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

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**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.

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## 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 km$^2$, 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-

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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.

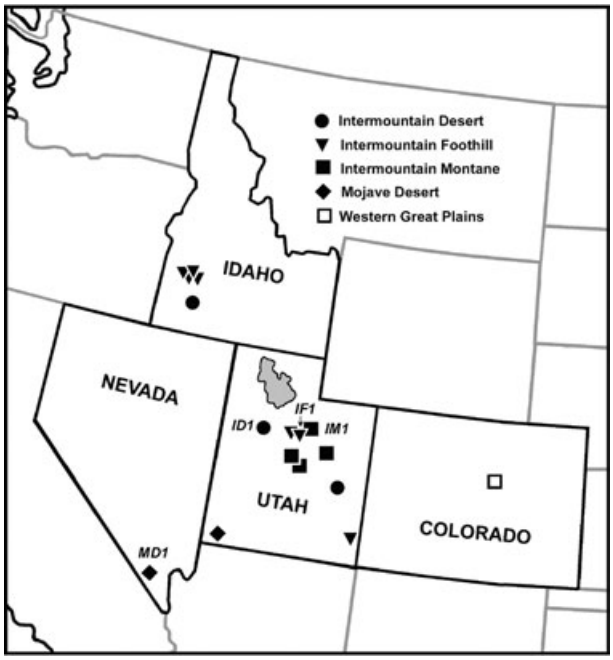


**Fig. 1** Parts of western USA showing the locations of the 17 survey populations. Intensively sampled populations are labeled (ID1, Whiterocks, Utah; IF1, Hobble Creek, Utah; IM1, Strawberry, Utah; MD1, Potosi Pass, Nevada).

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 Hobble 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 Hobble 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, Hobble 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 Hobble 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

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

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 | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 1998 | | | | | | | | 1999 | 2000 | 1999 | 2000 |
| Alleles | bp | Survey | 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), Hobble 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 Hobble 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

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

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 & Monsen 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.

$$d_{ij} = \sqrt{\sum_{k} \left(x_{ki} - x_{kj}\right)^2}$$

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 nonmetric 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

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

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 Hobble 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 Hobble 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 Hobble Creek was GCBB, which was not common in any other Intermountain population (Table 3). No common genotype at Hobble 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 Hobble Creek, placing it close to Hobble 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 Hobble 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 Hobble 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

| | Population/subpopulation | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Whiterocks | | Hobble Creek | | Strawberry | | Potosi Pass | | Whiterocks (a) | | Hobble Creek (a) | |
| Genotype | (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 |

