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ORIGINAL PAPER

Invasion genetics of *Senecio vulgaris*: loss of genetic diversity characterizes the invasion of a selfing annual, despite multiple introductions

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Abstract Genetic variation in invasive populations is affected by a variety of processes including stochastic forces, multiple introductions, population dynamics and mating system. Here, we compare genetic diversity between native and invasive populations of the selfing, annual plant *Senecio vulgaris* to infer the relative importance of genetic bottlenecks, multiple introductions, post-introduction genetic drift and gene flow to genetic diversity in invasive populations. We scored multilocus genotypes at eight microsatellite loci from nine native European and 19 Chinese introduced populations and compared heterozygosity and number of alleles between continents. We inferred possible source populations for introduced populations by performing assignment analyses and evaluated the relative contributions of gene flow and genetic drift to genetic diversity based on correlations of pairwise genetic and geographic distance. Genetic diversity within Chinese populations was significantly reduced compared to European populations indicating genetic bottlenecks accompanying invasion. Assignment tests provided support for multiple introductions with populations from Central China and southwestern China descended from genotypes matching those from Switzerland and the UK, respectively. Genetic differentiation among populations in China and Europe was not correlated with geographic distance. However, European populations exhibited less variation in the relation between G_{ST} and geographical distance than populations in China. These results suggest that gene flow probably plays a more significant role in structuring genetic diversity in native populations, whereas genetic drift appears to predominate in introduced populations. High rates of selfing in Chinese populations may restrict opportunities for pollen-mediated gene flow. Repeated colonization-extinction cycles associated with ongoing invasion is likely to maintain low genetic diversity in Chinese populations.

Keywords Gene flow · Genetic diversity · Genetic drift · Multiple introductions · Selfing

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Introduction

Comparisons of genetic diversity and differentiation at neutral genetic markers between native and invasive populations can provide insights into invasion

pathways and the amount of genetic variation introduced to the invasive range (Bossdorf et al. 2005; Prentis et al. 2009; Barrett et al. forthcoming). Genetic diversity at marker loci has also been used as a proxy for adaptive potential, with the amount of variation in introduced populations hypothesized to influence a species' capacity to adapt to novel environments, although this hypothesis has rarely been investigated rigorously (Martins and Jain 1979; Allendorf and Lundquist 2003). Investigating invasion histories and genetic structure by comparing genetic diversity in native and introduced populations using molecular markers has become an important area of research in the growing field of invasion genetics (Dlugosch and Parker 2008; Keller and Taylor 2008; Prentis et al. 2009; Li et al. 2012; Barrett 2015; Bock et al. 2015; Ferrero et al. 2015). Invasive plants offer valuable experimental systems for conducting such comparisons as populations are easily sampled and historical records are sometimes available concerning their introduction history.

Biological invasion may often be associated with dramatic founder events followed by periods of small population size, both of which can result in losses in genetic diversity (Novak and Mack 2005; reviewed in Barrett et al. 2008). But not all biological invasions exhibit this pattern and a survey of 80 species of plants, animals and fungi revealed that the overall average loss in allelic richness was only 15.5 % in introduced populations (Dlugosch and Parker 2008). Nevertheless, in invasive plants capable of uniparental reproduction (selfing or clonal propagation) sharp reductions in genetic diversity compared with the native range have been demonstrated in invasive populations (Kliber and Eckert 2005; Henry et al. 2009; Zhang et al. 2010; Barrett 2011; Ferrero et al. 2015). However, in some cases equal or even increased levels of genetic diversity have been reported in invasive populations in comparisons with native populations (Genton et al. 2005; Gillis et al. 2009; Li et al. 2012). Thus, caution should be exercised in assuming that all plant invasions are inevitably associated with genetic bottlenecks and a loss of adaptive potential. Indeed, neutral genetic markers may in some cases provide an unreliable proxy for the amount of genetic variation for ecological-relevant traits in introduced populations (Lewontin 1984; Steinger et al. 2002).

Multiple introductions from geographically diverse sources may help to explain higher than anticipated levels of genetic diversity in invasive populations relative to native populations (Novak and Mack 1993; Kolbe et al. 2004; Genton et al. 2005; Gillis et al. 2009; Kelager et al. 2013). During species invasions, multiple introductions may be of common occurrence rather than the exception (Roman and Darling 2007). But even in cases where there is marker-based evidence for multiple introductions to the introduced range there may still be significant reductions in genetic diversity in invasive populations. For example, a recent comparative study of genetic diversity in clonal *Oxalis pes-caprae* revealed that although invasive populations from the Western Mediterranean involve multiple introductions from the South African native range, they still exhibit dramatically reduced levels of genetic diversity owing to a predominance of asexual reproduction (Ferrero et al. 2015). Additional comparative studies of the population genetic structure of invasive and native populations are therefore desirable to evaluate the contributions of stochastic forces and multiple introductions to the patterns of genetic diversity in invasive species.

