GENETIC AND MORPHOLOGICAL VARIATION IN *TAENIATHERUM CAPUT-MEDUSAE* (MEDUSAHEAD): TAXOMONIC DIVERSITY, GEOGRAPHIC ORIGINS, MULTIPLE INTRODUCTIONS AND FOUNDER EFFECTS

by

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DEDICATION

This thesis is dedicated with love to Jeannette Irene Turman (1926-2011) and Paul Onil Frechette (1923-2011).

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ABSTRACT

Invasive species are novel to a region, thus their timely and accurate identification is a critical first step in recognizing and managing the threats that they may present in their new habitats. Accurate identification of an introduced species in its new range can prove difficult however for a species that displays taxonomic complexity in its native range, i.e. consists of multiple, morphologically similar subspecies.

Across its native range, *Taeniatherum caput-medusae* (medusahead) exhibits taxonomic complexity. Three subspecies have been recognized: *T. caput-medusae* ssp. *caput-medusae*, *T. caput-medusae* ssp.*asperum*, and *T. caput-medusae* ssp. *crinitum*. While subspecies *caput-medusae* is found in the western Mediterranean and subspecies *crinitum* occurs from eastern Europe to Central Asia, subspecies *asperum* is distributed across the geographic distribution of the species. Only subspecies *asperum* is believe to occur in the United States, where it is now invasive in portions of California, Idaho, Nevada, Oregon, Utah, and Washington. As part of ongoing research to better understand and manage this invasion, genetic analyses of both native and invasive populations of medusahead were conducted. An important prerequisite to these analyses is the proper identification of the three subspecies. In the current study, plants from each native population were grown in a greenhouse common garden, harvested at maturity, and measured using previously described morphological characters. After Bonferroni correction, three characters, glume length, glume angle, and

palea length, were found to be statistically significant. Thus, these three characters were quite useful in assigning plants to each of the three subspecies. I found that two other characters, lemma hairs and conical cells, were less informative. Differentiation among native populations of medusahead was further assessed using a molecular genetic marker. The results of a UPGMA cluster diagram based on allozyme data indicates that subspecies *crinitum* is genetically differentiated from the other two, some populations of subspecies *caput-medusae* and *asperum* co-occur within different clusters, and subspecies *asperum* is the most variable. Results of the analysis of multilocus genotypes are generally consistent with the UPGMA diagram (e.g., subspecies *caput-medusae* and *asperum* share six multilocus genotypes). This research confirms the need of such studies to disentangle the taxonomic complexity that can be found in the native range of invasive species.

The results of an earlier allozyme analysis were consistent with the genetic signature associated with multiple introductions, although this finding can only be confirmed with the analysis of native populations. In the current study, I compared allozyme diversity in native and invasive populations of medusahead to: identify the geographic origin(s) for the U.S. invasion, test the multiple introduction hypothesis, and determine the genetic consequences of these events. Five of the seven homozygous multilocus genotypes previously observed in the western U.S. have been detected in native populations. The geographic origins for these introductions appear to have been drawn from France, Sardinia, Greece, and Turkey, although additional analyses are ongoing. These findings provide support for the multiple introduction hypothesis. Results of this study have implications for the biological control of

medusahead: i) the search for effective and specific biological control agents will have to occur broadly across the species' native range, ii) multiple agents may be required to control invasive populations that are admixtures, and iii) because invasive population are genetically depauperate, highly adapted biocontrol agents are likely to be quite effective.

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Abstract

Invasive species are novel to a region, thus their timely and accurate identification is a critical first step in recognizing and managing the threats that they may present in their new habitats. However, accurate identification of an introduced species in its new range can prove difficult for a species that displays taxonomic complexity in its native range, i.e. consists of multiple, morphologically similar subspecies. Across its native range, *Taeniatherum caput-medusae* (medusahead) exhibits taxonomic complexity. Three subspecies have been recognized: *T. caput-medusae* subspecies *caput-medusae*, *T. caput-medusae* subspecies *asperum*, and *T. caput-medusae* subspecies *crinitum*. Only subspecies *asperum* is believe to occur in the United States, where it is now invasive in portions of California, Idaho, Nevada, Oregon, Utah, and Washington. As part of ongoing research to better understand and manage this invasion, the accurate identification of these three subspecies is a requisite first step. In the current study, plants from each native population were grown in a greenhouse common garden, harvested at maturity, and measured using previously described morphological characters. After Bonferroni correction, three characters, glume length, glume angle, and palea length, were found to be statistically significant. Thus, these three characters proved quite useful

in differentiating the three subspecies. I found that two other characters, conical cell prominence on the lemma and lemma surface hair location, were less informative. Differentiation among native populations of medusahead was further assessed using a molecular genetic marker. The results of a UPGMA cluster diagram based on allozyme data indicates that subspecies *crinitum* is genetically differentiated from the other two, some populations of subspecies *caput-medusae* and *asperum* co-occur within a cluster, and subspecies *asperum* is the most variable. Results of the analysis of multilocus genotypes are generally consistent with the UPGMA diagram (e.g., subspecies *caput-medusae* and *asperum* share six multilocus genotypes). Our findings confirm the need of such studies to disentangle the taxonomic complexity that can be found in the native range of invasive species.

Keywords: allozymes, invasive grass, multilocus genotype, taxonomic complexity, morphological characters, multiple subspecies, genetic relationships

Introduction

Human activities such as international trade and commerce have greatly increased the number and rate of biological invasions worldwide (Mack et al. 2000; Bossdorf et al. 2005; Ward et al. 2008). Invasive species often have negative ecological consequences such as loss of native biodiversity and community structure; modification of ecosystem processes such as nutrient cycling and trophic level interactions; and alteration of disturbance regimes, especially the frequency and intensity of wildfires (D'Antonio and Vitousek 1992; Wilcove et al. 1998; Mack et al. 2000, Sala et al. 2000; Allendorf and Lundquist 2003). Such events also have enormous economic costs (Pimentel et al. 2005). Thus, invasions are now considered one of the main drivers of global change (Vitousek et al. 1996; Sala et al. 2000).

Biological invasions occur when organisms are taken from their native range and transported to a new territory where they become established, proliferate and spread beyond their original point of introduction (Mack et al. 2000; Colautti and MacIsaac 2004; Lockwood et al. 2005). In fact, many invasions can be described as large-scale (intercontinental) biogeographical events (Groves and di Castri 1991; Mack et al. 2000; Hierro et al. 2005). Thus, in order to gain a better understanding of the invasion process, comprehensive analyses of invasive species must, by this characterization, adopt an equally large geographical scope. Two recent reviews (Bossdorf et al. 2005; Hierro et al. 2005) have emphasized the importance of studying invasive species in both their native and introduced ranges, and this approach has yielded considerable insights into the ecological, genetic, and evolutionary aspects of invasive species (for a review see Sax et

al. 2005).

Because introduced species are novel to a region, their timely and accurate identification is a critical first step in recognizing and managing the threats that they may present in their new habitats (Wittenberg and Cock 2005). Quick and reliable taxonomic identification of introduced/invasive species requires recognition of them by field personnel, sufficient diagnostic information, and accessible databases (Ricciardi et al. 2000; D'Antonio et al. 2004). Accurate identification of an alien can assist in predicting whether it will become invasive in its new range, based on its performance and impacts elsewhere (Reichard and Hamilton 1997; D'Antonio et al. 2004). However, accurate identification of an introduced species in its new range can prove difficult for a species that displays taxonomic complexity in its native range, i.e. consists of multiple, morphologically similar subspecies. Knowledge and recognition of the taxonomic complexity of an invasive species can be used to: i) differentiate between invasive and non-invasive subspecies in their native range (e.g., *Acacia nilotica,* Ali and Qaiser 1980; Kriticos et al. 2003, Wardill et al. 2005; Khatoon and Ali 2006, *Centaurea stoebe,* Hufbauer and Sforza 2008; Marrs et al. 2008 and *Codium fragile*, Trowbridge 1998, 2001; Provan et al. 2005), ii) identify native and non-native subspecies within the same region (e.g., *Phragmites australis* in North America, Saltonstall 2002; Saltonstall et al. 2004; Meyerson et al. 2009), iii) identify different invasive subspecies of the same species (*Lepidium draba*, Mulligan and Frankton 1962; Mummenhoff et al. 2001; Al-Shehbaz et al. 2002, Gaskin et al. 2005), iv) determine whether subspecies exhibit ecological differentiation, as described by Clausen and Hiesey (1958), in either their native and/or introduced ranges, and v) allows for the detection of a cryptic invasion

through the identification of a previously unrecognized invasive subspecies (Bickford et al. 2006). The accurate identification of invasive subspecies also aids in the search for the most specific and effective biological control agents in the native range of a species (Kriticos et al. 1999; Wardill et al. 2005; Bickford et al. 2006; Palmer et al. 2007).

Taeniatherum caput-medusae (L.) Nevski (medusahead, Poaceae), is a self-pollinating, diploid, annual, Eurasian grass that is invasive in the western United States (U.S.). In previous taxonomic treatments *Taeniatherum* had been included in *Elymus*, *Hordelymus* or *Hordeum* (for a review of the taxonomic history of *Taeniatherum* see Frederiksen 1986). Linnaeus (1753) originally recognized a single species placed in *Elymus* (*E. caput-medusae* L.), while Schreber (1772) named two species (*E. caput-medusae* and *E. crinitus* Schreb.), and Link (1827) named three species (*E. caput-medusae*, *E. crinitus* and *E. platyatherus* Link). Nevski (1934) established *Taeniatherum* and recognized three species: *T. caput-medusae* (L.) Nevski, *T. crinitum* (Schreber) Nevski, and *T. asperum* (Simonk.) Nevski. Although other taxonomic treatments have been proposed (e.g., Humphries 1978), probably the most widely accepted taxonomic revision of *Taeniatherum* recognizes three subspecies (Frederiksen 1986): *T. caput-medusae* (L.) Nevski subspecies *caput-medusae*, *T. caput-medusae* subspecies *crinitum* (Schreb.) Melderis and *T. caput-medusae* subspecies *asperum* (Simk.) Melderis. These three subspecies were differentiated by Frederiksen (1986) based on morphological characters associated with the spikes: glume length and spreading of glumes (glume angle) in seed stage, and several traits associated with the lemma and palea (e.g., palea length). Intermediate morphological forms were reported in regions where the geographic distributions of subspecies overlap (Frederiksen 1986).

All three subspecies have a diploid chromosome number ($2n = 14$), and exhibit the same karyotype (Frederiksen 1986; Frederiksen and von Bothmer 1986). Crossing experiments among the subspecies produced hybrids of low fertility, although a high amount of bivalent formation was observed during meiosis. The low fertility of these crosses, combined with observations concerning chromosome pairing behavior, led Frederiksen and von Bothmer (1986) to conclude that all three subspecies have similar genomes and that differences among them appeared to be genetically determined. The subspecies do exhibit different geographic distributions, although some overlap does occur. In general, subspecies *caput-medusae* is found in the western Mediterranean (Morocco, Portugal, Spain and France), subspecies *crinitum* occurs from eastern Europe and the eastern Mediterranean to central Asia (Kyrgyzstan, Tajikistan and Afghanistan) and subspecies *asperum* is found across almost the entire Eurasian native range of *Taeniatherum* [see Frederiksen (1986) for a map of the geographic distribution of *Taeniatherum*].

In the western U.S., *T. caput-medusae* occurs in disturbed sites in the 25-100 cm mean annual precipitation zones, and it can dominate sites with high clay content or well-developed soils (Dahl and Tisdale 1975; Hironaka 1994). The grass has invaded millions of hectares of semi-arid woodlands and shrub-steppe habitats in California, Idaho, Nevada, Oregon, Utah, and Washington (McKell et al. 1962; Young and Evans 1970; Young 1992, Pellant and Hall 1994, Miller et al. 1999, Blank and Sforza 2007). Based on the examination of plants in the native and invasive ranges, it is believed that the taxon introduced into the U.S. was *T. caput-medusae* subspecies *asperum* (Major et al. 1960; Young 1992; Kostivkovsky and Young 2000).

The ultimate goal of this thesis research is to determine the genetic and evolutionary consequences of the introduction of *Taeniatherum caput-medusae* into the western U.S. (see Chapter 2). However, given that, across its extensive Eurasian native range, *T. caput-medusae* occurs as three subspecies, gaining a better understanding of this taxonomic complexity is the requisite first step for achieving this goal. Thus, the specific objectives of the current study are to: 1) determine the utility of the morphological characters described by Frederiksen (1986) in distinguishing the three subspecies of *T. caput-medusae*, 2) assess morphological variation within and among native populations of the three subspecies of *T. caput-medusae*, and 3) determine the level of genetic differentiation among the three subspecies using a neutral molecular marker, enzyme electrophoresis.

Materials and Methods

Plant Collection

For this study, population samples were obtained from a total of 87 native range populations of *Taeniatherum caput-medusa* and these samples were collected over multiple years: 2002, 2004, 2005, 2007, 2009 and 2010 by Dr. René Sforza and Dr. Stephen J. Novak (Table 1.1, Figure 1.1). Field collected populations were assigned to a subspecies based upon the morphological characters described by Frederiksen (1986). Within each population, 30-35 intact spikes were sampled haphazardly 1 -3 m apart. In populations with fewer than 30 individuals, all individuals were harvested. Intact spikes were stored in individually labeled paper envelopes at room temperature. Many of the collections sites were along roadsides, adjacent to agricultural fields or in disturbed areas.

A total of 87 populations were collected from the native range. Two populations, Villaviciosa de Cordoba, Spain and Guzelkonak, Turkey consisted of two subspecies and were each separated into two "populations" for analysis, bringing the total to 89 "populations." Seventy-four populations were used in both the morphological and genetic analysis, seven populations in only the morphological analysis and eight populations in only the genetic analysis (Table 1). Thus, the total number of populations used in the morphological analysis is 81 and the total number used in the genetic analysis is 82. Samples from native populations were collected over multiple years by Dr. René Sforza and Dr. Stephen J. Novak (Table 1.1, Figure 1.1). The seven populations from Greece and Turkey collected in 2010 were analyzed only for genetic diversity and were not included in the morphological analysis. One population (Kars, Turkey) was not included in the morphological analysis because it did not successfully set seed. Seven populations were not used in the genetic analysis because of their geographic proximity to other populations that were included (see Table 1).

Samples for 22 of the populations included in this research were obtained as accessions from the USDA Western Region Plant Introduction Laboratory, Pullman, WA (USA); 13 from Turkey, seven from Afghanistan, two from Iran, and one from Kazahkstan (Table 1.1). Unfortunately, in some cases, the geographic location and collection date for these accessions are not provided. In addition, these accessions are the product of an unknown number of grow-outs in Pullman, WA, since they were first collected in their country of origin. Seeds from the Sterea Hellas, Greece population were kindly provided by Dr. Signe Frederiksen, Institute of Systematic Botany, University of Copenhagen, Denmark.

Greenhouse Common Garden

To determine which morphological characters are most useful for distinguishing the three subspecies, a greenhouse common garden was established at Boise State University in mid-winter 2009. For each population, two seeds from six randomly chosen individuals (maternal plants) were grown in a pot (V = 2000 mL) containing standard potting soil supplemented with ¼ cup granulated fertilizer. If both seedlings from each individual emerged, one of the two was randomly selected and discarded, leaving a maximum of six plants per pot. Plants were maintained under ambient growing conditions (e.g., no supplemental lighting), although the temperature within the greenhouse was not allowed to drop below 2 C or rise above 32 C. Plants were watered three times a week and additionally if needed. During the following summer (2010), mature plants were harvested and spikes from each population were placed in separate envelopes.

Morphological Measurements

Based on the species key developed by Frederiksen (1986), five morphological characters were selected to be used to distinguish between the three subspecies (Table 1.2). The five traits measured were: glume length, glume angle, palea length, conical cell prominence on the lemma, and lemma surface hair location. After harvest, measurements of the five traits were obtained for each individual in each population. The traits glume length and palea length were measured using a standard metric ruler and scored as continuous variables. Glume angle was measured with a True Angle ® protractor, and

was also scored as a continuous variable. Conical cell characteristics and the location of hairs on the lemma surface were determined using a Leica EZ4 Dissecting scope at various magnifications and scored categorically: conical cells not prominent = 1 and conical cells prominent = 2; hairs only at the margins of the lemma surface = 1; and hairs throughout the entire lemma surface = 2.

Enzyme Electrophoresis

Seeds were germinated in petri dishes on moistened filter paper and harvested 7 – 10 days following germination. Entire seedlings (shoot and root tissue) were macerated in a tris-HCl grinding buffer-PVP solution (pH 7.5). The starch concentration of each gel was approximately 12.5% (w/v). Enzyme electrophoresis protocols followed that of Soltis et al. (1983) with modifications described by Novak et al. (1991). A suite of 15 enzymes were stained and visualized using the following buffer systems : isocitrate dehydrogenase (IDH), glucose-6-phosphate dehydrogenase (G6PDH), and shikimate dehydrogenase (SKDH) using system 1 of Soltis et al. (1983); alcohol dehydrogenase (ADH), aldolase (ALD), glutamate dehydrogenase (GDH), and phosphoglucoisomerase (PGI) using system 6; glutamate oxalacetate transaminase (GOT), colorimetric esterase (CE), malic enzyme (ME), superoxide dismutase (SOD), and triosephosphate isomerase (TPI) using system 8; and malate dehydrogenase (MDH), phosphoglucomutase (PGM), and 6-phosphogluconate dehydrogenase (6PGD) using system 9.

Because medusahead is a diploid, the genetic basis of all allozyme variation observed was easily inferred based on the known subunit structure and compartmentalization of these enzymes (Gottlieb 1982, Weeden and Wendel 1989). Nomenclature for loci and alleles generally followed that of Novak et al. (unpublished

data), but also included modification based on the diversity detected in the current study. Across the 45 invasive populations of medusahead, Novak et al. (unpublished data) determined allelic diversity at 29 loci; but due to low gene expression and banding intensity, the genetic diversity of native populations of *T. caput-medusae* was assessed using 23 allozyme loci. For example, Novak et al. (unpublished data) scored six CE loci across invasive populations, but only two of these loci could be reliably scored among native populations: *Ce-2* and *Ce-4*. As new alleles were detected in native populations, the nomenclature for alleles had to be updated, with the most anodally migrating allele designated *a*, the next *b*, and so on.

Data Analysis

All continuous variables (glume angle, glume length, and palea length) were log transformed prior to statistical analysis because they were not normally distributed. I used the program SAS™ (SAS Institute 2002) to analyze the morphological data. Contingency tables were generated for the categorical variables (conical cell prominence and lemma surface hair location) and analysis of variance (ANOVA) was conducted on the log transformed continuous variables. To test for significant differences among subspecies morphology, Student-Newman–Keuls tests were run on each significant variable to determine which traits were significantly different for the three subspecies. Subsequently, a Bonferroni correction was performed to mitigate false positives when testing multiple hypotheses on the same data set (Rice 1989). Using only those morphological characters found to be significant, PROC GPLOT in SAS™ (SAS Institute 2002) was used to generate a three-dimensional (3D) scatter plot. The 3D scatter plot provides a visual representation of the data's ability to discriminate between

the three subspecies.

Because *T. caput-medusae* is broadly distributed across Eurasia and includes areas where the distribution of subspecies overlaps, populations with two subspecies and/or intermediate forms have been reported (Frederiksen 1986). For population instances in which populations were suspected of being composed of two subspecies, all individuals from the population were assigned to their respective subspecies and analyzed as two separate "populations." In cases of intermediacy, populations were classified as a certain subspecies based on the consensus derived when considering all available evidence (including the traits not found to be significantly different).

