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2023 grant amount: \$8000

Research Project Topic: Genetic diversity and fitness decline in kittentails (*Synthyris bullii*)

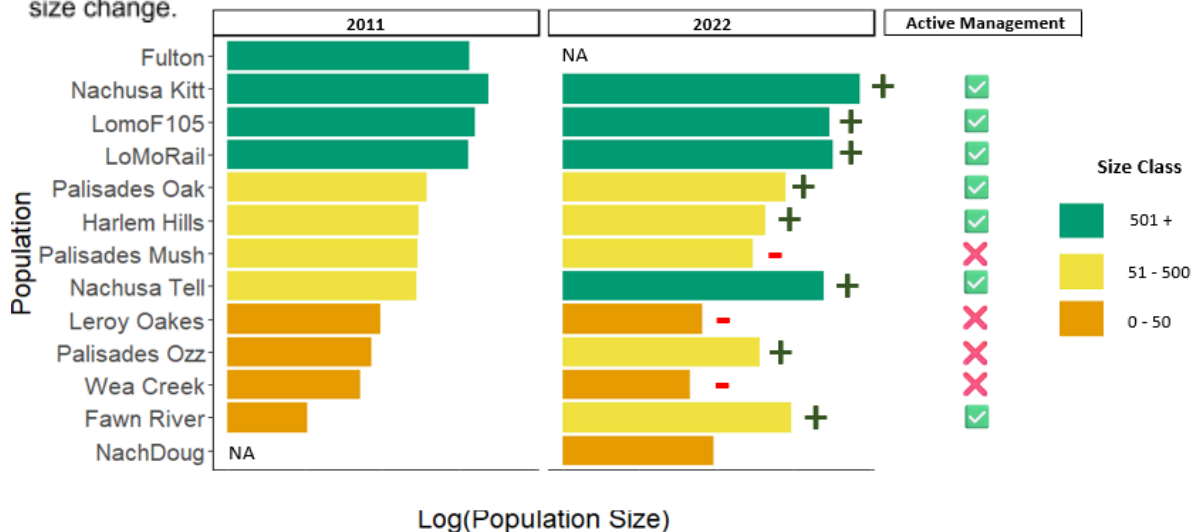
Research Project Purpose: For the state threatened endemic species, *Synthyris bullii*, this project aims to assess how population size has changed over an 11 year period, how population size relates to population genetic metrics of genetic diversity and inbreeding, and to determine how population size and population genetics correlate with various reproductive traits such as fruit to flower ratio, seed production, germination rate, and drought tolerance. Generally, small populations of rare species are thought to experience the greatest extinction risk, and this study aims to measure various traits associated with extinction potential to determine under which circumstances small populations of this rare species may be most at risk.

Research Project Outcomes to date:

Population Size

We found that from 2011 to 2022, all larger populations have grown or remained stable, as have all populations at Nachusa Grasslands. Moreover, across Illinois and Indiana, the three populations that have decreased in size are not being actively managed. This finding points to the efficacy of fire management, invasive species removal, and thinning of the canopy.

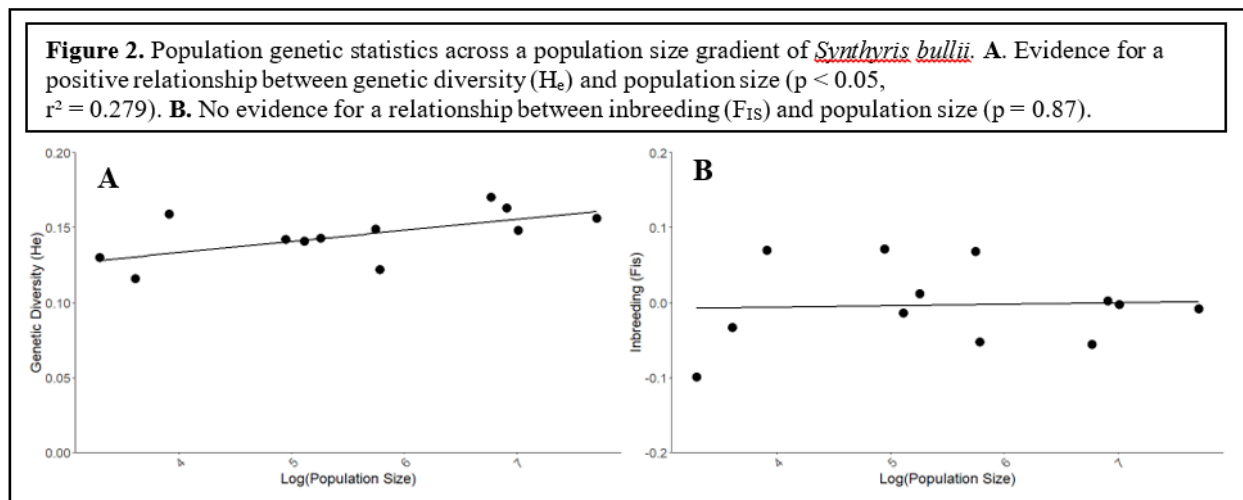
Figure 1: Population census data from 2011 and 2022, presented along a log scale. +/- denotes size change.



