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Pleistocene Glaciation Drove Shared Population Coexpansion in Eastern North American Snakes

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ABSTRACT

Glacial cycles during the Pleistocene had profound impacts on local environments and climatic conditions. In North America, some regions that currently support diverse biomes were entirely covered by ice sheets, while other regions were environmentally unsuitable for the organisms that live there now. Organisms that occupy these regions in the present day must have expanded or dispersed into these regions since the last glacial maximum, leading to the possibility that species with similar geographic distributions may show temporally concordant population size changes associated with these warming trends. We examined 17 lineages from 9 eastern North American snake species and species complexes to test for a signal of temporally concordant coexpansion using a machine learning approach. We found that the majority of lineages show population size increases towards the present, with evidence for coexpansion in five out of fourteen lineages, while expansion in others was idiosyncratic. We also examined relationships between genetic distance and current environmental predictors and showed that genomic responses to environmental predictors are not consistent among species. We, therefore, conclude that Pleistocene warming resulted in population size increases in most eastern North American snake species, but variation in environmental preferences and other species-specific traits results in variance in the exact timing of expansion.

1 | Introduction

Environmental and geological changes over space and time are major drivers of biological diversity (Bagley and Johnson 2014; Bidegaray-Batista, Ferrández, and Arnedo 2013; Harrington et al. 2018; Ivory et al. 2016; Pujolar et al. 2022; Weir and Schluter 2004). Sharp changes between ecoregions and physical barriers often delineate the boundaries between species and populations (Savage 1960). Climatic change can reduce the amount of suitable habitat for biological communities, causing population contraction and restriction to refugial habitat. In contrast, climatic changes can also result in species' ranges expanding to track favourable habitat, with concomitant population size increases (Devitt et al. 2013; Hewitt 1996; Nason, Hamrick, and Fleming 2002; Puckett et al. 2015). Different species may have unique biotic and abiotic niche tolerances that limit dispersal and population growth, and thus climatic changes often have varying effects on the population size and structure

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across communities (Moritz et al. 2008; Myers, Hickerson, and Burbrink 2017). However, if climatic or geological changes are drastic enough, ecologically similar species may share similar historical demographic changes (Burbrink et al. 2016; Lessa, Cook, and Patton 2003).

Species with similar life histories are most likely to have congruent signals of population expansion. Previous studies on demographic histories of snakes typically showed strong historical demographic responses to climate change during the Pleistocene (Burbrink et al. 2016; Fontanella et al. 2008; Guiher and Burbrink 2008; Ruane, Torres-Carvajal, and Burbrink 2015). This is expected given that all snakes share many broad morphological and physiological traits and therefore may show similar responses to changing climatic conditions. Specifically in the forested Eastern Nearctic (ENA), a large number of snake species occur near or in formerly glaciated areas. The ENA also presents several present-day sharp environmental changes, including precipitation and temperature gradients (Omernik 1987; Omernik and Griffith 2014), which may shape genetic structure in snakes and other organisms.

The glacial cycles of North America are a clear example of the types of broad-scale environmental changes that are expected to have shared effects on multiple taxa (Davis 1983; Hewitt 1996). Taxa with limited abilities to survive cold temperatures were extirpated from northern latitudes and, most dramatically, nearly all organisms were extirpated from regions covered by kilometres-thick glaciers, resulting in dramatic population declines in many species (Hewitt 2000). During the last glacial maximum, glaciers covered large portions of what is now the northeast and midwest of the USA, where the terminal moraine extended to Harbour Hill, Long Island, New York (39.7°) in the east and Shelbyville, Illinois (39.4°) in the Midwest (Kleman et al. 2010; Marshall, James, and Clarke 2002). Species presently occupying these regions must have expanded into these habitats after glaciers receded as these areas became more hospitable. Although it is expected that species will rapidly colonise habitat as it becomes suitable, unique life histories and dispersal abilities may lead to differing rates at which organisms expand their ranges (Burbrink et al. 2016).

Herein, we examine how climatic changes have influenced population sizes across nine species or species complexes of eastern North American snakes: *Agkistrodon contortrix, Diadophis punctatus, Farancia abacura, Farancia erytrogramma, Lampropeltis getula, Lampropeltis triangulum, Masticophis flagellum, Pantherophis guttatus*, and *Storeria dekayi*. We hypothesised that glacial cycles have had similar effects across these snake species and therefore we should see synchronous population expansion during the late Pleistocene as climate warmed and glaciers receded. Despite the broad-level similarities among snakes as ectothermic predators sharing a broadly similar body plan, there are differences in physiology, diet and habitat preferences among species; e.g., *A. contortrix* are heavy-bodied sit-and-wait predators that reach nearly a meter long and consume primarily mammals, whereas *Diadophis punctatus* are slender, semi-fossorial snakes typically less than 40 cm that feed mostly on invertebrates and ectothermic vertebrates (Conant and Collins 1998). We, therefore, expect that climatic variables will not have the same effects on genetic diversity across all species. To understand if changes in environment throughout the Pleistocene affected snake demography in similar ways, we examined the signal and timing of population coexpansion in nine snake species or species complexes. We then used a hierarchical simulation-based machine learning approach to simultaneously estimate the probability that species have coexpanded and the timing of expansion for expanding populations of snakes.