The mating systems of invasive populations also have the potential to influence the levels of within-population genetic diversity and among-population differentiation. First, selfing invaders may suffer more severe founder effects than outcrossers because uniparental reproduction facilitates population establishment by single individuals through reproductive assurance (Baker 1967; Pannell and Barrett 1998; Rambuda and Johnson 2004; Barrett et al. 2008; Petanidou et al. 2012; Pannell 2015). Propagule pressure may be less important for successful invasion in selfers than outcrossers as colony establishment at a minimum may involve a single propagule. Second, selfing and other forms of uniparental reproduction (e.g., asexual reproduction) restrict recombination and may magnify the influence of founder effects during invasion causing sharp reductions in genetic diversity (Nordborg 2000; Kliber and Eckert 2005; Zhang et al. 2010). Selective sweeps and background selection associated with selfing can further reduce heterozygosity and hence effective population size (Nordborg 2000; Charlesworth and Wright 2001). Third, invaders with very high selfing rates are subject to more serious limits on gene flow because seed and not pollen dispersal is the primary mechanism contributing to gene exchange among populations. Hence, owing to these various demographic and genetic processes we

might expect to observe reduced genetic diversity within introduced selfing populations and a greater differentiation among them in comparison with outcrossing invaders.

Owing to the reduced effective population size of selfing invaders they are especially vulnerable to the influence of genetic drift in eroding levels of genetic diversity and causing increased genetic differentiation. Correlations of pairwise genetic and geographic distance enable evaluation of the relative historical influences of genetic drift and gene flow on regional population structure (Hutchison and Templeton 1999). However, the potential effects of post-introduction gene flow on population genetic structure has only been evaluated in a few invasive species (e.g., Ray and Quader 2014). Moreover, few studies have examined the severity of genetic bottlenecks for invasions in which multiple introductions are suspected by direct ancestor–descendant comparisons of genetic variation between native and invasive populations.

Senecio vulgaris L. (Asteraceae), the common groundsel, is a highly selfing annual weed of open disturbed ruderal environments (Campbell and Abbott 1976; Marshall and Abbott 1982) that is likely native to southern Europe (Kadereit 1984) and currently has a near worldwide distribution (Mitich 1995; Robinson et al. 2003). Previous studies have investigated biological control (Frantzen et al. 2002; Grace and Müller-Schärer 2003), the evolution of increased competitive ability (Handley et al. 2008) and population genetic structure (Steinger et al. 2002; Haldimann et al. 2003; Handley et al. 2008) in *S. vulgaris*. Native populations in Europe exhibited significant genetic differentiation estimated by amplified fragment length polymorphic markers (AFLP) (Steinger et al. 2002; Haldimann et al. 2003; Handley et al. 2008). In addition, genetic differentiation between native (Switzerland) and invasive (North America and Australia) populations was relatively low, but populations sampled from the invasive region had higher molecular diversity, suggesting multiple introductions (Handley et al. 2008). A major goal of this study was to investigate patterns of genetic diversity in a different part of the introduced range—China—where *S. vulgaris* is distributed over a broad geographical area, and examine evidence for multiple introductions.

Here, we investigated patterns of genetic diversity and population genetic structure in a sample of 28 native and invasive populations of *S. vulgaris* using polymorphism at eight microsatellite loci. Our study addressed the following specific questions: (1) Is there evidence of reduced genetic diversity and greater population differentiation in introduced versus native populations? (2) Is there evidence of multiple introductions associated with the invasion of China and where might the likely original source regions be in Europe? (3) What is the relative importance of genetic drift and gene flow in structuring patterns of genetic diversity in native versus introduced populations? By comparing genetic diversity and differentiation between native and invasive populations our study provides insights into the role of stochastic forces during biological invasion.

Methods

Study species and sampled populations

S. vulgaris ($2n = 40$) has been considered to have an autotetraploid origin from *S. vernalis* Waldst. and Kit ($2n = 20$; Kadereit 1984). However, Ashton and Abbott (1992) suggested that *S. vulgaris* was allotetraploid, with the possibility that *S. vernalis* acted as one of its parents. There are two floral forms of *S. vulgaris*: the more common non-radiate form with disc florets only, and the less common radiate form with ray flowers around a central aggregation of disc florets. The non-radiate form had very low outcrossing rate (<1 %; Marshall and Abbott 1982). All populations in China that we have observed are non-radiate. *Senecio vulgaris* was first reported in the nineteenth century in northeast China and was most likely introduced as an accidental seed contaminant of crops. The source of this early introduction is unknown. The species has now spread widely in northeast and southwest China and typically occurs in ruderal and agricultural habitats (Xu and Qiang 2004).

We sampled population from nine European locations and from 19 locations in China (Table 1). The Chinese sampling sites were located from northeast to southwest China; samples from Europe were from sites in Germany, France, Switzerland and the UK. At each sampling site, leaf tissues were randomly collected from 17 to 32 flowering plants with a minimal distance between sampling points of 2 meters. The leaf tissue was preserved in silica gel for subsequent identification of genotypes at each site (Table 1).

