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ORIGINAL PAPER

Minimal genetic diversity in the facultatively outcrossing perennial pepperweed (*Lepidium latifolium*) invasion

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Abstract Perennial pepperweed (*Lepidium latifolium* L.) is a Eurasian plant species that is invasive in North America. The invasion often forms large, dense monocultural stands. We investigated the genetic diversity along transects in dense populations in the western USA using Amplified Fragment Length Polymorphisms. We also analyzed transect collections from the native Eurasian range for comparison. In addition, we conducted crossing studies to determine possible modes of seed production (sexual outcrossing vs. self-fertilization vs. apomixis). In our study of seed production we determined that self-fertilization and outcrossing both produce germinable seed in perennial pepperweed. Genetic diversity in the USA was unexpectedly low, with only three genotypes in 388 plants, and those three had genetic similarity of ≥98%. Up to 97% of the plants from Turkey and Russia transects were unique genotypes, while <4% of USA plants in a transect were unique. This lack of diversity in the USA samples suggests that perennial pepperweed, despite its success as an invader, is not well-positioned to adapt to new selective pressures, or to recruit pre-adapted genotypes that may vary in resistance or tolerance to disease or herbivory. Because 99% of the USA plants were genetically identical, we were unable to determine if increases in stand size were due to spread by rhizomes or seed derived from outcrossing between genetically identical parents or self-fertilization, as each of these methods produces shoots genetically identical to parental plants.

Keywords AFLPs · *Lepidium latifolium* · Perennial pepperweed · Genetic diversity · Transect

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Introductions of plant species to new regions often consist of isolated individuals or small populations, which are at high risk of local extinction (Radosevich et al. 2007). Within an introduced species, individuals and populations may vary in mode of reproduction, allocation of resources, levels of competitive ability, or resistance or tolerance to natural enemies and control

methods, all of which have important implications for invasion dynamics. Genetic diversity in isolated populations of introduced plants, while not a requirement for successful invasion, can enhance the ability to adapt and persist when encountering new selective forces (Lee 2002; Sakai et al. 2001). Genetic diversity in plant invasions can range from high in outcrossing species, where many individuals are unique genotypes (e.g., common ragweed, *Ambrosia artemisiifolia*; whitetop, *Lepidium draba*) to low in apomictic species (e.g., rush skeletonweed, *Chondrilla juncea*) or even non-existent in some clonally reproducing species (e.g., water hyacinth, *Eichhornia crassipes;* Japanese knotweed, *Fallopia japonica*) (Genton et al. 2005; Gaskin et al. 2005; Burdon et al. 1980; Zhang et al. 2010; Hollingsworth and Bailey 2000; respectively, and Ward et al. 2008a). Thus, the genetic diversity of an invasion may not be a good predictor of invasion success, but it can assist in management and control decisions (Lee 2002; Sakai et al. 2001). Invasions with high levels of genetic diversity have increased odds of containing pre-adapted phenotypes, or evolving phenotypes, that are resistant or tolerant to selective forces, such as herbicides, pathogens and herbivores used in invasion control programs (Dekker 1997). Even if there is no pre-adapted resistance or tolerance in a plant invasion, it can potentially evolve if there is adequate genetic diversity and a mechanism for creating new genotypic combinations, such as sexual outcrossing. Thus, not only is it important to understand the level of genetic diversity in an invasion (Roderick and Navajas 2003), but also the mode of reproduction, as that will affect ability to adapt to control methods.

Perennial pepperweed (*Lepidium latifolium* L.; family Brassicaceae) is a Eurasian species (Francis and Warwick 2007) that is invasive in the western and northeastern USA, as well as Quebec and western Canada, with smaller invasions in Mexico and Australia (Rios and Garcia 1998; Zouhar 2004; USDA 2005). Dense stands of perennial pepperweed interfere with regeneration of important native willows and cottonwoods along rivers (Young et al. 1995) and displace threatened, endangered, and rare species such as the salt marsh harvest mouse (*Reithrodontomys raviventris* Dixon) (Trumbo 1994) and Suisun Marsh aster (*Aster lentus* Greene) (Skinner and Pavlik 1994). The subsequent decrease in native plant diversity after pepperweed invasion lowers habitat quality and alters ecosystem functions (Zouhar 2004).

