MOLECULAR ECOLOGY

Molecular Ecology (2009) **18**, 4366–4379 doi: 10.1111/j.1365-294X.2009.04357.x

Genetic variation and local adaptation at a cheatgrass (*Bromus tectorum*) invasion edge in western Nevada

ELIZABETH A. LEGER,* ERIN K. ESPELAND,*† KEITH R. MERRILL‡ and SUSAN E. MEYER§

*Department of Natural Resources and Environmental Science, University of Nevada, Reno, 1664 N. Virginia Street, MS 370, Reno, NV 89557, USA, †USDA ARS NPARL, 1500 N. Central Ave., Sidney, MT 59270, USA, ‡Department of Plant and Wildlife Science, Brigham Young University, Provo, UT 84601, USA, §US Forest Service, Rocky Mountain Research Station, Shrub Sciences Laboratory, Provo, UT 84606, USA

Abstract

Cheatgrass (*Bromus tectorum*) is an invasive weed in western North America found primarily growing at elevations less than 2200 m. We asked whether cheatgrass is capable of becoming adapted to a marginal habitat, by investigating a population at a high elevation invasion edge. We used a combination of methods, including reciprocal field transplants, controlled environment studies and molecular analysis. High levels of SSR gene diversity (0.50 vs. 0.43) and comparable variation in phenotypic traits were observed at both the invasion edge and a low elevation, high-density population. Three heterozygotes were observed in the edge population, which is unusual in this predominantly self-pollinating plant. Plants from high elevations germinated more slowly in a growth chamber and had slower seedling growth rates. Survivorship was low at the edge (13%), compared with the low elevation site (55%), but surviving plants were of similar size and had equivalent reproductive output. Seed size positively affected survival and plant performance in the field and this trait was inherited. Emergence timing affected survival at the low elevation site and germination timing was also inherited. Local adaptation was seen in the low, rather than in the high elevation site, because of differential survival. While there was no evidence for local adaptation to the high elevation site observed in the field, family level and genotype-level differences in traits that affected field performance, high genetic diversity at the invasion edge, and evidence of outcrossing in this highly selfing species indicates that the potential for adaptation to a marginal habitat exists within this population.

Keywords: invasive species, local adaptation, natural selection, range limits, secondary invasion, selfing

Received 8 January 2009; revision received 12 May 2009; accepted 25 May 2009

Introduction

As the number of introduced species becoming aggressive invaders increases, the desire for a predictive model to anticipate invasion is growing (Mack *et al.* 2000). Impeding our efforts to predict where introduced species will invade is considerable variation in the population densities and dynamics of spread within invasive species, partially because invasive species are at different points in their colonization trajectories (Colautti & MacIsaac 2004; Suarez & Tsutsui 2008). For example, species in an initial expansion phase may, because of demographic constraints, cover very little of their potential habitat and thus appear less invasive, but they may be on their way to becoming very widespread invaders. It is possible to predict the potential range of introduced and invasive species by using their current distributions and known ecological tolerances to estimate their ecological niches (reviewed in Peterson 2003). This kind of modelling can help determine how close a species is to occupying its entire ecological niche, and alert managers that an invasion is likely, or that an incipient invader is likely to continue to spread

Correspondence: Elizabeth Leger, Fax: (775) 784 4789;
E-mail: eleger@cabnr.unr.edu

(Welk *et al.* 2002; Rouget *et al.* 2004; Thuiller *et al.* 2005; Mau-Crimmins *et al.* 2006).

One assumption of these species distribution models is that the fundamental niche of the invader will not change, and that tolerances in the home range or in the currently invaded range will predict future ones (Holt *et al.* 2005; Pearman *et al.* 2008; Whitney & Gabler 2008). This assumption is not borne out by all invasive species. For example, the climatic conditions of the area occupied by spotted knapweed (*Centaurea maculosa* L.) in North America is different than in its ancestral area of Europe, and predictions of the potential invasible range in North America based on the European niche would have been incorrect (Broennimann *et al.* 2007). Furthermore, some species initially spread quickly into a primary habitat type, followed by a secondary spread into additional habitats, which might be outside the described fundamental niche (reviewed in Dietz & Edwards 2006). For example, cheatgrass (*Bromus tectorum* L.) is a major invader of the Great Basin, with a range historically largely limited to low elevation sagebrush (*Artemisia tridentata* Nutt.) steppe habitats, but it appears to be rapidly expanding its range into more arid salt desert and warm desert shrub communities (Meyer *et al.* 2001; Ramakrishnan *et al.* 2006). If the niche of a species is capable of shifting, attempts to predict spread of species based on the characteristics of their primary invasion area will be inaccurate (Pearman *et al.* 2008).

During a secondary phase of an invasion, potential mechanisms for a shift in the types of habitats occupied may be evolutionary, i.e. the species persists at low densities until it evolves *in situ* to allow colonization of a new environment (e.g. Roy *et al.* 2000) or the introduction of a preadapted genotype allows range expansion (e.g. Neuffer & Hurka 1999). Alternatively, the mechanisms of secondary spread may be demographic, with repeated introductions or logistic growth leading to increases in propagule pressure and range expansion (Pysek & Hulme 2005). Finally, spread to a new habitat may be attributable to a change in type and/or frequency of disturbance, possibly associated with climate change (e.g. Ross *et al.* 2008). These mechanisms are the same as those used to explain primary expansion of invaders following a lag phase (the time between introduction and spread of an invasive species, Baker & Stebbins 1965; Sakai *et al.* 2001; Crooks 2005), with the exception that in a secondary invasion, the invasive species is already present at high densities in adjacent habitats. This might lead to greater ability to overcome demographic or evolutionary barriers compared with primary invasions (Holt *et al.* 2005). In these secondarily invaded environments, propagule pressure may be high, a factor that contributes to successful invasion (Rouget & Richardson 2003; Colautti *et al.* 2006), and hybridization between formerly isolated gene pools could promote adaptive evolutionary change (Ellstrand & Schierenbeck 2000). A constant propagule source from a core population may also promote the eventual colonization of edge habitats by maintaining high genetic diversity and viable population sizes in stressful environments (e.g. Harrison *et al.* 2001). Alternatively, high rates of gene flow could constrain adaptation of invasive species at range edges, swamping edge habitats with genotypes more suited to the core of the species' range (e.g. Nosil 2004).

The most direct way to address the role of adaptive evolution in the expansion of an invasive species' realized niche is to identify an ongoing invasion edge, where population densities of the invader are low, and to ask a series of questions about the edge population (Holt *et al.* 2005). First, is survivorship lower in the edge area than in the main invasion area? If yes, this indicates that factors other than propagule pressure are responsible for the smaller population size, and that the invasion edge corresponds to an ecological range limit (e.g. Pierson & Mack 1990; Rice & Mack 1991c; Lesica & Miles 2001; Chambers *et al.* 2007). Second, to determine if adaptive evolution can occur, one can ask: is there sufficient heritable variation in traits associated with fitness within the edge population (e.g. Cheplick & White 2002)? If so, natural selection may increase the frequency of more adapted genotypes, but if not, additional introductions or favourable mutations would be required before the species could adapt to the new environment (Lee 2002). Finally, is there evidence that natural selection has favoured particular genotypes in this new environment (e.g. Sexton *et al.* 2002)? Evidence for local adaptation could be either a shift in the mean of a particular trait in plants from the invasion edge compared with the core invasion area (e.g. Kao *et al.* 2008), or a genotype by environment interaction showing that invasion edge populations are locally adapted (e.g. Rice and Mack 1991a, b). A shift in the mean of traits is the weakest evidence for local adaptation, as genetic drift can also be responsible for trait shifts, while the direct demonstration of a genotype by environment interaction between a low-density edge population and a high-density population is the strongest evidence that adaptive evolution may be required for a species to successfully invade an edge habitat.

