Journal of Biogeography

WILEY

Journal of Biogeography

RESEARCH ARTICLE OPEN ACCESS

Heterogeneous Evolutionary History Defines the Rear Edge of the North American Herb *Campanula americana*

Antoine Perrier[1] | Keric S. Lamb[1,2] | Laura F. Galloway[1]

[1]Department of Biology, University of Virginia, Charlottesville, Virginia, USA | [2]Department of Integrative Biology, University of California, Berkeley, Berkeley, California, USA

Correspondence: Antoine Perrier (cdt9qe@virginia.edu)

Received: 25 November 2025 | **Revised:** 23 March 2026 | **Accepted:** 31 March 2026

Keywords: *Campanula americana* | eastern North America | glacial refugia | last glacial maximum | phylogeography | postglacial climate change | postglacial range expansion | rear edge | species distribution models | warmer range limit

ABSTRACT

Aim: Warmer range limits of species distributions offer a window into the ecological and evolutionary processes associated with long-term warming, as they often harbour relicts of refugial populations from the last glaciation. However, warm-edge populations vary in their refugial, evolutionary, and climatic histories, complicating their use as models. Here, we characterise this variation at the warmer range limit of the North American herb *Campanula americana*.

Location: Eastern North America.

Taxon: *Campanula americana*.

Methods: We evaluated the warmer range limit using species distribution model hindcasting, range-wide analyses of population structure, effective migration, and phylogenetic relationships.

Results: Warm-edge populations vary in their refugial history; most are relicts of historic refugial populations (i.e., rear edge), but some result from southward postglacial colonisation. Within the rear edge, populations exhibit pronounced genetic structure, forming highly isolated clusters despite contiguous geography and only modest landscape barriers. Distinct rear-edge clusters varied in climate history and gene flow among populations, with more southern populations experiencing greater habitat decline, fragmentation and isolation. Finally, only rear-edge populations from northern refugial areas contributed to postglacial range expansion.

Main Conclusions: Our study highlights the challenges of using warm-edge populations as ecological and evolutionary models of response to climate change. Even in a geographically simple setting, warm-edge populations exhibit refugial, genetic, and climatic heterogeneity. Characterising this variation is essential for accurately identifying rear-edge populations and inferring their evolutionary history. Knowledge of the contribution of warm-edge populations to range-wide genetic variation is key when considering how these populations may inform studies of adaptation to future climate change.

1 | Introduction

Understanding how changes in climate affect species distributions is a central challenge in ecology, evolution, biogeography, and conservation (Aguirre-Liguori et al. 2021; Lenoir and Svenning 2015; Nadeau and Urban 2019; Rubenstein et al. 2023; Urban 2024). This challenge can be addressed by studying how historical processes have shaped range limits (Davis and Shaw 2001; de Lafontaine et al. 2018). In temperate regions, Pleistocene glacial cycles repeatedly drove range contractions into refugia at low latitudes and elevations during cold periods, followed by expansions out of refugia in periods of warming (G. Hewitt 2000; G. M. Hewitt 2004). Much of the current range of temperate species thus reflects postglacial colonisation

This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

© 2026 The Author(s). *Journal of Biogeography* published by John Wiley & Sons Ltd.

("expanded range") after the Last Glacial Maximum (LGM, ~20 kya; Hughes et al. 2013). Warmer range limits often harbour populations that have been exposed to near continuous warming since the LGM, and are thus increasingly being used to study ecological and evolutionary outcomes of warming (Perrier et al. 2026; e.g., Masuda et al. 2023; Nielsen et al. 2024; Song et al. 2021; Tavares et al. 2024). However, these populations may vary in their refugial, evolutionary and climatic histories, both among and within species, and this complexity is underappreciated. Characterising this variation is key for using warmer range limits as models of climate response.

Warmer range limits vary in whether they represent former refugial populations. In temperate species, postglacial range expansion generally occurred by tracking shifts in suitable habitats, expanding from warmer refugia into areas that were previously too cold (G. Hewitt 2000; G. M. Hewitt 2004; Stewart et al. 2009). Contemporary populations at warmer range limits may thus represent descendants of historic refugial populations that persisted more-or-less in place, and that have given rise to populations in the expanded range. These descendants are termed "rear-edge" populations (Hampe and Petit 2005). The identity of rear-edge populations has often been assumed from their geographic position at the warmer latitudinal or elevational limit (Perrier et al. 2026). However, warmer range limits do not necessarily conform to these expectations and may include expansion populations (e.g., Bouchard et al. 2017; Korábek et al. 2023; Willi et al. 2018). The assumption that warm-edge populations represent a rear edge, i.e., ancestral and occurring in refugia, must therefore be tested if they are to serve as models of response to past climate change. Multiple lines of evidence can be used to test this assumption. The location of refugia can be inferred from species distribution model (SDM) hindcasting or from available fossil and pollen records, while ancestry can be inferred from phylogeographic analyses (Gavin et al. 2014).

Rear edges are also not uniform, even within a single species. For example, temperate species may have multiple, geographically distinct rear edges, corresponding to separate refugia isolated by major geographic barriers (e.g., Aradhya et al. 2017; Assis et al. 2016; G. Hewitt 2000; Provan and Maggs 2011; Zeng et al. 2015). Separate refugia create opportunities for independent evolution and genetic divergence. For example, climate history may vary among distinct rear edge regions. Refugia are often thought to have relatively stable climates (Gavin et al. 2014; e.g., Tzedakis et al. 2002), leading to long-term demographic stability and high genetic diversity in rear-edge populations (e.g., Erichsen et al. 2018; Neiva et al. 2020; Piotti et al. 2017). However, the continuous warming since the LGM may result in some rear-edge populations occurring in areas where suitable habitats are rare and of poor quality (Vilà-Cabrera et al. 2019), leading to genetic signatures of demographic decline and long-term isolation (e.g., Masuda et al. 2023; Sielezniew et al. 2015; Song et al. 2021; Tavares et al. 2024). Last, long-term environmental change at the rear edge may result in pronounced local adaptation (Bontrager et al. 2021), with unique adaptations to warming (e.g., Ghouil et al. 2020; Pelletier et al. 2023; Perrier, Turner, and Galloway 2025). Understanding such heterogeneity in evolutionary history is important as it affects the extent to which inferences of vulnerability and adaptive potential of rear-edge populations are broadly applicable under future warming.

Finally, the genetic contribution of rear-edge populations to the expanded region of a species range may vary. Postglacial range expansion carries the assumption that refugial populations are ancestral to populations elsewhere in the range. By extension, the genetic diversity in expanded portions of the range is expected to be a subset of that in refugial populations. However, this may not always be the case. For example, expansion may have had a limited origin, such as from populations at the colder edge of refugia or from cryptic mid-range refugia (e.g., Bemmels et al. 2019; Fernandez et al. 2021; Hantemirova et al. 2017; Lee-Yaw et al. 2008; Rull 2009), with limited contribution from ancestors of populations that now form the rear edge (Hampe and Petit 2005). Alternatively, in species with multiple rear edges, some edges may contribute to expansion while others do not, with variation linked to the presence of geographical barriers. For example, in Europe, high mountain ranges (e.g., Alps, Pyrenees) often divide refugia and expanded areas, limiting recolonisation (G. M. Hewitt 2004; Korkmaz et al. 2014; Petit et al. 2003). Evaluating the contribution of rear-edge populations to postglacial expansion is important for understanding the extent to which the rear-edge's genetic diversity, and therefore evolutionary potential, may be shared with the rest of the range.

Characterising populations at warmer range limits is important if we are to use them to understand response to past warming and to predict change from current warming. In this study, we explore the refugial, phylogeographic and climatic history of the warmer range limit of the North American herb *Campanula americana*. Specifically, we ask (1) Are populations at the warm edge of the range ancestral and located in refugial habitats, indicating rear-edge populations? (2) Does genetic complexity at the rear edge suggest multiple evolutionarily distinct refugia? (3) Are former refugial areas currently suitable habitats or have they become marginal under past warming, and has this affected genetic patterns at the rear edge? (4) What is the contribution of rear-edge populations to postglacial range expansion? We find that rear-edge populations can be highly heterogeneous even in a fairly small and largely contiguous geographic area. Our study provides a framework for evaluating and interpreting the heterogeneity of warm-edge populations and for informing our understanding of how to use them as models of species responses to environmental change.

