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Population Genetic Structure of *Bromus tectorum* in the American Desert Southwest

Desiree R. Lara

A thesis submitted to the faculty of
Brigham Young University
in partial fulfillment of the requirements for the degree of

Master of Science

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December 2013

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ABSTRACT

Population Genetic Structure of *Bromus tectorum* in the American Desert Southwest

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Following its introduction to North America in the late nineteenth century, *Bromus tectorum* L., an inbreeding invasive winter annual grass, has become dominant on millions of hectares of sagebrush steppe habitat throughout Intermountain Western North America. It appears that within the last 30-40 years, *B. tectorum* has expanded its range southward into the Mojave Desert and also into more climatically extreme salt desert environments. Previous research using microsatellite markers and experimental studies has suggested that lineages found in desert habitats are genetically distinct from those found in the sagebrush-steppe habitat and possess suites of traits that pre-adapt them to these environments. To provide additional support for our hypothesis that desert habitat-specific haplotypes dominate and are widely distributed across warm and salt desert habitats, we genotyped approximately 20 individuals from each of 39 *B. tectorum* populations from these habitats and adjacent sagebrush steppe habitats using 71 single nucleotide polymorphic (SNP) markers. Our data clearly demonstrate that populations throughout the Mojave Desert region, as well as in salt desert habitats further north, are dominated by a small number of closely related SNP haplotypes that belong to the desert clade. In contrast, populations from adjacent environments are largely dominated by haplotypes of the common clade, which is widely distributed throughout the North American sagebrush steppe. Populations across all habitats were usually dominated by 1-2 SNP haplotypes. This suggests that inbreeding *B. tectorum* lineages can often maintain their genetic integrity. It also explains the strong association between marker fingerprints and suites of adaptive traits in this species.

Keywords: *Bromus tectorum*, cheatgrass, single nucleotide polymorphism, SNP genotyping, Mojave Desert, Intermountain West, invasive species, ecological genetics

ACKNOWLEDGMENTS

This work would not be possible without the support of my family, especially my husband, Justin. I thank him for his love, encouragement, and patience. I also thank my parents who were remarkable examples of hard work and dedication and who instilled in me a love for education. I would like to thank my advisors, Dr. Craig Coleman and Dr. Susan Meyer, for providing me with the opportunity to work on this project and for their advice, guidance, and support. I also thank Dr. Jeff Maughan for serving on my graduate committee and for all he has taught me in classes and in the lab. I thank the other faculty and students in the department and lab who have helped me and encouraged me in my projects and my classes.

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INTRODUCTION

The mechanisms that enable an invasive species to expand its range and become dominant after a founder event are intensively studied in ecology. Invasions may be divided into two phases: primary and secondary. In the primary invasion phase, the recently introduced non-native species quickly becomes abundant in a resource-rich, disturbed habitat because of its pre-adapted traits and high propagule pressure (Lockwood et al. 2005; Dietz and Edwards 2006). In the secondary invasion phase, a species experiences invasion resistance due to competition or limiting conditions. However, it is able to further expand its ecological range through selection for increased competitive ability or, in the case of expansion into more extreme habitats, traits associated with stress-tolerance (Dietz and Edwards 2006). Even though the majority of invasive species reach a point where their boundaries become static, many are able to continue to expand their range (Lavergne and Molofsky 2007).

Bromus tectorum L. is one of the most successful and widespread invaders in North America. *Bromus tectorum* is a winter annual grass that was introduced to North America in the late 19th century. It is the most ubiquitous and sometimes most dominant species on western rangelands. It arrived into the interior Pacific Northwest by 1889, most likely as a grain contaminant. By 1930, *B. tectorum* had already largely occupied its current range in sagebrush steppe habitats of the Intermountain West (Mack 1981). *Bromus tectorum* is an example of a non-native plant that has undergone secondary invasion, as it has expanded its range into warm and salt desert habitats (Ramakrishnan et al. 2006; Merrill et al. 2012), apparently within the last 30 to 40 years.

Earlier studies using microsatellite (simple sequence repeat or SSR) markers have shown that *B. tectorum* populations from the apparently more recently invaded warm and salt desert habitats are genetically distinct from *B. tectorum* populations from sagebrush steppe habitats in the historic range (Ramakrishnan et al. 2006; Merrill et al. 2012). More recently it has been established that there are two distinct sets of related lineages of *B. tectorum* in western North America, the desert clade and the common clade (Meyer et al. 2013; Meyer, unpublished data).

Lineages within the common clade are generally better adapted to more mesic sagebrush steppe and upland communities while lineages of the desert clade are better adapted to more xeric warm and salt desert communities. One example of this is vernalization requirement, a trait predicted to be under genetic control. Vernalization requirement is the response to exposure of a plant to chilling conditions that induce or hasten the subsequent development of floral primordia (Chouard 1960). This is a common process in winter annuals because it ensures timely flowering in the spring. Meyer et al. (2004) sought to determine whether adaptively significant variation in vernalization response exists in Intermountain Western *B. tectorum* populations. They found that Mojave Desert lines did not require vernalization to flower, while lines from cold desert, foothill, and montane habitats showed incremental changes in the proportion of plants flowering within 20 weeks, weeks to initiation of flowering, and seed yield per plant as a function of vernalization period.

Merrill et al. (2012) investigated the mechanisms by which *B. tectorum* has achieved its recent secondary range expansion, specifically exploring the role of local adaption. They used patterns of distribution of neutral SSRs to study the roles of adaptively significant genetic variation, adaptive phenotypic plasticity, and the generation of novel genotypes through facultative outcrossing in the *B. tectorum* invasion. They demonstrated that the historic range of

B. tectorum is dominated by broadly adapted, generalist genotypes, while the apparently more recently invaded warm and salt desert range of *B. tectorum* is dominated by unique SSR genotypes that are restricted to specific environments. Common garden studies with these SSR genotypes have demonstrated that the desert specialist genotypes have traits, such as a minimal vernalization requirement and high seed dormancy under summer conditions, that pre-adapt them to warmer and more xeric environmental conditions (Meyer and Allen 1999; Meyer et al. 2004; Meyer et al. 2013).

Because Merrill et al. (2012) did not sample widely in the warm desert, they could not strongly demonstrate that the distribution pattern of the warm desert specialists was a result of sorting into an environment for which they were preadapted or was an accident of dispersal. Because of the small number of SSR loci genotyped and their potential for homoplasy, genetic differences may be masked and some individuals that appear to be highly related may be unrelated. Single nucleotide polymorphic (SNP) markers are a better choice for higher resolution genotyping. We tested our hypothesis that desert clade SNP haplotypes are widely distributed and dominant in warm and salt desert environments across the region, while SNP haplotypes belonging to the common clade are dominant in adjacent habitats. We used 71 neutral SNP markers to genotype approximately 20 individuals in each of 39 populations from across the Desert Southwest to test our hypothesis. We established that there was a genetic distinction between *B. tectorum* lineages from sagebrush steppe habitats and *B. tectorum* lineages from warm and salt desert habitats. We found SNP haplotypes in both sagebrush steppe and warm and salt desert habitats that were identical or nearly identical to SNP haplotypes of previously characterized SSR genotypes. We also confirmed that Mojave Desert populations are dominated by only a few haplotypes.

MATERIALS AND METHODS

Sample Selection and Tissue Production

Mature seed heads were collected from approximately 20 individuals in each of 39 populations in summer 2011 (Table 1). The 39 populations were selected by collecting in the general vicinity of locations where desert specialists had previously been discovered as well as in locations with similar habitats. Individuals were collected randomly, with the constraint that they be at least 1 m apart to reduce the chances of sampling full sibs. GPS coordinates were recorded for each location at the time of sampling. The seeds were allowed to lose dormancy under warm conditions. They were planted under greenhouse conditions and grown to the 4-leaf stage, at which time approximately 1cm^2 of the youngest leaf material was collected and stored at -80°C until DNA extraction.

DNA Extraction

DNA was extracted from the samples using a CTAB method modified from the conditions of Fulton et al. (1995). A microprep buffer was prepared once for every 192 reactions by combining 30 mL DNA extraction buffer (0.35 M sorbitol, 0.1 M Tris-base, 5 mM EDTA, pH 7.5), 30 mL of nuclei lysis buffer (0.2 M Tris, 0.05 M EDTA, 2 M NaCl, 2% CTAB), 12 mL 5% sarkosyl, and 0.172 g sodium bisulfite. Two 4 mm magnetic balls and 350 µL of microprep buffer were added to each sample, and a Geno/Grinder 2000 (OPS Diagnostics, Lebanon, NJ) was used to grind the tissue for 3 minutes at 500 strokes per minute. The samples were centrifuged for 2 minutes at 3500 x g. After they were incubated for 20 minutes at 65°C, 350 µL of chloroform:isoamyl alcohol (24:1) was added to each sample. The samples were shaken several times to mix the layers and centrifuged at 3500 x g for 15 min. After 140 µL of the aqueous phase was added to 100 µL of isopropanol, it was mixed by pipetting and centrifuged at 3500 x g for 20 minutes. The DNA pellet was washed with 200 µL of 70% ethanol and

centrifuged at 3500 x g for 5 minutes. After the sample wells were dry, 100 µL of TE buffer was added and the samples were incubated at 4°C overnight. The samples were stored at -20°C.

SNP Genotyping

A specific target amplification (STA) step was performed prior to SNP genotyping analysis to ensure proper quantities of DNA. For this pre-amplification, 2.5 µL 2X Multiplex PCR Master Mix (Qiagen, Germantown, MD), 0.5 µL 10X STA primers developed by Merrill (2011), 0.5 µL DNase-free water, and 1.5 µL of DNA were combined. The samples were placed in a thermal cycler with an initial denaturing step of 15 min at 95ºC, then 14 cycles of 15 sec at 95ºC, 4 min at 60ºC. After cycling, 1 µL of DNA product was added to 99 µL of DNA suspension buffer (10mM Tris-HCl, pH 8.0, 0.1 mM EDTA, pH 8.0). This final product was used in SNP genotyping analysis.

SNP markers were previously developed using sequence data from 454 pyrosequencing of normalized cDNA obtained from multiple contrasting SSR haplotypes and tissue types (Merrill 2011). A SNP had to meet two criteria to be considered for further analysis: 1) coverage depth at the SNP must be ≥ 10, and 2) the minor allele must represent at least 30% of the alleles observed. Additionally, only SNPs that did not have another SNP within 50 base pairs to either side were considered for possible assay development. Assay development, SNP validation and initial genotyping were carried out using the KASPar system (LGC Genomics, Teddington, England). Ninety-six working KASPar assays were converted for use with the Fluidigm EP1 platform using the 96.96 Dynamic Array™ IFC (Merrill 2011). These SNPs were later examined to determine the likelihood that they could be assumed to be neutral to selection. This resulted in the elimination of 25 markers because they were in coding positions within open reading frames. All individuals were genotyped with the remaining 71 neutral SNP markers using KASP assays

on the Fluidigm EP1 genotyping system following the protocol established by Fluidigm for KASP assays on the 96.96 dynamic array (Fluidigm, San Francisco, CA).