Population Size and Population Genetics

In line with population genetic theory, genetic diversity correlated strongly with population size across all populations (Figure 2A). The level of inbreeding in each population however, had no relationship with population size (Figure 2B). I am currently working on this genetic data - we just received the genome sequence data of *S. bullii* which I will be assembling over the summer before re-analyzing the sequence data from each population and aligning the sequences to the new genome.

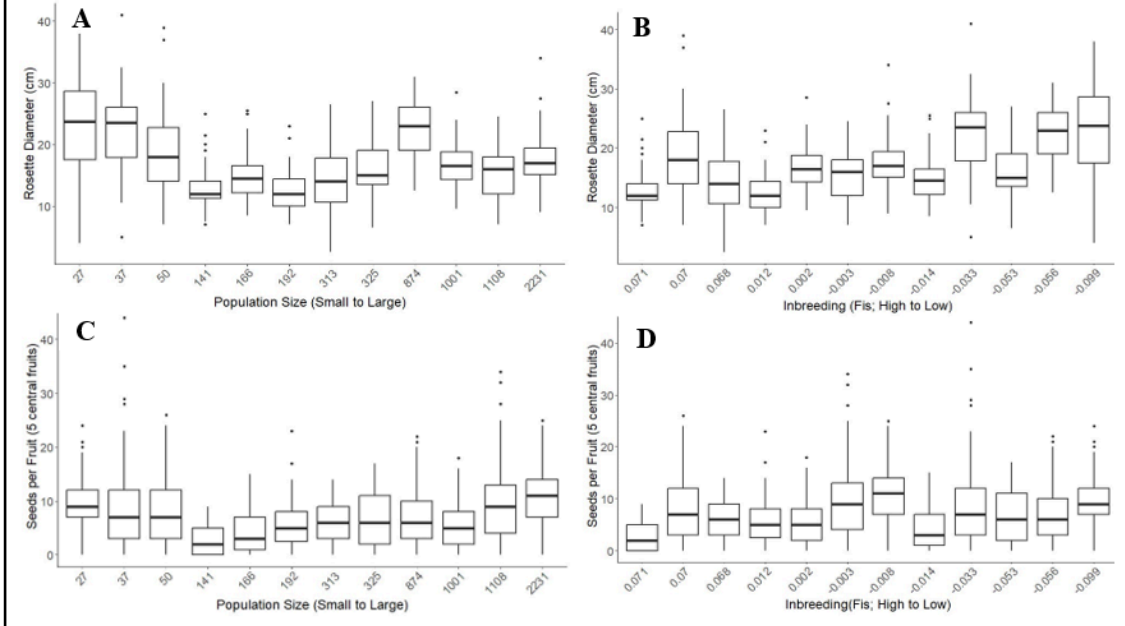
We observed higher genetic diversity in all *Nachusa* populations than in other populations across Illinois and Indiana, including in the small population at Doug's Knob. Gene flow between populations is likely maintaining genetic diversity for the small population at Doug's Knob. In contrast, a small population in Indiana, Wea Creek, is of a similar size but has much lower genetic diversity, and is isolated from other populations. The consequences of this isolation/connectivity is explored further below.