Allozyme data for 82 populations of *T. caput-medusae* were analyzed using POPGENE 1.32 (Yeh and Boyle 1997). The data were entered as individual multilocus genotypes and the populations were hierarchically arranged based on subspecies. Genetic diversity within subspecies of T. *caput-medusae* were expressed as the total number of alleles, the mean number of alleles per locus, the total number of polymorphic loci, the percentage polymorphic loci per subspecies, percentage of polymorphic populations, Nei's expected mean heterozygosity (H_{exp}), and the mean observed heterozygosity (H_{obs}). The expected mean heterozygosity was computed using the unbiased estimate method of Nei (1978), and the observed mean heterozygosity was determined using the Direct Count Method. F_{ST} and N_M were calculated using POPGENE 1.32 (Yeh and Boyle 1997).

The number and identity of multilocus genotypes (MLGs) for all three subspecies were determined using ARLEQUIN v.3.5.1.2 (Excoffier et al. 2005). The allozyme data were entered as psuedohaplotype frequencies for each population and structured

hierarchically according to subspecies. In addition, I used an analysis of molecular variance (AMOVA) to estimate the amount of genetic variation partitioned among and within subspecies. Individuals with missing data were deleted from the data file prior to analysis with ARLEQUIN.

The unweighted pair-group method with arithmetic averaging (UPGMA) algorithm (POPGENE 1.32) was used to generate a phenogram that displays the genetic relationship among populations of the three subspecies. Nei's 1978 unbiased genetic identity method (modified from the NEIGHBOR procedure of PHYLIP version 3.5c) was used to generate the UPGMA phenogram because it is best suited for use with data sets containing small sample sizes. The threshold for missing data was set at 0.05%.

Results

Morphological Traits and Variation Among Subspecies

After sequential Bonferonni correction (Rice 1989): three of the five morphological characters were found to be significantly different among the three subspecies: glume length ($F_{2,381}$= 369.41, p = 0.0003), glume angle ($F_{2,381}$ = 389.67, p = 0.0003), and palea length ($F_{2,381}$ = 339.28, p = 0.0003). Conical cell prominence (X^2 = 7.2527,d.f. = 2, p = 0.0532) and lemma surface hair locations (X^2 = 1.2403, d.f. = 2, p = 0.5379) were found to be non-significant among the three subspecies. Values for the statistically significant morphological characters for each of the three subspecies are given in Table 1.3. In general, subspecies *asperum* has moderately short glumes (mean = 28.3 mm), a mean glume angle of 63.8° and a palea length of 8.4 mm. Subspecies *caput-medusae* has the longest glumes (mean = 49.8 mm), an obtuse glume angle (mean =

122.5°) and a palea length of 8.3 mm. Subspecies *crinitum* has the shortest glumes (mean = 21.5 mm), an acute glume angle (mean = 36.8°) and the longest palea length (mean 11.1 mm) (Table 1.3). Results of the Student-Neuman-Keuls test reveal that the means for glume length and glume angle are significantly different among all three subspecies (Figures 1.2a and 1.2b); whereas only subspecies *crinitum* is significantly different for palea length (Figure 1.2c).

Three dimensional scatter plots were created with SAS™ (SAS Institute 2002) as graphical representations of the morphological variation found among the 81 populations and 385 individuals measured in this study (Figures 1.3a and 1.3b, respectively). At the population level, three distinct clouds of data points are apparent and clearly differentiate each of the three subspecies (Figure 1.3a). Conversely, some overlap among individuals associated with the three subspecies can be observed in Figure 1.3b. For instance, subspecies *asperum* and subspecies *caput-medusae* show overlap with regard to palea and glume length while subspecies *asperum* and subspecies *crinitum* show overlap for glume angle. No overlap in morphological characters can be seen between subspecies *caput-medusae* and subspecies *crinitum*.

Genetic Diversity

Estimates of genetic diversity and structure of native range *T. caput-medusae* are based on the analysis of 80 populations, two of these populations contained two different subspecies: Villaviciosa de Cordoba, Spain (subspecies *asperum* and *caput-medusae*) and Guzelkonak, Turkey (subspecies *asperum* and *crinitum*). Thus, individuals from each of these two populations were separated and assigned to the appropriate subspecies, for a total of 82 "populations" in the genetic analysis (~ 29 individuals per populations) (see

Figure 1.4 and Table 1.4). The 15 enzymes were coded for by 23 putative loci and across all three subspecies 16 of the 23 (70%) loci were polymorphic: *6Pgd-2, Adh, Ce-2, Ce-4, G3pdh-2, Gdh, Got-1, Got-2, Idh, Mdh-1, Mdh-2, Mdh-3, Pgi-2, Pgm-2, Skdh, Tpi-2.* Across the 82 "populations" analyzed, a total of 50 alleles were detected (2.17 alleles/locus) for all 23 loci. Each polymorphic locus has between 2 and 5 alleles. Three loci, *Ce-2, Mdh-3* and *Pgi-2,* had five alleles. Among all individuals analyzed, only two (one each in the Iran 1 population and Afghanistan 5 population) were found to be heterozygous at any of the 23 loci examined. Just over half (44 of 82, 53.7%) of the populations examined were genetically polymorphic with the remaining 37 populations being monomorphic across all loci (Table 1.4).

Subspecies *asperum* showed the highest amount of genetic diversity with 48 of the 50 alleles detected (2.09 alleles per locus) and 15 polymorphic loci (65.2% polymorphic loci) (Table 1.4). Subspecies *asperum* also had the highest expected mean heterozygosity values of any of the three subspecies (H_{exp} = 0.1408), and 23 of 34 (67.6%) subspecies *asperum* populations were polymorphic. Subspecies *caput-medusae* generally had intermediate values for genetic diversity parameters; whereas subspecies *crinitum* had the lowest values. For subspecies *caput-medusae,* 36 alleles (1.57 alleles per locus) and 10 polymorphic loci (43.5%) were detected. Half of the subspecies *caput-medusae* populations were polymorphic. Subspecies *crinitum* had 33 alleles (1.43 alleles per locus), nine polymorphic loci (39.3 %), and 11 of 28 (39.3%) populations were polymorphic.

Multilocus Genotypes

The program ARLEQUIN was used to identify all multilocus genotypes (MLGs)

across and within the three subspecies of medusahead. A total of 93 MLGs were detected across the three subspecies, with subspecies *asperum* having over two-thirds of these genotypes (66 out of 93) (Table 1.5). Subspecies *caput-medusae* had 22 MLGs and subspecies *crinitum* had 11 MLGs. Six MLGs were shared between subspecies *asperum* and subspecies *caput-medusae*, and neither subspecies shared MLGs with subspecies *crinitum*.

Genetic Relationships Among Subspecies of *Taeniatherum caput-medusae*

The UPGMA cluster diagram based on Nei's (1978) unbiased genetic identity values provides a graphic representation of the genetic relationships found among the three subspecies (Figure 1.4). Four distinct clusters of populations are apparent in the phenogram. Clusters 1 and 4 include 24 of the 34 (70.6%) populations of subspecies *asperum* included in this analysis. Cluster 2 includes all 20 populations of subspecies *caput-medusae* and eight populations of subspecies *asperum*, with these eight populations of subspecies *asperum* sampled from either Spain or Morocco. Cluster 3 contains all populations of subspecies *crinitum* and two populations of subspecies *asperum* (Orosei, Sardinia, Italy and Iran 1). Cluster 4 is composed of the three populations from the Italian mainland and three populations from Morocco.

Analysis of molecular variance (AMOVA, Table 1.6) reveals that 92.6% of the total genetic diversity for *T. caput-medusae* is partitioned among subspecies (48.38%) and among populations within subspecies (44.22%). The high level of genetic diversity partitioned among populations within subspecies is due to the high level of genetic differentiation among populations of all three subspecies especially subspecies *asperum*. Only 7.39 % of the total genetic diversity is partitioned within-populations and within-

individuals, indicating that populations possess little genetic diversity and individuals exhibit very low levels of heterozygosity.

Discussion

Taeniatherum caput-medusae exhibits taxonomic complexity in its native range. This study utilizes morphological characters and genetic data to gain a better understanding of this complexity and determine the level of differentiation among populations of the three subspecies of medusahead. These analyses will allow precise and valid comparisons of native and invasive populations. The morphological characters used to differentiate the three subspecies show overlap among traits for the subspecies but appear robust. The assessment of genetic differentiation among the subspecies shows some intermixing between subspecies, but generally reflects the relationships observed in the morphological analysis.

Morphological Trait Variation Among Subspecies

This study reveals that three morphological traits may be used to differentiate between the three subspecies of medusahead. The traits glume angle and glume length are significantly different among all three subspecies (Figure 1.2 b-c). Thus, these two characters are useful for subspecies differentiation. Palea length shows overlap between subspecies *asperum* and subspecies *caput-medusae* and was unable to differentiate between the two, but this trait clearly distinguishes these two subspecies from subspecies *crinitum* (Figure 1.2c). Variability among the three subspecies for palea length reflects the larger overall seed size associated with subspecies *crinitum* (Frederiksen 1986),

compared with the other two subspecies. While all three morphological traits, in combination, are effective in distinguishing the three subspecies of medusahead, some morphological overlap between individuals of subspecies *asperum* and *caput-medusae* and between individuals of subspecies *asperum* and subspecies *crinitum* does occur (see below).

Frederiksen (1986) found the prominence of conical cells and the density and location of lemma surface hairs to be diagnostic between the three subspecies. In my study, I did not find these characters to be diagnostic for distinguishing the three subspecies. When comparing my findings with that of Frederiksen (1986), some methodological differences may explain this discrepancy. First, Frederiksen (1986) used a scanning electron microscope (SEM) to analyze the prominence of conical cells and density and location of hairs found on the lemma surface, whereas I used a standard light microscope. Perhaps differences in magnification contributed to this discrepancy. Second, differences in sample preparations may also explain these different outcomes. Frederiksen (1986) conducted her study with herbarium specimens, while the measurements reported in the current study were made on plants grown in a greenhouse common garden. Finally, Frederiksen (1986) appears to have made her observations on a single individual per locality; whereas, I measured an average of 4.75 individuals per population (range = 2-6 individuals per population). Perhaps, my larger sample size per population increased the population-level variability detected for these traits.

The three dimensional scatter plots reveal different patterns at the population and individual levels. At the population level, three distinct groups of symbols, each associated with one of the three subspecies, can clearly be seen (Figure 1.3a). Because it

is based on the mean values of these three traits for individuals in each population (thus ignoring trait variation among individuals), this scatter plot better illustrates the morphological differentiation among the subspecies. The distinctiveness of these three groups, further demonstrates the usefulness of the morphological traits described by Frederiksen (1986). This result is not surprising, as characters associated with the spike or spikelet have repeatedly been shown to be diagnostic for members of the Triticeae (Dewey 1979; Baum and Bailey 1990; Murphy 2003; Frederiksen and Peterson 1997; Cabi and Dogan 2010. For example, Barkworth et al. (2009) evaluated 61 characters for their ability to assign herbarium specimens to one of 13 genera in the Triticeae. They found that specimens could almost always be identified to one of the genera using a single spike. At the species level, Kharazian and Rahiminejad (2005) showed that species within *Triticum* (Triticaeae) could be differentiated using two characters: the awns of the uppermost spikelet and the form of the glume.

The use of morphological characters, particularly those associated with spikes, panicles and/or caryopses (seeds) have widespread utility and are often diagnostic for species and subspecies identification within the Poaceae. For example, Saltonstall *et al*. (2004) used a combination of morphological traits (ligule length, lower glume length, upper glume length, and lemma length) and chloroplast DNA haplotype data to identify a new subspecies of *Phragmites* (Poaceae) that is native throughout much of North America.

Morphological overlap among some individuals belonging to these subspecies can be seen (Figure 1.3b), although this overlap occurs only among some individuals of subspecies *asperum* and subspecies *crinitum* and between subspecies *asperum* and

subspecies *caput-medusae*. Morphological overlap among certain individuals is not surprising considering that these three subspecies have been shown to be quite variable and intermediate morphological forms have been reported (Frederiksen 1986; Frederiksen and von Bothmer 1986). In addition, overlap occurs among individuals from populations in localities where the geographic distribution of subspecies also overlaps (e.g., Morocco, Spain, and France for subspecies *asperum* and *caput-medusae* and Sardinia and Sicily for subspecies *asperum* and *crinitum*). These morphological data suggest that hybridization may be taking place in areas where the distribution of the subspecies overlaps. Hybridization among the subspecies has previously been reported, although crossing experiments among subspecies produced hybrids of low fertility (Frederiksen 1986; Frederiksen and von Bothmer 1986). Depicting trait measurements at the individual level clearly blurs the morphological boundaries among some individuals of the three subspecies, however these results may signal important events (e.g., hybridization) that have occurred in the evolutionary history of populations of medusahead in regions where they co-occur.

The goal of this phase of my research was to better understand the taxonomic complexity of medusahead in its native range through an assessment of the morphological variation of the three subspecies. This work was not designed as a quantitative genetic study. Plants grown in the greenhouse common garden were derived from field-collected seeds, and I did not perform a preliminary grow-out in the greenhouse environment to purge maternal (environment) effects (Schaal 1984, Roach and Wulff 1987, Falconer and Mackay 1996). Maternal effects may contribute to some of the morphological trait variation and overlap observed among individuals of the three

subspecies included in this analysis. For example, seed size has been shown to be influenced by maternal effects, and seed size differences have an effect on germination characteristics, seedling size, and adult plant size (Roach and Wulff 1987). Thus, some of the morphological traits measured in this experiment may have been influenced by maternal carryover effect, which is possible even for plants grown in a greenhouse common garden. The influence of maternal effects on morphological trait variation in medusahead can only be resolved by future studies that utilize a quantitative genetic approach.

Genetic Diversity and Differentiation

Across all three subspecies (Table 4), allozyme diversity in medusahead (69.6% polymorphic loci and 2.17 alleles per locus) is higher than the mean values (50.5% polymorphic loci and 1.96 alleles per locus) reported for 473 plant species (Hamrick and Godt 1989). The level of allozyme diversity in subspecies *asperum* (65.2% polymorphic loci and 2.09 alleles per locus) is also higher than the mean values for all plant species reported by Hamrick and Godt (1989), whereas allozyme diversity across populations of subspecies *caput-medusae* and subspecies *crinitum* is lower than the mean values reported for all plants. In addition, allozyme diversity across all populations of subspecies *asperum* is generally higher or similar to the range of values (41.8 - 59.2 percent polymorphic loci and 1.69 – 2.38 alleles per locus) reported for selfing, early successional, annual, and monocot plant species (Hamrick and Godt 1989), while the same does not hold for subspecies *caput-medusae* or subspecies *crinitum*.

Genetic diversity and genetic differentiation vary among the three subspecies of medusahead. Subspecies *asperum* exhibits the greatest amount of allozyme diversity,

while subspecies *crinitum* possesses the least. The high diversity found within subspecies *asperum* may be related to its large geographic range (Frederiksen 1986). Species exhibiting large geographic distributions typically exhibit higher levels of genetic diversity, compared to congeners with smaller distributions (Karron 1989). The lower level of genetic diversity detected for subspecies *crinitum*, compared with the other two subspecies, may be explained, in part, by the type of plant collections mostly used to assess the diversity of this subspecies. Twenty-two (68.8%) of all subspecies *crinitum* population samples were obtained as accessions from the Western Regional Plant Introduction (PI) Station, Pullman, Washington (Table 1.1), and the use of this type of plant material may contribute to an underestimation of genetic diversity in subspecies *crinitum*. This underestimate of genetic diversity may occur because: 1) information about these accession is sparse and the original field collections may only have included a limited number of individuals and thus may not reflect the overall diversity of the sampled populations, 2) each accession has been through an unknown number of grow-outs since they were stored at the PI Station and this may further reduce diversity through genetic drift, and 3) grow-outs were carried out in Pullman, WA and selection for or against certain genotypes in a novel (non-native) environment may further reduce genetic diversity. However, some field-collected populations of subspecies *crinitum* (e.g., Avcilar, Dendril and Seydisehir, Turkey) also exhibit similarly low diversity, and some PI accessions (Uzumluk, Tukey, Afghanistan 4 and Karagali, Kazahkstan) do exhibit higher amounts of genetic diversity (data not shown). Thus, the low genetic diversity detected for subspecies *crinitum*, especially for some populations from eastern Turkey, Iran, and Afghanistan, may reflect a regional pattern, and not be due to factors associated

with how these populations were sampled. Resolving this issue can only be accomplished through the analysis of more populations of subspecies *crinitum* from this region.

Genetic differentiation among the three subspecies is illustrated by the UPGMA cluster diagram (Figure 1.4). Populations of subspecies *asperum* exhibit the highest amount of genetic differentiation as indicated by the occurrence of subspecies *asperum* populations throughout the cluster diagram. For example, Clusters 1 and 4 are exclusively composed of only populations of subspecies *asperum*, and these two clusters are highly diverged. Cluster 4 includes populations from Italy and Morocco, with populations from these two countries exhibiting relatively long branch lengths, suggesting that these populations are relatively well differentiated. Cluster 1 includes populations of subspecies *asperum* that possess genotypes that either match or are very similar to the genotypes detected in populations of medusahead (subspecies *asperum*) from western U.S. A comparison of genetic diversity in these native populations with that of western U.S. populations will provide insights into the geographic origins and genetic consequences (e.g., founder effects) of this invasion (Chapter 2).

Cluster 2 includes all 20 populations of subspecies *caput-medusae* included in this study as well as eight populations of subspecies *asperum* form either Spain or Morocco (Figure 1.4). Several of the populations of subspecies *caput-medusae* and subspecies *asperum* that co-occur in Cluster 2 share six multilocus genotypes (Table 1.5). Thus, genetic results are in agreement with morphological data, and suggest that certain subspecies *asperum* and subspecies *caput-medusae* populations (especially from Spain and Morocco) are morphologically and genetically similar. Taken together, these data

further support the possibility of hybridization occurring between these two subspecies in regions where their distributions overlap.

Cluster 3 primarily consists of populations of subspecies *crinitum* (Figure 1.4). The short branch lengths observed for 21 of the 28 populations of subspecies *crinitum* included in this analysis indicate a low level of genetic differentiation among these populations (Figure 1.4). Two populations of subspecies *asperum* (Orosei, Sardinia, Italy, and Iran 1) also occur in Cluster 3, indicating that both of these populations are genetically more similar to populations of subspecies *crinitum* than they are to other populations of subspecies *asperum*. This is true even though these two populations of subspecies *asperum* do not share any MLGs with populations of subspecies *crinitum* (Table 1.5). The population from Orosei, Sardinia, Italy is located in the UPGMA cluster diagram with the population of subspecies *crinitum* from Lunguaglossa/Mt. Etna, Sicily, Italy. Individuals from Orosei and Lunguaglossa/Mt. Etna exhibit morphological overlap (Figure 1.3b); and Orosei shares more alleles with populations of *crinitum* than it does with other populations of subspecies *asperum* (including other populations from Sardinia). As observed for populations of subspecies *asperum* and subspecies *caput-medusae* from Spain and Morocco, these data suggest that hybridization may be occurring between subspecies *asperum* and subspecies *crinitum* (or at least intermediate forms are present) in regions where their geographic distributions overlap.

The level of genetic differentiation among the three subspecies can be quantified through AMOVA (Table 1.6). Most of the total genetic diversity detected in this analysis is partitioned among the subspecies (48.4%) and among populations within subspecies (44.2%), indicating high genetic structure associated with the three subspecies. This

result is supported by data on the distribution of multilocus genotypes (Table 1.5): only six multilocus genotypes are shared between subspecies *asperum* and *caput-medusae* and the remaining 93 genotypes are found exclusively in one subspecies, or another. Additionally, very little diversity is partitioned within populations (7.2%) and within individuals (0.25%) (Table 1.6). The pattern by which genetic diversity is distributed among and within native subspecies and populations is typical with that reported for highly self-pollinating plant species (Hamrick and Godt 1989, 1996). Indeed, values for mean observed heterozygosity (H_{obs}) reported for the three subspecies (Table 1.4) suggest exceedingly high rates of self-pollination. Moreover, in an analysis of progeny arrays using allozyme genetic markers, Rausch (2004) found that 10 invasive populations of medusahead were 99.8% self-pollinating.

