Biol Invasions (2011) 13:933–944
DOI 10.1007/s10530-010-9880-y

ORIGINAL PAPER

Distinct invasion sources of common ragweed *(Ambrosia artemisiifolia)* in Eastern and Western Europe

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Received: 26 January 2010 / Accepted: 14 September 2010 / Published online: 2 October 2010

Abstract The common ragweed (*Ambrosia artemisiifolia* L.; Asteraceae) is a North American native that is invading Eurasia. Besides its economic impact on crop yield, it presents a major health problem because of its highly allergenic pollen. The plant was imported inadvertently to Europe in the eighteenth century and has become invasive in several countries. By analyzing French and North American populations, it was previously shown that French populations were best described as a mixture of native sources and that range expansion in France probably involved sequential bottlenecks. Here, our aim was to determine whether Eastern European populations of *A. artemisiifolia* originated from the previously established French populations or from independent trans-Atlantic colonization events. We used nuclear microsatellite markers to elucidate the relationships among populations from Eastern and Western Europe in relation to populations from a broad survey across the native North American range. We found that *A. artemisiifolia* from Eastern Europe did not originate from the earlier established French populations but rather represents multiple independent introductions from other sources, or introductions from a not yet identified highly diverse native population. Eastern European populations show comparable amounts of genetic variability as do previously characterized French and North American populations, but analyses of population structure clearly distinguish the two European groups. This suggests separate introductions in Eastern and Western Europe as well as divergent sources for these two invasions, possibly as a result of distinct rules for trade and exchange for Eastern Europe during most of the twentieth century.

Keywords Allergenic plant · Biological invasion · Invasive species · Multiple introductions · Population structure

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Introduction

Species introductions involve demographic and genetic bottlenecks when colonists are few and represent only a subset of the genetic variation

available in populations in the native range (Husband and Barrett 1991). However populations of introduced and invasive plants do not always lose genetic variation during invasion (see Bossdorf et al. 2005; Dlugosch and Parker 2008; Puillandre et al. 2008 for reviews). Some invasive populations originate from multiple introductions, thereby amassing allelic variation from a broad range of different populations from the native range (Bossdorf et al. 2005; Ciosi et al. 2008; Facon et al. 2005; Genton et al. 2005a; Hufbauer and Sforza 2008; Lavergne and Molofsky 2007), though multiple introductions do not always lead to an increase in genetic variation (Durka et al. 2005). Altogether, successful introductions, i.e. those leading to naturalization and invasion of a new geographical area, often appear to involve relatively small bottlenecks, with the best predictor of establishment success being propagule pressure, both in terms of number of introductions and number of individuals released (Simberloff 2009). Therefore many species that successfully establish and invade are likely those that lost least variation in the process, though invasions of single genotypes are not unknown (Grimsby et al. 2007; Okada et al. 2009).

Invasive populations may also show altered genetic structure compared to those in the native range, either less, with homogenised mixes of representatives of several differentiated populations (Le Roux et al. 2008), or more (Ciosi et al. 2008; Marrs et al. 2008), with invasive populations being particular subsamples of well mixed populations from the native range. Studies that compare genetic structures of invasive populations and populations in the native range can help pinpoint the area of origin as well as elucidating patterns of colonisation and pathways of spread (Le Roux et al. 2006). Do invaders come from areas with similar climatic characteristics? Have invaders followed a stepping-stone from an initial unique introduction (Amsellem et al. 2000)? Do newly colonised populations at the front of the species range originate from already established ones in the invaded range (Genton et al. 2005a) or do they represent independent colonization events from the native range (Ciosi et al. 2008)? These questions are relevant for practical purposes of risk assessment and the efficacy of control measures but also will provide important insights into the evolutionary ecology of invasive and other species undergoing range expansion.

Here we investigate the genetic structure of populations of the invasive *Ambrosia artemisiifolia* L. (Asteraceae), a North American native that is invading Eurasia, found particularly in sunflower and corn fields, abandoned fields, disturbed areas and along roadsides (Bassett and Crompton 1975). This wind-pollinated monoecious annual plant is self-incompatible, thus showing an outcrossing mating system, even in colonizing populations (Friedman and Barrett 2008). During the past 15–20 years, the spread of common ragweed has become a major problem in some parts of France and a number of Eastern European countries, including Hungary, Croatia, Ukraine, Russia and Serbia (Kiss and Béres 2007).