2 | Materials and Methods

2.1 | Data Generation

We obtained tissue samples from museum and personal collections for the following 7 species and species complexes: *Farancia abacura, Farancia erytrogramma, Agkistrodon contortrix, Diadophis punctatus, Masticophis flagellum, Pantherophis guttatus* and *Storeria dekayi* (Appendix 1). We focused on samples from the ENA only, and thus samples do not cover the full geographic ranges of many species. We combined data that we generated with published data for the *Lampropeltis triangulum* (Burbrink et al. 2022; using assembled data described below) and *Lampropeltis getula* species complexes (Harrington and Burbrink 2023; raw reads available on NCBI Sequence Read Archive under BioProject ID PRJNA889851). Species' ranges and sampling localities are shown in Figure 1.

We extracted DNA from all tissues using the Qiagen DNEasy kit following manufacturer protocol. We sent extracted DNA to the University of Wisconsin-Madison Biotechnology Center for ddRAD library preparation using enzymes PstI and Bfal and subsequent sequencing on an Illumina NovaSeq6000 to generate paired-end 150 bp reads. For the two *Farancia* species, which were prepped and assembled separately, library prep differed in that DNA was digested using PstI and MspI and libraries were sequenced on an Illumina HiSeq2500 sequencer. We generated 2.8 million reads per sample on average. We used ipyrad v0.9.63 to assemble the data (v0.7.28 for *Farancia* samples), using default settings for most parameters (see params files on Github at https://github.com/seanharrington256/Coexp_scripts). After generating preliminary assemblies, individuals with high amounts of missing data were removed before generating final assemblies that we used for all downstream analyses. We generated datasets that were 25% missing data or less by specifying the number of individuals required per locus separately for each assembly, depending on the number of total individuals. For the *L. triangulum* complex, we used the assembly generated by (Burbrink et al. 2022) and available on Dryad (https://datadryad.org/stash/dataset/doi:10.5061/dryad.g79cnp5qm) as file Data_D3_VCF_file_generated_for__Lampropeltis_triangulum_L._gentilis_and_L._elapsoides_from_ipyrad_filtered.vcf as well as an unfiltered version of this file, available as file milks_denovo-92.vcf on Dryad (https://doi.org/10.5061/dryad.9cnp5hqv5). This file is the raw output from ipyrad before the custom thinning and filtering applied by Burbrink et al. (2022).

2.2 | Population Structure

After assembling the data, we used the sparse nonnegative matrix factorization method (sNMF; Frichot et al. 2014) in the R

