IDENTIFYING THE GEOGRAPHIC ORIGINS FOR THE INTRODUCTION OF *TAENIATHERUM CAPUT-MEDUSAE* SUBSP. *ASPERUM* (MEDUSAHEAD) IN THE WESTERN UNITED STATES

by

Shane Kurtis Skaar

A thesis

submitted in partial fulfillment

of the requirements for the degree of

Master of Science in Biology

Boise State University

August 2015

BOISE STATE UNIVERSITY GRADUATE COLLEGE

DEFENSE COMMITTEE AND FINAL READING APPROVALS

of the thesis submitted by

Shane Kurtis Skaar

Thesis Title: Identifying the Geographic Origins for the Introduction of *Taeniatherum caput-medusae* subsp. *asperum* (Medusahead) in the Western United States

Date of Final Oral Examination: 08 June 2015

The following individuals read and discussed the thesis submitted by student Shane Kurtis Skaar, and they evaluated his presentation and response to questions during the final oral examination. They found that the student passed the final oral examination.

Stephen Novak, Ph.D.	Chair, Supervisory Committee
James F. Smith, Ph.D.	Member, Supervisory Committee
René Sforza, Ph.D.	Member, Supervisory Committee

The final reading approval of the thesis was granted by Stephen 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.

DEDICATION

To my family.

ACKNOWLEDGEMENTS

I am grateful for my Major Professor, Dr. Stephen Novak, and his willingness to energetically instruct and patiently mentor. Thank you for the time you invested in my instruction. I'm grateful for the hours spent advising me through the entirety of this research, including: plant collection trips, lab work, analysis, presentations and write up. I hope to one day follow in your footsteps and outpace anyone on the greenbelt and everyone in the workplace. I have had very few opportunities to associate with men that show such intelligence, kindness, and willingness to teach those who seek understanding. Thanks to my other committee members Dr. Jim Smith for your comments and support.

I am indebted to Dr. René Sforza for provisioning the samples that made this study possible and for his comments throughout. Thank you to Carly Prior and Alyssa Wepplo for assistance with the laboratory analysis.

I would like to give a special thanks to my wife, Krystal, for her patience and support during my time in the graduate program at BSU. She has played an integral role to my success here.

Finally, thanks to all of the organizations that contributed funding to this research: Boise State University and the United States Department of Agriculture European Biological Control Laboratory.

ABSTRACT

The use of molecular markers can provide insights into the demographic and evolutionary processes that have shaped the genetic diversity of native populations and can be used to identify an invasive species' geographic origins. *Taeniatherum caput-medusae* subsp. *asperum* (medusahead) is a cleistogamous, diploid, annual grass native to Eurasia that is now invasive in the western United States (U.S.). Enzyme electrophoresis methods (allozymes) have previously been used to analyze both native and invasive populations of medusahead. Results from these studies suggest that the invasion of medusahead in the western U.S. stems from multiple introduction events. In addition, 10 of 34 populations from across the native range of the species possessed multilocus genotypes that match some of those detected in invasive populations, with six of these putative source populations located in Greece and Northwestern Turkey. The overall objective of the current study is to better circumscribe the geographic origins for this invasion through allozyme analysis of 48 native populations of medusahead from Southeastern Europe (Albania, Bulgaria, Greece, Macedonia, Romania, Serbia, Northwestern Turkey, and Ukraine) and South-central Turkey. Among the 48 native populations I analyzed, a total of 35 multilocus genotypes were detected, with four of these genotypes matching those previously reported among invasive populations. Forty of the 48 (83.3%) native populations contained at least one individual with a multilocus genotype matching a genotype reported among invasive populations. The 48 populations from Southeastern Europe and South-central Turkey exhibit less genetic structure and

display lower levels of genetic diversity compared with the 34 native populations previously analyzed. Also, the genetic diversity of these 48 populations is not geographically structured; it does not conform to an isolation-by-distance pattern. Taken together, results from this study suggest that the geographic origins of this invasion occur broadly across the study region. In addition, the genetic diversity of these 48 native populations appears to be influenced by stochastic demographic processes in which an individual or individuals with various genotypes randomly colonizes disturbed sites and establishes a population. This process has led to an intermixing of genotypes within and among populations across the study area. Because allozymes typical underestimate the genetic diversity of populations, the findings of this study should be assessed using a molecular marker with greater resolving power (i.e., amplified fragment length analysis).

Keywords: allozymes, putative source populations, stochastic demographics, genetic diversity, genetic structure, multilocus genotype

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Biological invasions occur when organisms are introduced into a new range or habitat where they establish, proliferation, and spread (Mack et al. 2000). Human activities such as international commerce, trade, and migration have led to the deliberate or accidental introduction of species into novel locations around the world (Crosby 1986; Sakai et al. 2001). Invasions are often associated with severe ecological consequences such as loss of native biological diversity and community structure (and in extreme cases, the extinction of native species) (Mack et al. 2000; Allendorf and Lundquist 2003), modification of ecosystem processes such as nutrient cycling and productivity patterns (Vitousek and Walker 1989), and alteration of disturbance regimes, especially the frequency of wildfires (D'Antonio and Vitousek 1992). Invasive species also have enormous economic costs as a result of attempts to control these species, rehabilitate and restore damaged area, and reduced agricultural productivity (Chapin et al. 2000; Keller and Taylor 2010; Mack et al. 2000; Sakai et al. 2001). Pimentel et al. (2005) estimated that there are approximately 50,000 non-native species in the United States (U.S.), with the economic impacts of invasive species in the U.S. estimated to be $120 billion annually. In addition, the human health costs associated with biological invasions vary in effect and intensity and include increases in infectious disease vectors, allergic reactions, and smoke-induced asthma (D'Antonio and Vitousek 1992; Fumanal et al. 2005; Scholte et al. 2014). Thus, biological invasions, along with habitat destruction, are considered to be two of the main drivers of global change, and represent two of the greatest threats to

biodiversity around the globe (Wilcove et al. 1988). Moreover, Sala et al. (2000) suggest that areas with Mediterranean-like climates and grassland biomes are likely to experience some of the greatest losses in biodiversity because many global change drivers are occurring in these two ecosystems (Sala et al. 2000).

Due to their negative consequences, efforts have been undertaken to better understand biological invasions and to better predict which species will become invasive (Mack 1996; Rejmánek 2000; Richardson and Pysek 2006) and which communities are more likely to be invaded (Rejmánek et al. 2005; Shea and Chesson 2002). As summarized by Hierros et al. (2005), many hypothesis have been proposed to explain the success of introduced species in their new ranges: the enemy release hypothesis (Colautti et al. 2004; Liu and Stiling 2006), evolution of increased competitive ability (EICA, Blossey and Notzold 1995), evolution of invasiveness (Lee 2002), the empty niche hypothesis (Elton 1958; MacArthur 1970), and novel weapons (Callaway and Aschehoug 2000). Recent research with invasive species has focused on gaining a better understanding of the invasion process; consequently propagule pressure (the number of propagules arriving in the new range) has now emerged as one of the best predictors of establishment success of non-native species and a factor contributing to invasion (Kolar and Lodge 2001; Lockwood et al. 2005; Colautti et al. 2006; Novak 2011; Ricciardi et al. 2011; Simberloff 2009). In addition, the analysis of both native and invasive populations within the same experimental design can be used to assess various ecological, genetic, and evolutionary aspects of biological invasions (Bossdorf et al. 2005; Hierros et al. 2005; Novak 2007).

Most importantly, the combined analysis of native and invasive populations using genetic markers allow investigators to determine the geographic origins (source populations or regions) of an invasive species (Novak and Mack 1993, 2005; Facon et al. 2003; Maron et al. 2004; Novak 2007, 2011; Lavergne and Molofsky 2007; Ficetola et al. 2008; Wilson et al. 2009; Estoup and Guillemaud 2010; Gaskin et al. 2011, 2013; Lombaert et al. 2011). The accurate identification of the geographic origins of an invasive species provides researchers with 1) a clarification of taxonomy and evolutionary relationships, 2) the ability to identify a cryptic invasion (detection of closely related species that cannot be distinguished morphologically), 3) an assessment of the introduction dynamics of an introduced species (single vs. multiple introductions), 4) the relevant comparison for assessing the genetic consequences of introduction (e.g., founder effects), 5) detection of hybridization events, 6) the ability to study the ecology of source populations in their native habitat, and 7) information for developing effective management or control strategies (e.g., the search for biological control agents).

Taeniatherum caput-medusae (L.) Nevski (medusahead, Poaceae) is a primarily cleistogamous (self-pollinating), diploid (2n = 14), annual grass that is broadly distributed across Eurasia (Frederiksen 1986; Frederiksen and von Bothmer 1986). *Taeniatherum caput-medusae* is a member of the "wheat tribe" (Triticeae), and is therefore related to the common agricultural crops such as wheat, rye, and barley (Widmer and Sforza 2004). The native range of *T. caput-medusae* includes semi-arid habitats of southern Europe (Figure 1), the northern rim of Africa, the Middle East, and Central Asia (Frederiksen 1986; McKell et al. 1962). In its native range, three subspecies have been recognized (Figure 2) (Frederiksen 1986; Frederiksen and von Bothmer 1986):

T. caput-medusae (L.) Nevski subsp. *caput-medusae*, *T. caput-medusae* subsp. *crinitum* (Schreb.) Melderis, and *T. caput-medusae* subsp. *asperum* (Simk.) Melderis. The three subspecies do exhibit different geographic distributions, although some overlap does occur. In general, subsp. *caput-medusae* is found in the western Mediterranean (Morocco, Portugal, Spain, and France), subsp. *crinitum* occurs from Southeastern Europe and the Eastern Mediterranean to Central Asia (Kyrgyzstan, Tajikistan, and Afghanistan) and subsp. *asperum* is found across almost the entire Eurasian native distribution of the species (Frederiksen 1986).

Within its native range, *T. caput-medusae* appear to be a weedy, early-colonizing plants following disturbance and is an agronomic agricultural weed (Pineda et al. 1981; Kostivkovsky and Young 2000; Blank and Sforza 2007). Compared to the other two subspecies however, subsp. *asperum* may be the best of the three at colonizing following disturbance (Signe Frederiksen, personal communication). In its native range, the grass has been observed in disturbed places such as roundabouts or roadsides (René F. H. Sforza, personal communication). *Taeniatherum caput-medusae* displaces other native grasses in the low-density woodlands of South-central Spain and occurs in greater frequency than many other native grasses after a disturbance event (Pineda et al. 1981). The species is listed as a weed in vineyards in southern France, but apparently does not cause much crop damage (Blank and Sforza 2007). In Turkey, *T. caput-medusae* subsp. *asperum* was commonly found in non-disturbed soil, in wild areas or in abandoned agricultural fields (e.g., vineyards) (Sforza and Cristofaro 2002).

Based on the examination of plants in the native and invasive ranges, Major et al. (1960) suggested that the taxon introduced into and invasive in the western U.S. was *T.*

caput-medusae subsp. *asperum*, hereafter referred to as (medusahead) (Young 1992; Kostivkovsky and Young 2000). In the western U.S., medusahead occurs in disturbed sites in the 25-100 cm mean annual precipitation zones, and it can dominate sites with high clay content or well-developed soils (Dahl and Tisdale 1975; Hironaka 1994). The grass has invaded millions of hectares of semi-arid woodlands and shrub-steppe habitats in California, Idaho, Nevada, Oregon, Utah, and Washington (McKell et al. 1962; Young and Evans 1970; Young 1992; Pellant and Hall 1994; Blank and Sforza 2007). The grass has a well-known collection history (McKell et al. 1962; Young 1992); it was first collected in the western U.S. in Roseberg, OR in 1887. Medusahead is now rapidly spreading into areas where it did not previously occur, and it has degraded these newly infested rangelands (Horton 1991; Hironaka 1994). For example, the extent of medusahead in the 36 counties of Oregon increased from 18 to 31 between 1962 and 2004 (Davies and Johnson 2008) and the rangelands in Idaho infested by medusahead more than doubled between 1957 and 1992 (Hironaka 1994).

The genetic diversity of 45 invasive populations of medusahead from the western U.S. has been described by Novak (2004), and Novak and Rausch (2009), Novak and Sforza (2008). Over 1660 individuals were scored for their multilocus genotypes at 29 allozyme loci. A total of 7 homozygous multilocus genotypes (MLG) were detected, four of which were associated with early collection sites (1887, Roseburg, Oregon; 1901, Steptoe Butte, Washington; 1930, Rattlesnake Station, Idaho; 1944, Ladd Canyon, Oregon). Genetic diversity within populations was low, but 17 of 45 populations (37.8%) were genetically polymorphic. These results suggest that the invasion of this subspecies in the western U.S. stem from multiple introductions, and that some invasive

populations may be genetic admixtures. The allozyme analysis of 34 native populations of medusahead confirmed these findings (Peters 2014). In addition, ten of the 34 native populations analyzed in this study may be classified as putative source populations, with six populations from Greece and Northwestern Turkey possessing five of the seven multilocus genotypes previously detected in the western U.S. These data indicate that more comprehensive genetic analysis of medusahead populations from Southeastern Europe (Albania, Bulgaria, Greece, Macedonia, Romania, Serbia, Northwestern Turkey, and Ukraine) and South-central Turkey are required to better circumscribe the geographic origins of this invasion.

The overall goal of this study is to use allozyme data to more precisely identify the geographic origins of the invasion of medusahead in the western U.S. This goal will be accomplished by achieving the following specific objectives: 1) assess which of the 48 populations of medusahead from Southeastern Europe analyzed here has at least one individual that matches one of the seven multilocus genotypes previously reported within invasive Western U.S. populations, 2) determine the level of genetic diversity within these 48 native populations, and 3) determine the genetic and geographic structure of these native populations. Results of these analyses will provide insights into the demographic and evolutionary processes that have influenced the level and structure of genetic diversity within and among native populations of medusahead in Southeastern Europe and South-central Turkey.

METHODS

Plant Collections

Mature spikes with caryopses were collected from medusahead plants in 48 populations from eight countries in Southeastern Europe (Albania, Bulgaria, Greece, Macedonia, Romania, Serbia, Northwestern Turkey, and the Crimean Peninsula of the Ukraine) and South-central Turkey (Table 1, Figure 3). Samples from nine populations in Bulgaria were collected during October 2010, and samples for the remaining 39 populations were collected during June and July in 2011 and 2013 (Table 1). Spikes from each of 20-30 plants 1-3 m apart were sampled haphazardly. Sufficient spacing between collected plants reduces the possibility of collecting full siblings. In populations with fewer than 20 individuals, the spikes from all individuals were collected. Population sample sizes in this study ranged from seven individuals in the population from Sudak, Ukraine, to 38 individuals in the population from Seydishir, Turkey (Table 1). Spikes with caryopses from each plant were placed in individually numbered paper envelopes, and envelopes were stored at room temperature until laboratory analyses were conducted. Collections were generally made in disturbed areas (e.g., roadsides, dump sites, on the border of agricultural fields, within abandoned agricultural fields, and areas which have previously experienced wildfires), although the degree and frequency of disturbance appeared to vary among sites (S.J. Novak and R.F.H. Sforza, personal observations).