The first Eurasian records of this species are from a herbarium specimen from Central France from 1863, and the species showed a gradual but continuous spread in this region, demonstrating continuous presence in the area of Lyon, France, which seems to be the focus of its current French distribution (Chauvel et al. 2006). The earliest recorded Eastern European records of this plant appear first 40 years later from Orsova, Romania, and about 20 years after that from the south-western part of Hungary (Kazinczi et al. 2008; Makra et al. 2005; Csontos et al. 2010). It is therefore possible that Eastern Europe was colonized from the already established French invasive populations, though an independent introduction from the native range of this species is also possible. Over the last 20 years additional populations have appeared and become established in the intervening areas of Switzerland, northern Italy and Austria, but these are thought to represent recent range expansion from one or the other long established centres of spread. The conquest of Eastern Europe, however, has been dramatically more rapid and complete than that of the West, with a larger occupied range and far denser populations, as revealed by pattern of airborne pollen density across Europe (Fig. 1; Makra et al. 2004; Makra et al. 2005). This then poses the question of whether the Eastern European invasion is caused by other more competitive genotypes or those better adapted to the conditions in Eastern Europe.

Here we extend our previous investigation of the population structure of invasive populations in France (Genton et al. 2005a). We have previously shown that the French invasive populations of *A. artemisiifolia* originated from a mixture of sources. French

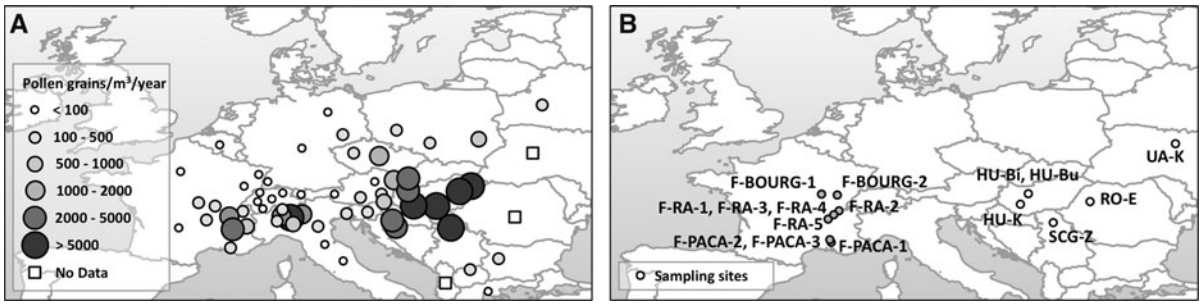

Fig. 1 Annual counts of *Ambrosia artemisiifolia* pollen grains (**a**) averaged over 1995–2007 (reproduced with permission from Regula Gehrig, MeteoSwiss; source: EAN European Aeroallergen Network) as a proxy for the local distribution and abundance of this plant; sampling sites of *A. artemisiifolia* (**b**) in Western Europe (previously presented in Genton et al. 2005a) and Eastern Europe (new to this study). *F* France; *HU* Hungary; *RO* Romania; *UA* Ukraine; *SCG* Serbia and Montenegro

populations showed even higher within-population diversity than did native North American populations, they contained mixes of rare alleles found in distinct North American populations and assignment tests failed to identify a single area of origin in North America (Genton et al. 2005a). Chun et al. (2010) showed that historical French populations, reconstructed from herbarium specimens dating from the late nineteenth to early twentieth century, appeared to harbour lower allelic and genetic diversity than recent populations. Altogether, this suggests that ragweed seeds were introduced repeatedly, or as mixtures, from different parts of North America to France. However within France, ragweed populations at the front of the invasion, far from the original area of introduction near Lyon, France, are genetically less diverse, indicating that ragweed range expansion probably involves sequential bottlenecks from the primary introduction rather than subsequent new introductions (Genton et al. 2005a). Here we test whether Eastern European ragweed populations could have been founded from French populations involving sequential bottlenecks or whether Eastern European ragweed populations were introduced independently.

We analysed Eastern European populations of this invasive plant using microsatellites to address the following questions: (1) Were Eastern European populations founded from French populations? Are they characterised by a subset of the allelic diversity already found in the French populations? (2) Did they come independently from similar sources in North America, characterized by a similar amount of variation and similar allelic profiles, or from elsewhere? Here we compare our new data from Eastern European ragweed populations with data previously presented in Genton et al. (2005a).

Methods

Sampling and DNA extractions

Ragweed populations were sampled in six localities in Eastern Europe (Fig. 1; Table 1). In each sampling site leaves were collected from 30 individual plants at approximately 1.5 m spacing, according to the sampling method of Genton et al. (2005a), air-dried, and kept as herbarium materials. DNA was extracted from 10 to 15 mg dried leaf tissue using DNeasy Plant Mini Kits (QIAGEN) and then stored at −20°C.

