Local-scale environmental variation generates highly divergent lineages associated with stream drainages in a terrestrial salamander, *Plethodon caddoensis*

Donald B. Shepard,a,b,* Frank T. Burbrinca,c

a College of Staten Island, City University of New York, Department of Biology, 2800 Victory Blvd., Staten Island, NY 10314, USA
b Sam Noble Oklahoma Museum of Natural History, University of Oklahoma, 2401 Chautauqua Ave., Norman, OK 73072, USA
c City University of New York, Biology Program, Graduate Center, 365 Fifth Avenue, New York, NY 10016, USA

**Abstract**

Spatial and temporal heterogeneity in environmental factors can have profound effects on diversification in species that are tightly linked to their environments. The Caddo Mountain Salamander (*Plethodon caddoensis*) inhabits a unique physiographic section of the Ouachita Mountains in central North America, a region in which Pleistocene climatic fluctuations have been implicated in driving lineage diversification in two other closely related salamanders. We examined *P. caddoensis* to determine whether it was similarly impacted by historic climatic changes and test whether physiographic features unique to the area also contributed to its diversification. We found that *P. caddoensis* is composed of four highly divergent, geographically distinct lineages that abut one another along an east–west axis. Phylogeographic structure was significantly related to both geographic distance and stream drainages, indicating that connectivity of streams and stream-associated habitats (e.g., talus) influence patterns of interpopulation gene flow. Lineages originated during the Middle Miocene and population size decreased in all lineages during the Pleistocene. Surface Geology and precipitation were the most important variables predicting the species distribution. Our results show that the unique physiographic features of the area coupled with species response to climatic factors have driven lineage diversification and phylogeographic structure in *P. caddoensis*. Variation in responses to historic climatic fluctuations among salamander species in this region underscores the importance of integrating species ecology with other factors such as geology and hydrology in order to better understand the effects of climate change on species with close associations to their environments.

© 2011 Elsevier Inc. All rights reserved.

1. Introduction

The climatic, geological, and hydrological history of a region has important implications for determining the processes that have generated present-day patterns of biodiversity (Gaston, 2000; Lomolino et al., 2006; Ricklefs, 1987). Given the geographic scale at which these factors usually function, their effects on speciation are typically observed over broad areas (Kozak et al., 2006a; Mayden, 1988; Pyron and Burbrink, 2010). However, when species ecology is intimately linked to these environmental factors and such variables are locally heterogeneous, they can also have profound effects on diversification at smaller spatial scales (Doebeli and Dieckmann, 2003; Huston, 1999; Kuchta et al., 2009).

During the warm climates of the Early Eocene (~50–52 Ma), much of central North America was mesic tropical forest, but subsequent cooling and aridification during the Miocene resulted in environmental conditions similar to the present (~6–8 Ma) (Axelrod, 1985; Cerling et al., 1997; Graham, 1993; Zachos et al., 2001). Since that time, this region has been characterized by a steep longitudinal moisture gradient that transitions from mesic conditions in the east to more xeric conditions in the west (Axelrod, 1985; Costa et al., 2008). During the climatic oscillations of the last 3.6 Myr associated with glacial cycles, the edge of this steep moisture gradient has fluctuated along an east–west axis, resulting in concomitant expansion and contraction of some species’ ranges along the moving front (Axelrod, 1985; Grundstein, 2009; Webb and Bartlein, 1992). The Ouachita Mountains and Ozark Plateau, collectively known as the Interior Highlands, occur at the eastern edge of this moisture gradient, and are hypothesized to have served as refugia for mesic-adapted organisms during these past climate fluctuations (Costa et al., 2008; Dowling, 1956). This region was not glaciated during the Late Pliocene and Pleistocene, but expansion and contraction of mesic forest in response to climatic fluctuations is predicted to have resulted in historic periods of population connectivity and isolation as well as changes in population size in resident organisms. The signatures of such demographic fluctuations in the past should be...

The Ouachita Mountains (Fig. 1) in west-central Arkansas and southeastern Oklahoma are of ancient origin (Pennsylvanian: \(~310\) Ma), and are inhabited by a number of endemic species including several salamanders in the genus *Plethodon* (Allen, 1990; Duellman and Sweet, 1999; Hatcher et al., 1989; Mayden, 1985). These salamanders are forest-dwelling, lungless ectotherms that require mesic environments for survival and reproduction; thus, their distributions are strongly influenced by moisture and temperature (Grover, 2000; Jaeger, 1971; Spotila, 1972). The *Plethodon ouachitae* complex is a monophyletic group of three currently recognized species endemic to the Ouachita Mountains (Duncan and Highton, 1979; Wiens et al., 2006). All three species are restricted to mesic forest and typically occur only at higher elevations and on north-facing slopes within their respective ranges (Shepard and Burbrink, 2008, 2009; Trauth and Wilhide, 1999). The parapatric sister taxa, *P. fourchensis* and *P. ouachitae*, are both distributed across multiple mountains where the unsuitable environmental conditions in the intervening valleys have created a sky island scenario in which each montane isolate is primarily occupied by a monophyletic lineage (Shepard and Burbrink, 2008, 2009). Lineage diversification within these two species occurred during the Pleistocene, supporting a role for climatic changes associated with glacial cycles in driving diversification (Shepard and Burbrink, 2008, 2009).

The third species in this group, *P. caddoensis* (Caddo Mountain Salamander), is proximate in distribution to *P. fourchensis* and *P. ouachitae* (\(~19\) km and \(~12\) km, respectively), but inhabits a geologically distinct area of the Ouachita Mountains called the Arkansas Novaculite uplift (Pope and Pope, 1951; Trauth and Wilhide, 1999). The mountains in this region are more numerous, but smaller in area, closer together, and lower in elevation (max 706 m) compared to the several large, high-elevation (max 817 m), continuous east–west ridgeline mountains occupied by *P. fourchensis* and *P. ouachitae*. At a smaller scale, surface habitats are also different between these montane areas due to their different geological origins. Surface rocks on mountains in the Arkansas Novaculite region (Devonian/Early Mississippian) are composed of chert, shale, slate, and novaculite, which typically form talus slopes of gravel-sized rock (Flawn et al., 1961; Foti and Bukenhofer, 1998; Hatcher et al., 1989). In contrast, the surface rocks on mountains occupied by *P. fourchensis* and *P. ouachitae* are composed of Carboniferous (Mississippian and Pennsylvanian) sandstone, shale, limestone, and conglomerates, which are often concentrated in large boulder fields on mountain tops and slopes (Flawn et al., 1961; Foti and Bukenhofer, 1998; Hatcher et al., 1989).

