A RAPD Assessment of Outcrossing and Invasion in Cheatgrass (*Bromus tectorum*)

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Abstract

Cheatgrass (Bromus tectorum) is a self-pollinating exotic invasive annual weed that typically, invades new habitats following disturbance whether natural or anthropogenic. Cheatgrass invasion of novel habitats in the absence of disturbance is rare. It has been hypothesized that obligate self-pollinators will outcross under ideal conditions, shuffling genotypes within a population and producing propagules that may be preadapted for novel environments. Once established it is expected that self pollination will resume, preserving successful genotypes. We documented a 1995 cheatgrass invasion of the Hot Springs Mountains, NV, in a sand over fossil lake bed soil, not typical cheatgrass habitat. We collected samples in 1995 and subsequent years. We used random amplified polymorphic DNA (RAPD), dominant markers, to evaluate 43 of the 1995 invaders for evidence of outcrossing (i.e.; genetic variation). Using Operon Technologies primer kits A, F, K, and P (n=80) we found 20 primers that produced a total of 115 repeatable bands, 30 polymorphic, yielding 39 phenotypes. Two phenotypes were shared by three individuals each, the remaining by single individuals. Null allele frequency within individuals ranged from 7 to 14 (mean = 10.65 ± 0.27) with average estimated heterozygosity of 0.322 ± 0.024). Pairwise phenotypic similarity for the total population ranged from 0.367 to 1.000 (mean = 0.798 ± 0.004). Genetic diversity was markedly higher than was previously found with isozymes for this species. We identified 7 RAPD phenotypes that could have given rise to all the observed combinations through out crossing. It is possible that a single source population in more typical cheatgrass habitat nearby could have accumulated the critical phenotypes as inbred matrilines through mutation and dispersal. We expect that RAPD profiles of subsequent generations will show a decrease in polymorphism as a result of a return to selfing. These results support the hypothesis that facultative out crossing by a self pollinating species is a potential means of facilitating invasion through production of novel gene combinations.

Introduction

Obligate self fertilizing species can benefit from occasional outcrossing by virtue of novel combinations of genetic traits within and between populations (Stebbins 1957). This is especially important in the evolution of new biotypes as such novel combinations may produce dispersers that are preadapted to novel environments (Stebbins 1957). Reversion to self fertilization could then act to preserve favorable combinations in the new environment (Stebbins 1957). Hybridization therefore, between species or populations, can facilitate invasiveness (Ellstrand et al. 2000). Even within a population of a self-pollinating plant such as cheatgrass (*Bromus tectorum*), outcrossing is effectively hybridization among inbred matrilines, each with their accumulated genetic variation (Ellstrand and Schierenbeck 2000), whether such variation arose *in situ* through mutation or by multiple introduction as traced by Novak and Mack (2001). Within plants these mutations may be neutral but may offer advantages when combined with traits held in other individuals.

Cheatgrass is a highly invasive annual grass that has spread across much of North America since its introductions in the late 19th century (Mack 1981), mainly from Mediterranean European stocks (Mack 2001). Arriving as a contaminant in wheat and straw for packing and livestock bedding, it often appeared in disturbed areas such as roadside and railway rights-of-way (Mack 1981). Even low densities of cheatgrass can dominate an area after an extreme disturbance such as overgrazing. Cheatgrass has become widely distributed across the intermountain west where its has altered many plant communities by reducing or eliminating native grasses, has become the dominant grass species in many communities, often forming dense monocultures, and has even shortened the natural fire interval in some areas (Knapp 1996).

Cheatgrass is generally a self-pollinating species and so can be expected to have little or no detectable genetic diversity (Novak et al. 1991). Indeed, an isozyme study of *B. tectorum* showed no variation within individuals and low levels of variation within populations (Novak et al. 1991). More recently, an expanded study of North American cheatgrass and old world source populations revealed higher levels of genetic diversity in naturalized over native populations and

Figure 1. Topographic representation of the Hot Springs Mountains study site and North Africa, Europe, a possible source population location.

Material and Methods

The primary study site is in the Hot Springs Mountains, NV, approximately W119°47'N39°43' near Fernley, NV (Figure 1). We collected samples in the North (n = 30) and Middle (n = 13) blocks of the site, >1 km apart. Whole plants were placed in individually marked plastic bags and stored at -80C until extraction. We extracted DNA from green tissue (excluding seed head and roots) ground in liquid nitrogen using a CTAB / 2-mercaptoethanol / chloroform:isoamyl alcohol(24:1) protocol based on Doyle and Dickson (1987).

