Research update: Diversification and population genetics of rare *Astragalus* in the Four Corners Region

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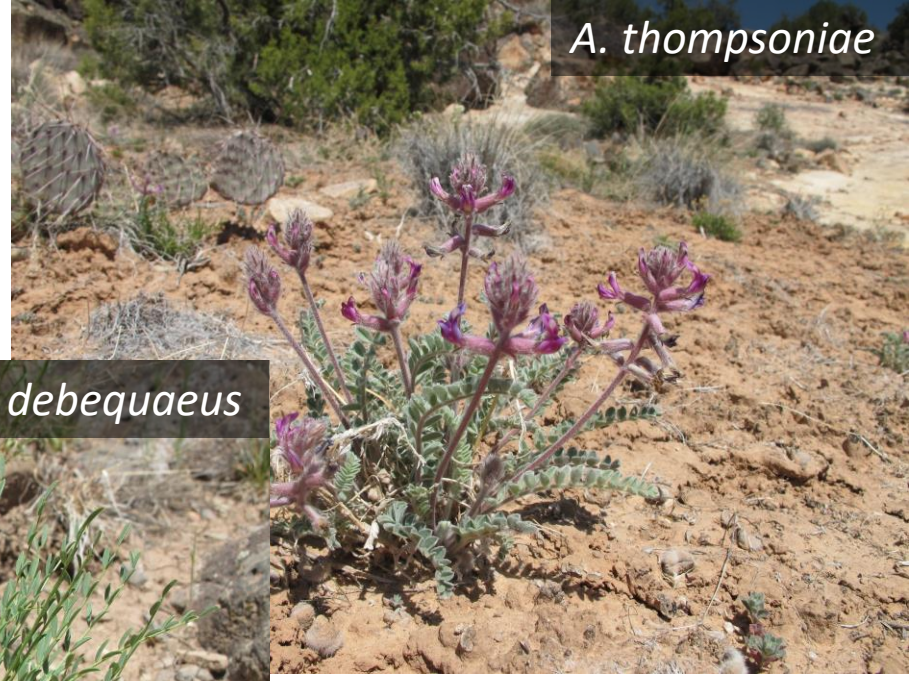
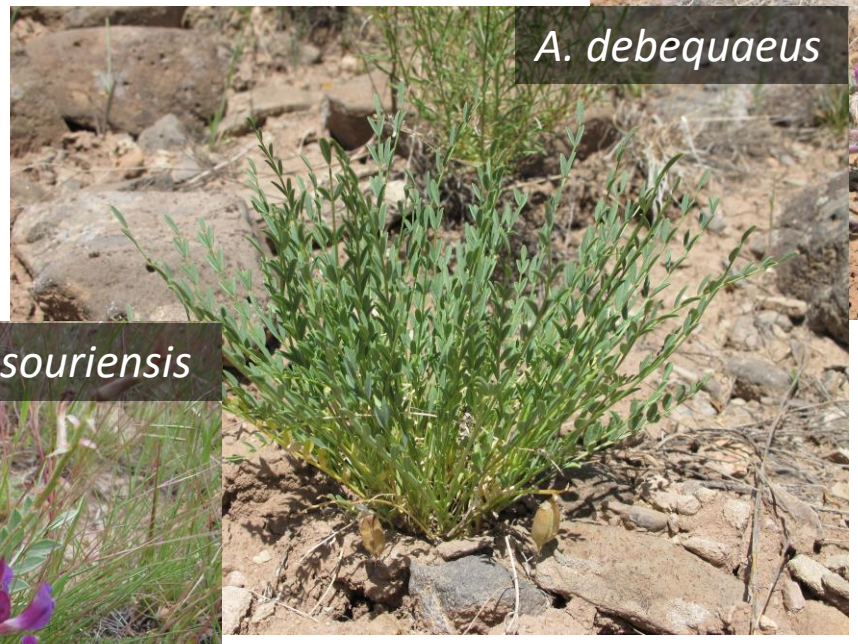
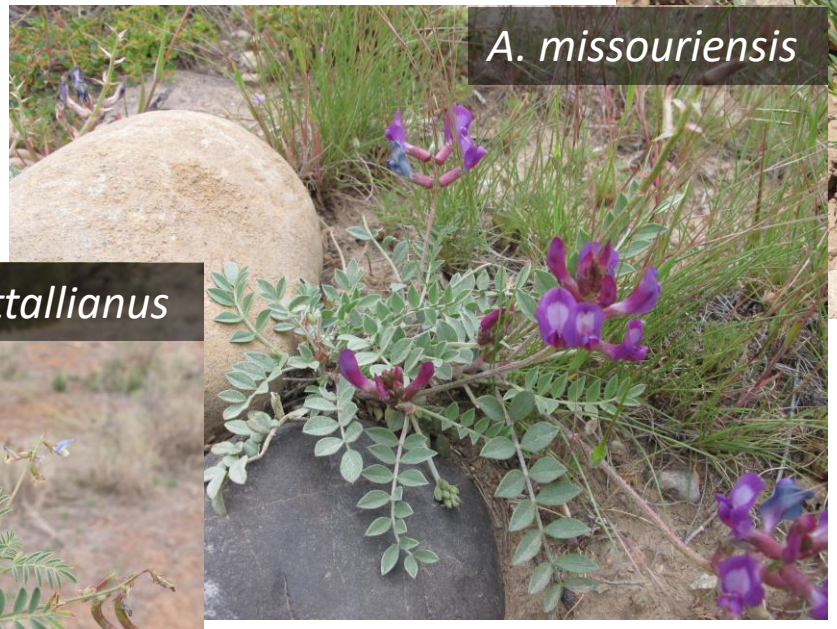
Astragalus in Colorado