Genetic data for a total of 12 North American and 10 French ragweed populations, obtained in a previously published study (Genton et al. 2005a), were also included in this work. The American populations sampled were located east of the Rocky Mountains, mostly from the East Coast and Great Lakes region of the USA and Canada, areas with a long history of commercial exchange with Western Europe. The French ragweed populations sampled were located in the Rhône-Alpes, Provence-Alpes-Côte-d'Azur and Bourgogne regions. The designations, locations and other data of the American and French ragweed populations included in this study are given in Genton et al. (2005a).

Microsatellite genotyping and analyses

We used the five nuclear microsatellite loci recently developed for *A. artemisiifolia* (Genton et al. 2005b).

Table 1 Origin, population structure and measures of genetic variation of the Eastern European ragweed populations analysed in this study

Populations	Location	Latitude	Longitude	A[a]	A_R[b]	R_S[c]	H_E[d]	H_O[e]	F_{IS}[f]	HW test[g]	F_{ST}[h]
HU-Bi	Biatorbágy, Hungary	47°46′N	18°81′E	10.6	8.2	9.7	0.80	0.19	0.76	***	
HU-Bu	Budaörs, Hungary	47°45′N	18°96′E	10.4	7.8	9.1	0.73	0.24	0.68	***	
HU-K	Keszthely, Hungary	46°76′N	17°25′E	9.0	5.2	8.4	0.78	0.18	0.79	***	
RO-E	Elesd, Romania	47°06′N	22°41′E	8.0	4.4	7.5	0.70	0.23	0.70	***	
UA-K	Kiev, Ukraine	50°43′N	30°51′E	5.8	2.8	5.3	0.60	0.25	0.47	***	
SCG-Z	Zenta, Serbia and Montenegro	45°92′N	20°07′E	8.8	4.8	8.5	0.78	0.20	0.75	***	
Mean ± SD				8.8 ± 1.7	5.5 ± 2.1	8.1 ± 1.5	0.73 ± 0.07	0.21 ± 0.03	0.69 ± 0.12		0.08 ± 0.05

[a] Mean number of alleles per locus

[b] Mean number of rare alleles per locus

[c] Allelic richness

[d] Expected heterozygosity

[e] Observed heterozygosity

[f] Inbreeding coefficient

[g] Results of exact Hardy-Weinberg tests

[h] Weir and Cockerham's F-statistics estimates

***$P < 0.001$

Polymerase chain reaction and allele resolution were carried out according to Genton et al. (2005a). Several samples from the study by Genton et al. (2005a), having the full range of alleles previously identified, were run on each gel to score allele sizes consistently between the previous and the present studies. New primers were designed for the locus Amb15 because of difficulties in amplification using the previous primers from Genton et al. (2005b). The new primer pair (Amb15-F2: aatccattccccacatcctt and Amb15-R2: gaggggttgggtcgagtaag) gave amplifications in a higher number of individuals.

Using FSTAT version 2.9.3.2 (Goudet 1995), we estimated (1) the means ± SD over all loci of the following genetic variation indices: allelic richness (R_S; El Mousadik and Petit 1996), observed heterozygosities and expected heterozygosities, respectively H_O and H_E (Nei 1987; the latter also referred to as Nei's gene diversity); (2) *F*-statistics (Weir and Cockerham 1984). 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 the number of alleles (A) and the number of rare alleles (A_R), i.e. with allelic frequencies below 0.1. 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).

Genetic distances among populations were measured with the POPULATIONS program (http://bioinformatics.org/~tryphon/populations/), using the chord distance of Cavalli-Sforza and Edwards (1967). The distance matrix was submitted to a Principal Coordinate Analysis (PCoA), as implemented in GENALEX (Peakall and Smouse 2006).

Comparison of genetic diversity and population differentiation

To compare the values of within-population genetic variation indices (A, A_R, H_E, F_{IS}) and population differentiation (F_{ST}) between the native range, France and Eastern Europe, we used FSTAT. For each index FSTAT computes the average over loci and populations for each group 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 three groups, keeping the number of populations constant in each group.

To compare the means of numbers of null genotypes among French, North American and Eastern European populations, pairwise mean comparisons were carried out using Student t-tests with the software JMP (SAS Institute Inc, SAS Campus Drive, Cary NC). The locus Amb15 was not included in these comparisons because different primer pairs had been used for genotyping Eastern European populations (see above), precisely because the number of null alleles was high using the first primer pair. Amb15 was however used in all other analyses as we could make the correspondence between the same alleles amplified using the two different primer pairs.

Analyses of molecular variance (AMOVAs) were performed using ARLEQUIN version 3.0 (Excoffier et al. 2005), with variation being partitioned within- and among-populations, and distance among genotypes calculated as the number of different alleles. This method was preferred over the method that uses the squared number of repeat difference between genotypes to calculate distances (Slatkin 1995) to avoid biases due to departures from the stepwise model of microsatellite evolution.