Enzyme Electrophoresis

Caryopses of medusahead were stored in the laboratory for approximately three months to allow for after-ripening. After this time period, one caryopsis from each individual in a population was germinated at room temperature in petri dishes lined with moistened filter paper. Most caryopses germinated in 24 h, and seedlings were harvested approximately 7-10 d after germination, when seedlings were about 5 cm tall. Entire seedlings (root and leaf tissues) were macerated in a Tris-HCl grinding buffer-PVP solution (pH 7.5). Starch concentration in the gels was 12% (w/v). Genetic analysis was performed using enzyme electrophoresis (allozymes), following the methods of Soltis et al. (1983), with modifications described by Novak et al. (1991) and Peters (2014). All plants were assessed for their allozyme diversity for 15 enzymes, which were visualized using four buffer buffer systems: buffer system 1, isocitrate dehydrogenase (*Idh*), glucose-3-phosphate dehydrogenase (*G6pdh*), and shikimate dehydrogenase (*Skdh*); buffer system 6, alcohol dehydrogenase (*Adh*), glutamate dehydrogenase (*Gdh*), and phosphoglucoisomerase (*Pgi*); buffer system 8, aldolase (*Ald*), glutamate oxalacetate transaminase (*Got*), colorimetric esterase (*Ce*), malic enzyme (*Me*), superoxide dismutase (*Sod*), and triosephosphate isomerase (*Tpi*); and buffer system 9, malate dehydrogenase (*Mdh*), phosphoglucomutase (*Pgm*), and 6-phosphogluconate dehydrogenase (*6Pgd*). Because medusahead is a diploid with low genetic diversity, the genetic basis of all allozyme variation observed was easily inferred based on the known subunit structure and compartmentalization of these enzymes (Gottlieb 1982; Wendel amd Weeden 1989). All individuals were assessed for variability at 23 putative loci, following the nomenclature

of the previous allozyme analysis of 34 native populations of medusahead conducted by Peters (2014).

Multilocus Genotype Assignment and Geographic Origins

Identifying the geographic origins (source populations) of an invasive species can be done with molecular markers using two methods: a phylogenetic approach and a multilocus genotype (MLG) approach (Roderick and Navajas 2003; Keller and Taylor 2008; Novak 2011). In this study, I will use the MLG approach. Based on the different alleles present at all polymorphic loci, each medusahead individual from Southeastern Europe analyzed in this study was assigned a MLG. The MLGs of these 48 native populations will be compared to the seven homozygous MLGs previously identified within the 45 invasive populations of medusahead from the western U.S. (Novak 2004; Novak and Sforza 2008; Novak and Rausch 2009). If any of the 48 native populations contains at least one individual with a MLG that matches one of the seven genotypes previously detected among western U.S. populations, they will be considered a putative source population.

DATA ANALYSIS

Genetic Diversity within Populations

Allozyme (genetic) diversity within the 48 medusahead populations from Southeastern Europe and South-central Turkey was analyzed with the program POPGENE 1.32 (Yeh and Boyle 1997). Allozyme data for each individual, in each population, were entered as multilocus genotypes. Genetic diversity was quantified using the following parameters: the mean number of alleles per locus (*A*), the number of polymorphic loci within each population (#*P*), the percent polymorphic loci per population *(%P*), the expected mean heterozygosity (H_{exp}), which was calculated using the unbiased estimate method of Nei (1978), the mean observed heterozygosity (H_{obs}), and the number of multilocus genotypes detected with each population (#MLG). The means of these genetic diversity parameters were used to describe the overall diversity within the 48 populations from the study area. Using the JMP Version (SAS Institute Inc.) statistical software package a non-parametric Spearman p-test was conducted to confirm that the sample size and mean number of alleles per locus were not correlated.

Genetic Structure Among Populations

FSTAT version 2.9.3.2 was used to calculate Nei's (1987) estimators of gene diversity and genetic differentiation (Goudet 2001). Allelic diversity within and among populations was calculated using the arithmetic mean of Nei's gene diversity statistics at each of the polymorphic loci. FSTAT calculated the total genic (allelic) diversity (H_T) and the value of H_T was partitioned into the within-population component (H_S) and the

among-population component (D_{ST}); thus, $H_T = H_S + D_{ST}$. H_S is calculated as the mean of H_e values over all populations, where H_e is the expected proportion of heterozygosity per individual (Nei 1973). G_{ST} is calculated from the total genetic diversity in pooled populations, H_T and the mean diversity within each population H_S, such that $G_{ST} = H_T - H_S/H_T$.

Analysis of molecular variance (AMOVA) was employed to estimate the amount of genetic variation partitioned within populations and among regions using the F-statistics method in ARLEQUIN v.3.1 (Excoffier et al. 2005). Allozyme data were entered as pseudo-haplotype frequencies for each population and structured geographically into eight sub-regions by country (Albania, n = 2; Bulgaria , n = 15; Greece, n = 5; Macedonia, n = 3; Romania, n = 4; Serbia, n = 1; Turkey, n = 11; Ukraine, n = 7), and two geographic regions. All individuals with missing data were removed from the analysis.

Neighbor-joining trees and unweighted pair-group method with arithmetic averaging algorithm (UPGMA) phenograms are two methods commonly used to graphically represent the genetic differentiation among populations. The UPGMA method assumes that all population or lineages evolve at the same rate, while the neighbor-joining tree does not assume equal evolutionary rates for each lineage, and thus does not force the branch lengths to be equal in length. Because I cannot accurately infer evolutionary rates from allozyme data, genetic differentiation among the 48 populations of medusahead analyzed in this study was displayed using an UPGMA phenogram. The phenogram was created based on Nei's (1978) unbiased genetic identity values calculated in POPGENE.

Allozyme data were employed to assess population genetic structure based on the Baysian clustering approach in STRUCTURE 2.3.X (Pritchard et al. 2000a) (Pritchard et al. 2000; Falush et al. 2003, 2007; Hubisz et al. 2009). The initial analysis to find an estimate for the "true" value of K was ran based on 10 replicates of values of K ranging from 1-10, and these runs had a burn in of 10,000 iterations, followed by parameter estimation over an additional 100,000 Markov Chain Monte Carlo (MCMC) repetitions. This initial analysis resulted in an equivocal estimate of K, so another analysis in STRUCTURE was conducted. This second analysis consisted of 10 replicates of values of K ranging from 1 to 6, with three replicates were run for values of K ranging from 7 to 14. This second run had a burn-in of 100,000 iterations, followed by parameter estimation over an additional 1,000,000 MCMC repetitions. A graphical representation of the STRUCTURE output was generated using the STRUCTURE HARVESTER software hosted on (http://taylor0.biology.ucla.edu/structureHarvester/#) (Earl and VonHoldt 2012). Two methods were employed in STRUCTURE HARVESTER to evaluate the most likely number of population clusters (K) based on allozyme data: calculating the delta K values (Evanno et al. 2005) for each value of K, and identifying the K value that maximizes the log probability of the data, lnP(D), for each value of K (Pritchard et al. 2000).

Results of the STRUCTURE analysis were visualized using DISTRUCT (Rosenberg 2004). The DISTRUCT output provides a convenient way of displaying genetic structure results by depicting each individual as a line segment within the population. The individual line segment is partitioned into K-colored components that represent the individual's estimated membership coefficient(s) for the number of clusters

detected. The estimated membership coefficients are extracted directly from the STRUCTURE population Q-matrix (Appendix A).

Geographic Structuring of Genetic Diversity

I tested for an isolation-by-distance (IBD) relationship among the 48 populations of medusahead analyzed in the study using a Mantel test of the correlation between genetic and geographic distances. Genetic distance values were based on the population pairwise F_{ST} values computed in ARLEQUIN v. 3.1 (Excoffier et al. 2005). ARLEQUIN computes pairwise F_{ST} values for all pairs of populations and from these values the program computes an index of dissimilarities (genetic distance), which describes the "short term" genetic distance between population pairs (Reynolds et al. 1983; Slatkin 1995). The geographic distance (km) between populations was exported from ArcGIS 10.2.2 software after using the point distance tool. Google Earth was used to confirm the location of population and distances between populations. The following parameters were used in ArcGIS: Projected coordinate system (Europe Lambert conformal conic), Projection (Universal Transverse Mercator (UTM)), false easting (500000.0), false northing (0.00), central meridian (27.00), and linear unit (meters). The projected coordinate system used was World Geodetic System 1984, Zone 35 North. The matrix of genetic distance (F_{ST}) and geographic distance (km) values for each population pair was uploaded into the Isolation by Distance Web Service (IBDWS, Version 3.23), and 30,000 randomization of the genetic distance and geographic distance data were run to produce a graph of the correlation between the two variables (Jensen et al. 2005).

RESULTS

Genetic diversity estimates for the 48 populations of medusahead from Southeastern Europe and South-central Turkey are based on the analysis of 1084 individuals (22.6 individuals per population), at 23 putative allozyme loci (Table 2). Population sample sizes in this study ranged from seven individuals in the population from Sudak, Ukraine, to 38 individuals in the population from Seydishir, Turkey. Among all 48 populations, 35 alleles were detected (1.52 alleles/locus) and seven loci (30.4%) are polymorphic: *Mdh-2, Mdh-3, Pgi-2, Got-1, Got-2, 6Pgd-2, and Idh* (Table 2). Across all populations, allelic diversity at the seven polymorphic loci varies from two alleles at *Mdh-3, Got-1*, *Got-2* and *Idh*, to three alleles at *Pgi-2* and *6Pgd-2*, and five alleles at *Mdh-2*.

Multilocus Genotype Diversity

Because seven of the 23 scored loci analyzed were polymorphic and no variability was detected for the remaining 16 loci (Table 2), only allelic variability at these seven loci contributed to the multilocus genotypes (MLGs) I detected. In addition, I did not observe any heterozygous individuals, thus I only detected homozygous MLGs. Among the 48 populations and 1084 individuals of medusahead from Southeastern Europe and South-central Turkey analyzed, a total of 35 MLGs were detected (Table 3). The most common MLGs in this study were #1 and #3, which occurred in 27 and 26 populations, respectively (Table 3, Table 4). Both MLGs #2 and #4 were detected in 14 different populations. Sixteen MLGs were unique, occurring in only a single population: #6, #8,

#11, #12, #13, #18, #19, #20, #24, #25, #26, #28, #29, #31, #34, and #35 (Table 3). These 16 unique MLGs occurred in 11 populations: Izorsko and Razlog, Bulgaria; Sapes, Greece; Akseki, Ipsala, Kesan, and Yalihuyuk, Turkey; Alushta, Izobilne, Pryvitne and Trudolybivka, Ukraine (Table 4). The remaining 15 MLGs occurred in two to nine populations.

The 48 populations of medusahead I analyzed contained, on average, 3.19 MLGs/population (Table 4). The population from Pryvitne, Ukraine, contained the largest number of MLGs (11), with five of 11 genotypes being unique to this population (MLG #25, 26, 28, 34, & 35) (Table 4). Eight different MLGs were detected in the Sozpol, Bulgaria, and the Ipsala, Turkey, populations; while 10 populations had just a single (but not the same) MLG: Korca and Struga, Albania; Kokinchoma, Greece; Sacele, Romania; Kladovo, Serbia; Corlu, Havsa, and Urunlu, Turkey; and Sudak and Yalta, Ukraine (Table 4). The 15 populations from Bulgaria had the most MLGs: an average of 4.2 MLGs/population. The populations from Ukraine (3.6 MLGs/population) and Turkey (3.1 MLGs/population) having the next highest number of MLGs; whereas, the two populations from Albania, and the one population from Serbia average only one MLG/population.

Geographic Origins: Identifying Source Populations

Putative source populations among the 48 populations of medusahead from Southeastern Europe and South-central Turkey can be identified when at least one individual has a MLG that matches one of the seven genotypes previously detected among the 45 invasive populations of medusahead from the western U.S. (Novak 2004; Novak and Sforza 2008; Novak and Rausch 2009). Four of the 35 MLGs detected among

these 48 native populations match an invasive MLG: MLG #1 corresponds to the Rattlesnake Station, Idaho genotype; MLG #2 corresponds to the Ladd Canyon, Oregon genotype; MLG #3 corresponds to the Steptoe Butte, Washington genotype; and MLG #4 corresponds to the Roseburg, Oregon genotype (Table 3). These four MLGs have the highest level of occurrence among the 48 native populations in the study area; and as a consequence of this widespread distribution, 40 of the 48 (83.3%) populations in this region are considered putative source populations (Table 4, Figure 4). Thus, the geographic origins for the invasion of medusahead in the western U.S. appear to have been drawn broadly from across the study region, and include one population from Albania, 13 populations from Bulgaria, four populations from Greece, three populations from Macedonia, four populations from Romania, 10 populations from Turkey, and five populations from the Crimean Peninsula of Ukraine. Sixteen of the 40 putative source populations (40%) contained one MLG that matched a genotype from the western U.S., 12 of 40 populations (30%) had two MLGs that matched genotypes from the invasive range, 8 of 40 populations (20%) had three MLG matches, and four of 40 populations (10%) contained four MLG matches (Table 4, Figure 4). Four of the 7 homologous MLGs detected in the invasive populations were found in four populations from Southeastern Bulgaria (Dripchevo, Harmanli, and Tenevo) and northwestern Turkey (Ipsala) (Figure 4).

The Rattlesnake Station and Steptoe Butte MLGs are generally found throughout the study area, with the exception that the Rattlesnake Station MLG, which was not detected in the three populations from South-central Turkey. The 14 populations with the Ladd Canyon MLG were only detected in Southeastern Bulgaria and Northwestern

Turkey; whereas the 14 populations containing the Roseburg MLG were found in Southeastern Bulgaria, Northwestern Turkey, South-central Turkey, and the Crimean Peninsula of Ukraine (Figure 4).

Genetic Diversity Within Populations

Thirty-eight of the 48 populations (79.1%) from the study area are genetically polymorphic (exhibit at least two alleles at one or more loci), while the remaining 10 populations are monomorphic at all 23 scored loci (Table 2, Table 5). On average, these 48 populations display 1.10 alleles per locus (*A*). The number of polymorphic loci in these populations range from 0 to 4 (mean = 2.08) and the values of %P range from 0.0 to 17.4, with an average of 9.05 per population (Table 5). The population from Pryvitne, Ukraine contained the highest genetic diversity (*A* = 1.30 and %*P* = 17.4). The genetic diversity of the populations from Sozopol, Bulgaria (*A* = 1.26 and %*P* = 17.4), and Razlog, Bulgaria, Ipsala, Turkey and Yorukler, Turkey are also quite high (*A* = 1.22 and %*P* = 17.4), compared with the other populations I analyzed.

Across all 48 populations, the expected mean heterozygosity (H_{exp}), which is also described as the expected genetic diversity within populations, is 0.029 (Table 5). The highest value of H_{exp} was detected in the Sozopol, Bulgaria population (H_{exp} = 0.077), with the populations from, Pryvitne, Ukraine, Yorkler, Turkey, Devnja, Bulgaria, and Galabets, Bulgaria also having relatively high values for expected mean heterozygosity (H_{exp} = 0.075, 0.065, 0.065, and 0.063, respectively). The lowest value of H_{exp} for populations with polymorphic loci was detected in Schela, Romania, and Trudolybivka, Ukraine (H_{exp} = 0.005). No heterozygotes were detected among the 1084 individuals that

were each scored at 23 loci; thus, the mean observed heterozygosity (H_{obs}) for the 48 populations was 0.000 (Table 5).