Second most speciose genus: 115 native species
Tracked by CNHP: 29 species – most in western Colorado
G1 – rated: 6 species







Astragalus in the Four-Corners

60 taxa in region
Ca. 12 are endemic

A. cutlerii

Photo: D. Roth

Photo: S. O'Kane

A. cronquistii

A. schmolliae

A. deterior

Photo: R. Sivinski

A. oocalycis

A. tortipes

Photo: A. Schneider

A. cottamii

A. humilimus

Photo: D. Roth

Photo: R. Sivinski

A. proximus

Photo: A. Schneider

A. chuskanus

Photo: D. Schleser

A. cliffordii

A. heillii

Photo: D. Roth

Mesa Verde National Park

Two G1 species of concern

Astragalus schmolliae

Astragalus deterior

View NE from Mesa Verde toward La Plata Mts.

Astragalus schmolliae

Only known from Chapin Mesa in MVNP and adjacent Ute Mountain Ute Tribe

Monitoring has occurred since 1980 – intensive monitoring by MVNP and CNHP since 2001

2002 Long Mesa Fire burned 38% of habitat

Fall 2020 recommended by FWS for listing as threatened under ESA with designation of 3635 acres of critical habitat in MVNP and UMUT

View south across Chapin Mesa

A. schmolliae genetic study

1) *What is the level and pattern of genetic diversity across the restricted range?*
   a) *Does the single contiguous occurrence behave as a single genetic population?*
   b) *Does fire history contribute to differences in population genetic diversity?*
   c) *Does population genetic diversity vary by year?*

2) *How does* A. schmolliae *fit in the phylogeny of regional* Astragalus*?*
   a) *Can this account for the pattern of narrow endemicity?*
   b) *Is there support for the hypothesis of hybrid speciation?*

2018

Very dry winter. Few plants emerged
Able to sample one population – Sun Temple

Developed microsatellite assay for population-level work

- Screened 19 potential primers from three North American species of *Astragalus*
- Evaluated consistency of amplification in one population and cross-amplification in 18 other taxa.
- 10-primer assay with mixture of di-, tri-, and tetranucleotide repeats

