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Patterns of introduction and adaptation during the invasion of *Aegilops triuncialis* (Poaceae) into Californian serpentine soils

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Abstract

Multiple introductions can play a prominent role in explaining the success of biological invasions. One often cited mechanism is that multiple introductions of invasive species prevent genetic bottlenecks by parallel introductions of several distinct genotypes that, in turn, provide heritable variation necessary for local adaptation. Here, we show that the invasion of *Aegilops triuncialis* into California, USA, involved multiple introductions that may have facilitated invasion into serpentine habitats. Using microsatellite markers, we compared the polymorphism and genetic structure of populations of *Ae. triuncialis* invading serpentine soils in California to that of accessions from its native range. In a glasshouse study, we also compared phenotypic variation in phenological and fitness traits between invasive and native populations grown on loam soil and under serpentine edaphic conditions. Molecular analysis of invasive populations revealed that Californian populations cluster into three independent introductions (i.e. invasive lineages). Our glasshouse common garden experiment found that all Californian populations exhibited higher fitness under serpentine conditions. However, the three invasive lineages appear to represent independent pathways of adaptation to serpentine soil. Our results suggest that the rapid invasion of serpentine habitats in California may have been facilitated by the existence of colonizing Eurasian genotypes pre-adapted to serpentine soils.

Keywords: adaptation, *Aegilops*, biological invasion, multiple introductions, serpentine soil

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Introduction

Biological invasions are characterized by the rapid spread of species that have been purposely or accidentally introduced outside their original native range. Invasions are an extreme case of range expansion, and understanding the role of evolutionary processes in establishing and maintaining range limits has important implications for species distributions and interactions (Holt 2009). A better understanding of processes of niche evolution at species range limits can be facilitated by studies on the role of selection and drift in the spatial spread of an invasive species in its new range (Sexton *et al.* 2009). Abiotic and biotic selective pressures thought to be important in setting native range limits have also been implicated in preventing or limiting the spread of invasive species (Lee 2002; Cox 2004). It seems paradoxical that an introduced species can displace resident species that have had a much longer time

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to adapt to the local environment (Baker 1965; Lee 2002; Suarez & Tsutsui 2008). The invader should be at a selective disadvantage and will likely experience demographic bottlenecks during initial establishment that reduce genetic variation necessary for evolutionary response to novel selection pressures in its new range (Chen *et al.* 2006).

Despite these demographic and evolutionary challenges, there is growing evidence that introduced species may adapt rapidly to conditions in their new range (Blossey & Nötzold 1995; Parker *et al.* 2003; Bossdorf *et al.* 2005; Blumenthal & Hufbauer 2007). Most invasive species undergo a demographic lag phase after establishment that may represent the time required for adaptation that may be a prerequisite for expansion (Sexton *et al.* 2002). Rapid adaptation in response to selection can only occur if there is genetic variation in ecologically important traits. Multiple introductions of genetically divergent individuals or populations could serve as important sources of this variation (Novak & Mack 1995).

Several studies suggest that multiple introductions may play a prominent role in explaining the invasion success of introduced species (Kolbe *et al.* 2004; Roman & Darling 2007; Lavergne & Molofsky 2007; but see Dlugosch & Parker 2008). It has been proposed that multiple introductions may facilitate the invasion process by (i) increasing propagule pressure (i.e. sheer numbers increase the likelihood of establishment; Lockwood *et al.* 2005), (ii) increasing the spatial range of introduction in the new range thus allowing for multiple expansion points and (iii) increasing the likelihood of increased genetic diversity as the number of individuals sampled from the native range increases.

Multiple introductions are likely to increase the number of evolutionarily independent genotypes and thus may lead to an increase in the adaptive potential of an invasive species. For example, sampling across populations in the native range that are adapted to different conditions should increase the likelihood of introduction of genotypes pre-adapted to selective challenges in the invasive range. The introduction of multiple pre-adapted genotypes might, in turn, facilitate the development of several independent invasion fronts. Alternatively, recombination between multiple introduced lines may give rise to new genotypes that might be favoured by selection in the new range. Under the first scenario, the lag phase may correspond to the time that is required for the introduction and establishment of pre-adapted genotypes, while under the second scenario the length of the lag phase is a potential indication of the efficacy of selection in producing newly adapted ecotypes.

The capacity to differentiate between these possibilities and to determine how multiple introductions provide genetic variation for responses to selection during introduction is probably highest during early stages of the invasion process. Invasive species that have been expanding and dispersing in their new range for long periods of time will show increasing admixture and recombination among independently introduced lines. While an explanation for observed admixture is that multiple introductions increase the invasive abilities of a species by providing the base for recombination, an alternative is that admixture is an inevitable consequence of range expansion and the eventual overlap of independent invasion events. The research reported on here illustrates a method for determining which of these explanations is more likely.

An exceptional opportunity to investigate the role of multiple introductions in plant invasions is provided by the invasion of *Aegilops triuncialis* (barbed goatgrass, Poaceae) into northern California, USA. *Aegilops triuncialis* is a predominately cleistogamous allotetraploid with a low outcrossing rate. It is native to the Mediterranean region and central Asia (van Slageren 1994). It was first recorded in California in 1914 from the foothills of the Sierra Nevada range (Kennedy 1928; Jacobsen 1929; Talbot & Smith 1930). Subsequent additional collections of this species during the 1960s from the Coast Range in northern California suggest independent establishment of at least two introductions; a scenario that had been recently confirmed using fingerprint analysis of nuclear loci (Meimberg *et al.* 2006).

Aegilops triuncialis invades low fertility soils in California and can reach high densities in this edaphically stressful environment (Peters *et al.* 1996). A special concern for conservation is that *Ae. triuncialis* is beginning to invade serpentine soil outcrops; edaphic habitats with a high number of endemic plant species adapted to low macronutrient availability, high levels of soil toxicity and significant soil moisture stress (Kruckeberg 1984). Because of these extreme edaphic conditions, serpentine soil habitats are generally less prone to invasion than nonserpentine soils (Huenneke *et al.* 1990; Harrison 1999; Harrison *et al.* 2001; Going *et al.* 2009).

We assessed patterns of introduction and the potential for genetic bottlenecks within *Ae. triuncialis* populations established in California by conducting a comparative study of microsatellite variation in both invasive and native range populations. In addition, we also tested for fitness differences among invasive and native range populations grown on loam soil as well as under serpentine edaphic conditions. With this combined molecular and ecological genetics approach, we investigated questions on (i) how many introductions of *Ae. triuncialis* into California have occurred and what

are the patterns of geographic spread; (ii) what are the relative levels of phenotypic variation among invasive and native range lineages and (iii) to what extent do invasive and native range lineages differ in adaptation to serpentine soil. With this multi-faceted approach, we examined whether multiple introductions provided pre-adapted genotypes that are currently invading serpentine soils or if admixture and recombination provided the adaptation for the observed invasion of serpentine.