Genetic Structure Among Populations

The mean value of Nei's (1987) total gene (allelic) diversity (H_T) averaged across all seven polymorphic loci is 0.248, the within-population component of gene diversity (H_S) is 0.100, the among-population component of gene diversity (D_{ST}) is 0.147, and the proportion of total diversity partitioned among populations (G_{ST}) is 0.417. These results indicate that 41.7% of the allelic diversity of the 48 populations analyzed in this study is distributed among populations (Table 6). *Mdh-2*, *Pgi-2*, and *Got*-1 are the three most polymorphic loci among the 48 populations sampled in the study region (Table 2), and consequently these loci have the highest values for the total gene diversity (H_T = 0.603, 0.493, and 0.359, respectively) (Table 6). In addition, most of the allelic diversity for these three loci is partitioned among populations (G_{ST} = 0.542, 0.667 and 0.621, respectively). For *Got-2* and *Idh* total gene diversity is low (H_T = 0.003 and 0.002), and most of the gene diversity at these two loci is partitioned within populations (G_{ST} < 0.014).

Analysis of molecular variance (AMOVA) in Arlequin 3.1 was used to partition genetic diversity within and among population and groups of populations from Southeastern Europe and South-central Turkey (Excoffier et al. 2005). In the first AMOVA analysis, genetic diversity was partition at three hierarchical levels, and the percentage of variation partitioned at the three levels was 0.00% within individuals, 40.5% within populations and 59.5% among populations (Table 7A). In the second AMOVA analysis, 38.8% of the genetic diversity was partitioned within populations,

49.6% of the diversity was partitioned among populations within the eight sampled countries, and 11.7% of the diversity was partitioned among the eight countries (Table 7B). For the final AMOVA analysis (Table 7C), genetic diversity was partitioned using natural physical barriers that may limit gene flow among populations among the two geographic regions. These natural barriers consist of three mountain ranges, the Balkan Gebirge (running east from the Black Sea, west to Sofia, Bulgaria), the Rhodope Mountains (which are located along the board of Greece and Bulgaria to the eastern-most portion of Greece) and the Rila Mountains (which run north to south and connect the Balkan Gebirge to the Rhodope Mountains), which separate populations into two geographic regions in the study area: populations from southeastern Bulgaria and northwestern Turkey and all other populations. This AMOVA analysis indicates that 39.3% of the genetic diversity is partitioned within populations, 59.6% of the diversity is among populations within the two regions, and 1.1% is partitioned among the two regions (Table 7C)

The UPGMA cluster dendrogram based on Nei's (1978) unbiased genetic identity values provides a graphic representation of genetic relationships among native populations of medusahead (Figure 5). The 48 populations in this analysis occurred in three distinct clusters; however, populations were not grouped based on their geographic locations. Cluster 1 includes populations from six countries (Bulgaria, Greece, Macedonia, Romania, Turkey, and Ukraine), and Cluster 2 has populations from seven countries (Albania, Bulgaria, Greece, Romania, Turkey, Serbia, and Ukraine). Both Cluster 1 and 2 contain a higher proportion of putative source populations compared with Cluster 3. Nine of the 14 (64%) populations with the Roseburg, Oregon MLG (MLG #4)

are found in Cluster 2 (Figure 5). In addition, Cluster 2 contains populations with unique MLG's, populations with 12 of the 16 (75%) unique MLG's detected in this analysis are found in Cluster 2 (see Table 4). The four populations in Cluster 3 are genetically distinct from populations in the other two clusters. For instance, the populations from Korca, Albania, and Kladovo, Serbia, are both fixed for MLG #15 (Table 4, Figure 5).

Genetic differences among the 48 populations were analyzed in STRUCTURE using a burn-in of 100,000 iterations, followed by parameter estimation over an additional 1,000,000 MCMC repetitions. The results of the log probability analysis suggest a K = 1 (Figure 6a), indicating that all 48 populations belong to the same genetic cluster; while the delta K analysis described by Evanno et al. (2005) supported K = 2 (Figure 6b). The K = 2 results from DISTRUCT display the proportion of each population that is assigned to the two genetic clusters (Figure 7). The 48 populations from the study area could not be consistently assigned to either of these two genetic clusters; however, the populations from Greece showed a high proportion of the genetic cluster shown in white.

Geographic Structuring of Genetic Diversity

A Mantel test was conducted to evaluate the correlation between F_{ST} values and geographic distance. The Mantel test was calculated with IBDWS and tested the null hypothesis that the correlation coefficient (R) is less than or equal to zero ($P<0.01$). The coefficient of determination (R^2) provided support that there was no correlation between the geographic distance and F_{ST} values for the 48 native populations ($R^2 = 0.0364$). The Isolation by distance correlation coefficient provided a very weak correlation between the two variables assessed during the Mantel test (R= 0.1908). These results indicate that

populations that are geographically close can have vastly different genetic diversity (e.g., Pryvitne, Ukraine has 11 MLGs and its neighboring population Sudak, Ukraine has only one MLG).

Comparison of Genetic Diversity Across and Within Native and Invasive Populations

Across populations, the 48 populations from Southeastern Europe and South-central Turkey exhibit fewer alleles and polymorphic loci compared to the 34 native populations previously analyzed by Peters (2014) (Table 8). Conversely, the 48 populations analyzed from the study area have a higher value for the "percentage of polymorphic populations" compared with the 34 native populations previously analyzed: 79.2 and 67.6, respectively. When compared with native populations, the 45 previously analyzed invasive populations consistently have lower values for all across-population genetic parameters. In general, similar patterns occur for the comparison of within-population genetic diversity parameters between the two groups of native populations and native and invasive populations (Table 9). However, the 48 populations I analyzed exhibited less genetic structure (G_{ST} 0.417), compared with either the 34 native populations previously analyzed (G_{ST} 0.745) or the 45 invasive populations (G_{ST} 0.906) (Table 9).

DISCUSSION

The combined analysis of native and invasive populations within the same experimental design provides insights into the invasion process (Bossdorf et al. 2005, Hierros et al. 2005; Novak 2011). Applying this approach using genetic marker data has allowed me to 1) identify the geographic origins (source populations) for the invasion of medusahead into the western U.S., 2) determine whether there is support for the multiple introduction of the grass in its new range, 3) assess the influence of founder effects in shaping the genetic diversity of medusahead in the western U.S., 4) generate a model describing a stochastic demographic processes that may have influenced the genetic diversity within and among native populations of the species in Southeastern Europe and South-central Turkey, and 5) obtain information that can be used to search for effective and specific biological control agents.

Geographic Origins: Identifying Source Populations

As a consequence of the analysis of these 48 populations of medusahead in Southeastern Europe and South-central Turkey, I identified 40 putative source populations in this region (Table 4, Figure 4). Collectively, these 40 populations contain four of the seven homologous MLG's previously reported among 45 invasive populations (Novak 2004; Novak and Sforza 2008; Novak and Rausch 2009). The Rattlesnake Station, Idaho MLG (MLG #1 in this study) and the Steptoe Butte, Washington MLG (MLG #3) were detected most often among putative source populations (Table 3, Table 4, Figure 4). Additionally, the Roseburg, Oregon MLG (MLG #4), which is the earliest

collection site of the grass in the U.S., has been detected in 14 populations within the study area. The Ladd Canyon, Oregon MLG (MLG #2) is associated with the invasive populations was also detected within 14 populations. While MLGs #1, #3, and #4 are distributed among populations across the study area (Figure 4), MLG #2 is primarily found within populations arrayed near the Bulgarian/Turkish border. Thus, putative source populations appear to be arrayed across much of Southeastern Europe and South-central Turkey. Alternatively, the four MLGs detected in this region also occur within each of four populations (Dripchevo, Harmanli and Tenevo, Bulgaria and Ipsala, Turkey); thus, only a handful of native populations could conceivably have served as source populations. While these results do not clearly indicate specific source populations for the invasion of medusahead in the western U.S., they do indicate that the geographic origins for this invasion may be broadly distributed across most of the study area.

Three MLGs detected within invasive populations in the western U.S., Malloy Prairie, Washington, Pullman, Washing and Salt Creek, Utah, each of which were found in only a single population, were not detected within the 48 populations analyzed in this study. While the Malloy Prairie MLG was not detected among the populations analyzed here, the allele that earmarks this MLG (*6Pgd-2a*) was detected within four populations from Bulgaria (Devnja, Harmanli, Izorsko, and Orizane) and one population from Macedonia (Umin Dol) (Table 2). The Pullman MLG was previously detected within the Ipsala, Turkey population by Peters (2014), but I did not observe this genotype among the individuals I analyzed from the very same locality. The individuals Peters (2014) analyzed were sampled during October 2010, and the individuals I analyzed were

sampled in July 2011 (Table 1). This difference may result from not sampling individuals in exactly the same places within this locality. Alternatively, these differences may indicate year-to-year genetic variability that can occur within small populations of an annual plant species (Ellstrand and Elam 1993).

In the previous analysis of 34 native populations broadly distributed across much of the native range of medusahead (Spain to Iran) (Peters 2014), five MLGs matching those introduced into the western U.S. were reported. Ten of these 34 (29.4%) populations were found to be putative source populations, with six putative source populations located in Greece and Northwestern Turkey. Results of my analysis are consistent with those reported by Peters (2014), and indicate that 40 of 48 (83.3%) of the populations from Southeastern European and South-central Turkey are putative source populations. The combined results of both studies suggest that populations from this region may be an area of high propagule pressure (Simberloff 2009) for the invasion of medusahead into the western U.S. The likelihood of identifying the geographic origins of an invasive species is greater for species with relatively low genetic diversity and high genetic structure (Novak and Mack 1993; Novak and Mack 2001; Facon et al. 2003; Goolsby et al. 2006; Keller and Taylor 2008; Novak 2011). This appears to be true for the study species because it produces cleistogamous flowers, which leads to a primarily self-pollinating mating system (S.J. Novak, in preparation).

Two MLGs, not yet detected in the invasive range of medusahead, were detected at relatively high frequency among the population analyzed in this study: nine of 48 (18.8%) populations within the study area had MLG #1 (0.22 of the individuals within these nine populations had this genotype), and eight of 48 (16.7%) populations within the

study area had MLG #5 (0.18 of the individuals within these eight populations had this genotype) (Table 3, Table 4). With continued sampling of individuals from the study area at high propagule pressure, I would predict that MLGs with relatively high frequency, such as these two genotypes, have the highest probably on being introduced into the western U.S. or other regions around the world.

Testing the Multiple Introduction Hypothesis

The allozyme diversity detected among the 45 invasive populations of medusahead (Novak 2004; Novak and Sforza 2008; Novak and Rauch 2009) is consistent with the pattern associated with multiple introduction events. The multiple introduction hypothesis however can only be confirmed when the MLGs detected among invasive population are also observed in native populations (Roderick and Navajas 2003; Novak and Mack 2005; Novak 2007, 2011). Taken together, results from the current study and the previous analysis of native populations (Peters 2014) provide support for the multiple introduction of medusahead into the western U.S. These results for medusahead join a growing body of literature that suggests that multiple introductions of invasive species are more common than previously reported (see Novak and Mack 1993; Facon et al. 2003; Maron et al. 2004; Lavergne and Molofsky 2007; Ficetola et al. 2008; Keller and Taylor 2008; Wilson et al. 2009; Estoup and Guillemaud 2010; Lombaert et al. 2011; Gaskin et al. 2013).

Genetic Diversity of Native Populations: Evidence for Founder Effects

Across populations, the 48 populations of medusahead analyzed in this study do not exhibit as much genetic diversity (total number of alleles and number of polymorphic loci) as the 34 native populations previously analyzed by Peters (2014) (Table 8). These

differences occur despite the fact that fewer populations were analyzed in the latter study, and a genetic parameter such as allelic richness is positively correlated with sample size (Leberg 2002; Landguth et al. 2012). Rather, these results likely reflect the geographic scale at which native populations were sampled in these two studies: in the current study populations were sampled across a distance of approximately 1,200 km; whereas, the 34 population previously analyzed were sampled across a distance of approximately 5,000 km (from Spain to Iran). The total number of alleles and polymorphic loci of the 45 invasive populations of medusahead from the western U.S. are lower than the values reported for either group of native populations (Table 8). These results indicate a reduction in the genetic diversity of invasive populations at the across-population level, and indicate that invasive populations of medusahead have experienced founder effects.

A similar pattern was observed for the number of MLGs detected among native and invasive populations. A total of 66 MLGs were detected among the 34 populations of medusahead previously analyzed (Peters 2014), whereas 35 MLGs were detected among the 48 populations from Southeastern Europe and South-central Turkey analyzed in this study (Table 3 and Table 4). A total of seven MLGs were detected among the 45 invasive populations of medusahead from the western U.S. These data indicate a reduction in the MLG diversity of invasive populations.

The level of genetic diversity at the within-population level reported in the current study (A = 1.10, %P = 9.05, He = 0.030) and the 34 native populations previously analyzed (A= 1.10, %P= 9.08, He= 0.025) are remarkably similar (Table 9). On average, the within-population genetic diversity of the 45 invasive populations from the western U.S. (A = 1.03, %P = 2.51, He = 0.006) is considerably lower than the within-population

genetic diversity of either group of native populations (Table 9). These data indicate founder effects at the within-population level for invasive populations of medusahead, and suggests that invasive populations are likely to have reduced evolutionary potential (Lavergne and Molofsky 2007).

Genetic diversity of medusahead has likely been influenced by multiple life-history characteristics; chief among these is the species' self-pollinating (selfing) mating system. Medusahead may be an extreme example of how a highly selfing mating system can influence genetic diversity: the level of diversity across and within native and invasive populations of the species (Table 5, Table 8 and Table 9) is low even when compared to other plants with a primarily selfing mating system (see Hamrick et al. 1979; Hamrick and Godt 1990).

Genetic Structure Among Native and Invasive Populations

High levels of genetic differentiation (structure) among populations can be facilitated by geographic (physical) barriers that reduce gene flow between populations, or the availability of suitable habitats, which results in a patchy distribution of populations across the landscape (Donnelly and Townson 2000; Gerlach and Musolf 2000; Palsson 2000; Tiedemann et al. 2000). The genetic structure of populations is also correlated with the geographic distribution of a species: species with restricted (smaller) geographic distributions typically exhibit less genetic structure and species with larger distributions posses more population genetic structure (Wright 1943; Hamrick et al. 1979; Loveless and Hamrick 1984; Hamrick and Godt 1990, 1996; Ward 2006). More importantly, life-history traits such as dispersal ability and mating system have long been recognized as key factors influencing the genetic structure of populations (Wright 1940;

Baker 1959, Allard et al. 1968; Jain 1975; Hamrick et al. 1979; Loveless and Hamrick 1984; Hamrick and Godt 1990, 1996). For instance, plant species with gravity dispersal of seeds generally have higher population genetic structure compared with species with seeds capable of attaching to fur, clothing, or machinery. Also, self-pollinating (selfing) plant species typically exhibit higher genetic structure when compared with outcrossing species (Hamrick et al. 1979, Loveless and Hamrick 1984, Hamrick and Godt, 1989, 1996).

