The lack of genetic bottleneck in invasive Tansy ragwort populations suggests multiple source populations

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The lack of genetic bottleneck in invasive *Tansy ragwort* populations suggests multiple source populations.

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Abstract

Jacobaea vulgaris (Asteraceae) is a species of Eurasian origin that has become a serious non-indigenous weed in Australia, New Zealand, and North America. We used neutral molecular markers to (1) test for genetic bottlenecks in invasive populations and (2) to investigate the invasion pathways. It is for the first time that molecular markers were used to unravel the process of introduction in this species.

The genetic variation of 15 native populations from Europe and 16 invasive populations from Australia, New Zealand and North America were compared using the amplified fragment length polymorphisms (AFLP's). An analysis of molecular variance showed that a significant part (10%) of the total genetic variations between all individuals could be explained by native or invasive origin.

Significant among-population differentiation was detected only in the native range, whereas populations from the invasive areas did not significantly differ from each other; nor did the Australian, New Zealand and North American regions differ within the invasive range. The result that native populations differed significantly from each other and that the amount of genetic variation, measured as the number of polymorphic bands, did not differ between the native and invasive area, strongly suggests that introductions from multiple source populations have occurred. The lack of differentiation between invasive regions suggests that either introductions may have occurred from the same native sources in all invasive regions or subsequent introductions took place from one into another invasive region and the same mix of genotypes was subsequently introduced into all invasive regions.

An assignment test showed that European populations from Ireland, the Netherlands and the United Kingdom most resembled the invasive populations.

Zusammenfassung

J. vulgaris, das Jakobskreuzkraut, ist eine eurasiatische Pflanzenart, die nach Nordamerika, Australien und Neuseeland eingeschleppt wurde und sich seither in diesen Gebieten invasiv ausgebreitet hat. Um 1) das Ausmass genetischer Flaschenhälse invasiver Populationen und 2) die Einführungsgeschichte dieser Art zu untersuchen, wurde die genetische Variation 15 einheimischer Populationen aus Europa und 16 invasiver Populationen aus Australien,

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Neuseeland und Nordamerika mittels neutraler molekularer Marker (AFLP's), die noch nie für die Untersuchung der Einführungsgeschichte dieser Art verwendet wurden, analysiert.

Eine Analyse der molekularen Varianz zeigte, dass ein signifikanter Anteil (10%) der gesamten genetischen Variation zwischen allen Individuen auf Unterschiede zwischen Ursprungs- und Invasionsgebiet zurückzuführen ist. Signifikante genetische Differenzierung zwischen Populationen wurde nur im europäischen Ursprungsgebiet gefunden, während sich die Populationen im Invasionsgebiet und auch die Regionen innerhalb des Invasionsgebiets (Australien, Neuseeland und Nordamerika) genetisch nicht signifikant voneinander unterschieden. Die signifikante genetische Differenzierung zwischen einheimischen, europäischen Populationen und das Fehlen von Unterschieden in genetischer Variation, gemessen als Anzahl polymorpher Banden, zwischen Ursprungs- und Invasionsgebiet weist darauf hin, dass *J. vulgaris* aus verschiedenen Ursprungspopulationen eingeschleppt wurde. Die fehlende Differenzierung zwischen Regionen innerhalb des Invasionsgebiets könnte darauf zurückzuführen sein, dass entweder Populationen aus denselben Ursprungsgebieten in das ganze Invasionsgebiet eingeschleppt wurden oder, dass Einschleppungen von einem Invasionsgebiet ins andere stattfanden. Ein Assignment-test weist auf europäische Populationen aus Irland, Großbritannien und den Niederlanden als wahrscheinlichste Ursprungsregionen hin.

Keywords: AFLP; Assignment test; Biological invasion; Multiple introductions; Pyrrolizidine alkaloids; Senecio jacobaea; Source populations

Introduction

The spread of introduced species in new environments offers the unique opportunity to study the evolution and adaptation of organisms to a changing environment, which is a key issue in biology (Sakai et al. 2001). A number of non-indigenous species become serious pests in the new environment (Mack et al. 2000) whereas they are not dominant in their native range. The reason why these species only become a pest in the introduced area remains intensively debated (e.g. Elton 1958; Callaway & Maron, 2006; Mortenson & Mack 2006).

