

Ecological significance of microsatellite variation in western North American populations of *Bromus tectorum*

ALISA P. RAMAKRISHNAN,*† SUSAN E. MEYER,* DANIEL J. FAIRBANKS† and CRAIG E. COLEMAN†

*USDA Forest Service, Rocky Mountain Research Station, Shrub Sciences Laboratory, 735 North 500 East, Provo, Utah 84606, United States of America and †Department of Plant and Animal Science, Brigham Young University, Provo, Utah 84602, United States of America

Abstract

Bromus tectorum (cheatgrass or downy brome) is an exotic annual weed that is abundant in western USA. We examined variation in six microsatellite loci for 17 populations representing a range of habitats in Utah, Idaho, Nevada and Colorado (USA) and then intensively sampled four representative populations, for a total sample size of approximately 1000 individuals. All loci were homozygous, indicating that the species is strongly selfing. Populations consisted of a few common genotypes and variable numbers of rare genotypes. Small sample sizes ($n = 10$ individuals) were adequate for distinguishing among populations, but larger sample sizes were needed to characterize more diverse populations, particularly in terms of genotype. Large populations contained more genetic diversity than small populations in terms of both number of alleles per locus and number of genotypes. Genetic distance among survey populations was much more strongly correlated with ecological distance (habitat) than with geographical distance, and was also strongly correlated with a suite of adaptively significant seed germination traits. This suggests that similar habitats across the range of *B. tectorum* in western USA select for specific self-pollinating lines from an array of widely distributed genotypes. Because all traits are effectively linked in this selfing organism, the distribution of adaptively significant genetic variation can be successfully inferred from an examination of microsatellite marker variation.

Keywords: adaptation, *Bromus tectorum*, ecology, genecology, genetics, microsatellite, selection, simple sequence repeat.

Received 24 August 2005; accepted 16 March 2006

Introduction

Bromus tectorum (cheatgrass or downy brome) is a relatively recent invader in western USA (Mack 1981). Originally introduced as a contaminant in grain seed in the late 1800s, it then spread through livestock-related vectors with amazing rapidity, reaching its current geographical distribution within 30 years (Mack 1981). *Bromus tectorum* has now come to occupy at least 40 million hectares in the Intermountain West alone (Rosentreter 1994). It is the dominant species on at least 200 000 km², making it the most common vascular plant species in the region (Mack 1989). The ecology of many

of the areas it inhabits has been permanently altered (Mack *et al.* 2000; Harrod 2001).

Bromus tectorum is most abundant in salt desert shrubland, sagebrush steppe and foothill woodland habitats, as well as in the winter cereal grain fields associated with these habitats. However, its ecological range is still increasing, both at higher elevations, where there is now significant invasion of ponderosa pine forest understory, and at lower elevations, where this species is becoming a common codominant with *Bromus madritensis* in the understory of warm desert shrubland communities in the Mojave Desert.

Investigations of characteristics contributing to the success of *B. tectorum* have led to a greater understanding of some of the processes involved in invasion biology. By combining historical record searches with genetic analy-

Correspondence: Susan E. Meyer
Email: smeyer@fs.fed.us

ses, a detailed picture of the introduction and spread of *B. tectorum* has been produced (Novak & Mack 2001). Historical evidence and extensive allozyme analysis of populations in the native and introduced ranges support a multiple introduction hypothesis (Mack 1981; Novak *et al.* 1991, 1993; Bartlett *et al.* 2002).

Many phenotypic traits important to survival are under strong genetic control, indicating that a degree of preadaptation is necessary for the spread of an invasive weed throughout a novel range. In *B. tectorum*, seed dormancy, regulation of flowering and resistance to the head smut fungus *Ustilago bullata* are important for the establishment and maintenance of viable populations (Meyer *et al.* 1997, 2001, 2004; Meyer & Allen 1999). Variation in these traits is highly correlated with molecular genetic variation on an individual plant basis (Ramakrishnan *et al.* 2004). The correlation is particularly high with microsatellite markers (simple sequence repeats or variable tandem repeats). This high correlation is due largely to the selfing mating system of *B. tectorum* (McKone 1985). Because all loci behave as if they are effectively linked in a self-pollinating species, high correlations among traits are not unexpected (Hedrick & Holden 1979). This presents an invaluable opportunity for research because variation in phenotypic traits leading to successful invasion and spread can be studied not only through direct observation, but also through observation of molecular marker variation.

In the current study, we used our previously developed microsatellite markers (Ramakrishnan *et al.* 2002, 2004) to examine the genetic structure of several Intermountain populations of *B. tectorum*. We addressed the following hypotheses: (i) *B. tectorum* is highly self-pollinating and exhibits low levels of heterozygosity; (ii) populations consist of measurable numbers of self-pollinating lines with identifiable molecular marker fingerprints; (iii) populations can be characterized using few molecular marker loci and relatively small sample sizes; (iv) molecular genetic variation and variation in adaptively significant phenotypic traits are correlated at the population level; and (v) both molecular genetic and adaptively significant phenotypic variation are more highly correlated at the population level with ecological distance (habitat variation) than with geographical distance.

Materials and methods

Populations

Our study populations were chosen from a series of populations included in previous genecological studies of *B. tectorum* (Meyer *et al.* 1997, 2001, 2004; Meyer & Allen 1999; Table 1, Fig. 1). Thirteen populations from Meyer *et al.* (1997) were included in our population survey at a low sampling intensity ($n = 10$), and we added four new

Table 1 Location data and sample sizes (n) for survey population collections

Population	Population code	n	Elevation (m)	Mean January temperature (°C)
Mojave desert				
Potosi Pass, Nevada†‡	MD1	10	1500	1.7
St. George, Utah‡	MD2	6	850	3.7
Intermountain desert				
Whiterocks, Utah†‡	ID1	10	1450	-2.3
E. Green River, Utah‡	ID2	8	1280	-5.2
Bruneau, Idaho	ID3	17	1080	-0.3
Western great plains				
Castle Rock, Colorado‡	WGP	6	1980	-1.9
Intermountain foothill				
Hobble Creek, Utah†‡	IF1	10	1530	-2.1
Provo Bench, Utah‡	IF2	5	1410	-1.3
Monticello, Utah‡	IF3	9	2100	-3.7
Arrowrock, Idaho	IF4	18	1150	-2.2
Boise Foothills, Idaho	IF5	7	950	-1.0
Black Creek Jct., Idaho	IF6	9	1035	-1.0
Boise, Idaho‡	IF7	7	870	-2.6
Intermountain montane				
Strawberry, Utah†‡	IM1	10	2400	-7.8
N. of Castlegate, Utah‡	IM2	4	2070	-4.7
Fairview Top, Utah‡	IM3	9	2770	-6.7
Nebo Pass, Utah‡	IM4	7	2850	-8.9

†Populations used for intensive sampling. ‡Populations in seed germination attribute dataset. Sample sizes (n) represent average number of samples amplified per locus.

