American Journal of Botany

American Journal of Botany 99(3): 529–537. 2012.

Population genetic analysis of *Bromus tectorum* (Poaceae) indicates recent range expansion may be facilitated by specialist genotypes[1]

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- *Premise of the study:* The mechanisms for range expansion in invasive species depend on how genetic variation is structured in the introduced range. This study examined neutral genetic variation in the invasive annual grass *Bromus tectorum* in the Intermountain Western United States. Patterns of microsatellite (SSR) genotype distribution in this highly inbreeding species were used to make inferences about the roles of adaptively significant genetic variation, broadly adapted generalist genotypes, and facultative outcrossing in the recent range expansion of *B. tectorum* in this region.
- *Methods:* We sampled 20 individuals from each of 96 *B. tectorum* populations from historically and recently invaded habitats throughout the region and used four polymorphic SSR markers to characterize each individual.
- *Key results:* We detected 131 four-locus SSR genotypes; however, the 14 most common genotypes collectively accounted for 79.2% of the individuals. Common SSR genotypes were not randomly distributed among habitats. Instead, characteristic genotypes sorted into specific recently invaded habitats, including xeric warm and salt desert as well as mesic high-elevation habitats. Other SSR genotypes were common across a range of historically invaded habitats. We observed very few heterozygous individuals (0.58%).
- *Conclusions:* Broadly adapted, generalist genotypes appear to dominate historically invaded environments, while recently invaded salt and warm desert habitats are dominated by distinctive SSR genotypes that contain novel alleles. These specialist genotypes are not likely to have resulted from recombination; they probably represent more recent introductions from unknown source populations. We found little evidence that outcrossing plays a role in range expansion.

Key words: *Bromus tectorum*; cheatgrass; downy brome; ecological genetics; inbreeding; invasive species; local adaptation; microsatellite; Poaceae.

A fundamental question in invasion ecology is how an introduced species is able to increase its range, including the range of habitats it can occupy, after initial establishment. Key conditions for successful invasion include (1) prior disturbance of native ecosystems in the invaded region (García-Ramos and Rodríguez, 2002), (2) high reproductive output or propagule pressure (Kolar and Lodge, 2001; Lockwood et al., 2005; Dawson et al., 2009), (3) capability for rapid dispersal resulting in range expansion (Moody and Mack, 1988), and (4) adaptive phenotypic plasticity (e.g., for biomass accumulation, seed production, tillering) under varying environmental conditions (Richards et al., 2006). While each of these conditions by itself has been associated with invasion success, cases where an invasion incorporated most or all of these elements have typically resulted in not only a high degree of invasion success but also widespread habitat destruction and high economic costs (Knapp, 1996; Pimentel et al., 2000). One such invader in the Intermountain Western United States (IMW) is *Bromus tectorum* L. (cheatgrass or downy brome; Poaceae) (Mack, 1981; Novak and Mack, 2001).

The population genetic structure of an invasive species, including breeding system, can have a strong impact on invasion dynamics, especially on the potential for local adaptation in recently established populations (Barrett et al., 2008; Kawecki, 2008; Alexander and Edwards, 2010). Local adaptation is defined as superior fitness of a local genotype when compared to a "foreign" genotype and is most convincingly demonstrated when both genotypes have highest fitness at their sites of origin in reciprocal designs (Kawecki and Ebert, 2004). Local adaptation is a common but by no means universal phenomenon (Leimu and Fischer, 2008). The forces of selection necessary to create local adaptation may be countered by high phenotypic plasticity, insufficient genetic variation in potentially adaptive traits, or continuing high levels of gene flow (Kawecki and Ebert, 2004); however, the extent to which each of these forces impacts the development of local adaptation depends on the intensity of selection. For example, high gene flow and intense selection pressures could result in the development of local adaptation, rather than preventing it.

[1]Manuscript received 22 February 2011; revision accepted 19 December 2011.

We thank the following individuals who provided seed collections for genetic analysis: M. Ashley and W. Longland (Agricultural Research Service, Reno, NV), R. Pendleton (U. S. Forest Service Rocky Mountain Research Station, Albuquerque, NM), D. Quinney and J. Weaver (Idaho Army National Guard, Boise, ID), J. Downs (Batelle Corp., Hanford DOE site), J. Beckstead and students (Gonzaga University), and current and former Shrub Sciences Laboratory employees O. Baughman, S. Carlson, S. Clement, D. Nelson, S. Inouye, K. T. Merrill, D. Smith, T. Stewart, and J. Stewart. We also thank K. Bean and other BYU Plant Genetics Laboratory participants for assistance and A. Ramakrishnan, E. Leger, and K. Crandall for helpful reviews of this manuscript. This research was funded in part through grants from the Joint Fire Sciences Program (2007-1-3-10 to S.E.M.) and the CSREES NRI Biology of Weedy and Invasive Species Program (2008-35320-18677 to S.E.M. and C.E.C.). We also thank the Idaho Army National Guard for generous financial support.

