Molecular Ecology (2005) **14**, 1697–1706 doi: 10.1111/j.1365-294X.2005.02521.x

Molecular evidence for multiple introductions of garlic mustard (*Alliaria petiolata*, Brassicaceae) to North America

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Abstract

Invasive species offer excellent model systems for studying rapid evolutionary change. In this context, molecular markers play an important role because they provide information about pathways of introduction, the amount of genetic variation introduced, and the extent to which founder effects and inbreeding after population bottlenecks may have contributed to evolutionary change. Here, we studied microsatellite variation in eight polymorphic loci among and within 27 native and 26 introduced populations of garlic mustard (*Alliaria petiolata*), a European herb which is a current serious invader in North American deciduous forests. Overall, introduced populations were genetically less diverse. However, considerable variability was present and when compared to the probable source regions, no bottleneck was evident. Observed heterozygosity was very low and resulted in high inbreeding coefficients, which did not differ significantly between native and introduced populations. Thus, selfing seems to be equally dominant in both ranges. Consequently, there was strong population differentiation in the native (F_{ST} = 0.704) and the introduced (F_{ST} = 0.789) ranges. The high allelic diversity in the introduced range strongly suggests multiple introductions of *Alliaria petiolata* to North America. Out of six European regions, the British Isles, northern Europe, and central Europe had significantly higher proportions of alleles, which are common to the introduced range, and are therefore the most probable source regions. The genetic diversity established by multiple introductions, and the lack of inbreeding depression in this highly selfing species, may have contributed to the invasion success of *Alliaria petiolata*.

Keywords: biological invasions, genetic differentiation, invasive species, microsatellites, population genetics, private alleles

Received 13 October 2004; revision received 12 January 2005; accepted 26 January 2005

Introduction

Invasive species offer some of the best model systems for studying rapid evolutionary change (Thompson 1998; Mooney & Cleland 2001; Sakai *et al*. 2001; Hänfling & Kollmann 2002; Müller-Schärer *et al*. 2004). First, introductions are often associated with genetic bottlenecks that may cause evolution by genetic drift and inbreeding (Brown & Marshall 1981; Barrett & Richardson 1986). Second, hybridization among native and introduced species, or among formerly distant invader genotypes, may create novel genetic material (Ellstrand & Schierenbeck 2000). Third, novel biotic or abiotic conditions in the introduced range may result in rapid adaptive evolution (e.g. Baker 1974; Blossey & Nötzold 1995; Bossdorf *et al*. 2004a; Maron *et al*. 2004). Some of these evolutionary processes could play a role in the success of invaders and possibly explain the frequent lag times between introduction and spread (Mack *et al*. 2000; Mooney & Cleland 2001; Sakai *et al*. 2001).

Molecular markers are important tools in this context because they provide information about pathways of introduction and the amount of genetic variation introduced (Barrett & Shore 1989). The latter, in particular, determines the species potential for postinvasion evolution and at the same time, the extent to which founder effects may have contributed to evolutionary change. In addition, it is often of great interest to identify the source regions, to learn both about dispersal routes and the extent to which invaders might have been 'pre-adapted' to their novel habitats

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(Neuffer & Hurka 1999; Milne & Abbott 2000; Novak & Mack 2001). Finally, spatial population genetic data from both ranges provide excellent opportunities to study gene flow, dispersal, and colonization on a geographical scale. However, apart from a handful of studies (Novak & Mack 1993; Neuffer & Hurka 1999; Amsellem *et al*. 2000; Squirrell *et al*. 2001; Maron *et al*. 2004), comprehensive analyses of molecular genetic variation in native vs. introduced populations are still surprisingly rare.

Garlic mustard, *Alliaria petiolata*, is a European herb that has become one of the most rapidly expanding current invaders in deciduous forests in North America (Nuzzo 2000; Welk *et al*. 2002). Recent common garden experiments found significant genetic differences between native and introduced populations in terms of competitive ability (Bossdorf *et al*. 2004a), allelopathic potential (Prati & Bossdorf 2004), and herbivore resistance (Bossdorf *et al*. 2004b). However, the roles that genetic drift vs. adaptive evolution have played in creating these patterns are still unclear, because we do not know to what extent garlic mustard has undergone a genetic bottleneck during its invasion. Nuzzo (1993) conducted a study of herbarium specimen that showed a westward spread of *A. petiolata* from presumably multiple founder populations. One previous study used molecular markers to analyse genetic variation in eight North American and three European *Alliaria* populations (Meekins *et al*. 2001), but the small sample size limited its ability to draw conclusions about genetic bottlenecks and pathways of introduction. Here, we have addressed these issues in a comprehensive study on population samples that covered the entire native and introduced range of *A. petiolata*. We used recently developed microsatellite markers (Durka *et al*. 2004) to analyse molecular genetic variation among and within native and introduced populations.

Specifically, we addressed the following questions: (i) How is genetic diversity in *A. petiolata* partitioned between individuals, populations, and continents? (ii) Can we identify the source populations from which garlic mustard invaded North America? (iii) Is there an overall reduced genetic diversity in the introduced range?