2 | Materials and Methods

2.1 | Study System

Campanula americana is a monocarpic herb of open forests found in the eastern USA across a broad climatic and geographic range, from the subtropical Gulf coast to the Great Lakes, and from west of the Mississippi to the Appalachian Mountains (Figure 1A). Populations belong to three geographically distinct genetic clades that diverged before the LGM, ca. 0.7–7.0 mya (Barnard-Kubow et al. 2015), that are in contact along the Appalachian Mountains (Lamb et al. 2024). We focus on the larger "Western" clade, comprising most populations of the species (~80%), and largely found at low elevations (< 600 m) west of the Appalachian Mountains. This clade is derived from the older "Appalachian" clade whose distribution is limited to the mountains. The Western

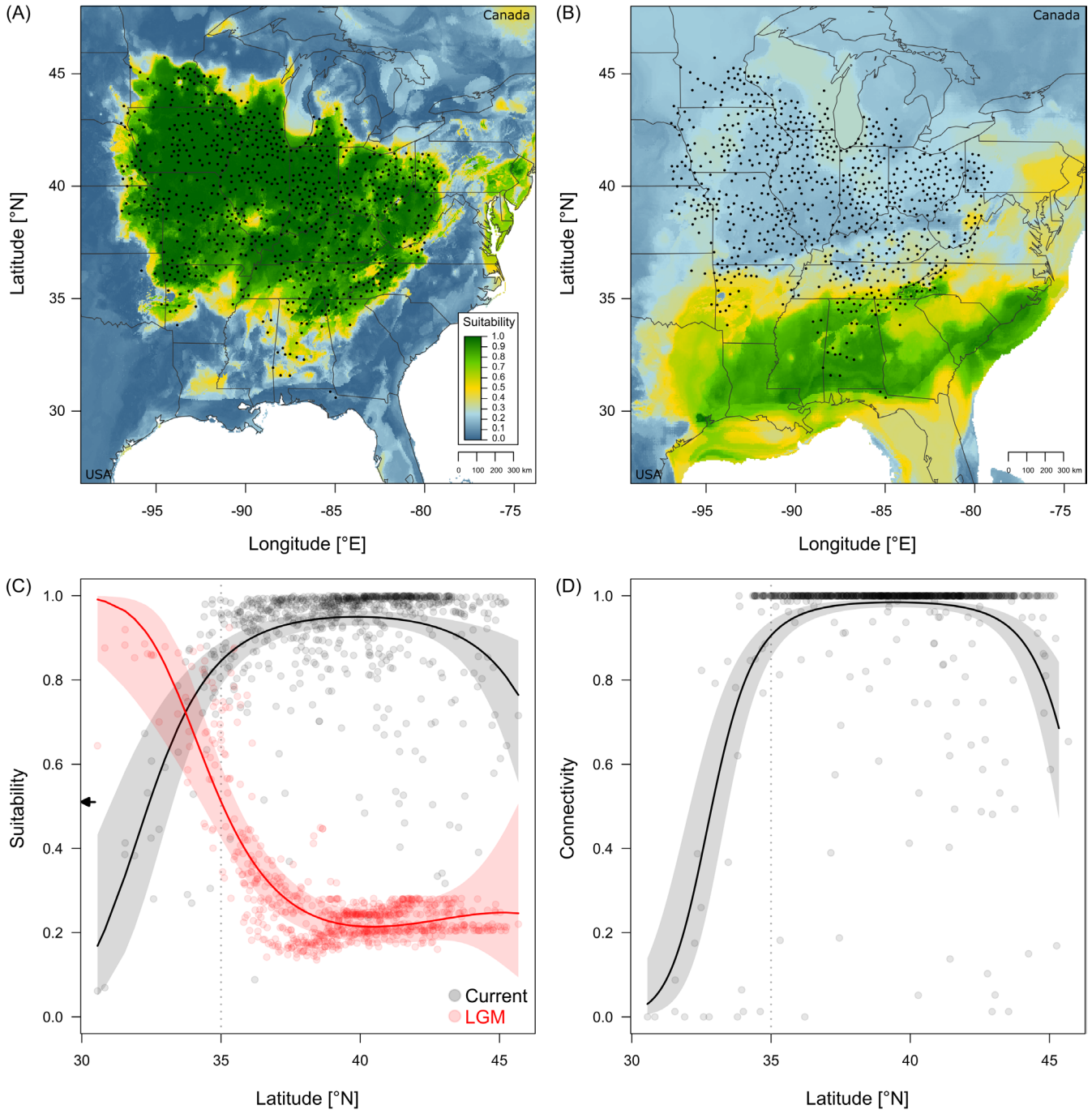


FIGURE 1 | Climatic history of *Campanula americana*. Species distribution model (SDM) showing predicted habitat suitability during the present (A) and the Last Glacial Maximum (B), with occurrences used to build the SDM as black dots. (C) Present-day (grey) and LGM (red) suitability, and (D) present-day connectivity estimated for all occurrences used to build the SDM. Vertical dotted line represents the geographic separation between the rear edge (< 35° N) and the expanded range. Lines represent the significant model-predicted relationship between each estimate and population latitude, with the 95% confidence interval indicated as shading. In (C), the black arrow represents the threshold where present-day suitability best matches presence and absence data. Test statistics reported in Table 1.

clade is characterised by a history of rapid postglacial colonisation with a mid-latitude origin estimated in eastern Kentucky (Barnard-Kubow et al. 2015; Koski et al. 2019; Prior et al. 2020). However, the phylogeography of the warmer range edge is unclear.

We define the warmer edge of *C. americana*'s Western clade as roughly the lower latitudinal third of the range (below 35° N, Perrier, Turner, and Galloway 2025). This area is characterised by subtropical climates, with mild winters and rare freezing events, in contrast to the temperate climate found throughout the rest of the range. Warm-edge populations harbour unique phenotypes, including populations that have lost the vernalisation requirement for flowering (Perrier, Turner, and Galloway 2025). Warm-edge populations broadly overlap with refugial areas and contain chloroplast haplotypes that, while not

TABLE 1 | Test of variation in present-day and past habitat suitability and connectivity across the latitudinal range of *Campanula americana*.

		Latitude					
Dependent variable	***N***	β_{linear}	β_{square}	β_{cubic}	χ^2		R^2
LGM suitability	763	−12.86	12.03	−4.02	56.08	***	0.35
Present-day suitability	763	6.65	−14.24	—	33.55	***	0.17
Present-day connectivity	763	1.67	−27.33	9.43	74.12	***	0.30

Note: Dependent variables were tested assuming binomial distribution. Test statistics include the slope of the effect of latitude (β) estimated for the linear, square and, if applicable, the cubic term (Table S2.1), the χ^2 value, and the McFadden's pseudo-R^2 of each model.
***$p < 0.001$.

unique to this area, are ancestral to the clade (Barnard-Kubow et al. 2015), suggesting these populations are the rear edge of the species distribution.

2.2 | Species Distribution Model (SDM)

We created a SDM of populations in *C. americana*'s Western clade to identify potential glacial refugia though hindcasting (Q1) and to assess how these habitats have changed under postglacial warming (Q3). We first generated a SDM based on contemporary climates using Random Forest with the package *ranger* (Wright and Ziegler 2017) in R (R Core Team 2025). The model was based on 763 occurrences obtained from iNaturalist (Figure 1A, www.inaturalist.org), and a 30 year average of 19 bioclimatic variables obtained from WorldClim 2.1 (1970–2000; Fick and Hijmans 2017). See Appendix 1 Method S1.1 in Supporting Information for details.

We used this SDM to develop a geographic projection of areas likely to have suitable climates based on the climate of present-day occurrences (WorldClim 2.1; Fick and Hijmans 2017). Suitability is defined as the relative likelihood a species will be present at a location based on the climate, and ranges from 0 (unsuitable) to 1 (suitable). We defined suitable habitat for *C. americana* as areas with a model predicted suitability ≥ 0.51. This value is the suitability threshold at which binarised SDM predictions best match presence-absence data used to generate the SDM (Method S1.1). It was calculated using the *optimal.thresholds* function from the R package *PresenceAbsence* (Freeman and Moisen 2008). We assessed suitability across the range during the LGM to identify putative refugia (Q1) by geographically projecting the SDM using downscaled paleo-climate data (i.e., hindcasting) as input (WorldClim 1.4, https://www.worldclim.org/data/v1.4/paleo1.4.html, accessed 04/11/2025). The location of putative glacial refugia was inferred as areas with suitable habitats during the LGM. Geographic projections of present-day and LGM suitability were then visually compared to assess differences in past and present suitability across the range (Q3).

We then estimated three habitat quality metrics for each iNaturalist occurrence from the SDM projections: LGM suitability, present-day suitability, and connectivity. Connectivity is a measure of habitat marginality and was estimated as the proportion of suitable habitat in a 25 km radius circle around each iNaturalist occurrence, and ranges from 0 (no suitable habitats, i.e., low connectivity) to 1 (full area is suitable, i.e., high connectivity). We tested for differences in LGM suitability, present-day suitability, and present-day connectivity across the warm range edge and the expanded range in separate linear models, using latitude as a proxy for range position. Preliminary analysis tested whether latitude was best described by a linear, second-degree or third-degree quadratic relationship (the latter allows variables to vary at both high and low latitudes). Results are reported for the best model based on AICc scores (Appendix 2 Table S2.1, Sugiura 1978). If multiple models performed equally well ($|\Delta AICc| \leq 2$), the simplest model was chosen. Dependent variables were tested assuming binomial distribution. For each model (and in all subsequent analyses), we tested and confirmed model assumptions.

2.3 | Sequencing and Genotyping

To determine phylogenetic relationships among populations (Q1, Q4) and population structure (Q2, Q3), we analysed sequence data from 5 individuals in each of 36 populations (four for OK61, 179 individuals total, Figure S2.1C, Table S2.2). These spanned the whole range of the Western clade of the species, excluding the Appalachian Mountains where gene-flow among clades may blur phylogeographic patterns (Lamb et al. 2024). We also included five individuals of Appalachian-clade population VA73 to serve as an outgroup in phylogenetic analyses. Sequence data was generated using Restriction-site Associated DNA sequencing (RAD-Seq; Andrews et al. 2016; Baird et al. 2008) as part of a larger project described in Perrier, Keenan, et al. (2025; Bioproject PRJNA1306192). Details of the plant material, DNA extraction, library preparation, and sequencing are provided in Method S1.2. In short, DNA extraction was performed by the Genomics & Cell Characterisation Core Facility at the University of Oregon (Eugene, OR, USA). Library preparation and sequencing was performed by Floragenex, INC (Beaverton, OR). Raw reads were processed into single nuclear polymorphism (SNP) calls following a modified STACKS v2 pipeline (Rochette et al. 2019) and using a haploid reference genome (Lopez-Caamal et al. unpublished data). The species is an old autotetraploid that has undergone cytological diploidisation (Lopez-Caamal et al. unpublished data.); prior phylogeographic studies have treated it as diploid (Barnard-Kubow et al. 2015; Koski et al. 2019; Prior et al. 2020; Lamb et al. 2024). Genotypes were thus called in diploid variant call format (VCF). SNPs were filtered for missingness (90%), minor allele count (5), sequencing depth (20×–70×), and indels and linkage, resulting in a final set of 40,195 SNPs.