The perennial pepperweed invasion has contrary evidence regarding both its level of genetic diversity and mode of seed production. The species was most likely introduced in the western USA in the early 1900s as a seed contaminant of sugar beet (*Beta vulgaris* L.) (Robbins et al. 1952; Renz and DiTomaso 2006), suggesting a large founding population and perhaps multiple introductions via seed shipments, and thus high levels of genetic diversity. This is supported by a RAPD (Randomly Amplified Polymorphic DNA) analysis, which found high diversity both within and between western USA populations (Printz 2000). This evidence contradicts preliminary work by Novak et al. (2001) which, using allozymes, found only two genotypes in six populations on the southern coast of France, where the species has recently naturalized. Regarding mode of seed production, the seeds are highly viable (>90%, Miller et al. 1986) and can travel by wind, water, or perhaps on waterfowl (Howald 2000). It has been suggested that perennial pepperweed can both self-fertilize and outcross, but this assumption has not been explicitly studied (Leininger and Foin 2009). Though there is evidence of high viability, in dry years seed set may be low, and in wet years seed set may be limited by infection with a white rust (*Albugo* sp.) (Young et al. 1997). Despite this potential for copious amounts of seed production and their spread (Howald 2000; Blank and Young 1997; Renz 2000), seedlings are reportedly rarely found in invasive populations (DiTomaso and Healey 2003; T. Dudley pers. comm.). Instead, perennial pepperweed is noted to spread via rhizomes (Francis and Warwick 2007), with ramet density in populations reaching 150 per square meter (Blank 2002; Blank and Young 1997), suggesting that the large monocultures often found in the western USA may contain low levels of diversity, as in southern France. Root fragments can also be transported via waterways or agricultural activities to establish new populations (Renz 2000) that are genetically identical to their source. The mucilaginous seeds are tolerant to salt, suggesting dispersal by tidal currents to new wetland areas (Forman Orth et al. 2006). The spread of perennial pepperweed has been modeled extensively in the San Francisco Bay Area, and studies suggest that the species invades the wetland-upland interface (Andrew and Ustin 2009) and areas with tidal action near open water (Vanderhoof et al. 2009). Long distance dispersal events appear more common in wet

years, with younger infestations having highest spread rates (Andrew and Ustin 2010).

Since the level of genetic diversity and mode of seed production are unclear in the USA perennial pepperweed invasion, we investigated the genetic diversity in dense populations in the western USA using highly variable molecular markers (AFLPs; Amplified Fragment Length Polymorphisms). We utilized transect collections in an attempt to determine genotype diversity within and between invasive plant stands. We further included analysis of transect collections from the native Eurasian range for comparison. In addition, we conducted crossing studies to determine possible modes of seed production (sexual outcrossing vs. self-fertilization vs. apomixis).

Materials and methods

Mode of seed production

In 2009 we grew plants from seed stored at 4°C since being collected by D. Jewett and M. Gaffri in 1999 from five locations in California, Nevada, Utah and Wyoming, USA (Table 1). Seeds were planted in our greenhouse in 5 × 5 × 5 cm pots in vermiculite, peat and perlite at a 2:2:1 ratio. AquaGro 2000G, a wetting agent, was added to the soil mix at a rate of 1:461 (7 cups:yd^3; 0.460 L:m^3). Throughout the experiment pots were watered as needed to retain soil moisture and subjected to 12 h/12 h light/dark. In a greenhouse at approximately 21°C, seeds germinated 6 days after planting (DAP) and were thinned to one plant per pot. At 40 DAP each seedling was transplanted to a 15 cm diameter × 14 cm depth pot containing the same soil. At 60 DAP plants were placed in a 4°C chamber with 12 h/12 h light regime for vernalization. One hundred and thirty-five DAP plants were returned to a greenhouse setting with 12 h/12 h light regime at approximately 21°C, and at approximately 160 DAP flowering began. Five plants each, i.e., one plant from each location, were randomly assigned to one of four treatments and spaced in the greenhouse so as not to pollinate each other by contact : (1) Apomixis; 10 unopened flowers on each plant were emasculated and their inflorescences then bagged with 37 hole/linear cm mesh netting (Organza Pouches, M.T.C. Trading Co., Brisbane, CA, USA) to prevent pollen movement by insects, (2) Unassisted self-fertilization; an inflorescence on each plant was bagged with 37 hole/linear cm mesh netting, (3) Assisted self-fertilization; an inflorescence on each plant was bagged with 37 hole/linear cm mesh netting, and as flowers were beginning to open, they were pollinated by hand with pollen collected from flowers about to open on that same plant, then inflorescences were re-bagged; and (4) Outcrossing; 10 unopened flowers on each plant were emasculated, pollinated by hand with anthers protruding from flowers about to open on a plant from a different location, then bagged with 37 hole/linear cm mesh netting. Fewer flowers were tested in the apomixis and outcrossing treatments ($n = 50$ for each treatment) due to the labor involved in flower emasculation. After plants were done flowering, number of pedicels (represents upper limit of number of possible pods), number of developed pods, and number of seeds produced were counted. Each perennial pepperweed fruit pod can produce up to two seeds, and based on this, the maximum number of seeds was calculated and compared to the realized number of seeds for each treatment. A subset of seed from each treatment was then tested for germination by placing 30 seeds per location per treatment (or fewer if 30 not available) on moist filter paper in a sealed Petri dish. Seeds were kept at 12 h/12 h of light/dark at approximately 21°C until 5 days after the last seed germinated.

