Rapid evolution or preadaptation in invasive Jacobaea vulgaris

Doorduin, L.J.

Chapter

6

Multiple introductions of the invasive species *Jacobaea vulgaris* and a reduced genetic diversity in its invasive area

Leonie Doorduin*, Jelle Zandveld*, Rolf Vossen§, Peter Klinkhamer*, Rene Glas* and Klaas Vrieling*,

* Institute of Biology, Section Plant Ecology and Phytochemistry, Leiden University, PO Box 9505, 2300 RA Leiden, the Netherlands

§ Leiden Genome Technology Center, Human and Clinical Genetics, Leiden University Medical Center, PO box 9600, 2300 RC Leiden, the Netherlands

Corresponding author: Leonie Doorduin, Tel.: +31715275117, fax: +310715274900
E-mail address: leoniedoorduin@hotmail.com and K.vrieling@biology.leidenuniv.nl

Introduction

Species have always invaded new areas. However, the rate and spatial scale has changed tremendously since the beginning of the industrial revolution 300 years ago (CS Elton, 1958). Human-assisted species invasion is considered a key threat to native biodiversity (DS Wilcove ,D Rothstein ,J Dubow *et al.*, 1998) because invasive species can alter species distributions, community structure and ecosystem processes. In turn these effects on biodiversity can lead to high economic costs (RI Carruthers, 2003). In the United States non-indigenous crop weeds cause an estimated reduction of 12% in crop yields. In economic terms this represents about 33 billion dollars in lost crop production annually (D Pimentel ,R Zuniga ,D Morrison, 2005).

Recently adaptive evolution has been highlighted as a key process in the success of invasive individuals (AR Kanarek ,CT Webb, 2010). Alternatively invasive individuals may have been preadapted such that individuals from the native area already contained traits that turned out to be beneficial in the new environment (KM Dlugosch ,IM Parker, 2007). In a number of species shifts were demonstrated in major traits and allocation patterns upon introduction in the new area (O Bossdorf ,H Auge ,L Lafuma *et al.*, 2005; J Joshi ,K Vrieling, 2005; EA Leger ,KJ Rice, 2003; E Siemann ,WE Rogers, 2001). To distinguish between the two possibilities mentioned above, it is necessary to compare traits of the exact source population with those from the invasive individuals (O Bossdorf ,H Auge ,L Lafuma *et al.*, 2005). This requires detailed information about the origin of the invasive populations which is mostly lacking. Preadaptation becomes less likely if multiple introductions from different areas have taken place, because there is little chance that multiple introductions from different source populations all contain "preadapted"indivduals. So the number of introductions, even as determination of (the) source population(s), can give insight if invasive species evolved after introduction or if they were already preadapted before introduction.

Whatever the mechanism, rapid evolution or preadaptation, the success of an introduced species depends on the amount of genetic variation introduced in the new area (NC Ellstrand ,KA Schierenbeck, 2000). A number of studies observed that genetic variation of invasive populations is decreased compared to the native populations (L Amsellem ,JL Noyer ,T Le Bourgeois *et al.*, 2000; A Grapputo ,S Boman ,L Lindstrom *et al.*, 2005; BC Husband ,SCH Barrett, 1991). Only a small part of the genetic variation of the native population is introduced and as a consequence not all alleles are present in the invasive area. Thus founder effects reduce the amount of genetic diversity in the invaded area compared to the native area (L Amsellem ,JL Noyer ,T Le Bourgeois *et al.*, 2000; S Lachmuth ,W Durka ,FM Schurr, 2010). A further decrease of genetic variation is brought about by initial small population sizes leading to inbreeding and genetic drift (H Meimberg ,JI Hammond ,CM Jorgensen *et al.*, 2006). If invasive individuals went through a bottleneck and the species was introduced once the level of genetic variation in the invasive area will be low. Than, it is unlikely this species becomes abundant due to reduction in genetic variation for most traits. Alternatively when invasive populations are founded multiple times and admixture of different source populations takes place in the invasive area genetic variation can be maintained or even increased and new combinations of traits may become invasive (BJ Genton ,JA Shykoff ,T Giraud, 2005; S Lavergne ,J Molofsky, 2007). It has been hypothesized that admixture may lead to problematic invaders capable of fast evolutionary response to selection pressure because of the increased genetic variation. (RA Hufbauer ,R Sforza, 2008; PJ Prentis ,JRU Wilson ,EE Dormontt *et al.*, 2008). In addition admixture can alleviate the inbreeding load (KFJ Verhoeven, M Macel, LM Wolfe et al, 2010).

Jacobaea vulgaris or tansy ragwort is a plant species that is native within Europe and western Asia and introduced circa 150 years ago into Australia, New Zealand and North America. Within the invasive areas this plant species received a pest status because of its toxicity to livestock and vigorous growth. Joshi and Vrieling (2005) showed that compared with native populations *J. vulgaris* from invasive areas grew bigger, produced more seeds and were better defended against generalist herbivores but less well defended against native specialist herbivores, which were absent in the areas where it was introduced (LJ Doorduin ,K van den Hof ,K Vrieling *et al.*, 2010; LJ Doorduin ,K Vrieling, 2011; J Joshi ,K Vrieling, 2005). The altered traits in the invasive area compared to the native area suggest that a fast evolution took place. However preadaption can not be excluded as traits of the invasive individuals stil show an overlap with traits in the native area. An AFLP study on 15 native- and 16 invasive populations of *J. vulgaris* showed that the amount of genetic variation did not differ between native and invasive populations (LJ Doorduin ,K van den Hof ,K Vrieling *et al.*, 2010). Furthermore the high levels of genetic variation in all studied invasive populations suggest that multiple introductions occurred followed by admixture (AV Suarez ,ND Tsutsui, 2008). However the source population(s) could not be clearly pinpointed due to limited sample sizes and the low resolution of AFLP markers (LJ Doorduin ,K van den Hof ,K Vrieling *et al.*, 2010). In this study we use a larger set of individuals to determine if and which multiple source populations founded the invasive populations. Furthermore we will use single nucleotide polymorphisms (SNPs) and microsatellites of the chloroplast genome. These markers have a higher resolution rate compared to nuclear markers used in the AFLP study because of maternal transmission only and the absence of recombination. To identify the source population(s) we need to know the genetic structure of individuals originating from the native area to pinpoint possible source populations which were not included in the set that was genotyped. Therefore we investigate with a Mantel test if genetic and geographical distances are correlated. We expect to find a higher degree of relatedness between individuals that are geographically closeby and less relatedness between individuals that are geographically widespread.

We address the following questions: 1) is ragwort introduced more than once and if so can preadaptation be excluded? 2) What is/are the source population(s) of the invasive ragwort populations? 3) are genetic distance and geographical distance positively correlated in the native area? 4) Does the genetic diversity between native populations differ? 5) Are ragwort populations in the native and the invasive areas genetically differentiated? 6) Is the genetic variation in invasive ragwort populations reduced compared to native populations?

Methods

Species description

Jacobaea vulgaris formerly known as *Senecio jacobaea*, is a monocarpic perennial plant species and belongs to the family of the Asteraceae. It is native to Europe and western Asia, ranging from Norway through Turkey, and from Great Britain to Siberia. In the 1850s this species was first reported from the east coast of Canada (Nova Scotia) (P Harris ,ATS Wilkinson ,ME Neary *et al.*, 1971) and shortly thereafter in New Zealand (1875) (GM Thomson, 1922) and Australia (L Schmidl, 1972). In 1900 *J. vulgaris* also invaded the west coast of North America (HM Gilkey, 1957) and in 1913 it was recorded on the

west coast of Canada (P Harris ,ATS Wilkinson ,ME Neary *et al.*, 1971). Control is difficult because the lifecycle can vary from annual to short-lived perennial and seeds remain viable in the soil for several years (E van der Meijden ,RE van der Waals-Kooi, 1979).

