AMPLIFIED FRAGMENT LENGTH POLYMORPHISM (AFLP) ANALYSIS OF *Taeniatherum caput-medusae*, AN INVADER OF WESTERN U.S. RANGELANDS

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The following individuals read and discussed the thesis submitted by student Elizabeth Catherine Kent, and they evaluated her presentation and response to questions during the final oral examination. They found that the student passed the final oral examination.

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ABSTRACT

Biological invasions are one of the main drivers of global change, and thus one of the main factors contributing to a loss of biodiversity world-wide. Introduced species can destroy habitat through predation, grazing, and competition for resources; spread disease; alter disturbance regimes; and disrupt ecosystem services. *Taeniatherum caput-medusae* subsp. *asperum* **(**medusahead) is a winter-annual grass native to Eurasia and invasive in the western United States. Medusahead dominates one million hectares of its invasive range and detrimentally affects the areas it inhabits through degradation of foraging value for livestock, increasing fire frequencies, and decreasing biodiversity. Previously, allozyme analyses have suggested this highly selfing species exhibits low genetic diversity within populations and high differentiation among populations in the invasive range. In this study, I used a dominant, multilocus molecular marker, amplified fragment length polymorphisms (AFLPs), to assess the genetic diversity and structure of 52 invasive populations of medusahead, evaluate the influence of propagule pressure and founder events during establishment, identify putative source regions, and compare my AFLP results to past allozyme results. Using 110 AFLP loci, 15 multilocus genotypes (utilizing an error rate of 3 loci) were detected among invasive populations, and I estimated that the number of independent introductions ranged from eight to 11. These data suggest moderate propagule pressure for the introduction of medusahead into the western United States. Despite moderate propagule pressure, my data revealed that invasive populations had relatively low genetic diversity and high genetic structure,

compared to plants with similar life-history traits (e.g., a highly selfing, gravity-dispersed, annual plant species). Moreover, the lower level of genetic diversity of invasive populations, compared with native populations, provides evidence that founder effects have influenced the diversity of invasive populations of medusahead. Putative source regions were narrowed to southern France and southeastern Europe. However, several lines of evidence clearly pinpoint seven populations from eastern Bulgaria, the Crimean peninsula, Russia, and central Greece as the most likely source populations for this invasion. My findings are generally similar to that of previous allozyme studies; although my estimates of genetic diversity are higher than the estimates using allozymes. Results of this study point to the additional insights into the invasion process that can be gained by using a more polymorphic molecular marker.

Keywords: AFLPs, medusahead, propagule pressure, founder effects, invasive species, multiple introductions, source populations

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INTRODUCTION

Human population growth and human activities such as international commerce and travel contribute to global change (United Nations 2015). Many deleterious environmental effects accompany global change including the loss of biodiversity. Five major drivers associated with global change and losses of biodiversity include land-use change, climate change, nitrogen deposition and acid rain, elevated carbon dioxide concentrations, and biotic exchange (Sala *et al.* 2000). With an increase in biotic exchange, the potential for biological invasions increases. Biological invasions occur when species are introduced in a nonindigenous area and are able to persist, flourish, and spread (Mack *et al.* 2000, Lockwood *et al.* 2013). Thus, invasions are a key component of global change and one of the main causes of declines in biodiversity (Vitousek *et al.* 1996). Few regions of the world are free of invasive species (Pyšek and Richardson 2010). Pimentel *et al.* (2001) estimated that over 480,000 invasive species have been introduced into six nations (the United States, Australia, Great Britain, South Africa, India, and Brazil). Fifty thousand alien plant and animal species are estimated to occur in the United States (U.S.) alone (Pimentel *et al.* 2001), with the number of invasive plants estimated in the country ranging from 20,000 (Pimentel *et al.* 2005) to 5,000 (Morse *et al.* 1995).

Invasive species can harm native species and destroy habitat through predation, grazing, and competition for resources; spread disease; alter disturbance regimes (e.g., alter the frequency and intensity of fires); and even eliminate natives through

hybridization (Mack *et al.* 2000). Ecosystem services are often disrupted, resulting in degradation to socioeconomic, cultural, and human health impacts (Pyšek and Richardson 2010). There are enormous monetary costs associated with these impacts and the attempts to control and minimize effects of invasions. The United States spends $120 billion a year on the harmful consequences and the control of invasive species (Pimentel *et al.* 2005). The previously mentioned six nation study estimated a total expenditure of over $314 billion per year in damages associated with invasions (Pimentel *et al.* 2001).

Whether introduced deliberately (such as for horticulture, agriculture, or biological control) or accidentally (occurring as a contaminant in global trade or associated with human movement), invasive species' exhibit a range of impacts. For instance, the brown anole lizard (*Anolis sagrei*) native to Cuba was accidentally introduced in the U.S., and while this invasive lizard has caused a behavioral change in the perching location of a native lizard species (Edwards and Lailvaux 2012) it has not negatively impacted biodiversity. Conversely, the brown tree snake (*Boiga irregularis*) is invasive in Guam (Richmond *et al.* 2015), and it has destroyed Guam's avifauna diversity (extirpating 13 of 22 native breeding birds) and has high monetary costs associated with increased shipping rates (trade fees), electrical powerline damage, and control of the snake (Rodda and Savidge 2007). Some intentional releases of non-native species for biological control have had some unintended negative consequences (Shine 2010, Veale *et al.* 2015), but Suckling and Sforza (2014) demonstrated that over 99% of biological control attempts on invasive weeds in the U.S. have had no non-target impacts on native plant populations.

For an invasion to take place, a series of steps referred to as the invasion process must occur (Kolar and Lodge 2001, Lockwood *et al.* 2005). Propagules of a species must be taken from their native range, transported via a vector (and survive) to a new area where they are introduced, naturalized, and then spread beyond their area of introduction (Kolar and Lodge 2001, Lockwood *et al.* 2005). Williamson and Fitter (1996) estimated general survivability of these species through three major transitional stages in the invasion process: escaping (from the native environment), establishing (in the nonindigenous environment), and becoming a pest (moving from the original establishment area). The "ten's rule" (Williamson and Fitter 1996) estimates only 10% of species complete any one transition stage in this process. Thus, a small percentage of those starting the journey will survive transport to a new range or establishment in that new range with little chance of becoming a pest.

Mating-system can contribute to establishment success and subsequent invasion with self-compatible species having greater probabilities of success (Baker 1955, 1967). This concept, called "Baker's Law" (Stebbins 1957), states that self-fertilizing species have higher likelihoods of establishment outside of their native range because they do not require a mate to achieve reproductive success. Thus, one individual has the potential to found a new population. As an example, a single individual of *Miconia calvescens*, an invasive tree established from multiple introductions throughout the Pacific Islands, has been shown to have been introduced and naturalized in Tahiti from a single individual in 1937 (Meyer 1996, Le Roux *et al.* 2008). Perhaps an oversimplification (see Cheptou 2012), Baker's law is the basis of much research on invasive populations and species (Barrett 2015).

The manner by which a nonnative species is introduced into an area will affect the genetic diversity and fitness of the individuals in founding populations (Gaskin *et al.* 2013) and there are various hypotheses of how alien species establish and invade new ranges (for a summary see Hierro *et al.* 2005). From these hypotheses, propagule pressure is now recognized as playing an important role in the establishment success of nonnative species, as well as range expansion during invasion. Propagule pressure encompasses two key components: propagule size (the number of individuals) and propagule number (the number of independent introductions/arrival rate) (Lockwood *et al.* 2005, Simberloff 2009). Propagule pressure also includes propagule richness, which refers to the number of unique genotypes introduced into an area or the number of taxa introduced into an area at one time (Ricciardi *et al.* 2011). The greater the number of individuals in a single introduction event and the greater the number of independent introductions, the higher the propagule pressure and the higher the probability of establishment and subsequent range expansion (Lockwood *et al.* 2005, Lockwood *et al.* 2009, Simberloff 2009, Blackburn *et al.* 2015). Evidence indicates increased establishment success with higher propagule pressure regardless of whether a species is deliberately introduced (*Galerucella calmariensis/G. pusilla*; Grevstad 1999), accidentally introduced (*Imperata cylindrica*; Lucardi *et al.* 2014), or escapes from captivity (*Myiopsitta monachus*; Goncalves da Silva *et al.* 2010).

Introduced species can originate from a single source population or from multiple source populations that are geographically separated in the native range (Lucardi *et al.* 2014). This may result in admixture within invasive populations, mating among formerly separated individuals within an invasive population (Lee 2002), and the generation of

novel recombinant genotypes (Novak and Mack 2005). In turn, small founder populations can reduce the likelihood of establishment and founder effects can reduce the genetic diversity within invasive populations and increase the genetic structure of populations throughout the new range. Founder effects occur when a small number of individuals establish a new population (Mayr 1942), leading to reduced genetic diversity relative to native populations. High propagule pressure (e.g., large founder populations) can lead to the establishment of populations with higher amounts of genetic diversity, thus reducing the likelihood of severe founder effects (Novak 2011).

The use of molecular markers can provide a better understanding of invasion dynamics, range expansion, and mechanisms of dispersal for invasive species (Novak 2004). Huttanus *et al.* (2011) summarized several genetic patterns associated with high propagule pressure: 1) a large number of genotypes or haplotypes in the invasive range, 2) comparable levels of genetic diversity within native and invasive populations, 3) genetic admixture that may not occur within native populations, and 4) if genetic admixtures are common in native and invasive populations, similar genetic structure will exist. A variety of molecular markers such as allozymes and amplified fragment length polymorphisms (AFLPs) can be used in the genetic analysis of invasive species (see Table 1 in Liu and Cordes 2004 for a summary). Allozymes, a codominant marker used since the 1960s, detect variability at a single locus but usually detect lower levels of genetic polymorphisms, compared with other markers. With a dominant marker such as AFLPs, many more loci can be included in the analysis (Liu and Cordes 2004; Vos *et al.* 1995). AFLPs use a multi-step process to create a unique banding pattern or “fingerprint” to assay genetic diversity without prior knowledge of an organism’s genome (Vos *et al.*

1995). AFLPs are an excellent technique for genetic analysis and have advantages over other markers due to their efficiency, high polymorphism content, and reproducibility (Jones *et al.* 1997).

Taeniatherum caput-medusae (L.) Nevski is a selfing, annual invasive grass that has negative ecological and economic consequences in the western U.S. Previous genetic analyses of invasive (S.J. Novak, unpublished data) and native populations (Peters 2013, Skaar 2015) of this species using enzyme electrophoresis (allozymes) have documented moderate propagule pressure through multiple introductions. Founder effects have led to a severe reduction in genetic diversity within introduced populations and high genetic structure among invasive populations. There have been few published studies on the genetic diversity of *T. caput-medusae* and more research on this species is needed to better understand this invasion (Rector *et al.* 2013). In this study using AFLPs, I will 1) evaluate the genetic diversity within invasive populations of *T. caput-medusae* in the western U.S.; 2) determine the genetic structure of invasive populations of *T. caput-medusae*; 3) assess the introduction dynamics (evidence for multiple introductions), propagule pressure, and founder effects of invasive populations; 4) identify putative source populations or regions by comparing my data to the AFLP data derived by the analysis of native populations; and 5) compare my results from AFLPs to the results of previous genetic analysis using allozymes. This genetic analysis will contribute to the overall body of knowledge of introduction dynamics and explore the impacts of propagule pressure, introduction dynamics, and founder effects on the genetic diversity of this invasive species. The ability to better understand the role of propagule pressure in the proliferation of an invasive species will lead to broader ecological and management

insights (Lockwood *et al.* 2005) and provide information to those attempting to manage or control this specific invasive plant (Novak 2004).

MATERIALS AND METHODS

Study Species

Taeniatherum caput-medusae (L.) Nevski **(**Poaceae, medusahead) is a winter-annual grass native to the western Mediterranean, Eastern Europe, and Central Asia (Frederiksen 1986) and invasive in the western United States (Nafus and Davies 2014). Three subspecies have been recognized: *T. caput-medusae* subsp. *caput-medusae*, *T. caput-medusae* subsp. *crinitum*, and *T. caput-medusae* subsp. *asperum* (Frederiksen 1986) with only *T. caput-medusae* subsp. *asperum* invasive in western U.S. rangelands. In its invasive range, medusahead germinates in the fall (Young 1992, Novak 2004) and sets seed by mid-July. It possesses cleistogamous flowers that lead to a primarily self-pollinating (selfing) mating system. Selfing rates in excess of 99% have been reported for native and invasive populations (S.J. Novak, unpublished data). First identified in 1884 near Roseburg, Oregon, the plant has a well-documented collection history that includes Oregon, Washington, Idaho, Utah, Nevada, and California (Novak 2004). Duncan and Jachetta (2005) estimated that medusahead is expanding its area of infestation at a rate of 12% per year. This species dominates one million hectares of the western United States (Duncan *et al*. 2004) and the plant was documented in Montana for the first time in 2013. Areas dominated by medusahead experience negative ecological and economic impacts, such as reduced foraging value for grazing animals due to its unpalatability (Lusk *et al.* 1961) and a reduction of up to 50-80% of grazing capacity for livestock (Hironaka 1961). Long, sharp awns are attached to the lemma, and these awns can cause injury to grazing

animals (Rice *et al.* 2005). Medusahead increases an invaded area's risk and frequency of wildfires (D'Antonio and Vitousek 1992). As a consequence of more frequent wildfires, unstable watersheds with increased soil erosion occur (Grey *et al.* 1995). Medusahead also greatly decreases biodiversity due to the thick layer of litter (thatch) the plant produces, which prevents emergence and growth of other plant species (Grey *et al.* 1995). Medusahead seeds are gravity dispersed, but long-distance dispersal may occur because the long awns can become attached to fur, clothing, and machinery (Davies 2008). Davies (2008) reported that 75% of all seeds land within 0.5 meters of the invasion front, with the majority of the remaining seeds dispersing no more than two meters.

Population Sampling and DNA Extraction

Spikes from medusahead plants have been collected over many years (1997 to 2014) over a wide range of locations spanning the invasive range of the grass in the western U.S. (Table 1, Fig. 1). Intact spikes from individual plants were haphazardly sampled in 52 distinct localities: 12 populations from Washington, nine populations from Oregon, 10 populations from California, one population from Nevada, three populations from Utah, 15 populations from Idaho, and two populations from Montana. Samples were stored in individual envelopes at Boise State University. Due to the age of some samples, I performed an initial feasibility study to test for the ability for seeds to germinate. I imbibed caryopses (hereafter referred to as seeds) from select individuals for 24, 48, and 72 hours. Older seeds did not germinate, but DNA extractions from the seeds proved successful as long as they were imbibed for at least 24 hours. DNA was extracted utilizing Qiagen DNeasy Plant Mini Kits (Valencia, CA) with a modification that

included incubation in a water bath at 65°C for 2 h. After extraction, total genomic DNA was stored in a freezer at -18°C.

AFLP Analysis

I performed AFLP procedures as outlined in Vos *et al.* (1995), utilizing the specific protocol described by Lucardi (2012). The AFLP technique includes four major steps: restriction/digestion, ligation, and two polymerase chain reactions (pre-selective and selective amplification). In restriction/digestion, the extracted DNA was double digested with restriction enzymes *EcoR*1 (Promega, Madison, WI) and *Mse*1 (New England Biolabs, Ipswich, MA) in a Bio-Rad PTC-200 Thermal Cycler (Hercules, CA) for 2 h at 37°C followed by 15 m at 70°C. Adapter pairs (Eurofins Operon, Huntsville, AL) *EcoR*1 (forward: *5'-CTCGTAGACTGCGTACC-3'*, reverse: *5'-AATTGGTACGCAGTCTAC-3'*) and *Mse*1 (forward: *5'-GACGATGAGTCCTGAG-3',* reverse: *5'-TACTCAGGACTCAT-3')* were ligated to restricted DNA fragments by incubating reactions at 37°C for 3 h. Following ligation, the first round of PCR was performed. Pre-selective amplification utilized the primer pair (Eurofins Operon, Huntsville, AL) *Eco+A* (*5'-GACTGCGTACCAATTC+A-3'*) and *Mse+C* (*5'-GATGAGTCCTGAGTAA+C-3'*) and used the following thermocycler protocol: 1 m at 94°C, 30 cycles of 30 s at 94°C, 1 m at 56°C, and 1 m at 72°C, and ending with 2 m at 72°C. I performed selective amplification on diluted (1:20) pre-selective products with two different primer pair combinations (Eurofins Operon, Huntsville, AL). The first primer pair consisted of *Eco+ACC* (*5'-GACTGCGTACCAATTC+ACC-3'*) and *Mse+CTC* (*5'-GATGAGTCCTGAGTAA+CTC-3'*). The second primer pair consisted of *Eco+ACT* (*5'-GACTGCGTACCAATTC+ACT-3'*) and *Mse+CAC* (*5'-*

GATGAGTCCTGAGTAA+CAC-3'). Both *Eco* primers were fluorescently labeled with 6-FAM (6-carboxyl fluorescein) in order to visualize bands during capillary electrophoresis. Reactions were heated for 2 m at 94°C, 10 cycles of 30 s at 94°C, 30 s at 65°C, and 1 m at 72°C, and ending with 30 s at 72°C.