Predictors of Fitness metrics

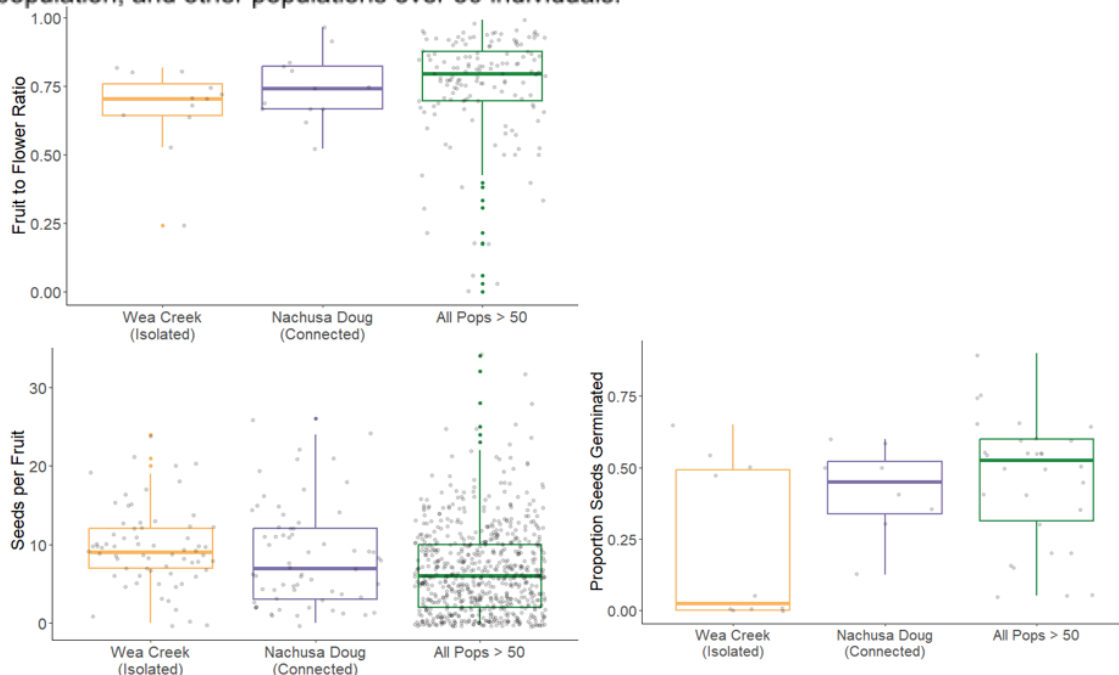
Population size and inbreeding are equally and strongly correlated with seed per fruit (Figure 3C and 2D). However, F_{IS} has a stronger correlation with plant size measured as rosette diameter than population size does (Figure 3A and 3B). These data suggest that while small populations may experience fitness decline due to Allee effects such as pollen limitation, not all small populations experience the same decline. Higher levels of inbreeding are known to lead to reductions in fitness, and we see this relationship for seed per fruit and plant size. Through this analysis, we can glean that while not all small populations may experience fitness declines, small and inbred populations likely will. However, *Nachusa* Doug is an interesting exception – this is a small population (56 individuals), yet has some of the most seed per fruit and largest plants. I compared the fitness metrics of this population with the small population in Indiana, Wea Creek, which has a similar number of individuals.

Figure 3. Correlations of plant size and seed production with population size and inbreeding (F_{IS}). **A.** No evidence for a linear relationship between population size and rosette diameter ($p = 0.655$). **B.** Strong evidence for a linear relationship between F_{IS} and rosette diameter ($p < 0.01$, $r^2 = 0.099$). **C.** Strong evidence for a linear relationship between population size and seeds per fruit ($p < 0.01$, $r^2 = 0.022$). **D.** Strong evidence for a linear relationship between F_{IS} and seeds per fruit ($p < 0.01$, $r^2 = 0.022$).



For Wea Creek, the small and isolated population, we observe slightly reduced fruit to flower ratio, little change in seed per fruit, and a significant decrease in germination rate compared to Nachusa Doug, the small and connected population (Figure 4). The significant decrease in germination (no germination at all for 6 of 10 maternal plants) could be explained by environmental differences, increased rates of selfing, or inbreeding depression. Future work in a common garden will explore this further.