J. vulgaris contains about 37 different hepatoxic PA's and it causes four million dollar losses annually to cattle poisoning in Australia alone (PD Roberts ,AS Pullin, 2007).

In Great Britain *J. vulgaris*. is attacked by 30 specialist and more than 40 generalist herbivores (JL Harper ,WA Wood, 1957) while in the invasive area mainly generalist herbivores are reported (KE Frick, 1972). In several invasive populations biological control agents has been introduced with mixed results (M Julien, 1987).

Chloroplast markers

To identify the source population often polymorphic markers from neutrally evolving areas of the genome are used because these will not be affected by natural selection (RA Marrs ,R Sforza ,RA Hufbauer, 2008). The chloroplast genome is transmitted as a single locus through the maternal line and does not recombine. Dispersal of chloroplasts genomes in the population is limited because they are only dispersed through the seeds. As a consequence the chloroplast genome has an effective population size of approximately one- fourth of the nuclear genome (RL Small ,RC Cronn ,JF Wendel, 2004). So chloroplast markers are more affected by genetic drift but genetic patterns will fade away more slowly compared to nuclear markers (DE McCauley ,JE Stevens ,PA Peroni *et al.*, 1996) due to the lack of recombination. The low mutation rate of single nucleotide polymorphisms of the chloroplast genome ($1.0 \times 10^{-9} – 3.0 \times 10^{-9}$) (KH Wolfe ,WH Li ,PM Sharp, 1987) and chloroplast microsatellites (3.2×10^{-5}- 7.9×10^{-5}) (J Provan ,N Soranzo ,NJ Wilson *et al.*, 1999) contributes furthermore to the conservation of genetic patterns. These characteristics make chloroplast markers good markers for finding (a) source population(s) in population studies if an appropriate number of polymorphic markers can be found. In other studies chloroplast microsatellites have already proven to be valuable for their polymorphisms (M Jakobsson ,T Sall ,C Lind-Hallden *et al.*, 2007; J Provan ,W Powell ,PM Hollingsworth, 2001; BA Richardson ,J Brunsfeld ,NB Klopfenstein, 2002).

Sampling

From each population, seeds of individuals growing at least 2 m apart were collected. From each maternal plant one seed was germinated and grown in a climate-room. Fresh leaf samples (5 leaf punches of 1 cm. diameter each) were collected from each individual and stored at -80 ºC. DNA was extracted using the CTAB protocol (JJ Doyle, JL Doyle, 1987).

In total 177 individuals were used for the analysis (see Table 1), 90 native individuals (11 populations) and 87 invasive individuals (29 populations). Since we expected to find less variation within populations of the invasive range, we chose to sample more populations but fewer individuals per population in the invasive range. However we were also interested in the genetic variation within invasive populations and therefore from every invasive area (New Zealand, Australia and North America) one population was sampled more intensively.

Detection of polymorphic loci on the chloroplast genome

In a previous study, the total chloroplast genomes of 12 native and 5 invasive individuals of *J. vulgaris* were sequenced using next generation sequencing techniques (LJ Doorduin ,B Gravendeel ,Y Lammers *et al.*, 2011). Comparison of the 17 chloroplast genomes with each other yielded 32 Single Nucleotide Polymorphisms (SNPs). In this study, eight SNPs were used to screen polymorphisms in 177 individuals. Two different methods were used; six SNPs were screened with high resolution melting and two SNPs, of which scoring by high resolution was difficult, showed good results with restriction mapping (Table 2).

In addition a total of 33 potential microsatellite regions with more than nine mononucleotide repeats were identified. Primers were developed to amplify the ten largest repeat regions (between 10 and 18 repeats). Nine of these amplified regions showed variation in repeat length between individuals (Table 3).

Table 1: Origin of sampled populations of *J. vulgaris*.
Pop. nr. = number of the population, AUS= Australia, CAN= Canada, NZ = New Zealand, USA = United States of America, N= number of individuals.

pop. nr.	country	location	latitude	longitude	N
	Invasive				
1	AUSba	Barramunga (Victoria)	E 143.41°	S 38.33°	3
2	AUSbe	Beech forest (Victoria)	E 143.33°	S 38.38°	2
3	AUSc	Cape Schank (Victoria)	E 144.54°	S 38.28°	3
4	AUSd	Dairy Plains (North Tasmania)	E 146.31°	S 41.38°	3
5	AUSf	Franklin (South Tasmania)	E 147.00°	S 43.05°	3
6	AUSt	Turton's Creek (Victoria)	E 146.14°	S 38.33°	12
7	AUSw	Wild Dog Road (Victoria)	E 144.15°	S 37.26°	2
8	CANcd	Cardigan	W 62.37°	N 46.14°	3
9	CANd	Dundas	W 62.31°	N 46.19°	3
10	CANg	Green Cables	W 63.24°	N 46.29°	3
11	CANc	Carvell	W 63.07°	N 46.15°	2
12	CANb	Beludere	W 63.07°	N 46.15°	4
13	CANe	Ellen's Creek	W 63.09°	N 46.15°	3
14	CANm	Marco Polo (Prince Edward Island)	W 63.20°	N 46.29°	2
15	NZms	Maruia (South Island)	E 172.13°	S 42.11°	1
16	NZmn	Mangatoki (North Island)	E 174.13 °	S 39.25°	1
17	NZcs	Craigieburn (South Island)	E 171.51°	S 43.06°	1
18	NZfs	Fox Glacier (South Island)	E 170.01°	S 43.28°	1
19	NZss	Southland (South Island)	E 167.55°	S 45.28°	2
20	NZhs	Haast (South Island)	E 169.02°	S 43.53°	7
21	USAh	Humboldt County (California)	W 123.52°	N 40.45°	10
22	USAs	Suprise Hill-Sylvia Lake (Montana)	W 115.21°	N 48.21°	2
23	USAin	Indian Creek (Oregon)	W 124.25 °	N 42.26°	2
24	USAi	Island Lake (Montana)	W 114.58°	N 48.14°	2
25	USAl	Larch Slope (Oregon)	W 121.50°	N 45.29°	1
26	USAn	No Bear (Oregon)	W 114.53°	N 48.14°	2
27	USAsi	Silvertown (Oregon)	W 122.47°	N 45.29°	2
28	USAsh	Surprise Hill (Montana)	W 114.59 °	N 48.15°	2
29	USAw	West Crest (Oregon)	W 121.50°	N 45.29°	3
	Native				
30	ENG	New Castle upon Tyne	W 01.36°	N 54.58°	10
31	FRA	Sainte Marguerite	E 00.50°	N 49.27°	10
32	GER	Halle	E 11.56°	N 51.28°	7
33	HUN	Lénárddaróc	E 20.22°	N 48.08°	7
34	IRE	Caherdaniel	W 10.06°	N 51.45°	6
35	NLv	Veluwe	E 05.24°	N 51.49°	8
36	NLw	Wageningen	E 05.39°	N 51.58°	8
37	NOR	Sør Trøndelag	E 63.00°	N 10.23°	7
38	POL	Warsaw	E 21.01°	N 52.13°	9
39	SPA	Porte de San Glorio	W 4.45°	N 43.04°	8
40	SWE	Uppsala	E 59.51°	N 17.38°	10

Table 2: Primers and probes used for SNP analysis in *J.vulgaris*
Primer = name of the primer, between brackets the used restriction enzyme, SNPpos = SNP position (bp) on cp genome *J. vulgaris* HQ234669, T = annealing temperature (°C), HRM = high resolution melting, HRMP = high resolution melting with probe, REST= restriction.

