Using genomic data to study population structure and adaptive traits of *Physaria globosa*

Megan G. Connor[1, 2], Wen-Hsi Kuo[1, 3], and Christine E. Edwards[1, 3]

[1]Center for Conservation and Sustainable Development, Missouri Botanical Garden, St. Louis, MO, United States,
[2]Department of Biology, Marist University, Poughkeepsie, NY, United States
[3]Department of Biology, Washington University, St. Louis, MO, United States

Address correspondence to M. Connor at this email: megangconnor0@gmail.com.

ABSTRACT

Natural selection drives local adaptation of a species to enhance its fitness to the local environment. Isolated populations have limited gene flow and migration resulting in genetic structure. A geographically isolated and small population are at risk of losses in genetic diversity, which is essential for maintaining resiliency and viability. Highly endangered species that have strong genetic structure and losses in genetic diversity due to geographic and environmental isolation are important to conserve the health of our ecosystem. The federally endangered short's bladderpod (*Physaria globosa*) is a key self-incompatible species to protect as it is geographically isolated and likely has limited seed dispersal. By performing landscape genomic analysis on 89 maternal lines collected across 8 locations spanning Tennessee, Indiana, and Kentucky, genetic structure, genetic diversity, and environmental associations can be examined. It was found that this species has strong genetic structure across its geographic range with a possible recent translocation from Franklin county to the Posey population and a recent translocation of two individuals from the Davidson region to Trousdale. The northern populations were found to be one distinct genetic cluster grouped together while the southern populations generally split up into their own distinct genetic clusters. High levels of inbreeding were found across the range of populations pointing to the possibility of self-compatibility despite. Significantly strong associations of isolation by environment for precipitation and temperature-related variables shows possibility of local adaptation. Highly fragmented populations, limited seed dispersal, low genetic diversity within populations, and majority of the genetic diversity partitioned among populations means each population may be genetically unique so it is necessary to protect many of the populations across the range to conserve as much genetic diversity as possible.

KEY WORDS

Climate change, landscape genomics, local adaptation, inbreeding, temperature, precipitation, short's bladderpod (*Physaria globosa*)

1 | INTRODUCTION

Local adaptation often occurs in plant species when populations genetically diverge, resulting in differences in phenotypic traits that enhance fitness to the local environment (Kawecki & Ebert, 2004). This process, driven by natural selection, shapes genetic variation within a species and contributes to the development of genetic structure (Sexton, Hangartner, & Hoffmann, 2014). Genetic structure can also arise from restricted dispersal and geographic isolation, limiting gene flow and migration (Avise, 2000). Understanding genetic structure is vital for recognizing the role of genetic diversity in a species' long-term stability, resilience, and ecological function (Hughes et al., 2008). Genetic diversity enhances a population's potential to evolve in response to environmental change and is positively correlated with individual fitness (Reed and Frankham, 2003). For endangered species, it is important to protect the full range of genetic diversity to ensure their resilience and persistence (Caballero and Toro, 2002).

Small and geographically isolated populations are at greater risk of experiencing losses in genetic diversity through genetic drift, genetic bottlenecks, and inbreeding. Genetic drift, which reduces genetic variation, is more pronounced in small population sizes (Ellstrand and Elam, 1993). When isolated populations lack gene flow, which helps to prevent losses of genetic diversity, they are more vulnerable to losses of genetic diversity due to genetic drift. Small populations are also vulnerable to inbreeding due to limited availability of unrelated mates. Because genetic diversity is essential for maintaining the resilience and viability of populations, conservationists must sample populations across the species' geographic range, assess existing genetic variation, and analyze its structure to effectively conserve the diversity of the endangered species. Genetic diversity loss may be mitigated through translocation efforts, where individuals are moved between populations to enhance genetic variation and increase effective population size (Edwards et al., 2023).

Local adaptation has also been increasingly investigated to identify genes involved in the divergent expression of known adaptive traits (Wadgymar et al., 2017) and examine the association between genetic variation and environmental gradients (Martins et al., 2018). As climate change and human activity increasingly threaten biodiversity, understanding local adaptation can inform conservation efforts for endangered species vulnerable to these habitat fluctuations. As environmental conditions shift rapidly, locally adapted populations may become mismatched with their changing environments, which is known as maladaptation. This can reduce survival and reproduction unless populations possess enough genetic variation to respond to new selective pressures.

The federally endangered Short's bladderpod (*Physaria globosa*) is a biennial or perennial plant species in the mustard family, *Brassicaceae* (Long et al., 2020). It has multiple stems, some branching at the base, which typically give the plant a low, bushy appearance (Long et al., 2020). The species produces yellow flowers that are pollinated by small insects, with bees and flies documented as floral visitors (Thacker et al., 2019; Rollins and Shaw, 1973). Self-incompatibility has been observed in greenhouse plants grown without pollinators, resulting in a lack of seed production (Baskin, 2012). While direct studies on seed dispersal are lacking,

small seed size (1.0-1.8 millimeters) and potential reliance on gravity and wind suggest dispersal is limited (Shea, 1993). Short's bladderpod typically inhabits steep, rocky, wooded slopes and talus areas along the tops, bases, and ledges of bluffs (Long et al., 2020). It is most frequently found on south-to-west-facing slopes adjacent to rivers or streams (Long et al., 2020). *P. globosa* occurs in four geographically isolated regions, including sites near the Kentucky River, near the Ohio River, and along the Cumberland River (Long et al., 2020). A combination of the geographical isolation of *Physaria globosa* and the species' limited seed dispersal, likely restricted by its small seed size and reliance on gravity and wind, suggests that the species may display adaptation to the local environment, resulting in genetic structure.