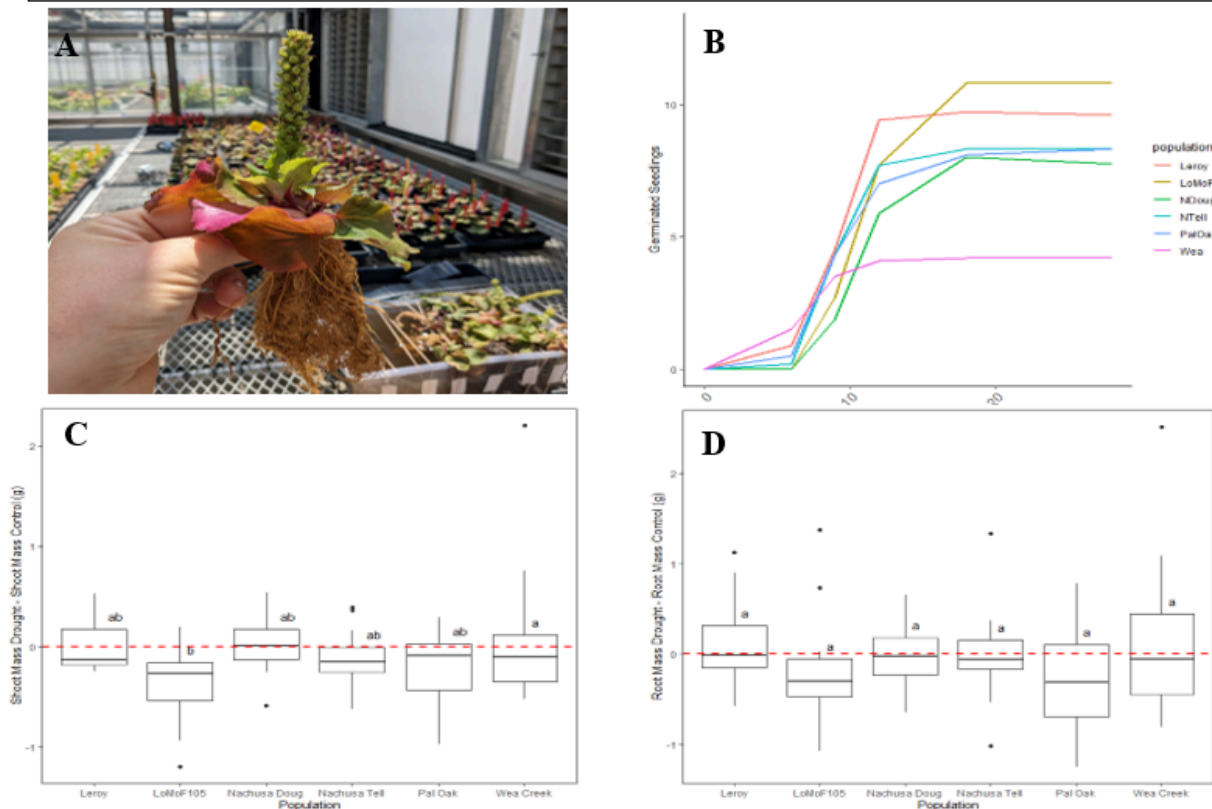
Figure 4: Fitness metrics measure for a small and isolated population compared to a connected population, and other populations over 50 individuals.



A drought experiment conducted in May/June 2023 contained seedlings that represent 10 maternal lines from each of three small populations (2 low and 1 high genetic diversity population) and three large populations (2 high and 1 low genetic diversity population). Germination measured by cotyledon emergence and seedling survival were tracked for each population prior to drought treatment. Half of the plants received weekly watering to maintain VWC above 15% and half of the plants were dried completely before rewatering over 4 phases of drying. During the drought experiment, rosette diameter was measured with a ruler and stomatal conductance was measured with a METER SC-1 porometer, and root and shoot biomass weighed on over half of plants.

