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Molecular evidence for an extreme genetic bottleneck during introduction of an invading grass to California

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Abstract

Although exotic species cause tremendous economic and ecological loss, we know relatively little about the post-introduction evolutionary dynamics of the invasive species themselves. Barbed goatgrass, *Aegilops triuncialis* L., is a cleistogamous annual grass with a native range throughout the Mediterranean Basin and Asia and introduced to California during the last century. It is considered a serious noxious range weed and is one of the few exotic plant species that is invading serpentine soil habitats. We examined whether patterns of molecular variation are consistent with a single or multiple introduction events into California and further, if individual populations show evidence for a genetic bottleneck during introduction. Fingerprinting patterns, using microsatellite loci derived from *Triticum aestivum*, were investigated for 57 Eurasian accessions, broadly spanning the native range and for 108 individuals from 11 localities in California. There is strong evidence for an extreme bottleneck in this species as it colonizes its new range because we detected only three multilocus genotypes occurring in California and 36 genotypes in Eurasia. In California one of the genotypes differs from one other by only one fragment and only occurs in one individual. This suggests two separate introductions. Each population is composed of highly uniform individuals and the two main genotypes are geographically separated. *A. triuncialis* is still expanding its range in California despite genomic uniformity after a strong bottleneck and its recently increased rate of spread is not correlated to a high within-population variability created by multiple introductions.

Introduction

Biological invasions are increasingly common and a costly worldwide phenomena affecting nearly every natural and managed habitat and ecosystem. Exotic species cause tremendous economic loss, threaten native diversity, and modify ecosystem functions (Vitousek 1986, 1990; D'Antonio and Vitousek 1992; Lodge 1993; Simberloff and Schmitz 1997; Enserink 1999; Pimentel et al. 2000; Meyerson and Reaser 2003). Although increasingly expensive post-introduction control techniques have been developed (Louda et al. 1998), we know relatively little about the post-introduction evolutionary dynamics of the invasive species themselves (Sakai et al. 2001; Lee 2002). Many invasive species are ideal systems to study the ecological genetics of range expansion; these species are invading entirely new habitats and thus the time course for any observed evolutionary changes can be estimated from existing historical records.

The capacity of invasive plants to spread into new habitats despite extreme genetic bottlenecks

during introduction is both interesting and problematic. Reductions in additive genetic variance should reduce the capacity of introduced species to adapt to the new environment. Long term adaptive responses in an invasive species are more likely to occur if there exists genetic variation in ecological traits for selection to act upon (Baker 1965; Lee 2002; Sexton et al. 2002). The most obvious mechanism for increasing heritable (i.e., additive) quantitative genetic variation following an initial bottleneck is by reducing the severity of the bottleneck through multiple introductions (Novak and Mack 1995). When two or more genotypes that differ by state (i.e., diverged at some subset of their loci) are introduced, there is a potential for recombination to produce variation (Falconer and Mackay 1996; Ellstrand and Schierenbeck 2000). The more diverged the genotypes are at loci affecting ecological traits, the more variation will be generated. This is particularly important given that introductions involve overcoming dispersal limitations, and may bring isolated lineages together that were effectively reproductively isolated by some barrier in the native range (Sexton et al. 2002, Gaskin and Schaal 2002).

Perceptions that reduced genetic variation should limit spread have led to management approaches that attempt to prevent introduction of new genotypes (Mack and Erneberg 2002). This management strategy of maintaining a genetic bottleneck will not work if adaptation and invasive spread do not depend on the continued infusion of genetic variation from multiple introductions. Widely scattered and isolated small populations may characterize the initial distribution of a newly introduced species and so the potential for multiple introductions at a particular site may be low. In addition, many weeds are cleistogamous and disperse effectively only by seed. In these newly established populations, lineages persist through selfing and therefore recombination is not effective in generating new genotypes because of the homozygous genome.

Molecular markers are frequently applied to investigate the population structure of invasive species, to reveal the introduction history and the degree of genetic variation (i.e. Lee 2002; Walker et al. 2003). Here we investigate the degree of molecular genetic variation within and among the populations of *Aegilops triuncialis* invading into serpentine soil communities in California.

