The demographic and ecological factors shaping diversification among rare Astragalus species

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Abstract

Aim: Evolutionary radiations are central to the origin and maintenance of biodiversity, yet we rarely understand how they are jointly shaped by demography and ecological opportunity. Astragalus is the largest plant genus in the world and is disproportionately comprised of rare species restricted to narrow geographic and ecological regions. Here, we explored the demographic and ecological mechanisms underlying patterns of diversification in a threatened Astragalus species complex endemic to a small desert region in the western United States.

Location: Southeast Utah, USA.

Methods: We used high-throughput DNA sequencing to infer genetic structure, genetic diversity, and demographic history (i.e., the timing of population divergence, effective population sizes and gene flow) among Astragalus taxa. We performed landscape genetic analyses to quantify the relationships between genetic differentiation, geographic distance, and ecological distance based on bioclimatic and soil variables. Finally, we identified putative adaptive loci that show higher genetic differentiation between taxa than expected based on our inferred neutral demographic model.

Results: We found evidence of low gene flow between three highly differentiated taxa (currently delineated as A. iselyi, A. sabulosus var. sabulosus and A. sabulosus var. vehiculus) that rapidly diverged from a small ancestral population near the beginning of the last glacial period. Genomic signatures revealed long-term effective population sizes are 2–10x larger than recent census sizes, perhaps due to the maintenance of standing genetic variation through seed banks. Consistent with limited dispersal and local adaptation, genome-wide patterns of differentiation are shaped by geographic distance (isolation-by-distance) and climate and soil variation (isolation-by-environment). Taxon-specific adaptation is further supported by uncovering putative adaptive loci.

Main Conclusions: Our findings suggest that interactions between demography (i.e., dispersal limitations and seeds banks) and ecological opportunity (i.e., spatial and temporal environmental heterogeneity) may promote diversification, endemism, and rarity among closely related Astragalus species and similar plant clades distributed across complex landscapes.
Evolutionary radiations, or the rapid diversification of clades, are central to the formation of new species and their coexistence across space and time (Gittenberger, 1991; Simões et al., 2016). A central model to emerge after nearly 100 years of research attributes rapid diversification to adaptation across unoccupied ecological niches (Schluter, 2001; Stroud & Losos, 2016) that often occurs in the face of substantial gene flow (i.e., adaptive radiation; Simões et al., 2016). However, a growing number of studies suggest that many radiations are primarily driven by demographic factors, including population fragmentation, bottlenecks and restricted gene flow (Kozak et al., 2006; Pru nier & Holsinger, 2010; Simões et al., 2016) and, in some cases, by intrinsic genomic characteristics (e.g., polyploidization; Van de Peer et al., 2017). In order to fully understand the mechanisms underlying evolutionary radiations, we must jointly examine the influence of demographic history and ecological opportunity on species diversification and persistence (Foote et al., 2016; Gavrilets & Vose, 2005).

Population size and gene flow are fundamental demographic variables influencing diversification, adaptation and persistence during evolutionary radiations (Gavrilets & Vose, 2005; Simões et al., 2016). As the efficacy of natural selection is positively related to long-term population size (Kimura, 1983), historically large populations may be more likely to rapidly adapt to and exploit ecological opportunities. Yet, evolutionary radiations are often associated with population bottlenecks linked to the colonization of a novel environment (Foxe et al., 2009; Särkinen et al., 2007; Wessel et al., 2013). In rare cases, genetic drift associated with founder events may actually trigger ecological diversification (Wessel et al., 2013, although see Barton & Charlesworth, 1984). However, if bottlenecks are severe, populations may lose substantial standing genetic variation (James, 1971), potentially limiting their ability to rapidly adapt to and persist in novel environments (Frankham et al., 1999). Gene flow between populations or species in early stages of colonization may counteract the effects of founder events by increasing adaptive genetic diversity (Dlugosch & Parker, 2008). Recent genomic studies of classic radiations (e.g., Darwin’s finches [Lamichhaney et al., 2015] and East African cichlids [Meier et al., 2017]) suggest gene flow played a crucial role in seeding genetic variation that ultimately facilitated adaptive divergence. On the other hand, local adaptation is likely to be swamped by the influx of maladapted alleles if rates of gene flow exceed a certain threshold (Wright, 1932), which may prevent the establishment of stable populations. Understanding the dynamics of genetic drift and gene flow during diversification therefore remains a fundamental research goal (Lawton-Rauh et al., 2007).

Spatial and temporal environmental heterogeneity facilitate the generation and maintenance of biodiversity by increasing opportunities for populations to experience aberrant climatic conditions (Rainey & Travisano, 1998; Williams & Jackson, 2007) or geographic isolation (Hewitt, 2000; Knowles & Massatti, 2017). The intermountain region of western North America (hereafter referred to as the Intermountain West) is a spatially complex landscape with a history of dramatic climate changes (e.g., repeated glacialiations altering regional climatic gradients throughout the Pleistocene) and therefore is an important system for investigating how demographic and ecological conditions interact to shape evolutionary radiations (Egan & Crandall, 2008; Tidwell et al., 1972). The Intermountain West hosts an abundance of endemic plant clades (e.g., Weber, 2003) that span sharp climate and soil gradients (Tidwell et al., 1972). Diversification of many of these plant clades has been linked to temporal environmental heterogeneity via glacial cycles (e.g., Egan & Crandall, 2008; Levens et al., 2012). Moreover, ongoing environmental changes (e.g., drought, grazing, wildfires and the spread of invasive species) continue to threaten native species and communities (Litt & Pearson, 2011; Winkler et al., 2019; Zedler et al., 1983). Uncovering the precise mechanisms that shape biodiversity in this region therefore likely has broader implications for evolution and conservation during periods of extreme environmental change (Thorpe et al., 2015).

To test how demographic and ecological processes affect evolutionary diversification, we combined population and landscape genomic analyses to study a rare group of Astragalus species narrowly distributed across a dryland ecosystem in the Intermountain West (see Methods for study system details). Astragalus (Linnaeus 1753; family Fabaceae) is the most species-rich plant genus in the world, comprising ~3,000 species mostly distributed across temperate regions of the Northern hemisphere, including at least 160 species restricted to the Intermountain West (Barney, 1989). Interestingly, a disproportionate number of Astragalus species are rare and endemic to narrow geographic or ecological regions, with approximately one third of extant species considered either vulnerable, endangered or critically endangered (IUCN, 2020; Rundel et al., 2015). Despite widespread interest in Astragalus evolution and conservation (Sanderson & Wojciechowski, 1996; Scherson et al., 2008; Stebbins, 1981; Wojciechowski et al., 1999), the lack of detailed genomic studies of closely related species has hindered our understanding of the mechanisms underlying patterns of diversification, endemism and rarity in this group (Rundel et al., 2015; although see Záveská et al., 2019).

