RESEARCH ARTICLE

AMERICAN JOURNAL OF BOTANY

Population genetic structure of *Bromus tectorum* in the mountains of western North America[1]

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PREMISE OF THE STUDY: Invasive species are often initially restricted to a narrow range and may then expand through any of multiple mechanisms including phenotypic plasticity, in situ evolution, or selection on traits preadapted for new habitats. Our study used population genetics to explore possible processes by which the highly selfing invasive annual grass *Bromus tectorum* has expanded into montane environments.

METHODS: We used 69 single nucleotide polymorphic (SNP) markers to genotype ca. 20 individuals from each of 38 montane cheatgrass populations from throughout the Intermountain West and to identify characteristic SNP haplotypes and examine their distribution.

KEY RESULTS: Five invariant SNP haplotypes were dominant in montane cheatgrass populations, making up 59% of genotyped individuals, with each haplotype present in 12 to 21 populations. Four of these were absent or present at low frequency in low elevation populations, while the fifth was also sometimes dominant at low elevation. Sixteen haplotypes made up 78% of all genotyped individuals. These haplotypes were distributed across several haplogroups within the clade that also includes most sagebrush steppe lineages.

CONCLUSIONS: The wide geographic distribution of several common haplotypes almost completely restricted to montane habitats suggests that dominant lineages in montane populations may possess adaptive syndromes that are preserved through reduced outcrossing rates or negative selection on outcrossed progeny. However, conclusive evidence of such local adaptation requires reciprocal seeding experiments and further characterization of adaptive traits and breeding system characteristics. Other lineages have likely risen to dominance in montane populations through selectively neutral processes.

KEY WORDS cheatgrass; downy brome; invasive species; local adaptation; Poaceae; pre-adaptation; range expansion; selfing; SNP genotyping; spatial sorting

Invasion by alien plant species has had large detrimental impacts on natural ecosystems worldwide (Mack et al., 2000; Pimentel, 2011; Vila et al., 2011). These impacts have often been especially severe with regard to invasive grasses, which can cause major ecosystem degradation and change due to their association with increased fire (D'Antonio and Vitousek, 1992). The winter annual grass *Bromus tectorum* L. has invaded tens of millions of hectares of semiarid rangeland in the western United States and is often the dominant species in postfire environments (Mack, 1981; Bradley and Mustard, 2005). Although the core habitat first occupied and dominated by this species was sagebrush steppe (Mack, 1981), it now occurs over a very wide range of habitats including salt deserts and warm deserts (Scott et al., 2010; Meyer et al., 2016). More recently, it has been documented as increasing in montane habitats as well (Brown and Rowe, 2004; Keeley and McGinnis, 2007; McGlone et al., 2009; Leger et al., 2009). Plant invasion into montane habitats has become a topic of considerable recent interest (Alexander et al., 2016). Mountain environments are often considered to be at relatively low risk of plant invasion, particularly by typical disturbance-responsive invaders, because they present abiotically stressful conditions (Alpert et al., 2000; Pauchard et al., 2009) or offer strong biotic resistance (Levine et al., 2004). One finding at the species level is that the alien flora of montane areas is more closely related to the flora of surrounding lowlands than to the flora of other mountains (Haider et al., 2010; McDougall et al., 2011). This relationship suggests that a species often must pass through a "lowland filter" to arrive in a particular montane environment, which may then constrain its ability to adapt to montane conditions (Haider et al., 2010). There is also evidence, however,

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https://doi.org/10.3732/ajb.1700038

that most montane invaders are generalists with distribution centers in the lowlands, even those found at the highest elevations (Alexander et al., 2011).

Relatively few studies examine patterns of within-species genetic variation in the context of invasion into mountains. Alexander et al. (2009) found contrasting patterns for two species of Asteraceae, with one showing reduced genetic diversity at high elevation, but neither species showed genetic structure related to elevation. In the present study, we examined patterns of genetic diversity and differentiation associated with *B. tectorum* invasion into montane environments in western North America.

Mechanisms for invasive expansion—Understanding the mechanisms by which an invasive species expands into new habitats is critical in reducing and preventing invasion. Invasive species can experience successful expansion through several processes. One process involves phenotypic plasticity, which allows for multiple phenotypic expressions through a single genotype (Richards et al., 2006; Davidson et al., 2011). In situ evolution of novel genotypes through mutation, recombination of existing genotypes through hybridization, or other genotype-altering events provides another expansion mechanism (Facon et al., 2006; Prentis et al., 2008). A third mechanism is facilitated through the presence of genotypes with preadapted traits (i.e., ecotypes) that increase fitness in particular habitats (Dlugosch and Parker, 2007; Bossdorf et al., 2008). The presence of multiple ecotypes with preadapted traits can result from multiple introductions, which have been shown to increase species invasive capability (Lavergne and Molofsky, 2007; Mimura et al., 2013). These invasion mechanisms are not necessarily mutually exclusive, but may also operate in tandem in a single species (Henery et al., 2010).

Processes other than selection can also operate to increase frequency of a particular allele or genotype in a population, sometimes to fixation, so that the presence of a genotype at high frequency is not a priori evidence that it is better-adapted than other genotypes. Genetic drift is the sum of random processes that can result in a change in the relative frequency of genotypes or alleles and that can ultimately result in fixation of a particular genotype or allele in a population (Suzuki et al., 1986). Genetic drift is most important in small populations where random processes are likely to have a relatively large effect. Genotype (or allele or mutation) "surfing" is a special case of genetic drift in populations undergoing range expansion (Excoffier and Ray, 2008). Modeling studies have shown that selectively neutral or even relatively maladapted genotypes or alleles are more likely to experience a chance increase in relative frequency at the "wave edge" of expansion and thus "surf" to high frequencies in small, newly founded populations (Travis et al., 2007). This process is essentially random and is unlikely to cause increased frequencies of the same genotype at multiple spatially separated invasion fronts.

Spatial sorting is another process that can result in an increase in frequency of a particular genotype at an expanding invasion front (Shine et al., 2011). In this case, genotypes with superior dispersal ability are disproportionately represented at the expanding invasion front, regardless of adaptive traits that might affect their fitness in terms of lifetime reproductive output. This process could cause disproportionate representation in multiple populations located at invasion fronts. For plants, dispersal ability is related to seed or diaspore traits. Smaller diaspore size has been experimentally shown in several studies to increase dispersal distances in both abiotically dispersed seeds (e.g., wind-dispersed seeds; Greene and Johnson, 1993) and those that are dispersed through epizoochory (Kiviniemi and Telenius, 1998). Adhesive structures (e.g., the barbed awns and retrorsely roughened hairs on the florets of *B. tectorum*) were also effective in increasing dispersal ability of epizoochorous seeds, but the negative effect of seed size on dispersal distance was of overriding importance (Tackenberg et al., 2006). An indirect test of the likelihood that particular *B. tectorum* genotypes have increased through spatial sorting at an expanding invasion front would be to examine whether the favored genotypes have smaller seed size and are therefore likely to have superior dispersal ability relative to common genotypes in the source population.

Selfing, adaptive syndromes, and invasion success—*Bromus tectorum* has a cleistogamous breeding system, meaning the plant self-pollinates before opening its flower, inhibiting pollination by exogenous pollen (McKone, 1985). This results in high levels of selfing, with reported outcrossing rates generally <1% and very low levels of observed heterozygosity (Meyer et al., 2013). Selfing mating systems have evolved numerous times in unrelated lineages during the course of angiosperm evolution, but high levels of selfing are considered to reduce evolutionary potential and therefore to be under negative selection in the long term (Wright et al., 2013). Several short-term selective advantages to selfing have been proposed, mostly involved with reproductive assurance when conditions for outcrossing are not met (Sicard and Lenhard, 2011; Shivanna, 2015). Shivanna (2015) pointed out that selfing is rarely absolute, so that evolutionary options are preserved.