Taxonomic Complexity and Invasions

The timely and accurate identification of introduced species is critical in their management (Wittenberg and Cock 2005). Taxonomic complexity arises when multiple, morphologically similar subspecies have been recognized within a species' native range, and this complexity is accentuated by the presence of intermediate morphological forms among these subspecies. These intermediate forms are likely to occur as a result of hybridization events.

Relationships among the three taxa of *Taeniatherum* were described and revised by Frederiksen (1986). Her analysis of morphological characters and crossing studies (Frederiksen 1986, Frederiksen and von Bothmer 1986) showed an indistinct boundary between the species and thus she recognized three subspecies, rather than the three species originally described by Nevski (1934). The combined analysis of morphological

traits and genetic diversity and differentiation among the three subspecies described here supports the taxonomic revision of Frederiksen (1986). Although crossing experiments among the three subspecies of medusahead produced hybrids with low fertility (Frederiksen 1986, Frederiksen and von Bothmer 1986), results of this study suggest that hybridization among these subspecies occurs in areas where their geographic distribution overlap in the native range.

In conclusion, results of this study indicate that i) the morphological traits associated with spikes and seeds are robust and useful to differentiate subspecies of medusahead, ii) the three subspecies of medusahead show genetic differentiation with a small amount of overlap, and iii) subspecies *asperum*, the subspecies with the broadest geographic distribution in the native range, is the most genetically diverse of the three subspecies. Through this understanding of the taxonomic complexity associated with the three subspecies of medusahead, further analyses comparing native and introduced populations of this species will likely lead to a better understanding of this invasion and how it may be managed.

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Figures and Tables

Table 1.1 Coordinates, date of collection, and analysis information for all populations analyzed in this study. Type of data collected: G indicates population included in the genetic analysis, M for those included in the morphological analysis, and B for populations included in both analyses. *Provided by Signe Frederiksen

Country	Location	Coordinates or Accession Number	Date Collected	Type of Data Collected
Spain	Alarba Village	N 41° 13' 43" W 01° 36' 56'	13-Jun-09	B
	Near Alarba	N 41° 13' 42" W 01° 35' 40"	13-Jun-09	M
	Canamares	N 41° 13' 42" W 02° 57' 27"	13-Jun-09	B
	Pedraza de la Sierra	N 41° 07' 51" W 03° 48' 27"	14-Jun-09	B
	Guadalupe	N 39° 29' 56" W 05° 39' 58"	18-Jun-09	B
	La Aliseda	N 39° 24' 20" W 06° 41' 24"	18-Jun-09	B
	Mijadas	N 39° 10' 20" W 05° 53' 50"	18-Jun-09	M
	Valdelabota	N 38° 58' 43" W 06° 55' 30"	18-Jun-09	M
	Cumbres Mayores	N 38° 03' 16" W 06°37' 25"	19-Jun-09	B
	Quintana	N 38° 41' 55" W 02° 57' 27"	19-Jun-09	M

Country	Location	Coordinates or Accession Number	Date Collected	Type of Data Collected
	Monesterio	N 38° 05' 45" W 06°12' 39"	19-Jun-09	B
Spain	Almonte	N 37° 12' 25" W 06° 30' 15"	21-Jun-09	B
	Villaviciosa de Cordoba (A and C)	N 38°04' 59" W 04° 58' 56"	22-Jun-09	B
	La Carolina	N 38° 19' 31" W 03° 35' 06"	22-Jun-09	B
	Robledillo	N 41° 32' 03" W 04° 56' 49"	14-Sep-09	B
	Castillejo de Martin Viejo	N 40° 41' 47" W 06° 39' 36"	15-Sep-09	B
Portugal	Freixo de Espada	N 41° 04' 38" W 06° 48' 41"	15-Jun-09	B
	Castelo Branco	N 39° 52' 00" W 07° 31' 42"	17-Jun-09	B
	Arronches	N 39° 09' 30" W 07° 19' 23"	17-Jun-09	B
	Juromenha	N 39° 43' 56" W 07° 17' 56"	17-Jun-09	B
	Torre de Moncorvo	N 41° 12' 18" W 06° 48' 41"	15-May-09	B
France	Rebourguil	N 43° 52' 20" E 02° 46' 43"	3-Jul-02	B
	Pezenas	N 43° 30' 02"	9-Jul-02	B

Country	Location	Coordinates or Accession Number	Date Collected	Type of Data Collected
		E 03° 30' 00"		
France	Aire de Beziers-Montblanc	N 43° 21' 41" E 03° 21' 11"	28-Jun-07	B
	Pezenas les Mines	N 43° 36' 11" E 03° 15' 45"	29-Jun-04	B
	Miramas	N 43° 37' 42" E 05° 01' 05"	29-Jun-09	B
	San Martin Plaine de la Crau	N 43° 34' 35" E 04° 46' 53"	29-Jun-09	B
	Le Cannet de Maures	N 43° 23' 00" E 06° 21' 34"	30-Jun-09	B
	Le Cannet de Maures (2)	N 43° 22' 33" E 06° 19' 59"	17-Jul-09	M
	Caux	N 43° 30' 02" E 23° 30' 00"	9-Aug-02	M
	Caux (2)	N 43 29' 44" E 03 23' 18"	17-Jul-09	B
	La Gardiole	N 47° 24' 25" E 23° 30' 00"	17-Jul-09	B
	Murviel-les-Montpellier	N 43° 36' 13" E 03° 45' 05"	17-Jul-09	M
Italy	Altamura	N 40° 56' 06" E 16° 30' 03"	3-Jul-09	B

Country	Location	Coordinates or Accession Number	Date Collected	Type of Data Collected
	Minervino Murge	N 41° 02' 43" E 16° 10' 57"	4-Jul-09	B
Italy	Poggorsini	N 40° 58' 35" N 40° 58' 35"	4-Jul-09	B
	Lodine, Sardinia	N 40° 09' 45" E 09°14' 10"	16-Sep-09	B
	Dorgali, Sardinia	N 40° 18' 18" E 09° 34' 18"	17-Sep-09	B
	Orosei, Sardinia	N 40° 23' 49" E 09° 43' 06"	17-Sep-09	B
	Lunguaglossa/Mt. Etna, Sicily	N 37° 50' 21" E 15° 06' 38"	3-Jun-07	B
Greece	Katharos, Crete	N 35° 09' 01" E 25° 33' 20"	8-Sep-05	B
	Sterea Hellas*	_	28-Jun-07	B
	Komotini	N 41° 05' 14" E 25° 44' 30"	6-Oct-10	G
	Xanthi	N 41° 00' 34" E 25° 10' 56"	6-Oct-10	G
	Askos/Filadelphio	N 40° 45' 27" E 23° 27' 11"	7-Oct-10	G
	Panorama	N 40° 35' 19" E 23° 02' 48"	7-Oct-10	G

Country	Location	Coordinates or Accession Number	Date Collected	Type of Data Collected
Morocco	Timahdite	N 33° 17' 02" W 05° 04' 33'	1-Oct-04	B
Morocco	Tizi n' tishka	N 31° 14' 14" W 07° 24' 51"	4-Oct-04	B
	Tizi n' test	N 30° 54' 59" W 08° 17' 34"	5-Oct-04	B
	Tafraoute	N 29° 44' 16" W 08° 50' 04"	6-Oct-04	B
	Tleta tassrit	N 29° 36' 59" W 08° 55' 24"	7-Oct-04	B
Turkey	Cat	N 39° 34' 56" E 40° 54' 13"	12-Sep-04	B
	Avcilar	N 39° 40' 58" E 39° 39' 48"	13-Sep-04	B
	Near Hafik	N 39° 51' 50" E 37° 37' 36"	14-Sep-04	B
	Dendril	N 39° 18' 34" E 35° 58' 49"	14-Sep-04	B
	Balikesir	N 39° 23' 35" E 27° 26' 27"	23-Jun-05	B
	Sarigol	N 38° 13' 26" E 28° 40' 03"	23-Jun-05	B
	Pamukkale	N 37° 56' 21" E 29° 08' 12"	24-Jun-05	B

Country	Location	Coordinates or Accession Number	Date Collected	Type of Data Collected
	Seydisehir	N 37° 29' 04" E 31° 49' 00"	25-Jun-05	B
Turkey	Karaman	N 37° 18' 26" E 33° 32' 18"	25-Jun-05	B
	Avanos	N 38° 41' 38" E 34° 50' 34"	27-Jun-05	B
	Kalecik	N 40° 02' 15" E 33° 26' 38"	28-May-05	B
	Havsa	N 41° 24' 05" E 26° 48' 41"	6-Oct-10	G
	Ipsala	N 40° 52' 27" E 26° 25' 10"	6-Oct-10	G
	Uzunkopru	N 41° 04' 10" E 26° 38' 21"	6-Oct-10	G
	Biloris	PI 561091	n/a	B
	Eruh	PI 561092	n/a	B
	Buldan Junction	PI 598389	n/a	B
	Aliaga	PI 577708	n/a	B
	Dakili Junction	PI 577709	n/a	B
	Guzelkonak (A and C)	PI 561093	n/a	B
	Tatvan	PI 561095	n/a	B
	Yeksekova	PI 561108	n/a	B

Country	Location	Coordinates or Accession Number	Date Collected	Type of Data Collected
	Uzumluk	PI 561109	n/a	B
	Pesan Stream	PI 577710	n/a	B
Turkey	Kars	PI 208075	n/a	G
	Zap River	PI 561094	n/a	B
Afghanistan	Oheh	PI 317476	n/a	B
	Sufed koh	PI 317475	n/a	B
	3	PI 220589	n/a	B
	4	PI 220590	n/a	B
	5	PI 220591	n/a	B
	6	PI 220592	n/a	B
	7	PI 222048	n/a	B
Kazahkstsan	Karagali	PI 314697	n/a	B
Iran	1	PI 227665	n/a	B
	2	PI 251387	n/a	B

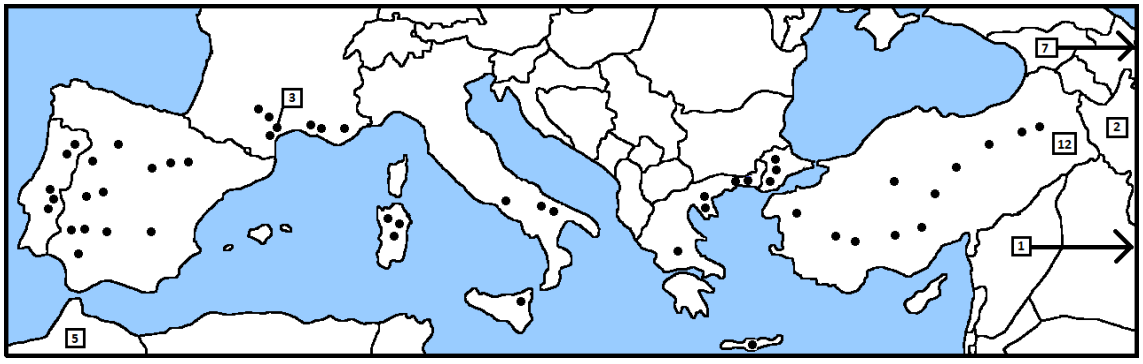

Figure 1.1 Map of 80 *Taeniatherum caput-medusae* native populations analyzed in genetic portion of this study

Table 1.2 Character traits measured to assess the morphological variation among among subspecies of *Taeniatherum caput-medusae* as described in Frederiksen 1986.

	ssp. *asperum*	ssp. *crinitum*	ssp. *caput-medusae*
Glume length	1.5 - 4.0 cm	1.5 - 3.5 cm	3.5 - 8.0 cm
Glume angle	Curved	Erect	Horizontal or reflexed downward
Palea length	5.0 - 9.5 mm	10.0 - 13.5 mm	5.0 - 8.5 mm
Lemma surface: hairs	Scabrous	Glabrous	Glabrous
Lemma surface: conical cells	Many prominent conical cells	Without prominent conical cells	Without prominent conical cells

Table 1.3 Summary statistics for the significant morphological variables for each subspecies of *Taeniatherum caput-medusae*. n is the sample size. Glume length and palea length are measured in millimeters. Glume angle is measured in degrees.

ssp. *asperum* (n=124)	Glume Length	Glume Angle	Palea Length
Mean	28.31	63.87	8.42
Median	27.50	65.00	8.00
Standard Deviation	7.43	19.94	0.94
Sample Variance	55.21	397.42	0.88
Minimum	15	21	7
Maximum	50	112	11

ssp. *caput-medusae* (n= 134)	Glume Length	Glume Angle	Palea Length
Mean	49.81	122.48	8.29
Median	49.00	129.50	8.00
Standard Deviation	12.87	31.14	0.65
Sample Variance	165.69	969.48	0.43
Minimum	22	40	7
Maximum	90	170	10

ssp. *crinitum* (n=124)	Glume Length	Glume Angle	Palea Length
Mean	21.54	36.83	11.08
Median	21.00	36.00	11.00
Standard Deviation	4.45	14.77	1.23
Sample Variance	19.78	218.04	1.51
Minimum	10	13	9
Maximum	31	74	15

a. Glume Length

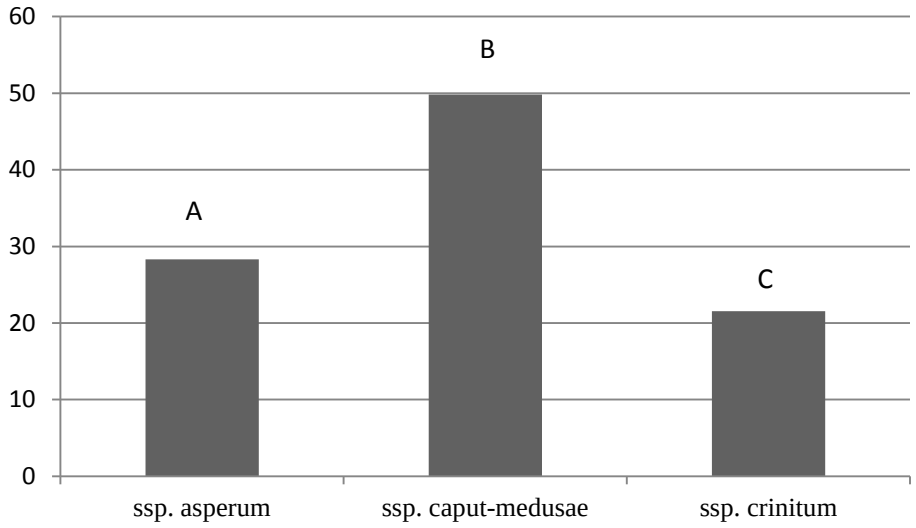

Figure 1.2a Histograms showing means and significant differences among subspecies of *Taeniatherum caput-medusae*. Letters above bars denote SKN groupings for significantly different means. All lengths are measured in millimeters, angles are measure in degrees.

b. Glume Angle

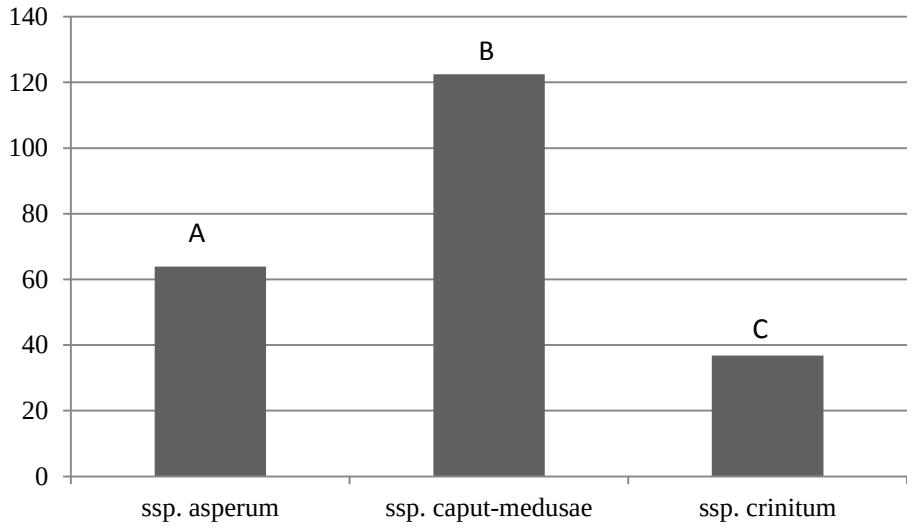

Figure 1.2b Histograms showing means and significant differences among subspecies of *Taeniatherum caput-medusae*. Letters above bars denote SKN groupings for significantly different means. All lengths are measured in millimeters, angles are measure in degrees.

c. Palea Length

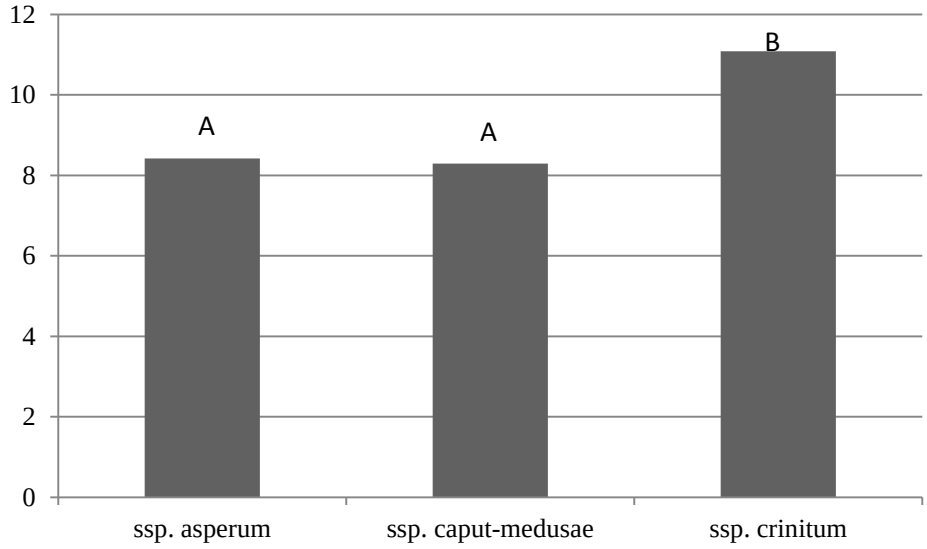

Figure 1.2c Histograms showing means and significant differences among subspecies of *Taeniatherum caput-medusae*. Letters above bars denote SKN groupings for significantly different means. All lengths are measured in millimeters, angles are measure in degrees.