Materials and methods

Study species

Garlic mustard, *Alliaria petiolata* (M. Bieb.) Cavara & Grande, is a hexaploid ($2n = 42$) member of the mustard family (Brassicaceae) native to the Eurasian temperate zone. The species has a mostly biennial life cycle. Plants typically germinate in early spring, form a rosette in the first year, overwinter as a rosette, develop flowering stems in the following spring, produce seeds in June or July, and die. Because *A. petiolata* is self-compatible and plants often self-pollinate before the flower is open (Anderson *et al*. 1996; O. Bossdorf, personal observation), selfing appears to be the predominant breeding system (see Cruden *et al*. 1996 for a different opinion). Dispersal is only by seeds (Cavers *et al*. 1979).

Alliaria petiolata contains glucosinolates, which are responsible for the species' garlic-like odour, act as a defence against generalist and a feeding stimulant for specialist herbivores (Renwick *et al*. 2001; Renwick 2002), and may be involved in allelopathic interference with other plant species (Vaughn & Berhow 1999; Roberts & Anderson 2001; Prati & Bossdorf 2004).

In Europe, garlic mustard occurs in mesic semishade habitats such as forest edges and moist woodlands. In the 19th century, the species was introduced to North America. Over the last few decades, it rapidly expanded its introduced range (Nuzzo 1993) and became one of the most serious invaders in the woodland habitats in North America (Fig. 1). It is now present in at least 34 US states and four Canadian provinces (Nuzzo 2000; Welk *et al*. 2002). Garlic mustard invades the understory of deciduous forests where it may displace native plant species (McCarthy 1997) or disrupt plant–insect associations (Porter 1994; Huang *et al*. 1995). As a consequence, the species has become the target of a recently established biocontrol program (Blossey *et al*. 2001).

Sampling scheme

We used plant material from 27 native European and 26 introduced North American populations of *A. petiolata* (Table 1). In both ranges, our collections covered the present geographical distribution fairly well (Fig. 1). In each population, mature siliques were collected from several mother plants chosen to represent the entire population extent. The seeds were cleaned and stored under cold dry conditions. In spring 2003, seeds from eight to 16 seed families per population were dark stratified at 4 °C for 100 days. After that, plants were cultivated in a climate chamber with a 12/12-hour light/dark cycle at 12/8 °C. One seedling per family was harvested when leaf area had reached approximately 1 cm^2 and instantly frozen at –25 °C. Altogether, we harvested 552 seedlings, 318 European and 234 North American, from 53 populations.

Microsatellite analysis

Eight microsatellite loci previously developed for *A. petiolata* (Durka *et al*. 2004) were analysed. DNA was extracted from whole frozen seedlings using the DNeasy 96 Plant Kit (QIAGEN). Polymerase chain reaction (PCR) was performed in a total volume of 10 μL with 2 μL of 1:10 diluted DNA solution and 2 pM of each fluorescence-labelled forward and unlabelled reverse primers using the methods previously described (Durka *et al*. 2004). PCR products were separated on an ABI 310 genetic analyser (Applied Biosystems) with