2.4 | Phylogeny and Population Structure

We assessed phylogenetic relationships among populations by constructing a phylogenetic tree with the Appalachian population VA73 as an outgroup using TreeMix v1.12 (Pickrell and Pritchard 2012). TreeMix was chosen over other population-level phylogenetic methods to account for the admixture identified in the population structure analysis (see below). TreeMix was run assuming one to 10 migration events (m). The optimal number of migration events was chosen as the m with the highest second-order rate of change (Method S1.2; Evanno et al. 2005). For this best m, TreeMix was run again with 100 bootstraps and visualised following the workflow of the *BITE* package in R (Milanesi et al. 2017). Higher m values only marginally improved the model fit (Methods S1.2) and were not investigated.

We inferred the genetic structure across 36 populations by determining individual-level genotype clustering using sparse nonnegative matrix factorisation (sNMF) implemented in the *LEA* package in *R* (Frichot and François 2015). The sNMF was run assuming one to 36 clusters (K, details in Method S1.2). Individual-level admixture, the proportion of an individual's genome assigned to genetic clusters, was generally consistent within a population (Figure S2.1B). We therefore focused on population-average admixture and assigned a main genetic cluster to each population (Method S1.2, Table S2.2).

We characterised geographic patterns of gene flow to complement the population structure analysis by estimating effective migration surfaces (Petkova et al. 2016) implemented in the python package *feems* (Marcus et al. 2021; Method S1.2). This method identifies geographic areas where gene flow between populations is higher or lower than expected under isolation-by-distance. It provides a spatially explicit visualisation of population structure, allowing for identification of geographic barriers and corridors of gene flow.

To infer the colonisation history of the species, we visually compared the results of the three phylogeographic analyses with the distribution of past and present-day suitability. We also compared these results with large geographical features across the range that could serve as barriers to infer their potential impact on colonisation history and population structure.

3 | Results

3.1 | Identification of Glacial Refugia

The SDM was a good match for the current range of the species (Figure 1A, Method S1.1), with a small error rate of 7.08% false negatives and 2.30% false positives, indicating it captures the climatic niche of the species well. Therefore, the SDM is appropriate for hindcasting the niche of the species to the LGM. Hindcasting the SDM suggests that the contemporary range above ~35° N was unsuitable during the LGM (Figure 1B). Populations in this area therefore likely established after the LGM, tracking shifts in suitable habitats. In contrast, the lower latitudinal range, below ~35° N, overlaps with an area of high LGM suitability extending from the northern Gulf coast up to the southern Appalachians, indicating it may have served as refugia during the LGM. Contemporary populations at *C. americana*'s warmer range edge occupy putative former refugial areas and thus likely represent remnants of refugial populations that have persisted more-or-less in place until the present day. LGM habitat suitability showed a significant third-degree quadratic relationship with latitude (Tables 1 and S2.1). Suitability was greatest at low latitudes and declined rapidly towards higher latitudes, with LGM habitats becoming unsuitable (<0.62 suitability) above ~34.5° N (Figure 1C), supporting inference from geographic projections.

3.2 | History of Postglacial Climate Change

Warmer range-edge habitats have deteriorated since the LGM. The SDM projection indicates that most populations across the species' distribution currently occur in suitable habitats, while southern parts of the range are less suitable and increasingly fragmented (Figure 1A). In line, present-day habitat suitability and connectivity both had significant negative quadratic relationships with latitude (second-degree and third-degree, respectively, Tables 1 and S2.1), with the highest values at mid latitudes and sharp declines towards low latitudes, particularly below 35° N (Figure 1C,D), and to a lesser extent at high latitudes. This relationship predicts that contemporary habitats become unsuitable (<0.51 suitability) below ~33° N. Only a small area along the northern edge of the putative refugia retains suitability today (Figure 1A,B).

3.3 | Phylogeography of the Rear Edges of *C. americana*

Most warm-edge and refugial populations belong to a distinct early-diverging clade. For the best migration ($m=1$) and topology, the phylogenetic tree largely recapitulated geography and shifts in habitats between the LGM and present-day. Populations belong to two major and geographically distinct clades (Table S2.2, Figure 2A). The "Refugia" clade is largely composed of populations occurring in putative refugia identified in the SDM analysis, except for the most basal one, TN2, occurring just beyond the northern edge of the refugia. In contrast, most populations of the "Expansion" clade occur in areas unsuitable during the LGM. The exception, OK61, occurs in the refugial area just south of the Ozark mountains, and is one of the most derived expansion populations, suggesting relatively recent recolonisation of refugial habitats. The overall topology and the split between the Refugia and Expansion clades was well supported by bootstraps. Within each clade, bootstrap support varied and was particularly low for the basal branches of the Refugia clade.

Warm-edge populations have substantial genetic structure. Range-wide genetic variation was best partitioned into five genetic clusters in the population structure analysis (best $K=5$, best run $=3$, Method S1.2, Figure S2.1A). The genetic clusters are geographically structured (Figure 2B, Table S2.2). Refugial populations in the southeastern part of the range belong to two "Refugia" clusters separated longitudinally (Refugia W, Refugia E). Further north, populations belong to a "Core" cluster that spans the putative refugial habitat and the expansion region of the range. Populations in the expanded western part of the range belong to two clusters, the large Expansion N cluster in the northwest and the smaller Expansion S cluster in the southwest of the range. Patterns of population structure generally recapitulated the phylogeny (Figure 2A).

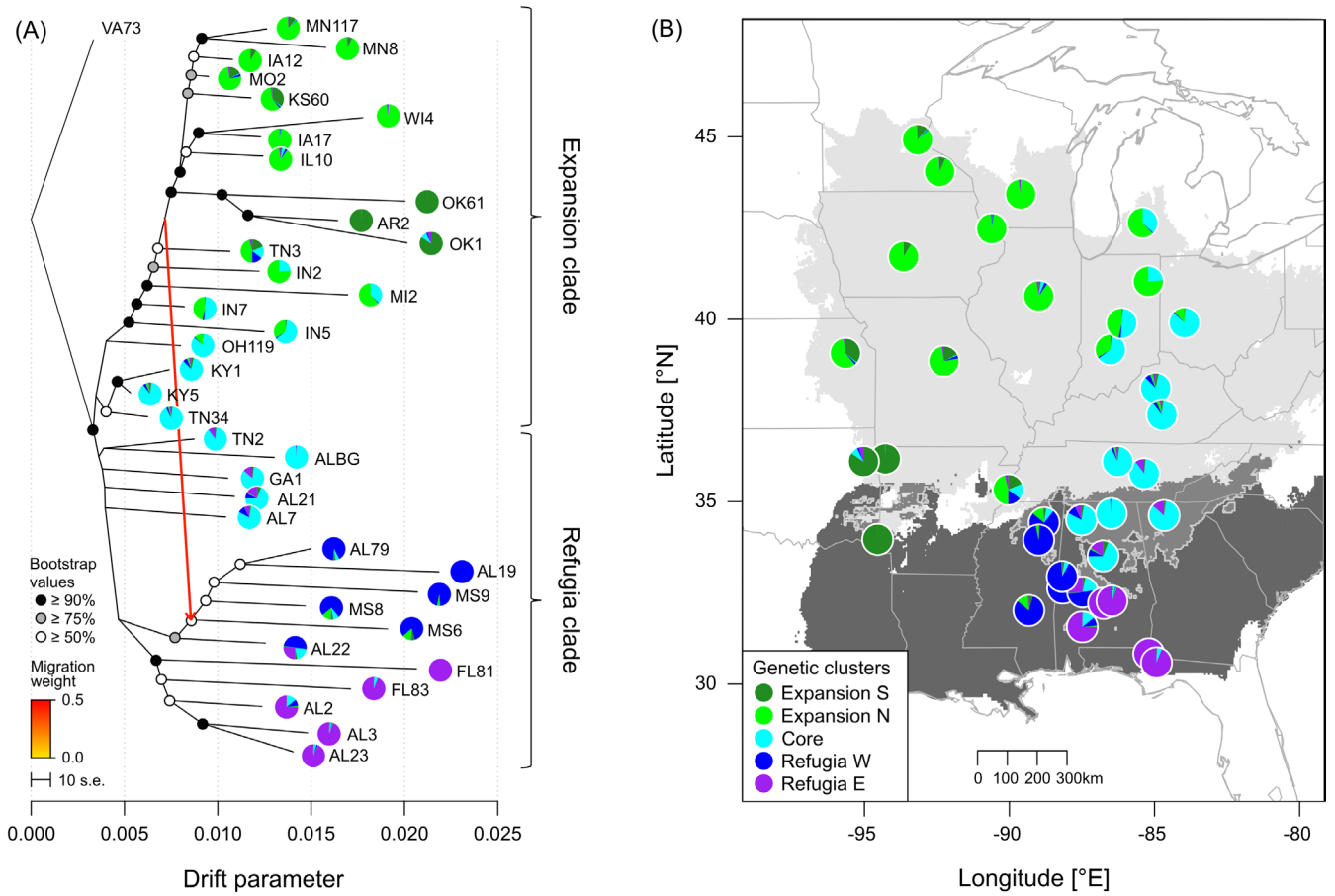


FIGURE 2 | Phylogeography of *Campanula americana*. (A) Phylogeny based on nuclear data (VA73 as outgroup) with pie chart colours indicating genetic clusters and polymorphism within populations. Brackets detail which populations belong to the Expansion and Refugia clade. Migration event and its inferred direction are displayed as a coloured arrow (see scale). The bootstrap value of each branch is indicated for values ≥ 50%, estimated from 100 bootstraps. The scale of 10× the standard error for the drift parameter is indicated. (B) Sequenced populations with genetic clustering as in (A). Light grey shading represents contemporary suitable habitat, dark grey represents the location of putative refugia, with intermediate hues representing areas suitable both during LGM and today.