Data Analysis

To characterize relationships among groups of identical or nearly identical individuals, a distance matrix for the 821 genotyped individuals was computed in PHYLIP software using the DNADIST program with the F84 method (Felsenstein 1989) based on the 71 SNP markers. This distance matrix was used to create a UPGMA tree using the default settings in the PHYLIP program NEIGHBOR (Felsenstein 1989) for preliminary assignment of individuals to haplotypes. Haplotypes were defined as groups of at least five individuals with F84 distances less than 0.03. Distances this small were determined to be within SNP genotyping error based on repeated genotyping of reference individuals; they represented differences or missing values at 1-2 SNP loci.

A UPGMA tree was constructed using the methods described above that included only the 19 haplotypes that met the five-individual criterion. SNP haplotypes for nine individuals of known SSR genotype (reference lines) were included in the UPGMA tree, with the objective of determining how high-frequency SNP haplotypes in the present study were related to the SNP haplotypes of known specialist SSR genotypes from Merrill et al. (2012). Each population was examined in terms of its haplotype composition. Groups of identical or nearly identical individuals that were comprised of <5 individuals were grouped together into common mixed and desert mixed haplotype groups in the analysis.

Principal components analysis (PCA; PRINCOMP Procedure, SAS V. 9.2) was used to examine variation in climate among the 39 collection sites. First, nineteen bioclimatic variables were calculated from the Worldclim database (Hijmans et al. 2005) based on the GPS

coordinates for each collection site. These variables were used as input for PCA and 39 collection sites were plotted on a two-dimensional ordination based on their scores on the first two principal components from the analysis. The collection site scores were also used on the first two principal components to calculate the Euclidian climate distance between sites (DISTANCE Procedure; SAS V. 9.2).

Gene diversity statistics were calculated for each population using the program Arlequin 3.5 (Excoffier et al. 2005). These statistics included mean nucleotide diversity and the percentage of polymorphic loci. Analysis of Molecular Variance (AMOVA; Excoffier et al. 1992) was calculated using the standard AMOVA computations in Arlequin 3.5 (Excoffier et al. 2005). AMOVA was calculated first with no secondary structure, then with the populations subdivided into groups in all possible combinations of geographic regions. The relationships among ecological, geographic, and genetic distance were examined using Mantel correlations, which were also calculated using Arlequin 3.5, with 1,000 permutations (Mantel, 1967; Smouse et al., 1986; Excoffier et al., 2005). The genetic distance measure was population pairwise FST from the AMOVA analysis described earlier, the geographic distance measure was calculated from collection site latitude-longitude coordinates using the online utility Geographic Distance Matrix Generator (Ersts 2013), and the ecological distance measure was the Euclidean climate distance described above.

A population tree was also calculated (UPGMA; PHYLIP, Felsenstein, 1989) using a genetic distance matrix based on population pairwise FST from AMOVA as the input matrix. For this analysis, the few slightly negative FST values were assigned a value of zero to avoid reverse branch lengths in the resulting tree.

RESULTS

Haplotype Analysis

The haplotype analysis established that 19 relatively common haplotypes, or groups of at least five individuals with F84 distances less than 0.03, collectively accounted for 81% of the 821 genotyped individuals. The UPGMA tree constructed using genetic distance with 19 haplotypes and nine reference lines from the SSR marker study confirmed the existence of two major clades, the desert clade and the common clade (Fig. 1; Meyer et al. 2013; Meyer, unpublished data). The reference lines included two common SSR genotypes (KCCB and DCBB), two montane SSR genotypes (DABB and GCCB), three salt desert SSR genotypes (IEBB, EZBY, and AEDA) and two warm desert SSR genotypes (FEDD and EBBF). Seven common clade SNP haplotypes (C1-C7) and one salt desert SNP haplotype (SD1) grouped within the common clade. Five salt desert SNP haplotypes (SD2, SD2a, SD3, SD3a, and SD4) and all six warm desert SNP haplotypes (WD1, WD1a, WD1b, WD2a, WD2b, and WD2c) grouped within the desert clade.

When compared with the nine reference lines from the SSR marker study that were included, it was clear that SNP haplotypes in the current study often corresponded directly with previously identified SSR haplotypes (Merrill et al. 2012; Meyer et al. 2013). The SD1 haplotype defined in this study was similar to the SNP haplotype of the IEBB salt desert reference line. It grouped within the common clade and was in the same large subgroup as six of the common clade haplotypes identified in this study and two representative common clade haplotypes from the earlier study. No individuals identical to the SNP haplotype of two montane specialist SSR reference lines (DABB and GCCB) were found, although the common clade haplotype C5

grouped more closely with the SNP haplotype of the GCCB montane reference line than with other common clade haplotypes (Fig. 1).

Within the desert clade, the haplotype defined as WD1 in this study had a SNP haplotype essentially identical to that of the reference line FEDD (Fig. 1). Two closely related haplotypes (WD1a and WD1b) also grouped most closely with the SNP haplotype of the FEDD warm desert reference line. We did not detect any individuals with the same SNP haplotype as the EBBF warm desert reference line, but three new Warm Desert 2 haplotypes (WD2a, WD2b, and WD2c) grouped most closely with this SNP haplotype. The SNP haplotype defined in this study as SD2 was identical to the SNP haplotype of the reference line EZBY, originally identified from populations in the Lahontan Basin of western Nevada. A closely related haplotype (SD2a) was also detected within several geographic regions in this study. The SNP haplotype defined here as SD3 was essentially identical to the SNP haplotype of the AEDA reference line from Cinder Cone Butte in Idaho (Meyer et al. 2013). A close relative of SD3 was designated haplotype SD3a. The haplotype SD4 was not found in the previous study and no haplotypes were found that were related to it to the same degree as other similar haplotypes (e.g. SD3 and SD3a) in the current study, though it clearly fell within the desert clade.

Haplotype distribution analysis revealed that nearly all populations from the Mojave Desert region were entirely composed of haplotypes from the desert clade (Table 2). The exception was the Mesquite Mountain Wilderness population which contained 14% individuals with common clade haplotypes. Six of the fourteen Mojave Desert populations (Kingman Highway, Kelso Junction, Laughlin, Mesquite Mountain Wilderness, Great Basin Highway 2, and South of Hoover Dam) were composed of at least 80% of one warm desert or salt desert

haplotype. The remaining eight Mojave Desert populations consisted of between 30 and 55% of a single warm or salt desert haplotype.

While populations from the Owens Valley region also consisted of mostly desert clade haplotypes, their haplotype distributions were more diverse (Table 2). The Whitney Portal population consisted entirely of the WD1 haplotype and three other Owens Valley populations (Big Pine, Benton, and Gilbert Pass) consisted of at least 74% of this haplotype. The Mono County Line and the Central Owens Valley populations contained a greater variety of haplotypes from both the common clade (19% and 43% respectively) and the desert clade. The Mojave fringe population haplotype distributions were similar to those for the Owens Valley populations (Table 2). Four of the populations, Goldfield, Tonopah, Lida Junction, and Lida Townsite, were dominated by the WD1 haplotype that accounted for between 67% and 95% of the individuals. The Hiko, White River Valley, and Rachel populations were more diverse, containing haplotypes from both the common (70%, 80%, and 28% respectively) and the desert clade. The two populations from the Columbia Basin region were also relatively diverse (Table 2). The Hanford ALE population consisted of 42% salt desert haplotypes. The Hanford Rattlesnake Spring population consisted of 80% salt desert haplotypes.

Haplotype distribution analysis demonstrated that the Chihuahuan fringe populations were dominated by common clade haplotypes (Table 2). Seven of the 10 populations did contain some desert haplotypes, but all had a greater proportion of common haplotypes (between 64% and 96%). The Belen 1 and Belen 2 populations consisted of at least 83% of one common clade haplotype while the remaining populations contained a more diverse array of haplotypes.

WD1 was the dominant haplotype (≥80% of individuals) in five Mojave populations, two Owens Valley populations, and three nearby Mojave fringe populations (Table 2). In all, WD1 was found in 22 of the 39 populations sampled. Two of the 10 Chihuahuan fringe populations contained this haplotype at low frequency, but it was otherwise confined to the Mojave Desert, Owens Valley, and Mojave fringe regions, where it was often the dominant haplotype. WD1 was by far the most frequent haplotype in the study, accounting for 270 individuals or 33% of the total sample. Two additional haplotypes closely related to WD1 were found only in the Mojave Desert region. Haplotype WD1a occurred at frequencies from 12 to 33% in five populations, whereas haplotype WD1b occurred at frequencies from 7 to 53% in eight populations. Three haplotypes closely related to WD2 were found in the Mojave Desert and Mojave fringe regions (Table 2). Haplotype WD2a was frequent (30% -55%) in three Mojave Desert populations. Haplotype WD2b only occurred in one Mojave Desert population (33%). Haplotype WD2c only occurred in two adjacent populations in the Mojave Fringe region.

SD2 was the dominant haplotype (≥ 80%) in one Mojave Desert population (Table 2). In all, SD2 was found in six of the 39 populations. One additional haplotype closely related to SD2, SD2a, was found in two Mojave Desert populations, one Mojave fringe population, four Owens Valley populations, and four Chihuahuan fringe populations. SD3 was found only in the two Columbia Basin populations, where it was moderately frequent (26% - 38%). A closely related haplotype, SD3a, was found in one population in the Mojave fringe region at a frequency of 33%. SD4 was restricted to and frequent in the two Columbia Basin populations (16% - 42%).

Of the common clade haplotypes, SD1 was present in moderate to high frequency (30% - 52%) in three Chihuahuan fringe populations, and one Owens Valley population (Table 2). It occurred in eight of the 39 populations. In addition to SD1, the common clade was represented

by seven related haplotypes. C1 was dominant (≥ 80%) in one Chihuahuan fringe population. It was frequent (48%) in another Chihuahuan fringe population and uncommon (5%) in one Owens Valley population. C2 was frequent (24 – 61%) in two Mojave fringe populations and uncommon (4 – 15%) in two Chihuahuan fringe populations and one Owens Valley population. C3 was dominant (88%) in one Chihuahuan fringe population. It was frequent (27 – 42%) in four Chihuahuan fringe populations and uncommon (4 - 12%) in one Chihuahuan fringe population and three Owens Valley populations. C4 was frequent (40%) in one Mojave fringe population. C5 was frequent (52%) in one Chihuahuan fringe population. C6 was uncommon (6 - 16%) in two Mojave fringe populations. C7 was frequent (32%) in one Chihuahuan fringe population and uncommon (3 – 6%) in two Chihuahuan fringe populations and one Mojave fringe population.

Haplotypes included in the desert mixed group (desert clade haplotypes present at a frequency of <5) accounted for 9.0% of the total sample and 13.7% of the desert clade sample (Table 2). Haplotypes in the common mixed group (common clade haplotypes present at a frequency of <5) accounted for 10.1% of the total sample and 29.4% of the common clade sample.

Genetic Diversity Statistics

The gene diversity analysis showed that populations dominated (≥ 80% of individuals) by one haplotype (Belen 1, Belen 2, Lida Townsite, Lida Junction, Tonopah, Gilbert Pass, Whitney Portal, Kingman Highway, Kelso Junction, Laughlin, Great Basin Highway 2, and South Hoover Dam) had small mean nucleotide diversity statistics (0.017 – 0.196; Table 3). Populations that contained many individuals from both clades such as Hanford ALE, Rachel, Gallup 1, Central Owens Valley, and Mono County Line had larger mean nucleotide diversity statistics (0.305 -

0.345). Populations with few haplotypes that all belonged to a single clade also tended to have lower frequencies of polymorphic loci though the pattern was not as strong as for gene diversity.