Population subdivision and assignment tests

We used STRUCTURE version 2.3.1 (Pritchard et al. 2000) to identify the source populations of Eastern European invasive populations and to examine population subdivision. The model implemented allowed information on sampling location (LocPrior model; Hubisz et al. 2009), admixture, and correlation in allele frequencies (Falush et al. 2003). Burn-in length was set at 150,000 iterations followed by a run phase of 750,000 iterations. We employed a hierarchical approach to detect all layers of population structure (Coulon et al. 2008; Evanno et al. 2005; Rollins et al. 2009). Analyses were first conducted on the total dataset, using the region of origin of individuals as prior information to assist clustering. We then repeated analyses on each of the K groups inferred in the previous step, using the population of origin of individuals as prior information. We set the number of populations (K) from 1 to 8, but not higher, as the results for the first level of analysis showed that

setting K values from 3 to 8 already led to clusters without geographical coherence and with admixed ancestry, which is typical of too high a cluster number. For all levels of analysis, we performed 30 independent runs for each value of K. Results were analysed with CLUMPP version 1.1.2 (Jakobsson and Rosenberg 2007) using the *Greedy* algorithm for $1 \leq K \leq 4$ and the *Fast-Greedy* algorithm for $K > 4$, with random input order and 10,000 permutations. Distinct modes among runs were identified by finding sets of runs with less than 85% similarity in the G′ pairwise similarity matrix ('modes' refer to distinct clustering solutions represented within the set of replicate cluster analyses). CLUMPP was used again to align outputs of the runs with the same clustering mode and to provide average cluster membership coefficients across aligned runs. The optimal K value was determined using the method of Evanno et al. (2005) based on the rate of change in the log probability of data between successive K values.

Distribution of private alleles

Because rare alleles can be powerful in identifying sources and routes of migrations, we also examined the distribution of private alleles, i.e. alleles unique to a single population or combination of populations. We used the program ADZE (Szpiech et al. 2008), which implements a rarefaction procedure for counting alleles private to populations while adjusting for differences in sample size across populations. We calculated the mean number of private alleles, averaging across loci, for each of three regional groupings of populations (North America, France, Eastern Europe) and each of three combined sets of two regional groupings. Calculations were performed using a standardized sample size of $n = 252$ (126 individuals times two chromosomes), corresponding to the smallest number of observations per regional grouping under consideration.

Results

Genetic diversity, F-statistics and Hardy Weinberg equilibrium

The five nuclear microsatellite loci had a total of 95 alleles in the six Eastern European populations analysed. Diversity indices, F-statistics estimates (Weir and Cockerham 1984) and results of exact Hardy-Weinberg tests (Guo and Thompson 1992) are presented in Table 1 for Eastern European populations and can be found in Genton et al. (2005a) for North American and French populations. Significant heterozygote deficiencies were detected in all populations, yielding significant positive F_{IS} values. These values were greater in Eastern European populations than in North American ones and in France ($P < 0.01$, Fig. 2). Tests for linkage disequilibria were all non-significant. Genetic variation, measured as number of alleles, number of rare alleles or expected heterozygosity, was not significantly different among the native range, France and Eastern Europe (Fig. 3).

To assess whether the higher level of F_{IS} in Eastern European populations could be due to the presence of more null alleles, we compared the proportion of null genotypes (i.e. giving no amplification in one or a few loci but giving bands in other loci; thus being assumed

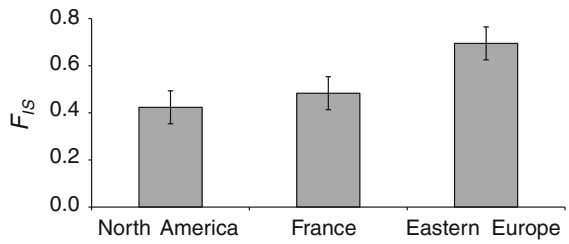

Fig. 2 Mean of the inbreeding coefficient (F_{IS}) in North American, French and Eastern European populations of *Ambrosia artemisiifolia*. *Error bars* represent standard error across loci

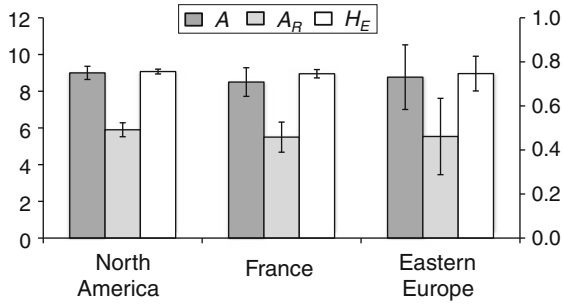

Fig. 3 Mean of allelic richness (A), number of rare alleles (A_R), and of the expected heterozygosity (H_E), in North American, French and Eastern European populations of *Ambrosia artemisiifolia*. *Error bars* represent standard error across loci

to be homozygotes for null alleles) among Eastern European, French and North American populations. Eastern European populations had a significant higher mean proportion of null genotypes (mean ± SE = 15.9 ± 2.0), than French (mean ± SE = 8.5 ± 1.5) or North American (mean ± SE = 10.2 ± 1.4) populations, which did not differ significantly from each other.