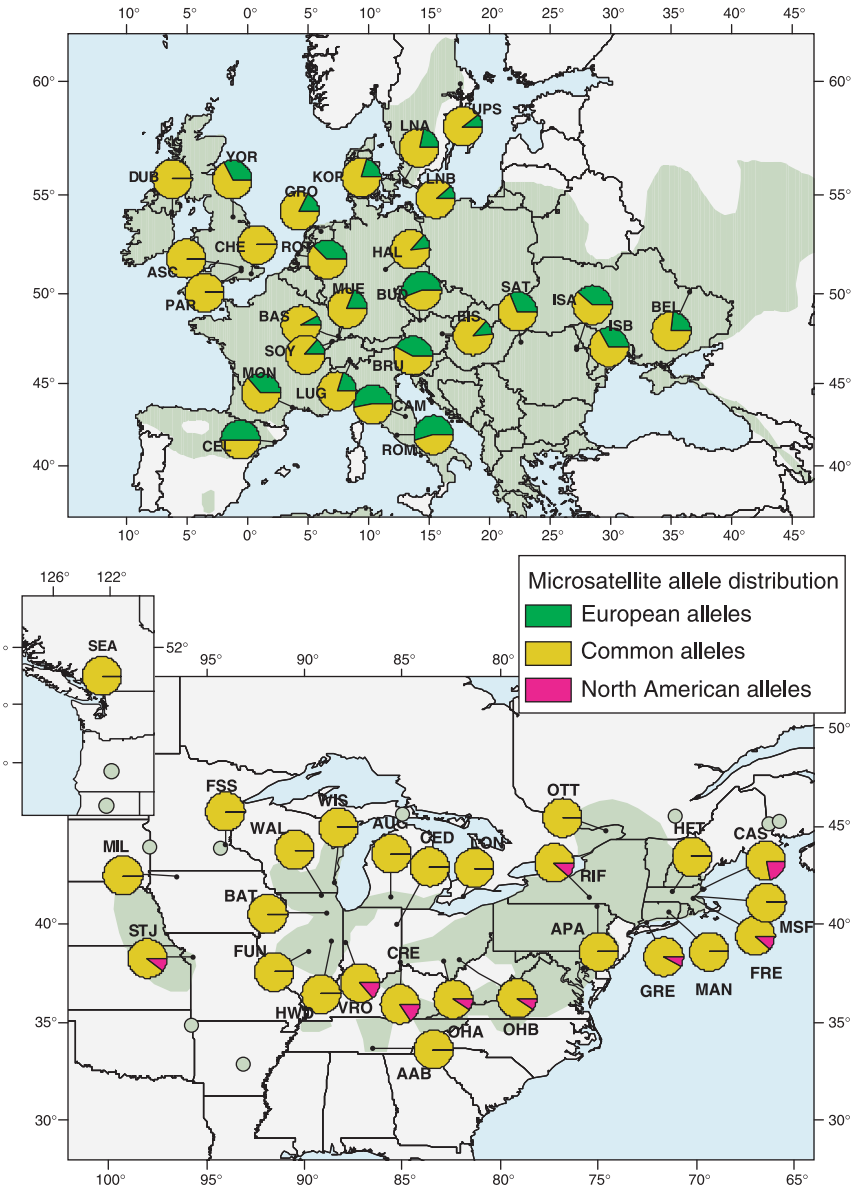

Fig. 1 Map of the native and introduced ranges of *Alliaria petiolata* with sampling locations and allele frequencies of European alleles found only in the native range (green), American alleles found only in the introduced range (pink), and common alleles (yellow) based on 552 individuals.

GeneScan 500 ROX as internal size standard. Individuals were genotyped using the GENOTYPER version 2.0 software (Applied Biosystems). To characterize genetic diversity at the population level, we calculated the mean number of alleles (A), observed heterozygosity (H_O), expected heterozygosity (H_E), and the number of multilocus genotypes (N_{GT}) using the software MSA version 3.0 (Dieringer & Schlötterer 2003). Inbreeding coefficients F_{IS} and their significance levels were calculated using FSTAT version 2.8 (Goudet 1995).

Statistical analysis

The global structure of genetic variability was described using F-statistics (Weir & Cockerham 1984) and the analysis of molecular variance (AMOVA; Excoffier *et al*. 1992) was carried out using ARLEQUIN version 2.000 (Schneider *et al*. 2000). To test for geographical structuring of genetic variability within continents, populations were grouped into geographical regions of approximately equal sample size prior to AMOVA (Table 1). Isolation by distance was tested by regressing pairwise F_{ST} over the log-transformed geographical distances between pairs of populations. Significance was tested by a Mantel test with 1000 randomizations performed using ARLEQUIN version 2.000. Rousset's (1997) method that uses pairwise $F_{ST}/(1 - F_{ST})$ estimates could not be applied because F_{ST} estimates were 1.0 in several cases. In addition, we tested for broad-scale geographic patterns in genetic diversity by calculating, separately for Europe and North America, the population-level correlations between genetic diversity and longitude and latitude.

Table 1 Geographical position of sampling sites with measures of genetic diversity at eight microsatellite loci: *N*, number of samples analysed; *A*, mean number of alleles; H_O, observed heterozygosity; H_E, expected heterozygosity; F_{IS}, inbreeding coefficient (**$P < 0.001$, *$P < 0.05$); N_{GT}, number of multilocus genotypes; and private alleles, number of European or North American alleles