Among Population Analysis

The AMOVA revealed that there is strong genetic structure in this group of populations, with a large amount of variance explained by among-population differences (Table 4). In the model with no geographic groups, among-population variance was 50.87% of the total variance while within population variation accounted for 49.13%. The data for the structure with two geographic groups, one consisting of the Mojave fringe, Owens Valley, and Mojave Desert regions and the other group consisting of the Chihuahuan fringe and Columbia Basin regions , is presented (Table 1). This structure resulted in the highest between-group variance. In the model with two groups, among-group variance accounted for 38.77% of the total variance, even though the second group contained two regions that are widely separated geographically. Separating these two groups in the analysis did not increase among group variance (data not shown). Among-population within-group variance accounted for 22.30% and within-population variance accounted for 38.94% of the total variance in this analysis. All components contributed significantly to the variance (P=0.00000) in each AMOVA analysis.

When the scores for each of the 39 population sites on the first two principal components from PCA based on 19 Worldclim bioclimatic variables (Hijmans et al. 2005) were plotted, clear patterns were displayed (Fig. 2). The first principal component accounted for 56.2% of the variation, with strong eigenvector loadings associated with generally decreasing temperature and increasing precipitation. The second principal component accounted for 16.5% of the variation, with strong eigenvector loadings associated with increasing temperature seasonality and increasing summer precipitation. The total spread of climates in this PCA was not very broad

because these climates are all temperate semi-desert climates (Table 5). The populations from the Mojave Desert region, whose climate is warmer and drier, were grouped in the upper left with a spread on the vertical axis that most likely represents variation in summer monsoon frequency (Fig. 2). The populations from the Chihuahuan fringe region clustered in the upper right. These populations have climates that are more cool and wet, with the strongest temperature seasonality and highest summer precipitation. The populations from the Columbia Basin region were found in the lower middle of the plot as they have dry summers and intermediate temperatures and precipitation levels. The populations from the Mojave fringe region and the Owens Valley region clustered in the middle, with the Owens Valley populations further down the vertical axis because their climates have drier summers. The exception to the Mojave fringe region clustering was White River Valley, NV, which was more similar to the Chihuahuan fringe region.

Ecological distance and geographic distance were significantly correlated ($R^2 = 0.284$) and both were significantly correlated with genetic distance (Table 6). The ecological distance variable accounted for 19.0% of the variation in genetic distance while the geographic distance variable accounted for 29.2%. To determine if ecological distance and geographic distance independently contribute some power to explain genetic distance, they were combined into a single analysis. Because they were themselves not highly correlated, this analysis explained somewhat more of the variation in genetic distance (32.3%). When both explanatory variables were included, ecological distance still accounted for 9.0% of the variation, while geographic distance accounted for 23.3%. All Mantel correlations were highly statistically significant (P=0.0000).

The UPGMA tree constructed using genetic distance based on pairwise FST with all 39 populations demonstrated the existence of two major groups (Fig. 3). The first group contained

the populations from the Chihuahuan fringe region and the Mojave fringe region that were dominated by common clade lines. The second group was comprised of three main branches. The first branch contained a single Mojave Desert population (Mercury), the only population in this study that was dominated by SD2. The upper main branch included the two populations from the Columbia Basin region that grouped together. They both contained high frequencies of the SD3 and SD4 haplotypes. Another sub-branch included mixed clade populations from the Owens Valley, Mojave fringe, and Chihuahuan fringe regions, as well as Mojave Desert populations dominated by the WD2 group of haplotypes (Jean, South Las Vegas, and Kelbaker Road). The lower main branch included the large group of Mojave Desert, Owens Valley, and Mojave fringe populations that were dominated by the WD1 group of haplotypes.

DISCUSSION

Our study provided further confirmation that *B. tectorum* lineages from warm and salt desert habitats are genetically distinct from *B. tectorum* lineages from sagebrush steppe habitats. The UPGMA tree constructed with 19 common haplotypes substantiated the existence of two major clades among *B. tectorum* individuals in the Intermountain Western United States (Fig. 1). We also found support for our hypothesis that desert clade SNP haplotypes are widespread throughout warm desert habitats in the Mojave Desert. Common clade SNP haplotypes are common in sagebrush steppe habitats in desert-adjacent regions.

In this study we confirmed desert clade haplotype distributions observed in earlier studies and discovered new examples of desert clade haplotypes widely distributed and dominant in warm and salt desert environments. The FEDD SSR genotype that was revealed as dominant in warm desert and transitional habitats in the Mojave and Chihuahuan Deserts in earlier studies

(Ramakrishnan et al. 2006; Merrill et al. 2012) was represented by a corresponding SNP haplotype that was common in 18 warm desert populations in the current study. We also discovered two previously uncharacterized haplotypes that were closely related to the SNP haplotype of the FEDD reference line in the Mojave Desert region. The SNP haplotype of the EBBF reference line, characterized as a warm desert specialist in an earlier study, was not found in the current study, but closely related haplotypes were common in five Mojave Desert and Mojave fringe populations. We found haplotypes that were identical and closely related to the SNP haplotype of the EZBY reference line characteristic of salt desert habitats in the Lahontan Basin of central Nevada in an earlier study (Merrill et al. 2012; Meyer et al. 2013) in moderate to high frequencies in two Mojave Desert populations and one Mojave fringe population. Merrill et al. (2012) identified the original individual with the AEDA SSR genotype at Cinder Cone Butte in Idaho, but did not discuss it specifically because of its rarity. It was later determined to be abundant and increasing in frequency at this location (Meyer et al. 2013). We found this SNP haplotype (SD3) to be common in both Columbia Basin populations. We also identified a new desert clade haplotype characteristic of salt desert habitats in the Columbia Basin (SD4).

We also confirmed common clade haplotype distributions observed in earlier studies and discovered new examples of common clade SNP haplotypes. We found a haplotype very similar to the SNP haplotype of the IEBB reference line characteristic of cold climate salt desert sites in earlier studies (Ramakrishnan et al. 2006; Scott et al. 2010; Merrill et al. 2012; Meyer et al. 2013) in Chihuahuan fringe, Mojave fringe, and Owens Valley populations. We confirmed the existence of common clade haplotypes related to the SNP haplotypes of SSR genotypes characteristic of sagebrush steppe and upland populations (Ramakrishnan et al. 2006; Merrill et al. 2012). We also found one haplotype closely related to the SNP haplotype of the GCCB

reference line that was common and widespread in montane environments in earlier studies (Ramakrishnan et al. 2006; Merrill et al. 2012) We did not detect any haplotypes related to the SNP haplotype of the DABB montane reference line (Ramakrishnan et al., 2006; Merrill et al., 2012).

In this study, we provided support for our hypothesis that xeric habitats throughout the Mojave Desert are dominated by a few inbreeding lineages of the desert clade. The gene diversity analysis reflected the fact that many populations in this study were strongly dominated by a small number of haplotypes from a single clade, whereas other populations consisted of both clades in fairly equal representation (Table 3). Almost all populations in our study were dominated by only a few haplotypes (up to 3 haplotypes). In the Mojave Desert populations, an average of 76.5% of the individuals within a population belonged to between one and three dominant haplotypes. In the Mojave fringe and Owens Valley populations, an average of 78.6% and 80.2% of the respective individuals within a population belonged to either one or two dominant haplotypes. This pattern was also present but usually less pronounced in populations with mixed or primarily common clade haplotypes. In the Chihuahuan fringe population group, an average 56.2% of individuals within a population belonged to either one or two dominant haplotypes, while one population did not have any dominant haplotypes. In the Columbia Basin populations, an average of 53.0% of individuals within a population belonged to one or two dominant haplotypes. Thus, most populations are made up of a small number of dominant haplotypes and variable numbers of less common haplotypes. Even with a 71-marker system, we were still able to detect stable inbreeding lines that have become dominant in each population. It is not probable that multiple individuals would have the same 71-marker SNP haplotype by chance; consequently they are likely descended from a common ancestor. Many of these stable

inbreeding lines with distinctive SNP haplotype fingerprints occurred in multiple populations, sometimes in widely separated geographic regions. This was observed both in populations dominated by the common clade and more dramatically in populations dominated by the desert clade.

One goal of our study was to demonstrate that the distribution pattern of the warm desert specialist haplotypes was a result of sorting into an environment for which they were preadapted. We demonstrated that SNP haplotypes were distributed according to habitat. Warm desert SNP haplotypes were mostly confined to warm desert habitats in the Mojave Desert, Mojave fringe, and Owens Valley regions.

It has been previously demonstrated that the SSR genotypes corresponding to the specialist SNP haplotypes in our study are associated with adaptive traits, including patterns of seed dormancy (Meyer and Allen 1999; Meyer et al. 2004; Scott et al. 2010). Meyer and Allen (1999) found that there was strong genetic control over seed germination in *B. tectorum* and that some genotypes were more plastic in their response to maturation environment than others. Meyer et al. (2004) demonstrated that unlike cold desert, foothill, and montane *B. tectorum* lines, Mojave Desert lines did not require vernalization to flower, a clear adaptive trait for warm desert habitats where winters are short and warm. Rice and Mack (1991) also found significant among-population genetic variation for flowering time within *B. tectorum* populations in eastern Washington. We determined in the present study that the salt desert population in the earlier study is dominated by SD3 and SD4 SNP haplotypes of the desert clade. It was the study of Rice and Mack (1991) that prompted us to include this population. Scott et al. (2010) found that seeds from a western Utah *B. tectorum* cold desert playa population dominated by the SD1 haplotype

were better adapted to high salinity compared to sagebrush steppe populations dominated by other common clade lines.

In our Mantel correlation analysis we found that both geographic and ecological distance variables combined best explained the patterns of genetic differentiation we observed; however, geographic distance contributed more to this explanation than ecological distance. Geographic distance and ecological distance were also significantly correlated with each other. The greater predictive power of geographic distance is probably largely an artifact of sampling because we did not have the full geographic distribution of the common clade in our sample. The Chihuahuan fringe populations were grouped together geographically, were generally similar genetically, and were among the few populations in the study dominated by common clade haplotypes. They also shared a climate closer to the steppe climates further north than to warm desert climates. If we had included more steppe populations from a wider geographic region, the association between ecological and genetic distance would not have been so confounded with geographic distance. We included these New Mexico populations in our sampling because desert clade lineages had been detected in this region in the earlier study (Merrill et al. 2012).

B. tectorum was not sufficiently abundant for rangeland managers to notice its secondary range expansion into warm and salt deserts until quite recently. We do not know, however, when the desert clade lineages that are now dominant in these habitats first arrived. Genotyping fresh material collected from extant populations has proven to be useful in elucidating the mechanism by which *B. tectorum* has invaded desert habitats. Nevertheless, inferences must be drawn about past genotype distributions based on current population structure. Herbarium plant material has recently been established as a valuable genetic resource (Lister et al. 2008). To gain further historical insight on the invasion of new habitats without relying on assumptions about genotype

distribution through time, *B. tectorum* herbarium specimens can be genotyped. A herbarium genotyping study is currently in process to establish the time line for the arrival and spread of these pre-adapted genotypes into the American Desert Southwest.

