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Investigation into the Genetic Provenance of Three Rare Plants with East-West Disjunction Patterns in Pennsylvania.

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Investigation into the Genetic Provenance of Three Rare Plants with East-West Disjunction Patterns in Pennsylvania



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Final Report for Grant Agreement WRCP-17571

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Acknowledgements

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Abstract

Rare plant conservation relies on an understanding of the natural history, biology and ecology, and real and potential threats to their populations to inform state regulations that serve to protect the species from extirpation. This work often involves extensive field surveys over several years to determine population sizes and whether those populations are seeing reductions in number of individuals necessary to maintain the genetic diversity within and between those populations. Species and populations with high genetic diversity are better equipped to withstand sudden changes to their habitats that derive from land use changes and changing climate. There are a variety of methods used to investigate population genetic diversity and next generation sequencing (NGS) methods allow for complete genomic coverage by analyzing single nucleotide polymorphisms (SNPs) and allowing for an estimation of population genetic parameters such as genetic variation (F_{ST}), the inbreeding coefficient (F_{IS}), and heterozygosity (H_O) (H_E). Population genomic investigations of *Baptisia australis*, *Chasmanthium latifolium*, and *Erigenia bulbosa*, plant species at the edge of their ranges in Pennsylvania and disjunct distributions within the state were performed for this study. All three species exhibited lower than expected heterozygosity and, with the exception of Chasmanthium, high levels of inbreeding. This information was incorporated into conservation rank status assessments and climate change vulnerability indices using the NatureServe Conservation Status Rank Calculator and the Climate Change Vulnerability Index tools. As a result, state ranks for Chasmanthium and Erigenia require formally proposed changes to the Department of Conservation of Natural Resources. Likewise, management recommendations are given as guidance on the steps likely necessary to preserve and potentially increase the genetic diversity for all species. Through these investigations, a long-term partnership between the Pennsylvania Natural Heritage Program at Western Pennsylvania Conservancy and Bucknell University was developed through which a pipeline of undergraduate and graduate students were, and will be, trained in both field-based natural heritage methods and new, innovative ways address the conservation of rare plants in Pennsylvania and beyond.

Objectives of the Study

The original objectives of this project were to compare genetic assessments of native populations with naturalized native cultivars, and non-native populations to determine the impacts on genetic diversity of the native populations of five edge of range species. However, due to lack of funding and locating adequate number of necessary populations of all species, the objectives were revised to explore the population genetics of three edge of range and/or disjunct species with respect to their regional genetic diversity. The revised objectives for this project are:

- Perform comparative population genetic assessments of native populations of *Baptisia australis, Chasmanthium latifolium*, and *Erigenia bulbosa*.
- Assess extant populations of *Baptisia australis, Chasmanthium latifolium*, and *Erigenia bulbosa* to determine conservation rank status and climate change vulnerability.
- Conduct quantitative vegetation assessments of rare riparian plant community.
- Develop long-term partnership between Pennsylvania Natural Heritage Program and Bucknell University to train undergraduate and graduate students in heritage field data collection standards and conservation genetics of rare plants in Pennsylvania.

Introduction and Justification

This project represents an excellent example of the power of collaboration between academic and non-academic partners to help close the science-practice information gap in the conservation of plants in Pennsylvania. We used interdisciplinary approach that introduced a post-doctoral associate, graduate and undergraduate students to problem-oriented botanical research with the goal of developing a long-term partnership between Bucknell University and PNHP. Through this partnership we applied the twin-win model of research goals that leads to published research papers and validated solutions that raises expectations through pursuit of discovery and innovation (Shneiderman 2018). Plant conservation relies on a multitude of information that spans a variety of sources including academic research and applied natural heritage research. Conservation practitioners are often tasked with making protection decisions without having the most recent or most important data crucial for the employment of actionable and responsible activities. Likewise, academic researchers often undertake conservation projects without knowing there exists a huge library of data collected over several years documenting natural heritage information. This science-practice information gap in conservation can be closed when professionals with experience-based information and professionals with evidence-based information communicate directly through direct exchanges (Fabian et al. 2019; Holderegger et al. 2019) Our work demonstrates it is possible achieve meaningful conservation outcomes, while contributing to the training and education of students through the use of new and innovative research tools.

The floristic complexity of Pennsylvania results from the ebb and flow of climatic changes over geological time as well as human modification to the landscape. These modifications have both directly and indirectly moved plant species into and out of their natural ranges within the state. There are 3195 vascular plant species known to occur in our state. Nearly three quarters of the flora is considered native to the state. Pennsylvania intersects eleven EPA Level 3 Ecoregions from the Eastern Great Lakes Lowlands along Lake Erie, through the Ridge and Valley in the central part of the state to the Mid-Atlantic Coastal Plain in southeast Pennsylvania (Figure 1). This diversity of ecological setting lends to having plant communities containing elements of both northern and southern floras as well as providing suitable habitats at the edges of species ranges and distributions.

Edge populations are created when habitats are altered is such a way that it fragments a larger population into a matrix of smaller, more isolated populations. This happens naturally over geologic time with the slow advance or retreat of plants to suitable habitats across the landscape. This migration and dispersal is largely driven by changing climate and weather patterns change the conditions of a given region (Davis & Shaw 2001; Parmesan & Yohe 2003; Engler et al. 2009). Reduction of large contiguous plant populations into smaller, relatively isolated populations through habitat fragmentation is another mode of creating edge populations (Oostermeijer 2003).

Plant populations that are peripheral to the core of the species range and distribution tend to exhibit certain qualities or characteristics that allow for adaptation to habitat or climatic differences (Hampe & Petit 2005; Sexton et al. 2011; Abeli et al. 2014). Edge populations can have higher genetic diversity than the central population with gene flow from other edge populations, while gene flow from the central population can homogenize the genetic diversity and swamp the adaptation potential of the edge populations (Sexton et al. 2011; Franks et al. 2014). Fragmented populations, especially small populations, are susceptible to loss of genetic diversity due to being unable to maintain mutation-drift balance and sufficient gene flow necessary for replacement of lost alleles in the population (Young et al. 1996; Honnay & Jacquemyn 2007). The smaller isolated populations have increased extinction risks, especially rare species, that utilize specialized habitats within the fragmented landscape (Lienert 2004).

The increased potential of local extinction for populations of rare plant species is a concern for the Department of Conservation and Natural Resources (DCNR), the agency responsible for regulating the conservation and protection of plant biodiversity in Pennsylvania. Conservation of rare species scattered across the landscape in relatively small, isolated populations with demonstrated low genetic diversity and/or high inbreeding coefficients relies on maintaining and potentially increasing the within population heterozygosity (Neale 2012). There are various proposed strategies for achieving this goal, habitat preservation, increase population sizes through augmentation, and ex situ methods that lead to admixing of genetic populations (St. Clair et al. 2020). However, understanding current state of genetic diversity of rare plant populations is the first step in developing strategies and recommendations for conserving the species in Pennsylvania.

The DCNR is currently updating the regulations for all species of conservation concern, especially those subjected to the environmental review process. Part of the regulation update process is to determine the conservation status for each species, which involves a suite of variables ranging from number and size of species occurrences, viability of those occurrences, and threats to long term viability and persistence of the species. This project focused on three species; *Baptisia australis* (Blue false indigo), *Chasmanthium latifolium* (River oats), and *Erigenia bulbosa* (Harbinger of spring). This project updates the state conservation ranks for each species with conservation recommendations to be presented to the Vascular Plant Technical Committee (VPTC) for consideration. The VPTC is a subcommittee of the Pennsylvania Biological Survey (PABS) that functions as the advisory committee for DCNR on status of plant species in Pennsylvania.

Each species is considered to be a single genetic unit (i.e. accepted species), shows an east-west disjunction pattern in the state, and appears to be at or near the edges of their natural range in Pennsylvania. The genetic diversity of species considered an important factor in understanding the state of populations and their relatedness to other populations. disjunct and at the edge of their native range.

The hypotheses tested in the genetic work include:

1. *Baptisia australis* subpopulations exhibit genetic structure
2. Gene flow among *Baptisia australis* populations follows the classic metapopulation model
3. *Baptisia australis* population in the Youghiogheny River is most genetically distinct due to geographic isolation from other populations on the Allegheny River.
4. Disjunction in the distribution of *Erigenia bulbosa* is reflected in its genetic history.
5. Distribution of *Chasmanthium latifolium* is reflected in its genetic history.

DNA was extracted and a genotyping-by-sequencing (GBS) approach was used to obtain many single-nucleotide polymorphisms (SNPs). This is a restriction enzyme–based approach appropriate for obtaining many loci from non-model organisms that has been used extensively in recent years (Seeb et al. 2011; Peterson et al. 2012; Schilling et al. 2014; Silliman 2019) A filtered SNP data set was used to estimate population genetic parameters such as genetic variation (F_{ST}), the inbreeding coefficient (F_{IS}), and heterozygosity (H_O) (H_E), visualize the spread of our data using a discriminant analysis of principal components (DAPC), examine population structure using sparse nonnegative matrix factorization (sNMF), infer a population network using a NeighborNet analysis, compare genetic variance within and among groups using an analysis of molecular variance (AMOVA), and examine whether there is a signature of isolation by distance (IBD).

Methods

Natural Heritage Data Collection and Analyses

A review of Natural Heritage data for each study species was performed to identify extant element occurrences (EOs) with adequate numbers of individuals in the populations that could be easily visited and sampled for the project. Survey sites were selected based on aerial photo interpretation of suitable habitat for each species. Surveys were performed for those EOs determined to have the highest probability of relocating them for inclusion in our analyses. The population data were compiled and used to perform updated conservation status rank assessments and develop climate change vulnerability indices.