The introduction of a species into a new environment can have different outcomes related to genetic variation in the native and invasive areas. Genetic variation can decrease by founder effects and genetic bottlenecks (Dlugosch & Parker 2008). However, multiple introductions, hybridisation (Ellstrand & Schierenbeck 2000) and the release of epistatic genetic variation (Dlugosch & Parker 2008) can lead to an increase in genetic variation in the new area compared to the native area.

A number of studies show that if introductions occur independently of each other and do not stem from the same source population, large differences in genetic variation among regions in the invasive range can be expected (Ellstrand & Schierenbeck 2000; Lavergne & Molofsky 2007).

To study whether life-history and other traits did change upon becoming a pest in the invaded areas, it is necessary to compare the traits of the invasive populations with those of the source populations in the native area (Hierro, Maron, & Callaway 2005). This, however, requires detailed information on the origin of the invasive populations.

In this study, we compared genetic variation, detected by neutral molecular markers (AFLPs), between and within native and invasive areas of *Jacobaea vulgaris,* (Tansy or Common Ragwort) *Asteraceae* (Pelser, Veldkamp, & van der Meijden 2006) (syn. *Senecio jacobaea). J.vulgaris* is a pest species in the invasive areas that is toxic to lifestock and humans caused by its pyrrolizidine alkaloids content (Witte, Ernst, Adam, & Hartmann 1992). This monocarpic perennial has been introduced into New Zealand, Australia and North America. In those days, there was a merchandising route between the three invasive regions (Morison 1912) and introductions, therefore, could also have occurred from one invasive region to the other.

In a previous study on the invasiveness of *J. vulgaris*, Joshi & Vrieling (2005) examined life-history traits, herbivory and chemical defence using common garden experiments. These experiments revealed that plants from invasive areas had a more vigorous growth and reproduction, were better protected against generalist herbivores, but less well defended against native specialist herbivores adapted to their main defence chemicals (Joshi & Vrieling 2005). Pyrrolizidine alkaloid (PA) concentration and composition varied considerably between populations from the native, but not from the invasive area.

In this study, we addressed the following questions: (1) Does the absolute amount of genetic variation differ between the native and invasive areas? (2) Is there genetic differentiation between (a) the native and invasive areas? (b) populations within the native and invasive areas, (c) the regions within the invasive area? (3) Can we identify the region in the native area, which most likely represents the potential source population(s)? (4) Were multiple source populations introduced?

Methods

Study species

J. vulgaris is a self-incompatible, allo-tetraploid, monocarpic perennial plant species (Harper & Wood 1957) that has become a serious pest in Australia, New Zealand, the United States and Canada. *J. vulgaris* was first recorded outside its native distribution area in the 1850s in Canada (Bain 1991), around 1874 in New Zealand (Poole & Cairns 1940) and Australia (McLaren, Ireson, & Kwong 2000) and in 1901 on the west coast of the USA (Rice 2003).

We used the same set of *J. vulgaris* populations as studied by Joshi and Vrieling (2005) (Appendix A): 15 native populations (Europe) and 16 invasive populations (Australia, New Zealand and North America). From each population, seeds of 5–20 individuals (growing at least 2 m apart from each other) were collected. Seeds were germinated and grown in a climate-room at the Leiden University and leaf samples were taken from these plants.

AFLP analysis

DNA was extracted from 38 native and 44 invasive individuals. Since we were primarily interested in interpopulation differentiation across the native and invasive range, we chose to sample as many populations as possible at the expense of less individuals per population. In this way most of the genetic variation in the area is estimated (Barbosa et al. 2003). Finally, we ended up with DNA from 1 to 4 offspring of different maternal genotypes per population. In the case of bulk samples, seeds were chosen at random from the sample (Appendix A). A fresh leaf was collected from each individual and stored at −80 °C until DNA isolation with the Qiagen DNeasy plant extraction kit.