Material and methods

Plant material

We sampled 480 individuals representing 20 California populations (20–30 individuals per population) that span the range of invasion sites into California (Table 1 and Fig. 1). In addition, we obtained 58 Eurasian accessions from the USDA National Small Grains Collection. Accessions covered the whole Eurasian range of the species, from Spain east to Afghanistan and north to Ukraine (Meimberg *et al.* 2006). Finally, to compare DNA polymorphism (i.e. within-population variation) between California and native range populations, we included field collections (between five and 10 individuals each) from four populations in Greece (Ioannina, Konitsa; Nemea, Korinthias; Ioannina, Ageloxori; Kavala, Orfani).

Seeds collected from California populations were stored at room temperature until used in the glasshouse experiments. Germination rates exceeded 90% and did not diminish noticeably with storage. Fresh leaf tissue samples from California populations and Eurasian accessions were frozen, lyophilized and ground for DNA extraction in a modified paint shaker using five 1.9 mm ball bearings in 2 mL microfuge tubes. DNA was isolated using the Plant Charge-Switch kit (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol.

Development of microsatellite markers

Microsatellite markers were identified from a motif-enriched library (Fischer & Bachmann 1998). Total DNA from five pooled *Aegilops triuncialis* samples from California was restricted with RSA I, and adapters from Fischer & Bachmann (1998) were ligated to the blunt end fragments within one reaction (33.8 μL containing 6 μg DNA, 3.3 μL T4 Ligase buffer (10 ×); 18 U RSA I; 6 U T4-Ligase; 10 mM ATP and 8.3 μM double-stranded adapter; incubated over night at 37 °C with subsequent heat inactivation). Twenty microlitre of the cleaned DNA fragment solution was used in a 65 μL hybridization assay, containing 19.5 μL of a 20 × SSC buffer (3 M NaCl; 0.3 M Na-Citrate; pH 7) and 0.15 μM each of the following biotin-labelled oligonucleotides: $(CA)_{10}$, $(CAA)_8$, $(GAA)_8$. The solution was incubated at 98 °C for 10 min and then incubated for 20 min at 74 °C. Subsequently, 35 μL of a Dynabead suspension (streptavidin coated in 2 × SSC, Invitrogen, Carlsbad, CA, USA) was added and incubated an additional 20 min at 74 °C. After incubation, the Dynabeads were immobilized using a magnetic particle collector and the supernatant removed and washed with 200 μL of 2 × SSC/0.1%

Table 1 List of Californian *Aegilops triuncialis* localities sampled. Lineage designation indicates the clustering of populations into invasive lineages as suggested by the microsatellite analysis

Number	Population name	CA County	*n* samples	Lineage	Soil type/Habitat
101	Hopland Foster	Mendocino	29	West	Serpentine grassland
102	Bear Valley	Colusa	27	West	Serpentine grassland
103	McLaughlin	Lake	25	East	Serpentine grassland
104	Snell Valley	Napa	27	West	Serpentine grassland
105	Jepson Prarie	Solano	28	West	Nonserpentine grassland
106	Sierra Field Station, Schubert Valley	Sierra	30	East	Nonserpentine grassland
107	Mather	Sacramento	29	East	Roadside
110	Nevada City	Yuba	25	East	Roadside
115	Yuba River	Yuba	28	East	Nonserpentine grassland
117	Malby crossing	El Dorado	29	East	Roadside
118	Red Hills	Tuolumne	22	South	Serpentine grassland
119	Waits Station	Amador	25	East	Serpentine outcrop
121	Oroville	Butte	27	East	Nonserpentine grassland
122	Yolo County Grassland	Yolo	26	West	Nonserpentine grassland
123	Cold Canyon Blue Ridge Trail	Solano	25	West	Nonserpentine grassland
124	Dove Ridge	Butte	25	East	Nonserpentine grassland
125	Bass Lake	El Dorado	28	East	Roadside
126	Old Gulch	Calaveras	25	South	Nonserpentine grassland

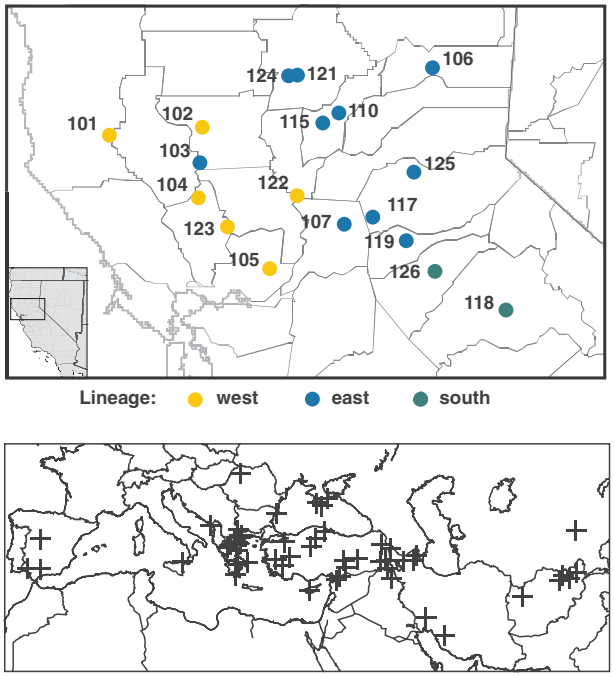

Fig. 1 Upper panel: Map of locations of seed collection sites of Californian populations, numbers correspond to population description provided in Table 1. Symbols indicate population clustering into lineages as determined by molecular marker data (see text). Lower panel: overview of localities of Eurasian accessions included in this study.

SDS and 200 μL 1 × SSC at room temperature. The fragments were eluted in 20 μL of 0.1 M NaOH/0.1 M NaCl incubated for 5 min. The supernatant was neutralized with 10 μL of 0.2 M HCl and 2.2 μL of 0.1 M Tris–HCl, pH 7.5 and cleaned using Microcon 30 Spin filter (Millipore, Billerica, MA, USA) according to the manufacturer's protocol. Of the resulting solution, 0.2 μL was used in a PCR containing 0.2 μM 21mer of the adapter as primer, 0.1 μM dNTPs and 1 U Taq polymerase and run for 28 cycles (94 °C, 48 s; 56 °C, 1 min; 72 °C, 2 min) with a final extension at 72 °C for 10 min. The PCR product was cleaned and used as a template for a second hybridization on Dynabeads. The resulting solution was concentrated using Microspin 100 filters (Millipore, Billerica, MA, USA) and subcloned using the pGEM-T Vector system (Promega, Madison, WI, USA) according to the manufacturer's protocol. Approximately, 500 clones were picked in 100 μL water and incubated at 95 °C, and subsequently a 1 μL aliquot was used for PCR amplification using the M13 primers. Ten microlitre of PCR products was denatured in 50 μL of 0.1 M NaOH and dot blotted on a nylon membrane. The membrane was neutralized, washed in 2 × SSC and prehybridized at 50 °C. After 45 min, 20 μL of the biotin-labelled oligonucleotides (10 μM) used for the enrichment was added and incubated over night. To reduce background, the membrane was washed in 2 × SSC/0.1 SDS 2 × 2 min at RT, 1 × 10 min at 50 °C and in 2 × SSC 1 × 2 min at RT. The membrane was cross-linked and developed colorimetrically using streptavidin–alkaline phosphatase (SA–AP) conjugate (Promega) and NBT/IPTG for visualization. One hundred positive clones were identified and sequenced bidirectionally using an ABI 3230xl system (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's protocol. Insert sequences that contained a microsatellite motif were used for primer design and tested for suitability as microsatellite marker. For subsequent genotyping, nine loci were selected that gave distinct bands after amplification in several samples. Clone sequences of these loci had been submitted to GenBank (accession numbers HQ231684–HQ231692), and primers and PCR conditions for each primer pair are listed as supplementary material.