As of 2019, *P. globosa* has been found in 33 occurrences, spanning Tennessee, Kentucky, and Indiana: 21 in Tennessee across seven counties, 11 in Kentucky across four counties, and 1 in Posey County, Indiana (Long et al., 2020). However, fewer than 100 individuals were present at 28 of the 33 sites (Long et al., 2020). All extant sites likely face one or more of the following threats: roadside maintenance, overstory shading, competition with invasive species, flooding and water level fluctuation, natural landslides, climate change, and stressors resulting from small, geographically isolated populations (Long et al, 2020). Owing to geographic isolation, limited seed dispersal, small population sizes, and the presence of threats, *P. globosa* may have faced reductions in genetic diversity, which can compromise the long-term resilience of the populations. Together, these factors indicate the potential for local adaptation in *P. globosa*; however, this has not been researched in previous studies.

This study aims to determine whether *Physaria globosa* exhibits genetic structure by performing population structure analyses, genetic diversity, and local adaptation by investigating the relationship between genetic distance and environmental variables. Understanding these patterns can help guide strategies to preserve genetic diversity and inform translocation or habitat restoration decisions.

2 | MATERIALS AND METHODS

2.1 | Sample collection and DNA extraction

Mature seeds from 89 maternal lines of wild *Physaria globosa* were collected across eight locations in Tennessee, Indiana, and Kentucky (see Figure 1 for sampling locations). Latitude and longitude were recorded for each accession. During collection, leaf tissue was placed into silica gel for subsequent DNA extraction (Edwards et al., 2023), and genomic DNA was extracted from the silica-dried leaf tissue using a standard CTAB-based protocol (Whitelock et al., 2008). Seeds were collected and stored in the Missouri Botanical Garden seed bank until germination began in December 2023. Seeds were plated on agar in Petri dishes (one per accession) and cold/dark stratified at 1°C for 15 weeks. On March 18, 2024, seeds were moved to germination conditions of 25/15°C with 12-hour light/dark cycles. Germinated seedlings were transferred to 72-cell trays with standard potting mix and maintained under the same temperature/light regime. On April 17, 2024, seedlings were moved to the Oertli Family Hardy

Plant Nursery in St. Louis, Missouri, and transplanted into 4" plastic pots on April 26, 2024. Plants were grown under standard greenhouse conditions (Wright et al., 2022), with mass irrigation every three to four days in fall and daily to every other day in spring. An automatic shade cloth maintained temperatures between 10°C and 24°C during the growing season. To induce dormancy, temperatures were reduced to 2–6°C in late November 2024 and increased again to 9–15°C in late February 2025.

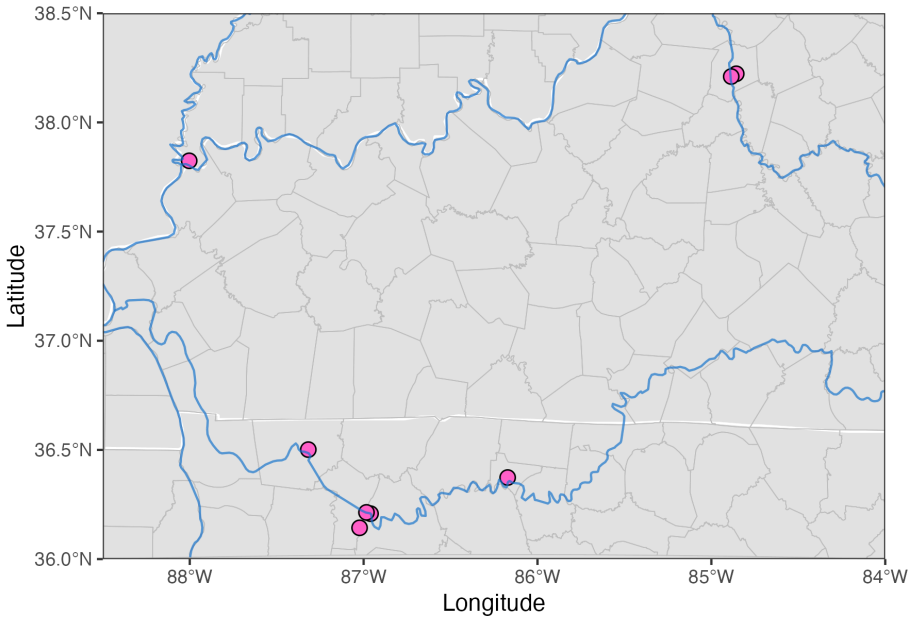

FIGURE 1. Short's bladderpod collections were used in this study. The 89 maternal lines were collected from 8 locations across Tennessee, Indiana, and Kentucky.

2.2 | Variant Filtering and Genotype Quality Control

Raw variant data were filtered to retain only biallelic SNPs using BCFtools v1.22 to ensure SNP quality. Genotypes with low confidence were classified as missing if they had genotype quality (GQ) below 10, read depth (DP) below 3, or DP above 50. SNPs were then filtered to keep variants with a minor allele frequency (MAF) ≥ 0.01 and <10% missing data across samples. The final VCF was annotated to include selected INFO fields, compressed, and indexed for further processing and analyses.

2.3 | Simple Imputation and SNP Pruning

Simple imputation for missing genotypes was performed using PLINK2 using the --fill-missing-a2 option, which imputes missing genotypes by assigning the second allele at each site. This method was used to ensure complete genotype matrices for future analyses. Linkage disequilibrium (LD) is influenced by admixture and population structure, as loci that are unlinked within each ancestral population may appear linked when analyzed jointly, potentially invalidating downstream analyses (Bercovich et al., 2024). LD pruning was then performed using a window size of 50 SNPs, a step size of 5, and an r^2 threshold of 0.2 to remove correlation

between markers. The pruned dataset was utilized for population structure and genetic structure analyses.