The gravelly talus slopes in the Arkansas Novaculite region serve as retreats for *P. caddoensis* during hot and dry summer months, and may have facilitated its survival in this region during the extended hot and/or dry climates of the past (Spotila, 1972). Talus is typically deepest and most abundant at the base of slopes.

**Fig. 1.** Topographic map of the United States showing the location of the Ouachita Mountains (A) and the mountains and main rivers within the range of *Plethodon caddoensis* (B). HUC6 stream drainage (blue) and HUC4 watershed (black) boundaries are overlaid on the Caddo Mountain region along with sampling localities color-coded by lineage (C). Lineages are: Brushy Creek (red), Cossatot/Little Missouri Rivers (green), Lower Caddo River (yellow), and Upper Caddo River (purple). Points comprised by two colors indicate both of those respective lineages were present. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)
and in particular the slopes above streams where moisture is also often most abundant (Pérez, 1991, 1998; D.B. Shepard, personal observation). The distribution of *P. caddoensis* is predicted to contract to talus habitats during extended hot and dry periods and individual movement is expected to be constrained by these habitats, thereby influencing patterns of population connectivity. Evidence for such events in the evolutionary history of *P. caddoensis* would be provided by a link between phylogeographic structure and particular physiographic features of the landscape correlated with connectivity of talus habitats such as stream drainages. Phylogeographic structure could also be present due to patterns of interpopulation gene flow independent from landscape features and/or historical periods of geographic isolation. Under this hypothesis, a positive relationship between genetic distance and geographic distance is predicted (i.e., isolation by distance; Hutchinson and Templeton, 1999; Slatkin, 1993; Wright, 1943). However, these hypotheses are not mutually exclusive as both could be important in generating phylogeographic structure.

Here we sample *P. caddoensis* throughout its range and use DNA sequence data in combination with GIS-based environmental niche analyses to test hypotheses related to factors that have limited its distribution and driven diversification. Studying *P. caddoensis* allows us to test whether physiographic features unique to the area in which it occurs have influenced its diversification and provides us with an opportunity to determine if all three closely related species of *Plethodon* in this region were similarly impacted by historic climatic changes. We first use statistical phylogenetic methods to infer phylogeographic structure within *P. caddoensis* and then test whether the observed structure can be explained by stream drainage basins (a correlate of talus habitat connectivity), geographic distance (isolation by distance), or by a combination of the two. Second, we use divergence dating to test whether the timing of lineage diversification within *P. caddoensis* is consistent with past climatic events that would have resulted in range contraction and fragmentation of populations. Third, given that the area of suitable environmental conditions for *P. caddoensis* is predicted to have contracted in the past, we test for historic decreases in effective population size (Nₑ). Lastly, we use ecological niche modeling to determine the environmental factors that potentially limit the distribution of *P. caddoensis*.

2. Materials and methods

2.1. Sampling and sequencing

We conducted extensive surveys throughout the Ouachita Mountains and surrounding region to establish the distribution of *Plethodon caddoensis* and collected 330 tissue samples from 66 unique localities (Appendix). We also included sequences of several closely related species, *Plethodon ouachitae* (N = 7; one of each lineage identified by Shepard and Burbrink, 2008), *Plethodon fourinchii* (N = 4; one of each lineage identified by Shepard and Burbrink, 2009), *Plethodon kiamichi* (N = 1), and *Plethodon albagula* (N = 1) to use as outgroups (Kozak et al., 2006b, 2009; Wiens et al., 2006; Appendix).

For each sample, we extracted whole genomic DNA from ethanol-preserved liver or muscle tissue using the DNeasy Blood and Tissue Kit (Qiagen), and amplified two mitochondrially encoded genes, cytochrome b (cytB) and NADH dehydrogenase 4 (ND4), and a portion of tRNA-His using Polymerase Chain Reaction (PCR). For PCRs, we followed the guidelines included with the Go-Taq Green Master Mix (Promega) scaled to a 10-μl reaction and used the primers PGlu4g2 and PThrR1 for cytB, and Ephist and ND4F (for ND4 (Wiens et al., 2006). We cleaned PCR products using 1 μl of ExoSAP-IT (USB Corp.) per 10 μl of PCR product, and sequenced them on a Beckman CEQ 8000 automated sequencer (Beckman–Coulter). For sequencing, we primarily used the primers PouachCytBf and PouachCytBr for cytB and PouachND4f and PouachND4r for ND4 as described by Shepard and Burbrink (2008); however, we also designed and used sequencing primers more specific to *P. caddoensis*: caddoCytBf (GTATTCACTTATYC TACC), caddoND4f (AGCAAYACACTATGARCGCA), and caddoND4r (GGGGGCGCATTATRATAGG). Sequencing reactions consisted of 2 μl of DTCS (Beckman–Coulter), 1 μl of 5-μM primer, 1–2 μl of PCR product, and 5–6 μl of H₂O. We assembled, edited, and aligned nucleotide sequences by eye using the program Sequencher v.4.5 (Gene Codes Corp.), and verified an open reading frame for these genes. Alignments were unambiguous and no indels were found in these genes in *P. fourinchii*, *P. ouachitae*, and *P. caddoensis*. A two-base pair indel, however, was present in the tRNA-His flanking region of the ND4 gene when compared to the outgroup taxa, *P. albagula* and *P. kiamichi*. We deposited all sequences in GenBank (Appendix).

2.2. Phylogeographic inference

We estimated phylogeographic relationships within *P. caddoensis* using both Maximum Likelihood (ML) and Bayesian Inference (BI) on the combined cytB, ND4, and tRNA-His sequences. Using Akaike's Information Criterion (AIC) in the program jModelTest (Posada, 2008), we determined that GTR + Γ was the best model of nucleotide substitution for cytB, ND4 and the entire concatenated dataset whereas HKY + Γ was the best model for tRNA-His. In order to incorporate evolutionary information specific to each gene and codon position, we evaluated three partitioning strategies prior to tree inference. The first strategy accounts for differences in evolutionary rates in each of the three codon positions of the cytB and ND4 genes and the sequences from tRNA-His. For this codon position-specific and tRNA-specific strategy, abbreviated 7Mp, a single tree was estimated for all seven partitions simultaneously, but all other model parameters were unlinked among partitions. The second strategy (3Mp) applied a separate model to each protein-coding gene and the tRNA with no partitioning among codon positions. The last strategy (1Mp) applied a single GTR + Γ model across both genes and the tRNA.

