

Local population differentiation in *Bromus tectorum* L. in relation to habitat-specific selection regimes

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Abstract A central question of invasion biology is how an exotic species invades new habitats following its initial establishment. Three hypotheses to explain this expansion are: (1) the existence of ‘general purpose’ genotypes, (2) the in situ evolution of novel genotypes, and (3) the dispersal of existing specialized genotypes into habitats for which they are pre-adapted. *Bromus tectorum* is a selfing exotic winter annual grass that has achieved widespread dominance in semiarid western North America and that is actively invading salt desert habitats. We examined mechanisms driving this invasion in three complementary studies. In reciprocal seeding experiments with ten populations from saline playa, salt desert shrubland, and upland sagebrush communities along a salinity gradient in western Utah, we found that seeds from the playa population were able to establish better than those of most other populations across all habitats, including two highly saline sites. Seeds of one of the two seed sources from upland big sagebrush communities established best on the site of origin, while the seeds of the other source established well across a range of non-saline sites. In a greenhouse experiment with playa and upland seed sources, we found evidence of adaptation to high salinity in the playa source. Playa plants were larger than upland plants in control treatments and invested less in root. They maintained vigor and seed production at high salinity better than upland plants. Molecular genetic analysis showed that the playa population was dominated by a single SSR genotype absent from upland big sagebrush populations, which were comprised of SSR genotypes generally rare or absent in populations from salt desert shrubland sites. Our results demonstrate local population differentiation in *B. tectorum*, resulting at least in part from differential selection on pre-adapted genotypes with characteristic marker fingerprints. We found little evidence for selection favoring novel genotypes.

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Introduction

There are few areas of biology where the intersection of ecology and evolutionary biology is more relevant than in the study of species invasions (Lambrinos 2004). Founding populations of invading species often experience genetic bottlenecks, so that the process of genetic drift is strongly implicated in invasion failure or success, especially initially (Dlugosch and Parker 2008). Founding populations have often been dispersed to novel environments that subject them to strong selection pressures that are most likely different from selection pressures in the habitat of origin. This combination of generally low genetic diversity in the founding population and low probability of dispersal to a suitable habitat explains why most introductions do not result in establishment. But some new arrivals establish successfully, and a small subset of these become abundant and widely distributed in their invaded ranges (Mack et al. 2000).

The process by which a species that has established small founding populations in one or more areas is then able to increase its range and abundance to become a regionally dominant organism is a key question in invasion biology. This question can sometimes be addressed in purely ecological terms, i.e., by considering an immigrant population as a static genetic entity that possesses some degree of adaptation to a new environment, with limits to invasion that are a consequence of ecological factors such as propagule pressure or disturbance regime. But more often the invasion process is likely to involve a complex interplay between evolutionary and ecological processes (Lambrinos 2004). Three alternative hypotheses have been proposed to explain the subsequent spread and persistence of a newly introduced and established species. The first emphasizes the importance of phenotypic plasticity. Baker (1965) proposed that some invasive species are able to achieve widespread success by virtue of ‘general purpose genotypes’ that are characterized by wide ecological tolerances, enabling the newly arrived population to quickly occupy a range of habitats. This invasion mechanism has been confirmed in subsequent studies, e.g., for *Verbascum thapsus* in California (Parker et al. 2003). Two other hypotheses stress the importance of genetic variation in successful invasion, and have been proposed in part to explain the often-observed lag time between a successful establishment event in a particular location and subsequent spread into adjacent areas (Williamson 1996). One idea is that an increase in both the relative and absolute abundance of formerly rare pre-adapted genotypes within the founder population can take place as selection operates over time on the array of genetic variation present. Alternatively, genetic recombination in populations of outcrossing species could result in the generation of novel genotypes, some of which may be better adapted to habitats in the introduced range and therefore able to spread more rapidly. In both cases, natural selection is responsible for increased frequency of certain genotypes; the difference lies in whether or not these genotypes result from in situ recombination.

The subject of our investigation is the winter annual grass *Bromus tectorum* L. (cheatgrass, downy brome). This very successful invader was introduced from Eurasia into western North America in the late 1800’s and has subsequently become perhaps the most common plant in the region, dominating tens of millions of hectares (Mack 1981). In extensive genetic studies using isozyme markers, Novak and Mack (2001) and their

colleagues (Novak et al. 1991; Novak and Mack 1993; Bartlett et al. 2002; Valiant et al. 2007; Schachner et al. 2008) have been able to map the distribution of different multilocus isozyme genotypes of *B. tectorum* in North America and to trace multiple introductions of this species back to their probable regions of origin in Eurasia. These studies have also demonstrated that *B. tectorum* generally exhibits low within-population genetic variation in terms of isozyme polymorphism. Populations in the introduced range tend to contain more variation than native populations, a reverse of the pattern for most introduced species, probably as a result of multiple introductions (Novak and Mack 2001; Dlugosch and Parker 2008).

Bromus tectorum exhibits tremendous phenotypic plasticity in response to variation in resource availability, lending credence to the idea that its range expansion might be due to the existence of broadly adapted general purpose genotypes (Rice and Mack 1991b). But this species also possesses adaptively significant genetic variation, especially in physiological traits that regulate biomass allocation, seed dormancy, and flowering time (Rice and Mack 1991a; Rice et al. 1992; Meyer and Allen 1999a; Rasmuson and Anderson 2002; Meyer et al. 2004). Both direct and indirect evidence indicate that local adaptation resulting from differential selection in contrasting habitats could be an important feature of the population genetics of this species in its western North American range (Rice and Mack 1991c; Meyer and Allen 1999a; Rasmuson and Anderson 2002; Meyer et al. 2004).

Bromus tectorum is known to be almost exclusively cleistogamous (McKone 1985), and heterozygous individuals are rarely found, supporting the idea that this species is highly selfing. A few heterozygotes have been encountered, however, suggesting that outcrossing can occasionally occur (Novak et al. 1993; Valiant et al. 2007; Ashley and Longland 2007; Kao et al. 2008; Schachner et al. 2008; Leger et al. 2009).

The development of microsatellite (SSR) markers for *B. tectorum* (Ramakrishnan et al. 2002) has enabled a more fine-scale examination of molecular genetic variation than has been possible with isozyme markers. Studies using SSRs have demonstrated that populations often consist of two to four common SSR genotypes and variable numbers of rare genotypes, and that genotypes may be common region-wide or restricted to particular habitats or subregions (Ramakrishnan et al. 2006).

Because of its highly selfing breeding system, it is possible to associate *B. tectorum* SSR genotypes with particular suites of adaptive traits (Ramakrishnan et al. 2004). There is also some support for the hypothesis that pre-adapted inbreeding lines with characteristic SSR marker fingerprints are favored by selection in similar environments over a wide geographic range (Ramakrishnan et al. 2006).

In the study reported here, we examined population differentiation and local adaptation in *B. tectorum* on a relatively small geographic scale, measured in tens of kilometers. We performed reciprocal seeding experiments across a salinity gradient in western Utah to look for evidence of local adaptation. In addition, we carried out greenhouse salinity tolerance experiments with playa and sagebrush steppe populations from opposite ends of the salinity gradient, and we examined population genetic structure of ten populations along the gradient using our previously developed SSR markers (Ramakrishnan et al. 2002). Our objectives were to determine: (1) whether local-scale population differentiation has taken place, (2) assuming we find population differentiation, whether differential selection in contrasting habitats is important in this differentiation (as opposed to stochastic processes like dispersal and genetic drift), and (3) assuming that differential selection is occurring, whether this differential selection is operating on pre-adapted genotypes with characteristic SSR fingerprints or on novel genotypes that have been generated in situ.

