Genetic analysis of native and introduced populations of *Taeniatherum caput-medusae* (Poaceae): implications for biological control

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Summary

Genetic analysis of both native and introduced populations of invasive species can be used to examine population origins and spread. Accurate delineation of an invasive species’ source populations can contribute to the search for specific and effective biological control agents. Medusahead, *Taeniatherum caput-medusae* (L.) Nevski, a primarily self-pollinating Eurasian annual grass that was introduced in the western USA in the late 1800s, is now widely distributed in California, Idaho, Nevada, Oregon, Utah and Washington. The goal of our current research is to assess introduction dynamics and range expansion of this grass in the western USA, and to identify source populations in the native range to facilitate the search for potential biocontrol agents. Across introduced populations, nine multilocus genotypes were detected, and we suggest a minimum of seven separate introduction events of *T. caput-medusae* in the western USA. Although range expansion appears to have occurred primarily on a local level, several introduced populations appear to be composed of admixtures of introduced genotypes. None of the native populations analysed to date possess the exact multilocus genotypes detected in introduced populations. We have recently begun screening Eurasian populations using intersimple sequence repeat (ISSR) genetic markers to determine whether this polymerase chain reaction–based technique can provide a higher degree of resolution for the identification of source populations.

Keywords: invasive grass, multilocus genotypes, multiple introductions.

Introduction

Experimental analyses of both native and introduced populations of invasive species can be used to assess various ecological, genetic and evolutionary aspects of invasion and the invasion process (Hierro *et al.*, 2005; Novak, 2007). For instance, comparison of the level and structure of genetic diversity within and among native and introduced populations can be used to determine whether the distribution of a species in its new range stems from single or multiple introduction events (Novak and Mack, 2001, 2005; Lavergne and Molofsky, 2007). Additionally, genetic analysis of native and introduced populations can be used to examine population origins and spread (Roderick and Navajas, 2003). Accurate delineation of an invasive species’ source populations (or regions) can contribute to the search for biological control agents. Indeed, the identification of areas of origin may reduce the economic cost of prospecting for agents and may result in the development of more specific and effective biological control agents (Gooolsby *et al.*, 2006a).

*Taeniatherum caput-medusae* (L.) Nevski, a member of the tribe Triticeae in the grass family, is considered a noxious weed in many western US states (e.g. Colorado, California, Oregon, Nevada and Utah). The grass was first collected in the USA in Roseburg, Oregon in 1887 (Fig. 1), and its collection history is well documented (McKell *et al.*, 1962; Young, 1992). The grass has now invaded millions of hectares of semi-arid ecosystems.
Genetic analysis of native and introduced populations of *Taeniatherum caput-medusae* (Poaceae) rangeland in the western USA (Young, 1992; Miller *et al*., 1999). It primarily occurs in areas disturbed by overgrazing and fire in the 25–100 cm annual precipitation zone, and it can become the dominant plant species at certain sites (Hironaka, 1961; Dahl and Tisdale, 1975; Young, 1992). Ominously, the species has probably not yet reached its ecological limit. If its ecological requirements approximate those of *Bromus tectorum* L., it has the potential to spread widely in the Great Basin of the USA and beyond. Different methods have been tried to control *T. caput-medusae* (burning, grazing, competition and herbicides), and all have generally resulted in failure (Horton, 1991).

The native range of *T. caput-medusae* includes much of Eurasia, where three distinct subspecies have been recognized (Frederiksen, 1986), but only *T. caput-medusae* ssp. *asperum* is believed to have been introduced into the USA (Young, 1992). Recently, foreign exploration was carried out for identifying candidates for biological control, and several plant pathogens were described, including the fungi, *Ustilago phrygica* Magnus and *Tilletia bornmuelleri* Magnus (Siegwart *et al*., 2003; Widmer and Sforza, 2004). A preliminary host range screening with *U. phrygica*, a systematic smut fungi that was collected in Turkey and attacks *T. caput-medusae*, has been conducted (Sforza *et al*., 2004).

Because natural enemy pressure can vary across genotypes (Evans and Gomez, 2003), populations and regions, the enemy release hypothesis is best tested by comparing introduced populations with native populations.

