

High Phenotypic and Molecular Variation in Downy Brome (*Bromus tectorum*)

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The invasive grass *Bromus tectorum* (cheatgrass, downy brome) has extensive ecological breadth in its introduced range, and is increasing in abundance at high elevations. As a predominantly selfing species with high potential population growth rates, it is a likely candidate for having general-purpose genotypes that facilitate its invasion into high elevations. We evaluated evidence for general-purpose genotypes by examining phenotypic and molecular variation in *B. tectorum* from low- and high-elevation sites in the Rocky Mountains. Common garden experiments revealed that plants from high elevations germinated faster, but no other phenological, morphological, or physiological differences were found between elevations. Leaf length was longer for low-elevation sites in a common garden using field-collected seeds, but this was not found in a common garden using greenhouse-grown seeds, suggesting maternal effects. For most traits, there was significant phenotypic variation among sites and individuals. Using microsatellites, we genotyped individuals from low- and high-elevation sites. We found no indication of genetic differentiation at these loci between high and low elevations, but there was significant variation within and among sites. No single genotype was common across sites. The success of *B. tectorum* is not due to a few general-purpose genotypes, but rather many genotypes associated with many different phenotypes. Understanding the mechanisms behind the success of invaders enhances our knowledge of basic evolutionary and ecological processes and is crucial to our ability to manage ongoing, and prevent future, invasions, especially into new habitats.

Nomenclature: Downy brome, *Bromus tectorum* L. BROTE.

Key words: Broad ecological distribution, *Bromus tectorum*, differentiation, general-purpose genotype, invasion.

Some invaders are extremely genetically variable (Ben-Shlomo et al. 2006; Genton et al. 2005; Kolbe et al. 2004; Lavergne and Molofsky 2007; Lee et al. 2004; Liebert et al. 2006) whereas others are genetically depauperate, including invasions of single clones (Mergeay et al. 2006; Terhivuo and Saura 2006; Wilen et al. 1995). Where a species lies along this continuum can influence the course of an invasion. Highly genetically variable invaders can evolve to adapt to novel aspects of their new range (Bossdorf et al. 2005; Butin et al. 2005; Novak and Mack 2005), whereas genetically depauperate invaders can have inherently broad tolerance to a variety of environmental conditions

(Lindholm et al. 2005; Poulin et al. 2005). Baker (1965) proposed that some introduced species become successful weeds by having “general-purpose genotypes” that enable them to spread across different environments. The term general-purpose genotypes has been extended to include invaders (Parker et al. 2003). Despite including the word “genotype” in the description of this phenomenon, little of the work on general-purpose genotypes actually characterizes populations at the genotypic level (but see: Dlugosch and Parker 2008; Geng et al. 2007).

The occurrence of general-purpose genotypes has been hypothesized to be associated with species that can self pollinate or reproduce apomictically and exhibit rapid population growth from a few introduced individuals (Baker 1965). *Bromus tectorum* L. (Poaceae, cheatgrass, downy brome) is a predominantly selfing grass invasive in North America. It can thrive under widely varying environmental conditions and recently has been observed to be increasing in abundance at high elevation (J. Connor, Rocky Mountain National Park, personal communication; Ramakrishnan et al. 2006). These characteristics make it a good candidate for having a general-purpose genotype found across low- and high-elevation habitats. Alternatively, because evidence exists for local adaptation in this

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Interpretive Summary

In managing downy brome, a key question is how to best allocate limited time and resources. In this paper we evaluated genetic diversity within and among populations and also examined evidence that downy brome from lower and higher elevations are genetically distinct. We found a high level of genetic diversity within and among populations, suggesting there is sufficient genetic variation available upon which selection could act. If this led to differentiation between elevations, it could be important to restrict movement of propagules within elevations, in particular to prevent the spread of preadapted individuals that already perform well at a given elevation. We found, however, that high- and low-elevation sites were not distinct, which suggests that resources need not be focused on movement of seed specifically within elevations, but rather movement of seeds more generally. Future research should focus on measuring propagule (seed) production and dispersal to understand when propagules are best controlled and what are the main pathways for dispersal. If propagule pressure is playing a role in the range expansion of downy brome, then preventing seed dispersal from the edge of the range could minimize its continued spread.

species (Rice and Mack 1991), there could be differentiation of plants found at different elevations. Here, we examine evidence for general-purpose genotypes from two perspectives. (1) We evaluated phenotypic variation within and among individuals from low- and high-elevation habitats grown in two common garden experiments: one with field-collected seed, and one with greenhouse-grown seed. This allowed us to determine if there are consistent phenotypic differences between high- and low-elevation sites or if most phenotypic variation lies at the site or individual level. (2) We measured molecular variation from the same individuals to determine whether and how that variation is structured geographically, and whether single or multiple genotypes are found at low- and at high-elevation sites.

Materials and Methods

Study Species. *Bromus tectorum* is an extremely well-studied and ecologically important species in western North America (Beckstead et al. 1996; McKone 1985; Meyer et al. 1997, 2004; Novak et al. 1991; Ogle et al. 2003; Rice et al. 1992; Rice and Mack 1991). It is a predominately selfing (McKone 1985), diploid, annual C_3 grass from Eurasia and the Mediterranean Basin that has spread into many regions worldwide, and its invasion is well documented in the western United States. Its winter annual life history results in flushes of germination and growth in fall and spring (Hulbert 1955). It can reduce the amount of available soil nitrogen (Evans et al. 2001; Rimer and Evans 2006; Sperry et al. 2006) and can alter ecosystem properties by increasing fire frequency and decreasing biomass production and decomposition rates (Ogle et al. 2003).