Table 1 Description of the populations of *Senecio vulgaris* used in this study, including sampling sites, sample size (*N*), expected heterozygosity (H_E) over all loci calculated by TETRA, average number of alleles per locus (AA), number of private alleles (AP), and number of 0/1 genotypes (NG). We divided the sampling region in China into four parts: south-western China (China-SW), central China (China-C), north-western China (China-NW), and northeastern China (China-NE)

Sampling site	Site code	Region	Altitude (m)	Latitude	Longitude	*N*	H_E	AA	AP	NG
Europe								8.9	24	126
Berne, Switzerland	BIE	Europe	437	47.134N	7.227E	30	0.517	3.4	0	13
Nuremberg, Germany	D1	Europe	416	49.116N	11.279E	30	0.521	4.1	2	12
Wurzburg, Germany	D2	Europe	226	50.888N	10.321E	29	0.501	3.9	1	8
Frankfurt, Germany	D3	Europe	125	50.128N	8.694E	29	0.513	4.3	2	11
Oxford, England	E	Europe	65	51.400N	0.634 W	30	0.562	4.3	1	20
Chaumont, France	F1	Europe	125	48.233N	4.036E	29	0.501	4.3	5	22
Paris, France	F2	Europe	41	48.852N	2.511E	30	0.456	3.5	1	17
Annecy, France	F3	Europe	1673	45.041N	6.338E	25	0.368	2.6	1	7
Neuchatel, Switzerland	LC	Europe	527	47.010N	6.961E	30	0.565	4.5	4	19
China								6.6	6	102
Dali	DL	China-SW	2396	25.695N	100.155E	32	0.362	2.6	0	9
Guiyang	GY	China-SW	1329	26.703N	106.654E	28	0.298	2.0	0	9
Kangding	KD	China-SW	2640	30.212N	101.963E	30	0.275	1.8	0	4
Kunming	KM	China-SW	1910	24.992N	102.622E	30	0.269	1.6	0	3
Luding	LD	China-SW	1609	29.825N	102.220E	30	0.210	1.6	0	2
Lijiang	LJ	China-SW	2406	26.881N	100.221E	30	0.415	2.3	0	6
Liupanshui	LP	China-SW	1816	26.594N	104.824E	30	0.296	2.4	0	7
Meigu	MG	China-SW	1951	28.331N	103.127E	26	0.252	1.9	0	4
Maoxian	MX	China-SW	1816	31.685N	103.899E	28	0.252	1.9	0	5
Guide	GD	China-NW	2180	36.033N	101.406E	30	0.337	2.1	0	3
Huangyuan	HY	China-NW	2539	35.992N	104.066E	30	0.288	2.5	0	5
Shennongjia	MY	China-C	1221	31.471N	110.390E	30	0.216	1.6	0	2
Benxi	BX	China-NE	124	41.337N	123.879E	30	0.324	2.6	0	4
Hulin	HL	China-NE	95	45.757N	132.932E	22	0.362	2.8	1	13
Jidong	JD	China-NE	196	45.231N	131.149E	23	0.356	2.4	0	10
Mudanjiang	MDJ	China-NE	214	44.598N	129.667E	17	0.426	2.3	0	8
Tonghua	TH	China-NE	371	41.739N	125.926E	31	0.391	2.6	0	9
Tumen	TM	China-NE	98	44.470N	129.977E	27	0.461	2.4	0	13
Yakeshi	YKS	China-NE	640	49.294N	120.729E	30	0.241	1.8	0	3

Molecular analyses

We extracted genomic DNA from each individual with the Plant Genomic DNA Kit (TIANGEN, DP305) and the DNA was stored at −20 °C. We used eight microsatellite loci (S13, S20, S26, V33, V34, V38, V44 and V45) identified by Liu et al. (2004) to genotype the samples. We conducted the polymerase chain reactions (PCR) following the protocol described by Liu et al. (2004). The forward primer of each locus was labeled with a fluorescent marker. We analyzed the PCR products on an ABI 3100, with Rox 500 as an internal marker standard, and scored bands using GENEMAPPER V.3.7 (Applied Biosystems).

Polyploidy can lead to complex banding patterns at microsatellite loci and *S. vulgaris* is tetraploid. There are four possible scenarios in tetraploids when genotypes at a specific locus are considered: (1) only one

allele giving one genotype (e.g., allele A giving genotype AAAA); (2) two different alleles giving three possible genotypes (e.g., allele A and B with possible genotypes ABBB, AABB or AAAB); (3) three different alleles giving three possible genotypes (e.g., alleles A, B, C giving genotypes AABC, ABBC or ABCC); (4) four different alleles giving one genotype (e.g., alleles A, B, C, D giving genotype ABCD). Because it was impossible to identify the specific genotype at the microsatellite loci with two or three different alleles, we coded the genotype only by the known alleles (e.g., AB or ABC). This coding scheme prevented straightforward calculation of several common statistics used in population genetic analysis software packages.

Genetic diversity

We calculated the expected heterozygosity, average number of alleles per locus, number of private alleles and number of 0/1 genotypes as measures of genetic diversity in each population. We used TETRA (Liao et al. 2008) and ATETRA (Van Puyvelde et al. 2010), software packages designed for the analysis of tetraploid microsatellite data, to calculate the expected heterozygosity and verify the reliability of our allele frequency estimation. The number of alleles per locus is the total number of alleles over all loci divided by the number of loci in each population. The number of private alleles is the number of alleles that only occur in a given population. Due to the uncertain genotypes of heterozygotes with two or three alleles, we transformed the microsatellite data into 0/1 data. We scored the presence/absence (1/0) of total alleles observed among the eight loci for each individual. The 0/1 data is analogous to AFLP data and enabled us to estimate the number of 0/1 genotypes in each population. This genotyping method will underestimate the genotypic diversity. We used analysis of covariance (ANCOVA) in R (R Core Team 2015) to compare the expected heterozygosity, average number of alleles, and number of 0/1 genotypes per population in Europe and China, with the region as the categorical explanatory variable and the number of individuals in populations as the continuous covariate. Because several populations had no private alleles, a generalized linear model with Poisson errors in R was used to compare the number of private alleles per population, with region and number of individuals in population as explanatory variables.