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doi:10.3732/ajb.1100085

Many early seral annual grass species, including *B. tectorum*, are highly inbreeding (McKone, 1985). In such species, high gametic phase disequilibrium creates strong associations between otherwise neutral genetic marker loci and loci governing the expression of adaptively significant traits ("hitchhiking"; Hedrick and Holden, 1979). Such an association was first described for *Avena barbata* in its introduced range in California (Allard et al., 1972), where two multilocus allozyme genotypes were found to be dominant in contrasting xeric and mesic habitats, both at regional and microenvironmental scales (Clegg and Allard, 1972; Hamrick and Allard, 1972). It was not clear whether the allozyme polymorphisms themselves were the direct substrate of selection or were maintained in association with adaptively important loci through linkage or high levels of inbreeding. In either case, *A. barbata* seemed to represent a clear example of local adaptation reflected in contrasting allozyme marker genotypes. These studies did not include a direct experimental measure of local adaptation, however. Detailed experimental studies subsequently failed to detect any evidence for local adaptation in these allozyme genotypes (Latta, 2009); instead, one genotype showed superior fitness across both habitats. This was interpreted to mean that the superior genotype was likely replacing the inferior genotype in a system still far from equilibrium.

Marker-distribution-based inferences have been made about local adaptation in several other inbreeding annual grass species, including *Hordeum spontaneum* (Nevo et al., 1979), *Aegilops peregrina* (Nevo et al., 1994), and *Triticum dicoccoides* (Li et al., 2000) in the eastern Mediterranean Basin. Subsequent reciprocal seeding experiments with *H. spontaneum* confirmed that populations of this species exhibit local adaptation, but the genetic variation associated with local adaptation was not correlated with allozyme variation (Volis et al., 2002a, b).

In this study, we used a marker-based approach to examine hypotheses about the invasion dynamics of *B. tectorum*. This species was accidentally introduced from Eurasia to the IMW in the late 1800s and is now the dominant plant species on tens of thousands of square kilometers of sagebrush steppe and cold desert habitat throughout the region (Bradley and Mustard, 2005). In addition, *B. tectorum* has continued to expand its range into salt and warm desert habitats that were formerly unoccupied (Scott et al., 2010). It has also extended its range into higher elevation foothill and montane communities (Pierson and Mack, 1990; Crawford et al., 2001; Keeley and McGinnis, 2007; Leger et al., 2009). Our primary objective was to elucidate the mechanisms by which *B. tectorum* has achieved this recent range expansion, including an examination of the possible role of local adaptation in the invasion process on a regional scale, by employing population genetic analysis of neutral microsatellite (SSR) molecular markers.

Rice and Mack (1991c) demonstrated the existence of local adaptation in *B. tectorum* in marginal salt desert and montane hemlock forest habitats at the upper and lower limits of its range, but not in historically invaded sagebrush steppe habitats. Similarly, Scott et al. (2010) showed that a salt desert *B. tectorum* population was more fit than populations from sagebrush habitat when planted into salt desert habitat, but the salt desert population also outperformed local populations in sagebrush habitats when planted without competition. Leger et al. (2009) demonstrated local adaptation in a population in historically invaded habitat in western Nevada but not in a nearby population at the upper elevational limit for *B. tectorum* in this region. These studies provide some experimental evidence that local adaptation is a feature of the population genetics of *B. tectorum* in its introduced range.

Another way to explore the potential for local adaptation is through experiments that examine among-population genetic variation in potentially adaptive traits in a common environment (Kawecki and Ebert, 2004). Such studies have revealed adaptively significant variation in *B. tectorum*, primarily in traits that control the timing of phenological transitions such as seed germination (Meyer and Allen, 1999) and flowering (Rice and Mack, 1991a; Meyer et al., 2004). Genetically based differences in seed mass (Rice and Mack, 1991a; Meyer and Leger, 2010), biomass allocation (Rice et al., 1992), response to salinity (Rasmuson and Anderson, 2002; Scott et al., 2010), and race-specific resistance to the pathogen *Ustilago bullata* (Meyer et al., 2005) have also been demonstrated. Many of the observed differences could readily be interpreted as adaptive in the habitat of origin.

Ramakrishnan et al. (2004) demonstrated that adaptively significant genetic variation in primary seed dormancy, vernalization response, and resistance to *U. bullata* was significantly correlated with a four-locus microsatellite (simple sequence repeat or SSR) genotype in *B. tectorum*. The hypothesis that preadapted genotypes may increase colonization success in novel environments was further supported by significant SSR genotype-by-habitat segregation in 17 populations of *B. tectorum* in Utah and Idaho (Ramakrishnan et al., 2006). One objective of the present study was to determine whether these inferred patterns of local adaptation were maintained when the size and geographic scope of sampling were greatly increased.

The apparent levels of adaptively significant genetic variation in *B. tectorum* are relatively high for an inbreeding species in its introduced range. Introduction events typically create founder effects, or low genetic diversity due to the introduction of a subset of the genetic variation present in the source region (Barrett and Kohn, 1991; Dlugosch and Parker, 2008). These founder effects are expected to be especially severe with inbreeding species due to limited opportunities for recombination and the strong effects of genetic drift in populations with small effective breeding sizes (Charlesworth, 2003; Barrett et al., 2008). High propagule pressure from multiple established populations may increase the chance of success for founding populations by increasing genetic variation (Allendorf and Lundquist, 2003). Allozyme studies have produced evidence for at least seven independent *B. tectorum* introductions into the IMW but have also indicated that extant genetic diversity remains low throughout the introduced range (Novak et al., 1991; Novak and Mack, 2001; Valliant et al., 2007; Schachner et al., 2008) when compared with populations in the native range (Novak and Mack, 2001). Allozyme diversity is generally low in this species, with a mean of 1.03 alleles per locus for polymorphic loci (Novak and Mack 1993).