An alternative hypothesis regarding the adaptive advantages of selfing focuses on the preservation of adaptive syndromes, i.e., complexes of adaptive traits that must occur in combination to confer maximum fitness (Allard et al., 1972; Allard, 1975). This hypothesis has been ignored in the recent reviews on selfing cited above, probably because of the low level of outcrossing required for recombination to disrupt such syndromes. However, there is evidence that there are genetically based differences in propensity to outcross among *B. tectorum* lineages, so that mean outcrossing rates at the population level do not reveal the true degree of outcrossing for a particular selfing lineage (Meyer et al., 2013). The likelihood of outcrossing as a pollen parent vs. an ovule parent varied among lineages in this study, suggesting multiple mechanisms for outcrossing suppression.

The association of particular neutral marker fingerprints with specific habitats has been frequently reported, particularly for inbreeding annual grasses of the eastern Mediterranean region (Nevo et al., 1979, 1994; Li et al., 2000; see Nevo, 2001 for review). This type of association was supported for *B. tectorum* by Merrill et al. (2012), who showed that certain four-locus single sequence repeat (SSR or microsatellite) haplotypes had strongly nonrandom distributions with respect to habitat.

In addition to providing the impetus for later studies demonstrating the existence of warm desert and salt desert specialist lineages, Merrill et al. (2012) produced some evidence for the existence of montane specialist lineages. Four-locus SSR haplotypes DABB and GCCB were found to be common in montane populations, but were found only at low frequencies in low-elevation populations. Haplotype DABB was very rare, occurring in only five of 77 low-elevation populations and never at a frequency >5%. Haplotype GCCB was equally rare in most low elevation habitats, but was present in 10 of 20 sagebrush steppe populations at a mean frequency

of 6%. Ramakrishnan et al. (2006) reported a similar result, showing that *B. tectorum* populations in montane and core sagebrush steppe habitats in the Intermountain West differed in SSR allelic composition.

A study of warm desert lineages in the Mojave Desert revealed large numbers of individuals across multiple warm desert populations over a wide geographic area that had an invariant 69-SNP (single nucleotide polymorphic) haplotype (Meyer et al., 2016). This haplotype was associated with a specific set of adaptive traits, such as lack of a vernalization requirement for flowering, that facilitate success in warm desert habitats. These results suggest selection for suppression of ability to outcross in conjunction with preservation of a specialist adaptive syndrome, as otherwise both the multilocus SNP haplotype and its association with specific adaptive traits would likely be disrupted by outcrossing.

Study objectives—In Merrill et al. (2012), only nine montane populations were sampled as part of a broad regional survey, so that the observed nonrandom distribution of putative montane lineages could have happened by chance. Our objective in this study was to greatly expand our sample of montane populations to learn whether the common SSR haplotypes previously detected corresponded directly to SNP haplotypes that occur at high frequency across a large number of montane sites. We tested the hypothesis that montane populations of *B. tectorum* in the Intermountain West are dominated by a few lineages with invariant SNP haplotypes, including the lineages represented by the SSR haplotypes DABB and GCCB. We further hypothesized that these dominant haplotypes would occur in multiple montane populations over a wide range but would be rare or absent in lower-elevation habitats in the region.

MATERIALS AND METHODS

Site selection and sample collection—Samples were collected from 38 sites across montane regions of Idaho, Nevada, Utah, Colorado, Arizona, New Mexico, and Washington (Table 1). These included the nine montane sites included in Merrill et al. (2012) as well as four foothill sites from that study that met our present criteria for inclusion. Mountain ranges and high plateaus in the Basin and Range, Columbia Plateau, and Colorado Plateau physiographic regions occur as islands surrounded by desert lowlands that are dominated by sagebrush steppe, salt desert, or warm desert vegetation. Collection sites were chosen based on elevation (>1850 m a.s.l.) or the presence of surrounding upland vegetation dominated by trees. Most sites met both criteria, but we included several populations from high latitude where forest vegetation occurs at much lower elevation. In addition, we included several high-elevation sites that were dominated by shrubland or meadow vegetation.

As a check on our definition of montane habitat, we also performed a climate ordination using 30-yr mean January temperature and mean annual precipitation data for each site from the Prism climate interpolator (Prism Climate Group, accessed 5 January 2017). We included the 38 montane sites in the present study (see climate data in Table 1) and also 77 lower elevation sites from Merrill et al. (2012). This ordination confirmed that climate at montane sites was distinct from climate at lower elevation sites, with very little overlap (Fig. 1). All but four of the 38 montane sites fell within the lower right quadrant of the graph, with mean January temperatures below 0°C and mean annual precipitation >400 mm. These values were chosen operationally to delineate the boundaries that best separated montane from lowland sites. Only five of 77 lower elevation sites fell within the montane quadrant, and these were relatively mesic steppe sites that did not meet the criterion for elevation or tree-dominated vegetation. Many low elevation sites were as cold as montane sites, but the combination of cold winters and high precipitation clearly defined climate at most of the sites that met the original criteria for inclusion. This classification is also concordant with the habitat classification based on a wider array of habitat characters presented by Merrill et al. (2012). All nine montane sites in that study fell within the climatic boundaries that we use to define montane habitat here. We also SNP-genotyped several foothill populations included by Merrill et al. (2012); these are represented as triangles in the climogram and fall somewhat outside our a posteriori climate boundaries. We chose to include these populations because they met our a priori elevation and vegetation criteria.

Even though sampled populations occupied sites surrounded by montane vegetation, most sites were at least moderately disturbed and lacked tree cover. Populations were generally small and often largely confined to road edges or other disturbances rather than occurring within established perennial vegetation.

Mature seed heads were collected from ca. 20 individuals per location for a total of 741 individuals. Plants were required to be >1 m from other sampled individuals to reduce the probability of sampling full siblings. Because *B. tectorum* is a highly selfing species, all seeds from each plant are considered essentially genetically identical; thus, the seeds collected from a plant represent a single line with a common maternal and paternal parent.

DNA extraction and genotyping—Seeds were allowed to lose dormancy under warm conditions and were planted in individual labeled pots in a greenhouse. At the 3–4-leaf stage, ~1 cm^2 of tissue was collected from each individual and stored at −80°C. We extracted DNA using the protocol of Fulton et al. (1995), with modifications to obtain higher DNA yields, and then performed SNP-based genotyping (Merrill et al., 2016). Ninety-five SNPs were originally obtained from deep sampling of cDNA libraries from widely divergent haplotypes previously identified using SSR markers (Merrill et al., 2012). All individuals were genotyped for the full set of SNPs, but a subset of 69 of these was selected for analysis. These 69 SNPs were located in neutral positions in open reading frames, decreasing the likelihood that they have experienced direct selection. Genotyping was performed using KASP chemistry (LGC Genomics, Beverly, Massachusetts, USA) on the EP1 platform (Fluidigm, South San Francisco, California, USA) for high-throughput SNP genotyping according to the manufacturer's instructions for the 96.96 dynamic array. Genotyping of the 741 individuals produced a neutral marker haplotype (a set of alleles for the 69 neutral SNP markers used in our analysis) for each individual.

Analysis of genotyping data—To identify identical or nearly identical lines, we used the PHYLIP software package (Felsenstein, 1989). First, a DNA distance matrix was created using the DNADist program with the default settings (F84 distance measure). We used the resulting DNA distance matrix as the input for the NEIGHBOR program in PHYLIP using the UPGMA (unweighted pair group method with arithmetic mean) clustering protocol, which produced a cluster dendrogram that we then visualized in the program Figtree (Rambaut, 2012). Using a threshold of 0.03 genetic similarity

TABLE 1. Collection site name, state, site code, number of lines genotyped, location, elevation, mean January temperature, mean annual precipitation, and associated vegetation for 38 montane *Bromus tectorum* collections included in the study. See Fig. 3 for map locations by site code.