Barbed goatgrass, *Aegilops triuncialis* L., is a cleistogamous annual grass with a native range throughout Southern Europe, the Mediterranean Basin, and Asia (Van Slageren 1994). There are numerous species of invasive Eurasian annual grasses in California and barbed goatgrass shares many characteristics with them including large seed size, distinctive adaptations to herbivory and animal dispersal, and winter–annual growth habits. *A. triuncialis* was first collected in California in 1914 (Kennedy 1928) in an area recently grazed by Mexican cattle and had since then continued its range expansion (Jacobsen 1929; Talbot and Smith 1930). It is now considered a serious noxious range weed due to its poor palatability for livestock (Peters et al. 1996). It is also one of the few exotic plant species that is invading and attaining high cover on serpentine soil habitats (McKay et al. in review). This is a concern from a conservation perspective, as serpentine habitats in California are islands of native plant endemism (Kruckeberg, 1984).

A. triuncialis is a allotetraploid derived from the diploid species *A. umbellulata* and *A. caudata* (Dubcovsky and Dvorak 1994; van Slageren 1994). It has been shown that *A. triuncialis* probably originated independently at least twice, strains with maternal *caudata* genome as well as strains with maternal *umbellata* genome are indicated (Murai and Tsunewaki 1986; Wang et al. 1997; Vanichanon et al. 2003). Allopolyploid species, especially with multiple origins can show a higher within species genetic diversity and may have greater ability to adapt through additive genetic variance or epigenetic change. Consistent with this is the observation that allopolyploid species tend to have a wider distribution than their diploid progenitors and have a higher ability to establish in more extreme habitats (Soltis and Soltis, 2000).

The genus *Aegilops* is closely related to cultivated wheat, *Triticum aestivum*. In many treatments, *Aegilops* and *Triticum* species are considered congeneric and the wild races of *Aegilops* species are treated as critical genetic resources for developing *Triticum* strains with greater pathogen resistance (Bandopadhyay et al. 2004). Unlike other *Aegilops* species, *A. triuncialis*

is not yet widely naturalized outside of its native range.

In this study, we have taken advantage of the large collections of goatgrass germplasm available to assess the reduction of genetic variation in California *A. triuncialis* populations relative to genetic variation in the native species range. We performed fragment analysis using primers designed for STRs in wheat to estimate the degree of genetic variability within *A. triuncialis* in California relative to the genetic diversity of this species in its Eurasian home range. A primary goal of this study was to determine if a strong genetic bottleneck, induced by founder effects during introduction, has occurred in Californian populations. Further, we examined whether patterns of molecular variation are consistent with single or multiple introduction events. This information will allow a better assessment of the importance of the infusion of new genetic variation in promoting the continued spread of *A. triuncialis* in its new range.

Materials and methods

Plant material sources

Fifty-seven Eurasian accessions from 13 countries that broadly span the native range of *A. triuncialis* were investigated and compared with 108 individuals from 11 localities in California. Eurasian samples were obtained as accessions from the USDA National Small Grains Collection while Californian samples were from field collections (Table 1 and Figure 1). Plants from these seed samples were grown in a common environment and fresh leaf tissue was collected, frozen, lyophilized, and ground for DNA extraction. For outgroups in the study, we included three samples of *A. caudata* and two of *A. umbellulata* (the two diploid progenitors of the allotetraploid *A. triuncialis*) as well as three samples from *Triticum aestivum*.

DNA extraction and amplification

Freeze-dried leaf tissue (3–4 young leaves) was ground for 10 min in a modified paint shaker using five 1.9 mm ball bearings in 2 ml Eppendorf tubes. After grinding, 250μl of extraction buffer (350 mM Sorbitol, 100 mM of Tris, and 5 mM EDTA) was added to approximately 30 mg of the ground leaf sample and gently mixed. To each sample, 250μl of lysis buffer (200 mM of Tris buffer, 50 mM EDTA, 2 M NaCl and 2% of CTAB) was added on ice and after short incubation 50 μl of 10% Sarkosyl was added. After samples were incubated in a 60 °C water bath for 45 min, 500μl of chloroform was added and phases were separated by spinning at 25,000 g for 20 min. The DNA was precipitated by adding 625 μl of isopranol and 3 μl of 3 M sodium acetate to the supernatant, left at least 12 h in −20 °C, and then centrifuged at 25,000 rpm at 4 °C for 15 min. The pellet was washed with 70% ethanol, dried and re-suspended in 250 μl of TE buffer (pH 8.0). DNA yield was quantified using an Eppendorf BioPhotometer and diluted to 50 ng/μl with dd H_2O. We used 1μl of this solution for amplification (50 ng of template DNA).