Separation of the AFLP fragments was performed by Genewiz Laboratories (South Plainfield, NJ) at both their New Jersey and Maryland locations. Genewiz conducted capillary electrophoresis on an Applied Biosystems ABI 3730 device (Foster City, CA) and produced an electropherogram for scoring. I scored the AFLP electropherograms using the software GeneMarker (SoftGenetics, LLC, State College, PA). I initially used the GeneMarker panel option to automatically select scorable bands with a minimum intensity of 75 relative fluorescent units (RFUs) and a size greater to or equal to 58 base pairs. Then, I manually selected or rejected each band based on consistent peak morphology. All electropherograms were autoscored by the "run wizard" at 40 RFUs with the resulting panel. At each locus, individuals were scored as "1" if the band was present and "0" if the band was absent. I manually inspected all peaks on the electropherograms after automated scoring to ensure accuracy. This scoring procedure was repeated for both sets of primer pairs and the resulting data sets were combined into a master data set consisting of 110 loci. AFLP amplifications and scoring procedures were repeated from extracted DNA on 20% of all individuals resulting in an error rate of 2.0097%, which translated to a three loci mismatch for the 110 loci.

Statistical Analysis of AFLP Data

Range-wide and within-population genetic diversity was primarily evaluated in AFLP-surv (Vekemans 2002). This is a software program designed specifically for the

analysis of AFLP data and was used to calculate the number of polymorphic loci (P), the percent of polymorphic loci (%P), and expected heterozygosity (H_e). The multilocus genotype (MLG) of each individual was determined in GenoType (Meirmans and Van Tienderen 2004). GenoDive (Meirmans and Van Tienderen 2004) was used to determine the Simpson's Genotypic Diversity Index (D_s), Simpson's Evenness (E_s), and the Shannon-Wiener Diversity Index (H') based on the MLG data from the GenoType output. The GenoType/GenoDive software requires users to include an error rate; this is the only analysis in my project that considered the error rate (3 bands). I employed AFLPdat (Ehrich 2006) source script to convert my AFLP data to the appropriate format for subsequent analysis in Hickory 1.1 (Holsinger and Lewis 2003). The range-wide selfing rate, *f*, a parameter equivalent to the inbreeding coefficient (F_{IS}), was estimated for invasive populations using Hickory 1.1 (Holsinger and Lewis 2003).

I calculated among-population genetic diversity and population genetic structure in accordance with the Lynch and Milligan (1994) method in AFLP-surv. Parameters measured include total gene diversity (H_t), the mean gene diversity within populations (H_w), and genetic differentiation among populations (H_b). The proportion of the total gene diversity partitioned among populations (F_{ST}) and pairwise F_{ST} was calculated for all populations sampled (1,000 permutations and bootstraps) to evaluate genetic structure. Analysis of molecular variance (AMOVA) was conducted using GenAlEx 6.5 (Peakall and Smouse 2006, 2012) to estimate the partitioning of genetic diversity within populations and among populations; a second AMOVA was conducted in which populations from each state were grouped into regions. I used the default settings in GeneAlEx to conduct both AMOVAs. I created a neighbor-joining tree using the

pairwise F_{ST} output file from AFLP-surv for invasive populations in PHYLIP 3.695 (Felsenstein 2008). The Bayesian-based assignment software STRUCTURE (Pritchard *et al.* 2000) was used to determine the number of genetic clusters (K) within the invasive range using five iterations of 100,000 burn-in and 300,000 Markov Chain Monte Carlo (MCMC) with no admixture assumed. I ran two simulations from K = 1-15 and then K = 1-8. In addition to the Pritchard *et al.* (2000) method, I employed the method of Evanno *et al.* (2005) to determine the most appropriate K value from the STRUCTURE results. The method of Prichard *et al.* (2000) provided equivocal estimates of K, while the method of Evanno *et al.* (2005) provided a much clearer estimate of K and this was the method I chose to determine K in all STRUCTURE analyses. STRUCTURE provided membership probabilities to all individuals assayed. Individuals with 97% or greater assignment probability to a given cluster were considered fully assigned to that cluster. Those individuals with 3% or greater membership probability to other clusters were considered to have mixed ancestry. This assignment threshold is higher than the membership probability thresholds employed by Lucardi *et al.* (2014) and Campitelli and Stinchcombe (2014). Linear regression was performed in R version 3.1.1 (R Core Team 2014) to examine patterns associated with expected heterozygosity and percent of polymorphic loci with distance from Roseburg, Oregon, the first locality where medusahead was collected and possibly its first introduction site in the western U.S.

I compared my data to that of a study examining genetic diversity within medusahead's native range (Guerdan 2016). This comparison allowed me to assess source populations, introduction dynamics and founder effects for this invasion. In this native range study, Guerdan (2016) surveyed 70 populations of medusahead (*T. caput-*

medusae subsp. *asperum* only) throughout its native range (Appendix A). Data from this study were obtained for populations from 13 countries including Albania (two populations), Bulgaria (16 populations), France (one population), Greece (six populations), Italy (six populations), Macedonia (three populations), Morocco (five populations), Romania (four populations), Russia (one population), Serbia (one population), Spain (four populations), Turkey (12 populations), and Ukraine (nine populations). Genetic diversity indices calculated in AFLP-surv for the native range were compared to my results from the invasive range. I compared mean expected heterozygosity and percent of polymorphic loci for significant differences using a Mann-Whitney U Test (a non-parametric two sample t-test) in R. I combined the results from several analyses in an attempt to provide the most clarity in examining putative source populations and to examine introduction dynamics. GenoType was employed considering the error rate to identify matching MLGs between populations in the native and invasive range. I repeated STRUCTURE analysis, as outlined above, with a simulation of K = 1-10 on a combined data set of both the native and invasive range populations to determine K for the entire species' range. A subsequent sub-structuring analysis was conducted with a simulation of K = 1-8. I used PHYLIP to create a neighbor-joining tree on the combined data based on pairwise F_{ST} produced by AFLP-surv to assess the genetic relationships of native and invasive populations of medusahead.

Finally, I conducted correlation tests to assess the relationship between allozyme and AFLP data in R using the Spearman rank test comparing expected heterozygosity, percent of polymorphic loci, and number of MLGs. Differences between these data sets were compared using the Mann-Whitney U Test in R.

RESULTS

In this study, I scored 417 individuals from 52 distinct populations of medusahead over 110 AFLP loci in the invasive range, an average of 8.02 individuals per population. These results reveal lower genetic diversity and higher genetic structure of invasive populations, compared with native populations; provided data for identifying the geographic origins of this invasion; and allowed for a comparison of results, for the same populations, obtained with a dominant and co-dominant molecular marker (AFLPs and allozymes, respectively).

Genetic Diversity

Range-wide genetic diversity estimates including the number of polymorphic loci (P), percent of polymorphic loci (%P), and the expected heterozygosity (H_e) are given in Table 2. Across invasive populations, 49 of the 110 assayed AFLP loci were polymorphic (%P = 44.5). The range-wide expected heterozygosity was 0.083 (S.E. ± 0.015). Within-populations, the number of polymorphic loci ranged from 1-16, averaging 6.0 per population (Table 3). The mean value of percent of polymorphic loci per population was 5.4, with values ranging from 0.9 to 14.5. The population with the highest number of polymorphic loci and percent of polymorphic loci was White Bird, Idaho (P = 16, %P = 14.5) followed by Threemile Creek, Washington (P = 13, %P = 11.8). The lowest number of polymorphic loci and percent of polymorphic loci was found in South Canyon Road, Utah and Chuck's Place, Montana, both having only one polymorphic locus and %P = 0.9. Populations from California averaged the highest mean number of polymorphic loci

(P = 6.6) and highest percent of polymorphic loci (%P = 6.0) and Montana had the lowest values (P = 1.5, %P = 1.4). Within-population expected heterozygosity ranged from 0.002 to 0.059 (S.E. ± 0.002-0.015) with an overall mean expected heterozygosity of 0.020 (S.E. ± 0.008). Consistent with the highest number of polymorphic loci and percent polymorphic loci, the population with the highest expected heterozygosity was White Bird, Idaho (H_e = 0.059, S.E. ± 0.015), followed closely by Loma Prieta, California (H_e = 0.045, S.E. ± 0.014) and Threemile Creek, Washington (H_e = 0.039, S.E. ± 0.011). South Canyon Road, Utah (H_e = 0.002, S.E. ± 0.002) had the lowest expected heterozygosity value, followed by Chuck's Place, Montana (H_e = 0.003, S.E. ± 0.003). The California populations had the highest mean expected heterozygosity values (H_e = 0.025, S.E. ± 0.009) and the lowest value (H_e = 0.004, S.E. ± 0.003) occurred in the Montana populations.

Considering an error rate of three bands, there were 15 unique AFLP MLGs among all invasive populations (Table 4); only two of those MLG (1 and 5) were shared among populations. Eighty-nine percent (89.2%) of all individuals (372 of 417) in the invasive range possessed MLG 1, the most common genotype (MCG) (Appendix B). Forty one of these 52 populations (78.8%) were monomorphic for the MCG. Seven populations contained more than one MLG with at least one individual possessing the MCG. Populations containing the MCG and other genotypes included Canby, California (MLG 4), Jepson Prairie, California (MLG 6 and 7), Pullman, Washington (MLG 8), Salt Creek, Utah (MLG 11), Old State Penitentiary, Idaho (MLG 12), Threemile Creek, Washington (MLG 13), and White Bird, Idaho (MLG 14 and 15). Polymorphic populations without the MCG included Al Black's Doghouse (MLG 2 and 3) and Quincy,

California (MLG 9 and 10). Both Montana populations (Chuck's Place and Nicholson Site) were monomorphic for MLG 5, which is only found in those two populations. California populations contained the most MLGs (1.4) and five MLGs were only detected among the populations from California. Oregon, Nevada, and Montana contained only one MLG per population and that genotype was monomorphic in all populations within each of these states. Overall, the invasive range contained an average of 1.2 MLGs per population.

Simpson's Genotypic Diversity Index (D_s) and the Shannon-Weiner Index (H') were zero for all 43 monomorphic populations (Table 4). I did not calculate Simpson's Evenness for these populations as they only possessed one MLG. Simpson's Genotypic Diversity was highest in Pullman, Washington ($D_s = 0.476$), followed by Jepson Prairie, California ($D_s = 0.464$) and White Bird, Idaho ($D_s = 0.417$), and ranged from 0.000 to 0.476. California populations had the highest Simpson's Genotypic Diversity Index values ($D_s = 0.125$), with populations from Montana, Nevada, and Oregon having no diversity ($D_s = 0.000$). The value of D_s averaged across all invasive populations was 0.060. Pullman, Washington had the highest Simpson's Evenness value ($E_s = 0.845$) and White Bird, Idaho had the lowest value (E_s=0.529). The mean Simpson's Evenness value for invasive populations which contained more than one MLG was 0.694, with populations from Washington ($E_s = 0.716$) having the highest average value and populations from Idaho (E_s=0.585) having the lowest. Shannon-Weiner Index values ranged from 0.000 to 0.320, with a mean value of 0.038 for all invasive populations. The highest value occurred in the population from Jepson Prairie, California (H'=0.320) and the lowest (H'=0.000) was found in each of the 43 monomorphic populations. California

populations had the highest value (H'=0.077), with the lowest value (H'=0.000) occurring in states with monomorphic populations (Oregon, Nevada, and Montana).

The value for the selfing rate, *f*, was estimated to be 0.979 across all populations of the invasive range (data not shown). Holsinger *et al.* (2002) were confident in Hickory's ability to estimate *f* from dominant markers, but now urge "extreme caution" when interpreting results of *f* due to discrepancies in some analyses. In their user manual, they recommend referencing previous work to determine consistency of inbreeding values before using Hickory results. The *f* estimate obtained from Hickory was consistent with the previous estimates of selfing (> 99%) in native and invasive populations of medusahead (S.J. Novak, unpublished data).

Population Genetic Structure

The total gene diversity (H_t) for invasive populations was 0.084 (S.E. ± 0.002) (Table 5). The mean value for the amount of total gene diversity partitioned within populations (H_w) was 0.020 (S.E. ± 0.003), which was three-fold less than the amount of the total gene diversity partitioned among population (H_b = 0.064; S.E. ± 0.024). The value of F_{ST} for all invasive population was 0.761, indicating that 76.1% of the total genetic diversity was partitioned among populations (Table 5). Results of the two AMOVA analyses were in close agreement with this value of F_{ST}. The first AMOVA analysis (Table 6a) revealed that 24% of the total genetic diversity was partitioned within populations and 76% of the total genetic diversity was partitioned among populations. With the addition of another hierarchical level, regions (states), the AMOVA results showed that 23% of the total diversity was partitioned within populations, 57% was partitioned among populations within regions, and 19% was partitioned among regions

(Table 6b). Linear regression analysis revealed no significant relationship between expected heterozygosity and Euclidian distance from Roseburg, Oregon ($F_{1,50} = 0.823$, $r^2 = -0.004$, $p > 0.36$) (Fig. 2a). No pattern was found for the relationship between percent of polymorphic loci and Euclidian distance from Roseburg, Oregon ($F_{1,50} = 0.541$, $r^2 = -0.009$, $p > 0.46$) (Fig. 2b).

I performed STRUCTURE analyses using two separate simulations (K = 1-15 and K = 1-8) to determine the appropriate number of genetic clusters (K). Using the method of Evanno *et al.* (2005), strong support for both K = 2 and K = 4 was obtained in the first simulation (K = 1-15). I performed an additional simulation, narrowing the range of possible K = 1-8, and the method of Evanno *et al.* (2005) resulted in the strongest support for K = 4 (Fig. 3a). The four clusters (blue, red, green, and yellow) are displayed graphically and mapped geographically (Fig. 4). Approximately 93% of individuals (387 of 417) were assigned to a cluster with greater than 97% assignment probability. Thirty individuals (approximately 7% of individuals) were assigned to multiple clusters implying these individuals had mixed ancestry. The majority of populations (39 of 52 = 75%) were monomorphic for a single genetic cluster and the remaining 13 populations (25%) were either polymorphic for individuals assigned to different clusters, contained admixed individuals, or both.

The genetic cluster indicated by the yellow color had the highest frequency of occurrence; it was observed in 14 monomorphic populations in the western U.S. and 121 individuals were fully assigned to this cluster (Fig. 4). The yellow genetic cluster dominated in Idaho; only two of 15 populations did not exhibit this cluster. This cluster also occurred in two of the three Utah populations (16 of 26 individuals). The genetic

cluster designated as green had the lowest frequency within invasive populations; it occurred in six monomorphic populations (66 individuals). Both populations (17 individuals) in Montana were monomorphic for the green genetic cluster. The red genetic cluster was monomorphic in 11 populations (103 individuals). It was most prevalent in populations from the state of Washington. Eight populations (97 individuals) were monomorphic for the blue genetic cluster and it was most prevalent in Oregon. At least one admixed individual occurred in 10 different invasive populations: three populations in California (Henry Coe State Park, Kelseyville, and Laytonville), three populations in Idaho (Lapwai, Black's Creek, White Bird), three populations in Oregon (Roseburg, Klamath Falls, and Emigrant Hill), and one population in Washington (Threemile Creek). Of the 30 admixed individuals, 29 individuals had membership to two clusters and one individual (found in White Bird, Idaho) had membership to three clusters. In both Henry Coe State Park and Kelseyville, all individuals within these populations were admixed and members of the same clusters (Fig. 4).

The neighbor-joining tree depicts genetic relationships among invasive populations based on pairwise F_{ST} values (Fig. 5). The populations from Kelseyville, California, White Bird, Idaho, Steptoe Butte, Washington, and Black's Creek, Idaho were excluded from assignment to any one cluster as they occur on their own branches, indicating that these four populations were highly diverged from the others. The remaining 48 populations formed nine clusters. Regional patterns emerged in some clusters. For example, Clusters 1 and 2 contained most populations from Idaho and Clusters 5a and 5b contained many populations from eastern Oregon and northern California. Other clusters revealed that populations from different regions co-occurred in

the same cluster. For example, Cluster 4a contained two California populations, two populations from Washington, and two populations from Oregon.