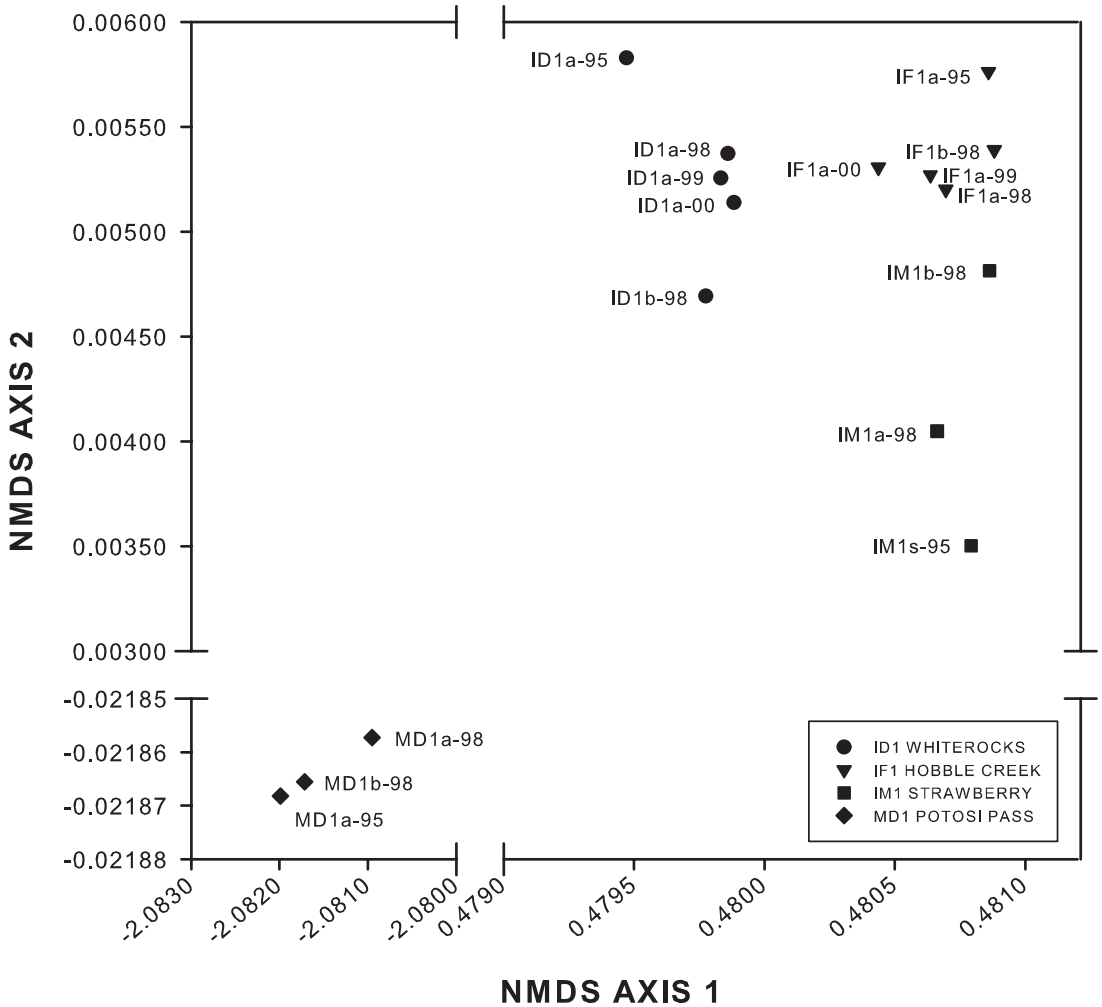


**Fig. 2** Non-metric multidimensional scaling analysis (NMDS) of the distance matrix based on allele frequencies for subpopulations and yearly samples from four principal populations. Collection years are indicated after the subpopulation letters. Note axis breaks and scale changes. Stress <0.001.

**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 Hobble 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 |
| Hobble 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.

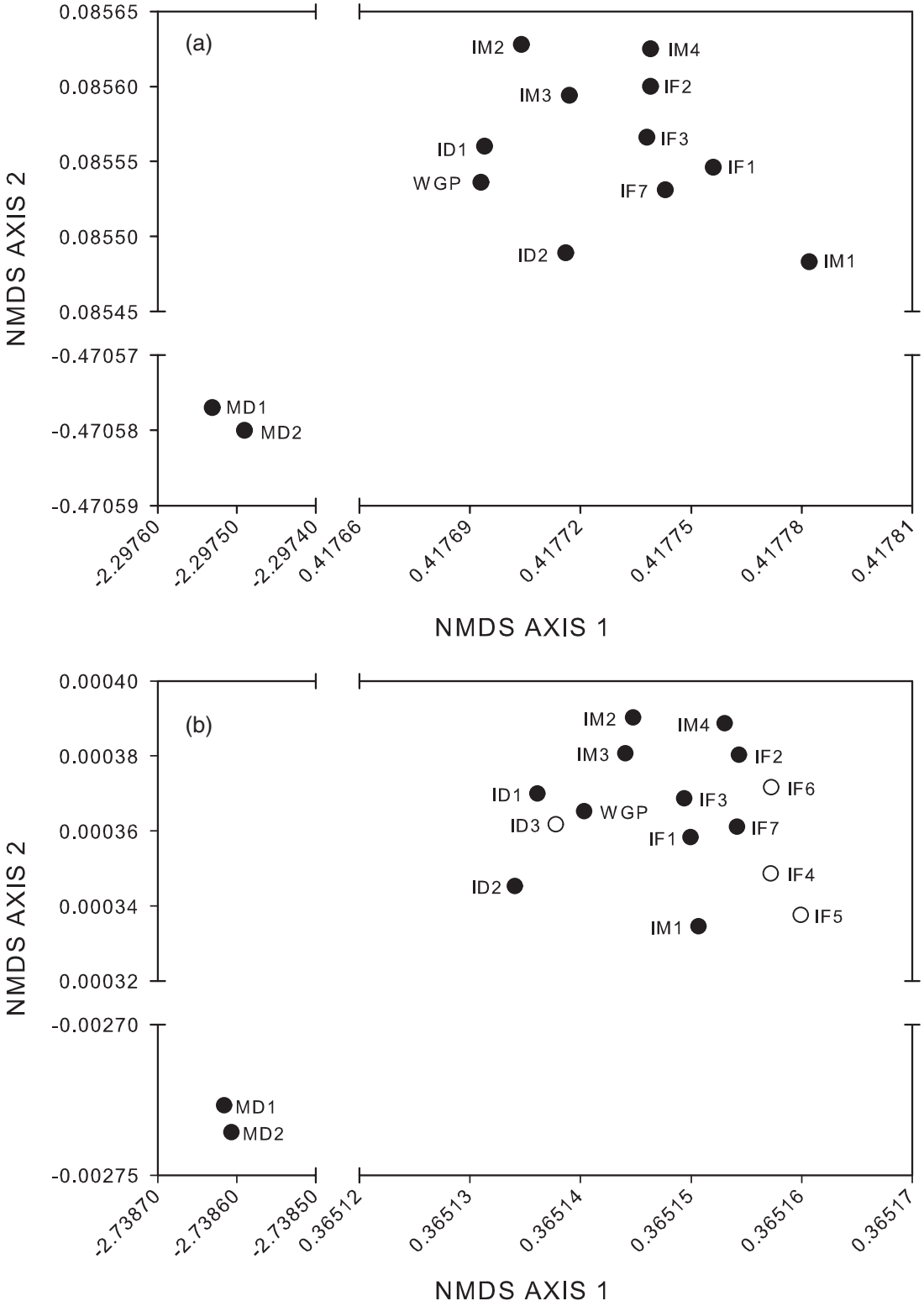


**Fig. 3** Non-metric multidimensional scaling analysis (NMDS) of (a) the seed germination attribute distance matrix based on data from Meyer *et al*. (1997) and (b) the microsatellite allele frequency distance matrix based on data from the 17 survey population samples. Population codes are found in 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).

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