

# Strong genetic differentiation in the invasive annual grass *Bromus tectorum* across the Mojave–Great Basin ecological transition zone

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**Abstract** *Bromus tectorum*, an inbreeding annual grass, is a dominant invader in sagebrush steppe habitat in North America. It is also common in warm and salt deserts, displaying a larger environmental tolerance than most native species. We tested the hypothesis that a suite of habitat-specific *B. tectorum* lineages dominates warm desert habitats. We sampled 30 *B. tectorum* Mojave Desert and desert fringe populations and genotyped 10–26 individuals per population using 69 single nucleotide polymorphic (SNP) markers. We compared these populations to 11 Great Basin steppe and salt desert populations. Populations from warm desert habitats were dominated by members of two haplogroups (87 % of individuals) that were distinct

from haplogroups common in Great Basin habitats. We conducted common garden studies comparing adaptive traits and field performance among haplogroups typically found in different habitats. In contrast to the haplogroup abundant in sagebrush steppe, warm desert haplogroups generally lacked a vernalization requirement for flowering. The most widespread warm desert haplogroup (Warm Desert 1) also had larger seeds and a higher root:shoot ratio than other haplogroups. In the field, performance of warm desert haplogroups was dramatically lower than the sagebrush steppe haplogroup at one steppe site, but one warm desert haplogroup performed as well as the steppe haplogroup under drought conditions at the other site. Our results suggest that *B. tectorum* succeeds in widely disparate environments through ecotypic variation displayed by distinct lineages of plants. Accounting for this ecotypic variation is essential in modeling its future distribution in response to climate change.

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## Introduction

The boundary between North American cold and warm deserts is a major ecological transition zone, or ecotone (Kent et al. 1997), where hundreds of native

plant species reach the limits of their natural ranges (Meyer 1978). Abiotic factors including temperature regime, degree of aridity, edaphic conditions, and seasonality of precipitation differentiate the Mojave Desert and Great Basin desert regions (Caldwell 1985; Ehleringer 1985). While plant species vary in their ecological amplitude (Slatyer et al. 2013), only a few are so broadly adapted that their distributions span the environmental shifts associated with these types of major ecological transition zones. Indeed, native plant communities in these two North American desert regions are notably different, with dominants shifting dramatically. Only a handful of native species, mostly dicot annuals, are found growing across the warm desert/cold desert boundary (Meyer 1978; Reveal 1980).

One particularly successful invasive species, *Bromus tectorum* L., has overcome the ecological barriers keeping most native species confined to either cold or warm deserts. Its distribution spans the Mojave Desert–Great Basin ecological transition zone (Young and Tipton 1990; Hunter 1991; Brooks 1999), occupying a range of environmental conditions well beyond the scope of most native plants. An inbreeding winter annual grass that was introduced to western North America in the late nineteenth century, *B. tectorum* is the most ubiquitous and sometimes most dominant species on western rangelands, largely occupying its current distribution in sagebrush steppe habitats of the Intermountain West by 1930 (Mack 1981). This species also occurs in more xeric, low elevation salt desert habitats (Young and Tipton 1990). *Bromus tectorum* is not the only invasive species to demonstrate wide ecological amplitude; some other widely distributed invasive species occupy environmental niches in their introduced ranges that extend beyond environmental conditions historically occupied (Lavergne and Molofsky 2007; Broennimann et al. 2007; Petitpierre et al. 2012; Early and Sax 2014).

How are some invaders able to grow across such a range of habitats? Extreme phenotypic plasticity, wherein one genotype can modify its phenotype and succeed in many environments (Sultan 2000), is one possible mechanism, and many invasive plants are highly plastic (Davidson et al. 2011). Rapid in situ evolution of novel genotypes adapted to new environments, either via mutation or through recombination of standing genetic variation, is another (Prentis et al.

2008). A third possibility is that pre-adapted genotypes arrive and persist in specific environments, and that wide ecological amplitude for the species as a whole is a consequence of introduction of multiple ecotypes, i.e., genotypes adapted to specific contrasting environments (e.g., Dlugosch and Parker 2007; Lachmuth et al. 2010).

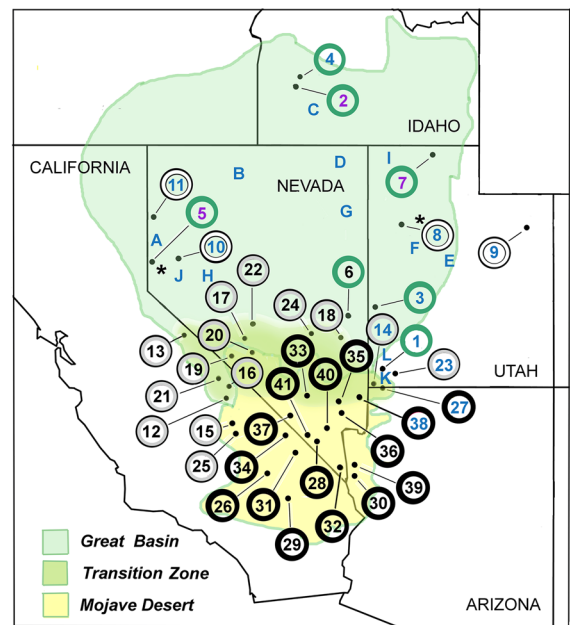
Previous ecological genetic research has suggested that *B. tectorum* lineages (groups of genetically similar individuals likely related by descent) commonly found in warm and salt desert habitats are genetically distinct from *B. tectorum* lineages that dominate more mesic sagebrush steppe habitats. In a common environment study of vernalization requirement for flowering, maternal lines (i.e., descendants of individual plants) collected from a Mojave Desert site where average winter temperatures remain above freezing did not require vernalization to flower, while maternal lines from colder salt desert, sagebrush steppe, foothill, and montane sites exhibited little or no flowering without some level of cold treatment (Meyer et al. 2004). Further, differences in seed dormancy have been observed between *B. tectorum* lines collected from different habitats, with slow rates of dormancy loss under summer conditions observed in lines from the Mojave Desert but not in lines from cold deserts (Meyer and Allen 1999). Finally, increased tolerance to salinity under greenhouse conditions relative to lines from sagebrush steppe sites has been observed in lines collected from salt desert habitats (Scott et al. 2010; Haubensak et al. 2014), and salt desert lines also performed better at salt desert sites in a reciprocal seeding experiment (Scott et al. 2010).

Molecular genetic evidence also suggests that lineages that dominate warm and salt deserts are distinct from those in sagebrush steppe and other more mesic habitats. A study designed to sample widely from across the range of *B. tectorum* in the western US demonstrated that simple sequence repeat (SSR) haplotypes (groups of individuals with identical SSR fingerprints) dominant in nine warm desert and desert fringe locations were almost completely absent in other habitats (Merrill et al. 2012).

In this study we ask whether *B. tectorum* lineages found across a wide range of warm desert locations share neutral marker fingerprints and ecological traits that differ from those of lineages abundant in highly invaded sagebrush steppe and salt desert environments. This question is important not only for

understanding the current distribution of *B. tectorum* in the western United States, but also because it provides crucial information on the environmental tolerances of this highly invasive plant, which could strongly affect the outcome of predictive modeling efforts under climate change scenarios (e.g., Bradley 2009). First, we conducted a population genetic study by characterizing neutral genetic variation using 69 single nucleotide polymorphic (SNP) markers developed for *B. tectorum* and generating a marker fingerprint (SNP haplotype) for each individual (Merrill 2011; Meyer et al. 2013). We sampled across the entire Mojave Desert region as well as in the ecological transition region to the north, including populations from southern Nevada, east-central California, southwestern Utah and northwestern Arizona (Fig. 1). We compared these populations to a representative group of populations from salt desert and sagebrush-steppe regions in the Great Basin (Merrill et al. 2012) using a genetic distance measure to classify individuals into haplogroups, i.e., groups of individuals with similar SNP haplotypes. Secondly, in a greenhouse study, we examined seed, seedling and flowering traits for members of haplogroups commonly found in these contrasting environments, and finally, in a field study, we measured performance in two sagebrush steppe common gardens. In our field study, we grew plants with and without competition from background *B. tectorum*, asking whether warm and salt desert lineages were capable of growing in sagebrush steppe conditions in the absence of potential competitive exclusion from sagebrush steppe lineages.

We hypothesized that lineages dominant in warm desert regions would be genetically and ecologically similar to each other but would differ from lineages characteristic of sagebrush steppe habitats. Further, we expected to see specific ecological traits in lineages from warm desert regions, including high maternal provisioning (larger seed size), lack of a vernalization requirement for flowering, and higher seedling root investment. Finally, we predicted that we would observe decreased survival and seed production of members of warm-desert and salt-desert haplogroups relative to members of sagebrush-steppe haplogroups when planted into more mesic steppe environments, with these differences increasing when plants were grown with ambient levels of competition from resident *B. tectorum* individuals.