All PCR reactions was set up in 25 μl volumes containing 2.5μl 10X Taq reaction buffer, 3 mM $MgCl_2$, 2.5 mM DNTPs, 0.4 μM of each primer, 0.5 unit of Taq DNA polymerase (Fa Qiagen) using the following temperature profile: initial step of 95 °C for 3 min, 35 cycles of denaturation for 45 s at 94 °C, annealing for 45 s at the respective annealing temperature (Table 2), elongation for 1 min at 72 °C, and a final extension step for 10 min at 72 °C. Aliquots from PCR reactions (10 μl) were loaded on a 4% Metaphor agarose gel and run for 2–4 h at 90 mV. Gels were stained for 20 min in ethidium bromide solution (10 mg/mL), de-stained in dd H_2O, and viewed using Kodak 1D software of the Gel Logic 100 system.

Primer selection and analysis of polymorphism

Of a preliminary screening of 100 primer pairs available from the Grainbank database (http://wheat.pw.usda.gov/GG2/index.shtml), nine primer pairs were selected as having discrete and reproducible bands (Table 2). Optimal annealing temperatures (Table 2) were determined for these primer pairs using gradient PCR. For fragment analysis, PCR bands were separated using 4% Metaphor agarose gels or on an ABI 3110 sequencer using labeled forward primers (for

Table 1. List of accessions, amount of samples, region of locality and sample names of Eurasian samples investigated in this study.

Country	N	Region	Accession	Plantid	Sample name
Afghanistan	7	Baghlan	276992	2506	E 50
		Badakhshan	215781	12819	E 53
		Faryab	220329	548	E 48
		Herat	317395	422	E 47
		Kondoz	220328; 317396	476; 1514	E 51–52
		Samangan	317399	1592	E 49
Bulgaria	1		574474	B92–37	E 16
Cyprus	3		483029; 483027; 483037	CC180; CC100; CC160	E 20–22
Greece	9	Macedonia	551195; 551205; 551199; 551196	JM-3690; JM-3730; JM-3705; JM-3692	E 04, E 8–10
		Thessaly	551193; 551182	JM-3674; JM-3626	E 03, E07
		Central Greece	551213	JM-3768	E 12
		Peloponnese	551250; 551239	JM-3981; JM-3902	E 05–06
Iran	5	East Azerbaijan	250696; 223321; 250908	K1512; 1636; K1596	E 42–44
		Fars	226501	14681	E 46
		Khuzestan	227291	14604	E 45
Iraq	2	North Iraq	219862; 219866	68; 69	E 39–40
Italy	1	Sicily	524957	3602	E 01
Romania	1	Cluj	361882		E 55
Spain[a]	4	Huelva			3536–3537
		Toledo			3535
		Sevilla			3538
Syria	2		487246; 487242	SY 20199; SY 20165	E 29–30
Turkey	18	Adiyaman	542345	84TK077-044	E 31
		Agri	486298	79TK085-440	E 41
		Ankara	573451	84TK329-009	E 23
		Bitlis	560548	Toslick	E 34
		Bursa	573455	84TK237-001	E 19
		Cankiri	573492	84TK302-001	E 24
		Denizli	542325	84TK158-034; 84TK157-007	E 15, E 17
		Elazig	554339	79TK019-084	E 33
		Hakkari	173615; 560769	7883; TU86-40-02	E 36–37
		Kars	486299	79TK094-479	E 35
		Malatya	486287	79TK014-065	E 32
		Manisa	542313; 542312	84TK151-021; 84TK150-003	E 13–14
		Sinop	573505	84TK314-002	E 26
		Usak	542310	84TK148-001	E 18
		Van	486280	79TK047-280	E 38
Ukraine	3	Krym	614632; 614630; 614635	UKR-99-112a; UKR-99-003; UKR-99-204b	E 25, E 27–28
Yugoslavia	1	Montenegro	374366	51/71	E 02

[a]Samples from Spain were provided from Consuelo Soler, Department of Biotechnology, INIA, La Canaleja, 28800-Alcalá de Henares

primers 526, 257 and 126). Fragment lengths for agarose gels were quantified by using the Kodak 1D software system (Scientific Imaging Systems, Eastman Kodak Company 1994–2002) while polyacrylamide gels were analyzed using the STRand – Nucleic Acid Analysis Software (http://www.vgl.ucdavis.edu/informatics/STRand/). As internal standards for each lane, a 100 bp ladder (New England BioLabs) was used for the agarose gels and a Rox-labeled standard ladder (Applied Biosystems) was used for the polyacrylamide gels. We attempted to sequence all polymorphic bands to estimate the molecular basis of the polymorphism.