2019 – early 2020

Very wet winter.
Largest emergence of plants ever recorded by CNHP

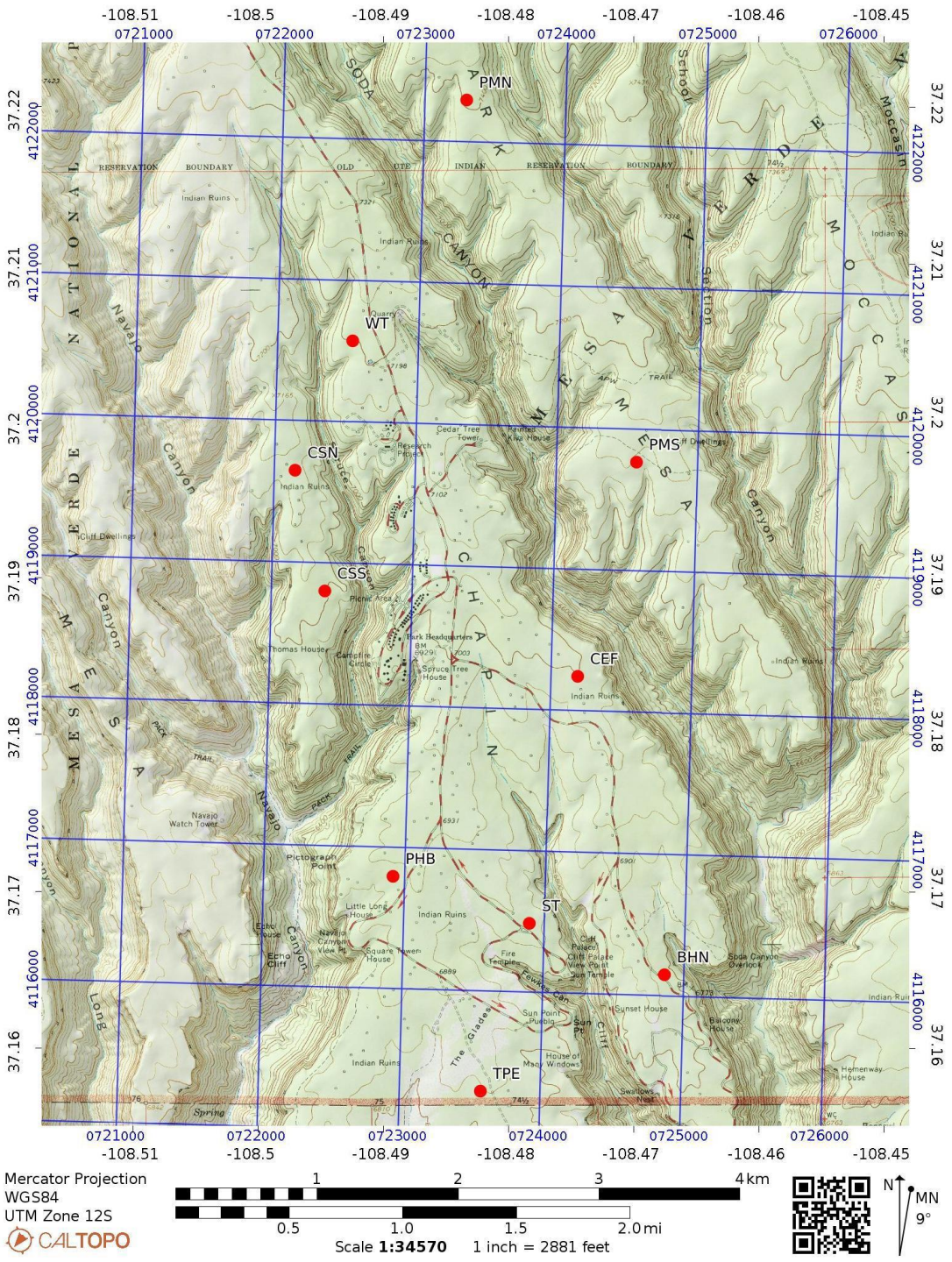
- Sampled 10 populations across entire range in MVNP

Began work on phylogenetic analysis

- Sampled 57 taxa known to occur in Four-Corners region using fresh and herbarium material from FLD and SJNM.

- Generated nuclear ribosomal internal transcribed spacer (ITS) and external transcribed spacer (ETS) sequences. Developed a new primer set for ETS optimized for North American species.

- To test potential hybridization and cytonuclear discordance a subset of 12 individuals also amplified for three chloroplast regions, *rpl32-trnL, psbA-trnH,* and *trnL-trnF.*



Results – Genetic Diversity

Genetic diversity was moderate to high across all populations – equal to that of other outcrossing species

No consistent differences noted between populations in old-growth PJ and burned areas

Measures of outcrossing similar among all populations

Levels equal across two years for the one multi-year sampled population – Sun Temple

Population (Abbreviation)	n	Na	Ne	Ap	P	H_o	H_E	F_{IS}
Sun Temple (ST_18)	20	6.375 (0.800)	3.395 (0.498)	1	100%	0.660 (0.109)	0.640 (0.070)	-0.031 (0.132)
Pithouse B (PHB)	20	6.750 (1.221)	4.166 (0.816)	1	100%	0.796 (0.078)	0.683 (0.068)	-0.173 (0.053)
Sun Temple (ST_19)	19	7.000 (1.309)	4.250 (0.752)	–	100%	0.777 (0.074)	0.702 (0.059)	-0.127 (0.088)
Park Mesa South (PMS)	20	6.000 (1.464)	4.231 (0.990)	2	100%	0.753 (0.098)	0.656 (0.085)	-0.151 (0.062)
Park Mesa North (PMN)	16	5.750 (0.648)	3.390 (0.499)	–	100%	0.762 (0.080)	0.643 (0.065)	-0.194 (0.067)
Chapin Spur South (CSS)	20	6.375 (1.133)	4.209 (0.729)	–	100%	0.855 (0.061)	0.704 (0.056)	-0.227 (0.048)
Chapin Spur North (CSN)	19	5.750 (1.130)	3.470 (0.589)	–	100%	0.796 (0.052)	0.655 (0.051)	-0.229 (0.046)
Tribal Park Entrance (TPE)	20	7.000 (1.239)	4.526 (0.730)	1	100%	0.865 (0.050)	0.727 (0.054)	-0.212 (0.057)
Chapin Mesa East Firescar (CEF)	19	6.750 (1.221)	4.067 (0.811)	2	100%	0.776 (0.085)	0.693 (0.056)	-0.133 (0.098)
Balcony House North (BHN)	20	6.875 (1.630)	4.293 (0.997)	–	87.5%	0.725 (0.122)	0.648 (0.099)	-0.134 (0.109)
Water Tank (WT)	20	6.375 (1.068)	4.044 (0.806)	–	100%	0.767 (0.105)	0.661 (0.086)	-0.156 (0.070)
Mean	19.364	6.455 (0.345)	4.004 (0.220)	0.636	98.86%	0.776 (0.025)	0.674 (0.020)	-0.161 (0.023)

