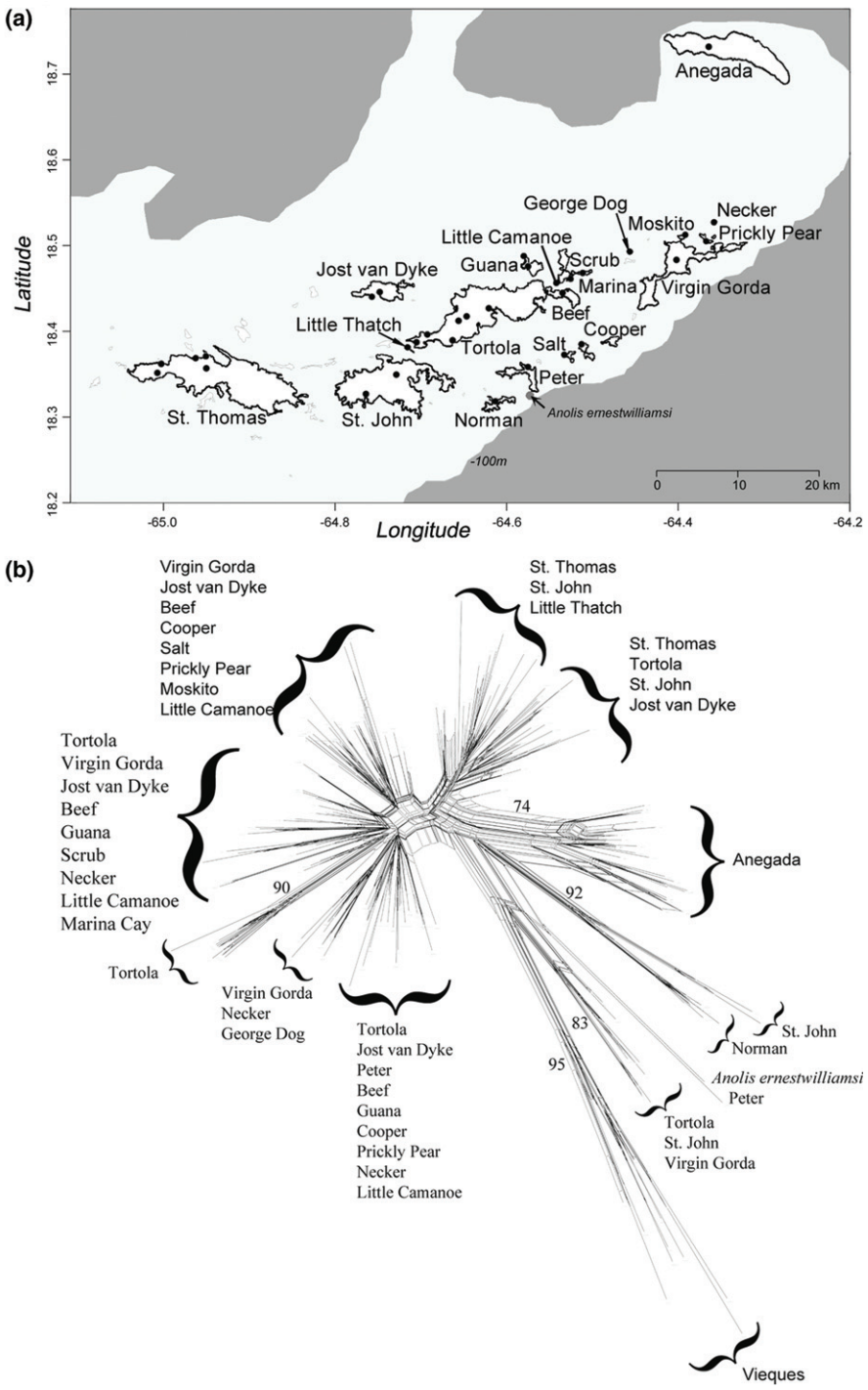

FIGURE 2 (a) The U.S. and British Virgin Islands showing sampling locations of *Anolis cristatellus* on each of 21 sampled islands (except Vieques Island, which is shown in Figure 1). (b) Network reconstruction of the entire mtDNA dataset for *Anolis cristatelllus* samples from the Virgin Islands. Island labels are ordered from largest (top) to smallest (bottom) area (km^2) in each list. Bootstrap values ≥70% are shown at major nodes. Also see Fig. S3 in Appendix S2