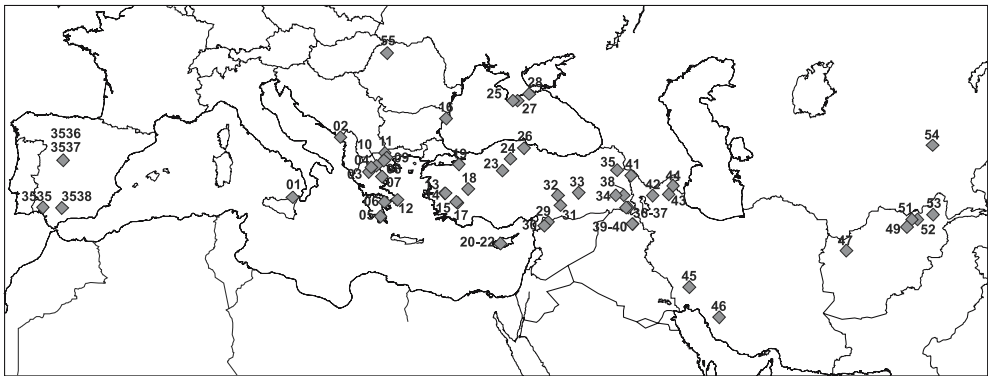

Figure 1. Map of Eurasia showing the location of Eurasian samples investigated. Sample names have the appendix E in the text.

Table 2. Californian Populations sampled.

Location	N
Bear Creek	3
Bear Valley	32
County line	3
Hopland	16
Jepson Prarie	3
Mather Field	3
Malby Crossing 1	3
Malby Crossing 2	3
McLaughlin	20
Sierra Shubert	3
Snell Valley	19

The fragments of all primers were scored as presence / absence data and cluster analysis was performed using the NTSysPC program package (Applied Biostatistics Inc.; Rohlf 2000). For distance estimation, the Dice-Coefficient (an equivalent metric to Nei and Li's Distance; Nei and Li 1979) was used (Simqual module) and distances among samples were determined by UPGMA. For the Eurasian samples, the Dice-Coefficient distance matrix was compared to a matrix of geographical distances using the matrix comparison module. Matrices were compared with Mantel test statistics that were calculated using NTSysPC.

Results

Transferability of markers

Markers used in this study were putative microsatellite containing amplicons from the wheat genome, with primers originally developed for *T. aestivum* (Roder et al. 1998; Thuillet et al. 2002). PCR products of the primers DWM 023, 642, 582, 645, and 312 were sequenced to determine if a microsatellite motif was also present in *Aegilops* and to confirm homology of the region to *T. aestivum* loci. None of the sequences obtained included a microsatellite motif. For most primer pairs it was not possible to verify the homology of amplified fragments to the original target loci. Only one of the loci studied is published as sequence (loci DWM 312, gi:9230766; Thuillet et al. 2002). The sequence of DWM 312 from *A. triuncialis* only differs in two positions from the *T. aestivum* sequence, but the microsatellite motif (GA_{30}) in *T. aestivum* is deleted and reduced to one single GA in *A. triuncialis*. In *T. aestivum* two fragments approximately 190 and 210 bp in length were determined in the fragment analysis. The fragments found for *Aegilops* were 175 and 170 bp long, whereby the 175 bp fragment has been used for sequencing. Thus, the length polymorphism within *A. triuncialis* is probably attributed to a polymorphism not correlated to the microsatellite motif. Primers 526 and 126 showed a pattern that indicated a length polymorphism typical for dinucleotide repeats. The length of fragments differed by a multiple of two bp from each other. In addition the length of the most frequent fragments was within the range of the length of *T. aestivum*. Primer 526 produced a fragment of 100 bp for *T. aestivum* and 118–148 for *Aegilops*, primer 126 produced fragments of 193 and 191 bp for *T. aestivum* and 185 and 181 for *Aegilops*. As no

sequence information was available from wheat for either marker, sequencing was not performed for the *Aegilops triuncialis* amplicons.

Patterns of genetic diversity

All of the primers studied generated reproducible banding patterns with up to ten fragments visible in a gel. Since at least one, and probably several of the markers studied did not contain a microsatellite motif, length polymorphism could result from more than a single deletion or insertion in a marker. As a result, the fragments were coded in a presence / absence matrix for similarity analyses. Between 2–10 polymorphic bands per primer were included in the matrix (Table 2) and the matrix consisted of 48 polymorphic positions and a total of 173 samples (165 *A. triuncialis* and eight outgroup samples).