Materials and methods

Study sites

The study was conducted on the US Army Dugway Proving Grounds, located in Tooele County, Utah, approximately 100 km west of Provo, Utah. Ten *B. tectorum* populations occupying semiarid sites that varied in elevation, topography, vegetation, and soils were selected for study, with the specific goal of including sites with a range of soil salinities (Table 1). The topography of the study area is typical of the Great Basin, with low but rugged mountain ranges skirted by alluvial deposits. These deposits have coarse, rocky texture on the upper slopes but grade to finer texture on the lower slopes. The coarse, well-drained upland soils are dominated by species of sagebrush, while the finer textured soils generally have higher salinity and support an overstory of more salt-tolerant species characteristic of salt desert shrublands. The valley floor has no external drainage and is

Table 1 Habitat data for ten *Bromus tectorum* populations at Dugway Proving Grounds, Utah, included in the study

Site	Code	Elevation (m)	Vegetation type	Dominant overstory species	Soil properties					
					EC ^a (dS-m ⁻¹)	pH	Organic matter (%)	Texture		
								% Clay	% Silt	% Sand
Fivemile Hill ^b	FMH	1469	Upland	<i>Artemisia tridentata</i>	0.43	8.02	3.94	22.6	30.6	46.8
MCM	MCM	1355	Upland	<i>Artemisia nova</i>	0.77	7.89	0.34	10.0	12.4	77.6
Dune	DUN	1334	Salt desert	<i>Sarcobatus vermiculatus</i>	0.88	8.06	0.91	16.6	30.3	53.2
Carr	CAR	1326	Salt desert	<i>Sarcobatus vermiculatus</i> - <i>Atriplex confertifolia</i>	5.25	7.82	1.64	22.0	34.2	43.8
Baker	BAK	1317	Salt desert	<i>Kochia americana</i>	5.60	7.55	0.69	34.6	29.3	36.2
Charait	CHA	1314	Playa	Bare	6.00	8.44	0.31	43.0	15.2	41.8
South Wig	SOW	1315	Salt desert	<i>Atriplex canescens</i> - <i>Kochia americana</i>	0.93	8.27	0.14	16.7	1.3	82.2
Wig	WIG	1326	Salt desert	<i>Sarcobatus vermiculatus</i>	0.75	8.14	0.81	12.6	31.3	56.2
Naval Gun	NAV	1499	Upland	<i>Artemisia nova</i> - <i>Atriplex confertifolia</i>	0.72	8.23	0.39	24.7	24.3	51.2
Cane Springs	CSP	1666	Upland	<i>Artemisia tridentata</i>	0.63	7.96	1.35	13.8	21.8	64.4

^a Electrical conductivity of the saturation extract, a measure of soil salinity

^b Sites are ranked by their order along the two legs of the salinity transect, starting at the upper (least saline) end of the Fivemile transect and ending at the upper end of the Cane Springs transect, with the shared Charait playa site (most saline) in the middle

occupied in its center by a dry lake or salt playa with highly saline, clay soil that supports few or no perennial plants. The sampling transect consisted of two legs, with the most saline site, on the edge of the salt playa, as the starting point for each leg.

The Fivemile Hill (FMH) leg of the transect extended southeast from the playa site (Charait) to a big sagebrush (*Artemisia tridentata*) site on Fivemile Hill, a distance of 19 km, while the Cane Springs leg of the transect extended north from the playa site to a big sagebrush community at Cane Springs, a distance of 10 km. The Fivemile Hill leg included populations from a toposequence of six plant communities characterized by the following dominant overstory species: Charait (salt playa), Baker (*Kochia americana* or gray molly), Carr (*Sarcobatus vermiculatus*–*Atriplex confertifolia* or greasewood-shadscale), Dune (*Sarcobatus vermiculatus* or greasewood), MCM (*Artemisia nova* or black sagebrush), and Fivemile Hill (*Artemisia tridentata*). The Cane Springs (CSP) leg included populations from a toposequence of five plant communities arrayed in a crescent to the north and east: Charait (salt playa), South Wig Mountain (*Atriplex canescens*–*Kochia americana* or fourwing saltbush-gray molly), Wig Mountain (*Sarcobatus vermiculatus* or greasewood), Naval Gun (*Artemisia nova*–*Atriplex confertifolia* or black sagebrush-shadscale), and Cane Springs (*Artemisia tridentata*). The maximum distance between sites was 27 km. All sites were accessible by roads regularly used by army personnel.

Bromus tectorum populations at the different sites varied in density and extent, with generally larger and probably older populations on less saline sites. More saline sites at Dugway, represented by Baker, Carr, and Charait, are known to have been unoccupied by *B. tectorum* as recently as 15 years ago (Meyer et al. 2001), while the more upland sites have probably been occupied at least to some degree for several decades. Soils were characterized at each site by collecting surface samples (0–40 cm) from five randomly located points and bulking them for analysis. Samples were obtained in summer 2000. The following soil properties were measured: texture (% sand, silt, and clay), organic matter content, and electrical conductivity (EC) and pH of the saturation extract (Table 1). Soil analysis was carried out at the Brigham Young University Plant and Soil Analysis Laboratory. Precipitation data from the Dugway, Utah NOAA reporting station (mean annual precipitation 198 mm), located near the Carr site at the center of the study area, were used in interpretation of results.

Reciprocal seeding experiments

Seeds (technically intact florets, the disseminule in this species) of *B. tectorum* for the reciprocal seeding study were collected from each of the ten study locations in June 1999 and June 2000. Seeds from at least 30 individuals were bulked for planting. Filled seeds were selected by hand from each bulked seed lot and allowed to after-ripen under laboratory conditions prior to planting in September each year (Christensen et al. 1996). An after-ripened subsample of each lot was placed in incubation to ensure germinability prior to planting each year; all lots germinated to high percentages.

Prior to seeding at each location each year, the in situ seed bank was eliminated by successive watering and weeding treatments and subsequent removal of the top 2 cm of litter and soil in a 1-m² area for each of six block replications. The two transect legs were treated as separate reciprocal seeding experiments. Twenty-five seeds of each of six populations (FMH transect leg) or five populations (CSP transect leg) were planted in a grid of circular subplots 10 cm in diameter within each of the six blocks (cleared 1-m² areas) at each site. Seed populations for each transect leg were randomly assigned to subplots within each block, with six subplots per block at the FMH transect sites and five

subplots per block at the Cane Springs transect sites. This resulted in a total of 216 seeded subplots on the FMH transect leg and 150 seeded subplots on the CSP transect leg each year. In 1999, the seeded subplots were marked with color-coded wire rings, while in 2000 the seeds were planted into color-coded 10-cm diameter PVC tubes buried 4 cm in the soil with a 2–3 mm lip, to reduce the chance of removal by wind. Seeds were broadcast into each plot and firmed into the roughened soil surface. Planting took place on September 23, 1999 and September 2, 2000.

Each plot was read a single time, in May 2000 for the 1999 planting and in May 2001 for the 2000 planting. At this time the plants were already beginning to drop their seeds, making it impossible to accurately measure reproductive output. Consequently, only number of plants surviving to reproduction in each subplot was recorded.

Data from the reciprocal seeding experiments were analyzed by year and transect using mixed model analysis of variance (PROC MIXED; SAS Institute, Cary NC, USA) for a nested block design. The response variable, proportion of seeds planted that established/survived to reproduction, was arcsine square-root transformed for analysis. Seed population and planting site were treated as fixed effects while block was treated as a random effect nested within planting site in each analysis.

Greenhouse salinity tolerance experiments

Bromus tectorum seeds were collected from the playa site at Charait and the *Artemisia tridentata* site at Cane Springs in June 2002. These sites were chosen to represent the end points of the salinity gradient (Table 1). Seed collections were cleaned and stored at 40°C for 6 weeks to overcome primary dormancy (Christensen et al. 1996). After-ripened seeds were then moist-chilled (stratified) at 2°C for 6 weeks in an attempt to satisfy vernalization requirements for flowering. Plants of most *B. tectorum* populations will not flower without a vernalizing cold treatment either as seeds or plants, and the length of the needed treatment varies among populations and inbreeding lines. Inadequately vernalized plants also show delayed flowering and reduced seed production (Meyer et al. 2004). The moist-chilled seeds, most of which had germinated in the cold treatment, were transplanted by 3's into pots filled with washed sand. The plants were grown in a hydroponic Hoagland's solution (Epstein 1972) without added salt for the first 3 weeks, so that all plants were at the 3-leaf stage prior to treatment. For each seed source, five pots containing three seedlings each were randomly assigned to each of two blocks for each of six salinity treatments. These pots were subirrigated in trays that contained six different Hoagland's-saline mix concentrations. A block consisted of a group of six trays, one at each salinity concentration. The target concentrations were 0 (control), 50, 75, 100, 125, and 150 mM NaCl, which corresponded to electrical conductivities of 1.5 (Hoagland's solution without added salt), 6.0, 8.5, 11.2, 13.7 and 15.3 dS-m⁻¹, as measured using a conductivity meter. The salinities were gradually increased one level each week for the first 5 weeks to avoid shock until the target level was reached. This process is similar to the natural increase in salinity as playa soil water evaporates gradually over the spring months. Once target salinities were reached, experimental conditions were maintained until plants had flowered and seeds had reached maturity, a total of 14 weeks, including the acclimatization period. The experimental design was a split plot design with two blocks, six salinities (main plot), two seed sources (sub plot), and five replicates per block for a total of 120 experimental units with 3 plants per experimental unit.