In the present study, we used patterns of distribution of SSR genotypes to indirectly address the respective roles of adaptively significant genetic variation, adaptive phenotypic plasticity, and the generation of novel genotypes through facultative outcrossing in the ongoing invasion of *B. tectorum* in the IMW, particularly into warm and salt desert habitats, both of which have only been invaded within the last 20 to 40 years. We used the SSR markers developed by Ramakrishnan et al. (2002) to genotype individuals collected from 96 populations representing diverse habitats throughout the region (Fig. 1). If *B. tectorum* SSR genotypes represent inbreeding lines with associated suites of adaptive traits, and if those inbreeding lines show local adaptation to specific habitats, then we expect to see dominance of characteristic SSR genotypes

in certain habitats, and low frequency of those same genotypes in other habitats regardless of geographical proximity. If invasion success is due to adaptive phenotypic plasticity, we expect to see a distribution of SSR variation across the region that is driven primarily by dispersal and genetic drift without regard for ecological barriers. If invasion into novel habitats is associated with the generation of new genotypes through facultative outcrossing, we would expect to find inbreeding lines that represent recombinant SSR genotypes to be the dominant genotypes in recently invaded habitats. The predictions generated by these hypotheses are not mutually exclusive, and all could potentially be supported to varying degrees by our data set.

MATERIALS AND METHODS

***Sample collection and preparation*—**Seed heads were collected from 20 individuals (maternal lines) in each of 96 populations of *B. tectorum* (Fig. 1; Appendix S1, see Supplemental Data with the online version of this article). Sampled individuals were separated from one another by at least 1 m to minimize the potential for sampling full siblings (Hulbert, 1955). Seeds from each population were collected in one of three years, 2005, 2006, or 2008. Geographical locations (GPS coordinates) for each population were obtained at the time of sampling. To investigate the effects of larger sample sizes on allele and genotype frequencies, we sampled 10 of the 96 populations at higher intensity (30–60 maternal lines; see Appendix S1 for identity of intensively sampled populations). We used a randomly selected subsample of 20 individuals from each of these more intensively sampled populations in the analysis and data presentation. A single plant was grown from each maternal line under greenhouse

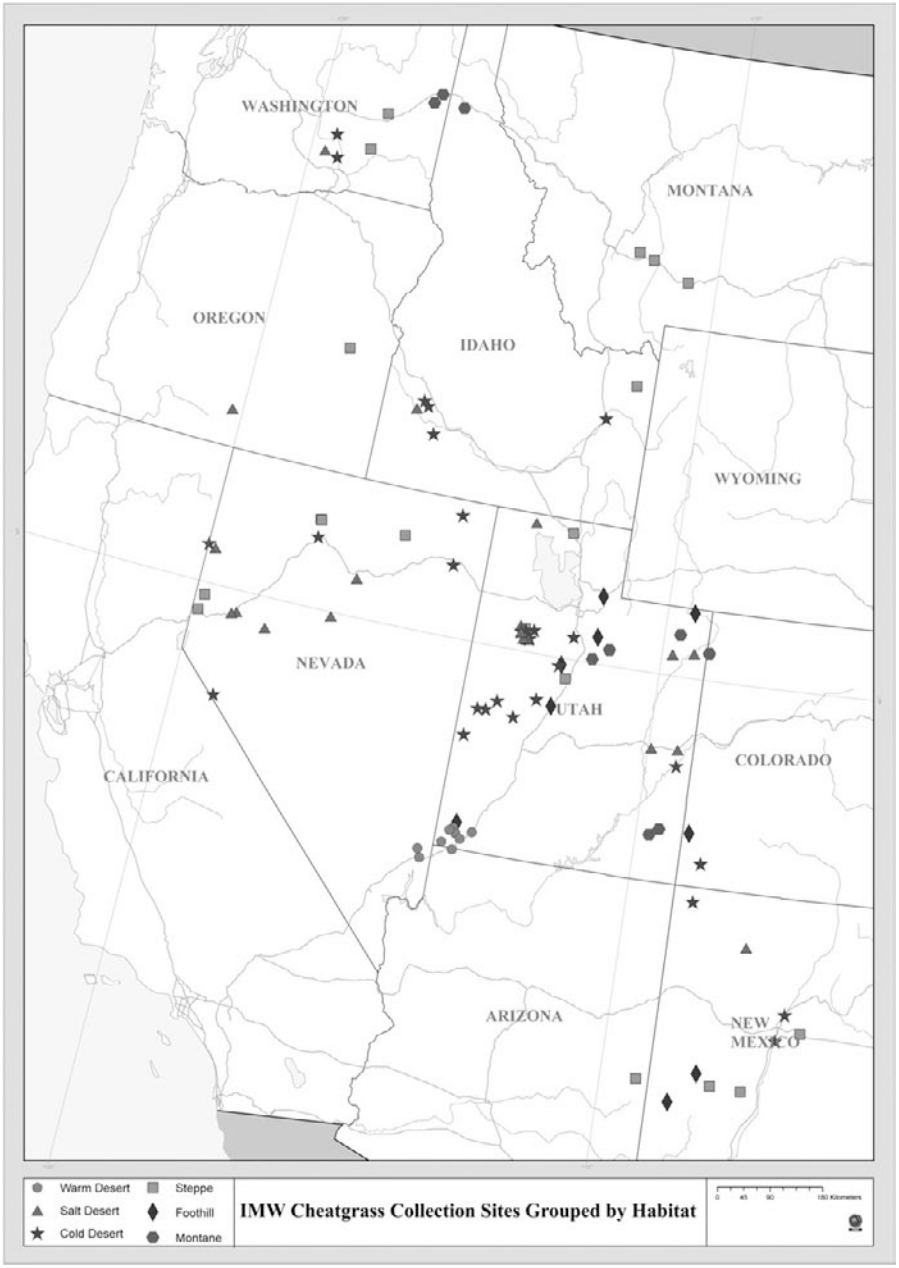

Fig. 1. Locations of 96 *Bromus tectorum* collection sites in the Intermountain Western United States included in this study, identified by ecological group (habitat).

conditions for DNA extraction. When plants were ca. 10 cm tall, 1–3 of the youngest leaves were collected from each plant and stored at −80°C. DNA was extracted using a DNeasy Plant Mini Kit (Qiagen, Germantown, Maryland, USA) or a modified CTAB extraction protocol (Fulton et al., 1995).