ID	Long name	Country/state (region)†	Latitude	Longitude	*N*	*A*	H_O	H_E	F_{IS}	N_{GT}	Private alleles
Europe											
ASC	Ascot	Great Britain-BI	51.25° N	00.41° W	8	1.13	0.000	0.063	1.00**	4	0
BAS	Basel	Switzerland-CE	47.33° N	07.36° E	6	3.00	0.000	0.591	1.00**	6	2
BEL	Belgorod	Russia-EE	50.38° N	36.36° E	8	2.00	0.067	0.290	0.62**	5	3
BRU	Bruck	Austria-SCE	47.18° N	12.49° E	15	4.50	0.008	0.573	0.99**	8	16
BUD	Budweis	Czech Republic-SCE	48.58° N	14.29° E	8	1.13	0.000	0.050	1.00*	2	5
CAM	Camerino	Italy-SE	43.07° N	13.04° E	16	1.75	0.047	0.134	0.39**	6	8
CEL	St Celoni	Spain-SE	41.47° N	02.28° W	8	1.33	0.000	0.122	1.00**	3	5
CHE	Cheapside	Great Britain-BI	51.24° N	00.38° W	8	1.00	0.000	0.000	n.d.	1	0
DUB	Dublin	Ireland-BI	54.14° N	06.15° W	8	1.00	0.000	0.000	n.d.	1	0
EIS	Eisenstadt	Austria-SCE	47.50° N	16.32° E	8	1.00	0.000	0.000	n.d.	1	1
GRO	Groningen	The Netherlands-NE	53.13° N	06.00° E	16	1.50	0.070	0.157	0.56**	11	2
HAL	Halle	Germany-CE	51.28° N	11.58° E	16	3.00	0.016	0.461	0.97**	11	3
ISA	Iasi 1	Romania-EE	47.09° N	27.38° E	16	3.38	0.031	0.526	0.96**	14	11
ISB	Iasi 2	Romania-EE	47.09° N	27.38° E	16	1.63	0.000	0.141	1.00**	7	5
KOP	Copenhagen	Denmark-NE	55.43° N	12.34° E	8	1.75	0.016	0.201	0.95**	5	3
LNA	Lund 1	Sweden-NE	55.42° N	13.10° E	16	2.25	0.039	0.249	0.73**	6	4
LNB	Lund 2	Sweden-NE	55.42° N	13.10° E	8	1.13	0.016	0.016	–0.03	2	1
LUG	Lugano	Switzerland-SCE	46.01° N	08.57° E	16	3.13	0.094	0.467	0.87**	14	5
MON	Montpellier	France-SE	43.36° N	03.53° E	16	2.25	0.109	0.463	0.70**	13	7
MUE	Müllheim	Germany-CE	47.48° N	07.37° E	16	2.63	0.070	0.216	0.79**	6	4
PAR	Parish church	Great Britain-BI	51.25° N	00.41° W	8	1.13	0.000	0.029	1.00	2	0
ROM	Rome	Italy-SE	41.57° N	12.28° E	16	2.38	0.158	0.459	0.63**	11	11
ROT	Rotterdam	The Netherlands-NE	51.55° N	04.29° E	14	2.00	0.028	0.234	0.75**	9	6
SAT	Satu Mare	Romania-EE	47.48° N	22.52° E	8	1.38	0.000	0.112	1.00**	5	4
SOY	Soyhieres	Switzerland-CE	47.24° N	07.22° E	16	1.63	0.016	0.186	0.86**	7	2
UPS	Uppsala	Sweden-NE	59.55° N	17.38° E	8	1.25	0.016	0.045	0.48	2	1
YOR	York	Great Britain-BI	54.08° N	01.15° W	8	1.13	0.000	0.029	1.00	2	3
North America											
AAB	Arnold Air Force Base (Tullahoma)	USA/Tennessee-S	35.21° N	86.12° W	8	1.13	0.031	0.050	0.36	3	0
APA	Appalachia	USA/New York-N1	42.05° N	76.08° W	8	1.38	0.000	0.117	1.00**	4	0
AUG	Augusta	USA/Michigan-N2	42.21° N	85.22° W	8	1.00	0.000	0.000	n.d.	1	0
BAT	Batavia	USA/Illinois-N2	41.51° N	88.20° W	8	1.50	0.141	0.243	0.41*	6	0
CAS	Castle Hill	USA/Massachusetts-E	42.41° N	70.51° W	8	1.13	0.000	0.029	1.00	2	2
CED	Cedarville Reserve, Fort Wayne	USA/Indiana-N2	41.12° N	85.02° W	8	1.00	0.000	0.000	n.d.	1	0
CRE	Crescent Spring	USA/Kentucky-S	39.05° N	84.58° W	16	3.00	0.128	0.504	0.74**	15	4
FRE	Fresh Pond	USA/Massachusetts-E	42.22° N	71.06° W	15	2.13	0.092	0.410	0.81**	5	2
FSS	Fort Snelling State Park (St Paul)	USA/Minnesota-W	44.54° N	93.12° W	8	1.13	0.000	0.029	1.00	2	0
FUN	Funk Forest	USA/Illinois-S	40.29° N	89.00° W	8	1.00	0.000	0.000	n.d.	1	0
GRE	Greenwich	USA/Connecticut-E	41.02° N	73.37° W	11	3.00	0.182	0.350	0.46**	8	2
HFT	Harvard forest	USA/Massachusetts-E	42.54° N	72.17° W	8	1.13	0.000	0.029	1.00	2	0
HWD	Hart woods	USA/Illinois-S	40.23° N	88.09° W	8	1.00	0.000	0.000	n.d.	1	0
LON	London	Canada/Ontario-N1	42.58° N	81.15° W	8	1.38	0.047	0.047	–0.03	3	0
MAN	Mansfield	USA/Connecticut-E	41.50° N	72.16° W	8	1.00	0.000	0.000	n.d.	1	0
MIL	Milford	USA/Iowa-W	43.19° N	95.10° W	8	1.13	0.000	0.029	1.00	2	0
MSF	Middlesex Fells	USA/Massachusetts-E	42.25° N	71.05° W	8	1.00	0.000	0.000	n.d.	1	0
OHA	Ohio I	USA/Ohio-S	39.20° N	82.83° W	8	1.25	0.016	0.078	0.48*	3	1
OHB	Ohio II	USA/Ohio-S	39.19° N	82.07° W	8	1.25	0.016	0.076	0.79*	4	1
OTT	Ottawa	Canada/Ontario-N1	45.25° N	75.43° W	8	1.00	0.000	0.000	n.d.	1	0
RIF	Richford	USA/New York-N1	42.21° N	76.11° W	8	1.13	0.016	0.016	–0.03	2	1
SEA	Seattle	USA/Washington-W	47.35° N	122.20° W	8	1.00	0.000	0.000	n.d.	1	0
STJ	St Joseph	USA/Missouri-W	39.46° N	94.52° W	8	1.13	0.000	0.029	1.00	2	1
VRO	Vermilion River observatory	USA/Illinois-S	40.09° N	87.37° W	16	2.38	0.221	0.503	0.58**	11	3
WAL	Walworth	USA/Wisconsin-N2	42.29° N	88.37° W	8	1.13	0.016	0.066	0.76*	3	0
WIS	Wisconsin	USA/Wisconsin-N2	43.05 °N	87.53° W	8	2.75	0.047	0.401	0.92**	7	0