Comparison of variability distribution among populations between North America, Western Europe and Eastern Europe

We first performed an analysis of molecular variance (AMOVA) on Eastern European populations to divide the genetic variance into within- and among population components. Results indicated that most genetic variation in Eastern Europe was within, rather than among, populations (Table 2), as was the case in the native range and in France (Genton et al. 2005a). The percentage of variation attributed to among-population differentiation was 8.79% in Eastern Europe (Table 2), i.e. higher than in France (4.81%) and in North America (6.39%).

Distribution of private alleles

The number of private alleles was calculated for regional groupings of populations and their combinations (Fig. 4). At the scale of regions, estimates were higher in Eastern Europe (mean ± SE = 5.29 ± 2.50) than in France (mean ± SE = 1.84 ± 0.95) and North America (mean ± SE = 0.73 ± 0.51). In analyses on combinations of regions, private allele richness was higher in the North America/France combination (mean ± SE = 4.14 ± 2.03) than in the North America/Eastern Europe (mean ± SE = 1.45 ± 0.72) and France/Eastern Europe (mean ± SE = 1.28 ± 0.72) combinations. The fact that the proportion of private alleles is the highest in North America and France when these regions are combined while it is the lowest when regions are analysed individually suggests that North American and Western European populations are more similar to each other in terms of allelic profiles than they are to Eastern European populations.

Genetic relationships among populations

Chord genetic distances were calculated among populations, and the resulting distance matrix was subjected to PCoA (Fig. 5). The first principal coordinate (explaining 41.7% of total variation) partitioned populations into two groups with populations from France and North America in one group, and populations from Eastern Europe in the other. The second and third principal coordinates (explaining 15.1 and 12.9% of total variation, respectively) did not reveal any obvious further correspondence between genetic distances and the geographical origin of populations. The only remarkable feature that emerges from the decomposition of variance along these axes is that the third coordinate separated the Ukrainian population from other Eastern European populations.

Table 2 Results of the analyses of molecular variance (AMOVA)

Source of variation	*d. f.*	Sum of squares	Percentage of variation	*P*-value
Eastern Europe				
Among populations	5	32	8.79	$<10^{-6}$
Within Populations	350	328	91.21	$<10^{-6}$
Total	355	360		
North America				
Among populations	9	63	6.39	$<10^{-6}$
Within Populations	550	796	93.61	$<10^{-6}$
Total	559	859		
France				
Among populations	9	45	4.81	$<10^{-6}$
Within Populations	488	694	95.19	$<10^{-6}$
Total	497	739		

Distance among multilocus genotypes is computed as the number of different alleles

