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ORIGINAL PAPER

Genetic diversity and population structure of sickleweed (*Falcaria vulgaris*; Apiaceae) in the upper Midwest USA

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Abstract Sickleweed (*Falcaria vulgaris*), an introduced species native to Europe and Asia, grows as an aggressive weed in some areas of the upper Midwest in the United States. We are reporting genetic diversity and population structure of sickleweed populations using microsatellite markers and nuclear and chloroplast DNA sequences. Populations showed high genetic differentiation but did not show significant geographic structure, suggesting random establishment of different genotypes at different sites was likely due to human mediated multiple introductions. Three genetic clusters revealed by microsatellite data and presence of six chlorotypes supported our hypothesis of multiple introductions. Chloroplast DNA sequence data revealed six chlorotypes nested into two main lineages suggesting at least two introductions of sickleweed in the upper Midwest. Some individuals exhibited more than two alleles at several microsatellite loci suggesting occurrence of polyploidy, which could be a post-introduction development to mitigate the inbreeding effects. High genetic variation in the introduced range attributable to multiple introductions and polyploidy may be inducing the evolution of invasiveness in sickleweed. Results of this study provide valuable insights into the evolution of sickleweed and baseline data for designing proper management practices for controlling sickleweed in the United States.

Keywords Chlorotypes · *Falcaria vulgaris* · Microsatellite · Polyploidy · *trnL-trnF* · *trnQ-rps16*

Introduction

Introduced species can have severe repercussions on global biodiversity (Sala et al. 2000; Lee 2002; Provan et al. 2005; Lachmuth et al. 2010; Alyokhin 2011); therefore, it is necessary to be vigilant about every plant species introduced outside of its range (Simberloff et al. 2011). Increasing international commerce has facilitated tremendous exchange of biotas (Mooney and Cleland 2001), a phenomenon expected to facilitate the spread of introduced species (Frankham 2005). Control of an introduced plant species can be a daunting task when the species is already invasive, but

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timely identification can ward off major ecological and economic impacts. It is thus essential to identify introduced plants that can become invasive as early as possible (Kolar and Lodge 2001) so that proper management can be instituted prior to their establishment and spread. Knowledge of the genetic structure and gene flow between founding populations is important to assess the long term persistence of an invading species (Nater et al. 2013).

Conservation biology theory suggests that a population with low genetic diversity tends to undergo extinction due to genetic drift and inbreeding depression (Ellstrand and Elam 1993; Young et al. 1996); however, there exists a genetic paradox for invasive species (Allendorf and Lundquist 2003). Introduced species that represent a subset of their source populations usually have reduced genetic diversity compared to the source population, but they may be capable of expanding their range in the novel habitat by outcompeting and replacing locally adapted native species (Allendorf and Lundquist 2003). A major obstacle for establishment of introduced species in the new range is to overcome negative effects of small population size and low genetic diversity (Leung et al. 2004; Taylor and Hastings 2005). Multiple introductions (Lavergne and Molofsky 2007), a large number of founder individuals (Allendorf and Lundquist 2003), gene flow among populations (Slatkin 1987) and polyploidy (Comai 2005) are some mechanisms whereby invasive species can offset the negative consequences of small population size and reduced genetic diversity.

High genetic diversity and gene flow among populations may promote successful spread of an established population (Sakai et al. 2001). Higher intra-specific genetic diversity provides greater opportunities for the creation of adapted genotypes through genetic reshuffling and recombination, and also more options for natural selection of genotypes better suited for the environment thus producing a population better able to exploit the novel conditions of the introduced range (Lavergne and Molofsky 2007). Therefore, investigation of genetic diversity and population structure and determination of number of introductions in the novel range can provide a strong framework for assessing invasiveness (Sakai et al. 2001; Valliant et al. 2007). At the same time, such studies may identify source populations (Novak and Mack 2001; Genton et al. 2005; Provan et al. 2005; Valliant et al. 2007) and help design effective control measures for an invasive species (Sakai et al. 2001).

Most studies are focused on the invasive plants with severe impacts (Pysek et al. 2008), and our knowledge of potentially invasive naturalized plants in the lag phase of establishment is very limited (Kowarik 2005). Recently naturalized plants need to be studied to determine their potential for invasion and to develop management strategies (Wilson et al. 2011). In this study, we report on the population genetic structure of sickleweed (*Falcaria vulgaris* Bernh.; Apiaceae) of the upper Midwest in the United States. Sickleweed is native to southern and central Europe and extends eastward through Central Asia and the Middle East. It is introduced in Africa, northern Europe, and North and South America (DAISIE 2008; Larina 2008). It has been reported in sixteen states in the USA (USDA, NRCS 2011) and exhibits disjunct distribution in the Midwestern, Southern and Eastern regions. It has been reported to occur abundantly in three states in the upper Midwest that include Iowa, Nebraska and South Dakota (Piya et al. 2012). In South Dakota, Fort Pierre National Grassland (FPNG) and Buffalo Gap National Grassland (BGNG) are the two grasslands where this plant has expanded in distribution and abundance (Korman 2011). A recent study by Butler and Wacker (2013) indicates that human disturbances created gaps in ground cover and altered competitive relationships among native species, which facilitated the spread of sickleweed at FPNG. We also observed several large populations of sickleweed along roadsides in Nebraska where the Nebraska Invasive Species Council has listed it as a potentially invasive plant (NISC 2011). The plant also occurs as much smaller populations in Onawa, Iowa and Brookings, South Dakota.