Of these 19 Fragments were amplified only in the outgroup samples and 14 only in *A. triuncialis*. Fifteen fragments were amplified in both the outgroups and *A. triuncialis*. Of the 29 fragments present in *A. triuncialis*, 19 were found in Californian samples and 28 in Eurasian accessions. The fragments generated unique banding patterns indicating a total of 38 different genotypes in our *A. triuncialis* survey. Of these genotypes, only three occurred in California and, except for the McLaughlin site, each California population contained only a single genotype. Within the McLaughlin population there was one sample (336) that had a reproducible autapomorphic fragment and was therefore treated as a unique genotype.

Distance Analyses

In the distance analysis, all the *A. triuncialis* samples formed a single cluster and were clearly separated from *A. caudata* and *A. umbellulata* samples. The Californian samples were split into two groups each with a zero distance within groups. One group included California population samples from Bear Valley, Hopland, Snell Valley, and Jepson Prairie as well as four individuals from Uzbekistan, Turkey, and Iran. The other group contained the remaining seven California population samples from McLaughlin, Bear Creek, County Line, Sierra Shubert, Math-

Table 3. Loci and Primers used in this study and number of polymorphic fragments scored in the analysis. All loci are according to Roder et al. (1998) and Thuillet et al. (2002) and available at the GrainGenes database.

Marker	Primer F	Primer R	Tm (°C)	N polymorphic Bands	N polymorphic Bands (*A. triuncialis*)
23	TTG CTC CGA TGT AAT AAG CG	GGC TAA GAA CAG ACT CAT TCA ACT G	55	3	1
312	ATC GCA TGA TGC ACG TAG AG	ACA TGC ATG CCT ACC TAA TGG	57	5	3
642	CAT GAA AGG CAA GTT CGT CA	ACG GCG AGA AGG TGC TC	55	9	5
582	TCT TAA GGG GTG TTA TCA TA	AAG CAC TAC GAA AAT ATG AC	51	5	3
526	CCA ACC CAA ATA CAC ATT CTC	CAA TAG TTC TGT GAG AGC TGC G	56	6	5
257	CCA AGA CGA TGC TGA AGT CA	AGA GTG CAT GGT GGG ACG	57	2	0
126	CAC ACG CTC CAC CAT GAC	GTT GAG TTG ATG CGG GAG G	58	10	7
645	TGA CCG GAA AAG GGC AGA	AGC TTT GCT CTA TTG GCG AG	56	6	3
666	TGC TGC TGG TCT CTG TGC	GCA CCC ACA TCT TCG ACC	58	2	2
Sum				48	29

er Field, and Malby Crossing 1 and 2. The single sample from the McLaughlin population that showed an autapomorphic fragment was positioned next to the main cluster in the UPGMA. Sequences of both clusters showed a similarity of 0.85 to each other (Figure 2), indicating that they were divided by five positions within three different primer assays. This cluster, which includes the original site of introduction of *A. triuncialis* in California is most similar to genotypes in Iran and Greece.

Diversity among the Eurasian samples was much higher. In all, 36 different genotypes were distinguished for the 57 samples with a minimum similarity of 0.50 (Dice-coefficient). Eurasian samples are placed within several clusters; one of these Eurasian clusters is positioned between the Californian clusters (Figure 2).

The Mantel test detected a significant correlation ($r = 0.25$; $p = 0.0003$) between similarity of banding pattern and geographical distance among Eurasian samples (Figure 3a). However, there is no obvious clustering of the genotypes according to geographical origin of the seed samples. Each cluster contains samples from all over the range of *A. triuncialis* and some sub-clusters with highly similar banding patterns contain individuals from a wide range of locations. Moreover, several samples with identical banding patterns originate from localities that are geographically distant from each other (Figure 3b). For example two genotypes were found both in Greece and in Southern Anatolia, suggesting a parallel establishment of different genotypic lineages.

In California the genotypes present are more highly restricted to geographical regions within the new range of *A. triuncialis*. Four of five populations situated in the coastal and delta regions along the western edge of the Central Valley all contain a single genotype. These localities (Bear Valley, Hopland, Snell Valley, and Jepson Prairie) are between 45 and 130 km from each other. All populations located in the Sierra foothills or eastern edge of the Central Valley, (Mather Field, County Line, Malby Crossing, Bear Creek and Sierra Shubert) all contain an alternate single genotype (Genotype 2). The only exception to this regional segregation of genotypes is the McLaughlin site that is located in the Coast Range but contains individuals with the western (Genotype 2) banding pattern (Figure 3c).