Significant differences in germination rate were detected between the 6 populations after 28 days (Figure 3B; $p < 0.05$, $r^2 = 0.201$). Wea Creek is likely driving this difference with the lowest germination of all populations. Peak germination occurred approximately 15 days after seeds were sown for all populations. There were slight reductions in both shoot and root biomass under drought for all populations, but root and shoot biomass

Figure 5. Drought experiment on 6 populations of *S. bullii*. **A.** Photo of cleaned roots on a control plant. **B.** Germination rate up to 28 days prior to drought experiment. **C.** Difference in shoot mass between drought and control plants. **D.** Difference in root mass between drought and control plants.



did not differ significantly across populations. This result could be due to an insufficiently stressful drought treatment. Nonetheless, drought tolerance of *S. bullii* appears to be high across all populations included in the experiment. Stomatal conductance and plant size data are still being assessed, as are biomass data.

Describe how the grant funds you have received from the Friends of Nachusa Grasslands have been used in regard to the above topic, purpose, and/or outcomes:

\$2000 - PI Stipend (\$20/hr for 100 hours)

As an unpaid masters student in the Spring and Summer of 2023, this funding allowed me to focus on completing a drought experiment, germination trials, and continuing bioinformatics work without relying on student loans or working on top of research. As of Fall 2023, I have been accepted as PhD student at Northwestern University and Chicago Botanic Garden and will be continuing work on kittentails with a PhD stipend.

*\$2500 - Genome sequencing of *Synthyris bullii**

Due to issues with mismatches in genetic data between populations of *S. bullii* that make calculating reliable inbreeding values difficult, and to get a finer resolution for further population genetics work, we decided to sequence the species genome instead of sequencing more individuals. I selected an individual grown from seed collected at Doug's Knob, and sent a fresh leaf to be sequenced at Mt. Sinai's Department of Genetics and Genomic Sciences. The genome data just arrived last week and I am about to begin assembling the genome to use it as a reference for aligning previously sequenced individuals.

\$600 - Soil Moisture Sensors x 4

Soil Moisture sensors were purchased as part of a set of soil moisture sensors for a drought experiment conducted during Spring/Summer of 2023. These sensors work with an internet router to record data continuously as well as take real time individual measurements. The sensors are currently being used by other students conducting drought experiments at the Chicago Botanic Garden this summer and will be a useful addition to our program's data collecting toolkit!

\$900 - Mileage for 2023 summer field work (1540 miles @ \$0.585/mile)

Field work in 2023 required revisiting all 12 populations across Illinois and Indiana to re-census each population. While there, I counted all flowering and non-flowering individuals, and measured soil moisture in situ before and after saturation. Soil moisture readings were taken to compare to soil moisture in the drought experiment I conducted in the Spring of 2023. Census data was recorded for a second year to account for year to year fluctuations in population size.

\$2000 - Publication costs for American Journal of Botany (open access) NOT SPENT

Once genome assembly and re-alignment with a reference genome have been completed, I will be writing up results that include wild population censuses (change in population size from 2010/11 to 2022/23), population demographic measurements (plant height and rosette diameter, fruit:flower ratio, seed per fruit, germination), and population genetics metrics (genetic diversity, inbreeding, and population structure). This publication will be the first chapter of my dissertation.

Describe how your project has benefited the work and goals of Nachusa Grasslands:

“The purpose of Nachusa Grasslands is to protect and restore rare habitats and to serve as an influence, inspiration, and resource for other conservation partners, researchers, and the public” - Nachusa Grasslands website.

My work has provided valuable information for the protection of a state-threatened midwestern endemic prairie species, *Synthyris bullii*. An understanding of the genetic diversity across the southern range edge helps us determine both which populations may need more conservation attention, and which populations harbor higher diversity. Protecting populations with high diversity is just as important as bolstering those without, if fitness is being impacted. Moreover, I have demonstrated the value in the restoration and protection of the rare habitats at Nachusa Grasslands in comparison to other unmanaged areas when we look at changes in population sizes over time. I have presented my work at multiple national conferences and at Nachusa Grasslands and have been given positive feedback on each occasion from volunteers, researchers, and land managers. I look forward to publishing this work and making recommendations to land managers on how to address populations of rare plant species with inbreeding depression.