Comparison of Genetic Diversity and Structuring with Native Populations

Data from an AFLP analysis of 70 native populations were compared to the results of this analysis of 52 invasive populations (Appendix A, Table 1). This comparison revealed that invasive populations have lower values for almost all genetic diversity parameters, when compared to native populations. For native populations, P = 104, %P = 94.5, and H_e = 0.166 (S.E. ± 0.013), while the value of these parameters were greatly reduced for invasive populations (P = 49, %P = 44.5, H_e = 0.083) (Table 2). The mean number of polymorphic loci per population (P) in the invasive range is 6.0 compared to 12.9 in the native range (Table 7). A Mann-Whitney U Test revealed significant reductions in H_e (0.020; $P < 0.001$) and %P (5.4; $P < 0.001$) for invasive populations compared to native populations (H_e = 0.049, %P = 11.7).

Fifteen MLGs (using the error rate) were detected among the invasive populations (with an average of 1.2 MLGs per populations), while 132 MLGs were detected among native populations (with an average of 2.5 MLGs per population). Simpson's Genotypic Diversity Index (D_s), Evenness (E_s), and the Shannon-Winer Index (H') were all reduced for invasive populations (D_s = 0.060, E_s = 0.694, H' = 0.038), compared with native populations (D_s = 0.358, E_s = 0.858, H' = 0.252).

The total gene diversity (H_t) for invasive populations was 0.084 (S.E. ± 0.002) compared with 0.171 (S.E. ± 0.004) for native populations (Table 5). Thus, invasive populations had lower total gene diversity compared with native populations. Additionally, invasive populations had slightly higher genetic structure than native

populations; the F_{ST} value of invasive populations was 0.761, while this value for native populations was 0.717.

Of the 15 MLGs detected among invasive populations and the 132 MLGs discovered among native populations, only one MLG, the MCG, was shared between the invasive and native ranges (see Appendix B). Forty eight of the 52 invasive populations contain at least one individual with the MCG, while 46 of the 70 native populations contain the MCG (55.6% of all native individuals possessed the MCG). The remaining 14 MLGs detected in the western U.S. were not found in any native individuals. Likewise, the unique 131 MLGs found in the native range did not correspond to any individuals sampled in the invasive range.

Identification of Source Populations or Regions

Four hundred and ninety five individuals from 70 native populations of medusahead were combined with the invasive populations to produce a data set consisting of 912 individuals from 122 populations. This combined data set was analyzed using the program STRUCTURE. The method of Evanno *et al.* (2005) found a K = 2 (Fig. 3b). Both genetic clusters occurred among native populations (Fig. 6a), but only one cluster was detected within invasive populations. Individuals assigned to the genetic cluster that only occurred within native populations were removed from the data set (they are indicated by the red color), and a subsequent sub-structuring analysis was performed to detect genetic differences within the remaining native and all invasive individuals. Overall, 91 individuals from the native range were removed and 821 individuals from across the invasive and native ranges were re-evaluated using STRUCTURE.

In the sub-structuring analysis, the method of Evanno *et al.* (2005) identified six subclusters (subK = 6) (Fig. 3c). Seventy three percent of individuals (605 of 821) were fully assigned to a cluster and 216 individuals exhibited admixture (Figs. 6b and 6c). White Bird, Idaho was the most diverse population in the invasive range and 17 invasive populations (one from each state) only contained one genetic cluster. Of the six subclusters identified in this analysis, only five were detected among invasive populations. The genetic subcluster indicated by the yellow color occurred in five native populations (Fig. 6b), but was not detected in any invasive populations (Fig. 6c). The most common genetic subcluster among invasive populations was indicated by the green color, and it occurred in 25 populations (144 individuals) (Fig. 6c). This genetic subcluster only had two fully assigned individuals in the native range (one from Staro Orjahovo, Bulgaria and the other from Pryvitne, Ukraine), and 24 admixed individuals in 12 other populations (in Bulgaria, Romania, Turkey and Ukraine) (Fig. 6b). The most common genetic subcluster among native populations is the one indicated in pink. This subcluster was detected in 187 individuals (91 fully assigned and 96 admixed) occurring in 43 populations in all countries except Serbia (Fig. 6b). In contrast, the pink colored subcluster was the least common subcluster among invasive populations. It was found in only four fully assigned individuals in Salt Creek, Utah and in an additional 46 admixed individuals in 18 populations (including Salt Creek) (Fig. 6c).

The genetic subcluster indicated by the red color appeared to be more common in invasive populations, compared to its distribution among native populations (Fig. 6c). In the invasive range, the genetic subcluster indicated in blue commonly co-occurred with the red subcluster. In the native range, the blue subcluster was distributed throughout

Eastern Europe and France, with most fully assigned individuals occurring in Ukraine. The light blue subcluster was detected in populations from the northern portion of the invasive range and in four populations from California. In the native range, light blue was present in one Italian population (Dorgali) and in Eastern Europe, especially in Turkey. Two populations in eastern Washington (Malloy Prairie and White Road) were monomorphic for admixed individuals containing the light blue and green subclusters (Fig. 6c). These two invasive populations were most similar to individuals found in Sarigol, Turkey.

Using pairwise F_{ST} values, I constructed a neighbor-joining tree for all 122 native and invasive populations of medusahead analyzed using AFLPs (Fig. 7). I identified 12 genetic clusters in this tree. Dorgali, Italy, Kokinochoma, Greece, and Lodine, Italy occurred on their own branches and were not assigned to any cluster. Populations from Italy, Spain, and Morocco appeared highly diverged from all invasive populations with the exception of Al Black's Doghouse, Washington (Clusters 1 and 2). Invasive populations clustered closely to one another in most instances indicating high similarity between populations within this range; Clusters 3e and 5b contained only invasive populations. However, several clusters contained both native and invasive populations, which may indicate potential source populations (or regions) for the invasion of medusahead into the western U.S. For example, the largest grouping of invasive populations (13) was nested most closely with Pryvitne, Ukraine (Cluster 5a), suggesting a close genetic relationship among these populations. Cluster 3a consisted of two populations, Goldendale, Washington and Askos, Greece, and indicated that these populations are more similar to each other than any other populations in the analysis.

Several other invasive populations were found nested closely with native populations in Cluster 4a, suggesting other close relationships between native and invasive populations.

Comparison of Allozyme and AFLP Data

AFLP analysis resulted in higher within-population genetic diversity parameters for invasive populations, compared with the parameters derived from the allozyme analysis of invasive populations (Appendix C). Mean within-population expected heterozygosity measured with allozymes ($H_e = 0.004$) was significantly lower ($p < 0.001$) than expected heterozygosity found using AFLPs ($H_e = 0.020$). The highest expected heterozygosity values with allozymes was in White Bird, Idaho, and Laytonville, California ($H_e = 0.034$). White Bird, Idaho also displayed the highest expected heterozygosity using AFLPs ($H_e = 0.059$) and Loma Prieta, California had the next highest value ($H_e=0.045$). Thirty-four of the 52 invasive populations had a H_e value of 0.000. This is in contrast to expected heterozygosity measured using AFLPs in which all populations had expected heterozygosity values greater than zero. No significant relationship was found between the data sets for expected heterozygosity ($r_s = 0.258$, $p > 0.06$) (Fig. 8a), or %P ($r_s = 0.155$, $p > 0.27$) (Fig. 8b). However, there was a significant relationship between these two data sets for the number of MLGs detected ($r_s = 0.324$, $p < 0.02$) (Fig. 8c). The mean value of within-population %P using AFLPs (%P = 5.4) was significantly higher ($p < 0.001$) compared to the value obtained using allozymes (1.8). The population from White Bird, Idaho had the highest value for percent polymorphic loci using AFLPs (%P = 14.5) followed by Threemile Creek, Washington (%P = 11.8), and nine populations exhibited the highest percent polymorphic loci using allozymes (%P = 6.9), including White Bird, Idaho. No significant difference ($p > 0.05$) was detected

between the average number of MLGs per population for allozymes (# MLG = 1.4) and AFLPs (# MLG = 1.2). Using allozymes, 18 of 52 (34.6%) invasive populations contained more than one MLG, with White Bird, Idaho containing the largest number of MLGs (# MLG = 4). Using AFLPs, nine of 52 (17.3%) invasive populations contained more than one MLG, with White Bird, Idaho and Jepson Prairie, California containing the most (# MLG=3).

DISCUSSION

In this AFLP analysis of medusahead, I examined 52 invasive populations throughout the western United States using 110 AFLP loci. These data yielded four major findings. First, invasive populations of medusahead had relatively low levels of genetic diversity and relatively high structure in comparison with other plant species analyzed using dominant molecular markers (Nybom 2004). Second, moderate propagule number (multiple introductions) was associated with the establishment of medusahead in the western U.S. Third, despite multiple introductions, invasive population of medusahead displayed reduced genetic diversity (founder effects) compared with native populations. Fourth, while the data indicated that 52 of the 70 native populations included in this analysis may have served as source populations for the invasion of medusahead in the western U.S., other lines of evidence point to seven populations as the most likely sources for this invasion. In addition to these findings, I detected variable results when I compared my AFLP data to the allozyme data from previous studies, however both data sets generally provide a similar picture about the invasion of medusahead into the western U.S.

Genetic Diversity and Genetic Structure

Medusahead has been widely studied, especially for ways that the plant can be managed, or controlled (e.g., Davies *et al.* 2015, DiTomaso *et al.* 2008, James *et al.* 2015, Kyser *et al.* 2013, Monaco *et al.* 2005). Despite this interest, few studies on the genetic diversity and/or genetic structure of this species exist; I am aware of two studies

assessing the genetic diversity of invasive populations. Rector *et al.* (2013) used bread wheat SSRs (simple-sequence repeats) to assess the utility of these markers in assaying medusahead, and S.J. Novak (unpublished data) used allozymes to assess the level and structure of 46 invasive populations of medusahead. Rector *et al.* (2013) found expected heterozygosity levels ranging from 0.0 to 0.539, while S.J. Novak (unpublished data) found much lower levels of expected heterozygosity (ranging from 0.0 to 0.034) and high genetic structure. While I found higher levels of genetic diversity using AFLPs compared with the results of S.J. Novak (unpublished data), my results are generally in agreement with the diversity previously reported using allozymes.

The AFLP data presented in this study reveal, on average, genetically depauperate invasive populations of medusahead, although the genetic diversity parameters for medusahead are in keeping with what has been reported for other self-pollinating (hereafter referred to as selfing) plants (Nybom 2004). Within-population expected heterozygosity for a selfing plant species with gravity-dispersed seeds using dominant markers such as AFLPs range from 0.12 - 0.19 (Nybom 2004). The mean within-population expected heterozygosity of invasive populations of medusahead ($H_e = 0.020$) (Table 3) was considerably lower than this range, and none of the expected heterozygosity values of the populations analyzed in this study exceeds the upper range values reported by Nybom (2004). Low genetic diversity, at both the range-wide and within-population levels, was also evident in the number of polymorphic loci, percent of polymorphic loci, and Simpson and Shannon-Weiner Genotypic Diversity Indices (Tables 2, 3, and 4). Furthermore, the predominance of the MCG, low total number of MLGs (15) detected among the 52 invasive population, and the low number of genetic

clusters co-occurring within a single population provides additional evidence for a lack of genetic diversity within invasive populations (Table 2, Fig. 4, Appendix B). Other predominantly selfing species that also exhibited low levels of genetic diversity throughout their introduced range include *Alliaria petiolata* (Durka *et al.* 2005), *Ceratocapnos claviculata* (Voss *et al.* 2012), and *Bromus tectorum* (Pawlak *et al.* 2015). The low variability found in medusahead certainly supports the idea that the lack of genetic diversity does not place a constraint on establishment success and invasion (Rollins *et al.* 2013), despite the apparently low evolutionary potential of such populations (Barrett and Schluter 2008).

I detected relatively high genetic structure among the 52 invasive populations of medusahead analyzed in this study. These results are also consistent with what has been previously reported for highly selfing plant species with low dispersal capabilities (Nybom 2004). Based on the results of my AMOVA analysis, 76% of the total genetic diversity of invasive populations was partitioned among populations, and 24% of the diversity was partitioned within populations (Table 6). The higher value for the amount of genetic diversity partitioned between populations ($H_b = 0.064$), compared to within populations ($H_w = 0.020$), and the value of F_{ST} (0.761) (Table 5), all indicate high amounts of genetic structure among invasive populations. These data, coupled with the results of my STRUCTURE analysis (Fig. 4) indicate a lack of genetic homogenization of these populations, and suggest widespread gene flow among populations has not occurred. An outcrossing mating system is often associated with higher levels of genetic diversity, compared with a selfing mating system (Novak and Mack 2005), but the high value for the selfing rate, or coefficient of inbreeding, (f=0.979) strongly indicates that

outcrossing is taking place at a very low rate. Other plant species with self-compatible mating systems exhibiting high structure among invasive populations include *Eichhornia paniculata* (Husband and Barrett 1991) and *Heracleum mantegazzianum* (Henry *et al.* 2009).

Propagule Pressure

Propagule pressure can be assayed through direct means (historical records) and/or indirect methods (results of molecular markers). Molecular markers allow for inferences on the role of propagule pressure on establishment success and during range expansion (Ricciardi *et al.* 2011, Simberloff 2009). Using historical information (presented here in Table 1 and Fig. 1), McKell *et al.* (1962) suggested that there was only a single introduction of medusahead into the western U.S. in 1884 (near Roseburg, Oregon), with range expansion occurring as plants spread from Oregon to Washington, Idaho, and California. A first approximation of propagule pressure (specifically, propagule number) can be made by determining the number of MLGs or haplotypes among invasive populations (Kolbe *et al.* 2004, Ficetola *et al.* 2008, Ross and Shoemaker 2008, Goncalves da Silva *et al.* 2010, Huttanus *et al.* 2011, Gaskin *et al.* 2013). Using molecular data, I found that propagule pressure played a role at different spatial scales (range-wide versus regional levels) during the establishment and range expansion of medusahead in the western U.S. Using the AFLP error rate, I detected 15 AFLP MLGs among the 52 invasive populations of medusahead I analyzed (Table 7). Thus, at the range level, my data indicate moderate levels of propagule pressure through multiple introduction events.

A second aspect of the scenario described by McKell *et al.* (1962) is that the distribution of medusahead in the western U.S. occurred via range expansion from its original point of introduction, Roseburg, Oregon. Assuming that this original introduction was associated with some genetic diversity, I would expect a negative relationship between the amount of genetic diversity within populations and their distance from Roseburg, Oregon. Results of the regression analyses assessing the relationship between H_e and %P and distance from Roseburg, Oregon, revealed no relationship between these parameters (Fig. 2). Taken together, results of this study do not support the McKell *et al.* scenario of a single introduction with subsequent range expansion (spread) from this locality. A similar result involving no clear pattern of genetic diversity among invasive populations has been documented in other invasive plant species, which exhibit a uniparental mode of reproduction, including the clonally reproducing plant *Imperata cylindrica* (Burrell *et al.* 2015) and the self-pollinating plant *Microstegium vimineum* (Baker and Dyer 2011).

Given that 15 AFLP MLGs were detected among populations of medusahead from the western U.S., I attempted to estimate the potential minimum and maximum number of separate introduction events using historical information and genetic data (Table 1, Figs. 1, 4, and 5). Based on this analysis, I estimate a minimum number of eight introductions, which is still in a range that would be consistent with moderate propagule pressure. Fully assigned individuals belonging to each of the four invasive genetic clusters were discovered in four separate sites associated with early collection localities (Table 1, Fig. 4): Roseburg, Oregon (blue color), 1884; near Yakima, Washington (White Swan, Washington, green color), 1899; near Yakima, Washington (Hubbard Road,

Washington, yellow color), 1899; and Steptoe Butte, Washington (red color), 1901. If each genetic cluster was introduced only once, corresponding to four separate introduction events, the presence of these genetic clusters in other populations would have been mediated by long-distance dispersal events during range expansion. Although such a scenario was proposed for the introduction and spread of *Ipomoea hederacea* in its invasive range (Campitelli and Stinchcombe 2014), I do not believe it explains the manner of range expansion of medusahead in the western U.S.

