INTRODUCTION AND SPREAD OF *BROMUS TECTORUM* (CHEATGRASS) INTO MIDWESTERN UNITED STATES: POPULATION GENETIC AND EVOLUTIONARY CONSEQUENCES

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ABSTRACT

High propagule pressure is correlated with invasion success, and has important implications for the genetic diversity and evolutionary potential of a species in its introduced range. Here, I examine an invasive annual grass *Bromus tectorum* and document the population genetic consequences that resulted from multiple introductions of genotypes native to different Eurasian regions into the North American Midwest. Herbarium collections showed that *B. tectorum* was first recorded near-contemporaneously throughout the Midwest in the late 1800s. Allozyme diversity data from 60 populations were used to assess the origin and frequency of introductions into the Midwest. Genetic variation and structure was compared to similar measures for the Eurasian native range and other North American regions to infer the introduction pathways of *B. tectorum* across the continent.

A minimum of four to five introduction events contributed to genetic diversity in the Midwest. A genetic bottleneck associated with introduction was detected across populations, yet more Midwestern populations were polymorphic (53%), and populations were more diverse ($H_T = 0.187$) and less structured ($G_{ST} = 0.582$) than native range populations, indicating high propagule pressure was associated with the invasion of *B. tectorum* into the Midwest. Within the Midwest, genetic diversity and structure values were compared for individual polymorphic loci to infer propagule pressure for individual

genotypes, and scenarios for their likely invasion pathways were drawn based on historical collections and the history of the region. The intermixing of previously allopatric genotypes within populations in the Midwest may have allowed the formation, via outcrossing, of a novel multilocus genotype found only in this region of North America.

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INTRODUCTION

Human activities such as migration, transport, and international commerce have, either accidentally or deliberately, contributed to an increase in the dispersal of organisms around the globe (Mack et al. 2000; Sakai et al. 2001). In a minority of cases (Williamson and Fitter 1996), these introduced species become established in new ranges in which they persist, proliferate and spread (Mack et al. 2000; Colautti and MacIssac 2004). Many studies have attempted to predict which introduced species will become invasive (Mack 1996; Rejmanek 2000; Richardson and Pysek 2006) and which communities are susceptible to invasion (Shea and Chesson 2002; Rejmanek et al. 2005; Didham et al. 2007). While some of these efforts have focused on identifying the characteristics or attributes of species and communities that are associated with invasiveness, another approach has focused on predicting the likelihood of invasion through an examination of the invasion process itself (Colautti and MacIssac 2004). Thus, propagule pressure has emerged as an important predictor of establishment and invasion success (Kolar and Lodge 2001; Lockwood et al. 2005; Colautti et al. 2006).

Propagule pressure is defined as the number of individuals transported to and introduced into a new range (Lockwood et al. 2005). With high propagule pressure, introduced species are buffered from the influence of stochastic events during all phases of the invasion process, and large founder populations and/or multiple introduction events would characterize the release of an introduced species in its new range. Propagule

pressure therefore not only holds demographic and ecological consequences for alien introductions, it also can have genetic consequences: with high propagule pressure the overall genetic and phenotypic diversity of a species in its new range would likely be increased, and the potential for severe genetic bottlenecks would likely decrease (Novak and Mack 2005; but also see Dlugosch and Parker 2008). Although propagule pressure is most often thought of in terms of long-distance (intercontinental) dispersal events, it can also play a role in range expansion during biological invasions (Lockwood et al. 2005; Colautti et al. 2006). Thus, propagule pressure can influence the course of an invasion in two ways: through the direct introduction of individuals from the native range and through the spread of individuals from previously established introduced populations during range expansion. Moreover, these events have genetic signatures, and the relative contribution of each of these mechanisms to invasion success can be evaluated through comprehensive population genetic analyses (Squirrell et al. 2001; Muirhead et al. 2008). Just such an approach has been taken in reconstructing the introduction and spread of *Bromus tectorum* L. in North America.

Bromus tectorum (cheatgrass or downy brome) is a diploid ($2n = 14$), predominantly cleistogamous, annual grass (Upadhyaya et al. 1986). Its native range encompasses most of Europe, the northern rim of Africa, and eastward to central Asia (Pierson & Mack 1990). It has been introduced into temperate regions around the world, including Japan, Australia, New Zealand, South America and North America (Mack 1981; Upadhyaya et al. 1986). The grass was probably introduced accidentally along the eastern seaboard of North America in contaminated seed lots, during pre-1800 European

settlement (Mack 1981). Subsequent range expansion of *B. tectorum* in North America has been facilitated by human disturbances, such as overgrazing and trampling by livestock, agriculture, and road and railroad development (Longman and Smith 1936; Yensen 1981).

Bromus tectorum was first reported in eastern North America ca. 1790 in Lancaster Co., Pennsylvania (Muhlenberg 1793), but the earliest herbarium specimen in this region was not collected until 1859 (Bartlett et al. 2002). In western North America, the plant was first discovered at Spences Bridge, British Columbia in 1889 (Valliant et al. 2007). By the early 1900s *B. tectorum* had been collected at several locales throughout the Intermountain West (Mack 1981), and in the following decades it spread rapidly and is now the region's most abundant vascular plant, occupying at least 40 million hectares (Mack 1981; Upadhyaya et al. 1986; Rosentreter 1994). In the western United States of America (USA) the ecological range of *B. tectorum* continues to expand into both higher elevations and desert shrublands (Ramakrishnan et al. 2006; Kao et al. 2008). Across the midcontinent of the USA, *B. tectorum* was reported as "common" throughout much of the early 20th century; however, since ca. 1950, the invasion of the grass in this region appears to have accelerated (Schachner et al. 2008).

Genetic diversity of the 51 native and 192 North American populations of *B. tectorum* analyzed to date is low in comparison to other diploid seed plants (Novak et al. 1991; Novak and Mack 1993, Bartlett et al. 2002; Valliant et al. 2007; Schachner et al. 2008). Across invasive populations, fewer alleles and polymorphic loci are detected compared to native populations, providing evidence for a genetic bottleneck associated

with the species' introduction into North America. In contrast, the level of genetic diversity within introduced populations is, on average, higher and genetic differentiation among introduced populations is lower than that reported for native populations (values summarized in Valliant et al. 2007). This pattern likely stems from multiple introductions into North America (Novak et al. 1993; Novak and Mack 2001; 2005), as well as the spread of genotypes from previously established introduced populations during range expansion. At least six to seven independent introductions of the grass occurred in western USA, two to three separate introductions may have occurred in eastern USA, and the number of introductions into Canada and the midcontinent region of the USA are somewhat intermediate to these other two regions. In addition, one multilocus genotype, *Pgm-1a* & *Pgm-2a*, occurs to varying degrees across North America (Novak et al. 1993; Novak and Mack 2001; Bartlett et al. 2002; Valliant et al. 2007; Schachner et al. 2008), suggesting that in the USA this genotype could have been spread during range expansion, most probably from eastern populations with European settlers.

Although populations of *B. tectorum* from eastern and midcontinental USA have been analyzed, the lack of adequate sampling from the Midwest region of the USA limits the full evaluation of the East-to-West pathway of range expansion. Obviously, this lack of sampling in the Midwest also limits determination of whether separate introductions may have occurred directly into this region. In the research reported here, I used enzyme electrophoresis to assess the population genetic and evolutionary consequences of the introduction and spread of *B. tectorum* in the Midwest region of the USA. Specifically, I determined 1) the amount of genetic diversity within Midwestern populations, 2) the

distribution of diversity within and among populations, 3) similarities (or differences) in genetic diversity and structure between Midwestern, other North American, and native populations of *B. tectorum*, 4) pathways associated with the introduction and spread in the Midwestern region and across North America, and 5) the geographic origins, in the native range, for the multilocus genotypes detected in Midwestern populations.

MATERIALS AND METHODS

Plant collections

Mature panicles were collected from plants in fifty-eight populations of *B. tectorum* from the Midwestern region of the USA in June 2005, with two additional populations sampled in July 2007. Samples were obtained from eleven states: Alabama, Illinois, Indiana, Kentucky, Michigan, Mississippi, Ohio, Pennsylvania, Tennessee, Wisconsin, and West Virgina (Figure 1). Early detection records were compiled from herbarium collections and regional floras (Appendix A), and localities at or near early collection sites were targeted for sampling. Other localities were sampled so that populations included in this analysis were distributed evenly across the study region, and to ensure continuity between Midwestern populations and those previously sampled in eastern USA by Bartlett et al. (2002) and midcontinental USA by Schachner et al. (2008). Most collection sites were along roadsides or railroad tracks, near grain elevators, or in vacant lots (Appendix B). At each locality, 30-35 plants were sampled haphazardly at 1-3 m intervals, depending on the size of the population. This distance reduces the chance of collecting full siblings. In populations with fewer than 30 individuals, the panicles from all individuals were collected. Panicles were stored in individually marked paper envelopes.

Enzyme electrophoresis

Caryopses were germinated at room temperature in petri dishes lined with moistened filter paper. Approximately 7-10 d after germination, when the seedlings were 3-10 cm tall, they were harvested and macerated in a tris-HCl grinding buffer-PVP solution. Starch concentration in the gels was 12% (w/v). Enzyme electrophoresis procedures generally followed Soltis et al. (1983), with modifications described by Novak et al. (1991). The 15 enzymes employed here were visualized using the following buffer systems: isocitrate dehydrogenase (IDH), glucose-6-phosphate dehydrogenase (G6PDH) and shikimate dehydrogenase (SKDH) using system 1 of Soltis et al. (1983); alcohol dehydrogenase (ADH), aldolase (ALD), glutamate dehydrogenase (GDH) and phosphoglucoisomerase (PGI) using system 6; glutamate oxalacetate transaminase (GOT), leucine aminopeptidase (LAP), malic enzyme (ME), superoxide dismutase (SOD) and triosephosphate isomerase (TPI) using system 8; and malate dehydrogenase (MDH), phosphoglucomutase (PGM) and 6-phosphogluconate dehydrogenase (6PGD) using system 9.

Nomenclature for the resulting 25 loci and all alleles followed Novak and Mack (1993). Multilocus genotypes designations were based on the identity of the allele at each locus. The most common genotype (MCG) refers to the most common combination of alleles at the 25 loci. The MCG has the highest frequency of occurrence across both native and introduced populations of *B. tectorum* (data not shown). Other multilocus genotypes were determined by specific alleles that deviated from the MCG at one or more loci.

Data Analysis

In order to determine the level and structure of genetic diversity within and among populations of *B. tectorum* in the midwestern region, allozyme data were analyzed using BIOSYS-1 (Swofford and Selander 1981). Thus, the same parameters could be used to compare genetic diversity in midwestern populations to that previously reported for native range populations of *B. tectorum* from Eurasia (Novak and Mack 1993), western USA (Novak et al. 1991), eastern USA (Barlett et al. 2002), midcontinental USA (Schachner et al. 2008), and Canada (Valliant et al. 2007). Allozyme data were entered into BIOSYS-1 as genotype frequencies, with populations arranged hierarchically based on their geographic location. Genetic diversity in *B. tectorum* was expressed as the mean number of alleles per locus (A), the percentage polymorphic loci per population using the 99% criterion *(%P*), and expected mean heterozygosity (H_{exp}) and the mean observed heterozygosity (H_{obs}). Expected mean heterozygosity is equivalent to the parameter expected gene diversity, and was computed using the unbiased estimate method of Nei (1978). Mean observed heterozygosity was determined by the direct count method. Means of these genetic diversity parameters were used to describe the overall diversity within populations of *B. tectorum* from the midwestern region.

Values for Wright's Fixation Index (F) at each polymorphic locus within all populations were calculated as the ratio of the number of observed to expected heterozygotes: $F = 1 - (H_{obs}/H_{exp})$ (Wright 1965). The significance of any deviation of H_{obs} from H_{exp} was determined using a Chi-square test (Workman and Niswander 1970).