Genetic structure

We calculated G_{ST} (Nei 1973) based on microsatellite data to estimate among-population genetic differentiation at the regional level within Europe, within China, and also between Europe and China using TETRA (Liao et al. 2008). We implemented Bootstrap (10,000 resampling runs) to obtain the 95 % confident interval (95 % CI) for each G_{ST}. To examine the structure of genetic variation we used analysis of molecular variance (AMOVA) on the transformed 0/1 data in ARLEQUIN version 3.5 (Excoffier and Lischer 2010). Variation was first partitioned between regions, among and within populations globally, then partitioned among and within populations within Europe and China.

The majority of populations of *S. vulgaris* that we sampled in China can be classified into two distinct, relatively isolated geographical regions (southwestern China and northeastern China), with the exception of three populations (GD, HY, and MY). The two regions are each comparable in extent to the sampling range in Europe. Therefore, we calculated pairwise G_{ST} using TETRA (Liao et al. 2008) and tested the relation between pairwise G_{ST} and geographic distance with the Mantel test using the ade4 package (Dray and Dufour 2007) in R for three regions: Europe, southwestern China, and northeastern China. We used permutation tests ($n = 5000$) to compare the variance of pairwise G_{ST} values between any two regions and limited the analyses to those pairwise populations sharing the same range of geographic distances. For each permutation, we calculated the difference between the variance of each region in pairwise comparisons and obtained the 5 % lower limit and 95 % upper limit of these randomized differences. If the observed difference was less than the 5 % lower limit or more than the 95 % upper limit, the variance of the region was significantly lower or larger than the other region.

Assignment analyses

We implemented two types of assignment tests on our microsatellite data. First, we tested whether individuals sampled in China could be directly assigned to any population that was sampled in Europe by analyzing the transformed 0/1 data using AFLPOP version 1.2 (Duchesne and Bernatchez 2002). Instruct,

an extension of STRUCTURE software that does not assume Hardy–Weinberg equilibrium within populations, is more suitable for our data given the high selfing rate of *S. vulgaris*, but unfortunately it cannot be used for polyploid genotype data (De Groot et al. 2012). Therefore, we analyzed our data in STRUCTURE version 2.3 (Falush et al. 2007). A total of 20 runs were implemented with 100,000 burn-in lengths and 200,000 MCMC repetitions for each possible K from 1 to 20. We used the admixture model with correlated allele frequencies and without a priori information on population origin. To infer the subdivided structures in the European native and Chinese introduced regions, the same analysis was, respectively, applied to populations in Europe and China with different K values (Europe: 1–9 and China: 1–19). We used the approach of Evanno et al. (2005) to determine the most likely number of clusters (K) and plotted the individuals' assignment probabilities using CLUMPP (Jakobsson and Rosenberg 2007) and DISTRUCT 1.1 (Rosenberg 2004).

Results

Genetic diversity

We identified a total of 77 alleles among the eight microsatellite loci in the 28 populations of *S. vulgaris*. Of these 77 alleles, 71 alleles were found in the nine European native populations and 53 alleles were evident in the 19 introduced Chinese populations. The number of alleles per locus per population varied from 2.6 to 4.5 in the European sample and from 1.6 to 2.8 in the Chinese sample (Fig. 1a; Table 1). The European populations had significantly more alleles per locus (mean $3.88 \pm$ SE 0.20) than the Chinese populations (2.17 ± 0.09, $F = 106.3$, $df = 1$, $P < 0.001$). We detected 24 private alleles in the European sample (alleles observed only in European populations, but probably shared by different European populations), of which 17 alleles (70.8 %) were private alleles at the population level. In contrast, six alleles were private to the Chinese range, of which just one allele (16.7 %) was private to population HL. A total of eight of the nine European populations had private alleles, but only one (HL) of the 19 Chinese populations had a private allele (Table 1). The number of private alleles in each European population (1.89 ± 0.54) was significantly higher than those in the Chinese populations (0.05 ± 0.05, $z = 3.48$, $P < 0.001$, Fig. 1b). The difference between values of the expected heterozygosity calculated by TETRA and ATETRA was virtually identical (ESM, Fig. S1). Therefore, we only compared the values of expected heterozygosity calculated by TETRA. The European populations (0.50 ± 0.02) had significantly larger expected heterozygosity compared with the Chinese populations (0.32 ± 0.02, $F = 54.87$, $df = 1$, $P < 0.001$, Fig. 1c).