We used a combination of methods, including field reciprocal transplants, controlled environmental studies and molecular analysis to address whether a high elevation site is indeed a marginal habitat, whether populations possess heritable variation for traits that affect fitness, and whether there is evidence for adaptive trait

shifts at a *B. tectorum* invasion edge on Peavine Mountain, Nevada, USA.

Materials and methods

Mountains are particularly good systems in which to investigate invasive species at the edges of their range (Dietz & Edwards 2006). Environmental conditions can shift dramatically over short distances and populations of invasive species found along this gradient are more likely to share an introduction history, and thus come from a more similar genetic background, than invasive species found over other types of gradients, such as latitudinal ones. The invasion of *B. tectorum* in the Great Basin is an ideal scenario to investigate the mechanism of secondary invasion, as one environment that remains relatively uninvaded by *B. tectorum* in the Great Basin is high-elevation sites (Bradley & Mustard 2006). *Bromus tectorum* is a highly selfing, facultative winter annual grass that may germinate any time from fall to early spring (Mack & Pyke 1983; Novak *et al*.1991; Novak & Mack 1993). Seeds mature in late spring to early summer. Potential reproductive output is high, with seed rain densities as high as 50 000 seeds-m^{-2} under favourable conditions (Smith *et al.* 2008). Seeds also have high dispersal potential, with wind and water dispersal typically carrying seeds about 1 m from the parent plant (Hulbert 1955), and exo-zoochory as the primary long distance dispersal mechanism (de Pablos & Peco 2007).

Bromus tectorum was studied in two populations, one high and one low elevation, on Peavine Mountain, Washoe County, NV. Peavine Mountain is a pair of peaks, with a maximum elevation of 2500 m, at the very western edge of the Great Basin. There is a gradient in rainfall from low to high elevations, with approximately 250 mm of rainfall at the lowest elevations to 760 mm at the highest elevations, and all communities spend some time under snow cover during the winter months (from 1 to 5 months, depending on the year and elevation, Klieforth 1992). Peavine is very close to the city of Reno, NV (11 km from downtown Reno to the top of Peavine Peak), and receives heavy recreational use, thus opportunities for weed introduction and seed movement are high.

We selected sites representing the densest *B. tectorum* populations at two contrasting elevations. Seeds were collected from the low elevation site on 8 June 2007 and from the high elevation site on 25 June 2007. Seeds were collected separately from 100 individual plants at high and low elevation sites (referred to as 'seed sources' or 'sources'), and stored at room temperature for approximately 3 months. Parent plants were separated by a minimum of 2 m in the field to minimize collection of seeds from sibling plants. The low elevation site (39°36′53.0″ N, 119°53′46.6″ W) is at approximately 1660 m, well within the known range of *B. tectorum*. The site is characterized by a diverse shrub community dominated by big sagebrush (*Artemisia tridentata* ssp. *wyomingensis* Beetle and A.M. Young) (Williams *et al.* 1992). Understory vegetation is dominated by *B. tectorum* (>40% cover), but there is a reasonable quantity of native perennial grasses, as well as various native and exotic annual forbs. The high elevation site (39°34′43.1″ N, 119°54′34.2″ W), at approximately 2100 m, is 4.2 km away from the low elevation site, and is at the edge of the current elevational range of *B. tectorum* (Bradley & Mustard 2006). The overstory is a stand of mountain mahogany (*Cercocarpus ledifolius* Nutt. var. *intermontanus* C.K. Schneid) and the understory is a combination of mostly native vegetation (Williams *et al.* 1992). *Bromus tectorum* is present in low densities at the high elevation (<5% cover across the landscape), and is primarily found under the canopy of *C. ledifolius*.

Molecular analysis

Genetic variation was compared between high and low populations using four microsatellite loci previously identified in *B. tectorum* (Ramakrishnan *et al.* 2002). Additional variable loci have been identified, but because *B. tectorum* is such a highly selfing organism including them does not typically increase the ability to differentiate between individuals (Ramakrishnan *et al.* 2006). Therefore, the four most informative loci were used in this study. Seedlings of 185 of the 200 source plants, 97 from the low elevation site and 88 from the high elevation site, were grown for molecular analysis. Tissue was collected from seedling shoots produced from individual seeds of each family and DNA was extracted from fresh tissue according to the methods of Fulton *et al.* (1995), modified to allow for extraction in a 96-well plate. We then amplified four microsatellite, or simple sequence repeat (SSR), loci (BT05, BT26, BT30 and BT33) in a single multiplexed PCR reaction using fluorescently labelled primers as described in Ramakrishnan *et al.* (2002). Fragment analysis was carried out on an ABI 3100 Genetic Analyzer (Applied Biosystems). Peak analysis was performed using 'Peak Scanner Software v.1.0' by Applied Biosystems. The visualization in the current study was carried out on a different analyser than the one used in earlier *B. tectorum* SSR studies (a Perkin-Elmer ABI 377 automated DNA analyser), therefore we included DNA from a set of ten *B. tectorum* reference lines from the earlier study (Ramakrishnan *et al.* 2004) along with the 182 unknowns to verify that the two analysers gave identical allele lengths for each of the SSR markers. In two replicate runs with these reference samples, the new

analyser always produced allele lengths 2 bp shorter than the older sequencer for loci BT05, BT30 and BT33, whereas allele lengths at BT26 were consistently 4 bp shorter than on the older machine. To make SSR genotypes in this study comparable with genotypes published earlier (Ramakrishnan *et al.* 2006), we have corrected for these differences in allele length in assigning letter codes to genotypes we report here. This correction does not change the conclusions of this study in any way, but makes it possible to directly compare genotypes across studies.

Field performance

Four seeds from each of the 200 maternal lines (hereafter referred to as 'families') were weighed and each glued to a separate toothpick (to aid in tracking individual seeds in the field) with Elmer's Washable School Glue (Elmer's Products Inc). Seeds were randomly assigned to one of four blocks, with each family represented with one seed in each block, for a total of 200 seeds per block. Two 36 cm × 76 cm blocks were planted in the low elevation site and two in the high elevation site (referred to as 'planting sites' or 'sites') on 8 October 2007, spaced approximately 150 m apart at the low site and 300 m apart at the high site. Seeds were planted 4 cm apart using a planting grid, and were located under the edge canopy of shrubs where *B. tectorum* was already established (low site, under *A. tridentata*, high site, under *C. ledifolius*).

Blocks in the low elevation site were monitored for emergence and survival on 15 November 2007, 3 March 2008, 6 April 2008, 2 May 2008 and 27 May 2008. The high elevation site was more difficult to access, and because of road impassibility, was monitored on a different schedule: 21 March 2008 (first day of access), 25 April 2008, 8 May 2008, 13 May 2008 and 27 May 2008. As the high elevation blocks were not surveyed until March, we did not measure any seeds that emerged in November and died before March: emergence is likely underestimated at the high elevation site. At each visit, we noted whether or not seedlings had emerged or seedlings had died, verifying the identity of seedlings by gently excavating along the toothpick until the glued seed could be observed. After the first observation, seedlings were encircled within the end-loop of a coloured, plastic-coated paperclip to facilitate plant identification on subsequent visits. Precipitation was extremely low during February, March and April, with essentially no measurable precipitation at the nearest weather station (Stead, Western Regional Climate Center, 7 km from low-elevation site) during these 3 months. This was the lowest recorded spring precipitation as data collection began at this weather station in 1985. To prevent potential catastrophic mortality, blocks were watered four times between 26 April 2008 and 16 May 2008, with 5 L of water per block. This may have improved survival, but had little effect on plant size, as *B. tectorum* planted in the blocks appeared identical to plants outside the blocks at the end of the experiment. Above-ground biomass of low-elevation plants was collected on 2 and 3 June 2008, and that of high-elevation plants was collected on 12 June 2008. Plants were dried at 40^0C for 5 days, and plant height, reproductive biomass and vegetative biomass were recorded for each plant. We were able to collect the majority of plants before they lost their seeds, but 14 of 257 surviving plants released some or all of their seeds prior to collection. Seed production for these plants was estimated using the regression equation from the relationship between seed mass and vegetative mass for the remaining plants (seed mass = 0.32 + 1.36(vegetative mass); $R^2 = 0.79$, $P < 0.0001$). Twenty-four toothpicks were missing at the end of the experiment because of unknown causes and the associated seeds were excluded from analysis.