Results of Nei's (1987) gene diversity statistics (G_{ST} = 0.417) (Table 6) and AMOVA, approximately 60% of the total genetic diversity was partitioned among populations or higher hierarchical levels (countries and regions) (Table 7), indicate that the 48 populations of medusahead from Southeastern Europe and South-central Turkey exhibit moderately high genetic structure. The genetic structure of these 48 populations is consistent with the values reported for other plants that posse the following life-history traits: self-pollination, annual species, early successional species, and gravity-dispersed seeds (Loveless and Hamrick 1984; Hamrick and Godt 1990, 1996). For instance, Welk et al. (2013) reported high genetic structure among native populations of two predominantly selfing grass species, *Stipa pennata* and *S. pulcherrima*. Conversely, relatively weak genetic differentiation among populations was reported for Eurasian populations of the two outcrossing grass species *Lolium rigidum* and *L. perenne* (Balfourier et al. 1998).

The genetic structure of the 48 populations analyzed in this study is much lower than that of the 34 populations of medusahead previously analyzed by Peters (2014): G_{ST} = 0.745 and AMOVA showed that approximately 70% of the total genetic diversity was

partitioned among populations and regions. Differences in these estimates of genetic structure for the same study species probably do not reflect differences in the life-history traits of the populations analyzed in these two studies. Rather, differences in these genetic structure parameters likely result from the different geographic scales at which populations were analyzed. In the current study, populations were sampled across a smaller region (Southeastern Europe and South-central Turkey), whereas Peters (2014) analyzed populations from across most of the geographic distribution of medusahead (Spain to Iran). As a consequence of this greater scale of sampling, the 34 populations analyzed by Peters (2014) possessed more overall genetic diversity compared with the 48 populations analyzed in this study (see above), but these 34 populations also exhibit higher genetic structure.

The genetic structure of the 45 invasive populations of medusahead from the western U.S. (G_{ST} = 0.906, S.J. Novak, unpublished data) is greater than that reported for either of the two groups of native populations of medusahead described above. Increased genetic differentiation among invasive populations of this grass is likely the result of multiple introduction events, combined with local or regional range expansion. Theoretical models indicate that these processes are reinforced by high rates of selfing within invasive populations (Brown and Marshall 1981; Wade and McCauley 1988; Whitlock and McCauley 1990; McCauley 1991; Novak and Mack 2005).

Geographic Structuring Among Population:

The Role of Stochastic Demographic Processes

While the 48 populations of medusahead analyzed in this study exhibit moderately high genetic structure, this genetic structure does not appear to be

geographically structured. Evidence for this conclusion is provided by the map showing the distribution of native populations with MLGs matching those previously detected among populations of medusahead from the western U.S. (Figure 4), the UPGMA cluster diagram (Figure 5), output from the STRUCTURE analysis (Figure 7), and the results of the isolation-by-distance analysis (Figure 8). These results indicate that there is no relationship between the genetic relatedness of the 48 populations analyzed in this study and their geographic distance: populations located close to each other are generally not genetically similar; and, in fact, such populations are likely to be genetically distinct.

This lack of geographic structuring among populations of medusahead from Southeastern Europe and South-central Turkey may be the result of stochastic demographic processes. Under this model, the genetic diversity of populations of the study species in this region is influenced by several demographic processes that proceed over time, and across the landscape: 1) habitats become available for colonization by medusahead following disturbances such as livestock grazing and agricultural abandonment, or at the margins or within agricultural fields, 2) because the grass has a predominantly selfing mating system, one seed may be sufficient for colonization and population establishment, 3) populations of the grass become established at different sites in a stochastic manner such that nearby populations may be colonized and established by seeds with different genotypes, 4) over time, succession occurs and the species is displaced by larger more competitive plant species, and some populations are extirpated, 5) across the landscape, other disturbances occur and other sites become available for colonization by the grass, and 6) over time, this process repeats itself. While this demographic model may most often be thought to influence the genetic diversity of

invasive populations, I believe that this model may also explain the pattern of genetic diversity among native populations of weedy, self-pollinating plant species such as medusahead.

Management Implications for Biological Control

Identification of the geographic origins of an invasive species can aid in the management of such species, especially in the development of a biological control program (Gaskin et al. 2011). For instance, the search for the most effective and specific biological control agents is thought to be facilitated by the accurate identification of source populations, or regions (Evans and Gomez 2004; Goolsby et al. 2006; Novak and Sforza 2008). Previous studies have identified natural enemies that were assessed for their use as biological control agents for medusahead (Seigwart et al. 2003; Widmer and Sforza 2004). The results of the current study and the previous analysis of native populations of medusahead (Peters 2014) point to Southeastern Europe and South-central Turkey as one of the geographic origins for the invasion of this grass in the western U.S., and therefore areas in which the search for biological control agents should be focused.

The combined analysis of native and invasive population of medusahead using the same genetic marker reveals that invasive populations stem from multiple introductions, and 17 of 45 (37%) invasive populations consist of genetic admixtures (i.e., they contain genotypes from different native populations). Thus, several biological control agents from different parts of the native range may be required for adequate control (Burdon and Marshall 1981). In addition, the analysis of native and invasive populations indicates that the genetic diversity of invasive populations has been reduced through founder effects.

This suggests that if biological control agents are found, they are likely to be quite effective (Muller-Scharer et al. 2003; Novak and Sforza 2008).

In conclusion, the analysis of native populations of medusahead described here provides information on the level and structure of genetic diversity within and among these populations. My results suggest that the genetic diversity in these populations appears to be influenced by stochastic demographic processes that have created a patchwork of genotypes across the study area. This analysis provides insights into the invasion of medusahead in the western U.S. through the accurate identification of source populations, and indicates that founder effects have played an important role in influencing the genetic diversity of the grass in its new range. Findings of this study indicate that putative source populations for this invasion occur throughout most of Southeastern Europe and South-central Turkey, and while this may be true, these results must be verified using a molecular marker with higher resolving power (i.e., a more polymorphic genetic marker such as amplified fragment length polymorphism, AFLP, analysis).

LITERATURE CITED

Allard RW, Jain SK, Workman PL (1968) The genetics of inbreeding populations. Advances in Genetics, ed. E. W. Caspari, 14: 55-131. New York: Academic

Allendorf FW, Lundquist LL (2003) Biological invasions by exotic grasses, the grass fire cycle, and global change. Conserv Biol 17: 24-30

Baker HG (1974) The evolution of weeds. Annual Review of Ecology and Systematics 5: 1-24

Balfourier F, Charmet G, Ravel C, (1998) Genetic differentiation within and between natural populations of perennial and annual ryegrass (*Lolium perenne and L. rigidum*) Heredity 81: 100-110

Blank RR, Sforza R (2007) Plant–soil relationships of the invasive annual grass *Taeniatherum caput-medusae*: a reciprocal transplant experiment. Plant Soil DOI 10.1007/s11104-007- 9308-3

Blossey B, Notzold R, (1995) Evolution of Increased Competitive Ability in Invasive Nonindigenous Plants: A Hypothesis. The Journal of Ecology, 83(5): 887-889

Bossdorf O, Auge H, (2005) Phenotypic and genetic differentiation between native nad introduced plant populations. Oecologia, 144: 1-11

Brown A, Marshall DR (1981) Evolutionary changes accompanying colonization in plants. In: Scudder GGE, Preveal JL (eds) Evolution today. Carnegie-Mellon University, Pittsburg pp 351-363

Burdon JJ, Marshall DR (1981) Biological Control and the Reproductive Mode of Weeds. Journal of Applied Ecology 18(2): 649-658

Callaway RM, Aschehoug ET (2000) Invasive plants versus their new and old neighbors: amechanism for exotic invasion. Science 290: 521-523

Chapin FS, Zavaleta ES, Eviner VT, Naylor RL, Vitousek PM, Reynold HL, Hooper DU, Lavorel S, Sala OE, Hobbie SE, Mack MC & Diaz S. (2000) Consequences of changing biodiversity. Nature 405: 234-242

Colautti RI, Ricciardi A, Grigorovich IA, MacIsaac HJ (2004) Is invasion success explained by the enemy release hypothesis? Ecology Letters 7(8): 721-733.

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

Crosby A (1986) Ecological Imperialism: The Biological Expansion of Europe, 900-1900. Cambridge University Press, Cambridge

Dahl BE; Tisdale EW (1975) Environmental factors related to medusahead distribution. Journal of Range Management. 28: 463-468.

D'Antonio CM, Vitousek PM (1992) Biological invasion by exotic grasses, the grass/fire cycle and global change. Annu. Rev. Ecol. Syst. 23: 63-87

Davies KW, Johnson DD (2008) Managing medusahead in the Intermountian West is at a critical threshold. Rangelands 30: 13–15

Donnelly MJ, Townson H (2000) Evidence for extensive genetic differentiation among populations of the malaria vector *Anopheles arabiensis*. Eastern Africa. Insect Molecular Biology 9: 357–367

Earl DA, VonHoldt BM. (2012) STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. Conservation Genetics Resources 4: 359-361

Ellstrand NC, Elam DR (1993) Population Genetic Consequences of Small Population Size Implications for Plant Conservation. Annual Review of Ecology and Systematics 24:217-242

Elton CS (1958) The Ecology of Invasions by Animals and Plants. Metheun, London.

Estoup A, Guillemaud T (2010) Reconstructing routes of invasion using genetic data: why, how and so what? Mol Ecol 19(19): 4113–4130

Evans KJ, Gomez DR (2004) Genetic markers in rust fungi and their application to weed biocontrol, In Genetics, Evolution and Biological Control (eds Ehler, L.E., Sforza, R. & Mateille, T.), 73-95. CABI Publishing, Oxon, UK.

Evanno G, Regnaut S, Goudet J, (2005) Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Mol Ecol. 14(8): 2611-2620

Excoffier L, Laval G, Schneider S (2005) Arlequin (version 3.0): an integrated software package for population genetics data analysis. Evol Bioinform 1: 47-50

Facon B, Pointier JP, Glaubrecht M, Poux C, Jarne P, David P (2003) A molecular phylogeography approach to biological invasions of the New World by parthenogenetic Thiarid snails. Molecular Ecology 12: 3027–3039.

Falush D, Stephens M, Pritchard JK (2003) Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. Genetics, 164: 1567–1587

Falush D, Stephens M, Pritchard JK (2007) Inference of population structure using multilocus genotype data: dominant markers and null alleles. Molecular Ecology 7: 895-908.

Ficetola G.F, Bonin A, Miaud C (2008) Population genetics reveals origin and number of founders in a biological invasion. Mol. Ecol. 17: 773–782

Frederiksen S (1986) Revision of *Taeniatherum* (Poaceae). Nord J Bot 6: 389-397.

Frederiksen S, von Bothmer R (1986) Relationships in *Taeniatherum* (Poaceae). Canadian J. Bot. 10: 2343-2347

Fumanal B, Roulain A, Gaujour E, Chauvel B, Bretagnolle F (2005) Estimated production of pollen and seeds of an invasive plant in France : *Ambrosia artemisiifolia* L. Proc. 17ème Colloque pluridisciplinaire de l'AFEDA, ISSBN 1271-3341. Ambroisie 22, 12

Gaskin J, Bon MC, Cock MJ, Cristofaro M, Biase A, Clerck-floate RD, Ellison CA. Hinz HL, Hufbauer RA, Julien M, Sforza René, (2011) Applying molecular-based approaches to classical biological control of weeds. Biological control 58: 1-21

Gaskin JF, Schwarzländer M, Kinter CL, Smith JF, Novak SJ (2013) Propagule pressure, genetic structure and geographic origins of *Chondrilla juncea* (Asteraceae): An invader on three continents. American Journal of Botany 100: 1871-1882.

Gerlach G, Musolf KF (2000) Fragmentation of landscape as a cause for genetic subdivision in bank voles. Conservation Biology 14: 1066–1074

Goolsby JA, De Barro PJ, Makinson JR (2006) Matching the origin of an invasive weed for selection of a herbivore haplotype for a biological control program. Molecular Ecology 15: 287-297.

Gottlieb LD (1982) Conservation and duplication of isozymes in plants. Science 454: 373-380

Goudet J. FSTAT, a program to estimate and test gene diversities and fixation indices (version 2.9.3). 2001. [Internet]. Lausanne, Switzerland: Lausanne University; [cited 2008 Nov 18]. Available from: URLhttp://www2.unil.ch/popgen/softwares/fstat.htm.

Hamrick JL, Linhart YB, Mitton JB (1979) Relationships between life history characteristics and electrophoretically detectable genetic variation in plants. Ann. Rev. Ecol. Svst. 10: 173-200

Hamrick JL, Godt MJ (1990) Allozyme diversity in plant species. In: Brown AHD, Clegg MT, Kahler AL, Weir BS (eds) Plant population genetics, breeding, and genetic resources. Sinauer Associates Inc., Sunderland, pp 43–63

Hamrick JL, Godt MJ (1996) Conservation genetics of endemic plant species. In: Avise JC, Hamrick JL, eds. Conservation genetics: case histories from nature. New York, NY: Chapman and Hall, pp 281–304.

Hierros JL, Maron JL, Callaway RM (2005) A biogeographical approach to plant invasions: the importance of studying exotics in their introduced and native range. Journal of Ecology 93: 1-15

Hironaka, M. (1994) Medusahead: natural successor to the cheatgrass type in the northern Great Basin. Pages 89–91 in , S. B. Monsen, and S. G. Kitchen, comps. Proceedings—Ecology and Management of Annual Rangelands. Ogden, UT: USDA–Forest Service, Intermountain Research Station

Horton WH (1991) Medusahead: importance, distribution, and control. In: James, Evans, Ralphs, and Childs (eds.) Noxious range weeds. Westview Press, Boulder, CO

Hubisz MJ, Falush D, Stephens M, Pritchard JK (2009) Inferring weak population structure with the assistance of sample group information. Molecular Ecology Resources. 9: 1322-1332

Jensen JL, Bohonak AJ, Kelley ST (2005) Isolation by distance, web service. BMC Genetics 6: 13-23 http://ibdws.sdsu.edu/

Keller SR, Taylor DR (2008) History, chance and adaptation during biological invasion: separating stochastic phenotypic evolution from response to selection. Ecol Letters 11: 852-866

Keller SR, Taylor DR (2010) Genomic admixture increases fitness during a biological invasion. J Evol Biol 23: 1720-1731

Kolar C, Lodge DM (2001) Progess in invasion biology: predicting invaders. Trends Ecol Evol. 16: 199–204.

Kostivkovsky V, Young JA (2000) Invasive exotic rangeland weeds: a glimpse at some of their native habitats. Rangelands. 22(6): 3-6.

Landguth EL, Cushman SA, Johnson NA (2012) Simulating natural selection in landscape genetics. Molecular Ecology 12(2):363-368

Lavergne S, Molofsky J (2007) Increase genetic variation and evolutionary potential drive the success of an invasive grass. PNAS 10: 3883-3888

Leberg PL (2002) Estimating allelic richness: Effects of sample size and bottlenecks. Molecular Ecology 11: 2445-2449

Lee CE (2002) Evolutionary genetics of invasive species. Trends Ecol Evol 17:386-391

Liu H, Stiling P (2006) Testing the enemy release hypothesis: a review and meta-analysis. Biological Invasions 8: 1535–1545.