(expansion or gene flow) for all islands except George Dog (one of the smallest islands sampled; Table 2). On George Dog only the sum of squared deviation (but not Harpending's index) indicated rejection of the null hypothesis of demographic stability. Both fixation indices (F_{IS}) and tests of HWE (heterozygote deficiency) for each island indicated departures from expectations of random mating for the majority of the islands (Table 3). Pairwise Φ_{ST} based on mtDNA haplotype frequencies was at, or close to, zero for many population comparisons, reflecting haplogroup sharing across islands (i.e. most islands are not monophyletic; Figs. S1, S2 in Appendix S1). In addition, Φ_{ST} and pairwise Tamura-Nei divergence indicate differential divergence among islands with respect to haplotype and genotypic divergences. For instance, smaller islands tend to show larger values for Φ_{ST} than larger islands, a signature of genetic drift, irrespective of isolation (Figure 3a; Table S7 in Appendix S2); while neither area nor isolation show clear patterns for Tamura-Nei distances (Table S7 in Appendix S2). Pairwise F_{ST} (microsatellite) values are generally low, although small islands such as Moskito and George Dog islands show higher F_{ST} values while larger islands tend to have lower F_{ST} values (Figure 3b, Table S4 in Appendix S2).

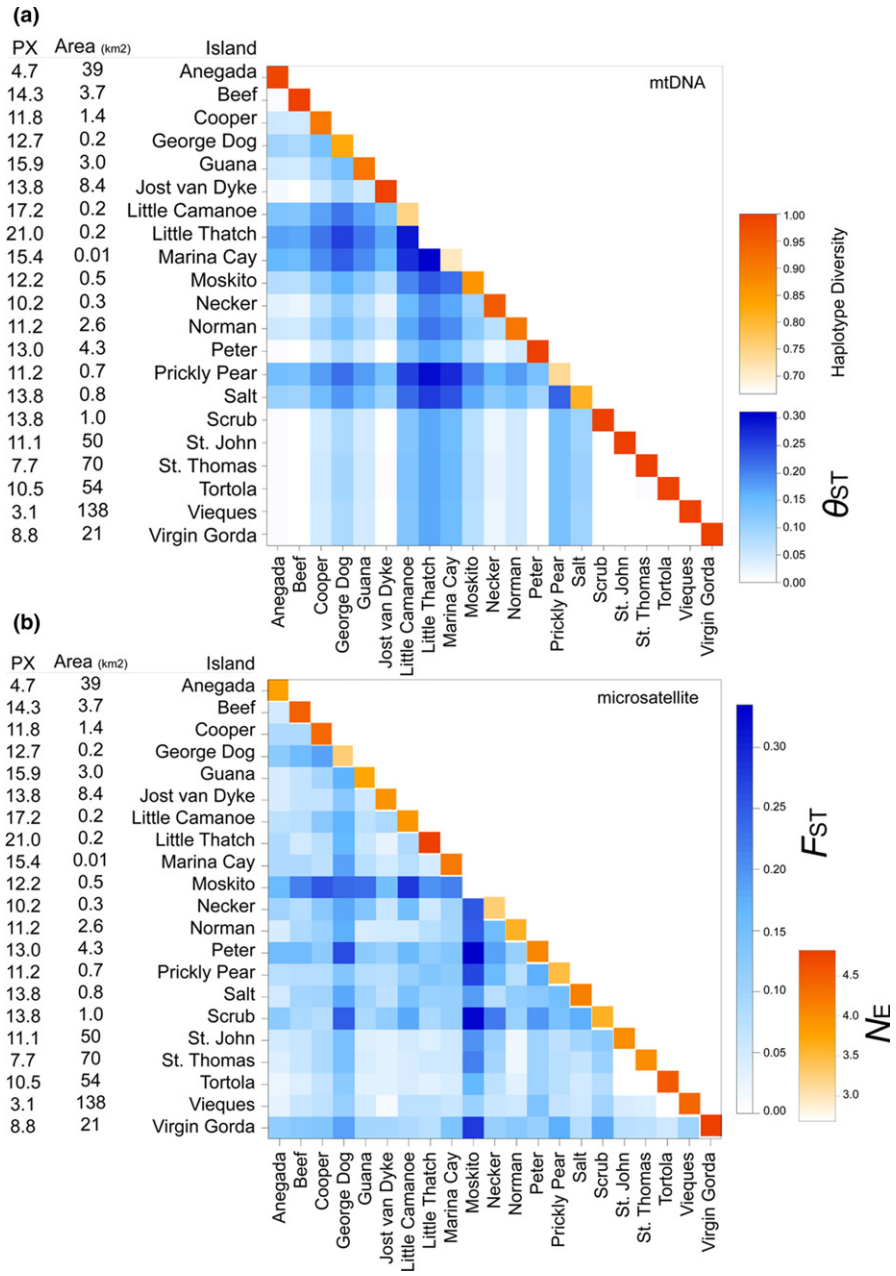

FIGURE 3 Population pairwise divergence among 21 island populations of *Anolis cristatellus* in the Virgin Islands based on 100,000 permutations. In both panels, cells below the diagonal represent between-island comparisons, while cells on the diagonal represent within-island diversity. (a) mtDNA Φ_{ST} estimates (below diagonal) and mtDNA haplotype diversity (on diagonal); (b) microsatellite F_{ST} comparisons (below diagonal) and effective number of alleles per island (N_E, on diagonal). Darker shades indicate increasing difference. To the left of island names are island characteristics including approximate area and a measure of relative isolation (*PX*, see text) [Colour figure can be viewed at wileyonlinelibrary.com]