In addition to weighing all seeds before planting, we measured two additional traits *ex situ*: germination timing in a laboratory setting and early seedling growth rates in a greenhouse setting. Two to three seeds per family were selected for germination trials, excluding six families that produced too few seeds. Seeds were placed on moistened Anchor Regular Weight germination paper (Anchor Paper Company) in Petri dishes and cold-stratified at 2 °C for 10 days. An alternating temperature regime was begun on day 11 (2 °C for 8 h, 15 °C for 16 h), and this regime was maintained for 3 weeks, when all but ten seeds had germinated. Visual inspection indicated that these ten seeds were not filled. This temperature regime mimics conditions in late fall, when emergence often takes place in these environments. Germinated seeds were planted in containers (Stuewe and Sons) and seedling growth rate measurements were taken by recording total shoot length 5 and 8 days after leaf emergence. Relative growth rate (RGR) was calculated as: (ln length at day 8) − (ln length at day 5) (Hunt 1990).

Data analysis

Gene diversity (a measure of the probability that two randomly chosen homologous alleles are different) averaged across loci and the distribution of genetic variation within and among populations were analysed with Arlequin 2.0 (Schneider *et al.* 2000). Analyses were carried out both by including family in our design, and by using four-locus SSR genotypes as an indication of a closely shared genetic background (hereafter referred to

as 'genotype'). While SSRs are assumed to be neutral, noncoding regions, in highly selfing species linkage disequilibrium is often extensive (Nordborg *et al.* 2002), and SSRs are likely to be associated with ecological and phenotypic traits (e.g. Ramakrishnan *et al.* 2004). Using SSR genotype, with individual families nested within genotype, as a way of categorizing genetic relatedness allowed us to look at the effects of genotypic variation while minimizing potential nonadditive effects (e.g. maternal effects *sensu* Roach & Wulff 1987). While using genotype to estimate additive genetic variation eliminates some of the error associated with the influence of parental environment on phenotypes, it introduces a different source of error because of the potential for homoplasy in SSR alleles (the independent appearance of similar sized alleles in unrelated individuals, Estoup *et al.* 2002). For these reasons, we used both family and genotype methods to estimate genetic relatedness between individuals. Models were run first on the whole data set, including family when possible, and run a second time on a truncated data set of ten common genotypes only, which included plants with genotypes collected from both high and low elevation sites and represented by at least four individual families. These different models did not change the significance of main effects or interactions, only the type of inference about genetic relatedness could be made. Details of each model are explained below.

Field survival from seed (the proportion of seeds planted that produced plants that survived until the end of the growing season) was analysed with logistic regression. Two models were run, the first on the entire data set, including family as a random factor (the entire model statement is shown in Table 2A). The second model was run on the common genotypes only, and contained the additional random factor 'genotype' (model in Table 2B). Significant planting site × seed source interactions were followed by separate analyses for each planting site. Observed emergence is reported for high and low elevation sites, but not compared statistically, because we lacked complete information on fall emergence at the high elevation site. Chi-squared tests were used to test for differences in the timing of field emergence at the low elevation site only, comparing the percentage of high and low elevation source seeds germinating in November, March, April and May. This model contained the factors block (random) and seed source. The effect of field emergence timing on seedling survival at the low elevation site was analysed with a chi-squared test, with survival as response variable, and emergence month and block as factors.

Field performance was analysed with mixed-model ANOVA. Height and reproductive biomass were log-transformed to meet the assumptions of ANOVA. Three models, rather than two, were run on field performance because survival at the high elevation site was low, and the inclusion of plant family in the overall model or the common genotype model restricted the sample size considerably. Therefore, the first set of models included no relatedness factors (genotype or family) (model in Table 3A). Genotype-level effects were analysed using common genotypes only (model in Table 3B). Initially, this model included a genotype x planting site interaction, but as it was always nonsignificant (all $P > 0.50$), this factor was not included in the final model. Finally, a family level analysis was conducted on data from the low elevation site only, because at the high elevation site, surviving plants were in all cases except one, the single representatives of a family (model in Table 3C). MANOVA was also conducted for each model shown in Table 3 with both field performance variables as response variables. The significance of all model factors in these MANOVAs was the same as those obtained with ANOVA, therefore these additional results are not presented.

Mixed model ANOVA was also used to analyse the three phenotypic traits measured *ex situ* (seed mass, growth chamber germination time and greenhouse RGR). Growth chamber germination rates were transformed using the Box-Cox method. The first model containing the factors seed source and family (nested within seed source, random). A second model was run on the common genotypes only, including the factors seed source, genotype and family (nested within genotype and seed source, random), with either seed mass, germination time, or RGR as response variables. Relationships between the three *ex situ* measurements and field performance were tested by including each of these three continuous variables as a covariate in a model that included planting site, block (random), seed source and a planting site × seed source interaction. When relationships were significant, we graphed the residual values of a model run with only the factor 'block' in the model, to remove variation among blocks. The results of linear regressions between *ex situ* phenotypic traits and the residuals of field performance traits are presented. Levene's test was used on all untransformed values of all response variables to determine if variation in traits was different between low and high elevation populations. All analyses were run in JMP 5.0.1a (SAS Institute).

Results

Molecular variation

The 185 families in this experiment were grouped into 29 SSR genotypes. There was a significant difference

in genetic variation between elevations; however, between-population variation accounted for only 9.8% of the total in analysis of molecular variance (Table 1). The low elevation sample contained 23 SSR genotypes, of which 11 were found only at the low elevation site, whereas the high elevation sample contained 18 genotypes, six of which were found only at the high elevation site (Fig. 1). Nine of 12 common genotypes (frequency > 0.05 in at least one population) were found at both sites, and 84% of the individuals at the high elevation site had SSR genotypes also found at the low elevation site. There were three heterozygous plants in the total sample of 185 individuals, all found at the high elevation site (Fig. 1).

Gene diversity at both sites was high: 0.50 (high elevation site) and 0.42 (low elevation site). Most alleles were shared between the two sites. The alleles BT05_C and BT26_D were present only at the low elevation site, whereas the alleles BT05_Z and BT26_Y were present only in a single heterozygous individual at the high elevation site. For alleles and allele lengths see Ramakrishnan *et al.* (2006). Three length alleles not reported in Ramakrishnan *et al.* (2006) were detected in this study and designated as follows: BT05_Z (length 168), BT26_Y (length 141) and BT26_X (length 147).

Field emergence and survival

Population differences. Greater emergence was observed at the low elevation site than at the high site (low site, 89%, 3.8 SE, high site, 42%, 3.5 SE). At the end of the growing season, very few plants survived at the high elevation site (survival from seed 13.3%, 0.7 SE), and there was much better survival from seed at the low site (55.5%, 1.88 SE), a difference that was statistically significant (Table 2A, B, Fig. 2). Plants from the low elevation seed source were significantly more likely to be present at the low elevation planting site at the end of the growing season compared with plants from the high elevation seed source (significant seed source × planting site interaction for survival from seed, Table 2A, B, Fig. 2), but survival of high and low source populations were not significantly different at high elevations.