Lockwood JL, Cassey P, Blackburn T (2005) Role of propagule pressure in explaining species invasions. Trends Ecol Evol 20: 223–228

Lombaert E, Guillemaud T, Thomas CE, Handley LJL, Li J, Wang S, (2011) Inferring the origin of populations introduced from a genetically structured native range by approximate Bayesian computation: case study of the invasive ladybird *Harmonia axyridis*. Mol. Ecol. 20: 4654–4670.

Loveless MD, Hamrick JL (1984) Ecological Determinants of Genetic Structure in Plant Populations. Annual Review of Ecology and Systematics, 15: 65-95

MacArthur RH (1970) Species packing and competitive equilibrium for many species. Theoretical Population Biology 1: 1-11

Mack RN (1996) Predicting the identity and fate of plant invaders: emergent and emerging approaches. Biological Conservation 78: 107–121

Mack RN, Simberloff D, Lonsdale WM, Evans H, Clout M, Bazzaz FA (2000) Biotic invasions: causes, epidemiology, global consequences, and control. Ecol Applic 10: 689-710

Major J, McKell CM, Berry LJ (1960) Improvement of medusahead-infested rangeland. Leaflet. California Agriculture Experimental Station, Ext Serv pp. 8

Maron JL, Vila M, Bommarco R, Elmendorf S, Beardsley P (2004) Rapid evolution of an invasive plant. Ecol Monogr 74: 261–280

McCauley DE (1991) Genetic consequences of local population extinction and recolonization. Trends Ecol Evol 1: 5-8

McKell CM, Wilson AM, Kay BL (1962) Effective burning of rangelands infested with medusahead. Weeds 10: 125–131.

Muller-Scharer H, Schaffner U, Steinger T (2003) Evolution in invasive plants: implications for biological control. Trends Ecol Evol 19(8) 322-343

Nei, M. (1973) Analysis of gene diversity in subdivided populations. Proceedings of the National Academy of Sciences, USA 70: 3321–3323.

Nei M (1978) Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 3: 583-590

Nei M (1987) Molecular evolutionary genetics. Columbia University Press, New York.

Novak SJ, Mack RN (1991) Genetic variation in Bromus tectorum (Poaceae): population differentiation in its North American range. Am. J. Bot. 78: 1150– 1161

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

Novak SJ, Mack RN (2001) Tracing plant introduction and spread: Genetic evidence from *Bromus tectorum* (Cheatgrass). Biosci 2: 114-122

Novak SJ (2004) Genetic analysis of downy brome (*Bromus tectorum*) and medusahead (*Taeniatherum caput-medusae*): management implications. Weed Tech 18: 1417-1421

Novak SJ, Mack RN (2005) Genetic bottlenecks in alien plant species: influence of mating systems and introduction dynamics. Pages 201-228 in Sax DF, Stachowicz JJ, Gaines SD (eds) Species Invasions: Insights into Ecology, Evolution and Biogeography. Sinauer, Sunderland, MA.

Novak SJ (2007) The role of evolution in the invasion process. Proceedings of the national academy of sciences of the United States of America. 10: 3671-3672

Novak SJ, Sforza R (2008) Genetic analysis of native and introduced populations of *Taeniatherum caput-medusae* (Poaceae): implications for biological control. Proceedings of the XII International Symposium on Biological Control of Weeds

Novak SJ, Rausch J (2009) Use of field surveys, distributional data and genetic analyses to monitor alien species: *Taeniatherum caput-medusae* as an example of the approach. *In*: Pyšek P, Pergl J [Eds]. Biological Invasions: Towards a Synthesis. Neobiota 8: 169–182

Novak SJ (2011) Geographic origins and introduction dynamics. Pages 273-280 *in:* Simberloff D, Rejmánek M (eds) *Encyclopedia of Biological Invasions.* University of California Press, Berkeley and Los Angeles, CA.

Palsson S (2000) Microsatellite variation in Daphnia pulex from both sides of the Baltic Sea. Molecular Ecology 9: 1075–1088

Pellant M, Hall C (1994) Distribution of two exotic grasses on public lands in the Great Basin: Status in 1992. In: Monsen, Stephen B.; Kitchen, Stanley G., comps. 1994. Proceedings--ecology and management of annual rangelands; 1992, May 18-22; Boise ID, Gen. Tech. Rep. INT-GTR-313. Ogden, UT: U.S. Department of Agriculture, Forest Service, Intermountain Research Station. 109-112.

Peters ML (2014) Genetic and Morphological Variation in *Taeniatherum caput-medusae* (Medusahead): Taxomonic Diversity, Geographic Origins, Multiple Introductions and Founder Effects. Boise State University Theses and Dissertations. Paper 717 (scholarworks.boisestate.edu/td/717/)

Pimentel D, Zuniga R, Morrison D (2005) Update on the environmental and economic costs associated with alien-invasive species in the United States. Ecol Econ. 52: 273-288

Pineda FD, Nicolas JP, Pou A, Galiano EF (1981) Ecological Succession in Oligotrophic Pastures of Central Spain. Vegetatio 44(3): 165-176

Pritchard JK, Stephens M, Donnelly PJ, (2000) Inference of population structure using multilocus genotype data. Genetics 155: 945-959.

Rejmanck M (2000) Invasive plants: Approaches and predictions. Austral Ecol. 25: 497-506

Rejmánek M, Richardson DM, Higgins SI, Pitcairn M.J, Grotkopp E (2005) Ecology of invasive plants: state of the art. *In* H. A. Mooney, R. N. Mack, J. A. McNeely, L. E. Neville, P. J. Schei, J. K. Waage, [eds.], Invasive alien species: a new synthesis, 104-161. Island Press, Washington, D.C., USA

Reynolds J, Weir BS, Cockerham CC (1983) Estimation for the coancestry coefficient: basis for a short-term genetic distance. Genetics 105: 767-779

Ricciardi A, Jones LA, Kestrup AM, Ward JM, (2011) Expanding the propagule pressure concept to understand the impact of biological invasions. In: Richardson, D.M. (Ed.), Fifty Years of Invasion Ecology: The Legacy of Charles Elton. Blackwell Publishing Ltd., pp. 225–235.

Richardson DM, Pysek P (2006) Plant invasions: merging the concepts of species invasiveness and community invasibility. Progr. Phys. Geogr. 30: 409–431

Roderick GK, Navajas M (2003) Genes in new environments: genetics and evolution in biological control. Nature Rev. Gen. 4: 889-899

Rosenberg NA (2004) Distruct: a program for the graphical display of population structure. Molecular Ecology Notes 4: 137-138

Sakai AK, Allendorf FW, Holt JS, Lodge DM, Molofsky J, With KA, Baughman S, Cabin RJ, Cohen JE, Ellstrand NC, McCauley DE, O'Neil P, Parker IM, Thompson JN & Weller SG (2001). The population biology of invasive species. Ann. Rev. Ecol. Sys. 32: 305-332

Sala OE et al. (2000) Global biodiversity scenarios for the year 2100. Sci 5459: 1770-1174

SAS Institute. 2002. SAS user's guide, version 9.1. SAS Institute, Inc., Cary, NC.

Scholte E, Petric D, Schaffner F, Rakotoarivony L, Bellini R (2014) Monitoring population and environmental parameters of invasive mosquito species in Europe. Parasites & Vectors 187: 1-14

Sforza R, Cristofaro M (2002) Search for biocontrol agents in Turkey against medusahead and yellow star thistle. Türkiye 5. Biyolojik Mücadele Kongresi. 4(7): 327-334

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

Siegwart M, Bon MC, Widmer TL, Crespy N, Sforza R (2003) First report of Fusarium arthrosporioides on medusahead (*Taeniatherum caput-medusae*) and preliminary tests for host-specificity. Plant Path 3: 416-416

Simberloff D (2009) The role of propagule pressure in biological invasions. Annu. Rev. Ecol. Syst. 40: 81-102

Slatkin M (1995) A measure of population subdivision based on microsatellite allele frequencies. Genetics 139: 457-462

Soltis DE, Haufler CH, Darrow DC, Gastony GL (1983) Starch gel electrophoresis of ferns: a compilation of grinding buffers, gel and electrode buffers, and staining schedules. Amer Fern J 73: 9-27

Tiedemann R, Hardy O, Vekemans X, Milinkovitch MC (2000) Higher impact of female than male migration on population structure in large mammals. Molecular Ecology 9: 1159–1163

Vitousek PM, Walker LR (1989) Biological invasion by *M. faya faya* in Hawai'i: Plant demography, nitrogen fixation, and ecosystem effects. Ecol. Monogr. 59: 247-265

Wade MJ, McCauley DE (1988) Extinction and recolonization: their effects on the genetic differentiation of local populations. Evol. 42: 995-1005

Ward S (2006) Genetic analysis of invasive plant populations at different spatial scales. Biological Invasions 8: 541-552

Welk E, Durka W, Constaze N, Rupercht E, Wagner V, Wesche K, Hensen I, (2013) Extreme genetic depauperation and differentiation of both populations and species in Eurasian feather grasses (Stipa). Plant Syst. Evol. 299: 259–269

Wendel JF, Weeden NF (1989) Visualization and interpretation of plant isozymes. In: Soltis DE, Sotlis PS (eds) Isozymes in plant biology. Dioscorides Press, pp. 5-45

Whitlock MC, McCauley DE (1990) Some population genetic consequences of colony formation and extinction – genetic correlations within founding groups. Evolution 44: 1717–1724

Widmer TL, Sforza R (2004) Exploration for plant pathogens against *Taeniatherum caput-medusae* (medusahead ryegrass). Symposium on Biological control of weeds.

Wilcove DS, Rothstein D, Dubow J, Phillips A, Losos E (1988) Quantifying threats to imperiled species in the United States. BioSci 48: 607-615

Wilson JR, Dormontt EE, Prentis PJ, Lowe AJ, Richardson DM (2009) Something in the way you move: dispersal pathways affect invasion success. Trends Ecol. Evol. 3: 136-144

Wright S (1943) Isolation by distance. Genetics 28: 114-138

Wright S (1940) Breeding structure of populations in relation to speciation. Am. Nat. 74: 232-48

Yeh FC, Boyle TJB (1997) Population genetic analysis of co-dominant and dominant markers and quantitative traits. Belgian Journal of Botany 129: 157.

Young J, Evans RA (1970 Invasion of medusahead (Taeniatherum caput-medusae) into the Great Basin. Weed Sci. 18:89-97

Young J (1992) Ecology and management of medusahead (*Taeniatherum caput-medusae* ssp. *asperum* [Simk.] Melderis). Great Basin Nature 52(3): 245-252

TABLES AND FIGURES

Table 1 Location and collection data for 48 populations of *Taeniatherum caput-medusae* subsp. *asperum* sampled from Southeastern Europe and South-central Turkey

Country	Population Number	Locality	Latitude/Longitude Coordinates	Elevation (m)	Collection date
Albania	1	Korca	40 40' 05" N 20 49' 20" E	871	7/14/2011
	2	Struga	41 04' 40" N 20 36' 25" E	1016	6/26/2011
Bulgaria	3	Beronovo	42 49' 39" N 26 42' 34" E	358	10/4/2010
	4	Devnja	43 13' 56" N 27 32' 33" E	128	10/4/2010
	5	Dripchevo	41 59' 41" N 26 11' 45" E	461	7/3/2011
	6	Galabets	41 49' 39" N 25 27' 03" E	322	7/4/2011
	7	Harmanli	41 58' 03" N 25 59' 42" E	241	10/3/2010
	8	Izgrev	42 08' 41" N 27 48' 38" E	137	10/5/2010
	9	Izvorishte	42 39' 31" N 27 26' 07" E	278	7/3/2011
	10	Izvorsko	43 16' 47" N 27 46' 57" E	323	7/2/2011
	11	Orizare	42 42' 43" N 27 37' 04" E	77	10/4/2010
	12	Razlog	41 53' 11" N 23 30' 05" E	834	10/2/2010

	13	Sozopol	42 22' 07" N 27 41' 07" E	50	10/5/2010
	14	Sredec	42 12' 49" N 27 02' 11" E	332	10/5/2010
	15	Staro Orjahovo	42 59' 11" N 27 47' 17" E	65	10/4/2010
	16	Tenevo	42 21' 38" N 26 34' 19" E	145	7/3/2011
	17	Zvezdel	41 28' 16" N 25 32' 24" E	572	7/4/2011
Greece	18	Askos	40 45' 27" N 23 27' 11" E	398	7/8/2011
	19	Edessa	40 47' 06" N 21 53' 20" E	587	6/25/2011
	20	Kokinochoma	40 55' 28" N 24 17' 24" E	73	7/7/2011
	21	Komotini	41 05' 14" N 25 44' 30" E	113	7/7/2011
	22	Sapes	40 59' 43" N 25 39' 41" E	84	7/7/2011
Macedonia	23	Bitola	41 02' 16" N 21 19' 10" E	645	6/25/2011
	24	Bitola North	41 03' 11" N 21 16' 49" E	748	6/26/2011
	25	Umin Dol	42 05' 21" N 21 36' 04" E	535	6/27/2011
Romania	26	Drobetia	44 48' 25" N 28 38' 45" E	100	6/28/2011

	27	Sacele	44 38' 30" N 22 37' 17" E	73	7/1/2011
	28	Schela	44 28' 45" N 28 38' 51" E	54	7/1/2011
	29	Slava Rusa	45 31' 59" N 27 49' 45" E	43	7/1/2011
Serbia	30	Kladovo	44 38' 01" N 22 33' 38" E	95	6/28/2011
Turkey	31	Akseki	37 07' 17" N 31 47' 49" E	1271	6/26/2013
	32	Corlu	41 03' 06" N 27 43' 56" E	19	7/6/2011
	33	Havsa	41 24' 05" N 26 28' 41" E	73	7/5/2011
	34	Ipsala	40 52' 47" N 26 25' 10" E	50	7/5/2011
	35	Kesan	40 44' 06" N 26 43' 21" E	104	7/6/2011
	36	Poyrali	41 37' 41" N 27 36' 20" E	329	7/6/2011
	37	Seydishir	37 24' 17" N 31 50' 06" E	1239	6/26/2013
	38	Urunlu	41 40' 27" N 26 59' 53" E	132	7/5/2011
	39	Uzunkopru	41 18' 57" N 26 34' 24" E	118	7/5/2011
	40	Yalihuyuk	37 18' 50" N 32 06' 18" E	1102	6/27/2013

	41	Yorukler	41 07' 07" N 27 14' 25" E	105	7/6/2011
Ukraine	42	Alushta	44 42' 17" N 34 25' 54" E	190	7/8/2013
	43	Bahate	45 01' 40" N 34 45' 57" E	303	7/8/2013
	44	Izobilne	44 42' 05" N 34 21' 02" E	217	7/9/2013
	45	Pryvitne	44 49' 19" N 34 43' 47" E	279	7/8/2013
	46	Sudak	44 53' 10" N 35 05' 40" E	176	7/8/2013
	47	Trudolybivka	44 46' 50" N 33 59' 51" E	190	7/10/2013
	48	Yalta	44 28' 52" N 34 07' 32" E	281	7/9/2013

Table 2 Allele frequencies for all polymorphic loci across the 48 populations of *Taeniatherum caput-medusae* subsp. *asperum*. Numbers in parentheses are population sample sizes.