Collection site	State	Site code	No. lines	Latitude (°)	Longitude (°)	Elevation (m a.s.l.)	Mean January temperature (°C)	Mean annual precipitation (mm)	Associated vegetation
Apache Creek	NM	APA	19	33.832755	−108.622106	2170	0.9	403	two-needle pinyon–alligator juniper
Aspen Grove	UT	ASP	21	40.404822	−111.602818	2094	−2.8	1012	aspen
Bicknell	UT	BIK	16	38.340165	−111.534630	2185	−3.9	235	mountain (mtn) big sagebrush
Brown's Mountain	WA	BRM	19	48.016668	−117.516673	666	−2.2	531	ponderosa pine
Buckskin Mountain	NV	BKS	19	41.781914	−117.547756	2480	−4.8	660	limber pine–mountain big sagebrush
Davidson Mountain	NV	DAV	21	39.318364	−119.669797	2369	−0.7	490	mtn big sagebrush–mountain meadow
Diamond Fork	UT	DFK	17	40.084307	−111.354818	1696	−4.0	623	gambel oak–bigtooth maple
Dinosaur	CO	DIN	18	40.387079	−108.995548	2341	−5.4	408	gambel oak–mtn big sagebrush
Dry Fork Face	UT	DFF	19	40.597595	−109.661622	2612	−6.5	551	aspen–gambel oak–mtn big sagebrush
Dry Wash	UT	DRW	18	37.765061	−109.531695	2299	−2.5	539	aspen–mtn big sagebrush
Dutch John	UT	DUJ	17	40.933166	−109.415652	1917	−4.4	288	two-needle pinyon–Utah juniper
Fish Lake Turnoff	UT	FLT	13	38.472584	−111.832748	2505	−3.7	432	mtn big sagebrush–mtn meadow
Flagstaff Arboretum	AZ	FLG	21	35.158442	−111.732564	2171	−1.7	556	ponderosa pine
Hancock Flat	UT	HCK	17	38.514234	−111.781243	2773	−3.8	688	aspen–mtn big sagebrush
Highland Mountains	NV	HIL	24	37.899248	−114.583361	2797	−3.1	462	subalpine fir-bristlecone pine
Huntington Canyon	UT	HUN	25	39.465569	−111.148598	2258	−7.3	456	aspen–gambel oak–mtn big sagebrush
Idaho City	ID	IDC	21	43.823793	−115.813752	1300	−3.6	544	ponderosa pine
Johnson Pass	UT	JPS	21	40.337344	−112.572678	1986	−2.3	516	Utah juniper–mtn big sagebrush
Koosharem Summit	UT	KSM	19	38.711884	−111.895386	2158	−2.9	394	Utah juniper–mtn big sagebrush
Lamoille Canyon	NV	LMC	22	40.663041	−115.438431	2260	−3.5	668	mtn big sagebrush–curlleaf mtn mahogany
Larb Hollow	UT	LRB	15	38.132359	−111.326003	2667	−3.9	416	gambel oak–mtn big sagebrush
Lewis Peak	NV	LEW	21	40.401058	−116.867492	2837	−4.3	657	mtn big sagebrush–mtn meadow
Milk Ranch	UT	MIL	18	37.665946	−109.719998	2188	−1.9	435	ponderosa pine
Mirror Lake Highway	UT	MLH	21	40.617518	−111.213698	1980	−5.1	584	gambel oak–mtn big sagebrush
More's Creek Summit	ID	MCS	21	43.931640	−115.668906	1890	−4.6	1110	subalpine fir–aspen
Mt. Charleston	NV	MTC	28	36.267188	−115.662365	2388	−1.5	602	subalpine fir–aspen
Nebo Loop	UT	NLP	20	39.873459	−111.684423	2700	−4.5	750	subalpine fir–aspen
North of Helper	UT	HEL	25	39.770134	−110.804420	2120	−5.5	458	gambel oak–mtn big sagebrush
Pietown	NM	PIE	14	34.298091	−108.132741	2370	−1.0	374	ponderosa pine
Pinecroft	WA	PCR	13	47.679614	−117.229015	608	−0.8	428	ponderosa pine
Red Horse Mountain	ID	RHR	11	47.549996	−116.648520	1209	−2.0	1165	grand fir–douglas fir
Rocky Mountain NP	CO	RMP	20	40.402408	−105.586183	2497	−4.1	592	ponderosa pine
Salina Summit	UT	SAS	24	38.873116	−111.550316	2089	−4.0	422	gambel oak–mtn big sagebrush
Soldier Summit	UT	SOL	25	39.929840	−111.086189	2269	−6.6	452	aspen–mtn meadow
Strawberry	UT	STR	19	40.227617	−111.118551	2333	−6.1	597	aspen–mtn meadow
Upper Peavine	NV	UPV	20	39.578639	−119.909500	2137	−0.5	640	mtn big sagebrush–curlleaf mountain mahogany
Virginia Peak	NV	VGP	18	39.760136	−119.466767	2517	−1.6	650	mtn big sagebrush–mtn meadow
Wild Horse Crossing	NV	WHS	21	41.726519	−115.894378	1870	−4.1	427	Utah juniper–mtn big sagebrush

on this dendrogram, we identified groups of individuals with identical or nearly identical neutral SNP haplotypes. This threshold was chosen because this degree of similarity was within the margin of error of our method as determined by genotyping the same reference samples on multiple Fluidigm runs. Most of the discrepancies were caused by different patterns of occasional missing data. Using this method, we identified 16 neutral SNP haplotypes that were provisionally named Montane 1 through Montane 16 (Fig. 2). Appendix S1 (see the Supplemental Data with this article) includes the dendrogram with branch tips labeled by collection location and line number. A named haplotype was operationally defined as a group of eight or more lines with identical or nearly identical SNP haplotypes found in one or more montane populations. Remaining haplotypes were present mostly as single individuals or rarely in groups of up to five, providing a clear threshold value for the definition of a putative montane haplotype.

We used two data sets to examine whether the putative montane haplotypes we had identified had been detected in lower elevation populations. First, we used cluster analysis (UPGMA) to compare reference lines from the 16 putative montane haplotypes with a larger set of 275 SNP-genotyped lines selected from the 96 populations of Merrill et al. (2012) to represent maximum habitat diversity, geographic range, and SSR polymorphism (K. R. Merrill, Monsanto, unpublished data). This method increased the likelihood of detecting a montane haplotype at low elevation because the reference data set included all known SSR haplotypes, whether common or rare. Detecting a match in this data set established the presence of a montane haplotype at low elevation but provided no information about its frequency. See Appendix S2 with the online Supplemental Data for location information for sampled low-elevation populations.

In the second analysis, we compared SNP haplotype composition of 18 montane populations with haplotype composition of 17 lowland populations (n = ca. 20 lines), each in reasonable proximity within the same region to at least one montane population. Appendix S3 presents location information for paired low-elevation

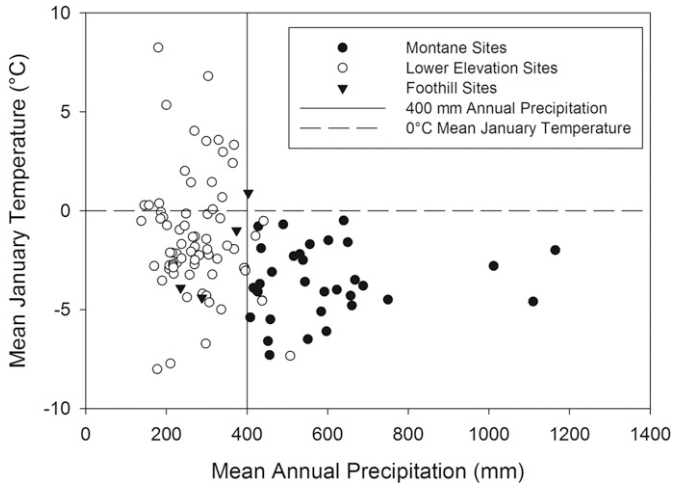

FIGURE 1 Climate ordination for 38 montane sites included in the present study and 77 low elevation sites from Merrill et al. (2012) using 30-yr mean January temperature and mean annual precipitation data for each site (Prism Climate Group, 2017). Solid vertical line represents montane annual precipitation threshold of 400 mm; dashed horizontal line represents montane mean annual temperature threshold of 0°C.

and montane populations. These low-elevation populations were not selected specifically for the current study, but instead represented populations that had been SNP-genotyped as part of other studies (Meyer et al., 2013, 2016; Lara, 2013; Merrill et al., 2016).