2.4 | Population Structure and Genetic Differentiation

Population structure was analysed based on the LD-pruned SNP datasets. Principal component analysis (PCA) and pairwise F_{ST} estimation were conducted via PLINK2 (Chang et al., 2015). Both PCA and F_{ST} results were plotted in R to analyze patterns of population structure across sampling groups.

Population structure was further assessed using ancestry estimation performed with ADMIXTURE v1.3.0, testing for ancestral populations (K) from 1 to 8, to represent the 8 sampling sites, in cross-validation mode (Alexander et al., 2009). Cross-validation (CV) errors were extracted from the ADMIXTURE log files. The CV error values were plotted in R to supplement the analysis of the error trend across all K values.

Ancestry estimations were visualized geographically by incorporating the ADMIXTURE outputs with the sample metadata. Pie charts were used to represent the ancestry proportions and were plotted on maps using the scatterpie and ggplot2 packages in R with coordinate transformations to EPSG:3857 to ensure spatial accuracy. Geographic layers, including state boundaries, county boundaries, and river systems, were added using the rnaturalearth package.

2.5 | Genetic Diversity

The inbreeding coefficient (F) was calculated for each individual using VCFtools v0.1.17. The F values were visualized in R using a histogram across all individuals and further analyzed using boxplots and violin plots grouped by county to compare the inbreeding levels across geographic locations.

2.6 | Environmental Associations

To explore the correlations between genetic differentiation, geographical distance, and environmental distance, we calculated pairwise Hudson's F_{ST} values between maternal lines. Linearized F_{ST}values were calculated as F_{ST} /(1−F_{ST}) to facilitate the Mantel test, which was then performed in R using the 'Vegan' package to assess correlations between genetic differentiation and each environmental variable. Environmental data for each sampling location were extracted from WorldClim v2.1 30. To explore structure in the climate dataset, we conducted Principal Component Analyses. Environmental distances based on scaled climate variables were calculated between populations and paired with genetic (F_{ST}) and geographic distances to explore how environmental variation aligns with genetic differentiation in PCA space.

3 | RESULTS

3.1 | Population Structure

According to ADMIXTURE analyses with assumed genetic clusters (K) ranging from K = 1 to 20, the lowest cross-validation error was observed at K = 13 (Figure 3). The optimal number K is 5 since there is no observable difference in error after this value.

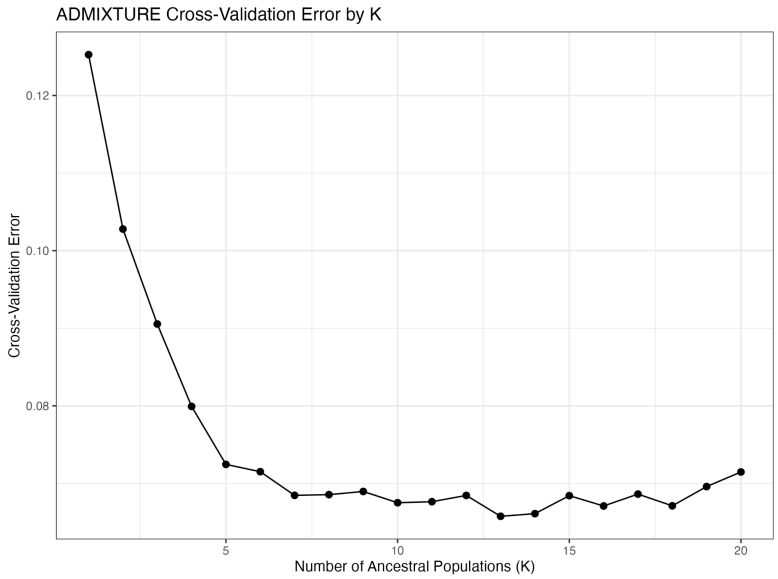

FIGURE 3. Cross-validation error of the ADMIXTURE analysis. The minimum cross-validation error occurs at K = 13. A significant difference in cross-validation error does not occur after K = 5.

The northernmost sample sites, Posey, Franklin1, and Franklin2, maintained a single ancestral population composition together (Figure 4a). The Franklin1 and Franklin2 sample locations are in close proximity to each other. The Posey sample location, on the other hand, is situated far downstream from the Franklin County populations (Figure 4b). As for the southern populations, each sample location is isolated into its own separate cluster except for Cheatham2 and Davidson, which are paired together in the same ancestral population (Figure 4a). Although in the Trousdale sample location, two individuals can be seen being part of the Cheatham2 and Davidson ancestral population, not the overall Trousdale genetic cluster (Figure4a). These southern populations are each located directly on the southern river, except for Cheatham1, which is adjacent to the river. Out of all the southern sample locations, Cheatham2 and Davidson are located in the closest proximity to each other (Figure 4b).