While sickleweed is abundantly found in some states of the Midwest, there are no recent reports of the species in the eastern USA (Piya et al. 2012). Several introduced populations may fail at different stages of invasion and may disappear (Richardson et al. 2000). This may have been the fate of sickleweed introduced in the eastern USA. It is also plausible that the plant may occur in this region but its frequency is so low that the plant has become inconspicuous. In Iowa, Nebraska and South Dakota, however, some populations have naturalized and are expanding. Therefore, in this study, we have included populations from these three states. Butler and Wacker (2013) reviewed that many naturalized associates of sickleweed from Eurasia have successfully established in similar

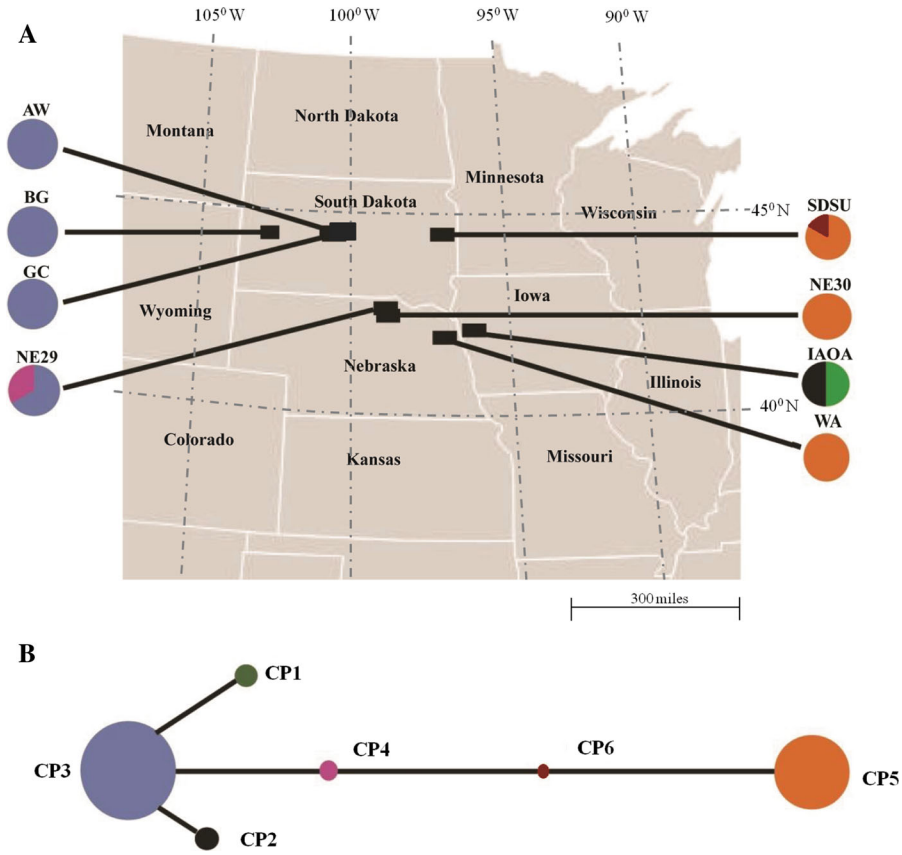

Fig. 1 Chlorotype variation in *F. vulgaris*. **a** Distribution map of chlorotypes in Iowa, Nebraska and South Dakota and **b** Chlorotype network. Each chlorotype is represented by a circle size proportional to the relative frequency of the chlorotype

habitats in North America and indicated that sickleweed might therefore be pre-adapted to the environmental conditions of Great Plains. The current sickleweed distribution pattern perhaps signifies the importance of species introductions at multiple sites with different conditions, whereby some populations overcome all barriers in the introduction-naturalization-invasion continuum (Lockwood et al. 2005; Simberloff 2009; Blackburn et al. 2011).

Sickleweed is described as annual, biennial or perennial (Clapham et al. 1987; Korman 2011). It produces protandrous andromonoecious flowers (Knuth 1908) and large numbers of seeds. The plant exhibits a characteristic seed dispersal mechanism: when the plant senesces, the stems break at the nodes and tumble in the wind to disperse the seed (Korman 2011). In addition, the plant can reproduce asexually by producing new shoots from its root stock (Gress 1923; Korman 2011). The plasticity in life history traits (i.e., habit, seed dispersal mechanism, and clonal reproduction) likely contributes to its emergence as an aggressive weed in the Midwest. In this study we used both chloroplast and nuclear DNA markers to achieve the following objectives: (1) study the genetic diversity and structure of upper Midwest populations of sickleweed in the United States, and (2) determine whether sickleweed populations in the United States were established from a single introduction or resulted from multiple introductions.

Methods

Sample collection

A total of 96 samples from eight populations were collected that represent three of the sixteen states where sickleweed has been reported in the United States (Fig. 1; Table 1). Fresh leaf tissues were collected in silica gel from 12 plants per population. Individuals growing more than 10 m apart were chosen to avoid sampling clones. Exceptions were made for two small populations (i.e. IAOA and SDSU; see Table 1 for population ID). The silica gel dried

Table 1 Geographic and genetic characteristics of sickleweed populations in the Midwest USA

S. no	Collection site	Population name	Latitude/longitude	A	A_e	H_o	H_e	F_{IS}
Nebraska								
1	Wayne	WA	42°14′7.18″ N 97°8′32.24″ W	17	1.88	0.36	0.37	0.05
2	Boyd	NE30	42°57′9.63″N 98°40′9.93″W	26	2.42	0.51	0.49	−0.04
3	Boyd	NE29	42°52′2.99″ N 98°45′7.22″ W	21	2.56	0.43	0.44	0.03
South Dakota								
4	Brookings	SDSU	44°18′53″ N 96°47′38″ W	19	2.41	0.34	0.31	−0.12
5	Grass Creek, FPNG	GC	44°11′45.35″ N 100°18′26.49″ W	24	2.47	0.57	0.59	0.04
6	Alkali West, FPNG	AW	44°10′57.85″ N 100°17′42.87″ W	27	2.84	0.57	0.61	0.08
7	BGNG	BG	43°55′56.12″ N 102°24′8.54″ W	28	3.05	0.61	0.54	−0.12
Iowa								
8	Onawa	IA	42°57′9.62″ N 96°7′34.68″ W	12	1.55	0.38	0.26	−0.05
Mean						0.47	0.55	−0.023

A = number of alleles per population, A_e = effective number of alleles, H_o = observed heterozygosity, H_e = expected heterozygosity, F_{IS} = inbreeding coefficient

leaf sample was ground to a fine powder using a mortar and pestle. DNA was isolated from ground leaf tissue using DNeasy Plant Minikit (Qiagen corp., Valencia, CA).