The "Refugia" clade is largely composed of populations from the two Refugia clusters, while the "Expansion" clade is mainly composed of populations from both Expansion clusters. Populations from the Core cluster are basal in both clades with the five southernmost populations at the base of the Refugia clade, and the six northernmost populations at the base of the Expansion clade. Both clades show substructure reflecting genetic clusters, with populations from the Expansion S and from the Refugia E and W forming distinct sub-clades.

Most refugial populations show high isolation among and within genetic clusters. The effective migration analysis identified limited migration between the clusters characterised by the population structure analysis (Figure 2C), with all clusters separated by areas of low gene flow. Within the Expansion clusters, populations were connected by areas of high gene flow, except for the northeasternmost population (MI2) in Expansion N. Core populations were also generally well connected, except for the three southernmost. In contrast, the two Refugia clusters showed reduced gene flow between populations within clusters.

Admixture between genetic clusters was common and largely reflects geographic proximity and ancestry (Figure 2B). Populations across the range showed large variation in admixture between genetic clusters, with the cumulative proportion of genetic variation assigned to secondary clusters ranging from 0.00 to 0.54 (mean = 0.17, Method S1.2). Admixture was homogeneous at the individual level (Figure S2.2B), suggesting potential gene exchange is old and not ongoing. Highly admixed populations mostly formed between geographically and phylogenetically close clusters and occurred at the geographic boundaries between clusters (Figure 2B). These patterns were also found in the effective migration analysis, evidenced by small breaks in the areas of low gene flow between clusters (Figure 3A). One notable exception was admixture between southern populations of the Expansion N (TN3) and S clusters (OK1) and three populations of the Refugia W cluster (MS6, MS8, MS9; Figure 2B). This was also identified in the phylogeny as a large admixture event from the Expansion clade into the Refugia clade (Figure 2A).

3.4 | Link Between Phylogeographic Patterns and Landscape Features

Phylogeographic patterns partially coincide with the extent of the LGM ice sheets. The three most basal populations of the Expansion clade (Core cluster, KY1, KY5, TN34; Figure 2A) are located just south of the ice sheet (Figure 3B) and form a

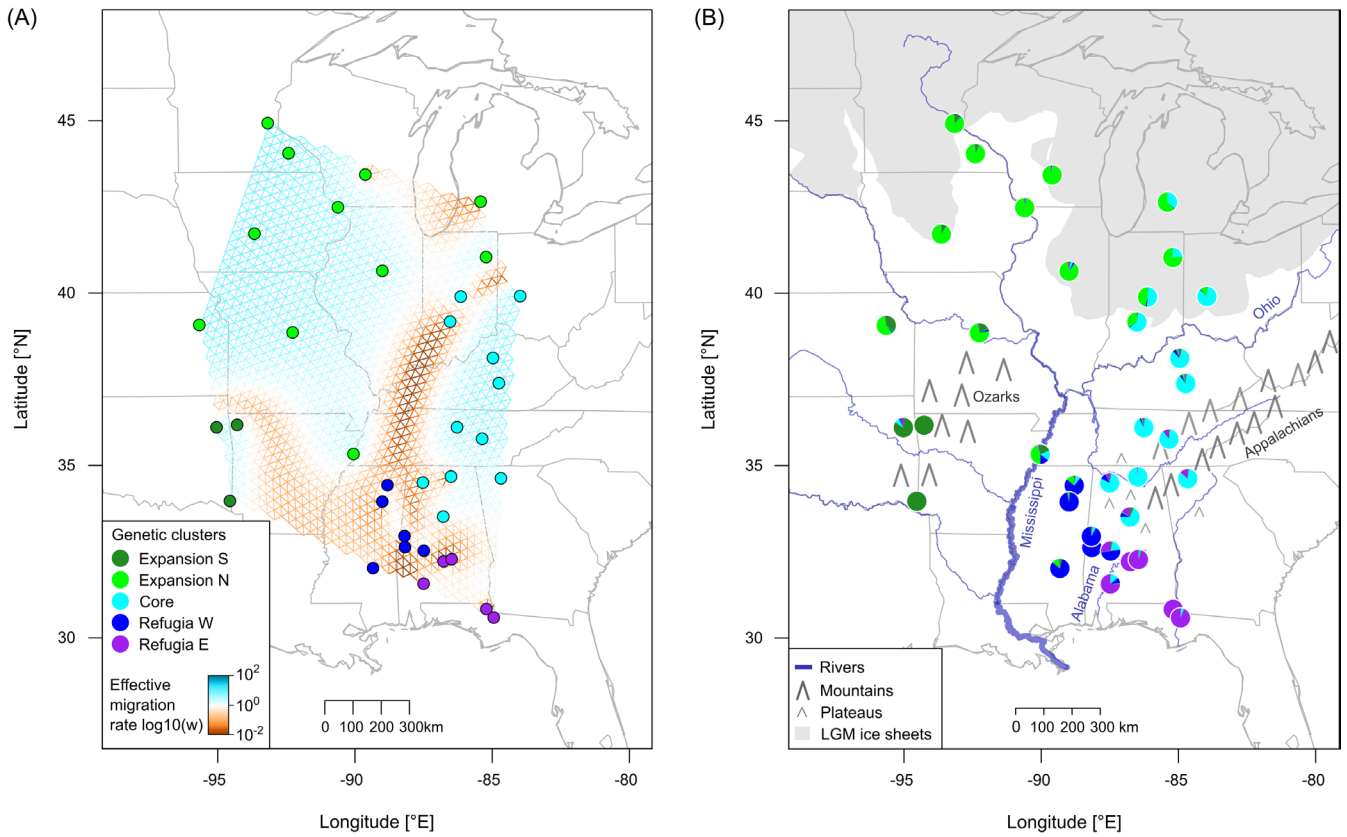


FIGURE 3 | Gene flow and geographic features across the range of *C. americana*. (A) Estimation of the effective migration surface between populations with populations coloured by main genetic cluster (as in Figure 2). Areas where gene flow is lower than expected under isolation-by-distance are represented in brown; areas where gene flow is higher are in blue. (B) Location of the sequenced populations, with pie chart colours indicating genetic clusters and polymorphism within populations (as in Figure 2). Depicted geographic features include the extent of the ice sheets during the Last Glacial Maximum (grey shading), major rivers crossing the sampled range (blue), and relevant mountains (grey triangles).

distinct subclade. In contrast, the next most derived populations along the phylogeny occur just to the north in areas covered by ice sheets during LGM (Figure 3B), suggesting range expansion after the retreat of the ice sheets.

Landscape features align with phylogeographic boundaries in the expanded range, but less so at the warmer range edge (Figure 3B). The Ohio and the lower Mississippi rivers separate both Expansion clusters from the rest of the range. The Expansion S cluster is partially separated from the Expansion N cluster by the Ozark Mountains. The Refugia clusters are separated by the Alabama river. In contrast, the Refugia and the Core clusters are not separated by defining geographic features, though they differ in physiographic regions. The southern Core populations occur in mountainous areas including the southern reaches of the Appalachian Mountains and uplands such as the Cumberland plateau and the southern Appalachian Piedmont, while Refugia population clusters occur in the Coastal plains.

4 | Discussion

4.1 | Refugial Clades and Southward Expansion Lead to a Complex Warmer Range Limit

The genetic composition of species' warmer range limits may be heterogeneous, including populations that vary in their refugial, phylogeographic, and climatic history. Most are expected to be rear-edge populations, relicts that persist in glacial refugia and are ancestral to populations derived from postglacial expansion. However, warmer edges and rear edges of species distributions are often conflated (Perrier et al. 2026), with "rear edges" identified by geographic position (i.e., equatorial edge), climate (i.e., warm edge), refugial overlap or phylogeographic history (i.e., basal). Depending on which criterion, or combination of them, is applied, different populations may be designated as the rear edge. We found that most populations at *C. americana*'s warmer range limit likely represent persistent refugial populations, i.e., stable rear edges (*sensu* Hampe and Petit 2005). Hindcasting SDM revealed that contemporary populations within the geographic rear edge (< 35° N) largely occur in putative LGM refugia along the northern Gulf Coast. These refugia are consistent with earlier findings in the species (Barnard-Kubow et al. 2015) and with proposed refugia for other Eastern North American (ENA) plants and animals (Lyman and Edwards 2022; Soltis et al. 2006; White et al. 2026), particularly species with distributions similar to *C. americana* (Bemmels and Dick 2018; Mohn et al. 2021; Walker et al. 2009; Wang et al. 2018; Wyatt et al. 2021; Xia et al. 2024). Populations occurring in putative refugial areas and east of the Mississippi also form a distinct Refugia clade that appears to have diverged early from populations restricted to areas unsuitable during the LGM. The combination of deep phylogenetic divergence and occurrence in putative long-term refugia provide evidence, albeit indirect,

supporting the inference that these warm-edge populations represent *C. americana*'s rear edge.

Postglacial recolonisation can lead to heterogeneity in warmer range limits, even among populations in refugial areas. For example, the southwestern OK61, although located in a putative refugial area, derives from northern populations of the Expansion clade, suggesting post-LGM recolonisation of refugia. Therefore, this population likely does not represent a rear edge. Unlike the Refugia clusters, it inhabits a relatively small putative refugial area west of the Mississippi. While postglacial expansion into warmer areas may seem counterintuitive, similar cases have been reported across the globe, including plants in ENA (Bouchard et al. 2017; Willi et al. 2018) and land snails in Europe (Korábek et al. 2023). We identified additional genetic signatures of the Expansion clade in refugial areas through an admixture event from the southern reaches of *C. americana*'s Expansion clade into the western-most populations of the Refugia clade. Together, these findings indicate that populations in warmer range limits are not always ancestral, even if they occur in putative refugia. Our study therefore highlights the limitation of using only SDMs to infer refugial history and underscores the importance of testing the assumption that warm edges are synonymous with rear edges with multiple lines of evidence.

4.2 | Multiple Contiguous but Isolated Refugia Lead to High Rear-Edge Population Structure

The rear edge of *C. americana* is highly structured despite occurring in a relatively restricted geographic area with limited topographic heterogeneity. It is composed of three largely independent genetic clusters—the southern populations of the Core cluster and the Eastern and Western Refugia clusters. These clusters are highly differentiated and isolated from one another, despite geographic proximity, suggesting that populations were confined to three independent refugia with limited gene flow among them during the LGM. In contrast, most of the expanded region of the Western clade has little structure and is occupied by a single cluster (Northern Expansion) with the Southern Expansion cluster modest in size.

Modest geographic features appear to have contributed to isolation between the distinct yet neighbouring rear-edge clusters. The Alabama River is a possible barrier separating the Refugia clusters. However, populations along this border show some admixture suggesting only partial isolation from the river. Rivers in this region experienced substantial changes during the late Pleistocene (Leigh 2006) that likely affected their capacity to act as a barrier. The separation of the southern Core cluster populations from the refugia clusters may be due to local topography. Habitats at the southern extent of the Core cluster consist of plateaus separated by deep valleys (e.g., Cumberland Plateau, Ridge and Valley, Appalachian foothills). Limited gene flow among the southernmost populations of the Core cluster suggests these physical features contributed to local isolation. In contrast to these modest geographic barriers, the genetic structure of the expanded range appears to have been shaped by more prominent present and past geographic features. The Ohio River and the southern extent of the LGM ice sheet overlap with the Core–Northern Expansion break, and the Ozark Mountains with the Northern–Southern Expansion break, suggesting these features as potential barriers.

Multiple genetically distinct and isolated rear edges are not uncommon. However, they typically involve well-separated refugia, divided by large distances or prominent landscape features. European species with refugia isolated in distinct Mediterranean peninsulas are classic examples (Havrdová et al. 2015; Jiménez-Mejías et al. 2012; Korkmaz et al. 2014; Petit et al. 2003; Svenning et al. 2008). In ENA, major landscape features such as the Mississippi river and the Appalachian Mountains have also been found to isolate refugia, but typically have been associated with breaks between distinct clades that diverged well before the LGM (Lyman and Edwards 2022; Soltis et al. 2006). In contrast, our study found the development of strong rear-edge genetic structure within a single clade and in a restricted geographic area, even in the absence of large-scale geographic barriers. This pattern is unusual, especially at such a small geographic scale and suggests greater attention is needed to the phylogeography of rear edges.

4.3 | Rear-Edge Populations Are Isolated

Populations in the rear edge are more isolated from each other than populations elsewhere in the range. At the rear edge, gene flow is limited among populations within clusters relative to expectations under isolation by distance. In contrast, populations in the Expansion clade, including northern Core populations and the Expansion clusters, have high levels of gene flow within clusters. The difference is particularly striking given that rear-edge populations were sampled more densely than the rest of the range. In the Expansion clade, substantial gene exchange within clusters but limited gene exchange between clusters that arise sequentially along the phylogeny suggests a serial range expansion process, with infrequent crossings of major geographic barriers and largely unobstructed gene flow within regions. This history contrasts with the rear edge, where populations did not experience range expansion and there are few barriers to explain isolation among populations within clusters. This pattern suggests other mechanisms underlie the high isolation at the rear edge.

Sustained postglacial warming over long periods of time may have contributed to increased isolation among rear-edge populations. While SDM hindcasting suggests that rear-edge habitats were once almost uniformly suitable for *C. americana*, present-day SDM projections indicate a steep reduction in habitat suitability, especially towards the southern range limit where the scarce patches of suitable habitat have low connectivity. This is expected to lead to small, isolated populations, expectations supported by the limited gene flow among populations in the southern clusters relative to expectations of isolation by distance. Therefore, progressive habitat decline and fragmentation under postglacial warming may have contributed to long-term isolation among rear-edge populations despite their geographic proximity. This pattern may be common in temperate taxa where rear-edge populations frequently occur in ecologically marginal habitats (Perrier et al. 2026; Vilà-Cabrera et al. 2019), near species' thermal and xeric physiological limits (Cahill et al. 2014).

Not all rear-edge habitats have been equally affected by postglacial warming. The northern part of the refugium has higher predicted contemporary suitability and connectivity than the southern portion, suggesting more modest postglacial warming with more habitat remaining within *C. americana*'s environmental tolerance. In line, northern refugial populations also show somewhat less isolation than more southern rear-edge populations where predicted suitability and connectivity are low. Variation in evolutionary response to past warming has been documented across several taxa, but often only among geographically distinct rear edges (e.g., Havrdová et al. 2015; Neiva et al. 2014; Provan and Maggs 2011). We find within a single, contiguous refugial region that rear-edge populations can differ in their climatic histories, with lasting consequences for patterns of genetic variation among populations.

4.4 | The Contribution of the Rear Edge to Range Expansion

A subset of rear-edge populations contributed to postglacial range expansion in *C. americana*. Only the southern populations in the Core cluster share ancestry with the expanded range, suggesting they served as the source of range expansion. In contrast, the Refugia clusters do not appear to have contributed to range expansion. While heterogeneity in the contribution to range expansion among rear-edge populations is common (Hampe and Petit 2005), it is typically found among geographically distinct rear edges (G. Hewitt 2000; Lee-Yaw et al. 2008; Petit et al. 2003; Zeng et al. 2015), where the presence or absence of geographical barriers limiting poleward range expansion is associated with the ancestry of populations in the expanded range (Korkmaz et al. 2014; Petit et al. 2003). Unglaciated ENA lacks comparable barriers, and most geographical features that have been associated with phylogenetic breaks run north to south (Lyman and Edwards 2022; Soltis et al. 2006). This suggests a relatively permissive corridor for range expansion (Bemmels and Dick 2018; Park and Donoghue 2019), with uniform expected contributions throughout the rear edge. Despite this, we find that rear edges may be composed of populations that differ in their contribution to the genetic composition of the expanded range and that this heterogeneity may be observed even among neighbouring populations.

The origin of postglacial expansion remains unclear in *C. americana*. Populations in northern, formerly unsuitable habitats, form a monophyletic clade, with northern and western populations at its tips and central populations in the east at its base (Tennessee, Kentucky), consistent with rapid westward range expansion from a mid-latitude origin (Koski et al. 2019; Prior et al. 2020). However, the predicted colonisation origin is north of the presumed refugia. A possible explanation for an origin in apparently unsuitable habitat is northern microrefugia, not identified by the SDM (see discussion in Prior et al. 2020). Postglacial colonisation from microrefugia in colder areas than main refugial habitats is common among temperate taxa (Gavin et al. 2014; Rull 2009; e.g., Fernandez et al. 2021; Hantemirova et al. 2017), including ENA species (Peterson and Graves 2016). Alternatively, the shared ancestry between northern Refugia-clade populations and Core-cluster populations that are basal to the Expansion clade implicates northern parts of the refugia as the origin of range expansion. In this case, the mid-latitude origin of the Western clade would represent a secondary expansion. In species with multiple refugia, greater contribution to postglacial colonisation from colder refugia has also often been reported (e.g., Lee-Yaw et al. 2008). Our findings indicate that colonisation from colder areas may also hold in the case of geographically contiguous refugia.

Which Western-lineage *C. americana* populations are oldest? In species or lineages that experienced postglacial range expansion, the oldest populations should be found at the rear edge (Hampe and Petit 2005; Park and Donoghue 2019). However, in contrast to the expected south-to-north gradient of ancestry (Barnard-Kubow et al. 2015), the base of *C. americana*'s Western clade is nested within the Core cluster in the phylogeny. The Core cluster populations are broadly located in the southern Appalachian Mountains where the Western clade of the species is thought to have emerged during the mid-Pleistocene (0.7–2.3 mya; Barnard-Kubow et al. 2015). One possibility is that the Core cluster is the closest to early Western populations, and that southern rear-edge populations represent an early split from this ancestor during (or before) LGM. However, the low bootstrap support of the split between southern core populations and the two refugial clades limits inference of this history. Poor bootstrap support of southern core populations also suggests that incomplete lineage sorting may obscure a clear phylogenetic signal. This is especially likely if populations in the core remained large and connected (Maddison and Knowles 2006). Indeed, we found high gene flow among populations in the core of the range, where refugial populations occur in climates that are also suitable today, suggesting demographic stability through time. Overall, these phylogenetic patterns highlight that rear edges differ not only in their contribution to range expansion but also in their ancestry, and suggest that distinct demographic and climatic histories contribute to their phylogenetic complexity.