For each partitioning strategy, we conducted two independent searches consisting of three "heated" and one "cold" Markov chain in MrBayes v.3.1.2 for 10 million generations with every 1000th sample retained (Ronquist and Huelsenbeck, 2003). Default priors were applied to all parameters. We assessed convergence of the MCMC using several diagnostics. First, we compared the variance across chains within a search to the chain variance among searches using Gelman and Rubin's "r" statistic (Gelman et al., 1995); searches were considered burned-in when values for "r" reached ~1.00. Second, we viewed trace plots of tree lnL values and other parameters in Tracer v.1.5 (Rambaut and Drummond, 2007). Lastly, we used the program AWTY (Nylander et al., 2008; Wilgenbusch et al., 2004) to view changes in the posterior probabilities of splits over the course of the MCMC run. Trees sampled prior to stationarity were discarded. To determine whether both independent runs for a given partitioning strategy converged on similar results, we examined the split standard deviation for −lnL tree values among chains; values <0.01 indicate convergence. We also viewed trace plots of −lnL values of the post-burn-in samples of each run in Tracer v.1.5 and viewed plots of the posterior probabilities of all splits for the two runs in AWTY. To evaluate the three different partitioning strategies, we compared the harmonic mean of the model likelihood, $f(X|M)$, using Bayes Factors (BF) for the equation $2\log(\text{BF})$ in Tracer v.1.5 (Newton and Raftery, 1994; Rambaut and Drummond, 2007). A $2\log(\text{BF})$ > 10 is considered strong evidence in favor of one model over another (Kass and Raftery, 1995).
Maximum Likelihood analysis was conducted using RAxML v.7.2.5 (Stamatakis, 2006; Stamatakis et al., 2008) under the GTR+GAMMA model (GTR + F) and the favored partitioning strategy. Support for nodes was obtained from 1000 nonparametric bootstrap pseudoreplicates under the preferred partitioning strategy using the GTR+CAT model. Trees from the BI and ML analyses were compared and the most credible inferences of relationship were restricted to nodes where the Bayesian posterior probability was >0.95 and the nonparametric bootstrap value was >70% (Felsenstein, 1985, 2004; Hillis and Bull, 1993).

2.3. Isolation by distance and drainage

Isolation by distance (IBD) occurs when gene flow among geographically proximate populations is greater than gene flow among geographically distant populations (Slatkin, 1993; Wright, 1943). Such disparity in interpopulation gene flow across a species’ range may result in phylogeographic structure; however, IBD is diagnosed by a positive correlation between genetic and geographic distance and discontinuities in this relationship indicate the influence of additional factors (Hutchison and Templeton, 1999; Slatkin, 1993; Wright, 1943). The hypothesis that phylogeographic structure within P. caddoensis is the product of historical patterns of population connectivity as dictated by stream drainages predicts that salamander populations occurring in different drainages should be more genetically divergent than populations occurring in the same drainage. As pointed out by Kozak et al. (2006a), causes of phylogeographic structure can be correlative or additive in their effects, thus potential factors must be considered simultaneously. For example, as the geographic distance between two populations increases so does the likelihood that the populations will occur in different drainages. Therefore, to test whether phylogeographic structure is influenced by stream drainages, it is necessary to determine whether the genetic divergence observed among populations in different drainages is greater than that expected based on the geographic distance separating them (Kozak et al., 2006a).

In order to evaluate the roles of IBD and stream drainages in generating phylogeographic structure, we used partial Mantel tests in accordance with the analytical framework of Kozak et al. (2006a). The partial Mantel tests for correlation between a dependent variable matrix and an independent variable matrix while holding other independent variable matrices constant and therefore allows us to test for an effect of stream drainages on genetic divergence while controlling for geographic distance. To generate matrices for correlation, we calculated all pairwise Maximum Likelihood estimates of genetic distance under the GTR model in PAUP* v.4.0 (Swoford, 2003) and all pairwise geographic distances in PASSaGE v.2 (Rosenberg and Anderson, in press). Next, we plotted each locality point on a digital elevation map (DEM) of the Ouachita Mountains and overlaid a map of hydrographic basins in ArcGIS v.9.3 (ESRI; Fig. 1). For each individual, we extracted the ID and geographic distance (HUC6) and watershed (HUC4) from which it originated, and then created two binary matrices corresponding to the different drainage levels where each pairwise comparison was coded as 0 if the comparison was between individuals within the same drainage/watershed or 1 if the comparison was between individuals from different drainages/watersheds (see Kozak et al., 2006a). In the end, this produced three independent variable matrices (geographic distance, HUC6 drainages, and HUC4 watersheds) with which to test for correlation with the dependent variable matrix (genetic distance). In an iterative manner, we calculated partial correlation coefficients between the genetic distance matrix and each independent variable matrix while holding the other independent variable matrices constant and tested their significance using the asymptotic t-test and 9999 matrix permutations in PASSaGE v.2 (Rosenberg and Anderson, in press). A significant result for geographic distance would support a role for IBD, significant results for HUC6 drainages and/or HUC4 watersheds would support a role for the connectivity of streams and/or stream-associated habitats, and significant results for all variables would indicate that each of these factors is important in explaining phylogeographic structure.

When IBD is present, the extent of its effects may range from creating phylogeographic structure within lineages to generating the lineages themselves. To determine whether IBD was the primary factor driving lineage diversification in P. caddoensis, we tested for correlation between genetic and geographic distance and performed reduced-major axis regression (RMA) in the web-based program IBDWS v.3.16 (Jensen et al., 2005). Because of non-independence among pairwise comparisons, we tested for correlation using a Mantel test with 10,000 randomizations. We then performed a linear regression on a scatterplot of all between-lineage pairwise comparisons of genetic and geographic distance and tested whether the Y-intercept passed through the origin (Good and Wake, 1992; Hutchison and Templeton, 1999). Because of non-independence of data points, we conducted 10,000 bootstraps of 66 samples each (the number of unique sampling localities) using a script in R (Filipiak, 2006; Development Core Team, 2010) to calculate a 95% confidence interval (CI) on the Y-intercept. We determined the origin to be the lowest genetic distance observed in a between-lineage comparison and rejected a role for IBD in lineage diversification if the origin fell outside the 95% CI of the Y-intercept.