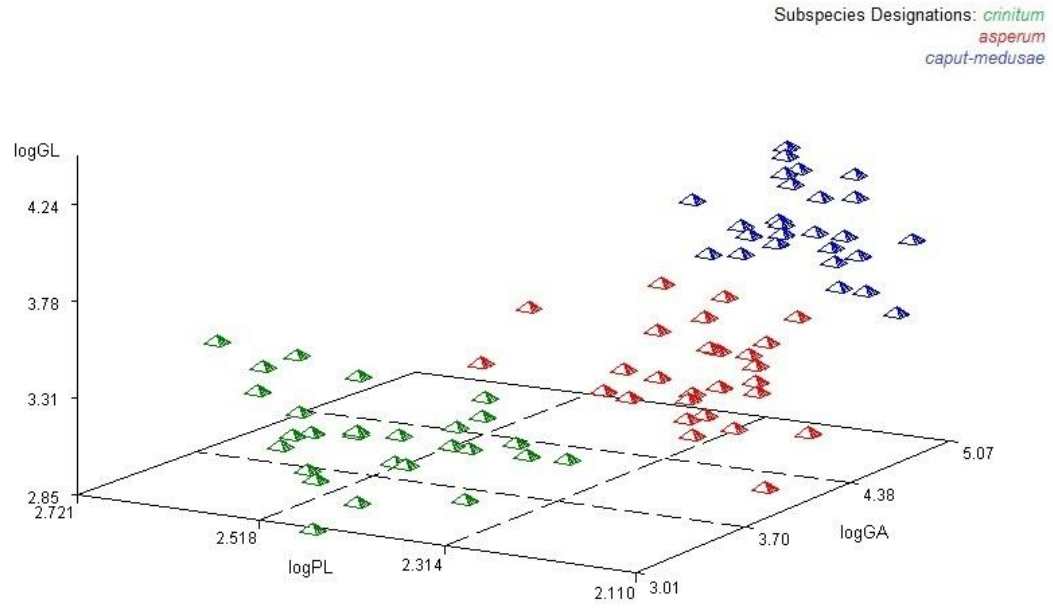

Figure 1.3a Three dimensional scatter plot of the mean values for the significant morphological traits measure for 81 populations of *Taeniatherum caput-medusae*

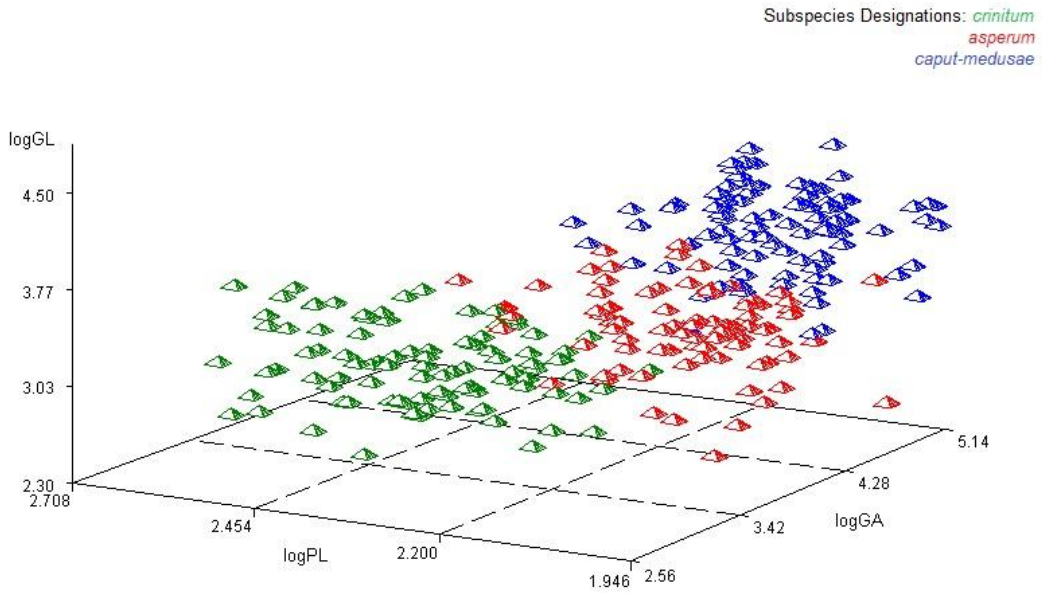

Figure 1.3b Three dimensional scatter plot of the significant morphological traits measured for 384 individuals of *Taeniatherum caput-medusae*

Table 1.4 Genetic diversity within and among the three subspecies of *Taeniatherum caput-medusae*

	ssp. *asperum* (n=34)	*ssp. caput-medusae* (n=20)	*ssp. crinitum* (n=28)	Overall
# Alleles	48	36	33	50
Alleles/Locus	2.09	1.57	1.43	2.17
# Polymorphic Loci	15	10	9	16
%Polymorphic Loci	65.22%	43.48%	39.13%	69.57%
%Polymorphic Populations	67.64%	50.00%	39.29%	53.66%
Nei's Expected Mean Heterozygosity	0.1408	0.0725	0.0258	0.1314
Mean Observed Heterozygosity	0.00003	0.00000	0.00003	0.00002
F_{ST}	0.8423	0.8663	0.8285	0.9081
N_M	0.0468	0.0386	0.0518	0.0253
# of Multilocus Genotypes	66	22	11	93

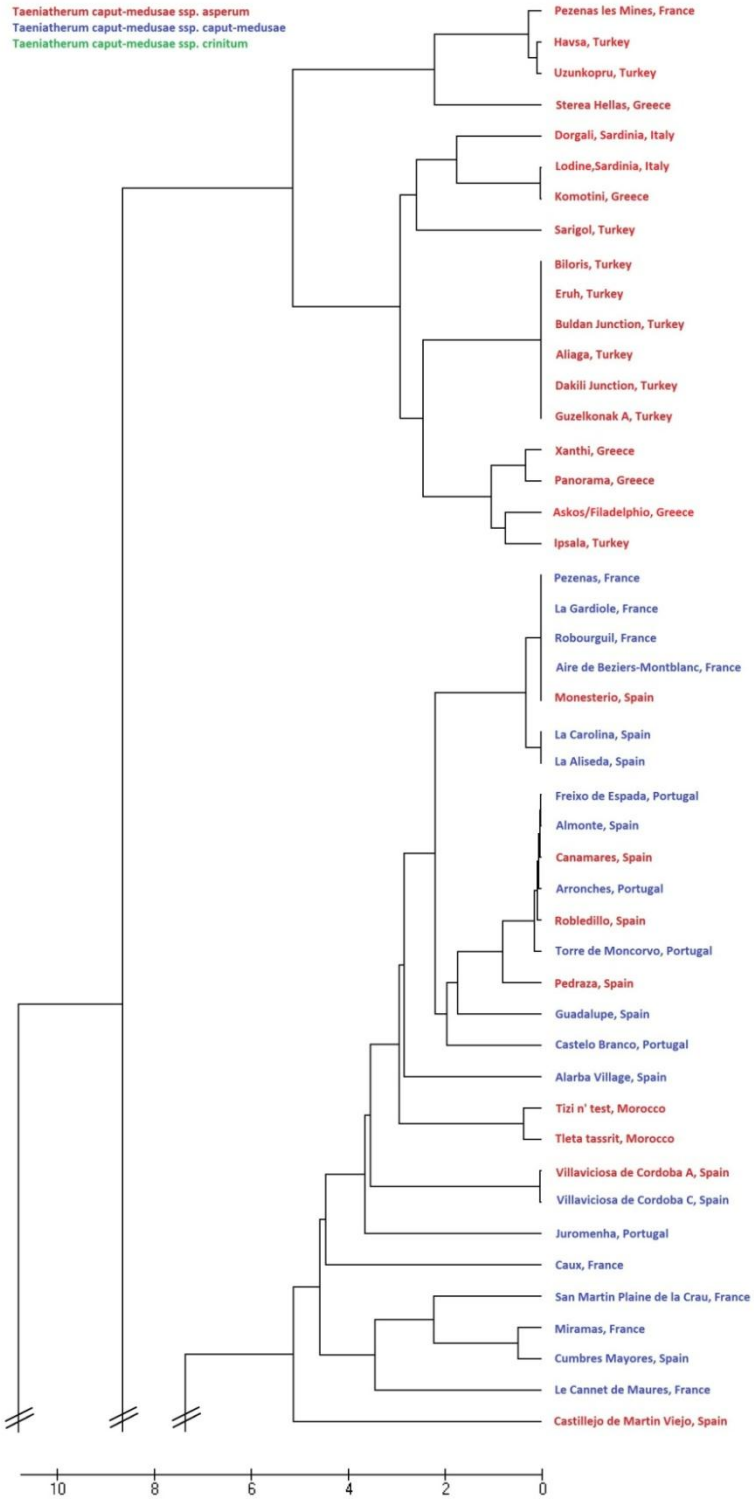

Figure 1.4a UPGMA cluster diagram of 80 native populations of *Taeniatherum caput-medusae*. Note: population samples from Villaviciosa de Cordoba, Spain and Guzelkonak, Turkey were each divided into 2 subspecies represented by an 'A' for subspecies *asperum* and a 'C' for subspecies *crinitum* thus the genetic relationships among 82 "populations" are shown.

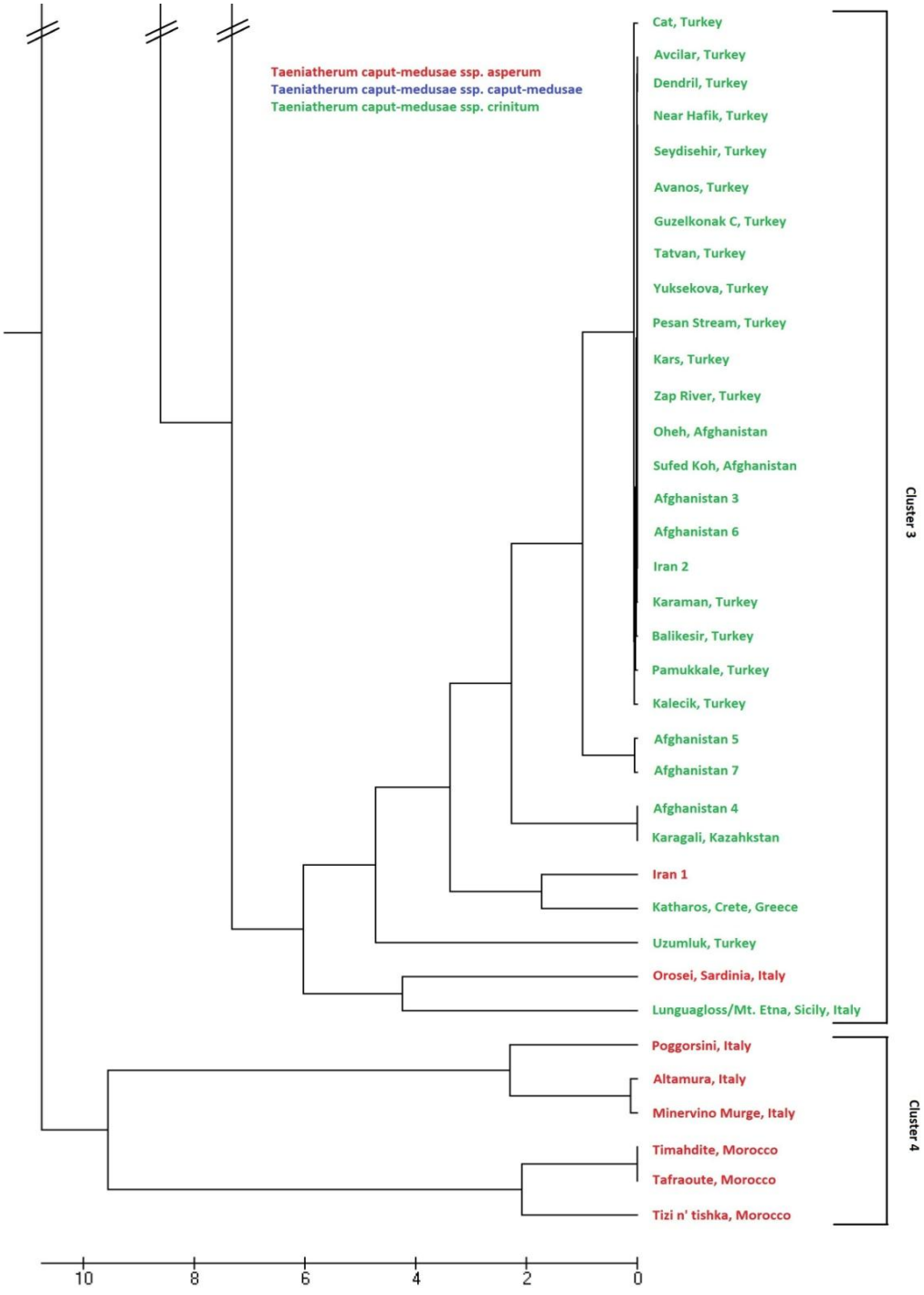

Figure 1.4b (continued) UPGMA cluster diagram of 80 native populations of *Taeniatherum caput-medusae*. Note: population samples from Villaviciosa de Cordoba, Spain and Guzelkonak, Turkey were each divided into 2 subspecies represented by an 'A' for subspecies asperum and a 'C' for subspecies crinitum thus the genetic relationships among 82 "populations" are shown.

Table 1.5 Multilocus genotypes detected in each of the three subspecies of *Taeniatherum caput-medusae*. See text for the order of loci used to generate multilocus genotypes. Letters represent different alleles at each of these loci.

Subspecies	ID	Multilocus Genotype
asperum	1	BCABABAAAAABBBBAADABABB
	2	BCABABAAAAAAABDAABAABBB
	3	BCABABAAAAAAABDAAAAABBB
	4	BCABABAAAAAAABDAABABBBB
	5	BAABABAAAAABBBBAACABBBB
	6	BCABABAAAAAABBDAAAABBBB
	7	BCABABAAAAAABBDAABABBBB
	8	BAABABAAAAABBBBAACABABB
	9	BAABABAAAAABBBAAACABABB
	10	BAABABAAAAAABBDAACABABB
	11	BCABABAAAAAABBDAABAABBB
	12	BCABABAAAAAABBBAADABBBA
	13	BCAAABAAAAAABBBAADABBBA
	14	BCAAABAAAAAABBBAADABBBB
	15	BCABABAAAAAABBBAADABBBB

Subspecies	ID	Multilocus Genotype
asperum	16	BCAAABAAAAAABACAADABBBB
	17	BCAAABAAAAAABBCAADABBBB
	18	BCAAABAAAAAABBCAADABBBA
	19	BCABABAAAAAABBCAADABBBA
	20	BCABABAAAAAABACAADABBBB
	21	BCABABAAAAAABACAAEABABB
	22	BCAAABAAAAAABACAADABBBA
	23	BCAAABAAAAAABBCAADABABA
	24	BCAAABAAAAAABACAAEABBBA
	25	BCABABAAAAAABACAAEABBBA
	26	BCAAABAAAAAABBCAADABABB
	27	BCACABAAAAAABBCAADABABB
	28	BCACABAAAAAABBCAAEABABB
	29	BCBBABAAAAAABBCAAEABABB
	30	BCBBAAAAAAAABBCAAEABABB
	31	BCBBAAAAAAAABBCAADABABB
	32	BCBBABAAAAAABBCAAEBBABB
	33	BCBBAAAAAAAABBCAAEBBABB

Subspecies	ID	Multilocus Genotype
asperum	34	BCABABAAAAAABBAAAEABABB
	35	BCAAABAAAAAABBEAAEABABB
	36	BCABAAAAAAAABBEAAEABABB
	37	BCACAAAAAAAABBCAAEABABB
	38	BEBBABAAAAAABBBAACABABB
	39	BCABABAAAAABBBBAACABABB
	40	BAABABAAAAABBBBAACAAABB
	41	BCABABAAAAAABBBAAAAAABB
	42	BDABABAAAAABBBBAACABABB
	43	BCABABAAAAAABABAAAABABA
	44	BDABABAAAAABBABAACABABB
	45	BDABABAAAAABBABAACABABA
	46	BCABABAAAAABBABAACABABB
	47	BCABABAAAAAABABAAAABABB
	48	BCBBABAAAAABBBBAADABABB
	49	BAABABAAAAAABBBAACABABB
	50	BCABABAAAAAABBAAADABABB
	51	BCABABAAAAAABCAAADABABB

Subspecies	ID	Multilocus Genotype
asperum	52	BAABABAAAAAABBBAADABABB
	53	BAABABAAAAABBBBAADABABB
	54	BAABABAAAAABBBBAAAABABB
	55	BCBBABAAAAAABBBAADABABB
	56	BDABABAAAAAABBBAADABABB
	57	BDABABAAAAABBBBAADABABB
	58	BAABABAAAAABBBBAACBBABB
	59	BDBBABAAAAABBBBAACABABB
	60	BDBBABAAAAABBBBAADABABB
	61	BCABABAAAAAABBBAAEABABB*
	62	BCABABAAAAAABBCAAEABABB*
	63	BCABAAAAAAAABBCAAEABABB*
	64	BCABAAAAAAAABBCAADABABB*
	65	BCABABAAAAAABBBAADABABB*
	66	BCABABAAAAAABBCAADABABB*
caput-medusae	1	BCABAAAAAAAABBDAACABABB
	2	BBABAAAAAAAABBDAACABABB

Subspecies	ID	Multilocus Genotype
caput-medusae	3	BCABABAAAAAABBDAADABABB
	4	BCABABAAAAAABADAADABABB
	5	BBABABAAAAAABADAADABABB
	6	BCABABAAAAAABADAACABABB
	7	BCABABAAAAABBBDAAEABABB
	8	BCABABAAAAAABBCAADABABA
	9	BCABAAAAAAAABBDAADABABB
	10	BCABABAAAAAABBDAAEABABB
	11	BCABAAAAAAAABBDAAEABABB
	12	BCABABAAAAAABBCAAEBBABB
	13	BCABABAAAAAABBCAAEABABA
	14	BEABABAAAAAABBCAAEABABB
	15	BCABABAAAAAABBCAAEABBBB
	16	ACABABAAAAAABBCAAEABABB
	17	BCABABAAAAAABBBAAEABABB*
	18	BCABABAAAAAABBCAAEABABB*
	19	BCABAAAAAAAABBCAAEABABB*
	20	BCABAAAAAAAABBCAADABABB*

Subspecies	ID	Multilocus Genotype
caput-medusae	21	BCABABAAAAAABBBAADABABB*
	22	BCABABAAAAAABBCAADABABB*
crinitum	1	BCABABAAAAAABAAAADABABB
	2	BCABABAAAAAABAAAADABAAB
	3	BCABABAAAAAABBBAAAABABB
	4	BCABABAAAAAABAAAAAABABB
	5	BCABABAABAAABAAAAAABABB
	6	ACABABAAAAAABAAAAAABABB
	7	BCABABAAAAAABAAAACABABB
	8	BCABABAAAAAAAAAAAAABABB
	9	BCABABAAAAAABAAAAAABABA
	10	BCABABAAAAAABBAAACABABB
	11	BBABABAAAAAABAAAAAABABB

* Multilocus genotypes shared between ssp. asperum and ssp. caput-medusae

Table 1.6 Analysis of Molecular Variance (AMOVA) using the pairwise differences distance method for the 82 native "populations" of *Taeniatherum caput-medusae*

	d.f	Sum of Squares	Variation Component	Percentage Variation
Among subspecies	2	2561.552	0.86506	48.38
Among populations within subspecies	75	3455.088	0.79073	44.22
Among individuals within populations	2194	571.854	0.12845	7.18
Within individuals	2272	8.500	0.00374	0.21
Total	4543	6686.994	1.78799	--

CHAPTER 2: COMPARISON OF NATIVE AND INVASIVE POPULATIONS OF *TAENIATHERUM CAPUT-MEDUSAE* SSP. *ASPERUM* (MEDUSAHEAD): GEOGRAPHIC ORGINS, EVIDENCE FOR MULTIPLE INTRODUCTIONS, AND FOUNDER EFFECTS

Abstract

The native range of *Taeniatherum caput-medusae* includes much of Eurasia, where three distinct subspecies have been recognized, but only *T. caput medusae* ssp. *asperum* (hereafter referred to as medusahead) is believed to occur in the United States (U.S.). Medusahead, a primarily self-pollinating annual grass, was introduced into western U.S. in the late 1800s. The results of an earlier allozyme analysis were consistent with the genetic signature associated with multiple introductions, although this finding can only be confirmed with the analysis of native populations. I compared allozyme diversity in native and invasive populations of medusahead to: identify the geographic origins of the U.S. invasion, test the multiple introduction hypothesis, and determine the genetic consequences of these events. Thirty-four native populations of medusahead were analyzed in this study, using enzyme electrophoresis. Five of the seven homozygous multilocus genotypes previously observed in the western U.S. have been detected in native populations. These results provide support for the multiple introduction hypothesis. The geographic origins of these introductions appear to have been drawn from France, Sardinia, Greece, and Turkey, although additional analyses are needed. Across native populations, 17 of 23 loci were polymorphic and a total of 48 alleles were

detected, while only five polymorphic loci and 28 alleles were found among invasive populations. On average, invasive populations possess reduced within-population genetic diversity, compared with those from the native range. While U.S. populations have experienced founder effects, 38% (17 of 45) these populations appear to be genetic admixtures (consisting of two or more native genotypes). Results of this study have implications for the biological control of medusahead: i) the search for effective and specific biological control agents will have to occur broadly across the species' native range, ii) multiple agents may be required to control invasive populations that are admixtures, and iii) because many invasive populations are genetically depauperate, highly adapted biocontrol agents are likely to be quite effective.