**Fig. 1** Map of collection sites showing the locations of the Mojave Desert and Great Basin biomes in western North America and the ecological transition zone between them. Numbers correspond to *Bromus tectorum* populations included in the population genetic analysis (Table 1); letters correspond to locations of lines used in common garden studies (Table 2). Numbers and letters in blue represent population samples from Merrill et al. 2012; numbers in purple represent population samples from Meyer et al. 2013; numbers in black represent population samples collected specifically for the current study. Stars show the locations of the two common gardens. Outer ring colors for populations used in the genetics study correspond to habitat types: dark green—sagebrush steppe, white—salt desert shrubland, gray—warm desert fringe, black—warm desert. Locations are approximate; see Tables 1 and 2 for GPS coordinates for each location

## Materials and methods

### Population genetic study

Populations were selected for genetic characterization based on geographic location and habitat (Table 1; Fig. 1). Habitat was characterized by composition of the surrounding shrubland: creosote bush [*Larrea tridentata* (DC.) Coville] = warm desert; blackbrush [*Coleogyne ramosissima* Torr.], indigobush [*Psoralea fremontii* (Torr. ex A. Gray) Barneby], other warm desert shrubs but not creosote bush = warm desert fringe; shadscale [*Atriplex confertifolia* (Torr. & Frém.) S. Watson], greasewood [*Sarcobatus vermiculatus* (Hook.) Torr.], gray molly

[*Bassia americana* (S. Watson) A.J. Scott], other halophyte shrubs = salt desert; big sagebrush (*Artemisia tridentata* Nutt.) = steppe.

Collection sites were also characterized in terms of mean annual temperature and precipitation and plotted on a two-way ordination using these two climate variables (Hijmans et al. 2005; Fig. 2). Mojave Desert sites occupy the warm, dry quadrant of the ordination, while sagebrush steppe sites from further north occupy the cool, wet quadrant, and salt desert sites occupy the cool, dry quadrant. Warm desert fringe sites occupied intermediate positions, showing considerable overlap with salt desert sites. Warm desert fringe sites were consistently either drier than steppe sites or cooler than Mojave Desert sites.

Seeds were collected at maturity from haphazardly-chosen individuals in the field, with the constraint of >1 m distance between individuals to reduce the chance of sampling full sibs. For each individual sampled, a single seed head was removed and placed in a packet with a unique code name. All progeny from each packet are subsequently referred to as belonging to the maternal line of that name, and thus represent a family with a common maternal ancestor. As this species is almost completely selfing and therefore highly homozygous (Meyer et al. 2013), most or all individuals in a line are genetically extremely similar to the maternal parent.

We genotyped between 10 and 26 individuals from 30 Mojave Desert and Mojave Desert fringe populations, and also included 11 populations from Great

Basin steppe and salt desert habitats for comparative purposes (Table 1). A majority of the Mojave Desert and fringe populations (N = 27) were sampled in spring and early summer 2011. Two Mojave Desert populations, two desert fringe populations, three sagebrush-steppe populations, and four salt desert populations sampled between 2005 and 2008 (previously SSR-genotyped for Merrill et al. 2012) were SNP-genotyped for the present study; this allowed us to link the current SNP study with the previous, larger-scale survey conducted with SSR markers (Online Resource 1). Finally, three sagebrush steppe populations sampled in 2010 that had been included in a previous study using SNP markers with much larger sample sizes (Meyer et al. 2013) were randomly sub-sampled for the present study to provide numbers comparable to less intensively sampled populations, for a total of 41 populations.

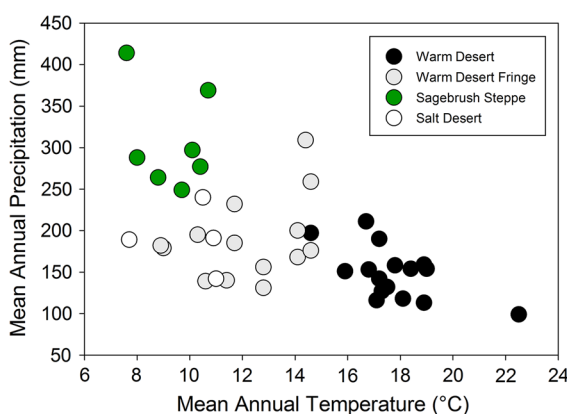
### SNP genotyping

To produce tissue for genotyping, seeds were allowed to lose dormancy under warm conditions. One seed from each maternal line was then planted in the greenhouse and grown to the 4-leaf stage, at which time approximately 1 cm<sup>2</sup> of the youngest leaf material was collected and stored at −80 °C. DNA was extracted using a modified CTAB protocol (Fulton et al. 1995).

SNP marker development from a cDNA library based on wide sampling of SSR haplotypes has been previously described (Merrill 2011; Meyer et al. 2013). For the present study, 71 SNPs were selected based on their location in non-coding positions in open reading frames, increasing the likelihood that they would behave as neutral markers not under direct selection. Two markers were eliminated from the data set because of excessive missing data, leaving a total of 69 SNP markers. Genotyping was carried out using KASP genotyping chemistry (LGC Genomics) on the Fluidigm EP1 system, a high-throughput SNP genotyping platform for allele-specific fluorescence amplification and detection, according to the manufacturer's specifications (see Lara 2013 for details). An average of 20 individuals per population (range 10–26) were successfully genotyped for a total of 813 individuals.

### SNP data analysis

We prepared a cluster dendrogram based on genetic distances between individuals for classification into



**Fig. 2** Climate ordination generated from data obtained for each *Bromus tectorum* collection site from Worldclim (Hijmans et al. 2005). Each site is plotted according to its mean annual temperature (x-axis) and mean total annual precipitation (y-axis)

**Table 1** Locations, elevations, and sample sizes for 41 collections from *Bromus tectorum* populations in the Intermountain West used for SNP analysis, separated into four habitat types

| Habitat type       | Population name                  | Latitude  | Longitude   | Elev (m) | Sample size | Map code |
|--------------------|----------------------------------|-----------|-------------|----------|-------------|----------|
| Sagebrush steppe   | *Baker Dam, UT                   | 37.383491 | −113.650686 | 1539     | 18          | 1        |
|                    | †Cinder Cone Butte, ID           | 43.221140 | −115.992781 | 1075     | 25          | 2        |
|                    | *Desert Experimental Range, UT   | 38.661941 | −113.848000 | 1834     | 20          | 3        |
|                    | *North Standifer, ID             | 43.287098 | −116.103010 | 1035     | 21          | 4        |
|                    | †Lower Peavine Mountain, NV      | 39.592872 | −119.870275 | 1712     | 25          | 5        |
|                    | White River Valley, NV           | 38.395764 | −115.036389 | 1637     | 23          | 6        |
|                    | †Whites Valley, UT               | 41.807674 | −112.303219 | 1489     | 25          | 7        |
| Salt Desert Shrub  | *Charait, UT                     | 40.263457 | −113.048527 | 1313     | 17          | 8        |
|                    | *Gusher, UT                      | 40.303181 | −109.772811 | 1570     | 20          | 9        |
|                    | *Hot Springs Mountains, NV       | 39.688937 | −119.065838 | 1269     | 26          | 10       |
|                    | *Lower Smoke Creek, NV           | 40.515594 | −119.829404 | 1233     | 20          | 11       |
| Warm Desert Fringe | Big Pine, CA                     | 37.128906 | −118.269433 | 1202     | 23          | 12       |
|                    | Benton, CA                       | 37.775708 | −118.463608 | 1614     | 20          | 13       |
|                    | *Castle Cliff, UT                | 37.065933 | −113.888004 | 1130     | 14          | 14       |
|                    | Central Owens Valley, CA         | 36.813464 | −118.211656 | 1203     | 19          | 15       |
|                    | Gilbert Pass, CA                 | 37.433017 | −117.949100 | 1934     | 26          | 16       |
|                    | Goldfield, NV                    | 37.794347 | −117.233972 | 1594     | 18          | 17       |
|                    | Hiko, NV                         | 37.459831 | −115.364406 | 1559     | 23          | 18       |
|                    | Lida Townsite, NV                | 37.444814 | −117.531353 | 2032     | 18          | 19       |
|                    | Lida Junction, NV                | 37.502394 | −117.185094 | 1434     | 21          | 20       |
|                    | Mono County Line, CA             | 37.471728 | −118.352547 | 1307     | 25          | 21       |
|                    | Tonopah, NV                      | 38.086389 | −117.091261 | 1668     | 20          | 22       |
|                    | *Toquerville, UT                 | 37.282021 | −113.309143 | 1167     | 19          | 23       |
|                    | Rachel, NV                       | 37.962700 | −116.059294 | 1488     | 18          | 24       |
|                    | Whitney Portal, CA               | 36.598528 | −118.091744 | 1262     | 22          | 25       |
| Warm Desert        | Baker, CA                        | 35.262014 | −116.077783 | 186      | 20          | 26       |
|                    | *Blackrock Exit, AZ              | 36.983300 | −113.646600 | 1128     | 17          | 27       |
|                    | Jean, NV                         | 35.811944 | −115.382222 | 1019     | 20          | 28       |
|                    | Kelbaker Road, CA                | 34.721944 | −115.678333 | 919      | 18          | 29       |
|                    | Kingman Highway, AZ              | 35.196111 | −114.433333 | 784      | 17          | 30       |
|                    | Kelso Junction, CA               | 35.176111 | −115.509167 | 1135     | 13          | 31       |
|                    | Laughlin, NV                     | 35.143611 | −114.579722 | 194      | 10          | 32       |
|                    | Mercury, NV                      | 36.574167 | −115.878611 | 1091     | 15          | 33       |
|                    | Mesquite Mountain Wilderness, CA | 35.610556 | −115.732778 | 977      | 21          | 34       |
|                    | Great Basin Highway 1, NV        | 36.507472 | −114.946222 | 825      | 22          | 35       |
|                    | Great Basin Highway 2, NV        | 36.534444 | −114.909444 | 805      | 19          | 36       |
|                    | Pahrump, NV                      | 36.263333 | −116.003333 | 813      | 19          | 37       |
|                    | *Riverside Exit, NV              | 36.779100 | −114.266500 | 665      | 20          | 38       |
|                    | South of Hoover Dam, AZ          | 35.490000 | −114.363611 | 909      | 21          | 39       |
|                    | South Las Vegas, NV              | 36.024722 | −115.173889 | 689      | 18          | 40       |
|                    | Sandy Valley Road, NV            | 35.819167 | −115.495000 | 1279     | 17          | 41       |