Nei's gene diversity statistics (1973, 1977) were used to partition total allelic diversity within and among populations, using the variance components from the output of the Wright-78 analysis of BIOSYS-1 (Swofford and Selander 1981). At each polymorphic locus, the total allelic diversity (H_T) was partitioned into a within-population component (H_S) and an among-population component (D_{ST}) using the expression $H_T = H_S + D_{ST}$. The proportion of genetic diversity partitioned among populations (G_{ST}) was determined using the equation $G_{ST} = D_{ST}/H_T$. Means of Nei's gene diversity statistics from all polymorphic loci were employed to describe the overall allocation of allelic diversity within and among populations for the mid-western region. Nei's (1978) unbiased genetic identity coefficients (I) were calculated for all possible pair-wise comparisons among all Midwestern populations using BIOSYS-1. The unweighted pair-group method with arithmetic averaging (UPGMA) algorithm (Swofford and Selander 1981) was used to generate a phenogram for the 60 Midwestern populations of *B. tectorum*, based on genetic identity (I) values.

RESULTS

Genetic Diversity of B. tectorum in the Midwest

Of the 2,009 panicles sampled across 60 Midwestern populations of Bromus tectorum, 1,905 (94.8%) caryopses successfully germinated. Thus, estimates of genetic diversity and structure of Midwestern populations of B. tectoum were based on an average of 31.75 individuals per population. Across all 60 populations at the 25 loci surveyed, a total of 30 alleles were detected (1.20 alleles/locus) and five loci (20%) were polymorphic: Got-4, Mdh-2, Mdh-3, Pgm-1, and Pgm-2 (Table 1). Each polymorphic locus had two alleles. The remaining 20 loci were monomorphic and their alleles did not differ from those of the MCG. When polymorphic, Mdh-2 and Mdh-3 displayed the same allele frequencies in each population, and a similar pattern was observed for Pgm-1 and Pgm-2 (Table 1). These results suggest that the alleles at each pair of loci are linked, most likely due to gametic disequilibrium (Novak and Mack, unpublished data). Across the entire Midwest region, the Pgm-1a and Pgm-2a alleles were detected in 34 of 60 (56.7%) populations with a mean frequency of 0.288, and these alleles were detected in populations from all four sub-regions (Figure 2; Table 1). The Mdh-2b and Mdh-3b alleles occur in five of 60 (8.3%) populations at a mean frequency of 0.026, and Got-4c appeared in three of 60 (5.0%) populations with a mean frequency of 0.007.

Slightly over half (32 of 60, 53.3%) of the populations of *B. tectorum* from the Midwest region were genetically polymorphic (exhibit multiple alleles), while the remaining 28 populations were monomorphic at the 25 scored loci (Figure 2; Table 2). On average, the 60 Midwestern populations displayed 1.05 alleles per locus (A) and 4.7% percentage polymorphic loci ($\%P$). Values for $\%P$ range from 0 to 20%, and the population at Jasper, Michigan (MI), had the highest level of within-population genetic diversity: 1.20 alleles per locus and five polymorphic loci ($\%P = 20.0$) (Table 2). The populations at Olney, Illinois (IL), and Porter Reach, Indiana (IN), were polymorphic at four loci ($A = 1.16$, $\%P = 16.0$), and the population from Athens, Tennessee (TN), was polymorphic at three loci ($A = 1.12$, $\%P = 12.0$). Across all 60 populations, the expected mean heterozygosity (H_{exp}), which is equivalent to the expected genetic diversity within populations, is 0.014 (Table 2). The highest value of H_{exp} was detected in the population from Olney, IL ($H_{exp} = 0.056$), and the lowest value for a population with polymorphic loci was detected in Memphis, TN ($H_{exp} = 0.005$).

The mean observed heterozygosity (H_{obs}) for all 60 populations was 0.00003 (Table 2). Just a single heterozygote was detected in the Midwest region: one individual in Valley Head, Alabama (AL) was heterozygous at both *Pgm-1* and *Pgm-2*. Progeny array analysis of several other maternal plants (from Tait's Gap, AL; Porter Reach, IN; Elk Rapids, MI; St. Johns, MI) revealed the presence of additional heterozygous individuals (data not shown), however these individuals were not included in the results reported here. Wright's fixation index (F) was 1.0 for 69 of 71 polymorphic loci detected all populations of *B. tectorum* from the Midwest region (Table 3), indicating complete

deviation from Hardy-Weinberg equilibrium. The fixation indices at *Pgm-1* and *Pgm-2* in the population from Valley Head, AL, were both 0.922 (Table 3). Thus, significant heterozygote deficiencies ($P < 0.001$) were detected at every polymorphic locus in all populations from the Midwest.

Population Differentiation of *B. tectorum* in the Midwest

Across all populations of *B. tectorum* from the Midwest region, *Pgm-1* and *Pgm-2* were the most polymorphic loci (Table 1; Figure 2), and consequently these loci had the highest value for total gene diversity ($H_T = 0.410$) (Table 4). The among-population component for *Pgm-1* and *Pgm-2* ($D_{ST} = 0.249$) was larger than the within-population ($H_S = 0161$), thus the proportion of the total gene diversity partitioned among populations (G_{ST}) was 0.607 at both loci. Even more of the total gene diversity at *Mdh-2* and *Mdh-3* ($H_T = 0.051$) was partitioned among populations ($G_{ST} = 0.739$). *Got-4* displayed the lowest value for total gene diversity ($H_T = 0.013$) and was the only polymorphic locus to have a greater within-population component of diversity ($H_S = 0.010$) than the among-population component ($D_{ST} = 0.003$). Consequently, the value of G_{ST} at *Got-4* was 0.216. The mean value of H_T for all polymorphic loci was 0.187, and the mean value of G_{ST} was 0.582, indicating that most of the genetic diversity (58.2%) for all 60 Midwestern populations was partitioned among populations (Table 4).

Mean values of Nei's (1978) unbiased genetic identity coefficients (I) for all possible pairwise population comparisons is $I = 0.976$ (data not shown), indicating a high level of genetic similarity among populations of *B. tectorum* from the Midwest. For

instance, because the MCG was detected in populations from all subregions, all intra- and inter-regional comparisons include population pairs that have a value of $I = 1.000$ (Table 5). Conversely, the mean values of all pairwise population comparisons that include populations from the Southern subregion are among the lowest, probably due to the high frequency of *Pgm-1a* and *Pgm-2a* in the populations from the Southern subregion.

The UPGMA cluster diagram based on Nei's (1978) unbiased genetic identity (I) values provides a graphic representation of the high level of genetic similarity among populations in the Midwest region of the United States (Figure 3). Genotypes of 27 of the 60 populations were grouped in the same cluster because these populations were either fixed for, or had a very high frequency of, the MCG. Other clusters were mainly defined by similar frequencies of *Pgm-1a* and *Pgm-2a*, or by similar frequencies of *Mdh-2b* and *Mdh-3b*. For instance, the position of the population from Huntington, West Virginia (WV), in the cluster diagram, occurred due to the relatively high frequency of *Pgm-1a*, *Pgm-2* and *Got-4c*. Similarly, the position of Washington, IN, and Daylight-Earle, IN, was based on the high frequency of *Mdh-2b* and *Mdh-3b* in these populations.

Genetic Diversity and Structure Among Introduced and Native Regions

Genetic diversity parameters within populations of *B. tectorum* from the Midwest region of the USA ($A = 1.05$, $\%P = 4.73$, $H_{exp} = 0.014$) were generally similar to those of the populations from midcontinental USA, western USA, and eastern Canada, and were slightly higher than the values reported for western Canada (Table 6). Mean genetic variability values in the Midwest were however higher than those reported by Bartlett et

al. (2002) in the adjacent region of the eastern USA (A = 1.01, $\%P$ = 1.05, H_{exp} = 0.002). A larger proportion of populations in the Midwest region were polymorphic (32 of 60, 53%) than either the adjacent eastern USA (7%) or midcontinental USA (48%) (Bartlett et al. 2002; Schachner et al. 2008). However, the level of polymorphisms of western USA (76%) (Novak et al. 1991) and Canadian populations (58%) (Valliant et al. 2007) were higher than that of Midwest populations. Genetic diversity within populations from the Midwest was also higher than populations within either region of the native range of *B. tectorum* (Table 6). In addition, only 16 of 51 (31%) native populations were found to be polymorphic (Novak and Mack 1993).

The value of total gene diversity for populations of *B. tectorum* from the Midwest (H_T = 0.187) is similar to that of populations from Southwest Asia (H_T = 0.201), but more than two-fold larger that that of populations from Europe (H_T = 0.069) (Table 6). The total gene diversity for populations from the Midwest is also more than two-fold larger than populations from the two adjacent regions: eastern USA (H_T = 0.075) and midcontinental USA (H_T = 0.084). In fact, the only introduced region in North America with a higher H_T value than the Midwest is eastern Canada (H_T = 0.265). The mean value of G_{ST} for all polymorphic loci in the Midwest is 0.582, indicating that the majority of the genetic diversity measured for all 60 Midwestern populations is partitioned among populations (Table 4). Only two other regions maintain a higher proportion of among-population diversity: the native range in Eurasia (G_{ST} = 0.754, Novak and Mack 1993) and the western United States (G_{ST} = 0.617, Novak et al. 1991), although the Midwestern

values are not much higher than those found in the eastern USA (G_{ST} = 0.560, Bartlett et al. 2002).

Shared Multilocus Genotypes

A total of six multilocus genotypes were detected in populations of *B. tectorum* from the Midwest region. The MCG occurred in 55 of 60 (92%) Midwestern populations, and it was the only multilocus genotype detected in 25 (41.7%) of these populations (Figure 2). Populations fixed for the MCG were distributed across the entire study area. Two of the four homozygous multilocus genotypes detected in the Midwestern populations match genotypes detected by Novak et al. (1993) and Novak and Mack (2001) in populations from the native range of *B. tectorum* (Table 6). The *Got-4c* multilocus genotype, detected in three Midwestern populations, has been found in Bayreuth, Germany, and Libochovice, Czech Republic, in the native range. The *Pgm-1a & Pgm-2a* multilocus genotype was detected in 33 of 60 (55%) Midwestern populations (Table 3), and was reported by Novak et al. (1993) in two populations in the native range (Vac, Hungary, and Bratislava, Slovakia). The *Mdh-2b* & *Mdh-3b* and the *Mdh-2b*, *Mdh-3b*, *Pgm-1a* & *Pgm-2a* multilocus genotypes were detected in four and three Midwestern populations, respectively. The geographic origins of these two genotypes in the native range of *B. tectorum* have not been identified. The sixth multilocus genotype, *Pgm-1ab & Pgm-2ab*, was detected in only one individual from Valley Head, AL, and was probably generated through an outcrossing event.

