

High genetic diversity in French invasive populations of common ragweed, *Ambrosia artemisiifolia*, as a result of multiple sources of introduction

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Abstract

Ambrosia artemisiifolia is an aggressive North American annual weed, found particularly in sunflower and corn fields. Besides its economic impact on crop yield, it represents a major health problem because of its strongly allergenic pollen. Ragweed was imported inadvertently to Europe in the 18th century and has become invasive in several countries, notably in the Rhône Valley of France. It has recently expanded in both the Provence-Alpes-Côte-d'Azur and Bourgogne regions. As first steps towards understanding the causes and mechanisms of ragweed invasion, genetic variability of French and North American populations was analysed using microsatellites. Overall genetic variability was similar in North America and in the Rhône-Alpes region, but within-population levels of genetic variability were surprisingly lower in native than in invasive French populations. French populations also exhibited lower among-population differentiation. A significant pattern of isolation by distance was detected among North American populations but not among French populations. Assignment tests and distribution of rare alleles did not point to a single origin for all French populations, nor for all individuals within populations and private alleles from different North American populations were found in the same French populations. Indeed, within all French populations, individual plants were roughly equally assigned to the different North American populations. Altogether, these results suggest that the French invasive populations include plants from a mixture of sources. Reduced diversity in populations distant from the original area of introduction indicated that ragweed range expansion probably occurred through sequential bottlenecks from the original populations, and not from subsequent new introductions.

Keywords: bio-invasion, microsatellite, plant, population structure

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Introduction

Biological invasions are now recognized as part of human-induced global change. They pose major threats to global and local biodiversity, ecosystem functioning, agriculture, fisheries and public health (Vitousek *et al.* 1997). Human activities promote bio-invasions by being worldwide efficient dispersal agents, and they may also facilitate establishment of introduced species by altering native habitats, making them more prone to invasions

(Williamson 1996). Although understanding of bio-invasions requires interdisciplinary studies, they have been studied mainly by population and community ecologists, receiving little attention from evolutionary biologists (Lee 2002). This is particularly astonishing considering that introduced species experience drastic changes in their selective regime. Even if they may occur in similar abiotic (e.g. climatic or edaphic) conditions in their native and introduced ranges, introduced species at least face new biotic environments (Williamson 1996). The success of invasive species may therefore depend on their ability to evolve in response to their new environments. For example, the Evolution of Increased Competitive

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Ability hypothesis stipulates that because introduced species escape many of their specialist enemies in moving to new areas (Keane & Crawley 2002; Maron *et al.* 2004), they can then reallocate resources saved from defence to growth or reproduction (Blossey & Nötzold 1995; but see Müller-Schärer *et al.* 2004; Genton *et al.* in press).

Genetic variability is required to permit an adaptive response of the introduced species to the new selective regime imposed by the invaded environment. However, introductions usually consist of a small number of founders, resulting in lower genetic variability in introduced populations compared to native ones (Nei *et al.* 1975). Losses of genetic diversity have indeed been detected both at selectively neutral markers (e.g. Villablanca *et al.* 1998; Amsellem *et al.* 2000; Estoup *et al.* 2001) and at loci under selective pressures (e.g. Eckert *et al.* 1996; Giraud *et al.* 2002). Colonization following introduction can involve sequential bottlenecks that would further reduce genetic diversity (Amsellem *et al.* 2000; Clegg *et al.* 2002; Estoup & Clegg 2003; Estoup *et al.* 2004). Loss of genetic diversity during introduction and subsequent range expansion should limit local adaptation possibilities in the new colonized areas (Lambrinos 2004). Nevertheless, invasive species seem to be able to rapidly adapt to the new conditions they face (reviewed in Reznick & Ghalambor 2001; Stockwell *et al.* 2003) when introduced (e.g. Lee 1999; Kinnison *et al.* 2001) as well as when expanding their range, leading to clinal and regional variation in the invaded range. For instance, plant vigour shows clinal variation with latitude in introduced *Solidago* sp. in Europe (Weber & Schmid 1998) and in introduced *Hypericum perforatum* in North America (Maron *et al.* 2004).