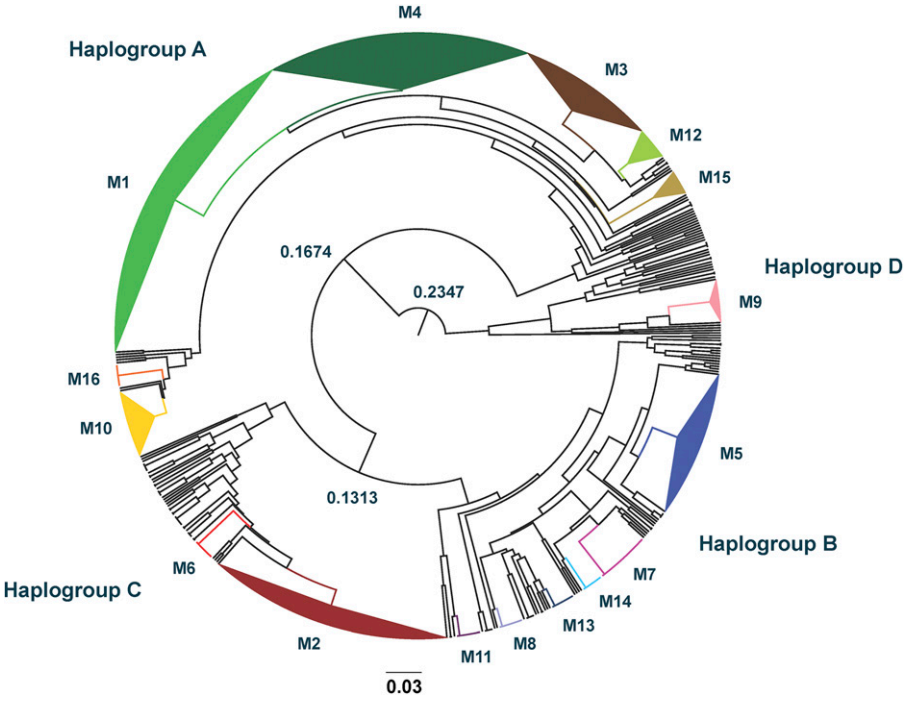

FIGURE 2 Dendrogram showing degree of similarity among genotyped lines (n = 741) from 38 montane populations of *Bromus tectorum* based on neighbor-joining analysis of 69 SNP loci. Groups of at least eight identical or near-identical lines (<0.03 genetic distance) are shown as a series of 16 color-coded putative montane haplotypes. Lines not included in a montane haplotype are shown in black. All lines fall within one of four major haplogroups (A–D) separated by genetic distances shown at the major nodes. (See Appendix S1 for the dendrogram with branch tips labeled by collection location and line number.)

There was some overlap of individuals in the two analyses because some of the populations of Merrill et al. (2012) that were the source of the reference lines in the first data set were later SNP-genotyped more intensively. This second analysis provided a comparison of putative montane haplotype frequencies in representative montane and lowland populations on a regional scale. We used the UPGMA procedure described earlier to search for haplotype matches in this data set.

To understand how the identified haplotypes from montane populations fit into the larger pattern of *B. tectorum* genetic variation, we performed a maximum likelihood phylogenetic analysis (DNAMLK in PHYLIP) using single reference lines taken from each putative montane haplotype identified in this study along with 13 reference lines from sagebrush steppe habitats selected at random from the larger data set used in the first matching analysis described above. We also included six known salt desert and warm desert specialist haplotypes identified in earlier work (Lara, 2013; Meyer et al., 2013, 2016). Online Appendix S4 presents identity and location information for the individual lines that were included.

Regional analysis—To examine the pattern of genetic differentiation among geographic regions, we performed a contingency table analysis (Proc Freq, Fisher's exact test with Monte Carlo probability calculation in SAS version 9.4; SAS Institute, Cary, North Carolina, USA). Our sampled montane populations were clustered into groups in six geographic regions: Colorado Plateau (11 populations), northern Utah and adjacent Colorado (12 populations), Nevada (9 populations), Washington and adjacent northern Idaho (3 populations), central Idaho (2 populations) and eastern Colorado (1 population; Fig. 3). We examined the frequency of occurrence in each region for haplotypes with >30% frequency in at least two populations. This included five common putative montane haplotypes (Montane 1–5) and Montane 10 (Table 2). We also included a group for all other montane haplotypes and a group for lines that did not group into any montane haplotype. The analysis used count data pooled across and within regions to compare the regional haplotype makeup with the overall haplotype composition. In the absence of any regional haplotype variation, we would expect each region's haplotype composition not to vary significantly from the haplotype frequency distribution across all regions. We used this approach rather than traditional population genetic analysis (i.e., analysis of molecular variance or genetic by geographic distance correlation) because our focus was on haplotype frequency distribution rather than allele frequency distribution.

Diaspore mass study—We examined whether the high frequency of two common and widely distributed montane haplotypes (Montane 1 and Montane 2) at expanding range edges in the mountains is associated with superior