Primer	SNP pos	Primer sequence (5'> 3')	T	Identification method
SNP 1F	4032	GACTTCGGTTTGCTCCCTTT	55	HRM
SNP 1R		CTTCTTTCACTTTTTCAATT		
SNP 2F	7837	AACCTTCGATTCAAACATTG	55	HRM
SNP 2R		CTCGCGGCTATCCGATAATT		
SNP 3F	11353	ATCAACTTCCTTTCATCTCC	55	HRM
SNP 3R		TTTATTTGTCAAGTCTACCT		
SNP 4F	18287	TTGTGGCTGCGGTGGCGACT	55	HRM
SNP 4R		GCAGCAATAACGCGTTGATC		
SNP 5F	60245	CAATCCAATTAATCAAGATA	55	HRM
SNP 5R		ATGAATATGACCCTCGTTGT		
SNP 6F	5555	TCATTTGTACTCATAACTCAAGTTCAA	60	HRMP
SNP 6R		CACGGATCCGAATCAAGAAT		
SNP6 probe		GATAGATATTTTTTTATTGAGTGGTCTTTAACCCC		
SNP 7F	118779	TGTCTTTACCACGAACAACTTTCCTTG	60	HRMP
SNP 7R		TTGTCCTATTTCCTTTATGTGGAAGA		
SNP7 probe		GTTTTACCAATATTTGCGGGTTCCTTAATTTTC		
SNP 1F (Dra I)	4032	GCCTTCCCGTATTGGGTACT	55	REST
SNP 1R	4032	GAAGCGAGACATTCGTCCAT		
SNP 3F (Sty I)	11353	CCATCTGATAGTAGGTTGCCAAA	55	REST
SNP 3R	11353	TTTATTTCCTAAGGGTGGTTGG		
SNP 8F (Xcm I)	39829	ACATGCCCAGTTGAGATGTG	55	REST
SNP 8R		GGCTAAGTGATACTGCACACCA		
SNP 9F (Bsr BI)	69567	CGAATTCAAAGTGCCATGCT	55	REST
SNP 9R		AAAAAGAATTGAAATCTACACATTGA		
SNP 10F (Bsa HI)	70234	TTTCTTGTTCTTAAACGAGCCTCT	55	REST
SNP 10R		CGTTTTCTCCCCAATCGAG		
SNP 11F (Ava I)	97496	CCAATTCCTTCCCGATACCT	55	REST
SNP 11R		GACTCACTAAGCCGGGATCA		

Table 3: Primers used for amplifying microsatellite regions in *J. vulgaris*.
Primer = primer name, Ms pos= start position of repeat (bp) in cp genome of *J. vulgaris* HQ234669, T = annealing temperature (°C), Label = used fluorescent label, Length of product = length of amplified PCR product in bp. Forward primers all contain the M13 primer sequence at the 5'end.

Primer	Ms pos	Primer sequence (5'> 3')	T	Label	Length of product
MS 1F	6705	CACGACGTTGTAAAACGACCGAAT TGT CAATGATG	50	Fam	133-137
MS 1R		TGTGAAAAATAATGAGCATCCCTA			
MS 2F	41459	CACGACGTTGTAAAACGACCAATCACGCGAGCAGACATTAGCTATTA	50	Tamra	242-246
MS 2R		TTGGGAAGAACCAACCAAAG			
MS 3F	79774	CACGACGTTGTAAAACGACCGAGCCCCACTGTTATCTGCT	50	Fam	239-247
MS 3R		AATAGCAGCGTCCAAAATGC			
MS 4F	24848	CACGACGTTGTAAAACGACCGGGCCACCATTGACTAGTTT	48	Hex	259-264
MS 4R		CAAAGGGAATTTTAGGAAAAAGA			
MS 5F	109743	CACGACGTTGTAAAACGACCTGGTCCTCCTCGAAAAACAC	48	Tamra	255-257
MS 5R		CCTTTTCCGTTTGAGTTTCA			
MS 6F	54013	CACGACGTTGTAAAACGACCTGGAATTCGAACCTGAACTCT	48	Hex	238-243
MS 6R		TCGAAATACCTAAAAATCACTCAAA			
MS 7F	27760	CACGACGTTGTAAAACGACCATTGGGGACGATGAAACAAA	52	Fam	185-189
MS 7R		GCTCAATACGTTCGCGGATA			
MS 8F	12459	CACGACGTTGTAAAACGACCTCCACCTATCTCATAGATTCCAGTC	52	Hex	208-212
MS 8R		TGTGGACATTGCGTCTATCC			
MS 9F	76775	CACGACGTTGTAAAACGACCAGATCCTGGGAGGCAATTCT	46	Tamra	215-217
MS 9R		TCCCGGAATTTGATTGATTT			

Single Nucleotide Polymorphisms (SNPs)

High resolution melting

For SNP genotyping, two high resolution melting protocols were used. The first protocol was used to identify nucleotide changes from A/T to G/C. When strands from the DNA duplex are separated, fluorescence rapidly drops. Different alleles result in different melting temperatures with lower temperatures for A/T genotypes compared to C/G genotypes (GH Reed ,JO Kent ,CT Wittwer, 2007) . For remaining cases where only the base pair orientation changed (A to T and G to C) a probe was added that allows fine discrimination of variants under the probe (LM Zhou ,AN Myers ,JG van der Steen *et al.*, 2004). A single base change will cause the probe to melt at a lower temperature than if the probe is completely complementary. Primers were developed in such a way that the SNP was located in the centre of the PCR product (total PCR product of 100-150 bp). Probes were developed including one of the SNP variant in its sequence. For primer and probe information see Table 2.

Four SNPs were identified with the first protocol using a PCR mixture of 25 µL containing 15 µL mineral oil, 6.45 µL water, 1.0 µL 10x PCR buffer with 20 mM $MgCl_2$, 0.3 uL 10 µM forward and reverse primer (Table 2), 0.8 µL 2.5 mM dNTPs mixture, 0.1 µL 10 µM low calibrator oligo's, 1 µL LC- Green Plus, 0.1 µL 5U/µL *Taq* polymerase (Roche, Woerden, the Netherlands) and 2.0 µL genomic DNA (circa 10 ng/ µL). Reactions were carried out in a 96 well plate covered with thermowell aluminium sealing tape to avoid evaporation. The PCR cycling conditions were as follows: 10 minutes at 95ºC for initial denaturation; following by 40 cycles of 20 seconds at 95 ºC, 30 seconds at 55 or 60 ºC and 40 seconds at 72 ºC; followed by a final extension for 5 minutes at 72ºC and a final denaturation step of 1 minute at 95 ºC. For the two SNPs that only had a change in base pair orientation, the same protocol was used but without adding oligo's (LM Zhou ,AN Myers ,JG van der Steen *et al.*, 2004) . Furthermore primers were added in different amounts, 0.05 µL forward primer (10 µM), 0.5 µL reverse primer (10 µM) with the probe added in the same amount as the reverse primer. To end up with 25 µL, 3.6 µL H2O was added in stead of 6.45 µL in the former protocol. The PCR cycling conditions were as follows: 10min at 95ºC for initial denaturation; following by 55 cycles of 20 seconds at 95 ºC, 30 seconds at 55 ºC and 40 seconds at 72 ºC; followed by a final extension for 5 minutes at 72ºC and a final denaturation step of 1 minute at 95 ºC. Melting analyses were performed on the LightScanner (Idaho Technology) at the Leiden Genome Technology Center. Melting curves were generated by monitoring the fluorescence of the saturating dye LC- Green Plus. Missing data of SNP 1 and SNP 3 were completed by restriction (see below for details).