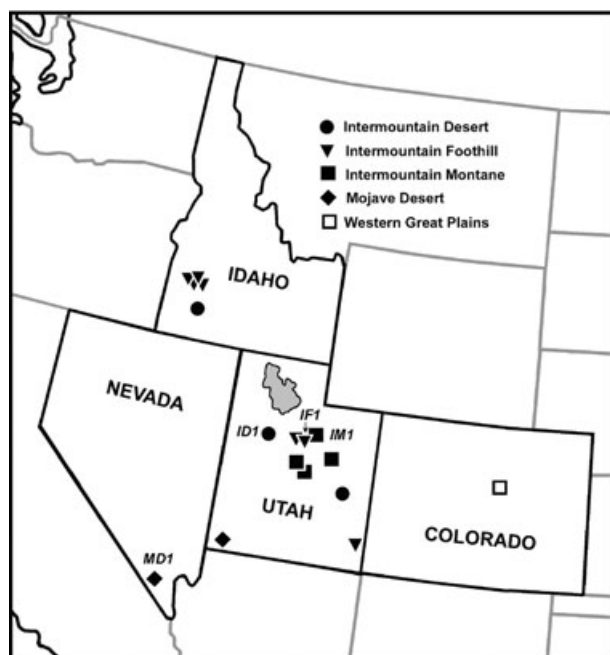


Fig. 1 Parts of western USA showing the locations of the 17 survey populations. Intensively sampled populations are labeled (ID1, Whiterocks, Utah; IF1, Hobbie Creek, Utah; IM1, Strawberry, Utah; MD1, Potosi Pass, Nevada).

populations at a similar sampling scale for a total of 17 (Table 1). Four populations that were examined in detail in earlier studies were chosen from the array of survey populations for more intensive sampling. These four populations represented the full range of habitats from warm desert to montane. Each of the four populations was represented by two subpopulations. The distance between the subpopulations was 6 km at Whiterocks, 0.3 km at Hobbie Creek, 0.5 km at Strawberry and 0.3 km at Potosi Pass. We collected among-year data from one subpopulation each at Whiterocks and at Hobbie Creek (Table 2). These were the largest and apparently most genetically diverse of the four populations based on preliminary information (Ramakrishnan *et al.* 2004).

Sample collection

For populations described in Meyer and Allen (1999; i.e. Whiterocks, Hobbie Creek, Strawberry and Green River, Utah; Potosi Pass, Nevada; and Castle Rock, Colorado), seeds were collected from 10 randomly selected plants in the field in 1995. For populations at Arrowrock and Bruneau, Idaho, seeds were collected from 20 randomly selected plants in the field in 1999. Ten seeds for each of the remaining populations in Table 1 were drawn at random from bulk seed collections (at least 100 individuals) made at each site in 1992. To obtain tissue for DNA

extraction, we grew plants from 10 seeds representative of each population in a greenhouse until they were approximately 10 cm tall, collected one to three leaves per plant, and stored the tissue at -80°C .

For the four intensively sampled populations, seeds were also collected in 1998 from 50 randomly selected plants in each subpopulation. These seeds were grown and tissues were sampled as described above. In 1999 and 2000 at Whiterocks and Hobbie Creek, leaf and stem tissue from randomly selected plants at least 1 m apart was collected in the field in coin envelopes and frozen on dry ice prior to storage at -80°C . Some samples were dried in an oven at 30°C or at room temperature prior to DNA extraction. The change in protocol to field-sampled tissue in 1999 and 2000 was necessary to fulfill design requirements for another objective of the study, namely evaluating changes in genotype frequencies of smutted and unsmutted subsets of each population across years (S. Meyer unpubl. data, 1999, 2000).

DNA extraction and molecular marker amplification

We extracted DNA using a modified CTAB procedure (Bult *et al.* 1992) and samples were stored at -20°C . We amplified microsatellite loci with the polymerase chain reaction (PCR) using primers for *B. tectorum* microsatellite loci BT03, BT04, BT05, BT12, BT26, BT30 and BT33 according to Ramakrishnan *et al.* (2002), with BT05, BT26, BT30 and BT33 in a single, multiplexed PCR reaction for each sample. We eliminated BT03 because, after an initial screening of 400 individuals, we found it to be fixed in all populations, with the only polymorphism being between the Mojave Desert and the Intermountain populations. The remaining six loci were amplified for all survey population samples and for the principal population samples collected in 1998. For the 1999 and 2000 samples, only the four multiplexed loci were examined.

Data analysis

To explore the differences among populations and subpopulations, we calculated genetic distances from allele frequencies using the distance measure

$$d_{ij} = \sum_{n=1}^k \frac{(x_{ki} - x_{kj})^2}{x_{ki} + x_{kj}}$$

from Balakrishnan and Sanghvi (1968), where d_{ij} is the distance between two populations or years, i and j , x is the frequency of allele k in a population, and n is the number of alleles in a pair of populations. This distance measure allows all alleles to be included in the distance calculation. We calculated distance matrices based on the dataset from the 17-population survey and based on all

Table 2 Allele sizes and frequencies for all populations, subpopulations (a and b) and years

Populations, subpopulations and years														
Alleles	bp	Survey	1998								1999	2000	1999	2000
			ID1a	ID1b	IF1a	IF1b	IM1a	IM1b	MD1a	MD1b	IDa		IFa	
BT05_A	163	0	0	0	0	0	0	0	0	0	0.005	0	0	0
BT05_B	165	0.048	0	0	0	0	0	0	0	0	0.021	0	0	0
BT05_C	167	0.143	0.026†	0.043	0.027	0.045	0	0.027	0	0	0.138	0.083	0.006	0.051
BT05_D	169	0.339	0.564†	0.681	0.324†	0.182	0.447†	0.459	0	0	0.319	0.619	0.416	0.576
BT05_E	170	0.024	0†	0.128	0.081	0.023	0	0	0	0	0.027	0	0.045	0.068
BT05_F	171	0.107	0	0	0	0	0	0	0.783†	1.000	0.005	0	0	0
BT05_G	173	0.095	0†	0.064	0.189†	0.455	0.191	0.432	0	0	0.096	0.071	0.214	0.119
BT05_H	174	0.012	0	0	0	0	0	0	0	0	0.005	0	0	0
BT05_I	175	0.089	0.282†	0.085	0	0	0.021	0	0.217	0	0.303	0.214	0.006	0.017
BT05_J	177	0.048	0.128	0	0.378	0.295	0	0.081	0	0	0.059	0.012	0.305	0.153
BT05_K	178	0.077	0	0	0	0	0.340†	0	0	0	0.021	0	0.006	0.017
BT05_L	180	0.018	0	0	0	0	0	0	0	0	0	0	0	0
BT26_A	136	0.150	0	0	0.108	0.159	0	0.257	0	0	0.005	0	0.068	0.214
BT26_B	150	0.006	0	0.021	0	0	0	0	0	0	0	0	0	0.014
BT26_C	152	0.630	0.791†	0.896	0.892†	0.841	0.957†	0.743	0	0	0.703	0.890	0.932	0.743
BT26_D	154	0.017	0	0	0	0	0.022	0	0	0	0	0	0	0
BT26_E	156	0.191	0.209†	0.083	0	0	0.022	0	1.000†	1.000	0.292	0.110	0	0.029
BT26_F	158	0.006	0†	0	0	0	0	0	0	0	0	0	0	0
BT30_A	112	0.011	0.023	0.245	0.023	0	0	0.024	0	0	0.021	0.022	0	0
BT30_B	114	0.807	0.955†	0.653	0.860†	0.913	0.378†	0.610	0.239	0	0.872	0.967	0.938	0.986
BT30_C	116	0.088	0.023	0.102	0.116	0.087	0.622†	0.366	0	0	0.108	0.011	0.063	0.014
BT30_D	118	0.094	0	0	0	0	0	0	0.761†	1.000	0	0	0	0
BT33_A	218	0.042	0.024	0	0.023	0.021	0	0	0	0	0	0	0.019	0.029
BT33_B	221	0.850	0.976†	0.708	0.930†	0.979	1.000†	0.974	0.217	0	0.969	0.916	0.975	0.971
BT33_C	222	0.006	0	0	0	0	0	0	0	0	0	0	0	0
BT33_D	223	0.084	0	0	0	0	0	0	0.783†	1.000	0	0	0	0
BT33_E	224	0.012	0†	0.292	0	0	0	0	0	0	0.031	0.084	0.006	0
BT33_F	225	0.006	0	0	0.047	0	0	0.026	0	0	0	0	0	0
BT04_A	108	0.153	0	0	0	0	0	0	0.733†	1.000	—	—	—	—
BT04_B	110	0.315	0.375†	0.057	0.200†	0.081	0	0	0.267	0	—	—	—	—
BT04_C	112	0.009	0	0	0	0	0	0	0	0	—	—	—	—
BT04_D	114	0.450	0.604†	0.943	0.800†	0.919	1.000†	0.970	0	0	—	—	—	—
BT04_E	116	0	0.021	0	0	0	0	0.030	0	0	—	—	—	—
BT04_F	118	0.072	0	0	0	0	0	0	0	0	—	—	—	—
BT12_A	252	0.546	0.500†	0.448	0.786†	0.976	0.972†	1.000	0	0	—	—	—	—
BT12_B	256	0.007	0	0	0	0	0	0	0	0	—	—	—	—
BT12_C	261	0.121	0	0.172	0	0	0.028	0	0.766†	1.000	—	—	—	—
BT12_D	263	0.326	0.500†	0.379	0.214†	0.024	0	0	0.234	0	—	—	—	—