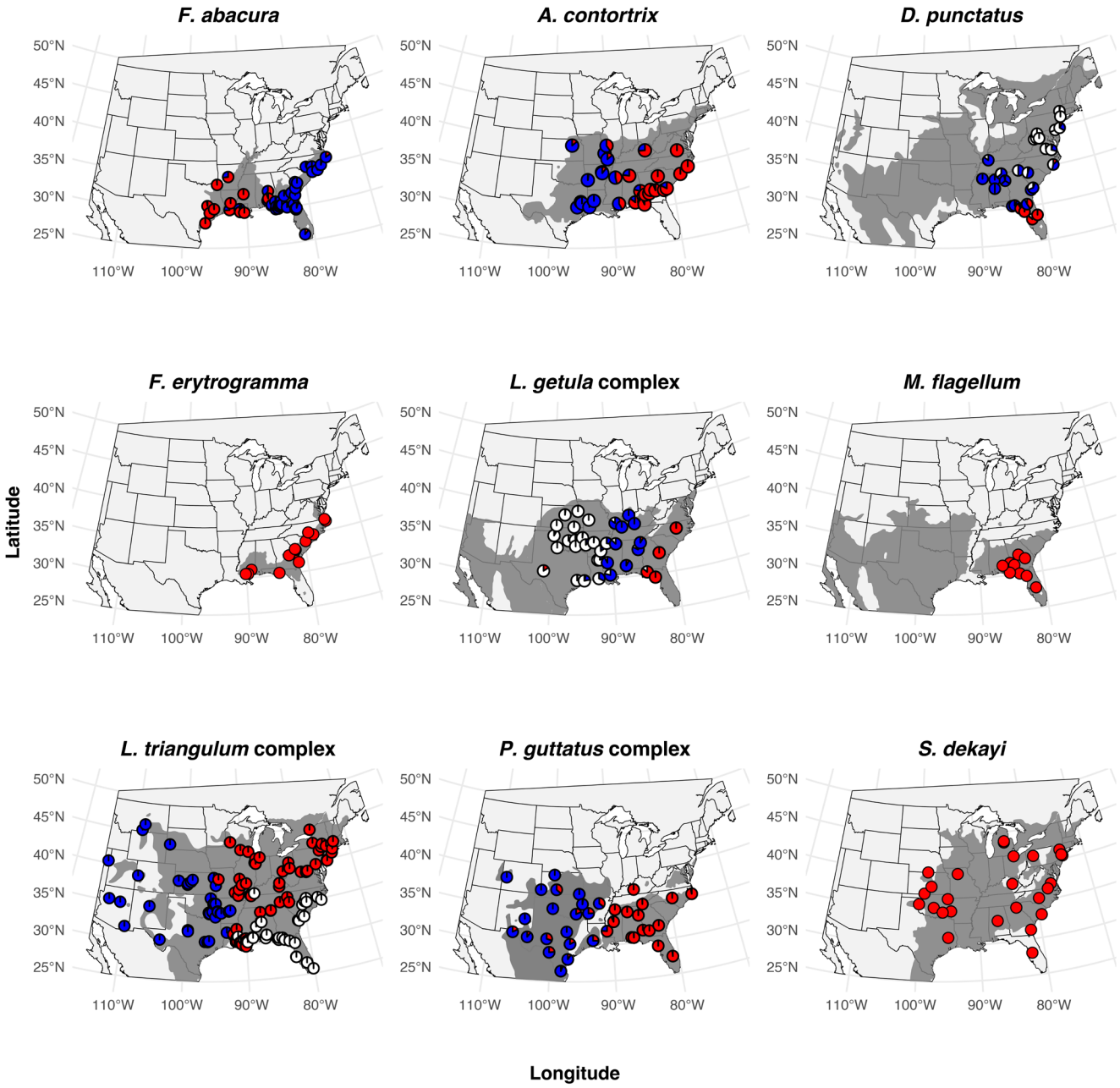

FIGURE 1 | Maps for each species/species complex showing the range in grey and samples as points. Points are coloured according to their estimated ancestry in clusters as determined by sNMF. Maps use the Albers equal area projection.

(v. 4.2.2; R Core Team 2023) package LEA v3.8.0 (Frichot and François 2015) to identify discrete population clusters within each species. This is an essential step because the methods we used to estimate population sizes changes through time assume that individuals are all drawn from a single, panmictic population. Therefore, including multiple discrete populations in a single analysis can strongly bias estimates. We identified the optimal value of K, the number of discrete populations, by running sNMF for values of K from 1–10 and then identifying which K value yielded the lowest cross-entropy score or the value of K that exhibited the sharpest drop followed by a plateau or increase in cross-entropy at higher values. For the *L. triangulum* complex, we used the population or species designations from the study that generated these data (Burbrink et al. 2022) available as file "Data_D6_DAPC_TESS_assignments_4_taxa.txt" on Dryad (https://datadryad.org/stash/dataset/doi:10.5061/dryad.g79cnp5qm).

2.3 | Environmental Correlates of Genetic Structure

Within each population, we then used generalised dissimilarity modelling (GDM) in the gdm v1.5.0–9.1 R package (Fitzpatrick et al. 2021) to identify significant geographic and environmental predictors of genetic distance within each population, therefore testing for both isolation-by-distance and isolation-by-environment simultaneously. We downloaded the Bioclim dataset (Hijmans

et al. 2005) and elevation data from the Worldclim database (Fick and Hijmans 2017) and combined these with the Envirem dataset (Title and Bemmels 2018) for our full set of environmental predictors. We removed highly correlated variables with $r > 0.8$, leaving us with 13 climatic and environmental variables (Bio1, mean annual temperature; Bio2, mean diurnal temperature range; Bio4, temperature seasonality; Bio5, maximum temp of the warmest month; Bio8, mean temperature of the wettest quarter; Bio9, mean temperature of the driest quarter; Bio12 annual precipitation; Bio15, precipitation seasonality; Bio18, precipitation of the warmest quarter; SAGA-GIS topographic wetness index; terrain roughness index; Thorn's aridity index; PET seasonality; elevation). For each species/species complex, we calculated pairwise distances among our samples for each variable and then ran a GDM including all environmental distances plus geographic distance as predictors of genetic distance (calculated across all SNPs). We visualised the relationship between geographic distance and genetic distance (i.e., isolation-by-distance; IBD) in each species/species complex using functions in the adegenet R package (Jombart 2008; Jombart and Ahmed 2011). We additionally visualised IBD using kernel density plots for subpopulations of *D. punctatus* to help determine how many populations to use for coexpansion analysis (see results).

2.4 | Single-Population and Assemblage-Level Demographic Inference

For analyses of population size change and coexpansion, we split each species/complex into populations as identified based on results from sNMF analyses and IBD plots. For each population, we generated vcf files using custom R scripts (available on Github https://github.com/seanharrington256/Coexp_scripts) and used easySFS (https://github.com/isaacovercast/easySFS) to generate site frequency spectra (SFS) as input for single-population (Stairway Plot 2) and assemblage-level (PTA) demographic analysis. For both types of analyses, we assumed a mutation rate of 6.6×10^{-9} mutations/site/generation, based on a mutation rate of 2.2×10^{-9} mutations/site/year (Gottscho, Marks, and Jennings 2014; Kumar and Subramanian 2002) and assuming an average generation time of 3 years for all taxa.