Microsatellite genotyping

Six microsatellite markers developed for *Daucus carota* were successfully transferred to sickleweed (Piya and Nepal 2013); therefore, we used these markers (Supplementary material table S1) to genotype individuals. Some of the microsatellite DNA sequence obtained using *Daucus* primer pairs had mutations in primer binding sites of two loci (forward and reverse primers of GSSR25 and reverse primer of BSSR53) in sickleweed; therefore, primers were redesigned for these two loci using http://primer3.wi.mit.edu/. For genotyping, PCR was carried out in a reaction mixture of 15 µl containing 50 ng genomic DNA, 3 µl of 5× buffer, 1.2 µl of dNTPs, 2 µl of 25 mM $MgCl_2$, 0.5 µl of 10 pM forward primer, 0.5 µl of 10 pM forward primer tagged with M13 tail, 1 µl of 10 pM reverse primers each and 2 units of *Taq* polymerase. The PCR conditions were an initial denaturation of 4 min at 94 °C followed by 40 cycles of 1 min denaturation at 94 °C, 20 seconds annealing temperature [varies with primer pairs; see (Piya and Nepal 2013)] and 1 min extension at 72 °C, and final extension of 5 min at 72 °C. The PCR products were genotyped using 3730×1 DNA Analyzer (Applied Biosystems) at the Iowa State University DNA Facility.

Data analyses

We used GeneMarker V2.4.0 (Softgenetics) to visualize the genotyping data and create allele reports. Possible genotyping artifacts such as stuttering, large allele drop-out and presence of null allele were tested using Micro-Checker (Van Oosterhout et al. 2004) and no evidence of scoring error due to stuttering, large allele drop-out and null alleles was noted. Absence of genotyping artifacts and null alleles suggests that any deviation from Hardy–Weinberg equilibrium (HWE)

is the result of change in allele frequency. Tests for linkage disequilibrium and population fixation indices (F_{IS}) for each locus were calculated using FSTAT V1.2 (Goudet 1995).Test for deviation from HWE was performed using the program Arlequin V3.0 (Excoffier et al. 2005). Markov chain randomizations based on 10,000 dememorizations and 100,000 iterations were carried out to assess the level of significance of the test. FSTAT V1.2 (Goudet 1995) was used to compute Nei's (1973, 1977) total gene diversity (H_T) which was partitioned into within population (H_S) and among population gene diversity (D_{ST}). G_{ST}, the proportion of genetic diversity that resides among populations, was computed as the ratio of D_{ST} to H_T. Genetic diversity within population was estimated in terms of total number of alleles per population (A), effective number of alleles (A_e), observed heterozygosity (H_o) and Nei's (1987) unbiased expected heterozygosity (H_e) using PopGene (Yeh and Boyle 1997). Private alleles for each population were determined using GENALEX 6.5 (Peakall and Smouse 2006). ARLEQUIN (Excoffier et al. 2005) was used to perform analysis of molecular variance (AMOVA) and calculate pair-wise genetic differentiation (F_{ST}). Mantel's test for isolation by distance (IBD) (Mantel 1967) was performed using IBDWS 3.23 (http://ibdws.sdsu.edu/~ibdws/) to test the relationship between natural log of geographical distance and genetic distance ($F_{ST}/1-F_{ST}$).

Bayesian analysis of genetic structure of the sampled populations was carried out in the program STRUCTURE Version 2.2 using admixture model with independent allele frequencies (Pritchard et al. 2000). We performed 20 independent runs for each value of K (=number of genetically distinct clusters) ranging from 1 through 11. Each run included 10,000 burn-in iterations and 50,000 replicates. The best value of K was determined by computing ΔK following Evanno et al. (2005). Each individual was assigned to the inferred clusters using a threshold proportion of inferred ancestry (q) *i.e.* $q \geq 0.70$, otherwise an individual was determined as admixed if the q value was less than 0.70.

