The impacts of habitat change on the genetic diversity of *Amsonia tharpii*, a rare plant species

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Introduction and Background

- Species are threatened by habitat fragmentation caused by land use change. Habitat fragmentation can lead to population decline.
- Inbreeding, genetic drift, and small population size can decrease genetic diversity. Low genetic diversity lowers species' ability to adapt, increasing extinction risk, as does small population size.
- *Amsonia tharpii* is found in 5 populations in New Mexico (4) and Texas (1) (Fig. 1a), which are threatened by gas and oil mining. Increased drought frequency due to climate change also imperils *A. tharpii*.
- To understand the impacts of habitat change on the historical genetic diversity and population structure of *A. tharpii*, it was compared to two other *Amsonia* species, *A. longiflora* and *A. fugatei*.
- *Amsonia longiflora* differs from the other two species by having a corolla tube that is almost twice as long (Fig. 1b). It also has the largest distribution of the three species, occurring in southern NM, Texas and Mexico.
- *Amsonia fugatei* is morphologically similar to *A. tharpii*, endemic to Socorro County, NM, and only known from three small populations (Fig. 1a).
- Results will help to inform the Fish and Wildlife service whether or not *A. tharpii* should be listed under the Endangered Species Act.

Figure. 1

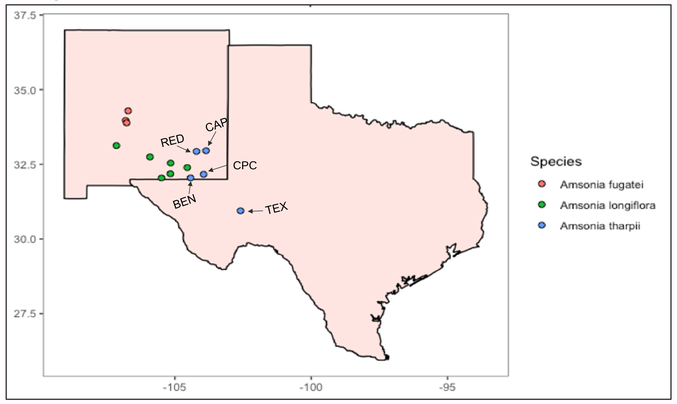

Fig. 1a

Fig. 1b

Fig. 1c

Fig. 1d

Figure 1. (a) Map of New Mexico and Texas, showing where each population of Amsonia (*A. fugatei*, *A. longiflora*, and *A. tharpii*) is located. **(b)** Flowers of *A. longiflora* **(c)** Flowers of *A. tharpii.* **(d)** Non-flowering *A. tharpii* plant with a Hwkmoth on the plant .

Objective and Hypothesis

We expect to find that the geographically closest populations of *A. tharpii* will be most closely related. We also expect *A. tharpii* to have less genetic diversity and higher inbreeding than the widespread *A. longiflora*. Conversely, we expect *A. tharpii* to have more genetic diversity and less inbreeding than *A. fugatei*.

Methods

- **Field Work:** Leaf tissue was collected and dried on silica gel in 2020 and 2022. We sampled all three populations of *A. fugatei*, all five of *A. tharpii*, and six of *A. longiflora.* A total of 10 individuals per population were used for genetic analyses.
- **DNA Extraction**: We used a modified CTAB protocol and quantified DNA with a Qubit.
- **Sequencing**: We used double digest Restriction-site associated DNA sequencing (ddRadSeq) to generate SNPs. Genomic libraries were prepared at CBG and sequenced at Northwestern University using a NovaSeq.
- **Analyses**: Raw sequences were demultiplexed, filtered, and aligned using Stacks. VCF files were filtered for missingness and sequence depth using vcftools. Hierfstat and dartR were used in RStudio to estimate genetic diversity and inbreeding statistics. ADMIXTURE was used to investigate population structure and to determine the best number of genetic clusters using the cross validation method.

