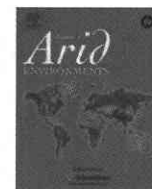




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Development of variable microsatellite loci and range-wide characterization of nuclear genetic diversity in the important dryland shrub antelope bitterbrush (*Purshia tridentata*)

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ABSTRACT

Antelope bitterbrush (*Purshia tridentata* Pursh DC; Rosaceae) is an arid-land shrub that occupies an important ecological niche in various fire-dominated communities across much of the western United States. Because of its importance as a browse for large mammals and a food source for granivores, *P. tridentata* is frequently planted by Federal agencies in arid-land revegetation. We are currently analyzing the range-wide genetic diversity of this species as part of a larger effort to develop seed movement guidelines. In this study, we describe the development of eight novel nuclear microsatellite loci and characterize the amount and apportionment of range-wide nuclear genetic diversity. The eight microsatellite loci exhibited a high level of polymorphism (13–33 alleles per locus) and in general, observed levels of heterozygosity did not deviate from Hardy–Weinberg expectations. An initial screen of 196 individuals from 12 widely distributed populations revealed a moderate amount of genetic differentiation ($F_{ST} = 0.09$, $p < 0.001$). Moreover, these loci successfully produced PCR products in cross-species amplifications with two closely related *Purshia* species. These results demonstrate the utility of these markers and provide useful measures of neutral genetic diversity and population differentiation.

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1. Introduction

Among native plant species used in habitat restoration, antelope bitterbrush (*Purshia tridentata* Pursh DC; Rosaceae) is arguably one of the most important shrubs in the western United States (U.S.). The native range of this species encompasses ~840 million hectares of rangeland and dry forest between the Cascades/Sierra Nevada and the Rocky Mountains (Young and Clements, 2002). Across this Intermountain Region, *P. tridentata* exhibits remarkable adaptability, spanning an elevation range of 60 to over 3500 m, and a precipitation gradient ranging from less than 25 cm yr^{-1} to over 125 cm yr^{-1} . This drought-adapted shrub figures prominently in the ecology of the Intermountain Region; it is a key forage for large mammals (mule deer, elk, and pronghorn antelope; Nord, 1965; Kufeld, 1973; Kufeld et al., 1973; Stuth and Winward, 1977; Guenther et al., 1993), an important seed source for granivores (Vander Wall, 1994; Young and Clements, 2002), and an early season pollen and nectar source for a diversity of insects (Furniss, 1983). In addition to these important roles, *P. tridentata* is one of few Rosaceous species that fix nitrogen through symbiosis with the actinomycete *Frankia* (Bond, 1976; Dalton and Zobel, 1977), making it an important contributor to soil nitrogen in arid-landscapes.

Although *P. tridentata* is naturally distributed across some of the most fire-prone ecosystems in the Northwest U.S., it is usually consumed by fire and it shows a low frequency of root sprouting relative to fire-resistant species like *Manzanita* or *Ceanothus*. While post-fire sprouting ability in *P. tridentata* is generally low, it does appear variable and may have a genetic component; for example, young and very old *P. tridentata* (<5 years, and >40 years) have been reported to root sprout in the Upper Snake River basin of Idaho (Blaisdell and Mueggler, 1956), while bitterbrush from central Oregon apparently lacks this trait (Clark et al., 1982). Rather than investing resources in below-ground, fire-resistant lignotubers, *P. tridentata* is a prolific seed producer that relies on the caching activities of granivores for fire avoidance (West, 1968; Sherman and Chilcote, 1972; Clark et al., 1982; Vander Wall, 1994). Below-ground burial by rodents and insects protects seed from fires, in addition to providing the cool-moist environment necessary to break seed dormancy (Young and Evans, 1976). In a study of 11 burned sites, Nord (1965) found that seedling recruitment was far more important than re-sprouting, with seedlings accounting for up to 80% of all new plants. Despite its importance, post-burn re-establishment of *P. tridentata* from seed is a slow process; at some sites, *P. tridentata* can require 10 years to reach flowering age (Nord, 1965). During this establishment period, wildlife is attracted to tender *P. tridentata* sprouts, and damage from browsing animals is a major cause of stand decline (Young and Clements, 2002).

