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Paleo-climates and past introgression explain the spatio-temporal distribution of genetic structure in *Triodanis perfoliata*

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Abstract

The dynamic nature of intrinsic (e.g., reproductive system, hybridization) and extrinsic factors (e.g., physical barriers to gene flow) across space and time generate complex biological processes that influence contemporary patterns of genetic diversity, highlighting the need for interdisciplinary studies. Using the widespread, mixed-mating annual *Triodanis perfoliata*, previous work demonstrated the important roles of breeding system, isolation by distance, and isolation by resistance in shaping patterns of population genetic diversity. Here we significantly build on this first step by incorporating paleoclimatic data, historical admixture, and estimating species divergence times across 18 populations of *T. perfoliata* spanning the contiguous US. This current study provides novel insights into factors driving patterns of intraspecific diversification that were not explained using only contemporary climate models. Specifically, these new analyses highlight the early Holocene (11.7 - 8.326 ka) and the Marine Isotope Stage M2 (ca. 3.3 Ma), as important time periods for explaining patterns of contemporary population genetic diversity, the latter of which appears to be an important time period for intraspecific divergence of *T. perfoliata*. In addition, we explored the influence of historical intrinsic factors, via admixture to explain patterns of population isolation and connectivity. The inclusion of an admixture analysis provided clarity through evidence of historical gene flow between populations that would have experienced suitable habitat connectivity in past climates. Our study illustrates the importance of incorporating historic, as well as contemporary data, into phylogeographic studies to generate a comprehensive understanding of patterns of population diversity, and the processes important in driving these patterns.

Highlights:

- The complex climatic and geologic history of the last three million years altered and shaped patterns of population connectivity and gene flow as organisms tracked climate and environmental changes.

- Landscape and genetic structure often change at different rates resulting in a disconnect between contemporary genetic patterns and the current landscape, emphasizing the importance of both current and past processes in shaping population genetic patterns, and in examining these factors through multidisciplinary research approaches.

- We present a combination of analyses that quantify historic and contemporary factors that influence modern patterns of genetic structure in *Triodanis perfoliata* across the contiguous United States.

- Our analyses highlight the importance of evaluating both landscape and phylogeographic drivers from historic and contemporary perspectives when investigating genetic structure.

Introduction

Contemporary patterns of intraspecific biodiversity are influenced by a range of factors both extrinsically (e.g., environmental, geospatial; Brown et al. 2016a, Alvarado-Serrano et al. 2019) and intrinsically (e.g., demography, breeding system; Chan et al. 2011, Toczydlowski and Waller 2019, Tackett et al. 2022). These factors, however, can vary across space and time, suggesting the potential influence of historic processes in shaping population genetic patterns. For example, patterns of lineage diversification and hybridization are directly impacted by decreased or increased population connectivity, which is spatially and temporally dynamic (Chan et al. 2011, Cruzan and Hendrickson 2020, Hellwig et al. 2022).

Traditional approaches to understanding spatial patterns of genetic diversity have primarily focused on contemporary environmental and resistance barriers as the cause of intraspecific genetic structure (Manel et al. 2003, Storfer et al. 2010, Wang 2010, Bohonak and Vandergast 2011, Manel and Holderegger 2013, Rissler 2016). Geospatial variation across the landscape may either facilitate or impede population connectivity (e.g., physical barriers to dispersal, climate suitability), and these factors have a strong influence on patterns of gene flow across populations (e.g., Ricketts 2001, Cushman et al. 2009, Tackett et al. 2022). However, it is important to also consider historic barriers, both physical and environmental, that may have played important roles in modern distributions and genetic patterns (Galbreath et al. 2009, Zellmer and Knowles 2009, Brown and Knowles 2012, Crowl et al. 2015).

Historic climate dynamism can influence population size and dispersal patterns as organisms track environmental changes, altering patterns of population connectivity and gene flow (Zellmer and Knowles 2009, Epps and Keyghobadi 2015, Rissler 2016). Due to the spatial mosaic of ecosystems, these changes occur at different rates creating a potential disconnect between present day genetic patterns and the landscape, referred to as time lag (Wu et al. 2015). This phenomenon can make it challenging to disentangle historic and contemporary influence on population genetic structure. In fact, most landscapes are highly dynamic and change more rapidly than population genetic patterns (Epps and Keyghobadi 2015, Wu et al. 2015, Rissler 2016).

Paleoclimates profoundly impacted the evolution and distribution of species on Earth (Rangel et al. 2018, Rahbek et al. 2019a, Rahbek et al. 2019b), and paleoclimates of the last interglacial cycle have been the traditional focus of biological investigations, largely because they correspond to times of temperature extremes in the northern latitudes (Hewitt 2000, Carstens and Richards 2007, Knowles et al. 2007). Nonetheless, there exists considerable variation in their ability to explain the distribution of biological diversity (for instance see: Raxworthy et al. 2003, Thomas et al. 2004a, Thomas et al. 2004b, Batalha-Filho et al. 2013, Brown et al. 2014, Smith et al. 2014, Brown et al. 2016a). Over the past decade, hundreds of studies have combined data on species occurrences with climate descriptions from interpolated weather-stations to model the distribution of animals and plants worldwide (Graham et al. 2004). When projected into paleo- and future-climatic scenarios, these models are widely used to investigate the historic and future distributions of biodiversity (Prates et al. 2016, Brown et al. 2016a, Brown et al. 2016b, Knowles and Alvarado-Serrano 2010, He et al. 2013). However, very few studies access paleoperiods outside of the last glacial cycle, with most studies focusing on the mid-Holocene (6 ka), Last Glacial Maximum (21 ka) and the Last Interglacial (130 ka). Moreover, for most taxa, speciation occurred well before the last interglacial cycle (ca. 130 ka to modern times, Rull 2008, Rull 2011) and climate dynamism throughout the last 0.8 ma mediated key diversification and extinction processes in the Americas (Rangel et al. 2018, Rahbek et al. 2019a, Rahbek et al. 2019b).