At each site, populations were visually assessed and documented using an iPad with ESRI Collector version 20.2.4, while walking the perimeter of the population. Images of the populations were recorded to show habitat context. All individuals were counted in small populations and for large populations, the number of individuals was estimated by counting a small portion and extrapolating that to the entire area covered by the population. The number of individuals was recorded as the EO size in FIND (Field Information Networked Database) a comprehensive heritage field data collection and reporting database that works with ESRI Collector to provide mobile access for data entry. In addition to the EO size, data for phenology, age structure, health/vigor, direct disturbances (natural or anthropogenic), site descriptions, habitat condition and landscape context were recorded in FIND. These data were reviewed and submitted to Biotics 5.12, a centralized NatureServe database that stores EO information for the purpose of providing a single source of data when determining state conservation ranks used for assigning regulatory protections.

NatureServe Conservation Status Rank Assessments

The conservation status, specifically the extinction risk of *Baptisia australis*, *Chasmanthium latifolium*, and *Erigenia bulbosa*, were assessed using the NatureServe Rank Calculator v3.2. This calculator is based on an automated, macro-enabled Excel workbook that ranks species statuses uses eight core rank factors that are organized into three categories (rarity, threats, trends). The factors are scaled and weighted relative to their risk impact, then combined by category resulting in an overall calculated rank (Faber-Langendoen et al. 2012). Element occurrence and source feature data were requested from PNHP data management. These data were compiled in Excel to determine range extent, area of occupancy, population size, and number of viable occurrences in Pennsylvania. A threats assessment was performed that assigned scope and severity values for 12 Level 1 threats that are broken down into more specific Level 2 threats (Master et al. 2012). The rank calculator tool currently lacks a threat category for genetic diversity, but this information was captured under “Other” for this study. The calculated ranks for each species were reviewed and assigned a final rank based on expert knowledge and understanding of the species in Pennsylvania.

Climate Vulnerability Analyses

A Climate Change Vulnerability Index (CCVI) was calculated for each study species using the NatureServe CCVI Tool. This tool uses a scoring system that integrates predicted exposure to modeled climate change variables (temperature, moisture availability) in Pennsylvania with three sets of factors associated with climate change sensitivity. These factors include indirect exposure to climate change, species-specific sensitivity and adaptive capacity factors, and documented response to climate change (Young et al. 2016).

Element occurrence points were analyzed for historical precipitation variation, historical temperature variation, annual predicted Hamon AET:PET moisture metric, and predicted temperature using ArcPro version ?? Values were extrapolated from the data using the raster to point tool in ArcPro and then exported to Excel to calculate the percentages of the population in the different variable ranges. For the indirect exposure to climate change variables, a review of scientific literature and EO habitat data in Biotics was performed to assign categories for the effect each factor has on the species vulnerability. Once all the data were entered, the result was exported to an Excel table that was used to draft justification summary documents. This was done using the mail merge function in Microsoft Word. Each justification document was reviewed for accuracy and developed into a stand-alone product.

Population Genetic Sampling and Analysis

Tissues were sampled from 24 *Baptisia* populations, 8 *Erigenia* populations, and 11 *Chasmanthium* populations throughout Pennsylvania. At each site, population sizes were assessed, voucher specimens were collected, and tissue from 10-20 individuals from throughout the populations were collected for DNA extraction. Voucher specimens were pressed, dried, and deposited in the Wayne E. Manning Herbarium at Bucknell University (BUPL).

DNA was extracted from silica-dried tissue samples using a variety of methods including modified CTAB or FastDNA kits from MP Biomedicals. Extracted DNA was quantified using the Qubit dsDNA BR Assay Kit and visualized on 1% agarose gels. Genomic DNA was sent to the University of Wisconsin Biotechnology Center (http://www.biotech.wisc.edu/services/dnaseq) for sequencing prior to analysis. Further testing revealed that single enzyme genotyping by sequencing was the preferred approach to maximizing the number and sizes of genome fragments. Sequencing was performed using 150-bp paired-end methods on a NovaSeq 6000 instrument (Illumina, San Diego, CA) (McDonnell et al. 2021). Raw sequence data were assembled using the Python software iPyrad version 07.30 (Eaton & Overcast 2020). Any samples with greater than 80% missing data were removed from the dataset prior to analysis.

Assembled sequence data were analyzed using R software (ver. 3.6.0) and various packages that calculate different genetic diversity metrics. Descriptive statistics including total genetic variance in a subpopulation (F_{ST}) and inbreeding coefficient (F_{IS}) were calculated using dartR and hierfstat (Goudet 2005; Gruber et al. 2018). These statistics are scaled 0 to 1 with values closer to 1 indicating high genetic diversity and high levels of inbreeding within a population. Observed heterozygosity (H_O) and expected heterozygosity (H_E) were calculated using R-packages pegas and adegenet (Jombart 2008; Paradis 2010). Bartlett's test was performed to compare variances of heterozygosity for statistical differences. R-package adegenet was also used to perform discriminant analysis of principal components (DAPC) that partitions variance into within- and between- group components that maximized discrimination between groups. Because DAPC requires predefined number of principal components (PCs), we tested between three and 120 PCs, then used xvalDapc to identify the optimal number of PCs that best fit the data. These data were transformed using principal components analysis (PCA) k-means clustering. The resulting clusters were identified with discriminant analysis (DA).

The number of ancestral populations, K value, was determined by calculating admixture coefficients using 100 replicates of 1000 iterations over a range of K values and comparing the cross-entropy values at each K value (McDonnell et al. 2021).

To estimate cyclic splits and visualized relationships within and among sampled individuals, the NeighborNet algorithm was used and a network was generated with SplitsTree5 version 5.0.0_alpha using filtered SNP VCF files (Huson 1998; Huson & Bryant 2006). The K2P model was used to generate a distance matrix and the splits network algorithm was used to estimate splits networks (Kimura 1980; Dress & Huson 2004).

The final analysis performed used dartR and ade packages to conduct Mantel tests looking for isolation by distance (IBD). AMOVAs were conducted using the popper package and plots were generated using the ggplot package (Excoffier & Smouse 1994; Wickham 2016). All R code used for this project is available at http://www.github.com/cheyennelmoore.

Products in Addition to this Final Report

The quantity and quality of data collected and analyzed resulted in several products throughout the duration of this project. Among these are poster and oral presentations at regional and national/ international botanical conferences, peer-reviewed publications in scientific journals, and a video highlighting a graduate student's research partially supported through this grant funding. The student research projects initially developed through this effort led to several student research awards from local, regional, and national botanical societies, that supplemented this grant funding. This additional funding allowed the students to attend conferences to share their research with the scientific community.

*International/national conference talks (*student author)*

Moore, C.L.*, A.J. McDonnell, S. Schuette, C.T. Martine. 2021. Population genomics of Pennsylvanian *Baptisia australis* var. *australis*: implications for conservation and understanding riparian metapopulation dynamics. *Society for Integrative and Comparative Biology Virtual Meeting*.

Moore, C.L.*, A.J. McDonnell, S. Schuette, C.T. Martine. 2020. Conservation and population genomics of Pennsylvania *Baptisia australis* var. *australis* (Fabaceae). *Botany 2020 Virtual meeting*.

McDonnell, A.J., C.L. Moore*, S. Schuette, and Martine, C.T. 2020. A harbinger of good things to come in academic and non-academic partnerships: population genomics and conservation of *Erigenia bulbosa* (Apiaceae) in Pennsylvania. *Society for Integrative and Comparative Biology 2020, Austin, TX*.

Schuette, S. and C.T. Martine. 2019. At the Intersection of Applied and Academic Botany: Fertile Ground for an Interdisciplinary Botanical Renaissance. *Botany 2019, Tucson, AZ*.

McDonnell, A.J., C.L. Moore*, S. Schuette, and Martine, C.T. 2019. A harbinger of good things to come in academic and non-academic partnerships: population genomics and conservation of *Erigenia bulbosa* (Apiaceae) in Pennsylvania. *Botany 2019, Tucson, AZ*.

Moore, C.L.*, A.J. McDonnell, S. Schuette, and Martine, C.T. 2019. Assessing the conservation status of *Baptisia australis* var. *australis* in Pennsylvania through natural history and metapopulation lenses. *Botany 2019, Tucson, AZ.*

*International/national conference posters (*student author)*

Hayes, J.*, T. Williams, R. Goad, C.T. Martine. 2020. Genetic diversity & connectivity of *Chasmanthium latifolium* (Poaceae) in Pennsylvania & the effect on conservation status. *Society for Integrative and Comparative Biology Virtual Meeting*.

Hayes, J.*, T. Williams, R. Goad, C.T. Martine. 2020. Genetic diversity & connectivity of *Chasmanthium latifolium* (Poaceae) in Pennsylvania & the effect on conservation status. *Botany 2020 Virtual meeting*.

Moore, C.L.*****, A.J. McDonnell, S. Schuette, and C.T. Martine. 2020. Prairies in Pennsylvania?: Conservation of a state-threatened species, *Baptisia australis* var. *australis*. *Society for Integrative and Comparative Biology 2020, Austin, TX.*

Moore, C.L.*, A.J. McDonnell, S. Schuette, and C.T. Martine. 2019. Prairies in Pennsylvania?: Conservation of a state-threatened species, *Baptisia australis* var. *australis*. *Ecology 2019, Louisville, KY*.

Davis, J.*, C.L. Moore, A.J. McDonnell, S. Schuette, C.T. Martine. 2019. What lies in the Dark: Cutting open *Baptisia australis* fruits in search of seed predators. *Botany 2019, Tucson, AZ*.

Moore, C.*, S. Schuette, A.J. McDonnell, C.T. Martine. 2018. Status of *Baptisia australis*(Fabaceae) in Pennsylvania and the potential impact of escaped cultivated genotypes: Preliminary observations. *Botany 2018, Rochester, MN.*

*Regional symposium talks (*student author)*

Hayes, J.*, T. Williams, R. Goad, C.T. Martine. 2020. Genetic diversity & connectivity of *Chasmanthium latifolium* (Poaceae) in Pennsylvania & the effect on conservation status. *2020 River Symposium, Lewisburg, PA*.