		Albania		Bulgaria		
Locus	**Allele**	**Korca (24)**	**Struga (15)**	**Bernovo (12)**	**Devnja (11)**	**Dripchevo (26)**
Mdh-2	a	-	1.000	0.167	0.455	0.154
	b	-	-	-	-	-
	c	1.000	-	0.833	-	0.846
	d	-	-	-	0.546	-
	e	-	-	-	-	-
Mdh-3	a	-	1.000	1.000	1.000	0.962
	b	1.000	-	-	-	0.039
Pgi-2	a	-	-	-	-	-
	b	-	1.000	-	0.546	0.692
	c	1.000	-	1.000	0.455	0.308
Got-1	a	1.000	-	0.250	-	-
	b	-	1.000	0.750	1.000	1.000
Got-2	a	-	-	-	-	-
	b	1.000	1.000	1.000	1.000	1.000
6pgd-2	a	-	-	-	0.455	-
	b	1.000	1.000	1.000	0.546	1.000
	c	-	-	-	-	-
Idh	a	1.000	1.000	1.000	1.000	1.000
	b	-	-	-	-	-

Locus	Allele	Galabets (25)	Harmanli (27)	Izgrev (17)	Izvorishte (27)	Izorsko (22)
Mdh-2	*a*	0.440	0.482	0.588	0.037	1.000
	b	-	-	-	-	-
	c	0.560	0.519	-	0.704	-
	d	-	-	0.412	0.259	-
	e	-	-	-	-	-
Mdh-3	*a*	1.000	1.000	1.000	1.000	1.000
	b	-	-	-	-	-
Pgi-2	*a*	-	-	-	-	-
	b	0.400	0.185	0.529	0.407	-
	c	0.600	0.815	0.471	0.593	1.000
Got-1	*a*	0.600	0.037	0.765	0.037	0.318
	b	0.400	0.963	0.235	0.963	0.682
Got-2	*a*	-	-	-	-	-
	b	1.000	1.000	1.000	1.000	1.000
6pgd-2	*a*	-	0.037	-	-	0.727
	b	1.000	0.963	1.000	1.000	0.273
	c	-	-	-	-	-
Idh	*a*	1.000	1.000	1.000	1.000	1.000
	b	-	-	-	-	-

Locus	Allele	Orizane (10)	Razlog (23)	Sozopol (17)	Sredec (17)	Staro Jahovo (15)
Mdh-2	*a*	0.500	0.739	0.471	0.059	0.600
	b	-	-	-	-	-
	c	0.500	-	0.294	0.941	0.400
	d	-	0.217	0.118	-	-
	e	-	0.044	0.118	-	-
Mdh-3	*a*	1.000	0.913	0.765	1.000	1.000
	b	-	0.087	0.235	-	-
Pgi-2	*a*	-	-	-	-	-
	b	0.100	0.304	0.177	0.059	0.733
	c	0.900	0.696	0.824	0.941	0.267
Got-1	*a*	0.900	0.217	0.353	-	0.400
	b	0.100	0.783	0.647	1.000	0.600
Got-2	*a*	-	-	-	-	-
	b	1.000	1.000	1.000	1.000	1.000
6pgd-2	*a*	0.500	-	-	-	-
	b	0.500	1.000	1.000	1.000	1.000
	c	-	-	-	-	-
Idh	*a*	1.000	1.000	1.000	1.000	1.000
	b	-	-	-	-	-

		Greece				
Locus	**Allele**	**Tenevo (30)**	**Zvezdel (25)**	**Askos (26)**	**Edessa (18)**	**Kokinochoma (25)**
Mdh-2	a	0.567	0.960	0.769	0.889	1.000
	b	-	-	-	-	-
	c	0.433	0.040	0.077	0.111	-
	d	-	-	0.154	-	-
	e	-	-	-	-	-
Mdh-3	a	1.000	1.000	1.000	0.889	1.000
	b	-	-	-	0.111	-
Pgi-2	a	-	-	-	-	-
	b	0.533	0.080	0.077	-	1.000
	c	0.467	0.920	0.923	1.000	-
Got-1	a	-	0.200	0.231	-	1.000
	b	1.000	0.800	0.769	1.000	
Got-2	a	-	-	-	-	-
	b	1.000	1.000	1.000	1.000	1.000
6pgd-2	a	-	-	-	-	-
	b	1.000	1.000	1.000	1.000	1.000
	c	-	-	-	-	-
Idh	a	1.000	1.000	1.000	1.000	1.000
	b	-	-	-	-	-

		Macedonia				
Locus	**Allele**	**Komotini (27)**	**Sapes (16)**	**Bitola (25)**	**Bitola North (26)**	**Umin Dol (30)**
Mdh-2	a	1.000	1.000	0.920	0.346	0.600
	b	-	-	-	-	-
	c	-	-	0.080	0.654	-
	d	-	-	-		0.400
	e	-	-	-	-	-
Mdh-3	a	1.000	1.000	0.920	0.346	1.000
	b	-	-	0.080	0.654	-
Pgi-2	a	-	-	-	-	-
	b	0.926	0.938	-	-	-
	c	0.074	0.063	1.000	1.000	1.000
Got-1	a	-	-	-		0.100
	b	1.000	1.000	1.000	1.000	0.900
Got-2	a	-	-	-	-	-
	b	1.000	1.000	1.000	1.000	1.000
6pgd-2	a	-	-	-	-	0.067
	b	1.000	0.688	1.000	1.000	0.933
	c	-	0.313	-	-	-
Idh	a	1.000	0.938	1.000	1.000	1.000
	b	-	0.063	-	-	-

		Romania				Serbia
Locus	**Allele**	**Drobetia (34)**	**Sacele (24)**	**Schela (30)**	**Slava Rusa (24)**	**Kladovo (26)**
Mdh-2	a	0.706	1.000	1.000	1.000	-
	b	-	-	-	-	-
	c	0.294	-	-	-	1.000
	d	-	-	-	-	-
	e	-	-	-	-	-
Mdh-3	a	0.706	1.000	1.000	1.000	-
	b	0.294	-	-	-	1.000
Pgi-2	a	-	-	-	-	-
	b	0.088	1.000	0.067	-	-
	c	0.912	-	0.933	1.000	1.000
Got-1	a	-	-	-	0.125	1.000
	b	1.000	1.000	1.000	0.875	-
Got-2	a	-	-	-	-	-
	b	1.000	1.000	1.000	1.000	1.000
6pgd-2	a	-	-	-	-	-
	b	1.000	1.000	1.000	1.000	1.000
	c	-	-	-	-	-
Idh	a	1.000	1.000	1.000	1.000	1.000
	b	-	-	-	-	-

		Turkey				
Locus	**Allele**	**Akeski (25)**	**Corlu (25)**	**Havsa (27)**	**Ipsala (23)**	**Kesan (16)**
Mdh-2	a	0.800	-	1.000	0.696	0.250
	b	-	-	-	-	0.063
	c	0.200	-	-	0.261	-
	d	-	1.000	-	0.044	-
	e	-	-	-	-	0.688
Mdh-3	a	1.000	1.000	1.000	0.826	0.250
	b	-	-	-	0.174	0.750
Pgi-2	a	0.080	-	-	-	-
	b	0.920	-	-	0.087	1.000
	c	-	1.000	1.000	0.913	-
Got-1	a	-	-	-	0.304	-
	b	1.000	1.000	1.000	0.696	1.000
Got-2	a	-	-	-	-	-
	b	1.000	1.000	1.000	1.000	1.000
6pgd-2	a	-	-	-	-	-
	b	1.000	1.000	1.000	1.000	1.000
	c	-	-	-	-	-
Idh	a	1.000	1.000	1.000	1.000	1.000
	b	-	-	-	-	-

Locus	Allele	Poyrali (25)	Seydishir (38)	Urunlu (25)	Uzunkopru (23)	Yalihuyuk (24)
Mdh-2	a	0.440	0.316	-	0.130	0.792
	b	-	-	-	-	-
	c	0.560	0.684	1.000	0.870	0.208
	d	-	-	-	-	-
	e	-	-	-	-	-
Mdh-3	a	0.960	1.000	1.000	1.000	1.000
	b	0.040	-	-	-	-
Pgi-2	a	-	-	-	-	0.125
	b	0.760	0.974	-	-	0.875
	c	0.240	0.026	1.000	1.000	-
Got-1	a	-	-	-	0.739	-
	b	1.000	1.000	1.000	0.261	1.000
Got-2	a	-	-	-	-	-
	b	1.000	1.000	1.000	1.000	1.000
6pgd-2	a	-	-	-	-	-
	b	1.000	1.000	1.000	1.000	1.000
	c	-	-	-	-	-
Idh	a	1.000	1.000	1.000	1.000	1.000
	b	-	-	-	-	-

		Ukraine				
Locus	**Allele**	**Yorukler (25)**	**Alushta (18)**	**Bahate (19)**	**Izobilne (24)**	**Pryvitne (30)**
Mdh-2	a	0.120	-	0.316	-	0.467
	b	-	-	-	-	-
	c	0.680	-	0.684	0.917	0.167
	d	-	0.722	-	0.083	0.300
	e	0.200	0.278	-	-	0.067
Mdh-3	a	0.800	0.722	1.000	1.000	0.833
	b	0.200	0.278	-	-	0.167
Pgi-2	a	-	-	-	0.042	0.100
	b	0.760	0.722	1.000	0.958	0.833
	c	0.240	0.278	-	-	0.067
Got-1	a	0.200	0.056	-	0.917	0.500
	b	0.800	0.944	1.000	0.083	0.500
Got-2	a	-	-	-	-	-
	b	1.000	1.000	1.000	1.000	1.000
6pgd-2	a	-	-	-	-	-
	b	1.000	1.000	1.000	1.000	1.000
	c	-	-	-	-	-
Idh	a	1.000	1.000	1.000	1.000	1.000
	b	-	-	-	-	-

Locus	Allele	Sudak (7)	Trudolybivka (17)	Yalta (19)	Overall Frequency (1084)
Mdh-2	a	-	-	1.000	0.530
	b	-	-	-	0.001
	c	-	1.000	-	0.353
	d	1.000	-	-	0.092
	e	-	-	-	0.024
Mdh-3	a	1.000	1.000	1.000	0.889
	b	-	-	-	0.111
Pgi-2	a	-	-	-	0.008
	b	-	1.000	-	0.411
	c	1.000	-	1.000	0.581
Got-1	a	1.000	-	-	0.214
	b	-	1.000	1.000	0.786
Got-2	a	-	0.059	-	0.001
	b	1.000	0.941	1.000	0.999
6pgd-2	a	-	-	-	0.027
	b	1.000	1.000	1.000	0.969
	c	-	-	-	0.005
Idh	a	1.000	1.000	1.000	0.999
	b	-	-	-	0.001

Table 3 Multilocus genotypes detected in 48 populations of *Taeniatherum caput-medusae* subsp. *asperum*. Letters represent different alleles at each locus. The order of the loci are as follows: *Mdh-1, Mdh-2, Mdh-3, Pgi-1, Pgi-2, Got-1, Got-2, 6pgd-1, 6pgd-2, Pgm-2, Adh, Gdh, Ald, Tpi-1, Tpi-2, Sod-1, Sod-2, Ce-2, Ce-4, Me, Idh, Skdh, G3pdh*. The first 4 multilocus genotypes listed in this table are found in the invasive range (RS-Rattlesnake Station, Idaho; LC-Ladd Canyon, Oregon; SB-Steppetoe Butte, Washington; R-Roseburg, Oregon)

ID	Shared between population	Multilocus Genotype
1 (RS)	27	BAAADBBABBABAAAAABBAABB
2 (LC)	14	BCAADBBABBABAAAAABBAABB
3 (SB)	26	BAAACBBABBABAAAAABBAABB
4 (R)	14	BCAACBBABBABAAAAABBAABB
5	8	BCBADBBABBABAAAAABBAABB
6	1	BABACBBABBABAAAAABBAABB
7	2	BDAADBBABBABAAAAABBAABB
8	1	BAAAABBABBABAAAAABBAABB
9	2	BAAADBBAABABAAAAABBAABB
10	9	BAAADABABBABAAAAABBAABB
11	1	BDBADBBABBABAAAAABBAABB
12	1	BEBADBBABBABAAAAABBAABB
13	1	BCAAABBABBABAAAAABBAABB
14	2	BCAADABABBABAAAAABBAABB
15	3	BCBADABABBABAAAAABBAABB
16	5	BDAACBBABBABAAAAABBAABB
17	5	BAAACABABBABAAAAABBAABB
18	1	BAAACBBACBABAAAAABBAABB

19	1	BAAACBBABBABAAAAABBABBB
20	1	BBBACBBABBABAAAAABBAABB
21	2	BEBACBBABBABAAAAABBAABB
22	2	BABACABABBABAAAAABBAABB
23	3	BCAACABABBABAAAAABBAABB
24	1	BCAACBAABBABAAAAABBAABB
25	1	BCBACABABBABAAAAABBAABB
26	1	BDAAABBABBABAAAAABBAABB
27	4	BDAADABABBABAAAAABBAABB
28	1	BAAAAABABBABAAAAABBAABB
29	1	BAAADABAABABAAAAABBAABB
30	2	BEBADABABBABAAAAABBAABB
31	1	BCAAAABABBABAAAAABBAABB
32	2	BCAADABAABABAAAAABBAABB
33	5	BDAACABABBABAAAAABBAABB
34	1	BEBACABABBABAAAAABBAABB
35	1	BEBAAABABBABAAAAABBAABB

Table 4 Frequency and type of Multilocus genotypes (MLG) detected within and among populations for *Taeniatherum caput-medusae* subsp. *asperum.* Red indicates that the MLG is found in only 2 populations, Green indicates that the MLG is unique to that population. MLG Identification number (ID) is the same as ID in Table 3.