DISCUSSION

Historical Information and Collections Records

Several of the earliest herbarium specimens of *Bromus tectorum* within the Midwest region of the USA are from the Great Lakes area (Appendix A, R. N. Mack, personal communication): in 1884 it was collected at Erie, Pennsylvania, and in 1886 at Cleveland, Ohio. One of the earliest collections in Illinois was near Lake Michigan, at Hilton, Illinois, in 1889 (Mohlenbrock 1972). By far the most specific description of an early introduction of *B. tectorum* into the Midwest comes from an account in a flora of the Grand Rapids, Michigan area (Cole 1901):

> *This grass was first noticed in the City on the S.E. Corner of Union St. and Wealthy Ave. in 1894, by C.W. Fallass. This is the first station reported in the State. The circumstances attending its appearance were carefully looked up by R.E. Matteson who reports the following: "The grass appeared shortly after a fruit dealer whose fruits were largely imported from Italy, occupied the barn on an adjoining lot. It was his custom to brush out his wagon while crossing this lot."*
>
> *This is a common grass in Southern Europe, and as it made its appearance immediately after the occupancy of the barn by the fruit dealer, and in such abundance, it seems conclusive that it must have been brought here in the packing material. When discovered it covered the lot and was confined to it. The wind carries the light feathery spikelets and spreads it with great rapidity; from this lot it has spread to all parts of the City, and is now invading the surrounding county.*

Although not so specific as the Grand Rapids account, descriptions from weed handbooks and regional floras of the central Midwest indicate that *B. tectorum* was, if not

abundant, then at least fairly common by the early 1900s. In southern Ohio, *B. tectorum* was collected at several different locations in 1899 (Appendix A), and is described in 1906 as a widespread weed (Selby 1906). As early as 1912, land managers in Indiana described *B. tectorum* as "liable to become a bad weed" (Blatchley 1912). Unfortunately, in West Virginia many early herbarium collections were lost, but *B. tectorum* appeared on the state checklist by 1930, and its considerable range suggests that it had been present in the state for some time (Strausbaugh and Core 1930). Due to repeated fires at the herbarium at the University of Kentucky, no early specimens from Kentucky could be found for inclusion in this study. A weed bulletin from 1914 describes *B. tectorum* only as "observed along railroads" (Garman 1914), but a number of specimens collected after 1935 were found, and their distribution suggests that *B. tectorum* was already widespread in Kentucky by the early 1930s (Appendix A).

The introduction history of *B. tectorum* in the southern region of the Midwest is more ambiguous because of few specimens (Appendix 1). In Tennessee, early collections of the plant were made in Knoxville (1890) and Nashville (1892). The southernmost early herbarium specimen was collected in Starkville, Mississippi, in 1892. No other herbarium records were found from the far south until 1939 in Cullman, Alabama. This collection history suggests either fewer introductions of *B. tectorum* into the far south, or slower increase in the abundance of the plant in this region, compared with populations in the northern portion of the study area.

Although these records are suggestive of an early introduction into the northern regions via lake ports, overland introductions from adjacent regions cannot be ruled out,

particularly from the east coast, where early collections of *B. tectorum* predate those in the Midwest (Bartlett et al. 2002). The near-contemporaneous collections throughout the Midwest in the 1890s suggest that *B. tectorum* may have been introduced into multiple areas within a few decades and expanded from several to many foci. The accuracy of this assessment is, of course, limited by several factors: uneven historical sampling effort, loss of potentially useful records, and incomplete discovery of early records. All of these factors combine to complicate the reconstruction of early introduction pathways; however, results from molecular markers support the hypothesis of multiple introductions into the Midwest (see below).

Source Populations

Bromus tectorum is a highly selfing plant species throughout its entire worldwide distribution (Novak and Mack 2001), therefore introduced populations appear to maintain the multilocus genotypes of their founders. Furthermore, probable source populations or regions in the native range can be confidently identified when native multilocus genotypes are highly structured (Novak and Mack 2001). Several regions within central Europe and the western Mediterranean have been previously identified as potential sources for North American populations (Novak et al. 1993, Novak and Mack 1993; 2001), and the results from this study are generally consistent with previous findings. Three of the multilocus genotypes preset in the Midwest have been detected previously in the native range. The MCG occurs across Eurasia, and therefore no geographically restricted donor region can be identified. However, the *Pgm-1a* & *Pgm-2a* multilocus

genotype is found in only two populations in the native range: Vac, Hungary, and Bratislava, Slovakia (Novak and Mack 2001), and this genotype was found in 33 of 60 Midwestern populations (Table 7). In the Midwestern region, the *Got-4c* multilocus genotype was detected in three populations; while in the native range this genotype was found in Bayreuth, Germany, and Libochovice, Czech Republic (Novak and Mack 2001). The *Mdh-2b & Mdh-3b* and *Mdh-2b, Mdh-3b, Pgm-1a & Pgm-2a* multilocus genotypes have not yet been detected in native Eurasian populations (Table 7); the geographic origins of these genotypes therefore can not be pinpointed. Thus, a minimum of four to five founder events are necessary to account for the genetic variability found in the Midwestern USA, and based on multilocus genotypes with exact matches, founders appear to have originated in central Europe.

Introduction and Spread in the Midwest Region

All multilocus genotypes detected in the Midwest have been previously detected elsewhere in North America, and therefore no Midwestern population can be identified as the unequivocal descendants of an introduction directly from the native range. However, given the current geographic distribution of genotypes in North America and historical records, herbarium specimens, and regional floras, some likely scenarios concerning the introduction and spread of *B. tectorum* in this region can be drawn.

The MCG has now been detected throughout the entire North American range of *B. tectorum*. This genotype occurs at the highest frequency along the eastern seaboard, and decreases in a westerly direction across the continent. This pattern has been attributed

to early introductions on the east coast (Novak and Mack 2001, Bartlett et al. 2002), with subsequent spread westward with European settlers (Mack 1981, Upadhyaya et al. 1986, Novak and Mack 2005). Results from the Midwest are consistent with this pattern. And although the MCG has been detected near some early collection sites (e.g., Erie, PA), it is difficult to determine whether this genotype was spread with settlers, or introduced directly into this region.

The *Pgm-1a & Pgm-2a* multilocus genotype was found in 33 of 60 midwestern populations (Table 7, Figure 2). This same genotype was reported in three populations in the southern portion of eastern USA (Bartlett et al. 2002), and was somewhat more common (24 of 54 populations) in the midcontinental USA (Schachner et al. 2008). Because *Pgm-1a & Pgm-2a* occurs at higher frequency in the southern subregion of the Midwest, it is plausible to suggest that it may have been spread overland from southern populations along the east coast, into the southern Midwest, and further into the midcontinental region. The *Pgm-1a & Pgm-2a* multilocus genotype occurs in only two populations from the western USA (Novak and Mack 1993), thus an East-West spread of this genotype seems more likely.

An alternate introduction pathway for the *Pgm-1a & Pgm-2a* multilocus genotype into the midwestern region may include Great Lakes commerce and shipping. Herbarium records from 1880-1900 document the early presence of *B. tectorum* in eastern Canada at Great Lakes ports or nearby railroads, and *Pgm-1a & Pgm-2a* is now common in southern Ontario (Valliant et al. 2007). Bi-directional shipping routes along the Mississippi and Ohio Rivers have long linked the Midwest and southeastern Canada

(Haites et al. 1975), and *B. tectorum* may have been dispersed repeatedly along this route. However, this avenue of spread cannot be adequately evaluated with these data.

The *Got-4c* multilocus genotype was detected in only three populations in the Midwest: it occurred at a frequency of 0.294 in the population from Huntington, West Virginia, and at much lower frequencies in Jasper, Michigan, and Athens, Tennessee (Figure 2; Appendix D). This result is notable because Huntington, WV is the furthest east this genotype has been detected in the USA. This genotype is common in western USA (Novak et al. 1991), and dispersal of this genotype into the Midwestern region may have occurred from areas of early introduction in the West (Novak et al. 1993) via transcontinental railroads which linked western and eastern regions of the USA by the 1880s (Meinig 1968; Porter 1991). The detection of this genotype in only one population from midcontinental USA (Schachner et al. 2008) however argues against this direction of spread. In addition, the genotype is found in eastern Canada at low frequency (Valliant et al. 2007), thus it may also have entered the Midwest from southern Ontario via Great Lakes and river commerce. Alternatively, the *Got-4c* genotype may have been introduced into the Midwest directly from native populations in central Europe. Regardless of the mechanism of introduction or spread, the low frequency of the *Got-4c* genotype in the Midwest suggests low propagule pressure for the introduction and spread of this genotype in the Midwest.

Results of the current study also support the possibility of an East-West dispersal pathway for the *Mdh-2b & Mdh-3b* multilocus genotype. This multilocus genotype was previously detected in two populations from the East (Bartlett et al. 2002) and three

populations from the midcontinental USA (Schachner et al. 2008). It has now been detected in four of 60 populations of *B. tectorum* from Midwestern USA: three populations from southern Indiana and Illinois, which are located approximately 80 km apart, and one population from Michigan (Figure 2; Table 7). The clustering of the *Mdh-2b & Mdh-3b* genotype in three populations in southern Indiana and Illinois suggests that it may have been introduced directly into this small geographic region. Alternatively, because populations with this genotype have now been detected in all four regions of the USA, settlers from the East may have spread this genotype westward.

The *Mdh-2b, Mdh-3b, Pgm-1a & Pgm-2a* multilocus genotype was detected in three populations from the Midwest (Figure 2; Table 7), and it occurred at highest frequency in the population from Daylight-Earle, IN (0.686). There is no direct match for this genotype among native populations (Novak and Mack 1993; 2001), and it had been previously reported in just one individual from Martin, South Dakota (Schachner et al. 2008). Schachner et al. (2008) suggested that this is a novel, recombinant multilocus genotype, and this view must now be reassessed. Because this genotype has now been detected in several populations across the USA, it may have been introduced directly into the USA from the native range (perhaps near Daylight-Earle, IN, because of its high frequency), with subsequent spread to just a few populations (Olney, IL, Porter Reach, IN, and Martin, SD). Given the long history of grain export from the central Midwest, particularly ca. l820-1860 (Cochrane 1993), it is plausible that seeds of *B. tectorum* with this genotype could have contaminated these shipments. Alternatively, the *Mdh-2b, Mdh-3b, Pgm-1a & Pgm-2a* multilocus genotype may have arisen *in situ* within Midwest

populations, as hypothesized for the individual in Martin, SD. Interestingly, three populations with this "recombinant" multilocus genotype (Daylight-Earle and Porter Reach, IN, and Martin, SD) do not contain the two multilocus genotypes contributing to it, *Mdh-2b & Mdh-3b* and *Pgm-1a & Pgm-2a*. Only the population from Olney, IL contains this "recombinant" type and the two "parental" multilocus genotypes, whereas the population from Jasper, MI, contains both "parental" genotypes, but not the "recombinant" type. Determining whether this multilocus genotype was introduced directly from the native range, or created *in situ* in North America, may be resolved by analyzing additional populations from the native range, especially areas in which previous sampling was limited, such as eastern Europe (Novak and Mack 1993).

The multilocus genotypes detected in the Midwest may be due to introductions directly from the native range as either a contaminant of grain or packing material, as illustrated by the account from Grand Rapids, MI (Cole 1901). The spread of these genotypes may be the result of propagule pressure associated with the movement of commodities within and out of the Midwest, and this spread may have been facilitated by several mechanisms. For example, the three populations in Indiana and Illinois are approximately 110 km from the Ohio River and the Wabash & Erie Canal. The population from Jasper, MI (which is the only population from the Midwest polymorphic at five loci), is located within 50 km of a major canal junction and ports on Lake Erie near Toledo, OH. By the 1830s, extensive canal systems linked Pennsylvania, Ohio, and Indiana to the east coast (Goldman 1983; Shaw 1993). The grain produced in the Midwest was transported almost exclusively through canal systems until the rise of

railroads in the 1850s; millions of bushels a year passed through the lake ports of Cleveland and Toledo, OH (Shaw 1993). Thus, results from this study highlight the importance of interstate commerce in the spread of invasive plants.

Genetic Diversity

Multiple demographic and evolutionary factors influence the genetic diversity of an alien in its new range, including mating system, founder events and populations bottlenecks (Barrett and Shore 1989; Barrett and Husband 1990; Barrett and Kohn 1991; Novak and Mack 2005). The amount of genetic diversity across and within population of *B. tectorum* from the Midwest (Table 2) and elsewhere (Table 6) is low, even compared to other plants with primarily a self-pollinating mating system (Brown 1979; Hamrick and Godt 1990). However, the genetic diversity of Midwestern populations is similar to the level of diversity reported for other introduced plants with a self-pollinating mating system, or that reproduce asexually (reviewed in Barrett and Shore 1989; Dlugosch and Parker 2008; Ward et al. 2009).

The genetic diversity of an alien species in its new range is greatly influenced by the specific details of its introduction: the location in the native range where individuals were sampled, size of the founder populations, and the number of independent introductions (Novak and Mack 2005). Founders drawn from a limited region of the native range may possess little of the diversity found across the entire native range (Tsutsui et al. 2000), and this situation would be compounded in predominantly self-

pollinating species (Brown and Marshall 1981, Barrett and Shore 1989, Novak and Mack 1993). At the 25 scored loci, Midwest populations of *B. tectorum* have fewer alleles (30 vs. 43) and polymorphic loci (5 vs. 13) compared with native populations (Novak and Mack 1993; 2005), indicating that these populations have experienced genetic drift through founder events and population bottlenecks during introduction and/or range expansion (Nei et al. 1975, Brown and Marshall 1981; Watterson 1984; Barrett and Husband 1990). Furthermore, results from the Midwest are consistent with a theoretical prediction of a genetic bottleneck associated with population bottlenecks or founding events (Nei et al. 1975, Watterson 1984): most of the rare alleles or multilocus genotypes of the native range, which predominantly occur in SW Asia and Morocco (Novak 1990), were not detected in populations from the Midwest.

Despite reduced genetic diversity across populations, Midwestern populations of *B. tectorum* possess, on average, higher within-populations diversity compared with native populations (Table 2; Table 6). Higher within-population diversity is a genetic consequence of multiple introductions from genetically distinct (and often geographically distant) source populations (Novak and Mack 2005; Kolbe et al. 2007, Ficetola et al. 2008, Kolbe et al. 2008, Suarez and Tsutsui 2008, and references therein). Based on the large percentage of populations from the Midwest that consist of two or more multilocus genotypes, many populations in this region, and other regions in North America (Novak et al. 1991; Valliant et al. 2007; Schachner et al. 2008), are genetic admixtures (discussed below).

Deviation from Hardy-Weinberg Equilibrium

Midwestern populations of *B. tectorum* exhibit an extreme violation of Hardy-Weinberg equilibrium (Table 3): only one heterozygote was detected in this region and the mean observed heterozygosity of Midwestern populations (H_{obs} = 0.00003) is over four hundred-fold smaller than the expected mean heterozygosity (H_{exp} = 0.014). Subsequent analysis of 21 other caryopses from this heterozygote revealed additional heterozygous individuals, and these results were consistent with segregation during selfing by a maternal plant that is heterozygous at both *Pgm* loci (T. Huttanus, unpublished data). Additional progeny arrays were carried out for other populations where outcrossing was suspected due to high levels of polymorphism or ambiguous initial scoring: these arrays revealed four other heterozygotes which were the only heterozygous individuals detected in the array. Thus, these four heterozygotes are likely the products of outcrossing events in the progeny generation (data not shown).

Although *B. tectorum* has been described as an obligate self-pollinator (McKone 1985) and several studies have detected no heterozygosity (Novak et al. 1991, Bartlett et al. 2002; Ramakrishnan et al. 2004; Ramakrishnan et al. 2006); results of the current study are consistent with others that have detected low levels of heterozygosity in North American populations using microsatellites (Ashley and Longland 2007; Kao et al. 2008) and allozymes (Valliant et al. 2007; Schachner et al. 2008). Outcrossing rates may be underestimated in populations characterized by a high proportion of one or a few homozygous multilocus genotypes (Siol et al. 2008), as is frequently the case with

predominantly selfing plants (Schoen and Brown 1991; Hamrick and Godt 1996; Charlesworth 2003; Duminil et al. 2007). Even in genetically polymorphic populations, unless outcrossing takes place between plants with different multilocus genotypes, most outcrossing events cannot be distinguished from selfing. Thus, considering that some cryptic outcrossing is taking place, the level of heterozygosity reported here undoubtedly represents an underestimate.

Plants with high rates of selfing may be at an advantage during the colonization, establishment, and range expansion phases of invasions. Self-pollination allows a single individual to overcome the demographic stochasticity associated with colonization and establishment because it reduces the need for a mate (Baker 1955) and can confer reproductive success, even during unfavorable environmental conditions (Stebbins 1957). Selfing is also an advantageous trait during the range expansion phase of invasion because new populations can be initiated following the dispersal of just one to a few propagules (Baker 1974). While selfing confers benefits to *B. tectorum* at short-term ecological time scales, even a limited amount of outcrossing may provide benefits at long-term evolutionary time scales. Only low levels of outcrossing are required to maintain genetic diversity and increase the evolutionary potential of inbreeding plant populations (Allard 1965).

Population Differentiation

With high propagule pressure, introduced populations consist of admixtures of genotypes from different native range populations, and the proportion of genetic diversity

partitioned among introduced populations is predicted to decrease relative to that of the native range (Brown and Marshall 1981, Barrett and Husband 1990, Novak and Mack 2005). Populations of *B. tectorum* from the Midwest possess less genetic structure (G_{ST} = 0.582) than either European populations (G_{ST} = 0.656) or Southwest Asian populations (G_{ST} = 0.735) (Table 4; Table 6). Furthermore, populations of *B. tectorum* in all regions of the introduced North American range exhibit less genetic structure than regions in the native range (Table 6). Thus, high levels of genetic diversity within North American populations and low genetic structure among populations, compared to native populations, likely reflect multiple introductions into most regions in North America and high rates of gene flow, via seed dispersal, during range expansion in these regions.

Within North America, inter-regional differences in genetic diversity and structure (Table 6) may reflect varying degrees of propagule pressure during introduction and population expansion. The mean total genetic diversity (H_T) in the Midwest is most similar to values reported for the western USA and western Canada, both regions that have experienced multiple introductions (Novak and Mack 1993; Valliant et al. 2007), and these values are only exceeded by the mean value for eastern Canada (H_T = 0.265). The magnitude of H_T is influenced by the range-wide proportion of polymorphic loci, the number of alleles per locus, and the evenness of allele frequencies averaged across the range (Loveless and Hamrick 1984). Despite having fewer polymorphic loci and lower allelic richness than the adjacent midcontinental region (Table 5, Schachner et al. 2008), the Midwest region has a higher value of H_T. Introduction dynamics and the pattern of range expansion in the Midwest and midcontinantal regions are probably quite similar,

but different from that of the eastern USA region. Thus, individual genetic diversity parameters may differ, but the composite picture obtained by these parameters can provide an evaluation of the genetic consequences of invasions.

Genetic identity values can also provide insights into introduction dynamics and patterns of range expansion into and among subregions of the Midwest. All values are high (Table 5) and indicate that populations within and among subregions are genetically similar; thus introduction dynamics and the pattern of spread throughout this region appear to be similar. The UPGMA diagram (Figure 3) graphically illustrates the lack of clustering by geographic subregion; most groups contain populations drawn from at least three and usually all four subregions. The notable exception is the population at Daylight-Earle, IN, which is the most genetically differentiated population in the Midwest because it contains only the *Mdh-2b* and *Mdh-3b* alleles. The high genetic similarity among populations from the Midwest is consistent with the results for other populations throughout the introduced range of *B. tectorum* in North American (Novak et al. 1991; Bartlett et al. 2002; Valliant et al. 2007; Schachner et al. 2008).

Invasion Success of *Bromus tectorum* in the Midwest

Invasion success in some species has been correlated with phenotypic plasticity (Richards et al. 2006). As a species, *Bromus tectorum* demonstrates a high level of phenotypic plasticity, allowing it to persist in a variety of environments (Hulbert 1955, Mack and Pyke 1983, Rice and Mack 1991). In addition, different genotypes of *B. tectorum* vary for ecologically important life-history traits and phenotypic plasticity,

indicating that chance sampling from the native range may influence whether establishment and invasion occurs (Kinter 2003). Thus, high propagule pressure has likely contributed to the invasion success of *B. tectorum* in the Midwest, either through repeated introduction of individuals with high phenotypic plasticity or by contributing to an increase in the genetic diversity within populations of this region.

Recombination among multilocus genotypes which were previously allopatric in the native range could lead to increased rates of local adaptation and invasiveness in the introduced range (Ellstrand and Schierenbeck 2000; Sakai et al. 2001; Novak and Mack 2005). The presence of a potentially novel, recombinant genotype *Mdh-2b, Mdh-3b, Pgm-1a* & *Pgm-2a* in the Midwest may signal post-immigration evolution. Although the possibility that this multilocus genotype was introduced directly from the native range cannot yet be discounted, the presence of this genotype in three Midwestern populations may provide the opportunity to study the fitness of a recombinant genotype in a predominantly selfing species. Multi-year analysis of these populations would reveal its rate of expansion in the field; thus these results potentially supply land managers with an early warning of a newly created, aggressive genotype. The detection of such low-frequency events, which may contribute to an acceleration of the invasion of *B. tectorum* in the Midwest, has only been possible through detailed, regional population genetic analyses.

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Table 1

Mean of Allele Frequencies at Polymorphic Loci for Populations of *Bromus tectorum* from Four Sub-regions, and for all 60 Populations, Collected in the Midwest Region of the USA that Vary Relative to the Most Common Genotype (MCG)

Locus	Allele	MCG	Northern (17)	Southern (12)	East-Central (15)	West-Central (16)	All 60 Midwest Populations
Got-4	*b*	1.000	0.998 (17)	0.994 (12)	0.980 (15)	1.000	0.993 (60)
	c	0.000	0.002 (1)	0.006 (1)	0.020 (1)	0.000	0.007 (3)
Mdh-2	*a*	1.000	0.996 (17)	1.000	1.0000	0.906 (15)	0.974 (59)
	b	0.000	0.004 (2)	0.000	0.0000	0.094 (3)	0.026 (5)
Mdh-3	*a*	1.000	0.996 (17)	1.0000	1.0000	0.906 (15)	0.974 (59)
	b	0.000	0.004 (2)	0.0000	0.0000	0.094 (3)	0.026 (5)
Pgm-1	*a*	0.000	0.240 (8)	0.464 (9)	0.245 (8)	0.246 (9)	0.288 (34)
	b	1.000	0.760 (17)	0.536 (10)	0.755 (14)	0.754 (16)	0.712 (57)
Pgm-2	*a*	0.000	0.240 (8)	0.464 (9)	0.245 (8)	0.246 (9)	0.288 (34)
	b	1.000	0.760 (17)	0.536 (10)	0.755 (14)	0.754 (16)	0.712 (57)

Note. Number of populations within each sub-region of the Midwest is given in parentheses. Numbers in parentheses beside the allele frequencies are the number of populations in each subregion, or across the region, which possess that allele.

Table 2

Measures of Genetic Diversity for 60 Populations of *Bromus tectorum* in the Midwestern USA

Pop. Num.	Locality	*N*	*A*	*%P*	H_{exp}	H_{obs}
1	Cullman, AL -- S*	26	1.08	8.0	0.006	0.000
2	Tait's gap, AL -- S*	33	1.08	8.0	0.034	0.000
3	Valley Head, AL -- S*	35	1.08	8.0	0.030	**0.002**
4	Carbondale, IL -- W*	29	1.08	8.0	0.040	0.000
5	Eureka, IL -- W*	33	1.08	8.0	0.040	0.000
6	Evanston, IL -- N	33	1.00	0.0	0.000	0.000
7	Greenville, IL -- W*	35	1.08	8.0	0.023	0.000
8	Olney, IL -- W*	29	1.16	16.0	0.056	0.000
9	Paxton, IL -- W	35	1.00	0.0	0.000	0.000
10	Toledo, IL -- W	31	1.00	0.0	0.000	0.000
11	Ballstown, IN -- W	35	1.00	0.0	0.000	0.000
12	Daylight-Earle, IN -- W*	35	1.08	8.0	0.035	0.000
13	Hendricks Township School, IN -- W*	35	1.08	8.0	0.033	0.000

Pop. Num.	Locality	*N*	*A*	*%P*	H_{exp}	H_{obs}
14	Pine Village, IN -- W	33	1.00	0.0	0.000	0.000
15	Porter Reach, IN -- N*	32	1.16	16.0	0.033	0.000
16	Washington, IN -- W*	30	1.08	8.0	0.039	0.000
17	Ashland, KY -- E*	34	1.08	8.0	0.013	0.000
18	Bowling Green, KY -- W*	32	1.08	8.0	0.021	0.000
19	Elizabethtown, KY -- W*	28	1.08	8.0	0.037	0.000
20	Lexington-Fayette, KY -- E*	35	1.08	8.0	0.026	0.000
21	Louisville, KY -- W	35	1.00	0.0	0.000	0.000
22	Madisonville, KY -- W*	30	1.08	8.0	0.019	0.000
23	Nicholasville, KY -- E*	39	1.08	8.0	0.029	0.000
24	Paducah, KY -- W	31	1.00	0.0	0.000	0.000
25	Paris, KY -- E	35	1.00	0.0	0.000	0.000
26	Somerset, KY -- E	32	1.00	0.0	0.000	0.000
27	Elk Rapids, MI -- N*	19	1.08	8.0	0.008	0.000
28	Grand Rapids, MI -- N*	26	1.08	8.0	0.021	0.000
29	Jasper, MI -- N*	34	1.20	20.0	0.034	0.000

Pop. Num.	Locality	N	A	$\%P$	H_{exp}	H_{obs}
30	Manchester, MI -- N*	32	1.08	8.0	0.018	0.000
31	Manistee, MI -- N*	23	1.08	8.0	0.007	0.000
32	Seney, MI -- N	30	1.00	0.0	0.000	0.000
33	St. Johns, MI -- N*	28	1.08	8.0	0.040	0.000
34	Starkville, MS -- S*	35	1.08	8.0	0.016	0.000
35	Athens, OH -- E	35	1.00	0.0	0.000	0.000
36	Belmont, OH -- E	32	1.00	0.0	0.000	0.000
37	Camden, OH -- E*	41	1.08	8.0	0.030	0.000
38	Conneaut, OH -- N	32	1.00	0.0	0.000	0.000
39	Lorain, OH -- N*	24	1.08	8.0	0.036	0.000
40	Painesville, OH -- N	35	1.00	0.0	0.000	0.000
41	Toledo, OH -- N	34	1.00	0.0	0.000	0.000
42	Washington Court House, OH -- E*	35	1.08	8.0	0.023	0.000
43	Zanesville, OH -- E*	26	1.08	8.0	0.012	0.000
44	Erie County, PA -- N	31	1.00	0.0	0.000	0.000
45	Pittsburg, PA -- E	30	1.00	0.0	0.000	0.000

Pop. Num.	Locality	N	A	$\%P$	H_{exp}	H_{obs}
46	Washington, PA -- E	38	1.00	0.0	0.000	0.000
47	Athens, TN -- S*	26	1.12	12.0	0.046	0.000
48	Camden, TN -- S*	34	1.08	8.0	0.036	0.000
49	Farragut, TN -- S	32	1.00	0.0	0.000	0.000
50	Franklin, TN -- S	25	1.00	0.0	0.000	0.000
51	Gallatin, TN -- S	29	1.00	0.0	0.000	0.000
52	Memphis, TN -- S*	30	1.08	8.0	0.005	0.000
53	Powell, TN -- S	35	1.00	0.0	0.000	0.000
54	Sparta, TN -- S	35	1.00	0.0	0.000	0.000
55	Fennimore, WI -- N	30	1.00	0.0	0.000	0.000
56	Gradiot, WI -- N	32	1.00	0.0	0.000	0.000
57	Ripon, WI -- N	23	1.00	0.0	0.000	0.000
58	Charleston, WV -- E	35	1.00	0.0	0.000	0.000
59	Huntington, WV -- E*	34	1.04	4.0	0.017	0.000
60	Morgantown, WV -- E	35	1.00	0.0	0.000	0.000
	Mean	31.75	1.05	4.7	0.014	0.00003

Note. The locality codes N, S, E, and W designate the Northern, Southern, East-central, and West-central subregions of the Midwest, respectively. *N* is the number of individuals sampled in each population, *A* is the mean number of alleles per locus, *%P* is the percent polymorphic loci, H_{exp} is the expected mean heterozygosity, H_{obs} is the mean observed heterozygosity. Values of H_{obs} are in bold in all populations where heterozygotes were detected.

* Midwestern United States populations of *B. tectorum* that possess genetic diversity.

Table 3

Wright's Fixation Indices (*F*) for each Polymorphic Locus in Populations of *Bromus tectorum* from the Midwest Region of the USA

Population	Locus	F*
25 Populations**	*Pgm-1*	1.000
	Pgm-2	1.000
Washington, IN	*Mdh-2*	1.000
	Mdh-3	1.000
Huntington, WV	*Got-4*	1.000
2 Populations***	*Pgm-1*	1.000
	Pgm-2	1.000
	Mdh-2	1.000
	Mdh-3	1.000
Athens, TN	*Pgm-1*	1.000
	Pgm-2	1.000
	Got-4	1.000

Population	Locus	F*
Jasper, MI	*Pgm-1*	1.000
	Pgm-2	1.000
	Mdh-2	1.000
	Mdh-3	1.000
	Got-4	1.000
Valley Head, AL	*Pgm-1*	0.922
	Pgm-2	0.922

*A value of 1.0 indicates complete deviation from Hardy-Weinberg expectations. All values are significant at $p < 0.001$.

**25 populations polymorphic at *Pgm-1* and *Pgm-2*: Cullman, AL; Tait's Gap, AL; Carbondale, IL; Eureka, IL; Greenville, IL; Daylight-Earle, IN; Hendricks Township School, IN; Ashland, KY; Bowling Green, KY; Elizabethtown, KY; Lexington-Fayette, KY; Madisonville, KY; Nicholasville, KY; Elk Rapids, MI; Grand Rapids, MI; Manchester, MI; Manistee, MI; St. Johns, MI; Starkville, MS; Camden, OH; Lorain, OH; Washington Court House, OH; Zanesville, OH; Camden, TN; Memphis, TN.

*** 2 populations polymorphic at *Pgm-1, Pgm-2, Mdh-2,* and *Mdh-3*: Olney, IL; Porter Reach, IN.

Table 4

Nei's (1973, 1977) Gene diversity statistics for 60 Populations of *Bromus tectorum* from the Midwest Region of the USA

Locus	H_T	H_S	D_{ST}	G_{ST}
Got-4	0.013	0.010	0.003	0.216
Mdh-2	0.051	0.013	0.038	0.739
Mdh-3	0.051	0.013	0.038	0.739
Pgm-1	0.410	0.161	0.249	0.607
Pgm-2	0.410	0.161	0.249	0.607
Mean	0.187	0.072	0.115	0.582

Note. H_T is the total gene diversity, H_S is the within-population component, D_{ST} is the across-populations component, and G_{ST} is the proportion of total genetic diversity partitioned across the populations.

Table 5

Nei's (1978) Unbiased Genetic Identity (I) Values Averaged Within and Between Subregions for *Bromus tectorum* in the Midwestern USA

Region	No. of Pops.	Northern	East-Central	Southern	West-Central
Northern	17	0.980			
		(0.928-1.000)			
East-Central	15	0.980	0.977		
		(0.920-1.000)	(0.916-1.000)		
Southern	12	0.974	0.972	0.972	
		(0.920-1.000)	(0.916-1.000)	(0.920-1.000)	
West-Central	16	0.979	0.977	0.971	0.976
		(0.881-1.000)	(0.876-1.000)	(0.881-1.000)	(0.881-1.000)

Note: The means of all within-region pairwise comparisons appear on the diagonal, and

the means of between-region comparisons appear below the diagonal. The range of genetic identity values for population pairs appears in parentheses.

Table 6

Measures of Genetic Diversity and Structure for *Bromus tectorum* in the Midwestern USA, other Regions in North America, and Two Regions in the Native Range

Region	No. pops.	N	A	$\%P$	H_{exp}	H_{obs}	H_T	G_{ST}
Midwestern USA	60	1905	1.05	4.73	0.014	0.00003	0.187	0.582
Eastern USA[a]	38	1248	1.01	1.05	0.002	0.0000	0.075	0.560
Midcontinent USA[b]	54	1624	1.04	4.07	0.009	0.0002	0.084	0.290
Western USA[c]	40	1830	1.05	5.36	0.015	0.0000	0.132	0.617
Eastern Canada[d]	16	495	1.05	5.00	0.013	0.0003	0.265	0.447
Western Canada[d]	44	1397	1.03	3.91	0.012	0.00005	0.131	0.316
Europe[e]	39	1246	1.02	1.64	0.005	0.0001	0.069	0.656
S.W. Asia[e]	12	484	1.04	3.67	0.005	0.002	0.201	0.735

Note. No. pops. = number of populations analyzed from each region. N = number of individuals sampled from each region. All other abbreviations are as given in Table 2 and Table 4.

[a]Bartlett et al. 2002

[b]Schachner et al. 2008

[c]Novak and Mack 1993

[d]Valliant et al. 2007

[e]Novak and Mack 1993

Table 7

Homozygous Multilocus Genotypes in Introduced Populations of *Bromus tectorum* from the Midwest Region of the USA and Populations from the Native Range in Eurasia

Multilocus Genotype	Native Range		Midwestern USA	
	Population	Freq.	Population	Freq.
Got-4c	Bayreuth, Germany	1.000	Jasper, MI	0.029
	Libochovice, Czech Republic	0.222	Athens, TN	0.077
			Huntington, WV	0.294
Mdh-2b & Mdh-3b	no match detected		Olney, IL	0.034
			Daylight-Earle, IN	0.314
			Washington, IN	0.400
			Jasper, MI	0.029
Mdh-2b, Mdh-3b, Pgm-1a & Pgm-2a	no match detected		Olney, IL	0.069
			Daylight-Earle, IN	0.686
			Porter Reach, IN	0.031
Pgm-1a & Pgm-2a	Vac, Hungary	0.280	Cullman, AL	0.962
	Bratislava, Slovakia	0.036	Tait's gap, AL	0.303

Multilocus Genotype	Native Range		Midwestern USA	
	Population	Freq.	Population	Freq.
			Valley Head, AL	0.229
			Carbondale, IL	0.552
			Eureka, IL	0.424
			Greenville, IL	0.171
			Olney, IL	0.345
			Hendricks Town. Sch., IN	0.286
			Porter Reach, IN	0.750
			Ashland, KY	0.914
			Bowling Green, KY	0.844
			Elizabethtown, KY	0.357
			Lexington-Fayette, KY	0.200
			Madisonville, KY	0.133
			Nicholasville, KY	0.231
			Paris, KY	1.000
			Elk Rapids, MI	0.947
			Grand Rapids, MI	0.154
			Jasper, MI	0.794
			Manchester, MI	0.125
			Manistee, MI	0.043
			St. Johns, MI	0.571

Multilocus Genotype	Native Range		Midwestern USA	
	Population	Freq.	Population	Freq.
			Starkville, MS	0.886
			Camden, OH	0.244
			Lorain, OH	0.667
			Washington C.H., OH	0.171
			Zanesville, OH	0.923
			Athens, TN	0.462
			Camden, TN	0.676
			Gallatin, TN	1.000
			Memphis, TN	0.033
			Powell, TN	1.000
			Huntington, WV	0.706

Note. Data for native range populations are from Novak et al. (1993) and Novak and Mack (2001).

Figure 1

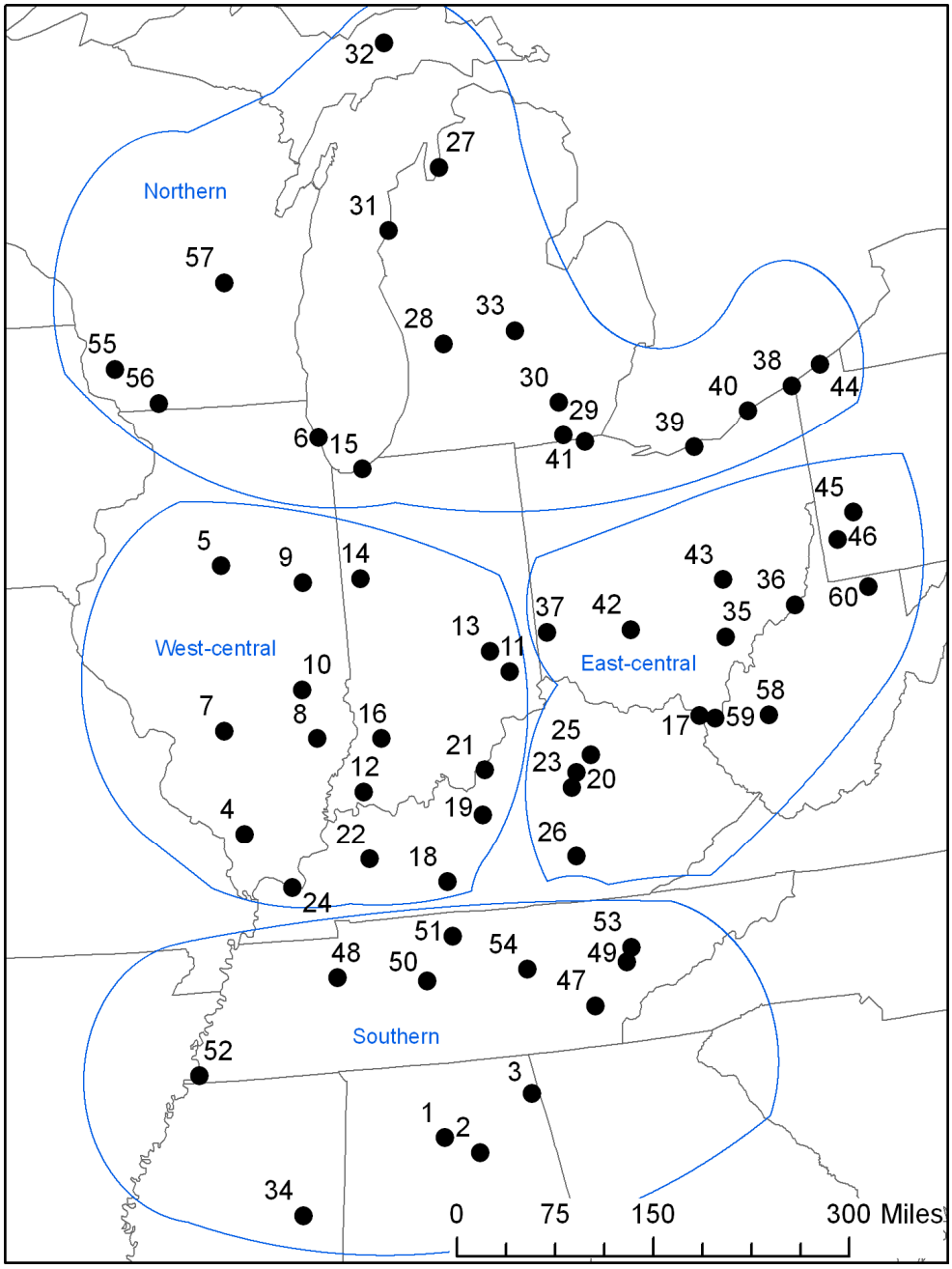


Figure 1. Subregion boundaries and collection localities for the 60 Midwestern USA populations of *Bromus tectorum* analyzed in this study.

Figure 2

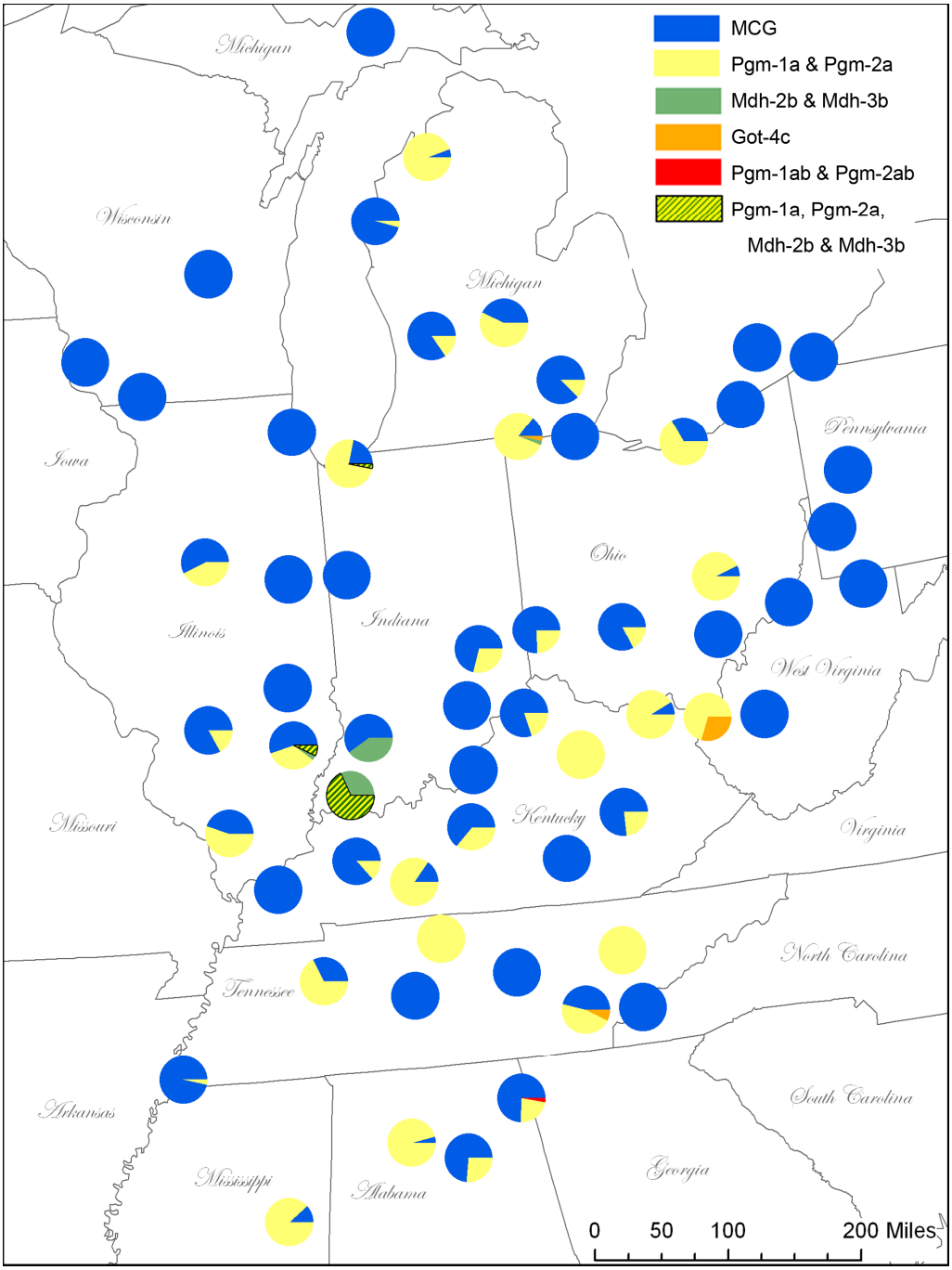


Figure 2. Multilocus genotype distribution in the 60 Midwestern USA populations of *Bromus tectorum* analyzed in this study.

Figure 3

(Part 1 of 2)

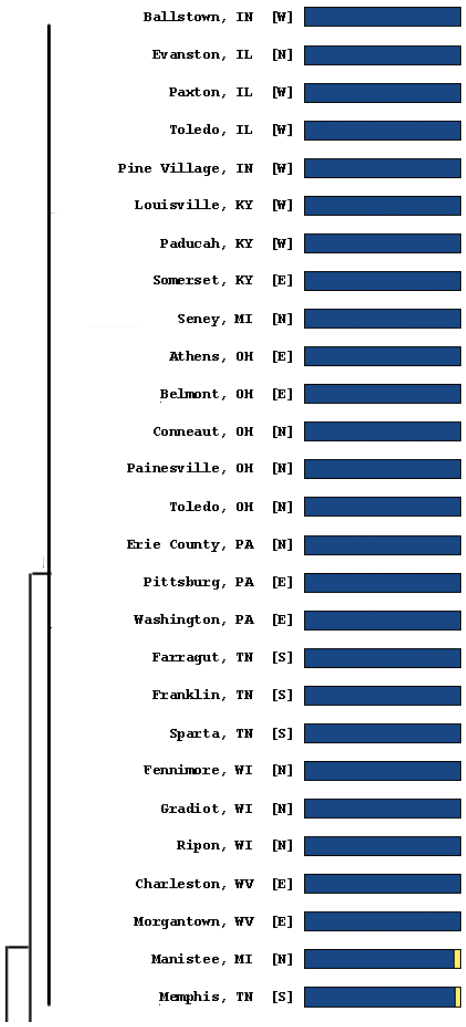
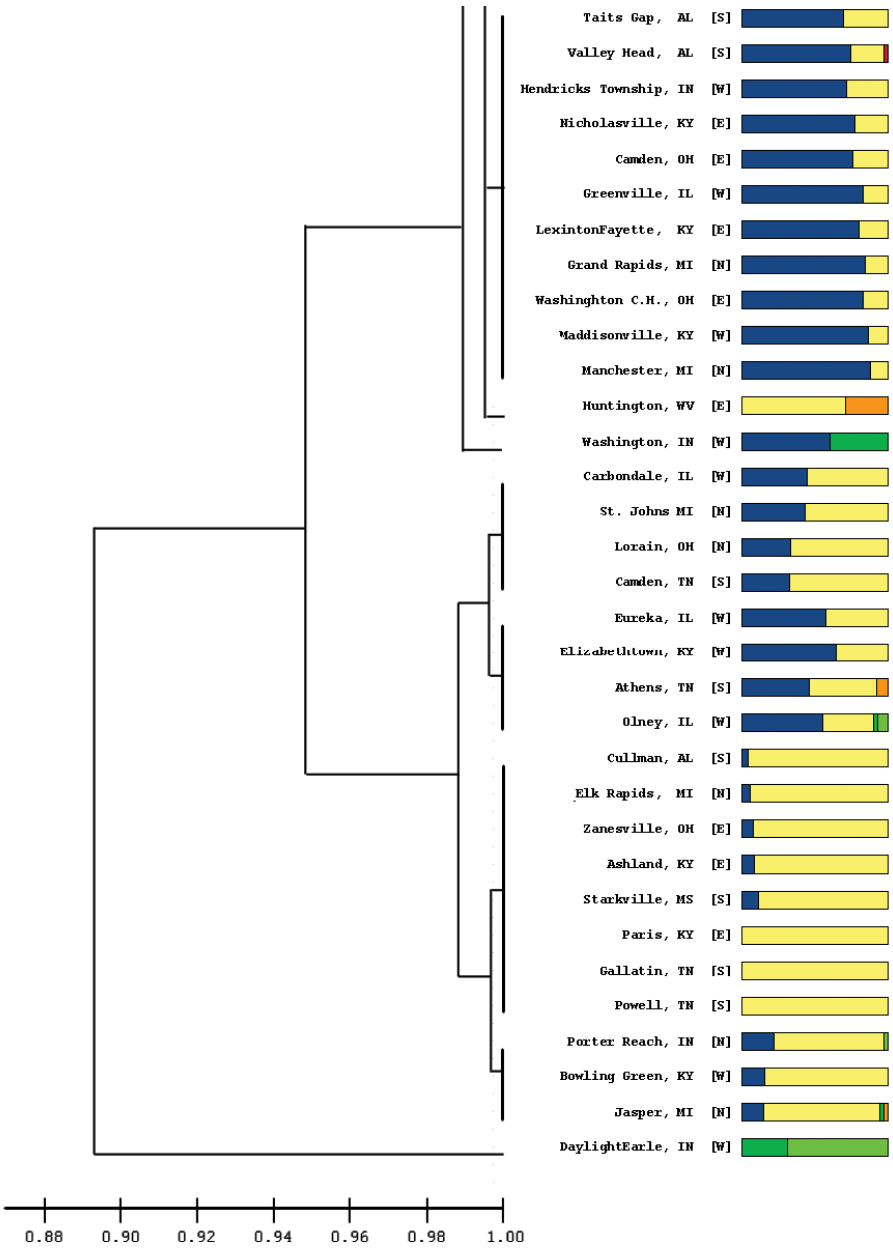


Figure 3. UPGMA phenogram based on Nei's genetic identity (I) values for 60 Midwest populations of *Bromus tectorum*

Figure 3

(Part 2 of 2)



APPENDIX A

Early Herbarium Specimen Collection Sites and Documented Occurrences for Populations of *Bromus tectorum* from the Midwest Region of the USA

Early Herbarium Specimen Collection Sites and Documented Occurrences for Populations of *Bromus tectorum* from the Midwest Region of the USA. *

State	Locality	Year	Source
Alabama	Cullman	1939	
Ilinois	Hilton (Tazewell Co.)	1888	UC
	Stark Co.	1905	UC
	Mt Carmel	1906	NY
Indiana	Putnam Co	1887	NY
	Putnam Co.	1889	NY
	Greencastle	1892	CM
	Pine	1896	MO
	Edgemoore	1897	MU
Kentucky	“along railroads”	1914	Garman 1914

State	Locality	Year	Source
	Ashland	1935	
	Maddisonville	1938	NY
	Poole	1938	NY
Michigan	Grand Rapids	1894	MN
	Adrian	1901	US
	Vestaburg	1903	MIN
	Kent Co.	1910	YU
Mississippi	Starkville	1892	NY
	Neely's Landing	1957	MISS
Ohio	Cleveland	1886	OS
	Painesville	1889	OS

State	Locality	Year	Source
	Alexandria	1890	OS
	Cleveland	1891	NY
	Painesville	1892	OS
	Toledo	1892	OS
	Columbus	1895	OS
	Cuyahoga Co.	1895	OS
	Logan (Hocking Co.)	1895	OS
	Coshocton	1896	OS
	Auglaize Co.	1899	MU
	Dayton	1899	OS
	St. Mary's	1899	MU

State	Locality	Year	Source
	Lorain Co.	1900	MU
Pennsylvania	Erie Co.	1884	CM
	Easton	1886	CM
	Bristol (Bucks Co.)	1898	CM
	Conneant Lake (Crawford Co.)	1901	CM
	Pittsburg	1902	CM
	Mt. Lebanon	1903	CM
Tennessee	Knoxville	1890	TENN
	Nashville	1892	US
	Knoxville	1896	US
Wisconsin	Manitowoc County	1910	MIL

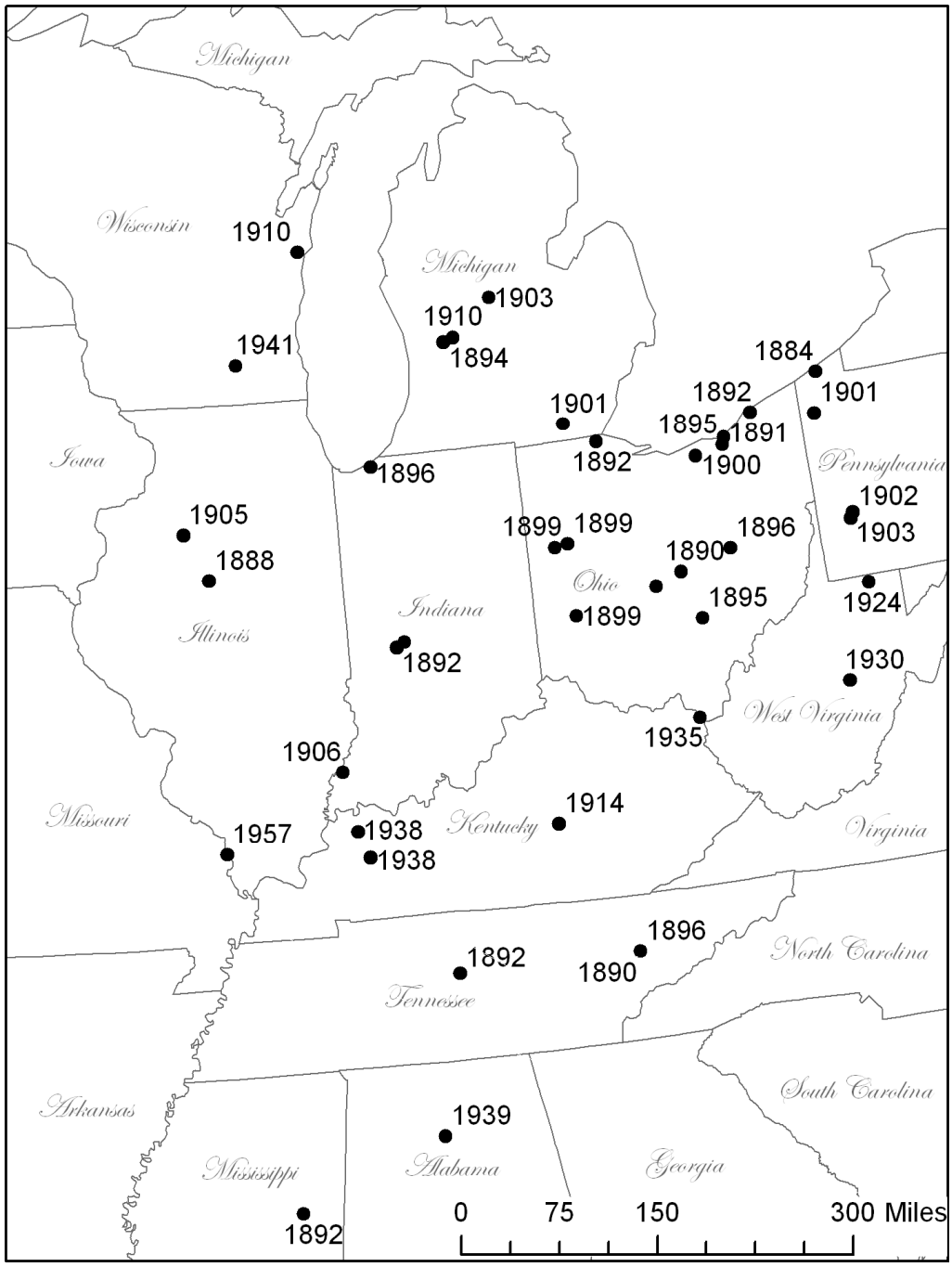
State	Locality	Year	Source
	Ft. Atkinson	1941	NY
West Virginia	Morgantown	1924	US
	unspecified	1930	Core 1940

*Note. Source for all herbarium records: Richard N. Mack, personal communication.

APPENDIX B

Early Herbarium Specimen Collection Sites and Documented Occurrences (from Appendix A) for Populations of *Bromus tectorum* from the Midwest USA

Early Herbarium Specimen Collection Sites and Documented Occurrences (from Appendix A) for Populations of *Bromus tectorum* from the Midwest USA.



APPENDIX C

Collection Localities and Habitat Descriptions for the 60 Midwestern USA Populations of *Bromus tectorum* Analyzed in this Study

Collection Localities and Habitat Descriptions for the 60 Midwestern USA Populations of *Bromus tectorum* Analyzed in this Study.

Population	Locality and collector information
1. Cullman, AL -- S	Vacant lot, disturbed sites near old depot (-86.8446, 34.1785) SJN
2. Tait's gap, AL -- S	Abandoned RR beds ~ 4.5 miles from Oneonta (-86.3950, 33.9752) SJN
3. Valley Head, AL -- S	4th Ave & Hwy 117 (-85.6147, 34.5573) SJN
4. Carbondale, IL -- W	Vacant lot/abandoned bldg near intersection of S. Washington & E. Elm St. Near RR tracks & Amtrack station (1 block from E. Walnut) (-89.2154, 37.7246) SJN
5. Eureka, IL -- W	Callendar & West Mill St. (-89.2741, 40.7233) RNM
6. Evanston, IL -- N	NW Univ. Sailing Center, along sidewalk at shoreline (-87.6703, 42.0522) RNM

Population	Locality and collector information
7. Greenville, IL -- W	Continuous population along RR tracks leading to Greenville, near intersection of Harris Ave & S. White Ave (~2 miles from I40 overpass) (-89.4028, 38.887) SJN
8. Olney, IL -- W	Along RR tracks near intersection of Railroad St & S. Whittle Ave (S. of downtown arks) (-88.0818, 38.7258) SJN
9. Paxton, IL -- W	Market St & Spruce St. (-88.0954, 40.4653) SJN
10. Toledo, IL -- W	Madison St & Maryland St. (-88.2394, 39.275) RNM
11. Ballstown, IN -- W	Roadside population beyond guardrail, two farms on either side of road (mile marker 7 on Hwy 229) (-85.2489, 39.2446) SJN
12. Daylight/Earle, IN -- W	Near intersection of Hwy 57 & Boonville-Harmony Rd. (-87.4936, 38.084) SJN
13. Hendricks Township School, IN -- W	Rt 44 ~1.2 mi W of Big Blue R. Hendricks Township School (abandoned) (-85.4936, 39.4961) RNM

Population	Locality and collector information
14. Pine Village, IN -- W	At grain elevator (-87.2544, 40.4502) RNM
15. Porter Reach, IN -- N	Vacant lot corner of Duneland Dr & Wabash St. (-87.0694, 41.6586) RNM
16. Washington, IN -- W	Along RR tracks near intersection of E 5th & Railroad St. (-87.1699, 38.6552) SJN
17. Ashland, KY -- E	In alleyways, parking lots & waste places near RR tracks (separated by a wall). Intersection of Greenup Ave & 17th St (-82.6372, 38.4797) SJN
18. Bowling Green, KY -- W	Along RR tracks near Western Kentucky University Campus -- at intersection of Adams & 15th St. (-86.4548, 36.9901) SJN
19. Elizabethtown, KY -- W	Along RR tracks near intersection of Hwy 62 & 31 W (-85.8594, 37.6936) SJN
20. Lexington-Fayette, KY -- E	Along RR tracks that cross Winchester Rd (Hwy 60W) - large population that had been cut and sprayed (near Jif factory) (-84.4749, 38.0416) SJN

Population	Locality and collector information
21. Louisville, KY -- W	Near the airport, intersection of Louisville Ave and Ottawa Ave. Along RR tracks. (-85.7567, 38.1883) SJN
22. Madisonville, KY -- W	Along RR tracks & sidewalk leading to train depot - near downtown off of W Central Street (large pop.) (-87.5006, 37.3283) SJN
23. Nicholasville, KY -- E	Roadside population at jct of 169 & Hwy 27 west of Nicholasville (-84.5715, 37.883) SJN
24. Paducah, KY -- W	Waste places near RR tracks behind Paduca Sheet Metal Bldg. near intersection of 10 & Monroe St. Industrial portion of town. (-88.6071, 37.0857) SJN
25. Paris, KY -- E	In the town along the sidewalks, waste places, near intersection of Main St and 2nd & Pleasant (-84.2483, 38.2135) SJN
26. Somerset, KY -- E	On Hwy 27, underneith and around guardrails heading into Glanes. Near Shell Station at intersection of Hwy 27 & 1575 (-84.6216, 37.1086) SJN

Population	Locality and collector information
27. Elk Rapids, MI -- N	Municipal Park near Bay Breeze Dr. (-85.4107, 44.9019) RNM
28. Grand Rapids, MI -- N	SE corner of Union St & Wealthy Ave (-85.6542, 42.9556) RNM
29. Jasper, MI -- N	0.5 mi S of Jasper (-84.0405, 41.7849) RNM
30. Manchester, MI -- N	Washtenaw Co. Rd. Comm (-84.0404, 42.1529) RNM
31. Manistee, MI -- N	0.5 mi N of Manistee (-86.3046, 44.2672) RNM
32. Seney, MI -- N	5 mi W of Seney & 1 mi W of M.28 Rest Area (-86.0604, 46.3446) RNM
33. St. Johns, MI -- N	N Clinton & E. Railroad (-84.5584, 43.0044) RNM
34. Starkville, MS -- S	Hogan & Jackston St (-88.8138, 33.4611) SJN
35. Athens, OH -- E	On offramp intersection of Hwy 50E & 33E. (-82.103, 39.3042) SJN

Population	Locality and collector information
36. Belmont, OH -- E	Corner of Jefferson & Shaver, beside Belmont Mills (-81.0392, 39.5349) RNM
37. Camden, OH -- E	Along RR tracks parallel E S. Main St (-84.6488, 39.6274) RNM
38. Conneaut, OH -- N	Along Norfolk & Southern RR tracks, between Sandusky St. & Mill St. (-80.5587, 41.95) RNM
39. Lorain, OH -- N	On Pearl St. at East 28th St. Cracks in sidewalk. (-82.1367, 41.4454) RNM
40. Painesville, OH -- N	Fairport Nursery Rd becomes 6th St. Along fence industrial area ~3mi from corner E East St (-81.2654, 41.7528) RNM
41. Toledo, OH -- N	7836 Central Ave. ~50m from corner of Shetland Rd. & Central Ave. (-83.7312, 41.6737) RNM
42. Washington Court House, OH -- E	Along street running behind a large grain silo/elevator. Near Intersection of S. Fayette st & Broad St. - near RR tracks (-83.4365, 39.5349) SJN

Population	Locality and collector information
43. Zanesville, OH -- E	Corner of South St. & 3rd St. along Muskingum R. (-82.0092, 39.9384) RNM
44. Erie County, PA -- N	0.2 mi on Elmwood Home Rd from jct. E Lynch Road; along RR tracks at x-ing (-80.0856, 42.1293) RNM
45. Pittsburg, PA -- E	between VanBraam St & Dinwiddie St, on 5th Ave (-79.9814, 40.4385) RNM
46. Washington, PA -- E	along abandoned RR siding corner of Green St. and Caldwell Ave (-80.2735, 40.171) RNM
47. Athens, TN -- S	Along RR tracks heading west on 39 out of Athens. Small pop. Turnoff for Old Riceville Rd. (-84.6191, 35.4394) SJN
48. Camden, TN -- S	Hwy 191 South out of Camden, on east side of overpass of RR tracks, roadside population (near Welcome to Camden sign) (-88.0912, 36.0483) SJN

Population	Locality and collector information
49. Farragut, TN -- S	Along RR tracks at intersection of Fox Rd & Canton Hollow Rd ~2 miles from intersection w/ Hwy S 11/70 (-84.1198, 35.8754) SJN
50. Franklin, TN -- S	Along RR tracks across Hwy 96, just before entering town (-86.8657, 35.921) SJN
51. Gallatin, TN -- S	On RR tracks just entering town on Hwy 25 (-86.4534, 36.3882) SJN
52. Memphis, TN -- S	Near intersection of Riverport & Mallary Ave, off Exit 9 of I55. Waste place near RR tracks (-90.0725, 35.0865) SJN
53. Powell, TN -- S	Along Rr tracks just after croswsing main into satin in town. Near community duct. (-84.0294, 36.0316) SJN
54. Sparta, TN -- S	On RR tracks leading into town off of Hwy 111 (-85.4822, 35.9318) SJN
55. Fennimore, WI -- N	Corner of Jefferson St. & 7th St. (-90.6547, 42.9855) RNM

Population	Locality and collector information
56. Gradiot, WI -- N	Around old abandoned train depot, about 50 m on Main St from JCT E RT 11 (-90.0235, 42.5793) RNM
57. Ripon, WI -- N	0.1 mi W of East Wind Rd on Hwy 23; in drainage ditch; 2.3 mi W of Ripon city limits (-88.9014, 43.8591) RNM
58. Charleston, WV -- E	Along RR tracks across from the Patrick St. Plaza (on Patrick St.). Small & scattered pop (-81.667, 38.3704) SJN
59. Huntington, WV -- E	Near building at end of 20th St., near RR tracks. Patch near intersection of 20th St & 2nd Ave, and down 2nd Ave (-82.4246, 38.4286) SJN
60. Morgantown, WV -- E	Beyond guardrail of Hwy 119S out of Morgantown, heading to I66 (-79.9581, 39.5955) SJN

Note. All populations were collected June 2005 except Elk Rapids, MI and Seney, MI which were collected in July 2007. The population codes N, S, E, and W designate the Northern, Southern, East-central, and West-central subregions of the

Midwest, respectively. Latitude and longitude are supplied in decimal degree notation. Habitat descriptions were transcribed from collection envelopes. Collector initials are as follows: RNM = Richard N. Mack; SJN = Stephen J. Novak.

APPENDIX D

Allele Frequencies at Polymorphic Loci for the 60 Midwestern USA Populations of *Bromus tectorum* Analyzed in this Study

Allele Frequencies at Polymorphic Loci for the 60 Midwestern USA Populations of *Bromus tectorum* Analyzed in this Study. Population Numbers follow Appendix 3. Number of Individuals Analyzed Appears in Parentheses.

		Population						
		1	2	3	4	5	6	7
Locus	Allele	(26)	(33)	(35)	(29)	(33)	(33)	(35)
Got-4	*b*	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	c	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Mdh-2	*a*	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	b	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Mdh-3	*a*	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	b	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Pgm-1	*a*	0.962	0.303	0.243	0.552	0.424	0.000	0.171
	b	0.038	0.697	0.757	0.448	0.576	1.000	0.829
Pgm-2	*a*	0.962	0.303	0.243	0.552	0.424	0.000	0.171
	b	0.038	0.697	0.757	0.448	0.576	1.000	0.829

Appendix D., continued.

		Population						
		8	9	10	11	12	13	14
Locus	Allele	(29)	(35)	(31)	(35)	(35)	(35)	(33)
Got-4	*b*	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	c	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Mdh-2	*a*	0.897	1.000	1.000	1.000	0.000	1.000	1.000
	b	0.103	0.000	0.000	0.000	1.000	0.000	0.000
Mdh-3	*a*	0.897	1.000	1.000	1.000	0.000	1.000	1.000
	b	0.103	0.000	0.000	0.000	1.000	0.000	0.000
Pgm-1	*a*	0.483	0.000	0.000	0.000	0.686	0.286	0.000
	b	0.517	1.000	1.000	1.000	0.314	0.714	1.000
Pgm-2	*a*	0.483	0.000	0.000	0.000	0.686	0.286	0.000
	b	0.517	1.000	1.000	1.000	0.314	0.714	1.000

Appendix D., continued.

		Population						
		15	16	17	18	19	20	21
Locus	Allele	(32)	(30)	(34)	(32)	(28)	(35)	(35)
Got-4	*b*	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	c	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Mdh-2	*a*	0.969	0.600	1.000	1.000	1.000	1.000	1.000
	b	0.031	0.400	0.000	0.000	0.000	0.000	0.000
Mdh-3	*a*	0.969	0.600	1.000	1.000	1.000	1.000	1.000
	b	0.031	0.400	0.000	0.000	0.000	0.000	0.000
Pgm-1	*a*	0.781	0.000	0.912	0.844	0.357	0.200	0.000
	b	0.219	1.000	0.088	0.156	0.643	0.800	1.000
Pgm-2	*a*	0.781	0.000	0.912	0.844	0.357	0.200	0.000
	b	0.219	1.000	0.088	0.156	0.643	0.800	1.000

Appendix D., continued.

		Population						
		22	23	24	25	26	27	28
Locus	Allele	(30)	(39)	(31)	(35)	(32)	(19)	(26)
Got-4	*b*	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	c	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Mdh-2	*a*	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	b	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Mdh-3	*a*	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	b	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Pgm-1	*a*	0.133	0.231	0.000	1.000	0.000	0.947	0.154
	b	0.867	0.769	1.000	0.000	1.000	0.053	0.846
Pgm-2	*a*	0.133	0.231	0.000	1.000	0.000	0.947	0.154
	b	0.867	0.769	1.000	0.000	1.000	0.053	0.846

Appendix D., continued.

		Population						
		29	30	31	32	33	34	35
Locus	Allele	(34)	(32)	(23)	(30)	(28)	(35)	(35)
Got-4	*b*	0.971	1.000	1.000	1.000	1.000	1.000	1.000
	c	0.029	0.000	0.000	0.000	0.000	0.000	0.000
Mdh-2	*a*	0.971	1.000	1.000	1.000	1.000	1.000	1.000
	b	0.029	0.000	0.000	0.000	0.000	0.000	0.000
Mdh-3	*a*	0.971	1.000	1.000	1.000	1.000	1.000	1.000
	b	0.029	0.000	0.000	0.000	0.000	0.000	0.000
Pgm-1	*a*	0.794	0.125	0.043	0.000	0.571	0.886	0.000
	b	0.206	0.875	0.957	1.000	0.429	0.114	1.000
Pgm-2	*a*	0.794	0.125	0.043	0.000	0.571	0.886	0.000
	b	0.206	0.875	0.957	1.000	0.429	0.114	1.000

Appendix D., continued.

		Population						
		36	37	38	39	40	41	42
Locus	Allele	(32)	(41)	(32)	(24)	(35)	(34)	(35)
Got-4	*b*	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	c	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Mdh-2	*a*	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	b	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Mdh-3	*a*	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	b	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Pgm-1	*a*	0.000	0.244	0.000	0.667	0.000	0.000	0.171
	b	1.000	0.756	1.000	0.333	1.000	1.000	0.829
Pgm-2	*a*	0.000	0.244	0.000	0.667	0.000	0.000	0.171
	b	1.000	0.756	1.000	0.333	1.000	1.000	0.829

Appendix D., continued.

		Population						
		43	44	45	46	47	48	49
Locus	Allele	(26)	(31)	(30)	(38)	(26)	(34)	(32)
Got-4	*b*	1.000	1.000	1.000	1.000	0.923	1.000	1.000
	c	0.000	0.000	0.000	0.000	0.077	0.000	0.000
Mdh-2	*a*	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	b	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Mdh-3	*a*	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	b	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Pgm-1	*a*	0.923	0.000	0.000	0.000	0.462	0.676	0.000
	b	0.077	1.000	1.000	1.000	0.538	0.324	1.000
Pgm-2	*a*	0.923	0.000	0.000	0.000	0.462	0.676	0.000
	b	0.077	1.000	1.000	1.000	0.538	0.324	1.000

Appendix D., continued.

		Population						
		50	51	52	53	54	55	56
Locus	Allele	(25)	(29)	(30)	(35)	(35)	(30)	(32)
Got-4	*b*	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	c	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Mdh-2	*a*	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	b	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Mdh-3	*a*	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	b	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Pgm-1	*a*	0.000	1.000	0.033	1.000	0.000	0.000	0.000
	b	1.000	0.000	0.967	0.000	1.000	1.000	1.000
Pgm-2	*a*	0.000	1.000	0.033	1.000	0.000	0.000	0.000
	b	1.000	0.000	0.967	0.000	1.000	1.000	1.000

Appendix D., continued.

		Population			
		57	58	59	60
Locus	Allele	(23)	(35)	(34)	(35)
Got-4	*b*	1.000	1.000	0.706	1.000
	c	0.000	0.000	0.294	0.000
Mdh-2	*a*	1.000	1.000	1.000	1.000
	b	0.000	0.000	0.000	0.000
Mdh-3	*a*	1.000	1.000	1.000	1.000
	b	0.000	0.000	0.000	0.000
Pgm-1	*a*	0.000	0.000	0.000	0.000
	b	1.000	1.000	1.000	1.000
Pgm-2	*a*	0.000	0.000	0.000	0.000
	b	1.000	1.000	1.000	1.000