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TABLES

Table 1. Locations and elevations of 39 *Bromus tectorum* collection sites in the Intermountain West separated into five geographic regions: Columbia Basin, Chihuahuan Fringe, Mojave Fringe, Owens Valley, and Mojave Desert.

Geographic region	Population Name	Latitude	Longitude	Elevation (m)
Columbia Basin Region	Hanford ALE, WA (ALE)	46.493000	-119.658000	186
	Hanford Rattlesnake Spring, WA (RSP)	47.128906	-118.269433	223
Chihuahuan Fringe Region	Belen 1, NM (BLN)	34.675814	-106.771692	1465
	Belen 2, NM (CIR)	34.654545	-106.778418	1466
	Gallup 1, NM (DNY)	35.528598	-108.667243	2017
	Gallup 2, NM (ELC)	35.527506	-108.722211	2004
	Grants, NM (DOW)	35.143402	-107.838666	1972
	Los Lunas, NM (LUN)	34.811533	-106.753898	1480
	Milan, NM (DOT)	35.189228	-107.900339	1999
	Prewitt 1, NM (EDI)	35.363436	-108.046764	2088
	Prewitt 2, NM (SCH)	35.365321	-108.053825	2093
	San Fidel, NM (SKY)	35.076028	-107.555127	2003
Mojave Fringe Region	Goldfield, NV (GDF)	37.794347	-117.233972	1594
	Hiko, NV (HIK)	37.459831	-115.364406	1559
	Lida Townsite, NV (LID)	37.444814	-117.531353	2032
	Lida Junction, NV (LIJ)	37.502394	-117.185094	1434
	Tonopah, NV (MNW)	38.086389	-117.091261	1668
	Rachel, NV (RCH)	37.962700	-116.059294	1488
	White River Valley, NV (WRV)	38.395764	-115.036389	1637
Owens Valley Region	Big Pine, CA (BGP)	37.128906	-118.269433	1202
	Benton, CA (BTN)	37.775708	-118.463608	1614
	Gilbert Pass, CA (GBP)	37.433017	-117.949100	1934
	Mono County Line, CA (MNL)	37.471728	-118.352547	1307
	Central Owens Valley (OWV)	36.813464	-118.211656	1203
	Whitney Portal, CA (WHP)	36.598528	-118.091744	1262
Mojave Desert Region	Baker, CA (BKC)	35.787222	-115.621944	186
	Jean, NV (JNN)	35.811944	-115.382222	1019
	Kelbaker Road, CA (KBK)	34.721944	-115.678333	919
	Kingman Highway, AZ (KMH)	35.196111	-114.433333	784
	Kelso Juction, CA (KSO)	35.176111	-115.509167	1135
	Laughlin, NV (LAU)	35.143611	-114.579722	194
	Mercury, NV (MCY)	36.574167	-115.878611	1091
	Mesquite Mountain Wilderness, NV (MMW)	35.610556	-115.732778	977
	Great Basin Highway 1, NV (MOH)	36.534444	-114.909444	805
	Great Basin Highway 2, NV (MOJ)	36.534444	-114.909444	805
	Pahrump, NV (PRP)	36.263333	-116.003333	813
	South of Hoover Dam, AZ (SHD)	35.490000	-114.363611	909
	South Las Vegas, NV (SLV)	36.024722	-115.173889	689
	Sandy Valley Road, NV (SVR)	35.819167	-115.495000	1279

Table 2. Frequencies of the 19 most common SNP haplotypes in 39 *Bromus tectorum* populations, separated into five geographic regions. The 19 most common SNP haplotypes are salt desert 1 (SD1), common 1-7 (C1-7), warm desert 1 (WD1), warm desert 1a (WD1a), warm desert 1b (WD1b), salt desert 2 (SD2), salt desert 2a (SD2a), salt desert 2b (SD2b), salt desert 3 (SD3), salt desert 3a (SD3a), salt desert 4 (SD4), warm desert 2a (WD2a), warm desert 2b (WD2b), and warm desert 2c (WD2c). Identical and nearly identical groups of individuals that were comprised of less than five individuals were grouped together into common mixed and desert mixed haplotype groups, labeled CM and DS.

Population	CM	SD1	C1	C2	C3	C4	C5	C6	C7	DS	WD1	WD1a	WD1b	SD2	SD2a	SD3	SD3a	SD4	WD2a	WD2b	WD2c	**Total CM**	**Total DS**
Columbia Basin Region																							
Hanford ALE, WA	45	0	0	0	0	0	0	0	0	13	0	0	0	0	0	26	0	16	0	0	0	**45**	**55**
Hanford Rattlesnake Spring, WA	8	0	0	0	0	0	0	0	0	13	0	0	0	0	0	38	0	42	0	0	0	**8**	**92**
Chihuahuan Fringe Region																							
Belen 1, NM	9	4	83	0	0	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0	**96**	**4**
Belen 2, NM	12	0	0	0	88	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**100**	**0**
Gallup 1, NM	8	52	0	0	4	0	0	0	0	4	16	0	0	0	16	0	0	0	0	0	0	**64**	**36**
Gallup 2, NM	17	3	48	14	0	0	0	0	3	3	3	0	0	0	10	0	0	0	0	0	0	**84**	**16**
Grants, NM	62	0	0	0	27	0	0	0	0	4	8	0	0	0	0	0	0	0	0	0	0	**88**	**12**
Los Lunas, NM	30	30	0	15	0	0	0	0	5	20	0	0	0	0	0	0	0	0	0	0	0	**80**	**20**
Milan, NM	4	4	0	0	33	0	52	0	0	0	0	0	0	0	7	0	0	0	0	0	0	**93**	**7**
Prewitt 1, NM	100	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**100**	**0**
Prewitt 2, NM	17	33	0	0	42	0	0	0	0	0	0	0	0	0	8	0	0	0	0	0	0	**92**	**8**
San Fidel, NM	26	0	0	0	42	0	0	0	32	0	0	0	0	0	0	0	0	0	0	0	0	**100**	**0**
Mojave Fringe Region																							
Goldfield, NV	11	0	0	0	0	0	0	0	0	0	67	0	0	11	11	0	0	0	0	0	0	**11**	**89**
Hiko, NV	9	0	0	61	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	30	**70**	**30**
Lida Townsite, NV	4	0	0	0	0	0	0	0	0	9	87	0	0	0	0	0	0	0	0	0	0	**4**	**96**
Lida Junction, NV	0	0	0	0	0	0	0	0	0	5	95	0	0	0	0	0	0	0	0	0	0	**0**	**100**
Tonopah, NV	5	10	0	0	0	0	0	0	0	5	80	0	0	0	0	0	0	0	0	0	0	**15**	**85**
Rachel, NV	17	0	0	0	0	0	0	6	6	6	33	0	0	0	0	0	33	0	0	0	0	**29**	**71**
White River Valley, NV	0	0	0	24	0	40	0	16	0	16	0	0	0	0	0	0	0	0	0	0	4	**80**	**20**
Owens Valley Region																							
Big Pine, CA	0	0	0	0	0	0	0	0	0	13	79	0	0	4	4	0	0	0	0	0	0	**0**	**100**
Benton, CA	0	0	0	0	0	0	0	0	0	16	74	0	0	5	5	0	0	0	0	0	0	**0**	**100**
Gilbert Pass, CA	4	0	0	0	4	0	0	0	0	0	92	0	0	0	0	0	0	0	0	0	0	**8**	**92**
Mono County Line, CA	0	4	0	4	12	0	0	0	0	12	42	0	0	0	23	0	0	0	4	0	0	**16**	**84**
Central Owens Valley	0	33	5	0	5	0	0	0	0	10	0	0	0	10	38	0	0	0	0	0	0	**43**	**57**
Whitney Portal, CA	0	0	0	0	0	0	0	0	0	0	100	0	0	0	0	0	0	0	0	0	0	**0**	**100**
Mojave Desert Region																							
Baker, CA	0	0	0	0	0	0	0	0	0	19	24	14	43	0	0	0	0	0	0	0	0	**0**	**100**
Jean, NV	0	0	0	0	0	0	0	0	0	25	0	0	20	0	0	0	0	0	55	0	0	**0**	**100**
Kelbaker Road, CA	0	0	0	0	0	0	0	0	0	22	0	33	11	0	0	0	0	0	0	33	0	**0**	**100**
Kingman Highway, AZ	0	0	0	0	0	0	0	0	0	6	94	0	0	0	0	0	0	0	0	0	0	**0**	**100**
Kelso Junction, CA	0	0	0	0	0	0	0	0	0	15	85	0	0	0	0	0	0	0	0	0	0	**0**	**100**
Laughlin, NV	0	0	0	0	0	0	0	0	0	20	80	0	0	0	0	0	0	0	0	0	0	**0**	**100**
Mercury, NV	0	0	0	0	0	0	0	0	0	7	0	0	7	87	0	0	0	0	0	0	0	**0**	**100**
Mesquite Mountain Wilderness, NV	14	0	0	0	0	0	0	0	0	19	0	24	43	0	0	0	0	0	0	0	0	**14**	**86**
Great Basin Highway 1, NV	0	0	0	0	0	0	0	0	0	0	45	0	0	0	25	0	0	0	30	0	0	**0**	**100**
Great Basin Highway 2, NV	0	0	0	0	0	0	0	0	0	8	83	0	0	0	4	0	0	0	4	0	0	**0**	**100**
Pahrump, NV	0	0	0	0	0	0	0	0	0	25	10	15	30	20	0	0	0	0	0	0	0	**0**	**100**
South of Hoover Dam, AZ	0	0	0	0	0	0	0	0	0	5	95	0	0	0	0	0	0	0	0	0	0	**0**	**100**
South Las Vegas, NV	0	0	0	0	0	0	0	0	0	21	37	0	11	0	0	0	0	0	32	0	0	**0**	**100**
Sandy Valley Road, NV	0	0	0	0	0	0	0	0	0	24	12	12	53	0	0	0	0	0	0	0	0	**0**	**100**

Table 3. Genetic diversity statistics for 39 *Bromus tectorum* populations included in the study. Nucleotide diversity values are means across loci ± standard deviations.