Laboratory Techniques

We first screened six cheatgrass samples with Qiagen-Operon decamer primer kits A, F, K, and P (n = 80) for strong band production. We then optimized PCR reactions (Figure 2) for those primers and screened the remainder of the samples for polymorphisms. We amplified DNA products with a Perkin Elmer GeneAmp 9600 PCR thermocycler, using Applied Biosystems Amplitaq Gold DNA polymerase and reagents. Reaction volume was 25µl consisting of 2.5 µl reaction buffer, 2-3 µl magnesium chloride, 2 µl dNTP mix (2.5 mM each dATP, dCTP, dGTP, and dTTp), 1-2 µM primer, 0.5 U DNA polymerase, 50 ng DNA template, and deionized water to volume in a 0.2 ml reaction tube. The PCR profile was, 45 cycles of 95 C (1 min.), 34 or 36 C (30 sec.), 7 minute ramp to 72 C (2 min.), preceded by a polymerase activation cycle of 95 C (10 min.) and followed by a final extension cycle at 72 C for 30 minutes.

We separated the PCR products on 1.4% agarose ethidium bromide-stained minigels at 75 volts for 20-30 minutes and at 100 V for 1-1.5 hrs relative to target band lengths. The separated products were visualized with a GDS-8000 Chemi System imager and length polymorphisms were scored using UVP LabWorks 3.0 software.

Data Analysis

We estimated null allele frequencies following Jorde et al. (1999) and mean heterozygosity following Lynch and Milligan (1994). We analyzed data for loci showing at least two null and two codominant marker bands, a data pruning strategy similar to that suggested by Lynch and Milligan (1994). We estimated diversity for the total population and the two blocks individually. We tallied phenotypes and estimated mean phenotypic similarity on the presence/absence of markers (1/0) for the population as a whole as well as within and between the two blocks using Nei and Li's (1979) similarity index (S):

Figure 2. Optimization products for Operon 10-mer primerP12. Optimizations were carried out for combinations of primer and magnesium chloride.

Results

Of the 80 primers screened, 33 (41.3%) produced strong repeatable bands. Twenty of those primers produced 115 strong repeatable bands, 51 (43.5%) polymorphic. Thirty (26.1%) of the polymorphic bands had dominant allele frequencies ranging from 5-95% (Table 1).

We found 39 phenotypes for the 43 individuals we characterized with only two phenotypes shared by 3 individuals each (Table 2). We identified seven phenotypes that could have given rise to all the observed combinations through outcrossing (Table 2).

Mean phenotypic similarity for the total population was 0.798 ± 0.004, ranging from 0.37 - 1.00 (Table 3). Pairwise phenotypic similarities were mostly normally distributed a long left kurtosis (Figure 3). Phenotypic similarity was quite close to that of the total sample for the two blocks with lowest mean similarity in the middle block (Table 4). Between block mean similarity differed only slightly from the total, 0.783 ± 0.006 (n = 390).

Null alleles per locus were proportionally the same for the blocks as for the total sample (Table 4). Likewise, null alleles per individual were virtually identical across and within blocks (Table 4). Mean estimated heterozygosity for the total population was moderate, 0.322 ± 0.017, with little difference between the two sampling blocks (Table 4).

Table 1. Operon Technologies primers (n = 20) used in the analysis and polymorphic band lengths.

Primer #	Sequence	Number of Bands	Number of Polymorphic Bands	Band Lengths (bp)
A 2	5'-TGCGCGCCTG-3'	4	1	880
A 4	5-AATCGGGCTG-3'	5	1	900
F 3	5-CCTGATCACC-3'	2	1	440
F 4	5'-GGTGATCAGG-3'	6	3	1040, 550, 490
F12	5-ACGGTACCAG-3'	6	1	700
F13	5-GGCTGCACAA-3'	8	2	860, 820
F16	5'-GGAGTACTGG-3'	9	2	725, 625
K 4	5'-CCGCCCAAAG-3'	10	2	1000, 950
K 7	5'-CCGCCCAAAG-3'	7	1	465
K 8	5'-GAACACTGGG-3'	5	2	1200, 1085
K14	5'-CCGTCTACAC-3'	7	2	860, 820
P 1	5'-GTAGCACTCC-3'	5	1	1400
P 2	5'-TCGCCACGCA-3'	3	1	1260
P 3	5-CTGATACGCC-3'	4	1	585
P 5	5-CCCCGGTAAG-3'	6	1	625
P 9	5-GTGGTCCGCA-3'	6	1	700
P10	5-TCCCGCCTAC-3'	5	2	1450, 650
P12	5-AAGGCGAGCT-3'	6	2	1100, 1000
P13	5-GGAGTGCCTC-3'	5	1	1000
P17	5-TGACCCGCCT-3'	6	2	2500, 650
		n = 115	30(26.1%)	

Table 4. Null alleles per locus, per individual, and estimated heterozygosity for the total population and the two collection blocks individually. Results are means ± S.E. and sample sizes (n).