Table 1 Locations of perennial pepperweed seed collections used in mode of seed production study

Seed source location	Latitude	Longitude
Elk Grove, California	38.286014	−121.455606
Dunnigan, California	38.84545	−121.931014
Sparks, Nevada	39.607219	−119.733825
Jensen, Utah	40.370344	−109.335547
Rock Springs, Wyoming	41.656897	−109.227467

Transect study

We collected fresh, disease free, undamaged leaf material from six populations in the native range (Turkey and Russia) and eight in the invaded range (California, Nevada and Wyoming, USA) and stored it in silica gel. A total of 201 and 388 plants were sampled in the native range and USA, respectively (Table 2). Plants were sampled every two meters along a transect of the longest axis of the stand. If no

Table 2 Information on perennial pepperweed populations sampled in the native range and in the USA, including number of samples (*N*), number of distinct genotypes (*G*), distinct genotypes per number of samples (*G/N*), Simpson's diversity index (*D*), Gene diversity (H_j), number of genotypes in a transect represented by only one shoot (singletons) and number of plants that share a unique genotype within a population

Transect location	Latitude	Longitude	*N*	*G*	*G/N*	*D*	H_j	No. of singletons	No. of plants sharing a genotype	Population identifier
Native range										
Turkey 1 (Topakkaya)	38.4720	33.8946	40	10	0.25	0.660	0.058	4	23, 4, 3, 2, 2, 2	TRLL8
Turkey 2 (Pozanti)	37.4243	34.8748	39	32	0.82	0.990	0.117	25	2, 2, 2, 2, 2, 2, 2	TRLL4
Turkey 3 (Kayseri)	38.8024	35.5960	44	35	0.80	0.989	0.114	26	2, 2, 2, 2, 2, 2, 2, 2, 2	11217
Russia 1 (Temryuk North)	45.2805	37.3844	32	31	0.97	0.998	0.073	30	2	11218
Russia 2 (Temryuk South)	45.2697	37.3668	27	26	0.96	0.997	0.078	25	2	9802
Russia 3 (Golubitskaya)	45.3312	37.2612	19	2	0.11	0.485	0.034	0	12, 7	9804
Mean (native range)			33.5	22.50	0.65	0.853	0.079			
Invaded range										
Fairfield, California	38.2006	−122.0274	49	1	0.02	0.000	0.000	0	49	10948
Tulelake, California	41.9065	−121.5650	50	1	0.02	0.000	0.000	0	50	10946
Wendel, California	40.3526	−120.3032	49	2	0.04	0.041	0.002	1	48	10196
Derby Dam, Nevada	39.5872	−119.4424	48	1	0.02	0.000	0.000	0	48	10195
Elko, Nevada	40.8215	−115.7702	50	1	0.02	0.000	0.000	0	50	10947
Fallon, Nevada	39.3872	−118.7091	46	1	0.02	0.000	0.000	0	46	10193
Reno, Nevada	39.4523	−119.7230	46	2	0.04	0.043	0.002	1	45	10194
Rock Springs, Wyoming	41.6560	−109.2277	50	2	0.04	0.078	0.004	0	48, 2	10900
Mean (invasive range)			48.5	1.38	0.03	0.020	0.001			

plant was within 1 m of the transect at the 2 m mark, nothing was collected and the location marked as a blank. At each site, 19–50 plants were collected per transect depending on stand size. We also collected leaf material from the 20 plants used in the mode of seed production test described above (Table 1), and from seeds ($n = 6$) produced in the outcrossing treatment (#4) that we were able to grow to the seedling stage.