TABLE 4 AMOVA results for *Anolis cristatellus* among islands in the Virgin Islands archipelago. Results are shown for both mitochondrial dataset and microsatellite (SSR) dataset

Marker	Grouping	Source of variation	*df*	Variance component	% total variance	*Φ* Statistic
mtDNA	All islands	Among islands	20	3.40	46.17	Φ_{ST} = 0.461*
		Within islands	326	3.96	53.83	
SSR	All islands	Among islands	20	0.16	7.30	F_{ST} = 0.073*
		Within islands	707	1.99	92.70	

*Significant at $p \leq .05$.

3.3.5 | Tests for migration and spatial expansion

Our explicit test of gene flow among eight islands indicated relatively low ($m_{[i][j]} \geq 0.01$) migration among most islands, although Tortola exhibited an order of magnitude higher level of emigration ($m_{[i][j]} > 0.20$) to other islands in the archipelago (Table S8). In contrast, we found far fewer immigrants ($m_{[i][j]} < 0.03$) moving to Tortola from the rest of the VI.

Our BSP supports a spatial expansion scenario, or a pattern of increasing N_ef over the last several hundred thousand years (Figure 4b).

TABLE 5 Multiple regression output for genetic diversity summary statistics by island population of *Anolis cristatellus* in the Virgin Islands regressed against island area and island proximity. Statistics for mtDNA are h = haplotype diversity and π = nucleotide diversity; statistics for microsatellite data are A_R = allelic richness and H_O = observed heterozygosity

Marker	Dependent variable	Independent variable	Coefficient	*p* value
mtDNA	h	Island area	0.031	.007*
		Proximity index	−0.004	.480
	π	Island area	1.2×10^{-3}	.002*
		Proximity index	-9.3×10^{-6}	.964
Microsatellite	H_O	Island area	0.025	.031*
		Proximity index	0.011	.110
	A_R	Island area	0.129	.002*
		Proximity index	0.035	.126

*Significant at $p \leq .05$.

Effective population size of VI *A. cristatellus* increased by three orders of magnitude, with two rate shifts apparent at 0.1 and 0.4 Ma. The units of this analysis are in coalescent time, such that we are estimating time based on the coalescent process and hence are likely overestimating the actual event times (Degnan & Rosenberg, 2009).