Photosystem II fluorescence values for apparently healthy leaves were recorded each week, once target salinities were reached, for plants in each experimental unit, as a relative

measure of plant vigor (Maxwell and Johnson 2000). A fluorescence meter emits light at 680 nm and after 10 s of exposure, a reading is taken to measure the 695 nm light energy being re-emitted by the plant (Fv/Fm). This provides a relative measure of vigor because photosystem II quantum efficiency is a reliable indicator of plant stress (Figuerola et al. 1997). For analysis, Fv/Fm values were regressed on time for each seed source-salinity combination. The resulting slopes were used as the response variable in analysis of covariance (PROC GLM; SAS Institute, Cary NC, USA) with seed source as the class variable and salinity as the continuous variable.

Survival and flowering were scored weekly during the course of the experiment. Seeds were harvested from the experimental plants just prior to shatter and were counted to calculate mean seed number per plant in each experimental unit. Seed collections were then bulked across blocks for each treatment combination, resulting in a single bulk sample for each treatment. Mean individual seed mass was quantified for the bulked seed samples by weighing the sample and dividing by the number of seeds. Seed viability was evaluated for bulk samples from each treatment combination by allowing the seeds to after-ripen (see above), then placing them in incubation for 4 weeks at room temperature (22C) and scoring the fraction of seeds that germinated. Nonviable seeds were generally only partially filled and tended to show obvious signs of deterioration during incubation. After seed harvest, plants were harvested, and root and shoot dry mass were determined for each experimental unit by washing roots on a sieve to remove clinging sand, then drying roots and shoots to constant weight at 60C. Shoot dry mass was determined after seed harvest and did not include the mass of the seeds.

We performed mixed model ANOVA (PROC MIXED; SAS Institute, Cary NC, USA) using absolute values of response variables in the control treatment (no added salt) to examine baseline differences between the two seed sources using analysis for a randomized block design. Response variables included proportion surviving, proportion flowering, total biomass, root biomass, shoot biomass, root:shoot ratio, and seeds per plant. Only plants that flowered were included in calculating seeds per plant. Proportional data were arcsine transformed prior to analysis. As described above, seed mass and viability were not determined on individual replicates and could not be analyzed statistically.

To examine differences between the two seed sources in response to salinity, we used analysis of covariance (PROC GLM; SAS Institute, Cary NC, USA) with seed source as the class variable and salinity as the continuous variable. Relative values for each response variable listed above were calculated by dividing the mean value in each treatment by the control value (Roundy et al. 1989). This approach showcases differences between seed sources in salinity response more clearly by standardizing values for the two seed sources in the control treatment.

Molecular genetic characterization

Bromus tectorum seeds were collected from each of the ten study populations in June 2006. Seeds from 20 randomly selected individual plants separated by at least 1 m were collected at each site and after-ripened over the summer. In September, a single seed from each of twenty plants per population was planted in a labeled cell in bookplanter boxes (Spencer-Lemaire, Edmonton, Canada) filled with a peat-based potting mix, for a total of 200 planted seeds. Plants were grown to the established seedling stage, and young single leaves were harvested and subjected to DNA extraction as fresh tissue (Fulton et al. 1995). The method was adapted for use in a 96-well plate format using a tissue lyser (Spex CertiPrep Geno/Grinder 2000, Spex CSP, Metuchen NJ), which enabled grinding and extraction of

192 samples simultaneously. We then amplified four SSR loci (BT05, BT26, BT30 and BT33) in a single multiplexed PCR reaction using fluorescently labeled primers as described by Ramakrishnan et al. (2002). We visualized PCR products on an ABI Prism 3100 automated capillary genetic analyzer (Applied Biosystems, Carlsbad CA) at the Brigham Young University DNA Sequencing Center and analyzed the data using Applied Biosystems Peak Scanner Software v.1.0.

Because the visualization in the current study was carried out on a different machine than the one used in our original studies (a Perkin-Elmer ABI 377 automated DNA analyzer), we included DNA from a set of *B. tectorum* reference lines from the earlier study (Ramakrishnan et al. 2004) along with the 200 unknowns, to verify that the two DNA analyzers gave identical allele lengths for each of the SSR markers. We encountered small but consistent differences in allele lengths at each locus for reference lines from the two analyzers. The new analyzer consistently produced allele lengths 2 bp shorter than the older analyzer for loci BT05, BT30, and BT33, while allele lengths at BT26 were consistently 4 bp shorter than on the older machine. To make SSR genotypes in this study comparable to genotypes published earlier (Ramakrishnan et al. 2006), we have corrected for these differences in allele length in assigning letter codes to genotypes we report here.

We classified each individual according to its four-locus SSR genotype, and calculated the frequency of each genotype in each of the ten populations. Allele frequency data from the ten populations were used as input for analysis of molecular variance (AMOVA) to test for population differentiation. This analysis was carried out in Arlequin (Excoffier et al. 1992). We also used allele frequency data from the ten populations to calculate a genetic distance matrix, which was used to test the hypothesis of isolation by distance using Mantel correlation (Mantel 1967). The isolation by distance analysis was carried out using IDWS software (Jensen et al. 2005).

Results

Reciprocal seeding experiments

Results of the reciprocal seeding study varied dramatically between the two study years. During the first year (1999–2000), the growing season (September–May) was a period of exceptionally low precipitation, especially in fall, early winter, and spring (Fig. 1). *Bromus tectorum* survival from seed to reproduction was consequently also very low (mean 0.023). The only significant effect on each transect leg was the site effect, with all seed sources showing significantly higher survival to reproduction at most upland sites than at any of the salt desert shrubland sites (data not shown). Many of the sites, including the playa site, had no surviving established plants. There was no significant effect of seed source on either transect leg and no significant source by site interaction.

In 2000–2001, overall *B. tectorum* survival from seed to reproduction was much higher (mean 0.185). The growing season in 2000–2001 included substantial precipitation events in September, October, and January, although early winter and spring precipitation were again below average (Fig. 1). The site main effect was significant on both transect legs, largely because of complete recruitment failure at the playa site (Table 2; Fivemile Hill transect leg: $F = 18.01$, $df = 5, 30$, $P < 0.0001$; Cane Springs transect leg: $F = 29.99$, $df = 4, 25$, $P < 0.0001$). On the CSP transect leg, survival to reproduction was similarly high at the four non-playa sites (range 0.26–0.29). On the FMH transect leg, survival to

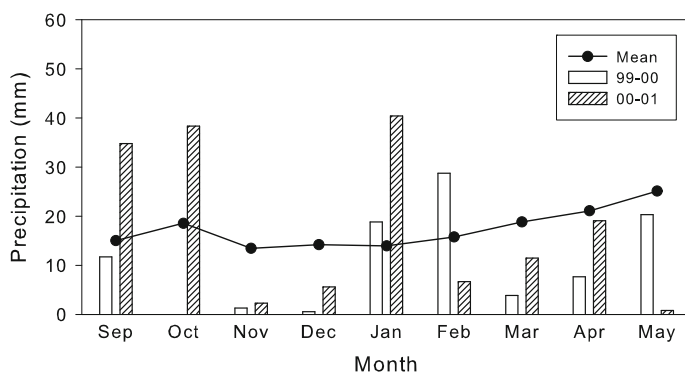


Fig. 1 Precipitation totals for *Bromus tectorum* growing season months (September–May) at the Dugway, Utah NOAA reporting station, including long term mean values and values for the 1999–2000 and 2000–2001 growing seasons, which correspond to the years of the reciprocal seeding study