We transformed the microsatellite data into 0/1 data and obtained 227 genotypes from the sample of 28 populations of *S. vulgaris*. There were 126 genotypes in the European range and 102 genotypes in the Chinese range. Only one genotype was observed in both a European population (F2) and a Chinese population (YKS). At the population level, the number of 0/1 genotypes in the European populations (14.3 ± 1.8) was significantly higher than the Chinese populations ($6.3 \pm 0.8, F = 28.81, df = 1, P < 10^{-3}$, Fig. 1d).

Genetic differentiation and gene flow

The G_{ST} values that we calculated globally, within Europe, within China, and between Europe and China, were significantly greater than zero (ESM, Fig. S2), demonstrating population structure in our data set at each geographical level. Genetic differentiation in China [$G_{ST} = 0.499$, 95 % CI (0.488, 0.523)] was higher than the genetic differentiation observed globally [$G_{ST} = 0.419$, 95 % CI (0.413, 0.444)], in Europe [$G_{ST} = 0.221$, 95 % CI (0.209, 0.267)] and between continents [$G_{ST} = 0.014$, 95 % CI (0.011, 0.019)]. These G_{ST} values were significantly different based on non-overlapping 95 % CI. The AMOVA results were in accord with this result in showing similar patterns of population structure (Table 2). Globally, there was no significant genetic differentiation between continents. The largest amount of genetic variation was observed among populations (59.40 %) in the total sample. However, when AMOVA was applied separately to the European and Chinese samples, different patterns of genetic differentiation were evident. In Europe, significantly more genetic variation occurred within (69.01 %) than among (30.99 %) populations. In contrast, this pattern was reversed in China with the largest component of genetic variation among

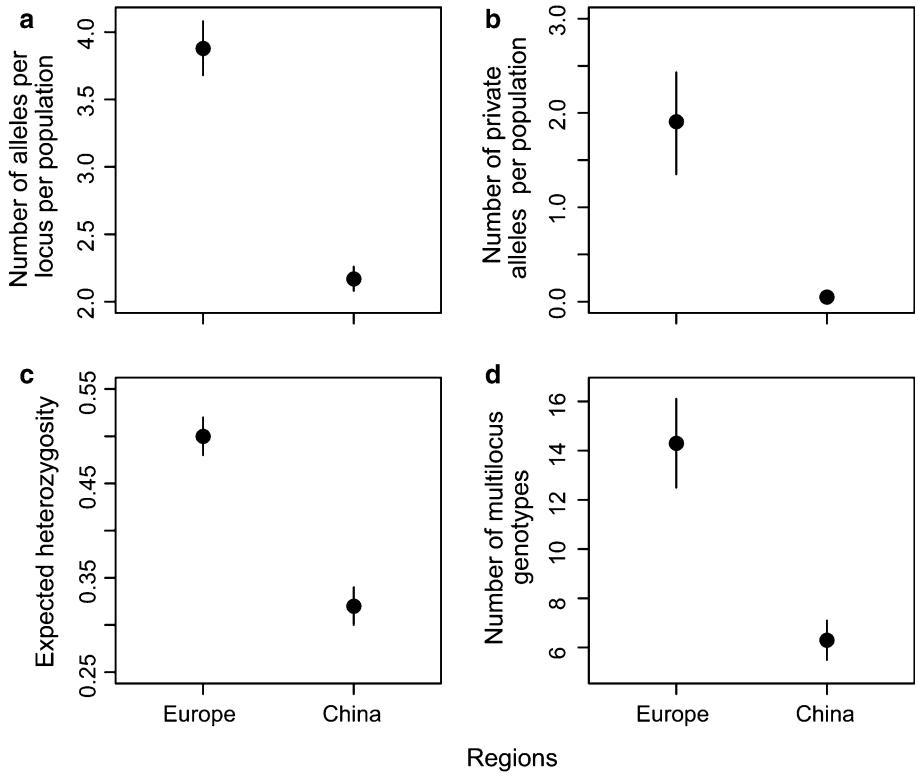

Fig. 1 Comparisons of genetic diversity between native (Europe) and introduced (China) populations of *Senecio vulgaris*: **a** number of alleles per locus per population, **b** number of private alleles per population, **c** expected heterozygosity, and **d** number of multilocus genotypes

Table 2 Results of the analyses of molecular variance (AMOVA) based on 0/1 data from studies of eight microsatellite loci in native and introduced populations of *Senecio vulgaris*

Source of variation	*df*	Sum of squares	Variance components	Percentage of variation	*P* value
Global					
Among continents	1	142.8	0.160	3.27	0.1
Among populations within continents	26	2197.2	2.912	59.40	<0.001
Within populations	768	1405.7	1.830	37.34	<0.001
Europe					
Among populations	8	373.8	1.491	30.99	<0.001
Within populations	253	840.2	3.321	69.01	<0.001
China					
Among populations	18	1823.4	3.568	76.47	<0.001
Within populations	515	565.4	1.098	23.53	<0.001

populations (76.47 %) compared to within populations (23.53 %).