†Alleles that were present in the survey sample for that subpopulation. Allele frequencies from the survey samples are averages across all populations. Alleles are indicated by letters following the locus identifiers. Populations are Whiterocks (ID1), Hobbie Creek (IF1), Strawberry (IM1) and Potosi Pass (MD1).

years and subpopulations for the four intensively sampled populations. We also calculated distance matrices based on the 1998 data alone for the four intensively sampled populations and on the among-year data for the subpopulations with multiple-year sampling at Whiterocks and Hobbie Creek.

To compare seed germination data with survey population allele frequency data, we reanalyzed data from Meyer *et al.* (1997), who found that ecotypic differentiation

in seed germination strategy in *B. tectorum* had already occurred in less than 100 years. In that experiment, freshly collected seeds from the 13 populations previously mentioned (as well as 10 additional populations for which seeds for molecular genetic characterization were no longer available) were subjected to five incubation temperature regimes and measured for germination percentage and mean germination time. Rates of dormancy loss during storage at 20°C were also calculated.

We used the original data to calculate the average taxonomic distance as

$$d_{ij} = \sum_{ij} \sqrt{\sum_{k=1}^n \frac{1}{n} (x_{ki} - x_{kj})^2}$$

where i and j are populations, n is the number of traits (k), and x is the value for a trait.

We used mean January temperature as a simple index of habitat variation in this study. We chose this index because it has been successfully used to relate variation in seed germination strategy to habitat variation for many Intermountain species (e.g. Meyer *et al.* 1989; 1995; Meyer & Mosen 1991). Use of more detailed habitat information for each population did not improve the correlations between seed germination traits and habitat in these studies. Ecological distance was calculated as Euclidean distance between two populations, i and j , using values (x) of mean January temperature (k) at the collection site (Table 1) for each population.

$$d_{ij} = \sqrt{\sum_k (x_{ki} - x_{kj})^2}$$

Geographic distance (Fig. 1) was calculated in MapPoint (Microsoft).

To visualize the content of genetic and phenotypic distance matrices, the matrices were used as input for non-metric multidimensional scaling (NMDS; Kruskal 1964). This technique iterates points on a 2-D plot in such a way as to maximize fit between the distances among points on the plot and the rank order of the dissimilarity matrix. Goodness of fit is measured as stress, which is minimized as the plot distances approach the distances in the dissimilarity matrix (Klahr 1969; Spence 1972).

For each subpopulation and year in the four intensively sampled populations, we calculated gene diversity averaged across loci as

$$GD = (1 - \sum p_i^2) / (n/n - 1)$$

where n is the number of samples in a subpopulation or year and p_i is the frequency of the i th allele in the subpopulation (Nei 1987). Gene diversity is equivalent to expected heterozygosity, and is often used as a measure of genetic variability in other papers on *B. tectorum* (Bartlett *et al.* 2002). A value of 1.00 indicates that every individual has a different allele, while a value of 0.00 indicates that every allele is fixed. We also calculated the mean number of alleles per locus (A) for the subpopulations and years in the four intensively sampled populations. We did not calculate gene diversity or mean alleles per locus for the survey population samples because of relatively small sample sizes.

To estimate the distribution of variance within and among the main populations, we calculated three analyses

of molecular variance (AMOVAs) (Excoffier *et al.* 1992) in Arlequin (Schneider *et al.* 2000): one for the 1998 data and one each for the among-year data from Whiterocks and Hobble Creek. The AMOVA procedure is analogous to a traditional hierarchical or nested analysis of variance. We treated our data as haplotypic because all samples were apparently homozygous. Several loci amplified alleles that differed in length by one basepair instead of the length of a single repeat, possibly because of mutations in flanking regions. Because of these alleles, we did not estimate the numbers of mutational steps between alleles of different lengths or compute R_{st} statistics, nor did we use such information in calculations of haplotypic distances. We did not calculate F_{st} statistics because *B. tectorum* populations are highly inbred and have undergone recent range expansion, making interpretation of this value in terms of gene flow extremely difficult (Neigel 2002; Pearse & Crandall 2004).

We used a Mantel correlation test to test for correlation between distance matrices calculated from the four multiplexed loci versus all six loci (Mantel 1967) using data from the eight subpopulations in 1998. To test for correlations between phenotypic distance (Meyer *et al.* 1997), geographical distance, ecological distance, and genetic distance, we conducted Mantel correlation tests on distance matrices calculated from germination attributes, geographic location, mean January temperature and allele frequencies for the 13 survey populations included in both studies. Distance matrices and Mantel tests were calculated using NTSYSpc (Exeter Software, Setauket, NY, USA).

We assembled genotypes from data on allelic variation at each locus for each individual, and calculated genotype frequencies from these data for subpopulations and years for the four principal populations. We did not calculate allele or genotype frequencies for the sample populations because of small sample sizes. We were unable to obtain data for one or more loci in some individuals. This meant that allele frequency values were sometimes calculated based on a larger dataset than genotype frequency values. In addition, distance matrices used data from all six loci when available, whereas genotypes were characterized using only the four multiplexed loci.

