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In situ genetic differentiation in a Hispaniolan lizard (*Ameiva chrysolaema*): A multilocus perspective

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ABSTRACT

A previous phylogeographic study of mitochondrial haplotypes for the Hispaniolan lizard *Ameiva chrysolaema* revealed deep genetic structure associated with seawater inundation during the late Pliocene/early Pleistocene and evidence of subsequent population expansion into formerly inundated areas. We revisit hypotheses generated by our previous study using increased geographic sampling of populations and analysis of three nuclear markers (*α-enolase* intron 8, *α-cardiac-actin* intron 4, and *β-actin* intron 3) in addition to mitochondrial haplotypes (*ND2*). Large genetic discontinuities correspond spatially and temporally with historical barriers to gene flow (sea inundations). NCPA cross-validation analysis and Bayesian multilocus analyses of divergence times (IMa and MCMCcoal) reveal two separate episodes of fragmentation associated with Pliocene and Pleistocene sea inundations, separating the species into historically separate Northern, East-Central, West-Central, and Southern population lineages. Multilocus Bayesian analysis using IMa indicates asymmetrical migration from the East-Central to the West-Central populations following secondary contact, consistent with expectations from the more pervasive sea inundation in the western region. The West-Central lineage has a genetic signature of population growth consistent with the expectation of geographic expansion into formerly inundated areas. Within each lineage, significant spatial genetic structure indicates isolation by distance at comparable temporal scales. This study adds to the growing body of evidence that vicariant speciation may be the prevailing source of lineage accumulation on oceanic islands. Thus, prior theories of island biogeography generally underestimate the role and temporal scale of intra-island vicariant processes.

1. Introduction

Mitochondrial DNA (mtDNA) has been the preferred locus for phylogeographic studies of animals because of its uniparental mode of inheritance, relatively small effective population size, and high rate of nucleotide substitution (Avise, 2000). These attributes make it an ideal locus for resolving population processes occurring over the past few hundred thousand to a few million years. One shortcoming of using a single locus, such as mtDNA, is that the inferred patterns represent a single realization of a complex population-genetic history. Additionally, mitochondrial loci could give a biased view of population history because they are transmitted only through the female parent. The use of nuclear DNA (nDNA) loci in phylogeographic studies is increasing, although possibilities of recombination and uncertain orthology of comparisons present analytical challenges (Hare, 2001). On average, nDNA loci evolve at a much slower rate than does mtDNA, and their effective population size is four times larger. The expected time frame over which nDNA polymorphisms coalesce is approximately four times longer than that for mtDNA, limiting precise resolution of the most recent events in a population's evolutionary history (Templeton, 2006).

The agreement, or lack thereof, between genetic patterns at multiple unlinked loci provides a powerful test of evolutionary hypotheses (Brumfield et al., 2003; Hey and Machado, 2003). Demographic phenomena should leave a common signature on all neutral loci whose evolutionary rates are appropriate for investigating those phenomena (Hare, 2001). Agreement among loci increases statistical power for testing alternative hypotheses. Recent developments in phylogeography provide rigorous tests for geographic fragmentation of populations. In Nested Clade Phylogeographic Analysis (NCPA, Templeton, 1998) one uses a series of nested groups in a haplotype genealogy to test historical explanations for geographic patterns of genetic variation. A rarely used and recent extension of this method cross-validates inferences from multiple loci using maximum likelihood (ML; Templeton, 2002, 2004). Other approaches use coalescent models in an ML or Bayesian framework to estimate historical demographic parameters (e.g., effective population sizes, migration rates, fluctuations

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in population size, population divergence times, etc.; Kuhner, 2006; Nielsen and Wakeley, 2001; Hey and Nielsen, 2004). Multilocus studies provide more precise estimates of population parameters, reduce variance caused by stochasticity of the coalescent process (Edwards and Beerli, 2000; Hudson and Turelli, 2003), and reduce error associated with stochastic sorting of haplotype lineages (Irwin, 2002; Kuo and Avise, 2005).

In a previous study we used mtDNA to evaluate the phylogeographic history of *Ameiva chrysolaema* (Gifford et al., 2004). *Ameiva chrysolaema* is a relatively large teiid lizard (max. SVL, 160 mm) distributed throughout most of the xeric lowlands of Hispaniola (Fig. 1). The species can be found in a variety of habitat types but is most commonly encountered in *Acacia* scrub and xeric woodlands, and occasionally on open beaches with some cover (Schwartz and Henderson, 1991). In our previous study maximum parsimony and Bayesian topologies revealed three deeply divergent lineages, representing older vicariant events, while Nested Clade Phylogeographic Analysis (NCPA; Templeton, 1998) revealed four geographically distinct lineages, which we called Northwestern, Barahona, and the Central/Southeast; the latter clade was partitioned into East and West sub-lineages. The morphologically cryptic East and West sub-lineages separated from each other during the late Pliocene to early Pleistocene, and made secondary contact in a narrow region along the southern coast of the Dominican Republic. The population-genetic inferences are geographically and temporally congruent with seawater transgressions and recessions that occurred episodically during the Pliocene and Pleistocene (Fig. 1). Sea inundation of the Azua Basin, the extreme topography of

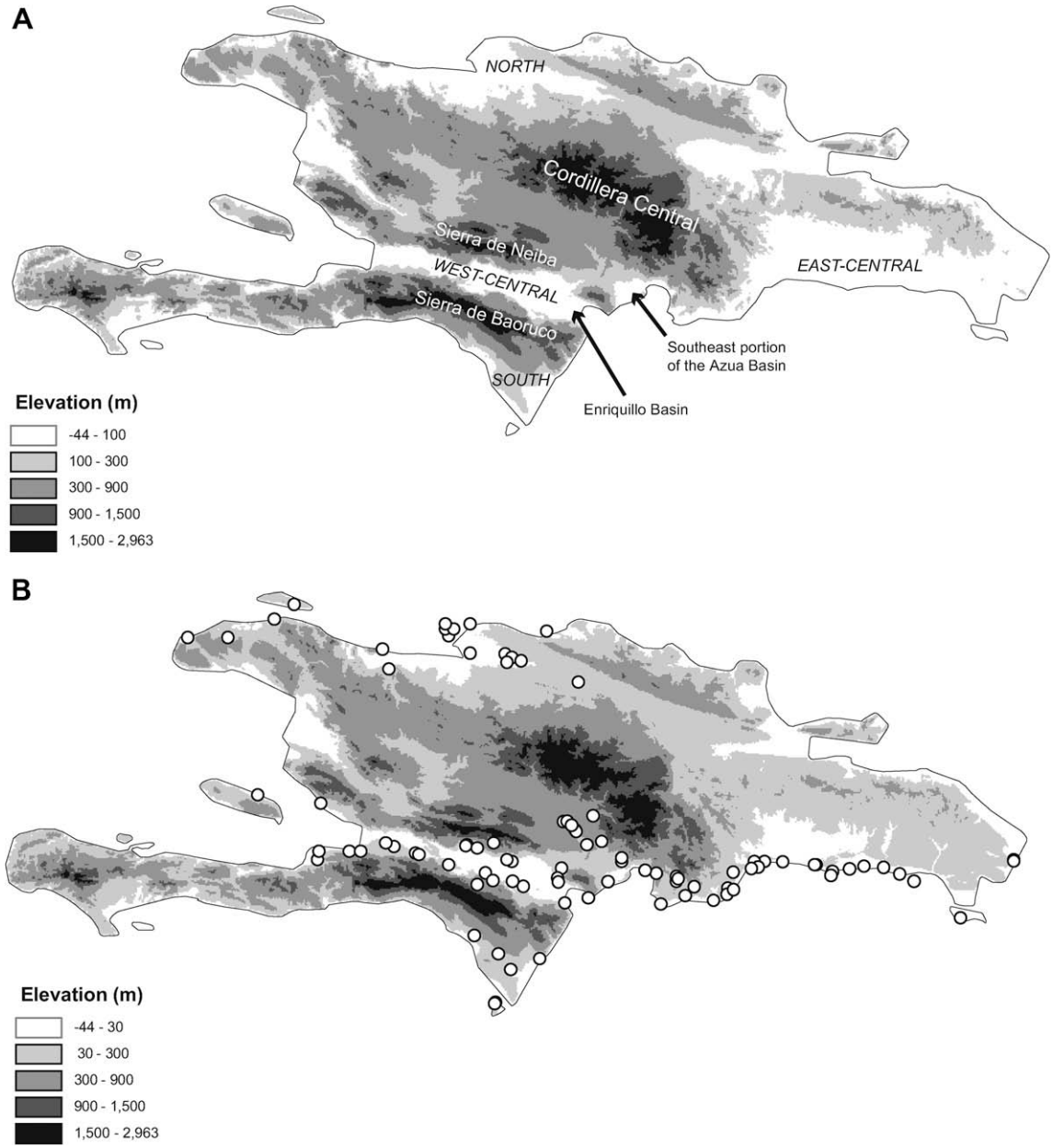

Fig. 1. Relief maps of Hispaniola depicting parts of the island that were emerged versus submerged during two periods of elevated sea-level: (A) early Pliocene, approximately 5.5–3 million years ago (mya) and (B) Pleistocene, approximately 1.1 mya. White areas in both the figures represent land submerged at the indicated time. Mountain ranges and biogeographic regions mentioned in the text are labeled in white and black, respectively in (A). The geographic distribution of *A. chrysolaema* is shown in (B) based on known presence localities from museum locality records. Specific geographic coordinates for presence localities are available from the lead author by request. Inundation of the Azua Basin would have likely restricted gene flow between East-Central and West-Central lineages as a consequence of the extreme topography of the southern Cordillera Central and the limited elevational distribution of *Ameiva chrysolaema*.

the southern Cordillera Central, and the restriction of *A. chrysolaema* to xeric lowlands together would have played a large role in isolating East and West sub-lineages. Divergence times among the Northern, Central/Southeast, and Barahona lineages represent older vicariant events. Major mountain ranges currently isolate these lineages, although the causes of their initial isolation remain unclear. In this study we refer to these lineages as the North (=Northwestern), South (=Barahona), and Central (=Central/Southeast) and the sub-lineages of the Central clade as East-Central and West-Central (Fig. 2).