Chloroplast and nuclear DNA sequence data

Six samples from each of the populations were randomly chosen to amplify the chloroplast *trnL* intron, *trnL-F* intergenic spacer and *trnQ-rps16* intergenic spacer. One sample per population was used to amplify the nuclear ribosomal Internal Transcribed Spacer (ITS) DNA sequence. The aforementioned nuclear and chloroplast DNA regions were amplified by PCR in a reaction mixture of 25 µl containing 50 ng genomic DNA, 5 µl of 5× buffer, 1 µl of dNTPs, 2.5 µl of 25 mM $MgCl_2$, 2.5 µl of 10 pM primers and 2 units of *Taq*. The PCR conditions were an initial denaturation of 5 min at 94 °C followed by 27 cycles of 1 min denaturation at 94 °C, 1 min of annealing at 55 °C and 2 min extension at 72 °C, and final extension of 10 min at 72 °C. The forward and reverse primer pairs used were TCCTCCGCTTATTGATATGC and GGAAGTAAAAGTCGTAACAAGG [White et al. (1990)] for ITS, CGAAATCGGTAGACGCTACG and ATTTGAACTGGTGACACGAG [Taberlet et al. (1991)] for the *trnL-trnF* region and GCGTGGCCAAGCGGTAAGGC and GTTGCTTTCTACCACATCGTTT [Shaw et al. (2007)] for the *trnQ-rps16* intergenic spacer region. The PCR products were purified using QIAquick PCR purification kit (Qiagen Corp., Valencia, CA). The purified PCR products were sent to the DNA facility at Iowa State University for sequencing using a 3730×1 DNA Analyzer (Applied Biosystems).DNA sequences were edited using the program Sequencher 5.0 (Gene Codes). Sequences were aligned using ClustalX (Larkin et al. 2007). Chloroplast haplotypes (chlorotypes) were identified using DnaSP5 (Librado and Rozas 2009). The program PAUP 4.0 (Swafford 2003) was used to perform maximum parsimony analysis of chloroplast DNA sequences using heuristic search with 2,000 replicates and *D. carota* sequence was used as the outgroup. Network tree of the chlorotypes were constructed using the program Network (http://www.fluxus-engineering.com/sharenet.htm). We treated each gap as the fifth base in both analyses.

Results

Sickleweed microsatellite and genetic diversity

All six microsatellite loci (Supplementary material Table S1) used in this study were characterized by Piya and Nepal (2013). Total gene diversity (H_T) across the six microsatellite loci averaged 0.553 with 0.456 within population gene diversity and 0.097

Table 2 Analysis of molecular variance (AMOVA) within and among populations

Source of variation	*df*	Sum of squares	Variance components	Percentage of variation	*p* value
Among populations	7	65.021	0.330	19.42	<0.001
Within populations	184	251.917	1.369	80.58	<0.001
Total	191	316.938	1.699		

among population gene diversity. Total allelic diversity partitioned among population (G_{ST}) was 0.175. The expected heterozygosity (H_e) ranged from 0.26 (IAOA) to 0.61 (AW) and the observed heterozygosity (H_o) ranged from 0.34 (SDSU) to 0.61 (BG). The number of alleles detected per population varied from 12 (IAOA) to 28 (BG) and the effective number of alleles ranged from 1.55 (IAOA) to 3.05 (BG; Table 1). Three populations in South Dakota (AW, GC and BG) and one population in Nebraska (NE30) exhibited higher genetic diversity than the other four populations. Two populations in Nebraska (NE29 and WA) exhibited moderate genetic diversity, and one population in both Iowa (IAOA) and South Dakota (SDSU) had the least genetic diversity (Fig. 1).

Population differentiation and structure

Private alleles were detected in five populations: one in AW, and three each in NE30, NE29, GC and BG (Supplementary material Table S2). A very high genetic differentiation was present among populations ($F_{ST} = 0.19$). Pair-wise genetic differentiation ranged from 0.054 (between GC and AW) to 0.344 (between NE29 and IAOA; Supplementary material Table S3). Of the total variation, 19.42 % variation was among populations and the remaining 80.58 % variation was within populations (Table 2). Mantel's test of IBD showed no correlation ($r = 0.15$, $p = 0.23$) between genetic distance and geographical distance ($F_{ST}/1-F_{ST}$) suggesting no IBD. In the STRUCTURE analysis, the highest value of ΔK was obtained at $K = 3$ suggesting three clusters (color coded: red [1], green [2] and blue [3]) of sickleweed as shown in Fig. 2. Ninety-four percent of the individuals were assigned to one of the three clusters based on the coefficient of inferred ancestry (Q-value). Six percent of the individuals were considered too admixed and were not assigned to any of the three clusters.

Detection of polyploidy

Multiple alleles were observed across five loci in four samples from NE30 populations (Supplementary material Table S4). However, none of the samples exhibited multiple alleles at BSSR53 locus. ESSR9, ESSR80, GSSR24 and GSSR154 had three alleles each while GSSR25 had four alleles. The presence of multiple alleles in a locus of these samples indicates at least partial genome duplication and firmly implies polyploidy. The amplified fragment size of these putative polyploid samples equaled to the amplicon size of diploid samples ruling out the possible amplification of random fragments from other regions within the genome. Samples exhibiting multiple alleles were not included in the genetic analysis.

DNA sequence variation

The total length of the *trnL* intron and *trnL-F* intergenic spacer was 909 bp (Genbank accessions—C995017–KC995064) and that of *trnQ-rps16* intergenic spacer was 884 bp (Genbank accessions—KC995065–KC995112; Supplementary material Table S5). The combined data matrix thus comprised of 48 × 1787 bp. This matrix contained 14 variable sites of which 13 were parsimony informative. Two types of sequence variations were observed in the aligned matrix: insertion/deletion (indels) and single nucleotide polymorphisms (SNPs). Among the variable sites, five were indels and the remaining eight were SNPs. The SNPs exhibited two-base polymorphisms. Based on these variable sites, six chlorotypes were identified among 48 sickleweed accessions. Distribution of these chlorotypes and a resulting network tree are shown in Fig. 1. We did not observe clear geographical pattern in the distribution of chlorotypes. However, CP3 was dominant in eastern populations while CP5 was dominant in western

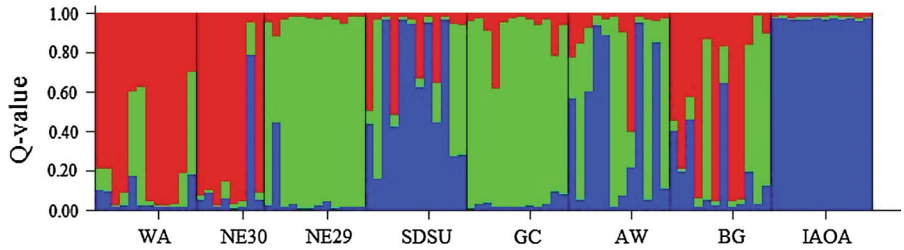

Fig. 2 Bayesian clustering of *F. vulgaris* individuals based on microsatellite data. Each column represents a different individual and each population is separated by vertical lines. Population name is along the horizontal line and the number in the vertical line indicates coefficient of inferred ancestry (Q). Each cluster is color-coded