†Europe: BI, British Isles; CE, central Europe; EE, eastern Europe; NE, northern Europe; SCE, south-central Europe; SE, southern Europe; North America: E, East coast; N1, northeast; N2, north-central; S, south; and W, west.

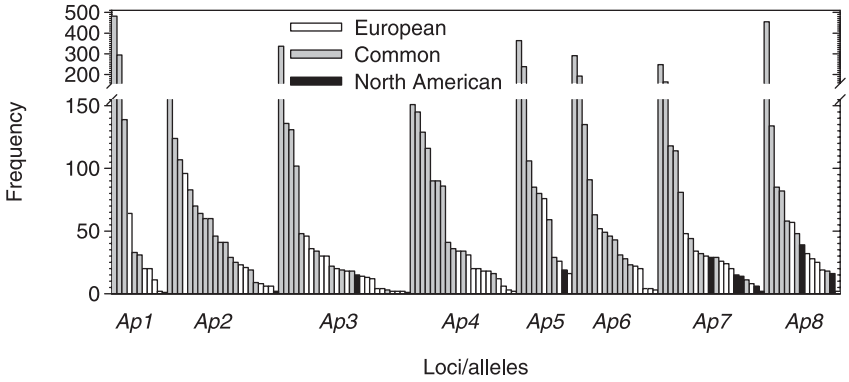

Fig. 2 Rank abundance plot and occurrence in Europe and North America of the 144 alleles found at eight microsatellite loci in 552 *Alliaria petiolata* individuals.

Neither the construction of phylogenetic trees nor the phenetic clustering methods was the appropriate method to detect invasion pathways. Because of the large genetic distances among populations, these methods yielded highly unresolved trees that did not allow any conclusions about the source regions. Instead, we attempted to track down invasion pathways by comparing the presence of alleles between North America and the six European regions (Table 1): British Isles (BI), northern Europe (NE); central Europe (CE), south-central Europe (SCE), eastern Europe (EE) and southern Europe (SE). For each region, we calculated the number and proportion of common alleles that occurred both in Europe and North America and the European alleles that were unique to Europe. Alleles that occur only in North America are referred to as American alleles. We tested for significance differences among numbers of common alleles among regions with a nonparametric chi-squared test.

To test for reduced genetic diversity in the introduced range, we calculated continental averages of population genetic diversity in terms of number of alleles A, number of multilocus genotypes N_{GT}, and expected heterozygosity H_E. This was carried out using both all individuals analysed and the eight randomly selected per population to assure equal sample size. We used t-tests to test for differences between European and North American populations, and between North America and the subset of the most probable source regions in Europe.

Results

Allelic diversity at the species level

In eight microsatellite loci analysed, we identified 144 alleles (mean of 17.3 alleles per locus). One hundred and thirty-three alleles (92% of the total) were present in Europe and 86 (60%) in North America (Fig. 1). There was a greater number of the European alleles than the North American alleles: 58 alleles (40%) were restricted to Europe, whereas only 11 (8%) were restricted to North America. The frequencies of the European alleles in the European samples ranged from 0.16% to 15.1% (mean of 3.39%), whereas those of the North American from 0.21% to 8.26% (mean of 3.04%) (Fig. 2). Among the 552 individuals analysed, there were 250 distinct multilocus genotypes, of which 68% occurred only once. The vast majority of genotypes (98%) were restricted to one population. Only five multilocus genotypes were found to occur in two or three populations, most of which were adjacent to each other and always on the same continent (1, sites ISA and ISB; 2, WAL, GRE; 3, WAL, FUN; 4, OHA, OHB, VRO; and 5, OHA, OHB).

Population differentiation and spatial genetic structure

In an analysis of molecular variance (AMOVA), the majority of genetic variation (71%) resided among populations, 25% within populations, and only 3.8% of the total genetic variation was due to the differences between continents (Table 2). When continents were analysed separately, population differentiation was slightly greater in the introduced range: 70% and 79% of genetic variation was partitioned among populations in the native and introduced ranges, respectively. In a hierarchical AMOVA, we analysed the partitioning of molecular variance among regions and populations within continents. In Europe there was more pronounced geographical structure as 10.8% of genetic variation resided among regions and 59.9% among populations within regions. In contrast, only 6.8% of genetic variation resided among regions and 71.4% among populations within regions in North America. Genetic distances (F_{ST}) among populations were large in both ranges [Europe: mean pairwise F_{ST} = 0.740 (SD 0.164) and North America: mean pairwise F_{ST} = 0.818 (SD 0.191)]. Thus, both native and introduced populations were highly structured, with the majority of genetic variation retained among populations.