No significant correlations were found between pairwise G_{ST} and geographical distance in Europe ($r = 0.11$, $P = 0.282$), southwestern China ($r = 0.24$, $P = 0.144$) or northeastern China ($r = 0.38$, $P = 0.141$, Fig. 2). However, both the southwestern and northeastern regions of China exhibited a larger degree of scatter compared to the European region. The difference in the respective

variances in G_{ST} values between southwestern China and the European region ($V_{CS}-V_E$) was 0.015 and was larger than the 95 % upper limit (0.006) determined by random permutation. Similarly, the difference in scatter between northeastern China and the European region ($V_{CN}-V_E = 0.022$) was much larger than the 95 % upper limit (0.009). However, the southwestern and northeastern China regions showed a similar degree of scatter ($V_{CN}-V_{CS} = 0.007$, 5 % lower limit $= -0.011$, 95 % upper limit $= 0.011$, Table 3).

Table 3 Comparison of variation in pairwise G_{ST} values among *Senecio vulgaris* populations from Europe (V_E), southwestern China (V_{CS}) and northeastern China (V_{CN})

	5 % lower limit	95 % upper limit
$V_{CS}-V_E = 0.015$	−0.006	0.006
$V_{CN}-V_E = 0.022$	−0.009	0.009
$V_{CN}-V_{CS} = 0.007$	−0.011	0.011

Assignment analyses

By analyzing the transformed 0/1 data using AFLPOP (Table 4) we detected 186 individuals belonging to 14 Chinese populations that could be assigned to four European populations (Oxford, UK, Neuchatel, Switzerland, Paris, France and Nuremberg, Germany) based on their inferred genotypes. A total of 141 of the 186 individuals were assigned to population E (Oxford, England). Of these 141 individuals, 139 individuals occurred in eight of the nine populations sampled from southwestern China. Notably, all of the 30 plants from population LD were assigned to the Oxford population. Additionally, all 30 individuals from the invasive population MY were assigned to population LC (Neuchatel, Switzerland), and 10 of the 180 northeast individuals were allocated to population F2 (Paris, France).

The model selection based on ΔK supported $K = 3$ (ESM, Fig. S3) in the STRUCTURE analysis of the full data set. The next closest model was $K = 2$. The ΔK values of the two models were very close ($K = 2$: 5.586; $K = 3$: 5.891). When $K = 2$, the populations in southwestern China (except DL) clustered together, while the other populations in China were in another cluster with some mixed individuals. When $K = 3$, the populations in southwestern China (except DL) also clustered together. For the other populations in China, there were two clusters and some populations were mixture of the two clusters. In contrast, no matter whether $K = 2$ or $K = 3$, European populations occurred in a mixture of the two or three clusters (Fig. 3). Within China, the most likely number of clusters was two, and the genetic structure pattern was analogous with the full data set assigned to two clusters (Fig. 3). Within Europe, five clusters were detected; however, most individuals were mixed with no particular patterns that were evident (Fig. 3).

Discussion

Our comparative study of genetic diversity and differentiation between native and invasive populations of *S. vulgaris* revealed several main findings. First, both the overall levels and average amount of genetic diversity within introduced populations in

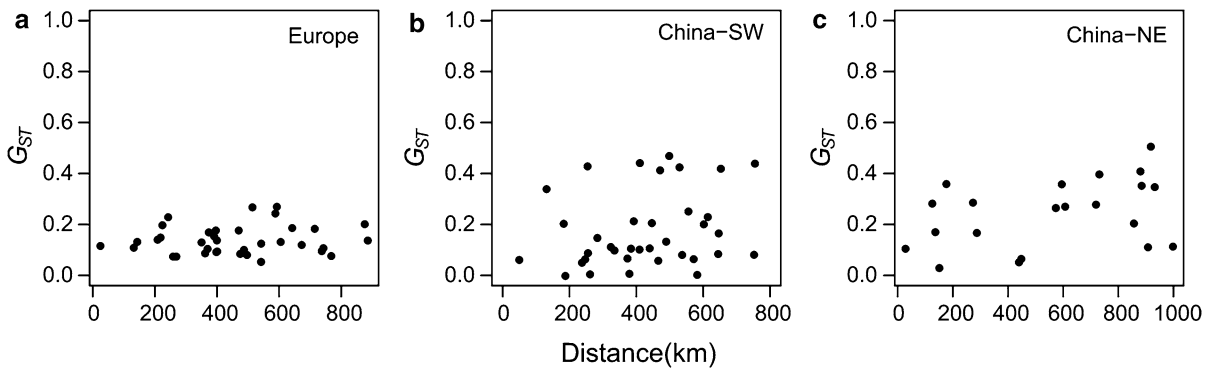

Fig. 2 Scatterplots of G_{ST} against geographic distance between each pairwise population of *Senecio vulgaris*: **a** Europe, **b** southwestern China and **c** northeastern China

Table 4 The number of individuals in Chinese invasive populations that were successfully assigned to European native populations by analyzing the transformed 0/1 data using AFLPOP

Population	D1	E	F2	LC	Percentage of individuals that were successfully assigned
GY		12			0.43
KD		17			0.57
KM		12			0.4
LD		30			1
LJ		18			0.6
LP		5			0.17
MG		24			0.92
MX		21			0.75
GD	5				0.17
MY				30	1
HL		1			0.05
JD			5		0.22
MDJ		1			0.06
YKS			5		0.17

China were significantly reduced compared to native European populations. Second, assignment tests provided evidence suggesting that there have been multiple independent introductions to China involving descendants originally from the native range. Finally, our investigations of correlations of pairwise genetic and geographic distance suggested that the post-introduction population dynamics of *S. vulgaris* is likely to have been dominated by genetic drift. Next, we consider in detail the historical, demographic and genetic processes that likely account for the differences in patterns of genetic variation between European and Chinese populations of *S. vulgaris* that were revealed by our study.