There were no differences in per cent emergence of seeds collected from high or low elevation sources at

Table 1 Summary of analysis of molecular variance. Differences among populations are significant, $P < 0.0001$

Source of variation	d.f.	SS	Variance components	Percentage of variation
Among populations	1	19.3	0.0996	9.83
Within populations	368	332.4	0.913	90.17

Table 2 Probability of survival from seed in the field experiment. Analysis (A) considers the entire data set and the second analysis (B) includes only 10 genotypes found at both elevations and represented by four or more separate families (45 of 200 families are not represented)

Factor	d.f.	χ^2	*P*
(A)			
Planting site	1	140.1	**<0.0001**
Block (planting site)	2	1.4	0.5071
Seed source	1	0.006	0.9391
Family (seed source)	198	81.8	1
Planting site × seed source	1	6.5	**0.0107**
(B)			
Planting site	1	110.3	**<0.0001**
Block (planting site)	2	2.0	0.3661
Seed source	1	2.6	0.1058
Genotype	12	12.4	0.4152
Family (genotype, seed source)	129	68.3	1
Planting site × seed source	1	4.8	**0.0287**

Block, family and genotype were treated as random effects. Bolded *P*-values highlight significant ($P < 0.05$) model effects.

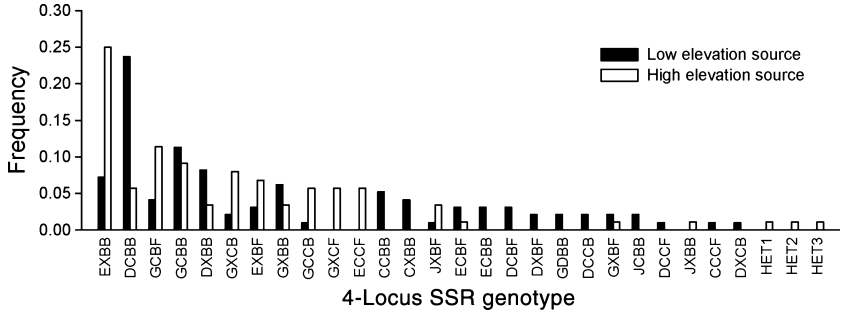

Fig. 1 Frequencies of 29 4-locus SSR genotypes found in low (97 individuals) and high elevation (88 individuals) seed sources from Peavine Mountain, Nevada, ranked from most to least frequent. Allele lengths for each locus follow the letter code naming convention in Ramakrishnan *et al.* (2006). The high elevation sample included three heterozygous individuals with the following genotypes: HET 1: G-C/X-C-B, HET 2: E/G-C/X-C-F and HET 3: E/Z-C/Y-C-F.

the low elevation site ($\chi^2 = 6.83$, d.f. = 1, $P = 0.2334$), nor was there a difference in the timing of field emergence ($\chi^2 = 3.3$, d.f. = 1, $P = 0.3492$). At the low elevation site, most plants had emerged in November regardless of source (high elevation source, 47%, 1 SE; low elevation source 55%, 1 SE). There was additional emergence between November and March (high elevation source, 31%, 0.5 SE; low elevation source 30%, 4 SE), and only a small amount of emergence in April (high elevation source, 6.5%, 3.5 SE; low elevation source, 3.5%, 0.5 SE).

Heritability. There were no family level or genotype-level differences in survival in the field, with either the full or common-genotypes-only data set (Table 2A, B).

Field performance

Population differences. At the end of the growing season, there was no difference in height or reproductive biomass between plants grown at the two different elevations: (Table 3A, B; plant height: low elevation site 10.5 cm, 0.7 SE, high elevation site 10.8 cm, 0.3 SE; reproductive biomass: low elevation site, 24.0 mg, 1.7 SE, high elevation site, 25.0 mg, 3.9 SE). Similarly, there was no effect of seed source on plant performance: plants from high and low elevation collections were of the same height and produced the same amount of reproductive biomass in the field, in both high and low elevation sites (Table 3A, B). There was also no evidence for local adaptation to high or low elevation sites for height or reproduction (no seed source × planting site interactions, Table 3A, B). Variation in plant height was not different between low and high elevation source populations ($P > 0.10$), but variation in inflorescence mass was higher in plants from the low elevation source ($P = 0.0283$, SD low = 25.6, SD high = 16.0).

Heritability. At the low elevation site (the only place where this analysis was possible), there were no differences between families in final height or reproduction (Table 3C). Field height differed by genotype (Table 3B), but this was because of one genotype (JXBF) that performed poorly in the field (Fig. 4A). There were no significant genotype × environment interactions for height or reproductive biomass (all $P < 0.0904$).

Phenotypic traits measured ex situ

Population differences. The weight of seeds collected from high and low elevation sites were not significantly different from each other (high elevation source, 2.52 mg, 0.03 SE, low elevation source 2.48 mg, 0.03 SE; $F_{1,599} = 0.82$, $P = 0.3658$). In the growth chamber study,

Table 3 ANOVA results of two measures of plant performance taken at the end of the growing season. Analysis (A) encompasses the entire data set, and genotype and family effects were analysed in separate models (B and C, respectively). Model (C) was run only at the low elevation

	Height		Inflorescence weight	
Factor	*F*	*P*	*F*	*P*
(A)				
Planting site	0.051	0.8321	0.21	0.7169
Block (planting site)	34.22	**<0.0001**	37.12	**<0.0001**
Seed source	0.031	0.8585	0.11	0.7981
Planting site × seed source	0.61	0.4266	0.11	0.7080
(B)				
Planting site	0.11	0.7225	0.11	0.7369
Block (planting site)	37.02	**<0.0001**	29.92	**<0.0001**
Seed source	0.11	0.7297	0.11	0.7380
Planting site × seed source	0.01	0.9255	1.91	0.1693
Genotype	2.69	**0.0010**	0.79	0.7002
(C)				
Low elevation block	22.72	**<0.0001**	27.92	**<0.0001**
Seed source	0.61	0.4273	0.61	0.4425
Family (seed source)	1.21	0.1912	1.01	0.4647

Block, family and genotype were treated as random factors. Bolded values highlight significant ($P < 0.05$) results.

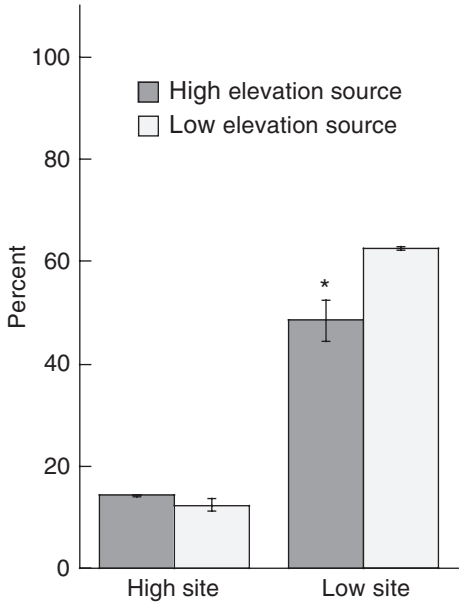

Fig. 2 Significant genotype by environment interaction for survival from seed. Bars indicate mean and standard errors of survival from seed (percentage of seeds that grew into adult plants) for the entire data set (Table 2A). Survival of different sources was not significantly different at the high elevation site ($\chi^2 = 0.15$, d.f. = 1, $P = 0.7031$). *Significant differences in survival at the low elevation sites ($\chi^2 = 8.04$, d.f. = 1, $P = 0.0046$).

there were significant differences in the timing of germination between plants from high and low elevations ($F_{1,201.8}$ = 42.22, P < 0.0001), with plants from the low elevation source germinating, on average, a little over 1 day faster than plants from the high elevation source (low elevation, 1.88 days ± 0.08 SE; high elevation, 3.12 days ± 0.13 SE). The low elevation seeds germinated more rapidly and synchronously, whereas the high elevation seeds germinated more slowly and less synchronously (Fig. 3). RGRs were significantly higher in plants from the low elevation source ($F_{1,225}$ = 12.78, P = 0.0004), with plants from low elevation growing on average 12.7% faster than plants from the high elevation source. Variance in seed mass and growth rate was equal between high and low elevation sources (P > 0.2), but germination timing was significantly more variable in the high elevation source ($F_{1,522}$ = 31.61, P < 0.0001, SD low = 2.1, SD high = 1.3).