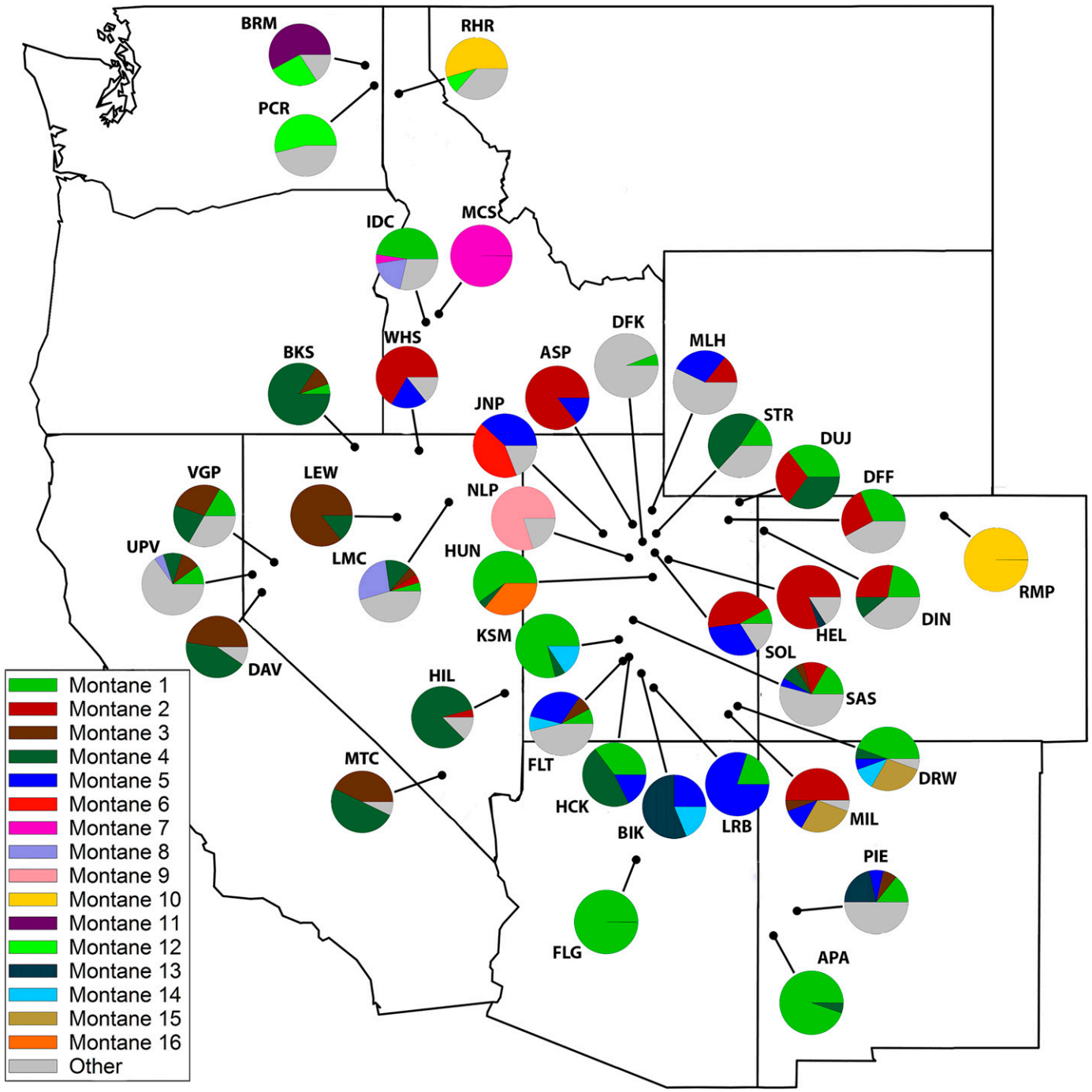

FIGURE 3 Frequency distributions of 16 putative montane haplotypes within 38 montane *Bromus tectorum* populations, with map location and site code (Table 1) shown for each population. Lines not belonging to one of the 16 defined haplotypes are shown in gray and labeled "other".

dispersal ability by using diaspore (floret) mass as a surrogate for dispersal ability as discussed earlier. We compared floret mass of lines of each of these two montane haplotypes from four populations with floret mass of a representative selection of eight lines belonging to different haplotypes from sagebrush steppe populations. Diaspores for each line were produced in cultivation in a common environment as part of other studies (Meyer et al., 2016). The lines were selected without reference to their diaspore mass. We used two replicate measures of 50 florets to characterize mass for each of the 16 lines. The data were subjected to mixed model analysis of variance for a nested design (SAS 9.4 Proc Mixed) with haplotype group (sagebrush steppe group, Montane 1, and Montane 2) as the fixed main effect and line within haplotype group as the nested random effect. This was a test of the spatial sorting hypothesis, which predicts that montane haplotypes should have smaller diaspores than haplotypes from the source area. Appendix S5 includes the experimental details and a more complete data presentation for this hypothesis test.

TABLE 2. Occurrence and frequency of sixteen 69-SNP multilocus haplotypes for which at least eight identical individuals (<0.03 genetic distance) were found in 38 montane populations included in this study. Haplotypes are arranged by haplogroup (see Fig. 2) and ranked within haplogroup by the number of populations in which they were found. Starred haplotypes occurred at a frequency of >0.07 in the overall sample of 741 individuals.

Haplotype	No. of populations	Fraction of total individuals	Mean frequency in populations of occurrence	Maximum frequency in populations of occurrence
Haplogroup A				
*Montane 1	21	0.179	0.321	1.000
*Montane 4	17	0.135	0.288	0.842
*Montane 3	12	0.075	0.231	0.857
Montane 12	3	0.017	0.297	0.538
Montane 10	2	0.034	0.772	1.000
Montane 15	2	0.013	0.278	0.278
Montane 16	1	0.012	0.360	0.360
Haplogroup B				
*Montane 5	12	0.071	0.241	0.800
Montane 14	4	0.012	0.133	0.188
Montane 13	3	0.017	0.272	0.562
Montane 7	2	0.029	0.524	1.000
Montane 8	2	0.015	0.232	0.273
Montane 11	1	0.015	0.579	0.579
Haplogroup C				
*Montane 2	13	0.126	0.371	0.857
Montane 6	1	0.012	0.429	0.429
Haplogroup D				
Montane 9	1	0.021	0.800	0.800

RESULTS

Montane haplotype distribution and abundance—The 16 provisional montane haplotypes identified collectively made up 78.2% of all individuals in montane populations, while just the five most common haplotypes (defined as haplotypes with >50 individuals, representing >7% of total individuals) accounted for 58.6%, supporting our hypothesis that montane *B. tectorum* populations are largely composed of a few common haplotypes (Fig. 2). Most haplotypes were found in multiple populations, often at high frequency (Table 2). The four haplotypes found in single populations also occurred at high frequency in their respective populations (36–80%).

The 16 haplotypes grouped into four distinct branches in the dendrogram and were assigned to haplogroups A–D (Table 2, Fig. 2). Montane A was the largest haplogroup (51.3% of total) and included seven putative montane haplotypes, three of which were among the most common. Montane 1 contained the largest number of individuals (17.8% of total) and was the most widely distributed (21 populations). For lines in montane populations that were also SSR-genotyped by Merrill et al. (2012), there was a perfect match between SNP haplotype Montane 1 and SSR haplotype GCCB. Montane 4 occurred in 17 populations and included 13.8% of total individuals, while Montane 3 included 7.3% of total individuals and was present in 12 populations. These three common montane haplotypes were found at frequencies averaging 23–32% in their populations of occurrence and were often found at very high frequencies (80–100%, Table 2, Fig. 3). Montane 10, though found in only two geographically widely separated populations, was dominant in these populations at frequencies of 55% and 100%.

Haplogroup B included six haplotypes and comprised 23.6% of the total sample (Fig. 2). Of this group, only Montane 5 was found to be widespread (12 populations) and common (7.7% of total; Table 2, Fig. 3). Montane 5 had an average frequency of 24.1% in its populations of occurrence.

Haplogroup C contained haplotypes Montane 2 and 6 and included 20% of total individuals (Table 2, Fig. 2). Montane 2 made up the majority of this haplogroup and comprised 12.8% of the total sample. It was present in 13 populations at an average frequency of 37.1% and occurred in several populations at high frequency (Fig. 3). For lines in montane populations also SSR-genotyped by Merrill et al. (2012), there was a near-perfect match between SNP haplotype Montane 2 and SSR haplotype DABB. The only exception was in the Strawberry population, where four individuals SSR-genotyped as GABB had the Montane 2 SNP haplotype.

Haplogroup D contained 5.1% of total individuals (Table 2, Fig. 2). This haplogroup included the Montane 9 haplotype from Nebo Loop, Utah, where it made up 80% of the population (Fig. 3). Most of the remaining lines in haplogroup D were from the Upper Peavine population in extreme western Nevada. These Upper Peavine individuals were genetically diverse and did not form any defined haplotype (Appendix S1).

Occurrence of montane haplotypes at lower elevation sites—When the 16 montane SNP haplotypes identified in this study were compared in a cluster analysis with a broad spectrum of lines from Merrill et al. (2012) representing a range of low elevation habitats, geographic areas, and four-locus SSR genotypes, seven montane haplotypes were found to have exact matches in nonmontane habitats. Matches for Montane 5 were found across a wide range of sagebrush steppe and salt desert sites, as discussed below. Of the other common montane haplotypes, Montane 1 was detected at four sagebrush steppe sites and Montane 2 at a single sagebrush steppe site, while Montane 3 and 4 were not detected at any low elevation site. Four of the 11 less common montane haplotypes had matches in nonmontane habitats. Montane 14, found at four montane sites, had two matches from nearby sagebrush steppe sites in central and southern Utah, while Montane 15, found at a single montane site in southeastern Utah, had one match from a nearby sagebrush steppe site. In the other two cases (Montane 7 and Montane 9), we found matches with lines from geographically distant low elevation sites. These results suggest that montane haplotypes may occur at low frequency in sagebrush steppe and other low elevation habitats, but that their presence was often below the limit of detection using our screening set.