Moore, C.L.*, A.J. McDonnell, S. Schuette, C.T. Martine. 2020. Population genomics of Pennsylvanian *Baptisia australis* var. *australis*: implications for conservation and understanding riparian metapopulation dynamics. *2020 River Symposium, Lewisburg, PA.*

McDonnell, A.J., C.L. Moore*, S. Schuette, and Martine, C.T. 2019. A harbinger of good things to come in academic and non-academic partnerships: population genomics and conservation of *Erigenia bulbosa* (Apiaceae) in Pennsylvania. *Susquehanna River Research Symposium, Lewisburg, PA*.

*Regional symposium posters (*student author)*

Hayes, J. *, T. Williams, A.J. McDonnell, R. Goad, C.T. Martine. 2019. Populations genetics of *Chasmanthium latifolium* (river-oats) in Pennsylvania. *Susquehanna River Symposium, Lewisburg, PA.*

Davis, J.*, C.L. Moore, A.J. McDonnell, S. Schuette, C.T. Martine. 2019. What lies in the Dark: Cutting open *Baptisia australis* fruits in search of seed predators. *Susquehanna River Symposium, Lewisburg, PA.*

Moore, C.*, S. Schuette, A.J. McDonnell, C.T. Martine. 2018. Status of *Baptisia australis* (Fabaceae) in Pennsylvania and the potential impact of escaped cultivated genotypes: Preliminary observations. *2018 Pennsylvania Botany Symposium, State College, PA.*

Moore, C.*, S. Schuette, A.J. McDonnell, C.T. Martine. 2018. Status of *Baptisia australis* (Fabaceae) in Pennsylvania and the potential impact of escaped cultivated genotypes: Preliminary observations. *2018 Three Rivers Evolution Event, Pittsburgh, PA.*

Moore, C.*, S. Schuette, A.J. McDonnell, C.T. Martine. 2018. Status of *Baptisia australis* (Fabaceae) in Pennsylvania and the potential impact of escaped cultivated genotypes: Preliminary observations. *2018 Susquehanna River Symposium, Lewisburg, PA.*

*Peer-reviewed journal articles (*student author)*

McDonnell, A.J., C.L. Moore*, S. Schuette, C.T. Martine. 2021. Population genomics and conservation of *Erigenia bulbosa* (Apiaceae), an edge-of-range species in Pennsylvania. International Journal of Plant Sciences. 182(5): 344-355. https://doi.org/10.1086/713917

Moore, C.L.*, A.J. McDonnell, S. Schuette, C.T. Martine. 2021. Lepidopteran granivory reduces seed counts in a rare species of riparian scour prairies. Natural Areas Journal: 41(1): 47-54. https://doi.org/10.3375/043.041.0107

Moore, C.L.*, A.J. McDonnell, S. Schuette, C.T. Martine. In prep. Population genomics of Pennsylvanian *Baptisia australis* var. *australis*: implications for conservation and understanding riparian metapopulation dynamics. Intended: *Molecular Ecology and Evolution*.

Hayes, J.*, T. Williams, R. Goad, S. Schuette, C.T. Martine. In prep. Genetic diversity & connectivity of *Chasmanthium latifolium* (Poaceae) in Pennsylvania & the effect on conservation status (*In prep*).

Video productions

Martine, C.T. and P. Frederick (Producers). 2020. Plants are Cool, Too! Episode 10: Allegheny Ice and the Blue False Indigo. Featured expert: Cheyenne Moore, Bucknell University/University of Pittsburgh. https://www.youtube.com/watch?v=b-XhtTw7VlY&t=7s

Student Research Awards

- Botanical Society of America Undergraduate Research Award (competitive grants program requiring submittal of project proposal and letter of reference)
    - 2020: Jon Hayes
- Botanical Society of America Graduate Student Research Award (competitive grants program requiring submittal of project proposal and letters of reference)
    - 2019: Cheyenne Moore
- Southern Appalachian Botanical Society Earl Core Graduate Student Research Award (competitive grants program requiring submittal of project proposal and letters of reference)
    - 2019: Cheyenne Moore
- Sigma Xi Scientific Research Honor Society – Grants In Aid of Research (competitive grants program requiring submittal of project proposal and letters of reference)
    - 2020: Jonathan Hayes
- Carnegie Museum of Natural History Student Research Travel Award (competitive grants program requiring submittal of project proposal and letters of reference)
    - 2019: Cheyenne Moore
- Yarnell Prize in Environmental Affairs, Bucknell University
    - 2020: Jon Hayes
- Botanical Society of America, Young Botanist Award

- 2021: Jon Hayes

- American Society of Plant Taxonomists Undergraduate Research Prize
  - 2021: John Hayes

Student Presentation Awards (might have another to add)

- Natural Areas Conference (national meeting):
  - 2018: Cheyenne Moore (2nd place, best student research poster)
- River Symposium (annual regional meeting):
  - 2020: Jonathan Hayes (best undergraduate oral presentation)
  - 2020: Cheyenne Moore (best graduate oral presentation)
- Pennsylvania Botany Symposium (bi-annual meeting)
  - 2018: Cheyenne Moore (2nd place, best graduate research poster)
- Botanical Society of America Ecology Section, Best Student Presentation
  - 2021: John Hayes

Results and Conclusions

Natural Heritage Conservation Status Rank and CCVI Assessments

Baptisia australis – Review of element occurrence data suggests that there are 25 historic and 8 extant EOs in Pennsylvania. These are restricted to the western part of the state in 4 watersheds, Allegheny River, Youghiogheny River, Clarion River, Red Bank Creek. The occurrences proximal to the Pittsburgh metro area on the Allegheny, Monongahela, and Ohio Rivers are presumed no longer present due to industrialization of the suitable habitats. A single historic occurrence from the Susquehanna River in eastern Pennsylvania near Wilkes-Barre is presumed no longer present. While there appears to be suitable habitat in the vicinity of the mapped location, a survey of the riparian corridor was unsuccessful in relocating the occurrence. For all other historic occurrences, no surveys were performed. Surveys were conducted at a total of 27 sites updating population data for 3 EOs. Most of the extant population data are within a single EO on the Allegheny River where populations range in size from just few individuals to 1000s of individuals. Population sizes seem to show a correlation to size of the available scour prairie habitat. The smallest populations are restricted to pockets of boulders in scour zones along the river while the largest populations are found on cobble fans below the confluence of tributaries with the Allegheny River (Figure 1).

Figure 1: Large population of Baptisia australis at the cobble fan below Bear Creek along the Allegheny River.

The conservation status rank calculator uses the population and occurrence data along with a threats assessment for each occurrence to develop an extinction risk for that species within the specified geographic area. *Baptisia australis* is restricted to the western third of the state in two Level 3 EPA Ecoregions, Western Allegheny Plateau and Northcentral Appalachians, with most of the populations in Western Allegheny Plateau. There are between 15,000 – 20,000 individuals estimated from previous and current survey work in western Pennsylvania. Nearly all of these are from the Allegheny River, Clarion River, and Red Bank Creek. Primary threats to these subpopulations are impacts from reduced seed production due to granivory, encroachment of invasive species, severe flooding during flower and fruit periods that reduce seed bank and new population establishment, changes in flow rates from dams, development of river banks for recreational purposes, and ecological succession towards closed canopy floodplain forest. Given the area of occupancy, population size, and threats, the conservation status rank for *Baptisia australis* is state-imperiled (S2), which qualifies for Pennsylvania Threatened within the regulation, Conservation of Pennsylvania Native Wild Plants.

Baptisia australis was assessed using the NatureServe Climate Change Vulnerability Index (CCVI) release 3.02 tool and determined extremely vulnerable (EV) to the changing climate (Young et al. 2016). Several factors ranged from somewhat increase (SI) to increase (I) vulnerability with most of these factors

related to the species sensitivity and adaptive capacity to climate change (Table 1). Natural populations of *B. australis* are confined to cobble scour prairies in four river corridors. Seed dispersal and movement to new locations within our outside of those rivers is limited by the presence of cobble scour prairies that experience seasonal floods that maintain early successional conditions. Although this species has a high degree of potential dispersal, the suitable habitats for establishment, reproduction, and long-term survival are reduced to scattered patches in any given river. This scenario of limited available habitat and high habitat fidelity combine to increase the vulnerability of *B. australis* to climate change.

Competition with native and especially non-native, invasive species is expected to increase the vulnerability of *Baptisia australis* to climate change. Without seasonal floods that scour away sediment build up, establishment and persistence of invasive species along the edges of riverbanks and within the cobble prairies of slower moving reaches is expected to impact populations of *B. australis* (Kui et al. 2014).

Several factors related to sensitivity and adaptive capacity are likely to somewhat increase vulnerability to climate change form *Baptisia australis*. These are generally related to reproductive capacity, genetic diversity, and the combination of disturbance frequency and intensity (Table 1). The concern about reproductive capacity is that primary pollinators for *Baptisia* are likely bumble bees, which are experiencing global declines due in part to changing climate (Soroye et al. 2020), and seed predation by invertebrates leading to potential reduction of genetic diversity due reduced seedbank inputs (Moore et al. 2021). In Pennsylvania, *B. australis* appears to have moderate genetic health with five distinct genetic populations with adequate heterozygosity and potential for healthy gene flow. However, the majority of individuals are found in the Allegheny River between Franklin and East Brady where the observed heterozygosity is much lower than expected suggesting elevated levels of inbreeding in these populations (Moore *pers comm*.).