Several different explanations, depending on the characteristics of the introduced species, the invaded environment and the invasion history, have been invoked to resolve this apparent paradox (Frankham 2005). First, adaptation may not always be a prerequisite for successful establishment of introduced populations (e.g. *Caulerpa taxifolia* in the Mediterranean Sea, Phillips & Price 2002). Second, rapid population expansion sometimes occurs just after introduction, retaining genetic diversity (e.g. Zenger *et al.* 2003) and facilitating later adaptation to local environment. Third, polyploidization and hybridization phenomena may rapidly produce novel diversity in the introduced area (Ellstrand & Schierenbeck 2000). Finally, multiple introductions may generate high level of genetic diversity in the introduced range (Neuffer & Hurka 1999; Maron *et al.* 2004), sometimes higher than in the native range (Kolbe *et al.* 2004).

Despite their importance for investigating evolutionary dynamics of bio-invasions, few studies to date have assessed the genetic diversity and structure in introduced populations and compared them to those in their native range (but see Giraud *et al.* 2002; Kolbe *et al.* 2004; Maron *et al.* 2004). Here, we compare genetic diversity between

North American native and French introduced populations of common ragweed to elucidate whether French populations of the Rhône Valley have a single or multiple North American source(s) and where is/are the source population(s). *Ambrosia artemisiifolia* L. (Asteraceae) is an aggressive North American annual weed, found particularly in sunflower and corn fields (Bassett & Crompton 1975). Besides its economic impact on crop yield, this wind-pollinated plant represents a major health problem since its pollen is a strong allergen and one of the main causes of hay fever. This plant was imported to Europe in the 18th century, probably in ballast or in seed lots. It became invasive in the Rhône Valley (France) in the middle of the 20th century, possibly after the Second World War (Bonnot 1967). It has recently expanded into both the Provence-Alpes-Côte-d'Azur (PACA) and Bourgogne (Burgundy) regions (Carvin *et al.* 2003). This species is also an invasive weed in other European countries such as Italy, Croatia, Hungary, Ukraine and Russia, in Asia (Japan, China) and even in Australia (Bass *et al.* 2000). However, it has not yet been studied using ecological and genetic approaches.

As first steps towards understanding the causes and mechanisms of ragweed invasion, we addressed more specifically the following questions: (i) Are there differences in neutral genetic diversity between France and North America, both within and among populations? (ii) Do French populations originate from a single or multiple North American sources? (iii) Where was (were) the source population(s) in North America? (iv) Do most recently established French populations (i.e. in the Bourgogne and PACA regions) exhibit lower genetic variability than older ones (i.e. in the Rhône-Alpes region), implying sequential bottlenecks during colonization?

Materials and methods

Sampling and DNA extractions

The distributions of *Ambrosia artemisiifolia* in North America and in France are shown in Fig. 1, together with sampling sites for the present study. Leaf tissue was collected in 2002 from 30 individuals in each of 10 French populations and 12 North American populations. Population sizes normally far exceed 100 individuals; however, recently established populations can be smaller. In the few populations with fewer than 30 individuals, all the plants were sampled. Five of the 10 French populations are located in the Rhône-Alpes region (Fig. 1) where *A. artemisiifolia* has been present for more than 30 years (Bonnot 1967). The other five French populations (3 in the PACA region and 2 in Bourgogne, Fig. 1) are thought to be more recently established (Carvin *et al.* 2003). North American populations were sampled on the East Coast and in the Great Lakes region of the USA and Canada (Fig. 1). These regions have a long history of

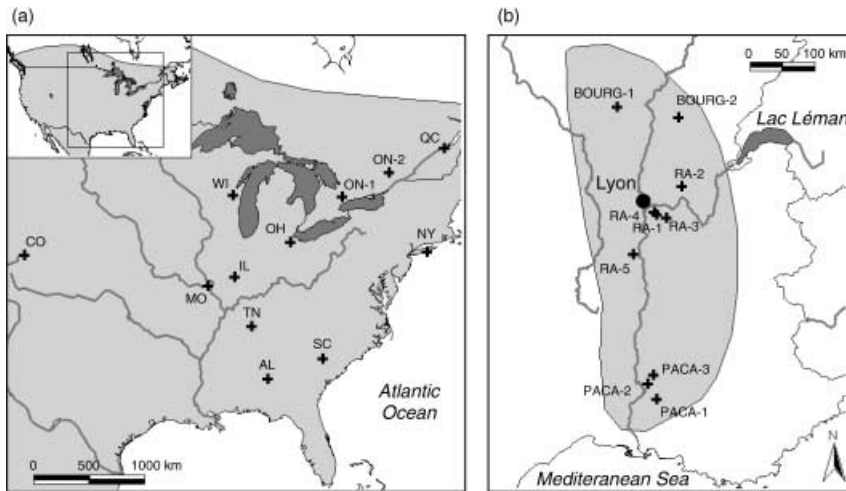


Fig. 1 Distribution of *Ambrosia artemisiifolia* in grey in North America (a) and in France (b) and sampling sites for the present study with their labels.

commercial exchanges with France. Leaf tissues were preserved in silica gel. DNA was extracted with QIAGEN plant DNA extraction kits and were then stored at -20°C .