To investigate historical demographic trajectories both within and among these co-distributed snake taxa, we undertook a two-stage inference approach. We first inferred population size change histories for each population independently using Stairway Plot 2 v2.1.1 (Liu and Fu 2020). For Stairway Plot 2 analyses, the numbers of haploid samples and numbers of SNPs retained in each population SFS after down projection in easySFS are shown in Table S1. The power of coexpansion analysis is the highest when populations that are contracting or have been at a stable population size throughout their history are excluded because the timing of an expansion event cannot be shared between a species that is expanding and one that is not, by definition. We, therefore, used Stairway Plot 2 results to bin populations as either expanding or not expanding. Populations determined to be expanding were then included in analysis of coexpansion, with the exception of *Lampropeltis gentilis*, the western member of the *L. triangulum* species complex. We excluded *L. gentilis* because it is distributed primarily in central and western North America, and we retain a focus on eastern taxa that are likely to have encountered similar climatic changes in the recent geological past. We implemented a hierarchical simulation-based machine learning approach (Phylogeographic Temporal Analysis; PTA; https://github.com/isaacovercast/PTA) to infer the temporal concordance of co-expansion and the timing of any shared co-expansion event.

PTA is a simulation-based comparative phylogeographic inference method (similar in spirit to previous methods, e.g. Chan, Schanzenbach, and Hickerson 2014; Xue and Hickerson 2017), which uses information from the SFS aggregated across all co-distributed populations (following Xue and Hickerson 2015) to estimate the proportion of co-expanding taxa (ζ) and the timing of co-expansion (τ_s). The required input data for PTA is one SFS per population (in *dadi* format; Gutenkunst et al. 2009), which is converted internally into a multiSFS (mSFS). A special consideration is that the dimension of each SFS must be identical to meet a requirement of the model to allow for exchangeability among SFS bins. We, therefore, used easySFS to project all populations down to 8 haploid samples, matching the sample size of the population with the smallest number of individuals. To increase computational tractability, we sorted the values of the mSFS bins in order of decreasing magnitude, a standard efficiency adopted by similar methods (Overcast, Bagley, and Hickerson 2017; Xue and Hickerson 2015).

PTA inference proceeds in three phases: 1) generating simulations (using msprime; Kelleher, Etheridge, and McVean 2016), 2) training and validating a machine learning (ML) model (scikit-learn; Pedregosa et al. 2011) and 3) parameter estimation and model adequacy checks using the empirical data. We generated 300,000 simulations while sampling from uniform priors on ζ (proportion of coexpanding taxa; 0–1), τ_s (timing of coexpansion; 1×10^4-1×10^6 years), N_e (contemporary effective population size; 2×10^5-5×10^6) and ε (expansion magnitude; 0.01–0.1). For the purpose of the simulation ζ is transformed into ζ_e (the effective number of co-expanding populations, which takes on integer values). Other model parameters were fixed to values which most closely represent the empirical data, including number of populations ($n = 14$), length of sequenced reads (150 bp) and mutation rate (6.6×10^{-9}). Next we implemented a random forest model (Breiman 2001), using 75% of simulations as training data and 25% as testing data. We also adopted a cross-validation approach to iteratively split simulations into multiple, independent train/test sets, to better evaluate model performance. We used random forest regression to evaluate the performance in estimating τ_s, N_e and ε, using R^2 as the performance criterion. We also used random forest classification to evaluate performance in estimating ζ_e, using average precision and recall as performance criteria. Finally, we used the trained ML model and the empirical mSFS to classify ζ_e and estimate model parameters for the empirical assemblage.

3 | Results

3.1 | Population Structure

Cross-entropy scores from sNMF support single populations within our samples of *A. contortrix*, *F. erytrogramma, L. getula, S. dekayi* and *M. flagellum* (Figure S1). Within *F. abacura* and *P. guttatus*, we find strong evidence for two populations

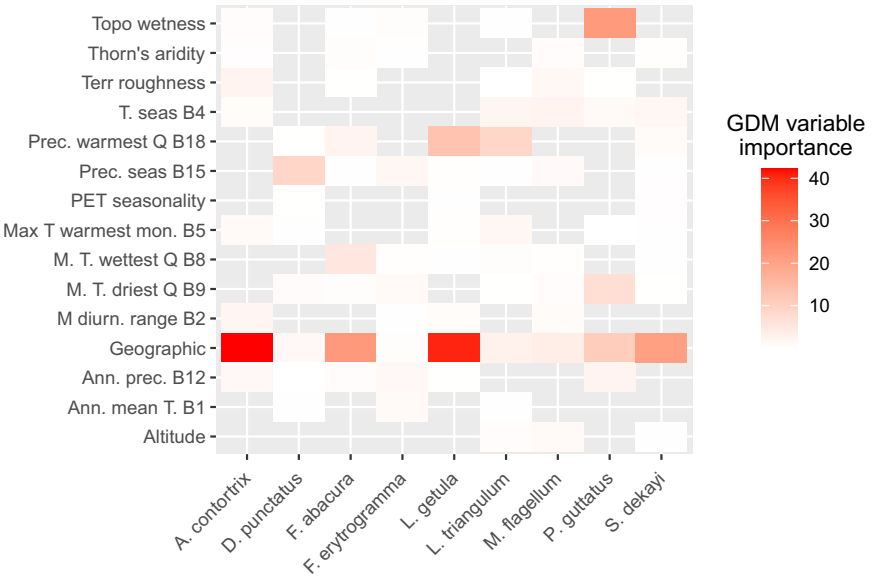

FIGURE 2 | Heatmap showing the variable importance of each predictor in generalised dissimilarity models for each species or complex. Absent cells (grey background) represent predictors that were not identified as part of the model for a given species.