Keywords: invasive grass, founder event, genetic diversity, multilocus genotype, propagule pressure

Introduction

Biological invasions are now considered to be one of the leading drivers of global change (Vitousek et al. 1996, 1997). Invasions occur when species are introduced to a new range, where they persist, proliferate, and spread beyond their initial points of introduction (Mack et al. 2000; Colautti and MacIsaac 2004; Keller and Taylor 2008). Humans have been transporting species to new regions for hundreds of years through exploration, colonization, international trade, and commerce, and these events have contributed greatly to the introduction of organisms around the globe (Crosby 2003, Mack et al. 2000; Bossdorf et al. 2005). Invasions often have negative ecological consequences including loss of native biological diversity and changes in community structure, modification to ecosystem or community processes, and alterations to disturbance regimes (Vitousek and Walker 1989; D'Antonio and Vitousek 1992; Vitousek 1994; Wilcove et al. 1998; Sala et al. 2000; Allendorf and Lundquist 2003). In addition, invaders cause great economic damage including decreases in agricultural productivity, increases in human-health costs and increases in annual costs for management and control programs (Pimentel et al. 2000, 2005). With the number of biological invasions ever increasing, determining how, when, and from where invaders are introduced becomes crucial to gaining a better understanding of the invasion process (Novak 2007; Wilson et al. 2009). Ideally, such information should be included in the management of invasive species, especially in the search for biological control agents within their native ranges (Kolar and Lodge 2001; Ward et al. 2008; Prentis et al. 2009).

According to Lockwood et al. (2005), biological invasions have five steps: an organism is taken up from its native range, transported to a new area, released into the new location, becomes established, and subsequently spreads beyond its initial introduction site. It is interesting to note, however, only a small fraction of the species sampled in their native range will ever become invasive (Williamson 1996; Williamson and Fitter 1996). Various factors have been investigated to better predict the identity and location of invasions. These factors include identifying the intrinsic properties of species that may be associated with invasiveness and the identification of the characteristics of invaded communities (Mack 1996; Rejmanek 2000; Shea and Chesson 2002; Rejmanek et al. 2005; Richardson and Pysek 2006; Didham et al. 2007). Thus far, the search for accurate predictors of invasiveness has proven to be elusive.

More recently, there has been a growing recognition of propagule pressure as a predictor of establishment success and the likelihood of invasion (Kolar and Lodge 2001; Colautti et al. 2009, Lockwood et al. 2005, 2009; Simberloff 2009). Propagule pressure is defined as the number of individuals in a propagule (propagule size), or the number of propagules that arrive in an area per unit time (propagule number), or both (Simberloff 2009). Recent literature has focused on the importance of propagule number (i.e. multiple introductions), and a growing body of evidence suggests that multiple introductions are the rule rather than the exception (Novak and Mack 2001, 2005; Kolbe et al. 2004, 2007, 2008; Wares et al. 2005; Novak 2007; Lavergne and Molofsky 2007; Dlugosch and Parker 2008; Keller and Taylor 2010).

While propagule pressure has demographic and ecological consequences (Mack et al. 2000, Lockwood et al. 2005), it also can influence the amount and distribution of

genetic diversity within and among invasive populations (Ficetola et al. 2008; Simberloff 2009; Goncalves da Silva et al. 2010). For instance, low propagule pressure would likely sample only a fraction of the total variation in a species' native range and thus would increase the potential for genetic drift in introduced populations through founder effects. Conversely, high propagule pressure would likely lead to the establishment of introduced populations with increased genetic and phenotypic diversity, compared with the diversity expected with low propagule pressure (Novak and Mack 2005; Wares et al. 2005; Kolbe et al. 2004, 2007; Lavergne and Molofsky 2007, but see Dlugosch and Parker 2008). Multiple introductions (high propagule pressure) can also lead to invasive populations that are genetic admixtures (i.e., combine the genetic diversity of two or more different native populations) (Kolbe et al. 2004; Huttanus et al. 2011). Finally, high propagule pressure, admixtures, and genetic recombination (Simberloff 2009; Schierenbeck and Ellstrand 2009) could set that stage for post-immigration evolution that result in local adaptation and increased invasiveness of introduced populations (Maron et al. 2004, Novak 2007; Lavergne and Molofsky 2007; Barrett et al. 2008; Colautti et al. 2009, Keller et al. 2009, Xu et al. 2010).

In addition to propagule pressure, the genetic diversity of introduced populations can also be influenced by the level and structure of genetic diversity within and among native source populations (Novak and Mack 2005; Taylor and Keller 2007; Novak 2011). If native populations exhibit low genetic structure, even limited propagule pressure may, by chance, introduce a large proportion of the genetic diversity found in the native range. Conversely, if native populations are highly structured, limited propagule pressure would lead to the introduction of only a fraction of the genetic diversity from the native range.

Alternatively, if propagule pressure is high, the genetic structure of native populations may not strongly influence the genetic diversity of introduced populations.

Taeniatherum caput-medusae (L.) Nevski (Poaceae) is a primarily self-pollinating, diploid (2n = 14), annual, Eurasian grass that is invasive in the western United States (U.S.). The native range of *T. caput-medusae* includes much of southern Europe, the arid north rim of Africa, the Middle East and central Asia (McKell et al. 1962, Frederiksen 1986). In its native range, three subspecies have been recognized (Frederiksen 1986; Frederiksen and von Bothmer 1986): *T. caput-medusae* (L.) Nevski ssp. *caput-medusae*, *T. caput-medusae* ssp. *crinitum* (Schreb.) Melderis, and *T. caput-medusae* ssp. *asperum* (Simk.) Melderis. The three subspecies do exhibit different geographic distributions, although some overlap does occur. In general, ssp. *caput-medusae* is found in the western Mediterranean (Morocco, Portugal, Spain, and France), ssp. *crinitum* occurs from eastern Europe and the eastern Mediterranean to central Asia (Kyrgyzstan, Tajikistan, and Afghanistan) and ssp. *asperum* is found across almost the entire Eurasian native range of *Taeniatherum* [see Frederiksen (1986) for a map of the geographic distribution of *Taeniatherum*]. *Taeniatherum* subspecies were differentiated by Frederiksen (1986) based on morphological characters associated with the spikes: glume length and spreading of glumes (glume angle) in seed stage, and several traits associated with the lemma and palea (e.g., palea length). Intermediate morphological forms were reported in regions where the geographic distributions of the subsepecies overlap (Frederiksen 1986).

In the western U.S., *T. caput-medusae* ssp. asperum (hereafter referred to as medusahead) occurs in disturbed sites in the 25-100 cm mean annual precipitation zones,

and it can dominate sites with high clay content or well-developed soils (Dahl and Tisdale 1975; Hironaka 1994). The grass has invaded millions of hectares of semi-arid woodlands and shrub-steppe habitats in California, Idaho, Nevada, Oregon, Utah, and Washington (McKell et al. 1962; Young and Evans 1970; Young 1992, Pellant and Hall 1994, Blank and Sforza 2007). Medusahead was first collected in the western U.S. in Roseberg, OR in 1887, and it has a well-known collection history (McKell et al. 1962; Young 1992). Based on the examination of plants in the native and invasive ranges, it is believed that the taxon introduced into the U.S. was *T. caput-medusae* subspecies *asperum* (Major et al. 1960; Young 1992; Kostivkovsky and Young 2000). However, it appears that no quantitative data has been reported to confirm this suggestion.

The level and structure of genetic diversity within and among 45 introduced populations of medusahead from the western U.S. has been previously described (Novak 2004; Novak and Sforza 2008; Novak and Rausch 2009). Over 1660 individuals were scored for their multilocus genotype across 29 loci. A total of 7 homozygous multilocus genotypes (MLG) were detected, four of which were associated with early collection sites (1887, Roseburg, OR; 1901, Steptoe Butte, WA; 1930, Rattlesnake Station, ID; 1944, Ladd Canyon, OR). Genetic diversity within populations was low, but 17 of 45 populations (37.8%) were genetically polymorphic. These data are consistent with a multiple introduction hypothesis for the invasion of medusahead in the western U.S., and suggest that some invasive populations may be genetic admixtures. These conclusions however cannot be rigorously evaluated without the genetic analysis of medusahead populations from the native range.

Before the genetic diversity of native and invasive populations of medusahead could be meaningfully compared to assess introduction dynamics and founder effects, I used the morphological characters described by Frederiksen (1986) to determine which of the three *Taeniatherum* subspecies described above were introduced into the western U.S. By doing this, I ensured that the same native and invasive taxon (or taxa) were being compared. As suggested by the literature (Major et al. 1960; Young 1992; Kostivkovsky and Young 2000), my hypothesis for this portion of the study was that all plants from the invasive range of medusahead would be ssp. *asperum*.

Herein, I report the results of a study assessing the genetic diversity of 34 populations of medusahead from across its native range. The specific objectives of this research were to: 1) determine the level and structure of allozyme diversity within and among native range populations of medusahead, 2) assess genetic relationships among native populations of medusahead, 3) compare the distribution of MLG within and among native and introduced populations to identify the geographic origins (source populations) for the invasion of medusahead in the western U.S., 4) evaluate the multiple-introduction hypothesis that has previously been proposed for this invasion, and 5) compare native and introduced populations to determine the degree to which the genetic diversity of invasive populations has been shaped by founder effects.

Materials and Methods

Plant Collections

In order to encompass as much of the native range genetic diversity of medusahead as possible, my goal was to sample populations widely across Eurasia. A

total of 34 native populations of medusahead were analyzed in this study, with populations ranging from Morocco and Portugal to Turkey and Iran (Table 2.1, Figure 2.1). For 26 of these populations, mature spikes were collected in the summers of 2002, 2004, 2009, and 2010 by Dr. René Sforza and Dr. Stephen J. Novak. Within each population, 30-35 intact spikes were sampled haphazardly 1 - 3 m apart. In populations with fewer than 30 individuals, all individuals were harvested. Intact spikes were stored in individually labeled paper envelopes at room temperature. Many of the collections sites were along roadsides, adjacent to agricultural fields or in disturbed areas.

Samples for seven other populations were obtained as accessions from the USDA-Plant Introduction Laboratory in Pullman, WA: six from Turkey and one from Iran. Unfortunately, in some cases, the geographic location and collection date for these accessions are not provided. In addition, these accessions are the product of an unknown number of grow-outs in Pullman, WA, since they were first collected in their country of origin. Seeds from the Sterea Hellas, Greece population were kindly provided by Dr. Signe Frederiksen, Institute of Systematic Botany, University of Copenhagen, Denmark.

Morphological Measurements

The 45 invasive populations of medusahead analyzed for their morphological characters were previously described by Novak and colleagues (Novak 2004, Novak and Sforza 2008; Novak and Rausch 2009). Based upon the morphological characters described by Frederiksen (1986), these 45 populations were assigned to subspecies *asperum*. Plants were grown in a greenhouse common garden at Boise State University in mid-winter 2009, as described in Chapter 1. Based on the species key developed by Frederiksen (1986), five morphological characters were used: glume length, glume angle,

palea length, conical cell prominence on the lemma, and lemma surface hair location. After harvest, measurements of the five traits were obtained for each individual in each population. The traits glume length and palea length were measured using a standard metric ruler and scored as continuous variables. Glume angle was measured with a True Angle ® protractor, and also scored as a continuous variable. Conical cell characteristics and the location of hairs on the lemma surface were determined using a Leica EZ4 Dissecting scope at various magnifications and scored categorically: conical cells not prominent = 1 and conical cells prominent = 2; hairs only at the margins of the lemma surface = 1 and hairs throughout the entire lemma surface = 2.

Enzyme Electrophoresis

Genetic diversity within and among the 34 native populations of medusahead was assessed using enzyme electrophoresis. Seeds were germinated in petri dishes on moistened filter paper and harvested 7 – 10 days following germination. Entire seedlings (shoot and root tissue) were macerated in a tris-HCl grinding buffer-PVP solution (pH 7.5). The starch concentration of each gel was approximately 12.5% (w/v). Enzyme electorphoresis protocols followed that of Soltis et al. (1983) with modifications described by Novak et al. (1991). A suite of 15 enzymes were stained and visualized using the following buffer systems: isocitrate dehydrogenase (IDH), glucose-6-phosphate dehydrogenase (G6PDH), and shikimate dehydrogenase (SKDH) using system 1 of Soltis et al. (1983); alcohol dehydrogenase (ADH), aldolase (ALD), glutamate dehydrogenase (GDH), and phosphoglucoisomerase (PGI) using system 6; glutamate oxalacetate transaminase (GOT), colorimetric esterase (CE), malic enzyme (ME), superoxide dismutase (SOD), and triosephosphate isomerase (TPI) using system 8; and malate

dehydrogenase (MDH), phosphoglucomutase (PGM) and 6-phosphogluconate dehydrogenase (6PGD) using system 9.

Because medusahead is a diploid, the genetic basis of all allozyme variation observed was easily inferred based on the known subunit structure and compartmentalization of these enzymes (Gottlieb 1982, Wendel and Weeden 1989). Nomenclature for loci and alleles generally followed that of Novak at al. (unpublished data), but also included modification described by Peters (Chapter 1). Across the 45 invasive populations of medusahead, Novak et al. (unpublished data) determined allelic diversity at 29 loci; but due to low gene expression and banding intensity, the genetic diversity of the 34 native populations of medusahead was assessed using 23 allozyme loci. For example, Novak et al. (unpublished data) scored six CE loci across invasive populations, but only two of these loci could be reliably scored among native populations: *Ce-2* and *Ce-4*. In this study, only these 23 loci are used when the genetic diversity of native and invasive populations was compared. As new alleles were detected in native populations, the nomenclature for alleles had to be updated, with the most anodally migrating allele designated *a*, the next *b*, and so on.

Data Analysis

Based on the statistical analyses presented in Chapter 1, the three native subspecies were found to be statistically, significantly different for three of five morphological characters (glume angle, glume length, and palea length). In the current study, I am comparing "invasive asperum" populations with the three native subspecies to determine which native subspecies "invasive asperum" plants most closely resemble. Thus, I am only making these comparisons for the three morphological characters

previously shown to be significantly different (Chapter1). To test for significant differences for these three morphological characters, among the four plant groups grown in the greenhouse common garden, Student-Newman–Keuls tests were conducted. Subsequently, a Bonferroni correction was performed to mitigate false positives when testing multiple hypotheses on the same data set (Rice 1989). Using only these three morphological characters found to be significant, PROC GPLOT in SAS™ (SAS Institute 2002) was used to generate a 3D scatter plot. The 3D scatter plot provides a visual representation of the data's ability to discriminate between the four plant groups.

Allozyme data was analyzed using the program POPGENE 1.32 (Yeh and Boyle 1997) to determine the level and structure of genetic diversity within and across native populations of medusahead. The data were entered as individual multilocus genotypes by population. Genetic diversity in medusahead was expressed as the mean number of alleles per locus (*A*), the percentage polymorphic loci per population *(%P*), expected mean heterozygosity (H_{exp}), and the mean observed heterozygosity (H_{obs}). Expected mean heterozygosity was computed using the unbiased estimate method of Nei (1978). The means of these genetic diversity parameters were used to describe the overall diversity within populations of medusahead from the native range.

Nei's gene diversity statistics (1973; 1977) were used to partition total allelic diversity within and among populations, using the variance components from the output of the Wright-78 analysis of BIOSYS-1 (Swofford and Selander 1981). At each polymorphic locus, the total allelic diversity (H_T) was partitioned into a within-population component (H_S) and an among-population component (D_{ST}) using the expression $H_T = H_S + D_{ST}$. The proportion of genetic diversity partitioned among

populations (G_{ST}) was determined using the equation $G_{ST} = D_{ST}/H_T$. Means of Nei's gene diversity statistics from all polymorphic loci were employed to describe the overall allocation of allelic diversity within and among populations for the study region. Nei's (1978) unbiased genetic identity coefficients (*I*) were calculated for all possible pair-wise comparisons using BIOSYS-1.

Analysis of molecular variance (AMOVA) using the F-statistics method was used to estimate the amount of genetic variation partitioned within populations and among regions using ARLEQUIN v.3.1 (Excoffier et al. 2005). The enzyme electrophoresis data were entered as psuedohaplotype frequencies for each population and structured geographically into four sub-regions (North Africa n = 5, Western Europe n = 7, Central Europe n = 11 Eastern Europe/Central Asia n = 11). All individuals with missing data were removed from the analysis.

Two methods are often used to visually represent genetic relationships among populations: the unweighted pair-group method with arithmetic averaging algorithm (UPGMA), which assumes that all lineages (populations) evolve at the same rate and a neighbor-joining tree, which does not assume equal evolutionary rates for each lineage. Because allozymes are considered to be selectively neutral molecular markers and I cannot infer evolutionary rate differences from these data, I have chosen to portray the genetic relationships among these populations using the UPGMA phenogram (Nei and Roychoudhury, 1993). A cluster UPGMA phenogram was generated using POPGENE. Nei's (1978) unbiased genetic identity values (modified from the NEIGHBOR procedure of PHYLIP version 3.5c) were used to generate the UPGMA phenogram because this parameter is best suited for use with data sets with small sample sizes. Seven invasive

populations, which possess the seven homozygous MLGs detected in the western U.S., were included in this analysis to assess the genetic similarity between populations of medusahead from the native and invasive ranges.

Geographic Origins

The geographic origins (source populations) of an invasive species can be determined with genetic markers using two approaches: the phylogenetic method or the multilocus genotype method (Roderick and Navajas 2003; Keller and Taylor 2008; Novak 2011). Because allozymes are being used to assess genetic diversity in medusahead, I am employing the multilocus genotype approach. Using this approach, I will identify native populations as being putative sources for this invasion when one or more individuals match one of the seven homozygous MLG previously detected in the western U.S. (Novak et al. unpublished data).

Results

Morphological Characters and Subspecies Comparisons

Of the 45 invasive populations, only 43 actually germinated, flowered, set seed and were analyzed. Native populations of ssp. *asperum* had moderately short glumes (mean = 28.3 mm), a mean glume angle of 63° and a palea length of 8.4mm (Chapter 1). Invasive populations of medusahead generally show similar trait means compared with their native counterparts, however native and invasive populations do exhibit statistically significant differentiation for two of these traits (glume length and palea length) (Figure 2.2).

The 3D scatter plot created with SAS™ (SAS Institute 2002) is a graphical representation of the morphological variation seen between native and invasive populations of medusahead. Invasive populations of medusahead (Figure 2.3a, indicated in light blue) largely overlap the populations of native ssp. a*sperum*, although some populations appear to exhibit a shift in morphology as revealed in Figure 2.2. Some invasive individuals of medusahead (Figure 2.3b) appear to overlap with individuals of all three native subspecies, indicating that individuals of medusahead from across the western U.S. are morphologically variable.

Genetic Diversity of Medusahead in the Native Range

Estimates of genetic diversity and structure of medusahead from its native range are based the analysis of 956 individuals in 34 populations (28.1 individuals per population). Across the 34 populations examined, 48 alleles were detected at the 23 scored loci (2.09 alleles/locus) (Table 2.2). Fifteen of the 23 loci (65.2%) were polymorphic: *Mdh-1, Mdh-2, Mdh-3, Pgm-2, 6Pgd-2, Got-1, Got-2, Ce-2, Ce-4, Pgi-2, Adh, Gdh, Idh, Skdh, G3pdh-2).* Allelic diversity at the 15 polymorphic loci ranged from two to five, with *Ce-4*, *Mdh-2*, and *Pgi-2* each having five alleles (Appendix A). Twenty-three of the 34 native populations (67.6%) analyzed in this study are genetically polymorphic (i.e., at least one locus in the population was variable), and the remaining 11 populations have no genetic diversity at any of the 23 scored loci (Table 2.1, Appendix A).

