

Microsatellite Markers and Polymorphism in Cheatgrass (*Bromus tectorum* L.)

Alisa P. Ramakrishnan
Craig E. Coleman
Susan E. Meyer
Daniel J. Fairbanks

Abstract—Cheatgrass (*Bromus tectorum*) individuals were genetically characterized using polymorphic microsatellite markers. Through analysis of alleles of five polymorphic loci, genotypes were constructed of individuals from four populations in Utah and Nevada. There were 15 different genotypes: Whiterocks, UT, had nine genotypes, Hobbie Creek, UT, had seven genotypes, Strawberry, UT, had four genotypes and Potosi Pass, NV, had one genotype that was unique among all areas studied. These results are similar to what has been found in previous germination phenology and head smut resistance tests. Further analysis of cheatgrass populations using these markers will assist in the analysis of cheatgrass head smut host/pathogen dynamics and in the development of a biological control agent for cheatgrass.

Introduction

Cheatgrass is an introduced annual grass that often spreads into disturbed areas, rapidly establishing itself as the dominant species. It inhibits reestablishment of native plants (Melgoza and others 1990) and, because cheatgrass dies early in the summer, sites with high cover of cheatgrass are prone to frequent fires. In the arid shrubland of the Great Basin, fires stimulated by the increase of cheatgrass heighten mortality of important shrubs such as *Artemisia tridentata* in addition to causing extensive economic damage (Young and Evans 1978).

Through analysis of cheatgrass germination phenology, Meyer and Allen (1999) showed that populations of cheatgrass exhibit adaptive genetic variation corresponding with habitat. This genetic variation is presumably the result of habitat-specific selection pressure acting on introduced genotypes. Because cheatgrass is an obligate inbreeder, populations are made up of an assortment of inbred lines, each with potentially unique genotypes (Novak and others 1991). Each unique genotype may be the result of a separate introduction of cheatgrass into a population (Novak and others 1991). Because genetic variation in traits such as

germination phenology or head smut resistance exists among the inbred lines, different lines in a population are variously susceptible to environmental controlling factors such as frost, drought, or disease. Meyer and others (2001) showed that resistance of cheatgrass to *Ustilago bullata* head smut is often genotype specific. This resistance polymorphism is one example of habitat-specific selection pressure. Characterization of the inbred line composition of a population will lead to a greater understanding of the processes leading to extensive colonization of an area by cheatgrass.

Isozyme studies have detected some among-population genetic polymorphism in cheatgrass, but isozyme techniques are not sensitive enough to detect inbred lines (Novak and others 1991). Microsatellite loci (loci with di- or tri-nucleotide repeats) have recently become popular in forensics and other studies where detailed genetic information is needed (Graham and others 2000). We have developed genetic markers using microsatellites, and they are variable enough to detect within-population variation in cheatgrass. Data generated from analysis of these markers will lead to an increased understanding of the invasion strategies of cheatgrass and may prove invaluable in developing biological control methods.

Materials and Methods

We designed oligonucleotide primer sets specific to microsatellite loci in cheatgrass by screening a cheatgrass λ -phage genomic library with dinucleotide repeat oligonucleotide probes, sequencing positive clones, and designing primers to flank sequences with tandem repeat areas. To construct the library, we extracted genomic DNA using a modified CTAB protocol (Bult and others 1992). We then digested the DNA with the restriction enzyme Sau3A and ligated the fragments into λ -phage using the ZAP Express Predigested Vector Kit (Stratagene: La Jolla, CA). The ligation yielded a genomic library of approximately 100,000 clones. We screened approximately 11,200 clones for short tandem repeats by lifting plaques onto nylon membranes and hybridizing the recombinant DNA with poly-CA and poly-CT oligonucleotide polymers labeled with DIG (Roche: Indianapolis, IN). Positive clones were detected by an antibody/color detection system (NBT/BCIP) and sequenced with a Perkin-Elmer ABI 377 automated DNA sequencer. We detected 23 positive clones through our screening process, and 10 of the sequences generated from these clones contained regions of six to 18 dinucleotide repeats. We

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Alisa P. Ramakrishnan is a Masters Student in the Botany Department at Brigham Young University, Provo, UT 84602. Craig E. Coleman and Daniel J. Fairbanks are Professors in the Botany Department, Brigham Young University, Provo, UT 84602. Susan E. Meyer is a Research Ecologist, USDA Forest Service, Shrub Sciences Laboratory, Provo, UT 84606.

Table 1—Population habitat and location information (Meyer and others 2000).

Population	Elevation	Plant community type	Climate			Location	
			Mean annual precipitation	Mean January temperature	Mean July temperature	County	State
	<i>m</i>		<i>mm</i>	-----°C-----			
Potosi Pass	1,850	Blackbrush-juniper (warm desert margin)	250	1.7	26.5	Clark	Nevada
Whiterocks	1,450	Shadscale (cold desert)	180	−2.3	25.8	Tooele	Utah
Hobble Creek	1,800	Sagebrush-gambel oak (foothills)	400	−2.1	24.8	Utah	Utah
Strawberry	2,400	Subalpine meadow	560	−7.8	16.1	Wasatch	Utah

designed primer sets to yield amplification products between 80bp and 300bp. We labeled one primer from each set with a fluorescent marker for analysis on the automated sequencer. Polymerase chain reaction (PCR) conditions were optimized using an Eppendorf Mastercycle Gradient thermocycler by varying temperature and magnesium concentration.