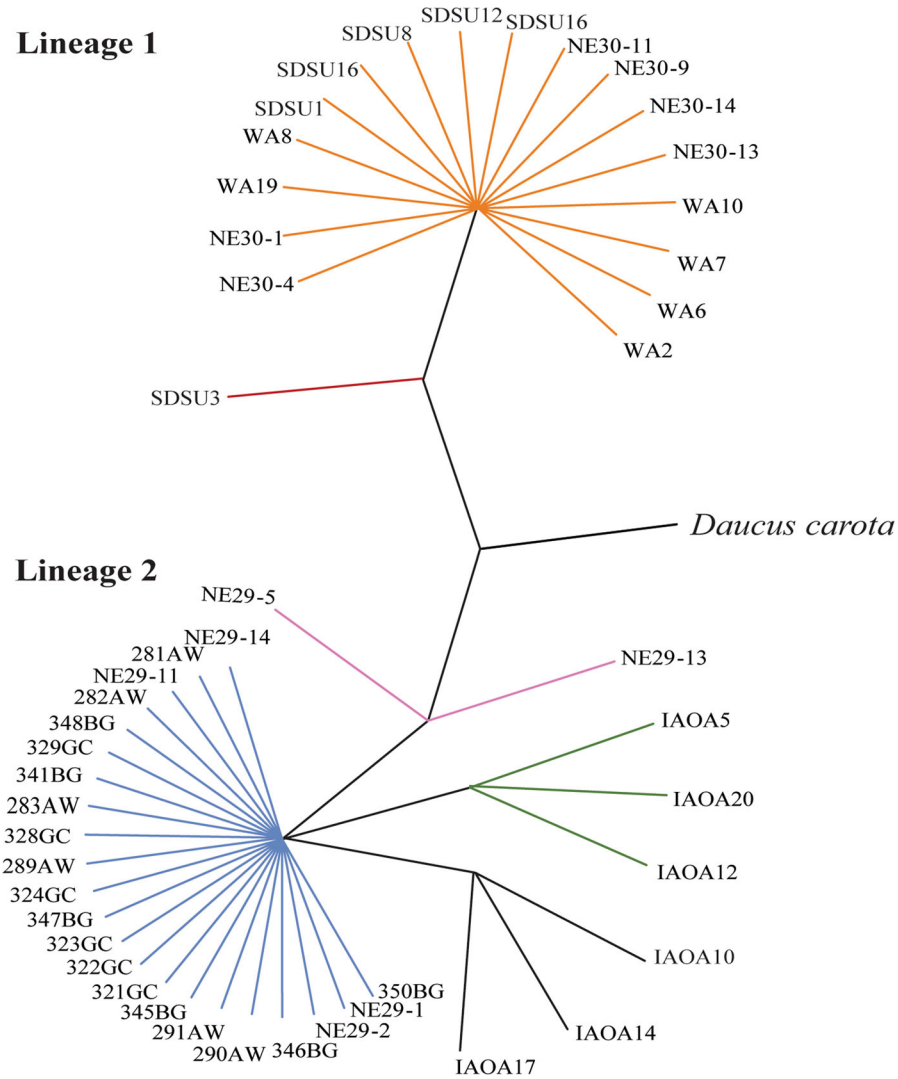

Fig. 3 Unrooted cladogram based on the combined data matrix of two chloroplast DNA sequence regions: *trnL-trnF* and *trnQ-rpl16*. *D. carota* was used as outgroup. Branches are color coded as in Fig. 1. See Table 1 for key to population source designation

populations. These chlorotypes were nested into two distinct maternal lineages as illustrated in Fig. 3. The total length of ITS sequences in *F. vulgaris* was 686 bp and the matrix was comprised of 8 × 686 bp (Genbank accessions—KC995009–KC995016; Supplementary material Table S5). The matrix contained

only one variable site as shown by the chromatogram in Fig. 3. The individuals that nested into lineage-1 had a "T" and those into lineage-2 had an "A" at the 513th position of the aligned ITS data matrix.

Discussion

Genetic diversity of sickleweed populations

Among the eight sampled populations, populations from FPNG (AW and GC) exhibited the greatest genetic diversity expressed as Nei's gene diversity and the number of alleles per population. These populations are the largest among the sampled populations. The population from BGNG (BG) is smaller and exhibited slightly lower genetic diversity than the two FPNG populations. These three populations share the same chlorotype. In spite of sharing the same chlorotype and similar genetic diversity, populations from FPNG to BGNG differ vastly in population size. Current sampling did not allow us to infer multiple introductions as the potential causes of higher genetic diversity at FPNG. Sickleweed populations in Nebraska are also large (NE29 is the largest followed by WA and NE30) and exhibited high genetic diversity. Comparatively, SDSU and IAOA populations are limited to small areas and these populations revealed the least genetic diversity. The trend seen from our microsatellite markers data is: larger populations of sickleweed have higher genetic diversity (higher number of alleles and higher observed and expected heterozygosity) than smaller sickleweed populations. However, our result cannot confirm whether high genetic diversity is necessary for successful range expansion. Nevertheless, previous studies have shown that high genetic diversity is a characteristic of many invasive plant populations (Kolbe et al. 2004; Lavergne and Molofsky 2007; Gaudeul et al. 2011). The nucleotide diversity of chloroplast DNA, however, did not correspond with the population size of the plant. Large populations (for e.g., AW, GC, BG) have only one chlorotype while small populations (for e.g., SDSU, IAOA) contain two chlorotypes each. Interestingly, the four larger populations (AW, GC, BG and NE29) shared the same chlorotype (CP3) which suggests that this genotype might be more adapted to the environmental conditions of the upper Midwest.