2.4. Divergence dating

We estimated the timing of lineage diversification and the age of the most recent common ancestor (MRCA) of each lineage within P. caddoensis using the relaxed clock method of Drummond et al. (2006) as implemented in BEAST v.1.5.3 (Drummond and Rambaut, 2007). For this analysis, we used the mean age estimate (12.0 Myr) and 95% Highest Posterior Density (HPD; 9.99–14.11 Myr) for the MRCA of P. caddoensis, P. fourchensis, and P. ouachitae from the dated Plethodontidae tree of Shepard et al. (in preparation) to place a lognormally distributed prior on the age of the root of a tree containing all samples of P. caddoensis and our outgroup samples of P. fourchensis and P. ouachitae. The study by Shepard et al. (in preparation) used DNA sequence data from three mitochondrial genes (cyt b, ND4, and ND2) and one nuclear gene (RAG1) for 352 salamander taxa (including 342 plethodontids) along with 11 fossil calibrations and included members of each lineage of P. caddoensis, P. fourchensis, and P. ouachitae. For our dating analysis of P. caddoensis, we simultaneously estimated topology and divergence dates by performing two independent runs of 30 million generations sampling every 3000th iteration with 20% of the initial samples discarded as burn-in. We used the same substitution models and partitioning strategy as the phylogenetic analyses described above and used an uncorrelated lognormal clock prior with a constant population size tree prior (Drummond et al., 2006). The dates and associated error for the MRCA of haplotypes within lineages were used in the historical demographic analyses described below so that changes N_e could be dated and related to geologic or climatic events in the past.

2.5. Historical demography

We examined past population dynamics of all phylogeographic lineages of P. caddoensis using multiple methods including Bayesian skyline plots (BSP; Drummond et al., 2005). This genealogical coalescent method permits the estimation of N_e through time without specifying an a priori demographic model (e.g., constant size, exponential growth, or logistic growth). For each lineage, we
applied a single HKY + Γ model to the concatenated dataset to construct BSPs in BEAST v.1.5.3 (Drummond and Rambaut, 2007); a simpler substitution model and partitioning strategy were required for these within-lineage analyses compared to phylogeographic and divergence dating analyses to achieve high ESS values (>200). We used 10 grouped coalescent intervals (m), a relaxed uncorrelated lognormal molecular clock, a Bayesian skytree line prior, and uniformly distributed priors for the phylogenetic model and population sizes. Additionally, to scale the time axis on BSPs, we used the mean and 95% HPD for the MRCA of all haplotypes in each lineage (obtained from divergence dating analysis described above) to set the lognormal mean and standard deviation of the tree root age prior. These analyses estimated genealogies and model parameters, and were sampled every 1000th iteration for 20 million generations with 10% of the initial samples discarded as burn-in. We visualized plots for each analysis using Tracer v.1.5 (Rambaut and Drummond, 2007).

To provide other estimates of historical changes in \( N_e \), we also calculated Tajima's \( D \) (Tajima, 1989) and Fu and Li's \( D' \) (Fu, 1997). Both Tajima's \( D \) and Fu and Li's \( D' \) are expected to be near zero if population sizes have been stable. Significant negative values are expected in populations that have undergone recent population expansion whereas significant positive values are expected in populations that have undergone recent population contraction (Fu, 1997; Tajima, 1989). We tested for significant deviations from zero using 10,000 coalescent simulations in DnaSP v.5.1 (Librado and Rozas, 2009; Rozas et al., 2003).

2.6. Ecological niche modeling

To determine the factors that potentially limit the distribution of \( P. \) caddoensis, we assembled a set of raster coverages for 30 environmental variables and conducted GIS-based analyses of the species' environmental niche (see Table 3 for variables and their sources). We clipped these coverages to a region that encompassed the entire Ouachita Mountain range and included most of eastern Oklahoma, western Arkansas, and parts of southern Missouri and northeastern Texas (33.18–36.93°N and 92.20–96.56°W), and projected them at 30 arc-seconds resolution (~1 km²). We used these 30 variables and the geographic coordinates of our 66 sampling localities to construct an ecological niche model for \( P. \) caddoensis using the default settings in the program Maxent v.3.3.2 (Phillips et al., 2006). Output from Maxent includes a measure of the overall performance of the model (Area Under the Receiver Operating Curve or AUC), an analysis of each variable's contribution to the model, and a grid map with each cell having an index of suitability between 0 (low) and 1 (high). We overlaid this grid map on a map of the Ouachita Mountains to examine visually how well the predicted distribution corresponds to the actual distribution. For viewing and presentation of the model results, we used the Equal Training Sensitivity and Specificity threshold to classify areas as unsuitable if they fell below this value and retained values above this threshold in order to group grid cells into classes representing different levels of predicted suitability (Fielding and Bell, 1997; Liu et al., 2005).

\( P. \) caddoensis is closely related and geographically proximate to \( P. \) fourchensis and \( P. \) ouachitae, but occupies a different physiographic region. Comparing environmental conditions between areas occupied by \( P. \) caddoensis and areas occupied by the other two species would determine whether environments for these salamanders differ between physiographic regions and help to identify the unique environmental factors that have influenced the distribution and diversification of \( P. \) caddoensis. To compare environments among species, we first extracted values for the environmental variables used in niche modeling from our 66 sampling localities for \( P. \) caddoensis; the 38 localities for \( P. \) fourchensis reported by Shepard and Burbink (2009) plus four additional localities (D.B. Shepard, unpublished data), and the 55 localities for \( P. \) ouachitae reported by Shepard and Burbink (2008). We excluded land cover (LANDCOVER) and Surface Geology (GEOL) from this analysis because they are categorical rather than continuous variables. Because many of the remaining 28 climatic variables are intercorrelated, we used Principal Components Analysis to reduce them to a smaller number of independent, composite variables. We retained principal components with eigenvalues >1 and that explained >10% of the variation. We used the factor scores for these principal components as dependent variables in a MANOVA to test for differences among species. A significant multivariate effect was followed by ANOVAs for each principal component and posthoc Tukey HSD tests to compare means among species. We examined loading factors for those principal components that were significantly different to determine the nature of the differences in environments among species.

3. Results

3.1. Phylogeography

We sequenced 1052 bp of the cytb gene, 723 bp of the ND4 gene, and 41 bp of the tRNA-His for 330 \( P. \) caddoensis and 13 outgroup taxa (1816 bp total). Bayes Factors (BF) strongly favored the more parameter-rich 7Mp partitioning strategy over the less parameterized 3Mp (\( 2\log(BF) = 1241.2 \)) and 1Mp (\( 2\log(BF) = 1967.6 \)) strategies. Therefore, we used the 7Mp strategy in the BI, ML, and divergence dating analyses. For the BI analysis, burn-in occurred at 4.5 million generations and both independent runs produced similar harmonic mean –lnL values for post burn-in trees with a difference in –lnL of 10.34. After discarding samples before burn-in and summing trees from the two runs, the posterior probability distribution contained 11,000 trees.