We expand our previous work with increased sampling (both individuals and localities) and with the addition of three nuclear loci. We test hypotheses generated by our previous study using multiple loci and new phylogeographic methods accommodating coalescent stochasticity. We make the following predictions for the population structure and historical demography of *A. chrysolaema*. (1) Genetic fragmentation of populations north and south of the major mountain ranges should predate Pleistocene sea inundations (~1.1 and 1.4 mya, Raymo et al., 2006) and be associated with the major episodes of Miocene mountain formation (~20–8 mya, Heubeck and Mann, 1991), the Miocene configuration of Hispaniola as two paleoislands (>8 mya, Graham, 2003) or Pliocene sea-inundation (~5.5–3 mya, Haq et al., 1987; Fig. 1); (2) fragmentation of populations in the Central region should be associated with Pleistocene sea-inundation of the Enriquillo and Azua Basins (~1.1 and 1.4 mya); and (3) East-Central and West-Central populations should show different phylogeographic patterns because sea inundations were more pervasive in the western region. Specifically, we predict that West-Central populations will show a stronger signature of population growth/expansion. These predictions allow a detailed test of hypotheses derived from our previous work (Gifford et al., 2004) and a more quantitative assessment of the role of vicariance as a major contributor to island biodiversity in the Greater Antilles (Losos and Schluter, 2000).

2. Methods

2.1. Sampling, laboratory techniques, and molecular data

Sampling for this study expands previous sampling (Gifford et al., 2004), and includes 166 individuals from 37 localities (avg. 4.6 individuals per locality, range 1–10; Fig. 2, Table S1). One individual of *Ameiva lineolata* served as an outgroup for all loci. Lizards were captured with a noose, blow pipe, or by hand, and tissue samples were taken non-destructively (~10 mm tail tip) or from voucher specimens (liver). All tissue samples were stored in 95% ethanol at −20 °C.

Genomic DNA was extracted using Qiagen DNeasy Tissue kits (Qiagen, Valencia, CA) or Viogene Blood & Tissue Genomic Mini kits (Viogene-Biotek, Sunnyvale, CA). Four genomic fragments (ca. 2500 bp total) representing one mitochondrial protein-coding gene and three nuclear introns were amplified using PCR (Saiki et al., 1985). The mtDNA ND2 protein-coding gene and $tRNA^{Trp}$ (~1094 bp) gene were amplified using primers listed in Gifford et al. (2004), the *α-cardiac actin* intron 4 (*ACA4*, ~600 bp) using primers described in Waltari and Edwards (2002), the *β-actin* in-

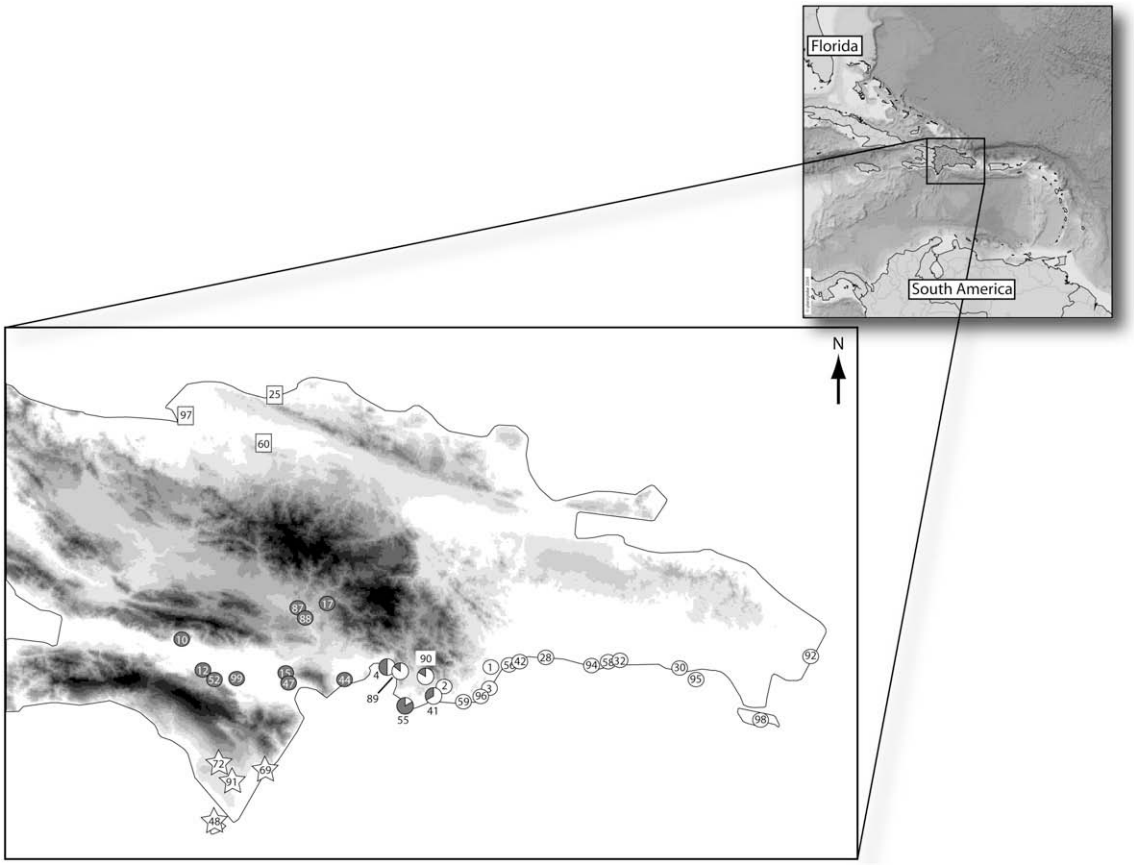

Fig. 2. Geographic sampling sites for *Ameiva chrysolaema* in the Dominican Republic. Symbols represent geographically cohesive haplotype clades identified in the Bayesian phylogenetic analysis of the combined data; squares, Northern Clade; stars, Southern Clade; circles, Central Clade. The Central Clade is further partitioned into Eastern (open circles) and Western (filled circles) parts. The zone of secondary contact of mtDNA haplotypes is represented with pie charts showing relative proportions of Eastern and Western haplotypes at those localities.

tron 3 (*BA3*, ~330 bp) using primers from Waltari and Edwards (2002), and the *α-enolase* intron 8 (*E8*, ~520 bp) using primers from Friesen et al. (1997). PCR was performed in 25 μl reactions containing 2.5 μl 10× buffer, 2.5 mM $MgCl_2$, 0.2 μM dNTPs, 0.2 μM each primer, 1 U Taq DNA polymerase, and 2–3 μl genomic DNA extract. Cycling conditions for all loci followed Gifford et al. (2004); the single exception was for *E8*, which had an annealing temperature of 55 °C. All other loci annealed at 50 °C. PCR products were purified using the Viogene Gel-M Extraction System (Viogene-Biotek, Sunnyvale, CA), sequenced using Big Dye Terminator chemistry (Perkin-Elmer, Wellesley, MA, USA), and visualized on an ABI 3130 automated capillary sequencer (Applied Biosystems, Foster City, CA). All sequences were aligned and manually adjusted using the alignment tool in MacClade 4.06 (Maddison and Maddison, 2003).

Heterozygous sites in nuclear data sets were recognized by the occurrence of two peaks in electropherogram traces. A site was considered heterozygous if the secondary peak reached at least 25% of the intensity of the primary peak. Certain individuals were heterozygous for alleles differing by length variation (indels), identified by otherwise clean electropherograms suddenly becoming unreadable due to the presence of multiple fragments of different sizes. The location of these transitions matched sites for which other individuals were homozygous at the indel site. Gametic phases of nuclear sequences with more than a single heterozygous site were estimated using a Bayesian approach implemented in the software program Phase 2.1 (Stephens et al., 2001; Stephens and Donnelly, 2003). Multi-base indels were collapsed to single-base polymorphisms for phasing analyses. All Phase analyses were run at least four times, each initiated using a different random-number seed. All analyses were run for 1000 iterations with a single thinning interval and 100 burn-in iterations. Consistency of results was checked by examining haplotype frequencies and coalescent goodness-of-fit measures estimated for each run, as suggested in the program documentation. The phases of some sites could not be confidently estimated at 90% posterior probability. These sites were coded as missing data or excluded from the dataset depending on the capacity of the other analytical programs to handle missing data. Less than 5% of all nucleotide characters could not be unambiguously phased.

2.2. Intraspecific phylogenetic analysis

Phylogenetic analysis of the combined data was performed using a partitioning strategy in MrBayes version 3.1 (Huelsenbeck and Ronquist, 2001). An appropriate model of sequence evolution for each locus was chosen using the Akaike Information Criterion (AIC, Akaike, 1974), implemented in the program ModelTest 3.7 (Posada and Crandall, 1998). Data were partitioned by locus, and branch support was measured by posterior probabilities. Two runs of five million generations with a sampling frequency of 1000 and a burn-in of 1000 samples were used to evaluate the consistency of analyses.

2.3. Multilocus phylogeographic and demographic analyses

2.3.1. Tests for recombination and neutrality

A major assumption of phylogeographic analyses is that there has been no recombination within a locus since the time of coalescence of the gene copies under study. Because recombination causes the merging of distinct haplotypes, a single haplotype can have segments that have experienced very different evolutionary histories. To discriminate homoplasies deriving from recombination and those from recurrent mutation we implemented a suite of recombination metrics (Max χ^2 [Maynard Smith and Smith, 2002], NSS [Jakobsen and Easteal, 1996], and PHI test [Bruen et al., 2006]) in the program PhiPack (Bruen et al., 2006, http://www.mcb.mcgill.ca/~trevor/) for each nuclear locus. We also examined refined incompatibility matrices to ensure that there were no blocks of site incompatibilities indicating deviations from the four-gamete test. Selective neutrality of each of the three nuclear regions was assessed with Hudson–Kreitman–Aguadé (HKA) tests (Hudson et al., 1987). These tests were performed using the software program HKA (J. Hey, Rutgers University, http://lifesci.rutgers.edu/~heylab/HeylabSoftware.htm) with 10,000 coalescent simulations.

2.3.2. Genealogies, NCPA, and cross-validation

Haplotype networks for all recombination-free loci were constructed using TCS 1.18 (Clement et al., 2000). The resulting haplotype networks were nested according to the rules of Templeton and Sing (1993) and Templeton (1998). Clade and nested-clade distances were calculated using GeoDis version 2.4 (Posada et al., 2000). Significance of associations between genealogy and geography was tested using 10,000 permutations. Possible scenarios responsible for significant associations were determined using the most recent inference key (http://darwin.uvigo.es/download/geodisKey_11Nov05.pdf).