Results

Molecular genetic structure of Bromus tectorum populations

We observed substantial variation at each of the six *B. tectorum* microsatellite loci we examined, with four to 12 alleles per locus (Table 2). In genotyping over 1000 *B. tectorum* individuals, we never observed an unequivocally heterozygous locus. This is strong evidence that the

species is highly self-pollinating. The potential number of six-locus genotypes that could be generated through recombination was very large (>7000). In 1998 we characterized six-locus genotypes for approximately 260 individuals belonging to four populations. We detected only 32 genotypes (data not shown). Similarly, only 50 four-locus genotypes (based on the four loci in the multiplexed PCR reaction) were observed in a total of almost 800 individuals from four populations (Table 3). This further supports the hypothesis that recombination events are rare in this species.

Only eight four-locus genotypes from 1998 were split into multiple genotypes when we added two additional loci (BT04 and BT12) to the dataset. The Mantel correlation between genetic distance matrices using four versus six loci was 0.955 ($P < 0.001$), and NMDS plots of genetic distances were virtually identical (data not shown). We concluded that for the purposes of population characterization in our studies, the four microsatellite loci that can be included in a single, multiplexed PCR reaction gave sufficient resolution.

A total of 38 alleles were detected in the course of our molecular genetic analysis. Interestingly, 36 of these alleles were detected when we genotyped small numbers of individuals from each of 17 survey populations (Table 2). Larger sample sizes and collections across multiple years resulted in the detection of very few new alleles.

Intensively sampled populations were composed of a few common (frequency >0.10) four-locus genotypes and variable numbers of rare (frequency <0.10) genotypes (Table 3). Whiterocks was the largest population and also the most diverse, with a total of 37 genotypes. But only four of these genotypes were ever present at frequencies >0.10 . The majority of genotypes at Whiterocks were represented by only one or two individuals detected in a single year. A number of the rare genotypes appeared to represent recombination products of common genotypes. These could be the result of outcrossing events in the past.

The Hobbie Creek population had three common genotypes and 10 rare genotypes, while the Strawberry population (sampled only in 1998) had four common genotypes and six rare genotypes. The Potosi Pass population (also sampled only in 1998) was the least diverse, with only two common genotypes. These differences in genetic diversity among the four principal populations were also evident in diversity statistics based on allele frequencies (Table 4).

The Mojave Desert Potosi Pass population was strongly genetically differentiated from the three Intermountain populations included in the 1998 study (Fig. 2). Both subpopulations were dominated by a single genotype, FEDD, which contains two unique alleles (Tables 2,3). The only other genotype found at Potosi Pass (IEBB) was also common at Whiterocks. The genetic distinctness of the Potosi

Pass population is further evidenced by its major impact on the among-population variance component in AMOVA (Table 5).

In general, subpopulations of the same population were more genetically similar to each other than to subpopulations of other populations. The variance accounted for by between-subpopulation differences was small relative to within-subpopulation variance (Table 5). Figure 2 shows that the montane Strawberry population had rather more subpopulation structure than the other populations, as indicated by the greater distance between subpopulations. This was primarily because of the presence of the common genotype KCCB in only one subpopulation. Several genotypes in common placed Strawberry closer to other Intermountain populations than to the Mojave population (Table 3). Strawberry shared seven genotypes with Hobbie Creek and four with Whiterocks. These four genotypes were present in all three Intermountain populations, but only one (DCBB) was common in all three.

A common genotype in both subpopulations at Hobbie Creek was GCBB, which was not common in any other Intermountain population (Table 3). No common genotype at Hobbie Creek was found exclusively in one subpopulation, resulting in two subpopulations that are close to each other on the NMDS plot (Fig. 2). The most common genotype in both subpopulations at Whiterocks was the widely distributed genotype DCBB. Whiterocks shared six genotypes with Hobbie Creek, placing it close to Hobbie Creek on the NMDS plot.

Genetic distances among the four principal populations were larger when calculated using the small 1995 survey samples than when calculated using larger sample sizes from later years, but the relationships remained essentially unchanged (Fig. 2).

When subpopulations at Whiterocks and Hobbie Creek were sampled in multiple years, differences among years accounted for only a small fraction of the variation (Table 6). This is because the same common genotypes tended to persist across years (Table 3), resulting in close proximity on the NMDS plot of population samples from different years for both Whiterocks and Hobbie Creek (Fig. 2).

Molecular markers and adaptively significant variation

The distance matrix calculated from seed germination data and the distance matrix based on allele frequency for survey populations common to the two studies were highly correlated (Mantel correlation test r -value = 0.720, $P < 0.001$; Table 7). The NMDS plots illustrate this high correlation (Fig. 3a,b). There are two main groups in each plot: the Intermountain group, containing all Intermountain Desert (ID), Intermountain Foothill (IF) and

Table 3 Four-locus genotype frequencies by subpopulation and year for four intensively sampled populations