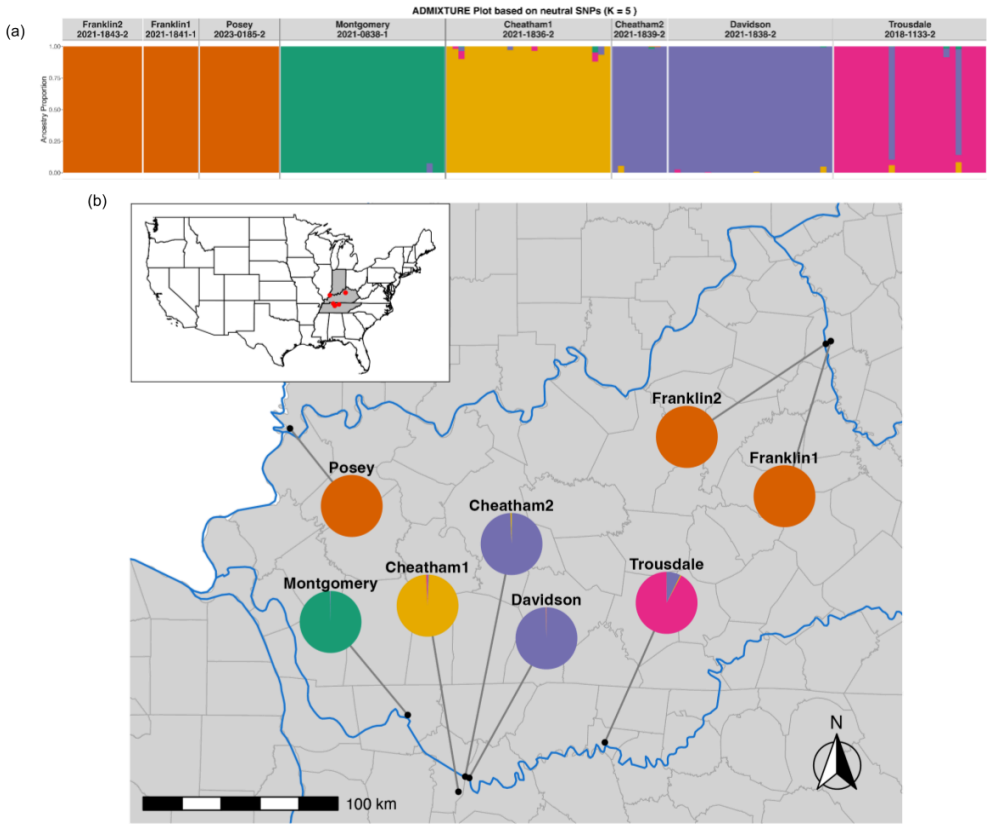

FIGURE 4. Population genetic analysis of *Physaria globosa* at 8 sampling sites across Tennessee, Kentucky, and Indiana. (a), ADMIXTURE plot for K (ancestral population) cluster value of 5, ordered from the northeast sampling sites to the northwest sampling site, then the sampling sites along the southern river from west to east. Each individual is represented by one line, and each color represents one population cluster. (b), Pie charts showing cluster percentages per sampling site using K = 5 from ADMIXTURE analysis shown in 3a. Pie charts have been manually situated to avoid overlap and are connected to their actual geographic locations by a black line. The inset figure gives geographic context for the sampling locations.

The PCA plot generally separated the Tennessee samples from the Kentucky and Indiana samples (Figure 5a). All of the northernmost populations, Franklin1, Franklin2, and Posey, were clustered together in genetic PCA space (left side of Figure 5a). The southernmost populations were clustered in 3 groups in the PCA plot. The furthest west population, Montgomery, was clustered separately and distinctly from all other populations (top-right corner of Figure 5a). The furthest east population, Trousdale, was clustered separately and distinctly from all other populations as well (bottom-right corner of Figure 5a). As for Cheatham1, Cheatham2, and Davidson, these populations were all grouped together in the PCA plot (Figure 5b).

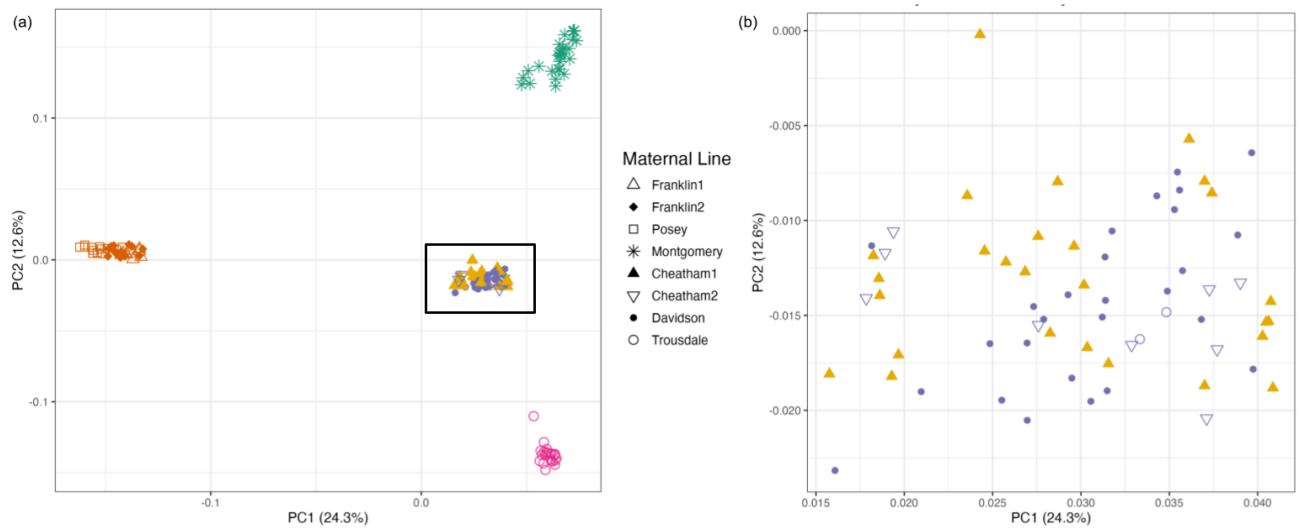

FIGURE 5. Principal Component Analysis colored by the corresponding ancestral population from ADMIXTURE analysis at K = 5 and shaped by maternal line. (a), PCA of individuals from each *P. globosa* sampling location. (b), Zoomed portion of the main PCA graph that only includes 2 ancestral populations, which spans across Cheatham1, Cheatham2, Davidson, and Trousdale. There are only 2 individuals from Trousdale in this portion, which are visible in the ADMIXTURE analysis.