SSR genotyping—Methods for SSR amplification and visualization for the four loci examined in this study (BT05, BT26, BT30, BT33; Ramakrishnan et al., 2002) were identical to those reported by Leger et al. (2009). The four SSR loci we used were selected for genotyping due to the ease of multiplexing in a single PCR reaction (Ramakrishnan et al., 2002). While the addition of two additional loci (BT04 and BT12) did add a few low-frequency genotypes in earlier studies, these four marker loci were concluded to provide sufficient resolution for population characterization (Ramakrishnan et al., 2006).

To make the SSR genotype classification in the present study consistent with previously reported studies (Ramakrishnan et al., 2002, 2004, 2006; Leger et al., 2009; Scott et al., 2010), we added the correction values of +2 base pairs to BT05, BT30, and BT33 and +4 base pairs to BT26, as described by Leger et al. (2009), to all allele lengths. This correction was necessary because we found systematic differences among DNA analyzers in specifying allele lengths for a set of reference lines (reference lines available upon request from the authors). Letter designations for allele lengths follow the pattern established by Ramakrishnan et al. (2006), as amended by Leger et al. (2009), with the addition of allele length 165 at BT26 (designated Z) and allele length 229 at BT33 (designated Y). Additional new alleles were encountered in this study (see Appendix S2 for a complete list of alleles for each locus). These were not assigned letters because they did not occur in common genotypes. Only the 14 most common SSR genotypes that individually accounted for >2.0% of total individuals sampled were included in the figures, with the remainder being combined into a "rare" category; however, all alleles were included in the molecular analyses.

Principal components analysis of habitat attributes—Each collection site was assigned to one of six ecological groups defined by plant community type. These consisted of warm desert (WD), salt desert (SD), cold desert (CD), steppe (ST), foothill (FT), and montane (MO) groups. These plant communities typically occur along an environmental gradient associated with increasing elevation, decreasing temperature, and increasing precipitation and are characterized by different dominant plant species (see Appendix S1 for detailed information on each population). To calculate ecological distances between sites, we included habitat characteristics for each site in principal components analysis (PCA) (SAS version 8.2; SAS Institute, Cary, North Carolina, USA). Yearly mean annual precipitation and mean January and July temperature between 1971 and 2007 for each collection site were obtained using the PRISM Climate Group website (http://prismclimate.org, Oregon State University, Corvallis, Oregon, USA [accessed May 2009]). Sites were also classified categorically as having saline soils (1) or nonsaline soils (2), and as having soil organic matter contents ranging from very low (1) to very high (6). Plant community type was also included as a rank order variable with numbers from 1 (warm desert) to 6 (montane), for a total of six environmental variables included in the PCA.

Genotype distribution among ecological groups—We used contingency table analysis (SAS version 8.2; SAS Institute, Cary, North Carolina, USA) to test whether SSR genotypes were nonrandomly associated with specific habitats (ecological groups). Frequencies of the 14 most common SSR genotypes and all rare genotypes (remaining genotypes pooled) were calculated for each population. Numbers of individuals amplifying each common genotype and pooled rare genotypes were summed across populations for each of six ecological groups. This summing across populations generated numeric genotype frequency distributions for each ecological group rather than proportional frequencies. Frequency distributions were then subjected to pairwise randomization tests with 100 000 iterations to determine whether pairs of frequency histograms were significantly different from each other. All pairwise comparisons were significantly different at $P < 0.0000$, making Bonferroni corrections to control the type I error rate unnecessary (Quinn and Keough, 2002).

Population genetic analysis—Analysis of molecular variance (AMOVA; Excoffier et al., 1992), and Mantel correlations (Mantel, 1967) were calculated using the program Arlequin 3.5 (Excoffier and Lischer, 2010). Both the AMOVAs and Mantel correlations were performed with 30 000 permutations and with the samples designated as haploid due to extremely high levels of homozygosity. For individuals that were heterozygous at one or more loci, one allele was chosen at random for inclusion in these analyses. AMOVA was used to test for differentiation among populations and ecological groups based upon allelic frequencies. The geographic distance matrix for the Mantel correlations was generated using the "Geographic Distance Matrix Generator" (Ersts, 2011). The ecological distance matrix was generated using the "dist" function in the program R 2.11.1 (R Development Core Team, 2008), which calculated Euclidean distances based on site scores on the first two principal components from the habitat PCA.

Genetic diversity indices for each population were also calculated using Arlequin 3.5 (Excoffier and Lischer, 2010). These included gene diversity at the haplotype level and gene diversity (mean expected heterozygosity, H_e) averaged across loci (Nei, 1987), as well as number of polymorphic loci and mean number of alleles per locus. Differences among ecological groups in mean values for each of these indices were tested in mixed model analysis of variance (ANOVA) with group as the fixed effect and population as the nested random effect (Proc Mixed; SAS version 8.2). We tested for differences among least squares means from the ANOVA using t tests ($P < 0.05$). We also calculated the among-population coefficient of variation for expected heterozygosity averaged across loci after arcsine square-root transformation of population values (Schoen and Brown, 1991).