Table 2 Proportion of planted seeds emerging and surviving to reproduction for seeds from six *B. tectorum* populations reciprocally planted at a series of sites on the Fivemile Hill transect leg and from five *B. tectorum* populations reciprocally planted at series of sites on the Cane Springs transect leg in September 2000 (see Table 1 for codes and site information)

Fivemile Hill transect leg

Site	Seed source						Mean
	FMH	MCM	DUN	CAR	BAK	CHA	
FMH	0.29	0.23	0.34	0.19	0.35	0.36	0.29a ^a
MCM	0.11	0.06	0.08	0.24	0.25	0.23	0.16b
DUN	0.12	0.12	0.07	0.11	0.11	0.35	0.15b
CAR	0.11	0.11	0.18	0.08	0.08	0.24	0.13b
BAK	0.02	0.10	0.07	0.22	0.07	0.33	0.14b
CHA	0	0	0	0	0	0	0c
Mean	0.11b	0.10b	0.12b	0.14b	0.14b	0.25a	

Cane Springs transect leg

Site	Seed source					Mean
	CSP	NAV	WIG	SOW	CHA	
CSP	0.32	0.18	0.23	0.29	0.38	0.28a
NAV	0.32	0.18	0.27	0.26	0.44	0.29a
WIG	0.33	0.17	0.26	0.30	0.38	0.29a
SOW	0.28	0.23	0.21	0.18	0.41	0.26a
CHA	0	0	0	0	0	0b
Mean	0.25ab	0.15c	0.19bc	0.21bc	0.32a	

The population at the playa site (CHA) was common to both transects

^a Main effects means on each transect leg that are followed by the same letter are not significantly different at $P < 0.05$, based on means separations from mixed model ANOVA

reproduction was about twice as high at the big sagebrush site (0.29) as at other non-playa sites (0.13–0.16).

The seed source main effect was also significant on both transect legs in 2000–2001 (Fivemile Hill transect leg: $F = 5.83$, $df = 5, 150$, $P < 0.0001$; Cane Springs transect leg: $F = 5.72$, $df = 4, 100$, $P = 0.0003$). The playa source showed the highest mean survival to reproduction across all sites on both transects (Table 2). On the FMH transect leg, sources from the other five sites did not differ significantly from each other and averaged about half the survival to reproduction of the playa source (0.10–0.14 vs. 0.25). On the CSP transect leg, survival to reproduction varied significantly among sources and ranged from a mean of 0.32 for the playa source to 0.15 for the black sagebrush-shadscale source.

The site by seed source interaction on the CSP transect leg in 2000–2001 was not significant, because all sources showed the same pattern of uniform survival to reproduction across all non-playa sites (Table 2; $F = 0.71$, $df = 16, 100$, $P = 0.7769$). The site by seed source interaction was significant on the FMH transect leg ($F = 1.96$, $df = 25, 150$, $P = 0.0071$). Only the playa source showed the pattern of uniform survival across all non-playa sites. The other five sources from the FMH transect generally showed highest survival to reproduction on the big sagebrush site, with lower survival at other sites along the transect.

The reciprocal seeding experiment demonstrated local population differentiation as evidenced by differences among seed sources in survival to reproduction in 2000–2001. But there was little direct evidence for local adaptation. The FMH big sagebrush source clearly performed best on its own site, while the playa seed source did better than other seed sources on saline sites at Baker and Carr (Tables 1, 2), even though it was unable to establish on its own site under conditions of the experiment.

Higher survival in general at non-playa sites on the CSP transect leg relative to the FMH transect leg could be due to lower soil salinity at valley sites on the CSP transect (Table 1). For big sagebrush seed sources, a regression of survival on soil salinity was significant when both transect legs were included ($df = 8$, $r = -0.796$, $P < 0.01$). On the other hand, the survival of the playa seed source was not significantly correlated with salinity ($df = 7$, $r = -0.481$, n. s.), as long as the playa site itself is excluded.

Greenhouse salinity study

Playa and upland seed sources showed many contrasting responses in the greenhouse study, both in the low-salinity control and in response to the gradient of increasing salinity. At low salinity, plants from the playa averaged nearly twice the dry biomass of plants from the upland source (Fig. 2; Table 3). Above-ground biomass was more than twice that of upland plants, while root biomass was significantly lower, resulting in a significantly lower root-shoot ratio in the playa source.

Survival in the control treatment was similarly high for both seed sources (Fig. 2). A significantly larger proportion of individuals from the upland source flowered in the control treatment, probably because their vernalization requirement for flowering was more completely met under these experimental conditions (Table 3). Mean number of seeds produced per flowering individual was also significantly higher for the upland source, probably for the same reason. Individual seed mass and seed viability in the control treatment appeared to be similar for the two sources but could not be tested for significance due to design constraints.

Plants from the playa seed source showed significantly higher survival than upland plants as salinity increased (Table 3; Fig. 3). They also tended to maintain vigor more

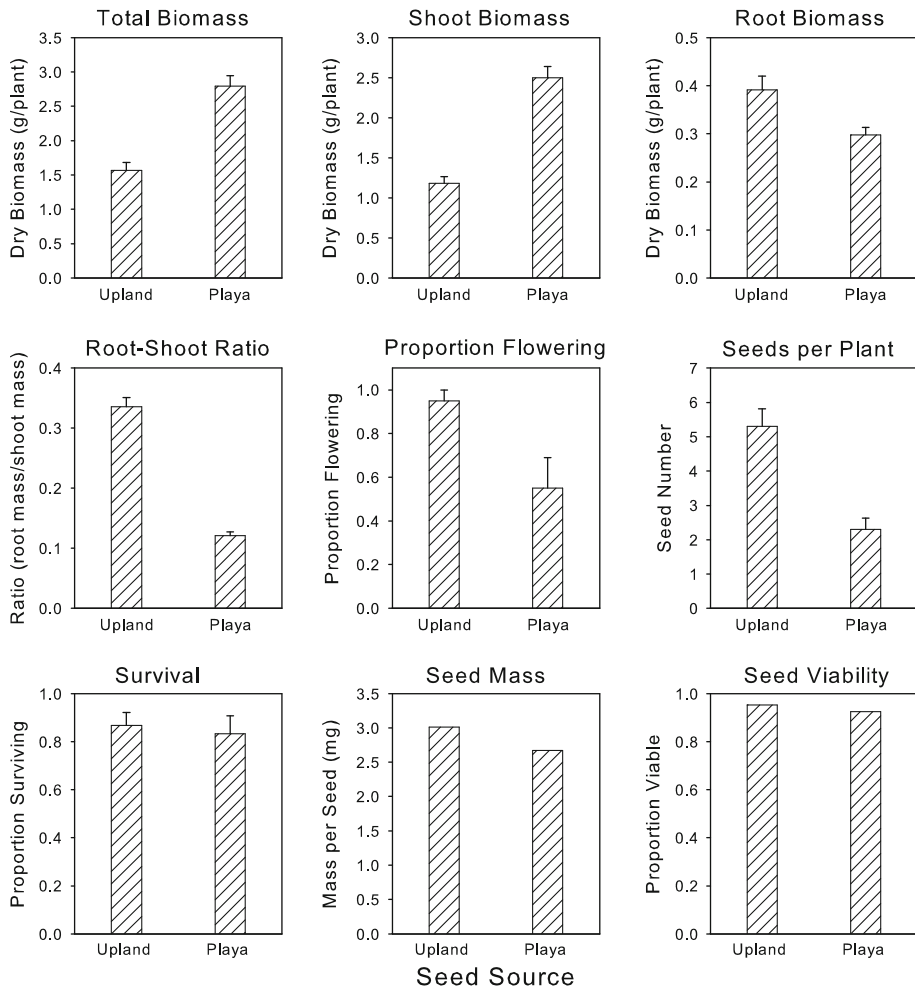


Fig. 2 Upland (Cane Springs) and playa (Charait) seed source mean values with standard errors in the control (no added salt) treatment for nine response variables measured in the greenhouse salinity experiment. See Table 3 for statistical analysis

successfully, as measured by the relationship between salinity and the slope of decrease in the fluorescence ratio (F_v/F_m) through time, with a steeper decline as a function of salinity for the upland source. Proportion of plants flowering also decreased significantly as a function of increasing salinity, but there was no significant difference between seed sources.