Restriction mapping

At four SNP positions primers were developed to amplify products of around 200 basepairs with the SNP position located at 2/3 of the amplicon. As mentioned two of the six SNPs, with position 4032 and 11353, were already identified with high resolution melting, but missing data were obtained by restriction. For two SNPs all individuals were screened with restriction mapping. Individuals of which the cp genome was already sequenced (LJ Doorduin ,B Gravendeel ,Y Lammers *et al.*, 2011) and which represented different nucleotides were used as a control. Primers and restriction enzymes that were used are indicated in Table 2. Amplification was carried out in a 25 µL PCR reaction containing 16.0 µL water, 2.5 µL 10x PCR buffer, 1.0 uL 25mM MgCl2, 1.0 µL 10 µM forward and reverse primer, 2.0 µL 2.5 mM dNTPs mixture, 0.5 µL 5U/µL *Taq* polymerase and 1.0 µL genomic DNA (about 1 ng/ µL). The PCR cycling conditions were as follows: 5 min at 95ºC for initial denaturation; following by 40

cycles of 30 seconds at 95ºC, 30 seconds at 55ºC and 30 seconds at 72ºC; followed by a final extension for 5 minutes at 72ºC. PCR products were checked on a 1% agarose gel and the following mix was added to 2.5 µL of the visualized PCR products;16.0 µL water, 1 µL 10x recommend buffer for enzyme (New England Biolabs) and 1.0 µL of the restriction enzyme (see Table 2). After spinning the mix was incubated in a PCR machine for 16 hours at 37ºC. After incubation 7.5 µL restriction mix was loaded on a 1.5% agarose gel next to a control (uncut PCR product). With the help of the negative and positive control all samples on the gel were analysed. For samples that did not have a PCR product, the whole protocol was carried out again but this time with approximately 10 ng of DNA per sample. For three invasive individuals we were unable to amplify one locus resulting in three missing data points for the SNP data.

Microsatellites

Microsatellites were amplified using the M13-tailed PCR protocol to label the PCR products with fluorescent dyes (I Boutin-Ganache ,M Raposo ,M Raymond *et al.*, 2001). A PCR mixture with a final volume of 10 µL containing 4.9 µL water, 1.0 µL 10x PCR buffer, 0.4 µL 10 µM M13- tailed forward primer, 0.4 µL 10µM reverse primer, 0.4 µL 10µM M13-labelled primer (CACGACGTTGTAAAACGAC), 0.8 µL 2.5 mM dNTPs mixture, 0.1 µL 5U/µL *Taq* polymerase (QIAGEN, Venlo, the Netherlands) and 2.0 µL genomic DNA (about 0.1 ng/ µL). M13 primers were labelled with Fam, Tamra and Hex fluorescent dyes. The PCR cycling conditions were as follows: 5 minutes at 94ºC for initial denaturation; following by 40 cycles of 1 minute at 94ºC, 1 minute at the annealing temperature of each primer pair and 1 minute at 72ºC; followed by a final extension for 10 minutes at 72ºC. Primer information can be found in Table 3. All nine amplified microsatellites were pooled because they differed in length and fluorescent label, and run on a MegaBACE sequencer (GE Health Care, Eindhoven, the Netherlands) with ROX 400 as internal standard. The allele size of each microsatellite locus was scored with Fragment Profiler, version 1.2 (Amersham Biosciences, 2003). Samples with weak signals or unclear peaks were amplified and run again to reduce errors. One invasive individual still had an unclear peak for one locus and therefore this data point was considered as a missing value. In total 0.13% of the SNP and microsatellite data are missing (4 out of 3179 data points).

Data analysis

Combining of SNP and microsatellite data

Single Nucleotide Polymorphisms were scored as nucleotides whereas for microsatellites the number of repeat nucleotides was scored. To analyze both SNPs and microsatellites, data were combined by converting fragment sizes into a sequence. Sequences for microsatellites were formed by taking the longest repeat and replacing bases by gaps for smaller repeats. For SNPs the alternative base for that particular SNP was inserted. All microsatellites and SNPs were concatenated to one sequence per individual and used for TCS (M Clement ,D Posada ,KA Crandall, 2000) and Arlequin (L Excoffier ,G Laval ,S Schneider, 2005).

In contrast Fstat and Geneclass were run with fragment sizes, because these programs only taken into account allele difference rather than allele length differences and because sequences could not be analysed. For SNPs allele length 101 was given to one variety whereas 102 was given to the alternative allele.

Genetic diversity

First the distribution of alleles was determined for SNP and microsatellite data separately, by counting the number of individuals sharing alleles. The Fisher exact test calculated if there were significant differences between the native and invasive area in the distribution of alleles per locus.

After combining the datasets, the total number of alleles per locus of the native and invasive area was also determined and significance was tested with an independent sample T- test.

Genetic diversity over all loci (Hs) for native and invasive areas was calculated with the program Fstat (J Goudet, 1995). Hs was calculated following Nei's F- statistic (1987) with 1000 permutations.

The number of private haplotypes and the number of non-private haplotypes were counted for both native and invasive areas and were tested with a Chi-square test to test for differences between the native and invasive area.

Genetic differentiation between populations, regions and areas.

Genetic differentiation among populations, among regions in the invasive area and among the native and invasive area was analyzed with an analysis of molecular variance (AMOVA) using Arlequin 3.5. Significance of variance was tested by comparing obtained values to a null distribution generated by 1000 permutations. The correlation between genetic distance (Fst) and geographical distance within the native area was tested with a Mantel test (N Mantel, 1967).

Within population genetic distance of native populations

To compare the within population genetic distances, in each population the number of basepair changes between a pair of individuals was calculated for all possible combinations within a population. The average within population genetic distance is the average of all possible combinations within a population. Differences between populations in average within population genetic distance were tested with an ANOVA with population as a random factor.

Haplotype networks

We used the program TCS 1.21 to construct haplotype networks of native and invasive individuals alone and of all individuals together. Pairwise differences were calculated and the default 95% connectivity limit was used as the maximum of mutational connections between pairs (M Clement ,D Posada ,KA Crandall, 2000). The number of independent haplotype networks gives an indication about the diversity within the native and invasive area. Furthermore this program shows shared haplotypes between native and invasive individuals which can indicate potential source populations.

Assignment analysis

Rannala and Mountain (1997) (B Rannala ,JL Mountain, 1997) developed a Bayesian method to calculate for each invasive individual the likelihood that it is related to a native individual. In Geneclass 2.0 (S Piry ,A Alapetite ,JM Cornuet *et al.*, 2004) this method was used and likelihood scores were calculated. Because missing data strongly affect the outcome of likelihood scores, only individuals without missing data were used. In total 4 invasive individuals with one missing locus were not included in the analysis. Total likelihood scores were ordered from high to low. Populations containing native individuals with highest likelihood scores were most probably involved in introduction of invasive individuals. Furthermore for invasive individuals that had low likelihood scores for every comparison with a native individual, the source population was most likely not sampled.

Results

Genetic diversity

All of the eight SNP loci were polymorphic in the native area and six were polymorphic in the invasive area. Furthermore the distribution of the alleles over native and invasive individuals differed significantly for four out of eight loci (Fisher-exact test P< 0.05). The microsatellite loci of native individuals were highly polymorphic for every locus. For invasive individuals eight microsatellite loci were polymorphic whereas one locus was monomorphic.

We recorded 46 alleles for nine microsatellite loci in the 40 native and invasive populations. For the invasive area the number of alleles per microsatellite locus ranged from one to six. In the native area the number of alleles ranged from three to six. In the native area forty-one microsatellite alleles in 11 populations with 90 native individuals were found, compared to 30 alleles in 29 populations with 87 individuals in the invasive area. Sixteen private microsatellite alleles were present in the native area compared to five in the invasive area. The distribution of alleles over native and invasive individuals differed for seven out of nine microsatellite loci (Fisher-exact test P<0.05).

For the combined dataset of SNPs and microsatellites the total number of alleles per locus averaged over all loci did not differ between the native and invasive area (independent sample T- test, $F_{1,32,}$= 2.432 p= 0,158).