AFLP fingerprints (Vos et al. 1995) were generated following the protocol from Kirk, Macel, Klinkhamer, and Vrieling (2004) using the AFLP core mix (Applied Biosytems) for PCR. A pre-selective PCR with one selective base pair (*Eco*RI+A and M*se*I+C) was carried out followed by selective amplification using six primer combinations on the *Mse*I side: CAA, CAG, CCG, CGT, CTG and CTT. The *Eco*RI primer (*Eco*RI-ACA) was labelled with the fluorescent dye 5-FAM. PCR products were separated with an ABI Prism^{TM} 310 capillary sequencer (Applied Biosystems, Rotkreuz, Switzerland) using Genescan ROX 500 as an internal standard. Electropherograms were scored using the Genographer 1.6.0 (Benham, Jeung, Jasieniuk, Kanazin, & Blake 1999). Fragments in the range of 100–500 base pairs were scored by two different people to test for repeatability. Fragments were only used for further analyses if the scoring differences were less than 5%. Repetition tests showed that the primers produced highly reproducible AFLP patterns.

Statistical analyses

Two populations of the native area were not used in the analyses because of only one individual (Rothenthurm) and because of missing values (Buggingen). So, all analyses were done on 34 native and 44 invasive individuals.

To test if fixation in the invasive area did occur, the percentage of polymorphic loci present in each population was calculated and analyzed with an analysis of variance testing differences among populations in native and invasive areas.

To estimate the genetic differentiation between invasive and native areas and between populations within the native and invasive area, an analysis of molecular variance (AMOVA) was carried out using Arlequin (Version 2.0; Schneider, Roessli, & Excoffier 2000). Analogous to an analysis of variance, an AMOVA partitions the total genetic variance into a part that can be attributed to differences between population and differences within populations. The software package Geneclass 2 (Piry et al. 2004) was used for an assignment test (Waser & Strobeck 1998), determining the most likely source population among the native populations sampled. Missing values seriously influenced the results of the assignment analysis. To eliminate this effect the dataset was pruned by omitting two primer combinations (EcoRI+ACA – MseI+CTT; EcoRI+ACA – MseI+CGT) so that no missing values were present in the native populations. As a result, 23 loci remained in the dataset. Since AFLP is a dominant marker, the second allele of the phenotype "band present" was scored as missing in the input files. Geneclass calculated for each invasive individual the likelihood that it is related to each native population using the Bayesian method of Rannala and Mountain (1997). Subsequently for each invasive individual the likelihood mass was calculated as: likelihood of each invasive individual related to a particular native population/sum of likelihoods for that invasive individual for all native populations. To see how each native population contributed to the likelihood mass of individuals of the invasive area, for each native population the likelihood masses were summed over all invasive individuals. This yielded for each native population a sum of likelihood masses. To obtain a relative likelihood mass for each native population, the sum of likelihood masses per native population was divided by the sum of the sum of likelihood masses for all native populations. The same procedure was carried out separately for the three regions within the invasive range (New Zealand, Australia and North America). The percentage likelihood mass obtained gives a relative ranking among the native populations how well they fit to the invasive area or region.

Finally, the percentage of shared bands was calculated for every native population to each invasive region (Appendix B).

Results

AFLP analysis

In the range of 100–500 base pairs for six primer combinations, 141 out of 197 bands (71.6%) were polymorphic. Of these bands, 39 were used for analysis because of their repeatability.

Amount of genetic variation

Polymorphic bands

All polymorphic bands found in the native area were also polymorphic within the invasive area indicating that the amount of neutral genetic variation did not differ between these areas. This suggests that the total amount of genetic variation among invasive populations was not reduced by severe bottlenecks and/or single introductions. Moreover, there was a significant correlation between both areas in the frequency of bands present at each locus ($r = 0.643$, $n = 39$, $P < 0.01$).

Invasive areas did not differ from native areas in the percentage of polymorphic loci per population (39.61 ± 5.51 vs. 36.79 ± 4.15; $F_{1,\,27} = 0.15$ $P > 0.7$). Some polymorphic bands were absent in some regions (2 in North America, 1 in New Zealand and 5 in Australia). One polymorphic band (EcoRI+ACA – MseI+CTG, 232bp) present in 83% of the plants from the British Isles was present in 89% of all invasive samples, while it was absent in all other European populations. All chosen loci were polymorphic at the level of the area for both the native and the invasive areas. None of the native populations contained all bands present in an invasive region (Appendix B). Baldoyle (Ireland) showed the highest percentage of shared bands with the invasive regions (average of 67%). This indicates that 33% of the bands still originated from (an)other native population(s).