Our modelling of changes in effective population size based on the microsatellite dataset under approximate Bayesian computation suggested that our Model 1, or expansion in N_e by 1–2 orders of magnitude, fits the empirical data far better (PP > 0.90) than other models (both PP < 0.1; Fig. S6 in Appendix S1). We obtained posterior predictive error rates from a linear discriminant analysis of only 0.041 (direct approach) and 0.019 (logistic approach) over either the 500 or 2,000 closest datasets from our simulations, respectively.

4 | DISCUSSION

Land-bridge archipelagos represent a challenge to inference of intraspecific historical demography owing to a potentially complex geologic history of island connection and separation. One approach, which we term an archipelagic genetics approach, includes defining a priori a set of predictions related to population genetic inference, followed by the use of genetic data to examine evidence for a variety of predicted outcomes related to genetic diversity and divergence. To empirically investigate divergence in the spatial context of an island land-bridge archipelago, we undertook an extensive survey of genetic variation in the widespread lizard *A. cristatellus* across Puerto Rico and the VI. We tested predictions related to alternate hypotheses for the influences of historical biogeographical processes operating in the VI archipelago.

4.1 | Origins of the Virgin Islands clade

Consistent with other studies (Brandley & de Quieroz, 2004; Kolbe et al., 2007; Revell et al., 2007; Rodríguez-Robles et al., 2007), we found four main mitochondrial clades of *A. cristatellus*: three on the main island of Puerto Rico (clades PR South [including *A. desechensis*], PR West and PR East) and one clade in the VI Archipelago (Figure 1b). We found that the VI mtDNA clade is sister to a clade containing both *A. desechensis* on Isla Desecheo and *A. cristatellus* (clade PR South) from the south of Puerto Rico, with the VI lineage sister to the PR east/west lineages in our multilocus analyses. Our inferred mtDNA coalescence time of 5.4 Ma for the VI and Puerto Rico clades indicates a coalescence that greatly predates Pleistocene sea level fluctuations, although we note that estimates of inferred coalescent time must predate, and thus overestimate, the actual time of lineage separation (e.g. Degnan & Rosenberg, 2009). Our multilocus analyses suggested a more recent divergence time of 1.3 Ma. Taken together, these indicate that the VI lineage of *A. cristatellus* has likely occupied the region throughout the recent history of bank inundation and island emergence in the Quaternary, as opposed to having been recently derived from elsewhere on the PRB.

4.2 | Archipelagic genetics in the Virgin Islands

We predicted that patterns of contemporary genetic diversity and divergence in the VI land-bridge archipelago would be consistent with one or more of three scenarios. An island allopatric scenario predicts that genetic diversity is influenced by isolation (despite island connections during periods of low sea level). A contiguous population scenario predicts that modern genetic diversity on islands is largely structured by historical connections among populations during intraglacial periods. Third, a spatial expansion scenario predicts that populations are derived from neither allopatric nor contiguous populations, instead arising from relatively recent expansion from a refuge or refugia in the archipelago.

Our results are consistent with our third hypothesized scenario (Table 1) that contemporary population genetic patterns in *A. cristatellus* in the VI Archipelago fit an historical spatial expansion scenario, with some minimal influence of contemporary island allopatry. With the exception of (1) a positive relationship between genetic diversity and island area (Table 5), our analyses indicate: (2) no relationship between island isolation and genetic diversity; (3) no IBD pattern; and (4) an absence of MMD equilibrium. Interestingly, this is a result that is largely consistent with that shown by *Eleutherodactylus antillensis*, another widespread terrestrial vertebrate in the archipelago (Barker et al., 2012), although our findings also suggest that *A. cristatellus* has likely persisted in the VI throughout much of the Quaternary rather than having expanded recently from mainland Puerto Rico. Taken together, these findings indicate that *A. cristatellus* likely found refuge on the VI, rather than on Puerto Rico. Such a situation is also consistent with the general lack of endemism among VI terrestrial herpetofauna (Heatwole & MacKenzie, 1967) and might suggest that other VI species, in addition to *A. cristatellus* and *E. antillensis*, could be subjected to similar historical patterns of spatial expansion which might slow the evolution of endemism in the region.