Population samples marked with \* were included in an earlier SSR study (Merrill et al. 2012); see Electronic Supplement 1 for a comparison between markers. Population samples marked with † were randomly subsampled from a larger SNP data set (Meyer et al. 2013). See Fig. 1 for locations based on map codes

haplogroups. A genetic distance matrix was prepared from the 69-SNP allelic data for each individual using the program DNADIST in the PHYLIP software package with settings at default values (Felsenstein 1989). Genotypes were treated as haploid, with very rarely occurring heterozygous loci assigned appropriate IUPAC ambiguity codes. The resulting distance matrix was input into the PHYLIP program NEIGHBOR and subjected to cluster analysis using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) clustering protocol (Sokal and Michener 1958) with settings at default values to determine major haplogroups based on genetic distance. The resulting dendrogram was visualized in Figtree software (Rambaut 2012) and used to classify the 813 individuals into five clearly defined haplogroups based on the node values for genetic distances separating the groups. We included one additional haplogroup, even though it was not strongly differentiated from a larger group, on the basis of evidence that it was genetically uniform, strongly habitat-specific, and showed evidence of specific adaptation to the salt desert habitat (Scott et al. 2010). Once this set of six haplogroups was defined, we determined, for each population, the proportion of individuals in each haplogroup (see Online Resource 1 for complete dendrogram with branch tips labeled by population and individual and color-coded by habitat).

We examined the relationships among ecological, geographic, and genetic distance using Mantel correlations, which were calculated using Arlequin 3.5 with 1000 permutations (Mantel 1967; Excoffier et al. 2005). The genetic distance measure was population pairwise  $F_{ST}$ , while the geographic distance measure was calculated from collection site latitude-longitude coordinates using the online utility Geographic Distance Matrix Generator (Ersts 2013). Ecological distance was defined as the Euclidean distance between pairs of sites using mean annual temperature and mean annual precipitation as x–y coordinates (SAS Proc Distance). Climate data were standardized before analysis to remove unequal weighting of variables.

### Greenhouse and field studies

We used field and greenhouse common garden studies to characterize ecological differences among lines from different haplogroups, conducting one

greenhouse study focused on differences in vernalization requirement, a second greenhouse study focused on root and shoot growth and biomass allocation of seedlings, and a field common garden study evaluating survival, growth, flowering, and seed production of lines belonging to different haplogroups in two sagebrush steppe locations.

### Selection of study lines

Twenty-four lines for the field and vernalization greenhouse studies were selected from a set of maternal lines that had been both SSR- and SNP-genotyped for other studies (K. Merrill, unpublished data) (Table 2). One line of each of two Mojave Desert specialist lineages and of two salt desert specialist lineages identified in Merrill et al. (2012) was selected from each of four different populations. The four lines for each specialist haplotype group had both identical SSR marker fingerprints and identical SNP haplotypes. These specialist lineages also corresponded to members of the four desert haplogroups identified in the current analysis (see Online Resource 1). The remaining eight lines belonged to the haplogroup identified here and in Meyer et al. (2013) as “Common” because it is the common haplogroup across the range of more mesic environments where *B. tectorum* is an important weed. These eight lines did not have identical SNP haplotypes, but instead were selected from eight populations representative of sagebrush steppe habitats across the Great Basin (Fig. 1; Table 2).

For the seedling greenhouse study, we selected 10 maternal lines each from the Warm Desert 1, Warm Desert 2, and Salt Desert 1 haplogroups, and 76 lines from the Common SNP haplogroup, from a total of 17 populations (Appendix 1, Table 5). The Salt Desert 2 haplogroup was not included because of insufficient seed. Subsequent SNP-genotyping confirmed the assignment of these lines to the currently defined SNP haplogroups.

### Greenhouse studies

Seeds for all field and greenhouse studies were grown for at least one generation in a greenhouse common garden environment, a process that can reduce maternal environment effects. To quantify seed size differences among lines, we weighed 2 replicate samples of

**Table 2** Maternal lines of *Bromus tectorum* used for field and vernalization greenhouse studies

| Habitat type/haplotype                                  | Line   | Population name         | Latitude  | Longitude   | Elev. (m) | Map code |
|---------------------------------------------------------|--------|-------------------------|-----------|-------------|-----------|----------|
| Sagebrush steppe (lineages from Common haplotype group) | BFL31  | Bedell Flat, NV         | 39.82327  | −119.80746  | 1612      | A        |
|                                                         | BRH04  | Bloody Run Hills, NV    | 41.27800  | −117.76000  | 1789      | B        |
|                                                         | BRU17  | Bruneau, ID             | 42.84344  | −115.75757  | 940       | C        |
|                                                         | DER09  | Desert Exptl. Range, UT | 38.661941 | −113.848000 | 1834      | 3        |
|                                                         | CON17  | Contact, NV             | 41.77098  | −114.75500  | 1645      | D        |
|                                                         | DOG11  | Dog Valley, UT          | 39.71638  | −111.95585  | 1713      | E        |
|                                                         | FMH10  | Five Mile Hill, UT      | 40.20129  | −112.83997  | 1474      | F        |
|                                                         | INV01  | Independence Valley, NV | 41.04098  | −114.74900  | 1743      | G        |
| Salt desert shrub (Salt Desert 1 haplotype)             | CHA01  | Charait, UT (CHA)       | 40.263457 | −113.048527 | 1313      | 8        |
|                                                         | GUS03  | Gusher, UT (GUS)        | 40.303181 | −109.772811 | 1570      | 9        |
|                                                         | SWR44  | Stillwater Refuge, NV   | 39.55709  | −118.40232  | 1213      | H        |
|                                                         | TMC11  | Tenmile Creek, UT       | 41.86489  | −113.13594  | 1453      | I        |
| Salt desert shrub (Salt Desert 2 haplotype)             | HSM04  | Hot Springs Mtns, NV    | 39.688937 | −119.065838 | 1269      | 10       |
|                                                         | LSC09  | Lower Smoke Creek, NV   | 40.515594 | −119.829404 | 1233      | 11       |
|                                                         | SWR10  | Stillwater Refuge, NV   | 39.55709  | −118.40232  | 1213      | H        |
|                                                         | TRM60  | Truckee Range, NV       | 39.65600  | −119.16001  | 1228      | J        |
| Mojave Desert (Warm Desert 1 haplotype)                 | ALB14  | Albuquerque, NM         | 42.52973  | −120.23211  | 1310      | –        |
|                                                         | R VX18 | Riverside Exit, NV      | 36.779100 | −114.26650  | 665       | 38       |
|                                                         | GSP05  | Green Springs Road, UT  | 37.15112  | −113.52627  | 914       | K        |
|                                                         | WNH15  | Winchester Hills, UT    | 37.22282  | −113.63555  | 1227      | L        |
| Mojave Desert (Warm Desert 2 haplotype)                 | BER19  | Bernalillo, NM          | 35.30190  | −106.53660  | 1566      | –        |
|                                                         | BRX12  | Blackrock Exit, AZ      | 36.983300 | −113.646600 | 1128      | 27       |
|                                                         | GSP11  | Green Springs Road, UT  | 37.15112  | −113.52627  | 914       | K        |
|                                                         | TOQ06  | Toquerville, UT         | 37.282021 | −113.309143 | 1167      | 23       |

Line designations are from Merrill et al. (2012). See Fig. 1 for locations based on map codes. New Mexico lines are not included on the map

50 seeds (florets) from individual greenhouse-grown progeny of each of the 24 lines used in the field and vernalization studies, resulting in a completely randomized design. For all analyses, when response variables were continuous and had a normal distribution of error, we used mixed model analysis of variance (ANOVA) for analysis. When response variables were binary, we used a generalized linear mixed model (GLMM) approach, and specified a binomial distribution of error (Dobson and Barnett 2008). Accordingly, differences in seed weight among haplogroups were analyzed using mixed model ANOVA with haplogroup as the fixed factor and line nested within haplogroup as the random factor.