Heritability. There was significant family level variation for field-collected seed mass ($F_{1,198}$ = 6.65, P < 0.0001). The average seed mass per family ranged from a low of 2.04 mg, 0.13 SE, to a high of 3.11 mg, 0.14 SE. There was also significant genotype-level variation for field-collected seed mass ($F_{9,108}$ = 2.4, P = 0.0147, Fig. 4B). In the growth chamber study, families differed significantly in their germination timing ($F_{194,328}$ = 3.5, P < 0.0001), ranging from all seeds germinating immediately after initiating temperature cycling (1 day after experiencing warmer temperatures) to an average of 12 days after warming. Common genotypes also differed significantly in their emergence timing ($F_{9,101}$ = 3.3, P = 0.0014, Fig. 4C). Finally, families differed significantly in their early seedling growth rates ($F_{186,276}$ = 1.45, P = 0.0024) in the growth chamber, but common genotypes did not ($F_{9,94}$ = 0.3, P = 0.9557).

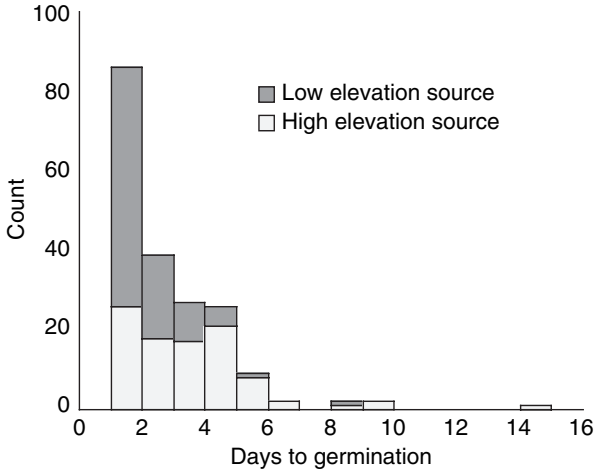

Fig. 3 Histogram of average germination times for 100 low elevation and 94 high elevation families. Seeds were incubated for 10 days at 2 °C, and a post-chilling fluctuating temperature regime of 15°/2 °C was initiated on day 1. Bars represent counts of the number of families with average germination times between 1 and 2 days, 2 and 3 days, etc.

Relationships between heritable traits and fitness measures

Seed weight positively affected every field response variable measured. Larger seeds were significantly more likely to emerge (χ^2 = 16.1, P < 0.0001) and to result in plants present at the end of the experiment (χ^2 = 13.4, P < 0.0001). Larger seeds produced taller plants (F = 9.6, P = 0.0022) that made more reproductive biomass (F = 10.8, P = 0.0012, Fig. 5). There were two pieces of evidence that timing of germination and emergence affected fitness. First, families that germinated faster in the growth chamber experiment produced significantly more reproductive biomass in the field, in both high and low environments (F = 7.9, P = 0.0054). Secondly, at the low elevation site, plants that emerged in November were significantly more likely to survive until reproduction (χ^2 = 15.66, d.f. = 3, P = 0.0013, Fig. 6). There were no significant relationships between average family seedling growth rates and field height or reproductive biomass (all P > 0.4).

Discussion

Observations of invasive species on the edge of their primary habitat can be useful for discerning the role of adaptive evolution in range expansion (Bridle & Vines 2007). Adaptation may be less likely on range edges than in core habitats. Strong selection in combination with small population sizes, low genetic diversity and propensity towards genetic drift increase the likelihood that the population will go extinct rather than adapt, and gene flow from the core population can overwhelm adaptation in edge populations (Holt & Gaines 1992; Kirkpatrick & Barton 1997; Lenormand 2002; Holt *et al.* 2005). Using a suite of methods, we confirmed that this high elevation site was marginal habitat for *B. tectorum.* However, local adaptation was seen within the low elevation core population, rather than in the marginal high elevation site. We found no evidence of current adaptation to the marginal site, but we observed differentiation from the core population and a large amount of heritable variation that could promote, rather than constrain, adaptation at the highest elevation of Peavine Peak, NV (Table 4).

The high elevation site was indeed a marginal habitat for *B. tectorum*, a result consistent with other studies

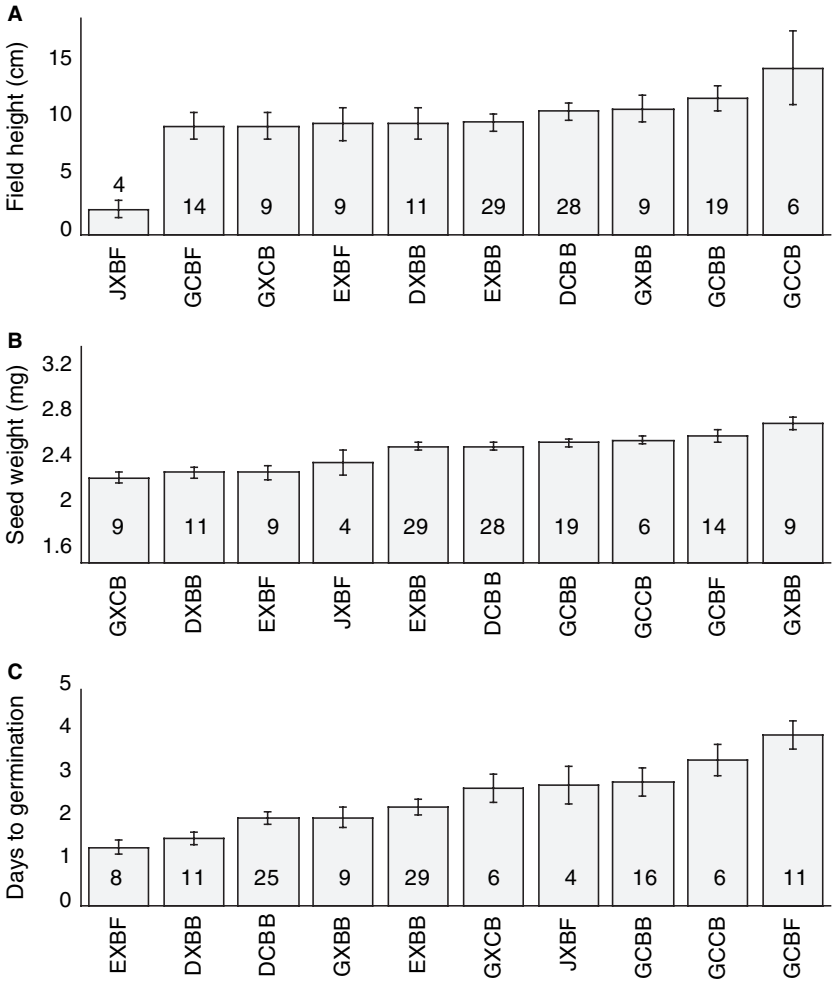

Fig. 4 Mean and standard errors of the average field height, seed weight and time to emergence for 10 common genotypes. Numbers on bars indicate the number of families represented with each genotype. Significance comparisons between bars are excluded for simplicity; however, there are significant differences between genotypes ($P = 0.0010$ for field height, $P = 0.0147$ for seed mass and $P = 0.0014$ for emergence time).

that have found that *B. tectorum* fails to establish well in high-elevation sites (Pierson & Mack 1990; Chambers *et al.* 2007; Adair *et al.* 2008; Kao *et al.* 2008). As high and low elevation environments were only represented by one site each in this study, we are cautious about drawing conclusions about the performance of cheatgrass in high elevation sites in general. For example, we cannot be sure that abiotic, rather than biotic, properties constrained growth at this site, as we did not manipulate these factors separately as part of the study. High elevation sites typically experience less disturbance, and thus retain more intact plant communities, than low elevation sites, and this might contribute to resistance to invasion. However, some evidence suggests that

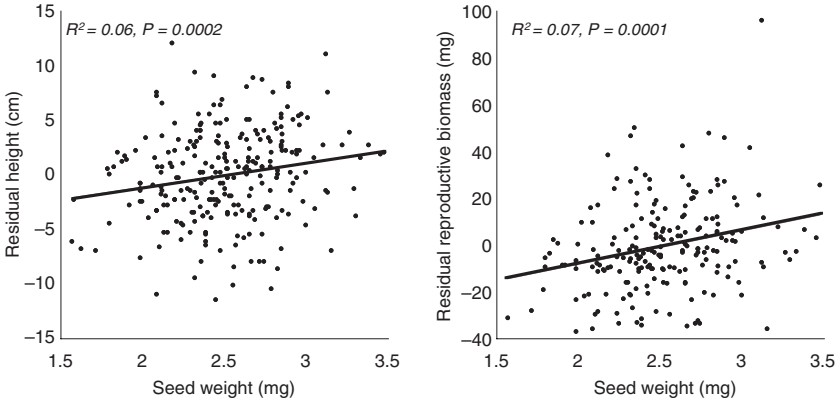

Fig. 5 Relationship between seed weight and two field performance measures: plant height and inflorescence weight. Values of plant height and inflorescence weight shown here are residuals from a model run with 'block' in the model, and thus some of these values are negative.