Genotype	Population/subpopulation								Whiterocks		Hobble Creek	
	Whiterocks		Hobble Creek		Strawberry		Potosi Pass		(a)		(a)	
	(a)	(b)	(a)	(b)	(a)	(b)	(a)	(b)	1999	2000	1999	2000
Common												
DCBB	0.486	0.422	0.194	0.098	0.359	0.235	0	0	0.249	0.526	0.346	0.415
GCBB	0	0	0.161	0.366	0	0.059	0	0	0.049	0.051	0.196	0.113
JCBB	0.135	0	0.355	0.220	0	0.029	0	0	0.038	0.013	0.288	0.132
DABB	0	0	0.065	0.098	0	0.206	0	0	0	0	0.039	0.151
FEDD	0	0	0	0	0	0	0.791	0.971	0	0	0	0
GCCB	0	0.022	0	0.073	0.231	0.382	0	0	0.022	0	0.020	0
ICBB	0.081	0	0	0	0	0	0	0	0.178	0.179	0.007	0
IEBB	0.216	0.089	0	0	0	0	0.209	0.029	0.092	0.026	0	0.019
KCCB	0	0	0	0	0.308	0	0	0	0	0	0	0
Rare												
CCBB	0	0.022	0	0.049	0	0	0	0	0.032	0.026	0.007	0.038
DCCB	0.027	0.022	0.097	0	0.077	0	0	0	0.005	0	0.026	0
JABB	0	0	0.032	0.049	0	0.059	0	0	0	0	0.020	0.038
ECBB	0	0.022	0	0	0	0	0	0	0.011	0	0.020	0.019
CEBB	0	0	0	0	0	0	0	0	0.092	0.064	0	0.019
DEBB	0.027	0	0	0	0	0	0	0	0.038	0.038	0	0
GABB	0	0	0.032	0.024	0	0	0	0	0	0	0	0.019
DBBB	0	0.022	0	0	0	0	0	0	0	0	0	0.019
DCAB	0	0.022	0	0	0	0	0	0	0.011	0	0	0
DCAE	0	0.089	0	0	0	0	0	0	0	0.026	0	0
DCBE	0	0.089	0	0	0	0	0	0	0.005	0.026	0	0
ECCA	0	0	0	0.024	0	0	0	0	0	0	0.020	0
IECB	0	0	0	0	0.026	0	0	0	0.011	0	0	0
KCBB	0	0	0	0	0	0	0	0	0.022	0	0.007	0.019
BCBB	0	0	0	0	0	0	0	0	0.016	0	0	0
CCAE	0	0.022	0	0	0	0	0	0	0	0	0	0
CCAF	0	0	0	0	0	0.029	0	0	0	0	0	0
DCBA	0.027	0	0	0	0	0	0	0	0	0	0	0
DCCE	0	0	0	0	0	0	0	0	0.011	0	0	0
ECAE	0	0.067	0	0	0	0	0	0	0	0	0	0
ECBF	0	0	0.065	0	0	0	0	0	0	0	0	0
ECCB	0	0.022	0	0	0	0	0	0	0.005	0	0	0
ECCE	0	0.022	0	0	0	0	0	0	0.005	0	0	0
GCAB	0	0.022	0	0	0	0	0	0	0.005	0	0	0
GCAE	0	0.022	0	0	0	0	0	0	0	0	0	0
GCBE	0	0	0	0	0	0	0	0	0	0.013	0	0
GCCE	0	0	0	0	0	0	0	0	0.005	0.013	0	0
GEBB	0	0	0	0	0	0	0	0	0.011	0	0	0
HCBB	0	0	0	0	0	0	0	0	0.011	0	0	0
ICCB	0	0	0	0	0	0	0	0	0.011	0	0	0
JCCB	0	0	0	0	0	0	0	0	0.016	0	0	0
BEBB	0	0	0	0	0	0	0	0	0.005	0	0	0
CECB	0	0	0	0	0	0	0	0	0.005	0	0	0
CECE	0	0	0	0	0	0	0	0	0.005	0	0	0
EABE	0	0	0	0	0	0	0	0	0	0	0.007	0
EEBB	0	0	0	0	0	0	0	0	0.005	0	0	0
FEBB	0	0	0	0	0	0	0	0	0.005	0	0	0
GECB	0	0	0	0	0	0	0	0	0.005	0	0	0
IEAB	0	0	0	0	0	0	0	0	0.005	0	0	0
JEBB	0	0	0	0	0	0	0	0	0.005	0	0	0

Common genotypes within each population are those with a maximum frequency >0.10. Loci for each genotype are listed in the order: BT05–BT26–BT30–BT33.

Table 4 Genetic information for intensively sampled populations: average number of samples that amplified per locus (n), average number of alleles per locus (A) and gene diversity averaged across loci

Population	Subpop./Year	Code	n	A	Gene diversity
Potosi Pass (Mojave Desert)	(a) 1998	MD1a98	46	1.8	0.306
	(b) 1998	MD1b98	39	1.8	0.114
Whiterocks (Intermountain Desert)	(a) 1998	ID1a98	44	2.6	0.349
	(b) 1998	ID1b98	43	3.0	0.401
	(a) 1999	ID1a99	175	4.7	0.452
	(a) 2000	ID1a00	88	2.8	0.352
Hobble Creek (Intermountain Foothill)	(a) 1998	IF1a98	37	2.8	0.332
	(b) 1998	IF1b98	43	2.5	0.228
	(a) 1999	IF1a99	143	3.1	0.319
	(a) 2000	IF1a00	68	3.2	0.255
Strawberry (Intermountain Montane)	(a) 1998	IM1a98	45	2.2	0.214
	(b) 1998	IM1b98	36	2.3	0.271

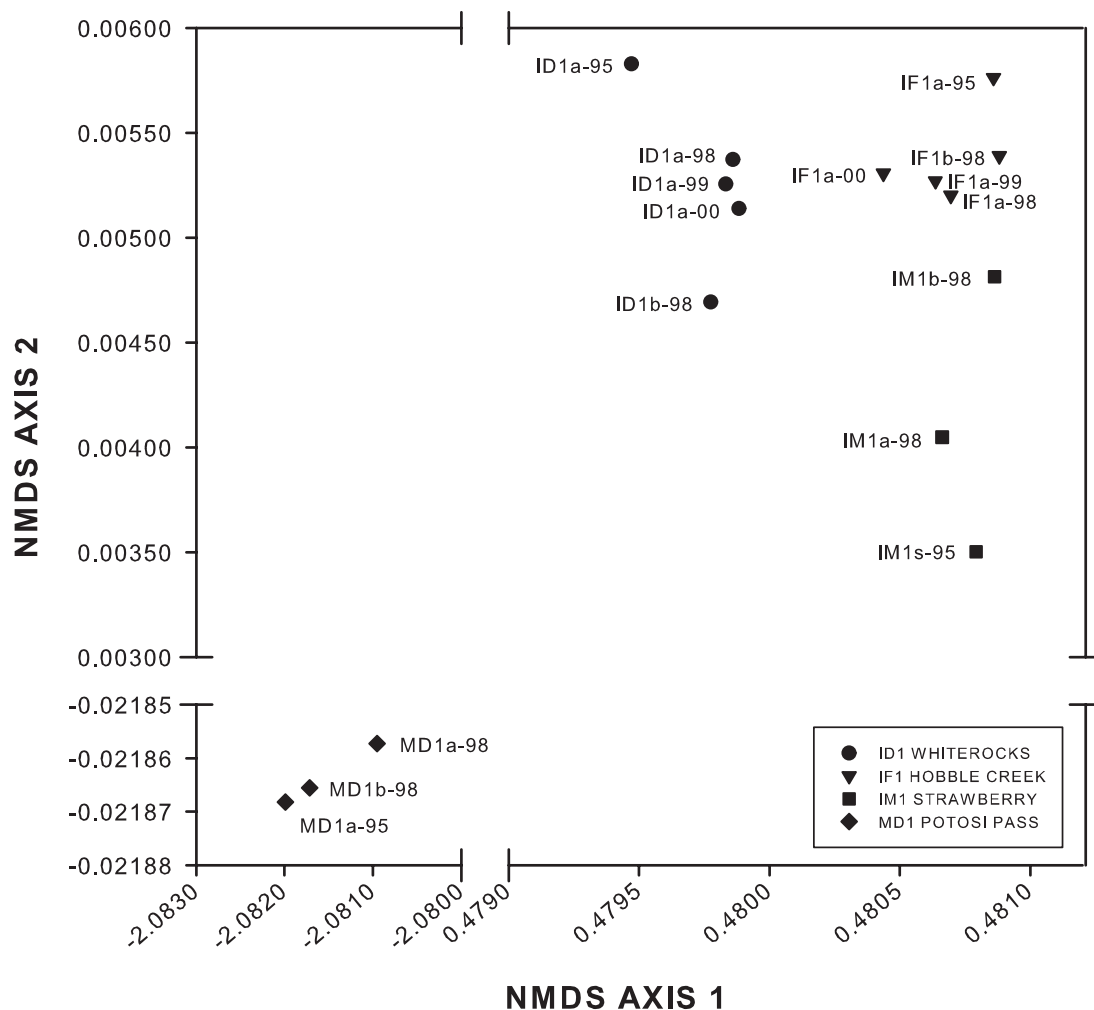
**Fig. 2** Non-metric multidimensional scaling analysis (NMDS) of the distance matrix based on allele frequencies for subpopulations and yearly samples from four principal populations. Collection years are indicated after the subpopulation letters. Note axis breaks and scale changes. Stress <0.001.