The pattern of genetic clustering in the STRUCTURE analysis at the regional level and the genetic relationship of populations in the neighbor-joining (NJ) tree was also consistent with four introduction events. These results appear to reflect local (or regional) range-expansion in western Oregon, central Idaho, northern Idaho, and eastern Washington (Figs. 4 and 5). In addition, based on the genetic clusters detected in populations from northern Idaho and southwestern Idaho (Fig. 4), two additional introduction events may have occurred. These two events increase my estimate of the number of introductions to six. Two populations in California (Henry Coe State Park and Kelseyville) contain genetically distinct admixture patterns (Fig. 4), which suggests that these two populations may be derived from independent introduction events from native populations. These two introductions increase my estimate of the minimum number of introductions to eight.

Based on the number and distribution of genetic clusters displayed in Fig. 4, a case can be made for additional introduction events. For instance, the genetic clusters detected in Salt Creek, Utah, Canby, California, and Quincy, California could have been derived from independent introduction events because the genetic clusters in these

populations occur outside the main geographical distribution of that cluster. The addition of these three potential introduction events would increase the potential maximum number of introductions to 11. I have tried to use a conservative approach in estimating the propagule pressure associated with the invasion of medusahead into the western U.S., thus I report here a range of possible introduction events (8-11).

Results of molecular markers can also be used to assess the role of propagule pressure during range expansion. Results of my study indicate that propagule pressure during range expansion of medusahead from its multiple points of introduction was relatively low. Evidence for this comes from the low level of genetic diversity detected, on average, within populations and a high level of genetic structure among invasive populations of medusahead. This is the exact pattern described above in the genetic diversity and genetic structure subsection of the discussion and the results shown in Fig. 4. Because low genetic diversity within populations and high genetic structure among populations is also associated with highly self-pollinating plant species, it is difficult to partition the relative contribution of mating system and low propagule pressure during range expansion. Thus, I believe that both processes have contributed to the pattern of genetic diversity reported here for invasive populations of medusahead.

Evidence for Founder Effects

Invasive populations often originate from small and genetically depauperate founder populations, which may result in founder effects (Allendorf and Lundquist 2003, Mayr 1942, Novak and Mack 2005). Common genetic signatures of founder effects include low levels of genetic diversity within invasive populations, compared with native populations. Such results have been reported for other invasive plant species: *Avena*

barbata (Crosby *et al.* 2014); *Heracleum mantegazzianum* (Henry *et al.* 2009); *Ardisia crenata* (Niu *et al.* 2012); and *Geranium carolinianum* (Shirk *et al.* 2014). Examining data for invasive populations alone, low levels of expected heterozygosity, low numbers of polymorphic loci, a small number of MLGs, and the low number of populations exhibiting admixture are consistent with the genetic consequences of founder effects (Tables 2, 3, and 4). The best evidence for founder effects during the introduction of medusahead into the western U.S. is provided by comparisons with the results from native populations. I detected reductions in range-wide genetic diversity parameters, within-population genetic diversity parameters, and among-population genetic diversity parameters for invasive populations, compared with the values of native populations (Tables 2, 5, and 7). A similar pattern of decreased genetic diversity in invasive populations was also observed in the combined analysis of genetic clusters/subclusters and total number of MLGs (Table 7, Fig. 6). Both STRUCTURE analyses reflect a reduction in the number of genetic clusters within invasive populations, compared to native populations. Fifteen MLGs were identified among the invasive populations, which is a small fraction of the 132 MLGs detected among the native populations. All of these results provide evidence that the genetic diversity of invasive populations has been reduced through founder effects.

Putative Source Regions

Tracing the geographic origins (source populations) of an invasion can be accomplished by the combined analysis of invasive and native populations using molecular markers (Novak 2011). Peters (2013) and Skaar (2015) used the distribution of allozyme MLGs within native and invasive populations to trace the geographic origins of

the invasion of medusahead into the western U.S. While some patterns emerged from their analysis, these two studies did not pinpoint source populations at a fine-scale. The same is true concerning the use of AFLP data to pinpoint source populations. Therefore, I used a combination of methods (MLGs, the genetic clusters identified by STRUCTURE, and the NJ tree) to identify the putative source populations/regions for this invasion. Because of their genetic uniqueness, I feel confident in eliminating populations from Spain, Morocco, and Italy from consideration as potential source populations. For the same reason, several other populations (Izvorishte and Tenevo, Bulgaria and Kakceveli, Ukraine) can also be eliminated as potential source populations. My results are consistent with the results of Peters (2013), which also eliminated populations from Spain, Morocco and four of six populations from Italy as putative source populations.

Based on this combination of methods, 52 of 70 (74.3%) native populations are candidates for being potential source populations (Figs. 6, 7, Appendix B). These 52 populations occur within two source regions: southeastern Europe and southern France. However, patterns emerging from the STRUCTURE and NJ tree analyses suggested that a subset of these 52 populations is more likely potential source populations. The most common genetic subcluster among invasive populations (indicated by the green color) was only detected in two fully assigned individuals from the Staro Orjahovo, Bulgaria and Pryvitne, Ukraine populations (Fig. 6b). Based on the predominance of fully assigned individuals to the green subcluster occurring in the western U.S., this suggests these two populations provided founding individuals for many invasive populations (Fig. 6c).

Similar patterns are revealed by the position of native and invasive populations within the same cluster in the NJ tree (Fig. 7). For example, the population from Pryvitne,

Ukraine occurs together in Cluster 5a with invasive populations from California, Idaho, Nevada, Oregon, Utah, and Washington. There are other instances in which a close genetic relationship between native populations and some invasive populations occurs (Clusters 3a and 4a of the NJ tree). These data suggest that five populations from southeastern Europe, specifically from Taman Bay, Russia, three populations from eastern Bulgaria (Orizare, Rudnik, and Sredec), and the population from Askos, Greece, could also be potential source populations for the invasion of medusahead into the western U.S. Using allozymes, Peters (2013) and Skaar (2015) also indicated that southeastern Europe and southern France may be the geographic origins for this invasion. Similar findings concerning the identification of source populations using both allozymes and AFLPs indicates that a molecular marker with greater resolving power than either of these two (e.g., next generation DNA sequencing) will be needed to more precisely pinpoint the source populations for this invasion.

Comparison of Results: AFLPs and Allozymes

A diverse array of molecular markers are available to researchers (e.g., allozymes, SNPs, RAPDs, AFLPs, microsatellites, and DNA sequencing) (Liu and Cordes 2004, Mueller and Woldenbarger 1999, Schlötterer 2004). Because these various molecular markers have different properties, studies using different marker systems have produced variable and often conflicting results (e.g., *Imperata cylindrica*, Burrell *et al.* 2015, Lucardi *et al.* 2014; *Pinus pinaster,* Mariette *et al.* 2001). The major findings in this study of medusahead using AFLPs were generally consistant with previous findings using allozymes (S.J. Novak unpublished data, Peters 2013, Skaar 2015), but the comparison of certain genetic parameters were variable between the two marker systems. In terms of

parameters describing genetic diversity, the AFLP-based values were generally higher. Conversely, allozyme and AFLPs both revealed relatively high genetic structure among invasive populations. In my study, I used almost four times the number of AFLP loci (110) as used in the previous allozyme (29) analysis of invasive populations. With the increased number of AFLP loci, I detected significantly higher mean values for expected heterozygosity and percent of polymorphic using AFLPs (Appendix C), even though there was not a statistically significant relationship between these parameters, as estimated using the two markers (Fig. 8). I attribute this lack of a relationship between the two markers to divergent results for some populations. For instance, White Bird, Idaho consistently exhibited the highest genetic diversity parameters using both markers; whereas, populations such as Loma Prieta, California and Ladd Canyon, Oregon did not possess any allozyme diversity, but these two populations were among the most diverse using AFLPs (Appendix C). In contrast, I did find a significant positive relationship between the number of MLGs per population estimated by the two markers (Fig. 8c), even though there was no significant difference for the mean values of this parameter for the two markers (Appendix C). Finally, it should be noted that the MLG data reported here based on AFLPs takes into account the error rate as described above, while no error rate calculation was applied to the allozyme MLG data.

Conclusion

My analysis of 52 invasive populations of medusahead using AFLPs provides insights into the genetic diversity and introduction dynamics of this destructive grass in rangelands in the western U.S. Several management strategies have been used to control medusahead (Davies *et al.* 2015, DiTomasso *et al.* 2008) and none have proven to be

uniformly successful, thus this species continues to spread and dominate in its invasive range. Potential biological control agents have been identified (*Fusarium arthrosporioides*, Siegwart *et al.* 2003; various spp., Widmer and Sforza 2004), but these agents are not likely to be released. Therefore, the search for more candidate biological control agents is warranted. The specific and smaller putative source regions I identified in southeastern Europe may serve as an opportunity to narrow search efforts for a biological agent that will be effective in the control of medusahead (Müller-Schärer *et al.* 2004, Novak and Sforza 2008). At a larger perspective, data from this study adds to the body of knowledge of biological invasions and provides further insights into introduction dynamics. Specifically, results of this study point to the importance of propagule pressure in establishment success and subsequent range expansion by assessing the genetic signatures of these steps in the invasion process. Future research should focus on sampling more broadly in poorly sampled areas of the native range (such as France) and analyze native and invasive populations using a more polymorphic genetic marker such as next generation sequencing to more precisely identify the source populations (or regions) from which the invasion of medusahead in the western U.S. stem.

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TABLES AND FIGURES

Table 1 Locality data for 52 invasive populations of *Taeniatherum caput-medusae* subsp. *asperum* from the western United States analyzed in this study. State, county, population name and number (corresponding to the numbers in Fig. 1), latitude, longitude, elevation (in meters), and year of first collection or report are provided. Populations in each state are arranged based on year of first collection or report.

State	County	Population	Latitude	Longitude	Elevation	Earliest Collection
California	Siskiyou	1. Klamathon	41° 53’ 54.05” N	122° 30’ 32.65” W	653	1903
	Santa Clara	2. Henry Coe State Park	37° 11’ 16.11” N	121° 32' 45.53” W	825	1908
	Santa Cruz	3. Loma Prieta	37° 06’ 18.35” N	121° 53’ 17.79” W	711	1908
	Modoc	4. Canby	41° 26’ 19.75” N	120° 52’ 46.39” W	1322	1935
	Trinity	5. Van Duzen River	40° 23’ 28.40” N	123° 30’ 49.91” W	826	1941
	Lake	6. Kelseyville	38° 58’ 28.43” N	123° 00’ 35.26” W	426	1942
	Plumas	7. Quincy	39° 56’ 17.80” N	120° 56’ 23.57” W	1043	1948
	Solano	8. Jepson Prairie	38° 16’ 30.71” N	121° 49’ 22.35” W	7	1949

	Lassen	9. Shaffer Mountain	40° 28’ 11.24” N	120° 26' 36.30” W	1355	1960
	Mendocina	10. Laytonville	39° 42’ 28.80” N	123° 29’ 20.71” W	517	1973
Idaho	Elmore	11. Rattlesnake Station	43° 11’ 43.55” N	115° 33’ 19.63” W	1165	1930
	Elmore	12. Mountain Home	43° 09’ 41.99” N	115° 33’ 19.63” W	1054	1930
	Payette	13. Payette Heights	44° 04’ 30.50” N	116° 52’ 55.18” W	729	1944
	Washington	14. Cherry Gulch	44° 09’ 47.50” N	115° 18’ 33.39” W	663	1944
	Gem	15. Montour	43° 55’ 06.11” N	116° 20’ 28.45” W	784	1945
	Nez Perce	16. Lapwai	46° 23’ 59.08” N	116° 50' 05.02” W	454	1946
	Elmore	17. Mayfield Road	43° 21’ 48.65” N	115° 49’ 26.51” W	1129	1950
	Washington	18. Crane Creek Reservoir	44° 21’ 45.10” N	116° 52’ 34.34” W	976	1950
	Washington	19. Rush Creek Road	44° 37’ 00.91” N	116° 41’ 26.02” W	900	1950
	Latah	20. Kendrick	46° 36’ 47.26” N	116° 50’ 06.03” W	415	1954
	Ada	21. Black's Creek Road	43° 28’ 05.08” N	116° 04’ 53.71” W	1040	1955
	Elmore	22. Bennett Mountain Road	43° 08’ 54.60” N	115° 18’ 33.39” W	1512	1972
	Ada	23. Old State Penitentiary	43° 36’ 13.13” N	116° 9’ 42.78” W	853	1972
	Ada	24. Seaman's Gulch	43° 41’ 55.26” N	115° 18’ 33.39” W	902	1972

	Idaho	25. White Bird	45° 46’ 51.89” N	116° 16’ 35.53” W	548	1977
Montana	Sanders	26. Chuck's Place	47° 13' 47.37" N	114° 12' 27.10" W	922	2013
	Lake	27. Nicholson Site	47° 13' 28.02" N	114° 11' 00.64" W	923	2013
Nevada	Washoe	28. Buckhorn Road	40° 55’ 26.15” N	119° 49’ 17.63” W	1662	1963
Oregon	Douglas	29. Roseburg	43° 14’ 58.67” N	123° 21’ 08.62” W	181	1884
	Josephine	30. Grants Pass	42° 26’ 16.58” N	123° 16’ 59.53” W	332	1909
	Lane	31. Goshen	43° 58’ 04.51” N	123° 00’ 35.26” W	169	1915
	Jackson	32. Emigrant Reservoir	42° 09’ 03.43” N	122° 37’ 19.64” W	220	1924
	Klamath	33. Klamath Falls	42° 15’ 31.85” N	121° 47’ 50.80” W	1291	1946
	Union	34. Ladd Canyon	45° 14’ 03.62” N	118° 00’ 55.43” W	881	1950
	Wasco	35. Juniper Flat	45° 08’ 18.46” N	121° 13’ 27.41” W	584	1955
	Umatilla	36. Emigrant Hill	45° 34’ 57.75” N	118° 35’ 24.24” W	1033	1976
	Umatilla	37. Birch Creek Road	45° 57’ 43.86” N	118° 15’ 58.06" W	467	1976
Utah	Box Elder	38. Salt Creek	41° 37’ 56.54” N	112° 15’ 28.67” W	1304	1988
	Box Elder	39. Tremonton	41° 45’ 02.86” N	112° 15’ 44.40” W	1379	1988
	Cache	40. South Canyon Road	41° 28’ 44.36” N	111° 49’ 29.38” W	1616	n/a

Washington	Yakima	41. White Swan	46° 24' 46.62" N	120° 45' 17.06" W	333	1899
	Yakima	42. Hubbard Road	46° 33' 40.51" N	120° 42' 52.74" W	492	1899
	Whitman	43. Steptoe Butte	47° 01’ 58.15” N	117° 18’ 17.16” W	929	1901
	Klickitat	44. Goldendale	45° 44’ 18.48” N	120° 49’ 13.13” W	523	1938
	Klickitat	45. Threemile Creek	45° 39’ 00.98” N	121° 08’ 45.85” W	149	1938
	Whitman	46. Pullman	46° 44’ 02.22” N	117° 11’ 12.04” W	737	1940
	Whitman	47. Al Black's Doghouse	46° 54’ 47.55” N	117° 15’ 48.11” W	703	1952
	Whitman	48. Hooper	46° 44’ 46.84” N	118° 08’ 26.64” W	388	1957
	Whitman	49. Rosalia	47° 15’ 43.20” N	117° 21’ 38.86” W	677	n/a
	Spokane	50. Cheney-Plaza	47° 22' 56.74" N	117° 34' 59.83" W	703	n/a
	Spokane	51. Malloy Prairie	47° 30’ 50.29” N	117 °42’ 50.47” W	722	n/a
	Spokane	52. White Road	47° 34' 48.24" N	117° 38' 32.99" W	732	n/a

Table 2 Range-wide genetic diversity estimates for the invasive and native ranges of *Taeniatherum caput-medusae* subsp. *asperum* calculated in AFLP-surv (Vekemans 2002). Parameters are the total number of populations sampled (n), the number of polymorphic loci (P), the percent of polymorphic loci (%P), the expected heterozygosity (H_e), and the standard deviation (S.E.(H_e)) for the expected heterozygosity values.

	n	*P*	*%P*	H_e	S.E.(H_e)
Invasive Populations	52	49	44.5	0.083	0.015
Native Populations	70	104	94.5	0.166	0.013

Table 3 Within-population genetic parameters for 52 invasive populations of *Taeniatherum caput-medusae* subsp. *asperum* sampled in the western United States calculated in AFLP-surv (Vekemans 2002). Parameters are based on 110 scored loci and include the number of individuals per population (n), the number of polymorphic loci (P), the percent of polymorphic loci (%P), the expected heterozygosity (H_e), and the standard deviation (S.E.(H_e)) for the expected heterozygosity values. The mean values of all parameters were calculated for all states except Nevada, where only one population was sampled.