The population genetic structure of *B. tectorum* has been examined with both allozymes and microsatellites. Allozyme loci reveal low variation within populations (Novak et al. 1991; Rice and Mack 1991) but significant structuring among populations. Likewise, across two states, Ramakrishnan et al. (2004) found only 15 different multi-locus genotypes using microsatellite loci. More recently, however, substantial microsatellite variation was found within some populations (Ramakrishnan et al. 2006). These studies did not find heterozygotes, supporting the idea that this plant is predominantly selfing. Only recently have heterozygotes been found using allozymes in three Canadian populations (Valliant et al. 2007). Rice and Mack (1991) also examined variation in quantitative traits, and despite the selfing habit and relatively low variation in allozymes and microsatellites, found substantial variation within and among populations, including local adaptation.

Collection Sites. We collected seeds from 15 individuals in each of six sites in the Rocky Mountains (Table 1; Figure 1). Three sites were designated as high elevation ($> 2,400$ m [7,874 ft]; Table 1) and three as low elevation ($< 1,500$ m). Given our focus within the Rocky Mountain region, the lowest low elevation site was just above 1,200 m. Thus, what we consider to be low elevation for the purpose of this study lies in the middle elevation range of the species, which extends to sea level.

Greenhouse Experiment I: Common Garden from Field-Collected Seeds. Variation in several phenotypic and life history traits was examined in a greenhouse common garden at Colorado State University. Three seeds from each field-sampled maternal plant were used as replicates in this experiment, for a total of 270 individuals (6 sites by 15 maternal plants by 3 replicates). For simplicity, we call the plants originating from each of the 90 maternal individuals “genotypes”; the replicates of those 90 genotypes are likely to have been produced by selfing, but it is conceivable that some might have been produced by outcrossing. The seeds were planted in standard commercial potting mix in containers¹ (164 cm³ [10 in³] soil volume) on March 3, 2006. On March 26, 2006, they were placed in a cold room for eight weeks until May 21, 2006. This vernalization period encourages flowering in the adult plants. Seedlings were then transplanted into 16.5 cm pots (1,888 cm³ soil volume).

The plants were randomly placed on two benches in the greenhouse and locations were rerandomized four times over the course of the experiment (June 2006 to February 2007). The average day temperatures were approximately 24.4 C (75.9 F) and the average night temperatures were approximately 17.2 C. The photoperiod was 16-hour daylength, and three 430-watt high-pressure sodium lamps provided enough lighting to induce the 16 hour photoperiod. The lights turned on based on natural light readings

Table 1. *Bromus tectorum* collection sites in Colorado, with latitude, longitude, elevation, and climate information.

Site	Latitude	Longitude	Elevation (m)	Category	Climate ^a				
					Mean January temp. min. (C)	Mean January temp. max. (C)	Mean July temp. min. (C)	Mean July temp. max. (C)	Mean Annual Precip. (mm)
1	40.36965	-105.57035	2,442	High	-9.0	3.6	7.8	25.6	350
2	37.68367	-106.62235	2,565	High	-14.2	1.8	8.9	25.8	251
3	37.96135	-107.30012	2,758	High	-18.8	1.3	7.0	25.1	572
4	40.28535	-103.69478	1,289	Low	-12.1	3.9	15.6	32.3	329
5	38.01120	-103.74630	1,332	Low	-8.1	9.2	17.5	35.1	393
6	39.18157	-109.01617	1,417	Low	-8.1	3.7	17.5	33.7	227

^aClimate data are from the Western Regional Climate Center, www.wrcc.dri.edu.

before sunset and turned off at 10:00 P.M. nightly. The lights also turned on during cloudy conditions, also based on natural light readings. The plants were watered by an automatic drip irrigation system for 16 min per day; additional watering was done in the heat of the summer to keep the plants from experiencing substantial drought stress. We used this experiment to measure water use efficiency and phenotypic and life history traits.

Water Use Efficiency. To determine differences in water use efficiency (WUE) between high- and low-elevation sites, we measured the ratio of ¹³C to ¹²C isotopes ($\delta^{13}\text{C}$) of senesced leaves. In a common garden experiment like this one, $\delta^{13}\text{C}$ reflects discrimination between carbon isotopes, with less negative values indicating higher water use efficiency over the lifespan of the leaf tissue (Dodd et al. 1998; Farquhar et al. 1989). We dried leaves from one replicate of each genotype to constant weight at 60 to 65 C, and ground to a fine powder. Carbon isotope composition

of the leaf tissue was measured using a Carlo Erba NA1500 elemental analyzer² coupled to a VG isochrom isotope ratio mass spectrometer.³ All values are reported with respect to $\delta^{13}\text{C}$ of Vienna Pee Dee Belemnite (VPDB).