The broader Astragalean clade does not appear to possess defining ecological traits (i.e., key innovations), and thus, Sanderson and Wojciechowski (1996) hypothesized that diversification within this group may be better explained by demographic factors, such as limited gene flow and population fragmentation, as opposed to ecological opportunity. However, the apparent prevalence of large genetic divergences and limited gene flow is not consistent with an ecological diversification model. To test how demographic and ecological processes affect evolutionary diversification, we combined population and landscape genomic analyses to study a rare group of Astragalus species narrowly distributed across a dryland ecosystem in the Intermountain West (see Methods for study system details). Astragalus (Linnaeus 1753; family Fabaceae) is the most species-rich plant genus in the world, comprising ~3,000 species mostly distributed across temperate regions of the Northern hemisphere, including at least 160 species restricted to the Intermountain West (Barney, 1989). Interestingly, a disproportionate number of Astragalus species are rare and endemic to narrow geographic or ecological regions, with approximately one third of extant species considered either vulnerable, endangered or critically endangered (IUCN, 2020; Rundel et al., 2015). Despite widespread interest in Astragalus evolution and conservation (Sanderson & Wojciechowski, 1996; Scherson et al., 2008; Stebbins, 1981; Wojciechowski et al., 1999), the lack of detailed genomic studies of closely related species has hindered our understanding of the mechanisms underlying patterns of diversification, endemism and rarity in this group (Rundel et al., 2015; although see Záveská et al., 2019).
of ecological specialization (particularly edaphic specialization) among Astragalus suggests that local adaptation may play a role in promoting the divergence and persistence of lineages (Rundel et al., 2015), although genetic evidence for local adaptation within Astragalus is lacking. In either case, environmental change may be predicted to play an important role in establishing the demographic or ecological conditions for divergence between Astragalus taxa. Here, we test predictions that rare and endemic Astragalus species (a) diversified during periods of major environmental change, (b) diversified largely in the absence of gene flow (according to Sanderson & Wojciechowski, 1996) and (c) are locally adapted across their narrow distributions. To test these predictions, we performed double-digest restriction-site associated DNA (ddRAD) sequencing and inferred patterns of population genetic structure, genetic diversity and inbreeding. Using these foundational data, we reconstruct the history of population size changes, gene flow and the timing of diversification (testing Predictions a and b) and examine genome-wide signatures of local ecological adaptation (testing Prediction c). Our analyses demonstrate a complex demographic and ecological history underlying Astragalus diversification, which we discuss in the context of the evolution and conservation of rare and endemic plants inhabiting heterogeneous landscapes.

2 | MATERIALS AND METHODS

2.1 | Study system

We studied two previously described Astragalus species in southeast Utah (Grand County and San Juan County): A. iselyi S. L. Welsh (Iselyi’s milkvetch; hereafter referred to as iselyi) and A. sabulosus M. E. Jones, which is comprised of A. sabulosus var. sabulosus (Cisco milkvetch; hereafter sabulosus) and A. sabulosus var. vehiculus S. L. Welsh (stage milkvetch; hereafter vehiculus). These taxa were originally described primarily on the basis of relatively minor morphological differences in flower size, flower colour and pod size (Welsh, 1974, 1998). For instance, pods are generally smaller in iselyi (25–32 mm long; Welsh, 1974) compared to vehiculus and sabulosus (20–48 mm long; Welsh, 1998). Astragalus species are reportedly all monoeious and some species show a high degree of self-fragmentation (Karron, 1989), although the degree of self-fragmentation is unknown among iselyi, sabulosus and vehiculus. Seed dispersal and pollination vectors in these taxa are also unknown, though research from other Astragalus species suggests seed dispersal is primarily gravity- or water-mediated (Morris et al., 2002; Vicente et al., 2011) and that various insects (particularly bees) serve as pollinators (Green & Bohart, 1975; Karron, 1987; Sugden, 1985). Each taxon is restricted to particular geological formations and altitudinal zones: iselyi occurs on seleniferous and uranium-rich soils of the Morrison, Paradox and Mancos formations between elevations of 1525–2010 m (Goodrich et al., 1999), sabulosus is restricted to the Mancos and Morrison formations between 1,300 and 1,600 m, and vehiculus is restricted to the Morrison formation between 1,370 and 1,465 m (Welsh, 1998).

In addition to their small ranges (Figure 1a), recent censuses revealed small population sizes ($N_c$; iselyi $N_c = 3,192$, sabulosus $N_c = 4,692$, vehiculus $N_c = 1,831$; Rita Reisor pers. comm.). Moreover, in addition to climate change, all taxa appear vulnerable to mining, oil and gas development, grazing, and off-highway vehicle use (Franklin, 2003) and are being petitioned for listing under the United States Endangered Species Act. We know of no published genetic or ecological studies in this group. Cytological studies suggest that polyploidy is extremely rare in New World Astragalus (Wojciechowski et al., 1993); thus, we assume these species are diploid.

![Figure 1](image-url)
2.2 Sample collection, DNA isolation and library preparation

In spring 2019, we systematically sampled iselyi (n = 42), sabulosus (n = 52) and vehiculus (n = 27) individuals at 13 locations (1,361 to 1,804 m) representative of the total ranges of these taxa (Figure 1A). At sites with fewer than 15 individuals, we sampled leaves from every individual (separated by at least 5 m). When more than 15 individuals were present at a site, we sampled a representative subset of as many as 18 individuals spaced up to 100 m apart. Leaf tissues were stored in silica desiccant prior to DNA extraction.

DNA was extracted using DNeasy 96 Plant Kits (Qiagen) following the manufacturer’s protocol. Genomic DNAs were individually barcoded and processed into ddRAD libraries (Peterson et al., 2012). Briefly, DNA was digested with EcoRI andMspI restriction enzymes, followed by the ligation of Illumina adaptor sequences and barcodes. Ligation products were pooled and amplified using 18 polymerase chain reaction cycles. A Pippin Prep (Sage Science) was used to select amplicons between 400 and 600 base pairs (bp). The resulting library was sequenced on a HiSeq 4000 (Illumina) at the University of Oregon’s Genomics and Cell Characterization Core Facility to generate single-end 100 bp reads.

2.3 Genomic data preprocessing

We processed and aligned raw sequence data with Stacks v2.41 (Rochette et al., 2019). Reads were removed if they contained un-called bases or if the average phred-scaled quality score fell below 22 within a sliding window of 15 bp. The last 5 bp were then removed from high-quality reads. We followed the procedure outlined by Rochette and Catchen (2017) to determine appropriate parameter settings in Stacks for de novo assembly. Briefly, we processed a test data set of 12 high-coverage samples (five sabulosus, two vehiculus, five iselyi) while varying the parameters M (the maximum nucleotide distance allowed between stacks) and n (the number of mismatches allowed between loci during catalog construction) from 1 to 9 and while keeping M = n and setting m = 3 (minimum depth of coverage to create a stack). Based on these tests, we chose to process the full data set with M = 3 and n = 3 because the total number of loci retained in 80% of individuals (r80) and the distribution of the number of single nucleotide polymorphisms (SNPs) per locus stabilized at these values (see Figure S1). However, we also tested several other filtering parameters to determine the robustness of our data set to filtering criteria (see Table S1). Using vcftools v1.14 ( Danecek et al., 2011), we filtered out variant sites represented by less than 50% of individuals and with a Hardy–Weinberg departure p-value < .0001. Singletonst were also excluded because they were more common in individuals used to generate the RAD loci catalog (cstacks program in Stacks), which is expected (Rochette & Catchen, 2017). Finally, we generated a separate “unlinked” data set, consisting of only one randomly selected SNP per locus, to minimize the number of non-independent SNPs.

2.4 Estimating population structure and diversity

Using our unlinked data set, we inferred genetic structure patterns with a principal components analysis (PCA) in EIGENSOFT v6.1.4 (Patterson et al., 2006) and a population cluster analysis in fastStructure v1.0 (Raj et al., 2014). For fastStructure, we tested K values (representing the number of population clusters) from 1 to 8 and selected the model with the highest log-marginal likelihood lower bound. Based on results from these analyses, we used vcftools to calculate pairwise genetic differentiation (FST) between genetic clusters both genome-wide and per-locus. For each genetic cluster, we calculated mean inbreeding coefficients (FIS) and nucleotide diversity (π) using the populations program in Stacks. To determine whether any genetic cluster represents an admixed or hybrid population, we performed FST tests in treemix v1.13 (Pickrell & Pritchard, 2012) and used a block-jackknife approach with sets of 100 random variants to estimate standard errors.