Genomic DNA was extracted from approximately 20 mg of silica-dried material using a modified CTAB method (Hillis et al. 1996). The AFLP method followed Vos et al. (1995) with these modifications: restriction and ligation were performed during a single step in an 11 µL reaction containing 500 ng genomic DNA, 2 U *Mse*I (New England Biolabs [NEB], Ipswich, MA, USA), 1 U *Eco*RI (NEB), 1X T4 DNA ligase buffer (NEB), 0.45 U T4 DNA ligase (NEB), 0.05 M NaCl, 0.5 X BSA, 4.5 µM *Mse*I adaptor, 0.45 µM *Eco*RI adaptor, and H_2O. The restriction-ligation product was incubated at room temperature overnight, then 5.5 µL of the product was diluted to 100 µL in TE (15 mM Tris and 0.1 mM EDTA). A pre-selective Polymerase Chain Reaction (PCR) was performed in a 20 µL reaction containing 4 µL of the diluted, restricted-ligated product, 1 X PCR buffer (Bioline, Taunton, MA, USA), 1.5 mM $MgCl_2$, 0.2 mM each dNTP, 0.2 µM of each pre-selective amplification primer (*Mse*I + C and *Eco*RI + A), 0.5 U Taq polymerase (Bioline) and H_2O. The pre-selective PCR conditions consisted of 20 cycles of: 30 s at 94°C, 60 s at 56°C, and 60 s at 72°C. 10 µL of the pre-selective amplification product was diluted to 200 µL in TE (15 mM Tris and 0.1 mM EDTA). The selective amplification was performed in a 20 µL reaction containing 3 µL of the diluted pre-selective amplification product, 1 X PCR buffer, 1.5 mM $MgCl_2$, 0.2 mM each dNTP, 0.1 µM *Mse*I selective primer, 0.05 µM *Eco*RI selective primer dye-tagged with 6-FAM (Integrated DNA Technologies, Coralville, IA, USA), 0.5 U of Taq polymerase and H_2O. The selective PCR conditions consisted of 120 s at 94°C; 10 cycles of: 20 s at 94°C, 30 s at 66°C (decreasing by 1°C each cycle), 120 s of 72°C; 25 cycles of: 20 s at 94°C, 30 s at 56°C, 120 s at 72°C. 0.25 µL of 600 base pair (bp) size standard and 9.25 µL of de-ionized formamide were heated to 95°C for 3 min, then 0.5 µL of each selective PCR product was added. This mixture was loaded into an Applied Biosystems (Foster City, CA, USA) 3130 Genetic Analyzer. In order to determine which AFLP primers were most informative, we analyzed all 15 selective primer combinations of *Mse*I + CAA, CAC, CAT, CTA, or CTA and *Eco*RI + AAG, ACC, or ACT for 8 plant samples from the USA and Eurasia. The two most polymorphic primer combinations were chosen (*Mse*I + CAT/ *Eco*RI + AAG and *Mse*I + CTA/ *Eco*RI + AAG). Loci were scored by the fragment analyzer software GeneMapper v 4.0 (Applied Biosystems) with a minimum of 50 relative fluorescent units (rfu). These bins were then manually screened for electropherogram shape, height, and isolation from nearby bins, making this a semi-automatic scoring process as suggested by Papa et al. (2005), further lowering the number of bins utilized for final analyses. To determine number of genotypes in a transect (G), we analyzed electropherograms using the program GeneMapper. We realize that GeneMapper may overestimate number of genotypes, thus we also visually compared electropherograms after GeneMapper analysis to confirm number of genotypes.

Statistical analyses

ANOVA was used to compare arcsine square-root transformed means of number of developed pods per pedicel, number of seeds per pod, and number of germinating seeds per seed deemed visually mature (full size and plump) for the four mode of seed production treatments. A Tukey HSD test was used to determine if pairs of means in pod and seed analyses were statistically different. Analyses for the transect study included the number of distinct genotypes in a transect (G) and the number of genotypes in a transect compared to the total number of plants in the transect (G/N), where $N =$ the number of plants sampled in a transect. Simpson's diversity index (D) corrected for sample size (Pielou 1969) was calculated with $D = 1 - \sum n_i(n_i - 1)/N(N - 1)$ for $i = 1$ to G, where n_i is the number of plants that share genotype i. Values of D can range from 0 to 1, with higher values corresponding to greater genetic diversity. Gene diversity within a transect (H_j; Nei 1978; Lynch and Milligan 1994) under an assumption of Hardy-Weinberg equilibrium was evaluated using AFLP-SURV 1.0 (Vekemans 2002). The Mann-Whitney U-test was used to compare means of G/N, D and H_j between populations.