In this study, we examine how both contemporary and historic factors influence patterns of population genetic structure in the common, widespread North- and South American annual *Triodanis perfoliata* (Campanulaceae/Campanuloidae). The distribution of *T. perfoliata* encompasses a large geographic range and multiple climatic regions (Gleason and Cronquist 1991, Weakley 2010) providing an excellent study system for broad scale phylogeographic questions. This species has also been the subject of numerous previous studies (Ansaldi et al. 2019, Berg et al. 2019, Berg et al. in press), specifically as a model to understand how contemporary habitat suitability, isolation by distance, habitat resistance, and breeding system influence patterns of genetic diversity in *Triodanis perfoliata* across the United States (Tackett et al. 2022). Building significantly on this framework we explore novel research questions concerning the roles of historic and current climatic factors, as well as signatures of past introgression in driving present-day genetic patterns of *T. perfoliata.* Including spatially explicit historic climate factors allows us to more fully elucidate how historic population connectivity influenced modern distributions and patterns of gene flow. This study dramatically expands the analyses of Tackett et al. 2022 which focused on univariate models of contemporary climate factors to explain landscape genetic patterns. Further, the phylogenetic results of Tackett et al. 2022 lacked estimates of divergence times, therefore could not provide context on how past periods influenced the diversification of *T. perfoliata.* Last, the population genomic analyses used by Tackett et al. 2022, failed to account for historical introgression. Here we improve upon the research of Tackett et al. 2022 and address novel research questions by (1) quantifying the timing of divergence of our study species, *T. perfoliata*, and describing patterns of past hybridization among populations. We predict that patterns of past introgression will reveal previously undescribed patterns of gene flow among our study populations. In addition, we (2) quantify the influence of environmental factors (e.g., soil and fire frequency) and historical and contemporary climate (e.g., temperature and rainfall) in driving patterns of population divergence and structure using mixed models.

We predict that by expanding our study to include historic factors, we can more fully explain patterns of population divergence. In conjunction, our analysis including both historical and modern processes is a powerful approach for addressing time lag patterns, providing more spatiotemporal context to fundamental biological processes. Therefore, we significantly expand on the preliminary framework of Tackett et al. (2022), which only included current climate data, and directly address issues of time lag by incorporating paleoclimate data and estimating historic patterns of introgression between populations. Overall, we (3) merge these results to better describe the spatial and temporally explicit factors important in driving current and past patterns of population genetic diversity for *T. perfoliata*.

Materials & Methods

Study system

Triodanis perfoliata (L.) Nieuwl. (Campanulaceae/ Campanuloideae) is a common, weedy, widespread annual herb, native to much of North- and South-America (Weakley 2010). Historically found in prairies, *T. perfoliata* is now found in a wide variety of habitats, including road sides, disturbed areas, rocky outcrops, prairies, and across a variety of climates (Gleason and Cronquist 1991, Weakley 2010). Seeds of *T. perfoliata* are very small (approx. Length = 0.5 mm, width = 1.3 mm) and may be dispersed by ants (McVaugh 1948, Shetler and Morin 1986). This taxon exhibits a mixed mating system through dimorphic cleistogamy. This breeding system consists of open self-compatible, chasmogamous, purple, five-petaled flowers that are ~1.5cm in diameter and closed, obligate selfing, cleistogamous flowers that lack a corolla (Trent 1940, Gara and Muenchow 1990, Goodwillie and Stewart 2013). The overall production of chasmogamous and cleistogamous flowers varies widely among populations of this species, and is associated with variation in abiotic conditions (i.e., climate and soil) as well as pollinator activity (Ansaldi et al. 2018a, Ansaldi et al. 2018b, Tackett et al. 2022). Notably, greater production of chasmogamous flowers on average is associated with increased population genetic diversity, emphasizing the importance of the breeding system in driving contemporary patterns (Tackett et al. 2022).

Description of Source Dataset

This study includes a previously published dataset that represents an expanded Campanuloideae genetic dataset, and is included here in order to estimate divergence times using available fossil data. This dataset was required because the population-level SNP dataset from Tackett et al. (2022) lacked species directly associated with fossil data. Thus, we first performed a phylogenetic analysis on a broadly sampled Campanuloideae dataset (Crowl et al. 2014) in order to include fossil data and obtain a divergence time estimate for the most-recent-common ancestor of *T. biflora* and *T. perfoliata*. The resulting age estimate of this ancestor was subsequently used in our second divergence time analysis using the population-level SNP dataset from Tackett et al. (2022). Sampling for the expanded Campanuloideae genetic dataset included the addition of T. biflora sequences generated for a Crowl et al. (2014) study. Crowl et al. (2014) amplified and sequenced four plastid regions (matK, petD, rbcL, and the atpB-rbcL spacer region) and two low-copy nuclear loci (PPR11 and PPR70) from the pentatricopeptide repeat (PPR) gene family.

For our landscape genetic research, we performed novel analyses using the open-source dataset from Tackett (et al. 2022, https://doi.org/10.5061/dryad. sf7m0cg98 and https://doi.org/10.5061/dryad. wh70rxwr9) and build from their framework of basic population genetic and landscape genetic results. Given these data are central to this study, as follows is a summary of methods used by Tackett et al. (2022). Leaf tissue was collected from individuals in 18 populations of *Triodanis perfoliata* (range: 1-6 individuals per population; average: 4.2 individuals per populations) across the contiguous United States (Fig. 1). A total of 76 samples were collected and used for genetic sequencing along with six accessions of *Triodanis biflora,* which was selected to serve as an outgroup because of its placement as sister to *T. perfoliata* (Tackett et al. 2022; Table S1).

RADseq (Restriction site Associated DNA sequencing) was performed by Floragenex Inc. (http://floragenex. com) to identify genetic variation within samples (Eaton 2014). The restriction enzyme Sbf1 was used and all samples were analyzed on the same flow cell with Illumina 1x91 bp sequencing. Sequencing, quality control, sequence alignment, and variant calling were conducted using Bowtie (Langmead and Salzburg 2012), BWA (Li 2011), Velvet (Zerbino 2010), and Samtools (Li et al. 2009) respectively. The resulting final dataset consisted of variant calls with a minimum sequencing depth of 15x, minimum Phred score of 20, and no more than 10% of missing genotypes; variant calling yielded 4705 SNPs (single nucleotide polymorphisms) observed in >90% of sequenced individuals of *T. perfoliata* (see Tackett et al. 2022 for more details about the genomics pipeline). The population-level SNP dataset from Tackett et al. (2022) was the primary genomic dataset used for all genetic analyses in this study (see divergence analysis a single exception).