In the paired analysis that included 18 montane sites and 17 low elevation sites and a total of 707 lines, haplotype frequencies were compared across low elevation and montane sites within and across regions (Appendix S3). This analysis detected the Montane 5 haplotype at low to high frequency in several low-elevation populations, as was expected from its wide distribution in the first analysis. We found only three additional haplotypes common to both low elevation and montane sites within a region or even across regions. One of these did not belong to any named montane haplotype; line PIE07 from northern New Mexico matched the low-elevation line GUS10 from the Uinta Basin in Utah. The only low-elevation population that contained any lines belonging to named montane haplotypes was the sagebrush steppe population at White's Valley in northern Utah. Montane 1 occurred in this population sample at 4%, similar to 10% frequency of the GCCB genotype detected at White's Valley by Merrill et al. (2012). We also detected the Montane 4 haplotype in this population sample at a frequency of 16%.

These two montane haplotypes were present in a larger set of SNP-genotyped individuals for White's Valley ($n = 375$) at frequencies of 6.1 and 8%, respectively (haplotypes L2 and L3; Meyer et al., 2013).

Eight of the lowland populations in proximity to montane sites in this analysis were salt desert, warm desert, or warm desert fringe sites, so it is perhaps not surprising that montane haplotypes were not detected, but montane haplotypes were also not present at eight of nine sagebrush steppe sites (Appendix S3). Their absence at sagebrush steppe sites confirmed that haplotypes provisionally designated as montane in this study generally occurred at frequencies below the limit of detection in the low-elevation populations we genotyped.

Relationship of montane haplotypes to* B. tectorum *haplotypes from other habitats—The cladogram from the maximum likelihood analysis of reference lines from a full range of habitats revealed that all montane haplotypes fell clearly into the common clade described in earlier work (Meyer et al., 2013, 2016) and were included in larger groups that also included haplotypes from core sagebrush steppe habitat (Fig. 4). This large-scale pattern with four principal haplogroups is evident in other analyses of common clade lineages (Meyer et al., 2016; K. R. Merrill, Monsanto, unpublished data). This analysis shows that the four groups of montane haplotypes identified in the present study are likely polyphyletic in origin. They are more similar to their core habitat common clade associates in the cladogram than they are to each other. Consistent with previous findings, the cladogram also showed a clear separation between common clade and desert clade haplotypes.

Haplotype Montane 5 was found to be identical to the Salt Desert 1 haplotype reported by Meyer et al. (2016) and identified in previous studies as SSR haplotype IEBB (Scott et al., 2010; Merrill et al., 2012). Meyer et al. (2016) showed that lines with the IEBB haplotype consistently exhibited Salt Desert 1 SNP haplotypes. The Montane 5/Salt Desert 1 haplotype grouped within Haplogroup B (Fig. 2). This well-defined haplotype, previously thought to be largely a salt desert specialist but with some presence in sagebrush steppe populations, is now known to be common across a wide range of different environments, including salt desert, sagebrush steppe, and montane habitats.

Montane 9, found only in an aspen–subalpine fir site in central Utah (Nebo Loop), clustered in Haplogroup D, which was strongly differentiated from other common clade haplogroups (Fig. 4). This haplogroup fits into a subclade that includes warm desert specialists and that was designated the Warm Desert 2 subclade by Meyer

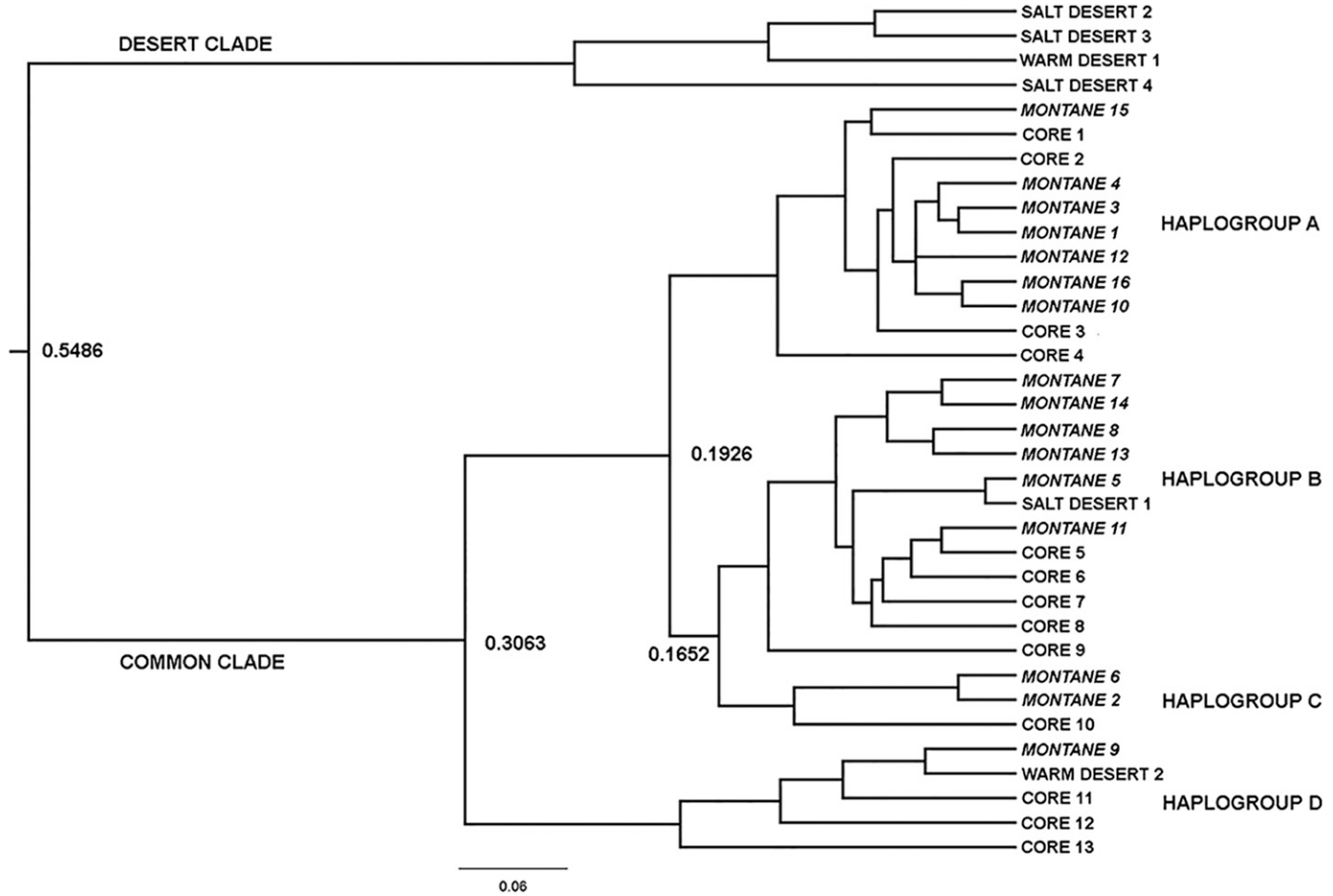

FIGURE 4 Cladogram based on maximum likelihood analysis of 69 SNP loci showing relationship of representative lines of each of 16 montane haplotypes to 19 representative haplotypes from core sagebrush steppe and warm and salt desert habitats. Node labels represent genetic distance between clades (desert clade vs. common clade; Meyer et al., 2016) and between haplogroups within the common clade as in Fig. 1. (See Appendix S4 for identity and location information for individual lines.)

et al. (2016). As mentioned earlier, Haplogroup D also included lines from a montane site on Peavine Mountain in western Nevada, where Meyer et al. (2016) also noted its presence in sagebrush steppe habitat.