For the vernalization experiment, plants were grown in two replicate blocks, with four lines per haplotype group (eight for the Common group), and

eight seeds per line per replicate (384 seeds total). Seeds were planted individually into Ray Leach Cone-tainers (3.8 cm × 21 cm SC10 Cone-tainers, Stuewe and Sons) in a soil-less greenhouse medium at the Shrub Sciences Laboratory, Provo, UT, greenhouse on February 3, 2014, and grown for 20 weeks under long days (naturally increasing day length) at a temperature that varied between 20 and 25 °C (well above the temperature range for vernalization). Each week, the number of individuals within each block and line that showed developing inflorescences was scored. Flowering was analyzed on a per plant basis using GLMM with binomial error distribution, with haplotype as a fixed effect. Line (nested within haplotype) and block were also included as random effects. Results are displayed as percentage of plants flowering.

For the seedling growth experiment, ten seeds per line, or 1060 seeds total, were planted in a topsoil/sand mixture in Ray Leach Cone-tainers in a fully randomized design at the University of Nevada, Reno, greenhouse in February 2010, with temperatures that varied between 5 and 20 °C. Pots were monitored daily for emergence. Plants were grown for 15 days after their day of emergence, at which point we measured total leaf length, separated roots from shoots, and dried and weighed biomass. Leaf length, root and shoot biomass, and root to shoot ratio in the greenhouse study were analyzed using mixed model ANOVA with haplogroup as a fixed factor and line as a random factor nested within haplogroup.

### *Field common garden studies*

Common garden studies were initiated at two sagebrush steppe sites, one at the base of Peavine Mountain, NV (39.5962 latitude, -119.8711 longitude, 1677 m elevation) and the other at the Davis Mountain study site in Skull Valley, UT (40.1203 latitude, -112.6633 longitude, 1582 m elevation). Precipitation data for these sites (long-term and during the study) were obtained from Prism Climate Group (<http://www.prism.oregonstate.edu/>) (Appendix 2, Fig. 7).

For each of the 24 lines chosen as described above, 100 seeds were glued to toothpicks with Tightbond II glue to aid with identification in the field (e.g., Leger et al. 2009), and distributed among 10 replicate plots at each site, with 5 seeds per line per replicate plot, and 1200 seeds total per site. Line placement within each replicate plot was random. Half of the replicate plots were assigned at random to a competition removal treatment, with resident *B. tectorum* weeded out at each census date, while the other half were left intact.

Seeding occurred Sep 28 2011 in Nevada and Oct 1 2011 in Utah, and plots were surveyed for emergence after the first rain and periodically throughout the growing season, as weather allowed (NV: Oct 17, Oct 27, Dec 1, Mar 5, Mar 29, Apr 17, Apr 27; UT: Oct 15, Nov 16, Dec 10, Mar 21, Apr 24). At the point when plants were beginning to senesce, we noted whether plants had flowered, collected all above-ground tissue, counted number of seeds produced, and dried and weighed total aboveground biomass and reproductive biomass.

Total reproductive biomass and seed number were very highly correlated ( $n = 963$ ,  $R^2 = 0.95$ ,

$P < 0.0001$ ), and thus only results for seed number are presented. Seed number and vegetative biomass, however, were not closely related ( $n = 1007$ ,  $R^2 = 0.46$ ,  $P < 0.0001$ ), primarily because not all plants set viable seed. For this reason, vegetative biomass data are also presented.

Differences in emergence, survival, and flowering among individuals on a per-seed basis were analyzed using GLMM with binomial error distribution, using a model that included garden location, competition treatment and haplogroup as fixed effects, and line (nested within haplogroup) and plot (nested within competition treatment) as random effects. Two and three way interactions between fixed effects were also included in the model. We also analyzed total seed output per seed planted (“seeds per seed”), a combined measure of survival and reproductive effort. Differences in vegetative biomass, seed number per plant, and seed output per seed were analyzed with ANOVA using the same mixed model described above, with significance of main effects and interactions determined by *F* tests based on type III sums of squares.

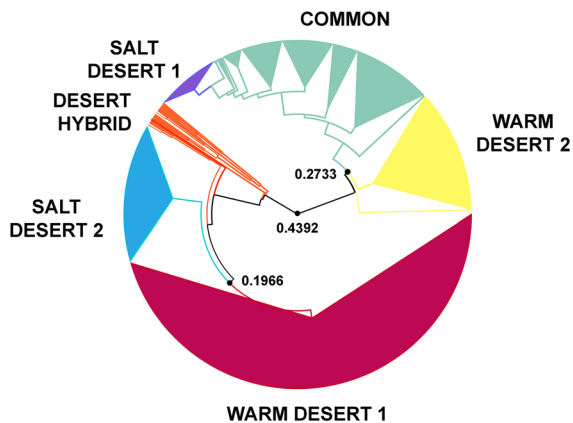
Continuous response variables were transformed as needed to improve data fit with the assumptions of ANOVA. Specifically, residuals were inspected for violation of assumptions of normality and homoscedasticity; transformations necessary for each response variable are listed in Table 4. When analyses indicated significant differences among treatment groups, post hoc comparisons among groups were conducted with Tukey’s HSD tests. GLMM models with binomial error distribution were analyzed with R package lme4, while continuous response variables with normal error distribution were analyzed with JMP version 11.0.0 (SAS Institute Inc., Cary, NC, USA).

## **Results**

### *Population genetic study*

#### *Classification of haplogroups*

Cluster analysis based on genetic distance for all SNP-genotyped individuals ( $n = 813$ ) belonging to 41 populations resulted in a dendrogram with clear groupings (Fig. 3). All individuals fell into one of two strongly differentiated genetic groups that



**Fig. 3** Results of cluster analysis based on 69 SNP loci for 813 *Bromus tectorum* individuals belonging to 41 populations from across the Mojave Desert–Great Basin transition zone. Colors represent six SNP haplotype groups readily identifiable in the dendrogram and named by primary habitat: the warm deserts of the Mojave region, the salt deserts of the Great Basin, and the sagebrush steppe habitats throughout western North America (labeled ‘common’). Numbers represent genetic distance for each major branch point in the dendrogram. (See Online Resource 1 for complete dendrogram and further explanation)

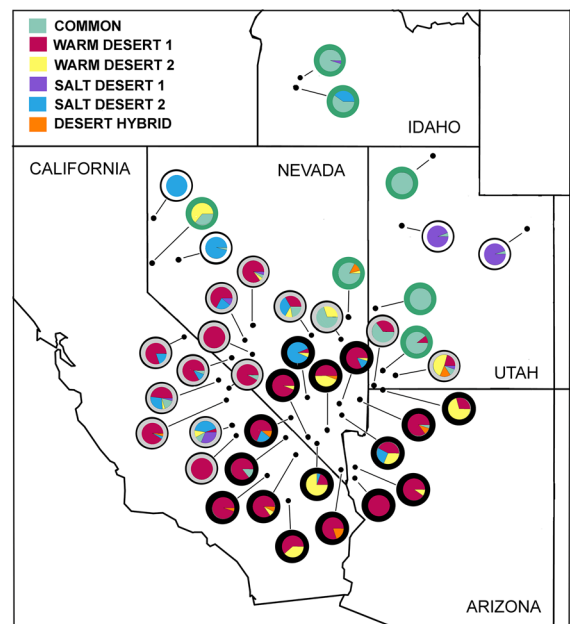
corresponded to two major clades identified in earlier work (Meyer et al. 2013) as the desert clade, containing haplotypes found almost exclusively in warm or salt deserts, and the common clade, containing primarily haplotypes that dominate sagebrush steppe habitats. These two major groups diverged in the dendrogram at a genetic distance of 0.4392. Within the desert group, there were two well-differentiated subgroups. The first of these, designated the Warm Desert 1 haplogroup, contained almost half of the individuals in the study (46 %), while the second subgroup, designated the Salt Desert 2 haplogroup, contained 13 % of the total. These two haplogroups diverged at a genetic distance of 0.1966. Also within the desert clade, but not closely similar to either of the two principal desert haplogroups or generally to each other, was a series of 24 individuals hypothesized to be inter-group hybrids (see Meyer et al. 2013); these were provisionally designated as the Desert Hybrid haplogroup.