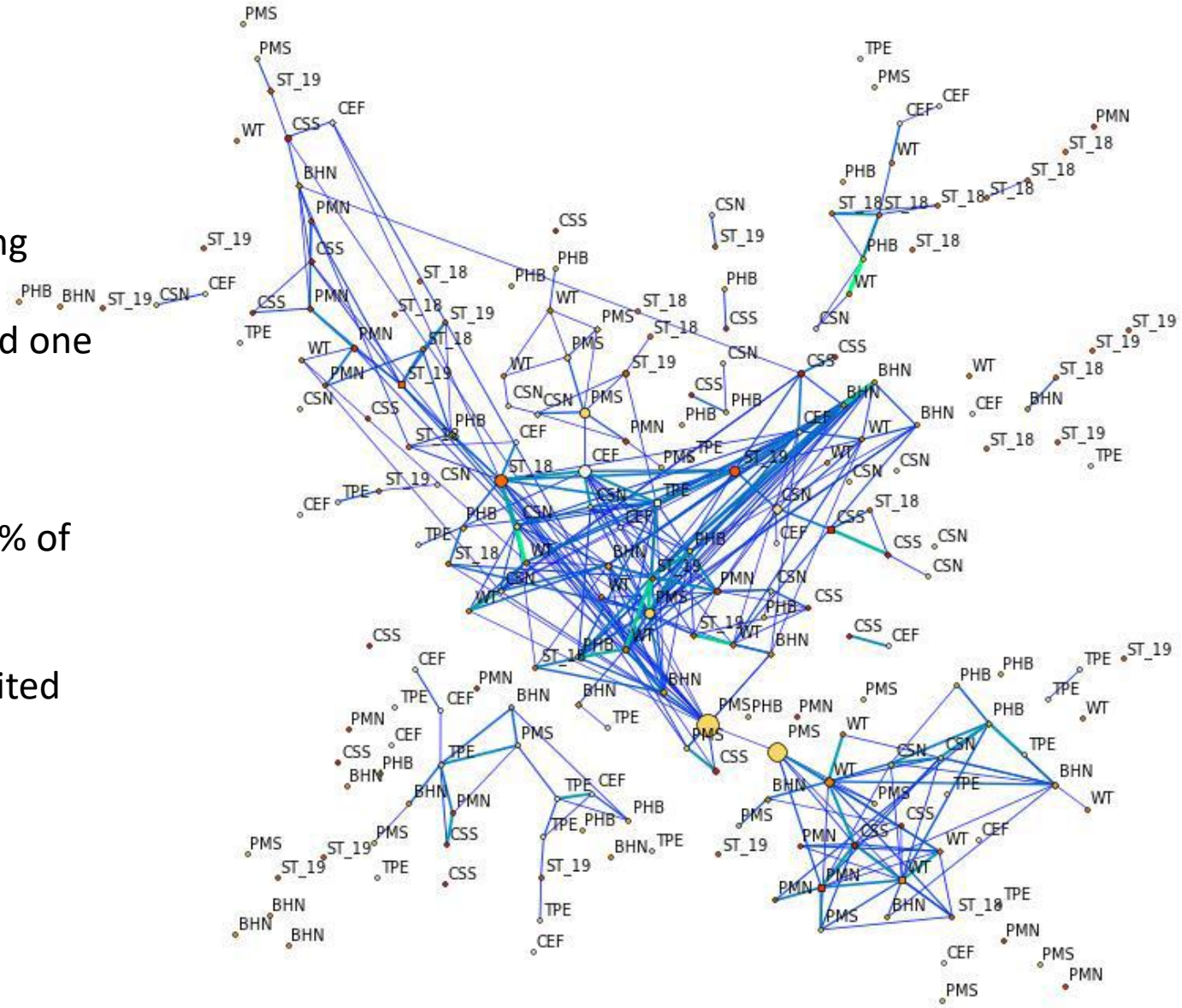
Results – Genetic Structure and Gene Flow

