Applied conservation genetics: GBS and building a genetic roadmap for the recovery of *Houstonia montana*, an imperiled high-elevation, southern Appalachian endemic.

Kira Lindelof
Advisor: Alexander Krings

Photo: *Houstonia montana* in flower.

Project Updates

Funding from the Tom and Bruce Shinn fund was critical for sampling and tagging populations of *Houstonia montana*. From July to October of 2021, I collected two cauline leaves from ca. 10% of each of four populations of *H. montana* across Ashe, Mitchell, and Watauga counties. I stored leaves and dried in coin envelopes with silica beads until DNA extraction. To track specific sampled clumps of *H. montana*, I secured aluminum tags to adjacent bedrock with drilled screws. I revisited populations in 2022 to evaluate the stability of the tags installed the previous year and to obtain additional representative leaf samples per population. Tags that were damaged between visits were replaced in the summer of 2022.

I am now in the process of extracting DNA from leaf tissue using Qiagen DNeasy Plant Mini Kits purchased with help from the Shinn Grant. The anticipated completion of DNA extraction and sample prep for genomic sequencing is expected for Fall 2023. In the early summer of 2023, I received an additional award to fund the library prep and genomic sequencing portion of this project with the intention to sequence in the Winter of 2023 through 2024.

Photo: A tagged clump of *Houstonia montana* in fruit.

Project Expansion

In regions where *H. montana* and its more common sister species, *H. purpurea* overlap, tetraploid populations of morphological "intermediates" between the two occur, suggesting some gene flow. Using microsatellite and AFLP markers, Glennon, Church, and Donaldson identified genetic admixture in two "intermediate" populations, suggesting past hybridization between *H. purpurea* and *H. montana* (Glennon, 2010; Glennon et al., 2011). While hybridization can be detrimental to a species like *H. montana* (e.g., loss of unique traits or of the species itself due to introgression - the introduction of genes from one species into the gene pool of another species), gene flow did not seem to be occurring between the overlapping populations of *H. montana* and *H. purpurea* (Glennon, 2010; Glennon et al., 2011). This was likely due to differences in ploidy between extant populations of the two species - *H. montana* is strictly diploid while most populations of *H. purpurea* are tetraploid (some diploids do occur but are out of the range of *H. montana*). An autopolyploidization event in *H. montana* may have yielded tetraploid individuals that could interbreed with *H. purpurea*, resulting in the hybrids. Originally thought to be an infrequent phenomenon in nature, autopolyploidization may contribute to the polyploids found not only in

Houstonia but in other genera as well (see Glennon & Church, 2015, and Soltis et al., 2007). Given the potential for more autopolyploidization events to occur, frequent monitoring of *H. montana* populations for hybrids was recommended by Glennon and colleagues (Glennon, 2010; Glennon et al., 2011).

While the combined works of Glennon, Church, and Donaldson provided much insight into the interactions between *H. montana* and *H. purpurea*, the practical question remains – how does one identify a hybrid in the field? That is, how can one discern whether an individual expresses expected variation of a particular species or exhibits morphological "intermediacy" between two species? Although Glennon (2010) examined morphology, only ten characters were studied between *H. montana*, *H. purpurea*, and the hybrids. A more exhaustive list of characters needs to be examined to identify distinctive morphology that can be used to reliably identify *H. montana* versus *H. purpurea* versus hybrids in the field. The loci examined by Glennon and colleagues also provide lesser power in comparison to current next-generation sequencing techniques like Genotyping-by-sequencing (GBS; Glennon, 2010; Glennon et al., 2011). To address this issue, I sampled from one hybrid population and two, newly identified "intermediate" populations in Ashe County, North Carolina, in the summer of 2022. The second hybrid population will be sampled in summer 2023. I will include these populations in my genomic study to examine their genetic (dis)similarity with *H. montana*. Additionally, a morphological analysis will be conducted to determine whether the individuals exhibit traits distinct to *H. montana*, *H. purpurea,* or a combination of the two.

Photo: Kira Lindelof with *Houstonia montana*

Literature Cited

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