AMOVA analysis

Significant genetic differentiation between the native (European) and invasive populations was detected by an AMOVA analysis (Table 1). Ten percent of all genetic variation was among the invasive and native areas and 5% of the total genetic variation was among populations within an area (Table 1). So, 10% percent of the allelic variation between individuals could be explained by native or invasive origin. Variation among populations within an area was only 5%. The remaining variation could be ascribed to allelic variation within

Table 1. Analysis of molecular variances (AMOVA's) for native populations (Baldoyle, Leiden, Wales, Chereng, l'Himelette, Plombieres, Meijendel, Westervoort, Zlin, Warsaw, Darmstadt, Gotland and Brocherbeck) and all invasive populations of *Jacobaea vulgaris*.

Source of variation	d.f.	Sum of squares	Percentage of variance explained
All populations combined ($n = 29$)			
Native vs. invasive	1	41.14	10.55**
Among populations within native/invasive areas	27	216.66	5.21*
Within populations	49	337.25	84.24**
Total	77	595.05	
Native populations only ($n = 13$)			
Among populations	12	105.63	13.26**
Within populations	21	132.17	86.74**
Total	33	237.79	
Invasive populations only ($n = 16$)			
Among regions	2	17.34	1.45
Among populations Within regions	13	93.69	−0.58
Within populations	28	205.08	99.14
Total	43	316.11	

The "all populations combined" AMOVA attributes the total genetic variance to the difference between native and invasive populations, differences among populations and variation within populations. In the "invasive population only" analysis, the invasive area is split up into three regions respectively Australia, New Zealand and North America.
(n = number of populations $^{**}p < 0.01$ $^{**}p < 0.05$).

Table 2. Percentage relative likelihood masses derived from the assignment test for invasive *Jacobaea vulgaris* individuals (see methods) for each invasive region. The percentages indicate how likely a native population is a source population relative to other native populations for a particular region. For the detailed calculation, see text.

Native population	Perecentage relative likelihood mass			
	North America	Australia	New Zealand	Invasive area
Ireland (Baldoyle)	28.88	24.21	21.73	24.94
Netherlands (Leiden)	28.29	18.69	14.52	20.50
United Kingdom (Wales)	6.30	16.17	15.20	12.56
France (Chéreng)	10.80	14.02	2.62	9.15
Switzerland (l'Himelette)	8.32	9.46	7.86	8.55
France (Plombieres)	6.68	5.35	7.17	6.40
Netherlands (Meijendel)	5.15	3.23	6.34	4.91
Netherlands (Westervoort)	0.69	4.80	8.72	4.74
Czech Republic (Zlin)	1.74	0.79	9.96	4.16
Poland (Warsaw)	0.85	0.53	5.50	2.29
Germany (Darmstadt)	1.39	0.38	0.03	0.60
Sweden (Gotland)	0.69	1.99	0.02	0.90
Germany (Brochterbeck)	0.23	0.37	0.32	0.31

populations of the native and invasive areas. When native and invasive populations and regions were analyzed separately, only native populations were significantly different from each other (Table 1). In contrast, no significant genetic differentiation between AFLP haplotypes was detected among different regions within the invasive range and populations within these regions (Table 1).

Assignment analysis

The percentage relative likelihood masses (Table 2) indicated that the populations from the Irish, UK and Dutch coast (Leiden) are the most likely source populations out of the 13 native populations used in this study. Interestingly, Baldoyle (Ireland) was the only native population with jacobine-type plants only, just as the invasive populations (see Appendix A), and had the highest likelihood mass (Table 2).

The pattern of the distribution of likelihood masses is largely congruent for three invasive regions. The UK population shows a high likelihood mass for Australia and New Zealand.

Discussion

Although it is generally assumed that genetic variation across introduced populations will increase/decrease compared to populations in native areas (e.g. Nei, Maruyama, & Chakraborty 1975, Novak & Mack 1993), there is no indication of such a pattern in our study. All polymorphic bands present in the native populations were also present in the invasive area. So the amount of neutral genetic variation of individuals from the native area was similar to individuals of the invasive area. Among populations, differentiation was detected only in the native range, whereas no significant genetic differentiation between AFLP haplotypes was detected among invasive populations within regions and not even among the different regions. The absence of genetic differentiation between regions is surprising considering the large geographical distance. Because of the small sample sizes it is possible that differences between populations in the invasive range were not detected.