4.5 | Integrating Rear Edge Complexity in Models of Climate Response

Characterising the multiple dimensions of variation in rear-edge populations is key to using them as ecological and evolutionary models. We found that not all warm-edge populations represent rear edges. Distinguishing evolutionary history is important because populations in refugia that result from recent range expansion may have reduced diversity due to serial founder events and bottlenecks relative to populations that persisted in the rear edge since the LGM (Excoffier et al. 2009). Similarly, populations with a long history of postglacial warming may have evolved adaptations to the warm climate while more recent arrivals may be less well adapted or have a different set of adaptations. Further, gene flow from populations in the expanded range into the rear edge may reduce fitness, swamping adaptation to warm climates, or increase fitness by providing novel variants (Aguirre-Liguori et al. 2021; Kottler et al. 2021; Sexton et al. 2011). Finally, the substantial genetic structure of the rear edge, with genetically distinct populations and clusters, provided the opportunity for independent evolution in response to postglacial warming, yielding possible distinctive adaptive

outcomes (e.g., Havrdová et al. 2015; Neiva et al. 2014; Provan and Maggs 2011). With this breadth of variation, assessing the diversity of the rear edge is key to using them as models of response to warming climates and to infer their potential contribution to ongoing change.

Finally, we found that not all rear-edge populations contribute equally to postglacial range expansion, even among neighbouring populations. Rear-edge populations often exhibit strong local adaptation (Bontrager et al. 2021), with unique traits that enable persistence under warming climates (e.g., Ghouil et al. 2020; Pelletier et al. 2023; Perrier, Turner, and Galloway 2025). If the underlying genetic variation is shared across the range, these adaptations could facilitate response to future warming in northern populations. However, if rear-edge populations did not contribute to range expansion, their adaptive variation may be regionally confined and contribute little to range-wide responses. For example, in *C. americana*, warmer winters reduce reproductive success in northern populations but have little effect on fitness in southern rear-edge populations (Perrier, Turner, and Galloway 2025). The limited contribution of these southern rear-edge populations to the expanded range raises concerns about the capacity of more northern populations to adapt to future warming. Identifying which populations historically contributed to range expansion, and which did not, will be essential for using rear-edge populations as models of range-wide responses to climate change.

Author Contributions

All authors contributed to the study design. K.S.L. built the SDM, and A.P. analysed the SDM output. K.S.L. and A.P. collected seeds in the field and raised plants for sequencing. A.P. prepared samples for sequencing, processed, and analysed the sequencing data. A.P. wrote the manuscript with input from L.F.G. and K.S.L.

Acknowledgements

This work was supported by the Swiss National Foundation (P2BSP3_195363), the National Science Foundation (NSF DEB-2140189), and the University of Virginia College of Arts and Sciences. For their help in raising plants, we thank C. Claussen, S. Cox, O. Keenan, A. López, H. Makowski, and M. Turner. We are also grateful to D. Brown, J. Collins, A. Diamond, L. Elliott, K. England, E. Galloway, F. Griffith, I. Guenther, J. Hansen, B. Hoagland, H. Horne, J. Kees, M. Kohout, R. Laporte, L. Michael, D. Reed, and B. Sutherland for seed and leaf material collection in natural populations. Collection permits were provided by the Florida Department of Environmental Protection (08312111). Collections were authorised by the Tennessee Department of Environment and Conservation and Missouri Department of Conservation. No permits were required for other localities.

Funding

This work was supported by Schweizerischer Nationalfonds zur Forderung der Wissenschaftlichen Forschung, P2BSP3_195363. National Science Foundation, NSF DEB 2140189. University of Virginia College of Arts and Sciences.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Following data is available on Zenodo (https://doi.org/10.5281/zenodo.19390090): Presence-absence data to generate the SDM, raster layers of Present and LGM projections of the SDM, suitability and connectivity data derived from these projections, list of sequenced samples, individual genetic cluster assignments, the code to filter the VCF and the final VCF. Read data are publicly available in the Sequence Data Archive (BioProject PRJNA1306192).

References

Aguirre-Liguori, J. A., S. Ramírez-Barahona, and B. S. Gaut. 2021. "The Evolutionary Genomics of Species' Responses to Climate Change." *Nature Ecology & Evolution* 5, no. 10: 1350–1360. https://doi.org/10.1038/s41559-021-01526-9.

Andrews, K. R., J. M. Good, M. R. Miller, G. Luikart, and P. A. Hohenlohe. 2016. "Harnessing the Power of RADseq for Ecological and Evolutionary Genomics." *Nature Reviews Genetics* 17, no. 2: 81–92. https://doi.org/10.1038/nrg.2015.28.

Aradhya, M., D. Velasco, Z. Ibrahimov, et al. 2017. "Genetic and Ecological Insights Into Glacial Refugia of Walnut (*Juglans regia* L.)." *PLoS One* 12, no. 10: e0185974. https://doi.org/10.1371/journal.pone.0185974.

Assis, J., N. C. Coelho, T. Lamy, M. Valero, F. Alberto, and E. Á. Serrão. 2016. "Deep Reefs Are Climatic Refugia for Genetic Diversity of Marine Forests." *Journal of Biogeography* 43, no. 4: 833–844. https://doi.org/10.1111/jbi.12677.

Baird, N. A., P. D. Etter, T. S. Atwood, et al. 2008. "Rapid SNP Discovery and Genetic Mapping Using Sequenced RAD Markers." *PLoS One* 3, no. 10: e3376. https://doi.org/10.1371/journal.pone.0003376.

Barnard-Kubow, K. B., C. L. Debban, and L. F. Galloway. 2015. "Multiple Glacial Refugia Lead to Genetic Structuring and the Potential for Reproductive Isolation in a Herbaceous Plant." *American Journal of Botany* 102, no. 11: 1842–1853. https://doi.org/10.3732/ajb.1500267.

Bemmels, J. B., and C. W. Dick. 2018. "Genomic Evidence of a Widespread Southern Distribution During the Last Glacial Maximum for Two Eastern North American Hickory Species." *Journal of Biogeography* 45, no. 8: 1739–1750. https://doi.org/10.1111/jbi.13358.

Bemmels, J. B., L. L. Knowles, and C. W. Dick. 2019. "Genomic Evidence of Survival Near Ice Sheet Margins for Some, but Not All, North American Trees." *Proceedings of the National Academy of Sciences* 116, no. 17: 8431–8436. https://doi.org/10.1073/pnas.1901656116.

Bontrager, M., T. Usui, J. A. Lee-Yaw, et al. 2021. "Adaptation Across Geographic Ranges Is Consistent With Strong Selection in Marginal Climates and Legacies of Range Expansion." *Evolution* 75, no. 6: 1316–1333. https://doi.org/10.1111/evo.14231.

Bouchard, J. R., D. D. Fernando, S. W. Bailey, J. Weber-Townsend, and D. J. Leopold. 2017. "Contrasting Patterns of Genetic Variation in Central and Peripheral Populations of *Dryopteris fragrans* (Fragrant Wood Fern) and Implications for Colonization Dynamics and Conservation." *International Journal of Plant Sciences* 178, no. 8: 607–617. https://doi.org/10.1086/693109.

Cahill, A. E., M. E. Aiello-Lammens, M. Caitlin Fisher-Reid, et al. 2014. "Causes of Warm-Edge Range Limits: Systematic Review, Proximate Factors and Implications for Climate Change." *Journal of Biogeography* 41, no. 3: 429–442. https://doi.org/10.1111/jbi.12231.

Davis, M. B., and R. G. Shaw. 2001. "Range Shifts and Adaptive Responses to Quaternary Climate Change." *Science* 292, no. 5517: 673–679. https://doi.org/10.1126/science.292.5517.673.

de Lafontaine, G., J. D. Napier, R. J. Petit, and F. S. Hu. 2018. "Invoking Adaptation to Decipher the Genetic Legacy of Past Climate Change." *Ecology* 99, no. 7: 1530–1546. https://doi.org/10.1002/ecy.2382.

Erichsen, E. O., K. B. Budde, K. Sagheb-Talebi, F. Bagnoli, G. G. Vendramin, and O. K. Hansen. 2018. "Hyrcanian Forests—Stable Rear-Edge Populations Harbouring High Genetic Diversity of *Fraxinus excelsior*, a Common European Tree Species." *Diversity and Distributions* 24, no. 11: 1521–1533. https://doi.org/10.1111/ddi.12783.

Evanno, G., S. Regnaut, and J. Goudet. 2005. "Detecting the Number of Clusters of Individuals Using the Software Structure: A Simulation Study." *Molecular Ecology* 14, no. 8: 2611–2620. https://doi.org/10.1111/j.1365-294X.2005.02553.x.

Excoffier, L., M. Foll, and R. J. Petit. 2009. "Genetic Consequences of Range Expansions." *Annual Review of Ecology, Evolution, and Systematics* 40, no. 1: 481–501. https://doi.org/10.1146/annurev.ecolsys.39.110707.173414.

Fernandez, M. C., F. S. Hu, D. G. Gavin, G. de Lafontaine, and K. D. Heath. 2021. "A Tale of Two Conifers: Migration Across a Dispersal Barrier Outpaced Regional Expansion From Refugia." *Journal of Biogeography* 48, no. 9: 2133–2143. https://doi.org/10.1111/jbi.14209.

Fick, S. E., and R. J. Hijmans. 2017. "WorldClim 2: New 1-Km Spatial Resolution Climate Surfaces for Global Land Areas." *International Journal of Climatology* 37, no. 12: 4302–4315. https://doi.org/10.1002/joc.5086.

Freeman, E. A., and G. Moisen. 2008. "PresenceAbsence: An R Package for Presence-Absence Model Analysis." *Journal of Statistical Software* 23, no. 11: 1–31. http://www.jstatsoft.org/v23/i11.

Frichot, E., and O. François. 2015. "LEA: An R Package for Landscape and Ecological Association Studies." *Methods in Ecology and Evolution* 6, no. 8: 925–929. https://doi.org/10.1111/2041-210X.12382.