On average, the 34 native populations of medusahead display 1.10 alleles per locus (*A*) and 9.08% polymorphic loci per populations (%*P*) (Table 2.1). Seven of 23 loci were polymorphic in the population at Sarigol, Turkey, thus it contained the highest

level of within-population genetic diversity (A = 1.39 and %P = 30.43). Four populations had five polymorphic loci: two from Morocco (Tizi n'tishka, A = 1.26 and %P = 21.74; Tleta Tassrit, A = 1.22 and %P = 21.74), one from Sardinia, Italy (Dorgali, A = 1.30 and %P = 21.71), and another from Turkey (Ipsala, (A = 1.22 and %P = 21.74). Averaged across all 34 populations, the expected mean heterozygosity (H_{exp}), which is equivalent to the expected genetic diversity, is 0.025 (Table 1). The highest value of H_{exp} was detected in the Sarigol, Turkey population (H_{exp} = 0.102) with the populations from Tleta Tassrit, Morocco, Ipsala, Turkey, Tizi n'tishka, Morocco, and Dorgali, Sardinia also having relatively high values for expected mean heterozygosity (H_{exp} = 0.073, 0.072, 0.065, and 0.061, respectively). The lowest value of H_{exp} for a population with polymorphic loci was detected in Villavicosa de Cordoba, Spain (H_{exp} = 0.007). The observed heterozygosity was 0.0000 for all populations with the exception of Iran 1 (H_{obs} = 0.0009).

Population Differentiation of Medusahead in the Native Range

Ce-4 and *Pgi-2* are the most polymorphic loci among all populations sampled in the native range (Appendix A), and consequently these loci have the highest value for total gene (allelic) diversity (H_T = 0.571 and 0.781, respectively) (Table 2.3). The among-population components for *Ce-4* and *Pgi-2* (D_{ST} = 0.098 and 0.633, respectively) were larger than the within-population (H_S = 0.047 and 0.085, respectively), thus the proportion of the total gene diversity partitioned among populations (G_{ST}) at each locus is 0.908 for *Ce-2* and 0.882 for *Pgi-2*. Even more of the total gene diversity at the loci *Got-2* and *Idh* is partitioned among populations (G_{ST} = 1.000 and 0.918, respectively). *6Pgd-2* displays the lowest value for total gene diversity (H_T = 0.033) and is the only

polymorphic locus with a greater within-population component of diversity than the among-population component. Consequently, the value of G_{ST} at *6Pgd-2* is 0.127. The mean value of H_T for all polymorphic loci is 0.262, and the mean value of G_{ST} is 0.745, indicating that most of the genetic diversity (74.5%) for all populations was partitioned among populations (Table 2.3).

Mean values of Nei's (1978) unbiased genetic identity coefficients (*I*) for all possible pair-wise population comparisons is $I = 0.864$ (data not shown), indicating a relatively high level of genetic similarity among native populations of medusahead analyzed in this study.

I further partitioned the genetic diversity of native populations of medusahead using analysis of molecular variance (AMOVA) in Arlequin 3.5.1.2 (Excoffier et al. 2005). Genetic differences among the four geographic regions accounted for 22.2% ($P < 0.00001$) of the total genetic variation, while 48.6% ($P < 0.00001$) of the total variability was partitioned among populations within geographic regions. The remaining 29.1% ($P < 0.00001$) of the variability is partitioned among individuals within populations.

The UPGMA cluster diagram based on Nei's (1978) unbiased genetic identity values provides a graphic representation of genetic relationships among native populations of medusahead (Figure 2.4). Native populations of medusahead in this analysis occurred in several distinct clusters. Cluster 4 is the most genetically distinct, and it consists of three populations from the Italian mainland and one population from Sardinia (Orosei). Cluster 3 is comprised of populations from Spain and Morocco, with three populations from Morocco forming a genetically distinct sub-group. The only population from Iran in this analysis is also found in Cluster 3. Clusters 1a, 1b, and 2

consist of native populations that have multilocus genotypes that either match or are similar to those genotypes previously detected among invasive populations. For instance, Cluster 1a is composed of the population from Pezenas les Mines, France, the population from Ladd Canyon, Oregon, two populations from Turkey and the population from Sterea Hellas, Greece. All individuals in Pezenas les Mines share the Ladd Canyon multilocus genotype, whereas only some of the individuals in the two Turkish populations have this genotype. None of the individuals in the Sterea Hellas population are an exact match for the Ladd Canyon multilocus genotype, although they differ from Ladd Canyon genotype at only one locus. This same pattern of exact and close matching of multilocus genotypes from the invasive range also explains the populations grouped together in Cluster 1b and Cluster 2. Because only three individuals in the Ipsala, Turkey population are a direct match to the Pullman, Washington, multilocus genotype, the populations from Ipsala is found in a cluster with the Rattlesnake Station, Idaho population rather than with Pullman.

Geographic Origins

The geographic origins (source populations) for the introduction of medusahead into the western U.S. can be identified when one or more individuals within a native population possess a multilocus genotype that matches on of the seven genotypes previously detected among invasive populations. To facilitate visualizing these matches, the seven multilocus genotypes detected across the invasive range were included in the UPGMA phenogram (Figure 2.4, color coded boxes). In some putative source populations, all individuals within the population were a match for the invasive multilocus genotype. As indicated above, the populations Pezenas les Mines, France and

Ladd Canyon, Oregon clustered together because they have a genetic identity of 1.0, indicating that all individuals in both populations share the same multilocus genotype. Similarly, populations from Lodine, Sardinia and Komotini, Greece form a cluster with the population from Steptoe Butte, Washington because they share the same multilocus genotype. Instances in which a match occurs but not all individuals in the native population share the same MLG are also indicated in the phenogram. For example, while many individuals in the population from Askos/Filadelphio, Greece are an exact match of the MLG detected in Rattlesnake Station, Idaho, only a few individuals in the population from Xanthi, Greece match this genotype. Based on the criteria provided here, a total of ten native populations of medusahead contain a multilocus genotype(s) that matches a genotype detected in the western U.S., and thus may be classified as a putative source population: Pezenas les Mines, France; Dorgali, Sardinia; Lodine, Sardinia; Askos/Filadelphio, Greece; Komotini, Greece; Xanthi, Greece; Ipsala, Turkey; Havsa, Turkey; Uzunkopru, Turkey; and Sarigol, Turkey (Table 2.1, Figure 2.5).

Comparison of Genetic Diversity in Native and Invasive Populations of Medusahead

Despite the fact that fewer native populations (n = 34) were sampled compared with invasive populations (n = 45), the level of genetic diversity both across and within native populations is higher than that of populations from the western U.S. For example, native populations possess more alleles (48) and three times as many polymorphic loci (Table 2.2). In addition, the percentage of native populations that are polymorphic (67.6%, 23 of 34 populations) is nearly double the value for invasive populations (37.8%, 17 of 45 populations). At the within-population level (Table 2.1), native range populations of medusahead display, on average, more genetic diversity (A = 1.10, %P =

9.08, He = 0.025) than invasive populations (A = 1.03, %P = 2.51, He = 0.006) (Novak *et al*. unpublished data). Genetic differences between native and invasive populations of medusahead, at the within-population level, are even more pronounced when only the 10 putative source populations are compared to those from the western U.S. (Tables 1 and 2).

Discussion

Based on the number of MLG, the invasion of *T. caput-medusae* ssp. *asperum* in the western U.S. may stem from a minimum of seven separate introduction events (Novak et al. unpublished data). However, reconstructing the introduction dynamics of medusahead in western U.S. requires the analysis of native population using the same molecular marker (Roderick and Navajas 2003; Keller and Taylor 2008; Novak 2011). The combined analysis of native and invasive populations allows me to identify the geographic origins of this invasion. Finally, results from this analysis also provide a framework for assessing the population genetic consequences of the introduction of medusahead into the western U.S.

Geographic Origins (Source Populations)

Based on the results for 34 native Eurasian populations, I have now accounted for five of the seven MLG previously detected among invasive western U.S. populations of medusahead. These five MLG were detected within 10 native populations, which represent putative source populations (Table 2.1, Figure 2.5). These putative source populations are arrayed across a wide geographic area and represent much of the Mediterranean native range of medusahead. Although these populations contain MLG

matches to those found throughout the western United States, these populations may not all represent actual source populations from which the invasion stemmed, but they more likely reflect source regions. Because six of the populations for which multilocus genotype matches were detected are clustered geographically, it is possible that northeastern Greece and the European portion of Turkey represent the source region for this invasion.

Of the seven MLG detected in the introduced range, five (Ladd Canyon, OR; Rattlesnake Station, ID; Steptoe Butte, WA; Roseburg, OR and Pullman, WA) were detected in the native range. The Rattlesnake Station (ID) and Steptoe Butte (WA) MLG occur most often among the putative source populations (Figure 2.5). For example, Rattlesnake Station was detected in Askos/Filadelphio and Xanthi, Greece; Havsa, Ipsala and Uzunkopru, Turkey. Similarly, the Steptoe Butte, WA genotype was detected in Lodine and Dorgali, Sardinia and Komotini, Greece (Figure 2.3). Additionally, the MLG associated with Roseburg, OR, which is the earliest collection of medusahead in the U.S., has been detected in two Turkish populations (Havsa and Sarigol). Two MLG detected in invasive populations, Malloy Prairie, WA and Salt Creek, UT, were not detected within the native populations analyzed thus far. Based on the findings presented here, additional analysis of eastern European populations is warranted.

Genetic Diversity of Medusahead in the Native Range

Across all 34 populations of subspecies *asperum* (Table 2.2), allozyme diversity (2.09 alleles per locus and 65.25% polymorphic loci) is higher than the mean values (1.96 alleles per locus and 50.5% polymorphic loci) reported for 473 plant species (Hamrick and Godt 1989). Conversely, allozyme diversity across the 10 putative source

populations (1.65 alleles per locus and 43.5% polymorphic loci) is lower than these mean values, but this comparison is confounded by large differences in sample size. Allozyme diversity across all populations of medusahead is generally higher or similar to the range of values (1.69 – 2.38 alleles per locus and 41.8 - 59.2% polymorphic loci) reported for selfing, early successional, annual, and monocot plant species (Hamrick and Godt 1989). Allozyme diversity of medusahead at the species level, stands in sharp contrast to the level of allozyme diversity within populations. On average, allozyme diversity of the 34 native populations of medusahead (A = 1.10, %P = 9.1 and H_{exp} = 0.025) and the 10 putative source populations (A = 1.15, %P = 12.6, and H_{exp} = 0.039) is lower than the mean values (A= 1.53, %P = 34.2, H_{exp} = 0.113) reported for populations of 468 plant species (Hamrick and Godt 1989). Allozyme diversity within populations of medusahead is also lower than the range of values (A = 1.31 - 1.66, %P = 20.0 - 40.3, H_{exp} = 0.074 – 0.144) reported for selfing, early successional, annual, and monocot plant species (Hamrick and Godt 1989). The relatively low level of within-population allozyme diversity of medusahead, compared with its across-population diversity, is a product of the highly selfing mating system of this species, as reflected in the very low values of H_{obs} provided in Table 2.1.

In addition, the number of MLG within native populations is also variable: some populations only have one MLG, and other populations contain many MLG. For example, the putative source population Pezenas les Mines, France contains only one MLG and is a match to the Ladd Canyon MLG found in the U.S. Conversely, the population from Sarigol, Turkey, which is also a putative source population, contains 14

MLG, only one of which matches a genotype detected in the invasive range (Table 2.1, Figure 2.5).

Seven populations included in this study were obtained as seed accessions from the USDA Plant Introduction Laboratory in Pullman, WA, and these accessions have limitations associated with their use in population genetic analyses (Table 2.1). While these samples provide insights into the genetic diversity found within specific geographic regions, this material likely underestimates the actual amount of genetic diversity in these populations. This underestimate of genetic diversity may be explained by: 1) data on the collections is sparse and the original field collections may have included a limited number of individuals that would provide an underestimate of genetic diversity, 2) the seed accessions have been through an unknown number of grow-outs from the time they were first collected and this may lead to a further reduction in genetic diversity through genetic drift, and/or 3) these grow-outs were carried out in Pullman, WA, and selection for or against some genotypes in a novel (non-native) environment may further reduce genetic diversity.

Population Differentiation

Predominately self-pollinating (selfing) species typically exhibit higher genetic structure compared with predominately outcrossing species (Hamrick and Godt 1989, 1996). This is especially true for predominantly selfing introduced plants, where in their area of introduction, such species would be expected to have a higher structure relative to native range (Brown and Marshall 1981; Wade and McCauley 1988; Whitlock and McCauley 1990, McCauley 1991, Novak and Mack 2005).

Results for medusahead are consistent with this expectation, and indicated that invasive populations exhibit higher genetic structure compared with native populations. The mean value of G_{ST} at all polymorphic loci for native populations is 0.745 (Table 2.4), whereas the mean value for G_{ST} reported for invasive populations of medusahead is 0.906 (Novak et al. unpublished data). A similar pattern occurs for values of genetic identity coefficients of native and invasive populations of medusahead. The mean genetic identity value of native populations is lower (I = 0.864) than the values previously determined for invasive populations (I = 0.964) (Novak et al. unpublished data). These finding indicate that the genetic similarity of native populations of medusahead is lower than that of invasive populations, a result supported by the genetic relationships observed among native populations depicted in Figure 2.5.

The pattern of genetic structure for medusahead is in contrast to values reported for the predominately selfing invasive grass *Bromus tectorum* (cheatgrass) (G_{ST} = 0.754 in the native range and 0.478 in the invasive range; Novak et al 1991, Novak and Mack 2005). Differences in the genetic structure of introduced populations of cheatgrass and medusahead may be due to differences in the number of introductions and/or the patterns of range expansion during these two invasions. The number of introductions of cheatgrass and medusahead in the western U.S., however, are quite similar; thus, the differences in genetic structure described above most likely reflects differences in the pattern of range expansion for the two species, with range expansion in medusahead mostly occurring at the local or regional level and more geographically widespread for cheatgrass.

Propagule Pressure and Founder Effects

Insights into the propagule pressure associated with the introduction of medusahead into the western U.S. vary depending on the scale at which the genetic diversity of native population is examined. For instance, 66 MLG were detected across the 34 native populations of medusahead analyzed thus far, yet only seven MLG (only 10.6% of the MLG found in the native range) were observed across invasive populations (Novak 2004; Novak and Sforza 2008; Novak and Rausch 2009). At the MLG level, therefore, these data suggest limited propagule pressure with only a handful of the genotypes of native populations being introduced into the western U.S. At the population level, however, moderate propagule pressure is indicated: of the 34 native populations analyzed in this study, 10 (29.4%) populations possess at least one individual that matches one of the MLG introduced into the U.S. The detection of 10 native populations that possess MLG that match those previously detected in the invasive range provides support for the multiple introduction hypothesis; and based on these data, individuals may have been sampled and introduced into the western U.S. from populations across the Mediterranean. Conversely, because five of the seven invasive range MLG were detected in as few as two to three populations from western Turkey, the geographical scale of sampling individuals in the native range may be much smaller. Reconciling this difference cannot be accomplished using allozymes, however another molecular marker (e.g., microsatellite or chloroplast DNA sequence data) may be more effective in estimating the propagule pressure of this invasion and the geographic scale at which source populations were sampled.

Founder effects during introduction can lead to invasive populations that exhibit reductions in average expected heterozygosity, or allelic richness (the average number of

alleles per locus), or both parameters, compared with native populations (Wright 1931; Nei 1975; Novak and Mack 2005). With sampling error, allele frequencies may be altered, and fixation or loss of alleles may occur (Nei et al 1975). Through this process, allelic richness may decline more rapidly than other genetic parameters, and therefore may be a better indicator that populations have experienced founder effects (Novak and Mack 2005). The invasion of medusahead into the western United States appears to be associated with founder effects because introduced populations exhibit a reduction in genetic diversity, compared with native populations (Table 2.1 and Table 2.2). Across native populations of medusahead, more alleles and a higher percentage of polymorphic loci (48 and 65.2%) were detected than for the introduced populations in North America (28 and 21.7%). When considering only putative source populations, the disparity in the genetic diversity across native and invasive populations of medusahead is even greater. Further evidence of founder effect can be seen when comparing the percentage of polymorphic populations among putative source populations (8 of 10, 80.0%) with that of invasive populations (17 of 45, 37.8%).

Within populations, all 34 native populations and the 10 putative source populations posses more genetic diversity compared with invasive populations (Table 2.1). Within all native populations of medusahead, the level of genetic diversity (%P = 9.08 and H_{exp} = 0.025) is greater than that of introduced populations (2.51% and 0.006, respectively). Within putative source populations of medusahead, the level of genetic diversity is, on average, even higher

The degree to which the genetic diversity of invasive populations is reduced relative to native populations can be determined for the parameters allelic richness (the

number of alleles across populations) and the expected heterozygosity. The proportional loss of expected heterozygosity within populations is calculated as [$F = (1 – H_i/H_{s'}) \times 100$], where H_i is the average expected heterozygosity across invasive populations and $H_{s'}$ is the average expected heterozygosity across the putative source populations (or regions). The proportional loss in allelic richness is calculated as [$A = (1 – A_i/A_{s'}) \times 100$], where A_i is the number of alleles among invasive populations and $A_{s'}$ is the number of alleles among the putative source populations (Wares et al. 2005; Dlugosch and Parker 2008).

Using these metrics, invasive populations of medusahead show an 84.1% reduction in expected heterozygosity and a 26.3% reduction in allelic richness, when compared with their putative source populations. Wares et al. (2005) reviewed 29 studies in which expected heterozygosity and the allelic richness were reported for both invasive and putative source regions. Their comparisons revealed that the loss of expected heterozygosity is typically small (an average of 17% across a broad range of taxa). They also concluded that founder effects have a similar, if not greater, effect on allelic richness (average of < 20%). The values reported in this study reveal a greater loss of genetic diversity than that observed for other invasive species (Wares et al. 2005), providing further support for the observation that the invasion of medusahead in the western U.S. is associated with founder effects.

Implications for Biological Control

Exploration for potential biological controls agents has already begun and has identified a several potential pathogens (Seigwart et al. 2003; Widmer and Sforza 2004; Novak and Sforza 2008). Data from the current study suggests that there is less genetic

diversity and increased structure within and among populations in the invasive range, compared with native populations. Reduced genetic diversity within invasive populations may render them more susceptible to attack from biological control agents. Furthermore, invasive populations may have reduced evolutionary potential, thus they would not be expected to quickly evolve to evade attack. The current study also reveals that putative source populations occur over a wide geographic area, much of the native Mediterranean distribution of medusahead. Thus, the search for biocontrol agents will need to emcompass a broad geographic area. Additionally, 17 of 45 invasive populations are composed of two or more native genotypes, therefore multiple agents will most likely be needed to control populations that are admixtures (Muller-Scharer et al. 2004, Novak and Sforza 2008).

In conclusion, this study highlights the insights that can be obtained through the combined analysis of native and introduced populations. The identification of putative source populations described here will also be useful in the search for effective and specific biological control agents for the management of this very destructive invasive plant.