Regressions of genetic distances over geographical distances were not significant for Europe (Mantel test, r^2 = 0.019, P = 0.105). They were significant, but very weak for North America (r^2 = 0.043, P = 0.002). Thus, garlic mustard did not show isolation by distance at the scales investigated.

In Europe, genetic diversity was significantly correlated with latitude and declined from south to north (Fig. 3). Although diversity was particularly low in the westernmost European

Table 2 Analysis of molecular variance (AMOVA) showing the distribution of genetic variation among continents, populations, and individuals

Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation
Native + introduced ranges				
Among continents	1	125.1	0.129***	3.74
Among populations within continents	51	2630.3	2.456***	70.72
Within populations	1045	927.0	0.887***	25.54
Total	1097	3682.5	3.472	
Native range				
Among regions	5	484.4	0.390***	10.82
Among populations within regions	21	1050.1	2.158***	59.87
Within populations	603	637.4	1.057***	29.32
Total	629	2171.9	3.606	
Introduced range				
Among regions	4	238.2	0.210**	6.79
Among populations within regions	20	807.6	2.209***	71.42
Within populations	427	287.8	0.674***	21.79
Total	451	1333.7	3.094	

***$P < 0.001$, **$P < 0.01$.

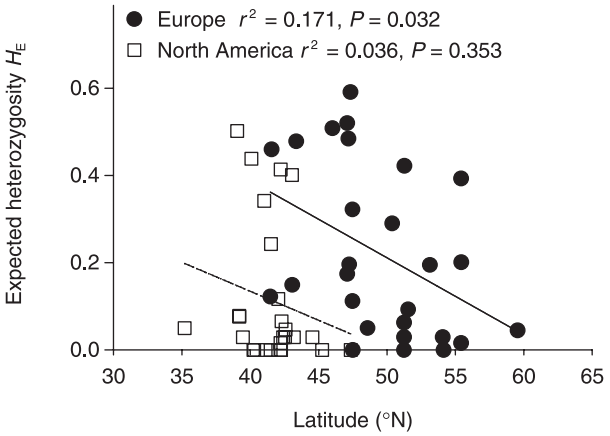

Fig. 3 The relationship between expected heterozygosity and latitude among 27 native European (black circles) and 26 introduced North American (white squares) populations of *Alliaria petiolata*.

sites (British Isles, Spain) no significant longitudinal cline was detected in Europe. No clines were found in the introduced range ($P > 0.6$).

Identification of source regions

Under a null hypothesis of random sampling, all European regions should have contributed the same proportion of their alleles to the introduced range. A higher proportion of common alleles, rather than their absolute number, will therefore identify the probable source regions. In contrast, high proportions of European alleles identify those regions that are unlikely to have been the sources of invasions.

The highest proportion of common alleles shared with North America was found in the British Isles (90%), followed by CE (81%) and NE (77%) without significant difference among each other (Table 3). SCE, SE, and EE had less than 65% of their alleles in common with North America. They had significantly less common alleles than the British Isles, and did not differ significantly among each other (Table 3).

Genetic diversity within populations

At the population level, genetic diversity varied considerably, ranging from fixation at a single multilocus genotype to high polymorphism at all loci investigated (Table 1). On average, introduced populations were genetically less diverse with respect to mean number of alleles per locus, expected heterozygosity, and number of multilocus genotypes (Table 4). These differences between the continents were either significant or marginally significant depending on whether we analysed all individuals (eight to 16) or an equal number of only eight randomly selected per population (Table 4). The total number of alleles at eight loci was higher in the European populations (mean $A = 15.7$, maximum $A = 37$) than that of North American (mean $A = 11.6$, maximum $A = 27$). Expected heterozygosity in Europe was twice as high as in North America. The number of multilocus genotypes was significantly reduced in North America (Table 2). In the native range, only three populations (11%) consisted of a single homozygous multilocus genotype, fixed for alleles at all loci, whereas there were eight such populations (31%) in the North American range. Thus, in a range wide comparison, introduced populations showed a genetic bottleneck. However, when we restricted the comparison to the most probable source regions and only included the British Isles, NE and CE, the genetic diversity at

Table 3 Total number of alleles in six European regions and number and proportion of common alleles shared with North America or restricted to Europe. For sites within regions, see Table 1

	British Isles (BI)		Northern Europe (NE)		Central Europe (CE)		South–central Europe (SCE)		Eastern Europe (EE)		Southern Europe (EE)	
	n	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%
Total no. of alleles	29	100.0	47	100.0	47	100.0	65	100.0	49	100.0	50	100.0
Alleles common with North America*	26	89.7[a]	36	76.6[ac]	38	80.9[ad]	42	64.6[bcd]	31	63.6[bcd]	25	50.0[b]
Alleles Restricted to Europe	3	10.3	11	23.4	9	19.1	23	35.4	18	36.7	25	50.0

*Values followed by the same letter are not significantly different at $P = 0.05$.