Genetic diversity (Hs) within populations was significantly higher in native populations (Hs =0.184) compared to invasive populations (Hs = 0.109) (Fstat, P < 0.05). Moreover the native area consisted of significantly more haplotypes than the invasive area, 63 versus 26 respectively (χ^2= 9.17 df=1, P< 0.01) despite equal sample size but a larger number of populations in the invasive area. The number of private haplotypes was also significantly higher in the native area, 46 versus 19 respectively (χ^2= 8.97 df=1, P< 0.01).

Within population genetic distance of native populations

The average within population genetic distance varied between 1.6 and 7.6 basepair changes with an average of 4.5 basepair changes for all populations. Hungary, France, Sweden and The Netherlands-Wageningen showed significantly higher within population genetic distances than the other populations (Fig. 1) suggesting that these are composite populations.

Genetic differentiation between populations, regions and areas.

Native and invasive populations were significantly genetically differentiated (Table 4). Of the total genetic variation, 23% was explained by native or invasive origin and 32% of genetic variation was due to differences among populations.

Within the native area only half of the total genetic variation was attributed to among population differences and the other half to variation within populations (Table 4).

Within the invasive area, the four regions (New Zealand, Australia, Canada east coast and North America west coast) accounted for only 14 % of the genetic variation, despite the large geographical distances. The among population genetic variation was 19% showing that the genetic differentiation in the invasive area is much smaller than the genetic differentiation between populations in the native area (Table 4). Variation among populations in the native area was not dependent on geographical distance (Mantel-test, Native area: r= -0.0918 n= 90, NS).

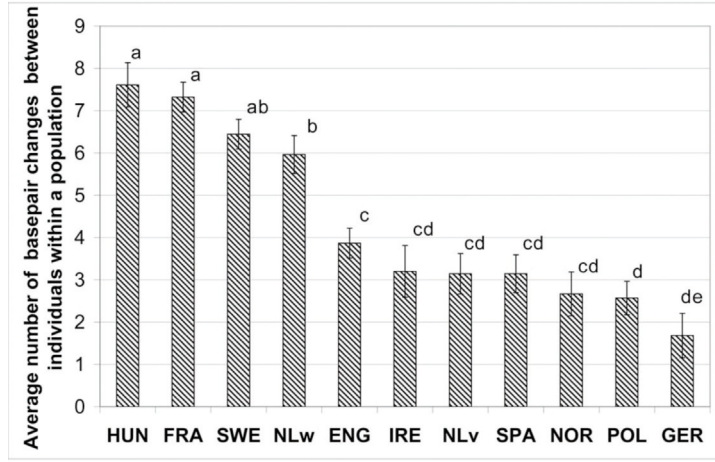

Fig. 1: Genetic distances within 11 native *J. vulgaris* populations. Different letters indicate significant differences. Bars indicate standard errors. Anova ($F_{10,646}$ = 22,98, p<0.001) For abbreviations of the populations see Table 1.

Haplotype network

Native area

For the native area we found 19 independent haplotype networks (Fig. 2). Thirteen of these networks consisted of single haplotypes and two networks consisted of two haplotypes from the same population (Fig. 2d). Four networks were found that consisted of 19,18,7 and 2 haplotypes, respectively (Figs. 2a,2b,2c,2d). The majority of the other native individuals were distributed over three large networks. One network, further on called the "North West European network" (Fig. 2a) consisted of all individuals from Ireland (6) and most individuals from Sweden (8), Norway (6) and The Netherlands-Veluwe (7), and one individual of The Netherlands-Wageningen. The second large network, further on called "South East European network" (Fig. 2b) consisted of all individuals from Germany (7) and most individuals from Poland (7), France (5), The Netherlands-Wageningen (6) and some individuals from Hungary (2) and England (1). The third network, further on called "England network" (Fig. 2c) consisted of individuals from England (7) Hungary (4) and The Netherlands-Wageningen (1). Most of the haplotypes in the native area are unique and only three haplotypes are shared by individuals from different native populations which is indicative of the large genetic variation found in the native area. The four networks were plotted on the geographic map showing the relatedness of these networks with particular geographic areas (Fig. 3).

Invasive area

In the invasive area five independent networks were detected by TCS. Three networks consisted of only one individual Carvell (CANc), Wild dog road (AUSw) and Humboldt County (USAh). One network consisted of two individuals namely Barramunga (AUSba) and Silvertown (USAs). The other network consisted of all other invasive individuals (Fig. 4).

The 19 independent networks of the native area and the five independent networks of the invasive area show that genetic diversity is higher in the native area compared to the invasive area. In contrast to the native area nine haplotypes are shared between individuals from different invasive populations. Surprisingly, shared haplotypes often contain individuals from the different invasive regions.

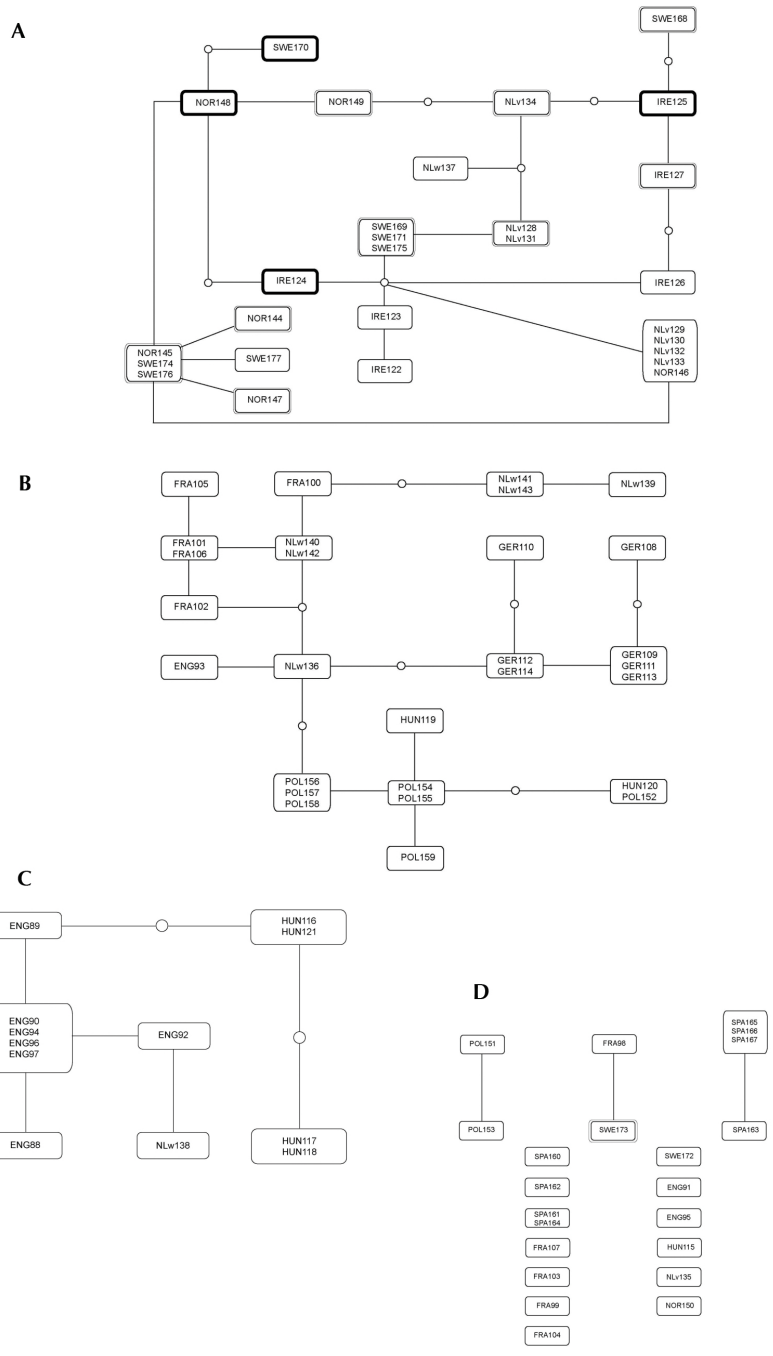

Fig. 2: Haplotype networks of *J. vulgaris* based on chloroplast microsatellites and SNPs in the native area.
Abbreviations are the same as in Table 1. Numbers indicate individuals. A bold line around a haplotype indicates that an identical haplotype is also present in the invasive network. A double line indicates a haplotype that is assigned to an invasive haplotype with a high likelihood score above the cut-of point (Fig. 5). a) North-west European network, b) The south European network, c) The England network and d) Independent networks consisting of a single or two haplotypes.