Tackett et al. (2022) assigned genetic clusters using STRUCTURE (Pritchard et al. 2000) and used STRUCTURE HARVESTER v6.0 (Earl and vonHoldt 2012) to estimate the number of genetic clusters (K). Briefly, ten independent runs were performed for each genetic cluster (K) and a burn in value of 40,000 were allowed before running 80,000 iterations per K. Admixture models and correlated allele frequencies were used. The values of ΔK were determined using the Evanno et al. (2005) method in STRUCTURE HARVESTER v6.0 (Earl and vonHoldt 2012). These analyses revealed that both K = 4 and K = 17 have high support (ΔK = 7.68 and ΔK = 6.28, respectively, these past results are summarized in Figs. 1 and 2). Tackett et. al (2022) also explored genetic clusters via sMNF (Frichot et al. 2014), which is robust to deviations from standard statistical assumptions; results were similar to STRUCTURE.
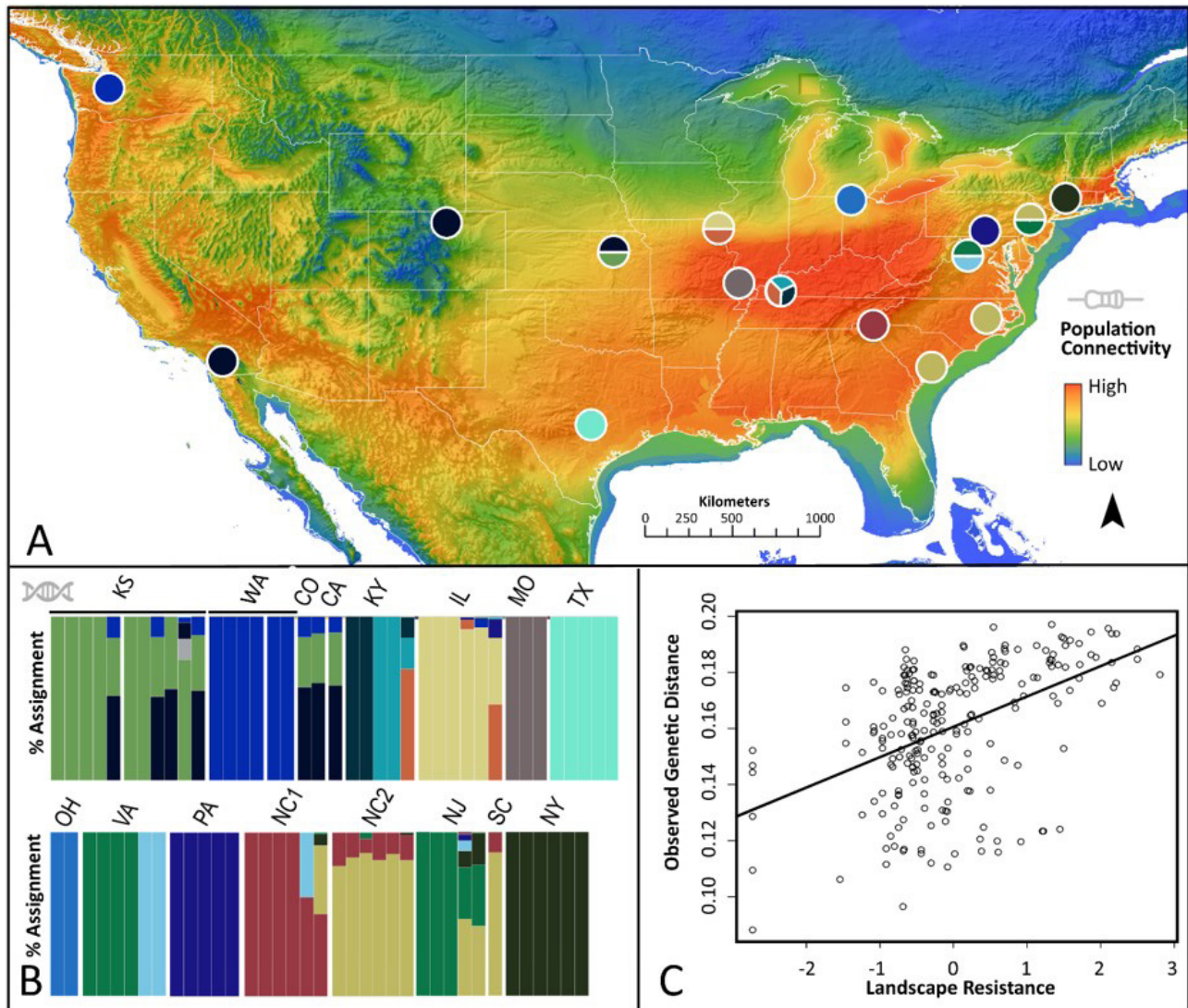
Figure 1. Results of mixed spatial models of landscape genetic connectivity including the 22 genetic groups depicted by colored dots. Areas of high population connectivity are represented with warm colors while those with poor connectivity with cool colors. (A) The highest-ranking composite model consisting of the early Holocene (11.7 - 8.3 ka) and the Marine Isotope Stage M2 period (ca. 3.3 Ma). Populations are colored to match cluster assignment from (B) STRUCTURE results (K=17) from a genomic SNP dataset, figure reproduced from Tackett et al. 2022. (C) Plot of highest-ranking mixed spatial model showing relationships between the predicted landscape friction and the observed genetic distance.