2.5 Inferring demographic history

Using the full data set, we next examined the history of divergence among taxa by inferring a maximum-likelihood phylogenetic tree with a general time reversible (+GAMMA) model in RAxML v8.2.8 (Stamatakis, 2014). We ran 1,000 replicates to estimate bootstrap support values for each node. With the unlinked data set, we then used the program þαδ (Gutenkunst et al., 2009) to estimate the timing of divergence, effective population sizes and levels of gene flow based on patterns of the folded site frequency spectrum (SFS). We used a folded SFS (i.e., major/minor allele calls rather than derived/ancestral) because we lacked a closely related reference genome. To determine the evolutionary relationships among taxa, we tested models of three possible bifurcating tree topologies. For each different topology, we tested a model with no migration and a model with one symmetrical migration rate (m, migrants per generation) between all taxa. Although migration rates may differ between taxa, we only tested a single migration rate in order to avoid over-parameterizing our model. Under each model, we estimated divergence times (t, in generations) between taxa, the ancestral population size (Nanc) and the population sizes for each taxon (N) estimated as the relative change in population size (ν) compared to Nanc (e.g., if population size increased 10x compared to Nanc, then ν = 10). Here, population size changes were modelled as instantaneous changes following divergence.

For each model, we performed 20 independent runs starting with parameter values sampled randomly across a uniform distribution (0.01 < ν < 100, 0 < 2Nanc · t < 20, 0 < 2Nanc · m < 5) and determined parameter values with the highest log-likelihood. We then examined which of the maximum-likelihood models produced the best overall fit to the data using a composite-likelihood ratio test with the Godambe Information Matrix (Coffman et al., 2016). Confidence intervals (CI) for each parameter were estimated with the Godambe Information Matrix with 100 bootstrap data sets.
comprising 50% of SNPs randomly selected from the full data set. As parameters \( t \) and \( m \) are reported in coalescent units, we scaled these parameters by \( \theta \), assuming the *Arabidopsis* genome-wide SNP mutation rate \((\mu = 7e-9; \text{Ossowski et al., 2010})\) and a 132:1 ratio of callable sites:SNPs (based on output from Stacks). We validated the final model by comparing the predicted SFS to the observed SFS for each population. We also simulated 10,000 SNPs under the final model with msms (Ewing & Hermisson, 2010) and generated expected per-SNP \( F_{ST} \) distributions with msstats (Thornton, 2003) to compare against the observed \( F_{ST} \) distributions calculated with vcftools.

### 2.6 Testing geographic and ecological correlates of genetic differentiation

To determine the relative roles of geography and ecology in shaping genome-wide patterns of differentiation within and among *Astragalus* taxa, we used BEDASSLE v1.5 (Bradburd et al., 2013) implemented in R (R Core Team, 2021). BEDASSLE is a Bayesian statistical approach that models the covariance in allele frequencies between populations as a function of geographic and ecological distance. Parameter values are estimated using a Markov Chain Monte Carlo (MCMC) algorithm. The crucial parameter is \( \alpha/E/\alpha D \), which measures the effect of ecological distance \( (\alpha E) \) on genetic differentiation relative to geographic distance \( (\alpha D) \). We created a genetic distance matrix based on sampling localities using pairwise Weir-Cockerham \( F_{ST} \) values estimated with the unlinked SNP data set in vcftools. We generated a geographic distance matrix between sampling localities using a publicly available script written by Peter Rosenmai (https://eurekastatistics.com/calculatin g-a-distance-matrix-for-geographic-points-using-r/). To generate ecological distance matrices, we first downloaded 11 physical and chemical soil variables at 250 m resolution from soilsgrid.org and 19 climatic variables from Worldclim.org (Hijmans et al., 2005; see Table S2 for variable information). We then tested for multicollinearity between all soil and climatic variables using a custom R script and removed highly colinear variables \((p < .01)\). The final set included nine uncorrelated soil and four uncorrelated climate variables (Figure S2). To further reduce the dimensionality of the data set, we performed a PCA in R \((prcomp \text{ in} “\text{stats}” \text{package}; \text{R Core Team, 2021})\) on the ecological variables scaled to have unit variance. We then generated ecological distance matrices in R based on the climate and soil variables.

Prior to running the final analyses with BEDASSLE, we performed short runs of 10,000 generations and tuned MCMC parameter values until acceptance rates were ~20%–50%. For our final analyses, we ran BEDASSLE with a beta-binomial model for two million generations, sampling every 1,000 generations. We performed three separate runs to ensure convergence on a single posterior distribution. We verified that parameter and log-likelihood values stabilized over the course of each run to evaluate model performance. Based on these evaluations, we discarded the first 1.5 million generations as burn-in and used the remaining samples to generate posterior distributions of parameter values.

### 2.7 Uncovering putative locally adaptive loci

To identify signatures of selection on SNPs within each population while accounting for the unique history of the clade, we identified \( F_{ST} \) and population branch statistic (PBS) outliers using a demographically informed null model. While SNPs with a high \( F_{ST} \) value between two populations may indicate a history of positive selection in either population, PBS polarizes the direction of allele frequency change (and thus the direction of selection) by comparing all pairwise \( F_{ST} \) values among three populations (Yi et al., 2010). Here, we used msms (Ewing & Hermisson, 2010) to simulate 10,000 SNPs under the inferred population history. Output from msms was analysed using the msstats software (Thornton, 2003) to generate pairwise \( F_{ST} \) estimates for each simulated SNP. We then calculated expected PBS distributions for each population as PBS_Pop1 = \( (−\log(1 − F_{ST}(\text{Pop1, Pop2})) + (−\log(1 − F_{ST}(\text{Pop1, Pop3}))) / 2 \) according to Yi et al. (2010). Finally, we identified SNPs with \( F_{ST} \) and PBS values that exceeded the 99% quantile predicted under the demographic model. Together, these methods provide a conservative estimation of putative adaptive loci that is not influenced by the demographic history among the focal taxa.

### 3 RESULTS

#### 3.1 Genetic structure and diversity

We obtained a total of 10,241 high-quality SNPs distributed across 5,269 loci. Our unlinked data set (one SNP per locus) therefore consisted of 5,269 SNPs. Across our final data set, we obtained a mean coverage of 23.1 ± 7.0 standard deviation (SD) per individual with an average missing data of 24.9 ± 9.0 SD per individual (see Table S3 for additional information). Data generated from this study are available at the USGS ScienceBase-Catalog (Jones et al., 2021).

We uncovered three distinct genetic clusters corresponding to previously described taxonomic groups *A. iselyi*, *A. sabulosus var. sabulosus* and *A. sabulosus var. vehiculius* with a PCA on unlinked SNPs (Figure 1b). We found strong evidence for the same genetic groups and no evidence of gene flow or continuous structure between them with fastStructure \((K = 3 \text{ best model}; \text{Figure 1c}; \text{see Figure S3 for additional K values})\). Genetic differentiation represented by mean Weir and Cockerham \( F_{ST} \) was relatively high between taxa (Figure 1b; *iselyi-vehiculius* \( F_{ST} = 0.189, \text{iselyi-sabulosus} F_{ST} = 0.190 \) and *vehiculius-sabulosus* \( F_{ST} = 0.187 \)) and \( F_{ST} \) statistics for each taxon were positive and small (Table 1), indicating that they show no signatures of being derived through admixture. However, between 23.4% and 49.1% of SNPs within taxa were shared with at least one other taxon (Table 2),
indicating shared ancestral polymorphism or gene flow. We also found relatively high genetic diversity (\(\pi \approx 0.002–0.003\)) and low inbreeding coefficients (mean \(F_{IS} \approx 0\)) within each taxon (Table 1).