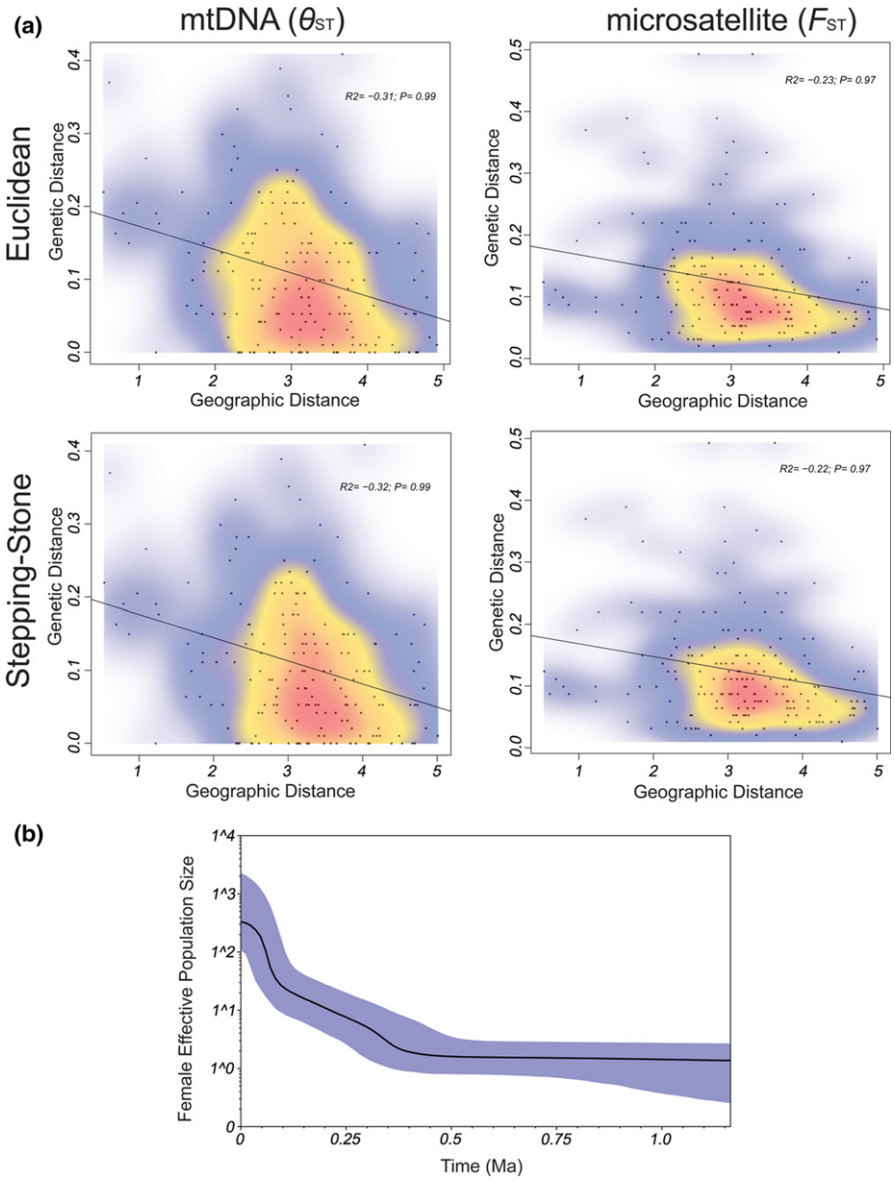

FIGURE 4 (a) Plots of isolation-by-distance for *Anolis cristatellus* in the Virgin Islands, with distances representing log-transformed distances between island centroids. Panels represent regressions of Rousset's Φ_{ST} for the mtDNA and Rousset's F_{ST} for the microsatellite data against both Euclidean distance and stepping-stone distance among island centroids. Dots represent pairwise comparisons, with a point density map overlaid using heat colours. (b) Bayesian skyline plot of the change in female effective population size through time in Virgin Islands *Anolis cristatellus*. Note two increases in rate of population size change corresponding to mtDNA coalescent times of 0.1 and 0.4 Ma [Colour figure can be viewed at wileyonlinelibrary.com]