Microsatellite analyses

We used the five nuclear microsatellite loci recently developed for *A. artemisiifolia* (Amb12, Amb15, Amb16, Amb30 and Amb82; Genton *et al.* 2005). Polymerase chain reaction (PCR) and allele resolution were carried out according to Genton *et al.* (2005). Using the FSTAT version 2.9.3.2 freeware (Goudet 1995), we estimated (i) the means $\pm$ SD over all loci of the following genetic diversity indices: allelic richness (R_s , El Mousadik & Petit 1996), observed heterozygosities and expected heterozygosities, respectively, H_o and H_e (Nei 1987), H_e being also called Nei's gene diversity, per population as well as over all populations; (ii) F -statistics (Weir & Cockerham 1984); (iii) the Goodman's estimator of R_{ST} (Goodman 1997), i.e. genetic differentiation accounting for variance in allele size and defined for genetic markers undergoing a stepwise-mutation model (Slatkin 1995). Deviations from Hardy-Weinberg proportions were assessed using FSTAT (Goudet 1995) with P values being corrected for table-wide significance levels ($\alpha = 0.05$) using the sequential Bonferroni method (Rice 1989). We also computed the means over all loci of (i) the number of alleles (A), (ii) the number of rare alleles (A_R), i.e. with allelic frequencies below 0.1, (iii) allele size variance (V), and (iv) the M ratio, i.e. the ratio of the number of alleles to the range in allele size (Garza & Williamson 2001). Allele size variance provides a valid estimate of genetic diversity if microsatellite systems conform to a stepwise-mutation model (Slatkin 1995). The M ratio is used to detect bottlenecks since it decreases after a population has undergone a size reduction (Garza & Williamson 2001). Indeed, as population size declines, number of alleles and

the range in allele size are diminished but not at equal rates for all alleles: rare alleles are most likely to be lost during episodes of genetic drift, decreasing total allele number while not necessarily decreasing the range in allele sizes. Finally, we tested for linkage disequilibria using Fisher exact tests in FSTAT (Goudet 1995) with P values being corrected for table-wide significance levels ($\alpha = 0.05$) using the sequential Bonferroni method (Rice 1989).

Comparison of genetic diversity and population differentiation between native and introduced ranges

To compare the values of within-population diversity indices (R_s , H_e , H_o , F_{IS}) and population differentiation (F_{ST}) between native and introduced ranges, we used the FSTAT freeware (Goudet 1995). For each index, FSTAT computes the average over loci and populations for North American and French samples and then the squared difference between these two averages. Significance of this difference is then assessed using a permutation test: the whole sample is allocated at random to the two groups, keeping the number of populations constant in each group. Since FSTAT did not allow comparisons of the M ratio nor of the Goodman's estimator of R_{ST} among groups of populations, we implemented similar tests in MATLAB version 4.1 (Wolfram Research, Inc.) using the J. F. Fernandez-Manjarres' files available at www.mathworks.com/matlabcentral/fileexchange. Because we wanted to get insights into introduction mechanisms, only data from French populations assumed to be near the original introduction site (i.e. of the Rhône-Alpes region) were used in these analyses.

AMOVA (analyses of molecular variance) were performed on the Rhône-Alpes and North American data using the ARLEQUIN version 2001 freeware package (Scheider *et al.*

2000; available at <http://anthropologie.unige.ch/arlequin>). These hierarchical analyses of variance divide the total variance into components due to interindividual differences and to interpopulation differences. AMOVA were run without the Amb15 locus and without two North American populations (AI and IL) because more than 5% of the data was missing for this locus and these populations.

Patterns of isolation by distance in France and North America were tested separately by computing Mantel tests between the half-matrices of pairwise genetic distances, estimated either as $F_{ST}/(1 - F_{ST})$ values or as $R_{ST}/(1 - R_{ST})$, and pairwise geographical distances (logarithm of the true geographical distances, Rousset 1997). Tests were performed using the GENETOP freeware (Raymond & Rousset 1995).