3.2 | Evolutionary history of divergence, population size and gene flow

Phylogenetic analyses revealed iselyi, sabulosus and vehiculus individuals each formed their own monophyletic clades with 100% bootstrap support values (Figure 2a). However, demographic modelling tests of alternative evolutionary relationships among taxa were ambiguous. Maximum-likelihood values were not significantly different between models with either iselyi (log-likelihood = −2648), sabulosus (log-likelihood = −2651), or vehiculus (log-likelihood = −2647) as the earliest diverging group. Moreover, parameter estimates were essentially identical between alternative models (Table S4).

Therefore, for the remainder of the paper, we present only results from the (iselyi, (sabulosus, vehiculus)) topology model. Although we cannot be certain of the precise tree topology for these taxa, a topology with sabulosus and vehiculus as reciprocally monophyletic is consistent with current taxonomy and the number of private and shared SNPs between taxa (see Table 2).

Under our best split scenario, we found support for a model with a low migration rate (\(m = 0.006\) migrants/generation; 95% CI: 0.005–0.008) over a model with no migration (log-likelihood = −3049; \(p = 0\), see Figures S4 and S5 for model fit). Divergence between the three taxa occurred almost simultaneously, with iselyi diverging 65,500 generations ago (95% CI: 49,280–83,696), followed by a split between sabulosus and vehiculus approximately 98 generation afterwards (95% CI: 0–5948 generations; Figure 2b). Given that the alternative topology models indicate similar split times, we surmise that taxa diverged from one another almost simultaneously. Finally, we estimate an ancestral population size of 1644 (95% CI: 1505–1784; Figure 2b), and that population size increased approximately 10.7× in vehiculus (\(N = 17,567\), 95% CI: 13,294–22,356), 6.7× in sabulosus (\(N = 11,069\), 95% CI: 7117–15,578), and 19.9× in iselyi (\(N = 32,856\), 95% CI: 28,120–37,954; Figure 2b) after divergence.

3.3 | Landscape genetics of population differentiation

We next explored differences in the environments occupied by Astragalus taxa. A PCA of climatic and soil variation showed that the first two principal components explained 33.2% and 21.7% of...
environmental variation between sampling localities (Figure 3a, see Figure S6 for separate climate and soil PCAs). PC1 separated iselyi from sabulosus and vehiculus, while PC2 largely separated vehiculus from iselyi and sabulosus. Temperature seasonality (bio4, the SD of mean monthly temperature) was most strongly correlated with PC1 (loading = 0.35, Figure 3a), indicating that iselyi occupies colder, wetter, and less seasonal climates compared to both sabulosus and vehiculus. However, many climatic and soil variables also loaded strongly on this axis. Silt content (SLTPPT) and sand content (SNDPPT) were largely correlated with PC2 (loading = 0.53 and −0.49, respectively; Figure 3a), indicating vehiculus grows in less silty and more sandy soils compared to iselyi and sabulosus. We focused subsequent analyses on temperature seasonality (bio4) and silt content (SLTPPT) to represent major axes of environmental variation between taxa.

We found a strong correlation between genetic differentiation and geographic distance (adjusted $R^2 = .71; p < 2.2e−16$; Figure 3b), temperature seasonality distance (adjusted $R^2 = .57; p < 2.2e−16$; Figure 3c) and silt content distance between sites (adjusted $R^2 = .15; p = 1.2e−7$; Figure 3d). However, geographic distance was strongly correlated with temperature seasonality (adjusted $R^2 = .70; p < 2.2e−16$).
and silt content (adjusted $R^2 = .05$; $p = .002$). Accounting for this correlation, the mean of the posterior distribution of the $\alpha E/aD$ parameter for temperature seasonality was 69.3 (95% CI: 67.6–72.9), 69.7 (95% CI: 66.1–71.5) and 84.4 (95% CI: 78.7–92.8) across three separate BEDASSLE runs (Figures S7a and S8). These results suggest that a ~0.66–0.93°C difference in temperature seasonality between sites has an equivalent effect on genetic differentiation as 1 km of geographic distance. Given that we observe ~6.5°C difference in temperature seasonality between iselyi localities and both vehiculus and sabulosus localities, these results suggest that the total effect of climate on genetic differentiation is equivalent to ~7–10 km of additional distance between iselyi and vehiculus localities (~17%–24% increase) and iselyi and sabulosus localities (~10%–14% increase). For silt content, the mean of the $\alpha E/aD$ posterior distribution was 0.3233 (95% CI: 0.3233–0.3238), 0.3381 (95% CI: 0.3373–0.3386) and 0.3976 (95% CI: 0.3973–0.3978; Figures S7b and S9), indicating that an approximately 0.3%–0.4% difference in soil silt content between sites has the same effect on genetic differentiation as 1 km. Therefore, the total effect on genetic differentiation is equivalent to an additional ~22–28 km of distance between vehiculus and sabulosus sites (9% difference in silt content) and ~17–21 km of geographic distance between vehiculus and iselyi sites (6.7% difference in silt content).

### 3.4 | Putative adaptive loci

We next used our “linked” data set to search for loci and SNPs with significant levels of differentiation between populations relative to our null demographic model. Using $F_{ST}$, we detected seven outlier loci (7 SNPs) between sabulosus and vehiculus, 19 outlier loci (28 SNPs) between sabulosus and iselyi, and 43 outlier loci (59 SNPs) between vehiculus and iselyi. With PBS, we found 29 loci (36 SNPs) in iselyi, 1 locus (one SNP) in sabulosus and seven loci (eight SNPs) in vehiculus (Figure 4). We identified putative locally adaptive loci for each population by restricting outlier loci to those identified using both $F_{ST}$ and PBS approaches. Based on these criteria, we identified a single putative adaptive locus in sabulosus, five putative adaptive loci in vehiculus and eight putative adaptive loci in iselyi. We ignored one locus that was identified in both vehiculus and iselyi, leaving a total of 13 loci that show unique signatures of local adaptation across taxa. These analyses provide evidence that adaptive differentiation may be important in the diversification or persistence of lineages; however, determining the specific genomic locations and functions of these candidate loci is beyond the scope of this study, in large part due to the lack of a closely related and annotated reference genome.

**FIGURE 4** Simulated and empirical distributions of the population branch statistic (PBS) for vehiculus (a), sabulosus (b), and iselyi (c). Black lines show the simulated PBS distribution under the maximum-likelihood demographic model while gold lines show the empirical distribution of values. Dashed vertical lines show the 99% quantiles for each distribution and outlier SNPs (>99% simulated quantile) are depicted as blue circles.
4 | DISCUSSION

To understand how demography and ecological opportunity influence the origin and maintenance of biodiversity, we studied the evolutionary history of an Astragalus species complex distributed across heterogeneous environments in the Intermountain West. Astragalus is a hyper-diverse genus that is prominent in montane and arid environments such as those that dominate the western United States. Across this complex region and similar areas worldwide, clades of closely related species with restricted distributions are abundant. As such, understanding how diversification has proceeded in one clade of Astragalus taxa may support a more general understanding of diversification in many other diverse plant clades with high rates of endemism in the western United States (e.g., Townsendia, Epilobium, Saxifraga, Carex, Mertensia, Poa, Oxytropis, Ericameria, Artemisia, Hilaria and Sporobolus). Our study suggests that the interactions between demography and spatial and temporal environmental variation likely contribute to patterns of diversification, rarity, and endemism of Astragalus and potentially other plant species inhabiting dynamic environments such as the Intermountain West.