Species	Natural barriers	Anthropogenic barriers	Dispersal/Movements	Historical thermal niche	Physiol. thermal niche	Historical hydrol. niche	Physiol. hydrol. niche	Disturbance	Dependence on ice/snow	Physical habitat restriction	Pollinator versatility	Other species dispersal	Natural enemies/pathogens	Competition	Interspecific interactions	Genetic variation	Phenological response	Index Score
Baptisia australis var. australis	U	SI	Inc	N	U	N	SI	SI	N	SI	SI	N	SI	Inc	N	SI	U	**EV**
Erigenia bulbosa	SI	SI	Inc	N	U	N	U	SI	N	N	N	N	N	Inc	U	SI	N	**EV**
Chasmanthium latifolium	N	N	N	N	U	N	U	SI	N	N	N	N	U	Inc	N	N	U	**LV**

Table 1: Factors influencing the species vulnerability. Orange shaded cells refer to indirect exposure. Yellow shaded cells refer to the species sensitivity and adaptive capacity to climate change. U= unknown; N= neutral; SI= Somewhat increase; I= Increase.

***Chasmanthium latifolium* –** Review of element occurrence data suggests that there are 12 historic and 15 extant EOs in Pennsylvania. These are found along the Cheat, Monongahela, Raystown Branch Juniata, and Susquehanna Rivers as well as along Conewago Creek. Nearly all occurrences in the Susquehanna are historic due habitat alteration and industrialization of the suitable habitats. Occurrences are mostly extant at Raystown Branch, Conewago Creek, Cheat, and Monongahela rivers. Surveys were conducted at a total of 11 sites updating population data for 5 of 15 extant EOs. Most of the extant populations range in size from just few individuals to several 1000s of individuals. Population sizes appear dependent on habitat condition, disturbance, and presence of invasive species. The smallest populations are in densely forested floodplains where there is high competition with other

species or at the base of steep shale barren slopes with very little suitable habitat due to lack of alluvial soils.

The conservation status rank calculator uses the population and occurrence data along with a threats assessment for each occurrence to develop an extinction risk for that species within the specified geographic area. *Chasmanthium latifolium* is restricted to the southern portion of the state in three Level 3 EPA Ecoregions, Western Allegheny Plateau, Ridge and Valley, and Northern Piedmont. There are an estimated 500 – 3000 genets and 5000 – 50000 ramets estimated including previous and current survey work. The largest populations are found along the Cheat, Monongahela, and Susquehanna rivers. Primary threats to these subpopulations include flooding frequency, intensity and duration, invasive species, and proximity to service and access roads. Each of these primary threats have the potential to displace *Chasmanthium* from its habitat. Given the area of occupancy, population size, and threats, the conservation status rank for *Chasmanthium latifolium* is state-vulnerable (S3), which qualifies for Pennsylvania Rare within the regulation, Conservation of Pennsylvania Native Wild Plants. *Chasmanthium latifolium* is currently listed as proposed state-endangered with a tentatively undetermined (TU) status within the regulation, Conservation of Pennsylvania Native Wild Plants. Therefore, based on our results from the conservation status review of extant EOs, a proposal will be presented at the 2022 Rare Plant Forum for consideration by the Vascular Plant Technical Committee (VPTC) to change the status rank from S2 to S3 with a recommendation to change the state regulatory status from TU to Pennsylvania Rare (PR).

Chasmanthium latifolium was assessed using the NatureServe Climate Change Vulnerability Index (CCVI) release 3.02 tool and determined to have low vulnerability (LV) under the current average climate change scenario (Young et al. 2016). Vulnerability is expected to somewhat increase (SI) with regard to naturally occurring disturbance regime and increase (I) when considering competition with invasive species. (Table 1). Natural populations of *C. latifolium* are predominantly found along edges of floodplain forests, but can occur in densely forested floodplains, along roadsides, and at the base of steeply sloped shale barrens. These floodplains are likely to experience more frequent, larger floods of longer duration that will impact portions of populations that are proximal to the river's edge and remain underwater for extended periods of time (Figure 2).

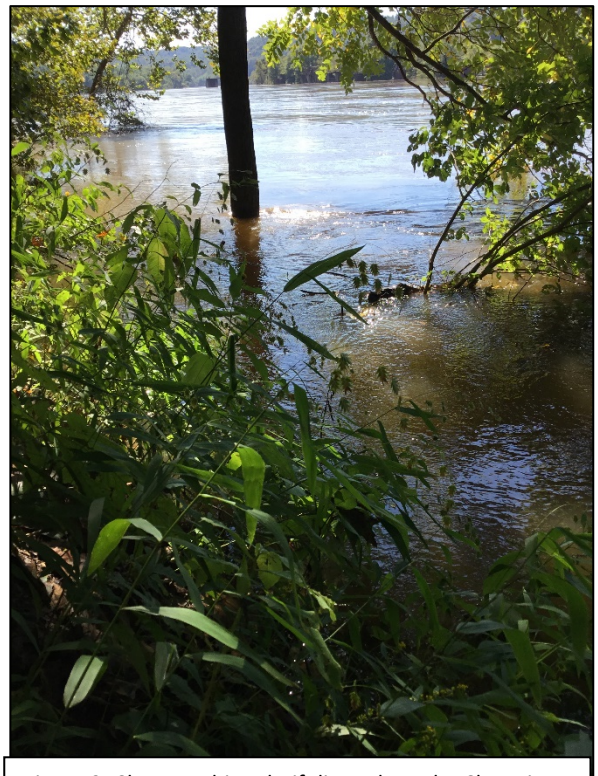

Figure 2: Chasmanthium latifolium along the Chet River during high flood waters above the confluence with the Monongahela River. Most of the population is underwater as indicated by the floodline.

Riparian corridors are often susceptible to herbaceous invasive species such as *Fallopia spp., Microstegium vimineum, Arthraxon hispidus, Phalaris arundinacea, Ligustrum spp.* and *Lonicera spp*. Although *C. latifolium* reproduces through both vegetative expansion via tillers and seed dispersal, it is at a competitive disadvantage when invasive species are present in the available suitable habitat (Greene & Blossey 2012). Seed dispersal and movement to new locations within or outside of riparian corridors is limited by the

presence of forested floodplain mesic forested slope habitats that experience seasonal floods and have relatively open conditions free of invasive species that compete with *C. latifolium*.

Erigenia bulbosa – Review of element occurrence data suggests that there are 13 historic and 42 extant EOs in Pennsylvania. There are 39 occurrences in the western part of the state and 3 occurrences in the Susquehanna watershed (Figure 3). The western occurrences are concentrated in the French Creek, Ohio River, Youghiogheny River, and Monongahela River watersheds. According to the EO data, several of the historic occurrences in western Pennsylvania were not relocated during multiple previous surveys and presumed destroyed due to logging and other development activities. For all other historic occurrences, no surveys were performed. Surveys were conducted at a total of 8 sites updating population data for 8 EOs encompassing all 3 occurrences in eastern Pennsylvania and 5 occurrences scattered across the distribution in the western part of the state.

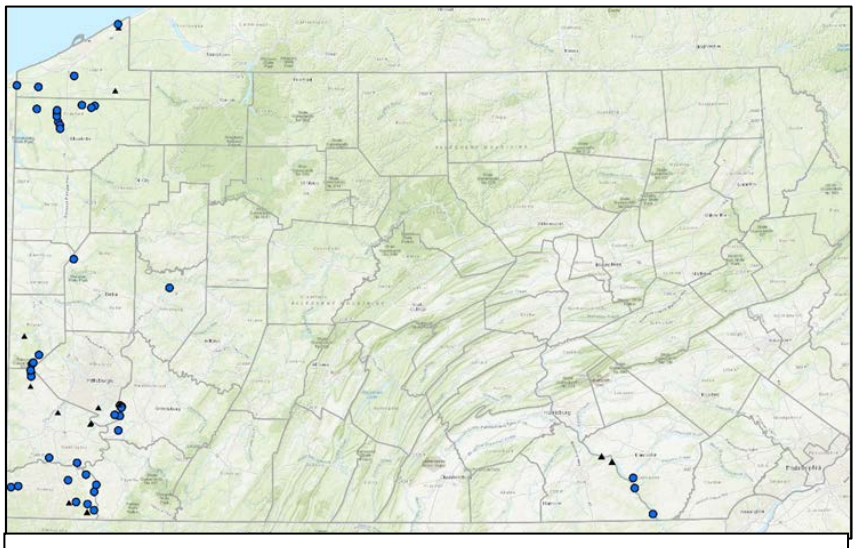

Figure 3: Statewide distribution of extant (blue circles) and historic (black triangles) element occurrences for Erigenia bulbosa showing the disjunct nature of the populations.

The conservation status rank calculator uses the population and occurrence data along with a threats assessment for each occurrence to develop an extinction risk for that species within the specified geographic area. *Erigenia bulbosa* is disjunctly distributed in western and eastern parts of the state seemingly separated by the Allegheny and Appalachian Mountains (Figure 7). This species is distributed across three Level 3 EPA Ecoregions, Northern Piedmont, Erie Drift Plain, and Western Allegheny Plateau, with most of the populations in Western Allegheny Plateau and Erie Drift Plain. There are between 10,000 – 30,000 individuals with an average population size between 200 and 600 individuals estimated from previous and current survey work. *E. bulbosa* grows in rich, well-drained soils of floodplain forests, mesic hardwood forests, and rich forested hardwood slopes. Primary threats to *Erigenia* populations are habitat conversion for agriculture and livestock grazing, limited dispersal abilities, competition with invasive species, long life cycle from germination to reproduction, and low genetic diversity coupled with high levels of inbreeding. Given the area of occupancy, population size, and threats, the conservation status rank for *Erigenia bulbosa* is state-rare (S3), which qualifies for Pennsylvania Rare within the regulation, Conservation of Pennsylvania Native Wild Plants.