Spatial congruence of NCPA inferences among multiple loci provides supporting evidence for these inferences. However, the temporal congruence of inferences is critical to testing biogeographic hypotheses. Templeton (1993) presented a method for temporally cross-validating phylogeographic inferences made with NCPA using multiple loci. More recently a likelihood framework for this procedure was introduced permitting tests of the null hypothesis that all loci are marking the same event using log-likelihood ratio tests (Templeton, 2002, 2004). The timing of an event or process inferred using NCPA can be estimated by the age of the youngest nested haplotype group that contributes in a statistically significant manner to the inference (Templeton, 2002, 2004). This particular age indicates the time during which all haplotype lineages affected by the event were present. Point estimates of these ages can be calculated with reference to a calibration point (10 mya in this study, based on a molecular divergence estimate between *A. lineolata* and *A. chrysolaema* from Hower and Hedges (2003), which was calibrated using immunological distance analysis) and the amount of nucleotide diversity within the group of interest relative to the number of nucleotide differences between that group and an outgroup (TLS estimator, Takahata et al., 2001). The TLS estimator provides a point estimate but does not account for error associated with the coalescent process. Kimura (1970) found that the distribution of fixation times (i.e., the inverse of time to coalescence) is approximated by a γ distribution. To accommodate stochastic error of the coalescent process, the estimated dates are fitted to a γ distribution with mean equal to the TLS estimator and variance conditioned upon the genetic diversity estimated for the group being dated (Templeton, 1993, 2004). The log-likelihood of the loci sharing a common age for an event is compared to the log-likelihood of the inferred events having separate ages using a log-likelihood ratio test with $n - 1$ degrees of freedom, where n is the number of loci being compared (i.e., those loci sharing a geographically concordant inference).

2.3.3. Multilocus coalescent-based divergence-time estimation and historical demographic analysis

The discrepancy between gene coalescent times and population divergence times varies depending on the demographic history of the diverging populations and the length of time since divergence (Edwards and Beerli, 2000; Rosenberg and Feldman, 2002; Arbogast et al., 2002; Jennings and Edwards, 2005). This discrepancy can become large especially for recently diverged populations and species (i.e., <5 my, Arbogast et al., 2002). In phylogeographic

studies, the main focus is on inference of population history rather than on the specific history of independently segregating loci. Accordingly, it has been suggested that details of population demography should be estimated from multiple loci using methods that incorporate information on historical demographic processes (Edwards and Beerli, 2000; Knowles and Maddison, 2002; Hey and Nielsen, 2004; Knowles, 2004). Multilocus data provide a powerful means of estimating divergence times among recently diverged populations because these data reduce the variance associated with estimating ancestral effective sizes (Edwards and Beerli, 2000; Arbogast et al., 2002). We used a coalescent approach to harness the power of multilocus data for estimating divergence times.

Because the relationships among major geographic haplotype clades are unresolved, we estimated divergence times for internal nodes of all three alternative resolutions of the population tree using Bayesian methods implemented in MCMCcoal version 1.2 (Rannala and Yang, 2003). This approach assumes neutrality of substitutions and no migration between species/populations after divergence. Thus, all cases of haplotype sharing among populations are treated as deep coalescence in this method. MCMCcoal estimates θ ($4N_e\mu$) for each extant population and each internal node in the population tree, as well as divergence times ($\tau = T\mu$) for each internal node; where N_e is the inbreeding effective size and μ is the mutation rate in substitutions site^{-1} year^{-1}. We accommodated rate variation among loci using the model of fixed relative rates as suggested in the program documentation. The mutation rates were scaled relative to the mean genetic distance between the ingroup and an outgroup across loci. This strategy produced an average scaled mutation rate of one, permitting the use of the mean mutation rate among loci to calculate parameters on a demographic scale. A heredity multiplier was used for the mitochondrial locus (=0.25) to ensure that effective sizes were analyzed on a comparable scale. Prior probability distributions were set by specifying α and β values (mean and variance of a γ distributed prior) for each parameter. Two independent analyses were initiated with different random-number seeds and run for 2×10^6 generations with a burn-in of 1×10^5 generations to check for convergence.

Our previous study identified geographic fragmentation of mtDNA lineages followed by secondary contact in the Central clade (Gifford et al., 2004). These lineages make secondary contact near the southeastern apex of the Cordillera Central. For the following analyses, West-Central populations include all sampling localities west of and including population #89; East-Central populations include all sampling localities east of and including population #55 (Fig. 2). This demographic history fits an isolation-with-migration model, so we fit this model to the data using IMa (Hey and Nielsen, 2007) to estimate the following demographic parameters all scaled by the neutral mutation rate: θ for each population and for the ancestral population; the population splitting parameter, τ; and bidirectional migration rates (m_1 and m_2) between East-Central and West-Central populations. We also recorded the timing and number of migration events to examine locus-specific contributions to the migration matrix. Priors, heating parameters, and the number of chains were optimized through several preliminary Markov Chain Monte Carlo (MCMC) runs. IMa runs included 40 chains, had a burn-in of 2×10^5 generations, and were continued for at least 4×10^6 generations until convergence diagnostics indicated stationarity. Effective sample size (ESS) values tend to be unstable (J. Hey, pers. comm.) and were often fairly low (minimum of 25), so convergence was accepted if repeat runs gave similar results and plots of parameter values by generation length showed no obvious directional trends. To convert parameters to a demographic scale, we used a generation length of two years based on published data from an ecologically similar and closely related species, *Ameiva exsul* (Rodriguez-Ramirez and Lewis, 1991).

We calibrated a mutation rate for the mtDNA locus based on an estimated divergence time of 10 my between *A. chrysolaema* and *A. lineolata* (Hower and Hedges, 2003). The resulting rate for this locus in *A. chrysolaema* is 1.334% uncorrected pairwise sequence-divergence per million years. This calibration agrees with other independent assessments for this locus in a wide variety of organisms [fish 1.3% (Bermingham et al., 1997); frogs 1.38% (Macey et al., 1998b); lizards 1.14–1.3% (Macey et al., 1998a; Macey et al., 1999); and salamanders 1.28% (Weisrock et al., 2001)]. Mutation rates of nDNA loci were calibrated in a similar fashion giving the following rates of pairwise sequence-divergence per my: 0.203% for *ACA4*, 0.117% for *BA3*, and 0.366% for *E8*.

Population structures within the East-Central and West-Central regions are predicted to differ because of the differential impact of sea inundations in each region. Much of the West-Central region lies below sea-level indicating that a rise in sea-level would have inundated much of this entire region. Alternatively, the East-Central region lies entirely above sea-level. We tested for these impacts on population demography in each region using Tajima's D (Tajima, 1989) and Fu's F_S (Fu, 1997) tests of neutrality. Significance of Fu's F_S and Tajima's D statistics were tested with 10,000 coalescent simulations in DnaSP (Rozas et al., 2003). We also tested for different levels of isolation by distance in each region using IBD version 1.52 (Bohonak, 2002). Significance of Mantel matrix correlations was evaluated with 10,000 permutations. Both of these analyses excluded five populations in the contact zone (#4, 89, 90, 55, and 41) to control for gene flow between lineages. For these analyses we used the mtDNA data because the dynamics of this locus make it more suitable than the slowly evolving nuclear loci for detecting recent demographic events.

3. Results

Unique haplotype sequences for each locus are deposited in GenBank (Accession Nos. *ACA4*, EU781033–EU781052, *BA3*, EU781053–EU781059, *ND2*, EU781060–EU781100, and *E8*, EU781101–EU781134. We obtained mtDNA sequences for the complete ND2 and partial $tRNA^{Trp}$ genes (1094 bp) from 166 individuals (64 from Gifford et al. (2004) and 102 newly reported) containing 79 haplotypes. The alignment contains no length variation and lacks premature stop codons. McDonald-Kreitman and Tajima's *D* tests indicate that the data do not deviate from neutral expectations (not shown).

Sample sizes, sequence information, and tests for recombination for the three introns are listed in Table 1. The NSS test of Jakobsen and Easteal (1996) for *ACA4* was significant, indicating possible recombination at this locus, but examination of the incompatibility matrix reveals no contiguous blocks of site incompatibilities. Furthermore, the NSS test has been shown to falsely infer the presence of recombination under some simple models of sequence evolution (Bruen et al., 2006). Because the Max χ^2 (Maynard Smith and Smith, 2002) and PHI test (Bruen et al., 2006) detect no recombination at this locus (Table 1) and no contiguous blocks of site incompatibilities occur (not shown), we treat *ACA4* as non-recombining in all analyses. An HKA test indicates that variation at nuclear loci is consistent with neutral expectations ($\chi^2 = 0.228$, df = 2, $P = 0.892$). The geographic distributions of haplotypes for all loci are included in the online supplementary materials (Tables S2–S5).

3.1. Partitioned multilocus phylogenetic analysis

Models of sequence evolution for each locus chosen by MODELTEST 3.7 (Posada and Crandall 1998) are shown in online Table S6. Bayesian analyses appeared to converge by 1×10^6 generations

Table 1
Sample size, sequence length, number of haplotypes, number and size of insertion–deletion (indel) events, number of variable sites (*s*), and tests for recombination (NSS, neighbor similarity score; Max χ^2, maximum χ^2; PHI, pairwise homoplasy index) for each locus used in this study

Locus	*N*	Length	Haplotypes	Indels	*s*	NSS	Max χ^2	PHI[†]
ACA4	148	599	20	2 (3,4)[*]	16	0.024	0.626	0.178
BA3	149	328	7	0	5	0.305	0.890	0.729
E8	144	503	34	2 (14,5)	24	0.470	0.261	0.187
ND2/tRNA^{Trp}	166	1094	79	0	187			

Table entries for recombination metrics are *P*-values for each test calculated with the software package PhiPack (Bruen et al., 2006).
[*] Indels restricted to three individuals.
[†] Significance of PHI test based on 1000 permutations.

based on inspection of the burn-in plot of log-likelihood and parameter values. We discarded the first 1000 samples (i.e., 1,000,000 generations) from each analysis, using two posterior distributions of 4001 samples each. A plot of the posterior probabilities of all splits from each analysis indicated no obvious non-linearity, suggesting that our analyses were not trapped in local optima.