RESULTS

Principal component analysis of habitat attributes—When scores for each of the 96 collection sites on the first two principal components from PCA based on habitat attributes were plotted, clear plant community groupings were displayed (Fig. 2). The first principal component (58% of variance explained) represents the elevational gradient, with strong eigenvector loadings for increasing precipitation, decreasing summer and winter temperature, and increasing soil organic matter. The second principal component (21% of variance explained) represents mostly a gradient of decreasing salinity and increasing winter temperature. Warm desert sites cluster in the upper left (warm winters, hot summers, low precipitation, and low salinity), while salt desert sites cluster in the lower left (cold winters, hot summers, low precipitation, and high salinity). Cold desert, steppe, foothill, and montane sites are

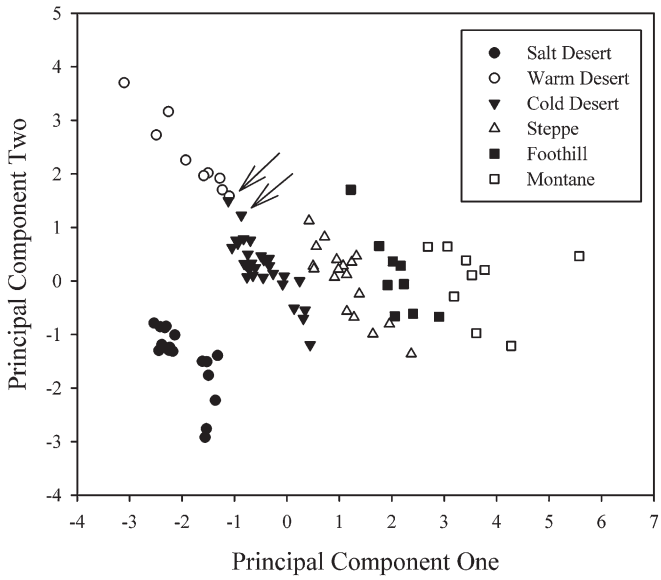

Fig. 2. Plotted scores on the first two components (accounting for 58% and 22% of total variance, respectively) from principal components analysis based on six habitat attributes for 96 *B. tectorum* seed collection sites included in this study. Ecological groups were assigned a priori and are indicated by different symbols. Collection sites marked with arrows are Albuquerque, NM, and Bernalillo, NM, which were classified as cold desert sites based on plant community but which had climates transitional to Chihuahuan Desert warm desert habitat.

located to the right of the more xeric warm and salt desert sites, and are generally arrayed in order from left to right along the gradient of increasing precipitation. Within each of these habitat groups, sites generally range from warmer and drier (upper left end) to cooler and wetter (lower right end). Warm and cold desert sites form a continuum with no clear break. Climatically transitional sites at the northern fringe of the Mojave Desert support a characteristic plant community and were classified as warm desert, whereas sites with this climatic regime at the northern fringe of the Chihuahuan Desert in New Mexico lack this characteristic plant community and were classified as cold desert. Warm desert transitional sites in New Mexico are indicated with arrows (Fig. 2).

***Genotypic distribution*—**In the 1884 maternal lines sampled, we found 131 four-locus SSR genotypes, with the 14 most common SSR genotypes collectively accounting for 79.2% of the total sample. Most populations were dominated by only one or a few common genotypes, with one or more of the most common SSR genotypes accounting for >55% of the genotypic composition in all but one population. Ten populations were dominated by a single genotype (≥90% frequency), and 85 of the populations had at least one genotype that accounted for ≥30% of the population. Fifty-seven genotypes were observed only once, and 101 were observed five or fewer times. The average number of genotypes in a given population was 5.25 with a high of 13 and a low of 2. The four loci, BT05, BT26, BT30, and BT33, had 12, 14, 5, and 9 alleles, respectively, for a mean of 10 alleles per locus (Appendix S2, see online Supplemental Data for a detailed list of alleles and genotypes within each population). The theoretical number of possible homozygous SSR genotypes was therefore 7560, while the total number of possible homozygous SSR genotypes including only alleles present in the 14 most common genotypes was 576. More intensive sampling of 10 populations yielded between 0 and 11 (2.88 average) additional low-frequency genotypes. Only one genotype frequency was altered by more than 10% (15%), and the average change in genotype frequency in more intensively sampled populations was ±0.04 (data not shown). Two populations yielded one or two additional heterozygotes with higher sampling intensity; however, this did not significantly alter the H_o in these populations (data not shown).

The six ecological groups (plant community types) had very different frequency distributions for the common SSR genotypes (Fig. 3). As mentioned earlier, randomization tests showed that every pairwise comparison for SSR common genotype frequency distributions from different ecological groups was significant at the $P < 0.0000$ level (df = 13, maximum likelihood ratio χ^2 values 172–610). Populations in marginal habitats at the lower end of the elevational range of *B. tectorum* were characterized by high frequencies of SSR genotypes that were largely confined to those habitats (Fig. 3). Genotypes FEDD, EBBF, and ECBB dominated warm desert and cold desert transitional habitats in both the Mojave and Chihuahuan Deserts, while each composed less than 3% of any population in other habitat types. Genotype IEBB was dominant in the salt desert habitat, but decreased in frequency with increasing elevation, and was almost completely absent from the montane habitat, while the salt desert genotype EZBY was not found outside of the salt desert habitat, even in geographically adjacent areas. Two SSR genotypes, DABB and GCCB, were characteristic of montane and foothill habitats near the upper elevational limit