Results

Mode of seed production study

The ANOVA revealed significant differences ($F = 17.48$, $df = 3$, $P < 0.0001$) in number of seed pods produced per pedicel. Specifically, both unassisted and assisted self-fertilization produced significantly more pods than both outcrossing and apomixis (Tukey HSD, $P < 0.01$) (Table 3). There were significant differences between treatments in the number of seeds actually produced compared to maximum possible number of seeds (apomixis treatment dropped from this ANOVA, since no mature seeds were produced, $F = 22.82$, $df = 2$, $P < 0.0001$). Assisted self-fertilization produced significantly more seeds than unassisted self-fertilization, outcrossing and apomixis ($P < 0.01$). The percentage of germinating seeds did not vary significantly (apomixis treatment dropped from this ANOVA, since no mature seeds were produced, $F = 1.78$, $df = 2$, $P = 0.214$), but was high in each case (>89%).

Transect study

The two AFLP primer pairs *Mse*I + CAT/*Eco*RI + AAG and *Mse*I + CTA/*Eco*RI + AAG, after manual screening, provided us with a total of 100 polymorphic fragments (52 and 48, respectively). In the USA, 384 of 388 individuals (99%) were genetically identical (Fig. 1). In five different USA locations (Fallon, Derby Dam, Tulelake, Elko and Fairfield), only one AFLP genotype was found. In the native range 136 genotypes were found and up to 35 different genotypes out of 44 plants tested were recorded at a single location. No genotypes were shared between native locations, or between native and invasive locations. The lowest genotype diversity in plants from a transect in the native range was two genotypes, however, at this location only 19 plants were sampled compared to at least 46 in the USA. On average, ratio of genotypes (*G/N*) per transect was lower in the USA than the native range (Table 2; Fig. 2, and Mann-Whitney *U*-test: mean, 0.03 vs. 0.65, $P = 0.001$). Simpson's diversity index (*D*) was also significantly lower in the USA compared to the native range (Mann-Whitney *U*-test: mean, 0.020 vs. 0.853, $P = 0.001$), as was gene diversity (H_j) (Mann-Whitney *U*-test: mean, 0.001 vs. 0.079, $P = 0.001$). Plants used in the study of mode of seed production from five different locations in the USA were all identical, representing the most common genotype found in the USA. The offspring resulting from outcrossing of three of these plants ($n = 6$ seeds from treatment #4 in the mode of seed production experiment) were also the same genotype. This common genotype was not found among the native genotypes recorded in this study.

Table 3 Results of mode of seed production study for perennial pepperweed

Treatment	No. pods/pedicel		No. full sized seeds/ no. seeds possible*		No. germinable seed/ visually mature seed	
	Total	Mean SE	Total	Mean SE	Total	Mean SE
Apomixis	11/50	0.220[a]	0/100	0.000[a]	NA	NA
		0.107		0.000		
Unassisted self-fertilization	1,909/1,909	1.000[b]	266/3,818	0.0672[a,b]	128/142	0.898[a]
		0.000		0.009		0.065
Assisted self-fertilization	452/452	1.000[b]	369/904	0.405	149/150	0.993[a]
		0.000		0.029		0.007
Outcrossing	21/50	0.420[a]	10/100	0.100[b]	10/10	1.000[a]
		0.159		0.035		0.000

See text for treatment details

NA not applicable (no seed available for analysis)

* Each perennial pepperweed seed pod can produce a maximum of two seeds

[a,b] Mean values with matching superscript letters are not significantly different ($P > 0.05$) within a treatment column, as determined by Tukey HSD test