Phenotypic and Life History Traits. Number of tillers and length of the longest leaf (leaf length1) were measured on June 12, 2006. On June 28, 2006, we measured the length (leaf length2) and width of the longest leaf and the length and width of the basal portion of the plant, and counted the number of culms. Relative growth rate was calculated using the formula: $\ln(\text{leaf length2}) - \ln(\text{leaf length1})$. Basal area was calculated using basal length and width as follows: $(\text{length}/2 * \text{width}/2) \pi$ (Fehmi et al. 2004). Plants were monitored daily to determine reproductive stage, allowing us to calculate time until flowering. Aboveground biomass and mass of all seeds (a measure of reproductive output) were measured after plants were harvested. The mass of individual seeds was estimated by weighing 20 seeds from one replicate of each genotype. To develop a relationship between total seed mass and number of seeds, we counted all of the seeds harvested from 20 random individuals.

Greenhouse Experiment II: Common Garden from Greenhouse-Grown Seeds. To evaluate germination and root-shoot ratio, we grew a second short-term common garden using greenhouse-grown seed. We also used this to evaluate leaf length and relative growth rate in plants from a common maternal environment. Two seeds from each genotype (from the 20 seeds weighed individually as described above) were planted in conetainers as in the above experiment, except with sand, on March 19, 2007. The greenhouse conditions were the same as in greenhouse experiment I. Plants were fertilized with 0.3 grams of slow-release Osmocote.⁴ Plants were watered manually once daily. Germination was monitored and recorded every other day. Pots were thinned to one plant within 1 wk of

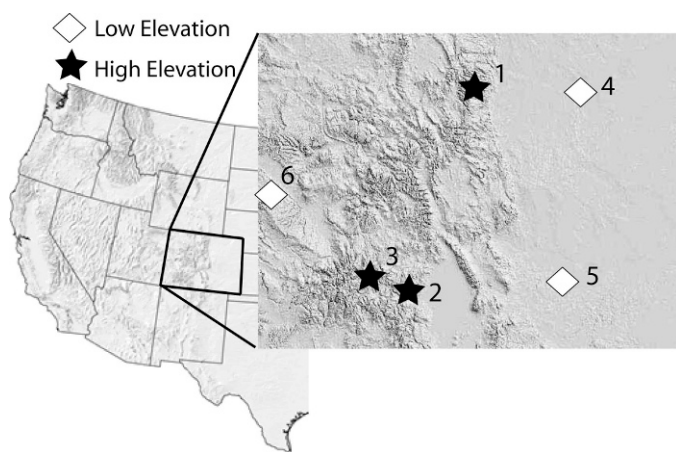


Figure 1. Location of six *Bromus tectorum* collection sites in Colorado. Sites 1 to 3 are high elevation, and sites 4 to 6 are low elevation.

germination. Growth measurements were taken in weeks two and three by measuring the longest leaf and used in the same way as above to calculate relative growth rate. Plants were harvested 31 d after germination. Above- and below-ground parts were separated, rinsed to remove sand, dried in a drying oven at 70 C, and weighed.

Neutral Molecular Genetic Variation. We evaluated the 90 genotypes described in Greenhouse Experiment I above for evidence of molecular genetic structure by site or elevation. Leaves from one replicate of each genotype were collected in the greenhouse and stored at -80 C until extraction. DNA was extracted following the methods of Doyle and Doyle (1987). We used six microsatellite loci (Bt04, Bt05, Bt12, Bt26, Bt30, Bt33; Ramakrishnan et al. 2002) with the primer labeling method of Schuelke (2000). PCR was performed in 10 μ l reactions (1 ng sample DNA, 1 μ l $10\times$ reaction buffer, 0.8 μ l 25 mM $MgCl_2$, 0.24 μ l 10 M labeled primer, 0.08 μ l 10 M forward primer, 0.24 μ l 10 M reverse primer, 0.08 μ l of 2.5 mM dNTP mix, and 0.2 μ l Taq DNA polymerase) with the following conditions: 95 C 15 min, 45 cycles (95 C 30 s, 60 C 30 s, 72 C 30 s), 8 cycles (95 C 30 s, 53 C 30 s, 72 C 30 s), followed by 5 min at 72 C. Fragments were separated on an ABI 3100 capillary instrument and scored using GeneMarker.⁵ We used the data from these microsatellite loci to elucidate population genetic structure and determine whether samples from high- and low-elevation sites were differentiated from each other.

Statistical Analyses. We used SAS 9.1⁶ to analyze variation in all traits measured from the greenhouse experiments. To evaluate whether water use efficiency differed in plants from low and high elevation when grown in a common environment, we ran a mixed model on the $\delta^{13}C$ data with elevation as a fixed effect and site nested within elevation as a random effect. For the first 10 of the 11 traits measured in greenhouse experiment I (Table 2), we measured differences between elevations using a mixed model with elevation as a fixed effect, site nested within elevation as a random effect, and genotype nested within site as a random effect. We calculated Q_{ST} for each of these traits following Spitze (1993): $Q_{ST} = \text{variance}_{\text{between sites}} / (\text{variance}_{\text{between sites}} + 2 * \text{variance}_{\text{within sites}})$. For the last trait measured in greenhouse experiment I, seed mass, we used a mixed model with elevation as a fixed effect and site nested within elevation as a random effect (this trait did not have replication at the genotype level). We used a paired t -test to analyze the differences in variances between high and low elevation, using each of the 11 traits in Table 2 as replicates. We used a linear regression to assess the relationship between total seed mass and the number of seeds, with number of seeds as the dependent variable. To measure differences between high and low elevations with maternal effects limited, we analyzed germination time, leaf

length, growth, aboveground biomass, belowground biomass, and the ratio of aboveground to belowground biomass using a mixed model with elevation as a fixed effect and site nested within elevation as a random effect. Germination time was log transformed to improve normality.