Four haplotypes from the native area (2 from Ireland, 1 from Sweden, 1 from Norway), all belonging to the North West European Network, were shared by invasive individuals in Australia, New Zealand, the east coast of Canada and the west coast of North America (Figure 2a and 4) . All shared haplotypes between native and invasive areas consisted of only one native individual shared with several individuals from different populations and regions, the exception being the Irish haplotype that is shared only with four Canadian populations. The Irish, Swedish and Norwegian populations sharing the haplotypes with invasive populations can therefore be considered source populations.

Furthermore the shared haplotypes in the invasive area with individuals from different invasive populations that are geographically far apart suggesting either multiple introductions or geneflow between regions.

Assignment analysis

Likelihood scores of how well a native individual matches an invasive individual were ranked. The first 40 likelihood scores are the pairs that share an identical haplotype between the native and invasive area (Fig. 5). That the log likelihood scores differ for these individuals with exactly the same haplotype is caused by differences in frequency of alleles. The graph shows a sudden drop after the 164th pair (representing 2.2 % of all invasive individuals). All the individuals in these pairs have near identical haplotypes between native and invasive individuals. All native individuals with these near identical sharing haplotypes belong to the North-Western European haplotype network reaffirming that possible source populations are from this particular native area (Figs. 2 and 4).

Invasive individuals with likelihood scores beyond the 164th pair probably originate from other source populations than the native individual they formed a pair with. These source populations are not represented in this study.

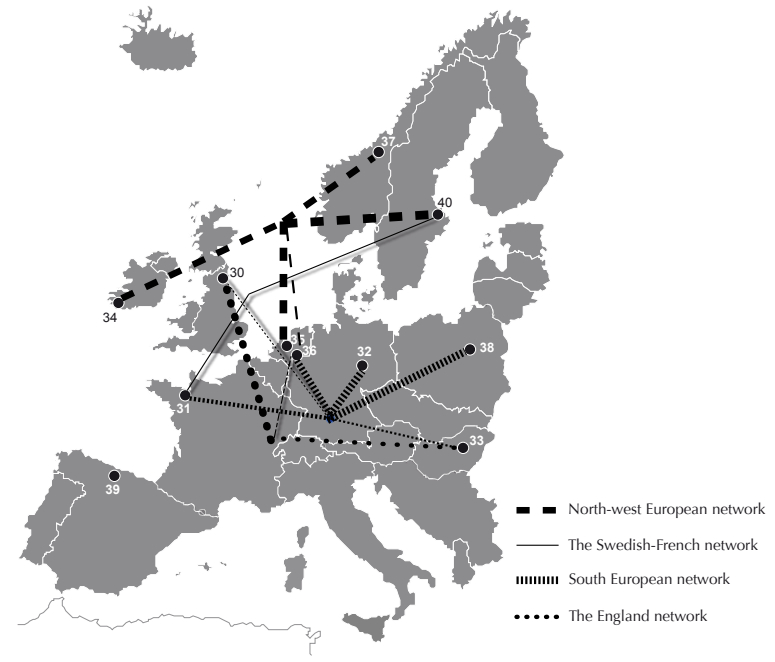

Fig. 3: Overview of four haplotype networks representing the native area in Europe. The thickness of the lines is proportional with the number of haplotypes in the network. Population number refers to Table 1.

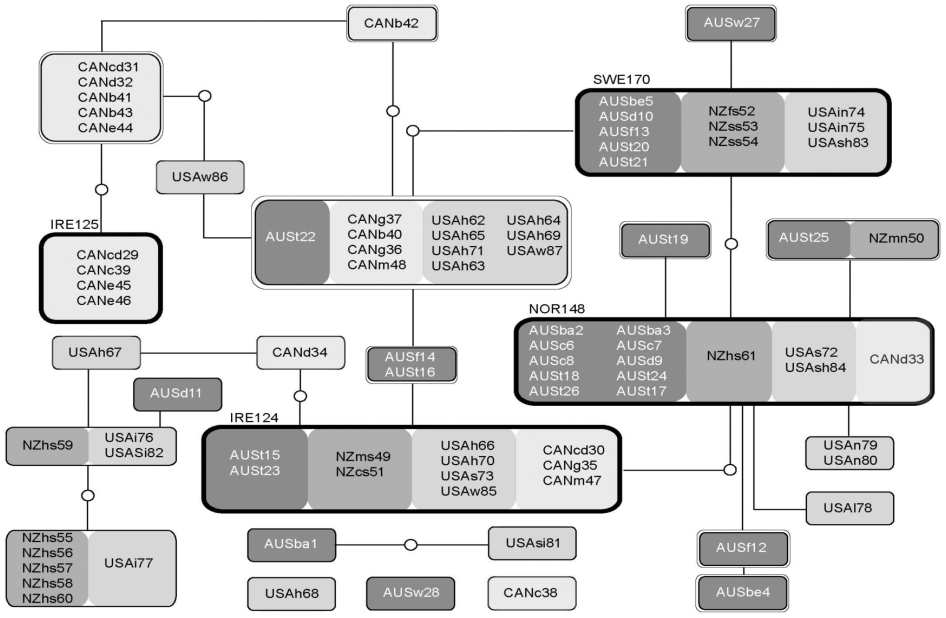

Fig. 4: Haplotype networks of *J. vulgaris* based on chloroplast microsatellites and SNPs in the invasive area.
Abbreviations are the same as in Table 1. Numbers indicate individuals. A bold line around a haplotype indicates that an identical haplotype is also present in the native network. A particular native haplotype is indicated above the box. A double line indicates a haplotype that is assigned to a native haplotype with a high likelihood score above the cut-of point (Fig. 5) Grades of shading indicate the four invasive regions from dark to light: Australia, New Zealand, North America west coast, North America east coast.

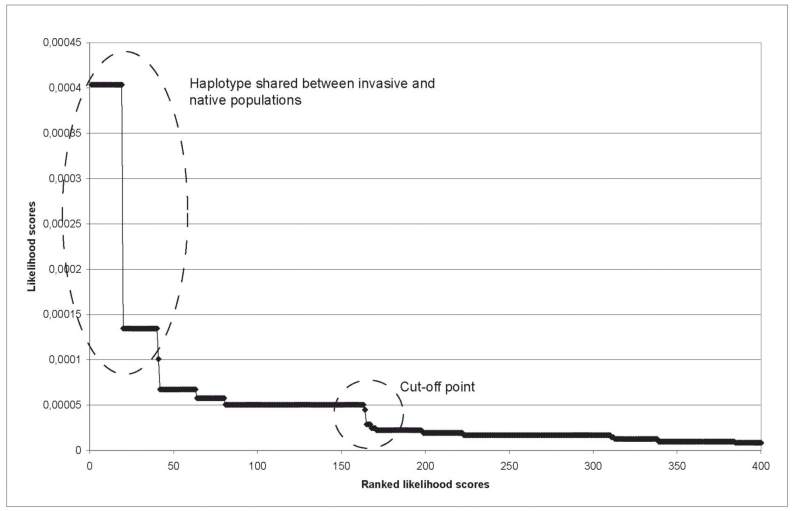

Fig. 5: The first 400 ranked likelihood scores of the assignment analysis between all possible pairs of invasive and native populations.
The likelihood scores in the circle on the left are likelihood scores for pairs with identical haplotypes in the native and invasive area. The likelihood scores for these pairs differ as the magnitude of the likelihood score depends on the allele frequencies. Individuals above the cut-off point have the highest chance of being the source population of (an) invasive individual(s). In total for 7470 pairs the likelihood scores were calculated, in the graph only the 400 highest likelihood scores are depicted.