Divergence Time Estimation

To provide a time estimate for the divergence between *T. perfoliata* and *T. biflora*, we carried out a Bayesian fossil-calibrated analysis. Fossil calibration was challenged by the limited fossil record for Campanulaceae, with no available fossils in our focal group. Therefore, we ran a BEAST2 (v.2.1.2; Bouckaert et al. 2014) analysis on a broad Campanuloideae-wide dataset (based on Crowl et al. 2014) that additionally included *Triodanis perfoliata* and *Triodanis biflora*. As in several previous studies (Cellinese et al. 2009, Roquet et al. 2009, Olesen et al. 2012, Crowl et al. 2014, 2015, 2016), we used the only reliable Campanulaceae fossil identified as seeds of Campanula paleopyramidalis and dated from the

Early-Middle Miocene (approximately 17–16 Ma; Lancucka-Srodoniowa 1977, 1979). We applied a lognormal prior distribution constraint to the node representing the most recent common ancestor of C. pyramidalis and C. carpatica, with mean = 5.0, stdev = 1.0, and offset = 16. An additional constraint was placed at the root of the Campanulaceae clade as a normal distribution with mean = 54.0 and stdev = 5.0 based on past studies (Bell et al. 2010, Crowl et al. 2016). This analysis was run under an uncorrelated lognormal model for 50 million generations, logging parameters every 1000 generations, and assuming a Yule process. Tracer v.1.6 (Rambaut et al. 2014) was used to verify effective sample sizes (ESS values >200) for estimated parameters and to determine burn-in.
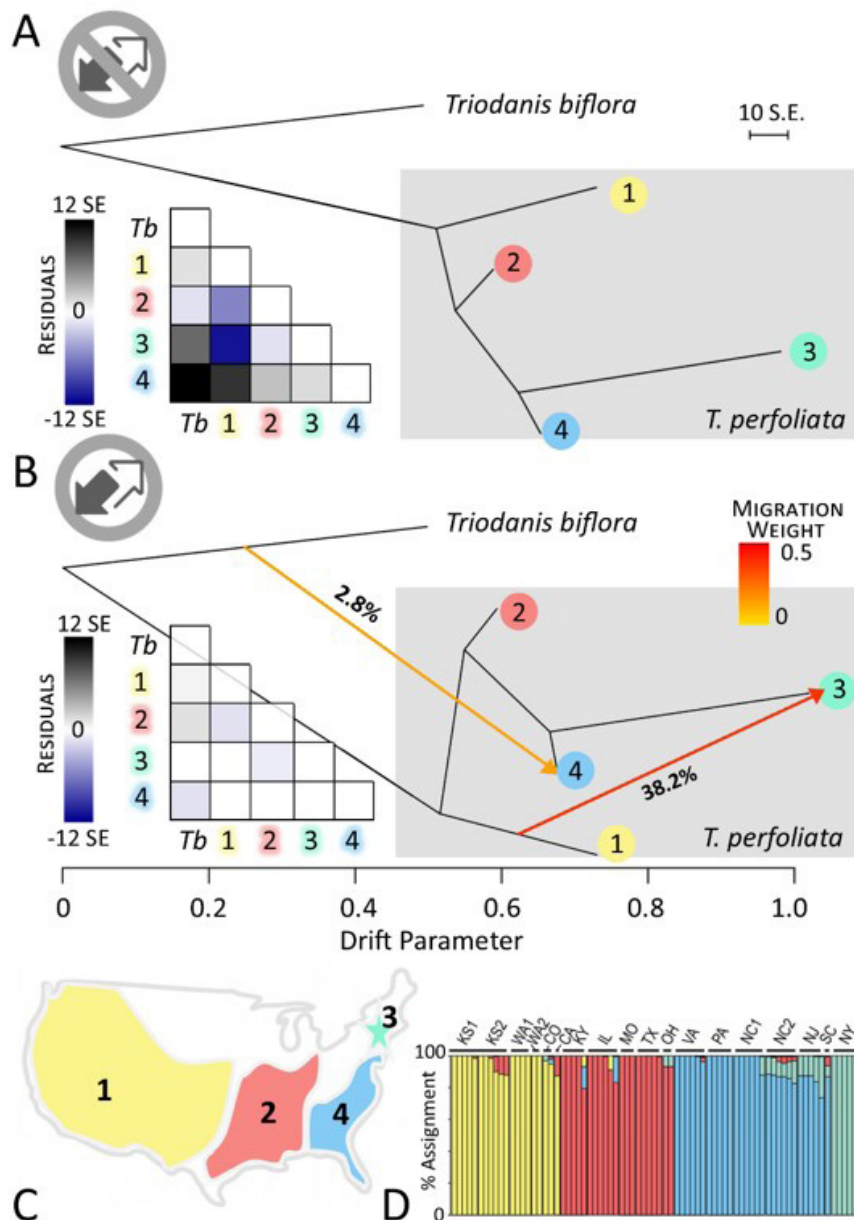
Figure 2. The admixture structure for *Triodanis perfoliata* populations (K = 4 from STRUCTURE), with colors corresponding to genetic clusters consistent with Figure 3, as well was cluster figures from Tackett et al. (2022), and an outgroup, *T. biflora*. Analyses with **A)** no migration events and **B)** two migration events. Migration arrows are colored according to their migration weight. The scale bar for the drift parameter shows ten times the average standard error of the entries in the sample covariance matrix. Residual fit plotted in the heatmap are comparisons of the residual fit from the maximum likelihood for each tree among each input population. Residuals deviating from zero identify populations that are not well-modeled under non-migration and thus are candidates for admixture events. **C)** Map of population distributions in the contiguous United States (K = 4). **D)** STRUCTURE results (K=4) from a genomic SNP dataset, figure reproduced from Tackett et al. 2022.