Plants from both sources showed a sharp drop in above-ground biomass at relatively low salinity, followed by a leveling-off at low values at higher salinities (Fig. 3). There was no significant source by salinity interaction (Table 3). This is in contrast to the pattern for root biomass, which showed a 60% decrease with increasing salinity for the upland source but not for the playa source, which showed only a 30% decrease. In spite of this difference in effect on roots, the effect of salinity on total dry biomass was not significantly different between sources. There was a larger shift in resource allocation to roots in the

Table 3 Results of mixed model ANOVA for the absolute response of plants from upland and playa seed sources in the control (no added salt) treatment in the greenhouse salinity gradient study and results of ANCOVA for the relative response of the two seed sources to the salinity gradient in the same study

Response variable	ANOVA Control treatment <i>df</i> = 1, 17			ANCOVA Salinity gradient					
	<i>df</i>	<i>F</i>	<i>P</i>	Overall model <i>df</i> = 2, 9		Salinity main effect <i>df</i> = 1, 9		Seed source × salinity interaction <i>df</i> = 1, 9	
				<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
Survival proportion	1.17	0.15	0.7029	18.56	0.0006	31.15	0.0003	5.98	0.0370
Root biomass	1.17	8.54	0.0095	9.98	0.0052	8.93	0.0152	11.03	0.0089
Shoot biomass	1.17	23.74	0.0001	15.24	0.0013	30.46	0.0004	0.02	0.9800
Total biomass	1.17	23.16	0.0002	13.78	0.0018	27.57	0.0005	0	0.9853
Root: shoot ratio	1.17	170.50	<0.0001	73.98	<0.0001	79.87	<0.0001	68.08	<0.0001
Flowering proportion	1.17	7.18	0.0153	10.71	0.0024	17.40	0.0024	4.02	0.0759
Seeds per plant	1.15	9.58	0.0074	8.08	0.0098	4.34	0.0668	11.82	0.0074
Individual seed mass ^a	–	–	–	29.10	0.0004	30.17	0.0004	28.04	0.0005
Seed viability ^a	–	–	–	203.50	<0.0001	310.78	<0.0001	96.22	<0.0001
Fluorescence ^b	–	–	–	166.86	<0.0001	312.23	<0.0001	21.49	0.0012

^a Variable not analyzed because data not collected on a per replicate basis

^b Variable not analyzed because fluorescence (Fv/Fm) values for the control (low stress) treatment did not change through time for either seed source

playa source. The root-shoot ratio of the playa source increased by a factor of four as salinity increased, while that of the upland source increased only by a factor of two.

Plants from the playa maintained their absolute shoot and total dry biomass advantage across all salinities, averaging 1.5 times the shoot biomass and almost twice the total biomass of upland plants even at the highest salinity. Even though plants of both seed sources increased their root:shoot ratios as salinity increased, absolute root:shoot ratios were generally low even at the highest salinity. The upland source maintained higher absolute root:shoot ratios across all salinities. The playa source root:shoot ratio increased from 0.12 in the control to 0.48 at the highest salinity, while the upland source root:shoot ratio increased from 0.34 to 0.67.

Plants from the playa source were able to maintain their reproductive output more successfully than upland plants as salinity increased (Table 3; Fig. 3). They showed only a 15% decrease in seed output per flowering plant at the higher salinities, while the upland plants showed a 42% decrease. Seeds from the playa source were also able to maintain quality at high salinities better than seeds from the upland source, with much smaller reductions in individual seed mass and viability.

Molecular genetic study

Molecular genetic analysis of 200 individuals using SSR polymorphism at four loci revealed the presence of 28 four-locus genotypes. Twelve of these genotypes were

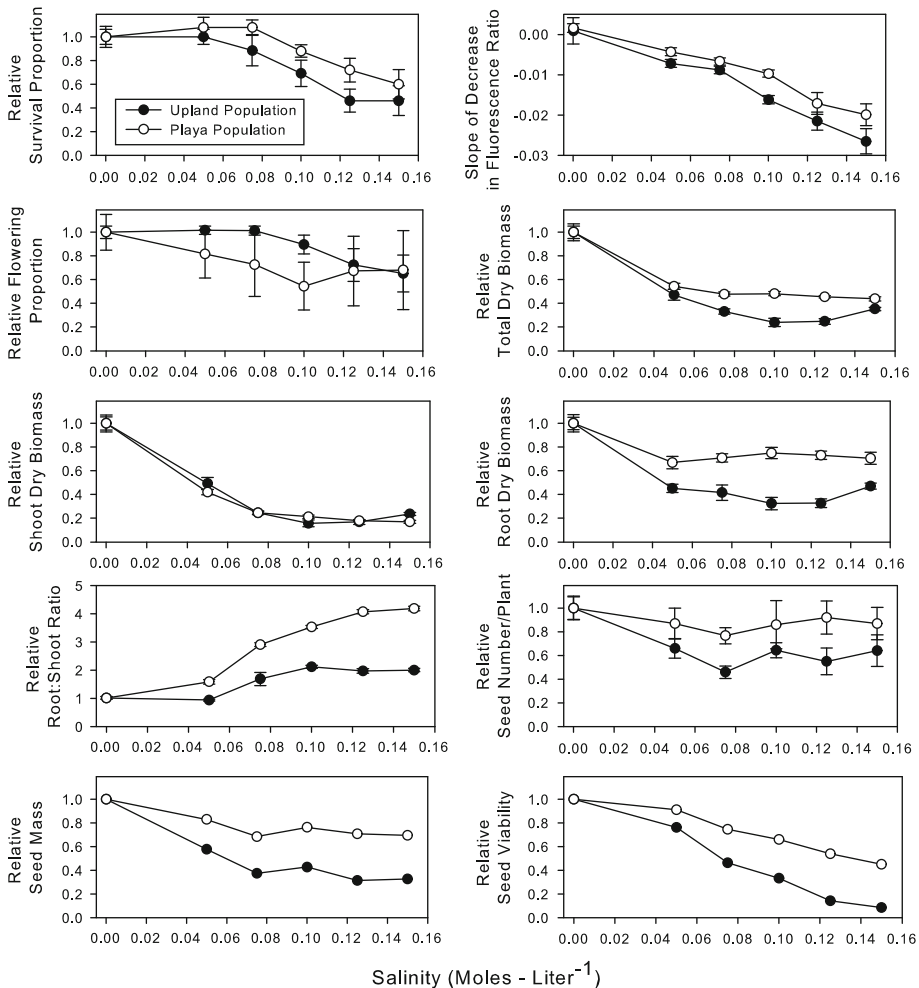


Fig. 3 Response to salinity for ten variables measured in greenhouse sand culture using plants grown from upland (Cane Springs) and playa (Charait) seed sources. Relative values were calculated by dividing each mean value by the mean value for that source in the control (no added salt) treatment. Error bars are standard errors from analyses based on full replication. No error bars are given for relative seed mass or viability because of design constraints (see text). See Table 3 for statistical analysis

encountered in single individuals and are not considered further. The remaining 16 SSR genotypes differed widely in abundance both within and among populations (Fig 4).

The most frequently encountered SSR genotype was IEGB (eight of ten populations, 31% of total individuals; Fig. 4). It dominated the playa site (frequency 0.85) and was relatively common across all salt desert shrubland habitats. It was generally less common in upland habitats and was not detected at all at either of the upland big sagebrush sites. In contrast, the second most common SSR genotype, DCBB (nine of ten sites, 13% of total individuals), was widely distributed but generally not very common. It showed no clear pattern with respect to habitat.