Monophyly of \( P. \) caddoensis was strongly supported in all analyses and both BI and ML indicated that \( P. \) caddoensis is composed of four geographically structured lineages (Figs. 1 and 2). All four lineages were strongly supported by both Bayesian posterior probabilities and ML bootstrap values (Fig. 2), and mean uncorrected sequence divergence (p distance) among lineages ranged from 8.3% to 10.9%. Geographically, lineages are structured along an east–west axis (Fig. 1). The western-most lineage occurs primarily within the Brushy Creek drainage of the Cossatot River and the Two Mile Creek drainage of the Mountain Fork River. The next lineage to the east occurs primarily within the Cossatot and Little Missouri River drainages. Northeast from there, the third lineage occurs primarily in the upper drainages of the Caddo River and the Big Fork Creek drainage of the Ouachita River. The fourth, eastern-most lineage occurs along a lower system of drainages on the Caddo River, spanning both sides of Caddo Gap, and eastward to the Mazarn Creek drainage of the Ouachita River. Both ML and BI analyses strongly supported a sister relationship between the Upper and Lower Caddo River lineages, but the placement of the Brushy Creek lineage differed between methods. The BI analysis placed the Brushy Creek lineage as sister to the Cossatot/Little Missouri Rivers lineage whereas the ML analysis placed it as sister to the clade comprised by the Upper and Lower Caddo River lineages; support was weak for both arrangements (Fig. 2). In spite of the short distances (<1 km) between adjacent lineages and our intensive sampling of potential contact zones, we found only one locality where individuals of two different lineages were present (Fig. 1).

3.2. Isolation by distance and drainage

Adjacent lineages abutted each other in the vicinity of drainage divides and occasionally spanned these divides for short distances
Fig. 2. Bayesian consensus tree for 330 individuals of *Plethodon caddoensis* and 13 outgroup taxa based on partitioned analysis of 1816 bp of the mitochondrial cyt b and ND4 genes and tRNA-His. Values above branches are Bayesian posterior probabilities and values below are percent support from 1000 nonparametric bootstraps on the Maximum Likelihood tree. Support values are not labeled on shallower nodes for simplicity of presentation. + indicates that node was not recovered in the Maximum Likelihood analysis and the stated bootstrap support value is for the node in the alternate topology (see text). Samples are labeled by locality and voucher number and major lineages are indicated (Appendix).
in high-elevation regions (Fig. 1). Significant partial correlation coefficients for geographic distance, HUC4 watersheds, and HUC6 drainages indicate that all of these factors are important in explaining phylogeographic structure in *P. caddoensis* (Table 1). Geographic distance has the highest correlation with genetic distance, but results clearly show that individuals in different drainages and watersheds are more genetically divergent than expected given the geographic distance between them (Table 1). Examination of the plot of geographic versus genetic distance shows the overall positive correlation (*r* = 0.41, *P* < 0.0001); however, high levels of genetic divergence are present across short distances (Fig. 3). Regression analysis of between-lineage pairwise comparisons resulted in a *Y*-intercept of 0.10732 with a 95% CI of 0.10718–0.10746 (Fig. 3). The origin was determined to be 0.08240, which is outside the 95% CI, thus rejecting a role for IBD in lineage diversification. Although HUC4 watersheds, the broadest drainage level examined, explained a significant amount of the phylogeographic structure in *P. caddoensis*, the four main lineages did not correspond exactly to these watersheds (Fig. 1). Together, these results suggest that additional factors have been important in generating the four phylogeographic lineages and that geographic distance and stream drainages largely influence phylogeographic structure within lineages.

### 3.3. Divergence dates

*Plethodon caddoensis* diverged from the common ancestor of *P. fourchensis* and *P. ouachitae* during the Middle Miocene (~11.73 Ma (95% CI: 10.15–13.38 Ma) and subsequent divergences that gave rise to the four main lineages occurred shortly thereafter still within the Middle Miocene (~9–11 Ma; Fig. 4). The MRCAs of haplotypes within lineages ranged from ~2.82 Ma (95% CI: 2.13–3.60 Ma) for the Cossatot/Little Missouri Rivers lineage to ~7.55 Ma (95% CI: 5.96–9.19 Ma) for the Upper Caddo River lineage (Fig. 4).

### 3.4. Historical demography

Both Tajima’s *D* and Fu and Li’s *D*′ indicated a significant decline in population size in the Brushy Creek lineage (Table 2). Additionally, Fu and Li’s *D*′ indicated significant declines in the Lower Caddo and Upper Caddo River lineages, although Tajima’s *D* was not significant. Both methods also failed to reject the null hypothesis of population stability for the Cossatot/Little Missouri Rivers lineage. Bayesian skyline plots (BSP) showed that population size has gradually declined in the Lower Caddo River lineage but gradually expanded in the Cossatot/Little Missouri Rivers lineage over the last 2.5 Myr (Fig. 5). Both lineages, however, showed marked declines in population size from ~300,000 years ago to the present (Fig. 5). Population sizes in the Brushy Creek and Upper Caddo River lineages were relatively stable from 2.5 to ~1.0 Ma and then have gradually decreased toward the present (Fig. 5).

### 3.5. Ecological niche modeling

The predicted distribution of *P. caddoensis* based on ecological niche modeling closely matched the species’ actual distribution (AUC = 0.995; Fig. 6). Most locality points fell within the area of highest predicted suitability; however, some peripheral points fell in areas of low predicted suitability, particularly the southwestern-most points along the Cossatot River. Of the 30 environmental variables used to construct the niche model, GEOL (Surface Geology) and BIO16 (Precipitation of the Wettest Quarter) had the highest contributions to the model (49.3% and 31.8%, respectively), with other precipitation-related variables making up the bulk of the remaining important variables (Table 3).