State	Population	n	*P*	%*P*	H_e	S.E.(H_e)
California	1. Klamathon	10	2	1.8	0.005	0.004
	2. Henry Coe State Park	10	5	4.5	0.017	0.008
	3. Loma Prieta	8	11	10	0.045	0.014
	4. Canby	9	10	9.1	0.036	0.012
	5. Van Duzen River	9	3	2.7	0.009	0.005
	6. Kelseyville	7	7	6.4	0.028	0.010
	7. Quincy	5	10	9.1	0.038	0.012
	8. Jepson Prairie	8	10	9.1	0.033	0.011
	9. Shaffer Mountain	8	2	1.8	0.008	0.005
	10. Laytonville	6	6	5.5	0.029	0.012
	California Mean	8	6.6	6	0.025	0.009
Idaho	11. Rattlesnake Station	8	5	4.5	0.017	0.008
	12. Mountain Home	8	7	6.4	0.025	0.009
	13. Payette Heights	9	5	4.5	0.013	0.006
	14. Cherry Gulch	9	8	7.3	0.023	0.008
	15. Montour	10	7	6.4	0.016	0.007
	16. Lapwai	8	4	3.6	0.014	0.008
	17. Mayfield Road	8	2	1.8	0.005	0.003
	18. Crane Creek Reservoir	10	7	6.4	0.017	0.007

	19. Rush Creek Road	4	5	4.5	0.024	0.011
	20. Kendrick	9	2	1.8	0.006	0.004
	21. Black's Creek Road	9	9	8.2	0.026	0.009
	22. Bennett Mountain Road	4	3	2.7	0.013	0.007
	23. Old State Penitentiary	8	10	9.1	0.035	0.011
	24. Seaman's Gulch	6	3	2.7	0.009	0.005
	25. White Bird	9	16	14.5	0.059	0.015
	Idaho Mean	7.9	6.2	5.6	0.020	0.008
Montana	26. Chuck's Place	7	1	0.9	0.003	0.003
	27. Nicholson Site	10	2	1.8	0.005	0.004
	Montana Mean	8.5	1.5	1.4	0.004	0.003
Nevada	28. Buckhorn Road	8	5	4.5	0.013	0.006
Oregon	29. Roseburg	8	9	8.2	0.035	0.012
	30. Grants Pass	6	6	5.5	0.024	0.010
	31. Goshen	7	5	4.5	0.020	0.009
	32. Emigrant Reservoir	8	3	2.7	0.013	0.007
	33. Klamath Falls	10	8	7.3	0.028	0.010
	34. Ladd Canyon	7	11	10	0.035	0.010
	35. Juniper Flat	8	2	1.8	0.007	0.005
	36. Emigrant Hill	7	7	6.4	0.020	0.007
	37. Birch Creek Road	8	4	3.6	0.009	0.004
	Oregon Mean	7.7	6.1	5.6	0.021	0.008
Utah	38. Salt Creek	10	11	10	0.035	0.011
	39. Tremonton	8	6	5.5	0.022	0.009
	40. South Canyon Road	8	1	0.9	0.002	0.002

	Utah Mean	8.7	6.0	5.5	0.020	0.007
Washington	41. White Swan	10	6	5.5	0.012	0.005
	42. Hubbard Road	10	7	6.4	0.023	0.009
	43. Steptoe Butte	5	3	2.7	0.011	0.006
	44. Goldendale	8	7	6.4	0.021	0.008
	45. Threemile Creek	8	13	11.8	0.039	0.011
	46. Pullman	7	6	5.5	0.025	0.010
	47. Al Black's Doghouse	7	8	7.3	0.027	0.010
	48. Hooper	7	5	4.5	0.023	0.010
	49. Rosalia	10	5	4.5	0.011	0.006
	50. Cheney-Plaza	9	6	5.5	0.025	0.010
	51. Malloy Prairie	7	2	1.8	0.005	0.004
	52. White Road	10	3	2.7	0.008	0.005
	Washington Mean	8.2	5.9	5.4	0.019	0.008
	Total Mean	8.0	6.0	5.4	0.020	0.008

Table 4 Multilocus genotype and genotypic diversity parameters for 52 invasive populations of *Taeniatherum caput-medusae* subsp. *asperum* scored over 110 loci sampled in the western United States. Parameters were calculated using the error rate of three bands in GenoType/GenoDive (Meirmans and Van Tienderen 2004). Parameters include the sample size per population (n), the number of multilocus genotypes detected in each population (# AFLP MLG), Simpson's Genotypic Diversity (D_s), Simpson's Evenness (E_s), and the Shannon-Wiener Index (H').

State	Population	n	# *AFLP MLG*	D_s	E_s	*H'*
California	1. Klamathon	10	1	0.000	-*	0.000
	2. Henry Coe State Park	10	1	0.000	-	0.000
	3. Loma Prieta	8	1	0.000	-	0.000
	4. Canby	9	2	0.389	0.764	0.230
	5. Van Duzen River	9	1	0.000	-	0.000
	6. Kelseyville	7	1	0.000	-	0.000
	7. Quincy	5	2	0.400	0.735	0.217
	8. Jepson Prairie	8	3	0.464	0.561	0.320
	9. Shaffer Mountain	8	1	0.000	-	0.000
	10. Laytonville	6	1	0.000	-	0.000
	California Mean	8.0	1.4	0.125	0.687	0.077
Idaho	11. Rattlesnake Station	8	1	0.000	-	0.000
	12. Mountain Home	8	1	0.000	-	0.000
	13. Payette Heights	9	1	0.000	-	0.000
	14. Cherry Gulch	9	1	0.000	-	0.000
	15. Montour	10	1	0.000	-	0.000
	16. Lapwai	8	1	0.000	-	0.000
	17. Mayfield Road	8	1	0.000	-	0.000
	18. Crane Creek Reservoir	10	1	0.000	-	0.000

	19. Rush Creek Road	4	1	0.000	-	0.000
	20. Kendrick	9	1	0.000	-	0.000
	21. Black's Creek Road	9	1	0.000	-	0.000
	22. Bennett Mountain Road	4	1	0.000	-	0.000
	23. Old State Penitentiary	8	2	0.250	0.640	0.164
	24. Seaman's Gulch	6	1	0.000	-	0.000
	25. White Bird	9	3	0.417	0.529	0.297
	Idaho Mean	7.9	1.2	0.044	0.585	0.031
Montana	26. Chuck's Place	7	1	0.000	-	0.000
	27. Nicholson Site	10	1	0.000	-	0.000
	Montana Mean	8.5	1.0	0.000	-	0.000
Nevada	28. Buckhorn Road	8	1	0.000	-	0.000
Oregon	29. Roseburg	8	1	0.000	-	0.000
	30. Grants Pass	6	1	0.000	-	0.000
	31. Goshen	7	1	0.000	-	0.000
	32. Emigrant Reservoir	8	1	0.000	-	0.000
	33. Klamath Falls	10	1	0.000	-	0.000
	34. Ladd Canyon	7	1	0.000	-	0.000
	35. Juniper Flat	8	1	0.000	-	0.000
	36. Emigrant Hill	7	1	0.000	-	0.000
	37. Birch Creek Road	8	1	0.000	-	0.000
	Oregon Mean	7.7	1.0	0.000	-	0.000
Utah	38. Salt Creek	10	2	0.200	0.610	0.141
	39. Tremonton	8	1	0.000	-	0.000
	40. South Canyon Road	8	1	0.000	-	0.000

	Utah Mean	8.7	1.3	0.067	0.610	0.047
Washington	41. White Swan	10	1	0.000	-	0.000
	42. Hubbard Road	10	1	0.000	-	0.000
	43. Steptoe Butte	5	1	0.000	-	0.000
	44. Goldendale	8	1	0.000	-	0.000
	45. Threemile Creek	8	2	0.250	0.640	0.164
	46. Pullman	7	2	0.476	0.845	0.260
	47. Al Black's Doghouse	7	2	0.286	0.662	0.178
	48. Hooper	7	1	0.000	-	0.000
	49. Rosalia	10	1	0.000	-	0.000
	50. Cheney-Plaza	9	1	0.000	-	0.000
	51. Malloy Prairie	7	1	0.000	-	0.000
	52. White Road	10	1	0.000	-	0.000
	Washington Mean	8.2	1.3	0.084	0.716	0.050
	Total Mean	8.0	1.2	0.060	0.694	0.038

*Values of E_s were not calculated for populations with one MLG

Table 5 Population genetic structure estimates for 52 invasive range populations and 70 native range populations of *Taeniatherum caput-medusae* subsp. *asperum* using the procedures of Lynch and Milligan (1994). Parameters were calculated in AFLP-surv (Vekemans 2002), and include the total number of populations sampled (n), the total gene diversity (H_t), the mean gene diversity partitioned within populations (H_w), the mean genetic diversity partitioned among populations (H_b), and the proportion of the total gene diversity partitioned among populations (F_{ST}).

	n	H_t	S.E.	H_w	S.E.	H_b	S.E.	F_{ST}
Invasive Populations	52	0.084	0.002	0.020	0.003	0.064	0.024	0.761
Native Populations	70	0.171	0.004	0.049	0.016	0.122	0.042	0.717

Table 6 Analysis of Molecular Variance (AMOVA) calculated for 52 invasive populations of *Taeniatherum caput-medusae* subsp. *asperum* sampled in the western United States using GenAlEx 6.5 (Peakall and Smouse 2006, 2012). AMOVA hierarchically partitioned genetic diversity (a) within and among populations, and (b) within populations, among populations within regions, and among regions (states). (P=0.001 for both analyses)

(a)

Source	d.f.	Sum of Squares	Variation Component	Percentage Variation
Among Populations	51	1487.26	3.50	76%
Within Populations	365	408.45	1.12	24%
Total	416	1895.71	4.62	--

(b)

Source	d.f.	Sum of Squares	Variation Component	Percentage Variation
Among States	6	453.09	0.93	19%
Among Populations	45	1034.17	2.74	57%
Within Populations	365	408.45	1.12	23%
Total	416	1895.71	4.80	--

Table 7 Within-population genetic diversity parameters and multilocus genotype/genotypic diversity measurements for the 52 invasive populations and 70 native populations of *Taeniatherum caput-medusae* subsp. *asperum* scored at 110 AFLP loci. Within-population genetic diversity parameters calculated in AFLP-surv (Vekemans 2002) include the number of populations sampled within the range (n), the mean number of polymorphic loci (P), the mean percent of polymorphic loci (%P), and the mean expected heterozygosity (H_e). GenoType/GenoDive parameters (Meirmans and Van Tienderen 2004) were calculated using the error rate of three bands, and include the total number of multilocus genotypes detected among native and invasive populations (Total MLG), the mean number of multilocus genotypes per population (MLG per pop), the Simpson's Genotypic Diversity Index (D_s), the Simpson's Evenness (E_s), and the Shannon-Wiener Index (H').

	n	*P*	%*P*	H_e	Total MLG	MLG per Pop	D_s	E_s	*H'*
Invasive Populations	52	6.0	5.4	0.020	15	1.2	0.060	0.694*	0.038
Native Populations	70	12.9	11.7	0.049	132	2.5	0.358	0.858*	0.252

* Values of E_s were not calculated for populations with one MLG

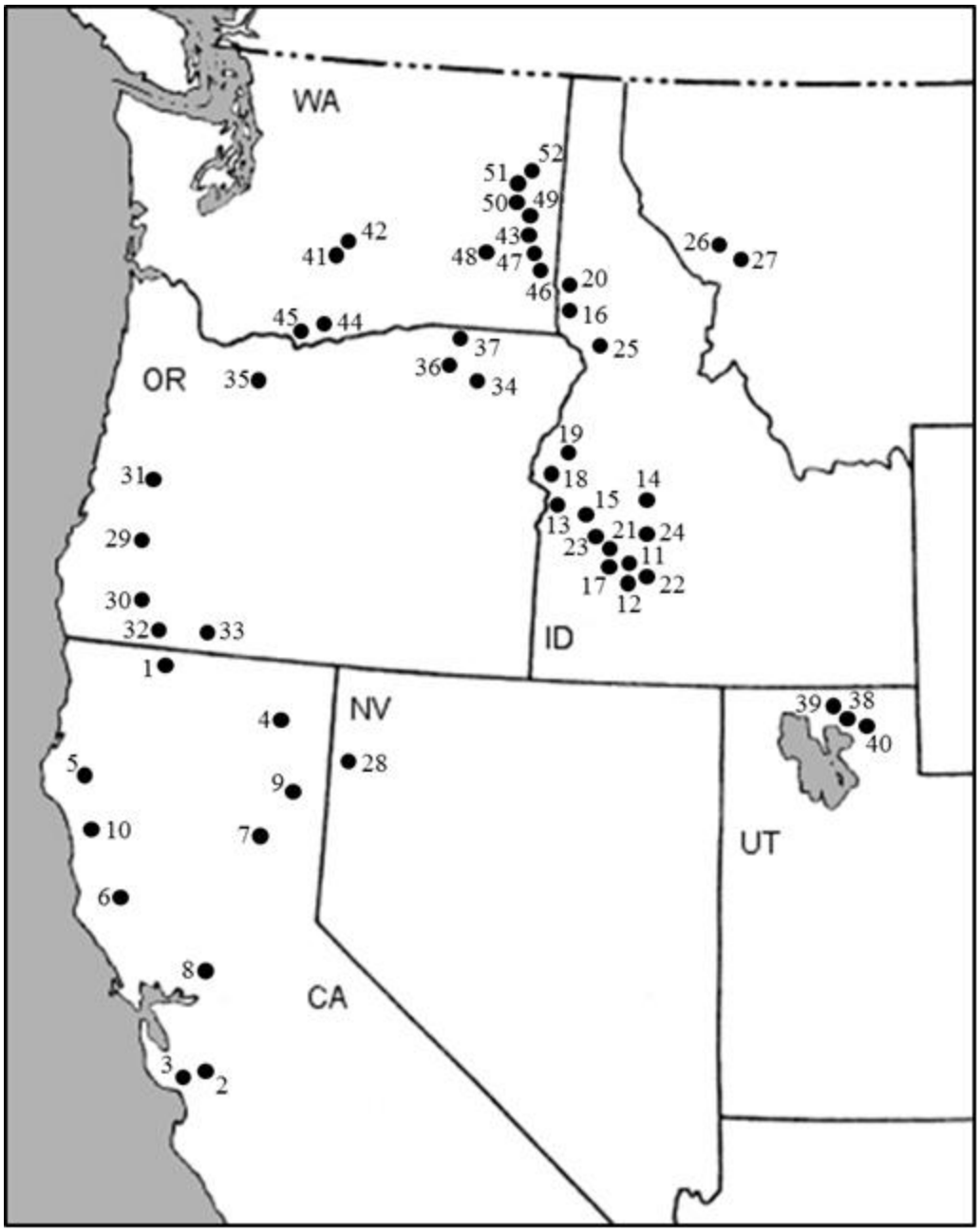
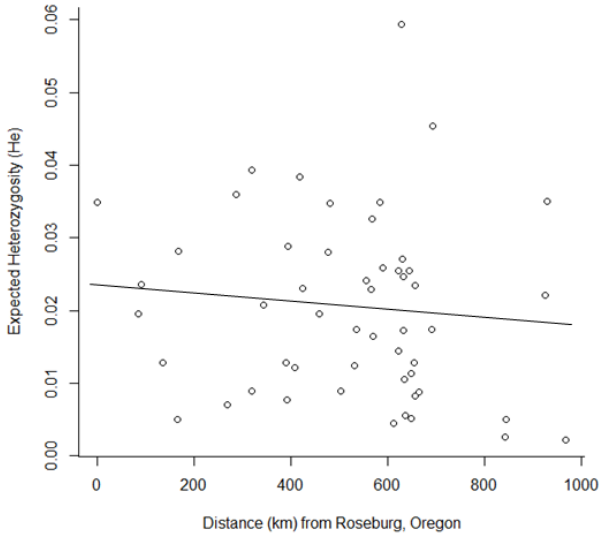


Figure 1 Collection locations for the 52 populations of *Taeniatherum caput-medusae* subsp. *asperum* from the western United States analyzed in this study. Population numbers correspond to the locality data provided in Table 1.