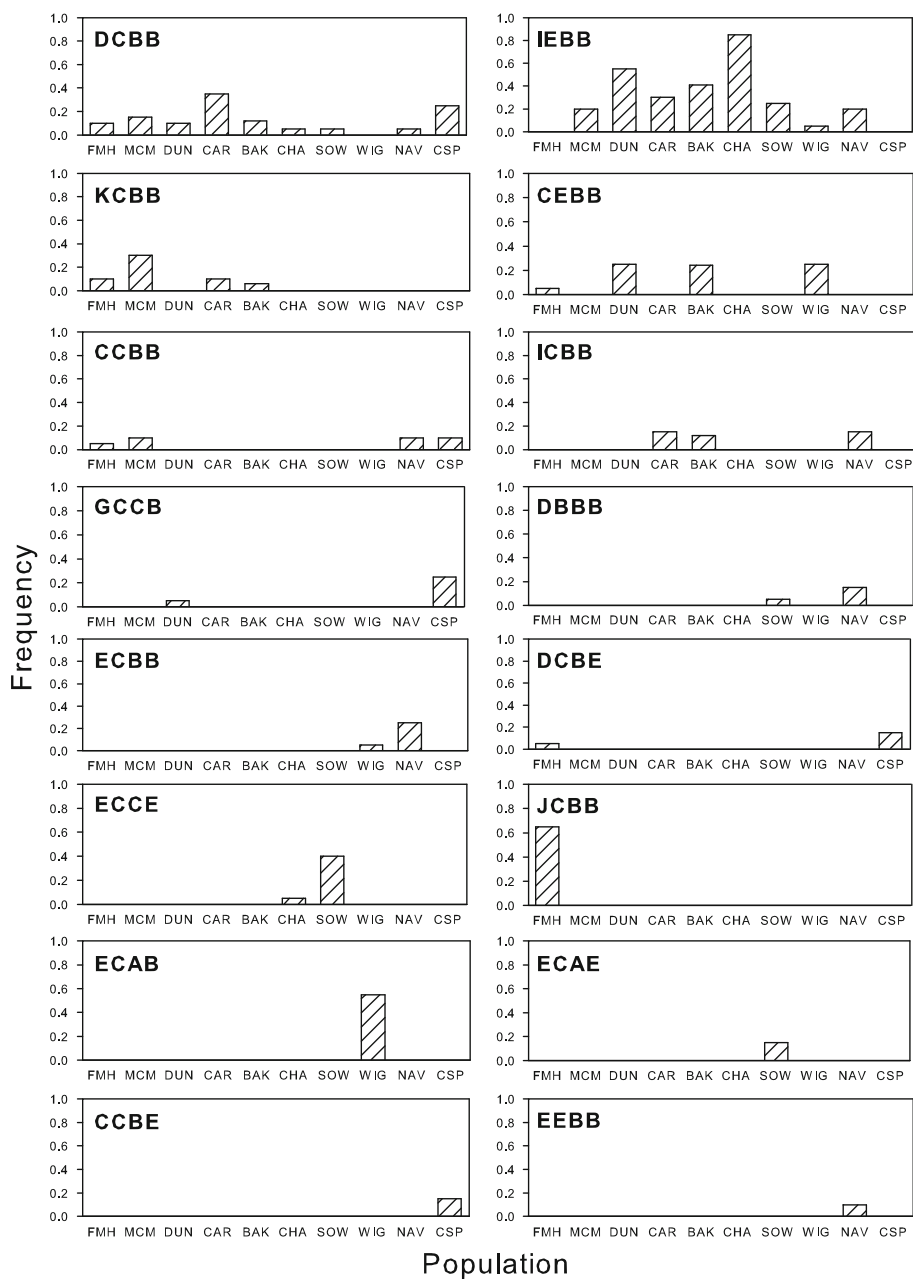


Fig. 4 The frequency of 16 four-locus SSR genotypes in each of ten *Bromus tectorum* populations included in the study. Genotypes are ranked from most to least common as indicated by the number of populations in which each was found. Populations are ordered as they are arrayed geographically in the field, from the Fivemile Hill (FMH) big sagebrush population at the top of the first transect leg down to the Charait (CHA) population at the playa edge and then up to the Cane Springs (CSP) big sagebrush population at the top of the second transect leg (see Table 1 for codes and site information)

The upland big sagebrush sites at Fivemile Hill and Cane Springs were generally occupied by SSR genotypes that were rare or absent in salt desert shrubland populations (Fig. 4). The Fivemile Hill population was dominated by SSR genotype JCBB (frequency 0.65). The Cane Springs population was more polymorphic, with SSR genotypes GCCB and DCBB each at a frequency of 0.25 and with three less common genotypes (DCBE at 0.15, CCBE at 0.15, and CCBB at 0.10). Two of these three less common genotypes were also present in the Fivemile Hill population, while none of the three were encountered in salt desert shrubland habitats. Of genotypes common (frequency > 0.10) at either big sagebrush site, only DCBB was also common at any salt desert shrub site.

Of the remaining nine SSR genotypes found in more than one individual, five were found at a single site or at two adjacent sites, two were found at two to four sites on a single transect leg, and two were found at three or four more widely separated sites (Fig. 4). All but one of these nine genotypes were rare or absent at upland big sagebrush sites. The exception was SSR genotype KCBB, which was found at the Fivemile Hill big sagebrush site (frequency 0.10), at the adjacent MCM black sagebrush site (frequency 0.35), and at low frequency at the Carr and Baker salt desert shrubland sites.

Most of the SSR genotypes encountered in this study were not novel. Eighteen of the 28 genotypes encountered were also found at Whiterocks, approximately 30 km northeast of the Dugway sites, by Ramakrishnan et al. (2006), and one additional genotype was found in that study at a more distant site. All but two of the 16 genotypes found in more than one plant in this study were also found at Whiterocks. Of the nine novel genotypes we encountered, only one, represented by a single individual, contained an allele (BT26, length 147 bp) not reported by Ramakrishnan et al. (2006).

Two of the novel genotypes encountered in this study were represented by single individuals heterozygous at one or more of the four SSR loci. The heterozygote at the MCM study site was apparently the result of a cross between two genotypes present at the site, CCBB and KCBB (genotype C/K-C-B-B). The second heterozygote (C-A/C-B-B), at the Cane Springs site, apparently had the common genotype CCBB as one parent, and a genotype not detected in the study, CABB, as the other parent. Only one of the ten homozygous genotypes that were encountered as single individuals was a possible recombination product of common genotypes recorded in the study.

Analysis of molecular variance (AMOVA) showed that a significant fraction of SSR allelic variation was distributed among populations ($F_{st} = 0.2887$; $df = 9$; $P = 0.00000$), supporting the idea that populations differed significantly in their genotypic makeup. Thirty-six of 45 pairwise population F_{st} 's were significant at $P = 0.00000$, and only five were not significant at $P < 0.05$. This demonstrates that populations were generally strongly genetically differentiated from one another. The five non-significant pairwise comparisons all involved sites at the lower end of the Fivemile Hill transect leg, but adjacent sites were generally not those with non-significant pairwise F_{st} 's, except in the case of the Carr and Baker sites, whose pairwise F_{st} had a marginally significant P value of 0.090.

The results of the isolation by distance analysis using a Mantel test of the correlation between a genetic distance matrix based on SSR allele frequencies and a geographic distance matrix revealed no significant correlation between genetic and geographic distance ($n = 45$, $r = 0.1827$, $P = 0.1142$). This indicates that adjacent populations are no more likely to be similar in their SSR allelic composition than more widely separated populations, a result expected from population pairwise F_{st} values from AMOVA.