Discussion

In this study we came a long way in establishing the source populations of invasive *J. vulgaris*. Our data strongly suggest that at least four but probably more introductions took place of *J. vulgaris*. The four native individuals from Ireland, Norway and Sweden sharing the same haplotype of invasive individuals are likely to be among the source population of the invasive individuals. All these populations belong to North-Western haplotype network. The assignment analysis showed that all native individuals with "near identical" haplotypes to invasive individuals were also only found in the North_Western European haplotype network. Collectively these results suggest that individuals from multiple source populations from North-Western Europe invaded Australia, New Zealand and North America. In the AFLP study of Doorduin et al 2010, populations from North-West Europe were the most likely source populations too. (LJ Doorduin ,K van den Hof ,K Vrieling *et al.*, 2010). A population from Ireland was assigned in both studies, Norway and Sweden were only assigned in this study and England only in LJ Doorduin ,K van den Hof ,K Vrieling *et al*, (2010). An explanation for this apparent discrepancy is that these samples, although originating from the same country, were sampled on different locations. This holds also true for Sweden, with high likelihood scores and an identical haplotype with invasive individuals in this study opposite to low assignment scores in the study of Doorduin et al (2010). In the invasive area nineteen private alleles were detected. Furthermore nine haplotypes were detected in the invasive area that were separated 2 or more mutations from the closest native individual. This indicates that we did not sample all native sources populations of the invasive individuals from this study. The latter shows that more source population are involved that have not yet been detected in the native area.

We found strong evidence that Irish populations were involved in the introduction of *J. vulgaris* to invasive areas. The Canadian individuals were sampled on the east coast of Canada. The introduction of *J. vulgaris* on the East coast of Canada was first recorded in 1850. Between 1845-1850 the potato famine took place in Ireland and as a response millions of Irishmen fled to Canada and also to North America (HPH Nusteling, 2009). This movement may have been responsible for the introduction of *J. vulgaris* from Ireland into the east coast of Canada and North America.

Subsequent introductions to one invasive region followed by admixture and spread to the other regions or independent introductions from the same source areas to all four regions, or both are likely scenarios because several haplotypes are shared between the invasive regions.

Although genetic diversity is lower in the invasive area, there are still many different haplotypes present and both the AFLP and this study indicate that populations and even different regions in the invasive area share their genetic diversity more than populations in the native area. In the common garden study of Joshi and Vrieling (2005) (J Joshi ,K Vrieling, 2005) individuals from the different invasive regions all showed the same changes in life history traits like growth, defence and reproduction. Because it is unlikely that all the introduced populations contained the same pre-adaptive traits we suggest that changes in life-history traits, herbivory and chemical defence of invasive individuals are caused by evolution rather than preadaptation.

We did not find a significant correlation between geographical and genetic distance suggesting that also native populations recently might have been admixed. Ragwort is a species from disturbed areas and often has ephemeral populations that exist in a metapopulation structure (E van der Meijden ,RE

van der Waals-Kooi, 1979). Within the native area most variation was detected in populations from Hungary, France, Sweden and The Netherlands-Wageningen. The significantly larger within population genetic distances in these four populations compared to the other populations suggests that these have been founded, or received immigrants, from different populations (Fig. 1). Although we did not find a correlation between genetic and geographical distance for all native populations, the two biggest independent networks showed, despite a small overlap, a significant distinction between populations from North- West Europe and South- East Europe (Fig 3).

Despite the fact that in the invasive area a similar number of individuals was analysed in more populations compared to the native area, more independent networks were found in the native area. This shows once more the larger genetic diversity of native populations compared to invasive populations.

The genetic variance among populations in the native area was 50% compared to 14% in the invasive area (Table 4). Genetic variance between populations was much higher in this study compared to the study of Doorduin et al 2010 using nuclear markers. This can be explained by the continuous recombination of nuclear markers that allow all possible combinations and therefore lead to a reduced differentiation between populations. These recombinations do not occur in chloroplast markers. The reduced genetic variation found between the invasive populations and even between different regions can be explained by admixture upon arrival in the invasive area. Both the AFLP data and the current data on microsatellite markers are in line with the admixture hypothesis in the invasive area of Verhoeven et al. (2010).

Table 4: Analysis of Molecular Variances (AMOVA's) for native and invasive populations combined, native population and invasive population. In the "invasive population only" analysis, the invasive area is split up in the four regions: Australia, New Zealand, west coast and east coast of America. n= number of populations ** $p < 0.01$ * $p < 0.05$.

Source of Variation	d.f.	Sum of Squares	Percentage of Variance
All populations combined (n=40)			
Native-vs.-invasive	**1**	**93.65**	**23.18****
Among populations within native/invasive areas	**38**	**280.83**	**31.91****
Within populations	**137**	**249.50**	**44.91****
Total	**176**	**623.98**	
Native populations only (n=11)			
Among populations	**10**	**185.18**	**49.54****
Within populations	**79**	**162.35**	**50.46****
Total	**89**	**347.52**	
Invasive populations only (n=29)			
Among regions	**3**	**30.00**	**13.84****
Among populations within regions	**25**	**65.65**	**18.63****
Within populations	**58**	**81.11**	**67.53****
Total	**86**	**168.93**	

In conclusion these results show that several populations from North-West Europe are the most likely source populations. The presence of alleles in the invasive area that were not found in the native area suggests that some source populations went undetected. Collectively the data suggest that multiple introductions have occurred. Furthermore the data show a reduced genetic variation in the invasive area. At last results show that upon arrival there has been a strong admixture before the invasive population spread over the different regions.

Acknowledgements

We are thankful to B. Stolk and N. Schidlo for helping with the SNP and microsatellite analyses and U. Schaffner, K. Wolff, J. van Alphen, H. Auge, A. Balogh, M. Bartelheimer, L. Joosten, N. Sletvold-Hommelvik and P. Olejnicrak for collecting seeds.

References

Afzal-Rafii Z, Dodd RS 2007 Chloroplast DNA supports a hypothesis of glacial refugia over postglacial recolonization in disjunct populations of black pine (Pinus nigra) in western Europe. Molecular Ecology 16 723-736.

Amsellem L, Noyer JL, Le Bourgeois T, Hossaert-McKey M 2000 Comparison of genetic diversity of the invasive weed Rubus alceifolius Poir. (Rosaceae) in its native range and in areas of introduction, using amplified fragment length polymorphism (AFLP) markers. Molecular Ecology 9 443-455.

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

Boutin-Ganache I, Raposo M, Raymond M, Deschepper CF 2001 M13-tailed primers improve the readability and usability of microsatellite analyses performed with two different allele-sizing methods. Biotechniques 31 24-+.

Carruthers RI 2003 Invasive species research in the United States Department of Agriculture - Agricultural Research Service. Pest Manag. Sci. 59 827-834.

Clement M, Posada D, Crandall KA 2000 TCS: a computer program to estimate gene genealogies. Molecular Ecology 9 1657-1659.

Dlugosch KM, Parker IM 2007 Molecular and quantitative trait variation across the native range of the invasive species Hypericum canariense: evidence for ancient patterns of colonization via preadaptation? Molecular Ecology 16 4269-4283.

Doorduin LJ, Gravendeel B, Lammers Y, Ariyurek Y, Chin-A-Woeng T 2011 The complete chloroplast genome of 17 individuals of pest species Jacobaea vulgaris: SNPS, microsatellites and barcoding markers for population and phylogenetic studies. DNA research 18 93-105.