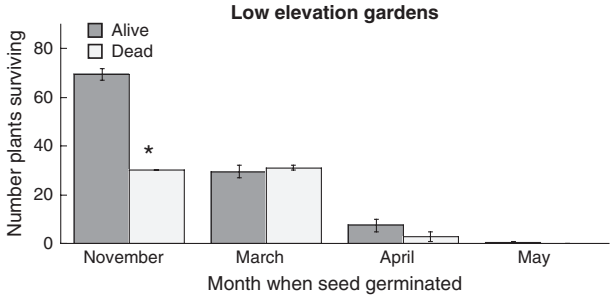

Fig. 6 Probability of survival of plants with observed emergence in different months at the low elevation site; significance ($P < 0.05$) is indicated with '*'.

climatic factors, more than competition with resident plants or lack of disturbance, limit the success of *B. tectorum* at higher elevation sites, as *B. tectorum* failed to establish well at high elevations even under neighbour removal and fire treatments, both of which facilitated establishment at lower elevations (Chambers *et al.* 2007). *Bromus tectorum* establishment may be facilitated by neighbouring plants (Adair *et al.* 2008), and we observed the highest densities of plants at high elevations under the canopies of shrubs (Meyer *et al.* 2001). However, there may be an interaction between disturbance and climatic conditions in high elevation sites, as neighbour removal has been shown to improve microsite conditions in other cold systems (e.g. tree removal can increase ground-level sunlight penetration and increase the growing season for *B. tectorum*, Pierson & Mack 1990; Rice & Mack 1991c).

Survival was the key factor limiting *B. tectorum* performance at this high elevation site on Peavine Mountain: there was a 24% decrease in survival at the high elevation relative to the low elevation site, but once plants survived performance was equivalent at high and low elevation sites. We note that our estimates of survival may be higher than actual field survival of nontarget plants, because we provided supplemental water to plants during a very dry spring. We do not expect that this water addition would have had differential impacts on high or low elevation blocks. Other studies have also seen survivorship as a key stage of population establishment in *B. tectorum*, as emergence has been shown to be far less variable than survival in marginal habitats (Pierson & Mack 1990; Rice & Mack 1991b, c). It follows that any adaptations that could increase survival at the high elevation would be likely to increase *B. tectorum* densities in high elevation environments.

Abundant genetic variation within a marginal population is likely to help, not hinder, adaptation (Holt & Gomulkiewicz 2004; Holt *et al.* 2005). The gene diversity we observed in this study was among the highest observed for *B. tectorum* in the Great Basin (0.50 high elevation, 0.42 low elevation). Gene diversities between 0.11 and 0.45 were found in populations sampled across Utah and southern Nevada (Ramakrishnan *et al.* 2006), and between 0.009 and 0.551 in four populations sampled in northern Nevada (Ashley & Longland 2007), including a population from the lower elevation of the south side of Peavine Mountain, which had a gene diversity of 0.330 using seven microsatellite markers. Although there was very low survivorship at the high elevation of Peavine Mountain, molecular genetic diversity was almost as great as in the low elevation site: while there were slightly fewer genotypes found at the high elevations (18 compared with 23), gene diversity

Table 4 Summary of results from field and laboratory studies

	Differences in				
	Family	Genotype	Seed source	Planting site	Variation between sources
Seed weight (field)	Yes	Yes	No	—	No
Germination rate (laboratory)	Yes	Yes	Yes*	—	Yes†
Growth rate (laboratory)	Yes	No	Yes‡	—	No
Survival (field)	No	No	Yes§	Yes¶	—
Height (field)	No	Yes	No	No	No
Inflorescence wt. (field)	No	No	No	No	Yes**

*Low elevation source had more rapid germination.
†High elevation source had higher variability.
‡Low elevation source had more rapid growth rates.
§Low elevation source survived better at low elevation site.
¶Survival was higher at low elevation site.
**Low elevation source had higher variability.

was greater at the high elevation site, corresponding to results that any observed difference in molecular diversity between core and edge populations is likely to be small (reviewed in Eckert *et al.* 2008). In addition to the similarity of molecular variation, variance in most phenotypic traits was identical and sometimes greater (e.g. variation in germination timing) in the high elevation population than in the low elevation population, indicating that evolutionary potential is nearly equivalent (McKay & Latta 2002) (Table 4). Similar diversity levels between these high and low elevation sites may be simply coincidental. However, the is a situation analogous to the similar genetic diversity found in some introduced and native populations (Lee *et al.* 2004; Dlugosch & Parker 2008), and parallel arguments can be made about the importance of diversity for invasive species colonization of secondary habitats. For example, similarities in diversity between core and marginal populations of invasive species could be causal: high diversity may be helpful in the colonization of new habitats, thus all low diversity satellite populations in marginal habitats have gone extinct, or high diversity could be found in marginal habitats because successful introductions require high propagule pressure.

We found evidence of heritability for multiple adaptive traits in *B. tectorum*. Of all traits measured, seed size and germination timing had the greatest effect on fitness, influencing both survival and performance in the field. These traits were heritable: both families and SSR genotypes differed significantly in seed weight and germination timing in the growth chamber (Table 4). Ramakrishnan *et al.* (2004) also found a significant correlation between SSR genotype and germination syndrome, among other adaptive traits, indicating that these genotypes are reasonable approximations of genetic relatedness in this highly selfing plant. A relationship between seed weight and genotype is noteworthy, as seed size is often determined by both genetic and maternal effects (Roach & Wulff 1987). As these maternal effects are minimized in a genotype-level analysis, seed size is likely to be controlled at least partially by genetic variation.

Differences observed in controlled environment studies were not present in the field study (Table 4). Unlike the *ex situ* measurements, we did not observe any family level differences in performance in the field, and only limited performance differences between genotypes in the field (genotypes differed in field height because of the performance of a single genotype). Family and genotype-level differences in germination timing in the laboratory were not matched with observations of differences in emergence timing in the field. Like families and genotypes, seed sources showed differentiation in controlled environments, particularly in germination timing (higher elevation populations germinated more slowly) and seedling growth rates (higher elevation populations grew more slowly). These differences were not observed in the field (there was no difference in emergence timing between seed sources in the field at the low elevation site). Higher variability in field environments and less resolution in the field (less frequent field measurements) may frustrate the detection of genetic differences in the field (Weinig & Schmitt 2004).