Sample	Phenotypic Similarity	Nulls/Locus	Nulls/Individual	Estimated Heterozygosity
Total	0.798 ± 0.004 (903)	15.3 ± 2.9 (43)	10.7 ± 0.3 (43)	0.311 ± 0.017 (43)
North	0.816 ± 0.005 (435)	10.8 ± 2.1 (30)	10.8 ± 2.1 (30)	0.300 ± 0.021 (30)
Middle	0.763 ± 0.011 (78)	4.5 ± 0.8 (13)	10.4 ± 0.6 (13)	0.350 ± 0.025 (13)

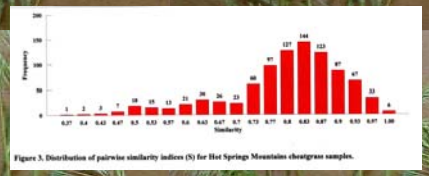

Figure 3. Distribution of pairwise similarity indices (S) for Hot Springs Mountains cheatgrass samples.

Table 2. RAPD phenotypes for the 43 individuals sampled from the Hot Springs Mountains 1995.

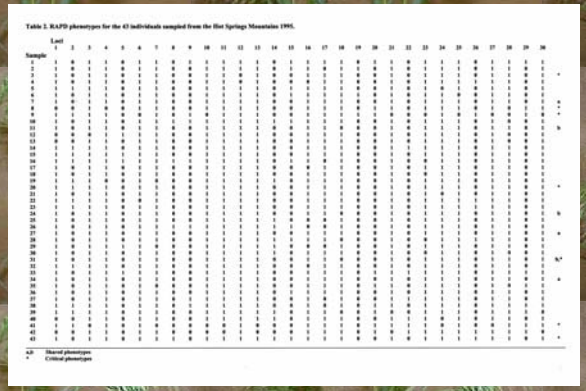

Table 3. Matrix of pairwise similarity indices for the 43 individuals sampled in 1995 from the Hot Springs Mountains.

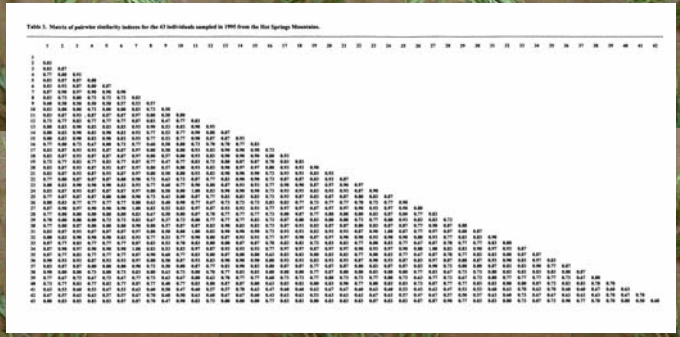

Table 5. Comparison of within-population levels of similarity for cheatgrass. Mean similarities were for the study population and populations sampled in four North American regions by Novak et al. (1991).

Study Area	N	Similarity	Range
Hot Springs Mountains[a]	1	0.798	0.367 - 1.000
East of Rocky Mountains[b]	14	0.975	0.890 - 1.000
Nevada-California[b]	14	0.983	0.951 - 1.000
Intermountain West[b]	22	0.989	0.960 - 1.000
British Columbia[b]	10	0.979	0.924 - 1.000

[a] RAPD analysis
[b] Isozyme analysis

Discussion

We found a large amount of phenotypic diversity in the newly invasive cheatgrass population at the Hot Springs Mountains study site (Table 2). This level of diversity gave rise to levels of similarity lower than those found for cheatgrass populations in North America by Novak et al. (1991) using isozymes (Table 5). The estimated heterozygosity we found for *B. tectorum* was moderate (0.322). Novak et al. (1991) reported no observed heterozygosity for their markers, however some evidence for extremely low levels of isozyme heterozygosity were alluded to by Novak and Mack (2001) in naturalized cheatgrass populations. The estimated heterozygosity we found is, however, comparable to two endemic Hawaiian tree species, *Colubrina oppositifolia* (0.389) and *Alphitonia ponderosa* (0.331) (Kwon and Morden 2002). We plan to extend this study to include both local populations, attempting to determine the source(s) of the invasion, and more widespread populations to develop a broader perspective of cheatgrass genetic diversity relative to this marker system. Additionally, a broad scale study may reveal population-specific markers that may be useful in tracing new habitat invasions and in identifying populations resistant to chemical or biological control measures.

Successful invasion is a rare event (Williamson 1993) and is often accompanied by a lag between establishment and invasiveness (Ewel et al. 1999). In some cases, this lag is punctuated by spontaneous hybridization (Ellstrand and Schierenbeck 2000). Obviously, hybridization alone cannot explain every case of invasiveness evolution. Even if hybridization occurs frequently and produces copious recombinations of genetic traits there is no guarantee that any will confer an advantage in novel habitats. Much depends upon the amount of genetic diversity present in the hybridizing populations, the fitness of the hybrids, the chance that they will be dispersed to favorable habitats, and their ability to establish amidst resident species even if they are capable of surviving in their new environs. Still, Ellstrand and Schierenbeck (2000) surveyed the literature and found 28 examples of post-hybridization evolution of invasiveness in plants and proposed a model for the evolution of invasiveness for inter- and intraspecific hybridization. Further examination of cheatgrass population genetics may provide additional insights into the spread of this species as well as the evolution of invasiveness in general.

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