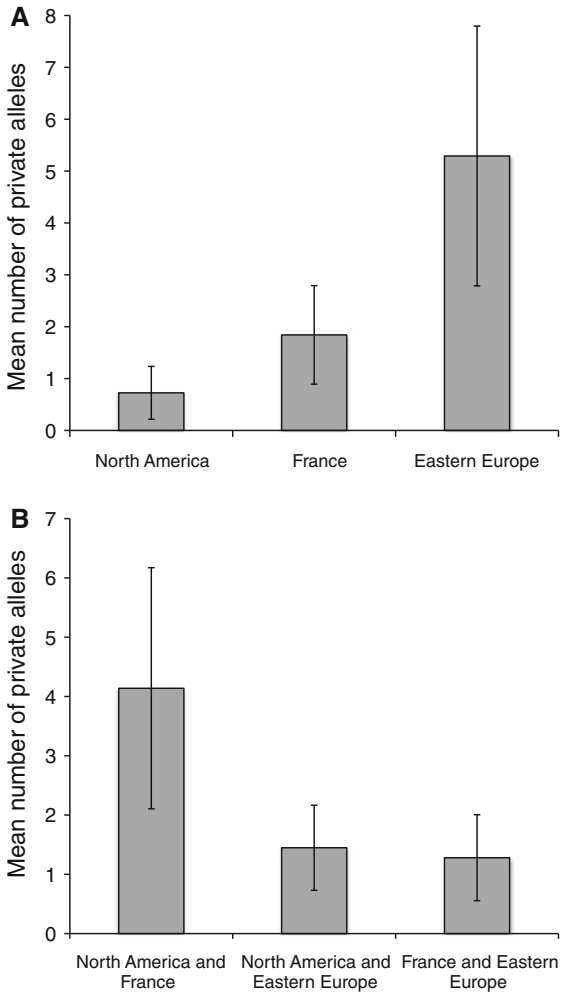

Fig. 4 Mean number of alleles private to regional groupings of populations (**a**) and their combinations (**b**). Estimates are based on a standardized sample size of 252 chromosomes (126 plants times two chromosomes). *Error bars* represent standard error across loci

In STRUCTURE analyses, the modal value of the ΔK statistic was found at $K = 2$, with North American and French populations in one cluster, and Eastern European populations in another (Fig. 6). Only a few individuals contradicted this pattern of clustering and had mixed membership in the two clusters or were not assigned to the cluster containing individuals from the region from which they were sampled. Generally, levels above $K = 2$ produced no new clusters corresponding to geographical structuring among North American and French genotypes, but instead introduced some heterogeneity in membership coefficients. Within Eastern European populations, no particular geographical pattern of clustering was detected, except that all individuals from Ukraine had consistently high membership in the same cluster and ended up individualizing in a separate cluster when K reached 6 (not shown). In the next level of analyses, we searched for possible additional layers of structure by repeating analyses on both of the $K = 2$ groups inferred in the previous step, using 'populations'—and not 'regions'—as prior information to assist clustering. In analyses of Eastern European samples, the modal value of ΔK was found at $K = 2$, with a secondary peak at $K = 5$. At $K = 2$, one cluster corresponded to individuals from Ukraine, and the other clusters grouped individuals from all other populations (not shown). At $K = 5$, individuals from populations from Serbia-Montenegro, Romania, and Ukraine (i.e. SCG-Z, RO-E, UA-K) had high membership proportions in separate clusters, individuals from Hungarian populations HU-Bi and HU-Bu had high membership in the same cluster, and individuals from Hungarian population HU-K had roughly equal membership in multiple clusters (Fig. 6). In analyses of French and North American samples, the modal value of ΔK was observed for $K = 6$, but neither $K = 6$ nor any other value of K yielded an obvious pattern of geographical clustering (Fig. 6). In support of a lack of population structure, average log posterior probabilities of data for $K = 1$ and $K = 2$ were very close (on average over runs from the main mode: $\mathrm{Ln}(P) =$ x$-10{,}512.2$ and $\mathrm{Ln}(P) = -10{,}512.9$ for $K = 1$ and $K = 2$, respectively), suggesting that a model with multiple populations is not significantly better than a model with a single population to represent data from French and North American samples.

Discussion

Invasion history of European ragweed

Overall population genetic variability of *A. artemisiifolia*, measured as expected heterozygosity or allelic richness, was similar in North America, Eastern and Western Europe. We found no evidence for the loss of genetic variation via sequential bottlenecks observed for other invasions (e.g. Amsellem et al. 2000; Henry et al. 2009; Puillandre et al. 2008). Therefore, in contrast to what was found in France, where populations at the current invasion front in the Bourgogne and PACA regions originate from the original