Country	Location	N	MLG ID	MLG Count	MLG Frequency
Albania	Korca	24	15	24	1.00
	Struga	15	3	15	1.00
Bulgaria	Bernovo	12	1	2	0.17
			2	7	0.58
			14	3	0.25
	Devnja	11	10	5	0.45
			16	6	0.55
	Dripchevo	26	1	2	0.08
			2	5	0.19
			5	1	0.04
			3	2	0.08
			4	16	0.62
	Galabets	25	1	2	0.08
			2	4	0.16
			3	4	0.16
			14	9	0.36
			17	5	0.20
			23	1	0.04
	Harmanli	27	1	11	0.41
			2	10	0.37
			3	2	0.07

		4	3	0.11
		32	1	0.04
Izgrev	17	1	3	0.18
		3	1	0.06
		10	3	0.18
		17	3	0.18
		27	2	0.12
		33	5	0.29
Izorsko	27	9	15	0.56
		10	6	0.22
		29	1	0.04
Izvorishte	22	1	1	0.05
		2	15	0.68
		4	4	0.18
		16	6	0.27
		33	1	0.05
Orizane	10	3	1	0.10
		10	4	0.40
		32	5	0.50
Razlog	23	1	16	0.70
		6	1	0.04
		21	1	0.04
		33	5	0.22
Sozopol	17	1	4	0.24
		2	3	0.18

			5	2	0.12
			3	2	0.12
			10	1	0.06
			17	1	0.06
			27	2	0.12
			30	2	0.12
	Sredec	17	2	16	0.94
			3	1	0.06
	Staro Jahovo	15	1	4	0.27
			3	5	0.33
			23	6	0.40
	Tenevo	30	1	11	0.37
			2	3	0.10
			3	6	0.20
			4	10	0.33
	Zvezdel	25	1	17	0.68
			2	1	0.04
			3	2	0.08
			10	5	0.20
Greece	Askos	26	1	20	0.77
			22	2	0.08
			27	4	0.15
	Edessa	18	1	16	0.89
			5	2	0.11
	Kokinchoma	25	17	25	1.00

	Komotini	27	1	2	0.07
			3	25	0.93
	Sapes	16	1	1	0.06
			3	9	0.56
			18	5	0.31
			19	1	0.06
Macedonia	Bitola	25	1	23	0.92
			5	2	0.08
	Bitola North	26	1	9	0.35
			5	17	0.65
	Umin Dol	30	1	13	0.43
			7	12	0.40
			9	2	0.07
			10	3	0.10
Romania	Drobetia	34	1	21	0.62
			5	10	0.29
			3	3	0.09
	Sacele	24	3	24	1.00
	Schela	30	1	28	0.93
			3	2	0.07
	Slava Rusa	24	1	21	0.88
			10	3	0.13
Serbia	Kladovo	26	15	26	1.00
Turkey	Akseki	25	3	20	0.80
			4	3	0.12

		13	2	0.08
Corlu	25	7	25	1.00
Havsa	27	1	27	1.00
Ipsala	23	1	9	0.39
		2	2	0.09
		5	2	0.09
		3	1	0.04
		4	1	0.04
		10	6	0.26
		11	1	0.04
		15	1	0.04
Kesan	16	3	4	0.25
		20	1	0.06
		21	11	0.69
Poyrali	25	2	5	0.20
		5	1	0.04
		3	11	0.44
		4	8	0.32
Seydishir	38	1	1	0.03
		3	11	0.29
		4	26	0.68
Urunlu	25	2	25	1.00
Uzunkopru	23	1	3	0.13
		2	3	0.13
		3	17	0.74

	Yalihuyuk	24	3	16	0.67
			4	5	0.21
			8	3	0.13
	Yorukler	25	2	1	0.04
			3	3	0.12
			4	16	0.64
			30	5	0.20
Ukraine	Alushta	18	12	5	0.28
			16	12	0.67
			33	1	0.06
	Bahate	19	3	6	0.32
			4	13	0.68
	Izobilne	24	4	1	0.04
			16	1	0.04
			23	20	0.83
			31	1	0.04
			33	1	0.04
	Pryvitne	30	1	2	0.07
			3	1	0.03
			4	3	0.10
			16	8	0.27
			17	9	0.30
			22	1	0.03
			25	2	0.07
			26	1	0.03

		28	1	0.03
		34	1	0.03
		35	1	0.03
Sudak	7	27	7	1.00
Trudolybivka	17	4	16	0.94
		24	1	0.06
Yalta	19	1	19	1.00

Table 5 Within-population genetic diversity parameters for the *Taeniatherum caput-medusae* subsp. *asperum* populations analyzed in this study. Parameters are mean number of alleles per locus (A), number of polymorphic loci per population (#P), percent polymorphic loci per population (%P), mean observed heterozygosity (Hobs), the expected mean heterozygosity which was calculated using the unbiased estimate method of Nei (1978) (Hexp), and the number of multilocus genotypes detected in each population(#MLG)

Country	Population #	Locality	N	A	# P	%P	H_{obs}	H_{exp}	# MLG
Albania	1	Korca	24	1.00	0	0.0	0	0.000	1
	2	Struga	15	1.00	0	0.0	0	0.000	1
Bulgaria	3	Bernovo	12	1.09	2	8.7	0	0.028	3
	4	Devnja	11	1.13	3	13.0	0	0.065	2
	5	Dripchevo	26	1.13	3	13.0	0	0.033	5
	6	Galabets	25	1.13	3	13.0	0	0.063	6
	7	Harmanli	27	1.17	4	17.4	0	0.041	5
	8	Izgrev	17	1.13	3	13.0	0	0.058	6
	9	Izorsko	22	1.09	2	8.7	0	0.036	3
	10	Izvorishte	27	1.17	3	13.0	0	0.043	5
	11	Orizane	10	1.17	4	17.4	0	0.059	3
	12	Razlog	23	1.22	4	17.4	0	0.058	4
	13	Sozopol	17	1.26	4	17.4	0	0.077	8
	14	Sredec	17	1.09	2	8.7	0	0.010	2
	15	Staro Jahovo	15	1.13	3	13.0	0	0.059	3
	16	Tenevo	30	1.09	2	8.7	0	0.043	4
	17	Zvezdel	25	1.13	3	13.0	0	0.024	4
Greece	18	Askos	26	1.17	3	13.0	0	0.038	3
	19	Edessa	18	1.09	2	8.7	0	0.017	2
	20	Kokinchoma	25	1.00	0	0.0	0	0.000	1

	21	Komotini	27	1.04	1	4.3	0	0.006	2
	22	Sapes	16	1.13	3	13.0	0	0.029	4
Macedonia	23	Bitola	25	1.09	2	8.7	0	0.013	2
	24	Bitola North	26	1.09	2	8.7	0	0.039	2
	25	Umin Dol	30	1.13	3	13.0	0	0.034	4
Romania	26	Drobetia	34	1.13	3	13.0	0	0.043	3
	27	Sacele	24	1.00	0	0.0	0	0.000	1
	28	Schela	30	1.04	1	4.3	0	0.005	2
	29	Slava Rusa	24	1.04	1	4.4	0	0.010	2
Serbia	30	Kladovo	26	1.00	0	0.0	0	0.000	1
Turkey	31	Akseki	25	1.09	2	8.7	0	0.020	3
	32	Corlu	25	1.00	0	0.0	0	0.000	1
	33	Havsa	27	1.00	0	0.0	0	0.000	1
	34	Ipsala	23	1.22	4	17.4	0	0.057	8
	35	Kesan	16	1.13	2	8.7	0	0.036	3
	36	Poyrali	25	1.13	3	13.0	0	0.041	4
	37	Seydishir	38	1.09	2	8.7	0	0.021	3
	38	Urunlu	25	1.00	0	0.0	0	0.000	1
	39	Uzunkopru	23	1.09	2	8.7	0	0.027	3
	40	Yalihuyuk	24	1.09	2	8.7	0	0.024	3
	41	Yorukler	25	1.22	4	17.4	0	0.065	4
Ukraine	42	Alushta	18	1.17	4	17.4	0	0.057	3
	43	Bahate	19	1.04	1	4.4	0	0.019	2
	44	Izobilne	24	1.13	3	13.0	0	0.017	5
	45	Pryvitne	30	1.30	4	17.4	0	0.075	11

46	Sudak	7	1.00	0	0.0	0	0.000	1
47	Trudolybivka	17	1.04	1	4.3	0	0.005	2
48	Yalta	19	1.00	0	0.0	0	0.000	1
	Overall Mean		1.10	2.08	9.05	0	0.03	3.19
	Source Pop. Mean		1.11	2.28	9.88	0	0.031	3.5
	Non-Source Pop Mean		1.05	1.13	4.89	0	0.02	1.63

Table 6 Nei's (1987) gene diversity statitics for native populations for *Taeniatherum caput-medusae* subsp. *asperum*. Refer to the text for the definitions of HT, HS, DST and GST.

Locus	H_T	H_S	D_{ST}	G_{ST}
Mdh-2	0.603	0.276	0.327	0.542
Mdh-3	0.190	0.078	0.113	0.592
Pgi-2	0.493	0.164	0.329	0.667
Got-1	0.359	0.136	0.223	0.621
Got-2	0.002	0.002	0.000	0.010
6pgd-2	0.084	0.044	0.040	0.474
Idh	0.003	0.003	0.000	0.014
Mean	0.248	0.100	0.147	0.417

Table 7 Analysis of Molecular Variance (AMOVA) using the F-statistics in ARLEQUIN v.3.1 (Excoffier et al. 2005) for the 48 native populations of *Taeniatherum caput-medusae* subsp. *asperum*. Letters (a,b,c) within the first column identify the three different hierarchical AMOVA analyses that were conducted. Refer to the text for an explanation of the three analyses.

		d.f	Sum of Squares	Variation Component	Percentage Variation
(a)	Among populations	47	1105.507	0.50634	59.55
	Within populations	1036	712.735	0.34398	40.45
	Within individuals	1084	0.000	0.00000	0.00
	Total	2167	1818.242	0.85032	--
(b)	Among countries	7	327.949	0.10138	11.69
	Among populations within countries	40	77.557	0.42988	49.56
	Within populations	2120	712.735	0.33620	38.76
	Total	2167	1818.242	0.86746	--
(c)	Among regions	1	34.247	0.00929	1.09
	Among populations within regions	46	1071.259	0.50964	59.60
	Within populations	2120	712.735	0.33620	39.32
	Total	2167	1818.242	0.85513	--

Table 8 Across-population genetic diversity parameters for *Taeniatherum caput-medusae* subsp. *asperum.*

	Number of Populations	Alleles	Alleles/ Locus	Number of Poly. Loci	Percentage of Poly. Loci	Percentage of Poly. Populations
This Study	48	35	1.52	7	30.4	79.2
Previously Analyzed Native Populations	34	48	2.09	15	65.2	67.6
Invasive Populations	45	28	1.22	5	21.7	37.8

Table 9 Within- and among-population genetic diversity parameters for *Taeniatherum caput-medusae* subsp. *asperum*. See Table 5 and Table 6 for an explanation of these parameters.

Country	Number of Populations	A	%P	H_{obs}	H_{exp}	Ht	Gst
This Study	48	1.10	9.05	0.00000	0.030	0.248	0.417
Previously Analyzed Native Populations	34	1.10	9.08	0.00003	0.025	0.262	0.745
Invasive Populations	45	1.03	2.52	0.00010	0.006	0.224	0.906

Figure 1 Photographs of *Taeniatherum caput-medusae* subspecies *asperum* populations in the native (a) and invasive (b) ranges. The photograph of the native population was taken at Izvorishte, Bulgaria, and the photograph of the invasive population was taken at Boise, Idaho

Figure 2 **Photographs of *Taeniatherum caput-medusae* subspecies of (a) *asperum* (b) *crinitum* (c) *caput-medusae*.**

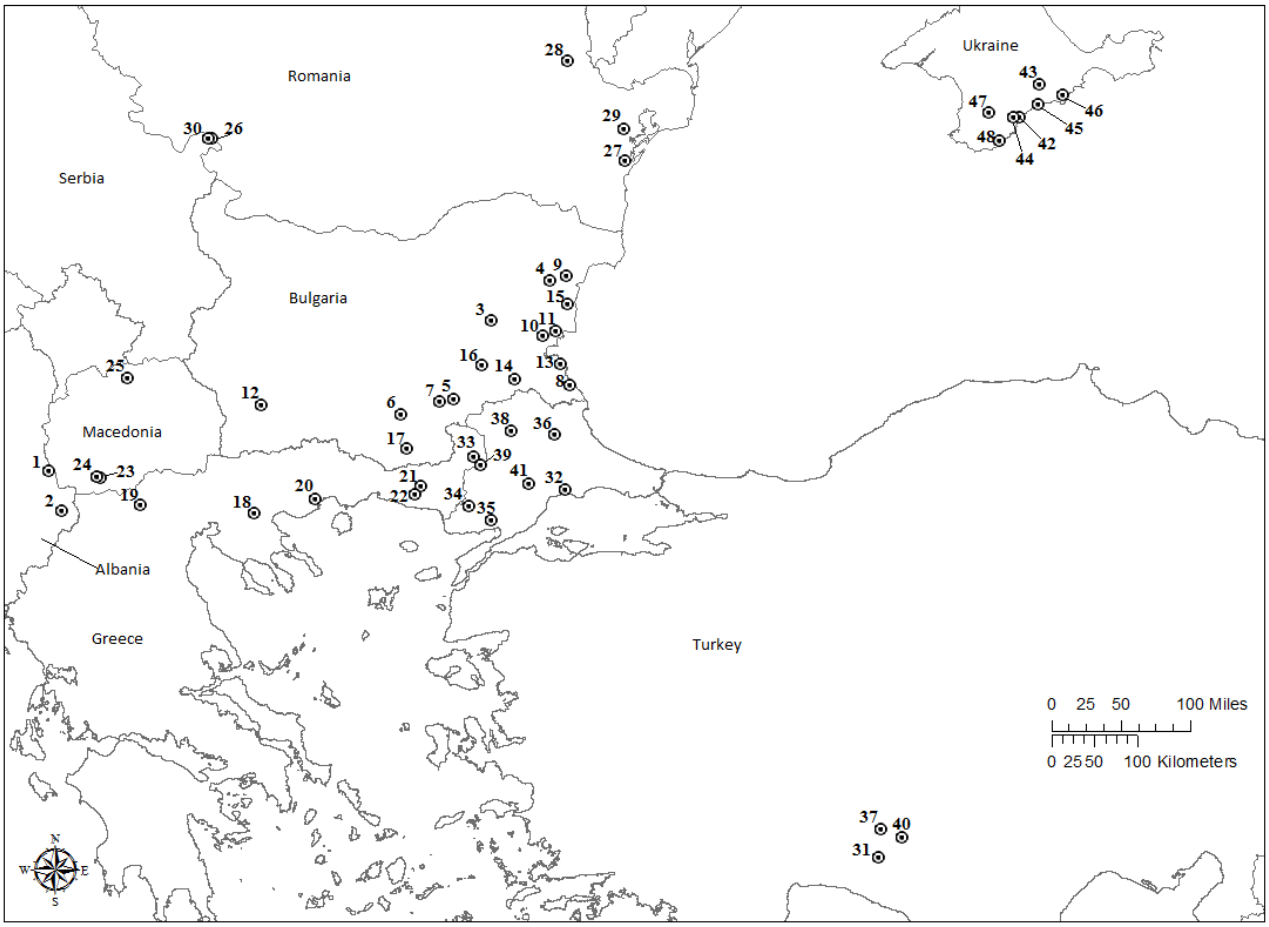


Figure 3 Map showing the distribution of populations of *Taeniatherum caput-medusae* subspecies *asperum* sampled in this study. Forty-eight (48) populations were analyzed in this study.