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**Figure 1.** Chronology of the spread of *Taeniatherum caput-medusae* in the western USA. This chronology was reconstructed using published accounts (see text), herbarium specimens and historical records.
that were the source of the invasion. When implementing biological control programs to reverse the release from natural enemies, knowledge of source populations or regions would increase the probability of finding highly specialized enemies (Goolsby et al., 2006b). In addition, evaluating the efficacy of candidate biological control agents should be done on the full range of genotypic diversity present within the introduced range (Gaskin et al., 2005), through the evaluation of specific polymerase chain reaction (PCR) primers when screening populations (Marrs et al., 2006).

The overall goals of our current research are to assess introduction dynamics and range expansion of *T. caput-medusae* in the western USA and to identify source populations in the species’ native range to facilitate the search for potential biocontrol agents. This research specifically addresses the following questions. (1) How many multilocus genotypes occur in western US populations of *T. caput-medusae*, and what is their geographic distribution? (2) What does the distribution of these genotypes indicate about range expansion of this species in its new territory? (3) How many genotypes occur in native range populations, and what is their geographic distribution? (4) Can source populations for the invasion of the grass in the western USA be identified?

**Methods and materials**

**Sampling of plant material**

One objective of our sampling has been to collect plant material from populations across the geographic distribution of *T. caput-medusae*, in both its invasive and native ranges. Another objective has been to obtain population samples at or near localities where the plant was first collected, or reported, during its invasion of the western USA (Fig. 1). Samples have been collected from a total of 45 populations in the states of California, Idaho, Nevada, Oregon, Utah and Washington, with several early collection localities represented.

Two groups of native range samples have been included in this study. The first group consisted of 23 populations, with 22 of these populations being accessions obtained from the USDA Plant Introduction Laboratory in Pullman, WA: 12 populations from Turkey, seven from Afghanistan, two from Iran and one from Kazakhstan. The personnel of the USDA Plant Introduction Laboratory variously classified these accessions to each of the three subspecies of *T. caput-medusae*. Only one of these 23 populations originated in Europe: Sterea Hellas, Greece. The second group consisted of 49 populations collected in August or September, 2002 and 2003, from across the grasses’ native range in Eurasia. For most populations, intact spikes were collected from 30 to 40 individual plants along a transect at approximately 1-m intervals and placed in separate envelopes. Seeds from the 49 Eurasian populations were brought back to the United States Department of Agriculture Agricultural Research Service European Biological Control Laboratory (USDA-ARS-EBCL) in Montpellier, France, on an official authorization (04LR011) granted by the French government. Seeds were stored in a quarantine greenhouse at the EBCL until further use.

**Enzyme electrophoresis**

The level and structure of genetic diversity within and among populations of *T. caput-medusae* in its invasive range in the western USA is based on the analysis of 1663 individuals from 45 populations. In the laboratory, one seed from each individual in a population was germinated on moistened filter paper in a Petri dish and harvested approximately 7 days after germination. Enzyme electrophoresis was conducted generally following the methods of Soltis et al. (1983), with modifications described by Novak et al. (1991). The 15 enzymes employed in this study were resolved with enzyme electrophoresis using four buffer systems (1, 6, 8 and 9), and these 15 enzymes were genetically encoded by 29 loci. Because *T. caput-medusae* is a diploid with low genetic diversity, the genetic basis of all allozyme variation observed was easily inferred based on known subunit structure and compartmentalization of these enzymes (Weeden and Wendel, 1989).

**ISSR analysis**

Five of the 49 Eurasian populations of *T. caput-medusae* mentioned above were selected for a preliminary analysis using intersimple sequence repeat (ISSR) genetic markers: one population from Morocco, Spain, France, Greece (Crete) and Turkey. For each population, five seeds were randomly selected from each of three plants located 5, 18 and 25 m along the transect from which they were sampled. In the EBCL quarantine greenhouse, seeds were germinated in Petri dishes with distilled water at 25°C, 80% relative humidity and 16:8 h light/dark. Ten days after germination, leaves were removed from the plants and frozen at -20°C. Total genomic DNA was extracted from frozen leaf material using DNeasy Plant Mini Kits according to the manufacturer’s instructions (Qiagen Inc., Valencia, CA). After extraction, DNA was amplified with the PCR using six ISSR primers described by Wolfe et al. (1998). Primer names and sequences are provided in Table 1. DNA amplifications were performed in 20 µl final reaction volumes containing 1 U of Taq DNA polymerase (Qiagen Inc.), 1x buffer (Qiagen), 1 mM MgCl₂, 0.2 mM of each deoxyribonucleotide triphosphates, 0.5 µM of a single primer and 2 µl of the template DNA. Amplifications were performed using the GeneAmp PCR System 9700 (Applied Biosystems, Forest City, CA) as follows: 94°C for 3 min, then 35 cycles at 94°C for 30s, 45°C for 45 s and and 72°C for...
for 1 1/2 min. A final extension was performed at 72°C for 7 min. Amplified products were electrophoresed on 1.0% agarose gels. Gels were stained with ethidium bromide, and DNA fragments were visualized with a UV transilluminator and photographed.