Three heterozygotes were observed in the high elevation population. Outcrossing is an extremely rare event in *B. tectorum*: a survey of 60 Canadian populations spanning the country found only three populations with evidence of outcrossing (Valliant *et al.* 2007), and J. W. Scott *et al.* (unpublished data) found less than 2% outcrossing rate in *B. tectorum* collected from Tooele County, Utah. Outcrossing is also rare within the home range of *B. tectorum*: a survey of 50 populations in Europe using allozymes (Oja 1999) uncovered no heterozygotes. Our result supports Ashley & Longland (2007), who also reported outcrossing on Peavine Mountain. Plasticity in outcrossing rates mediated by environmental conditions has been suggested for this species (Ashley & Longland 2007; Valliant *et al.* 2007) as well as for other *Bromus* species (Green *et al.* 2001). It is unlikely that outcrossing is triggered by environmental variables linked to high elevations: all the Canadian populations demonstrating outcrossing were at elevations less than 300 m, and the heterozygotes found in Tooele County collections were from relatively low elevation (1300–1600 m). While a shift from outcrossing to selfing has been suggested as a way for invasive species to succeed in colonizing new environments (Barrett *et al.* 2008), a shift from pure inbreeding to facultative outcrossing may have benefits to range expansion. Outcrossing accelerates rates of accumulation of genetic diversity, providing more phenotypes upon which selection can act (Hamrick & Godt 1996; Glemin *et al.* 2006), and thus could promote adaptation in the high elevation site.

Our combination of *ex situ* measurements, genotyping and reciprocal transplants showed that plants at the high-elevation site possessed heritable variation for adaptive traits, maintained high molecular genetic variation despite a low probability of survival and were capable of occasional outcrossing. Our ability to identify traits that conferred fitness at high elevation was restricted because of the small number of plants surviving at this elevation. However, early germination and larger seed sizes are traits that increased survival at the low elevation location. If these traits can shift to match a fitness optimum at high elevation, *B. tectorum* may be able to increase population densities and expand its range to include other high elevation sites on Peavine Mountain. Adapation to high elevation at Peavine could

provide a focal point for a secondary invasion of high elevation habitats across the west.

Acknowledgements

Funding was provided by the University of Nevada, Reno. We thank Hiroaki Zamma, Akiko Endo and Taraneh Emam for invaluable assistance in the laboratory and in the field, and Matthew Forister for a helpful manuscript review.

References

Adair EC, Burke IC, Lauenroth WK (2008) Contrasting effects of resource availability and plant mortality on plant community invasion by *Bromus tectorum* L. *Plant and Soil*, **304**, 103–115.

Ashley MC, Longland WS (2007) Microsatellite evidence of facultative outcrossing in cheatgrass (*Bromus tectorum*): implications for the evolution of invasiveness. *Plant Species Biology*, **22**, 197–204.

Baker HG, Stebbins GL (1965) *The Genetics of Colonizing Species*. Academic Press, New York, New York.

Barrett SCH, Colautti RI, Eckert CG (2008) Plant reproductive systems and evolution during biological invasion. *Molecular Ecology*, **17**, 373–383.

Bradley BA, Mustard JF (2006) Characterizing the landscape dynamics of an invasive plant and risk of invasion using remote sensing. *Ecological Applications*, **16**, 1132–1147.

Bridle JR, Vines TH (2007) Limits to evolution at range margins: when and why does adaptation fail? *Trends in Ecology & Evolution*, **22**, 140–147.

Broennimann O, Treier UA, Muller-Scharer H *et al.* (2007) Evidence of climatic niche shift during biological invasion. *Ecology Letters*, **10**, 701–709.

Chambers JC, Roundy BA, Blank RR *et al.* (2007) What makes Great Basin sagebrush ecosystems invasible by *Bromus tectorum*? *Ecological Monographs*, **77**, 117–145.

Cheplick GP, White TP (2002) Saltwater spray as an agent of natural selection: no evidence of local adaptation within a coastal population of *Triplasis purpurea* (Poaceae). *American Journal of Botany*, **89**, 623–631.

Colautti RI, MacIsaac HJ (2004) A neutral terminology to define 'invasive' species. *Diversity and Distributions*, **10**, 135–141.

Colautti RI, Grigorovich IA, MacIsaac HJ (2006) Propagule pressure: a null model for biological invasions. *Biological Invasions*, **8**, 1023–1037.

Crooks JA (2005) Lag times and exotic species: the ecology and management of biological invasions in slow-motion. *Ecoscience*, **12**, 316–329.

Dietz H, Edwards PJ (2006) Recognition that causal processes change during plant invasion helps explain conflicts in evidence. *Ecology*, **87**, 1359–1367.

Dlugosch KM, Parker IM (2008) Founding events in species invasions: genetic variation, adaptive evolution, and the role of multiple introductions. *Molecular Ecology*, **17**, 431–449.

Eckert CG, Samis KE, Lougheed SC (2008) Genetic variation across species' geographical ranges: the central-marginal hypothesis and beyond. *Molecular Ecology*, **17**, 1170–1188.

Ellstrand NC, Schierenbeck KA (2000) Hybridization as a stimulus for the evolution of invasiveness in plants? *Proceedings of the National Academy of Sciences of the United States of America*, **97**, 7043–7050.

Estoup A, Jarne P, Cornuet JM (2002) Homoplasy and mutation model at microsatellite loci and their consequences for population genetics analysis. *Molecular Ecology*, **11**, 1591–1604.

Fulton TM, Chunwongse J, Tanksley SD (1995) Microprep protocol for extraction of DNA from tomato and other herbaceous plants. *Plant Molecular Biology Reporter*, **13**, 207–209.

Glemin S, Bazin E, Charlesworth D (2006) Impact of mating systems on patterns of sequence polymorphism in flowering plants. *Proceedings of the Royal Society B-Biological Sciences*, **273**, 3011–3019.

Green JM, Barker JHA, Marshall EJP *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.

Hamrick JL, Godt MJW (1996) Effects of life history traits on genetic diversity in plant species. *Philosophical Transactions of the Royal Society of London Series B-Biological Sciences*, **351**, 1291–1298.

Harrison S, Rice K, Maron J (2001) Habitat patchiness promotes invasion by alien grasses on serpentine soil. *Biological Conservation*, **100**, 45–53.

Holt RD, Gaines MS (1992) Analysis of adaptation in heterogeneous landscapes – implications for the evolution of fundamental niches. *Evolutionary Ecology*, **6**, 433–447.

Holt RD, Gomulkiewicz R (2004) Conservation implication of niche conservatism and evolution in heterogeneous environments. In:*Evolutionary Conservation Biology* (eds Ferriere R, Dieckmann U, Couvet D), pp. 244–264. Cambridge University Press, Cambridge, UK.

Holt RD, Barfield M, Gomulkiewicz R (2005) Theories of niche conservatism and evolution: could exotic species be potential tests? In:*Species Invasions: Insights into Ecology, Evolution, and Biogeography* (eds Sax D, Stachowicz J, Gaines SD), pp. 259–290. Sinauer Associates, Sunderland, Massachusetts.

Hulbert LC (1955) Ecological studies of *Bromus tectorum* and other annual bromegrasses. *Ecological Monographs*, **25**, 181–213.

Hunt R (1990) *Basic Growth Analysis: Plant Growth Analysis for Beginners*. Uwin Hyman, London, 26 p.

Kao RH, Brown CS, Hufbauer RA (2008) High phenotypic and molecular variation in downy brome (*Bromus tectorum*). *Invasive Plant Science and Management*, **1**, 216–225.

Kirkpatrick M, Barton NH (1997) Evolution of a species' range. *American Naturalist*, **150**, 1–23.

Klieforth H (1992) Climate of Peavine Mountain. *Mentzelia*, **6**, 3–6.

Lee CE (2002) Evolutionary genetics of invasive species. *Trends in Ecology & Evolution*, **17**, 386–391.

Lee PLM, Patel RM, Conlan RS *et al.* (2004) Comparison of genetic diversities in native and alien populations of hoary mustard (*Hirschfeldia incana* [L.] Lagreze-Fossat). *International Journal of Plant Science*, **165**, 833–843.

Lenormand T (2002) Gene flow and the limits to natural selection. *Trends in Ecology & Evolution*, **17**, 183–189.

Lesica P, Miles S (2001) Tamarisk growth at the northern margin of its naturalized range in Montana, USA. *Wetlands*, **2**, 240–246.