Pairwise F_{st} values ranged from 0.05 to 0.51 (Figure 6). In line with the ADMIXTURE results, pairwise F_{st} values were generally lower in closely spaced population pairs than in other pairs of populations: Franklin1/Franklin2 = 0.12, Cheatham2/Davidson = 0.05, Posey/Montgomery = 0.51, Posey/Trousdale = 0.50 (Figure 6). However, Posey's relationship with both Franklin sampling locations were found to have low F_{st} values: Posey/Franklin1 = 0.21, Posey/Franklin2 = 0.24 (Figure 6). When comparing this to Cheatham1/Davidson, the F_{st} value was found to be 0.23 (Figure 6). Despite the similarity to the Posey/Franklin2 F_{st} value, Cheatham1 was not grouped with Davidson and Cheatham2 like Posey being grouped with Franklin1 and Franklin2 in the ADMIXTURE analysis at K = 5 (Figure 4a).

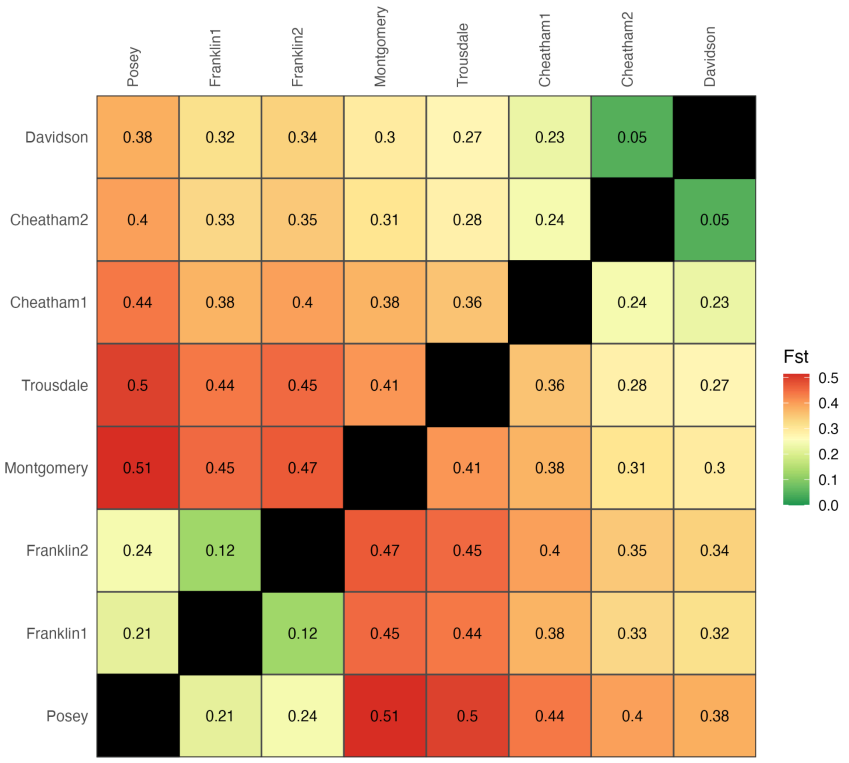

FIGURE 6. Pairwise F_{st} values between populations of *Physaria globosa*.

3.2 | Genetic Diversity

The average inbreeding coefficient, F_{is}, for all populations is positive, with the lowest average being 0.571 for Franklin1 and the highest average being 0.766 (Figure 2; Table 1).

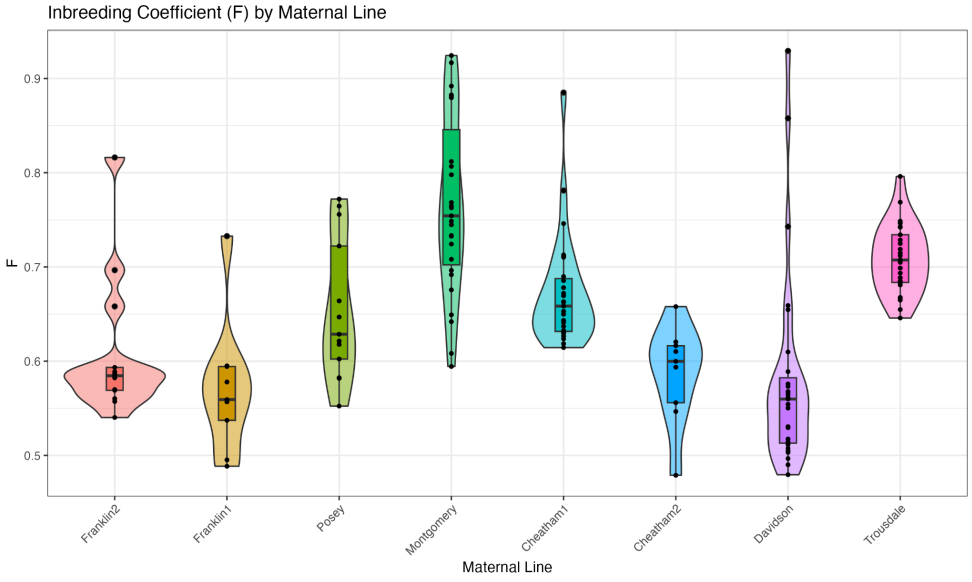

FIGURE 2. The F_{is}, inbreeding coefficient, measured the extent of inbreeding within each sampling population of *Physaria globosa*.