Network analyses showed high level of interconnectedness with no population clustering

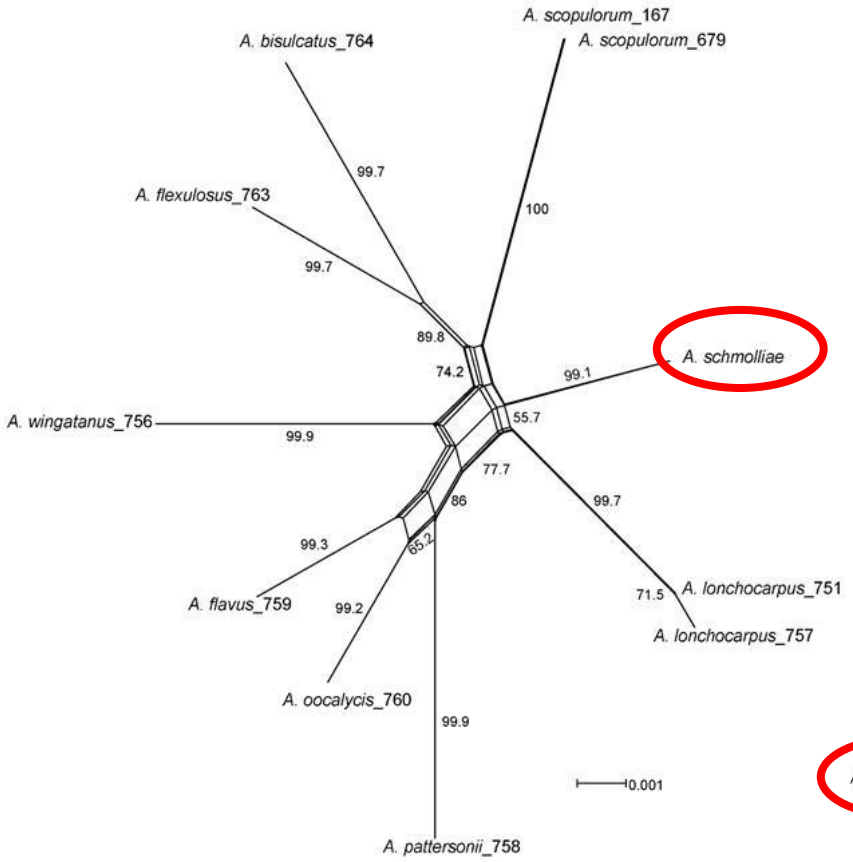
Baysian Structure analysis (not shown) suggested one genetic group

Analysis of Molecular Variance showed majority (88%) of variance among individuals with only 1% of variance occurring among populations.

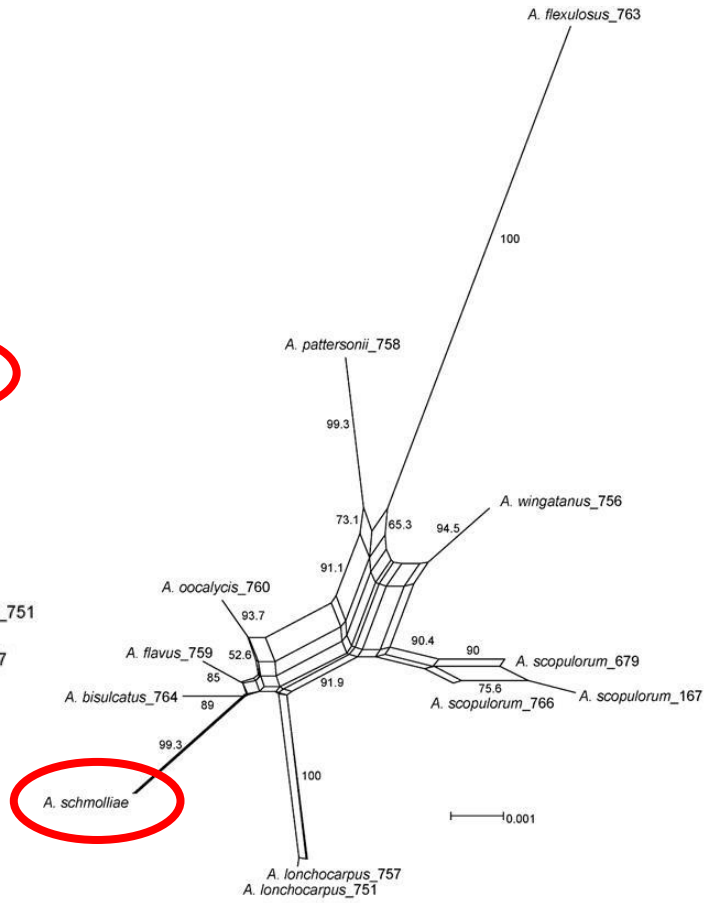
All metrics point to a single meta-population united through gene flow.