Measurement of microsatellite polymorphism

Microsatellite polymorphisms were determined for 480 Californian samples from 20 populations and for 64 Eurasian samples from 51 accessions (two samples each for 13 of these accessions). Primers were optimized for annealing temperature and annealing time (15 or 30 s) as well as for the number of touchdown cycles starting the amplification. The amplification of fragments was performed in 96-well PCR plates with each well containing Taq polymerase buffer, 125 nM dNTPS, 0.3 U Taq, 3.4 nM reverse primer, 0.85 nM of forward primer 5′ tailed with the M13 motif elongated by the bases GTCA (TGTAAAACGACGGCCAGTGTCA) and 2.56 nM 5′ labelled M13 primer (VIC, NED, PET or 6-FAM, Fa. ABI) in 15 μL volume. After PCR (up to four) amplicons were pooled, 2 μL of the pool was mixed with 10 μL HiDi formamide (Fa. ABI) and 0.1 μL Genescan Liz 500 internal size standard (Applied Biosystems) and subsequently run on an ABI 3230xl sequencer.

Glasshouse common garden and soil treatments

To compare phenotypic variation among populations and accessions, we grew plants from these same native and invasive populations in a randomized, factorial glasshouse common garden. For the glasshouse experiments, ten seeds from each of 58 Eurasian accessions were sown singly in 175-mL capacity pots (Supercell conetainers, Stuewe and Sons, Corvallis, OR, USA): five seeds in Yolo loam soil and five seeds in serpentine soil. Serpentine soil (Henneke and Montara soil series) was collected at the University of California McLaughlin Reserve (38°31′12″ N, 122°14′24″ W), and the soil was

disaggregated using a soil mixer before it was used in the glasshouse planting experiment. To adequately examine variation within California populations, seeds from 20 maternal families from each California population were also sown singly in 175-mL capacity pots (Supercell conetainers): 10 in Yolo loam soil and 10 in serpentine soil. After initial seedling establishment, watering of serpentine pots was reduced to one-third of that supplied to loam pots to simulate drier soil conditions measured in serpentine sites (McKay and Rice, unpublished data). Plants were grown to reproductive maturity in a glasshouse at the University of California-Davis campus (Davis, CA, USA). We measured flowering time, seed number per plant and total seed mass for each plant that reproduced. Individual seed mass was calculated from seed number and total seed mass.

Data analysis

Fragment lengths of microsatellite loci were calculated using Genemapper (Applied Biosystems) under manual control. Population genetic analyses were performed using Arlequin (Schneider *et al.* 2000) and Genepop (Raymond & Rousset 1995). The Shannon Index for genetic diversity was determined using Popgene (Yeh *et al.* 1997). The number of repeats for population genetic analysis using the stepwise mutation model was estimated by dividing the length of the fragment by the number of bases of the motif. One locus was apparently duplicated, probably because of the allotetraploid origin of *Ae. triuncialis* (Meimberg *et al.* 2009) and exhibited between two to four alleles for all samples. PCR amplicons from this primer pair were divided in two independent loci (loci 5 and 2) for population genetic analysis. The alleles fell in two distinct groups, one between 105 and 117 and the other between 136 and 138 repeats in a similar range of LD like the other pairs of loci.

Polymorphism data were analysed using NTSys (Applied Biostatistics Inc.; Setauket, NY, USA). Similarity analysis was performed as UPGMA using the Dice coefficient as a distance estimator and alleles coded as presence/absence data. F_{st} was calculated in Arlequin using both the number of different alleles and sum of size differences. Isolation by distance was determined using a mantel test in NTSys to compare pairwise F_{st} estimates and geographical distances between populations. Population structure for the Californian populations was further investigated by a STRUCTURE 2.2 analysis using 30 000 generations and a burn in of 10 000 generations assuming admixture and independent allele frequencies (Pritchard *et al.* 2000).

Phenotypic data from glasshouse experiments on loam and serpentine soil were analysed using JMP (Version 7; SAS, Cary, NC, USA). To test for significance between the observed means, mixed model nested analysis of variance was performed for the traits measured on plants growing in both loam and serpentine edaphic conditions. We tested for an effect of each of the following factors: invasive lineage in California (i.e. three invasive lineages were identified in the microsatellite analysis), population (nested within lineage), soil type and the interaction between population and soil type. Invasive lineage identity was added to the model, and populations were nested within lineage to test for differences among the three lineages as well as among populations within each lineage. Comparisons among all samples were performed to test the effect of continental source, (i.e. Californian vs. Eurasian), soil (serpentine vs. loam) and the interaction between continent and soil.

Results

Microsatellite variation

Variation of microsatellite loci was much higher in Eurasia accessions than in California populations. In total, the 64 individuals representing 51 Eurasian accessions contained 140 different alleles (7–22 per locus) compared to 77 (7–13 per locus) in 480 California samples. Each Eurasian accession studied represented a unique multilocus genotype. For 13 of the 51 Eurasian accessions, we sampled two seeds from the envelope to look for within accession variation. In eight (of 13) cases, the two samples within each accession showed different alleles at one to four loci. In five accessions, both samples showed the same genotype. In California, 79 different multilocus genotypes were found among the 480 samples representing 20 populations.

Analysis of genotype frequency

Similarity analysis divided Californian samples into three clusters, each made up of highly similar multilocus genotypes (Fig. 2). Several Eurasian accessions were more similar to Californian samples than Californian samples were to each other; as a result, Eurasian samples fall in between clusters of Californian samples in the cluster analysis. With few exceptions, the great majority of California samples showed a multilocus genotype from a single lineage within populations, so the three clusters correspond to three invasive lineages of essentially monomorphic populations (Fig. 2). Within each lineage, there is a high level of gene flow or similarity among populations (F_{ST} 0–0.2) but a low level of gene flow or similarity among the three lineages (F_{ST} 0.8–0.9; Fig. 3a). This pattern is further