---

**Table 1**

<table>
<thead>
<tr>
<th>Factor matrix</th>
<th>r</th>
<th>t</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Geographic distance</td>
<td>0.24</td>
<td>13.41</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>HUC4 watersheds</td>
<td>0.16</td>
<td>10.92</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>HUC6 drainages</td>
<td>0.11</td>
<td>15.08</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

---

*Fig. 3.* Plots of pairwise genetic and geographic distances used in tests for isolation by distance in *Plethodon caddoensis*. Note: Axes are extended beyond the origin in order to visualize the data points better.
P. ouachitae (38.16% and 29.52%, respectively; Table 3). MANOVA using the principal component scores for the two retained axes revealed that environmental conditions significantly differed among species (Wilk’s $\lambda = 0.098$, $F_{4,318} = 174.99$, $P < 0.001$). Species’ environments were significantly different along both the first ($F_{2,160} = 25.66$, $P < 0.001$) and second ($F_{2,160} = 346.33$, $P < 0.001$) principal component axes, and all species were significantly different from each other along both axes (all posthoc Tukey HSD tests $P < 0.05$). Based on the factor loadings (Table 3), the first axis represents a temperature gradient from cooler temperatures (low values) to higher temperatures (high values) whereas the second axis represents a moisture gradient from drier conditions (low values) to wetter conditions (high values). Environmental conditions where P. caddoensis occurs were hotter and wetter compared to conditions where P. fourchensis and P. ouachitae occur (Fig. 6).

4. Discussion

Niche conservatism is prominent in plethodontid salamanders and is an important factor driving population divergence and speciation (Kozak and Wiens, 2006, 2007, 2010). This phenomenon is most evident in montane regions where climatic changes may result in the fragmentation of a wide-ranging common ancestor into multiple isolated populations as individuals track their ecological niche; over time populations diverge and form reciprocally monophyletic groups (Wiens, 2004; Wiens and Graham, 2005). This scenario appears to be what generated high lineage diversity in the Ouachita Mountain endemic salamanders, P. fourchensis and P. ouachitae, because lineages are on mountains separated by well-demarcated valleys that act as barriers to gene flow (Shepard and Burbrink, 2008, 2009). We found that Plethodon caddoensis is composed of four highly divergent lineages; however, lineages do not appear to be separated by any barriers. Evidence indicates that P. caddoensis occupies a specific environmental niche, but how niche conservatism could have contributed to population divergence and lineage diversification in this species is not as clear.

<table>
<thead>
<tr>
<th>Lineage</th>
<th>$S$</th>
<th>$\pi$</th>
<th>$K$</th>
<th>Tajima’s $D$</th>
<th>Fu and Li’s $D^*$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brushy Creek</td>
<td>180</td>
<td>0.0358</td>
<td>57.241</td>
<td>1.933</td>
<td>1.565</td>
</tr>
<tr>
<td>Cossatot/Little Missouri</td>
<td>107</td>
<td>0.0216</td>
<td>17.916</td>
<td>–0.206</td>
<td>0.445</td>
</tr>
<tr>
<td>Lower Caddo</td>
<td>69</td>
<td>0.0226</td>
<td>21.738</td>
<td>1.125</td>
<td>1.255</td>
</tr>
<tr>
<td>Upper Caddo</td>
<td>230</td>
<td>0.0345</td>
<td>59.640</td>
<td>0.833</td>
<td>1.734</td>
</tr>
</tbody>
</table>

**Table 2**

Number of segregating sites ($S$), nucleotide diversity ($\pi$), average number of pairwise differences ($K$), and results of Tajima’s $D$, and Fu and Li’s $D^*$ for each lineage of Plethodon caddoensis calculated for all sites of the concatenated dataset. Results in bold rejected the null hypothesis of constant population size at $P < 0.05$. Fig. 4. Simplified tree showing mean divergence times and 95% Credible Intervals (Ma) for major nodes and the time to the most recent common ancestor (MRCA) for major lineages within Plethodon caddoensis.
are tightly linked to moisture and that moisture and talus
habitats would have similar effects in terrestrial plethodontids. Because terrestrial species are not restricted to aquatic habitats, phylogeographic structure in Plethodon caddoensis, P. fourchensis and P. ocellatus, the relative contribution of each variable to the niche model for P. caddoensis (Fig. 6).

We found that IBD contributes to phylogeographic structure in P. caddoensis, but we rejected it as having a role in lineage diversification. Likewise, stream drainages explained a significant amount of phylogeographic structure, but the four main lineages corresponded to groups of adjacent HUC6 drainages that were not all nested within the same HUC4 watershed (Fig. 1). Because drainage patterns may change over time, phylogeographic structure in some organisms may be better explained by historical drainage patterns rather than present-day patterns (Kozak et al., 2006a; Mayden, 1988). The historic stability of drainage patterns in the Caddo Mountains is unknown, but studies on multiple co-distributed fish species suggest that the major river systems have been established for a long time (Turner and Robison, 2006). These results imply that diversification of the four main lineages in P. caddoensis is likely due to some additional factor. Given the ecological niche of this terrestrial salamander, continuous high-elevation regions that span drainage divides, particularly on an east–west axis, may be important in connecting populations in different drainages. This interaction between stream drainages and topography could produce a pattern where each lineage occupies several adjacent HUC6 drainages that are connected by high-elevation regions which maintain genetic cohesion among populations on either side of drainage divides but that are not all nested within the same larger HUC4 watershed. Chance historical events that isolated common ancestors might also explain the existence of the four lineages with stream drainages and IBD having had more of an influence on phylogeographic structure within these lineages.

Table 3
Results from Principal Components Analysis on environmental variables used in comparison of environments among occurrence localities for Plethodon caddoensis, P. fourchensis and P. ocellatus, and the relative contribution of each variable to the niche model for P. caddoensis (Fig. 6).