3.3 | Environmental Associations

F_{st} was tested between the 8 sampling locations as a function of geographical distance or environmental divergence. Genetic differentiation is significantly correlated with geographical distance between locations (Mantel r = 0.476, p = 0.013) (Figure 8a). However, genetic differentiation displays stronger relationships with precipitation and temperature-related variables than with geographical distance alone (Figure 7). Among the environmental variables, Precipitation of Driest Quarter (mm), which helps determine the seasonal distribution of precipitation by identifying the 3 consecutive months with the least amount of rainfall, is the most effective in explaining genetic differentiation (Mantel r = 0.782, $p < 0.05$) (Figure 8b, Figure 7). Similarly, Annual Precipitation (mm) accounts for a substantial portion of genetic differentiation (Mantel r = 0.760, $p < 0.05$) (Figure 8c, Figure 7). Temperature-related variables also contribute to genetic differentiation, as shown with Maximum Temperature of the Coldest Month (ºC × 10), which shows a significant correlation (Mantel r = 0.684, $p < 0.05$) (Figure 8d, Figure 7).

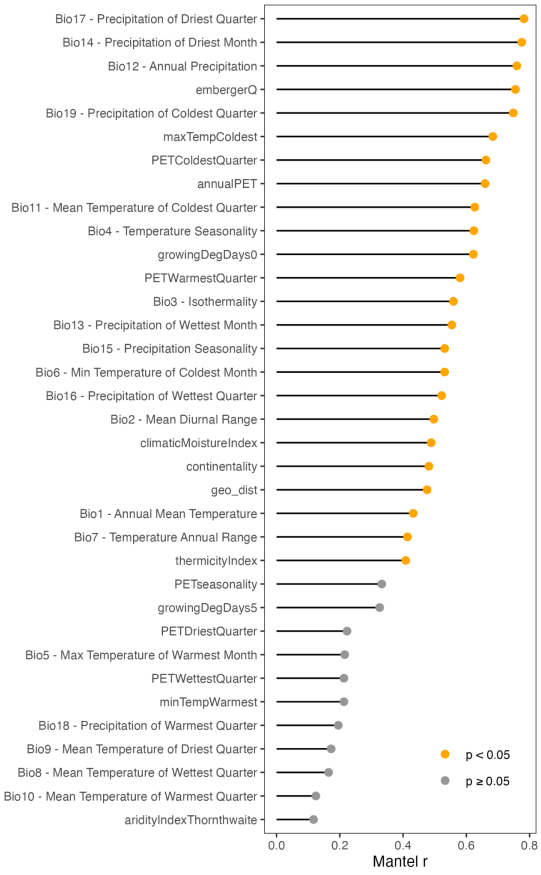

FIGURE 7. Mantel test for the correlations between the genetic distance ($F_{st}/1\text{-}F_{st}$) and the environmental variables. The p value was estimated from a permutation test of 5,000 times.

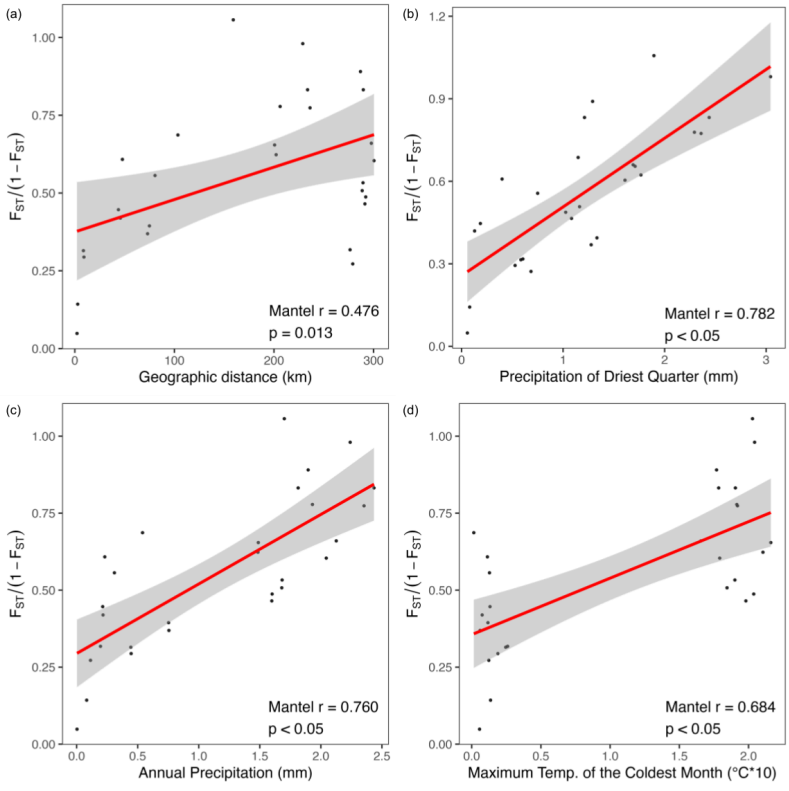

FIGURE 8. Geographical and environmental correlations. (a), Pairwise linearized genetic differentiation between locations ($F_{st}/1\text{-}F_{st}$) is significantly associated with geographical distance. (b), Euclidean distance of Precipitation of Driest Quarter (mm). (c), Euclidean distance of Annual Precipitation (mm). (d), Euclidean distance of Maximum Temperature of the Coldest Month (ºC*10).

4 | DISCUSSION

4.1 | Genetic Structure

The first goal of this study was to analyze how genetic variation is organized across the entire geographic range of the species. Genetic structure is created by limited gene flow and genetic drift. We found strong patterns of genetic structure in *Physaria globosa* seen in PCA and ADMIXTURE results. Examination of ADMIXTURE results at K = 5 shows clear assignment of individuals to clusters by sampling location and geography, except for the fact that all northern populations were grouped together and 2 of the southern populations were grouped together (Figure 4b). The clear assignment of individuals into clusters rather than admixed groups suggests a lack of gene flow between populations. The genetic clusters were: (1) the three northern populations, Posey, Franklin1, and Franklin2, (2) Montgomery, (3) Cheatham1, (4) Cheatham2 and Davidson, and (5) Trousdale. However, in the Trousdale population, two individuals are grouped with the 4th cluster, Cheatham2 and Davidson (Figure 4a). This shows a significant isolation by distance between northern and southern populations (Wright, 1943). This also shows a strong genetic structure between even proximal groups like Cheatham1 to

Cheatham2 and Davidson due to the small seed size and short dispersal radius, which is consistent with that of P. filiformis in a previous study (Edwards et al., 2021).