We ran PCR reactions in 15µl or 10µl total volume with 200µM dNTPs, 1.7 to 3.3 mM MgCl₂, 0.2µM each oligonucleotide of a primer pair, 0.008U/µl Taq DNA Polymerase (Promega), and 1X PCR buffer (2mM Tris-HCl (pH 8.0), 10mM KCL, 10µM EDTA, 100µM DTT, 5 percent glycerol, 0.05 percent Tween 20, and 0.05 percent Ninidet-P40, supplied by Promega). We performed cycling regimes on a Perkin-Elmer 9600 thermocycler with an initial 96 °C denaturing step for 3 min and a final extension step of 5 min at 72 °C. During the initial screening of the loci, we varied optimum annealing temperatures for the primers. After the initial screening, we switched polymerases to HotStarTaq (Qiagen). After switching to HotStarTaq, all polymorphic loci amplified well with an annealing temperature of 60 °C. Polymorphic loci included Bt03, Bt05, Bt26, Bt30, and Bt33.

Results and Discussion

We analyzed plants from Whiterocks (cold desert, UT), Strawberry (montane, UT), Potosi Pass (warm desert, NV), and Hobble Creek (foothills, UT) (table 1). These areas of study were chosen because studies by Meyer and others

(2001) focus on these areas. The first 10 plants from each population used in our study are from the same lines used by Meyer and Allen (1999) and Meyer and others (2001). Further research will analyze the correlation among different studies.

In a preliminary screen of 166 individuals from these populations, five loci were polymorphic, amplifying between two and seven different alleles (tables 2 and 3). We are in the process of developing more loci, and will use them to form a more complete picture of the cheatgrass populations. This paper offers a brief overview of the genotypes amplified by the current loci, but is not intended to be a thorough study of the cheatgrass populations. A complete analysis of the populations will be made at a later date when a representative number of loci and individuals have been sampled.

Of the 50 samples from Whiterocks, we identified nine different genotypes, with one genotype (B) representing half of the samples (table 4). The B genotype was found in only two of the 39 samples analyzed in Hobble Creek. The most abundant genotypes at Hobble Creek were E and I, both present in small numbers at Whiterocks. Five samples from Strawberry shared a genotype present in both Hobble Creek and Whiterocks. One Strawberry sample also shared a genotype present only at Hobble Creek and not Whiterocks. However, the most abundant genotype present in Strawberry was not found in either Hobble Creek or Whiterocks. Unlike the other three collection sites, Potosi Pass shared no genotypes with any other populations. Three of the five loci used to construct the genotypes amplified unique

Table 2—Summary of loci and alleles.

Locus	Core repeat	Size range	No. of alleles	Primer sequences
Bt03	(CA) ₇	112–114	2	Forward: GCATGTCCACCTCCATGGCCACGCC Reverse: CTGTCTTCTCCTCCCTCCCTCACTTGTGTTCC
Bt05	(CT) ₉	165–178	8	Forward: GGGAAAGCAAGCCGTTTCCGCGTTG Reverse: CCGTGGACGACGGGAAAGCGGAGCA
Bt26	(CT) ₁₈	152–156	2	Forward: ATCCGTCCCTCTTTCTTTGCGCTGC Reverse: GGAGGAAGAAGAATGACCGAGAGAG
Bt30	(GT) ₈	112–118	4	Forward: GCCACTTTTTTCCGAACAGACACC Reverse: CAAAAGCAGAGTGAGATGTAAATGAAATT
Bt33	(AG) ₃ (AG) ₇ (AGG) ₅	221–223	2	Forward: CTGCTATATCATGAGGCCATTGGGA Reverse: AGTTTGACAGCAGCCTGAGGCATG

Table 3—Genotypes: each number represents an allele of Bt03, Bt05, Bt26, Bt30, and Bt33, respectively.

Genotype	ID
11111	A
12111	B
12141	C
12211	D
14111	E
14121	F
15111	G
15211	H
16111	I
12121	K
13111	L
23232	M
16121	N
17111	O
17121	P
Total	15

Table 4—Genotypes (A-P, not J) of individuals in Whiterocks (WR), Hobbie Creek (HC), Potosi Pass (PP), and Strawberry (ST).

Population	Genotype															Total
	n	A	B	C	D	E	F	G	H	I	K	L	M	N	O	P
WR	1	25	8	4	1	1	1	5	4							50
HC	1	2				17	2			12	1	1		3		39
PP													52			52
ST			5								1				6	13
Total	2	32	8	4	18	3	1	5	16	2	1	52	3	6	13	166

alleles in Potosi Pass samples. Because both genotype composition and environmental conditions among the populations vary, the most successful genotypes in each population may be environmentally selected.

Potosi Pass is a recently invaded warm desert site with environmental conditions different than the other populations studied. Analysis of 52 samples from Potosi Pass showed that they were all the same genotype (M), which was not found in any other population studied. In Meyer and others (2001), the individuals from Potosi Pass were not susceptible to head smut collected from any of the other populations, but were 100 percent susceptible to head smut from Potosi Pass. Polymorphism among populations was also found by Meyer and Allen (1999) in their germination studies: Potosi Pass samples exhibited different germination patterns than Whiterocks, Hobbie Creek, or Strawberry. Meyer and Allen also found that Potosi Pass individuals had no vernalization requirement, whereas all other populations require vernalization to flower (Meyer, unpublished data). The data from these previous studies and the data presented in this paper all suggest that the Potosi Pass population is composed of one inbred line that was introduced separately from the other lines we identified and is unique to Potosi Pass.

If loci had mutated rapidly since the invasion of each site, we would expect to see a completely random assortment of

alleles in each population due to mutation, with 256 possible genotypes. However, we have only observed 15 genotypes, indicating that observed alleles are presumably the same as ancestral types.

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