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This broad ecological amplitude and desirability – combined with a limited ability to root sprout following fire (Clark et al., 1982; Young and Clements, 2002) – makes *P. tridentata* one of the most widely planted restoration shrubs in the Intermountain Region. Critical to the successful inclusion of *P. tridentata* in restoration projects is the use of appropriate, locally adapted seed sources. Ideally, locally adapted seed should produce viable and vigorous plants that will emerge to help mitigate soil erosion, compete with invasive exotics, and complement the local genetic composition of surrounding native stands (Lesica and Allendorf, 1999; Hufford and Mazer, 2003). Currently, genetic data do not exist for land managers to manipulate bitterbrush seed across the landscape. Moreover, the limited germplasm collections available for federal restoration projects often derive from a narrow genetic base and a limited geographic scale. In light of the enormous environmental heterogeneity across *P. tridentata*'s range and the limited public offerings for this species, maladaptive responses (poor establishment, weak growth, early stand decline) may be expected if restoration germplasm does not match (i.e., is not genetically appropriate) for a target location.

To guide the use of *P. tridentata* in restoration, we have developed novel nuclear microsatellite loci and have utilized them to provide baseline data on the magnitude and partitioning of neutral genetic variation. Microsatellites, or simple sequence repeats (SSRs) are short sequences composed of a simple repeated motif (e.g., CT_n, TGG_n) that are distributed throughout eukaryotic genomes (Jarne and Lagoda, 1996). Because of their high mutation rates (Jarne and

Lagoda, 1996, and references therein), they are highly polymorphic and have proven extremely useful in genetic studies addressing a broad range of questions at the population and intraspecific level (Cruzan, 1998; Hedrick, 1999). As a consequence of their presumed neutrality, microsatellites are particularly useful for providing estimates of the magnitude and direction of drift and migration (Jarne and Lagoda, 1996; Balloux and Lugon-Moulin, 2002). Additionally, we have tested these microsatellite markers for cross-species amplification in two close relatives, *Purshia glandulosa* (Curran) and *Purshia stansburiana* (Torr.) Henrickson. Successful cross-species amplification indicates that these markers will be informative for quantifying the frequency and symmetry of hybridization between *P. tridentata* and closely related species, and in conservation genetic applications for threatened or rare congeners such as *Purshia subintegra* (Kearney) Henrickson (Arizona cliffrose) and *Purshia pinkavae* Schaack (Pinkava's cliffrose).

2. Materials and methods

2.1. Tissue sample collection

Bitterbrush leaves were collected from 12 populations during the summer of 2005 from across the species range (Fig. 1). When possible, samples were collected from 20 individuals at a site, with individuals separated by > 5 m so as to minimize the likelihood of collecting half-sibs that originated from a common seed cache.