Data analysis

Allozyme multilocus genotypes were identified from enzyme electrophoresis data, and these genotypes were used to assess introduction dynamics and spread of *T. caput-medusae* in the western USA and to identify source populations of the grass in its native range. Allozyme multilocus genotypes are defined as the composite genotype over all loci examined and therefore are designated based on the identity of alleles at each scored enzyme locus. Populations were defined as genetically polymorphic if they contained two or more multilocus genotypes. As part of our preliminary analysis of native populations of *T. caput-medusae* using ISSR genetic markers, bands were not scored; however, we did qualitatively assess each primer to determine its utility for future analysis. Specifically, primers were evaluated based on whether they (1) did not generate bands in control reactions, (2) generated clear, distinct, darkly stained bands and (3) were polymorphic among test populations.

Results

Multilocus genotypes in the introduced range

Multilocus genotypes are named based on the populations in which they were first found. A total of nine multilocus genotypes were detected among all 45 populations: seven homozygous multilocus genotypes and two genotypes with one or two heterozygous loci (unpublished data, not shown). The seven homozygous genotypes were first detected in Roseburg, OR, Steptoe Butte, WA, Rattlesnake Station, ID, Ladd Canyon, OR, Pullman, WA, Malloy Prairie, WA and Salt Creek, UT. Five different multilocus genotypes were observed in the Palouse region of eastern Washington. The multilocus genotypes detected in Pullman, Malloy Prairie and Salt Creek appear to be restricted to just a single population. Heterozygous multilocus genotypes were found in two different populations: White Bird, ID contained two heterozygous genotypes and one of these genotypes was also detected at Emigrant Hill, OR. The level of polymorphisms within introduced populations was low: Only 17 of 45 populations (37.8%) contained two or more multilocus genotypes. Furthermore, of these 17 polymorphic populations, only three contained three or more multilocus genotypes.

Multilocus genotypes in the native range

Two distinct categories of multilocus genotypes were detected within the 23 Eurasian populations. The first group of genotypes were quite distinct and differed from those detected in the western USA at multiple loci. These genotypes were present in seven populations from Turkey, and all populations from Afghanistan, Iran and Kazakhstan. Based on their different enzyme banding patterns, plus their larger seed size, these populations probably all consisted of *T. caput-medusae* ssp. *crinitum*. The enzyme multilocus genotypes detected in the remaining populations in Turkey and the one from Greece were similar to those observed in the western USA, and these populations were tentatively assigned to *T. caput-medusae* ssp. *asperum*. However, none of the multilocus genotypes present in these 23 native populations was an exact match to those previously detected in the introduced range.

Preliminary ISSR analysis

Of the six ISSR primers that were screened in our preliminary analysis of native populations of *T. caput-medusae*, four met our selection criteria and will prove useful in future studies of both native and introduced populations (Table 1). Samples from the five countries (populations) display different DNA banding patterns with primer ISSR-17899A (Fig. 2), although no variability was detected among individuals within populations.

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**Table 1.** Identity and nucleotide sequences of the ISSR primers used in this preliminary analysis of *Taeniatherum caput-medusae* from its native range. ISSR primers used in this study were described by Wolfe et al. (1998). The utility of each primer, based on the criteria described in the text, is indicated: Y yes, N no.

<table>
<thead>
<tr>
<th>Primer</th>
<th>Primer sequence</th>
<th>Utility</th>
</tr>
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<tbody>
<tr>
<td>ISSR-17898A</td>
<td>(CA)7–AC</td>
<td>Y</td>
</tr>
<tr>
<td>ISSR-17898B</td>
<td>(CA)7–GT</td>
<td>N</td>
</tr>
<tr>
<td>ISSR-17899A</td>
<td>(CA)7–AG</td>
<td>Y</td>
</tr>
<tr>
<td>ISSR-17899B</td>
<td>(CA)7–GG</td>
<td>Y</td>
</tr>
<tr>
<td>ISSR-814</td>
<td>(CT)7–TG</td>
<td>Y</td>
</tr>
<tr>
<td>ISSR-HB15</td>
<td>GTG–GTG–GTG–GC</td>
<td>N</td>
</tr>
</tbody>
</table>
Discussion