each (Figure 1, Figure S1). Results for *D. punctatus* were similar, with a slightly lower cross-entropy score for K = 2 than K = 1. Examination of admixture plots for *D. punctatus* at K = 2 and K = 3 (Figure 1, Figure S1) shows that clusters are geographically cohesive. The kernel density plot of genetic distance versus geographic distance for all samples of *D. punctatus* showed disjunct clouds, with jumps in genetic distances at the same geographic distances, suggesting the presence of some discrete structure (Figure S2, S3). Because population structure is known to bias estimates of population sizes, we opted to split *D. punctatus* into three populations for analyses of population size change through time in Stairway Plot 2 and PTA. Similarly, we split our *L. getula* samples into three populations, despite evidence for a single population in our samples from our sNMF analyses here and a previous study (Harrington and Burbrink 2023). We did this because of the particularly steep relationship between genetic and geographic distances seen in the kernel density plot (Figure S2), and because these samples form geographically cohesive groupings, which closely correspond to divergent mitochondrial DNA clades (Pyron and Burbrink 2009). We additionally split *A. contortrix* into two populations for the same reason, as all samples combined show strong isolation-by-distance, and at K = 2, samples form geographically cohesive eastern and western clusters (Figures S1, S2) and also following previously identified lineage structure (Burbrink and Guiher 2015). After splitting *A. contortrix* and *D. punctatus*, we no longer see disjunctions in kernel density plots of isolation-by-distance (Figure S3). We additionally support splitting *D. punctatus* into three clusters and *A. contortrix* into two clusters on the basis that preliminary Stairway Plot 2 analyses using all samples for each dataset showed strong downward spikes in population sizes near the oldest time points (Figure S4). This pattern largely disappears when samples are split into different clusters, suggesting that this is an artefact of population substructure (Heller, Chikhi, and Siegismund 2013). Therefore, the splitting of *L. getula* and *D. punctatus* into three populations each is an attempt to more closely fit the assumptions of Stairway Plot 2 and PTA that populations are not substructured and approximate panmixia. These population designations resulted in a total of 17 populations that were treated as separate lineages for Stairway Plot 2 analyses.

3.2 | Environmental Correlates of Genetic Structure

Generalised dissimilarity models were significant ($p < 0.01$) for all species/species complexes, indicating that some combination of geographic and environmental distances are related to genetic distance in each case. Geographic distance stands out as the most important predictor of genetic distance for most species/complexes, consistent with kernel density plots of isolation-by-distance (Figure 2). Several precipitation variables also show strong importance in multiple models. When considering only variables that are individually significant in predicting genetic distance (Figure S5), we find that variables related to precipitation, topographic wetness and temperature are significant in some taxa, with none shared across two or more taxa. All nine taxa include multiple environmental variables in the best GDM model in addition to geographic distance.

3.3 | Single-Population and Assemblage-Level Demographic Inference

Stairway Plot 2 analyses show population size increases through time in all populations except the western population of *F. abacura*, *F. erytrogramma* and the western cluster of the *P. guttatus* complex corresponding to *Pantherophis emoryi* (Figure 3; see Figure S4 for confidence intervals for each population). All three of these clusters/species were estimated to have experienced recent population declines, rather than population size increases. Therefore, these three species were removed for the purpose of the downstream comparative phylogeographic PTA co-expansion analysis. We note that for some populations, such