The occurrence of the same chlorotype in AW, GC and BG suggests a single introduction from the same source population at these locations, or that one of the populations mentioned could be the source for the other populations. If there was a single source for these populations, then perhaps the founder population endured high propagule pressure either by single introduction of large propagule size or by repeated introduction events. High propagule pressure is often necessary for the establishment and spread of an introduced plant (Kolar and Lodge 2001). An alternative explanation might be multiple introductions in these populations followed by selection of adapted genotypes (i.e. chlorotype 3) and elimination of other chlorotypes from these populations at an early stage of introduction.

Nebraska sickleweed populations occurred along the roadsides. Roadsides often serve as corridors for the spread of weeds (Pysek and Prach 1993) and as a result, these populations are more likely to spread to other new sites. Among these populations, NE30 had the highest genetic diversity based on microsatellite data followed by NE29 ($\sim$25 miles away from NE30) and WA ($\sim$125 miles away from NE30). Also, three chlorotypes were detected from Nebraska (CP3 and CP4 from NE29, and CP5 from NE30 and WA). Populations NE29 and NE30 are geographically closer, but contain individuals genetically distinct from one another. Microsatellite data revealed that the NE29 population contained individuals of one genetic cluster while NE30 contained most individuals of another genetic cluster. Genetic admixture resulting from the exchange of propagules between these two populations with distinct chlorotypes could result in aggressive genotypes with high adaptive potential.

Two populations (IAOA and SDSU) are small, isolated and show low genetic diversity suggesting that these populations are affected by the founder effect. Such small isolated populations are assumed to be established by rare long distance dispersal (Austerlitz et al. 2000). Both populations, however, have two chlorotypes each. The SDSU population is a genetic mixture of two different genetic clusters as detected by Bayesian analysis using microsatellite data. This population of sickleweed, located on the South Dakota State University campus, has been mowed each year before they start flowering thus preventing crossing between plants with different genotypes. This population appears to be sustaining by

asexual reproduction through root-stocks as evidenced by recurrent multi-locus genotypes (five out of twelve samples).

Genetic structure of sickleweed in the United States

Tang et al. (2009) suggested that human mediated long distance dispersal might have contributed in the spread of *Parthenium hysterophorus* in China as they detected no geographical structure in genetic variation. Likewise, in our study, Bayesian clustering of the populations from different regions showed no pattern. Populations from different geographical regions clustered together. Similarly, Mantel's test of IBD in sickleweed populations showed no correlation ($r = 0.15$, $p = 0.23$) between genetic distance ($F_{ST}/1-F_{ST}$) and geographical distance suggesting that there is no genetic IBD. In addition, no distinct geographical pattern was observed in the distribution of chlorotypes. Absence of geographical structure in the distribution of sickleweed genotypes suggests chance establishment of different genotypes in different areas (Marrs et al. 2008) resulting from human mediated long distance dispersal (Gaudeul et al. 2011; Kelager et al. 2013). However, two populations of FPNG (AW and GC), which are less than two miles apart, had very low genetic differentiation ($F_{ST} = 0.05$). These two populations also share a common chlorotype and most likely have established by natural expansion through seed dispersal. Korman (2011) reported that sickleweed can effectively disperse its seeds with the help of wind: when the plants senesce, the stems break at the nodes and segments tumble in the wind while dispersing seed.

Multiple introductions of sickleweed in the United States

Bayesian analyses of the microsatellite data showed three distinct genetic clusters that are distributed without any geographical pattern suggesting multiple introductions of sickleweed in this region of the United States. This is also supported by our chloroplast sequence data. Six chlorotypes with two distinct maternal lineages were observed suggesting at least two introductions of sickleweed in this area of the Midwest. These two lineages differ in their ITS DNA sequences as well. Since the chloroplast gene regions used in this study are often conserved even at higher taxonomic levels, it is very unlikely that these different chlorotypes arose as a result of post-introduction evolution of sickleweed. The eastern populations contained chlorotypes of lineage 1 while lineage 2 was dominant in the western populations. If it is assumed two lineages represent two separate introductions, then the propagule size in each case should have been large as described by Gaskin et al. (2005) to include several chlorotypes. However, two chlorotypes of lineage 1 (CP1 and CP2) occur in the western region where they are geographically separated from other chlorotypes of lineage 1, suggesting that the individuals nested in lineage 1 might have resulted from multiple introductions. Genetically different populations at different sites strongly suggest multiple introductions of different genotypes from the native range through human mediated long range 'leapfrog' dispersal (Marrs et al. 2008).

Evidence of polyploidy

Microsatellite markers have been previously used to infer duplication of genomes in various plant species (Coyer et al. 2006; Besnard et al. 2008). Consistent presence of three or four alleles in a single locus across five of the six microsatellite loci strongly suggests occurrence of polyploidy (Coyer et al. 2006; Besnard et al. 2008) in sickleweed individuals. There is no previous report of polyploidy in this species, but there are several reports of genome duplication in invasive species (Pandit et al. 2006). Polyploidization in sickleweed could represent a post-introduction evolutionary development that could promote adaptation in the new environment and minimize inbreeding depression due to founder effect. However, it is important to explore sickleweed populations for polyploidy in the native range to determine whether polyploids may have been introduced or represent a post-introduction evolutionary development. Further investigation using flow cytometry backed by karyotyping is needed to verify polyploidy in sickleweed.