Diversity patterns within the invaded range

To test if the common ragweed populations that are located at the margin of the invaded area exhibit reduced genetic diversity, we compared within-population diversity indices (i.e. A , A_R , R_S , V , M , H_E , H_O , F_{IS}) between French populations from the Rhône-Alpes region and from the more recently invaded Bourgogne and PACA regions. We used similar permutation tests as described above to compare diversity indices between North America and the Rhône-Alpes region.

Population assignment tests and distribution of private alleles

To identify the source populations of French invasive populations, we assigned all sampled French individuals to North American sampled populations. We used methods based on the likelihood of multilocus genotypes (reviewed in Cornuet *et al.* 1999): methods of Paetkau *et al.* (1995) and of Rannala & Mountain (1997) that, respectively, use frequency and Bayesian criteria for likelihood estimation. These tests were performed using the GENECCLASS2 software (Piry *et al.* 2004). We tested the ability of this software to discriminate source populations by assigning North American individuals to North American populations.

Because rare alleles can be powerful in identifying source populations of invasions, we also examined the distribution of private alleles, i.e. alleles unique to a single population or a region. Because there were very few alleles unique to single populations, we considered all alleles present in only one or two North American populations as private alleles from North American populations. We then examined in which French populations these private alleles were found. We also recorded the number of French private alleles as the number of alleles found in only one or two French populations but absent from all North American populations.

Results

Overall genetic diversity, F-statistics and Hardy–Weinberg equilibrium

The five nuclear microsatellite loci had a total of 105 alleles in the 22 populations analysed. Among these 105 alleles, 90 were present in North America and 96 in France (92 in Rhône-Alpes region and 85 in Bourgogne and PACA regions), few alleles being private between continents. Diversity indices, F -statistics estimates, results of exact Hardy–Weinberg tests and number of private alleles are presented in Table 1. Significant heterozygote deficiencies were detected in all populations, yielding significant positive F_{IS} values. These values were the same for both French and North American populations. Tests for linkage disequilibria were all nonsignificant. Overall genetic diversities were similar in France and in North America.

Comparison of variability distribution among populations between the introduced and the native ranges

We compared here North America with the single Rhône-Alpes region, because it is assumed to host the first introduced French populations. The number of alleles (A), allelic richness (R_S) and variance of allelic size (V) were significantly greater within Rhône-Alpes populations than within North American ones (P values < 0.03 , Table 1). There were more rare alleles, i.e. with allelic frequencies below 0.1, per population in Rhône-Alpes than in North America, whereas no difference in the number of common alleles was detected. The M index, i.e. the ratio of the number of alleles to the range in allele size, was significantly greater in the North American populations than in the French ones. No significant differences in observed and expected heterozygosities were detected between French and North American populations.

Analyses of molecular variance (AMOVA; Table 2) indicated that genetic variation was poorly structured geographically both in the native and in the introduced range, with most genetic variation being within, rather than among, populations. This was however, particularly exacerbated in the introduced range: 85% of the genetic variance was found within population in the native North American range whereas 97% of the genetic variance was within populations in the Rhône-Alpes region. The same pattern, although less accentuated, was recovered from AMOVA using F_{ST} (not shown).

Permutation tests also showed that among-population differentiation measured as R_{ST} was significantly higher in North America than in France, whereas among-population differentiation measured as F_{ST} did not differ between France and North America (Table 1).

Table 1 Diversity indices (A , mean number of alleles per locus, A_R mean number of rare alleles per locus, i.e. with allelic frequencies below 0.1, R_S allelic richness, V allele size variance, M ratio of the number of alleles to the range in allele size, H_E expected heterozygosity, H_O observed heterozygosity), results of exact Hardy–Weinberg tests, Weir & Cockerham's F -statistics estimates and the Goodman's estimator of R_{ST} , i.e. genetic differentiation accounting for variance in allele size and number of private alleles. For each of the indices, values per population and mean $\pm$ SD for North America, France and the whole sample (France and North America) are given

