Distribution of Genotypes within and among *Taeniatherum caput-medusae* (Poaceae) Populations from Eastern Washington: Assessment of an Invasion at a Local Scale

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Abstract

Multiple introductions from many source populations can increase the chance of introducing preadapted genotypes, increase standing genetic variation, and increase the likelihood of adaptive evolution, all of which might contribute to invasion. Taeniatherum caput-medusae is an annual, highly self-pollinating grass that is invasive in the western United States. Through previous genetic analysis, five multilocus genotypes (MLG) were detected in the relatively small geographic area of eastern Washington, which suggests that multiple introductions have occurred in this region. Fifty-seven populations of T. caput-medusae from eastern Washington were assessed at 23 loci using starch gel electrophoresis. Based on these data, a sixth MLG was detected in the study area. The Steptoe Butte genotype, which was first introduced in 1901, has the largest range of the six genotypes and is creating polymorphic populations where it comes into contact with other genotypes. Founder effects appear to have reduced genetic diversity within and among populations from eastern Washington in comparison to native populations. The results of this study contribute to our understanding of the invasion process at a local spatial scale by reconstructing the introduction and range expansion of T. caput-medusae in eastern Washington.

Introduction

Biological invasions are defined as the introduction, establishment, spread, and proliferation of an organism into a new and often distant range. In the past 200 years, invasions have been increasing due to human-mediated dispersal (Mack et al. 2000, Sala et al. 2000, and Wilson et al. 2009). Humans not only provide efficient means of transport over increasingly large geographical distances, but also favor invasions through increased homogenization of the ecology among geographically remote areas and the destruction of native habitats (Estoup and Guillemaud 2010).

Human-caused invasions have both ecological and economic consequences. The invasive species can intensify natural disturbance regimes. For example, invasive grasses foster more frequent and intense fires, which key native species are not adapted to (Mack et al. 2000, Novak 2007). Invasive species can also alter community composition by eliminating dominant species and altering ecosystem functions, such as nutrient cycling and primary productivity, which in turn allows newly arriving invaders to establish and spread (Mack et al. 2000, Lockwood et al. 2005). These ecological consequences incur heavy economic costs; the total cost of invasive species in the U.S. (plants, animals, microorganisms) exceeds $138 billion per year (Mack et al. 2000, Pimentel et al. 2005).

Propagule pressure is defined as the number of individuals (propagules) introduced into a new range. There are two components associated with propagule pressure: (1) propagule size, the number of individuals associated with a single introduction event, and (2) propagule number, the number of distinct introduction events. Both propagule size and number influence establishment success and increase the likelihood of invasion (Lockwood et al. 2005, Simberloff 2009). While *en route* to a new range, many immigrants perish, and once they have arrived at a new range, many more perish due to abiotic conditions and/or biotic agents (Mack et al. 2000). High propagule pressure increases the likelihood of establishment for those immigrants.

Human-mediated dispersal tends to contribute to multiple introductions (invasions with high propagule pressure), which in turn leads to higher levels of within-population genetic diversity. A growing body of evidence suggests that multiple introductions may be the rule rather than the exception (Novak 2007, Gaskin et al. 2013, Genton et al. 2005). Multiple introductions may increase the evolutionary potential of invasive populations challenging the classical view of low genetic diversity due to founder effects (Facon et al. 2008). Multiple introductions increase the likelihood of pre-adaptation and post-immigration evolution occurring by increasing the likelihood of introducing a pre-adapted genotype, and/or increasing the genetic diversity of invasive populations (Lavergne and Molofsky 2007).

Taeniatherum caput-medusae (Poaceae) is a highly selfing, Eurasian annual grass that is invasive in the western U.S. This invasion is well documented, and the first instance was recorded in 1887 (Figure 1).

Figure 1: Introduction history of Taeniatherum caput-medusae.

There are three subspecies in its native range: subsp. *crinitum*, subsp. *caput-medusae*, and subsp. *asperum*; only subsp. *asperum* is believed to be invasive in the U.S. Through previous genetic analysis seven multilocus genotypes (MLG) were detected among 45 invasive populations from the western U.S., indicating multiple introductions of this species into its invasive range. Five MLG were detected in Eastern Washington alone, indicating multiple introductions into this relatively small geographic area (Figure 2).

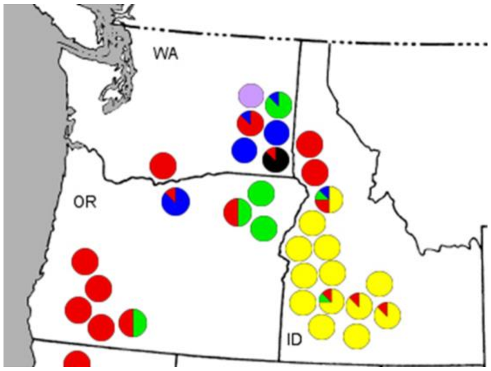

Fig. 2: Map showing the distribution of MLG in Washington, Oregon and Idaho. Note the presence of five colors (MLG) in Eastern Washington.