Population	Sample Size	Mean Nucleotide Diversity	% Polymorphic Loci
Columbia Basin Region			
Hanford ALE WA (ALE)	31	0.345±0.174	86.2
Hanford Rattlesnake Spring WA (RSP)	24	0.255±0.131	77.6
Chihuahuan Fringe Region			
Belen 1, NM (BLN)	23	0.085±0.047	59.4
Belen 2, NM (CIR)	19	0.031±0.020	14.1
Gallup 1, NM (DNY)	25	0.336±0.171	93.8
Gallup 2, NM (ELC)	29	0.229±0.117	98.4
Grants, NM (DOW)	26	0.194±0.101	92.1
Los Lunas, NM (LUN)	20	0.208±0.109	63.5
Milan, NM (DOT)	27	0.232±0.119	89.6
Prewitt 1, NM (EDI)	11	0.131±0.074	39.7
Prewitt 2, NM (SCH)	12	0.204±0.111	91.0
San Fidel, NM (SKY)	31	0.149±0.078	56.5
Mojave Fringe Region			
Goldfield, NV (GDF)	18	0.211±0.111	78.6
Hiko, NV (HIK)	23	0.213±0.111	63.5
Lida Junction, NV (LIJ)	20	0.030±0.020	11.1
Lida Townsite, NV (LID)	19	0.141±0.076	81.5
Rachel, NV (RCH)	18	0.311±0.161	90.3
Tonopah, NV (MNW)	21	0.196±0.103	76.9
White River Valley, NV (WRV)	24	0.172±0.090	78.5
Owens Valley Region			
Big Pine, CA (BGP)	23	0.079±0.045	54.1
Benton, CA (BTN)	20	0.126±0.068	50.8
Gilbert Pass, CA (GBP)	26	0.098±0.054	75.0
Mono County Line, CA (MNL)	25	0.305±0.155	90.6
Central Owens Valley, CA (OWV)	19	0.337±0.174	80.9
Whitney Portal, CA (WHP)	22	0.017±0.013	9.2
Mojave Desert Region			
Baker, CA (BKC)	20	0.123±0.067	48.4
Jean, NV (JNN)	20	0.231±0.120	65.1
Kelbaker Road, CA (KBK)	18	0.224±0.118	61.9
Kingman Highway, AZ (KMH)	17	0.032±0.021	19.4
Kelso Junction, CA (KSO)	13	0.114±0.064	58.2
Laughlin, NV (LAU)	10	0.185±0.103	58.2
Mercury, NV (MCY)	15	0.094±0.053	53.0
Mesquite Mountain Wilderness, NV (MMW)	20	0.190±0.100	63.5
Great Basin Hwy 1, NV (MOJ)	19	0.208±0.109	47.0
Great Basin Hwy 2, NV (MOH)	22	0.090±0.050	65.2
Pahrump, NV (PRP)	19	0.252±0.131	85.7
South of Hoover Dam, AZ (SHD)	21	0.077±0.043	59.1
South Las Vegas, NV (SLV)	18	0.243±0.127	78.1
Sandy Valley Road, NV (SVR)	17	0.134±0.073	57.8

Table 4. Analysis of molecular variance (AMOVA) with no secondary structure and with populations divided into two geographic groups, one that includes the Mojave Desert, Mojave fringe and Owens Valley regions, and the other that includes the Chihuahuan fringe and the Columbia Basin regions.

Structure	Source of variation	df	Sum of squared deviations	Variance components	Variation Percentage	Significance
No geographic groups	Among populations	38	4822.606	5.88169	50.87	0.000
	Within populations	766	4351.359	5.68063	49.13	0.000
Group 1: Mojave Desert, Mojave Fringe, and Owens Valley Regions. Group 2: Chihuahuan Fringe and Columbia Basin Regions.	Among groups	1	2140.141	5.65628	38.77	0.000
	Among populations within groups	37	2682.466	3.25307	22.30	0.000
	Within populations	766	4351.359	5.68063	38.94	0.000

Table 5. Climate variables from Worldclim (Hijmans et al. 2005) for 39 collection sites included in this study.

Geographic Region	Population	Mean Annual Temperature	Mean Annual Precipitation (mm)	Summer Precipitation (mm)	Mean Summer Temperature	Mean Winter Temperature
Columbia Basin Region	Hanford ALE, WA (ALE)	11.6	180	24	22.0	1.3
	Hanford Rattlesnake Spring, WA (RSP)	9.0	312	44	18.9	-0.7
	Region mean:	**10.3**	**246.0**	**34.0**	**20.5**	**0.3**
Chihuahuan Fringe Region	Belen 1, NM (BLN)	13.0	215	88	23.6	2.5
	Belen 2, NM (CIR)	13.0	214	88	23.6	2.5
	Gallup 1, NM (DNY)	9.6	278	102	20.2	-0.6
	Gallup 2, NM (ELC)	9.5	280	102	20.1	-0.6
	Grants, NM (DOW)	10.0	249	110	20.5	-0.5
	Los Lunas, NM (LUN)	12.9	216	87	23.7	2.4
	Milan, NM (DOT)	9.7	251	110	20.2	-0.8
	Prewitt 1, NM (EDI)	9.6	274	111	20.0	-0.5
	Prewitt 2, NM (SCH)	9.6	276	111	20.0	-0.5
	San Fidel, NM (SKY)	10.9	244	105	21.4	0.8
	Region mean:	**10.8**	**249.7**	**101.4**	**21.3**	**0.5**
Mojave Fringe Region	Goldfield, NV (GDF)	11.4	140	38	22.5	1.0
	Hiko, NV (HIK)	11.7	232	55	22.2	2.1
	Lida Townsite, NV (LID)	8.9	182	45	19.1	-0.5
	Lida Junction, NV (LIJ)	12.8	131	34	24.1	2.2
	Tonopah, NV (MNW)	10.6	139	40	21.4	0.6
	Rachel, NV (RCH)	11.7	185	46	22.5	1.7
	White River Valley, NV (WRV)	9.7	249	62	20.5	-0.4
	Region mean:	**11.0**	**179.7**	**45.7**	**21.8**	**1.0**
Owens Valley Region	Big Pine, CA (BGP)	14.1	168	15	24.3	4.4
	Benton, CA (BTN)	10.3	195	33	20.1	1.1
	Gilbert Pass, CA (GBP)	9.0	179	39	19.2	-0.2
	Mono County Line, CA (MNL)	12.8	156	20	22.9	3.4
	Central Owens Valley (OWV)	14.6	176	14	24.8	4.9
	Whitney Portal, CA (WHP)	14.1	200	17	24.3	4.6
	Region mean:	**12.5**	**179.0**	**23.0**	**22.6**	**3.0**
Mojave Desert Region	Baker, CA (BKC)	18.1	118	32	29.0	7.8
	Jean, NV (JNN)	16.8	153	41	27.4	6.8
	Kelbaker Road, CA (KBK)	18.9	159	45	29.0	9.6
	Kingman Highway, AZ (KMH)	19.0	154	38	29.5	9.2
	Kelso Junction, CA (KSO)	17.2	190	52	27.4	7.8
	Laughlin, NV (LAU)	22.5	99	23	33.6	11.8
	Mercury, NV (MCY)	15.9	151	38	26.5	6.1
	Mesquite Mountain Wilderness, NV (MMW)	17.2	142	39	27.8	7.2
	Great Basin Highway 1, NV (MOH)	17.5	132	32	28.7	7.1
	Great Basin Highway 2, NV (MOJ)	17.5	132	32	28.7	7.1
	Pahrump, NV (PRP)	17.1	116	27	28.0	6.7
	South of Hoover Dam, AZ (SHD)	17.8	158	41	28.4	8.1
	South Las Vegas, NV (SLV)	18.9	113	28	30.2	8.3
	Sandy Valley Road, NV (SVR)	14.6	197	54	25.0	5.0
	Region mean:	**17.8**	**141.2**	**36.5**	**28.5**	**7.7**

Table 6. Mantel correlations among genetic distance, ecological distance, and geographic distance for 39 populations of *Bromus tectorum* included in this study.

Predictor variable	Response variable	Correlation coefficient	Significance
Genetic distance	Ecological distance	0.190	0.000
Genetic distance	Geographic distance	0.292	0.000
Geographic distance	Ecological distance	0.284	0.000
Genetic distance	Geographic and ecological distance	0.323	0.000
	Ecological distance	0.090	0.000
	Geographic distance	0.233	0.000

FIGURES

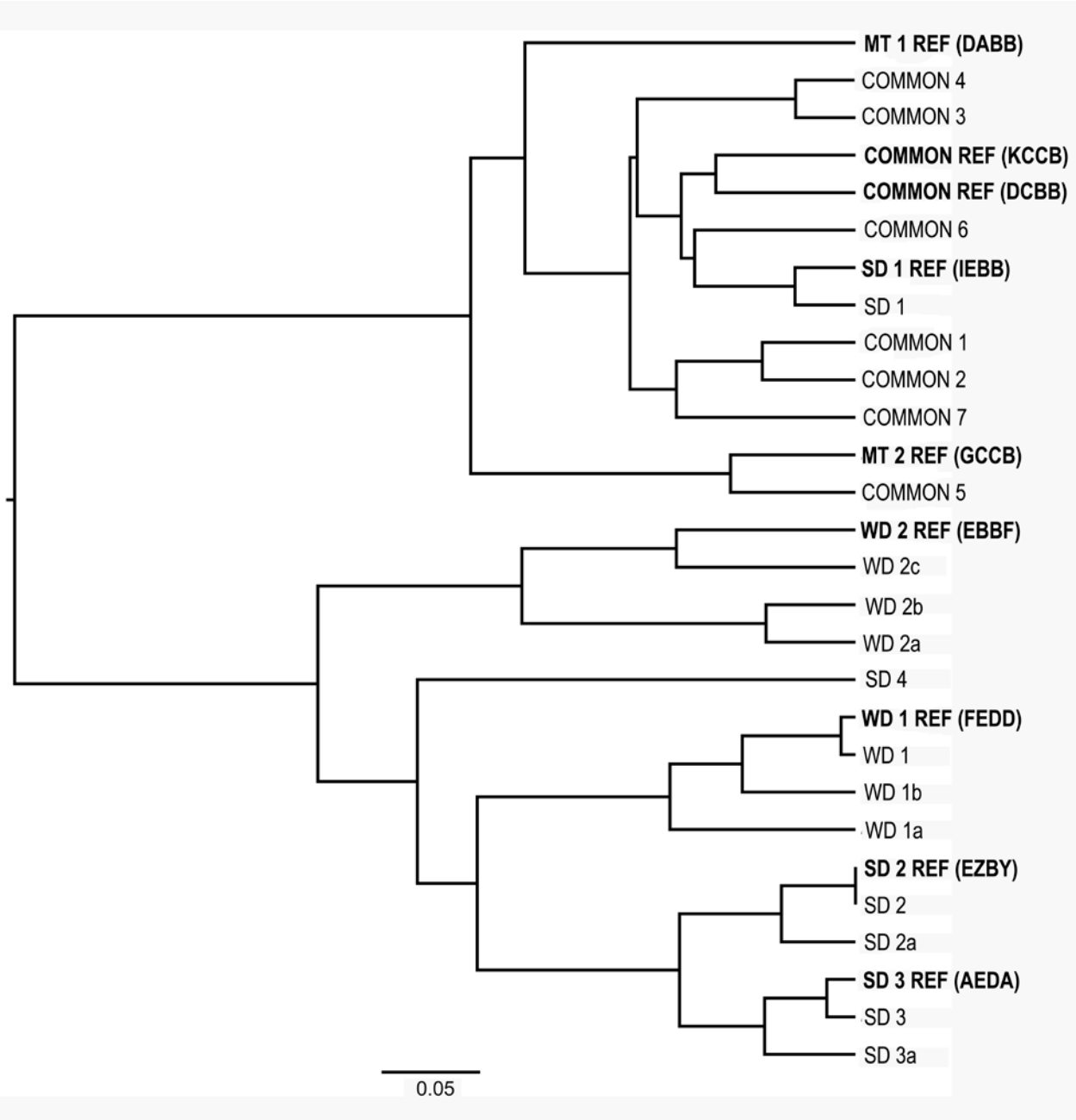


Figure 1. UPGMA tree constructed using genetic distance based on F84 branch length, showing the degree of similarity among 19 haplotype groups and 9 SNP haplotypes of reference lines from the SSR marker study (Merrill et al., 2012). The 19 most common SNP haplotypes are salt desert 1 (SD1), common 1-7 (C1-7), warm desert 1 (WD1), warm desert 1a (WD1a), warm

desert 1b (WD1b), salt desert 2 (SD2), salt desert 2a (SD2a), salt desert 2b (SD2b), salt desert 3 (SD3), salt desert 3a (SD3a), salt desert 4 (SD4), warm desert 2a (WD2a), warm desert 2b (WD2b), and warm desert 2c (WD2c). The SNP haplotypes of the reference lines are indicated in bold.