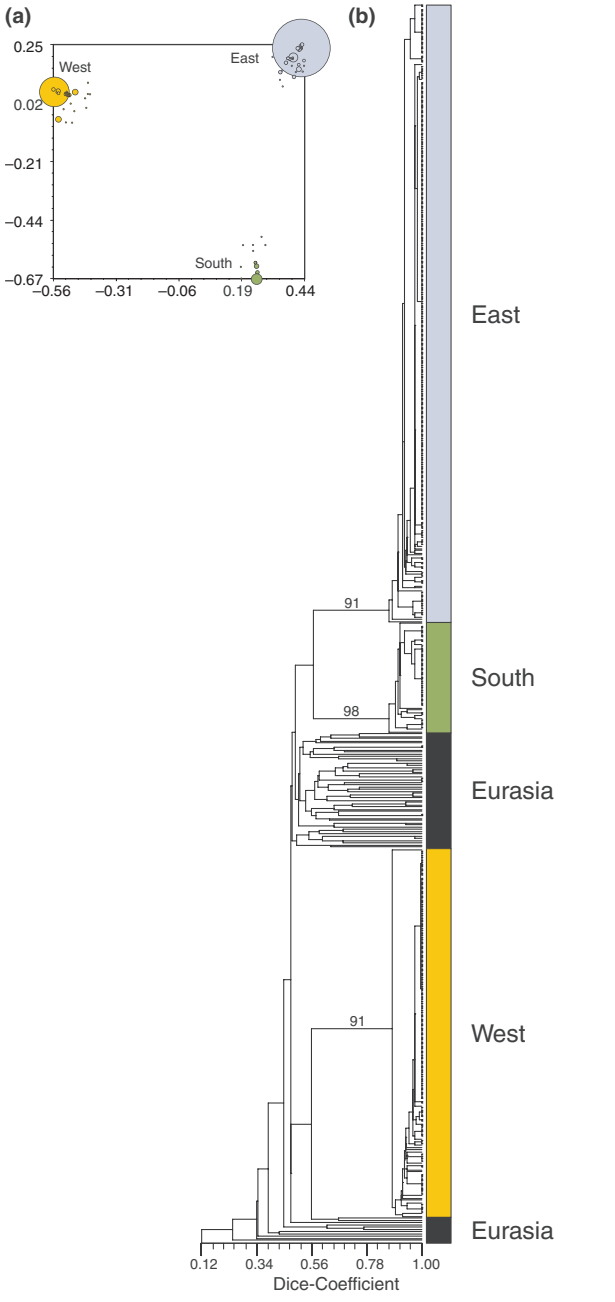

Fig. 2 Similarity analysis of multilocus genotypes with microsatellite alleles in a) a PCoA analysis of the Californian samples and b) an UPGMA analysis that included the Eurasian samples. In the PCoA analysis, the size of the dots is proportional to the frequency of the single genotypes in the sample. Bootstrap support values are indicated above branches. Colours indicate invasive lineages as determined by the analyses of molecular data (see text).

supported by an analysis of molecular variance (Excoffier *et al.* 1992) where 80% of the variation was explained by differences among the lineages, compared to small differences among populations within a lineage (18%) and very little within-population variation (2%). Under the assumption of a stepwise mutation model and using the sum of size differences as a distance estimate (Chakraborty & Nei 1976; Reynolds *et al.* 1983), the variation among lineages was 86%, within-lineage variation was 14%, and extremely low rates (0.2%) of within-population variation were detected. An increase in among-lineage variation and a decrease in within-lineage variation indicate that alleles responsible for within-lineage variation differ from each other by a smaller number of repeats than alleles explaining among-lineage variation. Overall, size differences of alleles within California and Eurasia were similar. The clustering of Californian populations into three invasive lineages can also be demonstrated by direct comparison of allele frequencies. For eight loci, alleles were found that had a very high frequency within a single lineage (loci 1, 4, 3, 10, 7, 8, 6, 9; Fig. S1 in Supporting information) but were rare or absent in other lineages. For one of these markers (locus 6), two lineages showed two alleles while the remaining lineage showed one, indicating that the locus was duplicated; both copies were amplified in some lineages, while another comprises a null allele. Another marker (locus 9) showed duplication for all samples. Allele frequencies indicated a fairly strong geographical pattern in the California invasion. Most of the eastern populations (Lineage East), western populations (Lineage West) and two of the southern populations (Lineage South) are characterized by similar allele frequencies. This spatial subdivision is also indicated by significant isolation by distance for the whole sampling area ($r = 0.404$; d.f. = 151; $P < 0.001$). No significant isolation by distance was found within each lineage.

In the STRUCTURE analysis, ln probability of the data was reaching a plateau at $K = 3$ with −2494 compared to −3977 for $K = 2$ and −2415 for $K = 4$, indicating that three or more clusters are best representing the genetic structure of *Ae. triuncialis* in California. With $K = 3$ in the STRUCTURE analyses, as well as in the genotype assignment tests implemented in Arlequin, single populations appear to be highly homogeneous. The vast majority of individuals are assigned unambiguously to the population from which they were collected with likelihoods above 0.95 (Fig. 4). In seven of 18 populations, evidence for admixture between invasive lineages was indicated by the existence of rare alleles in one lineage that have high frequencies in another lineage. This accounts for 12 individuals where the likelihood of assignment to the respective group was between 0.7 and 0.85 and therefore slightly lower than for the majority of the individuals. In two populations (Bass Lake and Cold Canyon), a single individual expressed a multilocus genotype most similar to multilocus genotypes of populations of another lineage,

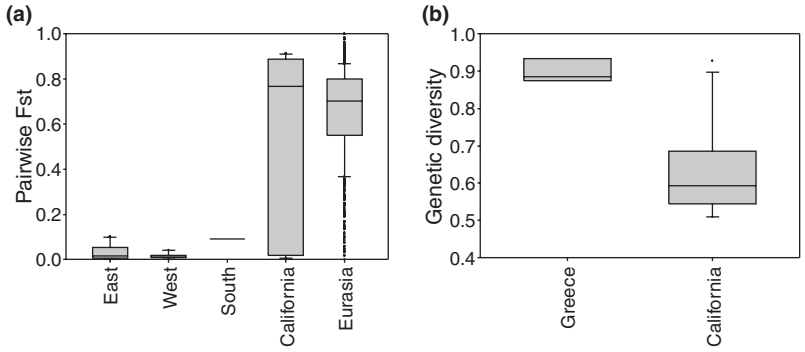

Fig. 3 (a) Comparisons of pairwise F_{st} values within the Californian lineages (i.e. East, West and South), among lineages within California and among the Eurasian accessions. (b) Genetic diversity within native *Aegilops triuncialis* populations collected from Greece and within invasive Californian populations ($F_{1,20} = 17.1$, $P = 0.0005$).

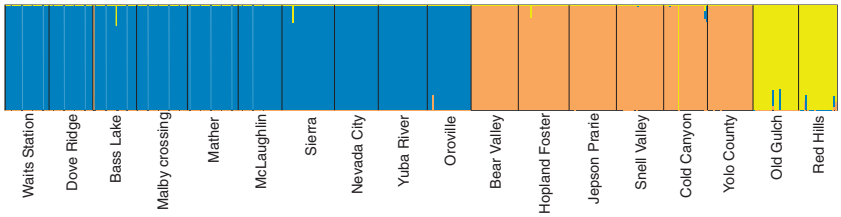

Fig. 4 Bar plot of STRUCTURE analyses ($K = 3$) of Californian populations of *Aegilops triuncialis*. Assignment of individuals to invasive Lineage East is indicated by blue, to Lineage West by red, and to Lineage South by green.

suggesting recent migration as seed. We found some evidence for gene flow within each lineage in the form of low frequency alleles that were shared among several populations. This pattern affects five of six populations for Lineage West, six of ten populations for Lineage East and both populations of Lineage South. Overall, the number of migrants as estimated according the method from Barton & Slatkin (1986) was low; Nm = 0.35–2.62 between populations within lineages and Nm = 0.07 among lineages.