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Figures and Tables

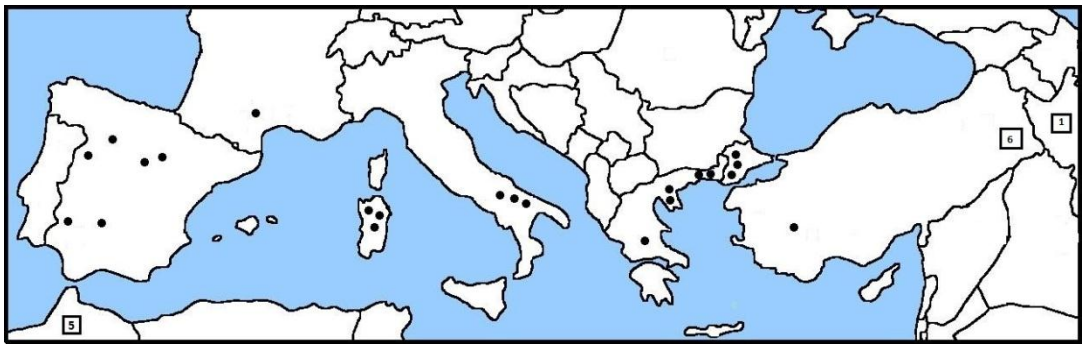

Figure 2.1 Distribution of the 34 native range populations of *Taeniatherum caput-medusae* used in the genetic analysis

Table 2.1 Within population genetic diversity for all populations of *Taeniatherum caput-medusae* ssp. *asperum* and for all putative source populations (indicated with *) of the invasion of the western United States. Samples obtained from the Plant Introduction Laboratory indicated by [PI]

Population	A	%P	H_{obs}	H_{exp}
Pezenas les Mines, France*	1.00	0.00	0.0000	0.0000
Dorgali, Sardinia, Italy*	1.30	21.74	0.0000	0.0609
Orosei, Sardinia, Italy	1.22	17.39	0.0000	0.0195
Lodine, Sardinia, Italy*	1.00	0.00	0.0000	0.0000
Poggorisini, Italy	1.09	8.70	0.0000	0.0157
Altamura, Italy	1.00	0.00	0.0000	0.0000
Minervino Murge, Italy	1.04	4.35	0.0000	0.0159
Timahdite, Morocco	1.09	8.70	0.0000	0.0221
Tizi n' tishka, Morocco	1.26	21.74	0.0000	0.0646
Tizi n' test, Morocco	1.17	17.39	0.0000	0.0301
Tafraoute, Morocco	1.09	8.70	0.0000	0.0278
Tleta Tassrit, Morocco	1.22	21.74	0.0000	0.0728
Monesterio, Spain	1.00	0.00	0.0000	0.0000
Canamares, Spain	1.13	13.04	0.0000	0.0168
Robledillo, Spain	1.13	13.04	0.0000	0.0285

Population	A	%P	H_{obs}	H_{exp}
Martin de Viejo, Spain	1.17	17.39	0.0000	0.0554
Villaviciosa, Spain	1.04	4.35	0.0000	0.0072
Pedraza de la Sierra, Spain	1.22	17.39	0.0000	0.0619
Sarigol, Turkey*	1.39	30.43	0.0000	0.1018
Biloris, Turkey[PI]	1.00	0.00	0.0000	0.0000
Eruh, Turkey[PI]	1.00	0.00	0.0000	0.0000
Buldan Junction, Turkey[PI]	1.00	0.00	0.0000	0.0000
Aliaga, Turkey[PI]	1.00	0.00	0.0000	0.0000
Dakili Junction, Turkey[PI]	1.00	0.00	0.0000	0.0000
Guzelkonak A, Turkey[PI]	1.00	0.00	0.0000	0.0000
Havsa, Turkey*	1.09	8.70	0.0000	0.0255
Ipsala, Turkey*	1.22	21.74	0.0000	0.0717
Uzunkopru, Turkey*	1.17	13.04	0.0000	0.0346
Sterea Hellas, Greece	1.00	0.00	0.0000	0.0000
Xanthi, Greece*	1.09	8.70	0.0000	0.0294
Panorama, Greece	1.09	8.70	0.0000	0.0155
Askos/Filadelphio, Greece*	1.09	8.70	0.0000	0.0271
Komotini, Greece*	1.09	8.70	0.0000	0.0096

Population	A	%P	H_{obs}	H_{exp}
Iran 1^{PI}	1.04	4.35	0.0009	0.0214
Native Population Mean	1.10	9.08	0.00003	0.0246
Putative Source Pop. Mean	1.15	12.56	0.00000	0.0390
Invasive Population Mean	1.03	2.52	0.00010	0.0060

a. Glume Length

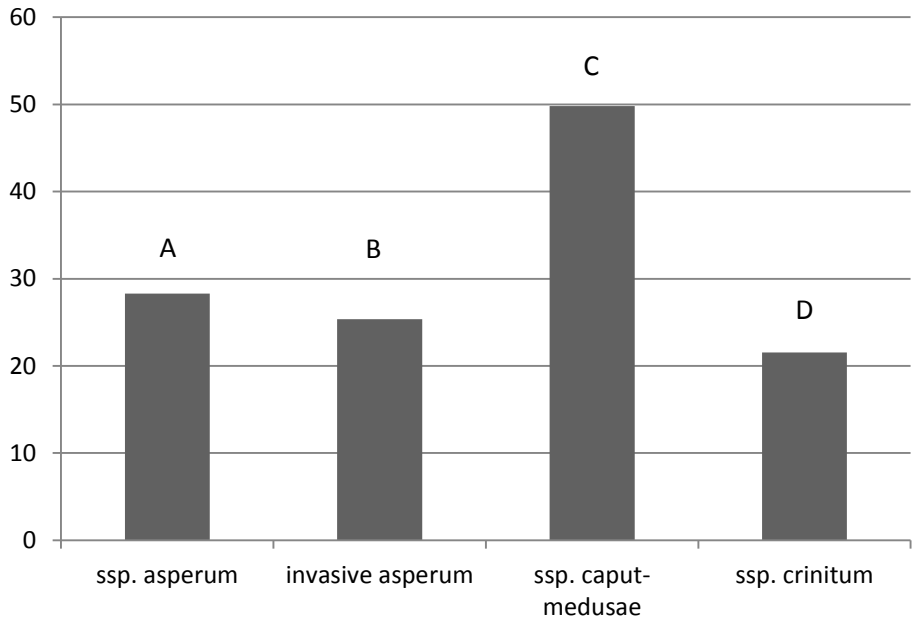

Figure 2.2a Histograms showing means and significant differences among subspecies of *Taeniatherum caput-medusae* and populations from the invasive range. Letters above bars denote SKN groupings for significantly different means. All lengths measured in millimeters, angles measured in degrees.

b. Glume Angle

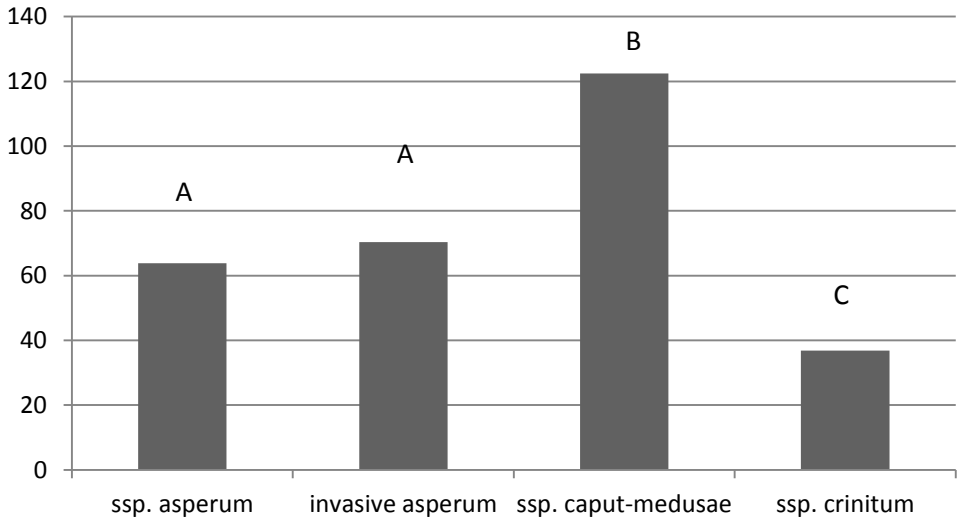

Figure 2.2b Histograms showing means and significant differences among subspecies of *Taeniatherum caput-medusae* and populations from the invasive range. Letters above bars denote SKN groupings for significantly different means. All lengths measured in millimeters, angles measured in degrees.

c. Palea Length

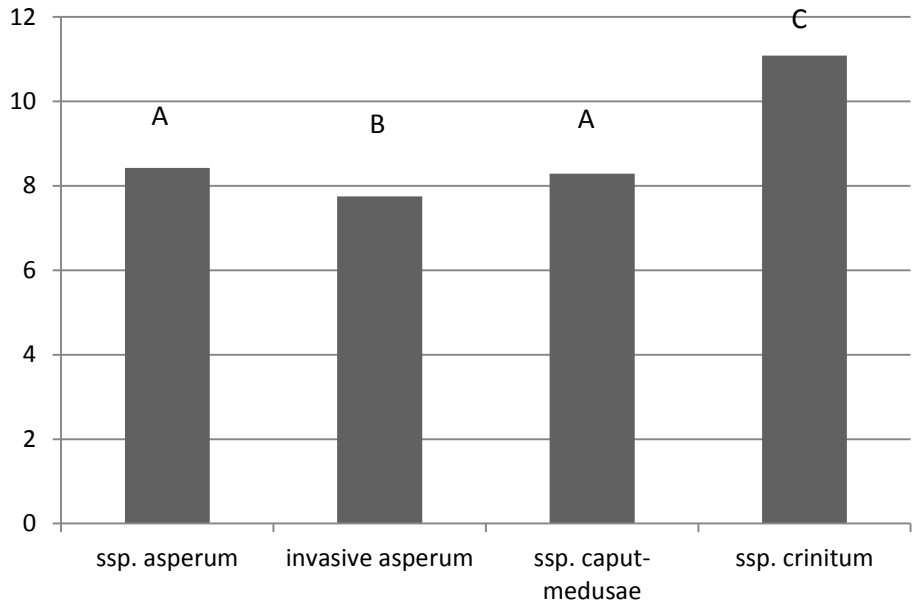

Figure 2.2c Histograms showing means and significant differences among subspecies of *Taeniatherum caput-medusae* and populations from the invasive range. Letters above bars denote SKN groupings for significantly different means. All lengths measured in millimeters, angles measured in degrees.

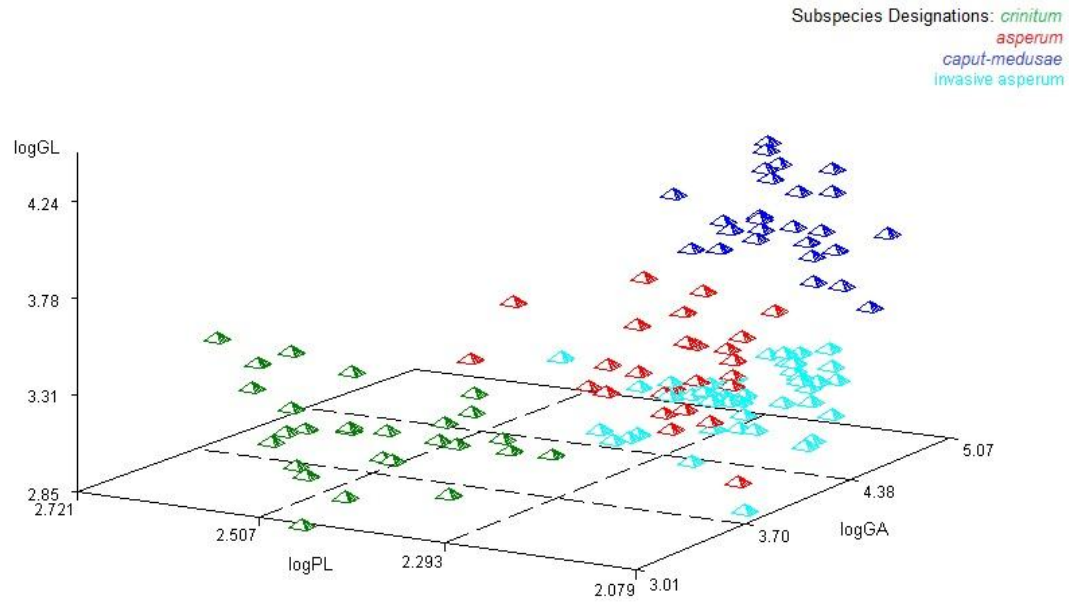

Figure 2.3a Three dimensional scatter plot of the mean values for the significant morphological traits measured for 82 native range and 43 invasive range populations of *Taeniatherum caput-medusae*

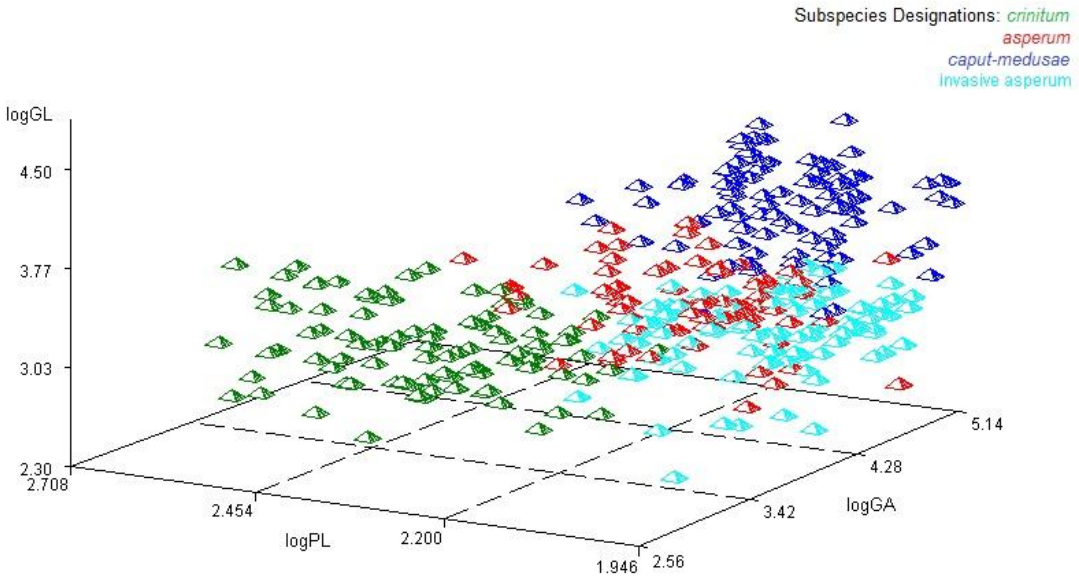

Figure 2.3b Three dimensional scatter plot of the significant morphological traits measured for all native and invasive individuals of *Taeniatherum caput-medusae*

Table 2.2 Across population genetic diversity for *Taeniatherum caput-medusae*

	Number of Populations	Alleles	Alleles/ Locus	Number of Poly. Loci	Percentage of Poly. Loci	Percentage of Poly. Populations
Native Populations	34	48	2.09	15	65.2	67.6
Putative Source Populations	10	38	1.65	10	43.5	80.0
Invasive Populations	45	28	1.22	5	21.7	37.8

Table 2.3 Nei's Gene Diversity statistics for native populations of *Taeniatherum caput-medusae*

Locus	H_T	H_S	D_{ST}	G_{ST}
Mdh-2	0.506	0.083	0.422	0.835
Mdh-3	0.137	0.026	0.110	0.807
Pgm-2	0.163	0.054	0.109	0.669
6pgd-2	0.033	0.029	0.004	0.127
Got-1	0.394	0.065	0.330	0.836
Got-2	0.057	0.000	0.057	1.000
Ce-2	0.144	0.047	0.098	0.676
Ce-4	0.571	0.052	0.518	0.908
Pgi-2	0.718	0.085	0.633	0.882
Adh	0.046	0.022	0.023	0.511
Gdh	0.147	0.020	0.127	0.864
Idh	0.344	0.028	0.315	0.918
G3pdh-2	0.144	0.049	0.094	0.657
Mean	0.262	0.043	0.194	0.745

Table 2.4 Analysis of Molecular Variance using the F-Statistics method. Only *Taeniatherum caput-medusae* ssp. *asperum* populations were included in this analysis. Populations were assigned to four geographic regions. Northwestern Africa (Morocco n= 5), Western Europe (all Spanish and French populations, n=7). Central Europe (all Greek and Italian populations, n=11), and Eastern Europe (all Turkish and Iranian populations, n=11).

	d.f	Sum of Squares	Variation Component	Percentage Variation
Among regions	3	172.366	0.010692	22.21
Among populations within regions	30	387.291	0.233780	48.57
Among individuals within populations	892	250.433	0.140110	29.11
Within individuals	926	0.500	0.000540	0.11
Total	1851	810.589	0.481350	--

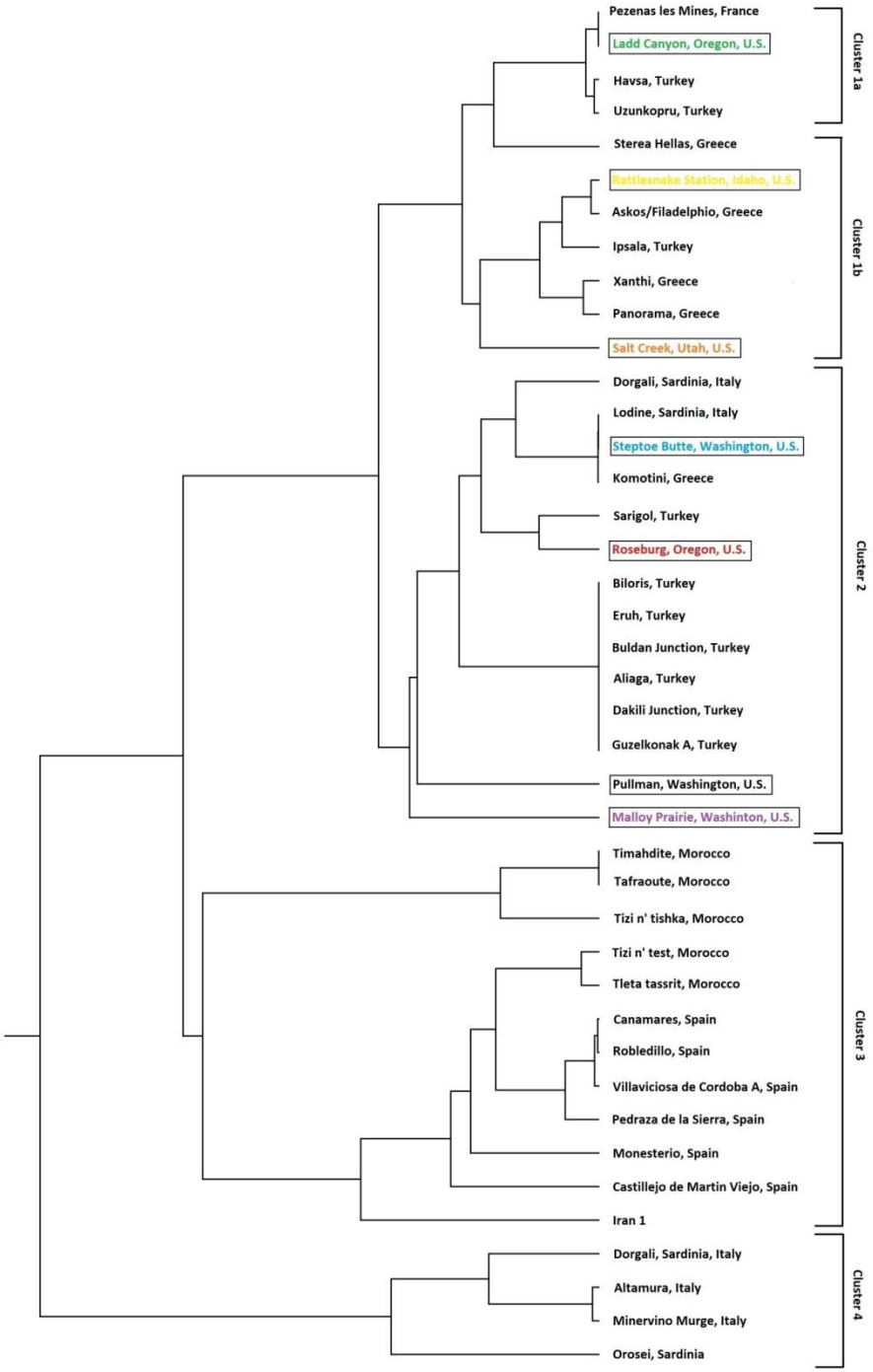

Figure 2.4 UPGMA cluster diagram of the genetic relationships of native range populations of *Taeniatherum caput- medusae* ssp. *asperum* and the 7 invasive range multilocus genotypes (indicated by boxes)