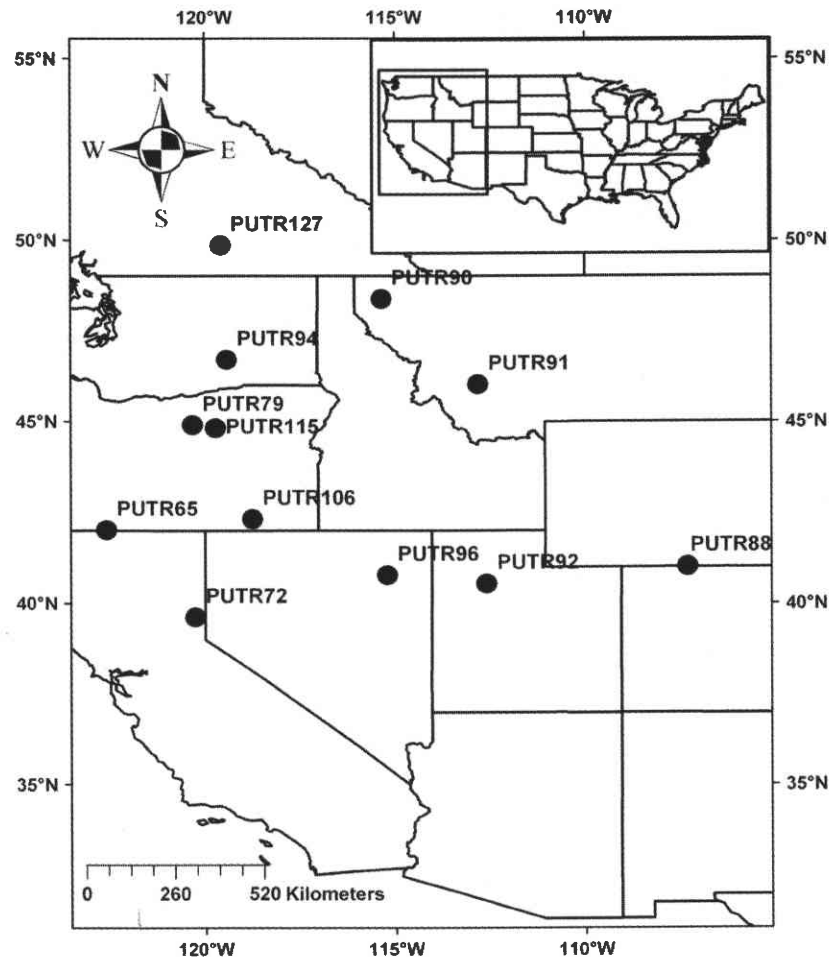


Fig. 1. Distribution of *Purshia tridentata* populations screened in this study. Small map inset outlines the location of the larger map image for reference.

Tissue was either frozen upon collection and stored at -20°C , or dried in the field and subsequently stored at -20°C . The former approach yielded higher quantities of DNA, but both methods yielded DNA of sufficient quality for genotyping. Leaves from *P. glandulosa* and *P. stansburiana* were collected from populations in NE Nevada for cross-species amplification tests of the microsatellite loci.

2.2. Genomic DNA isolation

Total genomic DNA was isolated from leaf tissue using the FastDNA[®] kit (MP Biomedicals; Solon, OH). To facilitate cell wall disruption, dried leaves were soaked for 4–18 h in 800 μL of CLS-VF solution prior to homogenization. For all samples, β -mercaptoethanol (1% v/v) was added to the extraction buffer. To remove traces of polysaccharide impurities, Nucleon Phytopure[™] resin (GE Healthcare; Piscataway, NJ, 2% v/v) was added to all DNA extracts.

2.3. Genomic library construction

Microsatellite loci were developed using the method of Hamilton et al. (1999), with minor modifications. Genomic DNA from one *P. tridentata* individual was isolated as described above, and then cleaned with a QIAquick[®] (Qiagen) PCR purification kit. Approximately 5 μg of DNA was digested with *Hae*III, *Rsa*I, *Alu*I, and *Nhe*I to produce a pool of random fragments 200–1000 bp in length. Genomic fragments were blunt-ended, 5'-dephosphorylated, and ligated to the SNX oligonucleotide linkers (Hamilton et al., 1999). Enrichment for repeat-containing fragments was performed with streptavidin paramagnetic particles (Promega) and 5'-biotinylated oligonucleotides (Integrated DNA Technologies; GT₁₅, CT₁₅, CCG₁₀, CTC₁₀, CAG₁₀, AAG₁₀, AAT₁₀, CAT₁₀, CAC₁₀, GGA₁₀). Single-stranded DNA was amplified by polymerase chain reaction (PCR) and ligated into the pBluescript[®] II phagemid vector (Stratagene). Ligations were transformed with XL2-Blue MRF⁺ ultracompetent cells utilizing blue-white screening.

2.4. Microsatellite locus identification and primer design

Plasmids from insert-containing colonies were amplified using rolling circle amplification (RCA) and sequenced at MCLab (San Francisco, CA) with T7 and T3 PCR primers using an Applied Biosystems 3730xl DNA analyzer. Resulting traces were evaluated using BioEdit (Hall, 1999), and plasmid vector sequence was removed using the program VecScreen (<http://www.ncbi.nlm.nih.gov/VecScreen/VecScreen.html>). Amplification primers were designed using the web-based software Primer3 (Rozen and Skaltsky, 2000), with the following parameters: minimum oligonucleotide length = 17 bp, optimal length = 19 bp; optimal annealing temperature = 55°C . We added a 5' motif (5'-GTTTCTT-3') to each oligonucleotide in a primer pair to reduce PCR amplification stutter and improve the accuracy of subsequent genotype interpretations (e.g., 'PIGtailing'; Brownstein et al., 1996). Fluorescently labeled oligonucleotides were 5'-end labeled with HEX (CT1-41, 1-18) FAM (CT2-21, CT1-48, CT2-18), NED (1-22, CT1-39), and PET (CT1-43). Oligonucleotides were purchased from Integrated DNA Technologies (HEX, FAM; Coralville, IA) or Applied Biosystems (NED, PET; Foster City, CA).