To infer the timing of diversification within *T. perfoliata*, we additionally estimated divergence times with MCMCTree in PAML v4.8 (Yang 2007) using our large SNP dataset with population-level sampling of *T. perfoliata*. Divergence time estimation from large genomic datasets can be computationally challenging, but MCMCTree allowed us to include all SNPs in our dataset and compute the output in a reasonable amount of time. We used the ML topology from Tackett et al. (2022) as a reference given it included all samples. We used an independent rates clock model for rate priors because we expect our broad intraspecific sampling will lead to branch-specific, heterogeneous rates of evolution, violating the rate homogeneity assumptions of a strict molecular clock (Wertheim et al. 2010, Brown and Yang 2011).
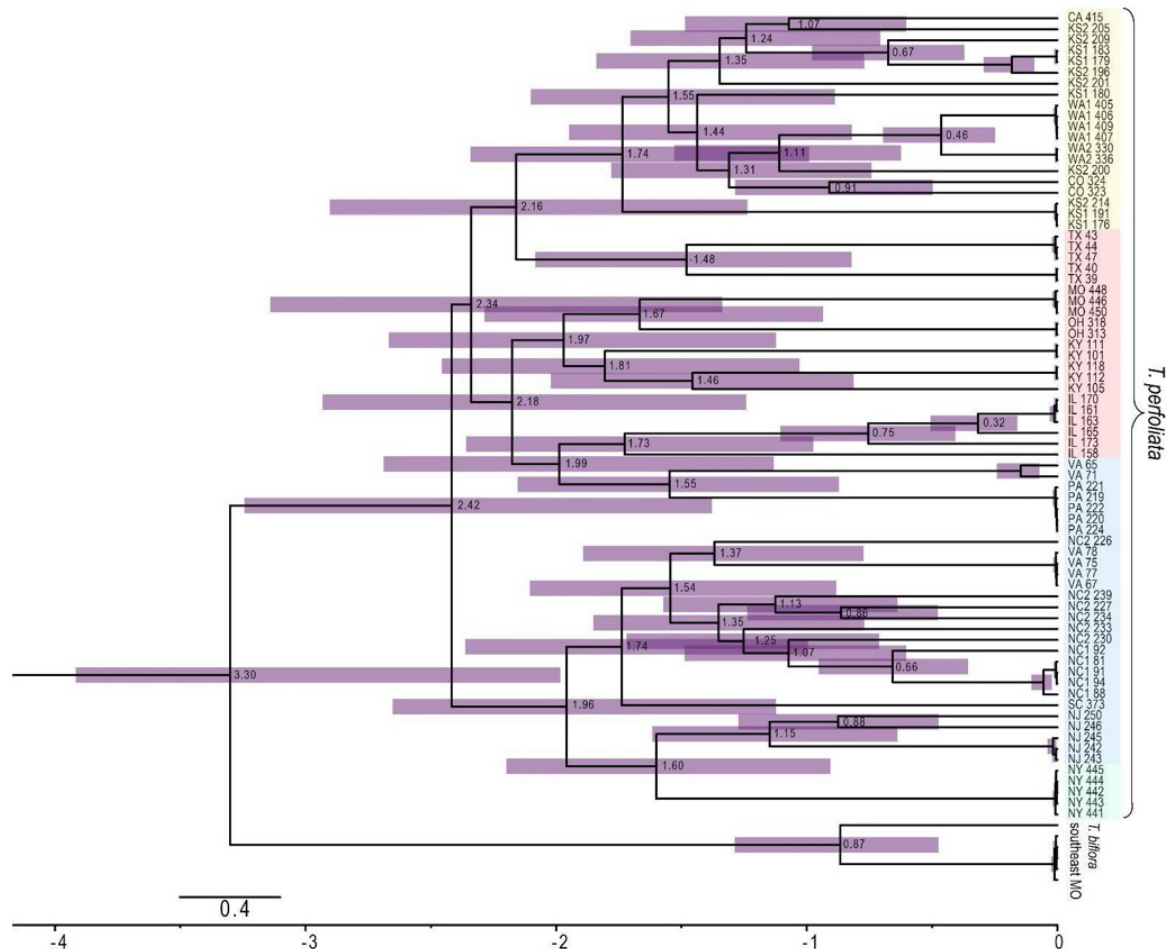
Figure. 3. *Triodanis perfoliata* MCMC time calibrated tree in millions of years with *T. biflora* as an outgroup using SNPs. Colors correspond to cluster assignments, consistent with Figure 2, as well as figures from Tackett et al. (2022); note that colored genetic clusters do not indicate monophyly. Purple Error bars represent bounds of 95% confidence intervals for each node, and time units are in millions of years. Our results estimate that *T. perfoliata* diverged from *T. biflora* approximately 3.3 mya. The extant populations of *T. perfoliata* surveyed shared a common ancestor of approximately 2.4 mya. After speciation, *T. perfoliata* continued to diverge, with the core lineages (*sensu* Tackett et al. 2022) diverging in the early Pleistocene.

We first ran the MCMCTree analysis without sequence data to assess whether our model parameters produced reasonable priors from our calibration. To expedite the analysis, we then implemented BASEML (also in PAML) to calculate approximate branch length values prior to running MCMCTree, under an HKY substitution model. We selected the HKY model due to computational constraints encountered when the parameter-rich GTR model was attempted and failed. We ran MCMCTree for 2,000,000 burn-in generations and subsequently, sampled every 1,000 generations until we obtained 20,000 samples across a total of 22,000,000 iterations. We input the age prior of 1.9 mya (95% HPD = 0.5 − 3.7) for the common ancestor of T. perfoliata, as estimated with our fossil-calibrated BEAST analysis. To assess convergence, we ensured ESS values for each node were over 200 using Tracer v1.7.1 (Rambaut et al. 2018), and ran our analysis twice from two different random starting seeds before confirming that both converged to similar posterior estimates.

Species Distribution Modeling

Species distribution models (SDMs) were generated using occurrence records previously curated and vetted in Tackett et al. (2022). Spatial biases were addressed by randomly selecting points clustered within a 10-km radius using SDMtoolbox 2.4 (Brown 2014). The final vetted dataset consists of 1735 occurrence records. Fourteen bioclimatic layers at a 30 arc-second resolution downloaded from WorldClim v2.0 (Hijmans et al. 2005; Bio 1, Bio 4, Bio 8-19). We tested for collinearity of all bioclimatic layers selecting variables with Pearson Correlation coefficients below 0.7 (Brown et al. 2017), which were then used to generate SDMs in MaxEnt 3.3.3k (Phillips et al. 2020). These Contemporary SDMs were projected into six key paleoclimatic periods spanning eight thousand years ago to three million years ago at 2.5 arc-minute resolution from Paleoclim.org (Brown et al. 2018; Early-Holocene, Bolling-Allerod, Last Glacial Maximum, Last Interglacial, Marine Isotope Stage M19, and Marine Isotope Stage M2, see Table S2 for more details).