Table 4 Genetic diversity of *Alliaria petiolata* at eight microsatellite loci among 26 invasive North American, 27 native European and a subset of 15 European populations representing the most probable source regions. *t*-tests for the comparison of North America and the two European data sets

	North America		Europe			Most probable European regions (BI, NE, CE)†		
	Mean	SD	Mean	SD	*t*-test *P*	Mean	SD	*t*-test *P*
All samples*								
Number of alleles, A	1.42	0.64	1.90	0.89	0.030	1.70	0.72	0.210
Number of genotypes, N_{GT}	3.54	3.43	6.07	4.10	0.018	5.00	3.42	0.196
Observed heterozygosity, H_O	0.04	0.06	0.03	0.04	0.633	0.02	0.02	0.307
Expected heterozygosity, H_E	0.12	0.17	0.22	0.19	0.052	0.17	0.17	0.376
Inbreeding coefficient, F_{IS}	0.68	0.34	0.80	0.26	0.203	0.77	0.30	0.438
Eight randomly selected samples per population								
Number of alleles, A	1.41	0.61	1.76	0.66	0.052	1.63	0.67	0.279
Number of genotypes, N_{GT}	3.00	2.15	4.41	2.19	0.022	4.07	2.34	0.147
Observed heterozygosity, H_O	0.04	0.07	0.03	0.05	0.868	0.02	0.03	0.414
Expected heterozygosity, H_E	0.11	0.16	0.22	0.19	0.034	0.17	0.18	0.283
Inbreeding coefficient, F_{IS}	0.67	0.35	0.79	0.28	0.199	0.77	0.31	0.412

*Number of individuals per population varies from 8 to 16 (mean = 11.8, SD = 4.2 for European and mean = 9.0, SD = 2.5 for North American populations).
†Means of the regions BI, British Isles; NE, northern Europe; and CE, central Europe, see Table 1.

population level did not differ significantly between native and introduced ranges (Table 4).

Observed heterozygosity was generally very low, as expected for a highly selfing species. All loci showed significant deficiency of heterozygotes. The average inbreeding coefficient was very high and did not differ significantly between native and introduced populations (Table 4). Levels of outcrossing calculated from these inbreeding coefficients according to Nei & Syakundo (1958) were $t = 0.12$ and 0.20, respectively, and indicate selfing rates above 80% in both ranges.

Discussion

The main results of this study are (i) garlic mustard populations are highly differentiated in both ranges; (ii) three European regions are likely the source regions; and (iii) although North American populations have reduced allelic variation compared to Europe, the substantial overall genetic variation indicates multiple introductions to North America. In the following, we will first discuss the numbers of introductions and their potential source regions, and then address the issue of genetic bottlenecks. Thereafter, we discuss implications for ecological differentiation and the success of this invasive species.

Potential source regions and numbers of introductions

Overall, garlic mustard's populations were highly differentiated and there was no indication of isolation by distance. Thus, gene flow among populations may be much lower than genetic drift, probably the result of the high selfing rates and the limited seed dispersal capability of garlic mustard.

Based on a limited set of native populations, Meekins *et al*. (2001) hypothesized that the North American populations

of garlic mustard may have originated from the British Isles. The microsatellite data presented here do not allow an analysis of geographical origin at the level of single populations. The large genetic distances among populations preclude detailed phylogenetic analyses. However, the distribution of common and European alleles proved to be a valuable source of information. Of the six European regions (Table 3), British Isles shared the highest proportion of their alleles with North America (90%), followed by CE (81%) and NE (77%). Significantly smaller proportions of common alleles were found in SCE, EE, and SE. In the British Isles, only one population (YOR) contained European alleles, whereas all other populations from other regions had European alleles (Fig. 1). Some populations from other regions, mainly NE and CE, had very few European alleles too (e.g. LNB and UPS in Sweden, Table 1). Taken together, our data support Meekins *et al*. (2001) in so far as the British Isles are a probable source of introduction of garlic mustard to North America. However, other, equally probable sources are CE and NE.

The microsatellite markers used here were highly polymorphic with up to 26 alleles per locus. High allelic diversity is attributed to the high mutation rate that these loci experience (Goldstein & Schlötterer 1999). Consequently, new alleles are expected to originate at relatively frequent rates both in the native and introduced ranges, and specific alleles are not necessarily homologous. Inference of source populations therefore must be made with caution and should not be based on single alleles. In our study, the large number of alleles (n = 144) allowed us to analyse proportions of shared or European alleles. We are confident in this analysis because there are (i) differentiation among regions in Europe, (ii) higher allelic diversity in SE, and (iii) the presence of a number of regional alleles, especially in the south. A latitudinal cline in genetic diversity (Fig. 3) is typical for species that recolonized Europe after the last glaciation (e.g. Durka 1999; Chauvet *et al*. 2004). Thus, although new microsatellite alleles may originate throughout the range at relatively frequent rates, historical patterns of allelic diversity are retained in the native range and thus can be used to identify potential source regions. Eleven alleles detected were unique to the introduced range. These North American alleles can be either novel alleles as a result of mutation after the introduction or of insufficient sampling of the native range.