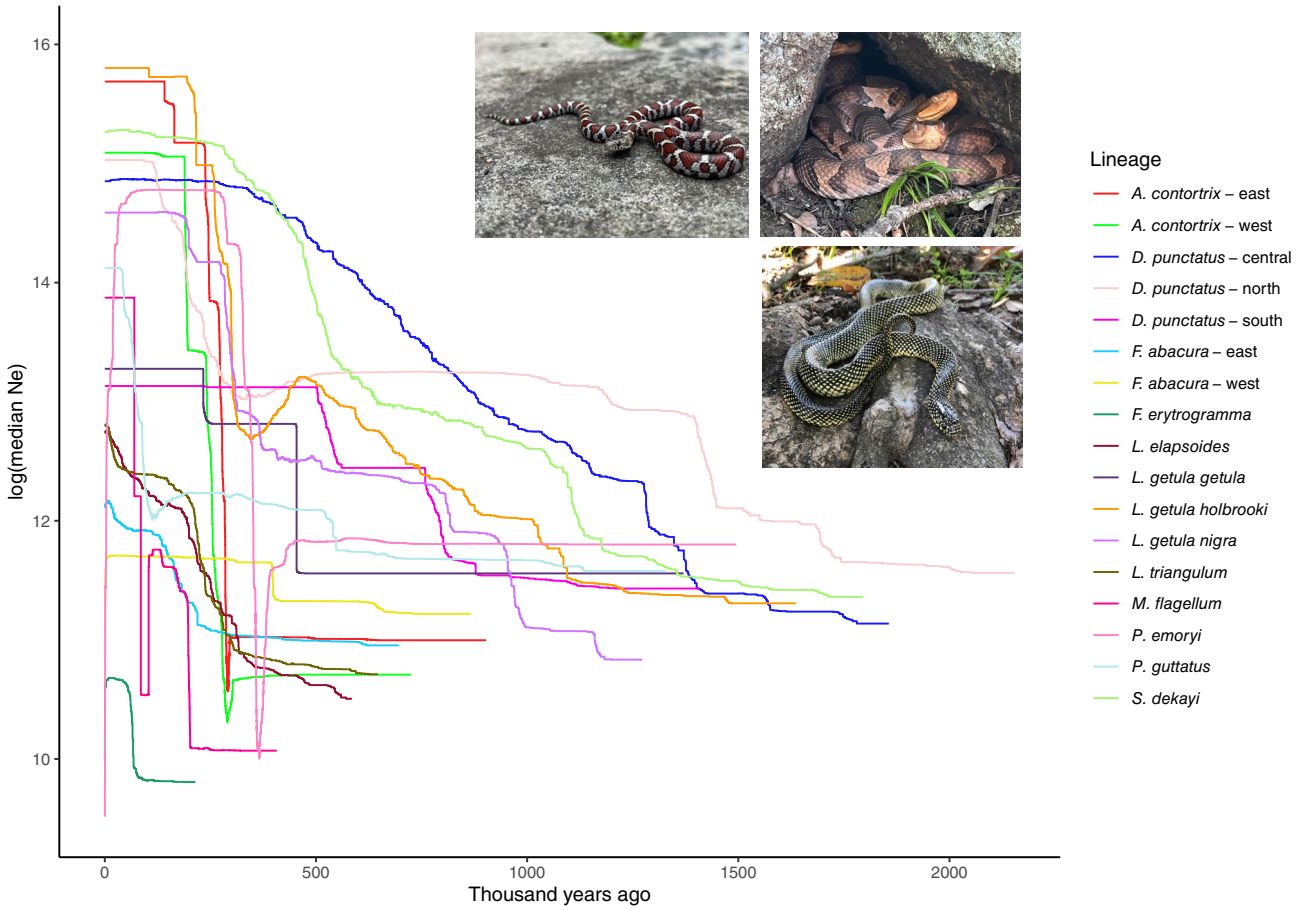

FIGURE 3 | Plot of log of median effective population size (N_e) through time for each lineage as estimated by Stairway Plot 2. Inset images show individuals of: *Lampropeltis triangulum* from Ulster Co., NY (top left); *Agkistrodon contortrix* from Westchester Co., NY (top right) and *Lampropeltis getula* complex from East Baton Rouge Parish, LA (bottom). All photos taken by FTB.

as northern *D. punctatus,* we do not have complete sample coverage across the northern part of the range, which may influence estimates. Inclusion of samples from poorly sampled northern parts of population ranges would likely increase the signal for recent expansion.

For the PTA co-expansion analysis, in evaluating the performance of the random forest classification scheme for ζ_e, we found the ML model produced adequate precision and recall with weighted average values of 0.31 and 0.32, respectively, which compares favourably to a random model with precision/recall of ~0.07. Importantly, visual inspection of the confusion matrix (Figure S6) demonstrated a key feature of the classification performance not readily apparent from the evaluation criteria, namely that the vast majority of incorrectly predicted ζ_e values were directly adjacent to correct ζ_e values. In other words, when the model failed to accurately classify ζ_e, it did so in a way that was not arbitrarily bad, rather it was often close to the true value. In evaluating random forest regression for parameter estimation, we found that some parameters were well estimated (e.g., τ_s, $R^2 = 0.47$; mean ε, $R^2 = 0.59$; see Figure S7), while others were quite poorly estimated (standard deviation of contemporary N_e; $R^2 = 0.12$). We focus on those parameters that are most accurately estimated, including the timing of co-expansion (τ_s) and the expansion magnitude (ε), which also happen to be the parameters of greatest interest

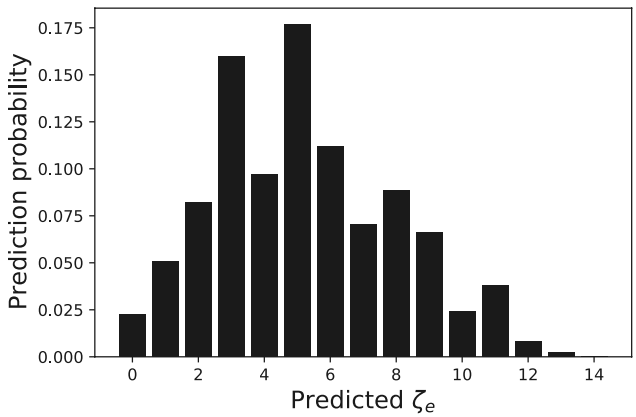

FIGURE 4 | Bar plot depicting the full distribution of predicted ζ_e values for the empirical dataset. The x-axis shows the predicted number of co-expanding taxa, and the y-axis indicates the prediction probability from the random forest machine learning classifier.