Mack RN, Pyke DA (1983) The demography of *Bromus tectorum*: variation in space and time. *Journal of Ecology*, **71**, 69–93.

Mack RN, Simberloff D, Lonsdale WM *et al.* (2000) Biotic invasions: causes, epidemiology, global consequences, and control. *Ecological Applications*, **10**, 689–710.

Mau-Crimmins TM, Schussman HR, Geiger EL (2006) Can the invaded range of a species be predicted sufficiently using only native-range data? Lehmann lovegrass (Eragrostis lehmanniana) in the southwestern United States. *Ecological Modeling*, **193**, 736–746.

McKay JK, Latta RG (2002) Adaptive population divergence: markers, QTL and traits. *Trends in Ecology & Evolution*, **17**, 285–291.

Meyer SE, Garvin SC, Beckstead J (2001) Factors mediating cheatgrass invasion of intact salt desert shrubland. In: *Shrubland Ecosystem Genetics and Biodoversity: Proceedings* (eds McArthur ED, Fairbanks DJ), pp. 224–232. June 13–15, 2000. USDA Forest Service, Rocky Mountain Research Station, Provo, Utah, RMRS-P-21.

Neuffer B, Hurka H (1999) Colonization history and introduction dynamics of *Capsella bursa-pastoris* (Brassicaceae) in North America: isozymes and quantitative traits. *Molecular Ecology*, **8**, 1667–1681.

Nordborg M, Borevitz JO, Bergelson J *et al.* (2002) The extent of linkage disequilibrium in Arabidopsis thaliana. *Nature Genetics*, **30**, 190–193.

Nosil P (2004) Reproductive isolation caused by visual predation on migrants between divergent environments. *Proceedings of the Royal Society of London Series B-Biological Sciences*, **271**, 1521–1528.

Novak SJ, Mack RN (1993) Genetic-variation in *Bromus tectorum* (Poaceae) – comparison between native and introduced Populations. *Heredity*, **71**, 167–176.

Novak SJ, Mack RN, Soltis DE (1991) Genetic-variation in *Bromus tectorum* (Poaceae) – population differentiation in Its North American range. *American Journal of Botany*, **78**, 1150–1161.

Oja T (1999) Allozyme diversity and interspecific differentiation of the two diploid bromegrass species, *Bromus tectorum* L. and *B. sterilis* L. (Poaceae). *Plant Biology*, **1**, 679–686.

de Pablos I, Peco B (2007) Diaspore morphology and the potential for attachment to animal coats in Mediterranean species: an experiment with sheep and cattle coats. *Seed Science Research*, **17**, 109–114.

Pearman PB, Guisan A, Broennimann O *et al.* (2008) Niche dynamics in space and time. *Trends in Ecology & Evolution*, **23**, 149–158.

Peterson AT (2003) Predicting the geography of species' invasions via ecological niche modeling. *Quarterly Review of Biology*, **78**, 419–433.

Pierson EA, Mack RN (1990) The population biology of *Bromus tectorum* in forests – effect of disturbance, grazing, and litter on seedling establishment and reproduction. *Oecologia*, **84**, 526–533.

Pysek P, Hulme PE (2005) Spatio-temporal dynamics of plant invasions: linking pattern to process. *Ecoscience*, **12**, 302–315.

Ramakrishnan AP, Coleman CE, Meyer SE *et al.* (2002) Microsatellite markers for *Bromus tectorum* (cheatgrass). *Molecular Ecology Notes*, **2**, 22–23.

Ramakrishnan AP, Meyer SE, Waters J *et al.* (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.

Ramakrishnan AP, Meyer SE, Fairbanks DJ *et al.* (2006) Ecological significance of microsatellite variation in western North American populations of *Bromus tectorum*. *Plant Species Biology*, **21**, 61–73.

Rice KJ, Mack RN (1991a) Ecological genetics of *Bromus tectorum* .1. A hierarchical analysis of phenotypic variation. *Oecologia*, **88**, 77–83.

Rice KJ, Mack RN (1991b) Ecological genetics of *Bromus tectorum* 2. Intraspecific variation in phenotypic plasticity. *Oecologia*, **88**, 84–90.

Rice KJ, Mack RN (1991c) Ecological genetics of *Bromus tectorum* 3. The demography of reciprocally sown populations. *Oecologia*, **88**, 91–101.

Roach DA, Wulff RD (1987) Maternal effects in plants. *Annual Review of Ecology and Systematics*, **18**, 209–235.

Ross LC, Lambdon PW, Hulme PE (2008) Disentangling the roles of climate, propagule pressure and land use on the current and potential elevational distribution of the invasive weed *Oxalis pes-caprae* L. on Crete. *Perspectives in Plant Ecology Evolution and Systematics*, **10**, 251–258.

Rouget M, Richardson DM (2003) Inferring process from pattern in plant invasions: a semimechanistic model incorporating propagule pressure and environmental factors. *American Naturalist*, **162**, 713–724.

Rouget M, Richardson DM, Nel JL *et al.* (2004) Mapping the potential ranges of major plant invaders in South Africa, Lesotho and Swaziland using climatic suitability. *Diversity and Distributions*, **10**, 475–484.

Roy S, Simon JP, Lapointe FJ (2000) Determination of the origin of the cold-adapted populations of barnyard grass (*Echinochloa crus-galli*) in eastern North America: a total-evidence approach using RAPD DNA and DNA sequences. *Canadian Journal of Botany*, **78**, 1505–1513.

Sakai AK, Allendorf FW, Holt JS *et al.* (2001) The population biology of invasive species. *Annual Review of Ecology and Systematics*, **32**, 305–332.

Schneider SD, Roesli D, Excoffier L (2000) *ARLEQUIN, Version 2.0: A software for population genetic data analysis*. Genetics and Biometry Laboratory, University of Geneva, Switzerland.

Sexton JP, McKay JK, Sala A (2002) Plasticity and genetic diversity may allow saltcedar to invade cold climates in North America. *Ecological Applications*, **12**, 1652–1660.

Smith DC, Meyer SE, Anderson VJ (2008) Factors affecting *Bromus tectorum* seed bank carryover in western Utah. *Rangeland Ecology and Management*, **61**, 431–436.

Suarez AV, Tsutsui ND (2008) The evolutionary consequences of biological invasions. *Molecular Ecology*, **17**, 351–360.

Thuiller W, Richardson DM, Pysek P *et al.* (2005) Niche-based modelling as a tool for predicting the risk of alien plant invasions at a global scale. *Global Change Biology*, **11**, 2234–2250.

Valliant MT, Mack RN, Novak SJ (2007) Introduction history and population genetics of the invasive grass *Bromus tectorum* (Poaceae) in Canada. *American Journal of Botany*, **94**, 1156–1169.

Weinig C, Schmitt J (2004) Environmental effects on the expression of quantitative trait loci and implications for phenotypic evolution. *BioScience*, **54**, 627–635.

Welk E, Schubert K, Hoffmann MH (2002) Present and potential distribution of invasive garlic mustard (*Alliaria petiolata*) in North America. *Diversity and Distributions*, **8**, 219–233.

Whitney KD, Gabler CA (2008) Rapid evolution in introduced species, 'invasive traits' and recipient communities: challenges for predicting invasive potential. *Diversity and Distributions*, **14**, 569–580.

Williams MJ, Howell JT, True GH *et al*. (1992) A catalogue of vascular plants on Peavine Mountain. *Mentzelia*, **6**, 3–74.

E. Leger's research focuses on applied evolutionary questions in the Great basin. E. Espeland studies environmental stress, plant–plant interactions, and their effects on evolutionary processes. K. Merrill conducted the SSR genotyping while an under graduate at Brigham Young University, and is now a Master's student with S. Meyer. S. Meyer's current research is focused on the evolutionary ecology of *Bromus tectorum* and its coevolutionary relationships with fungal pathogens.