2.5. Genotyping and fragment analysis

PCR reactions (10 μL) contained ~50 ng genomic DNA, 0.5 μM each forward and reverse primers, 1X PCR buffer, 200 μM dNTPs, 1.5 or 2.5 mM MgCl_2 (Table 2), 0.6 U *Taq* polymerase, polyvinyl propylene (PVP, 0.5% w/v), and 4 μg bovine serum albumin. PCR cycling was performed on a MJ Research PTC-100 thermocycler

with the following steps: 94°C for 5 min, followed by 30 cycles of 94°C for 1 min, 50°C or 55°C for 1 min (see Table 2), 72°C for 1 min, and an extension of 72°C for 5 min. Microsatellite loci were optimized for amplification success and polymorphism screening using single-locus PCR, but most loci were subsequently amplified in multiplex PCR reactions. Genotype detection was performed on an ABI 3100 automated capillary sequencer at Oregon State University's Center for Genome Research and Biocomputing (<http://corelabs.cgrb.oregonstate.edu/>).

2.6. Statistical analyses

Individual diploid (McArthur et al., 1983) genotypes were manually identified using Genotyper 3.7 (Applied Biosystems). To assess the reliability of these microsatellite loci, we tested each locus for deviations from Hardy-Weinberg equilibrium (HWE) to identify the presence of null alleles and other genotyping errors using the software program MICRO-CHECKER (van Oosterhout et al., 2004). For each locus we calculated the observed allele frequencies and the observed (H_o) and expected (H_e) levels of heterozygosity with Fisher's exact test using GenePop (Raymond and Rousset, 1995). Deviations from HWE were assessed using Markov Chain Monte Carlo simulations with the following parameters; dememorization = 1000, batches = 100, iterations = 1000. Genotypic linkage disequilibrium between loci was also assessed for single populations and across all populations using Fisher's exact test and the same chain parameters.

In our study, interspecific comparisons of two species, *P. glandulosa* and *P. stansburiana*, included eight individuals per species (four individuals from each of two populations). To determine if the number of observed alleles in these small samples were significantly different than those observed in *P. tridentata*, we created 1000 data sets of eight randomly selected individuals, and counted the number of unique alleles present in each locus for each jack-knife sample. The 95% credibility interval is defined as the number of alleles present at the 2.5 and 97.5 percentiles.

For *P. tridentata* populations, estimates of differentiation across multi-locus genotypes (Wright's F_{ST}) were computed via analysis of molecular variation (AMOVA; Peakall and Smouse, 2006). To determine if genetic divergence between populations was associated with their geographic distance, we conducted a Mantel matrix correlation test using genetic (Nei's D) and geographic (km) distance matrices. Additionally, principal coordinate analysis (PCoA) was used to explore multivariate relationships among inter-individual genetic distances and to identify a set of reduced-dimension traits (e.g., PC eigen vectors). These analyses were conducted using GenAlEx version 6 (Peakall and Smouse, 2006).

3. Results

3.1. Nuclear microsatellite locus characterization and genetic diversity

From our short genomic insert libraries, we isolated and sequenced 334 colonies, 68 of which contained inserts with unique repeat motifs. We designed primer pairs to amplify 67 of the putative loci; of this total, 26 did not amplify easily interpretable products and 30 appeared invariant on high resolution agarose electrophoresis. The remaining 11 polymorphic loci were tested using fluorescently labeled primers against a diversity panel including *P. tridentata* from across the range, *P. glandulosa*, and *P. stansburiana*. Resulting electropherograms from capillary electrophoresis indicated that three loci were invariant; these were dropped, leaving eight polymorphic microsatellites for the final analysis (Table 1).