Studies examining the introductions of an invasive species generally are conducted at a large spatial scale (e.g. Gaskin et al. 2013, Huttanus et al. 2011), I propose to analyze 57 populations of *T. caput-medusae* at a local spatial scale in eastern Washington to better understand the genetic and evolutionary consequences of multiple introductions.

In this study we use enzyme electrophoresis to address three main goals to aid in the understanding of the effects of multiple introductions at a local spatial scale: (1) determine the distribution of genotypes within and among the 57 populations, (2) examine the level of genetic diversity within and among the populations, and (3) compare the level of genetic diversity with the rest of the western United States, native populations, and putative source populations.

Materials and Methods

Seeds previously collected were germinated at room temperature in petri dishes lined with moistened filter paper. Approximately 7-10 days after germination, they were harvested and macerated in a tris-HCl grinding buffer-PVP solution. Enzyme electrophoresis procedures generally followed Soltis et al. (1983), with modifications described by Novak et al. (1991). We will use 15 enzymes (ALD, GOT, CE, GDH, G3PDH, IDH, MDH, ME, PGI, PGM, 6-PGD, SKDH, SOD, TPI, and ADH) using four buffer systems. This data was used to determine the MLG of each individual within each population from eastern Washington, specifically Whitman and Spokane counties. This will allow us to map the MLG and look at the geographic distribution of different MLG within the study region and provide genetic evidence for multiple introductions and admixture.

All individuals in the 57 populations were scored by hand at 21 loci. Multilocus genotypes are based on five variable loci (Adh, Mdh-2, Pgi-2, Pgm-2, 6Pgd-2). We mapped these MLG to identify the potential geographic structuring of the populations. To measure the within population genetic diversity of these populations we calculated the number of alleles present, the number of polymorphic loci, the number of polymorphic populations (defined as a population with two or more MLG present), and the percentage of polymorphic populations. For the among population genetic diversity, we calculated the ratio of alleles per locus, percent polymorphic populations, observed heterozygosity and expected heterozygosity. Both the within and the among population genetic diversity values were compared to other populations from the invaded range (western United States) to determine whether multiple introductions to the small spatial area of eastern Washington changed the genetic diversity of the 57 analyzed populations. These values were also compared to populations from the native range as well as populations considered putative sources for the invasion into the western United States and eastern Washington.

Results

Mapping the multilocus genotypes of the 57 populations revealed a slight structure among the populations (Figure 3).

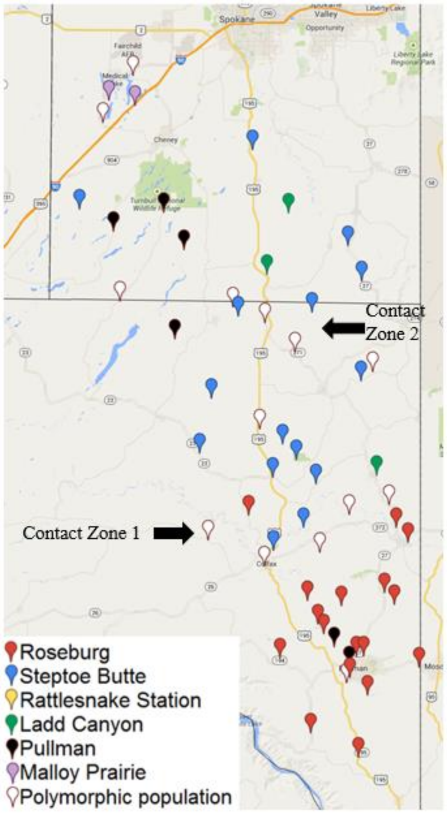

Fig.3: Map displaying the distribution of MLGs among 57 populations of *T. caput-medusae* analyzed in eastern Washington. The color of the marker corresponds with the populations' associated MLG. White markers correspond with polymorphic populations (populations with two or more MLGs present).

This map does reveal a possible dispersal corridor, U.S. Route 195, where the Ladd Canyon genotype, as well as several polymorphic populations, appear to be dispersed along the roadsides and adjoining areas. The Steptoe Butte MLG was likely introduced in 1901. The next documented introduction event was in Pullman in 1940, which most likely introduced the Roseburg genotype to eastern Washington. Roseburg and Steptoe Butte have expanded their ranges the most out of the six present MLG. At contact zone 1, there are four polymorphic populations, all of which have the Roseburg and Steptoe Butte MLG. At contact zone 2, there is a more complex array of polymorphic populations, all four of which contain the Roseburg MLG. Two also have the Steptoe Butte MLG, and the other two have the Ladd Canyon MLG. When the ranges of these genotypes expand and come into contact with one another, they will create polymorphic populations. The Malloy Prairie MLG is much more restricted and only occurs in the northwestern corner of our study area. This study revealed a sixth MLG, Rattlesnake Station, which was found in one population in Pullman, Washington, along State Route 27.