Discussion

Taken as a whole, this study has produced some support for the hypothesis of local population differentiation in response to habitat-specific selection pressures for *B. tectorum* across a toposequence of semiarid habitats in western Utah. The present distribution of genotypes across the landscape appears to have been produced by a combination of stochastic and selection-driven processes. Molecular genetic analysis showed that populations from playa and upland big sagebrush populations were largely comprised of different SSR genotypes. Seeds collected from the playa population showed significantly higher establishment and survival over most habitats. Specifically, seeds from the playa population performed better on two highly saline valley sites than seeds from the upland population planted at those sites. Playa and upland seed sources also showed contrasting life history strategies and contrasting responses to increasing salinity in a greenhouse salinity gradient experiment.

We did not find a significant correlation between site salinity and establishment of the playa seed source in our reciprocal transplant experiment as long as the playa site was excluded. The playa seed source established just as well at lower valley sites on the FMH transect, which were nearly as saline as the playa site, as they did at nonsaline valley and upland sites. This suggests that salinity per se was not the factor that prevented establishment of the playa seed source on the playa. A more likely explanation is that the experimental seeding treatment did not provide the safe sites in cracks that are apparently necessary for successful establishment and survival in the heavy clay playa soil, even for salinity-tolerant seedlings.

The significant negative correlation we obtained between site salinity and establishment of seed sources of *B. tectorum* from big sagebrush sites suggests that higher survival of the CSP seed source at valley sites on its transect leg may have been due to the less harsh nature of these sites rather than to higher fitness (e.g., salinity tolerance) of the CSP seed source relative to the FMH seed source. It is likely that both big sagebrush *B. tectorum* populations would be excluded from more saline sites.

The idea of invasion of new habitats by means of broadly adapted general purpose genotypes was marginally supported by our study. The SSR genotype DCBB was widely distributed across habitats in this study and is also widely distributed across a range of habitats in the region (Ramakrishnan et al. 2006; K. R. Merrill unpublished data). It was the most abundant genotype at Carr, one of the more saline sites, suggesting that it has wide ecological amplitude. Seeds from the playa population, dominated by the IEBB genotype, established and survived more successfully than seeds of any other population across all habitats in the reciprocal seeding experiment. This could be taken as evidence that IEBB genotype also represents a general purpose genotype. But IEBB generally showed a pattern of decreasing abundance in less harsh environments, and was absent from big sagebrush habitats. This genotype is common throughout the southern half of the Intermountain region on more xeric salt desert sites but is generally rare or absent on upland sites (Ramakrishnan et al. 2006; K. R. Merrill unpublished data).

The strong physiological differentiation between playa and upland populations in the greenhouse study also provided evidence for local adaptation, although the fact that seeds used in this experiment were field-collected rather than produced in a common environment makes it difficult to completely rule out maternal effects (Roach and Wulff 1987). Maternal effects have been detected for seed traits in *B. tectorum*, but the genetic component of variance was always much larger than the environmental component (Meyer and Allen 1999a, b). Kao et al. (2008) detected small differences in *B. tectorum* establishment

in a reciprocal seeding experiment for field-produced seeds but not for common garden-produced seeds, and attributed this result to maternal effects.

Rasmuson and Anderson (2002) found that a *B. tectorum* ecotype from a salt desert site in central Washington had twice the shoot growth rate of an ecotype from a big sagebrush community in eastern Idaho, at higher salinities as well as in controls. In contrast to our study, root:shoot ratios decreased as salinity increased for both ecotypes. Rice et al. (1992) found that root:shoot ratios were lower and shoot growth rates were higher in plants from more xeric environments than in those from mesic environments in Washington. Both these studies suggested that increased investment in shoots, enabling more rapid growth in xeric or saline habitats, is an adaptation to ensure successful maturation in a rapidly drying environment. Our study supports this conclusion, and indicates that adaptations to avoid water stress during maturation are more important than adaptations to endure water stress in this winter annual species. Plants from the playa population had faster growth rates (i.e., higher final biomass) and lower root:shoot ratios than plants from the upland population across a range of salinities, even though the relative shift to investment in root at higher salinity was greater for the playa seed source. The ability to produce high quality seeds in the face of increasing salinity is also an important adaptation exhibited by plants from the playa population.

It is likely that the salt desert seed populations used by Rice and Mack (1991a) and Rasmuson and Anderson (2002), both of which were collected from the Department of Energy Hanford ALE site in central Washington, represent a different SSR genotype than the IEBB genotype used in our study. We have not detected the IEBB genotype in salt desert shrubland populations from Hanford or other xeric habitats in central Washington (K. R. Merrill unpublished data). This could help explain why differences in root:shoot ratio response to salinity were observed in the different studies.

Adaptations for rapid growth, including a low root:shoot ratio, may place the playa genotype (IEBB) at a disadvantage in less harsh environments, where rapid maturation may not be under positive selection, and competition for belowground resources is a major factor in success. This could explain why the IEBB genotype apparently does not occur in more mesic environments in our study area and region wide, even though it performed well in steppe environments in our reciprocal seeding experiment. Each seed source was planted into its own plot at relatively low density in the seeding experiment, reducing the impact of intraspecific competition, which would be much more intense in dense, natural stands of mixed genotypes (Rice and Mack 1991b). A reciprocal seeding experiment in Washington produced some evidence of local adaptation for a salt desert shrubland (greasewood) population, but not for a sagebrush steppe population (Rice and Mack 1991c). Seeding rates were similar to those used in our study.

Rice and coworkers also found that populations from xeric sites flowered earlier than populations from mesic sites, both when plants were winter-vernalized in an unheated greenhouse and in the field (Rice and Mack 1991a, c; Rice et al. 1992). We vernalized seeds prior to planting, and obtained the counterintuitive result that individuals of the playa population were less likely to flower and also set fewer seeds in the control treatment than individuals of the upland population. The difference in flowering response between the two seed populations may have been an artifact of our methods, as the vernalization period was shorter than would be experienced under natural conditions and day length was not controlled (Meyer et al. 2004). This could also explain why seed set was generally low in our experiment. Plants grew normally in the hydroponic system we used, so that low seed set was not due to abnormally small plant size. We did not measure time to flowering in our experiment.

The case for generation of novel genotypes as a substrate for selection was not strongly supported by our study. We encountered two heterozygous SSR genotypes, a frequency of 1.0% overall. This is higher than frequencies reported in our earlier studies using this marker system (Ramakrishnan et al. 2006) but is similar to the frequency reported by Kao et al. (1.1%; 2008). It is lower than heterozygote frequencies reported by Ashley and Longland (2007) for two western Nevada populations. Infrequent recombination events could play a role in generating potentially better-adapted novel genotypes, but we saw little evidence for this. Instead, we encountered a limited number of SSR genotypes, and most did not appear to represent the result of recombination events. Populations were generally dominated by a few common genotypes. Selection or drift operating on pre-existing genotypes with characteristic marker fingerprints is the most parsimonious explanation for this pattern.

Dispersal is a stochastic process, but once present, an SSR genotype presumably becomes common in a population because it is adapted to the site, while poorly adapted genotypes remain rare and do not persist. In our earlier study at nearby Whiterocks, we detected several rare genotypes that appeared to be recombination products of common genotypes, but these did not persist across years, suggesting that they had lower fitness than their parents (Ramakrishnan et al. 2006).

It is undoubtedly true that a sizeable fraction of the genetic differentiation observed in our study was due to factors other than selection. The evidence for local adaptation in our reciprocal seeding study was not very strong. Several SSR genotypes were common at only one site, even though apparently similar sites were available for colonization. These locally restricted genotypes may represent more recent introductions, a likely scenario given the level of traffic from outside the area associated with military activities (Gelbard and Belnap 2003). These genotypes may not yet have had the opportunity to achieve their potential distribution in the area. The lack of correlation between genetic distance and geographic distance in our molecular genetic data for *B. tectorum* is not surprising, given the likelihood of repeated introductions of different genotypes, high dispersal potential, and the stochastic nature of the dispersal process itself. The fact that we were able to detect pattern even in the face of continuing influx and mixing of genotypes may be the best evidence for strong local selection as a factor in the genetic structuring of populations of this remarkably successful weed.