Table 1
Eight polymorphic microsatellite loci for *Purshia tridentata*

Locus (GenBank)	Repeat motif	Primer (5'-3')	Label	T_A (°C)	MgCl ₂ (mM)	Range (bp)	N_A	n	H_o (H_e)	HWE p
CT1-43 (EU293135)	(CT) ₁₅ TT(CT) ₈	F: GTT TCT TAT GTA GAG GTT TGG GCT TC R: GTT TCT TTG GTC TTG AAA CAG AAT GC	PET	55	1.5	196–240	20	189	0.79 (0.78)	0.77
CT1-41 (EU293136)	(GA) ₂₅	F: GTT TCT TCA ATG CCA GTC ACT CAT TC R: GTT TCT TCA TGA GGC ACC AGA TTG TA	HEX	55	1.5	169–223	21	190	0.72 (0.76)	0.16
CT2-21 (EU293137)	(GT) ₄ G(GAAA) ₇ (GA) ₂₀	F: GTT TCT TAG AAC ACC TGC ACA CAG AA R: GTT TCT TGT TCC CTA CAA CAG GAA CC	FAM	55	1.5	145–189	23	190	0.78 (0.81)	0.005
CT1-48 (EU293138)	(AG) ₄ G(GA) ₄ AG(GA) ₁₂	F: GTT TCT TAT GAC CTT GTG GAA AGA CC R: GTT TCT TGA GGA CTG CAA TGA TTG TG	FAM	55	1.5	241–287	19	191	0.65 (0.67)	0.42
CT2-18 (EU293139)	(GA) ₁₉	F: GTT TCT TTT CAT CTT CGT CCT CAG AA R: GTT TCT TAT TCC CTA ACT CCG TCA CA	FAM	50	2.5	160–188	15	170	0.83 (0.76)	0.93
1-22 (EU293140)	(GT) ₁₆	F: GTT TCT TTC CCT TTT TGC ATT TCT TT R: GTT TCT TTC TTT CAC ATC AAC CTT GG	NED	50	1.5	188–214	13	184	0.57 (0.55)	0.94
1-18 (EU293141)	(CT) ₁₉ (CA) ₈	F: GTT TCT TGT GCC ATG GAT GCT TAG AT R: GTT TCT TGC AAG TGG ATA CAT CCA TA	HEX	55	1.5	163–233	33	194	0.71 (0.82)	0.0014
CT1-39 (EU293142)	(AT) ₅ (AG) ₆ (CA) ₂ T(GA) ₂₂	F: GTT TCT TCT TCC TAG AAA TCC GCC TA R: GTT TCT TAA CTG GGG CAG ACA TTG	NED	55	1.5	211–253	19	185	0.80 (0.83)	0.04

For each locus name, the following information is provided: GenBank number, repeat motif, PCR amplification primers, fluorescent label, annealing temperature (T_A), MgCl₂ concentration, size range of amplicons (Range), number of alleles per locus (N_A), number of individuals genotyped per locus (n), and observed (H_o) and expected (H_e) heterozygosities. Tests for Hardy–Weinberg Equilibrium (HWE p) were conducted using Markov Chain Monte Carlo simulations.

Microsatellites included in this study exhibited a high level of polymorphism in *P. tridentata*, with 13–33 alleles per locus (Table 1). Observed heterozygosities averaged across populations were high for these loci, with values ranging from 0.57 to 0.83 (Table 1). No significant linkage disequilibrium was found between any locus pairs, but significant deviations from HWE were detected for three loci, CT1-39, CT2-21 and 1-18 (Table 1). Deviation was attributable to the following populations: PUTR94, PUTR106 (for locus CT1-39), PUTR88, PUTR92, and PUTR106 (for locus 1-18), and PUTR92, and PUTR96 (for locus CT2-21). Despite these deviations from HWE, we did not detect the presence of null alleles at any locus based on the algorithms described in van Oosterhout et al. (2004). This finding suggests that the deviations from HWE – while significant – are relatively minor, and most likely attributable to biological processes.

3.2. Preliminary assessment of population differentiation

AMOVA of multi-locus genotypes revealed that the majority of the observed genetic variation was partitioned within populations, with a moderate estimated global value of F_{ST} of 0.09 (S.E. = 0.015, $p < 0.001$). Pairwise estimates of F_{ST} ranged between 0.02 and 0.19 (Table 2), and all but two pairwise comparisons were statistically different from each other. Both comparisons involved population PUTR65 from Pilot Rock, CA; given our limited sample size of eight individuals, this population showed insignificant differentiation from PUTR72 (Loyalton, CA) and PUTR96 (Krenka Creek, NV, Table 2).

Analysis of inter-individual distances by principal coordinate analysis confirmed the absence of cohesiveness within populations and differentiation among populations, with the first and second axes explaining nearly equivalent levels of variation (25.8 and 21.4%, respectively; Fig. 2). Out of 12 sampled populations, three appeared relatively distinct from the remaining samples; PUTR94 from Grant, WA, PUTR127 from southern British Columbia, and PUTR92 from Tooele, UT. The overall relationship between among-population genetic distance (measured as Nei's D , Table 3) and geographic distance was weak (Mantel $r^2 = 0.08$; $p = 0.07$). Critically, excluding the geographically-divergent southern British Columbia source (PUTR127) returned a non-significant result (Mantel $r^2 = 0.04$; $p = 0.14$), indicating that a general pattern of

isolation-by-distance is not evident in this species, even over large geographic scales.