(a)



(b)

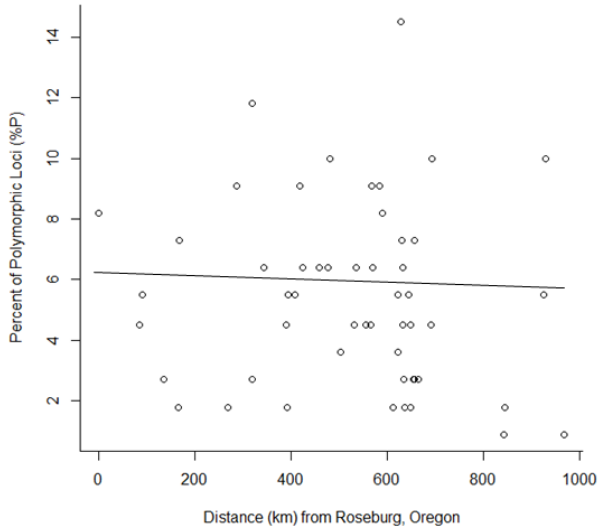
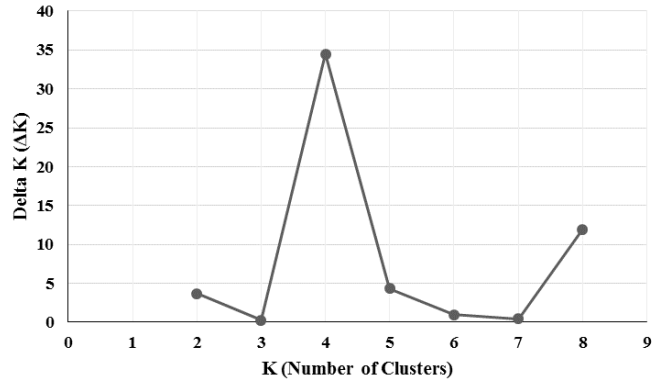
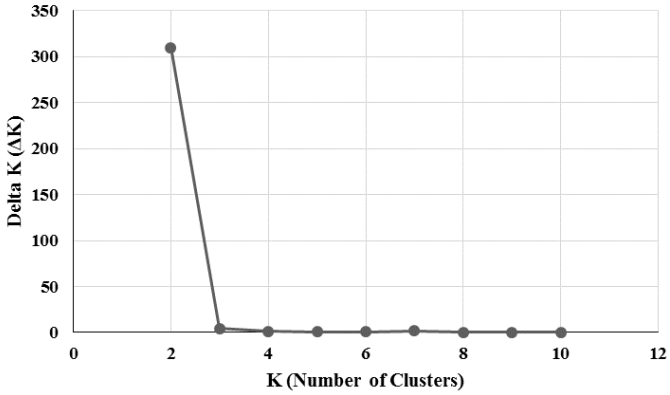


Figure 2 Linear regression analysis depicting the relationship between (a) expected heterozygosity and distance (km) from Roseburg, Oregon ($F_{1,50}$=0.823, r^2=-0.004, p>0.36,) and (b) percent of polymorphic loci and distance (km) from Roseburg, Oregon ($F_{1,50}$=0.541, r^2=-0.009, p>0.46) for the 52 populations of *Taeniatherum caput-medusae* analyzed in this study.

(a)



(b)



(c)

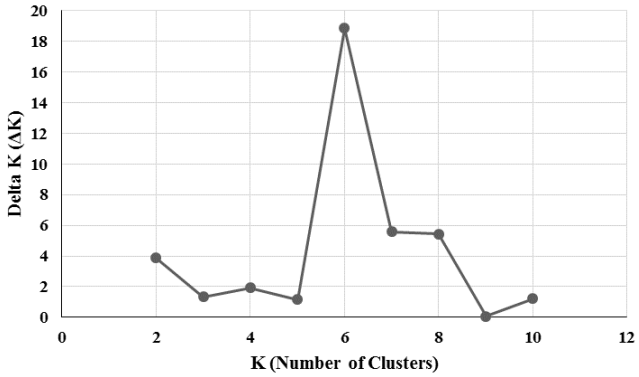


Figure 3 Graphs depicting the ΔK method of Evanno *et al.* (2005) used to determine the most likely number of genetic clusters (K) from STRUCTURE (Pritchard *et al.* 2000) results for (a) invasive population cluster analysis (K=4), (b) combined native and invasive population cluster analysis (K=2), and (c) combined native and invasive population sub-structuring analysis (subK=6).

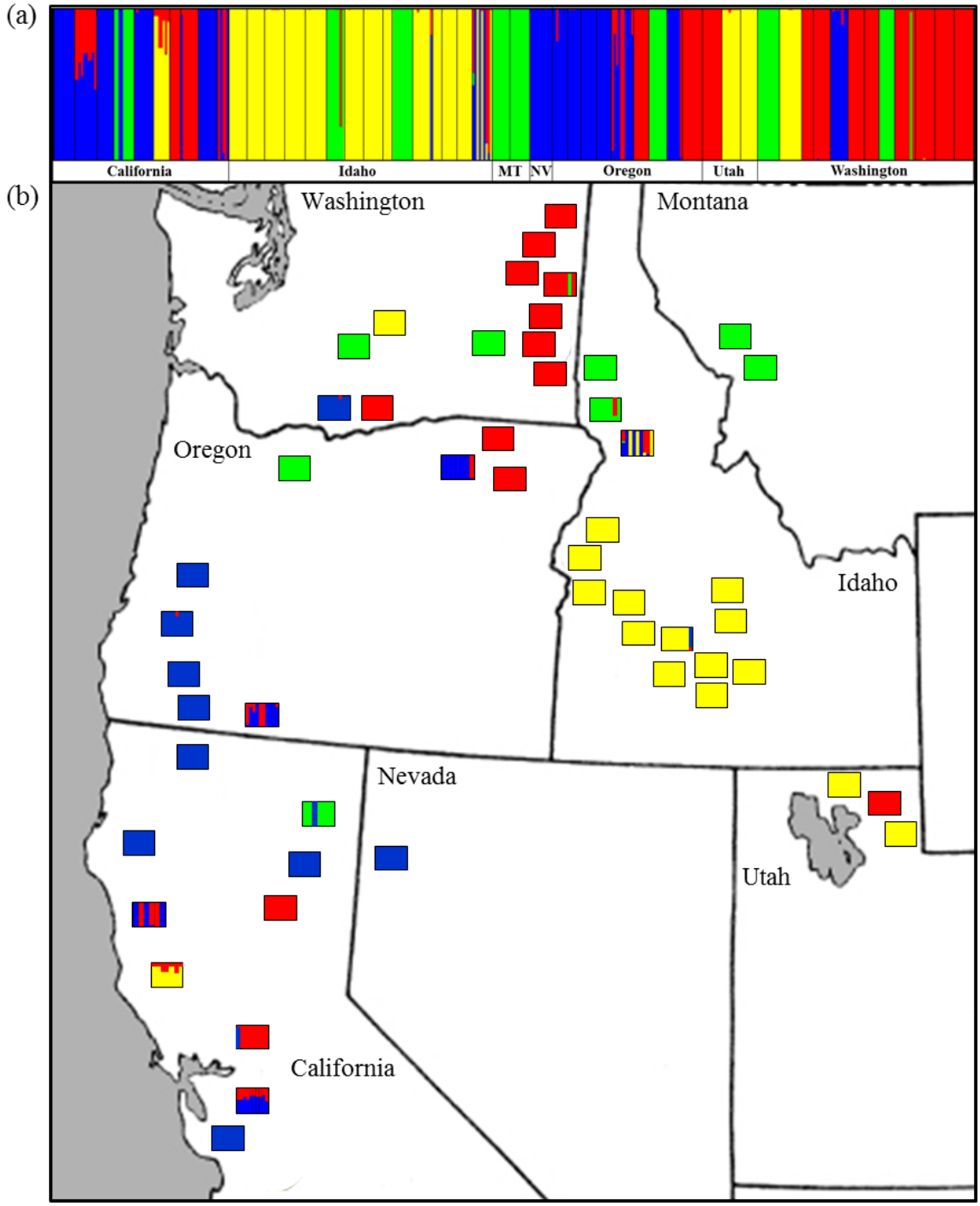


Figure 4 STRUCTURE (Pritchard *et al.* 2000) results of 52 populations of *Taeniatherum caput-medusae* subsp. *asperum* from the western United States (K=4). Vertical lines represent individuals and corresponding cluster assignments to the genetic clusters indicated by the blue, red, green, and yellow colors: (a) STRUCTURE bar plot of the four genetic clusters organized by state, and (b) the four genetic clusters mapped onto the 52 populations analyzed in this study.

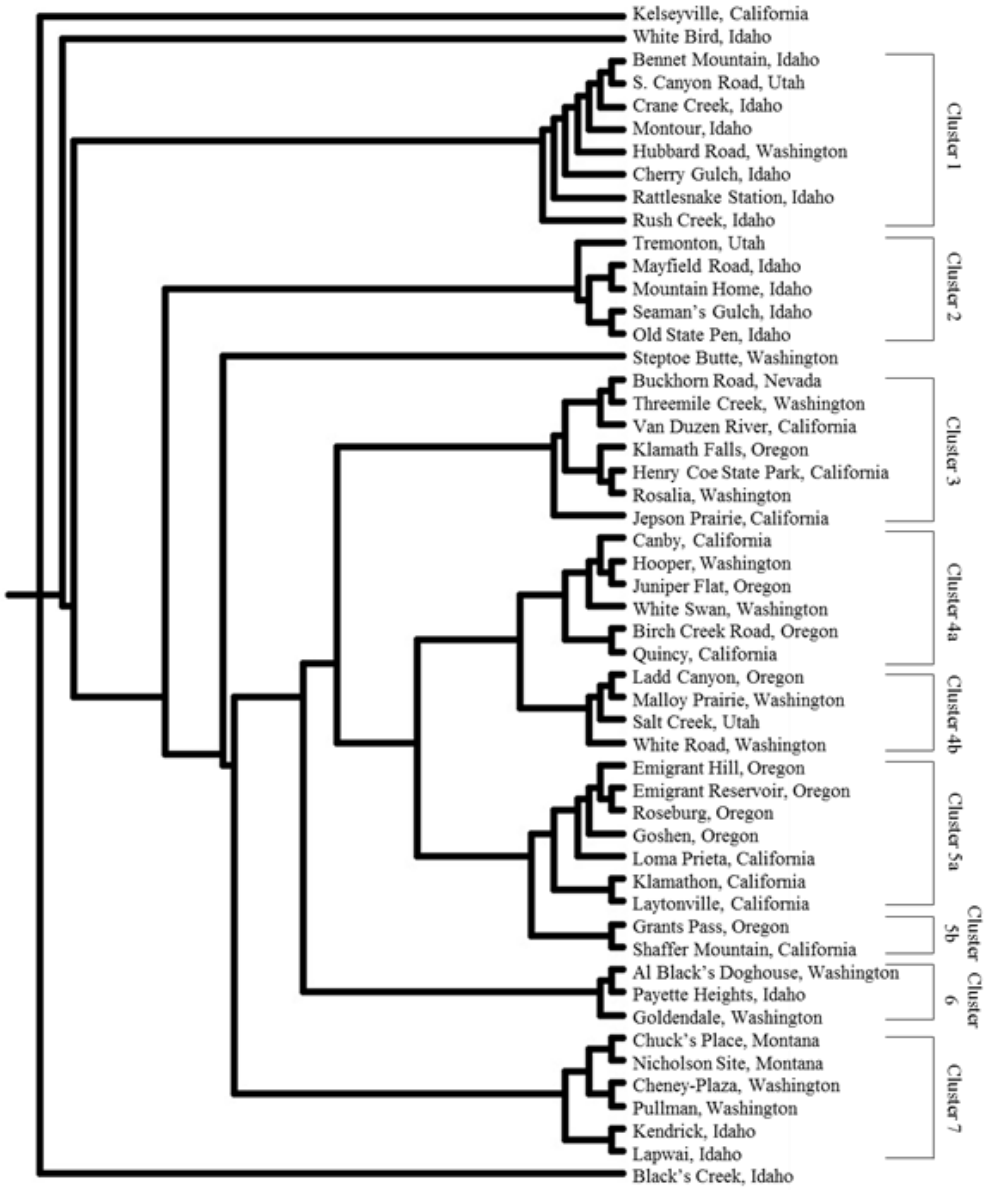
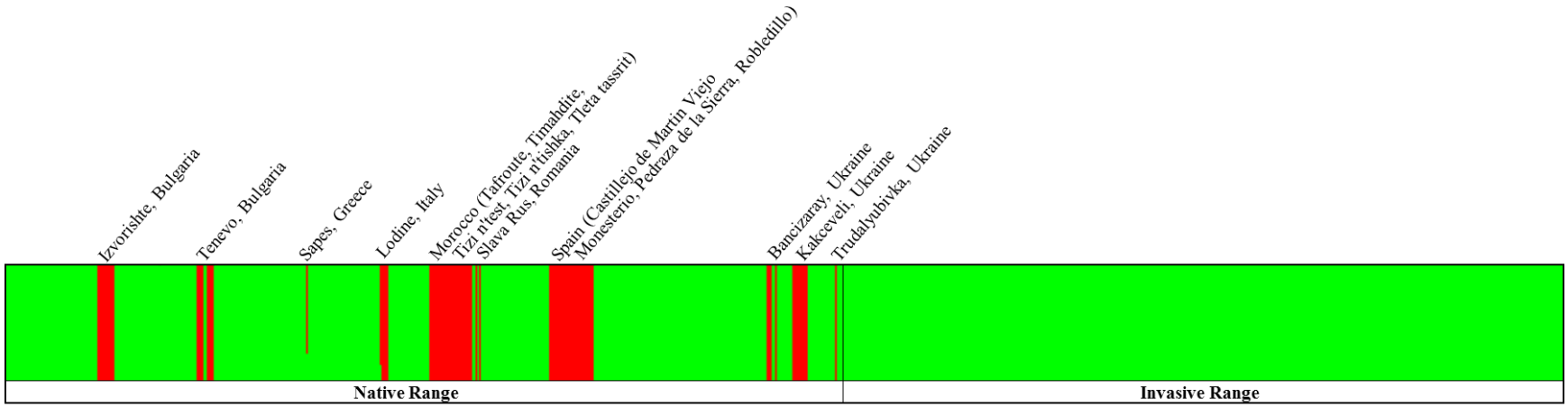
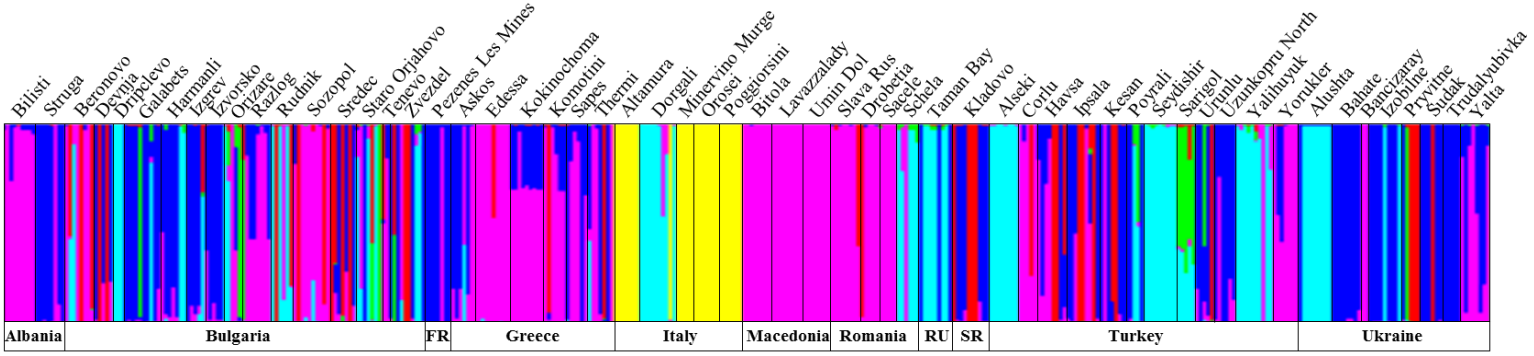


Figure 5 Neighbor-joining tree depicting genetic relationships among the 52 populations of *Taeniatherum caput-medusae* subsp. *asperum* from the western United States. Figure created using PHYLIP based on pairwise F_{ST} values.