The other principal group in the dendrogram also showed a major divergence into two distinct haplogroups, at a genetic distance of 0.2733 (Fig. 3). One of the two haplogroups included lines that clearly fell within a more narrowly defined common clade; these were designated as the Common haplogroup, which included 21 % of the total. Nested within this

haplogroup was a series of 48 closely similar individuals (6 % of the total) that were found primarily in salt desert habitats. Based on earlier work (Scott et al. 2010; Merrill et al. 2012), these were defined as the Salt Desert 1 haplogroup, even though they were not strongly divergent from other SNP haplotypes in the Common haplogroup. The other haplogroup contained mostly individuals from warm desert populations and was designated as Warm Desert 2, although it also included individuals from two of the southernmost sagebrush steppe populations. It comprised 11 % of the total sample.

#### Haplogroup frequency distributions

When haplogroup frequency distributions were plotted for each population, it was evident that the six haplogroups defined by SNP allelic composition were strongly associated both with particular habitat types and with geographic regions (Fig. 4). The Salt Desert 1 haplogroup was not found in any Mojave Desert



**Fig. 4** Frequency distributions in each of 41 populations of *Bromus tectorum* of each of the six haplotype groups identified in Fig. 1, with inner circles color-coded as described in the legend. Black points are approximate geographic locations of the populations referenced in the adjacent frequency histograms (green outer circle = sagebrush steppe habitat; white outer circle = salt desert habitat; gray outer circle = warm desert fringe habitat; black outer circle warm desert habitat)

population (black outer ring), while the Common haplogroup was found at low frequency at only two Mojave sites. Most Mojave Desert populations were strongly dominated by the Warm Desert 1 haplogroup, although several populations were characterized by relatively high proportions of Warm Desert 2. One Mojave Desert population, at Mercury NV, was dominated by the Salt Desert 2 haplogroup. Across all Mojave Desert populations, 87 % of individuals belonged to either Warm Desert 1 (68 %), or Warm Desert 2 (19 %). The remaining individuals belonged to the Salt Desert 2 (9 %), Desert Hybrid (3 %) or Common (1 %) haplogroups.

Mojave Desert Fringe populations that occupy the transitional area to the north of the warm desert were often also heavily dominated by the Warm Desert 1 haplogroup, though populations tended to have more mixed genetic composition than those further south (gray outer ring; Fig. 4). Some populations, including Central Owens Valley CA, Rachel NV, and Toquer-ville UT, had nearly equal representation of two or more haplogroups. Across all Mojave Desert Fringe populations, 62 % of individuals were from the Warm Desert 1 haplogroup, with the remainder distributed as follows: 8 % Warm Desert 2, 14 % Common, 4 % Salt Desert 1, 11 % Salt Desert 2, and 1 % Desert Hybrid. Warm Desert 1 was essentially completely restricted to warm desert and desert fringe populations, though it was found at very low frequency at Baker Dam, a sagebrush steppe site only a few miles north of the transition to creosote bush shrubland in southwestern Utah.

The haplotype pattern for representative sagebrush steppe populations (green outer ring; Fig. 4) found across the Great Basin to the north contrasted strongly with patterns for warm desert and warm desert fringe populations. Sagebrush steppe populations were generally dominated by members of the Common haplogroup, results that correspond with previous widespread surveys in this region (Ramakrishnan et al. 2006; Merrill et al. 2012; Meyer et al. 2013), although the Lower Peavine population in western Nevada also contained members of a subgroup of the Warm Desert 2 haplogroup at high frequency. The Cinder Cone Butte population in southern Idaho contained members of the Salt Desert 2 haplogroup at relatively high frequency, as reported earlier (Meyer et al. 2013).

Salt desert populations (Fig. 4; white outer ring) showed strongly contrasting genetic composition in

the Lahontan Basin of western Nevada versus the salt deserts of Utah. The Lahontan Basin populations were strongly dominated by members of the Salt Desert 2 haplogroup, while those in Utah were dominated by members of the Salt Desert 1 haplogroup. Each salt desert population was comprised almost entirely of individuals with identical SNP haplotypes (see Online Resource 1). Salt desert populations were genetically differentiated from sagebrush steppe populations and were also generally distinct from warm desert fringe and warm desert populations.

#### *Mantel correlation analysis*

Mantel correlation analysis showed highly significant but relatively weak correlations among genetic, geographic, and ecological distance for the populations included in the study. Because climate is directly tied to geography in this group of collection sites, distance matrices based on these two variables, namely ecological and geographic distance, were correlated ( $n = 820$ ,  $R^2 = 0.237$ ,  $P < 0.0001$ ). The correlations with genetic distance were significant for both geographic distance ( $n = 820$ ,  $R^2 = 0.199$ ,  $P < 0.0001$ ) and ecological distance ( $n = 820$ ,  $R^2 = 0.106$ ,  $P < 0.0001$ ), but combining these two predictor variables resulted in almost no net increase in variance accounted for ( $n = 820$ ,  $R^2 = 0.215$ ,  $P < 0.0001$ ), likely because of their correlation with each other. Because geographic distances between genetically closely similar populations were often as large as those between strongly dissimilar populations, the relationship of genetic distance with geographic distance was not very strong. The most abundant warm desert haplogroups were dominant across sites with a rather wide mean annual temperature and precipitation range (Fig. 2), so that the relationship of genetic distance with ecological distance based on climate variables was also not strong. The fact that there were many fewer steppe and salt desert reference sites than warm desert and desert fringe sites further decreased the likelihood of high correlations for genetic distance with either ecological or geographic distance.

#### *Greenhouse studies*

SNP haplogroups differed from each other in seed weight ( $F_{4,19} = 23.6$ ,  $P < 0.0001$ ), with the largest seeds observed in the Warm Desert 1 haplogroup, the

smallest seeds in the Salt Desert 2 haplogroup, and no differences among other haplogroups (Fig. 5a). SNP haplogroups also differed dramatically in vernalization requirement ( $\chi^2 = 14.0$ ,  $df = 4$ ,  $P = 0.0074$ ), with most plants of the Warm Desert 1 and the Salt Desert 2 haplogroups flowering without cold exposure, intermediate flowering without cold exposure in

the Warm Desert 2 haplogroup, and total lack of flowering in unvernallized plants of the Common and Salt Desert 1 haplogroups (Fig. 5b). Seedlings of different SNP haplogroups had similar leaf lengths ( $F_{3,102} = 1.7$ ,  $P = 0.1746$ ) and shoot biomass ( $F_{3,102} = 1.3$ ,  $P = 0.2656$ ), but differences in root biomass resulted in significant differences in R:S among haplogroups ( $F_{3,102} = 6.9$ ,  $P = 0.0003$ ), with significantly higher root allocation in Warm Desert 1 haplogroup seedlings (Fig. 5c).

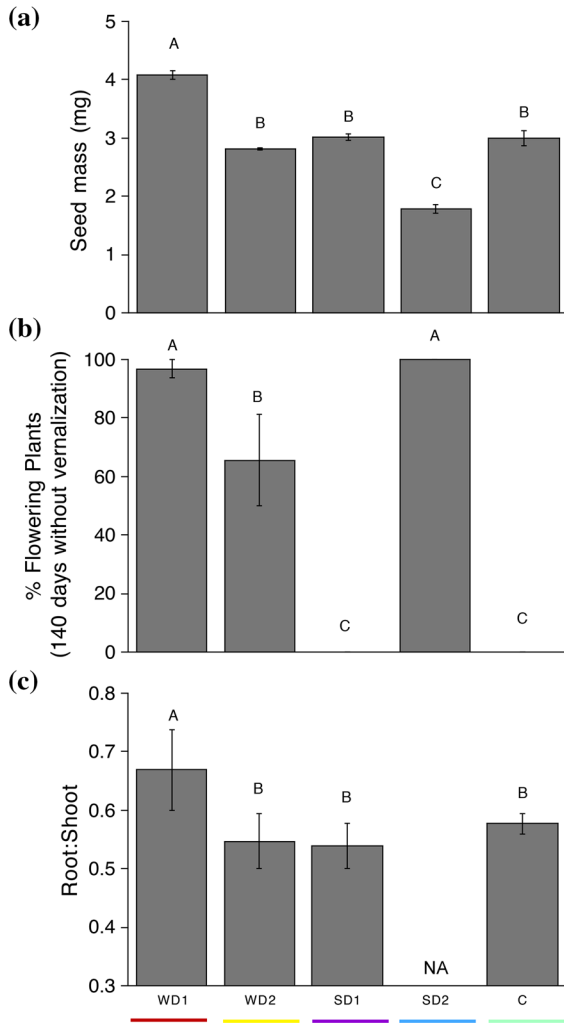
### Field studies

The 2011–2012 growing season (September 1–June 30) was extremely dry at the Nevada site, with 176 mm of precipitation relative to a growing season mean (1981–2010) of 378 mm. The germination-triggering rainfall episode in October was preceded by two dry months that provided little soil recharge, and there was no effective precipitation in November or December (Appendix 2). At the Utah site, precipitation was also below average, with 198 mm of growing season precipitation relative to a growing season average of 298 mm, but there was considerably more precipitation before the planting period in Utah than in Nevada, and at least one major storm in November provided follow-up moisture for establishment. Precipitation during winter and spring months was near or somewhat below average at both sites.