Five bioclimatic layers (Bio 2, Bio 3, Bio 5-7) were unavailable for several of our paleoclimatic datasets, and thus were not used in our initial, contemporary SDMs. Due to the limited georeferenced occurrence records available throughout the Neotropics, as well as the considerable taxonomic uncertainty of these individuals, we focused our models and model-based analysis on the contiguous United States.

SDMs were parameterized with SDMtoolbox v2.4 (Brown 2014) to evaluate the performance of various combinations of five feature classes (linear; linear and quadratic; hinge; linear, quadratic and hinge; and linear, quadratic, hinge, product and threshold), and five regularization multipliers (0.5, 1, 2, 3, 4; Radosavljevic and Anderson 2014) with the threshold set to the 10th percentile training presence. SDM performance built under each combination of parameters was assessed through a geographically structured k-fold cross-validation (i.e., the occurrence records were partitioned into k equal geographically clustered subsamples, here k = 3, and the models were trained with two of the groups and then evaluated with the excluded group until all group combinations were run). Model fit was assessed via the omission rate, area under the curve (AUC), and model feature class complexity (Brown 2014). After optimum model parameters were determined (those leading to the lowest omission rate, highest AUC, and lowest complexity, in the order listed), a final SDM was built with all occurrence sites and projected into the current climate across the contiguous US. The study was confined to the contiguous US as genetic sampling and available historic fire data (LANDFIRE 2020) was restricted to this region.

Spatial Hypothesis of Landscape Connectivity

For this study, we created ten spatial hypotheses for landscape connectivity. The first was the output from our best SDM model created for *T. perfoliata* in current time, which was subsequently projected into six paleoclimatic periods, each used here (Fig. S1). Each SDM represented areas of high habitat suitability bound by corresponding climates and, also, the shape of the terrestrial bounds of the North American landmass. We also generated three additional spatial hypotheses: climate stability, historic fire frequency, and three-dimensional topographic distance (Fig. S1). Climate stability through time has been shown to predict genetic and lineage diversity (Carnaval and Moritz 2008, Carnaval et al. 2009). We estimated climatic stability by averaging all the continuous paleoclimate SDMs and a current SDM that was projected in matching 2.5 arc-minute climate data from WorldClim v2.0 (Hijmans et al. 2005; Bio 1, Bio 4, Bio 8-19). Fire history, which is often an important factor in the distribution of North American prairie species (Anderson et al. 1999, Anderson 2006, Wagenius et al. 2020), was obtained by using the Mean Fire Return Interval dataset downloaded from LandFire database (LANDFIRE 2020). Three-dimension topographic distance was calculated in ArcGIS (ESRI 2022) using 30 arc-second SRTM dataset (downloaded from

Worldclim 2.0) (Hijmans et al. 2005), this hypothesis serves as a characterization of the null hypothesis of isolation by distance.

Mixed Spatial Models of Landscape Genetic Connectivity

To model the relationships between our observed genetic data and the environment, we used linear mixed effect models of the ten spatial hypotheses of landscape connectivity. Twenty two locations were included in this analysis. If individuals from the same genetic cluster (by majority assignment to a specific cluster, K=17; Tackett et al. 2022) occurred more than 200 km apart, then that cluster was represented at both locations, resulting in n = 22 genetic groups. Mixed effect models were run in R package, ResistanceGA (Peterman 2018) for every pair of the ten spatial hypotheses, as well as, each hypothesis run as a single predictor (totaling 37 unique modeled combinations. See Table S3 for details). Mixed models with three or more predictor variables were not evaluated as model groups to be compared were too numerous and thus, were not computationally feasible.

The ten spatial hypotheses were converted to resistance surfaces for mixed effect modeling using the R package, ResistanceGA (Peterman 2018). This program requires no *a priori* assumptions related to environmental response and instead utilizes a genetic algorithm to fit surface layers individually to pairwise genetic data to find the statistically best resistance layer(s) (Peterman 2018). ResistanceGA optimizes resistance surfaces based on pairwise genetic data and distances calculated using CIRCUITSCAPE. Since ResistanceGA provides a true unconstrained optimization there are no predefined resistance surfaces to assess, instead the optimization algorithm iteratively generates resistance surfaces exploring eight mathematical transformations and a broad array weighting regime until no improvement in predicting input genetic data can be made. This is coupled with maximum likelihood population effect (MLPE) model performance criteria to best objectively optimize resistance surface(s) (Peterman et al. 2019). Fit of regression between the environmental variables chosen and genetic data are used to determine the most likely migration corridors and the landscape features that are most probable in facilitating or inhibiting distribution (Van Strien et al. 2012). For these reasons ResistanceGA has become a popular method in addressing fundamental questions regarding landscape genetics and landscape ecology and was chosen for this study. Once all resistance surfaces were optimized, ResistanceGA (Peterman 2018) was run to assess the resistance of each layer individually and in pairs resulting in 37 total models. Models were ranked using marginal R^2.

Historical Patterns of Population Admixture and Divergence

The statistical model TreeMix v1.13 (Pickrell and Pritchard 2012) was used to infer historic patterns of population admixture and divergence where the frequency of alleles in present-day populations infers the structure of the maximum likelihood (ML) tree.

A SNP dataset for *T. perfoliata* was generated from a previous analysis in STRUCTURE which yielded four genetic clusters (Tackett et al. 2022) and used *T. biflora* as the outgroup (See Table S1). A covariance matrix was built to assess model fit for population relationships. TreeMix uses a Gaussian model to assess drift between ancestral and contemporary populations (Cavalli-Sforza and Edwards 1967). We estimated migration events from zero to three. For populations with the largest residuals migration edges are added and the graph is optimized for branch length and the weight of the migration edge. Weight of the migration edge indicates the allelic fraction of parental population inheritance. This process is repeated until the migration edge that most improves the likelihood is chosen. Then the relative weight of the migration edge is estimated, as well as its uncertainty, across the genomic dataset. Residuals deviating from zero identify populations that are not well-modeled under non migration scenarios and thus are candidates for admixture events to best explain the genetic data.