4.1 | The demographic history of Astragalus diversification

Diversification of angiosperms is hypothesized to be strongly shaped by population fragmentation and limited gene flow between local populations (Niklas et al., 1986), factors that may be strongly influenced by large-scale environmental change. We therefore tested whether the timing of divergence among Astragalus taxa coincides with known periods of environmental change, which would provide evidence for a role of temporal environmental heterogeneity in shaping diversification.

Our study indicates diversification of Astragalus taxa occurred ~130 thousand years ago (kya; assuming one generation every 2 years, as is common in many graminoids and forbs occupying extreme environments that do not reproduce in the year that seeds germinate and establish, e.g., Körner, 2003), roughly corresponding to the beginning of the Last Glacial Period (~110 kya) during the Pleistocene. The Pleistocene was a biogeographically dynamic epoch defined by climate change and cyclical glaciations, which facilitated large-scale shifts of species’ ranges, population sizes, and gene flow or hybridization between different populations or species (Galbreath & Cook, 2004; Graham, 1986; Hofreiter & Stewart, 2009; Swenson & Howard, 2005). Changes in both demographic and environmental conditions during this period provided opportunities for adaptive and non-adaptive diversification across diverse clades (Egan & Crandall, 2008; Galbreath et al., 2009; Graham, 1986; Knowles, 2001; Rickart, 2001; Spellman & Klicka, 2007). Thus, in agreement with our prediction, we find evidence that large-scale climate change may have played an important role in generating the demographic or ecological conditions for diversification of Astragalus taxa.

Plant diversification during environmental change can be triggered by numerous biogeographical processes including range fragmentation (i.e., vicariance; Kropf et al., 2006), range shifts (Knowles & Massatti, 2017) and rare long-distance dispersal (Särkkinen et al., 2007), leading to the geographic and genetic isolation of populations. To test whether such processes may have played a role in the diversification of Astragalus taxa (Prediction b, in agreement with Sanderson & Wojciechowski, 1996), we used demographic modelling to examine historic population sizes and rates of gene flow between iselyi, sabulosus and vehiculus. We found that divergence between the lineages leading to the present taxa occurred nearly simultaneously from a common ancestor with a small population size (N\text{anc} = 1,644; Figure 2b). We suggest these patterns may be best explained by founder events caused by fragmentation of the ancestral range into three parts, rather than other processes like independent long-distance dispersal events (e.g., see Schneeeweiß & Schönswetter, 2010). Consistent with this hypothesis, we find evidence of extremely low gene flow between Astragalus taxa (approximately 1 migrant every 167 generations; Figure 2b) and limited dispersal across the landscape (i.e., isolation-by-distance, Figure 3b). The substantial increases in population size following divergence may be the result of the opening of new habitat or local adaptation following environmental change (see below for more discussion of local adaptation). Although additional genomic research of this genus is needed, our findings are consistent with the hypothesis that vicariance events and genetic isolation of local demes contributes to rapid diversification of Astragalus following climate change (Gavrilets & Vose, 2005; Simões et al., 2016), similar to diversification patterns in other rare and narrowly distributed plant species (e.g., Backes et al., 2019; Godt et al., 1996; Kropf et al., 2006).

Under a vicariance model, we would generally expect strong genetic drift within small, isolated populations to decrease intrapopulation genetic diversity. Yet, in contrast to this prediction, we uncovered relatively high nucleotide diversity (Table 1: comparable to wild and cultivated soybeans; Lam et al., 2010), high shared ancestral polymorphism (Table 2), and large long-term effective population sizes (~7-20× larger than N\text{anc}; Figure 2b). In fact, studies have revealed relatively high levels of genetic diversity across many rare Astragalus taxa (Alexander et al., 2004; Baskauf & Burke, 2009; Harrison et al., 2019; Karron et al., 1988; Neel, 2008; Walker & Metcalf, 2008) and some rare plant populations more generally (Barrett & Kohn, 1991; Elstand & ELam, 1993). We suggest that aspects of Astragalus seed biology may lend important insights into these somewhat counterintuitive results.

Astragalus seeds are large, nutrient dense, and tend to exhibit high rates of predation and low rates of germination (Combs et al., 2013; Green & Palmhald, 1975; Kaye, 1999). Collectively, these characteristics are thought to lead to inefficient dispersal, low population growth rates and small population sizes (Combs et al., 2013; Kaye, 1999). This is consistent with the low census estimates of these populations (iselyi N\text{c} = 3,192, sabulosus N\text{c} = 4,649, vehiculus N\text{c} = 1,831) and low rates of gene flow across the landscape. Astragalus is also known to have genetically diverse
and strongly age-structured seed banks (Molnár et al., 2015; Morris et al., 2002; Van Buren & Harper, 2003), which may maintain genetic variation through time despite small census population sizes and limited gene flow. Seed banks are hypothesized to contribute to the maintenance of variation in rare plant species more generally (Ellstrand & Elam, 1993); however, this hypothesis remains largely untested. Alternatively or in addition, the high $N_e/N$ ratio among iselyi, sabulosus and vehiculus could be explained by recent population declines linked to habitat destruction (e.g., oil and gas development, livestock grazing, camping, and off-highway vehicle use; Ouren et al., 2007). Recent changes in population sizes are incredibly difficult to detect with the SFS (Beichman et al., 2018; Robinson et al., 2014), and our data set likely lacks the power to test this hypothesis. However, we found no signatures of inbreeding (Table 1), which may be expected with recent severe population declines (e.g., Jones et al., 2020). Although somewhat speculative, we suggest that limited seed dispersal and the maintenance of healthy seed banks are parsimonious explanations for widely observed patterns of rarity and high genetic diversity among Astragalus species.

4.2 | The ecological drivers of diversification

Given the apparent absence of adaptive innovations defining Astragalus (and the more-inclusive Astragalean clade), diversification of this genus has been largely attributed to demographic factors (Sanderson & Wojciechowski, 1996; Stebbins, 1981). Yet, many Astragalus species are restricted to narrow edaphic or climatic regions, suggesting they may be locally adapted ecological specialists (Baskin & Baskin, 1974, 2005; Rundel et al., 2015). In fact, rare plant species occupying spatially heterogeneous regions are often habitat specialists with narrow ranges (Cowling et al., 1996), suggesting the persistence of rare species may be facilitated by local adaptation. Patterns of limited gene flow and high genetic diversity that are relatively common in Astragalus and other rare species likely facilitate ecological adaptation (alternatively, ecological adaptation may contribute to limited gene flow); however, empirical tests of local adaptation are challenging and rare. Here, we leveraged landscape and population genomic approaches to test for signatures of local adaptation within Astragalus taxa.

Environmental variables are often the primary ecological factors shaping adaptive variation among a wide variety of plant species (Fischer et al., 2013; Manel et al., 2012), and Pleistocene glaciations are known to cause populations to experience novel environmental conditions (Jackson & Overpeck, 2000; Massatti & Knowles, 2020; Williams & Jackson, 2007). Soil and climate data show that iselyi, sabulosus and vehiculus occupy distinct ecological niches relative to one another (Figure 3a). We found evidence that iselyi (which occurs at higher elevations) occupies colder, wetter and less seasonal environments compared to sabulosus and vehiculus. Moreover, iselyi habitat appears to be differentiated from the other taxa based on certain soil characteristics, including higher soil organic carbon stock (OCSTHA; Figure 3a). Habitat for vehiculus appears to be comprised of soils with relatively high sand content and low silt content.