Discussion

The aim of this study was to access the level of genetic diversity in populations of *A. triuncialis* that are currently invading California and compare the genetic diversity of invading populations to the level of diversity in the native range of *A. triuncialis*. Based on this comparison, we found evidence for an extreme bottleneck in this species as it colonizes its new range in California. Pester et al. (2003) showed in a recent analysis of RAPDs and AFLPs on *A. cylindrica* L. and *A. triuncialis* that this method yielded only a very small number of polymorphic bands, possibly because these are recently formed allopolyploid species. Although RAPD markers in *A. triuncialis* were more polymorphic than *A. cylindrica*, we chose to investigate the utility of using clearly defined microsatellite markers from the intensively studied close relative, *T. aestivum* (Roder et al. 1998; Thuillet et al. 2002). Even if these sites in *A. triuncialis* do not contain microsatellites, the probability of a high intraspecific variability of these loci seems very likely with a lower probability of scoring homoplasic fragments than with methods based on a random amplification approach. We were able to create a marker presence / absence matrix that allowed a clear differentiation of most Eurasian accessions and this approach was also sufficient to determine genotypes within the introduced range in California. We are currently identifying additional polymorphic microsatellite loci as well as single nucleotide polymorphisms within *A. triuncialis* to refine our analysis of the potential Eurasian origins of barbed goatgrass introduced into California.

Distribution of genotypes in Eurasia

Our results delineate *A. triuncialis* as a distinct cluster of genotypes, clearly distinct from the presumed progenitor species *A. umbellulata* and *A. caudata*. Despite a correlation between the similarity matrix and a matrix of geographical distances, the occurrence of highly dissimilar