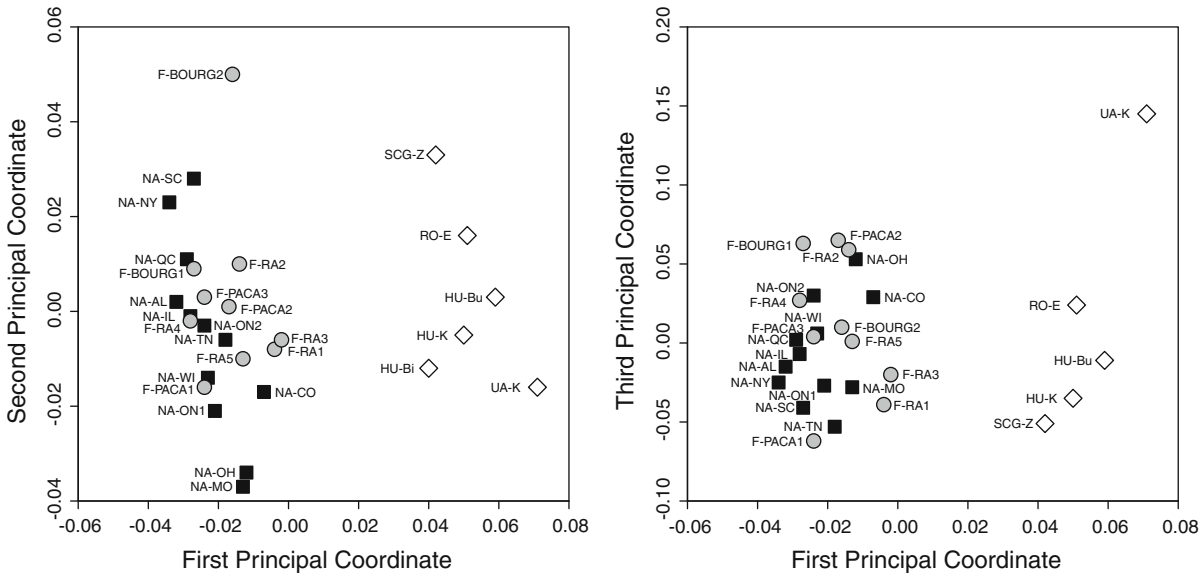

Fig. 5 Principal coordinate analysis of chord distance among populations. The first, second and third principal coordinates account for 41.7, 15.1 and 12.9% of the variation, respectively. *NA* North America; *F* France; *HU* Hungary; *RO* Romania; *UA* Ukraine; *SCG* Serbia and Montenegro. *Squares* represent US, *circles* West European and *diamonds* East European populations

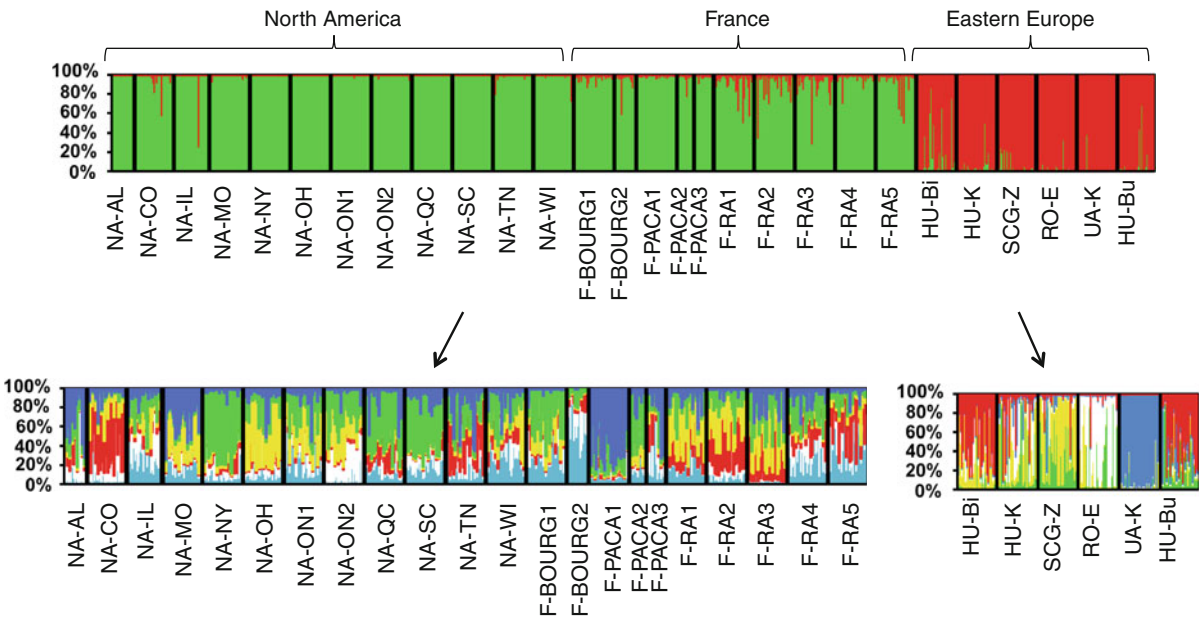

Fig. 6 Population structure of *Ambrosia artemisiifolia* inferred using the STRUCTURE program. The number of predefined clusters was $K = 2$ for the analysis of the total dataset. Subsequent hierarchical analyses (indicated by *arrows*) assumed $K = 6$ clusters for French and North American samples, and $K = 5$ clusters for Eastern European samples. Each individual is represented by a thin *vertical line* that is partitioned into two components according to the inferred membership in the two genetic clusters. *Black lines* separate genotypes from distinct populations. *Vertical axis* represents the membership proportions to the K clusters assumed, obtained using the CLUMPP program by averaging memberships across all runs corresponding to the main clustering mode. *NA* North America; *F* France; *HU* Hungary; *RO* Romania; *UA* Ukraine; *SCG* Serbia and Montenegro

populations of introduction in the East of Lyon (Genton et al. 2005a), Eastern European populations appear not to have originated from colonists from these older established French populations. Nor did Eastern European populations originate from a single source among the native populations sampled. The high genetic variability observed in Eastern European populations suggests either multiple sources of introduction, or introduction from a highly diverse source that we failed to sample (e.g. Muirhead et al. 2008). Multiple introductions have been inferred for many plant introductions studied to date (Bossdorf et al. 2005; Dlugosch and Parker 2008; Hufbauer and Sforza 2008), including the introduction of this same species to Western Europe (Genton et al. 2005a).