The small amount of migration between populations is also indicated by highly significant population differentiation for the whole sample ($P < 10^{-5}$ for all loci), and within lineages (Lineage West: $P < 0.008$; Lineage East: $P < 0.006$; Lineage South: $P < 0.0002$). In addition, we found that two populations have alleles that have gone to fixation (frequency of 1) that are either private (Amador) or occur only with low frequency in a neighbouring population (Tuolumne).

Diversity within Californian populations is significantly lower than within Greek populations sampled in the native range as indicated by gene diversity as implemented in Arlequin ($F_{1,20} = 17.1$, $P = 0.0005$). Californian populations exhibited, on average, a gene diversity of 0.63 (range of 0.50–0.92) compared to populations from Greece with an average gene diversity of 0.90 (range of 0.88–0.95, Fig. 3b). Californian populations exhibited lower diversity also using the Shannon Index, as implemented in Popgene ($F_{1,20} = 6.65$, $P = 0.018$).

Variation in trait means and plasticity to soil treatments

In glasshouse experiments, we compared flowering time and reproductive fitness (i.e. individual seed mass, total seed mass and seed number per plant) of all Californian populations and Eurasian accessions grown on both loam and serpentine soils. The results were analysed on three levels; invasive vs. native range (i.e. California vs. Eurasian collections), within each range, and within the invasive lineages in California.

On average, Californian individuals exhibited higher reproductive fitness in serpentine soil than on loam soil as measured by total seed mass and total seeds per plant (Fig. 5; Table 2). Interaction between soil and continent was significant indicating a differential response to serpentine conditions by Californian and Eurasian individuals. Within Eurasian accessions, there was a significant main effect of accession for all traits while soil type was not significant. In California, effects of both invasive lineage and soil type were significant for all traits, except individual seed mass. There was no interaction between soil type and lineage except in flowering time, suggesting that all three lineages responded similarly to soil condition in terms of

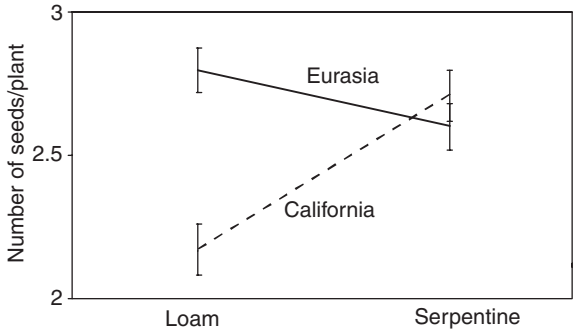

Fig. 5 Reproductive fitness differences (expressed as number of seeds per plant) between Eurasian accessions and Californian populations grown on loam and serpentine soils. Error bars indicate one standard error (interaction continent by soil: $F_{3,832} = 22.8$; $P = 0.0001$; Table 2).

reproductive fitness. Populations within lineages also showed a significant effect for flowering time. Thus, phenotypic responses to the serpentine conditions of populations within a lineage were similar for most traits.

There was a significant main effect of soil in both seed number and total seed mass when these data were averaged across the three lineages. Higher reproductive fitness was negatively correlated with flowering time because the earlier flowering individuals had higher seed mass ($r = -0.48$; $P < 0.0001$) and total number of seeds ($r = -0.38$; $P < 0.0001$). Flowering time differed significantly among lineages and there was also a significant interaction, indicating the lineages differ in the response of flowering time to serpentine soil. This interaction is because of a shift to early flowering under serpentine conditions in Lineage East and South (significant main effect of soil on flowering time within each of these two groups). This soil effect indicates phenotypic plasticity in flowering time. Populations of Lineage West flowered earlier than the other two lineages and did not exhibit a plastic flowering response to soil type (Table 2 and Fig. S2 in supporting information).

Potential trade-offs between seed size and seed number differed significantly among the invasive lineages. Plants in Lineage East exhibited a higher individual seed mass compared to the other lineages. In contrast, total number of seeds was higher in Lineage West compared to the other lineages. Individual seed mass increased to a greater extent in Lineage South under serpentine conditions than the other lineages, resulting in a significant interaction between soil and lineage in the pairwise comparison of Lineage South with Lineage West.

Variation in flowering time among Eurasian accessions was higher than for Californian samples. A few Eurasian accessions showed very early flowering on loam as well as on serpentine (i.e. between 70 and 90 days after germination). When comparing phenological and reproductive trait complexes between Eurasian and Californian samples, six of 57 Eurasian accessions were similar to Lineage East because they flowered between 120 and 145 days, exhibited earlier flowering under serpentine conditions, and produced a greater seed mass and total number of seeds per plant on serpentine. A different set of six Eurasian accessions were similar to the early flowering Lineage West, with flowering between 90 and 120 days, reduced plasticity in flowering time and higher seed mass and seed number per plant on serpentine. Finally, three Eurasian accessions were similar to Lineage South in their later flowering, between 130 and 145 days, plastic shifts to earlier flowering time on serpentine and an increase in seed mass and total seed number per plant on serpentine.

Discussion

Population genetic structure of Aegilops triuncialis

The expanded sampling in this study compared to our previous study (Meimberg *et al.* 2006) revealed evidence for three independent introductions into California, one more than previously identified. The occurrence of three independent introductions is supported by the finding that multilocus genotypes form three distinct lineages, each defined by a number of unique alleles with high frequencies within a multilocus genotype. In addition, Californian multilocus genotypes are more similar to some Eurasian accessions than to other Californian genotypes, and size variation of the microsatellites was comparable between California and Eurasia. According to the stepwise mutation model of microsatellites (Reynolds *et al.* 1983), divergence times are related to size differences between alleles. This model of mutation suggests that polymorphisms distinguishing lineages in California evolved in the native range, prior to introduction into California. In each of the three lineages of populations (and within populations nested within lineages), size variation and allelic diversity were very low; lower than the values measured for the populations from Greece. This is what would be expected if the invasive lineages in California all underwent an introduction bottleneck resulting in populations with reduced genetic variability. Furthermore, we found a geographic pattern in the occurrence of multilocus genotypes. *Ae. triuncialis* was first recorded in the Sierra Nevada foothills (Kennedy 1928). In the coastal range (i.e. Lineage West), the earliest records of occurrence are from the 1960s (Peters *et al.* 1996). The distribution of genotypes, with one lineage type predominately eastern and another predominately western, suggests that the 1960s species occurrence record in the Coast Range of northern California documented an independent intro-

Table 2 Results of analysis of variance of total number of seeds, total seed mass, individual seed mass and flowering time