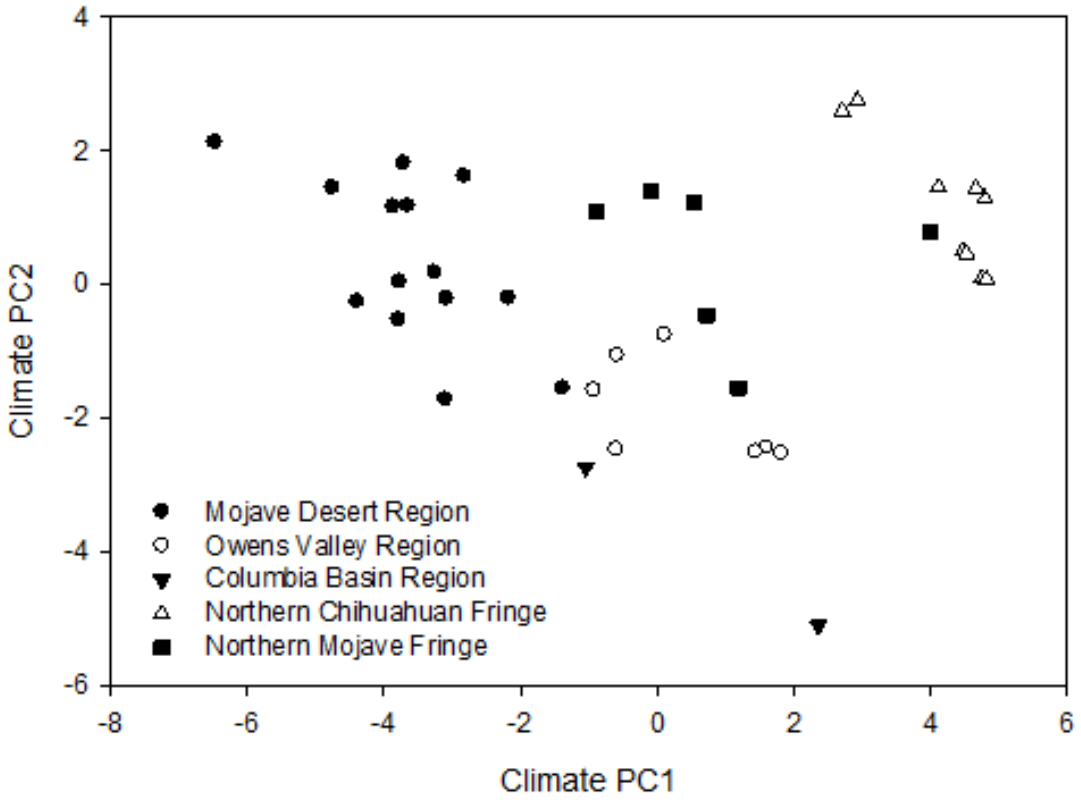


Figure 2. Scores of each of 39 population collection locations on the first two principal components from principal components analysis based on 19 Worldclim bioclimatic variables for each location (Hijmans et al., 2005).

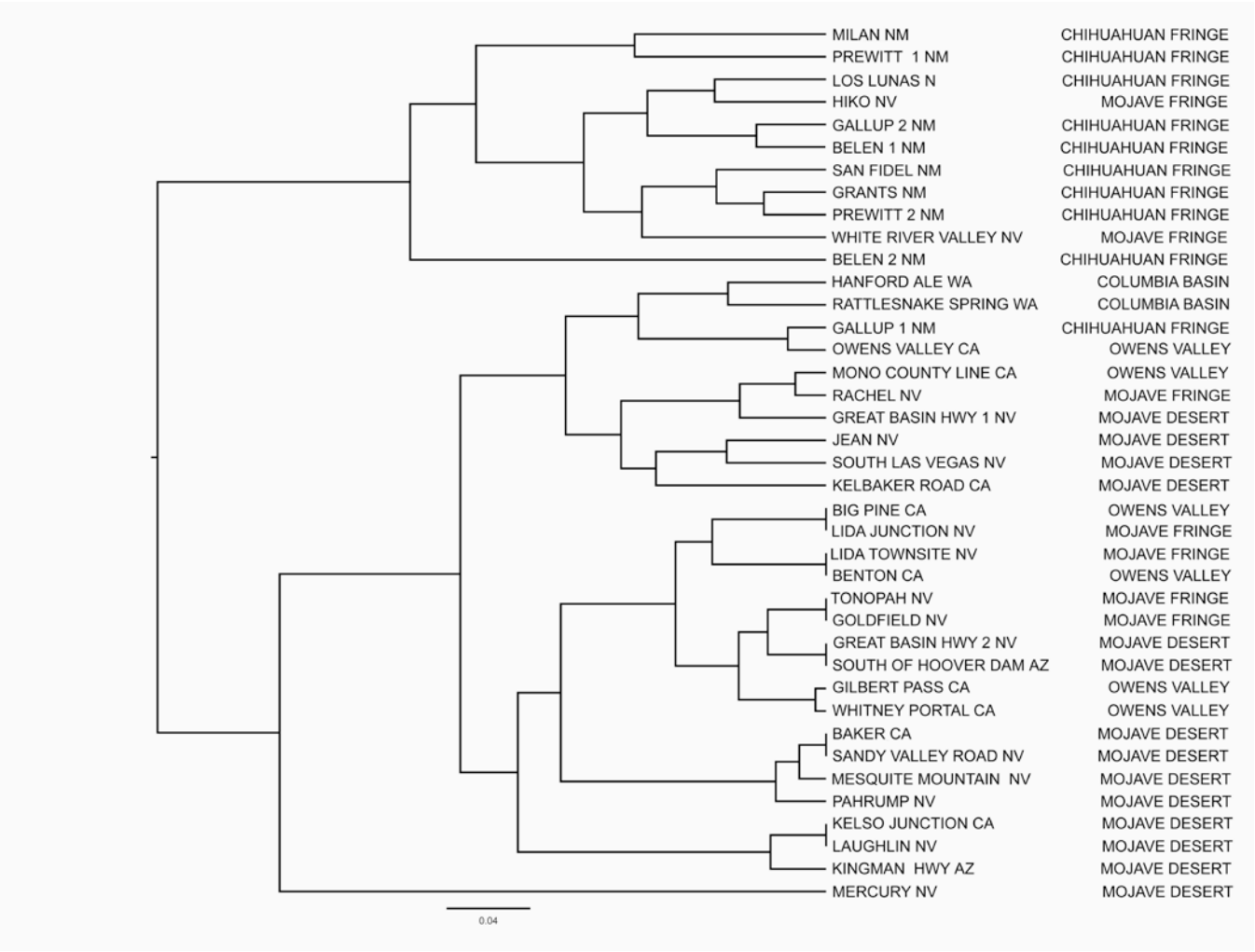


Figure 3. UPGMA tree constructed using genetic distance based on pairwise FST, showing the degree of genetic similarity among 39 *B. tectorum* populations from five geographic regions.

APPENDIX A

Bromus tectorum SNP Genotyping Master Protocol

This protocol was developed to eliminate inaccuracies when new students are introduced to our laboratory. It describes how our data is generated starting with DNA extraction through SNP genotyping.

DNA Extraction

Time: Approximately 4 hours.

Before beginning DNA Extraction you should have plant samples (about 1cm^2) collected into a 12x8 cluster tube rack (Corning: Fisher #07-200-321). Well H12 is usually left empty to serve as a negative control.

1. Turn on the incubator and set it to 65°C so it has time to warm up before you use it in step 7.
2. If two plates are being run, use the amount of reagents for 204X in the table below. If one plate is being run, use the amount of reagents for 102X. Pour the DNA extraction buffer into a beaker. Dissolve the sodium bisulfite completely in the DNA extraction buffer, and then add the nucleic lysis buffer and the 5% sarkosyl. Always make this mixture fresh on the day of extraction.

Reagents	1X	5X	102X	204X	306X
DNA extraction buffer	0.14706 mL	0.7353 mL	15.0 mL	30.0 mL	45.0 mL
Sodium bisulfite	0.000843 g	0.004215 g	0.086 g	0.172 g	0.257 g
Nucleic lysis buffer	0.14706 mL	0.7353 mL	15.0 mL	30.0 mL	45.0 mL
5% Sarkosyl	0.058824 mL	.29412 mL	6.0 mL	12.0 mL	18.0 mL

3. Add two 4 mm autoclaved magnetic balls to each cluster tube.

4. Add 350 µL DNA extraction-lysis mixture to each cluster tube using a multi-channel pipette. You can do four tubes at a time. Cap the tubes (Corning: Fisher #07-200-323) and place a folded piece of paper towel on top of the caps to cushion the tubes. Close the lid of the cluster tube rack and tape it shut.
5. Place the cluster tubes with racks in the GenoGrinder and grind the plant tissue at 500 strokes per minute for 3 minutes.
6. Weigh the two plates you are using (even if one is just to balance) and make sure they are within 2 grams of each other. If they are not within 2 grams of each other, add paper towel to the top of the cluster tubes until they are. Centrifuge at 3500xG for 2 minutes to reduce foam.
7. Incubate at 65°C for 20 minutes.
8. In the hood, carefully remove the caps because they will need to be reused. Add 350 µL of chloroform:isoamyl alcohol with a multi-channel pipette. Carefully add one 8-well row at a time to avoid melting the plastic.
9. Replace caps and place a folded piece of paper towel on top of the caps to cushion the tubes. Close the lid of the cluster tube rack and tape it shut. Shake several times to mix the layers.
10. Weigh the two plates to make sure they are within 2 grams of each other. Centrifuge at 3500xG for 15 minutes. Meanwhile, add 100 µL of isopropanol to each well of a 96-well round-bottom microtiter plate.
11. In the hood, transfer 140 µL of the aqueous phase to the round-bottom microtiter plate with the isopropanol. Mix well by pipetting up and down 3 to 5 times.
12. Centrifuge at 3500xG for 20 minutes. Decant the supernatant.

13. Wash DNA pellet with 200 µL of 70% ethanol. Centrifuge at 3500xG for 5 minutes.
14. Pour off the supernatant. Dry the plate on paper towel. Place in SpeedVac at 60°C for 15 to 20 minutes until all of the wells are dry.
15. Add 100 µL of 1/10 TE Buffer. Leave in 4°C fridge overnight to incubate. Keep sample in -20°C freezer until further use.
16. Quantify the amount of DNA in each sample using a NanoDrop Spectrophotometer. There must be at least 60 ng/µL of DNA for the Fluidigm reaction to work correctly. If the concentration of DNA is too low, run an STA reaction on the samples.

DNA Amplification

Time: Approximately 4.5 hours.