We evaluated the structure of genetic variation at microsatellite loci several ways. We performed an AMOVA using Arlequin 3.1 (Excoffier et al. 2005) to determine if there was genetic structure by elevation. In addition, we evaluated whether the samples showed isolation by distance (Isolation by Distance, Jensen et al. 2005). Finally, we visualized the microsatellite variation and evaluated whether our sampling sites represented distinct genetic clusters using Structure (<http://pritch.bsd.uchicago.edu/structure.html>; 100 K burnin, 100 K run, $K = 1$ to 12, 4 iterations, admixture model). Structure creates clusters of genetically similar individuals assuming Hardy-Weinberg equilibrium. With a predominantly selfing invasive species, sites are not in Hardy-Weinberg equilibrium, thus, the Structure analyses should be interpreted cautiously.

Results and Discussion

Greenhouse experiment I: Common Garden from Field-Collected Seeds. *Water Use Efficiency.* $\delta^{13}C$ values did not differ between elevations ($F_{1,88} = 0.36$, $P = 0.55$) or among sites ($\chi^2_1 = 0$, $P = 0.5$). $\delta^{13}C$ were -31.61 ± 0.80 (SD) and -31.69 ± 0.47 for high and low elevations, respectively. This suggests that individuals from high and low elevations have not differentiated in this important physiological trait linked to water use efficiency. This is consistent with work that evaluated instantaneous water use efficiency using gas exchange measurements that also detected no difference among populations (Rice et al. 1992).

Phenotypic and Life History Traits. Plants from lower elevations tended to have longer and wider leaves than those from high elevations, but none of the individual traits measured were significantly different between elevations after adjusting for multiple comparisons (Table 2). There was a significant amount of variation among sites and genotypes (Table 2). There was no significant difference between elevations for time until flowering, but there were differences among sites and genotypes (Table 2). Elevation was not a significant factor in total seed mass, but site and genotype were (Table 2). Total seed mass was a good predictor of the total number of seeds ($F_{1,17} = 124.51$, $r^2 = 0.87$, $P < 0.001$). There was no significant difference in the mass of 20 individual seeds between elevations, but seed mass did vary across sites (Table 2). There was no difference in the amount of variance for each trait between elevations (paired $t_{10} = 0.62$, $P = 0.55$).

Table 2. ANOVA results for fixed (elevation) and random (site and genotype) effects for traits measured in *Bromus tectorum* greenhouse experiment I. The P value for significance at the 0.05 level when controlling for multiple comparisons of the univariate tests is 0.005. Q_{ST} values are shown for each trait. The mean and standard deviation for each trait are shown for low and high elevations (length and width in cm, tiller and culm number, basal area in cm^2 , growth in cm/week, mass in g, time in d).

Trait	Effects in model						Trait values				
	Elevation		Site (elevation)		Genotype (site, elevation)		Q_{ST}	High-elevation genotypes		Low-elevation genotypes	
	F_{DF}	P	χ^2	P	χ^2	P		Mean	SD	Mean	SD
Leaf length1	20.7 _{1,4.01}	0.01	5.3	0.01	12.5	< 0.001	0.856	24.12	3.29	27.27	2.74
Leaf length2	6.4 _{1,4}	0.06	23.7	< 0.001	14.9	< 0.001	0.868	26.10	3.82	29.37	3.41
Leaf width	4.9 _{1,4}	0.09	16.3	< 0.001	9.9	0.001	0.815	0.57	0.01	0.64	0.11
Tillers	3.2 _{1,4.01}	0.15	1.8	0.09	8.6	0.002	0.547	15.53	3.91	14.39	3.02
Culms	0.2 _{1,4}	0.72	70.0	< 0.001	56.1	< 0.001	0.839	0.42	0.83	0.27	0.71
Basal area	0.4 _{1,4}	0.56	15.9	< 0.001	8.1	0.002	0.699	8.80	3.42	8.18	2.72
Growth	0.03 _{1,4}	0.88	16.2	< 0.001	4.3	0.02	0.706	0.08	0.10	0.07	0.08
Biomass	0.63 _{1,4.04}	0.47	7.2	0.004	1.4	0.12	0.620	24.66	5.40	23.61	5.22
Flowering time	0.16 _{1,4.01}	0.71	90.4	< 0.001	44.5	< 0.001	0.878	131.51	12.4	134.25	14.72
Total seed mass	0.15 _{1,4.01}	0.72	38.9	< 0.001	22.6	< 0.001	0.787	3.98	2.31	4.43	3.13
20 seeds mass	0.44 _{1,4}	0.54	8	0.002	— ^a	—	—	0.038	0.009	0.036	0.010