	Total seed number			Total seed mass			Individual seed mass			Flowering time		
Factors	SS	*F*	*P*-value	SS	*F*	*P*-value	SS	*F*	*P*-value	SS	*F*	*P*-value
Comparisons between Eurasian and Californian samples (DF = 3; Error = 832)												
Continent	14.51	11.8	0.0006*	0.000	1.1	0.299	4.2×10^{-4}	24.4	<0.0001*	6171	15.1	0.0001*
Soil	5.75	4.7	0.031*	0.001	6.7	0.009*	1.1×10^{-7}	0.01	0.935	2763	6.7	0.01*
Continent*Soil	28.01	22.8	<0.0001*	0.002	19.5	<0.0001*	1.2×10^{-5}	0.7	0.394	581	1.4	0.2341
Comparisons within California (DF = 20; Error = 344)												
Lineage	169.72	133.0	<0.0001*	0.002	10.4	<0.0001*	3.2×10^{-3}	107.6	<0.0001*	38677	188.6	<0.0001*
Population (Lineage)	11.44	1.2	0.273	0.001	1.2	0.263	3.5×10^{-4}	1.6	0.073	3407	2.2	0.006*
Soil	16.96	26.6	<0.0001*	0.002	22.3	<0.0001*	6.6×10^{-6}	0.4	0.505	2594	25.3	<0.0001*
Soil*Lineage	1.33	1.0	0.355	0.0003	1.7	0.178	7.8×10^{-5}	2.7	0.072	1483	7.2	0.0008*
Comparisons within Eurasia (DF = 155; Error = 680)												
Soil	0.18	0.2	0.653	8.4×10^{-9}	0.0001	0.991	4.1×10^{-6}	0.3	0.562	405	4.1	0.045*
Accession	364.95	5.3	<0.0001*	0.026	4.2	<0.0001*	5.5×10^{-3}	5.9	<0.0001*	258076	33.5	<0.0001*
Soil*Accession	94.60	1.4	0.02*	0.009	1.4	0.013*	8.2×10^{-4}	0.9	0.757	13892	1.8	<0.0001*
Lineage East (DF = 19; Error = 178)												
Soil	11.31	24.6	<0.0001*	0.002	16.2	<0.0001*	2.8×10^{-5}	1.2	0.274	2888	27.7	<0.0001*
Population	8.21	2.0	0.043*	0.001	1.2	0.277	3.0×10^{-4}	1.4	0.175	2949	3.1	0.002*
Population*Soil	8.97	2.2	0.026*	0.001	1.1	0.345	8.1×10^{-5}	0.4	0.942	599	0.6	0.764
Lineage South (DF = 3; Error = 28)												
Soil	2.24	6.6	0.016*	0.001	15.0	0.0005*	5.1×10^{-5}	3.9	0.058	1422	6.5	0.016*
Population	0.35	1.0	0.318	0.0001	2.8	0.103	6.3×10^{-6}	0.5	0.493	40	0.2	0.671
Population*Soil	0.20	0.6	0.443	0.00003	0.7	0.414	4.1×10^{-8}	0.003	0.955	345	1.6	0.218
Lineage West (DF = 11; Error = 123)												
Soil	16.24	17.0	<0.0001*	0.0003	4.1	0.045*	1.5×10^{-5}	4.2	0.042*	25	0.3	0.565
Population	3.73	0.8	0.565	0.0002	0.5	0.787	4.0×10^{-5}	2.2	0.055	301	0.8	0.552
Population*Soil	1.52	0.3	0.901	0.0001	0.3	0.904	7.2×10^{-6}	0.4	0.841	405	1.1	0.377
Lineage East vs West (DF = 17; Error = 315)												
Lineage	163.91	246.07	<0.0001*	0.0001	1.57	0.212	3.0×10^{-3}	201.14	<0.0001*	28587	312.3	<0.0001*
Population (Lineage)	11.15	1.20	0.277	0.001	1.15	0.318	3.5×10^{-4}	1.65	0.0642	3391	2.6	0.001*
Soil	29.05	43.61	<0.0001*	0.002	17.69	<0.0001*	4.4×10^{-4}	2.91	0.0889	1343	14.7	0.0002*
Soil*Lineage	1.31	1.96	0.163	0.0001	1.43	0.232	1.3×10^{-7}	0.01	0.9270	964	10.5	0.0013*
Lineage East vs South (DF = 13; Error = 216)												
Lineage	0.46	0.99	0.321	0.001	14.44	0.0002*	6.5×10^{-4}	30.23	<0.0001*	3334	28.1	<0.0001*
Population (Lineage)	7.88	1.69	0.084	0.001	1.47	0.153	3.1×10^{-4}	1.46	0.1572	3091	2.6	0.005*
Soil	6.82	14.66	0.0002*	0.001	16.88	<0.0001*	2.1×10^{-5}	1.00	0.3195	2935	24.7	<0.0001*
Soil*Lineage	0.02	0.04	0.837	0.00009	1.06	0.304	7.5×10^{-5}	3.50	0.0626	218	1.8	0.176
Lineage West vs South (DF = 7; Error = 157)												
Lineage	43.85	53.49	<0.0001*	0.002	26.02	<0.0001*	4.3×10^{-5}	8.31	0.005*	23100	225.3	<0.0001*
Population (Lineage)	3.85	0.78	0.585	0.0003	0.75	0.611	4.7×10^{-5}	1.51	0.1772	332	0.5	0.777
Soil	10.11	12.33	0.0006*	0.001	15.04	0.0002*	2.2×10^{-5}	4.27	0.040*	1227	12.0	0.0007*
Soil*Lineage	0.26	0.32	0.574	0.0002	3.76	0.054	6.8×10^{-5}	13.13	0.0004*	1015	9.9	0.002*

The model tests the effect of soil (serpentine condition vs. loam), population source and their interaction. For comparisons within California, lineage (according to the microsatellite analysis) was added to the model and populations were nested within lineage. *P* values on a significance level <0.05 are highlighted by an asterisk.

duction event from Eurasia and not a within-California dispersal event from the Sierra Nevada to the Coast Range. The two populations sampled from the Southeastern part of the invasive range in California likely represent a third introduction event. However, inferring details of the history and geography of invasion events from historical evidence and molecular data will always involve a great deal of uncertainty. Invasion biologists can take heed from attempts to track dispersal and range expansion of humans, where despite the tremendous effort and expense invested in the sampling of historical data by the determination of polymorphism in modern populations; many questions have yet to be answered with certainty (Cavalli-Sforza & Feldman 2003).

We did not find substantial migration or admixture between populations in California. In two populations, we found only one individual that differed in its genotypic assignment from the rest of the samples; indicating

recent seed dispersal from a different population. Alleles that were frequent in one lineage were very rare in another lineage and in most cases occurred only in single populations. This and the obvious east-west split of occurrences of the genotypes indicates that *Ae. triuncialis* is at an early state of range expansion and that all three lineages in California represent independent invasion events.

Adaptation to serpentine soil

Reproductive fitness, measured as either seed mass or seed number and averaged across the invasive California lineages, was significantly higher on serpentine soil than on loam soil in our glasshouse experiments. Variation in fitness was accompanied by a high variation among groups in average flowering time and phenotypic plasticity in flowering time. Lineage West flowered early and exhibited low plasticity in response to soil type. Lineage South also exhibited low plasticity but flowered later than the other two lineages. Flowering in Lineage East was phenotypically plastic because individuals in this group flowered earlier in serpentine soil compared to loam soil. An early flowering time appears to be adaptive under serpentine conditions, because coarse-textured serpentine soils dry out faster and have a lower water holding capacity (McKay and Rice, unpublished data), resulting in greater moisture stress in late spring/early summer (Kruckeberg 1984). Flowering phenology differed significantly among the three lineages, suggesting that the independently introduced genotypes may adapt to serpentine conditions via different phenological strategies.