Results – Cytonuclear Discordance



Nuclear ITS + ETS



Plastid *rpl32-trnL* + *trnH-psbA* + *trnL-trnF*

Hypothesis of hybrid origin: *A. lonchocarpus x A. scopulorum*

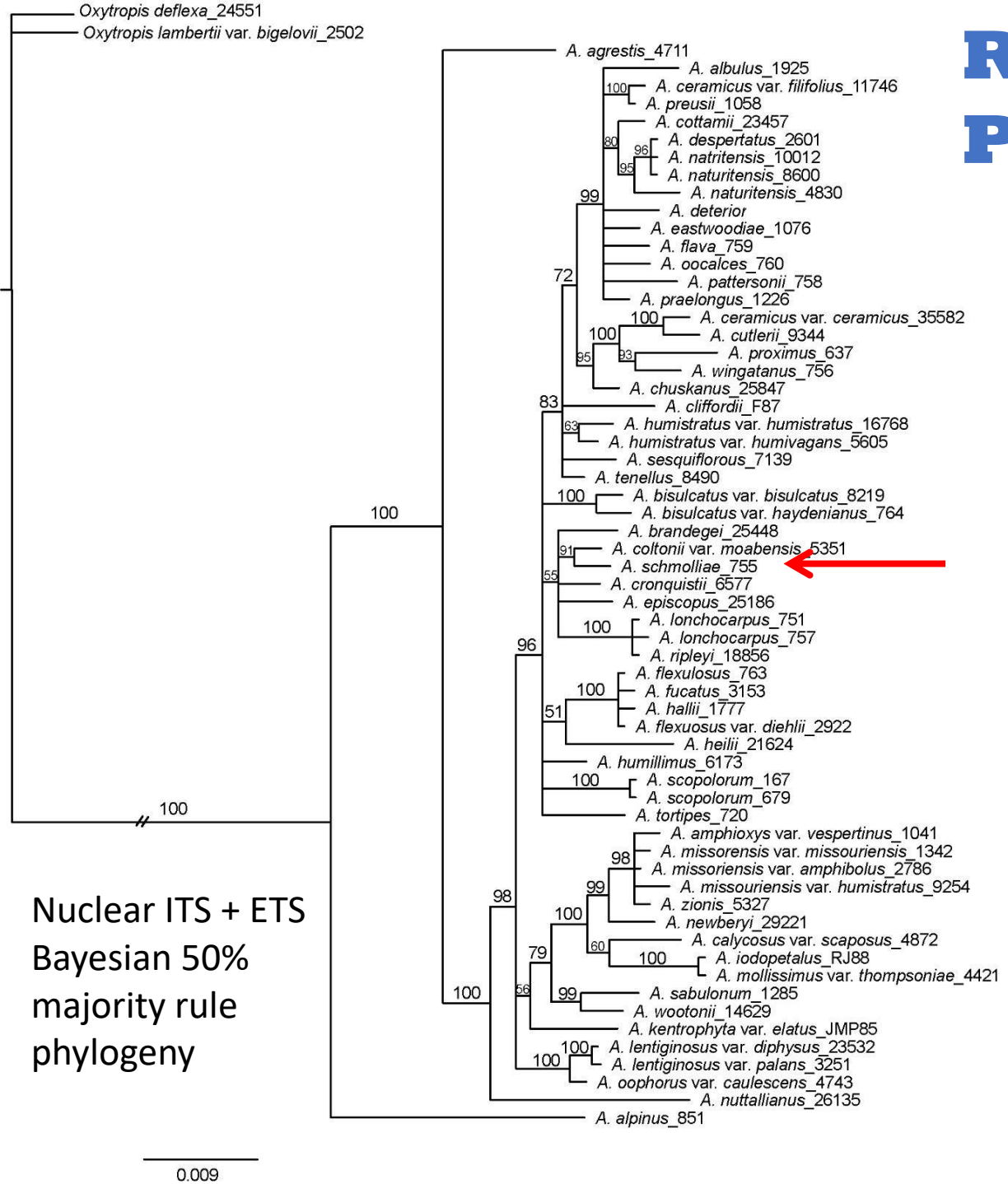
Nuclear network suggests intermediary position between putative parents

Plastid data shows a distant (3 node) relationship to one of the putative parents.