Regional analysis—We observed strong regional differentiation in haplotype composition, i.e., different montane haplotypes dominated populations in different regions. Each regional group in the contingency table analysis showed a significant difference in haplotype composition in a pairwise comparison with the haplotype composition of all regions combined (Fisher's exact test $P < 0.0001$ in each case; Fig. 5). Montane 1 dominated in populations in the Colorado Plateau region. In northern Utah, Montane 2 was the dominant haplotype, while Montane 3 and 4 were the dominant haplotypes in Nevada populations. Montane 5 was present at higher frequencies in the Colorado Plateau and northern Utah regions than it was overall across regions. Other montane haplotypes dominated populations in both the central Idaho and Washington regions, and Montane 10 was the only haplotype found in the one sampled eastern Colorado population. The five common montane haplotypes were well represented in the southern and western regions but, with the exception of Montane 1 at Idaho City, were absent from the more northern and eastern regions. These regions were dominated by either Montane 10 or by putative montane haplotypes that did not meet the criterion for individual consideration (30% frequency in at least two populations). Due to the small number of populations included from the Washington, central Idaho, and eastern Colorado regions, haplotype frequency distributions for these regions could change with increased sampling.

Diaspore mass study—In the indirect test of the spatial sorting hypothesis using diaspore mass as a surrogate for dispersal ability, the group main effect was not significant (df = 2, 13, $F = 2.80$, $P = 0.0973$), in spite of a relatively large difference between means (3.00 mg for the sagebrush steppe group vs. 3.53 and 3.33 mg for Montane 1 and 2, respectively). This lack of significance was due to high among-line variation. Examination of box plots showed that most among-line variation was within the sagebrush steppe group, whereas lines within each of the two montane haplotypes were closely similar in floret mass and were consistently at the high end of the floret mass range (Fig. 6). These results failed to support spatial sorting as an explanation for high frequencies of Montane 1 and 2 at range edges in the mountains. Their heavier diaspores would be predicted to have decreased dispersal ability rather than increased dispersal ability relative to most lineages in the core sagebrush steppe source area.

DISCUSSION

The hypothesis that montane populations would be dominated by a few common, widely distributed haplotypes was supported by the data. Most of the *B. tectorum* lines detected in the montane populations in this study (73.8%; Table 2) belonged to montane SNP haplotypes that occurred in multiple populations. All but one of the 12 haplotypes found in multiple populations (Montane 5, discussed further below) were largely restricted to montane habitats and were rarely or never found in lower-elevation populations. These results lend credence to the idea that one or more of these haplotypes, particularly those that are more common and widespread, could represent lineages that are locally adapted to montane habitats.

Spatial sorting, a selectively neutral process, represents an alternative hypothesis to explain large increases in frequency of the same haplotype in montane habitats at multiple widely separated locations. However, the heavier diaspores of two of the most common and widely distributed montane haplotypes likely have

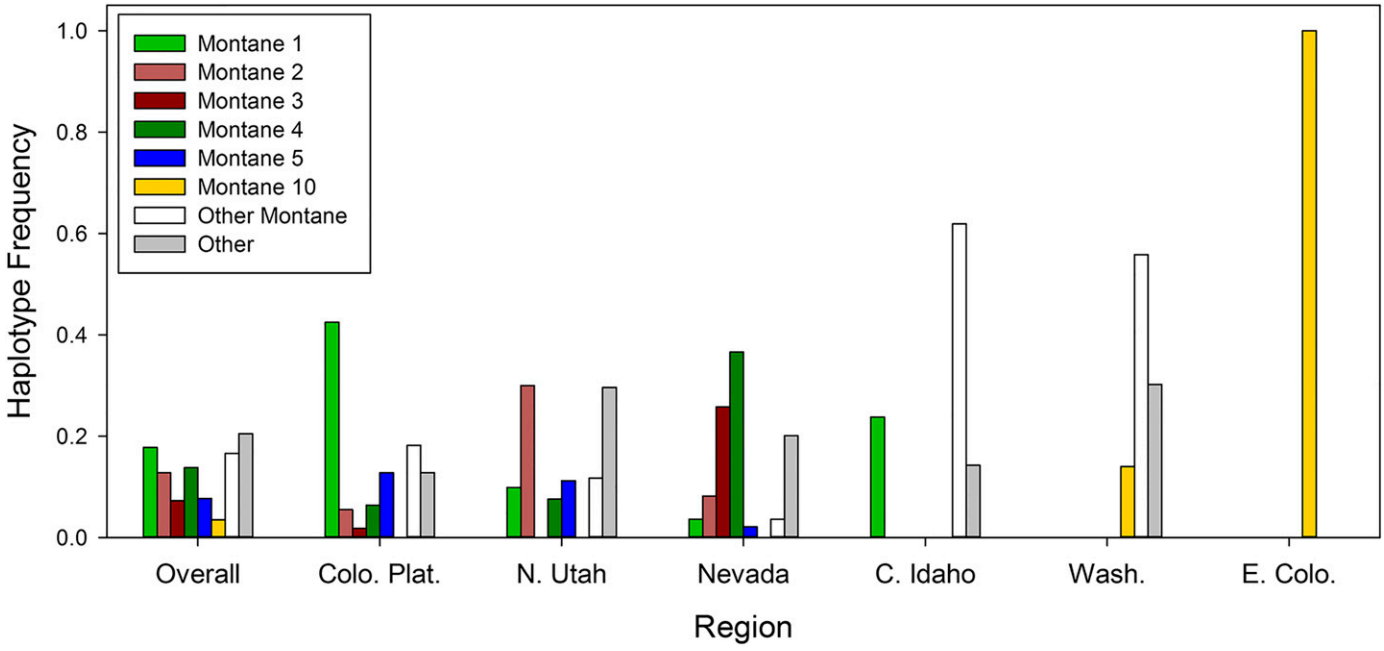

FIGURE 5 Relative frequency of six common montane haplotypes, a pooled category including all other montane haplotypes, and a category for lines not included in a montane haplotype, overall and in each of six geographic regions included in the study. Fisher's exact tests using the count data showed that frequency distributions for each region are significantly different from the overall frequency distribution at $P < 0.0001$.

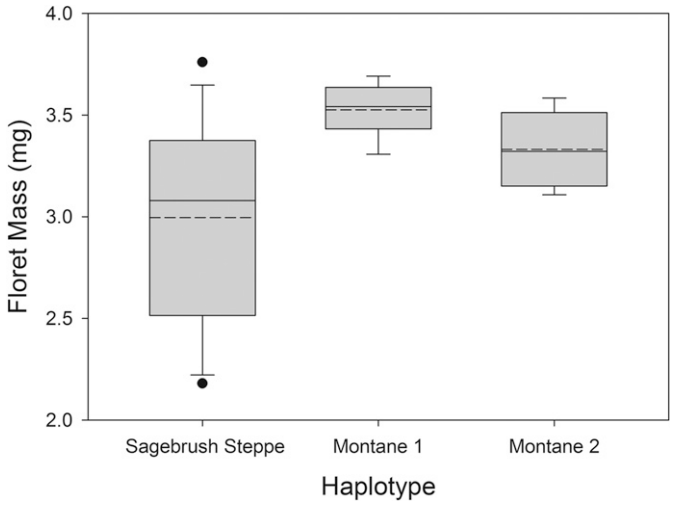

FIGURE 6 Boxplots showing the distribution of floret mass in three groups of *B. tectorum* lines produced in a common greenhouse environment: the sagebrush steppe group (8 lines), the Montane 1 group (4 lines), and the Montane 2 group (4 lines). Solid line = median value, dashed line = mean value, box = 25th and 75th percentiles, whiskers = 10th and 90th percentiles, dots = outliers. (See Appendix S5 for experimental description and detailed data presentation.)

inferior rather than superior dispersal ability, which would make them unlikely to rise to high frequency at a range edge through spatial sorting.