4.2.1 | Tests for influence of island characteristics

Some possible signature of large islands containing (relatively) higher genetic diversity might be expected, even under a spatial expansion scenario, owing to the range of island sizes present in the archipelago. Larger islands, such as Tortola (54 km^2), have the potential for larger effective population and census population sizes, while very small islands, such as George Dog (only 0.15 km^2), are likely under some influence from genetic drift owing to small effective population sizes. Samples from Anegada and Norman islands each form mtDNA clades (Figure 2; Figs. S1, S2 in Appendix S1), suggesting that these islands might have experienced mtDNA lineage sorting owing to demographic effects (e.g. founder effects), as we still recover evidence of microsatellite allelic similarity among these islands (Figure 3b; Table S8 in Appendix S2). Alternatively, strong sex-biased dispersal could give rise to a similar pattern, whereby females exhibit philopatry and males disperse widely. Nevertheless, all other islands are non-monophyletic, and we find no influence of isolation on measures of mtDNA or microsatellite genetic diversity or divergence (Table 5).

4.2.2 | Isolation by distance

Our analyses of two measures of geographical distance, Euclidean distance and stepping-stone distance, failed to identify a measurable signature of IBD among the VI populations of our study (Figure 4a). This apparent lack of correlation suggests that island *A. cristatellus* do not appear to represent vicariant populations which emerged from a landscape characterized by spatial substructure during intraglacial periods. It also suggests that island populations are not exchanging alleles at an exclusively local level during interglacial periods. Instead, this absence of pattern suggests possible spatial expansion or very high levels of gene flow over time-scales captured by both the mtDNA and microsatellite data. The effect of the latter would be to homogenize

populations across a large landscape while maintaining diversity on small islands (Pannell, 2003), an unlikely scenario given evidence for some drift on small islands, limited recent migration among islands and additional evidence for expansion discussed below.

4.2.3 | MMD equilibrium

Both mtDNA and microsatellite data indicate the presence of non-equilibrium dynamics in the VI. Our analyses of mitochondrial data suggested that at least eight of 21 island populations are not in MMD equilibrium based on Tajima's D and Fu's F_S tests. This suggests that processes such as natural selection, demographic fluctuation or gene flow might be occurring. Mismatch analyses indicated that most islands have experienced demographic fluctuation or immigration (Table 3). It has been shown that large variability in the coalescent process for a single locus might contribute to alternate signatures of demographic signal (Karl, Toonen, Grant, & Bowen, 2012), although our results are consistent other analyses suggesting non-equilibrium in most populations. Again, these results are interpretable either as spatial expansion or widespread gene flow without spatial structuring.

Both our BSP and ABC analyses support a spatial expansion scenario. Our BSP analysis shows a pattern of increasing N_ef of VI *A. cristatellus* over the last several hundred thousand years, with a dramatic increase during at least two bouts (Figure 4b). Thus, it is possible that these increased rates of growth in N_ef might coincide with periods of spatial expansion during intraglacial periods. Our ABC approach found strong support for a model incorporating a dramatic increase in N_e over the last 10^5 years relative to models of static or decreasing population sizes (Fig. S6 in Appendix S1).

We found evidence for a relatively high level of migration from Tortola to the rest of the archipelago (Table S8)—nearly an order of magnitude higher than migration rates among other islands or to Tortola. Tortola is centrally located relative to other islands, is the second largest island east of Vieques and has the highest peak in the VI (523 m a.s.l.). This suggests that Tortola might have been a source from which spatial expansion could have progressed. As we consider our estimates of migration to be relative, additional work using more individuals per island or more markers might better test for rates of migration at a smaller scale.

In a spatial context, AMOVA analyses showed that a similar amount of genetic variation occurs within islands relative to among islands (Table 4). This suggests that there is little spatial genetic structure, again suggesting that these populations are not the product of allopatric isolation nor phylogeographical structuring.