Results

Divergence Time Estimation

We estimated the mean divergence time for the node corresponding to the most-recent-common ancestor of *Triodanis perfoliata* and *Triodanis biflora* to be approximately 1.9 mya (95% HPD = 0.5 − 3.7) in the fossil-calibrated BEAST analysis (Fig. S2). This age range estimate approximates well with the age recovered by our MCMCTree results (mean of 3.3 MYA for the divergence of *T. perfoliata* from *T. biflora* and mean of 2.4 MYA for the crown clade containing all sampled *T. perfoliata* populations; Fig. 3). We estimate that the crown age of *T. perfoliata* populations surveyed in this study is approximately 2.4 mya (3.2-1.4). After speciation, *T. perfoliata* continued to diverge at a steady rate, with the core lineages (sensu Tackett et al. 2022) diverging in the early Pleistocene (2.4-1.5 Ma, Fig. 3; Appendix S1).

Mixed Spatial Models of Landscape Genetic Connectivity

The spatial model with the highest support (marginal r^2= 0.612) is a composite surface of the early Holocene (11.7 − 8.326 ka) and the Marine Isotope Stage M2 period (ca. 3.3 Ma; Fig. 1). The model with the second highest support (marginal r^2= 0.597) was a composite surface of the current period and the Marine Isotope Stage M2 period. Spatial models based only on contemporary climate (marginal r^2= 0.551) or only isolation-by-distance, performed more poorly than models with paleoclimate data (3D distance and 2D distance: marginal r^2= 0.546 and 0.485, respectively). The assessment of 2D distance is a default test within the ResistenceGA program (see Table S3 for the results of all models compared with corresponding AICc, marginal r^2, conditional r^2).

Historical Patterns of Population Admixture and Divergence

Our inference of historical patterns of population admixture and divergence best supported a scenario with a period of moderate introgression from the ancestors of *T. biflora* into the ancestors of *T. perfoliata* (2.8% of alleles inherited from *T. biflora* into Cluster 4, p < 0.001, Fig. 2). We also recovered an instance of high gene flow from the Cluster 1 into Cluster 3 (four genetic clusters in total; 38.2% of alleles inherited from Cluster 1 to Cluster 3, p < 0.001). Cluster 1 is currently distributed in the western US and extends eastward into Kansas and Cluster 3 is currently distributed in New York. The inclusion of historical population admixture and divergence better explains our observed genetic diversity, with the scenario of two migration events resulting in the highest support (see Fig. 2A and B, Table S4).

Discussion

Here we explore the roles of paleoclimate, climate stability and historic population admixture and divergence in shaping population genetic patterns in a widespread annual plant, *Triodanis perfoliata*. Our current study significantly expands previous research focused on the roles of contemporary climate, isolation by distance, and breeding system in shaping population connectivity (Tackett et al. 2022). Overall, this research is part of the growing call to use interdisciplinary approaches to elucidate drivers of population genetic patterns (Chan et al. 2011, Brown et al. 2016a, Shen et al. 2019, Dolby et al. 2022). A range of factors, both extrinsic (e.g., environmental, geospatial; Brown et al. 2016a, Alvarado-Serrano et al. 2019) and intrinsic (e.g., demography, physiological tolerances, seed and pollen dispersal, breeding system; Chan et al. 2011, Toczydlowski and Waller 2019, Tackett et al. 2022), influence patterns of genetic diversity. These factors are highly dynamic, varying across space and time, illustrating the potential influence of historic processes in shaping patterns of genetic diversity. Combining these processes with temporally explicit genetic data allows for a more complete understanding of population genetics (Carnaval et al. 2009, Bohonak and Vandergast 2011, Chan et al. 2011, Shen et al. 2019, Dolby et al. 2022).

Despite the importance of paleoclimates in shaping population genetic patterns, (Rangel et al. 2018, Rahbek et al. 2019a, Rahbek et al. 2019b), very few studies assess paleo-periods outside of the last interglacial cycle (ca. 130 ka to contemporary climates; Rull 2008, Rull 2011). This potential bias in studies has been, in part, a result of limited availability of older paleoclimatic datasets. In recent years, however, data from additional paleo-periods have become available (Lima-Ribeiro et al. 2015, Brown et al. 2018), although researchers continue to focus on relatively recent geological times. As such, there remains a need for studies that incorporate temporally relevant paleoclimatic data with genomic data to understand drivers of phylogenetics and population genomics (however see Evans et al. 2009, Prates et al. 2016, Rangel et al. 2018, Guillory and Brown 2021). The advantages of including these types of data can be seen through studies of several oak species in the Mexican highlands in which high genetic diversity but low genetic structure is explained through historical processes such

as range stability, elevational displacement, and dynamic gene flow between populations (Peñaloza-Ramírez et al. 2020, Sánchez-Acevedo et al. 2023). The dynamic nature of climate, especially over thousands or millions of years, alters the landscape and therefore the connectivity of populations and movement of individuals. These changes can have profound impacts on the genetic structure of affected populations (Taylor et al. 2006, Yannic et al. 2020). In *T. perfoliata*, we found that paleoclimatic periods both prior to and during the last interglacial to be significant in characterizing modern patterns of genetic diversity, highlighting the importance of diverse paleoclimatic data in these types of population genetic analyses. Our results support an important role for late Pliocene climates in structuring the population genetic diversity of *T. perfoliata* (Table S3). Our best mixed spatial model of landscape genetic connectivity had a marginal $r^2=0.612$, which is impressive given the observed introgression, as well as, the complicated population genomic clustering results in the center of the species' distribution, where multiple cluster groups coexisting within many populations. Each of these factors challenge the creation of a single 'genetic landscape', as these events likely occurred at multiple time periods that spanned many climatologies. Our genetic results (current and past) suggest that *T. perfoliata* underwent a complicated biogeography history, extending beyond a single expansion, and rather, likely experience multiple and repeated interactions among major lineages within *T. perfoliata.*

The late Pliocene, a key period in our best mixed spatial model, also coincides with the early divergence of *T. perfoliata* (2.4 mya [3.2-1.4]), suggesting a key role of this time period in shaping the phylogeography of this species (Fig. 3). It is worth clarifying that the 95% confidence intervals of the divergence estimate extend into the early Pleistocene (starting ca. 2.56 mya), thus, these mixed spatial modeling results and divergence time estimates are not perfectly aligned. However, given the fact that we only have paleoclimate data for time periods at 3.3 mya (late Pliocene) and 0.0787 mya (mid-Pleistocene), there certainly remains many key time periods unavailable that could better characterize additional historic periods important to the early divergence of *T. perfoliata.* However, these two datasets do share many climatic similarities to other temporally adjacent periods and might be producing causal correlations due to simular climatologies at other time periods, something that is most likely to occur in the matching epochs (e.g. mid-Pleistocene) corresponding to all input climatologies. Given we do not have a climate dataset for the early Pleistocene, we cannot compare the relative influence of this time period.