Inferences based solely on neutral marker genotype distribution cannot conclusively demonstrate local adaptation (Kawecki and Ebert, 2004). For example, Latta (2009) showed experimentally that habitat segregation of two multilocus allozyme genotypes of wild oat (*Avena barbata* L.), previously thought to represent local adaptation (Allard et al., 1972), likely represented a nonequilibrium situation where the genotype that performed better across both habitats was apparently in the process of replacing the competitively inferior genotype.

There is no reason to assume that any population of an invader with high genetic diversity and high dispersal capability is in an equilibrium condition, and major changes in *B. tectorum* population genetic composition through time have been detected (Meyer et al., 2013; S. E. Meyer, unpublished data). It is likely that there is a recurring influx of new *B. tectorum* lineages into montane habitats and that the genetic composition of a population at any point in time represents a complex interaction among factors associated with accidental dispersal, genetic drift, and selection. For example, at the time Nebo Loop was first sampled in 1995 by Ramakrishnan et al. (2006), Montane 2 was the dominant haplotype. The patch dominated by Montane 2 became locally extinct due to succession to perennial vegetation, while the patch now dominated by Montane 9 apparently represents a new introduction on a road disturbance a short distance from the original collection site. This example illustrates that perennial vegetation offers biotic resistance to *B. tectorum* invasion and can cause local extinction of populations established on disturbances regardless of their genetic composition. We observed a similar extinction/reintroduction scenario at two adjacent collection sites on quarry disturbances in the Strawberry area. Such random extinction events represent another form of genetic drift.

Many introductions fail to establish at all because of a mismatch between genotype and the abiotic environment. Even lineages that temporarily rise to dominance through genotype surfing or other neutral processes may be maladapted over the full temporal range of environmental conditions at a site, resulting in a presence that is only transient. This process may explain the current dominance of some putative montane haplotypes at only a single site. The local dominance of these haplotypes, though provisionally labeled as montane, could readily result from neutral processes rather than through selection on preadapted genotypes. Following changes in haplotype composition through time using repeated sampling, especially in marginal populations, could provide more clues as to the evolutionary processes involved.

There is mixed experimental evidence for local adaptation in montane populations of *B. tectorum*. Pierson and Mack (1990a, b) seeded a single steppe population into several montane habitat types. This seed source failed to establish and reproduce in montane environments, whether in shaded canopy or less light-limited disturbances, suggesting that it was not adapted to the short growing season and heavy snowfall of montane environments (i.e., genotype–abiotic environment mismatch). However, Rice and Mack (1991b) found evidence for local adaptation in a population collected from hemlock forest in a reciprocal seeding experiment; the local hemlock forest seed source outperformed lower-elevation seed sources at the hemlock site. Rice and Mack (1991a) also demonstrated genetic differences in flowering phenology, likely due to differences in vernalization requirement (Meyer et al., 2004), that could be related to fitness in different environments.

In another reciprocal seeding study, Leger et al. (2009) failed to detect local adaptation in a recently established montane population of *B. tectorum* on Peavine Mountain in western Nevada, but did find evidence for local adaptation in the adjacent sagebrush steppe population. They attributed this result to the nonequilibrium state of the invasion, i.e., that highly adapted genotypes had not yet arrived at the high elevation site or that selection had not yet had time to increase their numbers. A definitive test of whether any of the common and widely distributed haplotypes in the present study represent genotypes that are preadapted to montane environments would require reciprocal seeding studies combined with examination of the adaptive traits under positive selection.

We found one relatively common montane haplotype (Montane 5) that represents an apparent example of a broadly adapted genotype with high phenotypic plasticity, and more specifically an example of a "Jack of all trades, master of some" (Richards et al., 2006). Haplotype Montane 5 was first encountered as SSR haplotype IEBB in salt desert habitat on the Dugway Proving Grounds in west-central Utah (Scott et al., 2010). We demonstrated local adaptation of this haplotype in salt desert communities through reciprocal seeding studies (i.e., it outperformed core sagebrush steppe lineages in the salt desert environment) and also demonstrated experimentally that it was more salt-tolerant than lineages from the sagebrush steppe, suggesting that it was a salt desert specialist. We later found that it was a dominant haplotype across many salt desert sites in the eastern half of the Great Basin, the Uinta Basin, and the Colorado Plateau (Merrill et al., 2012). Interestingly, however, in the reciprocal seeding study at Dugway, this haplotype was as successful as core sagebrush steppe lineages in sagebrush steppe habitat, and this finding was confirmed in later common garden studies at two additional sagebrush steppe sites (Meyer et al., 2016). Merrill et al. (2012) noted that this haplotype (IEBB) was common at a few sagebrush steppe

sites and also detected it at several montane sites. In the present study, Montane 5 was widely distributed in montane populations across the geographic area where it has been frequently detected in lower elevation populations (Figs. 3 and 5). Its occurrence at numerous montane sites suggests that it has a phenotypic expression that allows it to persist at these sites and that it is not a transient that is only marginally adapted. Whether all lines that possess this SNP haplotype are ecologically equivalent is an open question; it is possible that evolution through mutation has taken place within this lineage to produce divergence in adaptive traits, even though the low propensity to outcross has preserved the neutral marker haplotype. A test of this hypothesis would require reciprocal seeding of salt desert and montane lines belonging to this haplotype.

The regional analysis of haplotype distribution showed that different geographic areas within the Intermountain West differed in relative frequency of the common montane haplotypes (Fig. 5). The simplest explanation for this is that dispersal limitation is an overriding factor in the composition of montane populations, so that populations in closer proximity are more likely to share haplotypes than those that are more widely separated. Some of the observed haplotype distributions indicate that dispersal over relatively long distances is a common occurrence, and certainly human-assisted jump dispersal is a probable mechanism for this. More intensive sampling of lower-elevation populations using the SNP marker system could give insight into how montane populations have arisen from the *B. tectorum* gene pool present in the region.

One puzzling aspect of the existence of locally adapted lineages in *B. tectorum,* whether in desert or montane environments, is the necessity for selection for the extreme selfing that would be needed to maintain the adaptive syndromes associated with fixed neutral marker haplotypes. It is possible that these haplotypes do experience rare outcrossing and that some of the lines we encountered that did not fall into defined haplotypes represent outcrossed progeny (Meyer et al., 2013, 2016). But there must be strong selection against such outcrossed progeny, because the existence of numerous individuals with identical haplotypes across many populations indicates that the genetic integrity of these lineages must somehow have been maintained. While complete cleistogamy and 100% suppression of outcrossing can be envisioned theoretically, it is almost never reported to occur (Shivanna, 2015). In cases where two lineages are only distantly related (e.g., in different clades), there may be postzygotic factors operating to reduce fertility, but montane lineages of *B. tectorum* are embedded within the common clade and have relatives that presumably exhibit outcrossing. The question of how these strongly selfing *B. tectorum* lineages are preserved is clearly a topic for further study.

In summary, we found that populations of *B. tectorum* from different mountain ranges in western North America were genetically more similar to each other than to adjacent lowland populations, a result that contrasts with earlier reports on montane invasions at both the species and population level (Alexander et al., 2009; McDougall et al., 2011). This pattern may be a consequence of the complex introduction history of *B. tectorum*, which has resulted in high genetic diversity in the introduced range, coupled with its exceptional capacity for human-aided, long-distance dispersal.

ACKNOWLEDGEMENTS

We thank Owen Baughman, Julie Beckstead, Beth Leger, Rosemary Pendleton, Duane Smith, and Bettina Schultz for making seed collections for this study. The study was funded in part through Joint Fire Sciences Program Grant Number 2011-S-2-6 to S.E.M., through funding to S.E.M. through the USDI Bureau of Land Management Cheatgrass Die-off Project, and through a grant to C.E.C. from the Sant Foundation. We also thank the handling editor and two anonymous peer reviewers for suggestions that greatly improved the quality of the manuscript.

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