Consistent with the results of Gifford et al. (2004), the Bayesian phylogeny indicates significant phylogeographic structure and recovers three well-supported, geographically cohesive haplotype clades (all posterior probabilities = 1, Fig. 3). The Northern clade contains haplotypes from the northwest portion of the Dominican Republic (localities 25 and 97). The Southern clade is restricted to

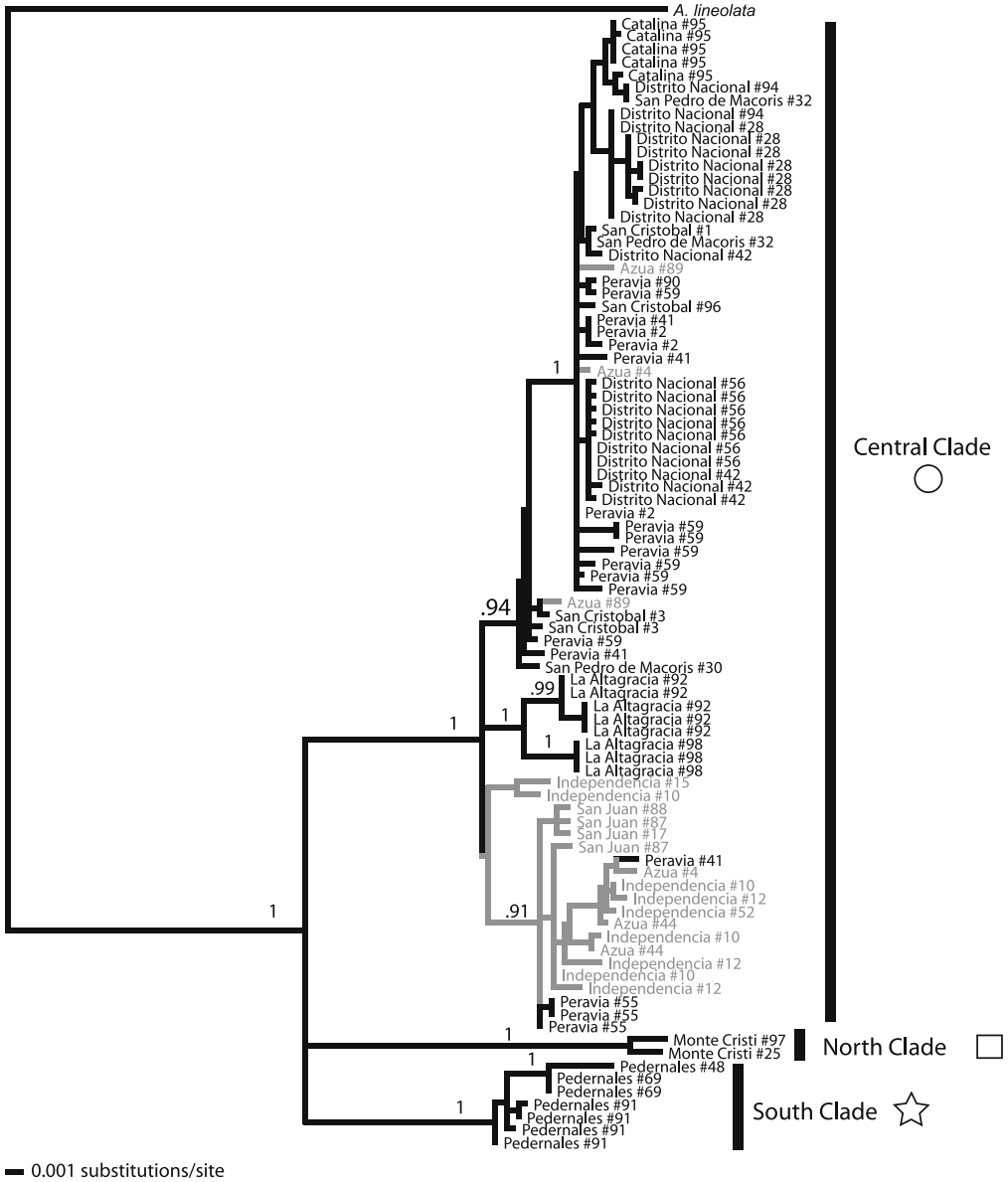

Fig. 3. Bayesian consensus phylogram derived from a partitioned analysis of nuclear and mitochondrial DNA sequences of *Ameiva chrysolaema*. Significant support for clades (posterior probability > 0.95) is depicted above branches. Well-supported, geographically cohesive haplotype clades are highlighted. Symbols are the same as those from Fig. 2. Within the Central Clade, branches are colored to represent the biogeographic distribution of haplotypes; gray, West-Central Region; black, East-Central Region (see text).

the Barahona peninsula and Isla Beata (localities 69, 72, 91, and 48). Haplotypes from all remaining localities form the Central clade. The Central clade shows considerable sub-structuring and partially segregates into largely East-Central and largely West-Central groups. Relationships among the Northern, Central and Southern geographic clades are ambiguous (Fig. 3).

3.2. Broad-scale phylogeography

3.2.1. mtDNA NCPA

ND2/tRNA^{Trp} haplotypes form three unconnected haplotype networks corresponding to the three geographically distinct clades identified in the Bayesian phylogenetic analysis (Fig. 4A, Online Table S3). In addition, the Central haplotype clade comprises two divergent haplotype groups separated by 25 mutational steps. These are the East-Central (nested haplotype group 5.1) and West-Central (nested haplotypes group 5.2) groups of haplotypes with some sharing among geographic regions (Fig. 4B).

Nested Clade Phylogeographic Analysis fails to reject the hypothesis of no geographic association of haplotypes for all but the highest nesting group (5.2) within the West-Central clade (Table 2). For nested group 5.2, a significantly small clade distance (D_c) with a corresponding significantly large interior-tip clade distance ($I - T_c$) suggests that the variation within this group is dominated by restricted gene flow with isolation by distance (Table 2). The East-Central group (5.1) shows a more complex population history. Significant associations between genetic structure and geography occur within nested groups 1.1, 2.1 and 2.3. Haplotype variation in these three groups suggests restricted gene flow with isolation by distance involving centrally located populations (#4, 89, 90, 41, 2, 59, 96, 3, 1, 56, 42, 3, 1, 56, 42, and 32), and an episode of long-distance dispersal to population #10 (nested group 1.1). Population fragmentation is inferred within nested groups 3.3 and 3.5. These inferences involve fragmentation between main-island populations and those on the satellite islands of Isla Catalina (#95) and Isla Saona (#98), respectively. The remaining inferences within the East-Central lineage (nested group 5.1) suggest restricted gene flow (nested groups 4.1, 4.2, and 5.1); however, 4-1 also indicates long-distance dispersal to the east coast (populations #92 and 98). Within nested group 6.1 we infer past genetic fragmentation between nested group 5.1 (East-Central) and nested group 5.2 (West-Central) followed by range expansion of 5.1 (Table 2); these inferences are geographically consistent with the history of sea inundation of the Azua Valley.

Genetic variation in the Southern lineage indicates a pattern of isolation by distance among main-island populations (#69, 72, and 91) and fragmentation between these populations and the population sampled from Isla Beata (#48; Table 2). Contingency analyses failed to reject the hypothesis of no geographic association of haplotypes within the Northern lineage. At the total-cladogram level, nested group 6.1 (i.e., the Central lineage) is designated the interior clade because the sum of the outgroup probabilities of the haplotypes within this group exceeds 0.95. Because haplotype groups do not overlap geographically, are separated by many mutational steps, and the species is absent in intervening areas between groups, we infer allopatric fragmentation to explain genetic variation at this level (Table 2).

3.2.2. nDNA NCPA

The haplotype network for the BA3 locus shows little variation and includes two geographically widespread haplotypes and five haplotypes with restricted geographic distributions (Fig. 5A, Online Table S4). Genetic variation at this locus suggests restricted gene flow with isolation by distance at all nesting levels and among all sampled populations (Table 2). AMOVA on haplotype-frequency data identifies significant partitioning of genetic variation among biogeographic regions (North, South, and Central; $\Phi_{CT} = 0.215$, df = 2, $P = 0.003$).

The haplotype network for *ACA4* shows little apparent geographic structure; however, haplotypes from the north and south regions tend to be associated with haplotypes from the West-Central region (Fig. 5B, Table S5). Four nested groups exhibit significant geographic haplotype associations (1.4, 2.1, 2.2, and total-cladogram; Table 2). Nested group 1.4 involves haplotypes from all geographic regions and indicates a contiguous range expansion to the North (#25). Sampling is inadequate to discriminate between short versus long-distance movements for nested group 2.1. Haplotype groups nested within 2.2 are significantly geographically structured but their distributions do not differ significantly in size from those expected. The total-cladogram level suggests a pattern of restricted gene flow with some long-distance dispersal to the east coast (populations #92 and #98; Table 2). AMOVA on haplotype frequencies indicates significant structure among biogeographic regions ($\Phi_{CT} = 0.427$, df = 2, $P < 0.0001$).

The *E8* haplotype network exhibits substantial geographic structure (Fig. 6, Online Table S6). Most haplotypes from the Northern region are separated from the remaining haplotypes by at least six mutational steps. Haplotypes from the east coast are at least three steps removed from the remaining haplotypes. The Southern region contains four divergent haplotypes, two unique to the Southern region and two found also within the East-Central and West-Central regions.

Significant geographic associations of haplotypes occur at all levels of nesting. Nested groups 1.1, 1.7, and 2.1 all suggest a genetic pattern of restricted gene flow, with isolation by distance in 1.7 and 2.1. Nested group 1.1 also exhibits a signature of long-distance dispersal to the west into populations 10 and 12 (Table 2). Haplotype groups nested within 3.1 and 3.3 indicate allopatric fragmentation of haplotypes in the Southern and Northern regions, respectively. Genetic variation within nested group 3.2 suggests restricted gene flow, with some long-distance dispersal to the east coast (populations #92 and #98). At the total-cladogram level, we infer restricted gene flow, with some long-distance dispersal over intermediate areas not occupied by the species, or past gene flow followed by extinction of intermediate haplotypes (Table 2). Consistent with NCPA inferences of fragmentation between biogeographic regions, AMOVA on haplotype frequencies suggests significant genetic structure among regions ($\Phi_{CT} = 0.180$, df = 2, $P < 0.0001$).