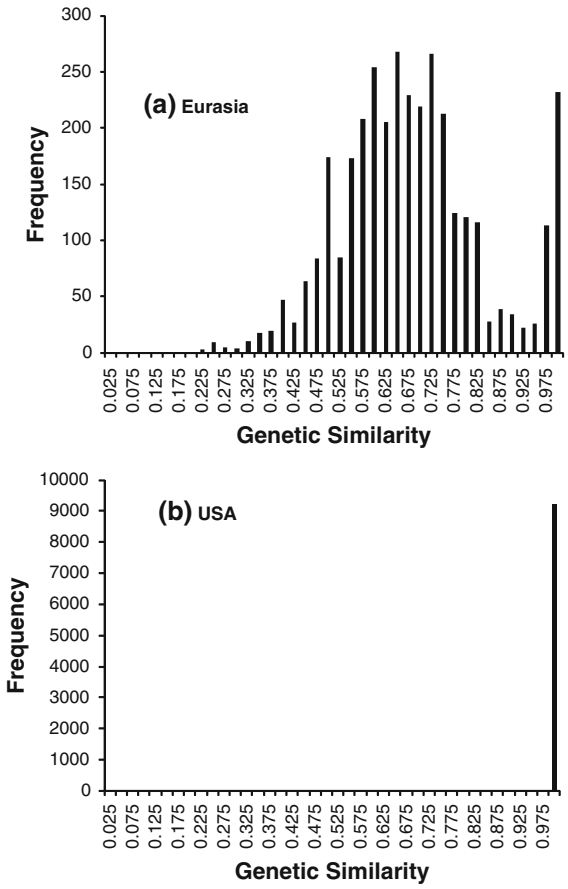

Fig. 1 Histogram of the pairwise similarities between all transect plants in **a** native range (Eurasia) and **b** invasive range (USA)

Discussion

Lower diversity in USA

Genetic diversity (*G/N*, *D*, and H_j) in the USA was significantly lower compared to the native range. Diversity in the invaded range was also low compared to other species capable of both vegetative reproduction and sexual outcrossing (e.g., Dong et al. 2006; Ward et al. 2008b; Sun et al. 2005; see Ward et al. 2008a), though direct comparisons are difficult due to differences in sampling, markers, and diversity measurements used. Unexpectedly, the genetic diversity of invasive perennial pepperweed populations was more similar to selfing or clonal aquatic invaders (e.g., Pester et al. 2003; Ye et al. 2004). The low diversity in perennial pepperweed could be due to founder effects or a strong bottleneck during or after introduction, and may have been enhanced by our sampling dense stands at USA locations, which may be more likely to contain identical clonal ramets. The low genetic diversity in the USA was also unexpected because the putative source of introduction was seed contamination (Howald 2000), which typically implies multiple introductions of multiple seeds, with each introduction likely to include multiple genotypes. For example, common ragweed, probably imported to Europe as contaminant in ballast or seed lots, showed similar genetic variation between the native USA range and the invasion in France (Genton et al. 2005). If perennial pepperweed did arrive in the USA as a seed contaminant, it suggests that the origin would be a native range population that was low in diversity like our Russia 3 population (Golubitskaya), in which we only found two genotypes. It should be kept in mind that this work is not meant to be an exhaustive analysis of genetic diversity in either the native or invasive range, or an analysis of origins, which would require more extensive collections.

In the USA transects, 384 of the 388 plants were identical AFLP genotypes. This suggests that on a region-wide scale (California, Nevada and Wyoming) there is possibly little genetic variation in the perennial pepperweed invasion. Even when USA transects contained more than one genotype, the different genotypes were genetically very similar, differing in character state at only one or two of the 100 loci ($\geq$97.8% Dice similarity; data not shown). In comparison, in the single native transect that had only two genotypes (Russia 3), these were quite distinct ($\leq$83% Dice similarity; data not shown). The histogram (Fig. 1) also illustrates how genetically different plants were in the native range compared to the invaded USA transects.

Conflict with earlier genetic study

The result of little or no diversity in USA transects is in agreement with the findings of Novak et al. (2001) in southern France, but is in direct conflict with results from the earlier RAPDs study performed on plants collected from 12 populations in western USA (Printz 2000). That study found significant mean genetic variation across all sites in the west ($H_W = 0.3603$), and also mean gene diversity averaged over all loci within each population (H_j: 0.276 to 0.4289), whereas our study found almost no gene diversity ($H_j \leq 0.004$)