Results

Table. 1

Species	Stacks parameters (m-M-n+r)	Loci retained/variant sites/ after filtering/ % missing	Ht	Fis
A. fugatei	2_4_4_r60	394/284/91 (75%)	0.153	0.496
A. longiflora	3_5_5_r60	7177/6029/441 (85%)	0.192	0.695
A. tharpii	2_3_3_r80	44547/38326/1114 (25%)	0.133	0.047

Table 1. Parameters used to filter data and results of STACKS and VCFTOOLS. Measures of genetic diversity (Ht) and inbreeding coefficients (Fis) for each species.

Table. 2

Population	Ho	He	Fis	ll	hl
CAP	0.113	0.119	0.050	0.011	0.085
CPC	0.110	0.115	0.047	0.009	0.086
TEX	0.110	0.115	0.041	0.003	0.079
RED	0.113	0.126	0.104	0.067	0.144
BEN	0.120	0.118	-0.010	-0.051	0.028

Table 2. Population level statistics for *A. tharpii* (Ho = observed heterozygosity, He = expected heterozygosity, Fis = inbreeding coefficient, ll-hl Bootstrap confidence interval for Fis).

Figure. 2

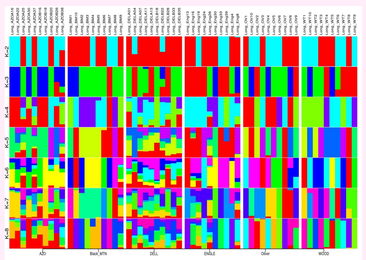

Fig. 2a

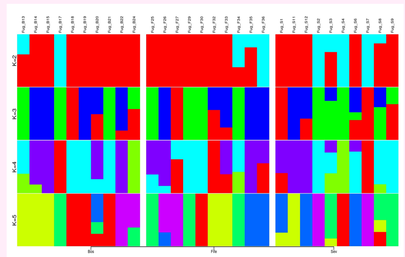

Fig. 2b

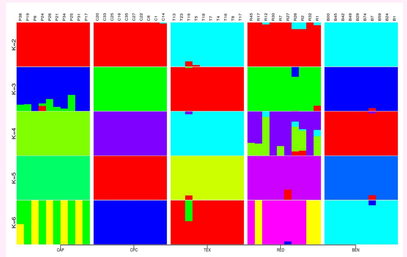

Fig. 2c

Figure 2. Results from ADMIXTURE for **(a)** *A. longiflora* (K = 2-8), **(b)** *A. fugatei* (K = 2-5), and **(c)** *A. tharpii* (K = 2-6). The best K barplot for each species was determined using the cross-entropy method, and is indicated with an arrow.

Discussion

Genetic Diversity

- *Amsonia tharpii* had the lowest genetic diversity and inbreeding coefficient (Table 1). Inbreeding was highest in *A. longiflora* and *A. fugatei*, but this was not reflected in their genetic diversity (Table 1).
  - Poor sequencing results may have inflated values for *A. longiflora* and *A. fugatei*.
- *Amsonia ludoviciana* has a narrow distribution in the southeastern U.S., and was found to have a genetic diversity (0.1918) and inbreeding coefficient (0.129), similar to that of *A. tharpii* (Table 1)[3].

Genetic Structure

- Pollination biology of *Amsonia* is unknown. The long corolla tubes of *A. longiflora* may be visited by hawkmoths, while the short corollas of *A. tharpii* and *A. fugatei* might be generalist pollinated.
- ADMIXTURE results suggest that hawkmoths may be facilitating pollen transfer for each species.
  - *Amsonia tharpii* populations were split into two clusters, BEN and TEX, and CAP, CPC, and RED, with little gene flow between the two (Fig. 2c).
  - This refutes our hypothesis that populations close together would be more related, since BEN is closer to CPC than TEX.

Preliminary conclusions

- *Amsonia tharpii* is likely self-incompatible.
- *Amsonia fugatei* and *A. longiflora* have high inbreeding.
- No strong population structure.
  - *A. fugatei* and *A. longiflora* show higher levels of gene flow than *A. tharpii*.

Future Directions

- Sequence *A. tharpii* offspring to determine contemporary genetic diversity.
- Resequence *A. longiflora* and *A. fugatei* for comparison
- Reproductive and pollination studies to determine *Amsonia* self compatibility and pollinators.

References

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