Describe how your findings can be applied to challenges in management practices for restoration effectiveness and species of concern:

Restoration effectiveness:

This study has focused on remnant prairie populations of *S.bullii* with the goal of understanding how populations have been impacted by fragmentation and habitat loss. The surviving populations of this endemic species have persisted in small pockets of sandhill prairies such as those at Nachusa Grasslands. Additionally, the restoration activity in the surrounding prairies have more than likely supported pollinator species that will maintain connectivity for *S. bullii* populations. The high genetic diversity and high overall fitness of plants in the small population on Doug’s Knob points toward this conclusion. Further genetic work to assess gene flow will provide more conclusive evidence for how connected each of the populations are across this part of the range.

Managing for species of concern:

Decline in 3 out of 4 unmanaged populations of *S. bullii* suggests that the restoration and maintenance of habitat is effective. All but 1 managed populations are stable. This is positive news for land managers focused on conserving kittentails, as burning, invasive species removal, and canopy thinning all seem to be effective. Anecdotally, I have observed that burning increases abundance of overall plants, but thinning the canopy will increase the abundance of flowering plants. This observation is worth further research, but is valuable information for managing *S. bullii*. With the additional research I have planned, I hope to provide land managers with a better understanding of what to do if a population is in decline, and how they can conduct a 'genetic restoration' appropriately.

Please list presentations/posters you have given on your research:

- Davies, A. 2024. **Conserving Kittentails: how valuable is connectivity for small populations of a rare endemic?** Botanical Society of America: Conservation Biology Section. Grand Rapids, MI. Oral Presentation.
- Davies, A. 2024. **Conserving Kittentails: an assessment of plant fitness and population genetics across a population size gradient.** Nachusa Grasslands Science Symposium. Franklin Grove, IL. Oral Presentation.
- Davies, A. 2023. **Does Size Really Matter? Implications of Small Population Size for Rare Plants.** Ecological Society of America: Conservation and Extinction Section. Portland, OR. Oral Presentation.
- Davies, A. 2023. **Does Size Really Matter? Implications of Small Population Size for Rare Plants.** Biological Society of America: Population Genetics Section. Boise, ID. Oral Presentation.
- Beck, I., Davies, A. 2022. **Small population size and isolation impacts on seed production of a midwestern rare species: *Synthyris bullii*.** Research Experience for Undergraduates Program. Chicago Botanic Garden. Chicago, IL. Poster Presentation.
- Bamford, V., Davies, A. 2022. **The effect of seed size on germination rate in *Synthyris bullii*.** BIOL 384, Plant Biology. Lake Forest College Department of Biology, Lake Forest, IL. Poster Presentation

Have you submitted manuscripts to scientific journals? If so, which ones? If not, do you anticipate doing so? (Please send digital copies of published articles to the Friends so that we can learn from your work.)

I haven't yet submitted to a scientific journal - once I have assembled the genome and finalized my genetic data I will be writing up and submitting to Conservation Biology or American Journal of Botany.

What follow-up research work related to this project do you anticipate (if any)?

With plants grown from seed collected in 2022, I am currently conducting a germination trial to determine if self-pollinated flowers produce seed with similar viability to seed from open pollinated flowers. These plants have been grown in a common garden sandbox at the Chicago Botanic Garden, where a total of 30 individuals from all populations were bagged before flowering and then self-pollinated once flowers were open. The fruits produced were then collected from the selfed plants to compare to fruits collected from open-pollinated plants. This experiment will help explain why we are seeing such low germination rates in certain populations - whether increased rates of selfing is resulting in low germination or whether there is an underlying genetic cause. I suspect that a small and isolated population in Indiana is experiencing inbreeding depression due to generations of selfing or mating with closely related individuals.

If we determine that inbreeding depression is the most likely cause of low germination rates at Wea Creek, then I will conduct a genetic rescue experiment next summer to assess at which genetic and geographic distances we can safely mix populations. This will be highly informative for land managers when trying to establish new populations and when looking to bolster genetic diversity of existing populations.

Finally, with the genome assembly complete and after reanalyzing sequence data, I plan to assess population structure using the program STRUCTURE. This will provide additional insight into gene flow between populations as well as how genetically dif

Optional: Suggestions for improving the application and award process for future Friends of Nachusa Grasslands Scientific Research Grants: