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

by

Temsha D. Huttanus

A thesis
submitted in partial fulfillment
of the requirements for the degree of
Master of Science in Biology
Boise State University

August 2009

BOISE STATE UNIVERSITY GRADUATE COLLEGE

# DEFENSE COMMITTEE AND FINAL READING APPROVALS

of the thesis submitted by

Temsha D. Huttanus

Thesis Title: Introduction and Spread of *Bromus tectorum* (cheatgrass) into Midwestern United States: Population Genetic and Evolutionary Consequences

Date of Final Oral Examination: 01 May 2009

The following individuals read and discussed the thesis submitted by student Temsha D. Huttanus, and they also evaluated her presentation and response to questions during the final oral examination. They found that the student passed the final oral examination, and that the thesis was satisfactory for a master's degree and ready for any final modifications that they explicitly required.

| | |
|---|---|
| Stephen J. Novak, Ph.D. | Chair, Supervisory Committee |
| Richard N. Mack, Ph.D. | Member, Supervisory Committee |
| James F. Smith, Ph.D. | Member, Supervisory Committee |

The final reading approval of the thesis was granted by Stephen J. Novak, Ph.D., Chair of the Supervisory Committee. The thesis was approved for the Graduate College by John R. Pelton, Ph.D., Dean of the Graduate College.

# ACKNOWLEDGEMENTS

I would like to thank my committee members, Steve Novak, Jim Smith, and Richard Mack, for their advice, encouragement, and careful editing. I would like to express my sincere gratitude to my advisor, Steve Novak, for his patience while teaching me the necessary laboratory and data analysis techniques and for his infectious enthusiasm and motivation throughout this project. I would like to thank Jim Smith for pointed discussions during our weekly seminars and for helpful advice on this manuscript. I am indebted to Richard Mack for sharing his experience, insights, and wealth of historical information.

I am grateful for the help of Nancy Mack and Lynda Leppert, who assisted Richard Mack and Steve Novak during the Midwestern sample collection. I thank my fellow graduate students for many thought-provoking discussions, and the Friday afternoon Bar Gernika crew for the reminder that science is fun and that there is life outside of the lab.

I owe my deepest gratitude to Dax Mickelson for encouragement, support, and pragmatic advice. I am deeply indebted to my parents and my strange and wonderful family for their unwavering support throughout the years.

This work could not have been completed without funding from Boise State University and the Idaho Army National Guard.

# 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.

## TABLE OF CONTENTS

# LIST OF TABLES

# LIST OF FIGURES

# 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 ($H_{\text{exp}}$) and the mean observed heterozygosity ($H_{\text{obs}}$). 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: $F = 1 - (H_{\text{obs}}/H_{\text{exp}})$ (Wright 1965). The significance of any deviation of $H_{\text{obs}}$ from $H_{\text{exp}}$ 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 ($H_{\text{exp}}$), which is equivalent to the expected genetic diversity within populations, is 0.014 (Table 2). The highest value of $H_{\text{exp}}$ was detected in the population from Olney, IL ($H_{\text{exp}}$ = 0.056), and the lowest value for a population with polymorphic loci was detected in Memphis, TN ($H_{\text{exp}}$ = 0.005).

The mean observed heterozygosity ($H_{\text{obs}}$) 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 ($A = 1.05$, $\%P = 4.73$, $H_{\text{exp}} = 0.014$) 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, $H_{\mathrm{exp}}$ = 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 ($H_{\text{obs}}$ = 0.00003) is over four hundred-fold smaller than the expected mean heterozygosity ($H_{\text{exp}}$ = 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.

# LITERATURE CITED

Allard, RW 1965 Genetic systems associated with colonizing ability in predominately self-pollinated  species. *in*: HG Baker, GL Stebbins eds. The genetics of colonizing species. Academic Press, New York.

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

Baker, HG 1955 Self compatibility and establishment after long distance dispersal. Evolution 9: 347-349.

Barrett, SCH, BC Husband 1990 The genetics of plant migration and colonization. *in*: AHD Brown, MT Clegg, AL Kahler, BS Weir eds. Plant population genetics, breeding and germplasm resources. Sinauer, Sunderland, Massachusetts.

Barrett, SCH, JR Kohn 1991 Genetics and evolutionary consequences of small population size in plants: Implications for conservation. *in*: DA Falk, KE Holsinger eds. Genetics and conservation of rare plants. Oxford University Press, New York, New York, USA.

Barrett, SCH, JS Shore 1989 Isozyme variation in colonizing plants. *in*: DE Soltis, SP S. eds. Isozymes in plant biology. Dioscorides, Portland, Oregon, USA.

Bartlett, E, SJ Novak, RN Mack 2002 Genetic variation in *Bromus tectorum* (Poaceae): Differentiation in the eastern United States. American Journal of Botany 89: 602-612.

Blatchley, WS 1912 The Indiana weed book. Nature Publishing, Indianapolis, Indiana.

Brown, AHD 1979 Enzyme polymorphisms in plant populations. Theoretical Population Biology 15: 1-42.

Brown, AHD, DR Marshall 1981 Evolutionary changes accompanying colonization in plants. *in*: GG Scudder, JL Reveal eds. Evolution today. Hunt institute of botanical documents. Carnegie-Mellon University Press, Pittsburg, Pennsylvania.

Charlesworth, D. 2003. Effects of inbreeding on the genetic diversity of populations.

Cochrane, WW 1993 The development of American agriculture: A historical analysis. U of Minnesota Press. 500 pp.

Colautti, RI, IA Grigorovich, HJ MacIssac 2006 Progaule pressure: A null model for biological invasions. Biological Invasions 8: 1023-1037.

Colautti, RI, HJ MacIsaac 2004 A neutral terminology to define 'invasive' species. Diversity and Distributions 10: 135-141.

Cole, EJ 1901 Grand rapids flora: A catalogue of the flowering plants and ferns growing without cultivation in the vicinity of Grand Rapids, Michigan. A. Van Dort, Michigan.

Didham, RK, JM Tylianakis, NJ Gemmell, TA Rand, RM Ewers 2007 Interactive effects of habitat modification and species invasion on native species decline. Trends in Ecology & Evolution 22: 489-496.

Dlugosch, KM, IM Parker 2008 Founder events in species invasions: Genetic variation, adaptive evolution, and the role of multiple introductions. Molecular Ecology 17: 431-449.

Duminil, J, S Fineschi, A Hampe, P Jordano, D Salvini, GG Vendramin, RJ Petit 2007 Can population genetic structure be predicted from life-history traits? American Naturalist 169: 662-672.

Ellstrand, NC, KA Schierenbeck. 2000. Hybridization as a stimulus for the evolution of invasiveness in plants? Proceedings of the National Academy of Sciences of the United States of America.

Ficetola, GF, A Bonin, C Miaud 2008 Population genetics reveals origin and number of founders in a biological invasion. Molecular Ecology 17: 773-782.

Garman, H 1914 Some Kentucky weeds and poisonous plants. Kentucky Agricultural Experiment Station Bulletin 183, State University, Lexington, Kentucky.

Goldman, M 1983 High hopes: The rise and decline of Buffalo, New York. SUNY Press.

Haites, EF, J Mak, GM Walton 1975 Western river transportation: The era of early internal development, 1810-1860. Johns Hopkins University Press, Baltimore, Maryland, USA.

Hamrick, JL, MJW Godt 1990 Allozyme diversity in plant species. *in*: AHD Brown, MT Clegg, AL Kahler, BS Weir eds. Plant population genetics, breeding, and germplasm resources. Sinauer, Sunderland, Massachusetts, USA.

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

Hulbert, LC 1955 Ecological studies of *Bromus-tectorum* and other annual bromegrasses. Ecological Monographs 25: 181-213.

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

Kinter, CL 2003 Comparative ecological genetics of *Bromus tectorum* (cheatgrass, downy brome) from native European, naturalized New Zealand, and invasive North American populations. Washington State University, Pullman.

Kolar, CS, DM Lodge 2001 Progress in invasion biology: Predicting invaders. Trends in Ecology & Evolution 16: 199-204.

Kolbe, JJ, A Larson, JB Losos 2007 Differential admixture shapes morphological variation among invasive populations of the lizard *Anolis sagrei*. Molecular Ecology 16: 1579-1591.

Kolbe, JJ, A Larson, JB Losos, K de Queiroz 2008 Admixture determines genetic diversity and population differentiation in the biological invasion of a lizard species. Biology Letters 4: 434-437.

Lockwood, JL, P Cassey, T Blackburn 2005 The role of propagule pressure in explaining species invasions. Trends in Ecology & Evolution 20: 223-228.

Longman, OS, WG Smith 1936 Investigation of the prevalence and distribution of cheat grass in southern Alberta. Alberta Department of Agriculture, Edmonton, Alberta, Canada.

Loveless, MD, JL Hamrick 1984 Ecological determinants of genetic structure in plant populations. Annual Review of Ecology and Systematics 15: 65-95.

Mack, RN 1981 Invasion of *Bromus tectorum* L. into western North America - an ecological chronicle. Agro-Ecosystems 7: 145-165.

Mack, RN 1996 Predicting the identity and fate of plant invaders: Emergent and emerging approaches. Biological Conservation 78: 107-121.

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

Mack, RN, D Simberloff, WM Lonsdale, H Evans, M Clout, FA Bazzaz 2000 Biotic invasions: Causes, epidemiology, global consequences, and control. Ecological Applications 10: 689-710.

McKone, MJ 1985 Reproductive-biology of several bromegrasses (*bromus*) - breeding system, pattern of fruit maturation, and seed set. American Journal of Botany 72: 1334-1339.

Meinig, DW 1968 The Great Columbia Plain. Seattle University of Washington Press, Seattle, Washington, USA.

Mohlenbrock, RH 1972 The illustrated flora of Illinois. Grasses: *Bromus* to *Paspalum*. Southern Illinois Univ. Press, Carbondale & Edwardsville; Feffer & Simons, London & Amsterdam.

Muhlenberg, H 1793 Index florae lancastriensis. Transactions of the American Philosophical Society of Philadelphia 3: 157-184.

Muirhead, JR, DK Gray, DW Kelly, SM Ellis, DD Heath, HJ Macisaac 2008 Identifying the source of species invasions: Sampling intensity vs. Genetic diversity. Molecular Ecology 17: 1020-1035.

Nei, M 1973 Analysis of gene diversity in subdivided populations. Proceedings of the National Academy of Sciences of the United States of America 70: 3321-3323.

Nei, M 1977 F-statistics and analysis of gene diversity in subdivided populations. Annals of Human Genetics 41: 225-233.

Nei, M 1978 Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 89: 583-590.

Nei, M, T Maruyama, R Chakraborty 1975 Bottleneck effect and genetic variability in populations. Evolution 29: 1-10.

Novak, SJ 1990 Multiple introduction and founder effects in *Bromus tectorum* L.: An analysis of Eurasian and North American populations. Washington State University, Pullman.

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

Novak, SJ, RN Mack 2001 Tracing plant introduction and spread: Genetic evidence from *Bromus tectorum* (cheatgrass). Bioscience 51: 114-122.

Novak, SJ, RN Mack 2005 Genetic bottlenecks in alien plant species: Influence of mating systems and introduction dynamics. *in*: DF Sax, JJ Stachowicz, SD Gaines eds.

Species invasions: Insights into ecology, evolution and biogeography. Sinauer Associates, Sunderland, Massachusetts.

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

Novak, SJ, RN Mack, PS Soltis 1993 Genetic variation in *Bromus tectorum* (Poaceae) - introduction dynamics in North America. Canadian Journal of Botany-Revue Canadienne De Botanique 71: 1441-1448.

Pierson, EA, RN Mack 1990 The population biology of *Bromus tectorum* in forests - distinguishing the opportunity for dispersal from environmental restriction. Oecologia 84: 519-525.

Porter, AN 1991 Atlas of British Overseas expansion. Routledge, London.

Ramakrishnan, AP, SE Meyer, DJ Fairbanks, CE Coleman 2006 Ecological significance of microsatellite variation in western North American populations of *Bromus tectorum*. Plant Species Biology 21: 61-73.

Ramakrishnan, AP, SE Meyer, J Waters, MR Stevens, CE Coleman, DJ Fairbanks 2004 Correlation between molecular markers and adaptively significant genetic variation in *Bromus tectorum* (Poaceae) an inbreeding annual grass. American Journal of Botany 91: 797-803.

Rice, KJ, RN Mack 1991 Ecological genetics of bromus-tectorum .2. Intraspecific variation in phenotypic plasticity. Oecologia 88: 84-90.

Rejmanek, M 2000 Invasive plants: Approaches and prediction. Austral Ec. 25: 497-506.

Rejmanek, M, DM Richardson, SI Higgins, MJ Picairn, E Grotkopp 2005 Ecology of invasive plants: State of the art. *in*: HA Mooney, RN Mack, JA McNeely, LE Neville, PJ Schei, JK Waage eds. Invasive alien species, a new synthesis. Island Press, Washington.

Richards, CL, O Bossdorf, NZ Muth, J Gurevitch, M Pigliucci 2006 Jack of all trades, master of some? On the role of phenotypic plasticity in plant invasions. Ecology Letters 9: 981-993.

Richardson, DM, P Pysek 2006 Plant invasions: Merging the concepts of species invasiveness and community invisibility. Progress in Physical Geography 30: 409-431.

Rosentreter, R. 1994. Displacement of rare plants by exotic grasses. In: SD Monsen, KS D. eds. Proceedings: ecology and management of annual rangelands, General Technical Report INT-GTR-313. USDA Forest Service, Intermountain Research Station, Ogden, UT.

Sakai, AK, FW Allendorf, JS Holt, DM Lodge, J Molofsky, KA With, S Baughman, RJ Cabin, JE Cohen, NC Ellstrand, DE McCauley, P O'Neil, IM Parker, JN Thompson, SG Weller 2001 The population biology of invasive species. Annual Review of Ecology and Systematics 32: 305-332.

Schachner, LJ, RN Mack, SJ Novak 2008 *Bromus tectorum* (poaceae) in midcontinental United states: Population genetic analysis of an ongoing invasion. American Journal of Botany 95: 1584-1595.

Schoen, DJ, AHD Brown 1991 Intraspecific variation in population gene diversity and effective population size correlates with the mating system in plants. Proceedings of the National Academy of Sciences of the United States of America 88: 4494-4497.

Selby, AD. 1906. A second Ohio weed manual. Vol. Bulletin 175. Ohio Agricultural Experiment Station, Columbus, Ohio.

Shaw, RE 1993 Canals for a nation: The canal era in the united states, 1790-1860. University Press of Kentucky, Lexington.

Shea, K, P Chesson 2002 Community ecology theory as a framework for biological invasions. Trends in Ecology & Evolution 17: 170-176.

Siol, M, JM Prosperi, I Bonnin, J Ronfort 2008 How multilocus genotypic pattern helps to understand the history of selfing populations: A case study in *Medicago truncatula*. Heredity 100: 517-525.

Soltis, DE, CH Haufler, DC Darrow, GJ Gastony 1983 Starch-gel electrophoresis of ferns - a compilation of grinding buffers, gel and electrode buffers, and staining schedules. American Fern Journal 73: 9-27.

Squirrell, J, PM Hollingsworth, RM Bateman, JH Dickson, MHS Light, M MacConaill, MC Tebbitt 2001 Partitioning and diversity of nuclear and organelle markers in native and introduced populations of *Epipactis helleborine* (orchidaceae). American Journal of Botany 88: 1409-1418.

Stebbins, GL 1957 Self fertilization and population variability in the higher plants. American Naturalist 91: 337-354.

Strausbaugh, PD, EL Core 1930 Some additions to the Millspaugh check-list of West Virginia spermatophytes. Proc. W. Va. Acad. Sci, West Virginia, USA. 38-48 pp.

Suarez, AV, ND Tsutsui. 2008. The evolutionary consequences of biological invasions. Molecular Ecology. 17: 351-360

Swofford, DL, RB Selander 1981 Biosys-1 - a FORTRAN program for the comprehensive analysis of electrophoretic data in population-genetics and systematics. Journal of Heredity 72: 281-283.

Tsutsui, ND, AV Suarez, DA Holway, TJ Case 2000 Reduced genetic variation and the success of an invasive species. Proceedings of the National Academy of Sciences of the United States of America 97: 5948-5953.

Upadhyaya, MK, R Turkington, D McIlvride 1986 The biology of Canadian weeds .75. *Bromus tectorum* L. Canadian Journal of Plant Science 66: 689-709.

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

Ward, SM, JF Gaskin, LM Wilson 2009 Ecological genetics of plant invasion: What do we know? Invasive Plant Science and Management 1: 98-109.

Watterson, GA 1984 Allele frequencies after a bottleneck. Theoretical Population Biology 26: 387-407.

Williamson, MH, A Fitter 1996 The characters of successful invaders. Biological Conservation 78: 163-170.

Workman, PL, JD Niswander 1970 Population studies on southwestern Indian tribes .2. Local genetic differentiation in Papago. American Journal of Human Genetics 22: 24-49.

Wright, S 1965 The interpretation of population-structure by f-statistics with special regard to systems of mating. Evolution 19: 395-420.

Yensen, DL 1981 The 1900 invasion of alien plants into Southern Idaho. Great Basin Naturalist 41: 176-183.

## 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 $\mathrm{H}_{obs}$ 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. | $N$ | $A$ | $\%P$ | $H_{\text{exp}}$ | $H_{\text{obs}}$ | $H_{\text{T}}$ | $G_{\text{ST}}$ |
|---|---|---|---|---|---|---|---|---|
| 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

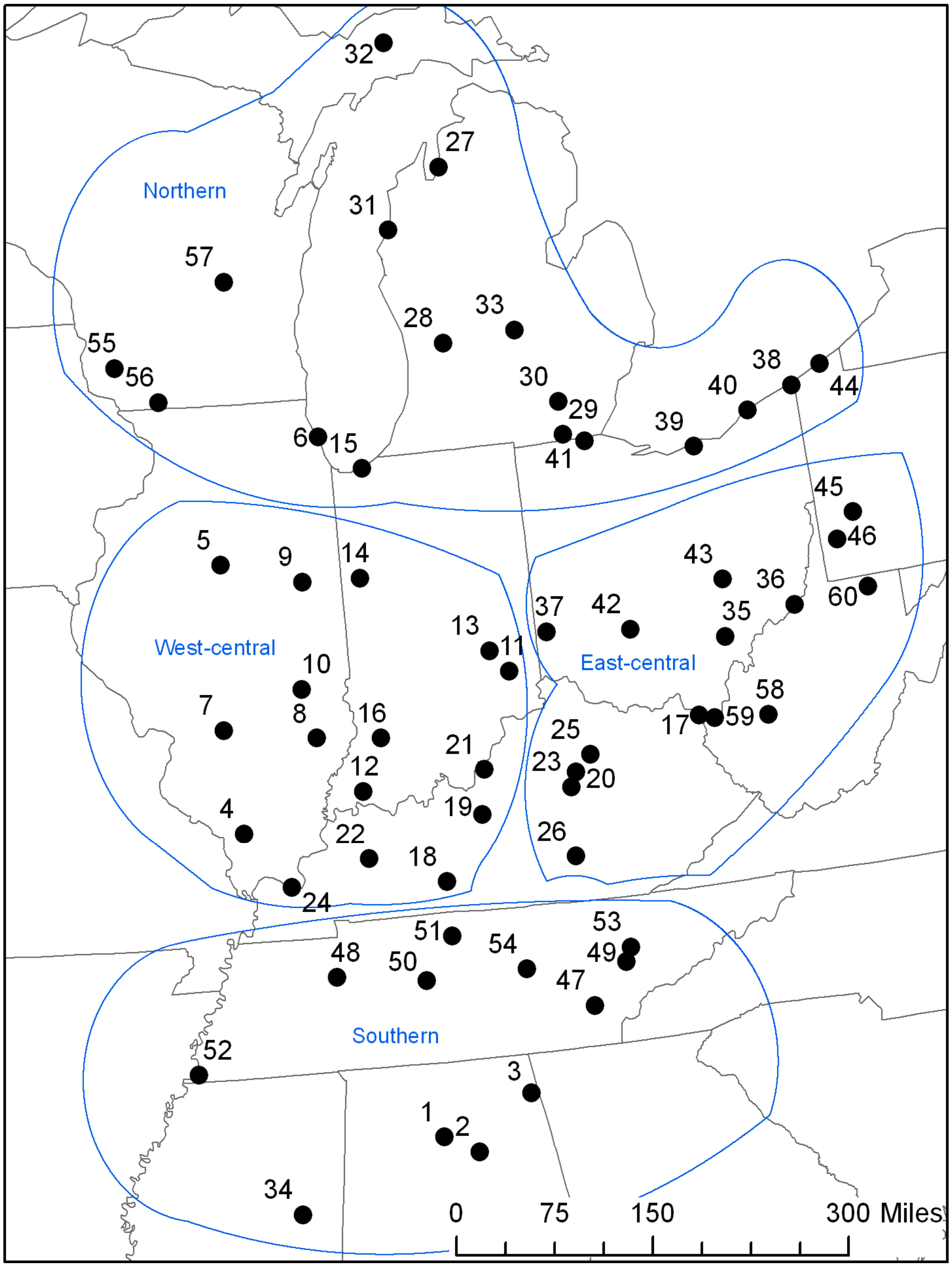


**Figure 1. Subregion boundaries and collection localities for the 60 Midwestern USA populations of *Bromus tectorum* analyzed in this study.**

## Figure 2

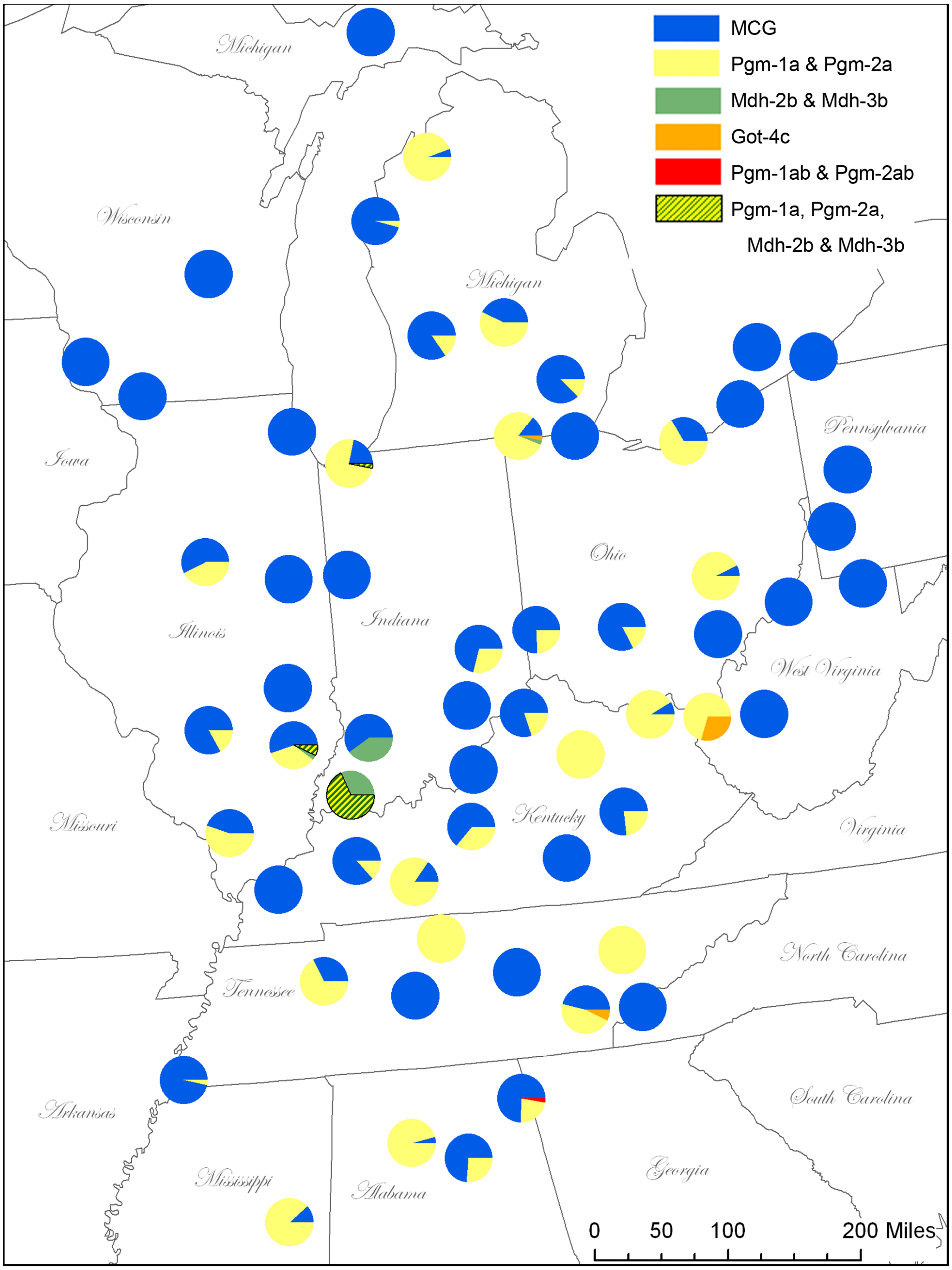


**Figure 2. Multilocus genotype distribution in the 60 Midwestern USA populations of *Bromus tectorum* analyzed in this study.**

# Figure 3

*(Part 1 of 2)*

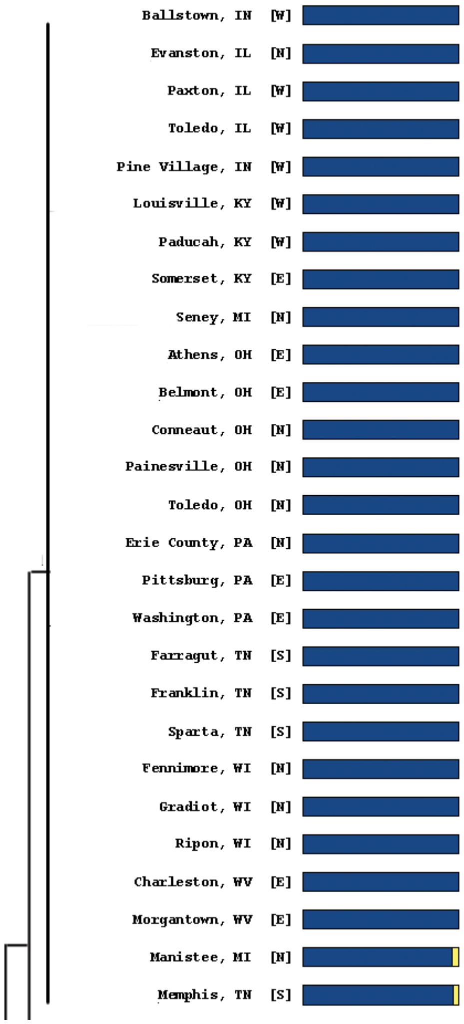
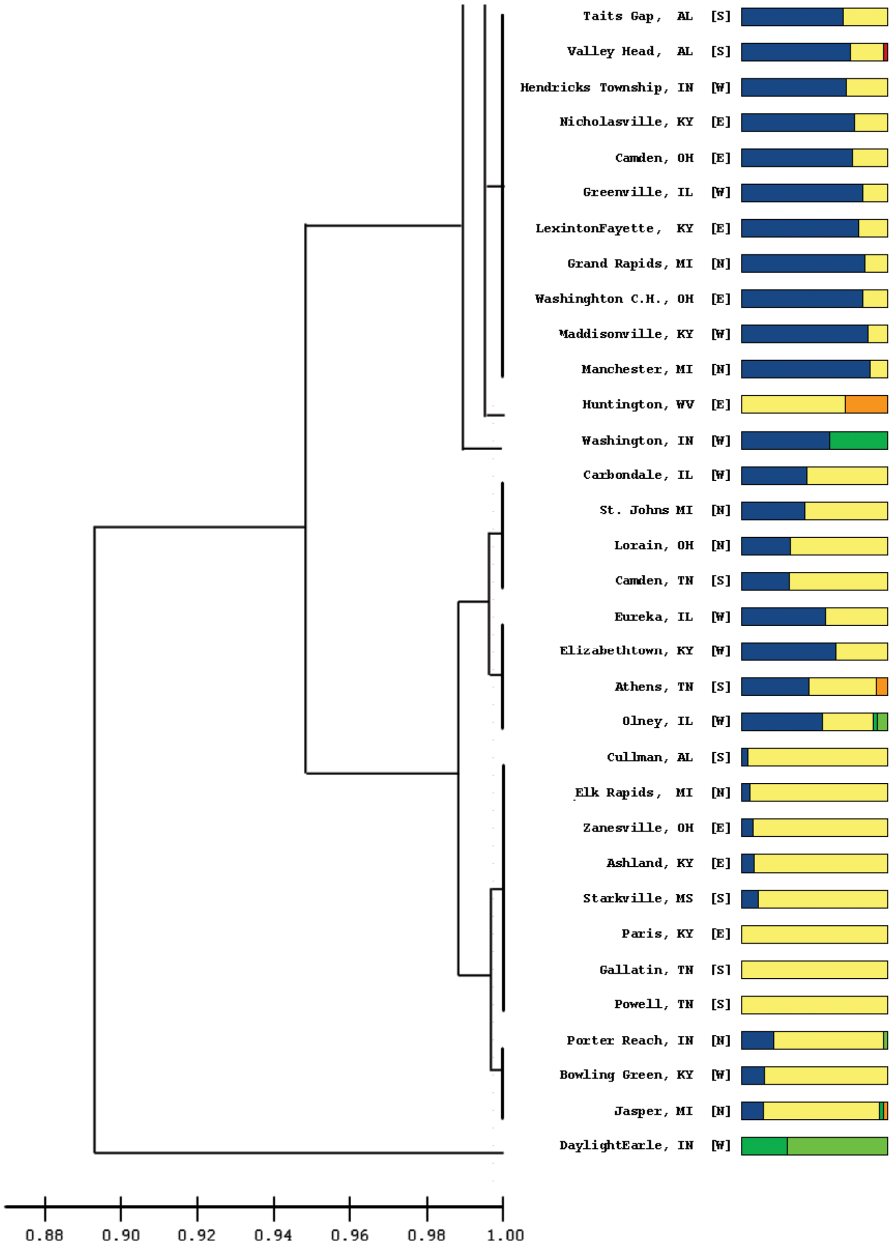


**Figure 3. UPGMA phenogram based on Nei's genetic identity (I) values for 60 Midwest populations of *Bromus tectorum***

# 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 |

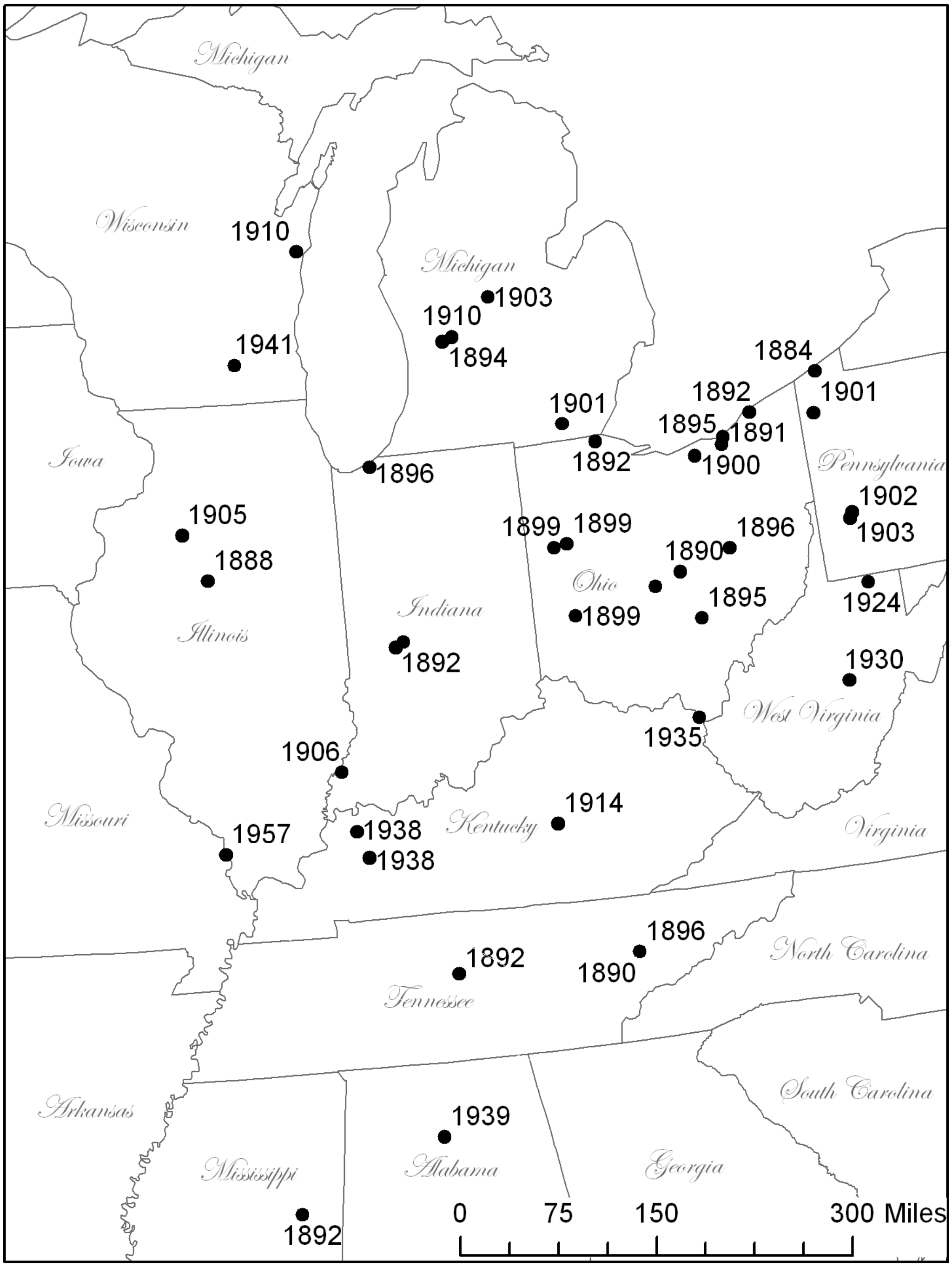
| State | Locality | Year | Source |
|---|---|---|---|
| | Ft. Atkinson | 1941 | NY |
| West Virginia | Morgantown | 1924 | US |
| | unspecified | 1930 | Core 1940 |

*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 |