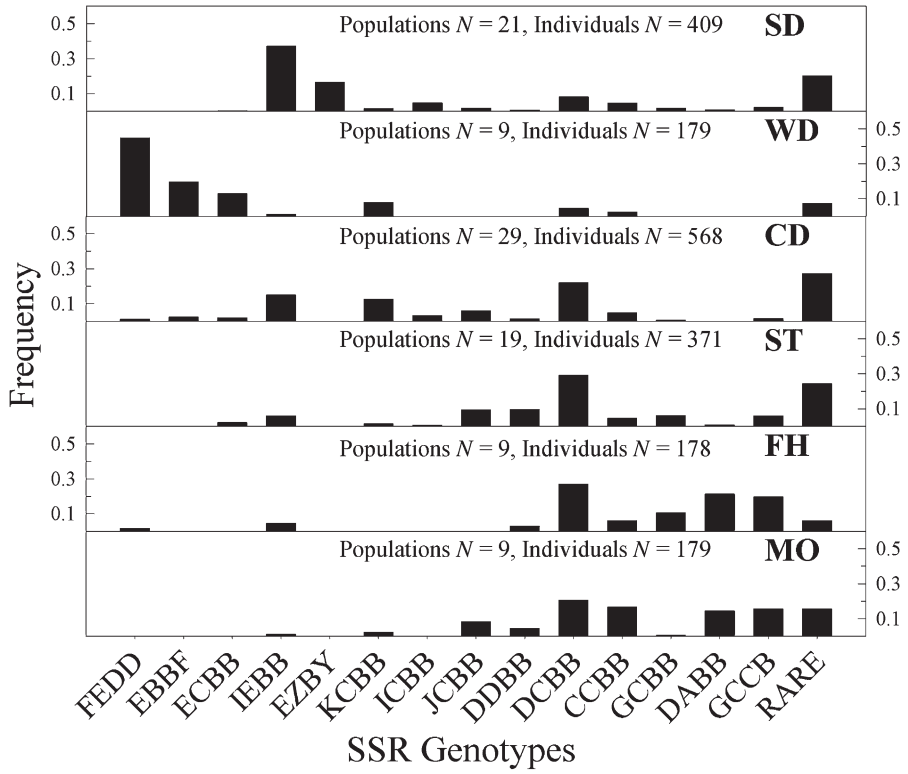

Fig. 3. Frequencies of the 13 most common SSR genotypes, and of all other SSR genotypes combined, within each ecological group. Frequency for each genotype represents the percentage of the total number of individuals pooled across populations for each ecological group (SD = salt desert; WD = warm desert; CD = cold desert; ST = steppe; FH = foothill; MO = montane).

of *B. tectorum*, but occurred at low to very low frequency in mid- to lower-elevation habitats.

Characteristic SSR genotypes were frequently found in specific habitats at geographically widely separated locations, making it unlikely that their association with these habitats is the result of dispersal limitation or genetic drift. For example, the warm desert genotypes FEDD and EBBF were both found at high frequency not only in the northeastern Mojave Desert, but also at warm desert transitional sites in central New Mexico, over 600 km from the Mojave Desert sites. Similarly, the salt desert genotype IEBB was dominant in salt desert communities across a distance of over 800 km, in northwestern New Mexico, central and northeastern Utah, southern Idaho, and central Nevada. In contrast, the other characteristic salt desert SSR genotype, EZBY, was restricted to the Lahontan Basin in western Nevada. Salt desert populations farther north in central Washington and Oregon possessed neither of these genotypes, and did not appear to have any specialist genotype at high frequency.

Most of the SSR genotypes from recently invaded low and high elevation plant communities contained one or more novel alleles not found in other common genotypes (Fig. 3). They are therefore not likely to represent recombination products of these genotypes, reducing the probability that they could have arisen through recent outcrossing events, although it is possible that they may have arisen through mutation.

At least one apparent "general purpose" genotype (DCBB) with wide ecological amplitude and geographic distribution was abundant across four of six ecological groups (montane, foothill, steppe, and cold desert); however, it appeared at much lower frequency in populations from salt and warm desert habitats (Fig. 3). Many of the other somewhat less common SSR genotypes, such as CCBB, DDBB, JCBB, and GCBB, were found mostly in habitats from middle to high elevation, while genotypes KCBB and ICBB were found mostly in middle to low elevation habitats.

Genetic analyses— The AMOVA analysis of allelic diversity revealed almost equal variation within and among populations, indicating a high degree of population differentiation and structure (Table 1). Secondary structure based on ecological groups accounted for 13.1% of the variation. The Mantel correlations provided further evidence for the importance of habitat in structuring genetic diversity (Table 2). Ecological distance was strongly and significantly correlated with genetic distance, while geographic distance was only weakly although significantly correlated. Ecological and geographic distances were not significantly correlated with each other, indicating that these two predictor variables were not confounded in the analysis. This conclusion was further supported by the Mantel correlation analysis including both predictors, which showed significant contributions of the two predictors that were essentially equivalent to their contributions when tested singly.

We encountered very low levels of outcrossing; mean H_e across all individuals was 0.31 (averaged across loci), but H_o was extremely low (0.0071). Of a total of 1884 individuals, 11 were found to be heterozygous at one or more loci. Heterozygotes were found in only three habitats, cold desert, salt desert, and steppe (3, 2, and 6 heterozygotes, respectively).