<table>
<thead>
<tr>
<th>Variable</th>
<th>PC1</th>
<th>PC2</th>
<th>Percent Contribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>BIO1 (Annual Mean Temperature)(^a)</td>
<td>0.958</td>
<td>-0.103</td>
<td>0</td>
</tr>
<tr>
<td>BIO2 (Mean Diurnal Range)(^a)</td>
<td>0.886</td>
<td>0.234</td>
<td>0</td>
</tr>
<tr>
<td>BIO3 (Isothermality)(^a)</td>
<td>0.451</td>
<td>0.306</td>
<td>0.1</td>
</tr>
<tr>
<td>BIO4 (Temperature Seasonality)(^a)</td>
<td>0.793</td>
<td>-0.130</td>
<td>0</td>
</tr>
<tr>
<td>BIO5 (Max Temperature of Warmest Month)(^a)</td>
<td>0.974</td>
<td>-0.117</td>
<td>0</td>
</tr>
<tr>
<td>BIO6 (Min Temperature of Coldest Month)(^a)</td>
<td>-0.405</td>
<td>-0.028</td>
<td>0</td>
</tr>
<tr>
<td>BIO7 (Temperature Annual Range)(^a)</td>
<td>0.927</td>
<td>-0.088</td>
<td>0</td>
</tr>
<tr>
<td>BIO8 (Mean Temperature of Wettest Quarter)(^a)</td>
<td>0.967</td>
<td>-0.073</td>
<td>0</td>
</tr>
<tr>
<td>BIO9 (Mean Temperature of Driest Quarter)(^a)</td>
<td>0.308</td>
<td>0.325</td>
<td>0.3</td>
</tr>
<tr>
<td>BIO10 (Mean Temperature of Warmest Quarter)(^a)</td>
<td>0.965</td>
<td>-0.154</td>
<td>0</td>
</tr>
<tr>
<td>BIO11 (Mean Temperature of Coldest Quarter)(^a)</td>
<td>0.803</td>
<td>0.103</td>
<td>0</td>
</tr>
<tr>
<td>BIO12 (Annual Precipitation)(^a)</td>
<td>-0.074</td>
<td>0.957</td>
<td>0.7</td>
</tr>
<tr>
<td>BIO13 (Precipitation of Wettest Month)(^a)</td>
<td>-0.028</td>
<td>0.080</td>
<td>4.4</td>
</tr>
<tr>
<td>BIO14 (Precipitation of Driest Month)(^a)</td>
<td>0.009</td>
<td>0.991</td>
<td>1</td>
</tr>
<tr>
<td>BIO15 (Precipitation Seasonality)(^a)</td>
<td>0.227</td>
<td>-0.864</td>
<td>0</td>
</tr>
<tr>
<td>BIO16 (Precipitation of Wettest Quarter)(^a)</td>
<td>-0.041</td>
<td>0.921</td>
<td>31.8</td>
</tr>
<tr>
<td>BIO17 (Precipitation of Driest Quarter)(^a)</td>
<td>0.052</td>
<td>0.986</td>
<td>5.0</td>
</tr>
<tr>
<td>BIO18 (Precipitation of Warmest Quarter)(^a)</td>
<td>-0.357</td>
<td>0.903</td>
<td>2.4</td>
</tr>
<tr>
<td>BIO19 (Precipitation of Coldest Quarter)(^a)</td>
<td>0.057</td>
<td>0.886</td>
<td>0</td>
</tr>
<tr>
<td>ASPECT (Slope Direction)(^b)</td>
<td>0.231</td>
<td>0.031</td>
<td>0.3</td>
</tr>
<tr>
<td>SLOPE (Angle of Slope)(^b)</td>
<td>-0.165</td>
<td>-0.616</td>
<td>0.1</td>
</tr>
<tr>
<td>WETINDEX (Upstream Drainage Area and Slope)(^b)</td>
<td>0.493</td>
<td>0.465</td>
<td>0.2</td>
</tr>
<tr>
<td>HV (Mean Daily Water Vapor Pressure)(^c)</td>
<td>0.917</td>
<td>-0.084</td>
<td>0</td>
</tr>
<tr>
<td>HVV (Standard deviation of HV)(^c)</td>
<td>0.892</td>
<td>-0.288</td>
<td>0</td>
</tr>
<tr>
<td>HEATDAYS (Annual No. Heating Degree Days)(^c)</td>
<td>-0.947</td>
<td>-0.067</td>
<td>0</td>
</tr>
<tr>
<td>PE (Annual Precipitation Event Frequency)(^c)</td>
<td>-0.014</td>
<td>0.872</td>
<td>3.3</td>
</tr>
<tr>
<td>PE (Mean Annual Precipitation per Event)(^c)</td>
<td>-0.637</td>
<td>-0.335</td>
<td>0.8</td>
</tr>
<tr>
<td>CANOPY (Percent Tree Canopy Cover)(^d)</td>
<td>-0.345</td>
<td>0.060</td>
<td>1.0</td>
</tr>
<tr>
<td>LANDCOVER (LANDCOVER Type)(^d)</td>
<td>NA</td>
<td>NA</td>
<td>0.2</td>
</tr>
<tr>
<td>GEOL (Surface Geology Category)(^d)</td>
<td>NA</td>
<td>NA</td>
<td>45.3</td>
</tr>
</tbody>
</table>

Eigenvalue                      10.69   8.26
% Variance explained            38.16   29.52

\(^a\) http://www.worldclim.org/.
\(^b\) http://eros.usgs.gov/#Find_Data/Products_and_Data_Available/gtopo30/hydro.
\(^c\) http://www.daymet.org/.
While *P. caddoensis* originated ~12 Ma, diversification into the four present-day lineages ~9–11 Ma was likely influenced by climatic change. This timing corresponds well to the period following the Middle Miocene Climatic Optimum (~15–17 Ma) in which warm and humid climates transitioned to cool and dry climates with increased seasonality (Flower and Kennett, 1994; Kohn and Fremd, 2008; Kürschner et al., 2008; Zachos et al., 2001). These climatic changes were especially pronounced at mid-latitudes and led to increased aridification and many faunal changes (Böhme, 2003; Kohn and Fremd, 2008; Kürschner et al., 2008). In central North America, this change in climate led to the expansion of grasslands, which occurred at the expense of forested habitats (Axelrod, 1985; Kohn and Fremd, 2008; Kürschner et al., 2008). High humidity environments are critical for plethodontid salamanders and increased aridification should have severely impacted their distributions (Spotila, 1972; Vieites et al., 2007). Isolated salamander species in New Mexico and the Edwards Plateau of Texas as well as fossils from Montana closely related to taxa with distributions suggest that plethodontids were more broadly distributed in the past (Petrakka, 1998; Tihen and Wake, 1981; Vieites et al., 2007). Species of the *P. ouachitae* complex persisted in the Ouachita Mountains during this period of climate change and likely survived because of the availability of moist microhabitats and retreats (e.g., high-elevations, north slopes, talus).

A Middle Miocene origin for the four lineages within *P. caddoensis* indicates that the climatic changes associated with Pleistocene glacial cycles did not drive lineage diversification in *P. caddoensis* as they did in *P. fourchensis* and *P. ouachitae* (Shepard and Burbrink, 2008, 2009). However, results from historical demographic analyses showed evidence for decreasing population sizes in all lineages at various points during the Pleistocene, suggesting that glacial cycle induced climatic fluctuations have influenced population size as well as structure. Although lineages within *P. caddoensis* were already present by the Pleistocene, range contraction and population fragmentation in response to climatic changes would tend to promote maintenance of pre-existing geographic and genetic distinctiveness. Further, given the inverse relationship between $N_e$ and the time required for ancestral genetic polymorphisms to sort, decreases in $N_e$ would facilitate lineage sorting (Avise, 2000). In contrast to our results for *P. caddoensis*, most studies on plethodontid salamanders have found evidence for recent population growth or stability (e.g., Carstens et al., 2004; Kozak et al., 2006a; Mahoney, 2004; Martínez-Solano et al., 2007; Shepard and Burbrink, 2008, 2009).