The significant response of California genotypes to soil type, whereby fitness was higher on serpentine, suggests the possibility that specialization to serpentine soils has occurred in California populations of *Ae. triuncialis.* Higher fitness on serpentine soils has been demonstrated for serpentine ecotypes and subspecies of native taxa, perhaps as a result of higher Mg requirements in serpentine specialists (Brady *et al.* 2005). Comparing the reproductive fitness of Californian and Eurasian *Ae. triuncialis* genotypes cannot conclusively determine whether the greater fitness of California plants on serpentine soil results from a *de novo* specialization that evolved after introduction into California or represents a case of pre-adaptation to serpentine conditions. The problem of not knowing the ancestral phenotypic state of the introduced genotypes is not unique to our study, but rather a general problem in studying the adaptation of invasive species. Determining the likely geographic origin of adaptive responses in invasive genotypes may be possible by comparing the phenotypic expression of invasive genotypes with those of the most similar genotypes from the native range (e.g. Maron *et al.* 2004).

Eurasian accessions demonstrated a high variability in their ability to grow under serpentine conditions. Although California genotypes exhibited higher fitness on serpentine, relatively few Eurasian accessions demonstrated this fitness increase on serpentine. This result at least suggests the possibility that serpentine specialization may have occurred after *Ae. triuncialis* had established in California. *Aegilops* is a prominent crop and range weed in its native range and has a high potential of being introduced as a seed contaminant (van Slageren 1994). It is possible that a very large number of diaspores were initially introduced that provided a range of genotypes that could have been selected for under the new environmental regime. The finding that the different phenotypic patterns of phenology and reproduction within the three California lineages are also expressed in a subset of the Eurasian genotypes suggests that lineages with an ancestral state similar to the Californian genotypes may have served as source for the successful introductions. At present, we lack evidence for this as none of the collections were noted as being from serpentine nor is *Ae. triuncialis* noted as occurring on serpentine in the European flora. However, although it is a rare occurrence, *Ae. triuncialis* can be found on serpentine sites within its native range (Meimberg, pers. observation).

Effects of multiple introductions on Aegilops triuncialis *invasion success*

Low additive genetic variance resulting from an introduction bottleneck should reduce the capacity for adaptation to a new environment and slow range expansion (Baker 1965). The most obvious mechanism for reducing the severity of genetic bottlenecks in an invasive species is multiple introductions (Novak & Mack 1995; Kolbe *et al.* 2004). When different genotypes co-occur, recombination increases variation by providing novel genotypes that can be tested under a new selection regime (Falconer & Mackay 1996; Ellstrand & Schierenbeck 2000). Because multiple introductions bring lineages into contact that are effectively isolated by geographic distance in the native range, such recombination can produce genotypes and phenotypes in the new range that are unlikely to occur in the native range (Gaskin & Schaal 2002; Sexton *et al.* 2002). Recent work suggests that reduction in founder effects via multiple introduction and subsequent recombination may be the rule in biological invasions rather than the exception. This effect is highly pronounced in organism groups with frequent introduction potential (Roman & Darling 2007). However, in most cases, it is difficult to show that high genetic variability of introduced populations causes invasiveness, or that invasive populations are the result of recombination

between lineages that became independently invasive and later came into contact. In a recent review, Dlugosch & Parker (2008) showed that naturalized species frequently become invasive despite genetic bottlenecks and concluded that the increase in genetic variation by gene flow does not necessarily facilitate an invasion (e. g. Wang *et al*. 2005; Kliber & Eckert 2005; Geng *et al*. 2007).

In *Aegilops triuncialis*, it is possible that the introduction of multiple genotypes, pre-adapted to serpentine habitats, may have facilitated invasion into California serpentine sites. However, in this case, it is not recombination among multiple introductions, but rather the repeated introductions of pre-adapted genotypes probably facilitated the formation of the multiple invasion fronts we observe. It is possible that the amount of genetic variation initially provided by these repeated introductions was subsequently reduced by selection.

In the future, recombination between *Ae. triuncialis* genotypes may further facilitate invasion by increasing the diversity of genotypes with different adaptive traits. While under a scenario of local adaptation and outbreeding depression, we might expect admixture to initially reduce the average fitness in populations, and the long-term result might be to add new genetic diversity to invasive populations and thus increase the evolutionary potential of these populations. By studying a species in the early stages of invasion, we have been able to examine some of the outcomes of multiple introductions on early population expansion and on initial adaptation to selective challenges in its new range.

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References

Applied Biostatistics Inc., 2002. NTSYSPc Version. 2.1.0w 1996–2002.

Baker HG (1965) Characteristics and modes of origin of weeds. In: *The Genetics of Colonizing Species* (eds Baker HG, Stebbins GL). pp. 147–172, Academic Press, New York.

Barton NH, Slatkin M (1986) A quasi-equilibrium theory of the distribution of rare alleles in a subdivided population. *Heredity*, **56**, 409–415.

Blossey B, Nötzold R (1995) Evolution of increased competitive ability in invasive nonindigenous plants—a Hypothesis. *Journal of Ecology*, **83**, 887–889.

Blumenthal DM, Hufbauer RA (2007) Increased plant size in exotic populations: a commongarden test with 14 invasive species. *Ecology*, **88**, 2758–2765.

Bossdorf O, Auge H, Lafuma L, Rogers WE, Siemann E, Prati D (2005) Phenotypic and genetic differentiation between native and introduced plant populations. *Oecologia*, **144**, 1–11.

Brady KU, Kruckeberg AR, Bradshaw HD (2005) Evolutionary ecology of plant adaptation to serpentine soils. *Annual Review of Ecology, Evolution, and Systematics*, **36**, 243–266.

Cavalli-Sforza LL, Feldman MW (2003) The application of molecular genetic approaches to the study of human evolution. *Nature Genetics*, **33**, 266–275.

Chakraborty R, Nei M (1976) Bottleneck effects on average heterozygosity and genetic distance with the stepwise mutation model. *Evolution*, **31**, 347–356.

Chen YH, Opp SB, Berlocher SH, Roderick GK (2006) Are bottlenecks associated with colonization? Genetic diversity and diapause variation of native and introduced Rhagoletis completa populations *Oecologia*, **149**, 656–667.

Cox GW (2004) *The Evolutionary Ecology of Exotic Plants, Animals, Microbes, and Interacting Native Species*, Island Press, Washington, DC.

Dlugosch KM, Parker IM (2008) Founding events in species invasions: genetic variation, adaptive evolution, and the role of multiple introductions. *Molecular Ecology*, **17**, 431–449.

Ellstrand NC, Schierenbeck KA (2000) Hybridization as a stimulus for the evolution of invasiveness in plants? *Proceedings of the National Academy of Sciences of the United States of America*, **97**, 7043–7050.

Excoffier L, Smouse P, Quattro J (1992) Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics*, **131**, 479–491.

Falconer DS, Mackay TFC (1996) *Introduction to Quantitative Genetics 4*, Longman Group Limited, Essex, England.

Fischer D, Bachmann K (1998) Microsatellite enrichment in organisms with large genomes (*Allium cepa* L.). *BioTechniques*, **24**, 796.

