

Genetic analysis of native and introduced populations of *Taeniatherum caput-medusae* (Poaceae)

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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. *Taeniatherum caput-medusae* (medusahead), a primarily self-pollinating Eurasian annual grass that was introduced into western United States 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 into western U.S., and to identify source populations in the native range to facilitate the search for potential bio-control agents. Across introduced populations, a total of nine multilocus genotypes were detected, and we suggest a minimum of seven separate introduction events of *T. caput-medusae* into western U.S. Although range expansion appears to have occurred primarily on a local level, several introduced population appear to be composed of admixtures of introduced genotypes. None of the native populations analyzed to date possess the exact multilocus genotypes detected in introduced populations. We have recently begun screening Eurasian populations using inter-simple sequence repeat (ISSR) genetic markers to determine whether this PCR-based technique can provide a higher degree of resolution for the identification of source populations.

Keywords: grass, multilocus genotypes; multiple introductions; *Taeniatherum caput-medusae*

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 (Goolsby et al. 2006a).

Taeniatherum caput-medusae, a member of the tribe Triticeae in the grass family, is considered a noxious weed in many western U.S. states (e.g. Colorado, California, Oregon, Nevada, and Utah). The grass was first collected in the U.S. in Roseburg, Oregon, in 1887 (Figure 1), and its collection history is well documented (McKell et al., 1962; Young, 1992). The abundance of *T. caput-medusae* increased greatly in the 1930s, and it has now invaded millions of hectares of semi-arid areas of intermountain rangelands in western U.S. (Young 1992). Its primary range includes areas disturbed by overgrazing and fire in the 25-100 cm annual precipitation zone, and it can become the dominant plant species on sites with high clay content and well-developed soils (Hironaka, 1961; Dahl and Tisdale, 1975; Young, 1992).

The grass can smother native plant species by producing a thick thatch, and its tissues accumulate high silica content. Consequently, it is virtually worthless as forage for cattle and sheep (Miller et al., 1999). *Taeniatherum caput-medusae* has substantially impacted

ecosystem functioning; its thick thatch leads to the production of high fine-fuel loads that have increased both the frequency and intensity of range fires. Following these events, *T. caput-medusae* and other non-native annual plants increase their dominance at a site. Ominously, the species has probably not yet reached its ecological limit. If its ecological requirements approximate those of *B. tectorum* it has the potential to spread widely in the Great Basin of the U.S., and beyond. Different methods have been tried to control *T. caput-medusae* including burning, grazing, plant competition, restoration of native plants, and herbicides; all have generally resulted in failure (Horton, 1991).

The native range of *T. caput-medusae* encompasses most of Europe, the arid northern rim of Africa, and southwest Asia through Turkey to Kazakhstan, Iran and Afghanistan. In Eurasia, three distinct subspecies of *T. caput-medusae* have been recognized (Frederiksen, 1986), but only *T. caput-medusae* ssp. *asperum* is believed to have been introduced into the U.S. (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* and *Tilletia bornmuelleri* (Siegwart et al., 2002; Widmer & Sforza, 2003). A preliminary host range screening with *Ustilago phrygica*, a systematic smut fungi that was collected in Turkey and attacks *T. caput-medusae*, was conducted in laboratory and field conditions and did not show any infectivity on barley, rye, oat, and wheat, but did control the weed (Sforza et al., 2004). Additional studies are required to verify specificity of these two pathogens. Because natural enemy pressure can vary across genotypes (Evans & Gomez, 2003), populations and regions, the enemy release hypothesis is best tested by comparing introduced populations with native populations 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 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* into western U.S., and to identify source populations in the species' native range in order to facilitate the search for potential bio-control agents. This research specifically addresses the following questions: 1) How many genotypes occur in western U.S. 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?, 5) Can source populations for the invasion of the grass in western U.S. be identified?

Methods and Materials

Sampling of plant material

One objective of our sampling has been to collect plant material from populations across the entire 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 western U.S. (Figure 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: 1) 23 populations, most of which consist of accessions from the USDA Western Region Plant Introduction Laboratory, Pullman, WA, and 2) 49 populations which were collected in August or September, 2002 and 2003, from across the grasses native range in Eurasia. For most populations, intact spikes were collected from 30-40 individual plants along a transect at approximately one-meter intervals, and placed in separate envelopes. Seeds from the 49 Eurasian populations were brought back to the USDA-ARS-EBCL laboratory 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

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 d 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 visualized using four buffer systems. 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 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, USA). Following extraction, DNA was amplified with the polymerase chain reaction (PCR) using six ISSR primers described by Wolfe et al. (1998). Primers names and sequences are provided in Table 1. DNA amplifications were performed in 20 µL final reaction volumes containing one unit of *Thermus aquaticus* DNA polymerase (*Taq*) (Qiagen Inc.), 10 X Qiagen *Taq* buffer, 1.0 mM MgCl₂, 0.2 mM dGTP, dATP, dTTP and dCTP each, 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, USA) set for 35 cycles of 30 s at 94°C, 45 s at 45°C and 1.5 min at 72°C. 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

western U.S., 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) generated clear, distinct, darkly stained bands, 2) were polymorphic among test populations, and 3) did not generate bands in control reactions.