Gavin, D. G., M. C. Fitzpatrick, P. F. Gugger, et al. 2014. "Climate Refugia: Joint Inference From Fossil Records, Species Distribution Models and Phylogeography." *New Phytologist* 204, no. 1: 37–54. https://doi.org/10.1111/nph.12929.

Ghouil, H., D. Sancho-Knapik, A. Ben Mna, et al. 2020. "Southeastern Rear Edge Populations of *Quercus suber* L. Showed Two Alternative Strategies to Cope With Water Stress." *Forests* 11, no. 12: Article 12. https://doi.org/10.3390/f11121344.

Hampe, A., and R. J. Petit. 2005. "Conserving Biodiversity Under Climate Change: The Rear Edge Matters." *Ecology Letters* 8, no. 5: 461–467. https://doi.org/10.1111/j.1461-0248.2005.00739.x.

Hantemirova, E. V., B. Heinze, S. G. Knyazeva, A. M. Musaev, M. Lascoux, and V. L. Semerikov. 2017. "A New Eurasian Phylogeographical Paradigm? Limited Contribution of Southern Populations to the Recolonization of High Latitude Populations in *Juniperus communis* L. (Cupressaceae)." *Journal of Biogeography* 44, no. 2: 271–282. https://doi.org/10.1111/jbi.12867.

Havrdová, A., J. Douda, K. Krak, et al. 2015. "Higher Genetic Diversity in Recolonized Areas Than in Refugia of *Alnus glutinosa* Triggered by Continent-Wide Lineage Admixture." *Molecular Ecology* 24, no. 18: 4759–4777. https://doi.org/10.1111/mec.13348.

Hewitt, G. 2000. "The Genetic Legacy of the Quaternary Ice Ages." *Nature* 405, no. 6789: 907–913. https://doi.org/10.1038/35016000.

Hewitt, G. M. 2004. "Genetic Consequences of Climatic Oscillations in the Quaternary." *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences* 359, no. 1442: 183–195. https://doi.org/10.1098/rstb.2003.1388.

Hughes, P. D., P. L. Gibbard, and J. Ehlers. 2013. "Timing of Glaciation During the Last Glacial Cycle: Evaluating the Concept of a Global 'Last Glacial Maximum' (LGM)." *Earth-Science Reviews* 125: 171–198. https://doi.org/10.1016/j.earscirev.2013.07.003.

Jiménez-Mejías, P., M. Luceño, K. A. Lye, C. Brochmann, and G. Gussarova. 2012. "Genetically Diverse but With Surprisingly Little Geographical Structure: The Complex History of the Widespread Herb *Carex nigra* (Cyperaceae)." *Journal of Biogeography* 39, no. 12: 2279–2291. https://doi.org/10.1111/j.1365-2699.2012.02740.x.

Korábek, O., T. Adamcová, M. Proćków, A. Petrusek, B. Hausdorf, and L. Juřičková. 2023. "In Both Directions: Expansions of European Land Snails to the North and South From Glacial Refugia." *Journal of Biogeography* 50, no. 4: 654–668. https://doi.org/10.1111/jbi.14531.

Korkmaz, E. M., D. H. Lunt, B. Çıplak, N. Değerli, and H. H. Başıbüyük. 2014. "The Contribution of Anatolia to European Phylogeography: The Centre of Origin of the Meadow Grasshopper, *Chorthippus parallelus*." *Journal of Biogeography* 41, no. 9: 1793–1805. https://doi.org/10.1111/jbi.12332.

Koski, M. H., N. C. Layman, C. J. Prior, J. W. Busch, and L. F. Galloway. 2019. "Selfing Ability and Drift Load Evolve With Range Expansion." *Evolution Letters* 3, no. 5: 500–512. https://doi.org/10.1002/evl3.136.

Kottler, E. J., E. E. Dickman, J. P. Sexton, N. C. Emery, and S. J. Franks. 2021. "Draining the Swamping Hypothesis: Little Evidence That Gene Flow Reduces Fitness at Range Edges." *Trends in Ecology & Evolution* 36, no. 6: 533–544. https://doi.org/10.1016/j.tree.2021.02.004.

Lamb, K., C. L. Debban, and L. F. Galloway. 2024. "Phylogeography and Paleoclimatic Range Dynamics Explain Variable Outcomes to Contact Across a Species' Range." *Molecular Ecology* 33, no. 15: e17450. https://doi.org/10.1111/mec.17450.

Lee-Yaw, J. A., J. T. Irwin, and D. M. Green. 2008. "Postglacial Range Expansion From Northern Refugia by the Wood Frog, *Rana sylvatica*." *Molecular Ecology* 17, no. 3: 867–884. https://doi.org/10.1111/j.1365-294X.2007.03611.x.

Leigh, D. S. 2006. "Terminal Pleistocene Braided to Meandering Transition in Rivers of the Southeastern USA." *Catena* 66, no. 1: 155–160. https://doi.org/10.1016/j.catena.2005.11.008.

Lenoir, J., and J.-C. Svenning. 2015. "Climate-Related Range Shifts—A Global Multidimensional Synthesis and New Research Directions." *Ecography* 38, no. 1: 15–28. https://doi.org/10.1111/ecog.00967.

Lyman, R. A., and C. E. Edwards. 2022. "Revisiting the Comparative Phylogeography of Unglaciated Eastern North America: 15 Years of Patterns and Progress." *Ecology and Evolution* 12, no. 4: e8827. https://doi.org/10.1002/ece3.8827.

Maddison, W. P., and L. L. Knowles. 2006. "Inferring Phylogeny Despite Incomplete Lineage Sorting." *Systematic Biology* 55, no. 1: 21–30. https://doi.org/10.1080/10635150500354928.

Marcus, J., W. Ha, R. F. Barber, and J. Novembre. 2021. "Fast and Flexible Estimation of Effective Migration Surfaces." *eLife* 10: e61927. https://doi.org/10.7554/eLife.61927.

Masuda, K., H. Setoguchi, K. Nagasawa, et al. 2023. "Rear-Edge Daylily Populations Show Legacies of Habitat Fragmentation due to the Holocene Climate Warming." *Journal of Biogeography* 50, no. 3: 551–563. https://doi.org/10.1111/jbi.14552.

Milanesi, M., S. Capomaccio, E. Vajana, et al. 2017. "BITE: An R Package for Biodiversity Analyses." *bioRxiv*. https://doi.org/10.1101/181610.

Mohn, R. A., N. H. Oleas, A. B. Smith, J. F. Swift, G. A. Yatskievych, and C. E. Edwards. 2021. "The Phylogeographic History of a Range Disjunction in Eastern North America: The Role of Post-Glacial Expansion Into Newly Suitable Habitat." *American Journal of Botany* 108, no. 6: 1042–1057. https://doi.org/10.1002/ajb2.1686.

Nadeau, C. P., and M. C. Urban. 2019. "Eco-Evolution on the Edge During Climate Change." *Ecography* 42, no. 7: 1280–1297. https://doi.org/10.1111/ecog.04404.

Neiva, J., J. Assis, F. Fernandes, G. A. Pearson, and E. A. Serrão. 2014. "Species Distribution Models and Mitochondrial DNA Phylogeography

Suggest an Extensive Biogeographical Shift in the High-Intertidal Seaweed *Pelvetia canaliculata*." *Journal of Biogeography* 41, no. 6: 1137–1148. https://doi.org/10.1111/jbi.12278.

Neiva, J., E. A. Serrão, C. Paulino, et al. 2020. "Genetic Structure of Amphi-Atlantic *Laminaria digitata* (Laminariales, Phaeophyceae) Reveals a Unique Range-Edge Gene Pool and Suggests Post-Glacial Colonization of the NW Atlantic." *European Journal of Phycology* 55, no. 4: 517–528. https://doi.org/10.1080/09670262.2020.1750058.

Nielsen, E. S., S. Walkes, J. L. Sones, et al. 2024. "Pushed Waves, Trailing Edges, and Extreme Events: Eco-Evolutionary Dynamics of a Geographic Range Shift in the Owl Limpet, *Lottia gigantea*." *Global Change Biology* 30, no. 7: e17414. https://doi.org/10.1111/gcb.17414.

Park, B., and M. J. Donoghue. 2019. "Phylogeography of a Widespread Eastern North American Shrub, *Viburnum lantanoides*." *American Journal of Botany* 106, no. 3: 389–401. https://doi.org/10.1002/ajb2.1248.

Pelletier, E., M. Couture, and G. de Lafontaine. 2023. "Hedging at the Rear Edge: Intraspecific Trait Variability Drives the Trajectory of Marginal Populations in a Widespread Boreal Tree Species." *Journal of Ecology* 111, no. 2: 479–494. https://doi.org/10.1111/1365-2745.14041.

Perrier, A., O. J. Keenan, J. W. Busch, and L. F. Galloway. 2025. "The Legacy of Past Climate Warming: Strong Local Adaptation in Rear-Edge Populations." *bioRxiv*. https://doi.org/10.1101/2025.08.15.669932.

Perrier, A., O. J. Keenan, and L. F. Galloway. 2026. "Revisiting Evolution at the Rear Edge." *Trends in Ecology & Evolution*. https://doi.org/10.1016/j.tree.2026.01.002.

Perrier, A., M. C. Turner, and L. F. Galloway. 2025. "Shifts in Vernalization and Phenology at the Rear Edge Hold Insight Into the Adaptation of Temperate Plants to Future Milder Winters." *New Phytologist* 246, no. 3: 1377–1389. https://doi.org/10.1111/nph.70005.