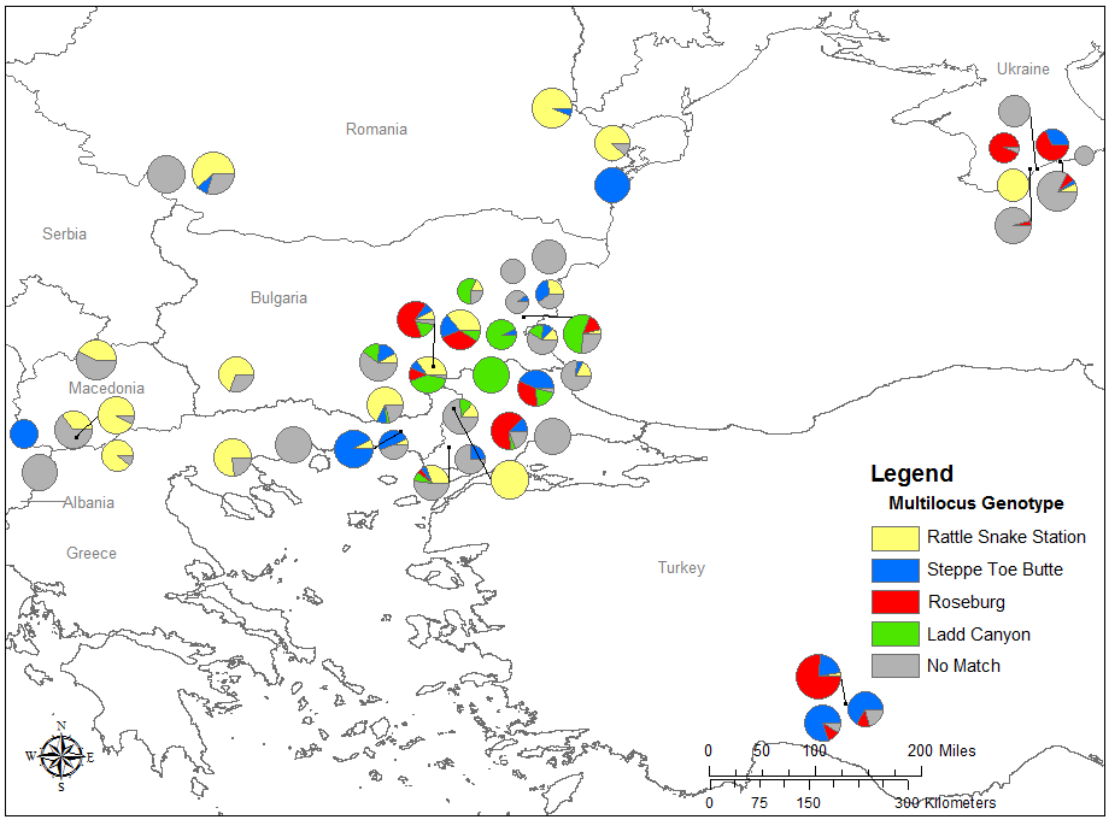


Figure 4 Map showing the distribution of multilocus genotypes (MLG) detected in native populations of *Taeniatherum caput-medusae* subspecies *asperum*. The identity of multilocus genotypes matching those detected among invasive populations are given in the legend, and non-matching genotypes are shown in grey.

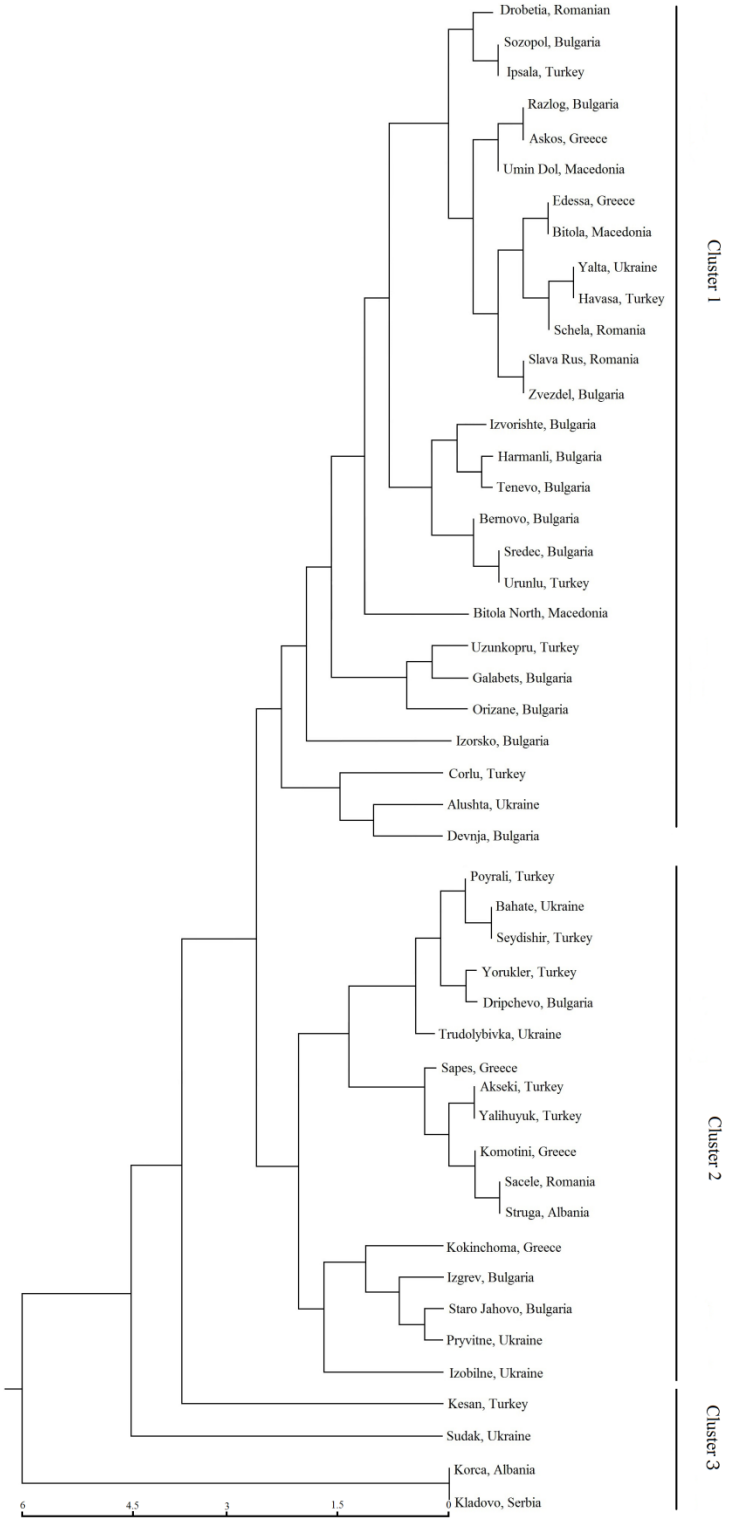
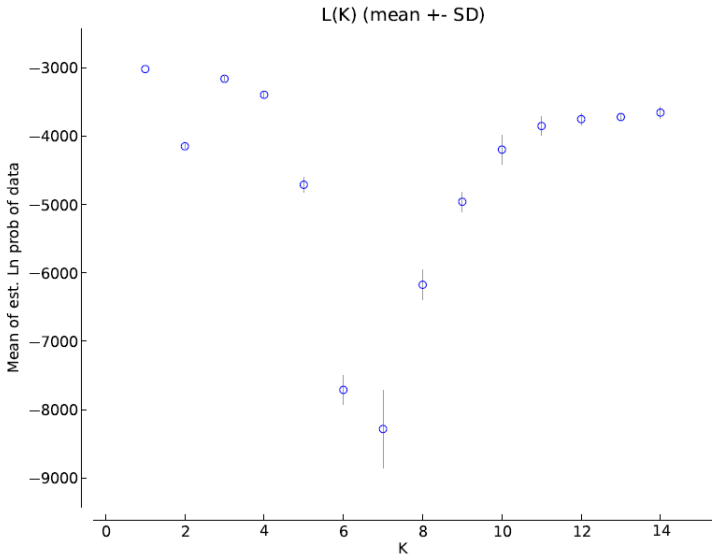


Figure 5 UPGMA cluster diagram showing genetic relationships among the 48 populations of *Taeniatherum caput-medusae* subsp. *asperum* analyzed in this study.

a) Final Parameter (100 thousand burn-in & 1 million iterations)



b) Final Parameter (100 thousand burn-in & 1 million iterations)

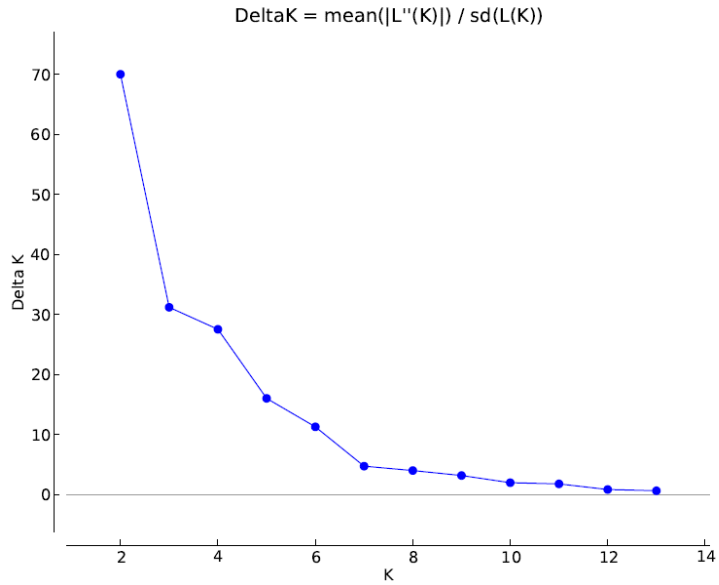


Figure 6 Results of the log probability (a) and delta K (b) analyses used to determine the number of genetic clusters (K) supported by the allozyme analysis of 48 populations of *Taeniatherum caput-medusae* subsp. *asperum*. These analyses were performed using 100 thousand burn-in iterations and a run consisting of 1 million iterations)

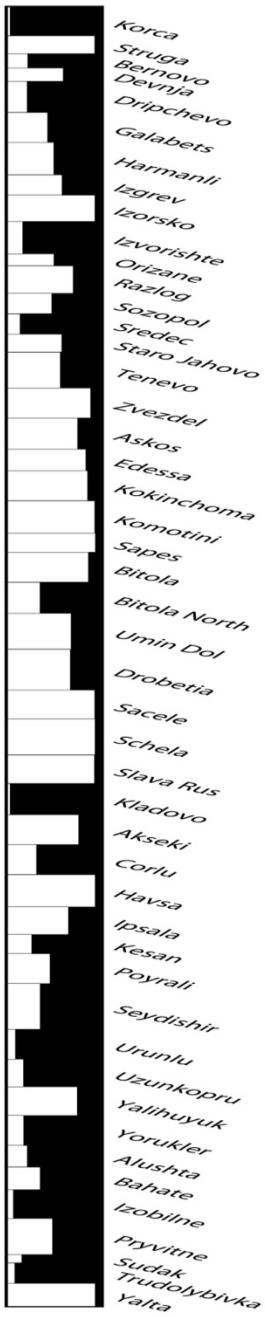


Figure 7 Genetic structure for the 48 populations of *Taeniatherum caput-medusae* subsp. *asperum*. This figure was prepared using the program DISTRUCT with a value of K=2

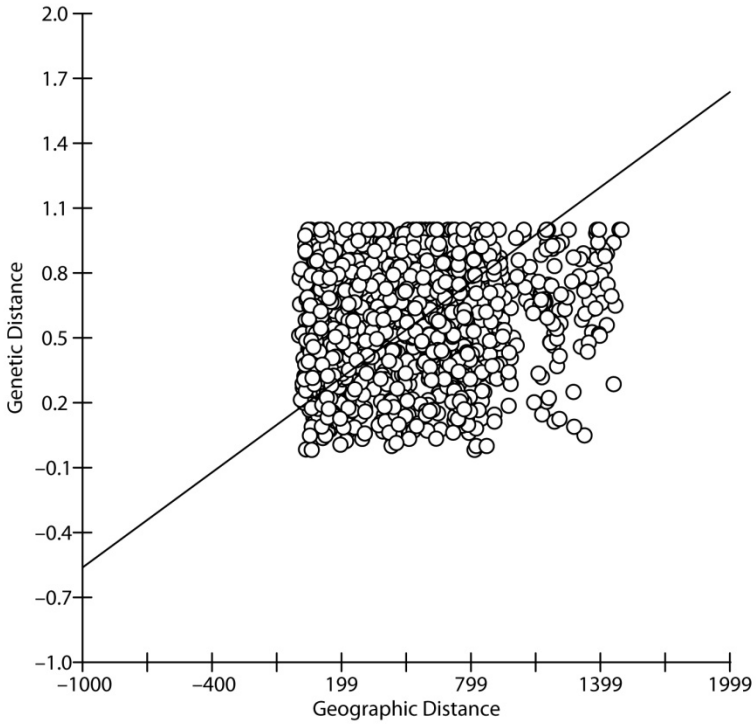


Figure 8 Isolation by Distance/Mantel test for the matrix correlation for pair-wise population values of genetic distance (pairwise FST) and geographic distance (km) for the 48 populations of *Taeniatherum caput-medusae* subsp. *asperum* analyzed in this study (r = 0.19, R2 =0.03, P=0.0128)

APPENDIX

Inferred Cluster Membership Coefficients from STRUCTURE Output

Table A.1 Inferred Cluster membership coefficients from STRUCTURE output.

K=2

Population #	Population Name	N	Inferred Cluster membership coefficients	
			1	2
1	Korca	24	0.023	0.977
2	Struga	15	0.937	0.063
3	Bernovo	12	0.216	0.784
4	Devnja	11	0.592	0.408
5	Dripchevo	26	0.208	0.792
6	Galabets	25	0.428	0.572
7	Harmanli	27	0.495	0.505
8	Izgrev	17	0.583	0.417
9	Izorsko	22	0.938	0.062
10	Izvorishte	27	0.158	0.842
11	Orizane	10	0.497	0.503
12	Razlog	23	0.702	0.298
13	Sozopol	17	0.471	0.529
14	Sredec	17	0.131	0.869
15	Staro Jahovo	15	0.581	0.419
16	Tenevo	30	0.566	0.434
17	Zvezdel	25	0.892	0.108
18	Askos	26	0.749	0.251
19	Edessa	18	0.84	0.16
20	Kokinchoma	25	0.86	0.14

21	Komotini	27	0.937	0.063
22	Sapes	16	0.944	0.056
23	Bitola	25	0.869	0.131
24	Bitola North	26	0.346	0.654
25	Umin Dol	30	0.679	0.321
26	Drobetia	34	0.674	0.326
27	Sacele	24	0.937	0.063
28	Schela	30	0.941	0.059
29	Slava Rus	24	0.932	0.068
30	Kladovo	26	0.023	0.977
31	Akseki	25	0.764	0.236
32	Corlu	25	0.3	0.7
33	Havsa	27	0.942	0.058
34	Ipsala	23	0.652	0.348
35	Kesan	16	0.257	0.743
36	Poyrali	25	0.454	0.546
37	Seydishir	38	0.348	0.652
38	Urunlu	25	0.081	0.919
39	Uzunkopru	23	0.168	0.832
40	Yalihuyuk	24	0.748	0.252
41	Yorukler	25	0.168	0.832
42	Alushta	18	0.203	0.797
43	Bahate	19	0.347	0.653
44	Izobilne	24	0.058	0.942
45	Pryvitne	30	0.479	0.521

46	Sudak	7	0.145	0.855
47	Trudolybivka	17	0.074	0.926
48	Yalta	19	0.941	0.059