^aGenotype was not replicated for this trait

Phenology and biomass allocation have previously been found to vary among populations (Rice et al. 1992), and we found the same pattern: almost every trait showed a significant amount of variation among sites. We found only one phenotypic difference between elevations; initial leaf length (leaf length1) was longer in individuals from low elevations than individuals from high elevations in greenhouse experiment I. It is important to note that the results from greenhouse experiment I included both genetic and maternal effects because we used field-collected seeds. This might bias us toward finding differences between elevations, yet we still found no difference in most traits. Genotypes collected from the same site were from plants that were likely to have experienced similar environments with respect to light, water, and nutrient levels; therefore, seeds from the same site were likely to be more similarly provisioned than seeds from different sites. Thus, the significant variation in genotypes nested within sites found in experiment I might have a genetic component. Although we found no clear evidence of differentiation in phenotypic traits between elevations in our common garden experiment, genotypes might have different quantitative trait values when tested across different environments (i.e., reciprocal transplant experiments), which was not tested here.

Greenhouse Experiment II: Common Garden from Greenhouse-Grown Seeds. Germination time was marginally longer for genotypes from low-elevation sites ($F_{1,42} = 6.90$, $P = 0.056$, Figure 2) but did not differ among

sites within elevation ($\chi^2 = 0.2$, $P = 0.33$). Variance in germination timing was larger for genotypes from low-elevation sites (90.25) than from high (66.59). Leaf length (week 2) did not differ between high (67.65 ± 15.56 cm) and low (61.63 ± 19.49 cm) elevation ($F_{1,59} = 1.8$, $P = 0.19$) nor among sites ($\chi^2 = 0$, $P = 0.5$). Growth did not differ by elevation ($F_{1,59} = 0.62$, $P = 0.43$) or site ($\chi^2 = 0$, $P = 0.5$). The growth rate of genotypes from high elevation was 0.32 ± 0.20 cm/week and of genotypes from low elevation was 0.39 ± 0.51 cm/week. There was no difference between genotypes from the two elevations for belowground biomass ($F_{1,65} < 0.01$, $P = 0.97$; high: 0.458 ± 0.284 g; low: 0.469 ± 0.341 g), aboveground biomass ($F_{1,3.77} < 0.01$, $P = 0.99$; high: 0.140 ± 0.063 g; low: 0.144 ± 0.073 g), or the ratio of belowground to aboveground ($F_{1,4.48} < 0.01$, $P = 0.98$; high: 3.81 ± 2.45 ; low: 3.59 ± 2.01). Additionally, there was no effect of site on any of these ($\chi^2 = 0$, $P = 0.5$; $\chi^2 = 2.3$, $P = 0.07$; $\chi^2 = 0.1$, $P = 0.38$, respectively).

The one trait that differed between genotypes from low and high elevation in experiment I was early leaf size. We did not observe this difference in experiment II, in which we minimized maternal effects by using greenhouse-grown seeds. This suggests that the difference in early leaf size found in experiment I was an effect of maternal environment rather than genetics. Similarly, the strong effects of site on all traits in experiment I were not found in those traits also measured in experiment II, suggesting that differences observed in experiment I in plants from different sites were also due to maternal effects.

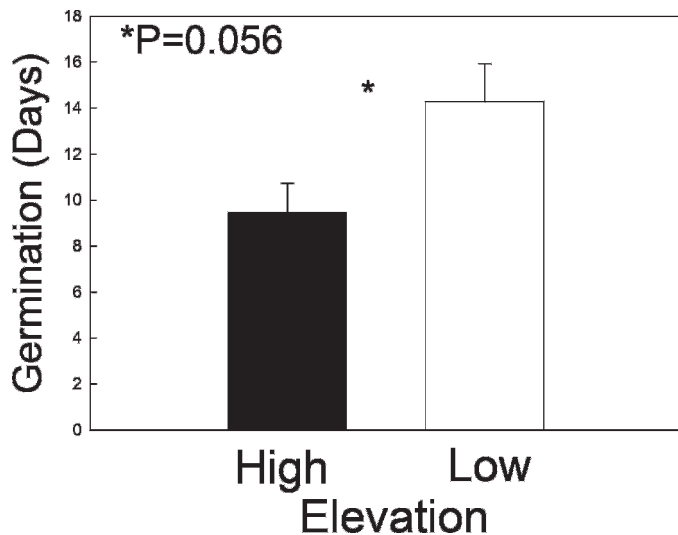


Figure 2. Number of days until germination for *Bromus tectorum* from high (black) and low (white) elevations, shown with 1 SE.

Delayed germination has been found to reduce competitive ability in *B. tectorum* (Rice and Dyer 2001), which could suggest that seeds from our high-elevation sites are more competitive. However, these previous results were with respect to differences in germination timing within a site, whereas the differences we found were among populations, where the timing of competition might be much different. Several studies have found a correlation between the strength of dormancy and increased environmental variation or unpredictability (Beckstead et al. 1996; Meyer and Allen 1999; Meyer et al. 1997). We hypothesize that the trend toward later germination of low-elevation plants in experiment II might reflect greater dormancy and be an adaptation to higher risk, more variable environments found at low elevation. Low-elevation sites have a more narrow window (approximately November through June) when adequate water is available for completion of the entire life cycle.