(all estimated model parameters and 95% prediction intervals are in Table S2).

The ML classifier for empirical PTA analysis predicted $\zeta_e = 5$ as most probable, with another peak of prediction probability at $\zeta_e = 3$. While the prediction probability was diffuse around these

intermediate values, there was little support for either total synchronous ($\zeta_e = 14$) or asynchronous ($\zeta_e = 0$) expansion (Figure 4). The ML regression model estimated the timing of co-expansion (τ_s) as 32,000 ybp (95% prediction interval: 19,000–45,000 ybp), with an expansion magnitude (ε) of 0.061 (95% prediction interval: 0.058–0.065). The average time of expansion of those taxa expanding asynchronously (i.e., not involved in the co-expansion pulse) was slightly older than the estimated τ_s (36,740 ybp; 95% prediction interval 30,364–43,539 ypb), though the prediction intervals somewhat overlapped. To evaluate goodness of fit, we generated posterior predictive simulations parameterized based on the model estimated parameter values and projected these into PC-space, along with the empirical data. The empirical mSFS fell within the cloud of posterior simulated mSFS, demonstrating the model and chosen parameter values were a good fit to the data (Figure S8).

4 | Discussion

When examining historical responses of eastern North American snakes to climatic fluctuations during the Pleistocene, we find that, as expected, most populations (14 out of 17, 82%) show population size expansions towards the present. The geographic distributions of each of these species are expected to have been restricted during glacial cycles compared to present day, with the most dramatic and obvious example being that the ranges of some populations presently extend into regions that would have been covered by ice sheets at the last glacial maximum (Conant and Collins 1998; Kleman et al. 2010; Marshall, James, and Clarke 2002). Two of the exceptions to this expansion pattern (western *F. abacura*, *F. erytrogramma*) are distributed primarily in the southern USA in regions that were never glaciated, and so may have experienced less severe population contractions and less subsequent opportunity for expansion than populations with more northern distributions. The third exception to the population expansion pattern, western *P. guttatus* complex corresponding to *P. emoryi*, does show a population expansion in recent history; however, it is followed by a more recent sharp population size decline and therefore we did not include it as one of the potentially coexpanding taxa.

We expected a signal of simultaneous population expansion, as the fourteen population expansions we detected are likely responses to the same climatic event in the same general geographic region. However, we found only moderate support for this hypothesis, with PTA estimating only 21%–36% of expanding taxa as showing a signal of temporally congruent coexpansion. The late Pleistocene was characterised by multiple 100,000 year glacial cycles over the span of ~800,000 years, and populations may have responded differently to any of these glacial cycles depending on a number of factors (Abe-Ouchi et al. 2013; Bintanja and van de Wal 2008; Hobart et al. 2023). Although all of the populations we include are distributed in the ENA, they do not have completely overlapping geographic distributions, with some having more southern or eastern distributions than others (Figure 1). These differences may affect the extent to which climatic factors in any given glacial cycle influenced population contraction and expansion. Populations that extend into regions covered by ice during the last glacial maximum could only have expanded into these regions since the retreat of glaciers, whereas more southern populations could have started to expand earlier as southern habitats became more hospitable, even while northern habitats were still glaciated. In each region, the timing of expansion can be further modulated by species-specific responses to environmental conditions. Temperatures did not warm to present conditions instantaneously, and therefore species better able to tolerate environments associated with lower temperatures may have been able to expand earlier into habitats that were still inhospitable for other taxa (Burbrink and Myers 2015). Population size change may also exhibit some lag when suitable habitat becomes available, and this lag will depend on dispersal and reproductive rates, among other factors (Blonder et al. 2017; Hewitt 1996; Sandel et al. 2011).

A role for species-specific responses to climate is supported by our analyses of the influence of present day environmental conditions on genetic distance. In these models, we found broadly varying environmental responses across the nine snake species/complexes. The most consistent response observed was that nearly all species show strong isolation-by-distance in eastern North America. This is an expected pattern in dispersal-limited organisms (Meirmans 2012; Wright 1943) and has been found in many previous snake phylogeographic studies (e.g., Harrington and Burbrink 2023; Harrington et al. 2018; Myers et al. 2019). However, we find varied importances of environmental variables, with no clear patterns, suggesting taxon-specific responses and tolerances. The taxa we have included vary widely in body size, prey base, foraging mode, and other traits. For example, *Diadophis punctatus* is a small (< 40 cm in eastern North America), secretive species that feeds on small vertebrates and invertebrates, whereas *Masticophis flagellum* is a large (typically > 1 m), diurnal species that actively forages for prey including many kinds of vertebrates and invertebrates (Conant and Collins 1998). Variation in these and other organismal traits can lead to different environmental variables being more important for some taxa than others in influencing genetic variation.