(a)


Izvorishte, Bulgaria
Tenevo, Bulgaria
Sapes, Greece
Lodine, Italy
Morocco (Tafroute, Timahdite, Tizi n'test, Tizi n'tishka, Tleta tassrit)
Slava Rus, Romania
Spain (Castilleio de Martin Viejo Monesterio, Pedraza de la Sierra, Robledillo)
Bancizaray, Ukraine
Kakceveli, Ukraine
Trudalyubivka, Ukraine
Native Range
Invasive Range

(b)


Bilisti
Struga
Beronovo
Devnja
Dripchlevo
Galabets
Harmanli
Izgrev
Izvorsko
Orizare
Razlog
Rudnik
Sozopol
Sredec
Staro Orjahovo
Tenevo
Zvezdel
Pezenes Les Mines
Askos
Edessa
Kokinochoma
Komotini
Sapes
Thermi
Altamura
Dorgali
Minervino Murge
Orosei
Poggiorsini
Bitola
Lavazzalady
Umin Dol
Slava Rus
Drobetia
Sacele
Schela
Taman Bay
Kladovo
Alseki
Corlu
Havsa
Ipsala
Kesan
Poyrali
Sevilishir
Sarigol
Urunlu
Uzunkopru North
Yalihuyuk
Yonukler
Alushta
Bahate
Bancizaray
Izobilne
Pryvitne
Sudak
Trudalyubivka
Yalta
Albania
Bulgaria
FR
Greece
Italy
Macedonia
Romania
RU
SR
Turkey
Ukraine

(c)

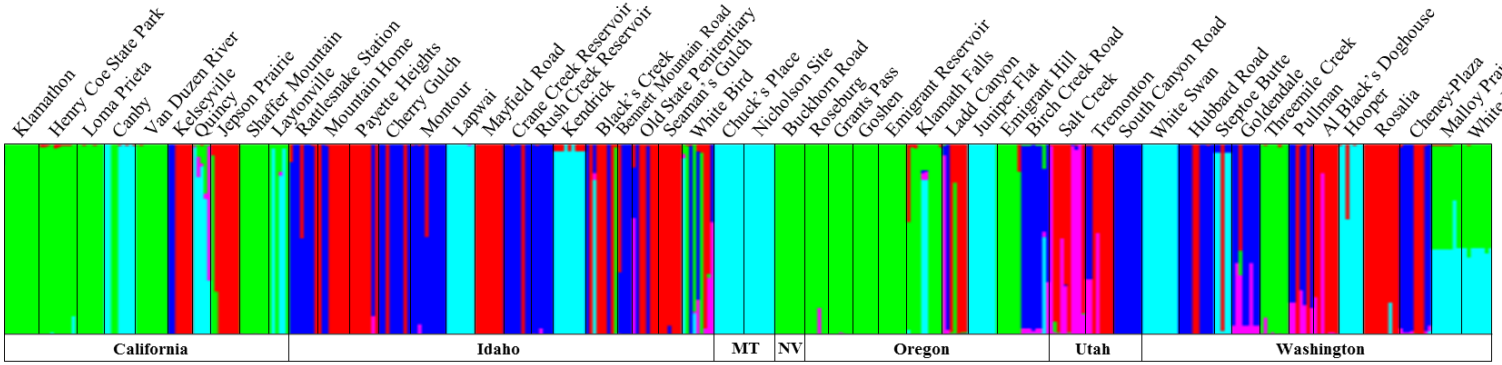
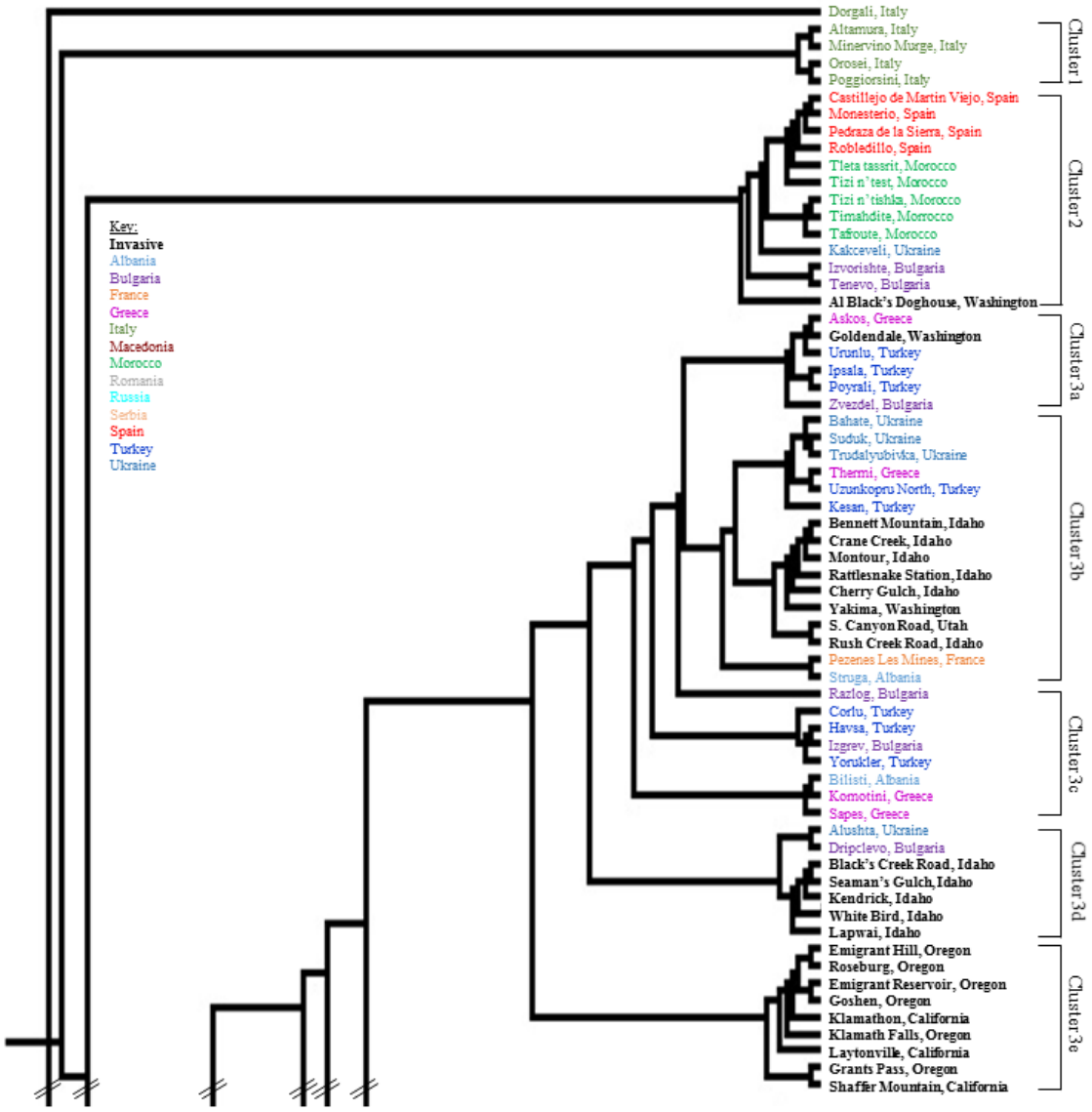


Figure 6 STRUCTURE (Pritchard *et al.* 2000) bar plots of the genetic clusters identified for populations of *Taeniatherum caput-medusae* subsp. *asperum* for (a) the initial combined analysis of 70 native and 52 invasive populations (K=2), (b) results for 58 native populations based on the sub-structuring analysis of 110 native and invasive populations (subK=6), and (c) results for 52 invasive populations based on the sub-structuring analysis of 110 native and invasive populations. Five of the six genetic subclusters were detected within these invasive populations

(a)


Key:
Invasive
Albania
Bulgaria
France
Greece
Italy
Macedonia
Morocco
Romania
Russia
Serbia
Spain
Turkey
Ukraine
Dorgali, Italy
Altamura, Italy
Minervino Murge, Italy
Orosei, Italy
Poggiorsini, Italy
Castillejo de Martin Viejo, Spain
Monasterio, Spain
Pedraza de la Sierra, Spain
Robledillo, Spain
Tleta tassrit, Morocco
Tizi n'test, Morocco
Tizi n'tishka, Morocco
Timahdite, Morrocco
Tafroute, Morocco
Kalcevali, Ukraine
Izvorishte, Bulgaria
Tenevo, Bulgaria
Al Black's Doghouse, Washington
Askos, Greece
Goldendale, Washington
Urunlu, Turkey
Ipsala, Turkey
Poyrali, Turkey
Zvezdel, Bulgaria
Bahate, Ukraine
Suduk, Ukraine
Trudalyubivka, Ukraine
Thermi, Greece
Uzunkopru North, Turkey
Kesan, Turkey
Bennett Mountain, Idaho
Crane Creek, Idaho
Montour, Idaho
Rattlesnake Station, Idaho
Cherry Gulch, Idaho
Yakima, Washington
S. Canyon Road, Utah
Rush Creek Road, Idaho
Pezenes Les Mines, France
Struga, Albania
Razlog, Bulgaria
Corlu, Turkey
Havsa, Turkey
Izgrev, Bulgaria
Yorukler, Turkey
Bilisti, Albania
Komotini, Greece
Sapes, Greece
Alushta, Ukraine
Dripclevo, Bulgaria
Black's Creek Road, Idaho
Seaman's Gulch, Idaho
Kendrick, Idaho
White Bird, Idaho
Lapwai, Idaho
Emigrant Hill, Oregon
Roseburg, Oregon
Emigrant Reservoir, Oregon
Goshen, Oregon
Klamathon, California
Klamath Falls, Oregon
Laytonville, California
Grants Pass, Oregon
Shaffer Mountain, California
Cluster1
Cluster2
Cluster3a
Cluster3b
Cluster3c
Cluster3d
Cluster3e

(b)

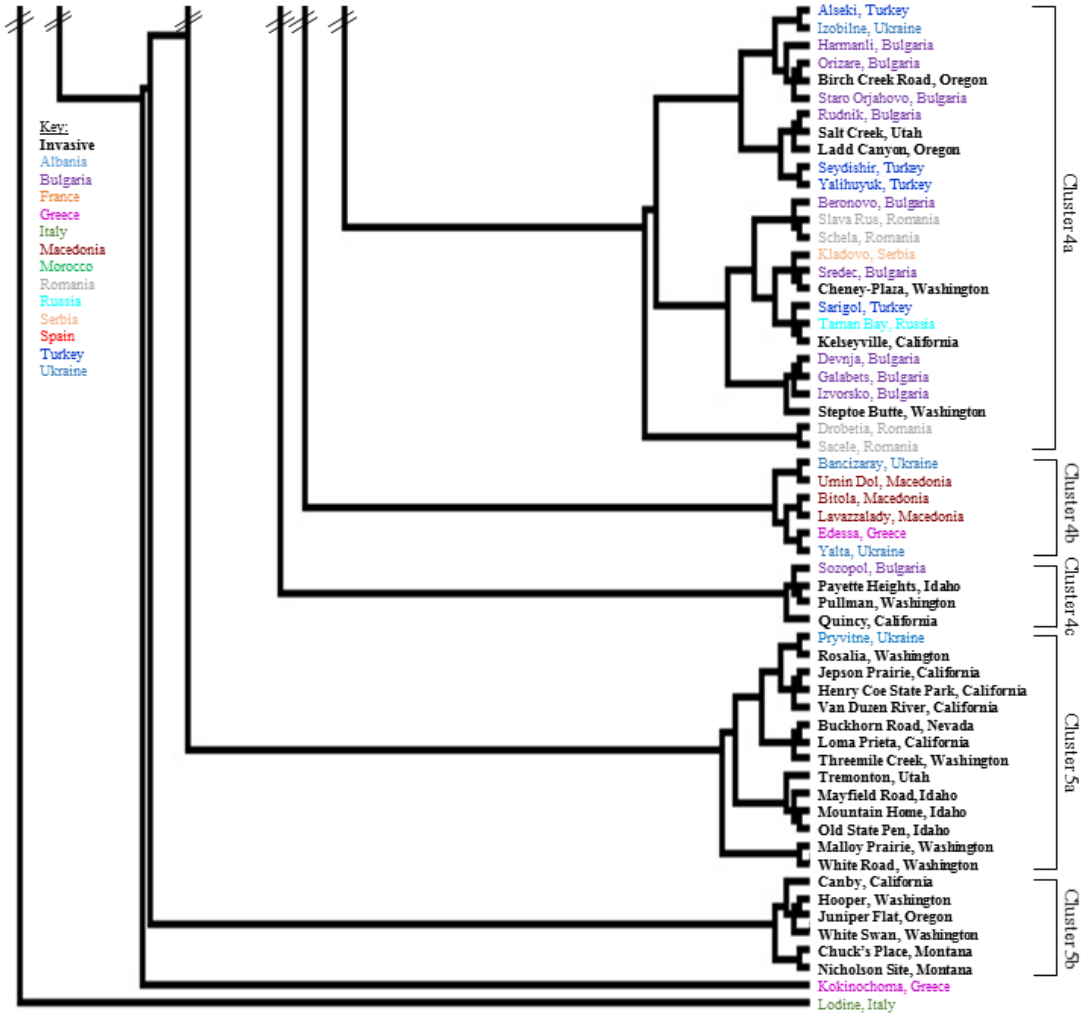
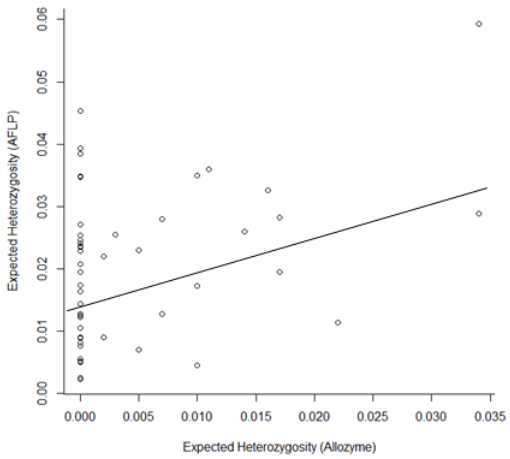
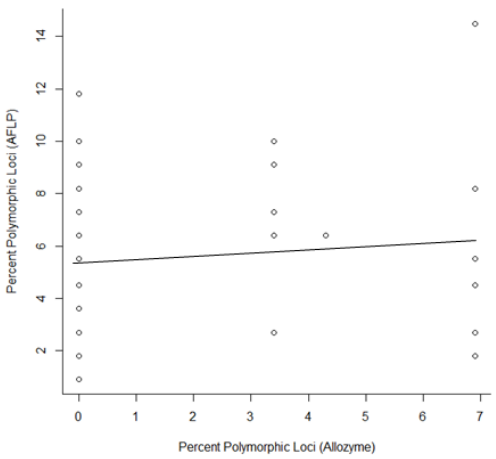


Figure 7 Neighbor-joining tree showing genetic relationships among 122 native and invasive populations of *Taeniatherum caput-medusae* subsp. *asperum*. Invasive populations are indicated by the black font and native populations are color coded according to country.

(a)



(b)



(c)

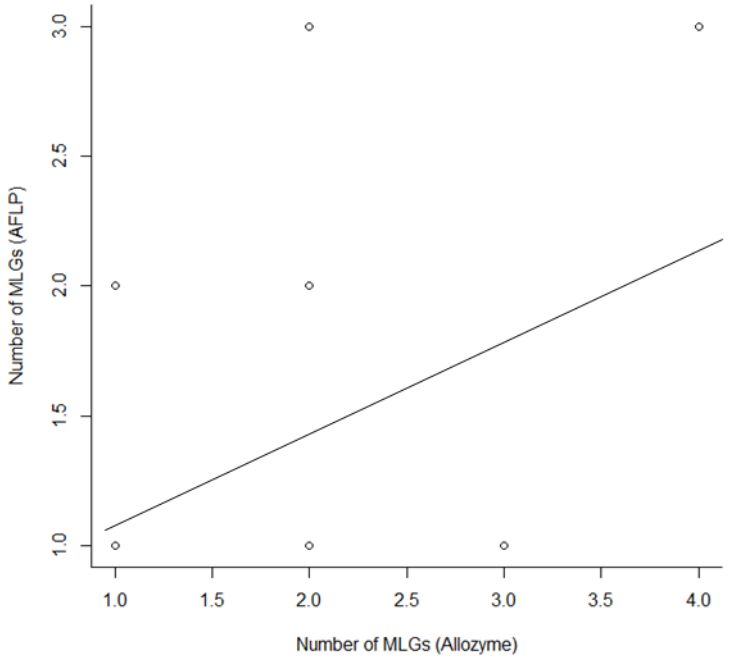


Figure 8 Linear regression analysis depicting the relationship between (a) allozyme expected heterozygosity and AFLP expected heterozygosity values (r_s=0.258, p>0.06), (b) allozyme percent polymorphic loci and AFLP percent polymorphic loci data (r_s=0.155, p>0.27), and (c) the number of MLGs detected using allozyme and AFLP data (r_s=0.324, p<0.02) for the 52 invasive populations of *Taeniatherum caput-medusae* subsp. *asperum* analyzed in this study.