### Site and competition effects

Emergence, survival, and flowering percentages differed between sites (Table 3), with all three measures lower in Nevada (NV, emergence 73.1 %, survival, 28.5 %, flowering, 19.4 % of seeds planted; UT, emergence 81.8 %, survival, 56.8 %, flowering, 49.3 % of seeds planted). Plants that survived in Utah were significantly larger than plants in Nevada (Table 4, NV,  $20.6 \pm 2.1$  mg; UT,  $38.8 \pm 1.7$  mg), but did not differ significantly in average seed number per plant (Table 4, seed number: NV,  $6.4 \pm 0.8$ ; UT,  $7.5 \pm 0.5$ ).

Though presence of background competition did not significantly affect emergence or survival in either garden, it did significantly affect all growth responses in both garden locations, decreasing average vegetative biomass and seed number by 59 and 63 %, respectively, and reducing flowering from 36.2 to



**Fig. 5** Differences in seed mass (a), flowering percentage for plants grown in greenhouse conditions without vernalization (b), and seedling root to shoot ratios (c) of *Bromus tectorum* lines belonging to different SNP haplotype groups. SD salt desert, WD warm desert, C Common. Values represent means and standard errors, and letters indicate significant differences among haplogroups based on Tukey's HSD tests. Vernalization requirement (panel b) was analyzed as binomial data, but is presented here as percentage. Values are means and standard errors. "N/A" indicate that lineages from SD 2 were not available for the seedling study

**Table 3** Results of generalized mixed model tests for differences in emergence, survival, and flowering among plants of different haplogroups grown in two common gardens, with significant ( $P < 0.05$ ) differences highlighted in bold

| Factor                                         | Emergence               |               | Survival                 |                  | Flowering               |                  |
|------------------------------------------------|-------------------------|---------------|--------------------------|------------------|-------------------------|------------------|
|                                                | $\chi^2$                | $P$           | $\chi^2$                 | $P$              | $\chi^2$                | $P$              |
| Garden location                                | <b>9.2<sub>1</sub></b>  | <b>0.0024</b> | <b>117.9<sub>1</sub></b> | <b>&lt;.0001</b> | <b>93.1<sub>1</sub></b> | <b>&lt;.0001</b> |
| Competition                                    | 0.0 <sub>1</sub>        | 0.8327        | 3.8 <sub>1</sub>         | 0.0513           | <b>5.1<sub>1</sub></b>  | <b>0.0039</b>    |
| Garden $\times$ competition                    | 0.1 <sub>1</sub>        | 0.7317        | 0.4 <sub>1</sub>         | 0.3746           | 0.0 <sub>1</sub>        | 0.9240           |
| SNP group                                      | <b>23.3<sub>4</sub></b> | <b>0.0001</b> | <b>13.9<sub>4</sub></b>  | <b>0.0076</b>    | <b>14.5<sub>4</sub></b> | <b>0.0057</b>    |
| SNP group $\times$ garden                      | <b>9.7<sub>4</sub></b>  | <b>0.0459</b> | <b>18.7<sub>4</sub></b>  | <b>0.0009</b>    | <b>20.6<sub>4</sub></b> | <b>0.0004</b>    |
| SNP group $\times$ competition                 | 1.6 <sub>4</sub>        | 0.8089        | 2.4 <sub>4</sub>         | 0.6584           | 2.6 <sub>4</sub>        | 0.6275           |
| SNP group $\times$ competition $\times$ garden | 4.3 <sub>4</sub>        | 0.3730        | 4.6 <sub>4</sub>         | 0.3264           | 2.4 <sub>4</sub>        | 0.6535           |

Subscript values indicate degrees of freedom

**Table 4** Results of mixed models comparing vegetative biomass, average seed production (seed number), and seed produced per seed planted of plants of *B. tectorum* from different haplogroups in two common garden sites in NV and UT, grown with and without competition from resident *B. tectorum*

| Factor                                         | Veg. mass <sup>log</sup>     |                  | Seed # <sup>log</sup>       |               | Seeds per seed <sup>bc</sup> |                  |
|------------------------------------------------|------------------------------|------------------|-----------------------------|---------------|------------------------------|------------------|
|                                                | $F$                          | $P$              | $F$                         | $P$           | $F$                          | $P$              |
| Garden location                                | <b>15.1<sub>1,16.9</sub></b> | <b>0.0012</b>    | 0.6 <sub>1,16.8</sub>       | 0.4559        | <b>15.8<sub>1,17.5</sub></b> | <b>0.0009</b>    |
| Competition                                    | <b>4.6<sub>1,16.0</sub></b>  | <b>0.0470</b>    | <b>5.7<sub>1,15.9</sub></b> | <b>0.0294</b> | <b>8.8<sub>1,17.1</sub></b>  | <b>0.0086</b>    |
| Garden $\times$ competition                    | 0.3 <sub>1,15.5</sub>        | 0.5631           | 0.1 <sub>1,15.1</sub>       | 0.7205        | 0.1 <sub>1,17.5</sub>        | 0.7760           |
| SNP group                                      | <b>9.7<sub>4,23.8</sub></b>  | <b>&lt;.0001</b> | <b>9.8<sub>4,22.2</sub></b> | <b>0.0001</b> | <b>20.5<sub>4,19</sub></b>   | <b>&lt;.0001</b> |
| SNP group $\times$ garden                      | 1.1 <sub>4,24.2</sub>        | 0.3659           | <b>7.6<sub>4,23.1</sub></b> | <b>0.0005</b> | <b>10.5<sub>4,19</sub></b>   | <b>0.0001</b>    |
| SNP group $\times$ competition                 | 1.8 <sub>4,28.8</sub>        | 0.1566           | 0.8 <sub>4,22.3</sub>       | 0.5155        | 0.9 <sub>4,19</sub>          | 0.4737           |
| SNP group $\times$ competition $\times$ garden | 1.5 <sub>4,24.8</sub>        | 0.1025           | 1.3 <sub>4,19.7</sub>       | 0.2960        | 2.0 <sub>4,19</sub>          | 0.1379           |

Subscript values indicate numerator and denominator degrees of freedom; significant ( $P < 0.05$ ) differences are highlighted in bold

<sup>log</sup> Log transformed, <sup>bc</sup> Box Cox transformed

32.8 % of seeds planted. These effects were relatively consistent between sites (no site  $\times$  competition interactions, Tables 3, 4). Contrary to our predictions, competition did not affect plants from different habitats differentially (no significant competition  $\times$  SNP haplogroup interactions, Tables 3, 4).

#### Differences among haplogroups in the field

Genetic background strongly affected plant performance in the field, with significant main effects of haplogroup for emergence, survival, flowering (Table 3, Appendix 3), vegetative biomass, seeds per plant, and seeds per seed (Table 4), and significant differences in SNP haplogroup performance between sites for all measures except biomass (SNP haplogroup