3.2.3. Concordant geographic NCPA inferences and ML-based cross-validation

Testing biogeographic hypotheses using molecular genetic data depends not only upon spatially congruent inferences but also a shared temporal scale. The cross-validation method of Templeton (1993, 2002, 2004) provides a framework for considering the temporal congruence of spatially concordant phylogeographic inferences. Considering NCPA results for all loci, four inferences are candidates for cross-validation: (1-2) allopatric fragmentation separating haplotypes among the Northern, Central and Southern regions, (3) restricted gene flow among centrally located localities with some long-distance dispersal to the west (populations #10 and #12), and (4) restricted gene flow in the East-Central region with long-distance dispersal or range expansion to the east coast. As recommended by Templeton (2002), we cross-validate only relatively old inferences (i.e., those at higher nesting levels) because the timing of relatively recent events is obscured by stochastic error associated with the coalescent process (Edwards and Beerli, 2000; Arbogast et al., 2002). As such, cross-validation of the inferences of westward long-distance dispersal are not attempted because they occur at the 1-step nesting level and involve a single (*ND2/tRNA^{Trp}*) or very few (*E8*) individuals. For the inference of allo-

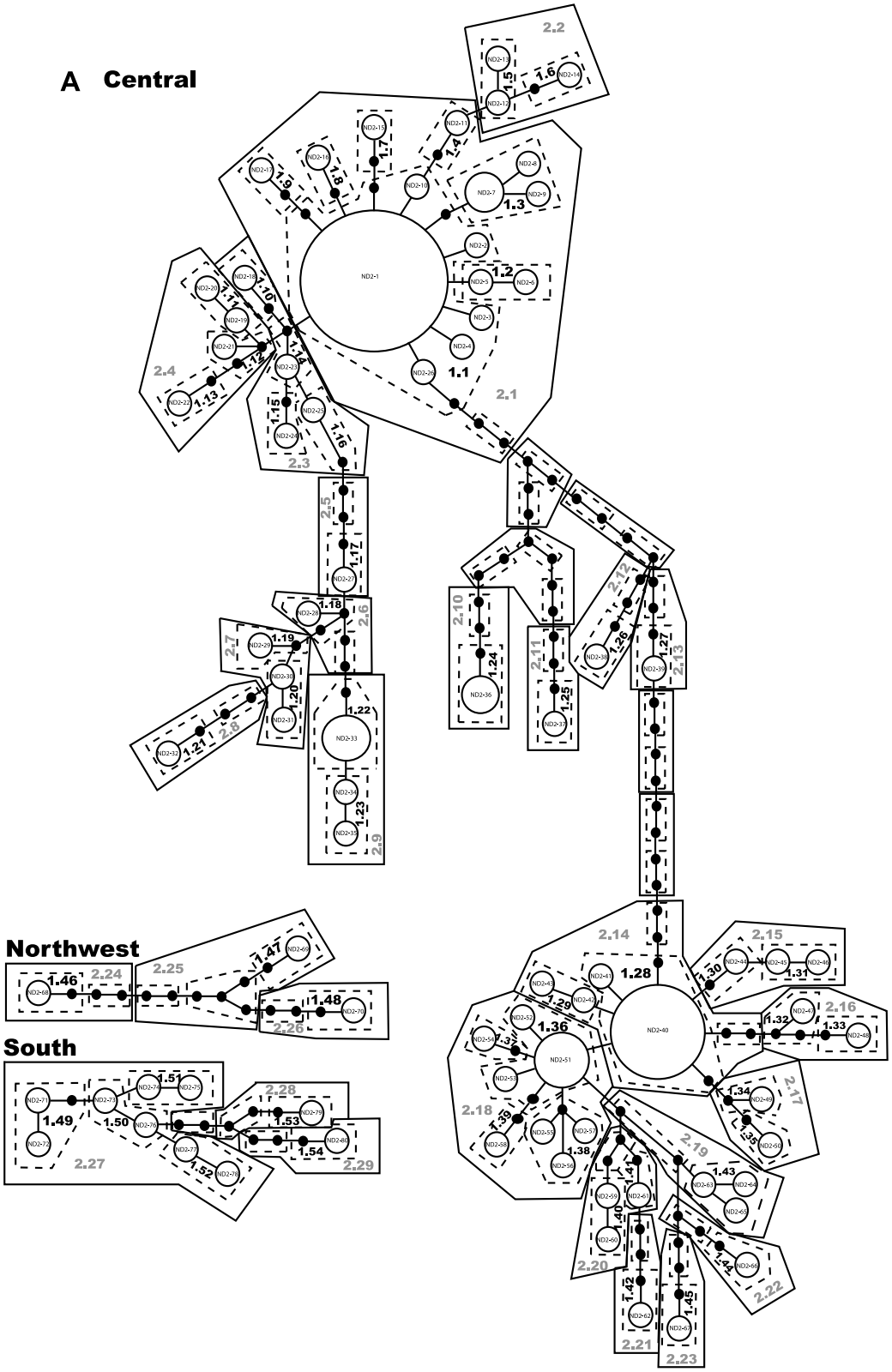

Fig. 4. Resolved 95% statistical parsimony network for *ND2* (mtDNA) haplotypes from *Ameiva chrysolaema*. Large circles represent observed haplotypes (relative size depicting relative haplotype-frequency). Small black circles represent unobserved haplotypes necessary to link observed haplotypes. Each line represents a single mutational step. Nested design for NCPA is shown.

patric fragmentation between the Northern and Central regions, the mean divergence times between haplotype groups estimated using each locus are very similar and have broadly overlapping 95% confidence intervals (Table 3). We were unable to reject the hypothesis of a single fragmentation event using a log-likelihood ratio test (LRT; Fig. 7A). Because we accepted the hypothesis of a single event, we used the pooled data to obtain a pooled divergence-time estimate of 4.87 mya with 95% confidence interval

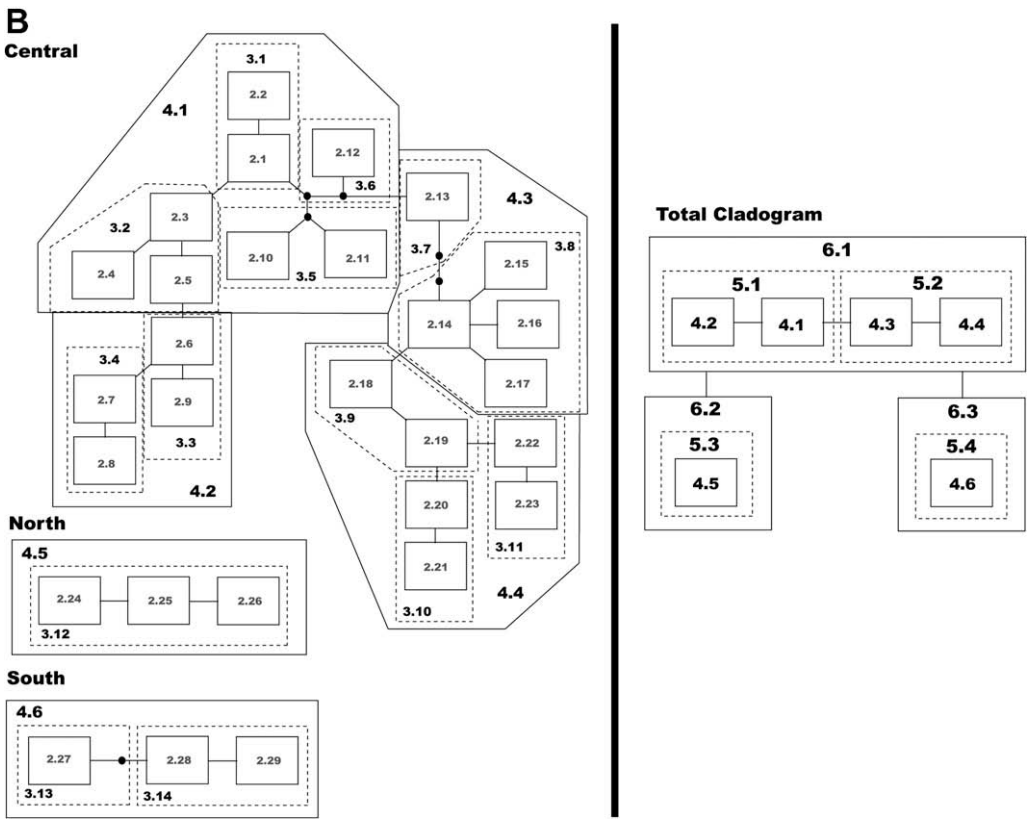

Fig. 4 (*continued*)

ranging from 3.87 to 5.98 mya. Similarly, for the allopatric fragmentation between haplotypes in the Southern and Central regions, the mean divergence times estimated using each locus differ, although 95% confidence intervals partially overlap (Table 3 and Fig. 7B). Again, we could not reject the null hypothesis of a single fragmentation event using an LRT (Fig. 7B). The pooled divergence-time estimate suggests an early Pliocene divergence, approximately 4.32 mya with 95% confidence interval ranging from 3.37 to 5.39 mya. The most recent concordant spatial inferences indicate population movement to the east coast of the Dominican Republic (range expansion and long-distance dispersal). The estimated times of this event for each locus are broadly overlapping (Table 3, Fig. 7C), and an LRT indicates that all loci reflect the same temporal event. The pooled estimate for the timing of this event is 0.48 mya with 95% confidence interval ranging from 0.24 to 0.79 mya.

The coalescence-based divergence times estimated from multilocus data using MCMCcoal 2.1 for each possible resolution of the population tree suggest divergence times among Northern, Central, and Southern populations ranging between 4 and 6 mya with confidence intervals ranging between 2.4 and 8.7 mya (Table 4). These divergence times are congruent with the estimates made using the NCPA cross-validation procedure.

3.3. Fine-scale population structure and historical demography

Recurring inundation of much of the Hispaniolan lowlands has had a major impact on the genetic structures and historical demographics of lowland lizards (Gifford, 2008a). We examine this impact on populations of *A. chrysolaema* located in the East-Central and West-Central biogeographic regions. First, widespread inundation of the central region of Hispaniola would likely have fragmented populations into eastern and western groups. Consistent with this prediction, NCPA of mtDNA reveals an historical fragmentation event between East-Central and West-Central population lineages. Bayesian coalescent analysis of multilocus data suggests that this fragmentation event occurred approximately 1.0 mya (Table 5), placing the fragmentation event in the Middle Pleistocene, coinciding with a period of elevated sea-level (Haq et al., 1987; Raymo et al., 2006).

IMa estimates of post-divergence gene flow from the East-Central into the West-Central region are approximately six times greater than in the opposite direction (Table 5), consistent with the mtDNA NCPA inference of post fragmentation range expansion of the East-Central lineage to the West. Within each sub-region we recovered a positive mtDNA signature of restricted gene flow with isolation by distance (East-Central, $Z = 24.08$, $r = 0.51$, $P = 0.006$; West-Central, $Z = 9.40$, $r = 0.39$, $P = 0.009$) and a signature of population growth/expansion in the West-Central sub-region (East-Central, $D = -0.77$, $P = 0.267$, $F_S = -3.24$, $P = 0.151$; West-Central, $D = -1.03$, $P = 0.137$, $F_S = -13.24$, $P < 0.0001$).

Divergence times reported here are lower than corresponding values from our previous study (Gifford et al., 2004), which were overestimated analytically by using maximum likelihood corrected genetic distances with a rate calibration estimated for uncorrected sequence divergence. The divergence times reported here are compatible with the expected rates of evolution of the loci analyzed (see Section 2) and should replace the estimates of the earlier study.