3.3. Cross-species amplification

Many microsatellite primer pairs designed from our *P. tridentata* library showed successful amplification in the related congeners *P. glandulosa* and *P. stansburiana*. Five loci (CT1-41, CT1-48, CT2-18, 1-22, CT1-39; Table 3) amplified products from all three *Purshia* species. Based on 1000 jackknife replicates of our *P. tridentata* dataset, allelic diversity revealed from these small samples (4 individuals/population \times 2 populations) was indistinguishable from *P. tridentata* for all but one locus; CT1-41 from *P. stansburiana* was the only locus to return a smaller number of alleles than predicted from the 95% jackknife confidence interval (Table 3). For the remaining three loci, CT1-43 and CT1-41 from *P. glandulosa* showed allelic diversity that was indistinguishable from *P. tridentata*, while these loci failed to amplify from *P. stansburiana*. Locus CT1-18 appeared to show the highest specificity to *P. tridentata*, because no bands were amplified from a potential pool of 32 chromatids. In total, these loci yielded a total of 46 alleles across seven loci for *P. glandulosa* and 28 alleles from 5 loci for *P. stansburiana* (Table 3); comparable jackknife sampling returned a total of 60 alleles at eight loci from *P. tridentata*.

4. Discussion

4.1. Characterization of novel microsatellite markers for *Purshia* and measures of genetic diversity

These markers are highly polymorphic with a total of 163 unique alleles observed in just this preliminary sample of individuals (Table 1). Even though several of these loci contain compound or imperfect repeats, for all loci except locus CT1-39, the intervals between observed allele sizes matched those expected from the microsatellite repeat motif and a step-wise mutation model. Based on observed allele sizes, locus CT1-39 may not follow a step-wise mutation model. However, the summary statistic we employ here (F_{ST}) does not require loci that follow a step-wise mutation model as required by R_{ST} . We did not detect the presence of null alleles in this preliminary screen and therefore did not adjust observed allele

Table 2
Pairwise differentiation by F_{ST} among *Parishia tridentata* populations (below diagonal), and interpopulation genetic distance by Nei's D (above diagonal)

	PUTR79	PUTR88	PUTR90	PUTR94	PUTR96	PUTR106	PUTR115	PUTR127	PUTR65	PUTR72	PUTR91	PUTR92
PUTR79	-	0.73	0.83	0.98	0.85	0.45	0.45	0.74	0.61	0.68	0.73	1.07
PUTR88	0.05**	-	0.76	0.97	0.79	0.73	0.66	1.00	0.78	0.80	0.83	0.70
PUTR90	0.07**	0.04**	-	0.82	0.85	0.64	0.70	0.56	0.72	0.77	0.87	1.28
PUTR94	0.12**	0.12**	0.10**	-	1.26	0.83	0.72	0.64	0.91	1.21	1.28	1.10
PUTR96	0.08**	0.06**	0.08**	0.15**	-	0.59	0.81	0.91	0.41	0.49	0.37	1.14
PUTR106	0.03*	0.07**	0.07**	0.12**	0.06**	-	0.69	0.54	0.41	0.57	0.54	1.41
PUTR115	0.04**	0.05**	0.06**	0.10**	0.09**	0.08**	-	0.58	0.72	0.68	0.81	1.10
PUTR127	0.13**	0.15**	0.11**	0.15**	0.16**	0.11**	0.12**	-	0.73	0.96	0.99	1.25
PUTR65	0.04**	0.04**	0.05**	0.11**	0.01 ^{ns}	0.02*	0.06**	0.12**	-	0.42	0.56	1.03
PUTR72	0.05**	0.05**	0.06**	0.14**	0.04**	0.05**	0.06**	0.16**	0.01 ^{ns}	-	0.66	1.10
PUTR91	0.07**	0.08**	0.09**	0.16**	0.03*	0.06**	0.10**	0.18**	0.04**	0.06**	-	1.29
PUTR92	0.09**	0.06**	0.11**	0.14**	0.11**	0.15**	0.11**	0.19**	0.09**	0.10**	0.13**	-

Shading below diagonal is proportional to magnitude of differentiation. Based on 999 permutations, F_{ST} values were either significant ($*0.05 > p > 0.01$) or highly significant ($**p < 0.01$) for all but two comparisons (denoted^{ns}).

frequencies to account for this phenomenon. Moreover, possible genotyping errors due to PCR amplification artifacts (e.g., stuttering) were minimized by utilizing the modified 'PIGtailing' PCR primer design approach as described above. As additional geographic regions are incorporated (i.e., additional unique populations), new currently unidentified alleles may be observed. Critically, our analyses indicate that these markers are in genotypic equilibrium (i.e., are unlinked) and are therefore providing independent measures of genetic diversity and differentiation.