Even in light of challenges associated with temporally connecting spatial and genetic data, this study elucidates the importance of including paleoclimates from key periods of diversification when examining how climatic drivers impact population genetic patterns. Distance between contemporary suitable habitats, for example, does not necessarily reflect past patterns of variability in habitat, which have led to modern patterns of genetic structure (Elith et al. 2006). For example, the origins of contemporary genetic diversity do not necessarily match the current species distribution, as such, past periods and regions of high gene flow might be better characterized using the methods proposed here. This statement is supported by the fact that an analysis based on isolation-by-distance had poorer performance (marginal r^2= 0.546 and 0.485) compared to our highest supported (marginal r^2= 0.612) based on the early Holocene and the Marine Isotope Stage M2 period).

Our study highlights the importance of historic climate in shaping patterns of genetic diversity in *T. perfoliata*. By gaining a detailed knowledge of geospatial patterns, both historic and contemporary, we are able to discern when and where barriers to gene flow may have influenced overall patterns in population genetics (Elith et al. 2006). For species with limited dispersal and widespread distributions, more recent climates likely have less impact on the broader spatial genetic patterns. Our highest performing mixed spatial model was one that included paleoclimates and it outperformed a model based only on contemporary climates (marginal r^2= 0.612 vs. marginal r^2= 0.551, respectively). Contemporary climates, however, have been repeatedly shown to effectively describe patterns of population genetic diversity at smaller spatial scales (e.g., Toczydlowski and Waller 2019, Tackett et al. 2022). For these reasons, considering both historic and present-day factors when discerning patterns of genetic diversity can provide novel insights into drivers of population genetic diversity.

Our inference of historical patterns of population structure shed light on an enigmatic cluster group that is currently found in New York state (Cluster 3, Fig. 2; Tackett et al. 2022). Tackett et al. (2022) placed this population in a separate cluster group from other East Coast populations (Cluster 4, Fig. 2), which all formed a cohesive group. We recovered an instance of high gene flow between a cluster group currently in the Western and Midwest of the US (Cluster 1) into our New York cluster group (Cluster 3). These results suggest a historic connection between the Midwest and Upper East Coast populations, where members of Cluster 1 introgressed into Cluster 3. Our mixed models of landscape connectivity also suggest a similar Midwestern corridor across the Ohio River drainage (Fig. 1). Given the current geographic isolation of the modern populations and the inferred directionality of the introgression, from the West into the North East, it is likely that the ancestral population sizes of Cluster 3 were small given the predominant genomic signature of Cluster 1 present in Cluster 3 (and not vice versa) and that this group remained somewhat isolated post-introgression. These results corroborate population genetic structure observed by Tackett et al. (2022). Tackett et al. (2022) suggested that *T. perfoliata* also experienced a complex demographic and evolutionary history, where they found breeding system, geography, and present-day environmental variables shape patterns of population genetics of *T. perfoliata*. Through integrative genomic and spatial analyses, we expand upon these findings, verifying the importance of both historic and present-day factors when discerning patterns of genetic diversity.

Conclusions

In this study, we take an interdisciplinary approach to comprehensively understand factors driving patterns of population genetics across geographic space and through evolutionary time in the widespread annual species, *Triodanis perfoliata*. Previous work examined the interplay of a novel, and likely highly inbreeding, reproductive system on patterns of population connectivity and genetic diversity (Tackett et al. 2022). This previous work quantified the roles of isolation by distance and breeding system in shaping the overall population genetic structure of this species, but also alluded towards the potential roles of demographic history and climate for influencing population connectivity. Here, we expand on this work by specifically elucidating the roles of past- and current climate and climatic stability in shaping population genetic patterns in these same populations. We found that past climatic periods, including those prior to the last interglacial, are important factors in driving observed patterns of genetic diversity. Our divergence time estimation coincides with our climate analyses, suggesting the late Pliocene as a key time period in the overall evolutionary history of *T. perfoliata*. Understanding demographic history and historic climate, provides insights as to the apparent genetic divergence among some populations that may otherwise appear connected by contemporary suitable habitat (i.e. NY population; Fig. 2A; Tackett et al. 2022). Taken together, our research in this study system, including Tackett et al. (2022) and the present work, emphasizes the importance of recognizing the impacts of both current and past processes in shaping population genetic patterns, and in examining these factors through multidisciplinary research approaches (see: Chan et al. 2011, Brown and Knowles 2012, Crowl et al. 2015, Brown et al. 2016a).

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Data Accessibility

Genomic data generated for this project are available via Dryad at https://doi.org/10.5061/dryad.sf7m0cg98 and https://doi.org/10.5061/dryad.wh70rxwr9 Genbank accession numbers for DNA sequences used in Crowl et al. (2014). https://doi.org/10.1371/journal.pone.0094199.s001

Supplemental Material

This material is available as part of the online article at https://escholarship.org/uc/fb
Figure S1. Model raster layers.
Table S1. Tackett et al. (2022) genetic cluster information.
Table S2. Paleoclimate citations.
Table S3. Scoring of all mixed spatial models of landscape genetic connectivity.
Table S4. TreeMix model scoring.
Figure S2. Chronogram from BEAST analysis of the Campanuloideae dataset from Crowl et al. (2014) with additional Triodanis perfoliata and T. biflora sampling.

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