The ability of *B. tectorum* to continue to invade new habitats appears to be a result of a complex set of strategies that includes broadly adapted ‘general purpose’ genotypes that can occupy a broad range of habitats as well as specialized genotypes that are apparently preadapted to occupy more extreme habitats. A role for outcrossing and the generation of novel genotypes in the invasion process was not strongly supported in this study of invasion of more xeric sites, but may yet prove to be important for *B. tectorum* in the invasion process at high elevation sites. Two studies of population genetic structure at the upper limit of *B. tectorum* distribution have revealed much higher levels of SSR genotype diversity than we encountered in salt desert shrublands on the Dugway Proving Grounds (Kao et al. 2008; Leger et al. 2009). This suggests that factors facilitating successful invasion of *B. tectorum* into salt desert versus high elevation marginal environments may differ in fundamental ways.

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References

- Ashley MC, Longland WS (2007) Microsatellite evidence of facultative outcrossing in cheatgrass (*Bromus tectorum*): implications for the evolution of invasiveness. *Plant Species Biol* 22:197–204
- Baker HG (1965) Characteristics and modes of origin of weeds. In: Baker HG, Stebbins GL (eds) *The genetics of colonizing species*. Academic Press, New York, pp 147–168
- Bartlett E, Novak SJ, Mack RN (2002) Genetic variation in *Bromus tectorum* (Poaceae): differentiation in the eastern United States. *Am J Bot* 89:602–612
- Christensen M, Meyer SE, Allen PS (1996) A hydrothermal time model of seed after- ripening in *Bromus tectorum* L. *Seed Sci Res* 6:155–163
- Dlugosch KM, Parker IM (2008) Founding events in species invasions: genetic variation, adaptive evolution, and the role of multiple introductions. *Mol Ecol* 17:431–449
- Epstein E (1972) *Mineral nutrition of plants: principles and perspectives*. Wiley, New York
- Excoffier L, Smouse P, Quattro J (1992) Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics* 131: 479–491
- Figuerola ME, Fernández-Baco L, Luque T, Davy AJ (1997) Chlorophyll fluorescence, stress and survival in populations of Mediterranean grassland species. *J Veg Sci* 8:881–888
- Fulton TM, Chunwongse J, Tanksley SD (1995) Microprep protocol for extraction of DNA from tomato and other herbaceous plants. *Plant Mol Biol Rep* 13:207–209
- Gelbard JL, Belnap J (2003) Roads as conduits for exotic plant invasions in a semiarid landscape. *Cons Biol* 17:420–432
- Jensen JL, Bohonak AJ, Kelley ST (2005) Isolation by distance, web service. *BMC Genetics* 6: 13. v.3.15 <http://ibdws.sdsu.edu/>
- Kao RH, Brown CS, Hufbauer RA (2008) High phenotypic and molecular variation in downy brome (*Bromus tectorum*). *Invasive Plant Sci Manag* 1:216–225
- Lambrinos JG (2004) How interactions between ecology and evolution influence contemporary invasion dynamics. *Ecology* 85:2061–2070
- Leger E, Espeland E, Merrill KR, Meyer SE (2009) Genetic variation and local adaptation at a cheatgrass (*Bromus tectorum*) invasion edge in western Nevada. *Mol Ecol* 18:4366–4379
- Mack RN (1981) Invasion of *Bromus tectorum* into western North America: an ecological chronicle. *Agroecosystems* 7:145–165
- Mack RN, Simberloff D, Lonsdale WM, Evans H, Clout M, Bazzaz FA (2000) Biotic invasions: causes, epidemiology, global consequences, and control. *Ecol Appl* 10:689–710
- Mantel NA (1967) The detection of disease clustering and a generalized regression approach. *Cancer Res* 27:209–220
- Maxwell K, Johnson GN (2000) Chlorophyll fluorescence—a practical guide. *J Exptl Bot* 59:659–668
- McKone MK (1985) Reproductive biology of several brome grasses (*Bromus*): breeding system, pattern of fruit maturation, and seed set. *Am J Bot* 72:1334–1339
- Meyer SE, Allen PS (1999a) Ecological genetics of seed germination regulation in *Bromus tectorum* L. I. Phenotypic variance among and within populations. *Oecologia* 120:27–34
- Meyer SE, Allen PS (1999b) Ecological genetics of seed germination regulation in *Bromus tectorum* L. II. Reaction norms in response to a water stress gradient imposed during seed maturation. *Oecologia* 120:35–43
- Meyer SE, Garvin SC, Beckstead J (2001) Factors mediating cheatgrass invasion of intact salt desert shrubland. p 224–232. In: McArthur ED, Fairbanks DJ (compilers). *Shrubland ecosystem genetics and biodiversity: proceedings: June 13–15, 2000, Provo, Utah*. RMRS-P-21. USDA Forest Service Rocky Mountain Research Station, Ogden, Utah
- Meyer SE, Nelson DL, Carlson SL (2004) Ecological genetics of vernalization response in *Bromus tectorum* L. (Poaceae). *Ann Bot* 93:653–663
- Novak SJ, Mack RJ (1993) Genetic variation in *Bromus tectorum*: comparison between native and introduced populations. *Heredity* 71:167–176
- Novak SJ, Mack RN (2001) Tracing plant introduction and spread: genetic evidence from *Bromus tectorum* (cheatgrass). *Bioscience* 51:114–122

- Novak SJ, Mack RN, Soltis DE (1991) Genetic variation in *Bromus tectorum* (Poaceae): population differentiation in its North American range. *Am J Bot* 78:1150–1161
- Novak SJ, Mack RN, Soltis PS (1993) Genetic variation in *Bromus tectorum* (Poaceae)—introduction dynamics in North America. *Can J Bot* 71:1441–1448
- Parker IM, Rodriguez J, Loik ME (2003) An evolutionary approach to understanding the biology of invasions: local adaptation and general-purpose genotypes in the weed *Verbascum thapsus*. *Cons Biol* 17:59–72
- Ramakrishnan AP, Coleman CE, Meyer SE, Fairbanks DJ (2002) Microsatellite markers for *Bromus tectorum* (cheatgrass). *Mol Ecol Notes* 2:22–23
- Ramakrishnan AP, Meyer SE, Waters J, Stevens MR, Coleman CE, Fairbanks DJ (2004) Correlation between molecular markers and adaptively significant genetic variation in *Bromus tectorum* (Poaceae), an inbreeding annual grass. *Am J Bot* 91:797–803
- Ramakrishnan AP, Meyer SE, Fairbanks DJ, Coleman CE (2006) Ecological significance of microsatellite variation in western North American populations of *Bromus tectorum*. *Plant Spec Biol* 21:61–73
- Rasmuson KE, Anderson JE (2002) Salinity affects development, growth, and photosynthesis in cheatgrass. *J Range Manag* 55:80–87
- Rice KJ, Mack RN (1991a) Ecological genetics of *Bromus tectorum*. I. A hierarchical analysis of phenotypic variation. *Oecologia* 88:77–83
- Rice KJ, Mack RN (1991b) Ecological genetics of *Bromus tectorum*. II. Intraspecific variation in phenotypic plasticity. *Oecologia* 88:84–90
- Rice KJ, Mack RN (1991c) Ecological genetics of *Bromus tectorum*. III. The demography of reciprocally sown populations. *Oecologia* 88:91–101
- Rice KJ, Black RA, Rademaker G, Evans RD (1992) Photosynthesis, growth and biomass allocation in habitat ecotypes of cheatgrass (*Bromus tectorum*). *Funct Ecol* 6:32–40
- Roach DA, Wulff RD (1987) Maternal effects in plants. *Ann Rev Ecol Syst* 18:209–235
- Roundy BA, Young JA, Evans RA (1989) Seedling growth of three Great Basin wildrye collections at reduced osmotic potential. *Agric Ecosyst Environ* 25:245–251
- Schachner LJ, Mack RN, Novak SJ (2008) *Bromus tectorum* (Poaceae) in midcontinental United States: population genetic analysis of an ongoing invasion. *Am J Bot* 95:1584–1595
- Valiant MT, Mack RN, Novak SJ (2007) Introduction history and population genetics of the invasive grass *Bromus tectorum* (Poaceae) in Canada. *Am J Bot* 94:1156–1169
- Williamson M (1996) Biological invasions. Chapman and Hall, London