The four gene-diversity indices examined were significantly correlated across populations ($r = 0.3012$–0.7698, with $P < 0.0001$ for all but one pair, namely, haplotype level gene diversity × number of polymorphic loci, $P = 0.0029$). Mean values (averaged across populations) were generally low (H_e averaged across loci 0.2930 ± 0.1441; haplotype level gene diversity 0.6024 ± 0.2283; number of polymorphic loci 3.021 ± 0.9011, of a maximum of 4; mean number of alleles per locus 2.307 ± 0.5722).

There were small but significant differences in gene-diversity indices among ecological groups (Table 3). H_e averaged across loci was lowest for salt desert populations and highest for warm desert populations, probably because the two warm desert genotypes with strongly contrasting allelic composition (FEDD and EBBF) were often found at similar frequencies in the same populations, whereas the comparable salt desert genotypes (EZBY and IEBB) were almost never found together. H_e values for the other four ecological groups were intermediate and not significantly different from each other.

The salt desert group also had the lowest haplotype level gene diversity, apparently because salt desert populations were usually dominated by a small number of SSR genotypes (Table 3). This value was also low for the warm desert group, because although allelic diversity was high, the number of genotypes per population was often low. This low haplotype gene diversity was especially characteristic of populations in low-elevation warm desert environments (two upper-left-most populations in Fig. 2; haplotype gene diversity = 0.10 and 0.19). In contrast, warm desert-fringe populations often had high haplotype gene diversity (0.70 - 0.80), because they contained characteristic warm desert genotypes as well as genotypes from adjacent cold desert or steppe communities. Higher mean haplotype gene diversity was found in the cold desert, steppe, and montane groups, but each group contained populations with a range of haplotype gene diversity values.

Diversity index values for number of polymorphic loci and number of alleles per locus generally followed the same pattern as H_e averaged across loci, with the warm desert group having significantly higher values and the salt desert group having lower values (Table 3). Every population of the warm desert group was polymorphic at all four loci.

The coefficient of variation (standard deviation/mean × 100) for H_e averaged across loci (as calculated from the mean and standard deviation across all populations using arcsine square-root-transformed population H_e values; Schoen and Brown, 1991) was 29.9%. The range of population values for H_e averaged across loci (untransformed) was 0.544, with a minimum of

TABLE 1. Analysis of molecular variance (AMOVA) with no secondary structure and with populations subdivided into six ecological groups. All *P* values are <0.00001.

Structure	Source of variation	df	Sums of squared deviations	Variance components	Percentage of variation
No secondary structure	Among populations	95	899.682	0.4530	43.59
	Within populations	1787	1047.303	0.5861	56.41
Six ecological groups	Among groups	5	245.801	0.1396	13.09
	Among populations within groups	90	653.882	0.3406	31.95
	Within populations	1787	1047.303	0.5861	54.96

TABLE 2. Mantel correlations among genetic distance, ecological distance, and geographic distance for 96 populations of *Bromus tectorum* from the Intermountain Western United States.

Predictor variable	Response variable	Correlation coefficient	% Variance explained	Probability
Simple correlation				
Ecological distance	Genetic distance	0.3793	14.40	0.000000
Geographic distance	Genetic distance	0.1400	1.96	0.015500
Geographic distance	Ecological distance	0.0743	0.55	0.066400
Multiple correlation				
Ecological distance	Genetic distance	0.3736	14.07	0.000000
Geographic distance	Genetic distance	0.1212	1.57	0.015800

0.047 and a maximum of 0.591. This coefficient of variation fell at the low end for inbreeding species in an earlier survey of genetic diversity in inbreeding vs. outbreeding species (Schoen and Brown, 1991), indicating a higher proportion of highly variable populations than is the norm for inbreeding species.

DISCUSSION

In this extensive survey of SSR variation in the IMW, we confirmed SSR genotype distribution patterns in recently invaded habitats that we observed in our earlier studies (Ramakrishnan et al., 2006; Meyer and Leger, 2010) and discovered new examples of these patterns. The warm desert genotype FEDD, found to be dominant in our earlier study in two eastern Mojave Desert populations, was found in all warm desert and warm desert fringe populations in southern Utah, southern Nevada, and central New Mexico, usually at medium to high frequency. The salt desert genotype IEBB, characteristic of low elevation, primarily salt desert sites in earlier studies (Ramakrishnan et al., 2006; Scott et al., 2010), was found to be present and often dominant at salt desert sites across a wide geographic range. We also found new genotypes characteristic of warm and salt desert habitats (EBBF and EZBY, respectively).

We confirmed that two of the three characteristic montane genotypes found in Utah in the earlier study (DABB and GCCB) were common and widespread in montane environments in the southern half of the region; the third montane genotype (KCCB) was not detected in the current study, even in a population closely adjacent to the now-extinct Strawberry UT population where the KCCB lines were originally collected. It appears that this third genotype was not able to persist, at least in numbers sufficient to permit detection at these sampling intensities. The fact that DABB and GCCB were also present in mid-elevation habitats, rather than being restricted to only montane habitats, may indicate that individuals with these genotypes are capable of exhibiting a higher level of phenotypic plasticity than other locally adapted genotypes, such as FEDD or EZBY.

The genotype-by-habitat segregation patterns we observed were strong, and we know that these SSR genotypes are associated with traits (such as minimal vernalization requirement for flowering in warm desert genotypes; Meyer et al., 2004) that are likely to be adaptive in the recently invaded habitats where they are found. We are aware, however, that we have only inferred the existence of local adaptation from the distribution of molecular marker genotypes, rather than conclusively demonstrating its existence (Kawecki and Ebert, 2004; Latta, 2009). Reciprocal seeding experiments to determine whether local adaptation has actually taken place in these genotypes are currently in progress.