Our analysis of among population genetic diversity revealed a lower number of alleles in the eastern Washington populations than in the western United States, and the invaded range has lower numbers of alleles than native and putative source populations (Table 1).

Table 1: Genetic Diversity among Populations

	# Populations	# Alleles	# Polymorphic Loci	# Polymorphic Populations	% Polymorphic Populations
Eastern Washington	57	27	4	14	24.6
Western U.S.	45	28	5	17	37.8
Native Populations	34	48	15	23	67.6
Putative Source Populations	10	38	10	8	80.0

This trend continues with the other parameters used to evaluate the among population genetic diversity. The within population genetic diversity values indicate an overall lower diversity than the other areas compared (Table 2).

Table 2: Genetic Diversity within Populations

	# Populations	Alleles/Locus	% Polymorphic Populations	H_{Obs}	H_{Exp}
Eastern Washington	57	1.01	1.4	0.00020	0.005
Western U.S.	45	1.02	1.9	0.00010	0.004
Native Populations	34	1.10	9.1	0.00003	0.025
Putative Source Populations	10	1.14	12.2	0.00000	0.036

Our analysis of within population genetic diversity revealed a lower percentage of polymorphic populations in eastern Washington than in the western United States (1.4% and 1.9%, respectively). While the expected and observed heterozygosity in eastern Washington (0.005 and 0.00020, respectively) was slightly larger than the western United States (0.004 and 0.00010, respectively), this is not significant. These results highlight the low level of genetic diversity in the invaded range compared to the native range of *T. caput-medusae*.

Discussion

As previously mentioned, the Steptoe Butte MLG was initially collected in 1901 and has the largest distribution of all the genotypes in this study area. The multilocus genotype map shows the expansion of the Steptoe Butte MLG and the Roseburg MLG (likely introduced into Pullman, Washington, in 1940) and their mixture where the expanded ranges come into contact with other genotypes (contact zone 1 and 2). This map also helped identify a potential dispersal pathway, U.S. Route 195. If the contaminated products being transported along this route could be identified, it could help curb the spread of at least the Ladd Canyon MLG. Another potential dispersal pathway is State Route 27, the main north-south road through Pullman, Washington, and the site of introduction for the sixth multilocus genotype found in eastern Washington, Rattlesnake Station. The closest population containing the Rattlesnake Station MLG is in White Bird, Idaho, and its presence in Pullman is likely a case of long distance dispersal. In the summer of 2014, we visited some of these sites in Pullman again and discovered that *T. caput-medusae* plants are no longer present in the location where the individuals with the Rattlesnake Station MLG were collected. Thus, it is likely that this MLG no longer occurs in the study area, although this result requires further testing.

Multiple introductions tend to increase genetic diversity due to an increase of propagule pressure. Our results indicate a decrease in genetic diversity in the invaded range, which may, in part, be due to *T. caput-medusae's* high selfing rates, keeping it from forming novel genotypes by way of outcrossing. The main driver, however, for the low within and among genetic diversity in eastern Washington populations and populations throughout the western United States is founder effects. Eastern Washington only represents 6 out of 66 (9%) genotypes (including Rattlesnake Station) present in the native range, which is a steep decrease. The rest of the western United States contains 7 out of 66 (11%) genotypes present in the native range, indicating founder effects for the invaded range as a whole.

The aggregate effects of human-caused invasions threaten efforts to conserve biodiversity, maintain productive agricultural systems, sustain functioning natural ecosystems, and also protect human health (Mack et al. 2000). Humans not only provide efficient means of transport over increasingly large geographical distances, but also favor biological invasions by decreasing ecological differences between geographically remote areas (Estoup et al. 2010).

The control of invasions is a costly activity, exceeding $138 billion per year (Mack et al. 2000), but with research like this study, we can mitigate those costs through increased knowledge about the invasive organism. Lack of genetic variation decreases the chances of establishment during an introduction, and both multiple introductions and secondary introductions from highly variable introduced populations may greatly favor the establishment and persistence of new introductions. As a result, invasive-species management, such as biological control, may need to account for genetically diverse biological invasions resulting from multiple introductions (Kolbe et al. 2004). The identification of all genotypes in an invasion, which is likely for species that engage in uniparental reproduction (e.g., self-pollination or clonal propagation), allows more complete testing of the host-specificity of potential biological control agents, thus lowering the risk that there will be unexpected resistance or tolerance to introduced biological control agents (Gaskin et al. 2013). Elucidation of the routes of introduction of undesirable organisms is essential for the development of effective management strategies and sustainable science-based policies (Estoup et al. 2010). With this study we increased our knowledge of the potential dispersal pathways in eastern Washington as well as what genetic consequences multiple introductions may hold for invasive and native species populations and the potential to implement genotype specific biological control agents to control this invasive species.

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