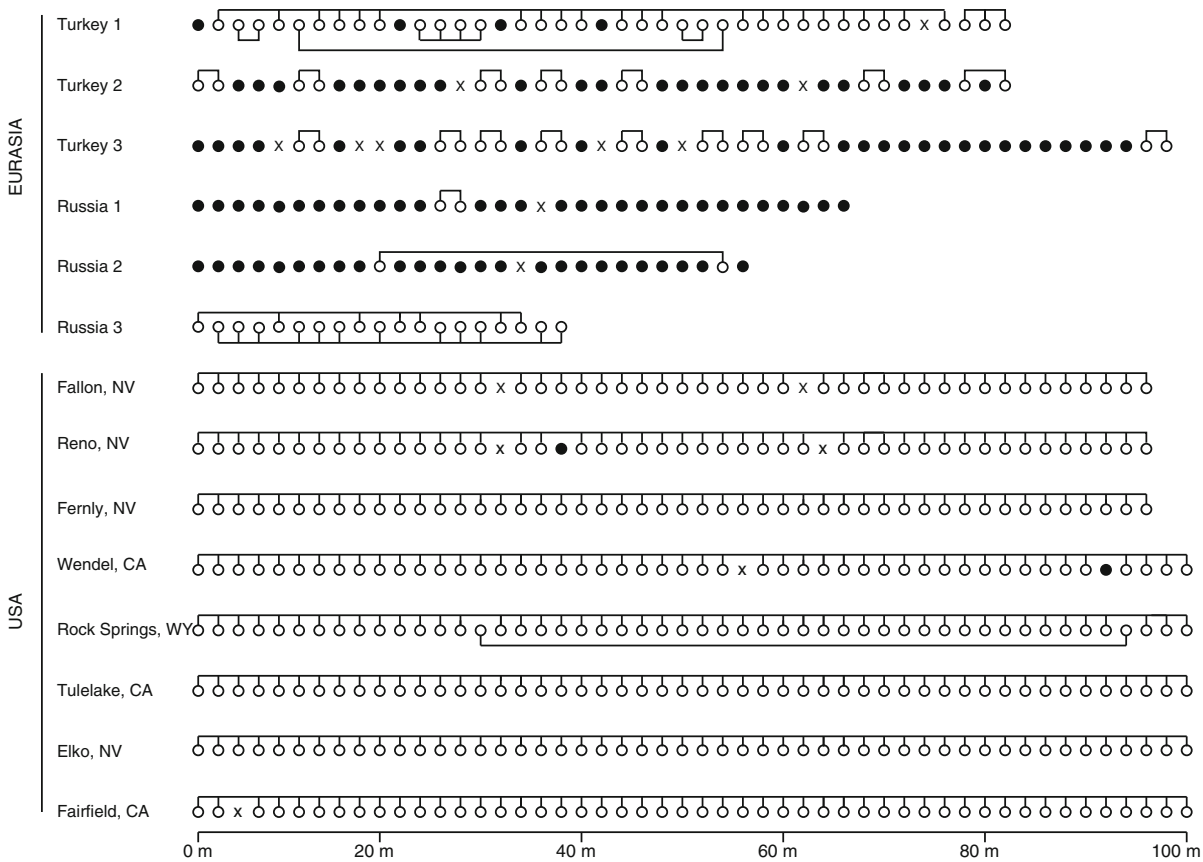

Fig. 2 Diagram showing the distribution of distinct genotypes in perennial pepperweed transects in the native range (Eurasia) and the invasive range (USA). *Empty circles* connected by *lines* are identical genotypes; *black circles* represent singleton genotypes, and x indicates no sample collected or successfully genotyped at that location on transect

within or between USA populations. This would indicate that either: (1) our genetic marker system provides much less resolution than RAPDs for this species; (2) the two studies sampled populations with vastly different levels of genetic diversity, or (3) the molecular markers used in the two studies have very different error rates. The first hypothesis seems unlikely as our AFLPs were able to distinguish up to 26 different genotypes out of 27 sampled plants in a native range transect (Russia 1), suggesting that the AFLPs used do have sufficiently high resolution to distinguish between perennial pepperweed individuals. The second hypothesis seems unlikely given that none of our invasive populations were genetically diverse, and all of the populations from the earlier RAPDs study exhibited significant diversity. The third hypothesis seems most plausible, as error rates for RAPDs are approximately 15% in genomes of both high and of low complexity (Halldén et al. 1996), whereas AFLP error rate is typically lower (2.0–2.6%; Bonin et al. 2004). AFLP analysis of a widespread collection, such as the samples collected by Printz (2000), could more fully resolve this conflict.

Mode of seed production

Our study revealed no evidence of apomixis in perennial pepperweed, i.e., no seeds were produced on plants assigned to this treatment. All seed produced in the outcrossing treatment germinated, but there were very few seeds produced (10 out of a possible 100 seeds). This is probably due to the methods used in the apomixis and outcrossing treatments. The experiments required emasculation of the very small, closed flowers (~3 mm wide), which led to visual flower desiccation and may have lowered pod or seed

production. Assisted self-fertilization produced an average of approximately six times more seed than unassisted self-fertilization, although both produced highly germinable seed. This indicates that movement of pollen from anthers to stigma within a flower may not consistently occur in this species without outside forces such as pollinators. It was already known that seeds of perennial pepperweed are highly viable (>90%, Miller et al. 1986), but now it is shown that viable seed can be produced from both self-fertilization and outcrossing.