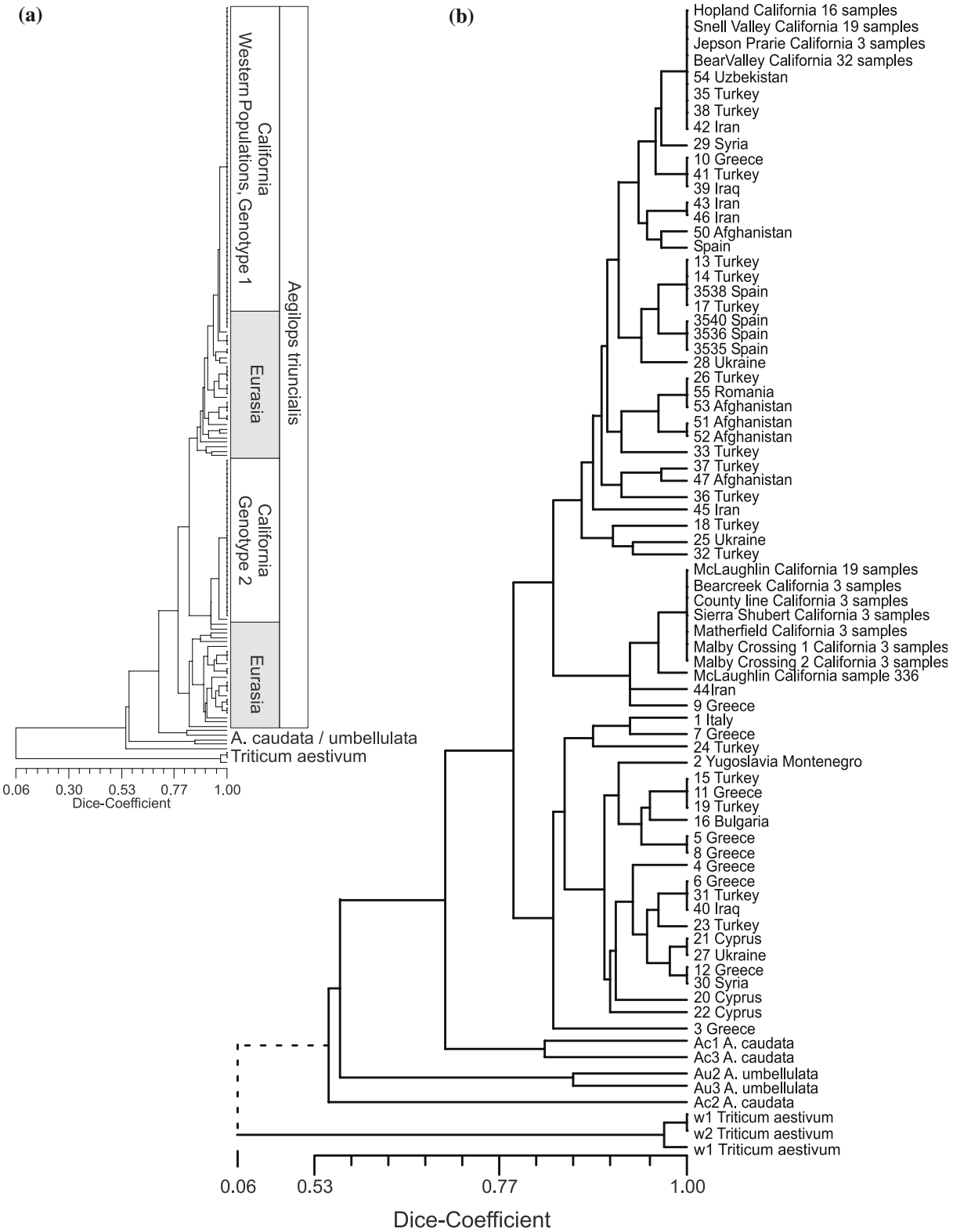

Figure 2. UPGMA of Dice similarity matrix derived from fragment analysis: (a) from every samples, (b) only one sample from each Californian Population shown.