Results

Multilocus genotypes in the introduced range

The level and structure of genetic diversity within and among populations of *T. caput-medusae* in its invasive range in western U.S. is based on the analysis of 1,663 individuals from 45 populations. Each individual was stained for 15 enzymes that were genetically encoded by 29 loci.

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. The heterozygous multilocus genotypes were found in two different populations: White Bird, ID, contained two heterozygous genotypes and one these genotypes was also detected at

Emigrant Hill, OR. The level of polymorphisms within introduced populations is low: only 17 of 45 populations (37.8%) contain 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

Twenty-three populations from the native range of *T. caput-medusae* were analyzed utilizing the same 29 enzyme loci as used in the analysis of introduced populations. Twenty-two of these populations consisted of 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. Only one population (Sterea Hellas, Greece) originated in Europe. The personnel of the USDA Plant Introduction Laboratory variously classified these accessions to each of the three subspecies of *T. caput-medusae*.

Two distinct categories of multilocus genotypes were detected within these 23 Eurasian populations. The first group of genotypes were quite distinct and differed from those detected in western U.S. 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 U.S., and these populations were tentatively assigned to *T. caput-medusae* ssp. *asperum*. However, none of the multilocus genotypes present in these 23 native populations were 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). Figure 2 is a photograph

showing PCR-amplified product from primer ISSR-17899A. Samples from the five countries (populations) display different DNA banding patterns with this primer, although no variability was detected among individuals within populations.

Discussion

Introduction dynamics and spread in western U.S.

The level of genetic diversity observed across and within western U.S. populations of *T. 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 U.S.

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 U.S. 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 U.S. (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 geneotypes observed in populations from Greece and several from Turkey are similar to those of the western U.S., 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* into the U.S., but the data clearly excludes many of the southwest and central Asian location from serving as source populations. These results probably stems 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 *T. caput-medusae* in the western U.S. 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 a PCR-based marker that possesses a high degree 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 (Widmer and Sforza, 2003; Siegwart et al., 2002). The genetic analyses described here are meant to compliment this effort, and results of these analyses are revealing concerning the likelihood for biological control of *T. caput-medusae*. Introduced populations of the grass are genetically depauperate, thus we would anticipate fast population build-up and spread of highly adapted biocontrol agents (Muller-Scharer et al, 2003). However, multiple introductions, the occurrence of some introduced populations that are genetic admixtures and the detection of low level of outcrossing within a few populations means that several biological control agents from different portions of the native range may be required (Burdon and Marshall, 1981). Thus, the accurate identification of source populations is needed to augment the exploration for biological control agents already taking place, and may result in the development of more specific and effective biological control agents for this highly destructive invasive species.