4. Discussion

Recent phylogeographic studies on Greater Antillean lizard taxa suggest a strong impact of sea inundations on lineage differentiation (Gifford et al., 2004; Glor et al., 2004). This is one of very few detailed demographic studies of a Greater Antillean taxon using phylogeographic analyses of data from multiple independently segregating loci (Glor et al., 2004; Townsend et al., 2007).

Table 2
Results of tests for geographic genetic structure in GeoDis 2.4 (Posada et al., 2000) for each locus

Locus	Nested clade	X^2	*P*	Inference	Localities involved
ND2	1.1	96.46	<0.0001	1-2-3-5-6-7-YES, RGF, some LDD	**1,2,3,4,41,89,90,96**—10,59,96
	2.1	163.5	<0.0001	1-2-3-4-NO, RGF, IBD	**1,2,3,4,10,41,59,89,90,96**—2,4,41,42,56,59,89
	2.3	1.00	<0.0001	1-2-3-4-NO, RGF, IBD	**32,42,56,96**—1,2,3
	2.27	12.00	0.01	1-2-3-4-NO, RGF, IBD	**91**—69,72,91
	3.2	36.00	0.006	1-19-20-2-3-5-15-16-18-NO, IS	
	3.3	17.00	0.001	1-19-NO, AF	**95**—28,94
	3.5	10.00	0.003	1-19-NO, AF	92,98
	4.1	191.40	<0.0001	1-2-3-5-6-7-8-YES, RGF, some LDD over intermediate areas not occupied OR Past GF followed by extinction of intermediate haplotypes	**1,2,3,4,10,15,30,32,41,42,55,56,59,89,90,96**—92,98
	4.2	14.32	0.004	1-2-3-4-NO, RGF, IBD	**28,94,95**—32,58,94,95
	4.6	14.00	0.02	1-19-NO, AF	**69,72,91**—48
	5.1	103.17	<0.0001	1-2-3-4-NO, RGF, IBD	**1,2,3,4,10,15,30,32,41,42,55,56,59,89,90,92,96,98**—28,32,58,94,95
	5.2	29.21	<0.0001	1-2-3-4-NO, RGF, IBD	**10,12,41,44,47,52,87,99**—4,17,41,44,55,87,88,90,99
	6.1	118.55	<0.0001	1-2-11-12-13-YES, Past FRAG, followed by RE	**1,2,3,4,10,15,28,30,32,41,42,55,56,58,59,89,90,92,94,95,96,98**—4,10,12,17,41,44,47,52,55,87,88,90,99
	Total	332.00	<0.0001	1-19-NO, AF	**1,2,3,4,10,15,28,30,32,41,42,55,56,58,59,89,90,92,94,95,96,98,4,10,12,17,41,44,47,52,55,87,88,90,99**—25,48,69,72,91,97
BA3	1.1	246.90	<0.0001	1-2-3-4-NO, RGF, IBD	**1,2,3,4,10,12,25,28,32,41,42,44,47,52,55,56,58,59,69,72,87,88,89,90,91,91,94,96,97,98,99**—12,15,17,48,52,87,88,99
	1.2	200.63	0.0063	1-2-3-4-NO, RGF, IBD	**1,2,3,4,10,12,15,28,30,32,41,42,44,47,55,56,58,59,87,89,90,94,95,96**—1,2,3,28,42,56,59,88
	Total	121.27	<0.0001	1-2-3-4-NO, RGF, IBD	**1,2,3,4,10,12,25,28,32,41,42,44,47,52,55,56,58,59,69,72,87,88,89,90,91,91,94,96,97,98,99,12,15,17,48,52,87,88,99**—1,2,3,4,10,12,15,28,30,32,41,42,44, **47,55,56,58,59,87,89,90,94,95,96**—1,2,3,28,42,56,59,88
ACA4	1.4	19.95	0.05	1-2-11-YES, CRE	**4,25,44,48,69,72,89,91,94**—25
	2.1	183.52	<0.0001	1-2-3-5-6-7-8-YES, RGF, some LDD over intermediate areas not occupied OR Past GF followed by extinction of intermediate haplotypes	**1,2,3,4,10,12,15,17,25,28,30,32,41,42,44,47,52,55,56,58,59,87,88,89,90,92,94,95,96,97,99**—12,99
	Total	421.57	<0.0001	1-2-3-5-6-7-YES, RGF, some LDD	**1,2,3,4,10,12,15,17,25,28,30,32,41,42,44,47,52,55,56,58,59,87,88,89,90,92,94,95,96,97,99**—4,10,12,25,44,47,48,59,69,72,87,89,90,91,92,94
E8	1.1	276.67	<0.0001	1-2-3-5-6-7-YES, RGF, some LDD	**1,2,3,4,28,32,41,42,44,52,55,56,58,59,72,87,88,94,95,99**—1,2,10,12,42,59
	1.7	169.60	0.01	1-2-11-17-4-NO, RGF, IBD	**2,3,10,12,15,17,30,41,42,44,55,59,87,88,89,91,96,99**—1,2,4,42,55,89
	2.1	190.10	<0.0001	1-2-3-4-NO, RGF, IBD	**1,2,3,4,10,12,28,32,41,42,44,52,55,56,58,59,72,87,88,94,95,99**—1,2,3,10,12,41,42,44,56,59,96
	2.3	46.06	<0.0001	1-2, Inconclusive Outcome	
	3.1	126.09	<0.0001	1-2-3-4-9-NO, AF	**3,48,69,72,87**—1,2,3,4,10,12,28,32,41,42,44,52,55,56,58,59,72,87,88,94,95,96,99
	3.2	167.83	<0.0001	1-2-3-5-6-7-YES, RGF, some LDD	**1,2,3,4,10,12,15,17,30,41,42,44,47,55,59,87,88,89,91,96,99**—1,2,3,28,44,88,92,98
	3.3	13.33	0.002	1-2-3-4-9-10-YES, AF	**10,12,44,97**—25,97
	Total	356.77	<0.0001	1-2-3-5-6-7-8-YES, RGF, some LDD over intermediate areas not occupied OR Past GF followed by extinction of intermediate haplotypes	**1,2,3,4,10,12,15,17,28,30,41,42,44,47,55,59,87,88,89,91,92,96,98,99**—1,2,3,4,10,12,25,28,32,41,42,44,48,52,55,56,58,59,69,72,87,88,94,95,96,97,99

Only significant associations between geography and genetic variation are shown. The path through the latest NCPA inference key (Templeton, 2004) is shown along with the associated demographic inference. The localities (see Fig. 2) involved in each significant inference are listed and coded according to whether their haplotypes were classified as interiors (bold) or tips (plain).

Multilocus analyses presented here corroborate our previous results and indicate that repeated episodes of sea inundation played a significant role in lineage formation and historical demography of *A. chrysolaema* in Hispaniola. Ancient vicariance dating as far back as the early Pliocene, a major source of biodiversity in continental faunas, also contributes significantly to lineage accumulation on islands (Heaney, 2007).

4.1. Mountain ranges, paleoisland configuration, early Pliocene inundation, and vicariance

The topographic structure of Hispaniola has been stable since the late Miocene to early Pliocene (~10–5 mya, Mann et al., 1991; McLaughlin et al., 1991), with the major orogenic events dating to the Early to Middle Miocene (~20–8 mya, Heubeck and Mann, 1991). If uplift of these mountain ranges fragmented *A. chrysolaema* populations, we would expect their divergence times to date to the Early to Middle Miocene (i.e., ~20–8 mya). Our NCPA and MCMCcoal analyses place the separations among the Northern, Central, and Southern lineages at 4–6 mya (Tables 1 and 2), thereby rejecting Miocene mountain-building processes as responsible for vicariant fragmentation of *A. chrysolaema* populations. Confidence intervals for divergence times from the NCPA cross-validation and for two of the three possible resolutions of the population tree from MCMCcoal entirely postdate the time interval during which the mountains formed. The configuration of Hispaniola as two separate paleoislands predicts colonization of the south paleoisland and divergence during the middle Miocene (a minimum of 8–9 mya). Our multilocus divergence time analyses likewise reject the hypothesis of paleoisland divergence for *A. chrysolaema*, a contrasting pattern to that found in *Calyptophilus* chat-tanagers (Townsend et al., 2007). Ancestral populations of *A. chrysolaema* most likely colonized geographically continuous lowland areas distributed around the mountains near the end of