Analyses of the distribution of genetic variability brought additional insights to the history of the ragweed invasion. AMOVA, PCoA and STRUCTURE analyses revealed contrasted patterns of population structure in the introduced range: while populations from France appeared less differentiated than populations from the native range, a higher level of geographical structure was observed among Eastern European populations. These differences may be related to the population structure in the native range and the genetic makeup of founding propagules. The shallow population structure of introduced French populations suggests that they were all 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 (Genton et al. 2005a). Such a transformation of among population genetic variation into within population genetic variation due to several introductions from similar multiple sources has been reported in several other cases of biological invasions with multiple introductions (e.g. Kolbe et al. 2004; Lavergne and Molofsky 2007). By contrast, the higher population structure observed in Eastern European populations of *A. artemisiifolia* may correspond to the introduction of genetically differentiated propagules resulting from independent samplings either from similar highly diverse populations or from separate differentiated populations. Such a pattern of higher population structure in the invasive range has been reported for several organisms (e.g. Ciosi et al. 2008; Marrs et al. 2008), though it seems less frequent than the opposite pattern (Bossdorf et al. 2005).

Different origins and structure for Eastern and Western European invasive ragweed populations

Several lines of evidence indicate that the Eastern and Western European invasive ragweed populations originate, at least in part, from separate mixes of different native populations. Analyses of population structure revealed that Western and Eastern European populations were differentiated, and French populations of *A. artemisiifolia* appeared genetically much more similar to the sampled North American populations than did Eastern European populations, as indicated by the number of alleles private to the combination of French and North American populations, patterns of genetic distance among populations and assignment tests. The higher F_{IS} values in Eastern Europe further support their distinctness. The plant is self incompatible and outcrossing in its native area (Friedman and Barrett 2008) and we have no indication of a breakdown of self incompatibility and shift in reproductive mode in Eastern Europe. Furthermore we found a higher proportion of null genotypes in these populations, so the significant F_{IS} values point strongly to the existence of null alleles caused by mutations in the flanking regions of the microsatellites that are expected to evolve more slowly than repeat number, implying a longer history of independent evolution. Because the microsatellite markers were cloned from French populations (Genton et al. 2005b), genetically distant populations should harbour more null alleles. The fact that more null alleles seem to be present in Eastern Europe than in North America and France thus further indicate that Eastern European populations are genetically distinct from both the sampled North American and French ones. We also note that a scenario in which Eastern European populations originate, at least in part, from different native populations than those analysed by Genton et al. (2005a) is likely given the geopolitics of the latter half of the twentieth century that facilitated neither commercial nor human exchanges between Eastern Europe and North America or Western Europe.

Conclusion

Introductions from multiple sources and separate introductions to different sites have previously been reported in the literature (see introduction), but the

pattern found for *A. artemisiifolia* is rarer, with two separate introductions on the same continent, different levels of population structure in different parts of the invasive range, and introductions from different but multiple sources each (see another example with the invasive grass *Bromus tectorum*; Novak and Mack 2001). This results in a similar level of variability in introduced and native populations, possibly yielding a considerable potential for rapid evolution in invasive populations. Nonetheless, we have little evidence for post-introduction adaptive evolution in this species. Genton et al. (2005c) investigated changes of introduced populations compared to native ones, but found no evidence for any evolutionary loss of defence against natural enemies despite strong enemy release in Europe, though there may have been an evolutionary change in the phenology of introduced populations, reflecting adaptation to higher latitudes in the introduced range.

Another remarkable aspect of the invasion of *A. artemisiifolia* in Europe is the possible role of the political context in the genetic structure and diversity on the invaded range. The wars and their consequences may indeed have set the stage for the introduction of genetically different sources in Western and Eastern Europe. The locations of the populations that gave rise to the Eastern populations remain to be identified.

Acknowledgments We are grateful to Tünde Jankovics and Vera Hayova for their help in collecting ragweed samples, to Bernard Clot from MeteoSwiss, and we thank anonymous reviewers for their suggestions. TG acknowledges a grant ANR 07-BDIV-003 and LK an Invited Professorship from University Paris-Sud. A part of this work was supported by grants of the Hungarian Scientific Research Fund (OTKA T046841 and IN67377).

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