Distribution patterns for the common SSR genotypes of historically invaded IMW environments in this study generally suggest that these genotypes occur over a range of habitats and are not specialists, though they are often largely excluded from the more extreme environments occupied by specialist genotypes, especially xeric warm and salt desert environments. They also tend to occur over a very wide geographic range, which is one reason that we found such a minimal effect of isolation by distance in the population genetic analysis. The species in general is known to exhibit high levels of phenotypic plasticity in response to resource variability, for example, as a function of plant density, and populations vary genetically in the degree of phenotypic plasticity they can express (Rice and Mack, 1991b). However, the study of Rice and Mack (1991b) did not reveal any adaptive significance that could be ascribed to the degree of phenotypic plasticity expressed by populations from different habitats. Thus, the idea that these particular genotypes are able to occupy a wide range of environments through adaptive phenotypic plasticity remains a tenable hypothesis, but we have produced no concrete evidence in its support.

One problem with interpretation of the distribution of the common SSR genotypes of historically invaded habitats is that most of the polymorphism is at the BT05 locus (Fig. 2). We know from sequencing multiple individuals with the same allele length that homoplasy in the form of sequence differences that result in the same allele length at this locus is not uncommon (Merrill et al., 2010). This indicates that, especially for SSR genotypes distinguished only at this locus, identical marker fingerprints do not necessarily indicate common descent. It is therefore quite possible that SSR genotypes that appear to be widespread or to exhibit generalist behavior in terms of the habitats they occupy may actually represent multiple unrelated lineages whose molecular genetic differences are masked by homoplasy or are not detectable with only four SSR loci. We are now developing a set of SNP (single nucleotide polymorphic)

TABLE 3. Summary statistics for ecological groups. Means separations for genetic diversity indices are from mixed model ANOVAs with ecological group as the fixed effect and population nested within ecological group as the random effect. Means followed by different letters are significantly different at $P < 0.05$.

Ecological group	Group code	No. populations	No. of individuals	No. of haplotypes	Mean H_e averaged across loci	Mean haplotype gene diversity	Mean no. polymorphic loci	Mean no. alleles/locus
Warm desert	WD	9	179	16	0.433 a	0.555 ab	4.00 a	2.53 a
Salt desert	SD	21	409	47	0.230 c	0.501 b	2.62 c	2.24 b
Cold desert	CD	29	568	65	0.309 b	0.663 a	3.14 b	2.48 ab
Steppe	ST	19	371	46	0.279 bc	0.624 ab	2.84 bc	2.25 b
Foothill	FH	9	178	14	0.256 bc	0.570 ab	3.00 bc	2.03 b
Montane	MO	9	179	25	0.313 b	0.679 a	3.00 bc	2.08 b

markers for *B. tectorum* to resolve these difficulties, as well as to examine the putative monophyletic origin of specialist SSR genotypes in recently invaded habitats (Merrill et al., 2010).

No evidence was found to support the hypothesis that facultative outcrossing is a mechanism for ongoing *B. tectorum* range expansion in the IMW. We detected a higher percentage of heterozygosity than that detected in several previous SSR studies with *B. tectorum* (Ramakrishnan et al., 2004, 2006; Scott et al., 2010), but lower than the percentage heterozygosity observed in others (Ashley and Longland, 2007; Leger et al., 2009). Additional molecular markers will be necessary to fully examine how much of a role outcrossing may have played in the past evolution of this species.

The existence of outcrossing makes recombination at least theoretically possible as a source of new genotypes with unique adaptive traits. However, novel alleles in the SSR genotypes of the lineages dominating recently invaded habitats make it unlikely that these lineages could have arisen through recombination from other extant genotypes. For example, the genotype represented by EZBY was first reported by Ashley and Longland (2007) from the same Lahontan Basin salt desert populations that were sampled in this study. They reported that *B. tectorum* was essentially absent from these habitats until quite recently and speculated on outcrossing as a potential mechanism for the origin of this adapted ecotype. However, the novel alleles BT26-165 (Z) and BT33-229 (Y) do not occur in any genotype found outside of the salt desert habitat of the Lahontan Basin, so EZBY is highly unlikely to be a recombination product of other extant genotypes in the area. It could perhaps have arisen by mutation, but there is no reason to expect that SSR mutations would be correlated with mutations conferring new adaptive traits. This same logic also applies to the other warm and salt desert specialist genotypes. We are currently undertaking a more extensive survey of salt desert habits in the southern Great Basin and the warm desert habitats to the south to determine the current limits of distribution of these apparent habitat-specific genotypes.

If specialist genotypes are facilitating the expansion of the *B. tectorum* range within the IMW, one question is why invasion into new habitats (montane, salt desert, warm desert) has occurred only in the last 40 years. There are two possible explanations for this recent expansion. Either specialist genotypes have been persisting at low frequency in other habitats and have only recently been dispersed into the habitats for which they are optimally adapted, or these specialist genotypes represent more recent introductions from the native range. The first explanation is possible, as we did find low to very low frequencies of some specialist genotypes (FEDD, IEBB) outside of their niche habitats, but it seems equally likely that these have invaded non-optimal habitats at low levels due to high propagule pressure from populations in adjacent optimal habitats. We therefore conclude that the most likely explanation given the present state of our knowledge is that the specialist genotypes that dominate in recently invaded habitats are the result of relatively recent introductions (within the last 20–40 years) from unknown source populations.

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