Although mean annual precipitation does not differ between high- and low-elevation sites (Table 1), the higher summer temperatures at low-elevation sites can lead to lower available moisture. In July and August, the months with the highest water stress in this region, low-elevation sites have a significantly lower chance of receiving 0.3 cm precipitation than high-elevation sites (probability of receiving 0.3 cm precipitation in July: high = 19.0% ± 1.0% [mean ± SE], low = 11.7% ± 1.9%, $t_4 = 3.48$, $P = 0.03$; August: high = 22% ± 2.9%, low = 10.7% ± 1.2%, $t_4 = 3.62$, $P = 0.02$; data from the Western Regional Climate Center, www.wrcc.dri.edu). If a seed germinates after a summer monsoon shower at low elevation, the seedling is likely to perish in subsequent dry conditions. At higher elevations, water is less limiting

because of more reliable afternoon thunderstorms through the summer and fall (Western Regional Climate Center, www.wrcc.dri.edu), and therefore selection on the timing of germination might be weak. Indeed, it has been found previously that seeds from some montane habitats germinate quickly and that this should not confer any disadvantage (Meyer et al. 1997). However, Meyer et al. (1997) also found a wide range of germination responses from montane habitats, suggesting that germination response patterns in recently established populations inherited from the source population might persist as long as there are not actively selected against.

Previous studies on *B. tectorum* showed greater variation in germination rates among populations from unpredictable environments and also found that water stress and high temperature can reduce seed dormancy (Allen and Meyer 2002). In this study, we found greater variation in germination timing at low elevation. Although we do not have a mechanistic explanation for the variation in germination timing found in our experiments, variation in germination response in *B. tectorum* is thought to have both genetic and environmental components (Beckstead et al. 1996), and here we confirm that germination timing does have a genetic component. Adjusting the timing of germination could be an important evolutionary change necessary for the range expansion of *B. tectorum*, as has been the case for at least one other species, *Xanthium strumarium* L. (Griffith and Watson 2006).

Neutral Molecular Genetic Variation. Most of the molecular genetic variation was found within sample locations (62.24%, $P < 0.001$). A significant amount of variation was also found among sites (30.68%, $P < 0.001$) but not between elevations (7.08%, $P = 0.33$). Site pairwise F_{ST} values were significantly different ($P < 0.05$) for all of the sites (pairwise F_{ST} range = 0.10–0.63, data not shown) except for between sites 4 and 5 (pairwise $F_{ST} = 0.09$, $P = 0.10$). There was no isolation by distance ($Z = 1465.50$, $r = 0.18$, $P = 0.25$).

An average of 1 to 3 alleles per locus was found in each of the sites (Table 3). There was complete homozygosity for all loci and individuals except one heterozygote at one locus from one site (Table 3; see also Appendix 1). To verify this result, DNA was re-extracted from the individual, and the locus was rerun. Although individuals were generally homozygous at a single locus, there were no dominant multi-locus genotypes. Only one multi-locus genotype was found in four sites, one in three sites, six in two sites, and the remaining 33 multi-locus genotypes were each found in only one site (see Appendix 1).

Site differentiation was also found in the Structure analysis, in agreement with the above AMOVAs. The negative log likelihood of K (the number of genetic clusters) reached a plateau at $K = 6$, with $K > 10$ showing

Table 3. The average number of alleles (A), expected heterozygosity (H_e), observed heterozygosity (H_o), and inbreeding coefficient (F_{IS}) for each *Bromus tectorum* site.

Site	A	H_e	H_o	F_{IS}
1	3.00	0.415	0	1*
2	2.67	0.282	0	1*
3	2.67	0.362	0	1*
4	2.50	0.399	0.011	0.968*
5	1.67	0.218	0	1*
6	2.67	0.409	0	1*

* $P < 0.001$.

a large amount of variation in values (Figure 3). Sites 4 and 5 are the most similar (agreeing with the pairwise F_{ST} analysis, Figure 4). Samples from the remaining sites grouped well with each other, with the mix of multi-locus genotypes within each site showing up visually as distinct patterns in the bar plots (Figure 4).

One of the most interesting aspects of our results is that the high level of phenotypic variation corresponds to high neutral genetic variation within and among sites but not between elevations. Our results are consistent with previous work that found relatively high within-population molecular genetic variation in the introduced range in North America (Novak and Mack 1993). Neutral molecular genetic variation in *B. tectorum* has been found to correlate more with habitat characteristics (mean annual precipitation) than with geographic distance (Novak et al. 1991; Ramakrishnan et al. 2006). This agrees with our finding of no significant isolation by distance. A lack of correlation between genetic and geographic distances at a landscape level likely means that new populations are founded in a pattern of leapfrogging dispersal, likely human- or animal-mediated. The high variability within sites suggests that populations are founded from several separate seed sources, or from a single genetically variable introduction. Indeed multiple introductions appear to be the rule in invasions (Bossdorf et al. 2005). In addition to the variation among individuals, we also found evidence of one heterozygote (at one locus). This is particularly interesting because heterozygotes have not been found in many previous studies of molecular variation in *B. tectorum* (Bartlett et al. 2002; Novak et al. 1991; Ramakrishnan et al. 2002, 2004, 2006) until recently in three Canadian populations using allozymes (Valliant et al. 2007). These recent findings suggest that *B. tectorum* might have more outcrossing than conventionally thought (this species is historically referred to as an “obligate selfer”). Even rare outcrossing events could contribute to the genetic variation within populations and also help further range expansion by aiding adaptation.