Genetic diversity was high in these loci with observed heterozygosities ranging from 0.57 to 0.82 (Table 1). Three loci did exhibit lower observed heterozygosities than expected from Hardy-Weinberg expectations (Table 1). However, these reductions are not due to the presence of null alleles as none were detected and are most likely due to the sampling strategy of the current screen. For example, some populations were determined to be out of HWE at multiple loci. Consequently, these deviations from HWE are most likely not due to locus-specific processes (i.e., presence of moderate frequency null alleles, large allele drop out) but rather to other population specific processes (i.e., localized inbreeding, or selection) that specifically affect a subset of the populations included in this screen.

4.2. Preliminary characterization of range-wide genetic structure

In general, our analyses revealed a moderate amount of range-wide population differentiation ($F_{ST} = 0.09$, $p < 0.001$). Even though this level of differentiation is considered moderate (Hartl, 1980), it is higher than anticipated given the reproductive biology (e.g., obligate out crosser, insect pollinated) of *P. tridentata*. This suggests that historical processes such as genetic drift among populations and migration during glacial cycles have played an important role in defining *P. tridentata*'s current genomic composition.

Interestingly, we have a case where two geographically proximate populations (PUTR79 and PUTR115) are significantly genetically differentiated ($F_{ST} = 0.04$, $p < 0.002$) to a higher magnitude than many other pairwise population comparisons over much greater geographic distances (Table 2). Pollinator movement among populations may be restricted in certain regions due to anthropocentric activities (Ries et al., 2001) and this may lead to reduced gene flow among populations and concomitant increase in population differentiation.

4.3. Cross-species utility

As is common with cross-species amplifications of microsatellite markers, we observed a marked reduction in PCR success as these primers were tested in *P. glandulosa* and *P. stansburiana*. Based on our limited sampling, *P. glandulosa* appeared to show the greatest success, with 46 alleles scored from seven loci. Locus 1-18 failed to work for this species, and locus 1-48 showed reduced, albeit insignificant (one-tailed $p = 0.13$; results not shown), allelic diversity. PCR success from *P. stansburiana* was further reduced, with 28 alleles scored from five variable loci. This reduction was anticipated, because this species has been historically treated as a member of a different genus (formerly *Cowania stansburiana* Torr.), and it is likely more genetically divergent.

Based on these results, we predict that the eight microsatellites developed for *P. tridentata* could be seamlessly integrated into studies of *P. glandulosa*, which is also an important restoration species. It seems likely that a subset of these markers (CT1-48, CT2-18, CT1-39, 1-18) may also have some utility in characterizing genetic diversity and population processes in *P. stansburiana* and its threatened relative, the Arizona cliffrose (formerly *Cowania sub-integra* Kearney; now believed to be a stabilized hybrid from *P. stansburiana* × *P. pinkavae*; (Travis et al., 2007). Because many of

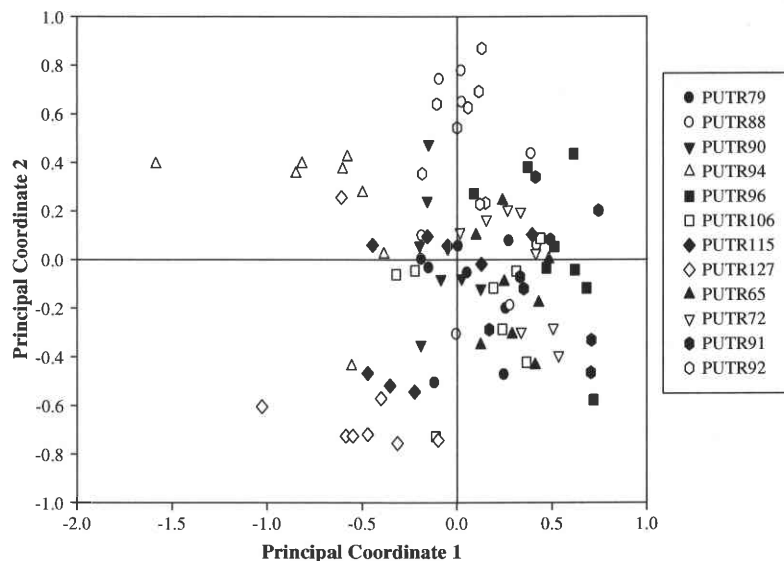


Fig. 2. Results of PCoA analysis. Plot shows the first two axes of the principal coordinate analysis (PCoA), PC1 and PC2 explain 25.8% and 21.4% of the observed variability respectively.