Populations	A	A_R	R_S	V	M	H_E	H_O	F_{IS}	HW test†	F_{ST}	R_{ST}	Private alleles‡	French populations with N. Am. private alleles
North America													
NA-AL	6.3	3.5	4.90	54.0	0.485	0.74	0.32	0.56	***			0	
NA-CO	9.6	6.8	6.51	69.2	0.462	0.79	0.44	0.44	***			9	F-RA-1, F-RA-2, F-RA-3, F-RA-4, F-RA-5, F-PACA-1, F-PACA-3
NA-IL	8.8	5.5	6.04	38.2	0.476	0.79	0.57	0.28	***			3	F-RA-1
NA-MO	9.4	6.4	5.94	66.3	0.408	0.75	0.45	0.40	***			5	F-RA-2, F-RA-4, F-PACA-2, F-PACA-3, F-BOURG-1
NA-NY	9.4	6.6	5.57	40.0	0.443	0.71	0.39	0.46	***			4	F-RA-1, F-RA-2, F-RA-3, F-RA-4, F-RA-5, F-PACA-1
NA-OH	8.6	5.8	5.62	29.8	0.456	0.71	0.32	0.55	***			3	F-RA-4, F-PACA-1, F-BOURG-1
NA-ON-1	8.8	5.6	5.75	44.0	0.438	0.76	0.38	0.50	***			1	F-RA-1, F-RA-3, F-RA-5
NA-ON-2	9.4	6.2	5.79	43.5	0.434	0.76	0.42	0.45	***			5	F-RA-1, F-RA-2, F-RA-3, F-RA-4, F-RA-5, F-PACA-3
NA-QC	9.8	7.0	5.97	40.1	0.538	0.74	0.43	0.41	***			1	F-RA-2, F-RA-5
NA-SC	7.0	3.2	5.05	48.6	0.497	0.73	0.54	0.26	***			5	F-RA-4, F-PACA-1
NA-TN	10.4	7.0	6.66	118.8	0.397	0.83	0.40	0.52	***			10	F-RA-1, F-RA-3, F-RA-4, F-RA-5, F-PACA-1, F-PACA-3
NA-WI	10.4	7.0	6.33	54.3	0.495	0.77	0.36	0.54	***			3	F-RA-1
Mean $\pm$ SD	9.0	5.9	5.85	53.9	0.461	0.756	0.42	0.45				4.1	
North America	($\pm$ 0.36)	($\pm$ 0.38)	($\pm$ 0.15)	($\pm$ 6.8)	($\pm$ 0.012)	($\pm$ 0.011)	($\pm$ 0.022)	($\pm$ 0.028)				($\pm$ 3.0)	
Total													
North America	18.4	15.6	6.64	60.5	0.534	0.76	0.41	0.47		0.052	0.080	10	

Table 1 Continued

Populations	A	A_R	R_S	V	M	H_E	H_O	F_{IS}	HW test†	F_{ST}	R_{ST}	Private alleles‡
France												
Rhône-Alpes												
F-RA-1	10.0	6.6	6.49	69.7	0.442	0.80	0.37	0.55	***			2
F-RA-2	11.0	8.0	6.62	69.8	0.399	0.80	0.48	0.40	***			2
F-RA-3	11.4	8.4	6.89	103.2	0.421	0.83	0.36	0.56	***			2
F-RA-4	10.8	8.0	6.53	57.6	0.435	0.77	0.40	0.48	***			1
F-RA-5	10.6	8.2	5.83	91.9	0.448	0.70	0.41	0.41	***			1
Mean $\pm$ SD	10.8	7.8	6.47	78.4	0.429	0.780	0.403	0.48				1.6
Rhône-Alpes	(± 0.23)	(± 0.32)	(± 0.17)	(± 8.3)	(± 0.009)	(± 0.022)	(± 0.021)	(± 0.033)				(± 0.5)
Total												
Rhône-Alpes	17.8	15.2	7.05	79.8	0.519	0.780	0.40	0.51		0.042	0.018	11
Bourgogne-PACA												
F-BOURG-1	7.0	4.5	4.83	71.7	0.438	0.67	0.31	0.54	***			2
F-BOURG-2	5.3	2.5	4.43	41.0	0.502	0.70	0.27	0.61	***			2
F-PACA-1	6.6	3.4	4.54	47.0	0.392	0.71	0.28	0.61	***			0
F-PACA-2	5.5	2.0	4.96	42.2	0.434	0.69	0.60	0.13	.			0
F-PACA-3	6.8	3.3	5.78	53.9	0.515	0.80	0.33	0.58	***			0
Mean $\pm$ SD	6.2	3.1	4.91	51.2	0.456	0.711	0.358	0.49				0.8
Bourgogne-PACA	(± 0.35)	(± 0.43)	(± 0.24)	(± 5.6)	(± 0.023)	(± 0.023)	(± 0.062)	(± 0.092)				(± 1.1)
Total	11.8	2.8	5.51	50.7	0.453	0.715	0.37	0.55		0.070	0.063	3
Bourgogne-PACA												
Mean $\pm$ SD	8.5	5.5	5.69	64.8	0.442	0.746	0.380	0.49				1.2
France	(± 0.78)	(± 0.82)	(± 0.30)	(± 6.6)	(± 0.012)	(± 0.019)	(± 0.032)	(± 0.046)				(± 0.9)
Total France	19.2	16.6	6.75	71.0	0.528	0.753	0.365	0.55		0.058	0.043	15
Mean $\pm$ SD												
Total sample												
(France + North America)	8.8	5.5	5.77	58.9	0.452	0.751	0.401	0.47				
	(± 0.40)	(± 0.42)	(± 0.15)	(± 4.8)	(± 0.008)	(± 0.010)	(± 0.019)	(± 0.026)				
Total*												
Total sample												
(France + North America)	21.0	18.4	2.6	6.77	64.9	0.538	0.76	0.50		0.054	0.064	