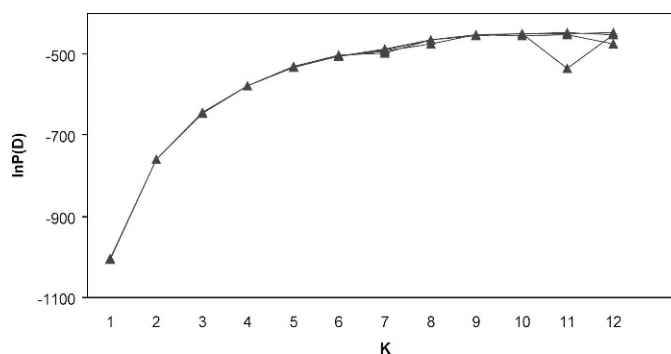


Figure 3. Likelihood rates for each number of groupings ($K = 1$ to 12) used in Structure analysis for four different simulations of *Bromus tectorum* microsatellite data. The value $\ln P(D)$ is the probability that K is the correct number of subdivisions given the data; the larger the log likelihood, the better the groupings fit the data.

Bromus tectorum is increasing in abundance in high elevations in North America and is successful in a broad range of habitats and ecological conditions. We set out to evaluate evidence for general-purpose genotypes in the Rocky Mountains, specifically exploring the amount of phenotypic and genetic variation present within and between high and low elevations. For most phenotypic traits measured, we did not find consistent differences between high- and low-elevation sites but found most variation to lie at the site and individual level. Although it has been suggested that the success of *B. tectorum* could be due to local adaptation (Meyer et al. 2004; Rice and Mack 1991), the invasion of *B. tectorum* in the Rocky Mountain United States is characterized by high levels of phenotypic and genetic variation per population. *Bromus tectorum* does not have a few general-purpose genotypes, but rather many genotypes associated with many different phenotypes. Similar to the results presented here for *B. tectorum*, the spread of *Verbascum thapsus* L. to higher elevations also is unlikely to have been driven by local adaptation; rather it was found to have high levels of phenotypic plasticity (Parker et al. 2003). Phenotypic plasticity could also be playing an important role in the success of *B. tectorum* but was not tested in these experiments.

More recent genetic analyses in *V. thapsus* also provide evidence that high genetic variability could contribute to the evolutionary potential of the populations but might not be necessary for invasion success (Dlugosch and Parker 2008). Our microsatellite data show that *B. tectorum* is successful without having one or a few common general-purpose genotypes, but instead has many. If this is the case, propagule pressure might play a larger role in the ongoing range expansion than adaptive evolution. Propagule pressure has been suggested to serve as a null model for invasions (Colautti et al. 2006) and should be a focus of future work in this system.

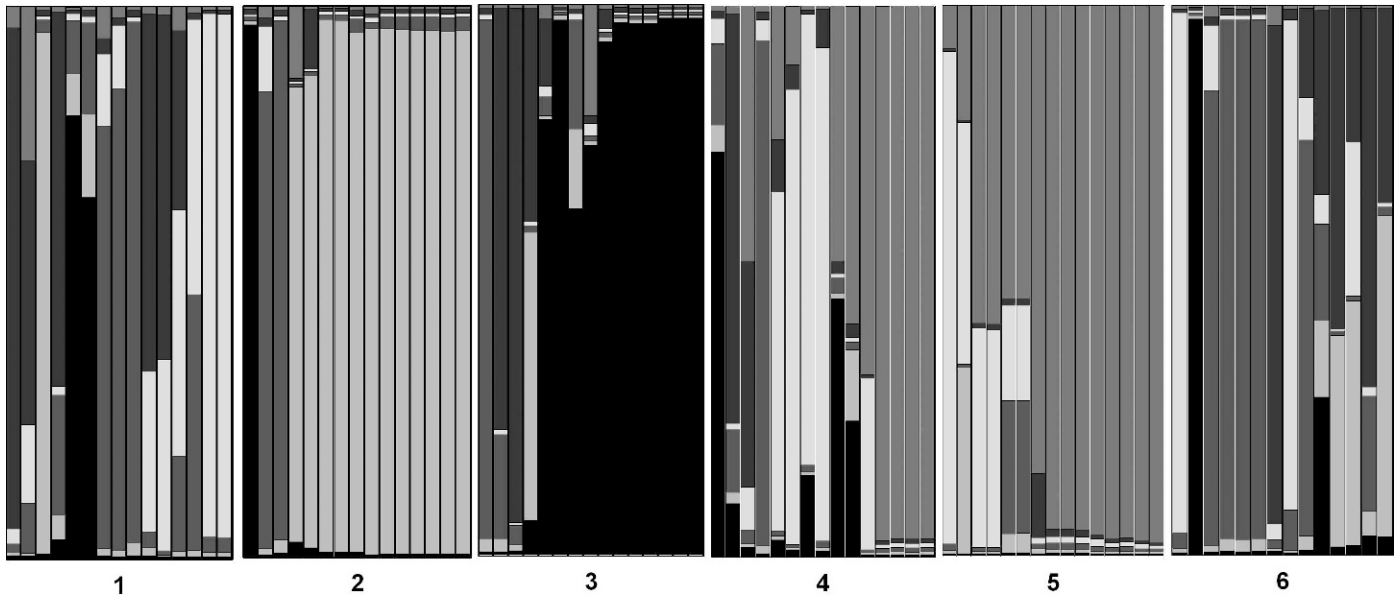


Figure 4. Structure results for *Bromus tectorum* from six microsatellite loci for $K = 6$. Each color represents a different cluster. Numbers on the bottom indicate sites; 1 to 3 are high-elevation sites and 4 to 6 are low-elevation sites.