APPENDIX A

Locality Data for the 70 Native Populations of *Taeniatherum caput-medusae* subsp. *asperum* Analyzed Using 110 AFLP Loci (Guerdan 2016). Country, Population, Latitude, Longitude, and Elevation (Meters) Data Is Provided. Populations Are Arranged Alphabetically by Country and Locality.

Country	Population	Latitude	Longitude	Elevation
Albania	1. Bilisti	40° 40' 05"N	20° 49' 20"E	878
	2. Struga	41° 04' 40"N	20° 36' 25"E	1016
Bulgaria	3. Beronovo	42° 49' 39"N	26° 42' 34"E	358
	4. Devnja	43° 13' 56"N	27° 32' 33"E	128
	5. Dripclevo	41° 59' 41"N	26° 11' 45"E	461
	6. Galabets	41° 49' 39"N	25° 27' 03"E	322
	7. Harmanli	41° 58' 03"N	25° 59' 42"E	241
	8. Izgrev	42° 08' 41"N	27° 48' 38"E	137
	9. Izvorishte	42° 39' 31"N	27° 26' 07"E	278
	10. Izvorsko	43° 16' 47"N	27° 46' 57"E	323
	11. Orizare	42° 42' 43"N	27° 37' 04"E	77
	12. Razlog	41° 53' 11"N	23° 30' 05"E	834
	13. Rudnik	42° 59' 10"N	27° 47' 18"E	75
	14. Sozopol	42° 22' 07"N	27° 41' 07"E	50
	15. Sredec	42° 12' 49"N	27° 02' 11"E	332
	16. Staro Orjahovo	42° 59' 11"N	27° 47' 17"E	65
	17. Tenevo	42° 21' 38"N	26° 34' 19"E	145
	18. Zvezdel	41° 28' 16"N	25° 32' 24"E	572
France	19. Pezenes Les Mines	43° 36' 11"N	03° 15' 45"E	361
Greece	20. Askos	40° 45' 27"N	23° 27' 11"E	398
	21. Edessa	40° 47' 06"N	21° 53' 20"E	587
	22. Kokinochoma	40° 55' 28"N	24° 17' 24"E	73
	23. Komotini	41° 05' 14"N	25° 44' 30"E	113
	24. Sapes	40° 59' 43"N	25° 39' 41"E	84

	25. Thermi	40° 34' 17"N	23° 03' 39"E	300
Italy	26. Altamura	40° 56' 06"N	16° 30' 03"E	507
	27. Dorgali	40° 18' 18"N	09° 34' 18"E	270
	28. Minervino Murge	41° 02' 43"N	16° 10' 57"E	572
	29. Orosei	40° 23' 49"N	09° 43' 06"E	26
	30. Poggiorsini	40° 58' 35"N	16° 15' 15"E	601
	31. Lodine	40° 09' 45"N	09° 14' 10"E	860
Macedonia	32. Bitola	41° 02' 16"N	21° 19' 10"E	645
	33. Lavazzalady	41° 03' 11"N	21° 16' 49"E	761
	34. Umin Dol	42° 05' 21"N	21° 36' 04"E	535
Morocco	35. Tafroute	29° 44' 16"N	08° 50' 04"W	1626
	36. Timahdite	33° 17' 02"N	05° 04' 33"W	1820
	37. Tizi n'test	30° 54' 59"N	08° 17' 34"W	1560
	38. Tizi n'tishka	31° 14' 14"N	07° 24' 51"W	1984
	39. Tleta tassrit	29° 36' 59"N	08° 55' 24"W	1670
Romania	40. Slava Rus	44° 58' 25"N	28° 38' 45"E	43
	41. Drobetia	44° 48' 25"N	28° 38' 45"E	100
	42. Sacele	44° 38' 30"N	22° 37' 17"E	73
	43. Schela	44° 28' 45"N	28° 38' 51"E	54
Russia	44. Taman Bay	45° 19' 40"N	36° 48' 35"E	22
Serbia	45. Kladovo	44° 38' 01"N	22° 33' 38"E	95
Spain	46. Castillejo de Martin Viejo	40° 41' 47"N	06° 39' 36"W	597
	47. Monesterio	38° 05' 45"N	06° 12' 39"W	745
	48. Pedraza de la Sierra	41° 07' 51"N	03° 48' 27"W	1039
	49. Robledillo	41° 32' 03"N	04° 56' 49"W	1230

Turkey	50. Alseki	37° 07' 17"N	31° 47' 49"E	1271
	51. Corlu	41° 03' 06"N	27° 43' 56"E	13
	52. Havsa	41° 24' 05"N	26° 28' 41"E	73
	53. Ipsala	40° 52' 47"N	26° 25' 10"E	50
	54. Kesan	40° 44' 06"N	26° 43' 21"E	104
	55. Poyrali	41° 37' 41"N	27° 36' 20"E	329
	56. Seydishir	37° 24' 17"N	31° 50' 06"E	1239
	57. Sarigol	38° 14' 53"N	28° 40' 12"E	311
	58. Urunlu	41° 40' 27"N	26° 59' 53"E	132
	59. Uzunkopru North	41° 18' 57"N	26° 34' 24"E	118
	60. Yalihuyuk	37° 18' 50"N	32° 06' 18"E	1102
	61. Yorukler	41° 07' 07"N	27° 14' 25"E	105
Ukraine	62. Alushta	44° 42' 17"N	34° 25' 54"E	190
	63. Bahate	45° 01' 40"N	34° 45' 57"E	303
	64. Bancizaray	44° 28' 58"N	34° 07' 30"E	180
	65. Izobilne	44° 42' 05"N	34° 21' 02"E	217
	66. Kakceveli	44° 24' 00"N	33° 57' 44"E	150
	67. Pryvitne	44° 49' 19"N	34° 43' 47"E	279
	68. Sudak	44° 53' 10"N	35° 05' 40"E	176
	69. Trudalyubivka	44° 46' 50"N	33° 59' 51"E	190
	70. Yalta	44° 28' 52"N	34° 07' 32"E	281

APPENDIX B

Distribution of AFLP Multilocus Genotypes (MLGs) Among the 52 Invasive and 70 Native Populations of *Taeniatherum caput-medusae* subsp. *asperum* Analyzed in This Study. Multilocus Genotypes Were Determined in GenoType (Meirmans and Van Tienderen 2004) Using the Three Band Error Rate (See The Text). Three Different MLG Categories Are Included: Monomorphic for the MCG, Polymorphic Including MCG, and Does Not Include the MCG.

MLG Category	Invasive Populations	Native Populations
Monomorphic for MCG	1. Klamathon, CA	4. Devnja, Bulgaria
	2. Henry Coe State Park, CA	6. Galabets, Bulgaria
	3. Loma Prieta, CA	8. Izgrev, Bulgaria
	5. Van Duzen River, CA	10. Izvorsko, Bulgaria
	6. Kelseyville, CA	15. Sredec, Bulgaria
	9. Shaffer Mountain, CA	19. Pezenes Les Mines, France
	10. Laytonville, CA	20. Askos, Greece
	11. Rattlesnake Station, ID	23. Komotini, Greece
	12. Mountain Home, ID	33. Lavazzalady, Macedonia
	13. Payette Heights, ID	45. Kladovo, Serbia
	14. Cherry Gulch, ID	50. Alseki, Turkey
	15. Montour, ID	52. Havsa, Turkey
	16. Lapwai, ID	55. Poyrali, Turkey
	17. Mayfield Road, ID	56. Seydishir, Turkey
	18. Crane Creek Reservoir, ID	58. Urunlu, Turkey
	19. Rush Creek Road, ID	59. Uzunkopru North, Turkey
	20. Kendrick, ID	62. Alushta, Ukraine
	21. Black's Creek Rd., ID	63. Bahate, Ukraine
	22. Bennett Mountain Road, ID	65. Izobilne, Ukraine
	24. Seaman's Gulch, ID	67. Pryvitne, Ukraine
	28. Buckhorn Road, NV	68. Sudak, Ukraine
	29. Roseburg, OR	70. Yalta, Ukraine
	30. Grants Pass, OR	
	31. Goshen, OR	

	32. Emigrant Reservoir, OR	
	33. Klamath Falls, OR	
	34. Ladd Canyon, OR	
	35. Juniper Flat, OR	
	36. Emigrant Hill, OR	
	37. Birch Creek Road, OR	
	39. Tremonton, UT	
	40. South Canyon Road, UT	
	41. White Swan, WA	
	42. Hubbard Road, WA	
	43. Steptoe Butte, WA	
	44. Goldendale, WA	
	48. Hooper, WA	
	49. Rosalia, WA	
	50. Cheney-Plaza, WA	
	51. Malloy Prairie, WA	
	52. White Road, WA	
Total Populations	41	22
Polymorphic including MCG	4. Canby, CA	1. Bilisti, Albania
	8. Jepson Prairie, CA	2. Struga, Albania
	23. Old State Penitentiary, ID	3. Beronovo, Bulgaria
	25. White Bird, ID	5. Dripclevo, Bulgaria
	38. Salt Creek, UT	7. Harmanli, Bulgaria
	45. Threemile Creek, WA	11. Orizare, Bulgaria

	46. Pullman, WA	12. Razlog, Bulgaria
		13. Rudnik, Bulgaria
		16. Staro Orjahovo, Bulgaria
		18. Zvezdel, Bulgaria
		21. Edessa, Greece
		22. Kokinochoma, Greece
		24. Sapes, Greece
		25. Thermi, Greece
		32. Bitola, Macedonia
		40. Slava Rus, Romania
		41. Drobetia, Romania
		43. Schela, Romania
		44. Taman Bay, Russia
		51. Corlu, Turkey
		53. Ipsala, Turkey
		54. Kesan, Turkey
		61. Yorukler, Turkey
		69. Trudalyubivka, Ukraine
Total Populations	7	24
Does not include the MCG	7. Quincy, CA	9. Izvorishte, Bulgaria
	26. Chuck's Place, MT	14. Sozopol, Bulgaria
	27. Nicholson Site, MT	17. Tenevo, Bulgaria
	47. Al Black's Doghouse, WA	26. Altamura, Italy
		27. Dorgali, Italy
		28. Minervino Murge, Italy

		29. Orosei, Italy
		30. Poggiorsini, Italy
		31. Lodine, Italy
		34. Umin Dol, Macedonia
		35. Tafroute, Morocco
		36. Timahdite, Morocco
		37. Tizi n'test, Morocco
		38. Tizi n'tishka, Morocco
		39. Tleta tassrit, Morocco
		42. Sacele, Romania
		46. Castillejo de Martin Viejo, Spain
		47. Monesterio, Spain
		48. Pedraza de la Sierra, Spain
		49. Robledillo, Spain
		57. Sarigol, Turkey
		60. Yalihuyuk, Turkey
		64. Bancizaray, Ukraine
		66. Kakceveli, Ukraine
Total Populations	4	24
Grand Total	52	70

APPENDIX C

Within-Population Genetic Diversity Parameters for 52 Invasive Populations of *Taeniatherum caput-medusae* subsp. *asperum* from the Western United States Analyzed Using Allozymes and AFLP. Parameters Include the Number of Individuals Per Population (n), Expected Heterozygosity (H_e), the Percentage of Polymorphic Loci (%P), and Number of Multilocus Genotypes (#MLG) Detected.

		Allozyme Data				AFLP Data			
State	Population	n	H_e	%*P*	#*MLG*	n	H_e	%*P*	#*MLG*
California	1. Klamathon	35	0.000	0.0	1	10	0.005	1.8	1
	2. Henry Coe State Park	41	0.000	0.0	1	10	0.017	4.5	1
	3. Loma Prieta	28	0.000	0.0	1	8	0.045	10.0	1
	4. Canby	32	0.011	3.4	2	9	0.036	9.1	2
	5. Van Duzen River	37	0.002	3.4	2	9	0.009	2.7	1
	6. Kelseyville	35	0.007	3.4	2	7	0.028	6.4	1
	7. Quincy	26	0.000	0.0	1	5	0.038	9.1	2
	8. Jepson Prairie	38	0.016	3.4	2	8	0.033	9.1	3
	9. Shaffer Mountain	35	0.000	0.0	1	8	0.008	1.8	1
	10. Laytonville	31	0.034	6.9	2	6	0.029	5.5	1
	California Mean	33.8	0.007	2.1	1.5	8.0	0.025	6.0	1.4
Idaho	11. Rattlesnake Station	40	0.010	6.9	2	8	0.017	4.5	1
	12. Mountain Home	40	0.000	0.0	1	8	0.025	6.4	1
	13. Payette Heights	39	0.000	0.0	1	9	0.013	4.5	1

	14. Cherry Gulch	35	0.000	0.0	1	9	0.023	7.3	1
	15. Montour	40	0.000	0.0	1	10	0.016	6.4	1
	16. Lapwai	35	0.000	0.0	1	8	0.014	3.6	1
	17. Mayfield Road	40	0.010	6.9	2	8	0.005	1.8	1
	18. Crane Creek Reservoir	35	0.000	0.0	1	10	0.017	6.4	1
	19. Rush Creek Road	35	0.000	0.0	1	4	0.024	4.5	1
	20. Kendrick	35	0.000	0.0	1	9	0.006	1.8	1
	21. Black's Creek Road	40	0.014	6.9	3	9	0.026	8.2	1
	22. Bennett Mountain Road	35	0.007	6.9	2	4	0.013	2.7	1
	23. Old State Penitentiary	40	0.000	0.0	1	8	0.035	9.1	2
	24. Seaman's Gulch	40	0.000	0.0	1	6	0.009	2.7	1
	25. White Bird	40	0.034	6.9	4	9	0.059	14.5	3
	Idaho Mean	37.9	0.005	2.3	1.5	7.9	0.020	5.6	1.2
Montana	26. Chuck's Place	28	0.000	0.0	1	7	0.003	0.9	1
	27. Nicholson Site	29	0.000	0.0	1	10	0.005	1.8	1
	Montana Mean	28.5	0.000	0.0	1.0	8.5	0.004	1.4	1.0

Nevada	28. Buckhorn Road	35	0.000	0.0	1	8	0.013	4.5	1
Oregon	29. Roseburg	40	0.000	0.0	1	8	0.035	8.2	1
	30. Grants Pass	34	0.000	0.0	1	6	0.024	5.5	1
	31. Goshen	35	0.000	0.0	1	7	0.020	4.5	1
	32. Emigrant Reservoir	35	0.000	0.0	1	8	0.013	2.7	1
	33. Klamath Falls	34	0.017	3.4	2	10	0.028	7.3	1
	34. Ladd Canyon	35	0.000	0.0	1	7	0.035	10.0	1
	35. Juniper Flat	40	0.005	6.9	3	8	0.007	1.8	1
	36. Emigrant Hill	44	0.017	3.4	2	7	0.02	6.4	1
	37. Birch Creek Road	36	0.000	0.0	1	8	0.009	3.6	1
	Oregon Mean	37.0	0.004	1.5	1.4	7.7	0.021	5.6	1.0
Utah	38. Salt Creek	40	0.010	3.4	2	10	0.035	10.0	2
	39. Tremonton	40	0.000	0.0	1	8	0.022	5.5	1
	40. South Canyon Road	35	0.000	0.0	1	8	0.002	0.9	1
	Utah Mean	38.3	0.003	1.1	1.3	8.7	0.020	5.5	1.3
Washington	41. White Swan	35	0.000	0.0	1	10	0.012	5.5	1

42. Hubbard Road	35	0.005	4.3	2	10	0.023	6.4	1
43. Steptoe Butte	50	0.000	0.0	1	5	0.011	2.7	1
44. Goldendale	35	0.000	0.0	1	8	0.021	6.4	1
45. Threemile Creek	38	0.000	0.0	1	8	0.039	11.8	2
46. Pullman	40	0.003	6.9	2	7	0.025	5.5	2
47. Al Black's Doghouse	35	0.002	3.4	2	7	0.027	7.3	2
48. Hooper	35	0.000	0.0	1	7	0.023	4.5	1
49. Rosalia	40	0.022	6.9	2	10	0.011	4.5	1
50. Cheney-Plaza	26	0.000	0.0	1	9	0.025	5.5	1
51. Malloy Prairie	35	0.000	0.0	1	7	0.005	1.8	1
52. White Road	32	0.000	0.0	1	10	0.008	2.7	1
Washington Mean	36.3	0.003	1.8	1.3	8.2	0.019	5.4	1.3
Total Mean	36.2	0.004	1.8	1.4	8.0	0.020	5.4	1.2