Doorduin LJ, van den Hof K, Vrieling K, Joshi J 2010 The lack of genetic bottleneck in invasive Tansy ragwort populations suggests multiple source populations. Basic and Applied Ecology 11 244-250.

Doorduin LJ, Vrieling K 2011 A review of the phytochemical support for the shifting defence hypothesis. Phytochem Rev 10 99-106.

Doyle JJ, Doyle, J.L. 1987 A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochemistry Bulletin 19 11-15.

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

Elton CS 1958 *The Ecology of Invasions by Animals and Plants* Chicago: University of Chicago press.

Excoffier L, Laval G, Schneider S 2005 Arlequin (version 3.0): An integrated software package for population genetics data analysis. Evolutionary Bioinformatics 1 47-50.

Frick KE 1972 Third List of Insects That Feed Upon Tansy Ragwort, Senecio-Jacobaea, in Western United-States. Annals of the Entomological Society of America 65 629-&.

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. Molecular Ecology 14 4275-4285.

Gilkey HM 1957 Weeds of the Pacific Northwest. Oregon State College, Corvallis 382-383.

Goudet J 1995 FSTAT (Version 1.2): A computer program to calculate F-statistics. Journal of Heredity 86 485-486.

Grapputo A, Boman S, Lindstrom L, Lyytinen A, Mappes J 2005 The voyage of an invasive species across continents: genetic diversity of North American and European Colorado potato beetle populations. Molecular Ecology 14 4207-4219.

Harper JL, Wood WA 1957 Senecio-Jacobaea L. J. Ecol. 45 617-637.

Harris P, Wilkinson ATS, Neary ME, Thompson LS 1971 Senecio jacobara L. Tansy ragwort (Compositae) in: Biological control programmes against insects and weeds in Canada 1959-1968. 97-104.

Hufbauer RA, Sforza R 2008 Multiple introductions of two invasive Centaurea taxa inferred from cpDNA haplotypes. Diversity and Distributions 14 252-261.

Husband BC, Barrett SCH 1991 Colonization History and Population Genetic-Structure of Eichhornia-Paniculata in Jamaica. Heredity 66 287-296.

Jakobsson M, Sall T, Lind-Hallden C, Hallden C 2007 Evolution of chloroplast mononucleotide microsatellites in Arabidopsis thaliana. Theoretical and Applied Genetics 114 223-235.

Joshi J, Vrieling K 2005 The enemy release and EICA hypothesis revisited: incorporating the fundamental difference between specialist and generalist herbivores. Ecology Letters 8 704-714.

Julien M (1987) Biological control of weeds: a world catalogue of agents and their target weeds, Brisbane.

Kanarek AR, Webb CT 2010 Allee effects, adaptive evolution, and invasion success. Evol. Appl. 3 122-135.

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. Molecular Ecology 19 3952-3967.

Leger EA, Rice KJ 2003 Invasive California poppies (Eschscholzia californica Cham.) grow larger than native individuals under reduced competition. Ecology Letters 6 257-264.

Mantel N 1967 Detection of Disease Clustering and a Generalized Regression Approach. Cancer Research 27 209-&.

Marrs RA, Sforza R, Hufbauer RA 2008 Evidence for multiple introductions of Centaurea stoebe micranthos (spotted knapweed, Asteraceae) to North America. Molecular Ecology 17 4197-4208.

McCauley DE, Stevens JE, Peroni PA, Raveill JA 1996 The spatial distribution of chloroplast DNA and allozyme polymorphisms within a population of Silene alba (Caryophyllaceae). American Journal of Botany 83 727-731.

Meimberg H, Hammond JI, Jorgensen CM, Park TW, Gerlach JD, Rice KJ, McKay JK 2006 Molecular evidence for an extreme genetic bottleneck during introduction of an invading grass to California. Biol. Invasions 8 1355-1366.

Nusteling HPH 2009 How Many Irish Potato Famine Deaths? Toward Coherence of the Evidence. Hist. Methods 42 57-80.

Pimentel D, Zuniga R, Morrison D 2005 Update on the environmental and economic costs associated with alien-invasive species in the United States. Ecological Economics 52 273-288.

Piry S, Alapetite A, Cornuet JM, Paetkau D, Baudouin L, Estoup A 2004 GENECLASS2: A software for genetic assignment and first-generation migrant detection. Journal of Heredity 95 536-539.

Prentis PJ, Wilson JRU, Dormontt EE, Richardson DM, Lowe AJ 2008 Adaptive evolution in invasive species. Trends Plant Sci. 13 288-294.

Provan J, Powell W, Hollingsworth PM 2001 Chloroplast microsatellites: new tools for studies in plant ecology and evolution. Trends in Ecology & Evolution 16 142-147.

Provan J, Soranzo N, Wilson NJ, Goldstein DB, Powell W 1999 A low mutation rate for chloroplast microsatellites. Genetics 153 943-947.

Rannala B, Mountain JL 1997 Detecting immigration by using multilocus genotypes. Proceedings of the National Academy of Sciences of the United States of America 94 9197-9201.

Reed GH, Kent JO, Wittwer CT 2007 High-resolution DNA melting analysis for simple and efficient molecular diagnostics. Pharmacogenomics 8 597-608.

Richardson BA, Brunsfeld J, Klopfenstein NB 2002 DNA from bird-dispersed seed and wind-disseminated pollen provides insights into postglacial colonization and population genetic structure of whitebark pine (Pinus albicaulis). Molecular Ecology 11 215-227.

Roberts PD, Pullin AS 2007 The effectiveness of management interventions used to control Ragwort species. Environmental Management 39 691-706.

Schmidl L 1972 Biology and control of ragwort, senecio- jacobaea L, in Victoria, Australia. Weed research 12 37-&.

Siemann E, Rogers WE 2001 Genetic differences in growth of an invasive tree species. Ecology Letters 4 514-518.

Small RL, Cronn RC, Wendel JF 2004 Use of nuclear genes for phylogeny reconstruction in plants. Australian Systematic Botany 17 145-170.

Stegelmeier BL, Edgar JA, Colegate SM, Gardner DR, Schoch TK, Coulombe RA, Molyneux RJ 1999 Pyrrolizidine alkaloid plants, metabolism and toxicity. Journal of Natural Toxins 8 95-116.

Suarez AV, Tsutsui ND 2008 The evolutionary consequences of biological invasions. Molecular Ecology 17 351-360.

Thomson GM 1922 The naturalisation of animals and plants in New Zealand. Cambridge University Press, London.

Vandermeijden E, Vanderwaalskooi RE 1979 Population Ecology of Senecio-Jacobaea in a Sand Dune System .1. Reproductive Strategy and the Biennial Habit. J. Ecol. 67 131-153.

Verhoeven KJF, Macel M, Wolfe LM, Biere A 2010 Population admixture, biological invasions and the balance between local adaptation and inbreeding depression. Proceedings of the Royal Society B-Biological Sciences 278 2-8.

Wilcove DS, Rothstein D, Dubow J, Phillips A, Losos E 1998 Quantifying threats to imperiled species in the United States. Bioscience 48 607-615.

Witte L, Ernst L, Adam H, Hartmann T 1992 Chemotypes of 2 Pyrrolizidine Alkaloid-Containing Senecio Species. Phytochemistry 31 559-565.

Wolfe KH, Li WH, Sharp PM 1987 Rates of Nucleotide Substitution Vary Greatly among Plant Mitochondrial, Chloroplast, and Nuclear Dnas. Proceedings of the National Academy of Sciences of the United States of America 84 9054-9058.

Zhou LM, Myers AN, Vandersteen JG, Wang L, Wittwer CT 2004 Closed-tube genotyping with unlabeled oligonucleotide probes and a saturating DNA dye. Clinical Chemistry 50 1328-1335.