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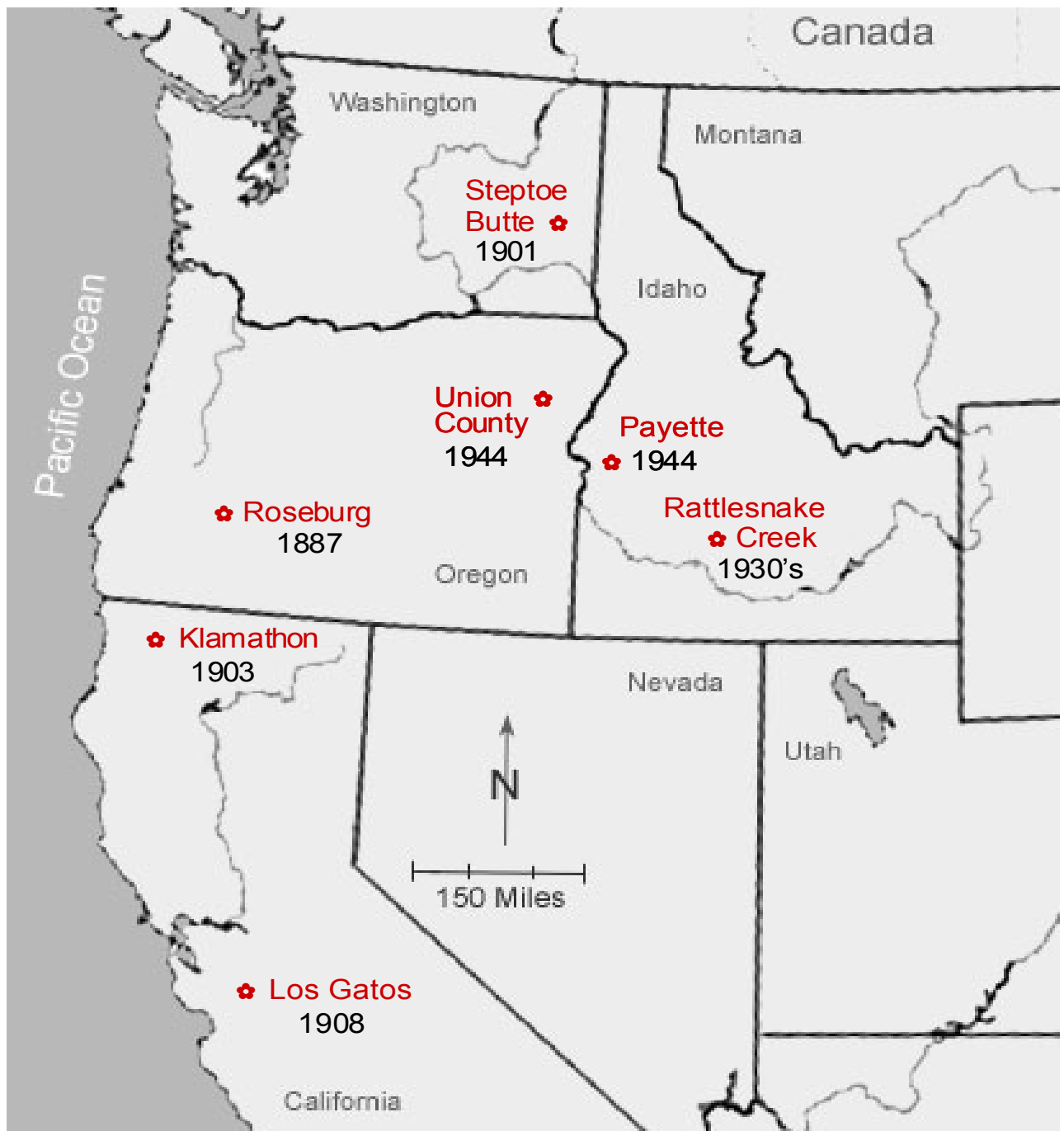


Figure 1. Chronology of the spread of *Taeniatherum caput-medusae* in the western U.S. This chronology was reconstructed using published accounts (see text), herbarium specimens, and historical records.

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, and N = no.

Primer	Primer sequence	Utility
ISSR-17898A	(CA) ₇ – AC	Y
ISSR-17898B	(CA) ₇ – GT	N
ISSR-17899A	(CA) ₇ – AG	Y
ISSR-17899B	(CA) ₇ – GG	Y
ISSR-814	(CT) ₈ – TG	Y
ISSR-HB15	GTG – GTG – GTG - GC	N

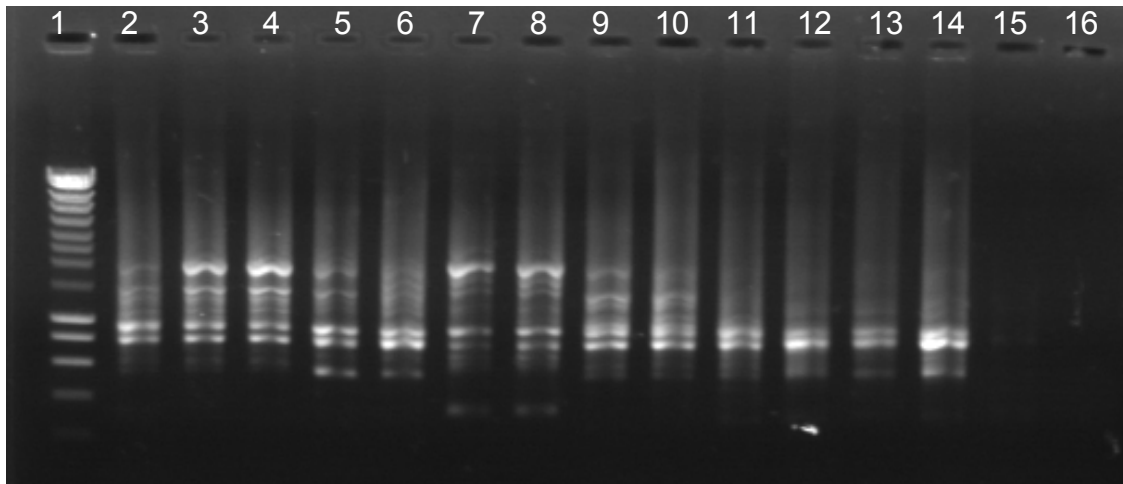


Figure 3. Photograph of DNA banding patterns obtained using ISSR primer 17899A. Note the different banding patterns for populations of *Taeniatherum caput-medusae* from different countries. Contents of the lanes on this gel are as follows: 1, 1 Kb ladder; 2 to 4, France; 5 and 6, Greece; 7 and 8, Morocco; 9 to 11, Spain; 12 to 14, Turkey; 15, PCR control; 16, extraction control.