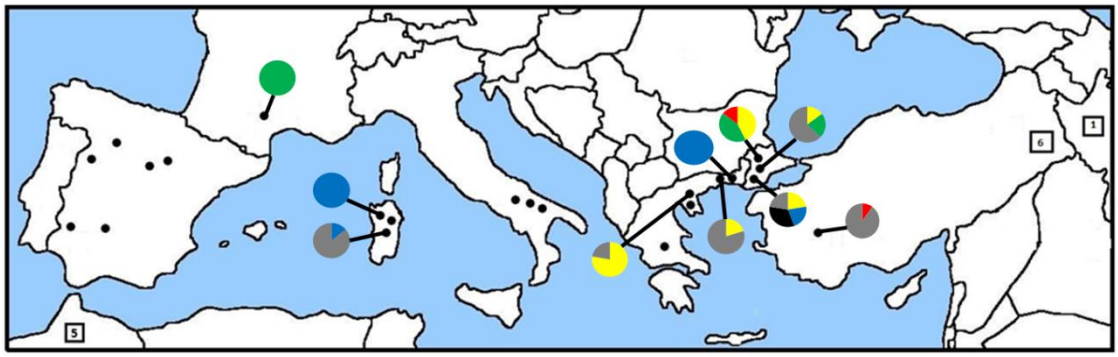

Figure 2.5 Native range map showing the distribution of invasive range multilocus genotypes found in native populations of *Taeniatherum caput-medusae*

APPENDIX

Allele Frequencies for All Polymorphic Loci Found in Native Populations of *Taeniatherum caput-medusae*

Alleles Found in Invasive Populations are Indicated with an Asterisk (*)

Locus	Allele	Pezneas les Mines, France	Poggorsini, Italy	Dorgali, Sardinia, Italy	Orosei, Sardinia, Italy	Lodine, Sardinia, Italy
MDH-1	*a*	-	-	-	-	-
	*b**	1.0000	1.0000	1.0000	1.0000	1.0000
MDH-2	*a**	-	-	0.8621	0.0400	1.0000
	b	-	-	-	-	-
	*c**	1.0000	1.0000	0.1379	0.9600	-
	d	-	-	-	-	-
	e	-	-	-	-	-
MDH-3	*a**	1.0000	1.0000	1.0000	1.0000	1.0000
	b	-	-	-	-	-
PGM-2	*a**	-	-	-	-	-
	*b**	1.0000	1.0000	1.0000	1.0000	1.0000
	c					
6PGD-2	*a*	-	-	-	-	-
	*b**	1.0000	1.0000	1.0000	1.0000	1.0000
GOT-1	*a*	-	1.0000	0.1379	1.0000	-
	*b**	1.0000	-	0.8621	-	1.0000
GOT-2	*a*	-	1.0000	-	-	-
	*b**	1.0000	-	1.0000	1.0000	1.0000
CE-2	*a*	-	-	-	-	-
	*b**	1.0000	1.0000	1.0000	1.0000	1.0000
	c	-	-	-	-	-
CE-4	*a*	-	-	0.7586	-	-
	*b**	1.0000	-	0.1034	1.0000	1.0000
	c	-	-	-	-	-
	d	-	1.0000	0.1379	-	-
	e	-	-	-	-	-
PGI-2	*a*	-	0.1000	0.1034	0.8800	-

Locus	Allele	Pezneas les Mines, France	Poggorsini, Italy	Dorgali, Sardinia, Italy	Orosei, Sardinia, Italy	Lodine, Sardinia, Italy
	b	-	0.9000	0.0345	0.0800	-
	*c**	-	-	0.8621	0.0400	1.0000
	*d**	1.0000	-	-	-	-
	e	-	-	-	-	-
ADH	*a**	1.0000	1.0000	1.0000	1.0000	1.0000
	b	-	-	-	-	-
GDH	*a*	-	0.9000	-	-	-
	*b**	1.0000	0.1000	1.0000	1.0000	1.0000
IDH	*a**	1.0000	-	0.8276	0.0400	1.0000
	b	-	1.0000	0.1724	0.9600	-
SKDH	*a*	-	-	-	-	-
	*b**	1.0000	1.0000	1.0000	1.0000	1.0000
G3PDH-2	*a*	-	-	-	-	-
	*b**	1.0000	1.0000	1.0000	1.0000	1.0000

Locus	Allele	Altamura, Italy	Minervino Murge, Italy	Timahdite, Morocco	Tizi n' tishka, Morocco	Tizi n' test, Morocco
MDH-1	*a*	-	-	-	0.0476	-
	*b**	1.0000	1.0000	1.0000	0.9524	1.0000
MDH-2	*a**	-	-	-	-	-
	b	-	-	-	-	-
	*c**	1.0000	1.0000	1.0000	1.0000	1.0000
	d	-	-	-	-	-
	e	-	-	-	-	-
MDH-3	*a**	1.0000	1.0000	1.0000	1.0000	1.0000
	b	-	-	-	-	-
PGM-2	*a**	-	-	0.1333	0.9524	-
	*b**	1.0000	1.0000	0.8667	0.0476	1.0000
	c					

Locus	Allele	Altamura, Italy	Minervino Murge, Italy	Timahdite, Morocco	Tizi n' tishka, Morocco	Tizi n' test, Morocco
6PGD-2	*a*	-	-	-	-	-
	*b**	1.0000	1.0000	1.0000	1.0000	1.0000
GOT-1	*a*	1.0000	1.0000	1.0000	1.0000	1.0000
	*b**	-	-	-	-	-
GOT-2	*a*	-	-	-	-	-
	*b**	1.0000	1.0000	1.0000	1.0000	1.0000
CE-2	*a*	-	-	-	0.0476	0.9091
	*b**	1.0000	1.0000	1.0000	0.8095	0.0909
	c	-	-	-	0.1429	-
CE-4	*a*	-	-	-	-	-
	*b**	-	-	1.0000	0.5882	0.1111
	c	-	-	-	0.4118	0.8889
	d	1.0000	1.0000	-	-	-
	e	-	-	-	-	-
PGI-2	*a*	-	-	-	1.0000	-
	b	1.0000	1.0000	-	-	-
	*c**	-	-	-	-	-
	*d**	-	-	1.0000	-	0.0909
	e	-	-	-	-	0.9091
ADH	*a**	1.0000	1.0000	1.0000	1.0000	1.0000
	b	-	-	-	-	-
GDH	*a*	1.0000	0.7586	-	-	-
	*b**	-	0.2414	1.0000	1.0000	1.0000
IDH	*a**	-	-	-	-	0.9091
	b	1.0000	1.0000	1.0000	1.0000	0.0909
SKDH	*a*	-	-	-	-	-
	*b**	1.0000	1.0000	1.0000	1.0000	1.0000
G3PDH-2	*a*	-	-	0.8333	0.5238	-
	*b**	1.0000	1.0000	0.1667	0.4762	1.0000

Locus	Allele	Tafraoute, Morocco	Tleta tassrit, Morocco	Monesterio, Spain	Canamares, Spain	Castellijo Martin de Viejo, Spain
MDH-1	*a*	-	-	-	-	-
	*b**	1.0000	1.0000	1.0000	1.0000	1.0000
MDH-2	*a**	-	-	-	-	-
	b	-	-	-	-	-
	*c**	1.0000	1.0000	1.0000	1.0000	1.0000
	d	-	-	-	-	-
	e	-	-	-	-	-
MDH-3	*a**	1.0000	1.0000	1.0000	1.0000	0.3143
	b	-	-	-	-	0.6857
PGM-2	*a**	0.2000	0.2083	-	-	-
	*b**	0.8000	0.7917	1.0000	0.8750	1.0000
	c			-	0.1250	-
6PGD-2	*a*	-	-	-	0.1250	0.2000
	*b**	1.0000	1.0000	1.0000	0.8750	0.8000
GOT-1	*a*	1.0000	1.0000	1.0000	1.0000	1.0000
	*b**	-	-	-	-	-
GOT-2	*a*	-	-	-	-	-
	*b**	1.0000	1.0000	1.0000	1.0000	1.0000
CE-2	*a*	-	0.8333	-	-	-
	*b**	1.0000	0.1667	1.0000	1.0000	1.0000
	c	-	-	-	-	-
CE-4	*a*	-	-	-	-	-
	*b**	1.0000	-	-	-	-
	c	-	1.0000	1.0000	1.0000	1.0000
	d	-	-	-	-	-
	e					
PGI-2	*a*	-	-	-	-	-
	b	-	-	-	-	-
	*c**	-	-	-	-	-

Locus	Allele	Tafraoute, Morocco	Tleta tassrit, Morocco	Monesterio, Spain	Canamares, Spain	Castellijo Martin de Viejo, Spain
	*d**	1.0000	0.1667	1.0000	0.1250	0.0286
	e	-	0.8333	-	0.8750	0.9714
ADH	*a**	1.0000	1.0000	1.0000	1.0000	0.3714
	b	-	-	-	-	0.6286
GDH	*a*	-	-	-	-	-
	*b**	1.0000	1.0000	1.0000	1.0000	1.0000
IDH	*a**	-	0.7500	1.0000	1.0000	1.0000
	b	1.0000	0.2500	-	-	-
SKDH	*a*	-	-	-	-	-
	*b**	1.0000	1.0000	1.0000	1.0000	1.0000
G3PDH-2	*a*	0.8000	0.2917	-	-	-
	*b**	0.2000	0.7083	1.0000	1.0000	1.0000

Locus	Allele	Villaviciosa de Cordoba, Spain	Robledillo, Spain	Pedraza de la Sierra, Spain	Biloris, Turkey	Eruh, Turkey
MDH-1	*a*	-	-	-	-	-
	*b**	1.0000	1.0000	1.0000	1.0000	1.0000
MDH-2	*a**	-	-	-	1.0000	1.0000
	b	-	-	-	-	-
	*c**	1.0000	1.0000	1.0000	-	-
	d	-	-	-	-	-
	e	-	-	-	-	-
MDH-3	*a**	1.0000	1.0000	1.0000	1.0000	1.0000
	b	-	-	-	-	-
PGM-2	*a**	0.3333	-	0.3333	-	-
	*b**	0.6250	0.8750	0.6250	1.0000	1.0000
	c	0.0417	0.1250	0.0417	-	-
6PGD-2	*a*	0.1667	0.1250	0.1667	-	-

Locus	Allele	Villaviciosa de Cordoba, Spain	Robledillo, Spain	Pedraza de la Sierra, Spain	Biloris, Turkey	Eruh, Turkey
	*b**	0.8333	0.8750	0.8333	1.0000	1.0000
GOT-1	*a*	1.0000	1.0000	1.0000	1.0000	1.0000
	*b**	-	-	-	-	-
GOT-2	*a*	-	-	-	-	-
	*b**	1.0000	1.0000	1.0000	1.0000	1.0000
CE-2	*a*	-	-	-	-	-
	*b**	1.0000	1.0000	1.0000	1.0000	1.0000
	c	-	-	-	-	-
CE-4	*a*	-	-	-	-	-
	*b**	-	-	-	1.0000	1.0000
	c	0.5417	1.0000	0.5417	-	-
	d	-	-	-	-	-
	e	0.4583	-	0.4583	-	-
PGI-2	*a*	-	-	-	-	-
	b	-	-	-	-	-
	*c**	-	-	-	1.0000	1.0000
	*d**	0.0833	0.1250	0.0833	-	-
	e	0.9167	0.8750	0.9167	-	-
ADH	*a**	1.0000	1.0000	1.0000	1.0000	1.0000
	*b**	-	-	-	-	-
GDH	*a*	-	-	-	-	-
	*b**	1.0000	1.0000	1.0000	1.0000	1.0000
IDH	*a**	1.0000	1.0000	1.0000	1.0000	1.0000
	b	-	-	-	-	-
SKDH	*a*	-	-	-	-	-
	*b**	1.0000	1.0000	1.0000	1.0000	1.0000
G3PDH-2	*a*	-	-	-	-	-
	*b**	1.0000	1.0000	1.0000	1.0000	1.0000

Locus	Allele	Buldan Junction, Turkey	Aliaga, Turkey	Dakili Junction, Turkey	Guzelkonak A, turkey	Sarigol, Turkey
MDH-1	*a*	-	-	-	-	-
	*b**	1.0000	1.0000	1.0000	1.0000	1.0000
MDH-2	*a**	1.0000	1.0000	1.0000	1.0000	0.0312
	b	-	-	-	-	-
	*c**	-	-	-	-	0.3750
	d	-	-	-	-	0.4062
	e	-	-	-	-	0.1875
MDH-3	*a**	1.0000	1.0000	1.0000	1.0000	0.8125
	b	-	-	-	-	0.1875
PGM-2	*a**	-	-	-	-	-
	*b**	1.0000	1.0000	1.0000	1.0000	1.0000
	c	-	-	-	-	-
6PGD-2	*a*	-	-	-	-	-
	*b**	1.0000	1.0000	1.0000	1.0000	1.0000
GOT-1	*a*	1.0000	1.0000	1.0000	1.0000	0.2812
	*b**	-	-	-	-	0.7188
GOT-2	*a*	-	-	-	-	-
	*b**	1.0000	1.0000	1.0000	1.0000	1.0000
CE-2	*a*	-	-	-	-	0.3750
	*b**	1.0000	1.0000	1.0000	1.0000	0.6250
	c	-	-	-	-	-
CE-4	*a*	-	-	-	-	-
	*b**	1.0000	1.0000	1.0000	1.0000	1.0000
	c	-	-	-	-	-
	d	-	-	-	-	-
	e	-	-	-	-	-
PGI-2	*a*	-	-	-	-	0.0938
	b	-	-	-	-	-
	*c**	1.0000	1.0000	1.0000	1.0000	0.9062
	*d**	-	-	-	-	-

Locus	Allele	Buldan Junction, Turkey	Aliaga, Turkey	Dakili Junction, Turkey	Guzelkonak A, turkey	Sarigol, Turkey
	e	-	-	-	-	-
ADH	*a**	1.0000	1.0000	1.0000	1.0000	1.0000
	*b**	-	-	-	-	-
GDH	*a*	-	-	-	-	0.0625
	*b**	1.0000	1.0000	1.0000	1.0000	0.9375
IDH	*a**	1.0000	1.0000	1.0000	1.0000	1.0000
	b	-	-	-	-	-
SKDH	*a*	-	-	-	-	-
	*b**	1.0000	1.0000	1.0000	1.0000	1.0000
G3PDH-2	*a*	-	-	-	-	0.1250
	*b**	1.0000	1.0000	1.0000	1.0000	0.8750

Locus	Allele	Ipsala, Turkey	Uzunkopru, Turkey	Havsa, Turkey	Xanthi, Greece	Sterea Hellas, Greece
MDH-1	*a*	-	-	-	-	-
	*b**	1.0000	1.0000	1.0000	1.0000	1.0000
MDH-2	*a**	0.8889	0.2308	0.3750	1.0000	-
	b	-	-	-	-	-
	*c**	0.1111	0.6923	0.6250	-	1.0000
	d	-	0.0769	-	-	-
	e	-	-	-	-	-
MDH-3	*a**	0.8889	0.8462	1.0000	1.0000	-
	b	0.1111	0.1538	-	-	1.0000
PGM-2	*a**	-	-	-	-	-
	*b**	1.0000	1.0000	1.0000	1.0000	1.0000
	c	-	-	-	-	-
6PGD-2	*a*	-	-	-	-	-
	*b**	-	-	1.0000	1.0000	1.0000
GOT-1	*a*	0.3889	-	0.0625	0.6667	-

Locus	Allele	Ipsala, Turkey	Uzunkopru, Turkey	Havsa, Turkey	Xanthi, Greece	Sterea Hellas, Greece
	*b**	0.6111	1.0000	0.9375	0.3333	1.0000
GOT-2	*a*	-	-	-	-	-
	*b**	1.0000	1.0000	1.0000	1.0000	1.0000
CE-2	*a*	-	-	-	-	-
	*b**	1.0000	1.0000	1.0000	1.0000	1.0000
	c	-	-	-	-	-
CE-4	*a*	-	-	-	-	-
	*b**	1.0000	1.0000	1.0000	1.0000	1.0000
	c	-	-	-	-	-
	d	-	-	-	-	-
	e	-	-	-	-	-
PGI-2	*a*	-	-	-	0.1333	-
	b	-	-	-	-	-
	*c**	0.5000	0.0385	-	-	-
	*d**	0.5000	0.9615	1.0000	0.8667	1.0000
	e	-	-	-	-	-
ADH	*a**	0.8333	1.0000	1.0000	1.0000	1.0000
	*b**	0.1667	-	-	-	-
GDH	*a*	-	-	-	-	-
	*b**	1.0000	1.0000	1.0000	1.0000	1.0000
IDH	*a**	1.0000	1.0000	1.0000	1.0000	1.0000
	b	-	-	-	-	-
SKDH	*a*	-	-	-	-	-
	*b**	1.0000	1.0000	1.0000	1.0000	1.0000
G3PDH-2	*a*	-	-	-	-	-
	*b**	1.0000	1.0000	1.0000	1.0000	1.0000

Locus	Allele	Askos/ Filadelphio, Greece	Panorama, Greece	Komotini, Greece	Iran 1
MDH-1	*a*	-	-	-	-
	*b**	1.0000	1.0000	1.0000	1.0000
MDH-2	*a**	0.8333	0.8667	1.0000	-
	b	-	-	-	-
	*c**	-	0.1333	-	1.0000
	d	0.1667	-	-	-
	e	-	-	-	-
MDH-3	*a**	1.0000	0.9333	1.0000	1.0000
	b	-	0.0667	-	-
PGM-2	*a**	-	-	-	-
	*b**	1.0000	1.0000	1.0000	1.0000
	c	-	-	-	-
6PGD-2	*a*	-	-	-	-
	*b**	1.0000	1.0000	1.0000	1.0000
GOT-1	*a*	0.2222	1.0000	0.0588	1.0000
	*b**	0.7778	-	0.9412	-
GOT-2	*a*	-	-	-	-
	*b**	1.0000	1.0000	1.0000	1.0000
CE-2	*a*	-	-	-	-
	*b**	1.0000	1.0000	1.0000	0.5612
	c	-	-	-	0.4388
CE-4	*a*	-	-	-	1.0000
	*b**	1.0000	1.0000	1.0000	-
	c	-	-	-	-
	d	-	-	-	-
	e	-	-	-	-
PGI-2	*a*	-	-	-	-
	b	-	-	-	-
	*c**	-	-	0.9412	-
	*d**	1.0000	1.0000	0.0588	1.0000

Locus	Allele	Askos/ Filadelphio, Greece	Panorama, Greece	Komotini, Greece	Iran 1
	e	-	-	-	-
ADH	*a**	1.0000	1.0000	1.0000	1.0000
	*b**	-	-	-	-
GDH	*a*	-	-	-	-
	*b**	1.0000	1.0000	1.0000	1.0000
IDH	*a**	1.0000	1.0000	1.0000	1.0000
	b	-	-	-	-
SKDH	*a*	-	-	-	-
	*b**	1.0000	1.0000	1.0000	1.0000
G3PDH-2	*a*	-	-	-	-
	*b**	1.0000	1.0000	1.0000	1.0000