We explored whether these environmental features are associated with patterns of genome-wide differentiation, which would be indicative of local adaptation. As predicted, we found that genome-wide differentiation across the landscape appears to be strongly shaped by both climatic and soil variation (i.e., isolation-by-environment; Wang & Bradburd, 2014; Figures 3c,d and S7). We should note that, although genome-wide differentiation is associated with contemporary environmental data, it remains possible that differentiation could be more strongly associated with historic environments. Given our ignorance about the historic distributions of these taxa, this is difficult to test and we therefore assume that the associations we uncovered reflect recent or contemporary adaptation. In support of local adaptation, we also identified several highly differentiated, putative adaptive loci in the focal taxa, even after controlling for the demographic and diversification history of the clade (Figure 4). Additional research is needed to validate these selective signatures and uncover the possible functional genetic elements and phenotypes associated with these genetic changes; however, given the nature of the reduced representation data set used herein, there are likely many more putative adaptive loci to be uncovered using targeted approaches (Jones & Good, 2016). The evidence of local adaptation we uncovered does not necessarily support a role of ecological opportunity in initiating divergence between Astragalus, as adaptation may have occurred well after taxa became isolated from one another. Regardless, spatial climatic and soil variation appear to be important factors shaping local adaptation and possibly endemism within Astragalus species.

Our study demonstrates that a combination (and potentially an interaction) of demographic and ecological forces have contributed to diversification of Astragalus taxa. The timing of divergence coincides with a major historic climate change event, which potentially contributed to the geographic or genetic isolation of populations in novel environments. Moreover, the demographic factors shaping low gene flow and high genetic diversity in Astragalus (e.g., seed biology) may have also been conducive to allowing local adaptation to these novel environments, which could further reduce gene flow. However, our insights into the diversification process of this clade are relatively coarse, and further research is needed to more rigorously test these hypotheses.

4.3 | Conservation implications

Our investigation of the iselyi-sabulosus-vehiculus clade in southern Utah revealed evidence for three highly differentiated genetic clusters that represent the currently defined taxonomic units (Figure 1; Barneby, 1989; Welsh, 1974, 1998). Demographic modelling
suggests that these taxa diverged almost simultaneously from a common ancestor (Figure 2b). More detailed ecological and phylogenetic studies (including closely related congeners) are needed to determine whether these taxa should each be considered their own species or as varieties of a single species; other genetic studies of Astragalus clades suggest that either scenario may be acceptable based on observed values of genetic differentiation (e.g., Alexander et al., 2004; Baskauf & Burke, 2009; Massatti et al., 2018; Travis et al., 1996).

Regardless of taxonomic classification, conservation and management efforts may best be served by maintaining the unique genetic and adaptive histories of each taxon. While they do not appear to currently face high risk of population decline due to genetic factors (e.g., low genetic diversity or inbreeding depression), all taxa face potential risks of decline due to land use (Franklin, 2003). A crucial goal of future research will be to discern whether small census sizes are typical for these taxa or are the result of recent population declines. In addition, modelling habitat availability in anticipation of climate and land use change may help to establish conservation priorities by identifying whether certain Astragalus populations are disproportionately vulnerable. Although future research is required, Astragalus seed banks may provide a crucial natural resource for conservation and restoration of vulnerable species. Collectively, our study provides critical insights into the concerted influence of demography and adaptation during the diversification of an endemic clade, the details of which likely have important implications for the conservation of many rare species across the region.

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PEER REVIEW

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DATA AVAILABILITY STATEMENT

Soil and climate variables (found in Table S2) can be downloaded from the Soil Grids database (http://www.soilgrids.org) and Worldclim database (http://www.worldclim.org). Files used in analyses are archived in ScienceBase (Jones, Winkler, et al., 2021).

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REFERENCES

Alexander, J. A., Liston, A., & Popovich, S. J. (2004). Genetic diversity of the narrow endemic Astragalus oniciformis (Fabaceae). American Journal of Botany, 91(12), 2004-2012. https://doi.org/10.3732/ajb.91.12.2004
Backes, A., Mäder, G., Turchetto, C., Segatto, A. L., Fregonesi, J. N., Bonatto, S. L., & Freitas, L. B. (2019). How diverse can rare species be on the margins of genera distribution? AoB PLANTS, 11(4), plz037. https://doi.org/10.1093/aobpla/plz037
Barney, R. C. (1989). In A. Cronquist, A. H. Holmgren, N. H. Holmgren, J. L. Reveal, & P. K. Holmgren (Eds.), Intermountain Flora, Vol. 3, Part B. Fables. Columbia University Press.
Barton, N. H., & Charlesworth, B. (1984). Genetic revolutions, founder effects, and speciation. Annual Review of Ecology and Systematics, 15, 133–164. https://doi.org/10.1146/annurev.es.15.110184.001025
Barrett, S. C. H., & Kohn, J. R. (1991). Genetic and evolutionary consequences of small population size in plants: Implications for conservation. In D. E. Falk, & K. E. Holsinger (Eds.), Genetics and conservation of rare plants (pp. 3–30). Oxford University Press.
Baskauf, C. J., & Burke, J. M. (2009). Population genetics of Astragalus bibilatus (Fabaceae) using AFLPs. Journal of Heredity, 100(4), 424–431. https://doi.org/10.1093/jhered/esp033
Baskin, C. C., & Baskin, J. M. (1974). Responses of Astragalus tennesseensis to drought—Changes in free amino acids and amides during water stress and possible ecological significance. Oecologia, 17(1), 11–16. https://doi.org/10.1007/BF00345091
Baskin, J. M., & Baskin, C. C. (2005). Ecology of two geographically restricted Astragalus species (Fabaceae), A. bobilatus and A. tennesseensis, of the eastern United States. Brittonia, 57(4), 345–353.
Beichman, A. C., Huerta-Sanchez, E., & Lohmueller, K. E. (2018). Using genomic data to infer historic population dynamics of nonmodel organisms. Annual Review of Ecology, Evolution, and Systematics, 49, 433–456. https://doi.org/10.1146/annurev-ecolsys-110617
Bradburd, G. S., Ralph, P. L., & Coop, G. M. (2013). Disentangling the effects of geographic and ecological isolation on genetic differentiation. Evolution, 67(11), 3258–3273. https://doi.org/10.1111/evol.12193
Coffman, A. J., Hsieh, P. H., Gravel, S., & Gutenkunst, R. N. (2016). Computationaly efficient composite likelihood statistics for demographic inference. Molecular Biology and Evolution, 33(2), 591-593. https://doi.org/10.1093/molbev/msv255
Combs, J. K., Lambert, A. M., & Reichard, S. H. (2013). Predispersal seed predation is higher in a rare species than in its widespread sympatric congener (Astragalus, Fabaceae). American Journal of Botany, 100(11), 2149–2157. https://doi.org/10.3732/ajb.1300238
Cowling, R. M., Rundel, P. W., Lamont, B. B., Arroyo, M. K., & Arianoutsou, M. (1996). Plant diversity in mediterranean-climate regions. Trends in Ecology & Evolution, 11(9), 362–366. https://doi.org/10.1016/1013-4217(96)01044-6
Danecek, P., Auton, A., Abecasis, G., Albers, C. A., Banks, E., DePristo, M. A., Handsaker, R. E., Lunter, G., Marth, G. T., Sherry, S. T., McVean, G., & Durbin, R. (2011). The variant call format and VCFtools. Bioinformatics, 27(15), 2156–2158. https://doi.org/10.1093/bioinformatics/btr330
Dlugosch, K. M., & Parker, I. M. (2008). Founding events in species invasions: Genetic variation, adaptive evolution, and the role of multiple introductions. Molecular Ecology, 17(1), 431–449. https://doi.org/10.1111/j.1365-294X.200703538.x
Egan, A. N., & Crandall, K. A. (2008). Divergence and diversification in North American Psoraleae (Fabaceae) due to climate change. BMC Biology, 4(1), 1-13. https://doi.org/10.1186/1741-7007-6-55