Coupled with observations of frequent co-occurrence of putative parents with no hybrids the hybrid origin is unlikely

Hybridization and introgression has likely played a role in evolution of the group locally.

Results – Regional Phylogeny



Nuclear ITS + ETS Bayesian 50% majority rule phylogeny

Shows close association with *A. coltonii* var. *moabensis*

Photo: A. Schneider

Similar habitat and leaf structure but differing flower color

A. schmolliae – Conservation Implications

1) While demographic trends suggest reduction in population size, genetic diversity remains high.
   a) Long age – up to 50 years – allows for mixed-age structure in populations.
   b) Pattern likely buffers stochastic demographic changes within the natural 300-year fire cycle.

2) Large interconnectedness of meta-population allows managers to treat occurrence as a single large population.

Current work – *A. schmolliae*

May 2021: Sampled three populations near the southern end of Chapin Mesa on the Ute Mountain Ute Reservation

Populations part of an almost continuous distribution in old growth forest across southern end of mesa

Currently extracting DNA with genotyping and analysis to be completed by the end of 2021

Astragalus deterior

Only known to occur on the southern escarpment of Mesa Verde in MVNP – may extend on to Ute Mt. Ute lands

Almost complete specialization to rim rock

Sporadic monitoring suggests reduction in population sizes

Southern escarpment of Mesa Verde, west of Cliff Palace

Typical rim rock habitat
Cliff Palace – Chapin Mesa

Mug House – Wetherhill Mesa

Atypically can establish below cliffs in canyon bottoms

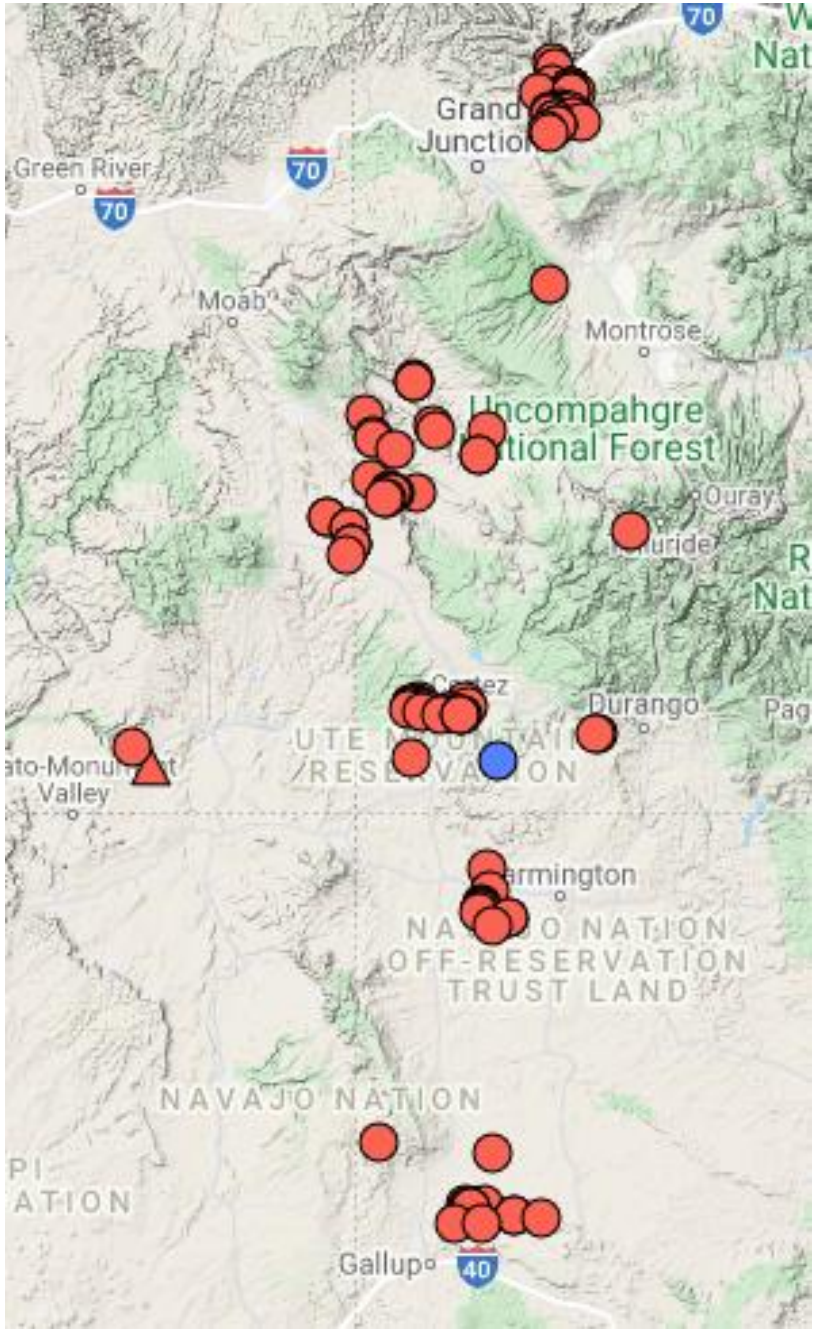
While atypical – canyon bottom occurrence shows very high abundance