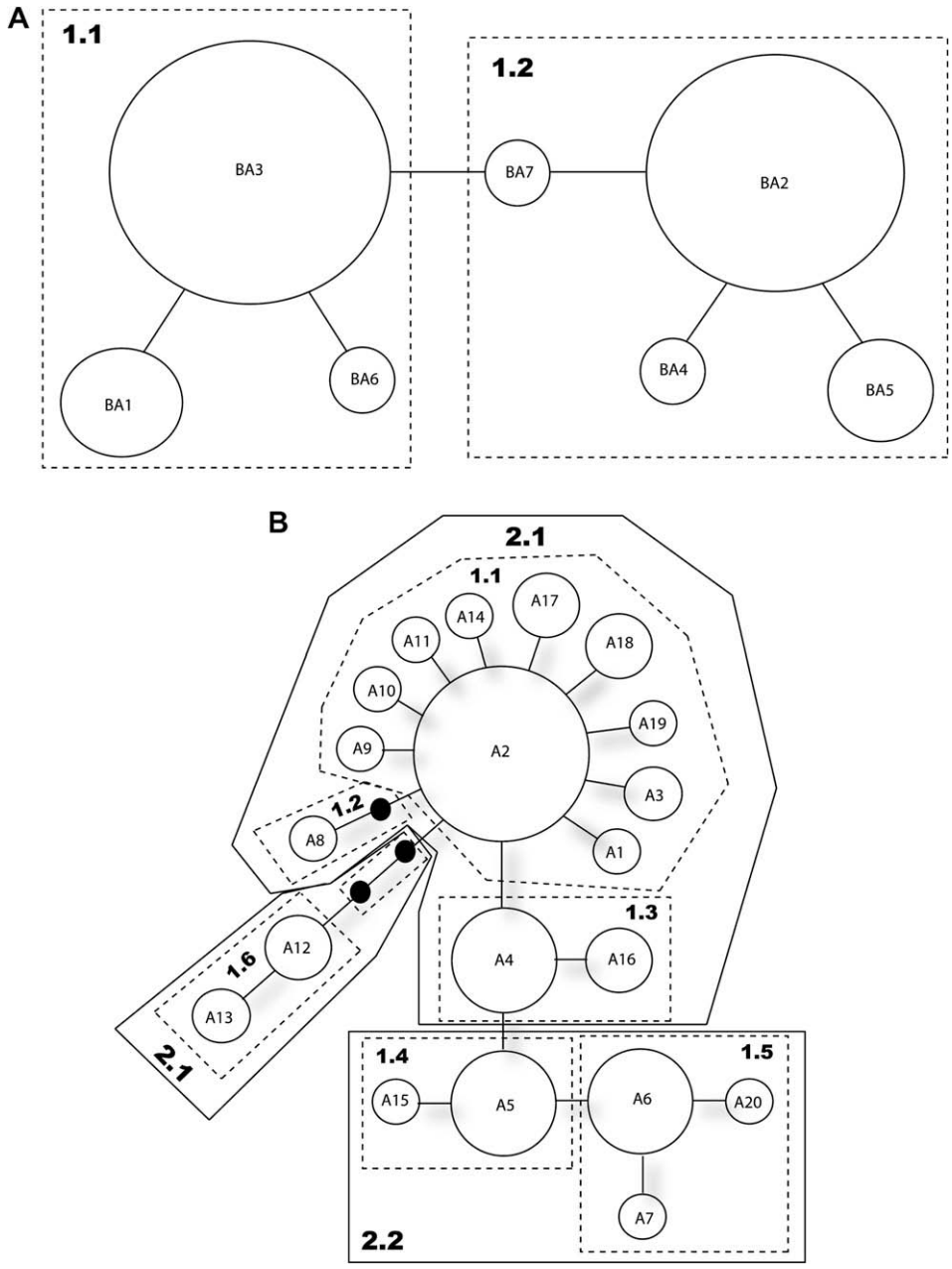

Fig. 5. Resolved 95% statistical parsimony network for *BA3* (A) and *ACA4* (B) haplotypes from *Ameiva chrysolaema*. Large circles represent observed haplotypes (relative size depicting relative haplotype-frequency). Small black circles represent unobserved haplotypes necessary to link observed haplotypes. Each line represents a single mutational step. Nested design for NCPA is shown.

the Miocene, followed by vicariant fragmentation of populations by subsequent localized sea inundations. Global sea-levels during the early to middle Pliocene are estimated to have been ca. 50–100 m higher than at present (~5.5–3 mya, Haq et al., 1987), which would have sundered Hispaniola into at least two separate islands. The hypothesis that vicariant fragmentation by Pliocene sea-inundation separated the North, South, and Central populations predicts divergence times of approximately 5.5–3 mya, consistent with our molecular results.

4.2. Pleistocene sea-inundation, population structure, and demography

Much of south-central Hispaniola lies at low elevations (<100 m) with most of the Enriquillo Basin below sea-level. These areas nearly perfectly mirror the current distribution of *A. chrysolaema* (Schwartz and Henderson, 1991). Climatic fluctuations during the Pleistocene produced episodic fluctuations in global sea-levels. Two particular episodes of sea-level fluctuations occurred during Pleistocene interglacial periods approximately 1.1 and 1.4 mya (Raymo et al., 2006). At these times, sea-levels are estimated to have been 10–20 m above current levels, high enough to have inundated much of the West-Central region. As such, we expect a substantial signature of these events on the structure and demography of populations in these areas. Our multilocus population divergence-time estimate for the East-Central/West-Central split is 1.0 mya, geographically associated with the southeastern reaches of the Cordillera Central. Inundation of lowland regions to the east and west of this range likely pushed populations into geographically distinct areas, the locations of which are unclear because the current geographic distribution of the species no longer includes pre-

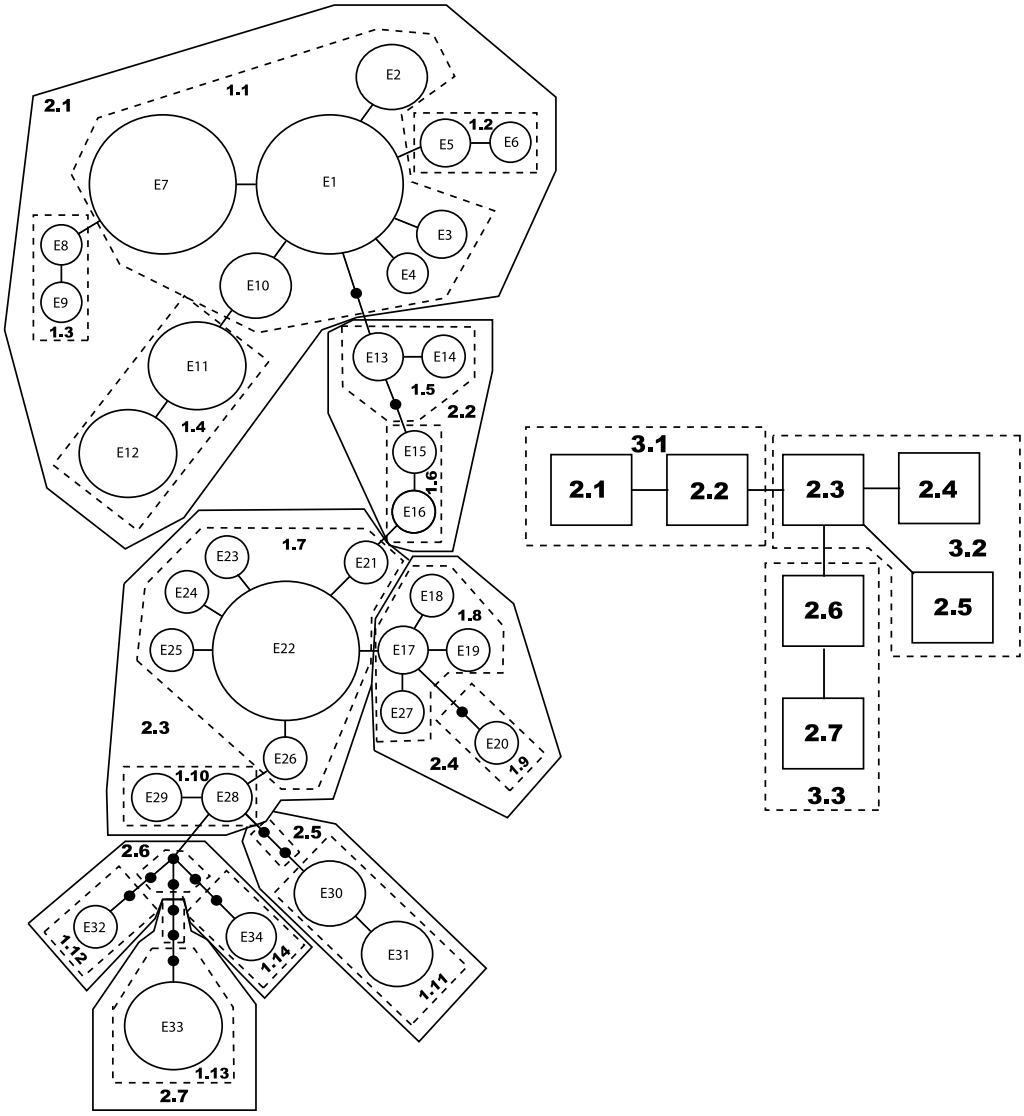

Fig. 6. Resolved 95% statistical parsimony network for *E8* (nDNA) haplotypes from *Ameiva chrysolaema*. Large circles represent observed haplotypes (relative size depicting relative haplotype-frequency). Small black circles represent unobserved haplotypes necessary to link observed haplotypes. Each line represents a single mutational step. Nested design for NCPA is shown.

Table 3
Time (t_i) and 95% confidence intervals estimated for each locus for each significant inference using Templeton (2004) maximum-likelihood cross-validation procedure

Inference*	t_i	95%$_{(lower)}$	95%$_{(upper)}$	$T_{(pooled)}$	95%$_{(lower)}$	95%$_{(upper)}$
C-N FRAG				4.87	3.87	5.98
ND2/tRNA^{Trp}	4.93	3.86	6.12			
E8	4.36	1.99	7.64			
C-S FRAG				4.32	3.37	5.39
ND2/tRNA^{Trp}	4.48	3.47	5.62			
E8	2.25	0.744	4.58			
RE East				0.48	0.24	0.79
ND2/tRNA^{Trp}	0.48	0.20	0.86			
ACA4	0.40	0.03	1.26			
E8	0.55	0.07	1.52			

Because the null hypothesis of a single event was not rejected for any case, pooled estimates ($T_{(pooled)}$) and confidence intervals for each event are also presented. All units are in millions of years.

* Fragmentation of Central and Northern populations (C-N FRAG), fragmentation of Central and Southern populations (C-S FRAG), and eastward range expansion of Central populations (RE East).

dicted areas of emergence during this time (Fig. 1B). These inferences are strengthened by the fact that a narrow mtDNA contact zone (~45 km in width, Gifford, 2008b) is centered at the southeastern apex of the Cordillera Central (Fig. 2, Gifford et al., 2004).

Multilocus estimates of migration rates suggest that soon after their separation, East-Central and West-Central populations initiated gene flow asymmetrically; migration from East-Central into West-Central regions was substantially higher than in the opposite direction. Because the West-Central region lies largely below sea-level, populations in this region would have been restricted to geographic refugia during sea inundations. The East-Central region lies entirely above sea-level; thus, populations in this region likely would have remained closer to their current distribution and population size. Upon recession of sea water the East-Central populations could have entered the previously inundated areas, and the incumbency of East-Central populations may have prevented West-Central populations from moving eastward.