Overall, our study suggests multiple introductions and gene flow in sickleweed populations of the upper Midwest probably as a result of human mediated propagule dispersal that has now resulted in higher genetic diversity in the introduced populations. The ability of sickleweed to reproduce asexually by root sprouting can foster rapid selection of such adapted genotypes. Collectively, the results of our study

indicate that sickleweed in the Midwest has the potential to emerge as an invasive species. This study provides baseline data to further explore invasion dynamics of sickleweed. Evolutionary changes that sickleweed has undergone during its establishment and expansion could be used to prioritize eradication measures that reduce further gene flow among genetic clusters or chlorotypes. A comprehensive study that uses a large number of molecular markers and a large number of samples from native and introduced ranges could reveal invasion routes and post-introduction evolution of sickleweed.

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References

Allendorf FW, Lundquist LL (2003) Introduction: population biology, evolution, and control of invasive species. Conserv Biol 17:24–30

Alyokhin A (2011) Non-natives: put biodiversity at risk. Nature 475:36

Austerlitz F, Mariette S, Machon N, Gouyon PH, Godelle B (2000) Effects of colonization processes on genetic diversity: differences between annual plants and tree species. Genetics 154:1309–1321

Besnard G, Garcia-Verdugo C, De Casas RR, Treier UA, Galland N, Vargas P (2008) Polyploidy in the olive complex (*Olea europaea*): evidence from flow cytometry and nuclear microsatellite analyses. Ann Bot 101:25–30

Blackburn TM, Pysek P, Bacher S et al (2011) A proposed unified framework for biological invasions. Trends Ecol Evol 26:333–339

Butler JL, Wacker SD (2013) Sickleweed on the Fort Pierre National Grassland: an emerging threat. Prarie Nat 45:28–38

Clapham AR, Tutin TG, Moore DM (1987) Flora of the British Isles. Cambridge University Press, Cambridge Cambridgeshire, New York

Comai L (2005) The advantages and disadvantages of being polyploid. Nat Rev Genet 6:836–846

Coyer JA, Hoarau G, Pearson GA, Serrao EA, Stam WT, Olsen JL (2006) Convergent adaptation to a marginal habitat by homoploid hybrids and polyploid ecads in the seaweed genus *Fucus*. Biol Lett 2:405–408

DAISIE (2008) *Falcaria vulgaris*. European Invasive Alien Species Gateway. http://www.europe-aliens.org/speciesFactsheet.do?speciesId=21090. Accessed 4 April 2012

Ellstrand NC, Elam DR (1993) Population genetic consequences of small population size: implications for plant conservation. Annu Rev Ecol Syst 24:217–242

Evanno G, Regnaut S, Goudet J (2005) Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Mol Ecol 14:2611–2620

Excoffier L, Laval G, Schneider S (2005) Arlequin (version 3.0): an integrated software package for population genetics data analysis. Evol Bioinform 1:47–50

Frankham R (2005) Invasion biology—resolving the genetic paradox in invasive species. Heredity 94:385

Gaskin JF, Zhang DY, Bon MC (2005) Invasion of *Lepidium draba* (Brassicaceae) in the western United States: distributions and origins of chloroplast DNA haplotypes. Mol Ecol 14:2331–2341

Gaudeul M, Giraud T, Kiss L, Shykoff JA (2011) Nuclear and chloroplast microsatellites show multiple introductions in the worldwide invasion history of common ragweed, *Ambrosia artemisiifolia*. Plos One 6:e17658

Genton BJ, Shykoff JA, Giraud T (2005) High genetic diversity in French invasive populations of common ragweed, *Ambrosia artemisiifolia*, as a result of multiple sources of introduction. Mol Ecol 14:4275–4285

Goudet J (1995) FSTAT (Version 1.2): a computer program to calculate F-statistics. J Hered 86:485–486

Gress EM (1923) *Falcaria rivini*, a plant new to the United States. Rhodora 25:13–14

Kelager A, Pedersen JS, Bruun HH (2013) Multiple introductions and no loss of genetic diversity: invasion history of Japanese Rose, *Rosa rugosa*, in Europe. Biol Invasions 15:1125–1141

Knuth P (1908) Handbook of flower pollination. Clarendon, Oxford

Kolar CS, Lodge DM (2001) Progress in invasion biology: predicting invaders. Trends Ecol Evol 16:199–204

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

Korman BL (2011) Biology and ecology of sickleweed (*Falcaria vulgaris*) in the Fort Pierre National Grassland of South Dakota. Biology and microbiology. South Dakota State University, Brookings, South Dakota, p 78

Kowarik I (2005) Time lags in biological invasions with regard to the success and failure of alien species. In: Pysek P, Prach K, Rejmanek M, Wade M (eds) Plant invasions: general aspects and special problems. SPB Academic Publishers, Amsterdam

Lachmuth S, Durka W, Schurr FM (2010) The making of a rapid plant invader: genetic diversity and differentiation in the native and invaded range of *Senecio inaequidens*. Mol Ecol 19:3952–3967

Larina SY (2008) *Falcaria vulgaris* Bernh. Interactive agricultural ecological atlas of Russia and neighboring countries. In: Afonin AN, Greene SL, Dzyubenko NI and Frolov AN (eds) Economic plants and their diseases, pests and weeds. http://www.agroatlas.ru/en/content/weeds/Falcaria_vulgaris/. Accessed 15 March 2012

Larkin MA, Blackshields G, Brown NP et al (2007) ClustalW and clustalX version 2.0. Bioinformatics 23:2947–2948

Lavergne S, Molofsky J (2007) Increased genetic variation and evolutionary potential drive the success of an invasive grass. Proc Natl Acad Sci USA 104:3883–3888