Erigenia bulbosa was assessed using the NatureServe Climate Change Vulnerability Index (CCVI) release 3.02 tool and determined extremely vulnerable (EV) to the changing climate (Young et al. 2016). Several factors ranged from somewhat increase (SI) to increase (I) vulnerability with most of these factors related to the species sensitivity and adaptive capacity to climate change (Table 1). Natural populations of *E. bulbosa* are found in floodplains and lowland mesic forests with rich soils that are generally separated by steep, dry, forested hills inconducive to seedling establishment. In addition to these natural barriers, anthropogenic barriers such as agriculture and residential developments prevent

Erigenia from dispersing to suitable habitat leading to somewhat increased vulnerability to the predicted climate change conditions in Pennsylvania.

Several factors related to sensitivity and adaptive capacity are likely to somewhat increase vulnerability of *Erigenia* to climate change. Changes to the natural disturbance regimes such as increased frequency, intensity, and duration of floods will likely alter existing habitats in floodplains (Andersen & Marshall Shepherd 2013; Kuo et al. 2015). Western and eastern populations are both genetically isolated and exhibit low within population genetic diversity and moderate levels of inbreeding (McDonnell et al. 2021). Very limited distance of seed dispersal from parent plants restricts movement to new locations within our outside of existing habitats. The inability to disperse widely increases this species vulnerability to climate change. Likewise, competition with native and especially non-native, invasive species is expected to increase the vulnerability of *Erigenia bulbosa* to changing climate conditions (Pattison et al. 2019).

Population Genetic Analyses

Baptisia australis: Leaf tissue samples were analyzed from 24 populations in four Pennsylvania watersheds (Allegheny River, Clarion River, Red Bank Creek, and Youghiogheny River), and one West Virginia watershed (Greenbrier River). Attempts were made to acquire tissue samples from the last remaining population in the Ohio watershed, but surveys for that population were unsuccessful due to it likely being extirpated from the state. The samples from West Virginia populations were acquired via Ernst Seed Company nursery stock that have been in cultivation for several years (Table 2).

Table 2 (adapted from (Moore 2020): Baptisia population sites sampled with general location information and sampling density.

Watershed	County	Site Name	Site Abbreviation	Collector Name & Number	Collection Date	# of Plants Sampled
Allegheny River	Venango	Fisherman's Cove	FC	C. L. Moore 69	7/8/2019	5
Allegheny River	Venango	Gas Pipeline	GP	C. L. Moore 70	7/8/2019	10
Allegheny River	Venango	Robert's Run	RR	C. L. Moore 85	7/11/2019	15
Allegheny River	Venango	Wood Hill	WH	C. L. Moore 1	7/15/2018	15
Allegheny River	Venango	Mill Creek	MC	C. L. Moore 9	7/13/2018	15
Allegheny River	Venango	Meadowsweet Run	MR	C. L. Moore 14	7/13/2018	15
Allegheny River	Butler	Butler County	BCO	C. L. Moore 21	7/14/2018	15
Allegheny River	Clarion	Clarion Island	CI	C. L. Moore 26	7/14/2018	15
Allegheny River	Clarion	Clarion Island Mix	CIM	C. L. Moore 31	7/14/2018	15
Allegheny River	Clarion	Parker Island	PI	C. L. Moore 71	7/9/2019	15
Allegheny River	Clarion	Bear Creek	BC	C. L. Moore 67	9/6/2018	25
Allegheny River	Clarion	Heck Drive	HD	C. L. Moore 77	7/9/2019	10
Allegheny River	Clarion	Black Fox Island	BFI	C. L. Moore 79	7/10/2019	15
Allegheny River	Clarion	Bald Eagle Island	BEI	C. L. Moore 83	7/10/2019	15
Allegheny River	Crawford	Ernst	EPA	C. L. Moore 88	7/11/2019	10
Allegheny River	Armstrong	River's Edge	RE	C. L. Moore 89	7/12/2019	15
Clarion River	Clarion	Grassy Flats	GF	S. Schuette 2204	8/9/2018	15
Clarion River	Clarion	Clarion River	CR	C. L. Moore 60-66;68	9/5/2018	15
Red Bank Creek	Clarion	Lawsonham A	LA	S. Schuette 2245	8/28/2018	15
Red Bank Creek	Clarion	Lawsonham B	LB	S. Schuette 2246	8/28/2018	15
Red Bank Creek	Clarion	Red Bank Station	RBS	C. L. Moore 58	9/4/2018	20

Youghiogheny River	Fayette	Layton	YR	C. L. Moore 92	7/26/2019	11
Greenbrier River	Crawford	Ernst	EWV	C. L. Moore 87	7/11/2019	10
Greenbrier River	Union	Ernst @ BU	EWVC	C. L. Moore 93	7/25/2019	5

This represents complete sampling of all extant Pennsylvania populations of *Baptisia australis* allowing for analysis of overall genetic diversity, genetic structure within populations, and gene flow among populations to test the classic metapopulation model.

Genetic Diversity

Tests for genetic diversity compare expected heterozygosity (H_e) with observed heterozygosity (H_o) based on the number of differences between single nucleotide polymorphisms (SNPs) across populations. The proportion of total genetic variance in a subpopulation relative to the total genetic variance (F_{ST}) measures population differentiation due to genetic structure. Values greater than 15% in subpopulations of plants of the same species is considered significant differentiation (Frankham et al. 2010). Based on 11,323 SNPs from 317 individuals collected from 24 populations there is significant differentiation between most populations of B. australis in Pennsylvania with an overall global F_{ST} = 0.185, 95% confidence interval. Global genetic diversity of *B. australis* populations show H_e higher than H_o (global H_e=0.037 global H_o=0.031). The variance between H_o and H_e is significant between all populations except Grassy Flats along the Clarion River, Lawsonham B along Red Bank Creek, and River's Edge along the Allegheny River (Table 3). Lower H_o than H_e indicates there is less genetic variability than expected suggesting some level of inbreeding among the populations. This is supported by the global *F*is = 0.173 with a range of 0.0292 to 0.2201, where *F*is measures the proportion of the variance in the subpopulation contained in an individual. High levels of inbreeding were found among populations along the Clarion River, Youghiogheny River, and Red Bank Creek (Table 3). This is unsurprising as these populations are smaller, and spatially and genetically isolated from the mainstem Allegheny River where the populations are larger and more or less contiguous.

Table 3: Inbreeding coefficient (F_{IS}) and expected and observed heterozygosity (H_e and H_o), fixation index $F_{ST,}$ and watershed of Baptisia australis populations (adapted from (Moore 2020))

	H_o	H_e	F_{IS}	F_{ST}
Global	0.031	0.037	0.173	0.185
FC	0.073	0.084	0.088	Allegheny
GP	0.073	0.080	0.062	Allegheny
RR	0.074	0.079	0.072	Allegheny
WH	0.086	0.095	0.084	Allegheny
MC	0.085	0.087	0.003	Allegheny
MR	0.096	0.103	0.059	Allegheny
BCO	0.075	0.083	0.085	Allegheny
CI	0.073	0.085	0.129	Allegheny
CIM	0.052	0.068	0.215	Allegheny
PI	0.052	0.065	0.193	Allegheny
BC	0.054	0.068	0.213	Allegheny
HD	0.052	0.065	0.194	Allegheny
BFI	0.062	0.079	0.210	Allegheny
BEI	0.066	0.089	0.266	Allegheny
EPA	0.049	0.058	0.153	Allegheny
RE	0.064	0.074	0.140	Allegheny
GF	0.124	0.150	0.163	Clarion
CR	0.120	0.155	0.206	Clarion
LA	0.056	0.073	0.207	Red Bank
LB	0.066	0.076	0.104	Red Bank
RBS	0.059	0.076	0.208	Red Bank
YR	0.074	0.089	0.151	Youghiogheny
EWV	0.093	0.113	0.154	Greenbrier
EWVC	0.061	0.109	0.320	Greenbrier

Population Structure

There is an upstream to downstream separation among populations along the Allegheny river with principal components analysis (PCA) resulting in separation between the four Pennsylvania watersheds. (Figure 4). Discriminant analysis of principal components (DAPC) supports K=6 genetic populations with each river clustering separately, the Allegheny River having three metapopulations, and the Greenbrier populations loosely grouping with the Youghiogheny population suggesting greater distinction of those populations from those along the Allegheny and its tributaries. Analysis of ancestry coefficient proportions based on K=6 suggests five distinct genetic populations of *Baptisia australis* including Greenbrier River (WV), Clarion River, Youghiogheny River, and Allegheny River with Red Bank Creek. Allegheny River populations separate into two clusters, one consisting of the upstream individuals and one consisting of individuals downstream and the Red Bank Creek individuals (Figure 5).

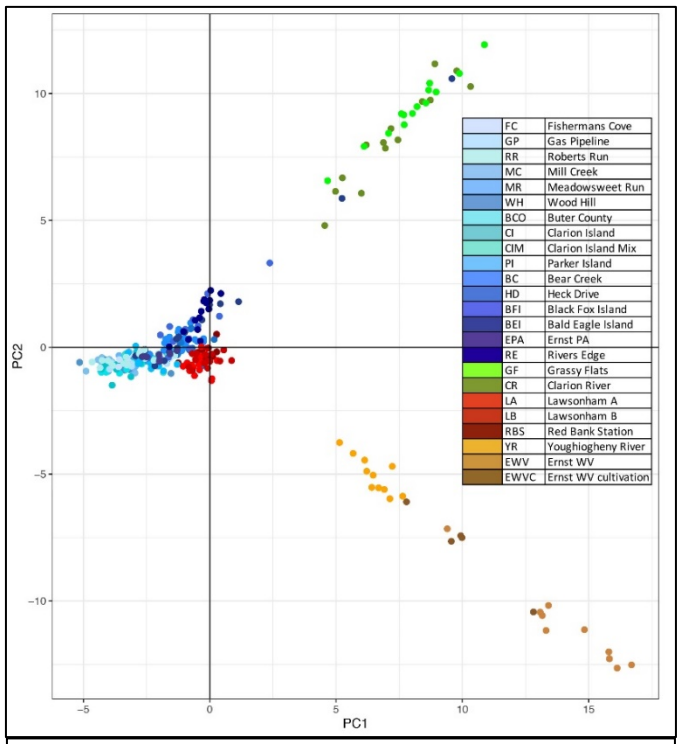

Figure 4: Principal Components Analysis of Baptisia australis SNPs shows distinct separation of Greenbrier (WV) (brown), Youghiogheny River (orange), and Clarion River (green) populations from the Allegheny River (blue) and Red Bank Creek (red) populations. Allegheny River samples appear to separate from upstream (light blue) to downstream (dark blue).

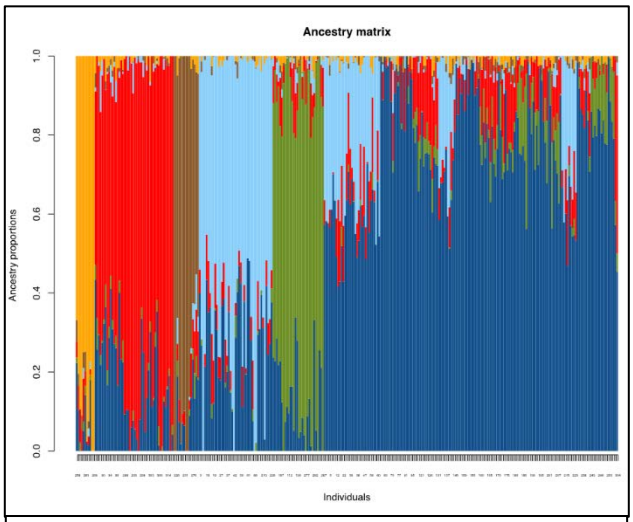

Figure 5: Ancestry plot for 24 sampled populations of Baptisia australis shows five distinct genetic populations; Greenbrier (WV) (brown), Youghiogheny River (orange), and Clarion River (green), and the Allegheny River (blue) plus Red Bank Creek populations. Light blue represents upstream populations on the Allegheny River, while dark blue represents the downstream section plus Red Bank Creek.

Chasmanthium latifolium: Leaf tissue samples were analyzed from 133 individuals from 11 populations in four Pennsylvania watersheds Monongahela River, Raystown Branch of the Juniata River, Susquehanna River, and Conewago Creek (Table 4). This sampling encompassed the disjunct distribution of populations and over 70% of element occurrences in Pennsylvania (Hayes 2021).

Table 4: Chasmanthium population sites sampled with general location information and sampling density. (adapted from (Hayes 2021)

Watershed	County	Site Name	Site Abbreviation	Collector Name	Collection Date	# of Plants Sampled
Susquehanna River	Lancaster	Haines	H	C.T. Martine	9/13/2018	15
Susquehanna River	Lancaster	North of Fisherman Run	NFR	C.T. Martine	9/13/2018	16
Susquehanna River	Lancaster	South of Fisherman Run	SFR	C.T. Martine	9/13/2018	12
Susquehanna River	Lancaster	Chickies Ridge	CR	C.T. Martine	9/13/2018	11
Conewago Creek	York	Erney Creek	EC	T.M. Williams	9/5/2018	12
Raystown Branch, Juniata River	Bedford	Raystown Branch	RB	S. Schuette	9/24/2018	15
Monongahela River	Fayette	Cheat River 1N	C1N	G. Malone	9/27/2018	8
Monongahela River	Fayette	Cheat River 1S	C1S	G. Malone	9/27/2018	7
Monongahela River	Fayette	Cheat River 2	C2	S. Schuette	9/27/2018	8
Monongahela River	Fayette	Friendship Hill 1	FH1	G. Malone	9/28/2018	14
Monongahela River	Fayette	Friendship Hill 2	FH2	G. Malone	9/28/2018	15

Genetic Diversity

Table 5: Inbreeding coefficient (F_{IS}) and expected and observed heterozygosity (H_e and H_o), fixation index F_{ST}, and region of Chasmanthium latifolium populations. All populations have significantly greater than expected genetic diversity and no inbreeding. (adapted from (Hayes 2021)

	H_o	H_e	F_{IS}	F_{ST}
Global	0.659*	0.397	-0.622*	0.113
H	0.7103	0.3837	-0.8512	East
NFR	0.7355	0.3944	-0.8649	East
NFS	0.5154	0.4097	-0.2580	East
CR	0.7388	0.3962	-0.8517	East
EC	0.7400	0.3985	-0.8569	East
RB	0.6440	0.4047	-0.5915	Central
C1N	0.6969	0.3958	-0.4978	West
C1S	0.6212	0.4147	-0.4978	West
C2	0.5507	0.3863	-0.4258	West
FH1	0.6847	0.3909	-0.7517	West
FH2	0.6119	0.4035	-0.5166	West

Tests for genetic diversity compare expected heterozygosity (H_e) with observed heterozygosity (H_o) based on the number of differences between single nucleotide polymorphisms (SNPs) across populations. The proportion of total genetic variance in a subpopulation relative to the total genetic variance (F_{ST}) measures population differentiation due to genetic structure. Values greater than 15% in subpopulations of plants of the same species is considered significant differentiation (Frankham et al. 2010). Based on 999 SNPs from 133 individuals collected from 11 populations there is moderate differentiation between all sampled populations of *C. latifolium* in Pennsylvania with an overall global F_{ST} = 0.113, 95% confidence interval. Global genetic diversity for sampled populations had significantly higher H_o than H_e (global H_e=0.3969 global H_o=0.6590). (Table 5). Higher H_o than H_e indicates there is more genetic variability than expected suggesting that inbreeding is effectively absent among the populations. This is supported by the global *F*is = -0.6219, where *F*is measures the proportion of the variance in the subpopulation contained in an individual. There were high levels of gene flow among the Monongahela populations in the west and high genetic differentiation of the eastern Susquehanna populations from the western

populations. The central populations show some admixture of genetic diversity from both eastern and western populations (Figure 6).

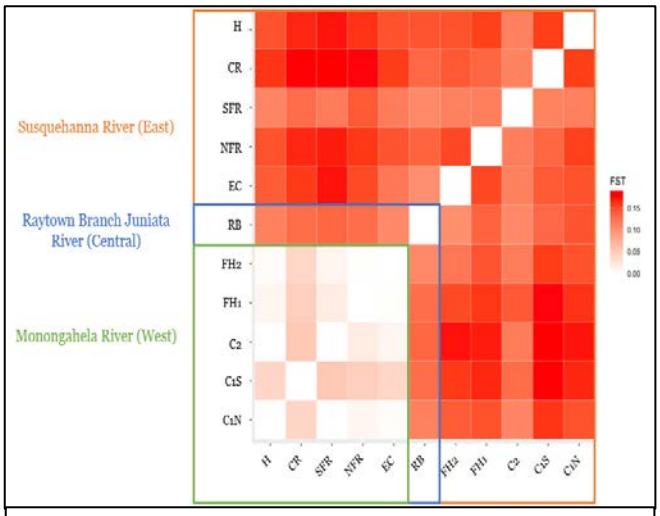

Figure 6: Heatmap of pairwise *F*ST values. Site abbreviations correspond to Table 5. No genetic differentiation within western populations, while eastern and central populations showed genetic differentiation (*F*ST > 0.15). (Figure borrowed from Hayes, 2021).

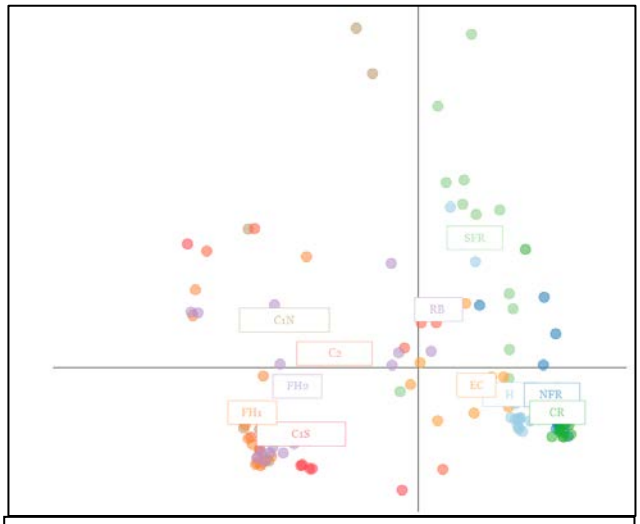

Figure 7: Principal components analysis of SNPs from sampled *C. latifolium* showing western populations (C1N, C1S, C2, FH1, FH2) clustering together, the eastern populations (H, NFR, SFR, CR, EC) clustering together, the central population (RB) intermediate between east and west populations (Figure borrowed from Hayes, 2021).

Population Structure

Principal components analysis of the SNPs shows eastern populations clustering together, western populations clustering together, and the central population clustering between the eastern and western populations (Figure 7). Genetic structuring and diversity are supported by K=5 ancestral populations with the eastern populations genetically different from each other and the central population and significantly different from the western populations, which appear to be a single genetic unit (Figure 8).

Figure 8: STRUCTURE analysis plot for K=5 genetic units. Eastern populations appear different from each other and western populations. The central population is genetically similar to EC, and western populations appear as one genetically

Erigenia bulbosa: Leaf tissue samples were analyzed from 118 individuals from 8 populations, 5 in western Pennsylvania and 3 in eastern Pennsylvania (Table 6). This sampling encompassed the disjunct distribution representing 20% of the known populations in the state. With only 3 extant populations in eastern Pennsylvania, there was complete representation from that portion of the distribution.

Table 6: Erigenia population sites sampled with general location information and sampling density. (adapted from (Hayes 2021)

County	Site Name	Collector Name & Number	# of Plants Sampled
York	Peach Bottom	McDonnell 366	20
York	York Furnace	McDonnell 367	14
York	Safe Harbor	McDonnell 368	9
Westmoreland	Braddock's Trail Park	Schuette 2095	15
Westmoreland	Cedar Creek Park	Schuette 2094	15
Butler	Slippery Rock Creek Natural Area	Isaac 10420	15
Beaver	Raccoon Creek State Park Wildflower Reserve	Isaac 3813	15
Greene	Ryerson Station State Park	Schuette 2096	15

Genetic Diversity

Tests for genetic diversity compare expected heterozygosity (H_e) with observed heterozygosity (H_o) based on the number of differences between single nucleotide polymorphisms (SNPs) across populations. The proportion of total genetic variance in a subpopulation relative to the total genetic variance (F_{ST}) measures population differentiation due to genetic structure. Values greater than 15% in subpopulations of plants of the same species is considered significant differentiation (Frankham et al. 2010). Based on 14,350 SNPs from 118 individuals collected from 8 populations there is moderate differentiation between all sampled populations of *C. latifolium* in Pennsylvania with an overall global F_{ST} = 0.518, 95% confidence interval. Global genetic diversity for sampled populations had significantly higher H_e than H_o (global H_e=0.152 global H_o=0.055). (Table 7). Higher H_e than H_o indicates there is lower than expected genetic variability suggesting higher than expected homozygosity likely caused from inbreeding within the populations. This is supported by the global *F*is = 0.642, where *F*is measures the proportion of the variance in the subpopulation contained in an individual. Inbreeding is slightly higher in eastern populations with a range of 0.558 to 0.749 (mean 0.647) than western populations ranging from 0.565 to 0.735 (mean 0.638) (McDonnell et al. 2021).

Table 7: Inbreeding coefficient (F_{IS}) and expected and observed heterozygosity (H_e and H_o), fixation index $F_{ST,}$ and region of the populations. All populations have significantly greater than expected genetic diversity and no inbreeding.

	H_o	H_e	F_{IS}	F_{ST}
Global	0.055	0.152	0.642	0.518
Peach Bottom	0.032	0.126	0.749	East
York Furnace	0.037	0.103	0.635	East
Safe Harbor	0.027	0.062	0.558	East
Braddock's Trail Park	0.095	0.219	0.565	West
Cedar Creek Park	0.053	0.145	0.633	West
Slippery Rock Creek Natural Area	0.089	0.232	0.617	West
Raccoon Creek State Park Wildflower Reserve	0.029	0.108	0.735	West
Ryerson Station State Park	0.080	0.221	.0.640	West

Population Structure

Discriminant analysis of principal components (DAPC) is a multivariate analysis of the SNPs uses a priori-defined clusters derived from k-means cluster analysis that shows the spatial relationship of the eight populations. The analysis loosely grouped the grouped the populations according to their geographic location in the east and west. However, there is clear separation between all populations regardless of sharing a broad geographic region, except for the Peach Botton and York Furnace. eastern populations somewhat clustering together (Figure 9). This suggests some shared genetic ancestry when these populations were historically connected in the Susquehanna watershed or a founder event after dispersal from one population to the other ((McDonnell et al. 2021).

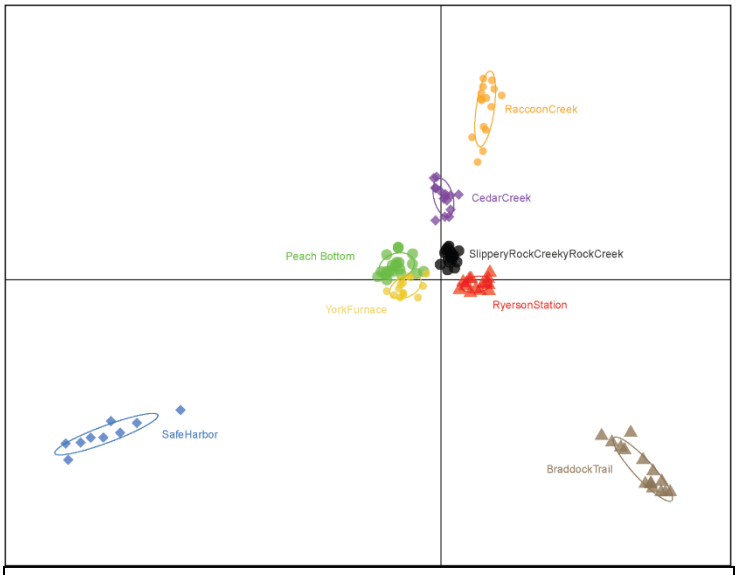

Figure 9: Discriminant analysis of principal components showing spatial relationship of sampled populations. Western Pennsylvania populations fall to the right of the vertical axis, while eastern populations fall to the left of the vertical axis.

The shared ancestry was supported in a NeighborNet plot where Peach Botton and York Furnace share many edges while the other populations show strong geographic separation (Figure 10). This graph also indicates that no recent gene flow has occurred between populations in the east and west as well as within those populations, suggesting they are isolated by distance (IBD). Test for IBD among all populations were significant suggesting accrued local genetic variation in eastern and western populations (McDonnell et al. 2021).

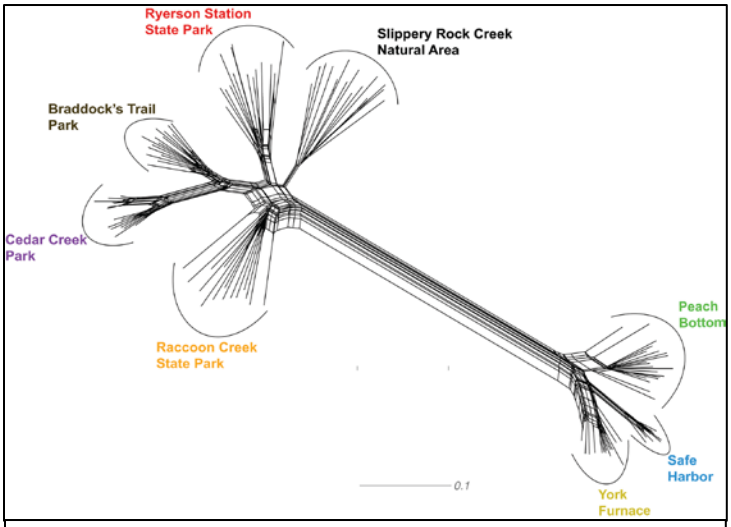

Figure 10: NeighborNet network estimated by Splits Tree5 showing shared ancestry of Peach Botton and York Furnace and isolation by distance of all sampled populations

Summary of Conclusions

This project tested five hypotheses comparing the genetic diversity and population structures for three plant species of concern that are at their range edges in Pennsylvania; *Baptisia australis*, *Chasmanthium latifolium*, and *Erigenia bulbosa*. Each species was considered a priori to be a single genetic unit with showing shared ancestry between populations. Our results based on sampled populations indicate that *Baptisia* consists of 4 genetic units (4 Pennsylvania, 1 West Virginia), *Chasmanthium* is likely 7 genetic units, and *Erigenia* is 7 genetic units with two populations with some shared ancestry.

Baptisia exhibited an upstream to downstream genetic differentiation in the Allegheny River partially supporting the classic metapopulation model. The Clarion River and Youghiogheny populations are both genetically distinct and spatially separated from the Allegheny River populations, which supports our hypotheses that the Youghiogheny population is distinct from all other populations of *Baptisia* due spatial separation and genetic isolation by distance and there is genetic structure of the populations.

Genetic diversity was lower than expected and inbreeding was present in most of the sampled populations. Genetic diversity was lowest and inbreeding highest in *Erigenia*, while these values are highest and lowest, respectively in *Chasmanthium*. Although populations of these species are genetically separated when comparing western and eastern populations. This result supports our hypothesis that their distribution is reflected in their genetic history.

Discussion and Management

The overall genetic health varies for populations of the three species in this report with *Baptisia australis* having moderate genetic diversity and slightly observable inbreeding, *Chasmanthium latifolium* having good genetic diversity and no observable inbreeding, and *Erigenia bulbosa* having low genetic diversity and high levels of inbreeding.

Baptisia australis, a globally vulnerable (G3) species in Pennsylvania consists of five distinct genetic populations that correspond to the watersheds in which they are found; Upper Allegheny, Lower Allegheny, Red Bank Creek, Clarion River, and Youghiogheny River. There is adequate gene flow, especially in the Allegheny River/Red Bank Creek populations where the genetic structure is most pronounced. Overall there is little inbreeding in all populations, but the highest values are found in the downstream populations of the Allegheny River below the confluences with the Clarion River and Red Bank Creek.

Genetic differentiation between upstream and downstream populations along the Allegheny River is present with populations around Red Bank Creek showing admixture. Genetic differences across these *Baptisia* populations could be caused in part by habitat differences such as development of surrounding areas that has led to some level of isolation of the populations. All upstream populations have less nearby development (e.g. homes, boat docks, bank erosion from alterations to the floodplain) than the downstream populations and as a result have lower levels of inbreeding (Table 3). These more developed areas are likely experiencing less ice and flood scour due the subsequent flood controls imposed by the presence of dams. Scouring of these habitats is important to maintain their open, early successional conditions suitable for establishment and persistence of *Baptisia* populations (Lind et al. 2014; Bywater-Reyes et al. 2015).

As mentioned, populations from the Clarion River and Youghiogheny Rivers are significantly distinct from the Allegheny Populations with the Youghiogheny population the most distinct sharing little to no gene flow with all other populations of *Baptisia australis* in Pennsylvania. There is concern regarding recruitment of new individuals at this location. The 11 sampled plants were the only remaining individuals in the last remaining population along the Youghiogheny River. This population, despite its small size and isolation, maintains relatively healthy levels of genetic diversity and low levels of inbreeding (Table 3). These factors make it a population of distinct conservation value (Ellstrand & Elam 1993). As a population on the edges of the Pennsylvania distribution, the Youghiogheny River population might also be expected to exhibit lower levels of genetic diversity and higher measures of genetic differentiation, perhaps related to genetic drift, founder effects, inbreeding, and other bottlenecks in the future (Eckert et al. 2008). Therefore, it is especially important to protect and conserve the population. Isolated populations in danger of increased inbreeding and genetic drift are ideal candidates for facilitated gene flow, through pollination or seeding from outside sources (Frankham et al. 2017) Seed collection from sites could also be important if facilitated gene flow is ineffective and *ex situ* conservation of these genotypes is required.

While the genetic status of Pennsylvania populations may not currently be dire, these edge-of-range populations are valuable for many reasons. The species currently faces reduction and degradation of suitable habitat in Pennsylvania. *Baptisia australis* is typically found in threatened cobble scour prairies that rely on regular, periodic disturbance in the form of ice and/or flood scouring events. Ice break up is an important disturbance regime analogous to fire in several habitats, while flood scouring is analogous to bison grazing to keep competition for available resources in prairie systems (Rood et al. 2007; Elson & Hartnett 2017). Metapopulations, which can be considered multiple management units must be maintained in order to sustain the long-term persistence of species and genetic viability through

facilitated gene flow in all populations (Hanski & Gilpin 1997; Funk et al. 2012; Frankham et al. 2017; Coates et al. 2018). The idea of management units can be applied to the concept of NHP Elements of Occurrence (EO). The EO is considered to represent populations that, if conserved, contribute to the survival or persistence of the species (NatureServe 2021). Currently the Clarion River, Red Bank Creek, and Allegheny River are considered one EO. Number of EOs needs reevaluated based on genetic data, and recognizing the Clarion River, Allegheny River, and Youghiogheny River listed as separate EOs so they can be utilized as management units. Coates et al., (2018) argues for management of species based on conservation units as more useful for preserving diversity in the long term. Maintaining genetic diversity in these units is important, as they may become valuable for genetic rescue in the future (Frankham et al., 2017). Benefits to preserving genetic diversity in *Baptisia australis* includes mitigating invasive species establishment and protecting important pollinator habitats to facilitate pollen gene flow (Van Geert et al. 2010). In addition to maintaining these populations we should also consider how we can facilitate connectivity. This could come in the form of seed collection from all populations and reciprocally planted to ensure admixture of the management units. If this prove ineffective, then an ex situ approach can be implemented to conserve each of the population genotypes.

Chasmanthium latifolium is a globally secure (G5) species at the edge of its range in Pennsylvania. Within the state, this species exhibits an east-west disjunct distribution where the populations are found in three major watersheds along the Monongahela, Raystown Branch Juniata, and Susquehanna Rivers. These populations may be impacted by several factors such as decrease in seed production due to isolation along river corridors and increased sensitivity to changing climate conditions (Jump & Woodward 2003; Jump et al. 2009; Abeli et al. 2014). The central marginal hypothesis predicts edge-of-range species will exhibit low genetic diversity and show genetic differentiation due to historical genetic drift, founder, inbreeding, and/or bottleneck events (Eckert et al. 2008).

The life history and biology of *C. latifolium* likely influences inbreeding and genetic differentiation of Pennsylvania populations. *Chasmanthium* is wind pollinated, which is traditionally been assumed to limit the efficiency of long-distance pollen transfer (Rognli et al. 2000; Friedman & Barrett 2009). The likelihood of inbreeding was presumed relatively high within *C. latifolium* due to the presence of cleistogamous florets and potential limited long-distance dispersal of pollen and seeds, which aligns with the central marginal hypothesis (Eckert et al. 2008). However, contrary to the central marginal hypothesis, our results suggest that *C. latifolium* populations show no evidence of inbreeding and genetic diversity is high, despite significant genetic isolation between the two waterways and among the populations along the Susquehanna River and its tributaries (Figures 6-7).

Susquehanna River populations cluster together, yet show some genetic structuring, and are separate from the Monongahela River populations, which are genetically different from all populations in the eastern side of the state. This suggest the possibility of the eastern populations having diverged from each other more recently than the western populations. This seems a plausible explanation considering the geographic barrier that the Allegheny and Appalachian Mountain ranges pose between the two waterways, ultimately limiting gene flow between the two regions.

Along the Susquehanna River, there significant genetic isolation between populations along the Susquehanna River and the centrally located Raystown Branch population that may have been due to a founder event with little subsequent gene flow (Eckert et al. 2008). Alternatively, it's possible that there were intermediary populations between Raystown Branch and the Susquehanna that have since been extirpated and removing any gene flow. A plausible explanation for the isolation within the eastern populations is due to a bottleneck effect resulting from habitat alterations and the low probability of long-distance gene flow. As observed in other systems, unidirectional down-stream gene flow through

water-dispersion would be observed through genetic similarity and connectivity between sites along a river, with populations further downstream having increased heterozygosity (Moore 2020). However, all *C. latifolium* populations along the Susquehanna River were shown to be genetically isolated, indicating that there is very limited down-stream gene flow within this system (Love et al. 2013).

Management of *Chasmanthium latifolium* to effectively increase genetic diversity is of less concern in Monongahela River populations. They are genetically diverse, have no inbreeding, and experience gene flow. However, the Susquehanna populations may be of greater concern. Although these sites are genetically diverse and not yet inbred, there is very limited gene flow between populations. Given the genetic isolation of these populations inbreeding may be a future concern (Hayes 2021). Likewise, potential negative effects of genetic drift could have a greater impact on the populations along the Susquehanna River and its tributaries. While crossbreeding that may occur between cultivars and native individuals could limit the potential for inbreeding, it could also inundate native populations with traits maladapted for the harsh Pennsylvania winters. Therefore, facilitated gene flow via seeds or seedings from other Pennsylvania sites may be an effective way to maintain adaptive genetic diversity and limit the potential for inbreeding (Hayes 2021).

Erigenia bulbosa is a globally secure (G5) species at the edge of its range in Pennsylvania. Within the state, this species exhibits an east-west disjunct distribution where a majority of the populations are scattered throughout western Pennsylvania from Greene County to Erie County. The eastern populations are restricted to relatively small geographic area along the Susquehanna River. All populations sampled are genetically distinct from each other and have much lower than expected heterozygosity, i.e. genetic diversity, and high levels of inbreeding (McDonnell et al. 2021).

The life history and biology of E. bulbosa may support our results suggest that populations are highly structured and evolving separately from each other. This species is very slow to reach reproductive maturity, only producing its first flowers on average 6 to 7 years after germination (Buddell II & Thieret 1985). The flowers bloom for a short period in the early spring each year and are pollinated by a number of early emergent insects (Dailey & Scott 2006). Seeds are very small and have limited dispersal capability and may contribute to genetic structuring in these populations through genetic drift without selection (Tero et al. 2005).

Inbreeding in plant populations can decrease genetic diversity over time and have impacts on the effective populations size, i.e. the number of reproductive individuals that have the same genetic response to random processes as the real population size (Ellstrand & Elam 1993; Charlesworth 2009). Our results suggest the observed heterozygosity is very low, this isn't direct evidence for inbreeding depression. However, given that most genetic variation is between populations and there is significant isolation by distance, it's likely these populations are still at risk. Without corridors connecting the populations, the potential negative effects from genetic drift in combination with low dispersal ability, low genetic diversity, potential threats of land use changes due to development activities, and the ongoing effects from climate change may decrease the likelihood that E. bulbosa will successfully adapt to future conditions (Waples 2010; McDonnell et al. 2021). For these reasons, all populations in the state warrant conservation considerations to help protect these isolated populations from additional reductions in size and number. This would effectively make regulatory decisions easier for DCNR to define and justify in the environmental review process be eliminating the need to justify partial regulation for this species.

The DCNR is in the process of updating the regulations for all tracked plant in the state and part of those updates is to assess them with NatureServe rank calculator to ensure that each species element occurrence data are viewed objectively taking into consideration the threats currently impacting the

rare plant diversity in Pennsylvania. One of the major threats to plant species is climate change (Engler et al. 2009). A number of factors were identified and incorporated into the Climate Change Vulnerability Index Tool and used to determine the impacts of this on our plant species vulnerabilities (Young et al. 2016). Prior to this project, *Baptisia australis*, *Chasmanthium latifolium*, and *Erigenia bulbosa* had not received formal NatureServe Rank Calculator Assessments or Climate Change Vulnerability Assessments.

Of the three species, only *Baptisia australis* has the appropriate state regulatory status of Pennsylvania Threatened (PT) based on our rank calculator assessment. *Chasmanthium latifolium* is currently listed as Tentatively Undetermined (TU) according to the state regulation. However, our rank calculator assessment shows this species likely qualifies for Pennsylvania Rare (PR) based on the number and sizes of the extant populations in the state. Likewise, Erigenia bulbosa is currently listed as PT according to the state regulations, but based on our assessment of the populations, qualifies for PR.

The process of formally changing the state regulatory status of plants requires that each species have a proposed change presented at a public forum (PA Rare Plant Forum) to allow botanists statewide the opportunity to provide input on the proposed changes. The proposals are brought forward to the Vascular Plant Technical Committee, the advisory committee on rare plant statuses to DCNR, for an official vote using the information provided in the public proposal presentation and discussion.

Erigenia bulbosa was taken through the entire proposal to vote to recommendation process during the 2021 PA Rare Plant Forum that indicated that the species is need of statewide protections due to limited dispersal abilities, potential loss of habitat, and combined low genetic diversity coupled with high inbreeding. This changes the way DCNR will treat *Erigenia* moving forward requiring that all instances of environmental review hits statewide be given equal conservation measures. Prior to this project DCNR required conservation measures for only the eastern populations because there are so few still extant in that part of the state. The remaining two species will have proposals put forward to correct their state regulatory statuses at the 2022 PA Rare Plant Fourm.

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