This step is optional and is used to determine if your extracted DNA is of good enough quality that it is able to be amplified. If it is able to be amplified, it is likely able to be genotyped using a Fluidigm chip.

PCR

1. Make assay mix:

Component	Volume per reaction	Total Volume (x100 for ease of pipetting)
Qiagen 2X Multiplex PCR Master Mix (Cat No. 206143)	5 µL	500 µL
10X STA Primers	1 µL	100 µL
DNase-free Water	1 µL	100 µL

2. Combine 7 µL of assay mix with 3 µL of DNA in each well.
3. Run in the thermal cycler with the following conditions:
    a. Hold: 15 minutes at 95°C
    b. Cycle 35 times: 15 seconds at 95°C, 4 minutes at 60°C

Gel Electrophoresis

96 samples can be observed on three 100 mL agarose gels.

1. Mix 1 g agarose with 100 mL buffer. Microwave in 30 second increments until boiling.
2. Add 5 µL of ethidium bromide.
3. Let liquid cool to 60°C. Meanwhile, tape a gel box so no liquid will leak out.
4. Pour cooled liquid into box and add combs. Let it sit for 20-30 minutes until it has completely solidified.
5. If a ladder has not already been mixed with loading dye, it can be prepared using the following quantities:

Components	Volume
Ladder	1.5 µL
Loading Dye	1 µL
ddH_2O	3.5 µL
Total	6 µL

6. Add 6 µL of ladder to the first well of each line in the gel.
7. For each 5 µL of sample, add 1 µL of loading dye. Mix well by pipetting. Then add 8 µL of sample to the wells of the gel.
8. After the gel is loaded, place the agarose gel into the electrophoresis unit. Make sure that the gel is covered by buffer. Run the gel for 25-30 minutes at 120 V until the dye line is approximately 70-80% of the way down the gel.
9. Open the QuantityOne software on the gel imaging computer, choose “Basic” on the window that appears, and under the File menu select “GelDoc”.

10. Slide the gel out of the box onto the bottom section of the Bio-Rad imaging device. Open the top portion and position the gel so you will be able to see the whole thing on the screen. You can zoom in and out with the buttons on the machine. Close the top portion and turn on the UV light on the Bio-Rad imaging device.
11. To view the gel, choose either the auto expose or the manual expose option and change the amount of time under "Exposure Time" until the gel exposure appears and is clear.
12. After enough exposure time, you can select "Video Print" to automatically print a copy of the gel. To save the image, select "Save" and then under the File menu choose Export to "TIFF."

Specific Target Amplification (STA) Reaction
Time: Approximately 2-2.5 hours.

Specific Target Amplification (STA) is done to prepare the DNA for the Fluidigm reaction. It amplifies the specific regions surrounding the SNPs using STA primers.

1. Make the STA pre-mix:

Component	Volume	Mix for 96-well plate with overage (x110)
Qiagen 2X Multiplex PCR Master Mix (Cat No. 206143)	2.5 µL	275.0 µL
10X STA Primers	0.5 µL	55.0 µL
DNase-free water	0.5 µL	55.0 µL
Total	**3.5 µL**	**385.0 µL**

2. Combine the 3.5 µL of STA pre-mix with 1.5 µL of your genomic DNA into a 96-well PCR plate.

3. Thermal Cycle:

	Hold	**14 Cycles**	
Temperature	95°C	95°C	60°C
Time	15 minutes	15 *seconds*	4 minutes

4. Add 99 µL of 1/10 TE Buffer to each well in a 96-well PCR plate. When thermal cycling is complete, add 1 µL of DNA sample to the corresponding well in the new PCR plate.

Fluidigm Run

Time: Approximately 6 hours.

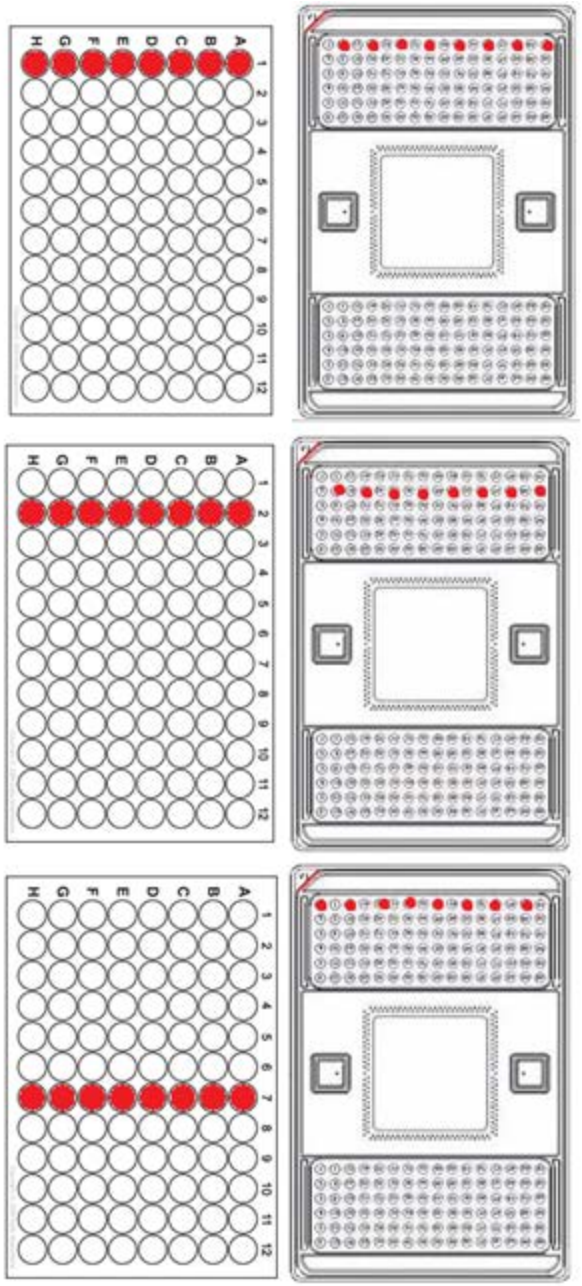
1. Prepare the pre-assay mix. It is important for the reagents to be completely thawed and vortexed.

Component	Volume per inlet + overage	Mix for 96-well plate with overage (x110)
2X Assay Loading Reagent (Fluidigm, PN 85000736)	5.0 µL	550.0 µL
DNase-free water	3.5 µL	396.0 µL
Total	**8.5 µL**	

2. Combine 8.5 µL of the pre-assay mix with 1.5 µL of each of the 96 SNP Primers, which are labeled "KASP Assay Primers October 2013" in the -20°C freezer, for a total of 10 µL of 10X assay mix. This 10X assay mix can be used for two Fluidigm chips. Make sure there are no bubbles in any of the wells of the 96-well plate.
3. Insert control-line fluid into the wells with rubber stoppers on either side of the chip.
4. Insert the chip into the Fluidigm Loader and prime the chip. This will take approximately 20 minutes.
5. While the chip is being primed, prepare the pre-sample mix. It is important for the reagents to be completely thawed and vortexed.

Component	Volume per inlet + overage	Mix for 96-well plate with overage (x110)
KASP 2X Reagent Mix (KBioscience, PN KBS-1004-001)	3.0 µL	330.0 µL
GT Sample Loading Reagent (20X) (Fluidigm, PN 85000741)	0.3 µL	33.0 µL
DNase-free water	0.2 µL	22.0 µL
Total	**3.5 µL**	

6. Combine 3.5 µL of the pre-sample mix with 2.5 µL of DNA for a total of 6 µL of sample mix. Make sure there are no bubbles in any of the wells of the 96-well plate.
7. Thoroughly mix the 10X assay mix and the sample mix before pipetting into the chip inlets.
8. Pipette 4.5 µL of 10X assay mix into each assay inlet. When pipetting, only go down to the first stop to avoid introducing air into the chip inlets. The first row (A1-H1) should be pipetted into every other well in the first line of assay inlets. The second row (A2-H2) should be pipetted into every other well in the second line of assay inlets. Continue pipetting this way for the first 6 lines, then the seventh row (A7-H7) should be pipetted into every other well, starting with the second well, in the first line of assay inlets. Continue pipetting this way for the remaining 5 lines. (See figure below).



9. Pipette 5.5 µL of sample mix into each of the sample inlets in the same pattern as in the assay inlets.

10. Make sure there are no bubbles in any of the chip inlets. They will cause loading failures. Bubbles can be removed using clean pipette tips or alcohol vapor.

11. Make sure the surface of the chip is clean. Dust can be removed using scotch tape. Liquid or dried liquid can be cleaned using a damp kimwipe.
12. Insert the chip into the Fluidigm Loader and load the chip. This will take approximately 90 minutes.
13. After the chip has finished in the Fluidigm Loader, insert it into the Fluidigm Thermal Cycler and set the program to "KASP Touchdown -8". This will take approximately 2 hours.
14. Read the chip using the EP1 machine:
    a. Open the "EP1 Data Collection" Program. Click on "Start a New Run". The tray in the EP1 machine should open.
    b. Load chip into the EP1 machine. Click on "Load". The tray in the EP1 machine should close. Click "Next".
    c. Enter a New Chip Run Name and choose where you want your chip run data to be saved. Click "Next".
    d. Choose the probes manually. Choose "FAM_MGB" for probe 1 and "VIC-MGB" for probe 2. Click on "Next".
    e. Make sure the box next to "Auto Exposure" is checked. Click "Next".
    f. Click on "Start Run". This will take 6-7 minutes. If the lamp is not warmed up, allow it to warm up.
15. After reading the chip, insert it into the Fluidigm Thermal Cycler again and set the program to "KASP Touchdown Extra Cycles". This will take approximately 15 minutes.
16. Repeat steps 14 and 15 three more times to get a total of four reads to compare.

Reading and Exporting Fluidigm Data

Reading Fluidigm Data

1. Open the "Fluidigm SNP Genotyping Analysis" Program. Click on "Open a Chip Run" on the left side of the screen, choose the run that will be analyzed, and open the "ChipRun.bml" file associated with that run.
2. Set up the samples. Click on "Sample Setup" on the left side of the screen. Select "New" and a new window will pop up. The container type should say SBS Plate and the Container format should say SBS96, and then click "Update". Under "Mapping" select the "…" icon. A new window will appear. Choose "M96-Sample-SBS96". You can then input the names of your samples by double clicking on the corresponding square on the plate in the right window.
3. Set up the assays. Click on "Assay Setup" on the left side of the screen. Select "Import" and then choose the file "Assay Plate 10.15.2013.plt". This file can be found on the M drive under Dr. Coleman's Cheatgrass folder in Desiree's folder.
4. To observe the ROX, click on "Detail Views" and then select "Image View" from the top menu. Then choose "ROX" from the drop down menu and 1 from the drop down menu next to it. The red grid lines can be turned off by clicking the button that has a square with 4 red Xs in the corners. You can then determine if the ROX looks uniform. If there are lines that are significantly brighter than others, the reagents were not adequately mixed and they can be marked as invalid. If there are lines or wells that are missing, then there was a loading failure and they can be marked as invalid.
5. To begin calling the samples, you can select the SNP you want to look at on the left side of the screen. Make sure to click the "Analyze" button on the left side of the screen before calling samples. If the reaction worked properly, there should be an

NTC in the bottom left hand corner of the grid with up to three clusters of data points: one to the right, one up, and one diagonal from the NTC. Select each cluster by circling the data points and call them as XX if they are further along the x-axis, YY if they are further along the y-axis and XY if they are diagonal from the NTC.