Gaskin JF, Schaal BA (2002) Hybrid Tamarix widespread in US invasion and undetected in native Asian range. *Proceedings of the National Academy of Sciences of the United States of America*, **99**, 11256–11259.

Geng Y-P, Pan X-Y, Xu C-Y *et al.* (2007) Phenotypic plasticity rather than locally adapted ecotypes allows the invasive alligator weed to colonize a wide range of habitats. *Biological Invasions*, **9**, 245–256.

Going BM, Hillerislambers J, Levine JM (2009) Abiotic and biotic resistance to grass invasion in serpentine annual plant communities. *Oecologia*, **159**, 839–847.

Harrison S (1999) Local and regional diversity in a patchy landscape: native, alien, and endemic herbs on serpentine. *Ecology*, **80**, 70–80.

Harrison S, Rice KJ, Maron J (2001) Habitat patchiness promotes invasion by alien grasses on serpentine soil. *Biological Conservation*, **100**, 45–53.

Holt RD (2009) Up against the edge: invasive species as testbeds for basic questions about evolution in heterogeneous environments. *Molecular Ecology*, **18**, 4347–4348.

Huenneke LF, Hamburg SP, Koide R, Mooney HA, Vitousek PM (1990) Effects of soil resources on plant invasion and community structure in a California serpentine grassland. *Ecology*, **71**, 478–491.

Jacobsen WC (1929) Goatgrass—a weed pest of the range. *The Monthly Bulletin, Dept. of Agriculture, State of California*, **18**, 37–41.

Kennedy PB (1928) Goatgrass or wild wheat (*Aegilops triuncialis*). *Journal of the American Society of Agronomy*, **20**, 1292–1296.

Kliber A, Eckert CG (2005) Interaction between founder effect and selection during biological invasion in an aquatic plant. *Evolution*, **59**, 1900–1913.

Kolbe JJ, Glor RE, Rodrıguez Schettino L, Chamizo Lara A, Larson A, Losos JB (2004) Genetic variation increases during biological invasion by a Cuban lizard. *Nature*, **431**, 177–181.

Kruckeberg AR (1984) *California Serpentines: Flora, Vegetation, Geology, Soils and Management Problems*, University of California Press, Berkeley.

Lavergne S, Molofsky J (2007) Increased genetic variation and evolutionary potential drive the success of an invasive grass. *Proceedings of the National Academy of Sciences of the United States of America*, **104**, 3883–3888.

Lee CE (2002) Evolutionary genetics of invasive species. *Trends in Ecology and Evolution*, **17**, 386–391.

Lockwood JL, Cassey P, Blackburn T (2005) The role of propagule pressure in explaining species invasions. *Trends in Ecology and Evolution*, **20**, 223–228.

Maron J, Vilà M, Bommarco R, Elmendorf S, Beardsley P (2004) Rapid evolution of an invasive plant. *Ecological Monographs*, **74**, 261–280.

Meimberg H, Hammond JI, Jorgensen CM *et al.* (2006) Molecular evidence for an extreme genetic bottleneck during the introduction of barbed goatgrass, *Aegilops triuncialis*, to California. *Biological Invasions*, **8**, 1355–1366.

Meimberg H, Rice KJ, Milan NF, Collins C, Njoku CC, McKay JK (2009) Multiple origins promote the ecological amplitude of allopolyploid *Aegilops* (Poaceae). *American Journal of Botany*, **96**, 1262–1273.

Novak SJ, Mack RN (1995) Allozyme diversity in the apomictic vine *Bryonia alba* (Cucurbitaceae)—potential consequences of multiple introductions. *American Journal of Botany*, **82**, 1153–1162.

Parker IM, Rodriguez J, Loik ME (2003) An evolutionary approach to understanding the biology of invasions: local adaptation and general-purpose genotypes in the weed *Verbascum thapsus*. *Conservation Biology*, **17**, 59–72.

Peters A, Johnson DE, George MR (1996) Barb goatgrass: a threat to California rangelands. *Rangelands*, **18**, 8–10.

Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. *Genetics*, **155**, 945–959.

Raymond M, Rousset F (1995) GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. *The Journal of Heredity*, **86**, 248–249.

Reynolds J, Weir BS, Cockerham CC (1983) Estimation of the coancestry coefficient: basis for a short-term genetic distance. *Genetics*, **105**, 767–779.

Roman J, Darling JA (2007) Paradox lost: genetic diversity and the success of aquatic invasions. *Trends in Ecology and Evolution*, **22**, 454–464.

Schneider S, Kueffer J-M, Roessli D, Excoffier L (2000) *Arlequin Version 2.000: A Software for Population Genetic Analysis*, Genetics and Biometry Laboratory, University of Geneva, Geneva, Switzerland.

Sexton JP, McKay JK, Sala A (2002) Plasticity and genetic diversity may allow Saltcedar to invade cold climates in North America. *Ecological Applications*, **12**, 1652–1660.

Sexton JP, McIntyre PJ, Angert AL, Rice KJ (2009) Evolution and ecology of species range limits. *Annual Review of Ecology Evolution and Systematics*, **40**, 415–436.

van Slageren MW (1994) Wild wheats: a monograph of Aegilops L. and Ambylopyrum (Jaub.& Spach) Eig (Poaceae). *Wageningen Agricultural University Papers*, **94**, 37–380.

Suarez AV, Tsutsui ND (2008) The evolutionary consequences of biological invasions. *Moecular Ecology*, **17**, 351–360.

Talbot MW, Smith LS (1930) The goatgrass situation in California. *The Monthly Bulletin, Dept. of Agriculture, State of California*, **19**, 40–46.

Wang BR, Li WG, Wang JB (2005) Genetic diversity of *Alternanthera philoxeroides* in China. *Aquatic Botany*, **81**, 277–283.

Yeh FC, Yang RC, Boyle TB, Ye J, Mao J (1997) *POPGENE, the User-friendly Shareware for Population Genetic Analysis*, Agricultural Food and Forestry Molecular Biology Centre, University of Alberta, Canada.

H.M. investigates the mechanisms of speciation and adaptive diversifications in the context of biological invasions, polyploidy and insular radiations. N.M. is interested in host–parasite evolution, specifically the molecular basis of how host immunity, parasite virulence, and other biological factors can influence the interaction. M.K. studies rangeland ecology and ecophysiology, the physiological mechanisms of adaptation under biotic or abiotic stress, in particular drought conditions. E.K.E. applies evolutionary mechanisms of effective population size and maternal effects to improving techniques in invasive species control, conservation of native species, and restoration. J.K.M. investigates the ecology, evolution and genetics of local adaptation in natural plant populations and crops. K.R. studies the role of evolutionary ecology and ecological genetics in the dynamics of plant invasions and the ecological restoration of native plant communities.

Supporting information

Additional supporting information may be found in the online version of this article.

Fig. S1 Allelic frequencies per locus for *Ae. triuncialis* populations in California. Grouping of populations is indicated in reference to Fig. 1. Locus names are given in the upper left corner of each separate locus map.

Fig. S2 Variation in flowering time, total seed mass, total seeds number per plant and individual seed mass for the Californian lineages and Eurasian accessions grown in loam and serpentine soils. Shown is a box plot indicating the median and the 75% and 25% quantiles. Bars correspond to the 90% and 10% quantiles.

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