Peterson, B. J., and W. R. Graves. 2016. "Chloroplast Phylogeography of *Dirca palustris* L. Indicates Populations Near the Glacial Boundary at the Last Glacial Maximum in Eastern North America." *Journal of Biogeography* 43, no. 2: 314–327. https://doi.org/10.1111/jbi.12621.

Petit, R. J., I. Aguinagalde, J.-L. de Beaulieu, et al. 2003. "Glacial Refugia: Hotspots but Not Melting Pots of Genetic Diversity." *Science* 300, no. 5625: 1563–1565.

Petkova, D., J. Novembre, and M. Stephens. 2016. "Visualizing Spatial Population Structure With Estimated Effective Migration Surfaces." *Nature Genetics* 48, no. 1: 94–100. https://doi.org/10.1038/ng.3464.

Pickrell, J. K., and J. K. Pritchard. 2012. "Inference of Population Splits and Mixtures From Genome-Wide Allele Frequency Data." *PLoS Genetics* 8, no. 11: e1002967. https://doi.org/10.1371/journal.pgen.1002967.

Piotti, A., C. Leonarduzzi, D. Postolache, et al. 2017. "Unexpected Scenarios From Mediterranean Refugial Areas: Disentangling Complex Demographic Dynamics Along the Apennine Distribution of Silver Fir." *Journal of Biogeography* 44, no. 7: 1547–1558. https://doi.org/10.1111/jbi.13011.

Prior, C. J., N. C. Layman, M. H. Koski, L. F. Galloway, and J. W. Busch. 2020. "Westward Range Expansion From Middle Latitudes Explains the Mississippi River Discontinuity in a Forest Herb of Eastern North America." *Molecular Ecology* 29, no. 22: 4473–4486. https://doi.org/10.1111/mec.15650.

Provan, J., and C. A. Maggs. 2011. "Unique Genetic Variation at a Species' Rear Edge Is Under Threat From Global Climate Change." *Proceedings of the Royal Society B: Biological Sciences* 279, no. 1726: 39–47. https://doi.org/10.1098/rspb.2011.0536.

R Core Team. 2025. *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing.

Rochette, N. C., A. G. Rivera-Colón, and J. M. Catchen. 2019. "Stacks 2: Analytical Methods for Paired-End Sequencing Improve RADseq-Based Population Genomics." *Molecular Ecology* 28, no. 21: 4737–4754. https://doi.org/10.1111/mec.15253.

Rubenstein, M. A., S. R. Weiskopf, R. Bertrand, et al. 2023. "Climate Change and the Global Redistribution of Biodiversity: Substantial Variation in Empirical Support for Expected Range Shifts." *Environmental Evidence* 12, no. 1: 7. https://doi.org/10.1186/s13750-023-00296-0.

Rull, V. 2009. "Microrefugia." *Journal of Biogeography* 36, no. 3: 481–484. https://doi.org/10.1111/j.1365-2699.2008.02023.x.

Sexton, J. P., S. Y. Strauss, and K. J. Rice. 2011. "Gene Flow Increases Fitness at the Warm Edge of a Species' Range." *Proceedings of the National Academy of Sciences* 108, no. 28: 11704–11709. https://doi.org/10.1073/pnas.1100404108.

Sielezniew, M., D. Patricelli, R. Rutkowski, M. Witek, S. Bonelli, and M. M. Buś. 2015. "Population Genetics of the Endangered Obligatorily Myrmecophilous Butterfly *Phengaris* (=Maculinea) *Arion* in Two Areas of Its European Range." *Insect Conservation and Diversity* 8, no. 6: 505–516. https://doi.org/10.1111/icad.12129.

Soltis, D. E., A. B. Morris, J. S. McLachlan, P. S. Manos, and P. S. Soltis. 2006. "Comparative Phylogeography of Unglaciated Eastern North America." *Molecular Ecology* 15, no. 14: 4261–4293. https://doi.org/10.1111/j.1365-294X.2006.03061.x.

Song, X.-H., J. Assis, J. Zhang, et al. 2021. "Climate-Induced Range Shifts Shaped the Present and Threaten the Future Genetic Variability of a Marine Brown Alga in the Northwest Pacific." *Evolutionary Applications* 14, no. 7: 1867–1879. https://doi.org/10.1111/eva.13247.

Stewart, J. R., A. M. Lister, I. Barnes, and L. Dalén. 2009. "Refugia Revisited: Individualistic Responses of Species in Space and Time." *Proceedings of the Royal Society B: Biological Sciences* 277, no. 1682: 661–671. https://doi.org/10.1098/rspb.2009.1272.

Sugiura, N. 1978. "Further Analysts of the Data by Akaike' s Information Criterion and the Finite Corrections: Further Analysts of the Data by Akaike' s." *Communications in Statistics - Theory and Methods* 7, no. 1: 13–26. https://doi.org/10.1080/03610927808827599.

Svenning, J.-C., S. Normand, and M. Kageyama. 2008. "Glacial Refugia of Temperate Trees in Europe: Insights From Species Distribution Modelling." *Journal of Ecology* 96, no. 6: 1117–1127. https://doi.org/10.1111/j.1365-2745.2008.01422.x.

Tavares, A. I., J. Assis, L. Anderson, et al. 2024. "Past and Future Climate Effects on Population Structure and Diversity of North Pacific Surfgrasses." *Journal of Biogeography* 51, no. 10: 1999–2010. https://doi.org/10.1111/jbi.14964.

Tzedakis, P. C., I. T. Lawson, M. R. Frogley, G. M. Hewitt, and R. C. Preece. 2002. "Buffered Tree Population Changes in a Quaternary Refugium: Evolutionary Implications." *Science* 297, no. 5589: 2044–2047. https://doi.org/10.1126/science.1073083.

Urban, M. C. 2024. "Climate Change Extinctions." *Science* 386, no. 6726: 1123–1128. https://doi.org/10.1126/science.adp4461.

Vilà-Cabrera, A., A. C. Premoli, and A. S. Jump. 2019. "Refining Predictions of Population Decline at Species' Rear Edges." *Global Change Biology* 25, no. 5: 1549–1560. https://doi.org/10.1111/gcb.14597.

Walker, M. J., A. K. Stockman, P. E. Marek, and J. E. Bond. 2009. "Pleistocene Glacial Refugia Across the Appalachian Mountains and Coastal Plain in the Millipede Genus *Narceus*: Evidence From Population Genetic, Phylogeographic, and Paleoclimatic Data." *BMC Evolutionary Biology* 9: 25. https://doi.org/10.1186/1471-2148-9-25.

Wang, W. J., F. R. Thompson III, H. S. He, J. S. Fraser, W. D. Dijak, and M. A. Spetich. 2018. "Population Dynamics Has Greater Effects Than Climate Change on Tree Species Distribution in a Temperate Forest Region." *Journal of Biogeography* 45, no. 12: 2766–2778. https://doi.org/10.1111/jbi.13467.

White, E., P. S. Soltis, and D. E. Soltis. 2026. "Origins of Diversity in the Eastern Gulf Coastal Plain of North America: High Endemism Within a Biodiversity Hotspot." *Molecular Ecology* 35, no. 5: e70276. https://doi.org/10.1111/mec.70276.

Willi, Y., M. Fracassetti, S. Zoller, and J. Van Buskirk. 2018. "Accumulation of Mutational Load at the Edges of a Species Range." *Molecular Biology and Evolution* 35, no. 4: 781–791. https://doi.org/10.1093/molbev/msy003.

Wright, M. N., and A. Ziegler. 2017. "Ranger: A Fast Implementation of Random Forests for High Dimensional Data in C++ and R." *Journal of Statistical Software* 77, no. 1: 1–17. https://doi.org/10.18637/jss.v077.i01.

Wyatt, G. E., J. L. Hamrick, and D. W. Trapnell. 2021. "Ecological Niche Modelling and Phylogeography Reveal Range Shifts of Pawpaw, a North American Understorey Tree." *Journal of Biogeography* 48, no. 4: 974–989. https://doi.org/10.1111/jbi.14054.

Xia, M., Y. Luo, Y. Suyama, et al. 2024. "Genetic Divergence and Ecological Adaptation of an Eastern North American Spring Ephemeral *Sanguinaria canadensis*." *Diversity and Distributions* 30, no. 4: e13813. https://doi.org/10.1111/ddi.13813.

Zeng, Y.-F., W.-T. Wang, W.-J. Liao, H.-F. Wang, and D.-Y. Zhang. 2015. "Multiple Glacial Refugia for Cool-Temperate Deciduous Trees in Northern East Asia: The Mongolian Oak as a Case Study." *Molecular Ecology* 24, no. 22: 5676–5691. https://doi.org/10.1111/mec.13408.

Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Appendix S1:** Supplementary Methods **Method S1.1:** Species distribution model **Method S1.1 Figure A:** Final set of presence (black) and pseudo-absences (blue) used to generate the species distribution model. **Method S1.1 Figure B:** Comparison of Global Climate Models (GCM) for paleoclimate hindcasting. **Method S1.1 Figure C:** Uncertainty estimation of contemporary and paleoclimate species distribution model (SDM) projections. **Method S1.2:** Phylogeography **Method S1.2 Table A:** Second-order rate of change in likelihood (Δm) for each migration edge (m). **Method S1.2 Table B:** Cross-entropy per ancestral populations K and independent analysis runs for each K of the sNMF population structure analysis. **Method S1.2 Table C:** Cross-validation error for each value of the smoothing regularisation parameter λ. **Appendix S2:** Supplementary Results **Table S2:1:** Model comparisons testing the linear and 2nd or 3rd degree quadratic effect of latitude. **Table S2:2:** Populations used, genetic cluster assignment and population-average admixture per genetic cluster. **Figure S2:1:** Parametrization of the population structure analysis, individual cluster assignments and populations sequenced.