Table 5 AMOVA for populations and subpopulations in 1998 samples

Source of variation	d.f.	Sums of squared deviations	Variance components	Percentage of variation
Among populations	3 (2)	236.2 (27.6)	0.784 (0.109)	53.0 (13.3)
Among subpopulations	4 (3)	14.0 (9.6)	0.060 (0.053)	4.0 (6.4)
Within subpopulations	376 (286)	238.7 (188.0)	0.635 (0.657)	43.0 (80.3)

Values in parentheses are for AMOVA excluding the Mojave Desert Potosi Pass population. $P < 0.01$.

Table 6 AMOVAs for 3 years of data from Whiterocks (ID1a) and Hobbie Creek (IF1a) calculated separately

Subpopulation	Source of variation	d.f.	Sums of squared deviations	Variance components	Percentage of variation
Whiterocks (ID1a)	Among years	2	6.1	0.026	4.29
	Within years	332	195.7	0.590	95.7
Hobbie Creek (IF1a)	Among years	2	2.8	0.012	2.6
	Within years	284	123.3	0.434	97.4

$P < 0.003$ for each AMOVA. Four instead of six loci were used for the last two collection years in each population, so all data are analyzed from four loci

Table 7 Mantel correlation tests comparing distance matrices calculated for 13 populations from germination phenotypes, genetic (allele frequency) data, geographical distance and habitat (mean January temperature)

	Germination	Genetic	Geographical
Genetic	0.720*		
Geographical	0.275	0.378	
Habitat	0.550*	0.641*	0.076

* $P < 0.05$.

Intermountain Montane (IM) populations, and the Mojave Desert group containing Potosi Pass (MD1) and St George (MD2). The Mojave Desert group is an extreme outlier on both plots, and this fact undoubtedly contributes to the high r -value.

Even within the Intermountain populations, there is a high degree of congruency between the plot based on seed germination data and the plot based on allele frequency data (Fig. 3a,b). The Intermountain Montane populations are split into two groups on both plots: IM2, IM3 and IM4 at the top and IM1 (Strawberry) closer to the bottom. The desert populations cluster towards the left of the Intermountain group in both plots, and the foothill populations cluster towards the right. When we added desert and foothill populations to the allele frequency data that were not included in the seed germination data, those populations were grouped with the other Intermountain Desert and Intermountain Foothill populations, respectively, on the NDMS plot (Fig. 3b, open circles).

The Mojave Desert populations were quite different from all other populations in seed germination attributes

in that they exhibited unusually high levels of dormancy at high incubation temperatures, but not at low temperatures (Meyer *et al.* 1997). These populations were also very different from all other populations in their allelic composition because they share the predominant genotype FEDD. These major differences explain why they are outliers on both plots.

Within the Intermountain Montane population group, Strawberry (IM1) differs from the other three populations in both seed germination attributes and allele frequency data. The other montane populations had much higher levels of seed dormancy than Strawberry, whose seeds were non-dormant across a wide range of temperatures (Meyer & Allen 1999) and the Strawberry survey population sample contained a high-frequency allele that was not present in other montane populations.

Microsatellite-based genetic distance measures for the survey populations were much more closely correlated with habitat than with geographical distance (Table 7). Geographically, the Idaho foothill and desert populations are nearest to each other (Fig. 1), yet the Idaho desert population is more similar genetically to the Utah desert populations than to the Idaho foothill populations, both in terms of its seed germination attributes and its allelic composition (Fig. 3). The same is true of the foothill populations, which occur over a wide geographical range but are more similar to each other than to adjacent populations in contrasting habitats. Coupled with the close association between molecular marker and seed germination data, this suggests that specific self-pollinating lines with characteristic adaptive syndromes occur in given habitats at widely separated locations.

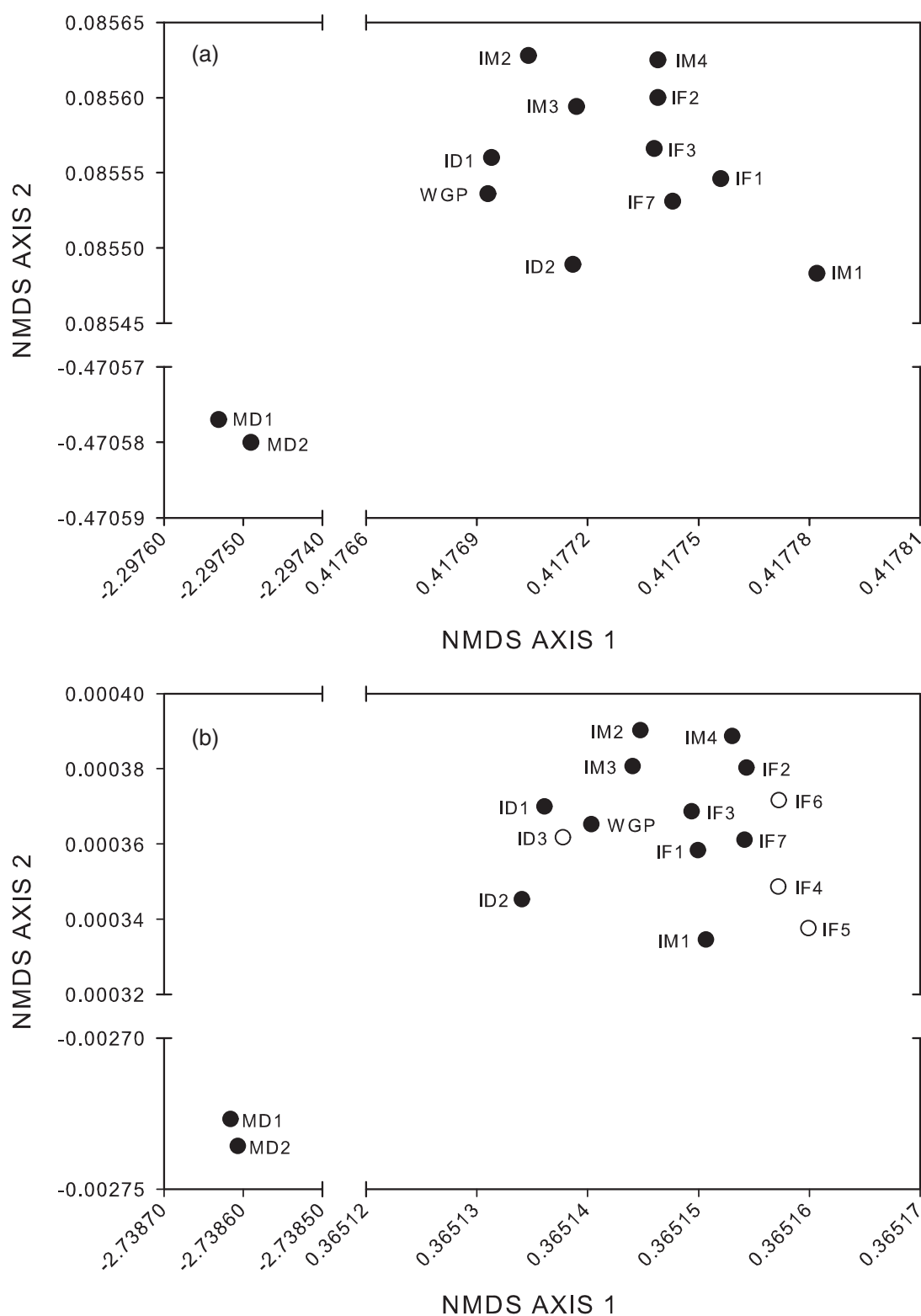


Fig. 3 Non-metric multidimensional scaling analysis (NMDS) of (a) the seed germination attribute distance matrix based on data from Meyer *et al.* (1997) and (b) the microsatellite allele frequency distance matrix based on data from the 17 survey population samples. Population codes are found in Table 1. Note that four new populations were added to the allele frequency data (○). Also note axis breaks and scale changes. Stress <0.001.