The grouping of the 3 northern populations together in ADMIXTURE analysis and in the PCA plot points to a possible recent translocation from the Franklin populations to Posey county, whether that was done via natural forces, like the river, or through human interaction. Despite the considerable distance between these sets of northern populations, they show similar genetic structure. This is supported with the F_{st} data where the relationship between Franklin1 and Franklin2 (F_{st} = 0.12) is similar to that of Posey to each Franklin population (Posey/Franklin1 = 0.21, Posey/Franklin2 = 0.24) (Figure 6).

At K = 5, the grouping of Cheatham2 and Davidson into one genetic cluster is supported with the F_{st}value being 0.05 between them (Figure 6). However, Cheatham1 does not get grouped into this genetic cluster, despite the F_{st} values being Cheatham1/Davidson = 0.23 and Cheatham1/Cheatham2 = 0.24 (Figure 6), which are similar to the Posey and two Franklin populations relationships. Also, Cheatham1 individuals are found grouped close to Cheatham2 and Davidson in genetic space (Figure 5). This may be due to the fact that the F_{st} value is smaller for Cheatham2/Davidson than that of Franklin1/Franklin2. However, you would expect due to the proximity that Cheatham1 has to Cheatham2 and Davidson, that it would be genetically closer to that group and grouped together in the ADMIXTURE than Posey has to the Franklin populations.

When comparing northern populations to southern populations, F_{st} values were highest (Figure 6), which is in concordance with the general pattern that increasing genetic distance increases with geographic distance. However, the relationship between Posey and the Franklin populations should be analyzed further to determine why they are so closely related despite their distance apart.

4.2 | Factors Affecting Genetic Diversity

The second goal of this study was to measure levels of genetic diversity in *P. globosa* and infer whether populations show evidence of reductions in genetic diversity as a result of inbreeding. While further analysis needs to be performed on genetic diversity, all of the *P. globosa* populations exhibited greater-than-expected inbreeding coefficients. Given that this species has previously been shown to be self-incompatibility (Baskin, 2012), it was expected that these populations would show little evidence of inbreeding, similar to that of P. filiformis which is a self-incompatible species (Edwards et al., 2021). Surprisingly, the populations showed average inbreeding coefficients ranging from 0.571 and 0.766 (Figure 2). This raises the question on whether these populations could actually be self-compatible.

More importantly, however, is whether this inbreeding has been detrimental to these populations. High levels of inbreeding increases homozygosity within a genome, increasing the likelihood of inbreeding depression, which occurs when individuals are homozygous for recessive deleterious alleles (Charlesworth and Willis, 2009). Since *P. globosa* is typically outcrossing and has demonstrated self-incompatibility, these populations may be particularly

vulnerable to the negative effects of inbreeding depression. Further research needs to be conducted to evaluate whether inbreeding depression is occurring and how it influences the viability of these populations.

4.3 | Environmental Associations Reveals Local Adaptation

While there is significant genetic isolation by distance across the sampled range (Figure 8a), results indicate a stronger signal of isolation by environment rather than by distance, which suggests local adaptation. The specific climatic variable that has been found to best describe isolation by environment, Precipitation of Driest Quarter (mm), helps determine the seasonal distribution of precipitation by identifying the 3 consecutive months with the least amount of rainfall. Its significantly high r value compared to that of the pairwise linearized genetic differentiation between locations displays the stronger influence that precipitation has on genetic structuring than distance does. The Maximum Temperature of the Coldest Month (ºC*10) also has a significantly high r value compared to that of geographic distance displays the stronger influence that temperature has on genetic structuring. This points to local adaptation in these populations.

5 | CONCLUSION

Due to the strong genetic structure found across sampling locations, multiple populations should be conserved throughout its geographic range to conserve genetic variation. Due to the grouping of northern populations in ADMIXTURE analysis, all 3 populations do not need to be fully conserved unless there is considerable difference in environmental factors in each region. As for southern populations, each population is important to conserve since they are genetically distinct from each other. However, since Cheatham2 and Davidson are genetically similar, it may not be as important to fully conserve both populations rather just proportions of each. Continued public protection and habitat management of these multiple populations within each ecoregion is integral to the survivability of this species. Further analysis should be conducted on whether the optimal K value was chosen in this study to determine if this many populations need to be conserved in order to preserve the range of this species.

The strong isolation by environment relationship suggests that there may be local adaptation found throughout these unique populations. It is important to understand these ecoregions and these possible adaptations in a changing environment to determine if transplanting varieties to better suited regions can result in higher viability in this endangered species. The northern and southern populations are crucial genetic sources for climatic adaptation in other regions as climate shifts over time.

ACKNOWLEDGEMENTS

We thank the researchers and graduate students working at the Center for Conservation and Sustainable Development at the Missouri Botanical Garden, including Matthew Albrecht, Burgund Bassüner, Jordan Hathaway, Brock Mashburn, Jordyn Riehn, Binoshi Hettihewa,

Eduardo Aguirre Mazzi, Maia Jones, and Jillian Cieslik. We thank Devin Rogers, Noah Dell, and Emily Powell for collecting the seeds. We thank the staff at Oertli Family Hardy Plant Nursery for providing plant care. Finally, we would like to especially thank Mónica Carlsen for running the Research Experiences for Undergraduates program at the garden.