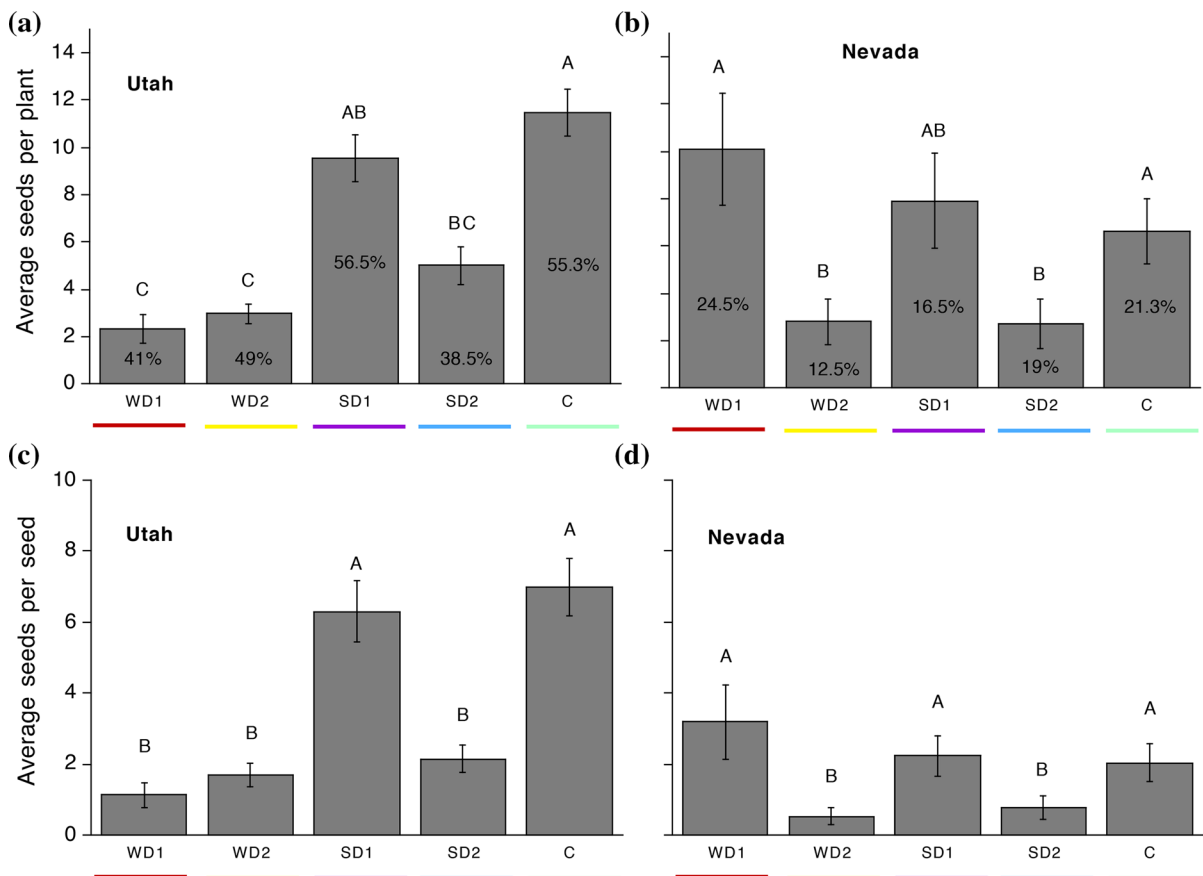
$\times$  garden interactions, Tables 3, 4). At the Utah site, plants in the Common and Salt Desert 1 haplogroups were always among the top performers for percent emergence, survival, and flowering, with Salt Desert 2 haplogroup plants always among the worst (Appendix 3, Fig. 8). At the Nevada site, most SNP haplogroups had similar emergence, survival, and flowering percentages, with the exception of lower emergence for Salt Desert 2 haplogroup plants and lower survival and flowering for Warm Desert 2 haplogroup plants (Appendix 3, Fig. 8).

The most pronounced differences among haplogroups were seen in seed production responses. At the Utah site, plants of the Common haplogroup typically found in sagebrush steppe habitats, and also plants of the Salt Desert 1 haplogroup, made more

seeds per plant than other haplogroup plants, with plants belonging to the Warm Desert 1 and 2 haplogroups making the fewest seeds per plant (Fig. 6a). At the Nevada site, plants of the Common and Salt Desert 1 haplogroups again made more seeds than plants of the Warm Desert 2 haplogroup; but, in this garden, plants of the Warm Desert 1 haplogroup made as many seeds per plant as those from the Common haplogroup (Fig. 6b). When considering seeds produced per seed planted, an integrative performance measure that combines emergence, survival of emerged seeds, and fecundity of surviving plants, patterns were similar, with more seeds per seed produced for plants of the Common and Salt Desert 1 haplogroups in Utah, and with the Warm Desert 1, Salt Desert 1, and Common haplogroups producing the most seeds in Nevada (Fig. 6c, d).

## Discussion

The molecular and ecological genetic results presented here provide strong evidence that *B. tectorum*, a widespread invasive species, has expanded its introduced range across a major ecological transition zone through differential success of pre-adapted ecotypes. Multiple introductions of pre-adapted genotypes are also known to have assisted the range expansion of some other cosmopolitan weeds (e.g., Neuffer and Hurka 1999; Bossdorf et al. 2008; Simberloff 2009; Henery et al. 2010; Mimura et al. 2013). While previous studies have suggested that multiple introductions of *B. tectorum* occurred in North America (Novak and Mack 2001), this work is the first to demonstrate strong genetic differentiation between *B. tectorum* populations across the Mojave Desert and



**Fig. 6** Average seeds produced per plant (**a**, **b**) and seeds produced per seed planted (**c**, **d**) for *Bromus tectorum* lineages belonging to five SNP haplotype groups grown in common gardens in Utah (**a**, **c**) and Nevada (**b**, **d**). Values are means and standard errors, and letters indicate significant differences

among haplotype groups within each garden according to Tukey's HSD tests; % in panels **a** and **b** indicates the percentage of seeds planted that produced flowering plants. Haplogroup codes as in Fig. 5

those in Great Basin sagebrush steppe and salt desert habitats to the north. Recent work with less variable allozyme markers (Pawlak et al. 2015) did not reveal differences among genotypes across this transition zone, highlighting the importance of a robust set of polymorphic markers for addressing plant population genetic questions of this kind.

The strong relationship between geography and habitat evident in Fig. 1 makes it difficult to prove unequivocally that the spatial distribution of haplogroups we observed has an ecological basis. This contention is supported by additional lines of evidence, however. First, lineages of the Warm Desert 1 haplogroup, and to some extent the other haplogroups found in the Mojave Desert (Warm Desert 2, Salt Desert 2), possess ecologically relevant adaptive traits that increase their fitness in warm desert habitats. The almost complete lack of a vernalization requirement for flowering in these haplogroups is clearly adaptive in habitats with warm winters. Lack of a vernalization requirement has also been reported for the warm desert invader *Bromus rubens* L. (Hulbert 1955), further supporting its importance for successful colonization of the warm desert.

Warm Desert 1 lineages also differed from those of other haplogroups in having higher maternal provisioning (larger seeds) and higher root investment (larger root:shoot ratio; Fig. 4), both potential adaptations for survival of this annual plant in harsh, short-season environments (Venable and Brown 1988; Chapin et al. 1993; Lloret et al. 1999; Moles and Westoby 2004). Increased root investment for montane collections of *B. tectorum* relative to steppe collections has also been reported (Rice et al. 1992). Finally, Warm Desert 1 seeds lose dormancy at high temperature much more slowly than Common haplogroup seeds, possibly preventing premature germination in response to monsoonal storms (Meyer and Allen 1999). Taken together, this is solid evidence that *B. tectorum* lineages that dominate the Mojave Desert possess a suite of divergent and potentially adaptive traits.

An additional line of evidence that the spatial distribution of haplotypes we observed represents ecotypic differentiation comes from the field common garden experiments. When lineages belonging to the three haplogroups common in warm deserts were planted into a sagebrush steppe environment during a near-average precipitation year at Davis Mountain in

central Utah, they performed very poorly relative to lineages from sagebrush steppe habitats, and often were barely able to replace themselves. This poor performance was not the result of lower competitive ability, as the removal of resident individuals affected all *B. tectorum* haplogroups similarly. Their reduced fitness was more likely the result of a poor match between environmental cues and physiological responses that affect phenology. This supports the idea that their exclusion from steppe habitats is climatically mediated. At the Nevada common garden, extreme fall drought conditions resulted in much lower survival and reproductive success overall. Here, the stress adaptations of Warm Desert 1 were apparently advantageous, as it performed as well as the Common haplogroup at this site. In contrast, Warm Desert 2 and Salt Desert 2, which were observed to lack some potential stress adaptations (large seeds, high root investment), performed poorly at both the Utah site under near-average conditions and the Nevada site under drought conditions. Ideally, these common garden studies would have included reciprocal planting, in order to ask if warm desert lineages perform better than sagebrush steppe lineages when planted into their own environment and whether the lack of Common lineages in warm desert habitats is due to their reduced fitness in those habitats, possibly due to their vernalization requirement.

The fact that a warm desert ecotype was able to succeed in a sagebrush steppe environment in western Nevada in an exceptionally dry year may have important implications for future distribution of this species in response to climate change. To date, the assumption in modeling efforts has been that *B. tectorum* is essentially genetically uniform across its range, and that the current climate of the sagebrush steppe is the only relevant climate for predicting future occupancy (e.g., Bradley 2009). The existence of distinct warm desert ecotypes that can survive and even thrive under climate scenarios predicted for the Great Basin in the future calls for a more genetically-informed approach to bioclimatic envelope modeling for this, and other, highly invasive species. The current distribution of warm desert haplogroups suggests that some northward expansion of warm desert-adapted ecotypes may already be taking place (Fig. 4).

Results reported here represent a confirmation and an extension of earlier results on the population genetic structure of *B. tectorum* in the Intermountain

West obtained using SSR markers (Ramakrishnan et al. 2004, 2006; Merrill et al. 2012). The unusual Mojave Desert genotype described in our early studies (Meyer and Allen 1999; Meyer et al. 2004) is demonstrated here to belong to a group of closely related lineages that are widespread and dominant across the Mojave Desert and into the desert fringe region, namely the Warm Desert 1 haplogroup (see Online Resource 1 for a full discussion of the relationship between these two marker sets).