these species are known to hybridize and intergrade (Stutz and Thomas, 1964; Travis et al., 2007), some care is warranted in applying these markers in hybrid zones, as amplification success will be highly dependent upon the extent of introgression for these loci, and null alleles could be frequent. In this context, locus 1-18 may be the most informative single marker for examining long-standing questions of the frequency and direction of introgression between *P. tridentata* and other sympatric *Purshia* species.

4.4. Implications for arid-land management and restoration

Because of the frequent use of *P. tridentata* in revegetation projects and the fact that most germplasm collections are composed of wild collected seed, there is an urgent need for seed movement guidelines for land managers in the western U.S. We are currently addressing this need, and the molecular markers we describe in this study provide essential measures of genetic diversity and differentiation. The pattern of neutral variation inferred from microsatellites (e.g., F_{ST}) can be integrated with quantitative trait variation (e.g., Q_{ST} from common garden studies) to reveal quantitative traits that are adaptive under different environmental conditions (Spitze, 1993; Latta, 1998; McKay and Latta, 2002). Although we have characterized a relatively small number of microsatellite loci, they are highly variable, and as such can provide a statistically robust estimate of F_{ST} (Kalinowski, 2002) as a measure of neutral genetic differentiation on the landscape (see below). In a common garden study we are currently evaluating quantitative traits of >100 *P. tridentata* populations that are exemplary of a variety of environments that *P. tridentata* inhabits. Preliminary

results suggest that the magnitude of among-population differentiation is relatively high ($Q_{ST} \sim 0.57$, R. Johnson, R. Cronn, and M. Horning, unpubl. data) for flowering (transformed as growing degree days). Moreover, the date of seed shatter is highly variable and strongly related to elevation and latitude (Johnson and Berrang, 2007). Upon further analysis these traits may be used to construct seed zones, however, in the context of our preliminary measurements of F_{ST} , additional quantitative traits will need to have high Q_{ST} estimates to be considered adaptive. The relatively high preliminary estimates of F_{ST} reported here have significant implications for adaptive trait identification and subsequent seed zone delineation.

Models of future climate variability and widely anticipated global warming scenarios predict warmer temperatures, reduced precipitation, and increased fire frequency over much of the western U.S. within the next century (Joyce et al., 2001; Bachelet et al., 2003; Lenihan et al., 2007). The predicted effect of these trends will most likely expand the extent of arid-land ecosystems and subsequently increase the range of *P. tridentata* into geographic regions that are currently unoccupied (Joyce et al., 2001). For example, *Pinus ponderosa* arid-forest communities in which *P. tridentata* is an ecologically dominant understory shrub are predicted to expand into areas currently occupied by other conifer and grassland communities (Joyce et al., 2001). Moreover, an accelerated disturbance regime caused by increased wild fire frequency will develop in many western regions (Joyce et al., 2001) and because of *P. tridentata*'s weak fire tolerance, management efforts will intensify. Consequently, the frequency at which *P. tridentata* will be planted (e.g., in post-fire revegetation) will most surely

Table 3

Results of cross-species PCR amplification of eight nuclear microsatellite primer pairs isolated from a *Purshia tridentata* genomic library and tested on *Purshia glandulosa* and *Purshia stansburiana*

Species	Locus							
	CT1-43	CT1-41	CT2-21	CT1-48	CT2-18	1-22	1-18	CT1-39
<i>P. glandulosa</i>	8	8	6	4	8	6	0*	6
<i>P. stansburiana</i>	0*	4*	0*	8	7	3	0*	6
95% CI	5–11	5–11	5–11	3–9	4–10	2–7	6–12	5–11

The number of alleles resolved from a sample of eight individuals per species (four from each of two populations) is shown. The 95% confidence interval derived from *P. tridentata* samples (1000 jackknife replicates) are shown, and results differing from our sample of *P. tridentata* are indicated by an asterisk.

increase and the demand for appropriate germplasm will follow. Therefore, it is essential that we characterize potential germplasm sources for use in restoration. The markers described in this study can be used to define the genetic base of germplasm collections (i.e., quantify the genetic diversity contained within a collection), and in comparisons to the genetic database we have generated, may identify the origins of founding populations.

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