Lee CE (2002) Evolutionary genetics of invasive species. Trends Ecol Evol 17:386–391

Leung B, Drake JM, Lodge DM (2004) Predicting invasions: propagule pressure and the gravity of allee effects. Ecology 85:1651–1660

Librado P, Rozas J (2009) DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. Bioinformatics 25:1451–1452

Lockwood JL, Cassey P, Blackburn T (2005) The role of propagule pressure in explaining species invasions. Trends Ecol Evol 20:223–228

Mantel N (1967) The detection of disease clustering and a generalized regression approach. Cancer Res 27:209–220

Marrs RA, Sforza R, Hufbauer RA (2008) When invasion increases population genetic structure: a study with *Centaurea diffusa*. Biol Invasions 10:561–572

Mooney HA, Cleland EE (2001) The evolutionary impact of invasive species. Proc Natl Acad Sci USA 98:5446–5451

Nater A, Arora N, Greminger MP et al (2013) Marked population structure and recent migration in the critically endangered Sumatran Orangutan (*Pongo abelii*). J Hered 104:2–13

Nei M (1973) Analysis of gene diversity in subdivided populations. Proc Natl Acad Sci USA 70:3321-3323

Nei M (1977) F-statistics and analysis of gene diversity in subdivided populations. Ann Hum Genet 41:225–233

Nei M (1987) Molecular evolutionary genetics. Columbia University Press, New York

NISC (2011) Invasive plants of Nebraska. http://snr5.unl.edu/invasives/pdfs/Invasive%20Plant%20Lists/NE%20Invasive%20Plants%20List%20Only%204-14-11.pdf. Accessed 12 Jan 2012

Novak SJ, Mack RN (2001) Tracing plant introduction and spread: genetic evidence from *Bromus tectorum* (Cheatgrass). Bioscience 51:114–122

Pandit MK, Tan HTW, Bisht MS (2006) Polyploidy in invasive plant species of Singapore. Bot J Linn Soc 151:395–403

Peakall R, Smouse PE (2006) GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. Mol Ecol Notes 6:288–295

Piya S, Nepal MN (2013) Characterization of nuclear and chloroplast microsatellite markers for *Falcaria vulgaris* (Apiaceae). Am J Plant Sci 4:590–595

Piya S, Neupane A, Larson GE, Butler J, Nepal MN (2012) Inferring introduction history and spread of *Falcaria vulgaris* Bernh. (Apiaceae) in the United States based on herbarium records. Proc SD Acad Sci 91:113–119

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

Provan J, Murphy S, Maggs CA (2005) Tracking the invasive history of the green alga *Codium fragile* ssp tomentosoides. Mol Ecol 14:189–194

Pysek P, Prach K (1993) Plant invasion and the role of riparian habitats: a comparison of four species alien to central Europe. J Biogeogr 20:413–420

Pysek P, Richardson DM, Pergl J, Jarosik V, Sixtova Z, Weber E (2008) Geographical and taxonomic biases in invasion ecology. Trends Ecol Evol 23:237–244

Richardson DM, Pysek P, Rejmanek M, Barbour MG, Panetta FD, West CJ (2000) Naturalization and invasion of alien plants: concepts and definitions. Divers Distrib 6:93–107

Sakai AK, Allendorf FW, Holt JS et al (2001) The population biology of invasive species. Annu Rev Ecol Syst 32:305–332

Sala OE, Chapin FS, Armesto JJ et al (2000) Biodiversity-Global biodiversity scenarios for the year 2100. Science 287:1770–1774

Shaw J, Lickey EB, Schilling EE, Small RL (2007) Comparison of whole chloroplast genome sequences to choose noncoding regions for phylogenetic studies in angiosperms: the tortoise and the hare III. Am J Bot 94:275–288

Simberloff D (2009) The role of propagule pressure in biological invasions. Annu Rev Ecol Evol Syst 40:81–102

Simberloff D, Alexander J, Allendorf F et al (2011) Non-natives: 141 scientists object. Nature 475:36

Slatkin M (1987) Gene flow and the geographic structure of natural populations. Science 236:787–792

Swafford DL (2003) PAUP4. Phylogenetic analysis using parsimony. Sinauer Associates, Sunderland

Taberlet P, Gielly L, Pautou G, Bouvet J (1991) Universal primers for amplification of three non-coding regions of chloroplast DNA. Plant Mol Biol 17:1105–1109

Tang SQ, Wei F, Zeng LY et al (2009) Multiple introductions are responsible for the disjunct distributions of invasive *Parthenium hysterophorus* in China: evidence from nuclear and chloroplast DNA. Weed Res 49:373–380

Taylor CM, Hastings A (2005) Allee effects in biological invasions. Ecol Lett 8:895–908

USDA, NRCS (2011) The plants database. National Plant Data Center, Baton Rouge. http://plants.usda.gov. Accessed 16 April 2011

Valliant MT, Mack RN, Novak SJ (2007) Introduction history and population genetics of the invasive grass *Bromus tectorum* (Poaceae) in Canada. Am J Bot 94:1156–1169

Van Oosterhout C, Hutchinson WF, Wills DPM, Shipley P (2004) MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. Mol Ecol Notes 4:535–538

White TJ, Bruns T, Lee S, Taylor JW (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. Academic Press, New York

Wilson JRU, Gairifo C, Gibson MR et al (2011) Risk assessment, eradication, and biological control: global efforts to limit Australian acacia invasions. Divers Distrib 17:1030–1046

Yeh FC, Boyle TJB (1997) Population genetic analysis of co-dominant and dominant markers and quantitative traits. Belg J Bot 129:157

Young A, Boyle T, Brown T (1996) The population genetic consequences of habitat fragmentation for plants. Trends Ecol Evol 11:413–418