FUNDING INFORMATION

This research in botany and conservation biology at the Missouri Botanical Garden was funded by the National Science Foundation through the Research Experiences for Undergraduates program (Grant No. DBI-2243824). Any opinions, findings, and conclusions or recommendations expressed in this material are those of the author(s) and do not necessarily reflect the views of the National Science Foundation.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

RESOURCES

Alexander, D. H., Novembre, J., and Lange, K. (2009). Fast model-based estimation of ancestry in unrelated individuals. Genome Research, 19(9), 1655–1664.

Avise, J. C. (2000). *Phylogeography: The History and Formation of Species*. Harvard University Press. https://doi.org/10.2307/j.ctv1nzfgj7

Baskin, C. C. (2012). Personal Communication. Email to Geoff Call, Fish and Wildlife Biologist. re: Short's bladderpod reproductive biology. University of Kentucky. December 10, 2012.

Bercovich, U., Rasmussen, M. S., Li, Z., Wiuf, C., and Albrechtsen, A. (2014). Measuring linkage disequilibrium and improvement of pruning and clumping in structure populations. bioRxiv, doi: https://doi.org/10.1101/2024.05.02.592187.

Caballero, A., and Toro, M. A. (2002). *Analysis of genetic diversity for the management of conserved subdivided populations*. Conservation Genetics, *3*, 289-299.

Chang, C. C., Chow, C. C., Tellier, L. C., Vattikuti, S., Purcell, S. M., and Lee, J. J. (2015). Second-generation PLINK: Rising to the challenge of larger and richer datasets. GigaScience, 4, 7.

Charlesworth, D., and Willis, J. H. 2009. FUNDAMENTAL CONCEPTS IN GENETICS The genetics of inbreeding depression. Nature Reviews Genetics 10: 783-796.

Edwards, C. E., Landon, C., and Albrecht, M. A. (2023). *Rangewide conservation genetics of Physaria globosa (Short's bladderpod)*. Unpublished Report. Center for Conservation and Sustainable Development, Missouri Botanical Garden.

Edwards. C. E., Tessier, B. C., Swift, J. F., Bassüner, B., Linan, A. G., Albrecht, M. A., and Yatskievych, G. A. (2021). *Conservation genetics of the threatened plant species Physaria filiformis (Missouri bladderpod) reveals strong genetic structure and a possible cryptic species*. PLOS ONE, *16*(3).

Ellstrand, N. C., and Elam, D. R. (1993). *Population genetic consequences of small population size: implications for plant conservation*. Annual Review of Ecology and Systematics, *24*, 217-242.

Hughes, A. R., Inouye, B. D., Johnson, M. T. J., Underwood, N., and Vellend, M. (2008). *Ecological consequences of genetic diversity*. Ecology Letters*, 11*, 609-623.

Kawecki T. J., and Ebert, D. (2004). *Conceptual issues in local adaptation*. Ecology Letters, *7*(12), 1225–1241. https://doi.org/10.1111/j.1461-0248.2004.00684.

Long, Q., Albrecht, M., and Call, G. (2020). *Species Status Assessment Short's Bladderpod (Physaria globosa)*. US Fish and Wildlife Service.

Li, H., and Durbin, R. (2009). Fast and accurate short read alignment with burrows–wheeler transform. Bioinformatics, 25(14), 1754–1760.

Martins, K., Gugger, P. F., Llanderal-Mendoza, J., Gonzalez-Rodrıguez, A., Fitz-Gibbon, S. T., Zhao, J. L., Rodrıguez-Correa, H., Oyama, K., and Sork, V. L. (2018). *Landscape genomics provides evidence of climate-associated genetic variation in Mexican populations of Quercus rugosa*. Evolutionary Applications, *11*(10), 1842–1858.

Reed, D. H., and Frankham, R. (2003). *Correlation between fitness and genetic diversity*. Conservation Biology, *17*(1), 230-237.

Rollins, R. C. and Shaw, E. A. (1973). *The Genus Lesquerella (Cruciferae) in North America*. Harvard University Press.

Sexton, J. P., Hangartner, S. B., and Hoffmann, A. A. (2014). *Genetic isolation by environment or distance: Which pattern of gene flow is most common?* Evolution, *68*(1), 1–15. https://doi.org/10.1111/evo.12258.

Shea, M. M. (1993). *Status Survey Report on Lesquerella globosa (Desv.) Wats*. Unpublished Report. Kentucky State Nature Preserves Commission.

Thacker, J. H., Krosnick, S. E., Maynord, S. C., Call, G. P., and Perkin, J. S. (2019). *Pollination biology and reproductive phenology of the federally endangered endemic Physaria globosa (Brassicaceae) in Tennessee*. The Journal of the Torrey Botanical Society, *146*(1), 27–40. https://doi.org/10.3159/TORREY-D-17-00039.1

Wadgymar, S. M., Lowry, D. B., Gould, B. A., Byron, C. N., Mactavish, R. M., and Anderson, J. T. (2017). *Identifying targets and agents of selection: innovative methods to evaluate the processes that contribute to local adaptation*. Methods in Ecology and Evolution, *8*(6), 738–749. https://doi.org/10.1111/2041-210X.12777.

Whitlock, R., Hipperson, H., Mannarelli, M., and Burke, T. (2008). A highthroughput protocol for extracting high-purity genomic DNA from plants and animals. Molecular Ecology Resources, 8(4), 736–741.

Wright S. *Isolation by distance*. Genetics. 1943; 28:114–38. PMID: 17247074

Wright, S. J., Goad, D. M., Gross, B. L., Muñoz, P. R., and Olsen, K. M. (2022). Genetic trade-offs underlie divergent life history strategies for local adaptation in white clover. Molecular Ecology, 31(14), 3742–3760.