In summary, we conclude that the extraordinary ability of *B. tectorum* to bridge the Mojave–Great Basin transition zone and succeed in both cold and warm desert environments is due neither to phenotypic plasticity of generalist genotypes nor to in situ evolution of locally adapted genotypes. Warm desert and sagebrush steppe ecotypic characteristics are associated with strongly contrasting molecular genetic marker fingerprints, indicating that these ecotypes likely have a long evolutionary history. This is strong evidence that the SNP haplotypes that have become abundant and widespread in the Mojave Desert represent pre-adapted ecotypes that were introduced independently from the native range. Tracing the

history of the origin, introduction, and spread of pre-adapted desert ecotypes of *B. tectorum* will require further study, including molecular-genetic characterization of historic herbarium collections as well as genetic characterization of populations from throughout the native range.

**Acknowledgments** This work was supported in part by grants from the USDA Cooperative State Research Service (2008-35320-18677 to S.E.M. and C.E.C.), the Joint Fire Sciences Program (JFSP-2007-1-3-10, JFSP-2011-S-2-6 to S.E.M.), the Bureau of Land Management (Integrated Cheatgrass Die-off Research Project to S.E.M. and E.A.L.), and the University of Nevada Reno (start-up funds to E.A.L.). Thanks to Phil Allen, Joshua Nicholson, David Salman, and Bettina Schultz for seed collection, to Keith Merrill and Sam Decker for help with the SNP analysis, to Suzette Clement and Joshua Nicholson for assistance with the field study in Utah, to Sandra Li, Owen Baughman, Bryce Wehan, and Erin Goergen for field and greenhouse assistance in Nevada, and to Bettina Schultz for preparing the color graphics.

## Appendix 1

See Table 5.

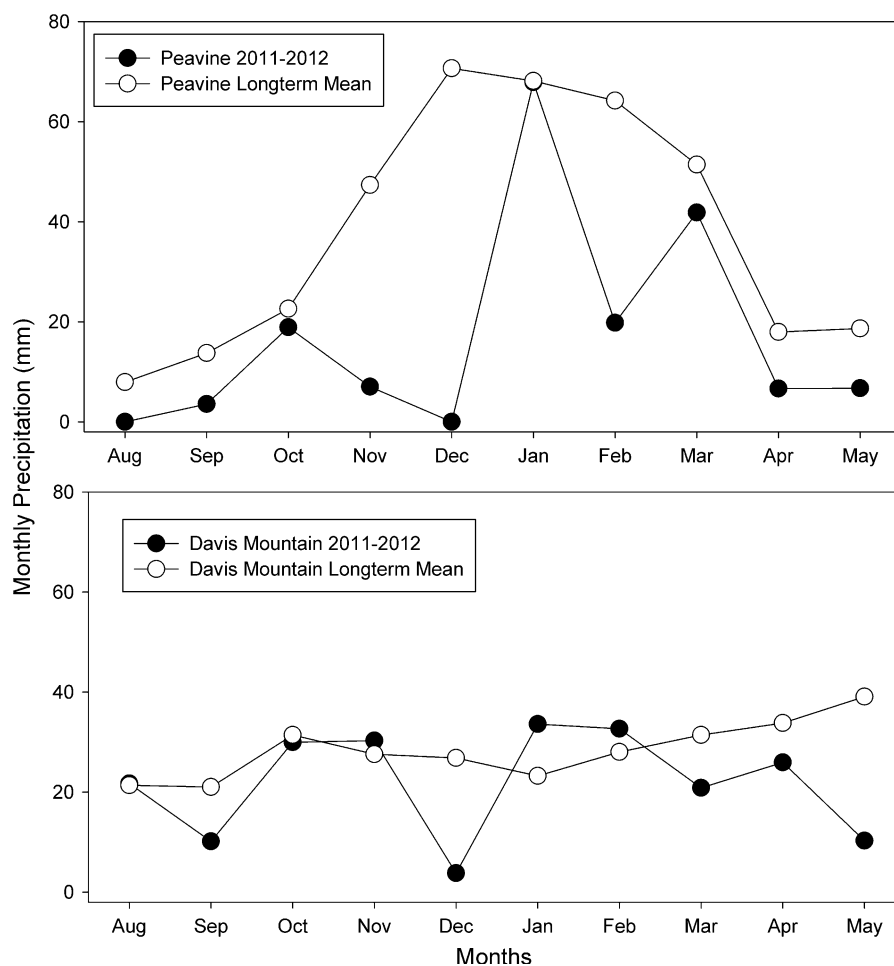
**Table 5** Seed sources for 106 *Bromus tectorum* lines from Merrill et al. (2012) included in the seedling greenhouse study, identifying the SNP haplotype group, population name, number of lines used, and collection location information

| Haplotype group | Population name               | # of lines | Latitude | Longitude  | Elevation (m) |
|-----------------|-------------------------------|------------|----------|------------|---------------|
| Common          | Bloody Run Hills, NV          | 10         | 41.27800 | −117.76000 | 1789          |
|                 | Dr. Lefcourt, WA              | 1          | 48.01667 | −117.51667 | 678           |
|                 | Five Mile Hill, UT            | 10         | 40.20129 | −112.83997 | 1474          |
|                 | Gunlock Reservoir, UT         | 2          | 37.25594 | −113.77000 | 1108          |
|                 | Naval Gun, UT                 | 3          | 40.31030 | −112.95560 | 1601          |
|                 | Pinecroft, WA                 | 5          | 47.67961 | −117.22902 | 688           |
|                 | Peavine Mountain, NV          | 10         | 39.59287 | −119.87028 | 1712          |
|                 | Red Horse Mountain, ID        | 10         | 47.55000 | −116.64852 | 963           |
|                 | Upper Sand Cove Reservoir, UT | 2          | 37.29950 | −113.69705 | 1380          |
|                 | Confusion East, UT            | 10         | 39.08197 | −113.49199 | 1377          |
|                 | Wallsburg, UT                 | 10         | 40.38046 | −111.41026 | 1766          |
|                 | Winchester Hills, UT          | 3          | 37.22282 | −113.63555 | 1227          |
| Salt Desert 1   | Charait, UT                   | 10         | 40.26346 | −113.04853 | 1313          |
| Warm Desert 1   | Gunlock Reservoir, UT         | 2          | 37.25594 | −113.77000 | 1108          |
|                 | Upper Sand Cove Reservoir, UT | 3          | 37.29950 | −113.69705 | 1380          |
|                 | Toquerville, UT               | 4          | 37.28202 | −113.30914 | 1167          |
|                 | Winchester Hills, UT          | 1          | 37.22282 | −113.63555 | 1227          |
| Warm Desert 2   | Green Springs Road, UT        | 4          | 37.15112 | −113.52627 | 914           |
|                 | Toquerville, UT               | 6          | 37.28202 | −113.30914 | 665           |

## Appendix 2

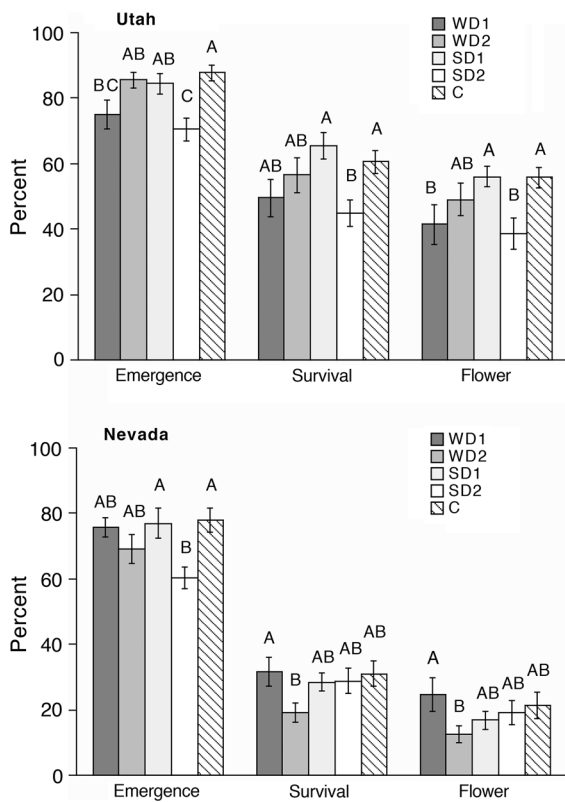
See Fig. 7.

**Fig. 7** Precipitation during field studies at the Peavine, Nevada and Davis Mountain, Utah field sites. Values are monthly totals immediately preceding and during the field study period, and 30 year averages (1981–2010) for each site, from the Prism Climate Group (<http://www.prism.oregonstate.edu>)



## Appendix 3

See Fig. 8.



**Fig. 8** Differences in percent emergence, survival, and flowering among SNP haplotype groups for *Bromus tectorum* plants at the Utah and Nevada common gardens, presented as the percentage of seeds planted that emerged, survived, and flowered (means and standard errors). Data were analyzed on a per seed basis, as described in the main text, and letters indicating significant differences among haplotype groups are from Tukey's HSD tests from those analyses

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