Reduction in genetic diversity and multiple introductions

Reductions in allelic richness and expected heterozygosity associated with biological invasions are commonly reported in the literature, although the magnitude of losses in genetic diversity are highly variable among species (Dlugosch and Parker 2008). Our comparisons of genetic diversity in native and introduced populations of *S. vulgaris* are consistent with the common finding of reduced diversity in the invasive range (Fig. 1). All four of the indices that we measured (alleles per locus, expected heterozygosity, number of multilocus genotypes and private alleles) each showed a significant reduction in our sample of Chinese invasive populations (Fig. 1; Table 1). The overall level of genetic diversity in the Chinese invasive range was also lower than in Europe despite the fact that the European sample involved less than half the number of populations (China—19; Europe—9). A larger sample of populations from Europe would have almost certainly accentuated the differences that we detected between the levels of genetic diversity in the two regions.

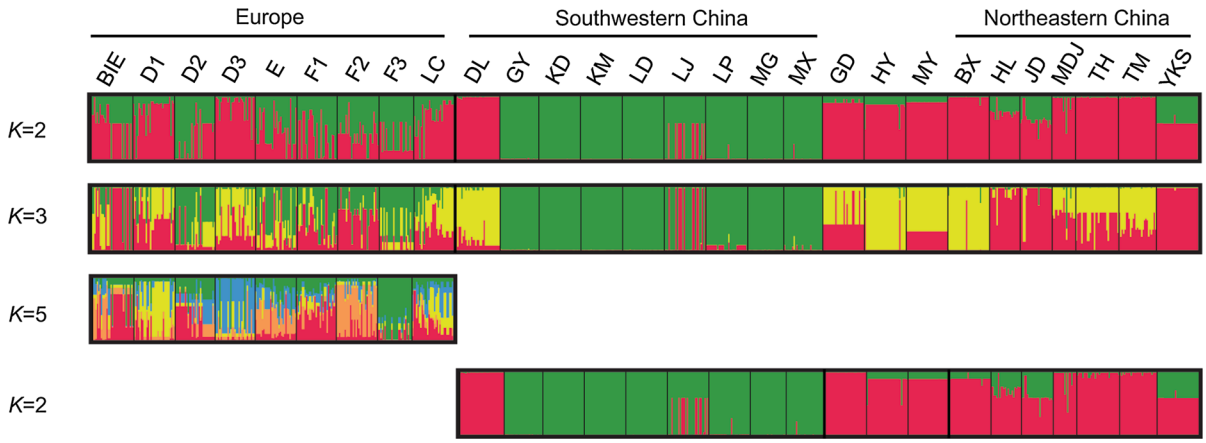

Fig. 3 Population structure of *Senecio vulgaris* inferred using the program STRUCTURE. The first two rows are the results of all individuals in Europe and China when $K = 2$ and $K = 3$. The third row is the analysis of the European samples when $K = 5$. The fourth row is the analysis of the Chinese samples when $K = 2$

Our results support the hypothesis that the Chinese invasion of *S. vulgaris* has involved multiple introductions. The assignment analyses using AFLPOP revealed that all of the plants in population MY (Central China) matched those from Neuchatel, Switzerland, whereas plants in population LD (southwestern China) matched those from a population sampled from Oxford, UK. Obviously it is not possible to say that these particular populations represent the precise sources from which the two populations in China are derived, and indeed this is highly unlikely and the true source populations may be elsewhere in Europe, or from other parts of the alien range because *S. vulgaris* has also been introduced into Asia, America, South Africa, New Zealand and Australia (Robinson et al. 2003). Thus, with our data it is not possible to determine whether the invasion of China by *S. vulgaris* came directly from Europe, or involved secondary invasions from other parts of the introduced range. However, the results from the assignment analysis strongly support that the introductions to China ultimately involve descendants from different source areas and populations in Europe. With the exception of population DL, most of the individuals from the eight other southwestern populations in China were assigned to the Oxford, UK population. In the northeastern populations, five plants (22 %) from JD and five plants (17 %) from YKS were assigned to Paris, France, whereas all of the other plants sampled from China were not assigned to any of the nine native populations. This suggests that at least some of these populations are derived from additional undetected introduction events. As Muirhead et al. (2008) proposed, the ability to correctly match introduced individuals to their source populations will increase as more source populations are surveyed. Therefore, a much larger sample of populations spanning a greater proportion of the native European range, and also other parts of the introduced range, would be required to narrow down likely source regions for the Chinese invasion and enable more accurate estimates of the number of separate introductions. Despite our limited sample of European populations, the STRUCTURE analyses also supports the hypothesis that there were multiple introductions of *S. vulgaris* to China. Populations in this region clustered into main two groups (Fig. 3), a pattern consistent with separate invasion events.