Climatic changes induced by glacial advance and retreat fragmented geographic distributions of many organisms and provide powerful explanations of geographic genetic structures and population demography in northern latitudes (e.g., Hewitt, 2000, 2001). Similarly, volcanic eruptions in the Canary and Hawaiian Islands have had a strong influence on vicariant evolution in various taxa

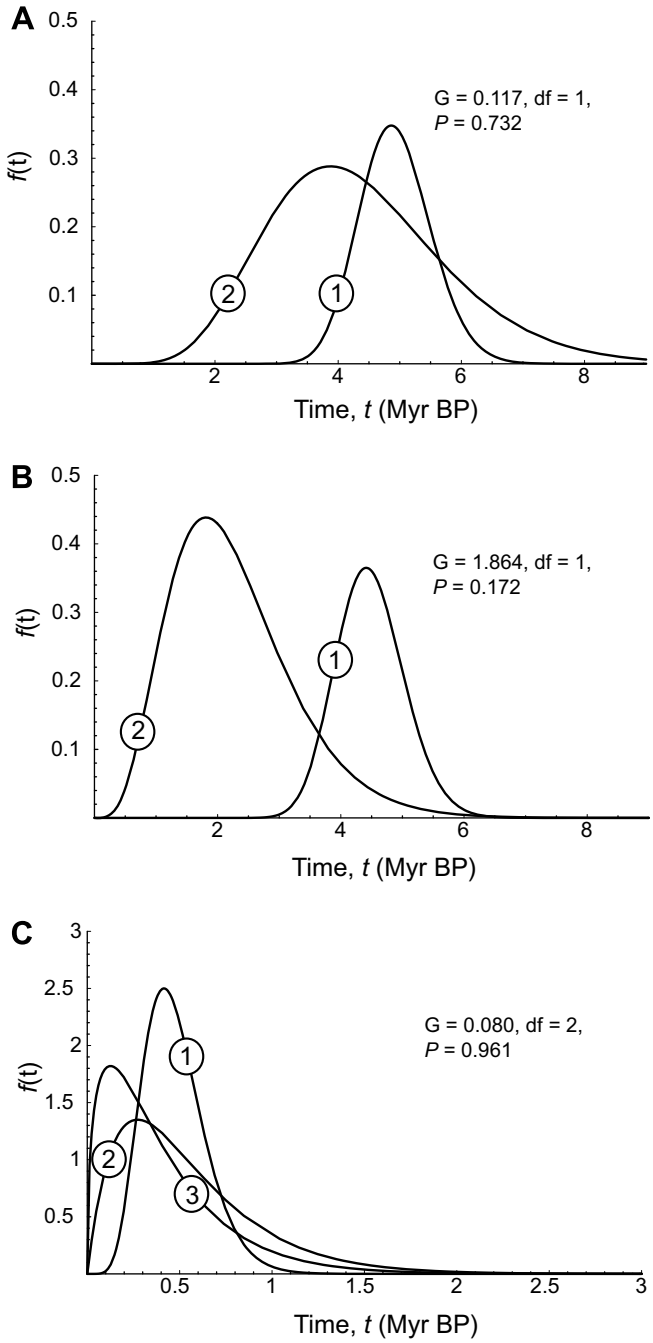

Fig. 7. NCPA cross-validation tests of temporal concordance of phylogeographic inferences. Distributions of ages of the clades contributing to a significant inference of fragmentation for mtDNA (1) and *E8* (2) between the Northern and Central regions (A) and for mtDNA (1) and *E8* (2) between the Southern and Central regions (B). Distributions of ages of the youngest clade contributing to a significant inference of population movement to the east (range expansion or long-distance dispersal) for mtDNA (1), *E8* (2), and *ACA4* (3) are shown in (C). Results of log-likelihood-ratio tests of the null hypothesis that all loci infer the same event are shown in the upper right of each graph.

(Roderick and Gillespie, 1998; Juan et al., 2000). A common pattern observed in these studies is a genetic signature of population growth and/or range expansion into affected areas after removal of a barrier. We used complementary phylogeographic methods to test predicted patterns of recent population growth and/or geographic expansion into formerly inundated lowland areas. Geographic variation at three loci suggests expansion of populations into habitats at the eastern extreme of the Dominican Republic. A log-likelihood ratio test cannot reject the hypothesis that all three loci mark the same expansion event. We estimate that this expansion occurred approximately 0.48 mya (95% CI = 0.24–0.79 mya). Consistent with the prediction that sea inundation displaced the West-Central population to a greater degree than the East-Central population we detect a significant signature of population expansion in the West-Central population but not in the East-Central population (significantly negative Fu's F_S in the West-Central lineage and a non-significant value for the East-Central lineage). Populations in both regions, however, seem to have maintained sufficient geographic breadth for genetic sub-structure (i.e., isolation by distance) to be marked by variation in mtDNA.

Our previous study presented an evolutionary history of *A. chrysolaema* characterized by ancient divergence (ca. 10 mya) among three geographic lineages distributed in the lowlands of the Dominican Republic, labeled Northwest, Barahona, and Central/Southeast. The Central/Southeast lineage was further separated into eastern and western haplotype lineages with a signature of secondary contact near the southeastern reaches of the Cordillera Central. Finally, we inferred population growth for each sub-lineage of the Central/Southeast clade. This study utilized detailed analyses of multilocus DNA sequence data to test the hypotheses generated in our previous study. The analysis of multiple loci has strengthened some hypotheses generated in our previous study (Gifford et al., 2004) and provided new and more detailed insight into the phylogeography of *A. chrysolaema*. First, multilocus analysis of divergence times among major geographic lineages (North, Central [synonymous with the Central/Southeast clade from Gifford et al. (2004)], and South [synonymous with the Barahona clade from Gifford et al. (2004)]) using two different approaches (i.e., NCPA cross-validation and coalescence-based MCMCcoal) recover younger divergence times than our previous study, implicating Pliocene sea inundation rather than Miocene divergence prior to the unification of the North and South paleoislands and Miocene mountain-building. Furthermore, the agreement among analyses indicating similar divergence times and confidence intervals suggests that the data are robust to the method of analysis employed. Second, multilocus analysis using IMa builds upon our previous work providing a more detailed picture of the post-divergence population-genetic interactions between East-Central and West-Central lineages. Finally, the increased sampling of individuals and molecular markers provides insight unavailable from our previous work. Multilocus NCPA recovers a signature of Pleistocene dispersal (ca. 0.48 mya) within the East-Central lineage to the east coast. The fidelity of inferences among analytical methods and cross-validation among loci within methods confirm the utility of a combined approach to phylogeography utilizing methods with different assumptions to maximize the extraction of information from molecular data. Despite recent criticism (Panchal and Beaumont, 2007; Petit, 2008, see Templeton, 2008 for response), NCPA performs well in identifying major phylogeographic patterns recovered from the other analyses and can be used to inform which aspects of the multilocus data are responsible for the inferred general patterns.

4.3. In-situ differentiation and biogeography on islands

Allopatric fragmentation associated with Miocene, Pliocene and Pleistocene vicariance is a common phylogeographic pattern among geographically widespread continental faunas (see Avise, 2000). Recent studies of a wide variety of organisms have identified similar patterns of historical genetic fragmentation on oceanic islands (e.g., Gifford et al., 2004; Glor et al., 2004; Juan et al., 2000; Brown et al., 2000, 2006; Thorpe and Malhotra, 1998; Báez and Brown, 1997) suggesting that *in situ* spatial processes are important in lineage proliferation (see Losos and Schluter, 2000). Inter-

Table 4
Results from the coalescence-based analysis (MCMCcoal) estimating divergence times among Northern, Central, and Southern lineages

Parameter	Population topology		
	((N,S),C)	((S,C),N)	((N,C),S)
τ_{ABC}	**0.0052 (0.0034, 0.0075)**	**0.0076 (0.0038, 0.0109)**	**0.0053 (0.0034, 0.0083)**
T_{ABC}	4.1 my (2.7 my, 5.9 my)	6.0 my (3.0 my, 8.7 my)	4.2 my (2.7 my, 6.6 my)
τ_{AB}	**0.0051 (0.0033, 0.0074)**	**0.0052 (0.0029, 0.0078)**	**0.0051 (0.0033, 0.0074)**
T_{AB}	4.0 my (2.6 my, 5.8 my)	4.1 my (2.4 my, 6.2 my)	4.1 my (2.6 my, 5.9 my)

Parameter estimates of the population-splitting parameter, $\tau = T\mu$ are in bold. Values directly below parameter estimates are converted to a demographic scale assuming a generation time of two years and $\mu = 1.26 \times 10^{-9}$ substitutions^{-1}, site^{-1}, year^{-1}. Ninety-five percent credibility intervals are indicated in parentheses. Subscripted letters A, B, and C refer the alternative population topologies such that ((A,B),C).

Table 5
Results from two replicate isolation-with-migration (IMa) analyses estimating divergence times and demographic parameters between East-Central and West-Central lineages

Parameter	Analysis 1	Analysis 2
τ	**0.47 (0.25, ??)**	**0.43 (0.13, ??)**
T	1.02 my (0.54 my, ??)	0.93 my (0.28 my, ??)
q_{EC}	**2.1 (1.2, 3.3)**	**2.1 (1.2, 3.3)**
Ne_{EC}	567,610 (329,754, 881,147)	567,659 (329,783, 892,036)
q_{WC}	**3.42 (2.0, 5.5)**	**3.42 (2.1, 5.5)**
Ne_{WC}	924,394 (556,799, 1,475,788)	924,474 (556,847, 1,486, 728)
qA	**4.95 (0.05, 87.7)**	**4.95 (0.05, 87.9)**
Ne_A	1,527,143 (13,514, 23,853,159)	1,338,055 (13,515, 23,747,100)
$m_{WC \rightarrow EC}$	**0.29 (0.03, 1.3)**	**0.31 (0.03, 1.3)**
$Nem_{WC \rightarrow EC}$	0.5 (0.0, 2.4)	0.6 (0.0, 2.4)
$m_{EC \rightarrow WC}$	**1.68 (0.8, 3.4)**	**1.68 (0.8, 3.4)**
$Nem_{EC \rightarrow WC}$	3.1 (1.4, 6.4)	3.1 (1.5, 6.3)

Analyses were initiated with different random-number seeds. Parameter estimates of the population-splitting parameter, $\tau = T\mu$; $\theta = 4N_e\mu$; and bidirectional migration rates, $m = m\mu$ are in bold. Values directly below parameter estimates are converted to a demographic scale assuming a generation time of two years and the geometric mean of the independent locus mutation rates, $\mu = 4.62 \times 10^{-7}$ substitutions^{-1}, gene^{-1}, year^{-1}, as suggested in the program documentation. Ninety-five percent credibility intervals are indicated in parentheses. Both analyses produced posterior density curves that had flat tails of equal probability at increasing values of τ. Thus, the maximum of the 95% credibility interval could not be estimated for this parameter.

estingly, the geographic factors responsible for lineage formation on islands are spatially and temporally comparable to those on continents (e.g., mountain ranges, continental ice sheets versus island sea inundations). Here, we demonstrate that the evolutionary history of *A. chrysolaema* is dominated by allopatric fragmentation and dispersal from geographic refugia, with the temporal and spatial scales of events being similar to those recorded for continental taxa. This is in stark contrast to a view that oceanic islands are relatively simple systems whose diversity is largely maintained by colonization and extinction dynamics (MacArthur and Wilson, 1967). However, the fine-level spatial fragmentation of both continental and island faunas revealed by molecular genetic data postdating MacArthur and Wilson (1967) theory could not have been anticipated by them because so many of the lineages are morphologically cryptic. Although colonization and extinction processes are no doubt important, it is clear that island diversity must be examined from a broader temporal perspective with explicit considerations of within-island vicariance (Losos and Schluter, 2000; Lomolino and Weiser, 2001), an emerging new paradigm for oceanic island biogeography (Heaney, 2007).

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ympev.2008.06.003.

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