We found that pairwise genetic distances were generally low among islands (Figure 3) and that all but two islands contain non-monophyletic populations for mtDNA (Figs. S1, S2 in Appendix S1). Small islands have slightly higher estimates of genetic distances, potentially owing to under-sampled allele frequencies given the small sample sizes from these islands, or a stronger effect of genetic drift in islands with relatively small effective population sizes. Large islands tend to have somewhat lower F_{ST} values, again suggesting that genetic drift might be increasing genetic divergence among populations on small islands. For the microsatellite data, 13 of 21 populations were found to deviate from HWE (Table 2) and pairwise F_{ST} was generally low (Figure 3b). Most populations found to deviate from HWE were also from islands with the largest sample sizes, suggesting that our power to detect HWE might be dependent on our sample sizes or the sizes of the islands. We do not necessarily expect that departures from expectations of random mating in both the fixation indices (F_{IS}) and tests of HWE would owe to a Wahlund effect on smaller islands, although it might be possible on larger islands despite our finding little evidence for population substructuring on large islands (Fig. S3 in Appendix S1). Nevertheless, overall findings of departures from HWE and generally low F_{ST} values further suggest non-equilibrium owing to spatial expansion or extensive gene flow.

4.2.4 | Vieques Island and human introduction

Interestingly, two main mtDNA clades of *A. cristatellus* co-occur on Vieques Island—the VI clade and the PR East clade (Figure 1b). The haplotypes in the VI clade lizards from Vieques are reciprocally monophyletic with respect to the rest of the VI, which might reflect the isolation of Vieques (PX = 3.1) relative to other islands in the study. *Anolis cristatellus* has been suggested to exhibit a colonizing phenotype (Hertz, 1983; Williams, 1969) and is capable of colonizing small islands (Heatwole & Levins, 1973) as well as establishing in the face of novel competitors (e.g. Eales, Thorpe, & Malhotra, 2010). Hence, it is possible that the PR East lineage colonized Vieques via human-facilitated introduction, as Perry et al. (2006) observed *A. cristatellus* hitchhiking in potted plants on boats. Such boats might be a vector carrying lizards between islands, although large N_e on large islands means new alleles have a low probability of going to fixation. In addition, we found only one compelling example of an introduced lizard—a haplotype from Prickly Pear Island was identical to haplotypes on Little Camanoe Island. These islands are separated by ~18 km as well as other islands such as Great Camanoe and Virgin Gorda, suggesting the Prickly Pear haplotype was introduced. A more likely scenario for Vieques Island is that the presence of two lineages represents a zone of secondary contact in the absence of geographical barriers, as is seen in many other studies of West Indian *Anolis* lizards (e.g. Geneva et al., 2015; Kolbe et al., 2004), although this requires additional study.

5 | CONCLUSIONS

Since the estimated divergence of VI *A. cristatellus* around the start of the Pliocene, the PRB has been a contiguous land mass for long periods, permitting connectivity among subpopulations presently restricted to islands (Donn, Farrand, & Ewing, 1962; Heatwole & MacKenzie, 1967). Our archipelagic approach attempts to reconstruct the influence of this historical land-bridge island structuring on contemporary population genetic structure. Taken together, our results suggest that genetic diversity and divergence of VI

populations of *A. cristatellus* are minimally influenced by allopatry or spatial genetic structure, as might be predicted given the current distribution of the species on islands or recent land-bridge connectivity that persisted for nearly 1×10^5 years. Instead, we find evidence for population spatial expansion producing a relatively homogenous distribution of haplotypes and alleles across the archipelago. Island archipelagos are often considered to be good examples of the influence of islands themselves on the distribution of genetic diversity (e.g. Johnson et al., 2000), yet our results suggest that land-bridge islands might be subject to unanticipated demographic dynamics, such as spatial expansion, that serve to obfuscate traditional predictions of island diversity. Thus, we suggest an archipelagic genetics approach in an a priori predictive framework when attempting to characterize intraspecific genetic diversity and divergence on land-bridge archipelagos.

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DATA ACCESSIBILITY

Alignments and phylogenetic trees archived at Dryad (https://doi.org/10.5061/dryad.4m3r4). R code is available from GitHub (https://github.com/caribbeanboas).

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BIOSKETCHES

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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