Mating systems and genetic bottlenecks

Historical processes are important determinants of the patterns of genetic diversity in invasive species, particularly in highly selfing species. Inbreeding tends to preserve multilocus associations and linkage disequilibrium following colonizing events (Golding and Strobeck 1980; Brown 1984; Husband and Barrett 1991). Subsequent opportunities for erasing the signature of founder events by outcrossing and genetic admixture are highly dependent on mating patterns and gene flow after colonization. Selfing rates in *S. vulgaris* are strongly influenced by whether or not individuals produce ray florets and thus attract pollinators (Marshall and Abbott 1982). These authors found that the outcrossing rates of individuals with ray florets ranged from 13 to 20 %, whereas in plants with only disc florets outcrossing rates never exceeded 1 %. Significantly, all of the populations that we investigated in China were composed exclusively of plants with only disc florets. Therefore it is probable that Chinese populations are highly selfing, although this hypothesis needs to be confirmed by marker gene estimates of mating patterns. High selfing in *S. vulgaris* following separate introductions to different regions of China would preserve the signature of founder events, and further spread accompanied by frequent genetic bottlenecks may have led to additional erosion of genetic diversity in invasive populations.

Our results from the assignment tests can be used to assess the extent of genetic bottlenecks accompanying invasion by performing "ancestor–descendant" comparisons of genetic diversity. For example, by comparing levels of genetic diversity between population MY and LC and between LD and E, two population pairs that are clearly genetically related and could serve as ancestor–descendant pairs, we can gauge the magnitude of genetic erosion. While in reality the two pairs of populations are highly unlikely to be related as true ancestors and descendants, comparisons are nonetheless instructive. The number of alleles per locus, number of private alleles, expected heterozygosity, and number of multilocus genotypes from the "ancestor" population LC (4.5, 4, 0.565, and 19, respectively) were each significantly higher than comparable estimates those from the "descendant" population MY (1.6, 0, 0.216, and 2, respectively, Table 1). Similarly, all four indices from the

"ancestor" population E were also much higher than those from the "descendant" population LD (Table 1). The results of these two comparisons are therefore consistent with the hypothesis that selfing species are especially vulnerable to genetic bottlenecks during invasion.

Effects of post-introduction population dynamics on genetic diversity

Post-introduction population dynamics can also affect genetic diversity once introduced species have established in their invasive ranges. Neutral genetic diversity in invasive populations will be affected by the relative contributions of gene flow and stochastic forces, particularly genetic drift (Hutchison and Templeton 1999). Our results suggest that neither European nor Chinese populations of *S. vulgaris* are at equilibrium because we found no significant correlations between pairwise G_{ST} and geographical distance in either region (Fig. 2). This finding may reflect the recent shared evolutionary history of populations in Europe or China and suggests that the populations may not have existed long enough for regional patterns of isolation-by-distance to have been achieved (McCauley 1993; Hutchison and Templeton 1999). Our data are consistent with the AFLP study of *S. vulgaris* by Handley et al. (2008) who also found no relation between pairwise genetic and geographical distance within Europe, Australia and North America.

It has been suggested that European populations of *S. vulgaris* originated from southern Europe and are assumed to have migrated north to occupy their current habitats since the Quaternary Ice Age (Kadereit 1984). In contrast, based on herbarium records, invasive populations in China were first recorded in the region approximately 200 years (Xu and Qiang 2004). Thus, populations in native versus introduced regions have had vastly different amounts of time to saturate available habitats and for demographic and genetic equilibrium to have been reached. European populations exhibited much less scatter in the relation between G_{ST} and geographical distance compared to the Chinese sample of populations (compare Fig. 2a vs. 2b, c). Therefore, we infer that gene flow has been of greater historical importance in European native populations. We do not know how common the ray floret morph of *S. vulgaris* is among European populations. However, it seems probable that more outcrossing occurs in Europe than China and this would likely serve to promote some degree of pollinator-mediated gene flow between populations, in addition to the pappus-mediated wind-dispersal of seeds that occurs in this species. In contrast, high rates of selfing and the pervasive influence of stochastic forces associated with colonization bottlenecks and genetic drift are likely to be of greater importance in Chinese invasive populations.

Populations of *S. vulgaris* in both Europe and China are often small and occupy disturbed habitats such as roadsides and agricultural fields. However, in contrast to Europe, populations in China are often isolated from one another and it is clear that in this region non-equilibrium conditions prevail and that many suitable habitats have as yet not become occupied. Low levels of gene flow in the highly selfing Chinese populations of *S. vulgaris* are unlikely to counter the random loss of alleles from populations by genetic drift (Gillespie 2004). Despite early proposals for future research on the genetics of migration and colonization in invasive plants (reviewed in Barrett and Husband 1990), and the pioneering work of Hutchison and Templeton (1999) on the influences of gene flow and drift on the distribution of genetic variability in populations, few researchers have investigated the correlation of pairwise genetic and geographic distance in invasive plants to evaluate the effects of gene flow and genetic drift on genetic diversity. More studies would be desirable on the effects of post-introduction population dynamics on patterns of genetic diversity in invasive plants to determine the extent to which demographic factors promoting genetic erosion are balanced by genetic admixture following multiple introductions from the native range.

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