Spring House Trail – April 2021 – 100s of individuals in footpath!

A. deterior – Taxonomic Confusion

Potentially conspecific with G2 ranked *A. naturitensis*

A. deterior separated based on rudimentary septum on fruit, more diminutive stature, and occurrence within Mesa Verde N.P.



Map - SEINet

Sandy edge of slickrock – Canyons of the Ancients
Montezuma Co.

Sandy/gravel alluvium – Big Wash
Mesa Co.

Gravely canyon top – Dolores River Canyon
San Miguel Co.

A. deterior – Current Work

Spring 2021

- Sampled four *A. deterior* populations – MVNP
- Sampled four *A. naturitensis* populations – western Colorado

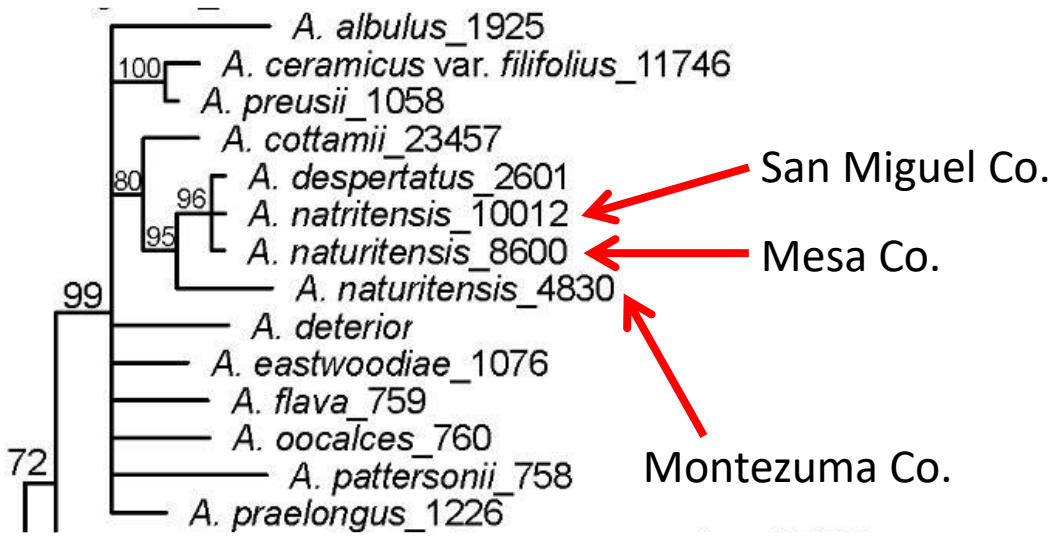
Summer/Fall 2021

- Extracting DNA and microsatellite genotyping
- Improving phylogenetic resolution
  - Adding cyclic nucleotide-gated channel 4 (CNGC4) gene to ITS/ETS phylogeny

Spring/Summer 2022

- Sampling of *A. naturitensis* from Navajo Nation populations in New Mexico

A. deterior – Preliminary findings



Nuclear ITS + ETS
Bayesian 50% majority rule phylogeny

Regional phylogeny suggests that *A. naturitensis* and *A. deterior* are not conspecific

A. naturitensis may be showing regional segregation and may be conspecific with *A. despertatus*

Future plans with Four-Corners *Astragalus*

Collaboration with MVNP

- Initiate study of pollination and reproductive biology of *A. deterior*
- Planned to start in Spring 2021 – try again for Spring 2022

Collaboration with Ute Mountain Ute Tribe

- Field survey of potential habitat for *A. deterior*
- Genetic study of endemic *A. tortipes*

Further phylogenetic analysis

- Couple detailed phylogenetic analysis with distribution and habitat to examine patterns of speciation and endemism

Acknowledgements

Research Team

Ellie Porter – Development of initial phylogeny

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