Ellstrand, N. C., & Elam, D. R. (1993). Population genetic consequences of small population size: Implications for plant conservation. Annual Review of Ecology and Systematics, 24, 217-259. https://doi.org/10.1146/annurev.es.24.110193.001245

Ewing, G., & Herminsson, J. (2010). MSMS: A coalescent simulation program including recombination, demographic structure and selection at a single locus. Bioinformatics, 26(16), 2064-2065. https://doi.org/10.1093/bioinformatics/btq322

Fischer, M. C., Rellstab, C., Tedder, A., Zoller, S., Gugerli, F., Shimizu, K. K., Holderegger, R., & Widmer, A. (2013). Population genomic footprints of selection and associations with climate in natural populations of Arabidopsis halleri from the Alps. Molecular Ecology, 22(22), 5594-5607. https://doi.org/10.1111/mec.12521

Foote, A. D., Vijay, N., Ávila-Arcos, M. C., Baird, R. W., Durban, J. W., Fumagalli, M., Gibb, R. A., Hanson, M. B., Korneliussen, T. S., Martin, M. D., Robertson, K. M., Sousa, V. C., Vieira, F. G., Vinar, T., Wade, P., Worley, K. C., Excoffier, L., Morin, P. A., Gilbert, M. T. P., & Wolf, J. B. W. (2016). Genome-culture coevolution promotes rapid divergence of killer whale ecotypes. Nature Communications, 7, 11693. https://doi.org/10.1038/ncomms11693

Foxe, J. P., Slotte, T., Stahl, E. A., Neuffer, B., Hurka, H., & Wright, S. I. (2009). Recent speciation associated with the evolution of selfing in Capsella. Proceedings of the National Academy of Sciences of the United States of America, 106(13), 5241-5245. https://doi.org/10.1073/pnas.0807679106

Frankham, R., Lees, K., Montgomery, M. E., England, P. R., Lowe, E. H., & Briscoe, D. A. (1999). Do population size bottlenecks reduce evolutionary potential? Animal Conservation, 2(4), 255-260. https://doi.org/10.1111/j.1469-1795.1999.tb00071.x

Franklin, M. A. (2003). 2001-2002 Survey Results: Astragalus iselyi Welsh.

Galbreath, K. E., & Cook, J. A. (2004). Genetic consequences of Pleistocene glaciations for the tundra vole (Microtus oeconomicus) in Beringia. Molecular Ecology, 13(1), 135-148. https://doi.org/10.1046/j.1365-294X.2003.02026.x

Galbreath, K. E., Hafner, D. J., & Zamudio, K. R. (2009). When colder is better: Climate-driven elevation shifts yield complex patterns of diversification and demography in an alpine specialist (American Pika, Ochotona princeps). Evolution, 63(11), 2848-2863. https://doi.org/10.1111/j.1558-5646.2009.00803.x

Gavrilets, S., & Vose, A. (2005). Dynamic patterns of adaptive radiation. Proceedings of the National Academy of Sciences of the United States of America, 102(50), 18040-18045. https://doi.org/10.1073/pnas.0506330102

Gittenberger, E. (1991). What about non-adaptive radiation? Biological Journal of the Linnean Society, 43(4), 263-272. https://doi.org/10.1111/j.1095-8312.1991.tb00598.x

Godt, M. J. W., Johnson, B. R., & Hamrick, J. L. (1996). Genetic diversity and population size in four rare southern Appalachian plant species. Conservation Biology, 10(3), 796-805. https://doi.org/10.1046/j.1523-1796.1996.10030796.x

Goodrich, S., Armstrong, L., & Thompson, R. (1999). Endemic and endangered plants of Pinyon Juniper communities. In USDA Forest Service Proceedings RMRS-P-9, pp. 260–268.

Graham, R. W. (1986). Response of mammalian communities to environmental changes during the late Quaternary. In J. Diamond & T. J. Case (Eds.), Community Ecology (pp. 300–313). Harper & Row.

Green, T. W., & Bohart, G. E. (1975). The pollination ecology of Astragalus cibarius and Astragalus utahensis (Leguminosae). American Journal of Botany, 62(4), 379–386. https://doi.org/10.2307/2442091

Green, T. W., & Palmibald, I. G. (1975). Effects of insect seed predators on Astragalus cibarius and Astragalus utahensis. Ecology, 56(6), 1435-1440. https://doi.org/10.2307/1934711

Gutenkunst, R. N., Hernandez, R. D., Williamson, S. H., & Bustamante, C. D. (2009). Inferring the joint demographic history of multiple populations from multidimensional SNP frequency data. PLoS Genetics, 5(10), e1000695. https://doi.org/10.1371/journal.pgen.1000695

Harrison, J. G., Forister, M. L., McKnight, S. R., Nordin, E., & Parchman, T. L. (2019). Rarity does not limit genetic variation or preclude subpopulation structure in the geographically restricted desert forb Astragalus lentiginosus var. piscinensis. American Journal of Botany, 106(2), 260–269. https://doi.org/10.1002/ajb2.1235

Hewitt, G. (2000). The genetic legacy of the Quaternary ice ages. Nature, 405, 907-913. https://doi.org/10.1038/35016000

Hijmans, R. J., Cameron, S. E., Parra, J. L., Jones, P. G., & Jarvis, A. (2005). Very high resolution interpolated climate surfaces for global land areas. International Journal of Climatology, 25(15), 1965-1978. https://doi.org/10.1002/joc.1276

Hofreiter, M., & Stewart, J. (2009). Ecological change, range fluctuations and population dynamics during the Pleistocene. Current Biology, 19(14), R584–R594. https://doi.org/10.1016/j.cub.2009.06.030

IUCN. (2020). The IUCN Red List of Threatened Species. https://www.iucnredlist.org.

Jackson, S. T., & Overpeck, J. T. (2000). Responses of plant populations and communities to environmental changes of the late Quaternary. Paleobiology, 26(s4), 194–220.

James, J. W. (1971). The founder effect and response to artificial selection. Genetics Research, 16(3), 241–250. https://doi.org/10.1017/S0016672300002500

Jones, M. R., & Good, J. M. (2016). Targeted capture in evolutionary and ecological genomics. Molecular Ecology, 25(1), 185-202. https://doi.org/10.1111/mec.13304

Jones, M. R., Mills, L. S., Jensen, J. D., & Good, J. M. (2020). The origin and spread of locally adaptive seasonal camouflage in snowshoe hares. The American Naturalist, 196(3), 316–332. https://doi.org/10.1086/710022

Jones, M. R., Winkler, D. E., & Massatti, R. (2021). Astragalus species complex genetic data from southeast Utah (Grand County and San Juan County), USA: U.S. Geological Survey data release. https://doi.org/10.5066/P93SRC7M

Karron, J. D. (1987). The pollination ecology of co-occurring geographically restricted and widespread species of Astragalus (Fabaceae). Biological Conservation, 39(3), 179–193. https://doi.org/10.1016/0006-3207(87)90033-4

Karron, J. D. (1989). Breeding systems and levels of inbreeding depression in geographically restricted and widespread species of Astragalus (Fabaceae). American Journal of Botany, 76, 331–340. https://doi.org/10.1002/j.1537-2197.1989.tb11319.x

Karron, J. D., Linhart, Y. B., Chaulk, C. A., & Robertson, C. A. (1988). Genetic structure of populations of geographically restricted and widespread species of Astragalus (Fabaceae). American Journal of Botany, 75(8), 1114–1119. https://doi.org/10.2307/1357197.1988.tb08823.x

Kaye, T. N. (1999). From flowering to dispersal: Reproductive ecology of an endemic plant, Astragalus australis var. olympicus (Fabaceae). American Journal of Botany, 86(9), 1248–1256. https://doi.org/10.2307/2656772

Kimura, M. (1983). The Neutral Theory of Molecular Evolution. Cambridge University Press.