A previous study using mitochondrial DNA datasets to examine population coexpansion across multiple eastern North American tetrapod groups, including snakes, found that most snake lineages expanded, but with considerable variance in the timing of expansion, similar to our findings (Burbrink et al. 2016). Burbink et al. (2016) demonstrated that other vertebrate taxa exhibit signatures of expansion in the region, but that the timing of expansions varies particularly widely among higher taxonomic groups (e.g., among birds, mammals and snakes) with very different physiologies and evolutionary histories. At a more restricted taxonomic scale, but wider geographic scale Bai et al. (2018) found wide variation in population size trajectories among species in the walnut genus *Juglans* across temperate regions of the northern hemisphere, including but not limited to North America. In contrast, Kuhn et al. (2022) found synchronous expansion in most lineages across reptiles and amphibians only in the humid rainforest biome of eastern Madagascar, but not in other biomes. Other studies have shown synchronous population expansion across reptiles and amphibians in the Caatinga biome of Brazil (Gehara et al. 2017) and that expansion dynamics in marine turtles vary depending on ocean basin and other traits (Reid et al. 2019). Taken together, these studies and our own results suggest that patterns of expansion may be most likely to be similar in species with broadly similar

physiology and restricted geographic distributions within single distinct biomes. However, while these are potentially necessary conditions for coexpansion, they are not fully predictive for the potential of community responses. For example, unique physiology, specific adaptations, life history, evolutionary history, biogeographic origins and underlying genetic diversity may have unpredictable consequences on population sizes through time when acting synergistically, even among ecologically similar species within the same biome.

Though such trait variation may drive increasing variation in the timing of expansion events, the degree at which such events should be considered synchronous or asynchronous is at some level a function of the temporal scale at which synchrony is evaluated (Gehara et al. 2017). Within the PTA model, a necessary simplification assumes the synchrony of co-expansion is absolute (i.e., instantaneous co-expansion at a single time point). In contrast, our Stairway Plot 2 analyses show population size increases over many thousands of years in some cases (Figure 3). Taking a more biologically relevant approach and sampling expansion times from within a temporal co-expansion 'window' might better capture some of the natural variability in demographic responses, allowing for a more general concept of synchrony. Taking a broad enough temporal view, we could consider all 14 expanding snake lineages in our study as having synchronously expanded during the late Pleistocene, despite PTA results indicating that only up to 36% of the populations expanded at the same time. The average time of expansion of non-coexpanding taxa is ~37 kya, which is not much older than the point estimate of the time of coexpansion at ~32 kya, suggesting that most taxa expanded at similar, but not identical, times. This might indicate that all taxa were responding to the same environmental events occurring over thousands of years. Both of these times predate, but are relatively similar to, the last glacial maximum of ~20 kya (Clark et al. 2009). Additionally, estimates of the timing of past events from genetic data are often subject to even more uncertainty than is present in confidence intervals returned by most methods, as these intervals only include the sources of uncertainty that are built into the models. PTA treats the mutation rate and generation time parameters as fixed values known without error that do not vary across taxa or through time, assumptions that are never fully met (e.g., Baer, Miyamoto, and Denver 2007). Given that the confidence intervals from PTA are likely overly precise, we consider the timing of most expansions to be broadly congruent with the LGM and suggest that most lineages of snakes in eastern North America expanded as climate warmed following the LGM.

5 | Conclusions

We show that there is a consistent pattern of population expansion in populations of eastern North American snakes, as expected given the glacial and climatic history of the region. We show only a moderate degree of synchrony in these expansions, suggesting idiosyncratic responses to glacial cycles through the Pleistocene, rather than a single, concerted response to warming since the last glacial maximum. Consistent with this, we find that the effects of present day environmental variables on genetic distances vary widely across species, suggesting niche and life history variation that may drive different genetic responses to climatic fluctuations. However, timings of expansions are broadly similar across most taxa and roughly correspond to the timing of the last glacial maximum, suggesting the possibility that eastern North American snake taxa have generally expanded as climate has warmed since the LGM. However, estimates of population sizes through time for individual taxa show much longer, protracted expansions that start earlier in the Pleistocene with no discernable relation to glacial cycles. Future studies aggregating data across diverse regions and species could examine the effects of different traits on the degree to which species do or do not coexpand, giving further insight into the mechanisms driving genetic metrics of population size.

Author Contributions

S.H. and F.T.B. designed the study. S.H. and E.A.M. performed lab work. S.H. and I.O. analysed data. All authors contributed to writing the manuscript.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Raw sequence reads generated for this study are available on the NCBI Sequence Read Archive under BioProject ID PRJNA1189052. Note that data for *L. getula* and *L. traingulum* complexes were obtained from previous publications as described in the Methods section. Data files used as input for analyses are all available on Dryad at https://doi.org/10.

5061/dryad.9cnp5hqv5. All scripts used for data processing and analyses are available at: https://github.com/seanharrington256/Coexp_scripts.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.