*The values in the 'Total' rows were calculated pooling all the populations from North America, from France, or all populations from France and North America.

†For the Hardy–Weinberg test, the P -values are indicated as : < 0.1 ; * : < 0.05 ; ** : < 0.01 ; *** : < 0.001 .

‡For North American populations we indicate the number of private alleles, i.e. those found in only one or two North American populations and those French populations that sharing private alleles with that North American population. For French populations private alleles we indicate the number of private alleles, i.e. those found in only one or two French populations and in none of the North American populations. In the 'Total' rows, private alleles correspond to alleles unique to a region or to a continent.

Table 2 Results of the analyses of molecular variance (AMOVA) using R_{ST} . (A) North American populations; (B) French populations from the Rhône-Alpes region

A

Source of variation	d.f.	Sum of squares	Percentage of variation	P value
Among populations	9	20 975.969	14.24	< 0.000001
Within populations	550	124 498.024	85.76	< 0.000001
Total	559	145 473.99		

B

Source of variation	d.f.	Sum of squares	Percentage of variation	P value
Among populations	4	18.137	3.33	< 0.000001
Within populations	295	435.817	96.67	< 0.000001
Total	299	453.953		

Isolation by distance and diversity pattern within continents

A significant pattern of isolation by distance was detected among North American populations when using R_{ST} ($r = 0.51$, $P = 0.012$), but not among French populations ($r = 0.20$, $P = 0.179$).

Permutation tests revealed significant decreases in the number of alleles (A), allelic richness (R_S) and variance of allelic size (V) between French populations from the Rhône-Alpes region and more recent French populations from Bourgogne and PACA regions (P values < 0.03). The lower number of alleles in populations far from Lyon was mainly due to the presence of fewer rare alleles. In particular, there were more private alleles in the Rhône-Alpes region than in the two other French regions (Table 1). In

contrast, no significant differences were detected between the core region of the introduction and later colonized regions in observed heterozygosities (H_O , $P = 0.09$), gene diversity (H_E , $P = 0.09$) and M index (Table 1).

Population assignment tests

Although population differentiation was not very high in North America, control assignment tests placed American plants in their correct North American populations for 65% of the individuals using the method of Rannala & Mountain (1997) and for 58% of the individuals using the method of Paetkau *et al.* (1995). French individual plants were assigned roughly equally to the different North American populations sampled (Fig. 2). Some plants from within each single French population were assigned to different North American populations that were not the closest geographically (Fig. 2). The two assignment methods (Paetkau *et al.* 1995; Rannala & Mountain 1997) gave similar results.

Distribution of total numbers of alleles and of private alleles

There were several private alleles in North America, i.e. alleles unique to one or two North American populations (Table 1). Alleles unique to different North American populations were found in the same French populations and there were only 10 alleles found exclusively in North America. These observations indicate that French populations result from a mixture of several North American sources.

The mean number of alleles per locus in Rhône-Alpes (17.8) was much higher than in any single North American populations (maximum of 10.4), also indicating that the French invasion did not originate from a single or a few North American sources. Some private alleles were found in France, i.e. were present in only one or two French populations and not in North American populations.