Knowles, L. L., & Massatti, R. (2017). Distributional shifts—not geographic isolation—as a probable driver of montane species divergence. Ecography, 40(12), 1475-1485. https://doi.org/10.1111/ecog.02893
Särkinen, T. E., Newman, M. F., Maas, P. J. M., Maas, H., Poulsen, A. D., Harris, D. J., Richardson, J. E., Clark, A., Hollingsworth, M., & Toby Pennington, R. (2007). Recent oceanic long-distance dispersal and divergence in the amphi-Atlantic rain forest genus Renealmia L.f. (Zingiberaceae). Molecular Phylogenetics and Evolution, 44(3), 968-980. https://doi.org/10.1016/j.ympev.2007.06.007

Scherson, R. A., Vidal, R., & Sanderson, M. J. (2008). Phylogeny, biogeography, and rates of diversification of New World Astragalus (Leguminosae) with an emphasis on South American radiations. American Journal of Botany, 95(8), 1030–1039. https://doi.org/10.3732/ajb.0800017

Schrötter, D. (2001). The ecology of adaptive radiation. Oxford University Press.

Schneeweiss, G. M., & Schönswetter, P. (2010). The wide but disjunct range of the European mountain plant Androsace lactea (Primulaceae) reflects Late Pleistocene range fragmentation and post-glacial distributional stasis. Journal of Biogeography, 37(10), 2016–2025. https://doi.org/10.1111/j.1365-2699.2010.02350.x

Simões, M., Breitkreuz, L., Alvarado, M., Baca, S., Cooper, J. C., Heins, L., Herzog, K., & Lieberman, B. S. (2016). The evolving theory of evolutionary radiations. Trends in Ecology and Evolution, 31(1), 27–34. https://doi.org/10.1016/j.tree.2015.10.007

Spellman, G. M., & Klicka, J. (2007). Phylogeography of the white-breedt nuthatch (Sitta carolinensis) Diversification in North American pine and woodlands. Molecular Ecology, 16(8), 1729-1740. https://doi.org/10.1111/j.1365-294X.2007.03237.x

Stamatakis, A. (2014). RAxML version 8: A tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics, 30(9), 1312-1313. https://doi.org/10.1093/bioinformatics/btu033

Stebbins, G. L. (1981). Why are there so many species of flowering plants? Molecular Phylogenetics and Evolution, 1740. https://doi.org/10.1111/j.1365-294X.2007.03237.x

Wang, J. I., & Bradburd, G. S. (2014). Isolation by environment. Molecular Ecology, 23(23), 5649–5662. https://doi.org/10.1111/mec.12938

Weber, W. A. (2003). The middle Asian element in the southern Rocky Mountain flora of the western United States: A critical biogeographical review. Journal of Biogeography, 30(5), 649–685. https://doi.org/10.1046/j.1365-2699.2003.00864.x

Welsh, S. L. (1974). Utah plant novelties in Astragalus and Yucca. Great Basin Naturalist, 34(5), 305–310.

Welsh, S. L. (1998). Astragalus (Leguminosae): Nomenclatural proposals and new taxa. Great Basin Naturalist, 58(1), 45–53.

Wessel, A., Hoch, H., Asche, M., Von Rintelen, T., Steibrink, B., Heck, V., Stone, F. D., & Howarth, F. G. (2013). Founder effects initiated rapid species radiation in Hawaiian cave planthoppers. Proceedings of the National Academy of Sciences of the United States of America, 110(23), 9391–9396. https://doi.org/10.1073/pnas.1301657110

Williams, J. W., & Jackson, S. T. (2007). Novel climates, no-analog communities and ecological surprises. Frontiers in Ecology and the Environment, 5(9), 475–482. https://doi.org/10.1890/070037

Winkler, D. E., Belnap, J., Hoover, D., Reed, S. C., & Duniway, M. C. (2019). Shrub persistence and increased grass mortality in response to drought in dryland systems. Global Change Biology, 25(9), 3121-3135. https://doi.org/10.1111/gcb.14667

Wojciechowski, M. F., Sanderson, M. J., Baldwin, B. G., & Donoghue, M. J. (1993). Monophyly of aneuploid Astragalus (Fabaceae): Evidence from nuclear ribosomal DNA internal transcribed spacer sequences. American Journal of Botany, 80(6), 711–722. https://doi.org/10.2307/2445441

Wojciechowski, M. F., Sanderson, M. J., & Hu, J.-M. (1999). Evidence on the monophyly of Astragalus (Fabaceae) and its major subgroups based on nuclear ribosomal DNA ITS and chloroplast DNA trnL intron data. Systematic Botany, 24(3), 409. https://doi.org/10.2307/2419698

Wright, S. (1932). The roles of mutation, inbreeding, crossbreeding and selection in evolution. Proceedings of the 6th International Congress of Genetics, 1, 356–366.

Yi, X., Liang, Y., Huerta-Sanchez, E., Jin, X., Cuo, Z. X. P., Pool, J. E., Xu, X., Jiang, H., Vinckenbosch, N., Korneliussen, T. S., Zheng, H., Liu, T., He, W., Li, K., Luo, R., Nie, X., Wu, H., Zhao, M., Cao, H., ... Wang, J. (2010). Sequencing of 50 human exomes reveals adaptation to high altitude. Science, 329(5987), 75–78. https://doi.org/10.1126/science.1190371

Záveská, E., Maylandt, C., Paun, O., Bertel, C., Frajman, B., Consortium, T. S., & Schöchner, P. (2019). Multiple auto- and allopolyploids marked the Pleistocene history of the widespread Eurasian steppe plant Astragalus onobrychis (Fabaceae). Molecular Phylogenetics and Evolution, 139, 106572. https://doi.org/10.1016/j.ympev.2019.106572

Zedler, P. H., Gautier, C. R., & McMaster, G. S. (1983). Vegetation change in response to extreme events: The effects of a short interval between fires in California chaparral and coastal scrub. Ecology, 64(4), 809–818. https://doi.org/10.2307/1937204
Biosketches
The authors comprise the Genetics for Western Restoration and Conservation (GWRC) research group at the USGS Southwest Biological Science Center. The GWRC group applies state-of-the-art genome sequencing technologies and advanced bioinformatic and analytical tools to study genetic variation of rare, threatened, ecologically important species across the Western United States. Specifically, our research focuses on inferring the evolutionary and ecological processes shaping genomic variation across species' distributions and developing informed strategies to preserve or increase the genomic health of plant and animal populations. We also conduct field-based experiments and landscape genomic analyses to support the development of native plant materials for restoration across the Western United States. GWRC website: https://www.usgs.gov/sbsc/gwrc

SUPPORTING INFORMATION
Additional supporting information may be found online in the Supporting Information section.

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