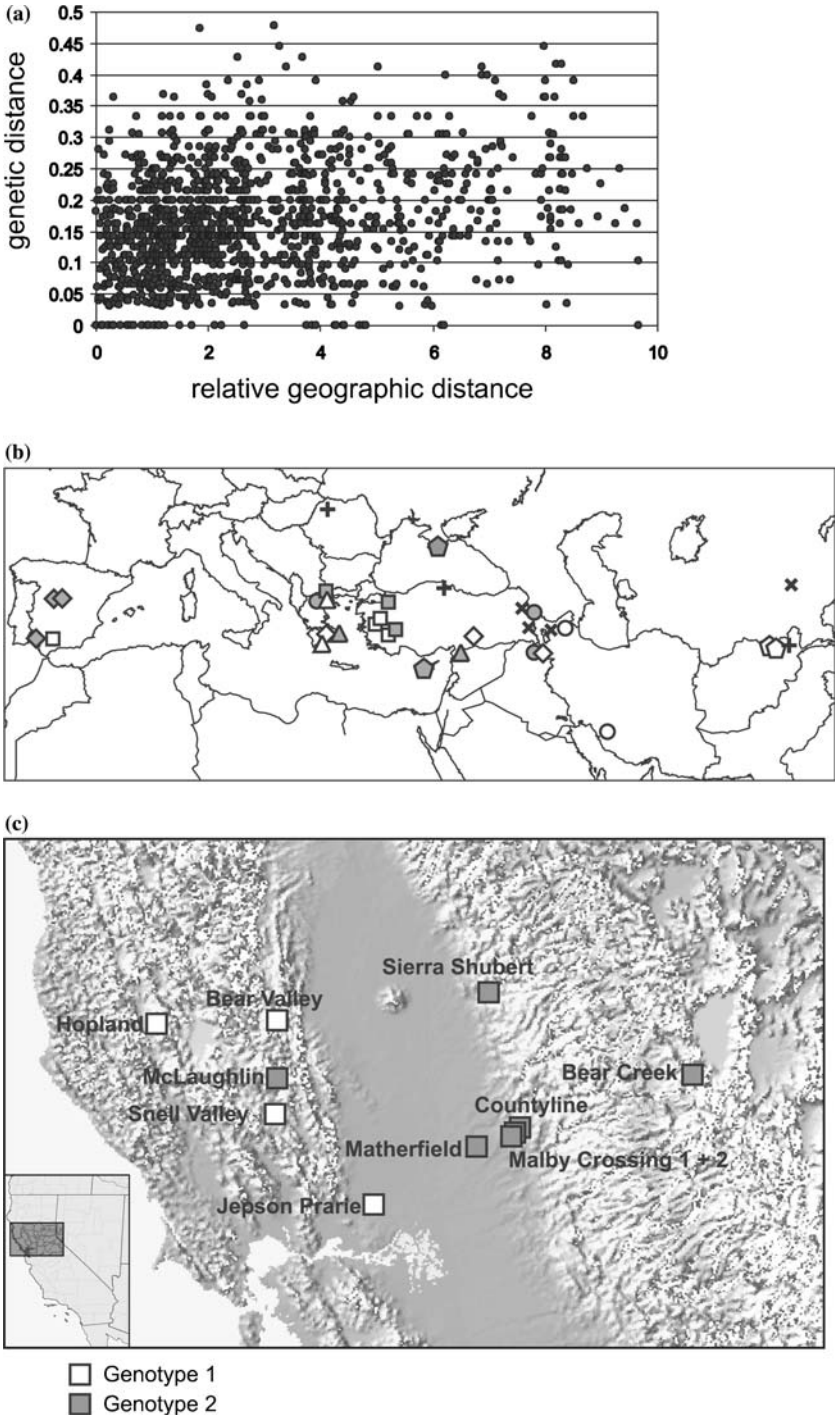

Figure 3. (a) Matrix correlation blot of geographical distances (relative) and dissimilarity (according to Dice) of the Eurasian samples. Correlation coefficient is $r = 0.25$. (b) Geographical distribution of genotypes found in Eurasia, which samples had 0 distance to each other. (c) Geographical distribution of genotypes of *A. triuncialis* found in California.

genotypes in the same region and the wide geographic distribution of some very similar genotypes was pronounced within our samples. For example, identical genotypes occurred in Cyprus, the Ukraine, Northern Afghanistan, Rumania, Western Turkey, and Spain. This indicates that different distinguishable genotypes have expanded their range independently and have remained distinct from each other. Polyploid *Aegilops* species such as *A. triuncialis* are considered to be predominately, if not obligatory, inbreeders (Hedge et al. 2000). The wide distribution of distinct lineages we detected for Eurasian accessions is an expected pattern for a selfing species that has been dispersed widely in its home range by human agricultural activity and trade.

Multiple introductions during goatgrass invasion

Out of 38 *A. triuncialis* genotypes we identified, 35 were found only in the native range, a single genotype was found both in its new range in California and Eurasisa and two very similar genotypes were found only in California. The McLaughlin population contained two genotypes, one individual out of the 20 sampled differed from the others by only one autapomorphic band. Because this band was not found in any Eurasian samples, this is most likely due to a new mutation that occurred in that lineage in California since the introduction. Overall, the distribution pattern of both genotypes in California suggests two independent introductions. The first record of *A. triuncialis* occurrence in the US was from one of our eastern-most collection sites in the foothills of the Sierra Nevada (1914 at Malby Crossing) and subsequent discoveries of *A. triuncialis* were relatively close to this first collection site (Talbot and Smith 1930). The next collection in 1922 was made about 90 km south of the Malby Crossing site and in 1923 an additional population was discovered about 100 km north of Malby Crossing. These first introductions were all in areas of wheat farming and cattle and sheep grazing along major roads leading to the gold mining districts of the Sierra Nevada foothills. As a result of these human activities, there may have been a relatively simultaneous introduction of these populations followed by rapid dispersal at the very early stages of the introduction. The 1923 population was apparently eradicated but the 1914 and 1922 populations spread rapidly into the nearby foothills (Talbot and Smith 1930). According to Calflora (http://www.calflora.org/), *A. triuncialis* was discovered in California's northern Coast Ranges approximately 40 years later. West of the initial introduction sites in the Sierra Nevada foothills, these Coast Range populations represent a second period of rapid range expansion in *A. triuncialis*. Overall, the pattern of the distribution of the genotypes found in California indicates that eastern populations in the Sierra Nevada foothill have descended from the original introduction while the Coast Range populations represent a separate, later introduction.

The ability of a species to become invasive after initial establishment has been frequently explained by multiple introductions. Multiple introductions can create populations of high genetic variability in the new range, often higher than populations in the native range (Kolbe et al. 2004; Maron et al. 2004). With multiple introductions, founder effects that produce genetic bottlenecks are prevented and the genetic diversity necessary to respond to novel selective challenges in the new range is retained. Although we initially suspected that the recent invasive spread of *A. triuncialis* would have resulted from the recombination of genotypes from multiple introductions, our results suggest that this has not occurred. The two separate introductions indicated by the molecular data are composed of highly uniform populations that are still quite distinct geographically. The capacity of *A. triuncialis* to expand its range in California despite this strong genetic bottleneck suggests that phenotypic plasticity may be important for adaptation in this species. In addition to studies on adaptive plasticity in *A. triuncialis*, we are also exploring the possibility that mutation in quantitative traits may represent an *in situ* source of genetic variation within *A. triuncialis* populations. Genome-wide rates of mutation in fitness traits have been shown to be quite high ($U = 0.04$) in a related tetraploid species of *Triticum* (Bataillon 2000). For inbreeding invasives such as barbed goatgrass, the possibility that the evolutionary response in bottlenecked founding populations may be facilitated by mutation in quantitative traits represents an intriguing phenomenon that deserves further study.

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