Several lines of argumentation suggest multiple independent introductions of *Alliaria petiolata* to North America. When pooled over all populations, the total number of alleles in North America is clearly much higher than in any single native population. A single introduction from one European population can therefore be excluded. Both the amount of differentiation among populations and the occurrence of various multilocus genotypes in North America suggest introductions from several native populations. Given that populations in the putative source regions had a low genetic diversity, a substantial number of introductions must have occurred to create the allelic diversity currently found in the introduced range. It is well known that the species was traditionally used as spice (it is also called 'sauce-alone' in the British Isles) and medicinal plant in Europe. It is likely therefore that European settlers could have introduced garlic mustard for personal use during the 19th century from various source populations.

Bottlenecks and genetic diversity in the introduced range

Populations of invasive species often have low levels of genetic diversity within and among populations (Baker 1974; Morgan *et al*. 1981; Barrett & Richardson 1986; Warwick & Black 1986; Rapson & Wilson 1988; Wang *et al*. 1995; Williams *et al*. 1995). In the extreme, single genotypes of clonal species may invade large geographical areas by vegetative reproduction (Amsellem *et al*. 2000; Hofstra *et al*. 2000; Hollingsworth & Bailey 2000; Xu *et al*. 2003). The degree to which biological invasions are accompanied by genetic bottlenecks (Barrett & Richardson 1986) depends on the species breeding systems and is expected to be lowest in highly selfing species or those that reproduce vegetatively (Brown & Marshall 1981). Outbreeding species, in contrast, often display high levels of genetic diversity in both native (Hamrick & Godt 1996) and introduced ranges (Godt & Hamrick 1991; Pappert *et al*. 2000). There are, however, only a small number of studies that explicitly compared genetic diversity in native vs. introduced populations (e.g. Warwick *et al*. 1987; Maron *et al*. 2004). In fact, most of these did not find pronounced genetic bottlenecks (Bossdorf *et al*. 2005).

Here, we found substantial molecular genetic variation within and among introduced garlic mustard populations. Similar patterns have been found in other invasive species (e.g. McCauley *et al*. 2003; Maron *et al*. 2004). Overall, introduced populations had a slightly, but significantly, reduced genetic diversity (Table 4). Still, when we compared introduced populations to those in European regions, where they most likely originated from, no significant differences were found. We therefore conclude that there is no pronounced bottleneck in the introduced range. On the other hand, several North American populations consisted of a single multilocus genotype (Table 1**)**, which suggests that some North American populations may have served as source of origin for other North American populations, and thereby created repeated bottlenecks in the introduced range (Amsellem *et al*. 2000). Estimated inbreeding coefficients did not differ between native and introduced populations (Table 4). Thus, in garlic mustard there is no indication of an evolutionary shift towards a more selfing breeding system,

which has been suggested by Barrett & Richardson (1986) as a general evolutionary pattern in invasive species.

Implications for ecological differentiation and invasion success

Recent experimental studies with *A. petiolata* have demonstrated genetic differentiation between native and introduced populations in a number of potentially adaptive traits (Bossdorf *et al*. 2004a, b). Introduced populations showed reduced competitive ability, allelopathic potential, and reduced resistance against specialist herbivores. Such pattern could be explained by adaptive evolution or by the effects of reduced genetic diversity or drift after a population bottleneck. However, we found little evidence for a genetic bottleneck in invasive garlic mustard populations. Moreover, our molecular data suggest that levels of inbreeding are equally high in native and introduced populations. Thus, inbreeding depression is unlikely to have arisen during colonization because genetic load should have been purged during the evolution of the species in its native range (Husband & Schemske 1996). In conclusion, it seems unlikely that reduced genetic variability at the individual or population level after population bottlenecks was the primary cause for evolutionary change in introduced populations of *A. petiolata*. In contrast, multiple introductions may have created considerable genetic variability in the introduced range, thereby increasing the evolutionary potential and the invasion success of this invasive species.

Acknowledgements

The authors thank Ina Geyer and Martina Herrmann for their technical assistance. This work would not have been possible without the generous help of many seed collectors: Debora Arlt, Bernd Blossey, Yvonne Buckley, Enzo Colonelli, E. Davidian, Alecu Diaconu, Elizabeth Gault, Thomas Hickler, Betsy Hickey, Hariet Hinz, Jan Jedlicka, Johannes Kollmann, Kristin Lewis, Domenico Lucarini, Ross McCauley, Forrest Meekins, Dick Pegtel, Staffan Roos, Mark Schwarzländer, Leo Soldaat, Montserrat Vilà, Mark Williamson, and Rüdiger Wittenberg.

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This work is part of a series of papers of the research group on the ecology and evolution of invasive species. Walter Durka is interested in applying molecular methods to problems of conservation and invasion biology. Oliver Bossdorf is interested in the ecological genetics of invaders, plant–plant interactions, and phenotypic plasticity. Daniel Prati is a plant population biologist particularly interested in clonal plants and biological invasions. Harald Auge is interested in plant population ecology with a particular focus on plant–insect interactions and evolutionary ecology.