Spread by seed versus rhizomes

It is clear that perennial pepperweed can produce high numbers of viable seed which may contribute to its local spread. It is, however, also possible that the large infestations found in the USA were the result of spread via rhizomes. We sampled 100 m across many infestations of perennial pepperweed, but these infestations can be even larger (JFG, pers. observ.). In the invasive range, infestations of this species can grow at a rate of 1–2 m per year at their leading edge (Renz 2002), thus a 100 m infestation could be created from an individual via rhizomes in as little as 25 years. Perennial pepperweed has been in the western USA since 1932 (Rice 2005), and most recently expanded its presence since 1980 (Young et al. 1998), giving ample time for production of large infestations without requiring spread by seed.

Our ability to draw conclusions on the relative contributions of seeds versus rhizomes to the spread of invasive perennial pepperweed at the local level is limited due to the unexpected lack of genetic diversity found in the USA transects. High genetic differentiation in transects strongly suggests that reproduction by seed is predominant, as was seen in most sites in the native range (Fig. 2). Conversely, populations with genetically identical individuals, as found in the USA, suggests clonal reproduction. It is, however, also possible for genetically identical plants to originate from self-fertilization or outcrossing of genetically identical and highly homozygous plants. We observed this in our greenhouse outcrossing studies, where parental plants created offspring identical to each other and the parents. Unfortunately, our data set does not allow for statistical tests to distinguish clonal from sexual recombination since 99% of USA plants from transects were genetically identical (Stenberg et al. 2003, see review in Halkett et al. 2005). The observation that few if any seedlings are ever seen in the USA strengthens the hypothesis of spread by rhizomes, at least at the local level, and modeling studies suggest that tidal action and episodic flooding events are capable of spreading rhizome fragments long distances (Andrew and Ustin 2009; Vanderhoof et al. 2009).

Implications for management

Our study of eight perennial pepperweed populations in the USA indicates that there is almost no genetic diversity in the areas that we sampled. Further collections and analysis may alter that result, but it currently appears that the diversity levels are similar to those found by Novak et al. (2001) in naturalized populations in southern France. This lack of diversity suggests that the perennial pepperweed invasion is not well-positioned to adapt to selective pressure from control methods, or to recruit pre-adapted genotypes that may vary in resistance or tolerance to disease or herbivory, though the species currently invades a wide range of habitats and climates, most likely made possible by high levels of phenotypic plasticity. If the genetic diversity in North America is greater than our data suggest, the demonstrated ability for this species to produce viable seed via outcrossing indicates that adaptation could occur when genetically diverse populations come in contact.

We found evidence that stand expansion most likely occurs via rhizomes. Although recruitment from seedlings cannot be excluded, our data suggest that any management strategy should aim at disrupting clonal growth to control local expansion. Reduction of seed output will nevertheless be important to inhibiting long distance dispersal. Because conventional control techniques have not adequately provided permanent reduction of population density of perennial pepperweed (Young et al. 1998; Renz and DiTomaso 2006), a biological control program has been initiated in 2005 (Hinz et al. 2008). The host-specificity and efficacy of one root-feeding weevil (*Melanobaris* sp. pr. *semistriata* Boheman), a collar gall-weevil (*Ceutorhynchus assimilis* (Paykull, 1792)), three stem-attacking insects (*Ceutorhynchus marginellus* Schultze, *Phylotreta reitteri* Heikertinger, *Lasiosina deviata* Nartshuk) and one gall mite (*Metaculus lepidifolii* Monfreda & De Lillo) are currently being investigated. Also

underway is a study characterizing the natural enemies attacking perennial pepperweed in its introduced range. Such studies are often conducted with little or no knowledge of the genetic diversity existing in their host plant populations in the invasive range. It is common practice to use just one or a few populations in tests to represent all of the invasive populations that often occur over a large geographic range. The low genetic diversity we found in invasive perennial pepperweed will simplify these studies, as it will be sufficient to use the one extremely common genotype for any host-specificity and efficacy trials. In addition, if origins of this genotype can be determined, that may help in finding potential agents that have co-evolved with the invasive genotype.

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