Discussion

Many weeds are clonal or highly inbreeding, which assists their ability to maintain viable populations with few founders (Baker 1955). We detected no unequivocal heterozygotes in *B. tectorum*, which supports previous research demonstrating little or no detected outcrossing or heterozygosity (McKone 1985; Novak *et al.* 1991; Bartlett *et al.* 2002). The presence of rare genotypes that appear to represent recombination products of common genotypes in some populations could be the result of rare outcrossing events. The fact that these genotypes are rare and ephemeral could indicate that such putative outcrossing events produce genotypes that are not as well adapted as common parental genotypes. Such a hypothesis could be tested using field fitness trials of parental and experimentally produced outcrossed progeny genotypes.

Our measures of genetic diversity were slightly lower than those reported for microsatellites in other obligately self-pollinating species (Innan *et al.* 1997; Sun 1997; Dje *et al.* 1999; Li *et al.* 2000; Green *et al.* 2001). They were significantly higher, however, than diversity measures for isozyme data in *B. tectorum*, which exhibited an average of only 1.05 alleles per polymorphic locus (Novak *et al.* 1991). Microsatellite loci contain sequences that are highly repetitive and that mutate quickly. These markers are known to be more variable than isozymes in many species (e.g. *Anisantha sterilis*; Green *et al.* 2001).

Because *B. tectorum* is a highly self-pollinating species, we found relatively small numbers of genotypes compared to the numbers that could occur if alleles recombined randomly. Each population was dominated by only a few genotypes, and other genotypes were present at low frequencies if at all. The amount of within-population genetic variation we observed differed markedly among the extensively sampled populations. The very large, contiguous population at Whiterocks had numerous genotypes, while the small, isolated Mojave Desert population at Potosi Pass had only two. The low diversity in the Mojave Desert could be attributed to founder effects. However, because of the strong correlation with phenotypic and environmental variables, it is more likely that the paucity of genotypes at Potosi Pass is related to the stringency of the selection regime there, which would permit survival of very few genotypes. This idea is supported by the fact that the almost equally small and isolated montane population at Strawberry, a more favorable habitat, was much more diverse than the Potosi Pass population. Our dataset overall suggests that the rate of dispersal of an array of genotypes into these and other areas should not present much of a bottleneck to genetic diversity, although this conclusion requires a more rigorous test.

The self-pollinating breeding system of *B. tectorum* leads to high correlation between molecular and phenotypic variation (Table 7), often called 'hitchhiking' (Hedrick & Holden 1979). Our previous work correlating microsatellite variation and adaptively significant variation in *B. tectorum* was carried out at the individual level (Ramakrishnan *et al.* 2004). In the present study, we describe a similar correlation at the population level using allele frequency and seed germination data for 13 populations. Our results in this paper support our previous assertion that it may be possible, in a highly self-pollinating species such as *B. tectorum*, to use molecular genetic variation as a surrogate for adaptively significant variation in population genetic studies.

One four-locus genotype (DCBB) was common in all three Intermountain populations and may represent a broadly adapted (general purpose) genotype. A more typical scenario in our dataset was the presence of high-frequency genotypes that were nearly or completely confined to single populations. Because of the lack of geographical correlation and the high correlation with habitat and adaptive traits, these genotypes are likely to have achieved abundance in specific habitats through selection on their associated adaptive traits. For example, high frequency genotypes in the montane Strawberry population showed phenotypic traits consistent with adaptation to the high-montane environment, whereas the predominant genotype at Potosi Pass showed phenotypic traits consistent with adaptation to the warm desert environment (Meyer & Allen 1999; Meyer *et al.* 2004; Ramakrishnan *et al.* 2004). The FEDD genotype was also the predominant genotype at St George, a Mojave Desert site over 200 km away, supporting the idea that its associated traits represent adaptations for survival in the warm desert. This genotype may represent a separate and more recent introduction from a warm desert environment in the Old World range. New introductions from different parts of the Old World range may have been more or less continuously occurring during the century since initial introduction, adding to the diversity of genotypes present in western USA.

Because populations from similar habitats share adaptive traits and are also similar in terms of microsatellite allele frequencies, it is likely that populations are composed of self-pollinating lines that possess particular suites of adaptive traits. These lines are apparently selected out repeatedly from a broad array of widely dispersed genotypes in response to specific selection regimes in similar habitats across the western North American range of *B. tectorum*.

Clearly the dispersal vehicles necessary for the continued spread of *B. tectorum* are already in place (Mack 1981), and given the results of our research, it appears likely that various genotypes are being introduced into different

environments more or less continuously. Further research specifically quantifying seed dispersal rates among populations, although difficult, would be valuable to our understanding of the dispersal patterns of *B. tectorum*; however, we suspect that dispersal rates are generally high. When an optimally preadapted genotype is dispersed into a new location it is likely to establish a viable population, due in part to the self-pollinating mating system of this remarkably successful weed. This would suggest that *B. tectorum* in western USA has the capacity to continue to expand its range (Schemske 1984; Werth *et al.* 1984).

Acknowledgments

Thanks to Mitchell B. Cruzan for comments, and to Bettina Schultz for drafting Figure 1. This research was supported in part by the US Department of Agriculture, Cooperative State Research, Extension, and Education Service, National Research Initiative Competitive Grants Program: Grant 98-35303-6957 from the Plant Pathology Program and Grant 2000-35319-09920 from the Biology of Plant-Microbe Interactions Program.