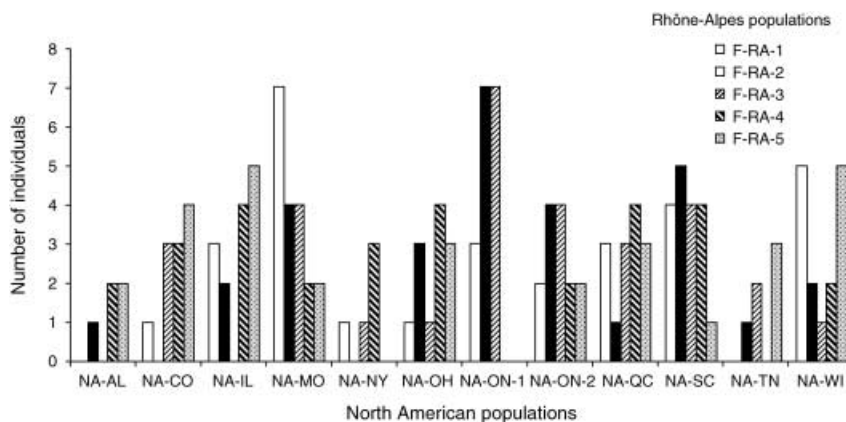


Fig. 2 Results of assignment tests of French populations of *Ambrosia artemisiifolia* to North American populations, following the method of Rannala & Mountain (1997): number of individuals from each of the five populations sampled in the Rhône-Alpes region that were assigned to the different North American populations.

These alleles were mostly in the Rhône-Alpes populations, a few were in the Bourgogne region and none in the PACA region.

Discussion

Multiple sources for the introduction of common ragweed in France

Overall population genetic variability of *Ambrosia artemisiifolia* was found to be similar in North America and in the Rhône-Alpes region. However, our results indicated that within-population levels of genetic variability were lower in native than in invasive French populations. No significant difference could be detected in Nei's gene diversity, but the number of alleles and the variance of allele size were greater within French populations of the Rhône-Alpes region than within North American ones, due to a larger number of rare alleles within French populations. These results contrast with most previous plant invasion studies that detected a reduced genetic diversity within introduced populations (e.g. Husband & Barrett 1991; Amsellem *et al.* 2000) or found no clear differences (e.g. Neuffer & Hurka 1999; Maron *et al.* 2004). A few studies on plant invasions however, also demonstrated an increase in genetic variability in the introduced range (Squirell *et al.* 2001; DeWalt & Hamrick 2004; Bossdorf *et al.* 2005).

If French populations had come from a single source population in North America, one would have expected a reduced number of alleles in France compared to native populations. Introduced populations indeed generally lose some rare alleles because of the genetic bottleneck occurring at introduction (Nei *et al.* 1975). French populations should also, then, have exhibited a lower variance of allele size than North American ones (e.g. Estoup *et al.* 2001). The higher number of alleles and variance of allele size observed in the Rhône-Alpes region suggest in contrast the existence of multiple sources for invasive populations of common ragweed in France.

The origin of French populations as a mixture from several North American sources is further supported by the approximately twofold higher mean number of alleles per locus in the total French sample than in any single North American population. Clearly, the combined French sample contains more individuals than any one North American population, which can explain part of this allelic richness. Nonetheless no single source population could account for this high number of alleles, with as many found in France (96) or even only in the Rhône-Alpes region (92) as in North America (90).

The *M* index was however, lower in French populations than in North American ones, as is actually expected after a bottleneck from a single source population, because the

number of alleles then decreases more than the size range (Garza & Williamson 2001). The finding that *M* has decreased after introduction of ragweed in France despite the lack of bottleneck can be explained by a larger increase of the range of allele size than of the number of alleles in introduced populations. The French populations have indeed more rare alleles, coming from different North American populations, and it is likely that these rare alleles span a larger size range than the most common ones.

Analyses of the distribution of genetic variability brought additional insights into the history of ragweed invasion in France. R_{ST} comparisons between North America and the Rhône-Alpes region revealed a greater among-population differentiation in North America than in France. Results of the AMOVA also pointed to genetic variation being more geographically structured in the native range than in the French introduced range. Moreover, there was a significant pattern of isolation by distance among North American populations but not among French populations. The higher within-population diversity of French introduced populations of *A. artemisiifolia* together with their lower among-population differentiation and lack of isolation by distance suggest that the French introduced populations result from a mixture of different native populations and that they may not be at migration-drift equilibrium, in contrast to the North American populations. The low among-population differentiation of introduced French populations further suggests that all of them together were founded by genetically similar sources, either by a single introduction of mixed propagules followed by dissemination or by multiple introductions coming from similar mixtures of sources. Such a transformation of among-population genetic variation into within-population genetic variation due to several introductions from the same multiple sources has also been reported in Cuban lizard invasions (Kolbe *et al.* 2004).