Introduction dynamics and spread in the western USA

The level of genetic diversity observed across and within western US populations of *Taeniatherum caput-medusae* is lower than the mean value reported for other self-pollinating plant species (Hamrick and Godt, 1990) but similar to that of other invasive plants that exhibit a uniparental mode of reproduction such as selfing (Novak et al., 1991). Yet, despite its lack of genetic diversity, at least at the loci examined in this study, this species is now invasive over much of the semi-arid portions of the western USA.

The occurrence and geographic distribution of multilocus genotypes can provide insights into introduction dynamics and spread of invasive species (Novak and Mack, 2001, 2005). Multilocus genotype results for western US populations of *T. caput-medusae* are consistent with the pattern often associated with multiple introductions. Based on just the number of homozygous multilocus genotypes detected across all populations, we suggest a minimum of seven independent founder events. This conclusion is bolstered by the observation that four of the localities where these genotypes were detected are at or near early collection sites of the plant: Roseburg (1887), Steptoe Butte (1901), Rattlesnake Station (1930) and Ladd Canyon (1944).

The detection of five different multilocus genotypes in eastern Washington suggests that multiple introductions can occur within a relatively small geographic area. Because the plant was not collected in Utah until 1988, the detection of a unique multilocus genotype in Salt Creek, Utah may be evidence for a relatively recent introduction event. If so, these data indicate that introduction of this grass is ongoing. Our results for *T. caput-medusae* join a growing body of information indicating that, for invasive plant species, multiple introduction may be the rule rather than the exception (Novak and Mack, 2005) and may contribute to invasiveness (Allendorf and Lundquist, 2003; Lavergne and Molofsky, 2007; Novak, 2007).

The low level of polymorphisms observed within introduced populations of *T. caput-medusae* indicates that gene flow or dispersal among these populations is low. Thus, we conclude that spread or range expansion of the species has occurred mostly at the local or regional level and certainly has not been widespread. However, the detection of populations that appear to be admixtures of different introduced genotypes, as seen at several locations in the western USA (data not shown), suggests that intermixing of genotypes can take place if multiple introductions have occurred within the same region. Moreover, the detection of heterozygous multilocus genotypes suggests that plants with different genotypes and potentially originating from different introduction events have recently mated. Such hybridization events have been suggested to contribute to increased invasiveness in introduced species (Ellstrand and Schierenbeck, 2000).

Source populations

Although the multilocus genotypes observed in populations from Greece and several from Turkey are similar to those of the western USA, no exact matches were found among native populations. Thus, our allozyme analysis did not reveal the source populations (or regions) for the introduction of *T. caput-medusae* in the USA, but the data clearly excludes many of the southwest and central Asian locations from serving as

Figure 2. Photograph of DNA banding patterns obtained using ISSR primer 17899A. Note the different banding patterns for populations of *Taeniatherum caput-medusae* from different countries. Contents of the lanes on this gel are as follows: 1 1 kb ladder; 2–4 France; 5 and 6, Greece; 7 and 8, Morocco; 9–11, Spain; 12–14, Turkey; 15 PCR control; 16 extraction control.
source populations. These results probably stem from insufficient sampling in the native range, especially Europe: Only one of 23 native populations was from Europe, and the other 22 populations were collected from southwest or central Asia.

Additional analysis of European populations will be required before source populations, and introduction dynamics of the invasion of Taeniatherum caput-medusae in the western USA can be more confidently described. To this end, our future plans include allozyme analysis of the 49 additional Eurasian populations, which have already been collected. In addition, our preliminary analysis of native range populations of the grass using ISSR genetic markers is very promising and will hopefully provide us with PCR-based markers that possesses high degrees of polymorphism and resolution for identifying source populations.

Prospects for biological control

Foreign exploration for the identification of possible biological control agents has already led to the identification of several promising plant pathogens (Siegwart et al., 2003; Widmer and Sforza, 2004). The genetic analyses described in this paper are meant to complement this effort, and results of these analyses reveal the likelihood for biological control of Taeniatherum caput-medusae. Introduced populations of the grass are genetically depauperate; thus, we would anticipate fast genetic depauperate; thus, we would anticipate fast

Acknowledgements

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References