Temperatures in the Arkansas Novaculite region commonly exceed the preferred environmental temperature of *P. caddoensis* (~18 C) and the critical thermal maximum of most *Plethodon* spp. (~34 C), especially during summer months (Spotila, 1972). The gravelly talus slopes that characterize this region provide subterranean retreats where *P. caddoensis* can escape harsh surface conditions and thus, are hypothesized to be important in determining its distribution. In support of this hypothesis, ecological niche modeling indicated that Surface Geology was the most important variable predicting the species’ distribution. Niche modeling results also indicated the importance of several moisture-related variables, which is expected because plethodontid salamanders require high humidity environments to avoid dehydration (Spotila, 1972). The higher temperatures where *P. caddoensis* occurs compared to localities where *P. fourchensis* and *P. ouachitae* occur mean that more moisture (water vapor) must be present in the air to achieve the same relative humidity. The higher amount of precipitation in areas occupied by *P. caddoensis* would compensate somewhat for the hotter temperatures to create occasional periods of high relative humidity. Climatic changes that result in decreased relative humidity (e.g., increased temperature and/or decreased precipitation) should negatively impact all three species, but could be most severe for *P. caddoensis* given its smaller body size and thus higher rate of dehydration (Spotila, 1972).

Althoogh lineages within *P. caddoensis* exhibit a high level of mean uncorrected sequence divergence (average 8.3–10.9%) and are geographically distinct, it is unknown whether gene flow occurs where lineages come into contact or if any lineages are reproductively isolated. Hybridization is common among closely related species of *Plethodon* and the frequency of occurrence is related to time since divergence (Weisrock et al., 2005; Weisrock and Larson, 2006; Wiens et al., 2006). The ages of the MRCAs of lineages within *P. caddoensis* (9–11 Myr; Fig. 4) are within the range of divergence

![Fig. 5. Bayesian skyline plots showing the demographic history of the four lineages of *Plethodon caddoensis* over the last 2.5 Myr (Pliocene/Pleistocene boundary to the present). The central line represents the median value for the log$_{10}$ of the population size (N, s - r) and the shaded area represents the 95% Highest Posterior Density. The generation time (τ) is in units of millions of years (e.g., a 3-year generation time equates to τ = 3 × 10$^{-4}$).](image-url)
Fig. 6. Ecological niche model (A) for *Plethodon caddoensis* constructed using 30 environmental variables at 30 arc-seconds resolution (≈1 km²) and our 66 sampling points. Areas of predicted suitability range from low to high: green (0.07–0.2), yellow (0.2–0.4), orange (0.4–0.6), and red (>0.6). Locality points for *P. caddoensis* (yellow), *P. fourchensis* (green), and *P. ouachitae* (red) used in comparison of environmental conditions among species plotted on a map of all elevations > 400 m (B) and the means ±95% Confidence Intervals for each species from the Principal Components Analysis (C). Environmental conditions occupied by *P. caddoensis* are significantly warmer, receive more precipitation, have higher seasonality in temperature, and lower seasonality in precipitation compared to *P. fourchensis* and *P. ouachitae*. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)
observed in some reproductively isolated species of Plethodon, although patterns are variable (Wiens et al., 2006). A small amount of hybridization in contact zones is not usually enough to obscure the historic effects of prolonged isolation in Plethodon (Chatfield et al., 2010; Weisrock et al., 2005; Weisrock and Larson, 2006), but how lineages within P. caddoensis have remained geographically distinct over the last several million years without any clear physical or environmental barriers separating them warrants further investigation. Data from nuclear markers should provide a more comprehensive view of the extent of gene flow among lineages and provide the necessary information to make decisions on species status of lineages.

5. Conclusion

We have shown here that the unique physiographic features of the region coupled with species response to climatic factors have generated four highly divergent lineages in P. caddoensis over a small geographic area. More generally, our study demonstrates the importance of integrating species biology with other factors such as climate, geology, and hydrology in order to better understand the factors driving lineage divergence and speciation. Even among the closely related and geographically proximate species of the P. ouachitae complex, P. caddoensis showed different responses to historic climatic changes than P. fourchensis and P. ouachitae (Shepard and Burbrink, 2008, 2009, this study). These differences underscore the importance of individual responses to climate change (Hewitt, 1996, 2000, 2004; Stewart, 2009; Sullivan et al., 2000), and illustrate how interactions among multiple intrinsic and extrinsic factors over small spatial scales can generate broad-scale patterns of biodiversity via different mechanisms. Integrating knowledge from diverse sources is often necessary in order to understand the processes that generate and maintain biodiversity (Wiens and Donoghue, 2004), and will be crucial in forecasting the effects of future climate change on environmentally sensitive species.

Acknowledgments

We thank K. Irwin (Arkansas Game and Fish Commission) for considerable assistance with fieldwork, logistics, and countless other valuable contributions to this project. We also thank R. Barton, N. Clay, T. Colston, G. Costa, S. Filipek, D. Filipek, A. Fink, T. Gendusa, T. Guicher, R. Lewis, S. Martin, K. Roberts, S. Ruane, B. Timpe, and the Arkansas Herp Society for help with fieldwork. R. Bastarache, S. Cochran, B. Crump, J. Davis, and E. Sharp of the Ouachita National Forest, US Forest Service, helped facilitate our project. We also thank R. Pyron for help with laboratory work and analyses, K. Kozak and B. Weinstein for advice and assistance with GIS and analyses, R. Chong for comments on an earlier version of this paper, and T. Taggart for tissue samples from the Sternberg Museum of Natural History collection (MHP). Funding was primarily provided by a State Wildlife Grant from the Arkansas Game and Fish Commission. All collecting was done under permits to DBS and/or FTB from the State of Oklahoma, State of Arkansas, and US Forest Service. This work was conducted under the approval of the Animal Care and Use Committees of the University of Oklahoma (#RO6-007) and College of Staten Island (12-Y3-08).

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ympev.2011.03.007.

References

Flawn, P.T., Goldstein Jr., A., King, P.R., Weaver, C.E., 1961. The Ouachita System. Bureau of Economic Geology, University of Texas, Austin.