The finding that the originally introduced populations of *A. artemisiifolia* were founded by multiple, similar North American sources is further supported by the distribution of private alleles and assignment tests. Private alleles from different North American populations were found within the same French populations and assignment tests placed individual plants from each French population roughly equally across the different North American populations. These assignment tests appeared to be valid because American plants were most often assigned to their populations of origin. The significant differentiation among population and pattern of isolation by distance in North America provide ideal conditions for assignment tests. Isolation by distance should indeed allow assignment of individuals to the correct region, even if the exact population of origin had not been sampled.

We predominantly sampled eastern populations from North America, considering that the traffic between eastern

North America and Europe was greater than that for western North America. Of course, we cannot exclude that our inability to assign populations to a North American source stems from our failing to have sampled the appropriate region, but we consider it most likely that the ragweed invasion was indeed sourced in the east. The finding of French private alleles suggests either that we did not sample all the North American sources of the French populations or that new and unique alleles have arisen *de novo* within the French populations.

Range expansion of common ragweed in France

We observed reduced allelic richness and variance of allele size in populations distant from the putative original area of introduction, the east of Lyon. However, since these reductions were due to losses of rare alleles, no decrease in Nei's gene diversity was detected. This pattern, together with the distribution of allelic richness, with 92 alleles and 11 private alleles found in the Rhône-Alpes region but only 85 and 3 private alleles in the combined Bourgogne and PACA regions, supports the east of Lyon as being the original area of introduction of common ragweed populations present in France. This pattern of allelic diversity indicates that the ragweed range expansion probably occurred through sequential bottlenecks from the original populations, and not from subsequent new introductions. Contrary to expectations (Garza & Williamson 2001), the *M* index was, however, not lower in these recent invasive populations that have been subjected to bottlenecks. This may be due to French populations from the Rhône-Alpes region being a mixture of different North American populations not yet at equilibrium, thus resulting in a non-normal allele size distribution.

Heterozygote deficiency

All populations deviated from Hardy–Weinberg equilibrium at all loci, presenting heterozygote deficiencies. These may be due to the presence of null alleles (Genton *et al.* 2005) or to some degree of selfing since *A. artemisiifolia* is a self-compatible species (Bassett & Crompton 1975). A Wahlund effect (i.e. reduction in observed heterozygosity because of pooling discrete subpopulations with different allele frequencies that do not interbreed as a single randomly mating unit) is not likely because plants were sampled very close to each other within populations and because *A. artemisiifolia* is wind pollinated with an extended flowering period so that temporal isolation is highly unlikely. Although the French populations could possibly still represent mixtures from different source populations that had not yet attained genotypic equilibrium, generating a kind of Wahlund effect, this is unlikely given their presence in at least the Rhône-Alpes region for several decades. Further,

such nonequilibrium mixtures cannot be the explanation for the heterozygote deficiencies in North American populations. On the other hand, the distribution of genetic variation, with most variation found within, rather than among populations, particularly for the invasive French populations, and low values of F_{ST} and R_{ST} , points to an outcrossing wind-pollinated species (Loveless & Hamrick 1984).

Further, French populations did not show higher heterozygote deficiencies than North American ones, which would indicate a shift towards increased levels of self-fertilization in invasive populations of *A. artemisiifolia*, in contrast to what has been observed in some other invasive plant species (Baker 1955; Husband & Barrett 1991). This may not be surprising because selfing rate is expected to increase after invasion only if plants are at low density in the introduction range, which may not have been the case for common ragweed invasion in Europe. Indeed, the high level of genetic diversity found in France suggests that many individuals were introduced. Alternatively, the French invasion may be old enough that plants have evolved back to lower levels of selfing from a post-introduction high. No direct estimates of reproductive system parameters for this species are available to date but measures of selfing and outcrossing rates from progeny arrays would allow us to refine hypotheses for reconciling the inconsistent patterns of the distribution of genetic variation within (heterozygote deficit) vs. among individuals and populations.

Conclusion

Our results are consistent with a scenario of multiple sources for the introduction of common ragweed in the Rhône-Alpes region, followed by colonization of new areas to the north (Bourgogne region) and south (PACA region) from this initial introduction, through sequential bottlenecks. Further, our study provides an interesting solution to the paradox outlined by Frankham (2005), that introduced species are able to invade their new environment despite expected reduced genetic diversity. In the case of the invasion of *Ambrosia artemisiifolia* in France, no actual loss of genetic diversity has been detected. In contrast, within-population diversity is even higher in introduced populations than in source populations. More studies comparing genetic characteristics of native and introduced populations of invasive species are needed to get more insights into the genetic changes that accompany bio-invasions (Hierro *et al.* 2005).

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