References

- Baker H. G. (1955) Self-compatibility and establishment after "long-distance" dispersal. *Evolution* **9**: 347–349.
- Balakrishnan V. & Sanghvi L. D. (1968) Distance between populations on the basis of attribute data. *Biometrics* **24**: 859–865.
- Bartlett E., Novak S. J. & Mack R. N. (2002) Genetic variation in *Bromus tectorum* (Poaceae): differentiation in the eastern United States. *American Journal of Botany* **89**: 602–612.
- Bult C., Kallersjö M. & Youngbae S. (1992) Amplification and sequencing of 16/18S rDNA from gel-purified total plant DNA. *Plant Molecular Biology Report-ISPMB* **10**: 273–284.
- Dje Y., Forcioli D., Ater M., Lefebvre C. & Vekemans X. (1999) Assessing population genetic structure of sorghum landraces from North-western Morocco using allozyme and microsatellite markers. *Theoretical and Applied Genetics* **99**: 157–163.
- Excoffier L., Smouse P. & Quattro J. (1992) Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics* **131**: 479–491.
- Green J. M., Barker J. H. A., Marshall E. J. P. *et al.* (2001) Microsatellite analysis of the inbreeding grass weed Barren Brome (*Anisantha sterilis*) reveals genetic diversity at the within- and between-farm scales. *Molecular Ecology* **10**: 1035–1045.
- Harrod R. J. (2001) The effect of invasive and noxious plants on land management in eastern Oregon and Washington. *Northwest Science* **75**: 85–90.
- Hedrick P. W. & Holden L. (1979) Hitch-hiking: an alternative to coadaptation for the barley and slender wild oat examples. *Heredity* **43**: 79–86.
- Innan H., Terauchi R. & Miyashita N. T. (1997) Microsatellite polymorphism in natural populations of the wild plant *Arabidopsis thaliana*. *Genetics* **146**: 1441–1452.
- Klahr D. (1969) A Monte Carlo investigation of the statistical significance of Kruskal's nonmetric scaling procedure. *Psychometrika* **34**: 319–331.
- Kruskal J. B. (1964) Multidimensional scaling by optimizing goodness of fit to a nonmetric hypothesis. *Psychometrika* **29**: 1–27.
- Li Y. C., Fahima T., Peng J. H. *et al.* (2000) Edaphic microsatellite DNA divergence in wild emmer wheat, *Triticum dicoccoides*, at a microsite: Tabigha, Israel. *Theoretical and Applied Genetics* **101**: 1029–1038.
- Mack R. N. (1981) Invasion of *Bromus tectorum* into western North America: an ecological chronicle. *Agroecosystems* **7**: 145–165.
- Mack R. N. (1989) Temperate grasslands vulnerable to plant invasions: characteristics and consequences. In: Drake J. A., Mooney H. A., di Castri F., *et al.* (eds). *Biological Invasions: A Global Perspective*. John Wiley and Sons, New York, pp. 155–179.
- Mack R. N., Simberloff D., Lonsdale W. M., Evans H., Clout M. & Bazzaz F. A. (2000) Biotic invasions: causes, epidemiology, global consequences, and control. *Ecological Applications* **10**: 689–710.
- McKone M. K. (1985) Reproductive biology of several brome-grasses (*Bromus*): breeding system, pattern of fruit maturation, and seed set. *American Journal of Botany* **72**: 1334–1339.
- Mantel N. A. (1967) The detection of disease clustering and a generalized regression approach. *Cancer Research* **27**: 209–220.
- Meyer S. E. & Monsen S. B. (1991) Habitat-correlated variation in mountain big sagebrush (*Artemisia tridentata* ssp. *vaseyana*: Asteraceae) seed germination patterns. *Ecology* **72**: 739–742.
- Meyer S. E. & Allen P. S. (1999) Ecological genetics of seed germination regulation in *Bromus tectorum* L. I. Phenotypic variance among and within populations. *Oecologia* **120**: 27–34.
- Meyer S. E., McArthur E. D. & Jorgensen G. (1989) Variation in germination response to temperature in *Chrysothamnus nauseosus* (Asteraceae) and its ecological significance. *American Journal of Botany* **76**: 981–991.
- Meyer S. E., Kitchen S. G. & Carlson S. C. (1995) Seed germination timing patterns in Intermountain *Penstemon*. *American Journal of Botany* **82**: 377–389.
- Meyer S. E., Allen P. S. & Beckstead J. (1997) Seed germination regulation in *Bromus tectorum* (Poaceae) and its ecological significance. *Oikos* **78**: 475–485.
- Meyer S. E., Nelson D. L. & Clement S. (2001) Evidence for resistance polymorphism in the *Bromus tectorum*—*Ustilago bullata* pathosystem: implications for biocontrol. *Canadian Journal of Plant Pathology* **23**: 19–27.
- Meyer S. E., Nelson D. L. & Carlson S. L. (2004) Ecological genetics of vernalization response in *Bromus tectorum* L. (Poaceae). *Annals of Botany* **93**: 653–663.
- Nei M. (1987) *Molecular Evolutionary Genetics*. Columbia University Press, New York.
- Neigel J. E. (2002) Is *Fst* obsolete? *Conservation Genetics* **3**: 167–173.
- Novak S. J. & Mack R. N. (2001) Tracing plant introduction and spread: genetic evidence from *Bromus tectorum* (Cheatgrass). *Bioscience* **51**: 114–122.
- Novak S. J., Mack R. N. & Soltis D. E. (1991) Genetic variation in *Bromus tectorum* (Poaceae): population differentiation in its North American range. *American Journal of Botany* **78**: 1150–1161.

- Novak S. J., Mack R. N. & Soltis P. S. (1993) Genetic variation in *Bromus tectorum* (Poaceae)—Introduction dynamics in North America. *Canadian Journal of Botany-Revue Canadienne de Botanique* **71**: 1441–1448.
- Pearse D. E. & Crandall K. A. (2004) Beyond Fst: analysis of population genetic data for conservation. *Conservation Genetics* **5**: 585–602.
- Ramakrishnan A. P., Coleman C. E., Meyer S. E. & Fairbanks D. J. (2002) Microsatellite markers for *Bromus tectorum* (cheatgrass). *Molecular Ecology Notes* **2**: 22–23.
- Ramakrishnan A. P., Meyer S. E., Waters J., Stevens M. R., Coleman C. E. & Fairbanks D. J. (2004) Correlation between molecular markers and adaptively significant genetic variation in *Bromus tectorum* (Poaceae), an inbreeding annual grass. *American Journal of Botany* **91**: 797–803.
- Rosentreter R. (1994) Displacement of rare plants by exotic grasses. In: Monsen S. B. & Kitchen S. G. (Compilers). *Ecology and Management of Annual Rangelands*. Ogden, UT, USDA. Forest Service Intermountain Research Station General Technical Report INT-GTR-313.
- Schemske D. W. (1984) Population structure and local selection in *Impatiens pallida* (Balsaminaceae), a selfing annual. *Evolution* **38**: 817–832.
- Schneider S., Roessli D. & Excoffier L. (2000) *Arlequin*, Ver. 2.0 ed. Genetics and Biometry Laboratory, University of Geneva, Switzerland.
- Spence I. (1972) A Monte Carlo evaluation of three nonmetric multidimensional scaling algorithms. *Psychometrika* **37**: 461–487.
- Sun M. (1997) Population genetic structure of yellow starthistle (*Centaurea solstitialis*), a colonizing weed in the western United States. *Canadian Journal of Botany-Revue Canadienne de Botanique* **75**: 1470–1478.
- Werth C. R., Riopel J. L. & Gillespie N. W. (1984) Genetic uniformity in an introduced population of witchweed (*Striga asiatica*) in the United States. *Weed Science* **32**: 645–648.

This document is a scanned copy of a printed document. No warranty is given about the accuracy of the copy. Users should refer to the original published version of the material.