Sources of Materials

- ¹ Conetainers, Steuwe and Sons, Corvallis, OR 97333.
- ² Elemental analyzer, C. E. Elantech, Milano, Italy.
- ³ VG isochrom isotope ratio mass spectrometer, G. V. Instruments, Manchester, United Kingdom.
- ⁴ Slow-release Osmocote, Scotts, Marysville, OH 43041.
- ⁵ GeneMarker, SoftGenetics, LLC, State College, PA 16803.
- ⁶ SAS 9.1, SAS Institute Inc., Cary, NC 27513.

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Appendix 1. Number of individuals of *Bromus tectorum* per site for each six-locus genotype. Individuals are homozygous for all loci except for one individual at locus bt5, shown in boldface type.

Loci						Sites						Total
bt4	bt5	bt12	bt26	bt30	bt33	1	2	3	4	5	6	
1, 1	1, 1	3, 3	6, 6	2, 2	2, 2	1						1
1, 1	2, 2	2, 2	2, 2	2, 2	2, 2						1	1
1, 1	3, 3	2, 2	1, 1	1, 1	2, 2	1						1
1, 1	3, 3	2, 2	2, 2	2, 2	2, 2	1	1	1			3	6
1, 1	3, 3	2, 2	2, 2	3, 3	2, 2		6					6
1, 1	3, 3	2, 2	3, 3	1, 1	2, 2	1						1
1, 1	3, 3	2, 2	5, 5	3, 3	3, 3		1					1
1, 1	3, 3	3, 3	2, 2	2, 2	2, 2	1	1				1	3
1, 1	3, 3	3, 3	4, 4	2, 2	2, 2					2		2
1, 1	4, 4	2, 2	3, 3	2, 2	2, 2				1			1
1, 1	4, 4	3, 3	4, 4	2, 2	1, 1					1		1
1, 1	4, 4	3, 3	4, 4	2, 2	2, 2				4	2		6
1, 1	4, 5	3, 3	6, 6	2, 2	2, 2				1			1
1, 1	5, 5	1, 1	3, 3	3, 2	3, 3			1				1
1, 1	5, 5	2, 2	2, 2	2, 2	2, 2	1					1	2
1, 1	5, 5	2, 2	2, 2	3, 3	2, 2						1	1
1, 1	5, 5	2, 2	3, 3	1, 1	2, 2		1	3				4
1, 1	5, 5	2, 2	4, 4	1, 1	2, 2			1				1
1, 1	5, 5	2, 2	5, 5	3, 3	2, 2						1	1
1, 1	5, 5	3, 3	5, 5	2, 2	2, 2	1					1	2
1, 1	6, 6	2, 2	3, 3	1, 1	3, 3			1				1
1, 1	7, 7	2, 2	2, 2	2, 2	2, 2			1				1
1, 1	7, 7	2, 2	5, 5	2, 2	2, 2			1				1
2, 2	5, 5	2, 2	2, 2	3, 3	2, 2						1	1
2, 2	5, 5	3, 3	2, 2	2, 2	2, 2	1						1
3, 3	2, 2	3, 3	2, 2	2, 2	2, 2						1	1
3, 3	3, 3	3, 3	2, 2	2, 2	2, 2	2					1	3
3, 3	3, 3	3, 3	3, 3	2, 2	2, 2				1			1
3, 3	3, 3	3, 3	4, 4	2, 2	2, 2					1		1
3, 3	3, 3	3, 3	4, 4	3, 3	1, 1					1		1
3, 3	4, 4	3, 3	4, 4	2, 2	2, 2				1			1
3, 3	5, 5	2, 2	2, 2	3, 3	2, 2						1	1
3, 3	5, 5	3, 3	2, 2	2, 2	2, 2				1			1
3, 3	5, 5	3, 3	4, 4	2, 2	2, 2				1			1
3, 3	7, 7	3, 3	5, 5	2, 2	2, 2	1						1
4, 4	3, 3	2, 2	2, 2	3, 3	2, 2		2					2
4, 4	3, 3	2, 2	3, 3	2, 2	3, 3				1			1
4, 4	4, 4	2, 2	2, 2	3, 3	3, 3		1					1
4, 4	4, 4	2, 2	4, 4	2, 2	2, 2				1			1
4, 4	5, 5	2, 2	2, 2	2, 2	2, 2						1	1
4, 4	5, 5	2, 2	3, 3	1, 1	2, 2			3			1	4
Individuals						11	13	12	12	7	15	70