Exporting Fluidigm Data

Fluidigm will export the data into a .csv file that can be opened in Microsoft Excel. The data can then be copied and pasted into a format that is easier to read. To export, analyze the data a final time and then choose "Export" under the File menu.

Preparing Reagents

Before proceeding with any steps in the process, it is important to be sure that you have enough of each necessary reagent. In most cases if there is not enough, you can just order more, but some of the reagents we can make ourselves.

DNA Extraction Reagents

Extraction Buffer:

	Per Liter:
0.35 M Sorbitol	63.75 g
0.1 M Tris	12.10 g Tris-base
0.005 M EDTA	1.86 g

- Adjust pH to 7.5 with HCl.
- Add Sodium Bisulfite (3.8 g/L or 0.02M) just before use.
- Do not autoclave.
- Store in 4°C refrigerator.

Nucleic Lysis Buffer:

	Per Liter:
0.2 M Tris	24.2 g Tris-base
0.05 M EDTA	18.6 g
2.0 M NaCl	116.9 g
2% CTAB	20.0 g Hexadecyltrimethylammonium bromide

- Adjust pH to 7.5 with HCl.
- Autoclave.
- Store at room temperature.

5% Sarkosyl:

Mix 5 g of N-lauryl sarcosine per 100 mL ddH2O. Sterilize by filtration.

Cloroform:isoamyl alcohol:

In the hood mix isoamyl alcohol and chloroform in a ratio of 4 mL isoamyl to 100 mL chloroform.

1/10 TE Stock:

	Per 100 mL:	Per 200 mL:
10 mM Tris (pH 7.5-8)	1 mL 1M Tris-base, pH 7.5	0.2423 g Tris
0.1 mM EDTA	0.2 mL 0.5 M EDTA	80 µL 25 M EDTA

STA Reaction Reagents

10X STA Primers:

Component	Volume (µL)
100 µM STA primer (for each of the 96 assays)	2 (x96=192 total)
100 µM Constant primer (for each of the 96 assays)	2 (x96=192 total)
TE Buffer	16
Total	400

Here is a list of the constant primers on the left and the STA primers on the right and in which primer plate they can be found.

CR Primers	Plate	Common Reverse Well	STA Primers	Plate	Well
SNP_997_C1	Bt-plate 001	C12	SNP_1_sta	KM1	B9
SNP_751_C1	Bt-plate 001	D9	SNP_1013_sta	KM1	B10
SNP_1930_C1	Bt-plate 001	E6	SNP_1058_sta	KM1	B11
SNP_433_C1	Bt-plate 001	E12	SNP_1064_sta	KM1	B12
SNP_1724_C1	Bt-plate 001	G9	SNP_1125_sta	KM1	C1
SNP_882_C1	Bt-plate 002	A9	SNP_1203_sta	KM1	C2
SNP_2166_C1	Bt-plate 002	C12	SNP_1204_sta	KM1	C3
SNP_2521_C1	Bt-plate 002	F6	SNP_1211_sta	KM1	C4
SNP_129_C1	Bt-plate 002	H6	SNP_1270_sta	KM1	C5
SNP_2773_C1	Bt-plate 003	A9	SNP_129_sta	KM1	C6
SNP_1489_C1	Bt-plate 003	B3	SNP_13_sta	KM1	C7
SNP_2120_C1	Bt-plate 003	B12	SNP_1352_sta	KM1	C8
SNP_887_C1	Bt-plate 003	C12	SNP_1383_sta	KM1	C9
SNP_637_C1	Bt-plate 003	D12	SNP_1388_sta	KM1	C10
SNP_1438_C1	Bt-plate 003	E9	SNP_1398_sta	KM1	C11
SNP_1450_C1	Bt-plate 003	G3	SNP_14_sta	KM1	C12
SNP_1470_C1	Bt-plate 003	H6	SNP_1400_sta	KM1	D1
SNP_696_C1	Bt-plate 004	A3	SNP_1407_sta	KM1	D2
SNP_2834_C2	Bt-plate 004	B4	SNP_1413_sta	KM1	D3
SNP_2807_C1	Bt-plate 004	C7	SNP_1438_sta	KM1	D4
SNP_175_C1	Bt-plate 004	E7	SNP_1450_sta	KM1	D5
SNP_468_C1	Bt-plate 004	E11	SNP_1470_sta	KM1	D6
SNP_505_C1	Bt-plate 004	F3	SNP_1489_sta	KM1	D7
SNP_1383_C1	Bt-plate 004	F7	SNP_1507_sta	KM1	D8
SNP_1640_C1	Bt-plate 004	G11	SNP_1586_sta	KM1	D9
SNP_1352_C1	Bt-plate 004	H7	SNP_1635_sta	KM1	D10
SNP_2142_C2	Bt-plate 005	A4	SNP_1640_sta	KM1	D11
SNP_1400_C1	Bt-plate 005	A7	SNP_1647_sta	KM1	D12
SNP_2409_C1	Bt-plate 005	A11	SNP_1652_sta	KM1	E1
SNP_663_C1	Bt-plate 005	C3	SNP_1724_sta	KM1	E2
SNP_2704_C1	Bt-plate 005	D3	SNP_175_sta	KM1	E3
SNP_904_C1	Bt-plate 005	D7	SNP_1818_sta	KM1	E4
SNP_1652_C1	Bt-plate 005	F7	SNP_1832_sta	KM1	E5

SNP_1058_C1	Bt-plate 005	F11	SNP_1873_sta	KM1	E6
SNP_402_C1	Bt-plate 005	H3	SNP_19_sta	KM1	E7
SNP_853_C1	Bt-plate 006	A3	SNP_1907_sta	KM1	E8
SNP_229_C1	Bt-plate 006	B7	SNP_1930_sta	KM1	E9
SNP_1873_C1	Bt-plate 006	C3	SNP_2120_sta	KM1	E10
SNP_1013_C1	Bt-plate 006	C7	SNP_2141_sta	KM1	E11
SNP_973_C1	Bt-plate 006	C11	SNP_2142_sta	KM1	E12
SNP_2918_C2	Bt-plate 006	D8	SNP_2166_sta	KM2	A1
SNP_1125_C1	Bt-plate 006	D11	SNP_229_sta	KM2	A2
SNP_1413_C1	Bt-plate 006	F3	SNP_232_sta	KM2	A3
SNP_1586_C1	Bt-plate 007	A7	SNP_2399_sta	KM2	A4
SNP_2919_C1	Bt-plate 007	A11	SNP_2409_sta	KM2	A5
SNP_854_C1	Bt-plate 007	B3	SNP_2436_sta	KM2	A6
SNP_2877_C1	Bt-plate 007	B11	SNP_2521_sta	KM2	A7
SNP_583_C1	Bt-plate 007	D7	SNP_2704_sta	KM2	A9
SNP_601_C2	Bt-plate 007	E4	SNP_2773_sta	KM2	A10
SNP_697_C1	Bt-plate 007	E7	SNP_2795_sta	KM2	A11
SNP_1832_C1	Bt-plate 007	E11	SNP_2807_sta	KM2	A12
SNP_874_C1	Bt-plate 007	F7	SNP_2834_sta	KM2	B1
SNP_1818_C2	Bt-plate 007	F12	SNP_2850_sta	KM2	B2
SNP_1388_C1	Bt-plate 007	G7	SNP_2877_sta	KM2	B4
SNP_3142_C1	Bt-plate 007	H3	SNP_2918_sta	KM2	B5
SNP_1507_C1	Bt-plate 008	B11	SNP_2919_sta	KM2	B6
SNP_790_C1	Bt-plate 008	C3	SNP_3025_sta	KM2	B8
SNP_1407_C1	Bt-plate 008	C7	SNP_3049_sta	KM2	B9
SNP_1398_C1	Bt-plate 008	C11	SNP_3142_sta	KM2	B12
SNP_1647_C1	Bt-plate 008	D3	SNP_3285_sta	KM2	C1
SNP_2795_C1	Bt-plate 008	F7	SNP_4_sta	KM2	C2
SNP_1794_C1	Bt-plate 008	G3	SNP_402_sta	KM2	C3
SNP_1270_C1	Bt-plate 008	G7	SNP_433_sta	KM2	C4
SNP_2148_C1	Bt-plate 008	H7	SNP_448_sta	KM2	C5
SNP_605_C2	Bt-plate 008	H12	SNP_449_sta	KM2	C6
SNP_1907_C2	Bt-plate 009	A2	SNP_468_sta	KM2	C7
SNP_1204_C2	Bt-plate 009	A5	SNP_505_sta	KM2	C8
SNP_449_C2	Bt-plate 009	A7	SNP_583_sta	KM2	C9
SNP_2436_C2	Bt-plate 009	A8	SNP_605_sta	KM2	C10
SNP_992_C2	Bt-plate 009	A10	SNP_637_sta	KM2	C11
SNP_1203_C2	Bt-plate 009	B4	SNP_657_sta	KM2	C12
SNP_818_C2	Bt-plate 009	B6	SNP_663_sta	KM2	D1
SNP_2399_C2	Bt-plate 009	B7	SNP_696_sta	KM2	D2
SNP_3025_C2	Bt-plate 009	B9	SNP_697_sta	KM2	D3
SNP_3285_C2	Bt-plate 009	C7	SNP_7_sta	KM2	D4
SNP_657_C2	Bt-plate 009	C11	SNP_751_sta	KM2	D5
SNP_1211_C2	Bt-plate 009	D6	SNP_775_sta	KM2	D7
SNP_2141_C2	Bt-plate 009	D7	SNP_790_sta	KM2	D8
SNP_1654_C2	Bt-plate 009	D8	SNP_818_sta	KM2	D9
SNP_448_C2	Bt-plate 009	E9	SNP_82_sta	KM2	D10
SNP_232_C2	Bt-plate 009	F1	SNP_853_sta	KM2	D11
SNP_775_C2	Bt-plate 009	F2	SNP_854_sta	KM2	D12
SNP_1635_C2	Bt-plate 009	F6	SNP_874_sta	KM2	E1
SNP_1064_C2	Bt-plate 009	F11	SNP_882_sta	KM2	E2
SNP_3049_C2	Bt-plate 009	G2	SNP_887_sta	KM2	E3
SNP_2869_C2	Bt-plate 009	G4	SNP_9_sta	KM2	E4

SNP_82_C2	Bt-plate 009	G9	SNP_904_sta	KM2	E5
SNP_2850_C2	Bt-plate 009	G10	SNP_973_sta	KM2	E6
SNP_718_C2	Bt-plate 009	G12	SNP_992_sta	KM2	E7
SNP_4_C2	Bt-plate 009	H2	SNP_997_sta	KM2	E8
SNP_9_C2	Bt-plate 009	H4	SNP_718_sta	Tube	
SNP_13_C2	Bt-plate 009	H8	SNP_601_sta	Tube	
SNP_19_C2	Bt-plate 009	H12	SNP_2869_sta	Tube	
SNP_1_C1	Tube	Tube	SNP_2148_sta	Tube	
SNP_14_C1	Tube	Tube	SNP_1794_sta	Tube	
SNP_7_C1	Tube	Tube	SNP_1654_sta	Tube	