Different scenarios of the route of introduction can explain these findings: (1) a single introduction from one population in Europe representing all genetic variation of native populations used in this study into different regions in the invasive area or into one invasive region followed by subsequent introductions to the other regions (Fig. 1A) However, the existence of one European population representing all genetic variation of all European populations is very unlikely; (2) introductions from different native populations, together representing all the genetic variations of native populations used in this study into all different regions in the invasive area or into one invasive region followed by subsequent introductions to the other regions (Fig. 1B). We consider the second scenario more likely because there is a very little chance that the same native populations were introduced independently to all three invasive regions.

Joshi and Vrieling (2005) analyzed pyrrolizidine alkaloid (PA) patterns in native and invasive populations and only found populations of the

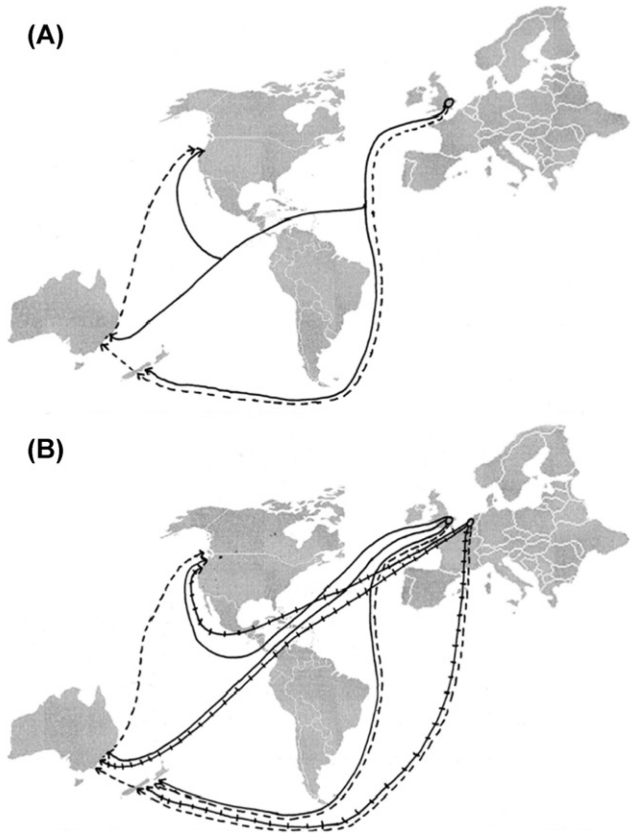

Fig. 1. Different scenarios of the route of introduction from native European *Jacobaea vulgaris* individuals to invasive regions Australia, New Zealand and North America. (A) A single introduction from one population in Europe into all different regions in the invasive area (solid lines) or into one invasive region followed by subsequent introductions to the other regions (dashed lines); and (B) introductions from different populations in Europe into all different regions in the invasive area. One introduction from a European population is indicated with solid lines; the introduction from another European population is indicated with solid lines with strokes. Because of clarity, the example is given for only two European introductions. Dashed lines indicate the invasion of different European populations into one invasive region followed by subsequent introductions to the other regions.

jacobine-chemotype in the invasive range. The bouquet of PA's from *J. vulgaris* plants from Baldoyle (Ireland) was most similar to the PA composition pattern found in the invasive range. In our study, the assignment test indicated that out of the 13 populations used for this study, Baldoyle (Ireland), Wales (U.K) or Leiden (The Netherlands) were the populations with the highest genetic similarity to *J. vulgaris* populations. It should be kept in mind that the exact source population(s) cannot be pinpointed due to the limited sample size in the analysis. However it suggests that if multiple source populations were introduced, populations from Ireland, the UK and the Netherlands are the most likely source population(s) out of the European populations analyzed.

In conclusion, the present study shows that the invasion of Australia, New Zealand, and North America by *J. vulgaris* did not involve strong bottleneck events. AFLPs identify populations from the United Kingdom, Ireland and The Netherlands as putative source populations. The homogeneity of the genetic variation between populations in the invasive area suggests a common origin.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.baae.2010.01.001.

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