

Multiple introductions boosted genetic diversity in the invasive range of black cherry (*Prunus serotina*; Rosaceae)

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• **Background and Aims** Black cherry (*Prunus serotina*) is a North American tree that is rapidly invading European forests. This species was introduced first as an ornamental plant then it was massively planted by foresters in many countries but its origins and the process of invasion remain poorly documented. Based on a genetic survey of both native and invasive ranges, the invasion history of black cherry was investigated by identifying putative source populations and then assessing the importance of multiple introductions on the maintenance of gene diversity.

• **Methods** Genetic variability and structure of 23 populations from the invasive range and 22 populations from the native range were analysed using eight nuclear microsatellite loci and five chloroplast DNA regions.

• **Key Results** Chloroplast DNA diversity suggests there were multiple introductions from a single geographic region (the north-eastern United States). A low reduction of genetic diversity was observed in the invasive range for both nuclear and plastid genomes. High propagule pressure including both the size and number of introductions shaped the genetic structure in Europe and boosted genetic diversity. Populations from Denmark, The Netherlands, Belgium and Germany showed high genetic diversity and low differentiation among populations, supporting the hypothesis that numerous introduction events, including multiple individuals and exchanges between sites, have taken place during two centuries of plantation.

• **Conclusions** This study postulates that the invasive black cherry has originated from east of the Appalachian Mountains (mainly the Allegheny plateau) and its invasiveness in north-western Europe is mainly due to multiple introductions containing high numbers of individuals.

Key words: Microsatellites, putative sources, invasive tree species, phylogeography, population genetics, black cherry, *Prunus serotina* var. *serotina*.

INTRODUCTION

The success of non-indigenous plants in areas distant from their native range have caused considerable economic and ecological costs worldwide (e.g. Wilcove *et al.*, 1998; Forman, 2003; Simberloff, 2003; Pimentel *et al.*, 2005). Different stages are usually recognized in the invasion process, ranging from the introduction of a species to its spread into the new range (With, 2002; Lockwood *et al.*, 2005; Theoharides and Dukes, 2007). Because invasive species are introduced by human activities either intentionally or unintentionally, introduction modes can greatly vary among species. Propagule pressure constitutes a particularly important factor in the first steps of invasion. This composite term accounts for both the number of individuals – propagule size – within a single introduction event and the number of independent introductions – propagule number (Lockwood *et al.*, 2005). Both propagule size and number play a major role in the dynamics of species' establishment. The introduction of a small number of individuals in a new environment can potentially limit the establishment of a species if there is not enough genetic variation for the adaptive

evolutionary changes required by the new selection pressures to take place (Suarez and Tsutsui, 2008; Okada *et al.*, 2009). On the other hand, multiple introductions and subsequent gene flow between populations may maintain part or all genetic variation present in native populations (Carlton, 1996; Novak and Mack, 2001; Lockwood *et al.*, 2005; Facon *et al.*, 2008; Prentis *et al.*, 2008; Henry *et al.*, 2009; Wilson *et al.*, 2009). The influence of these multiple introductions has not yet been fully resolved and a recent review has pointed out that the influence of propagule number on the ability of a species to spread is dependent on both spatial and temporal aspects of the multiple introductions (Dlugosch and Parker, 2008). Nevertheless, multiple introductions from genetically differentiated source populations have been increasingly found in successful plant invasions (Hufbauer and Sforza, 2008; Prentis *et al.*, 2009). The time needed for populations resulting from different introductions to become connected is then dependent on the initial size of the introduced populations and the geographical distance separating them.

Key steps in the understanding and management of biological invasions are the frequency with which a species is

introduced into a specific area, the size of each introduction and the subsequent pattern of spread across the new range. Several population genetic studies have been devoted to answer these questions by identifying source populations (e.g. Carlton, 1996; Meekins *et al.*, 2001; Besnard *et al.*, 2007; Schlaepfer *et al.*, 2008), comparing genetic structure of invasive populations with those in the native range (e.g. Eckert *et al.*, 1996; Prentis *et al.*, 2009), unravelling invasion histories (e.g. Carlton, 1996; Neuffer and Hurka, 1999; Meekins *et al.*, 2001; Gaskin *et al.*, 2005; Le Roux *et al.*, 2008; Henry *et al.*, 2009), or seeking the role played by evolutionary processes in establishment and spread (for reviews, see Prentis *et al.*, 2008; Suarez and Tsutsui, 2008).

Combining molecular markers with different modes of inheritance can be highly useful to assess population structure in invasive species (e.g. Gaskin *et al.*, 2005; Grapputo *et al.*, 2005). Chloroplast DNA (cpDNA) is generally maternally inherited in angiosperms, and cpDNA markers thus record gene flow coming from seed movement only, whereas nuclear markers record gene flow of both pollen and seeds (Devos *et al.*, 2003). In addition, polymorphisms in haploid genomes are more affected by genetic drift than those from the nuclear genome (Petit *et al.*, 1993). Patterns of the spatial distribution of cpDNA polymorphism established by seed dispersal during range expansion should therefore be more slowly eroded by subsequent gene flow, when compared with spatial patterns in nuclear genetic markers, especially in trees (e.g. McCauley *et al.*, 2003; Petit and Hampe, 2006).

Trees have long been transferred across countries and continents without being considered as potential threats (Petit *et al.*, 2004; Bucharova and van Kleunen, 2009). In most cases, invasive trees have a long history of successive introductions before being eventually classified as problematic aliens. Among these trees is the black cherry (*Prunus serotina*; Rosaceae), which was one of the first North American tree species to be introduced into Europe (Starfinger *et al.*, 2003). Five varieties are usually recognized in its natural range that covers most of eastern north America (i.e. var. *serotina*, *eximia*, *rufula*, *virens* and *salicifolia*; McVaugh, 1951). The most common and widespread variety *serotina* produces valued furniture wood, but only in a limited portion of its range on the Allegheny plateau of Pennsylvania, New York and West Virginia (Marquis, 1990). The first record of the species in Europe dates back to 1623 when the tree was planted for ornamental purposes near Paris (Starfinger, 1997). Until the late 18th century, *P. serotina* was sparsely planted in parks and gardens in several European countries. Recommended in 1810 for silvicultural practices, it was then more intensively planted for growing high quality timber trees and increasing forest production mainly on poor sandy soils (Muys *et al.*, 1992). These experimental plantations rarely produced good quality wood. Between 1900 and 1930, black cherry was planted for multiple uses such as wind and firebreaks, improving the sandy soils under coniferous plantations, or providing shelter (Starfinger, 1997; Starfinger *et al.*, 2003; Pairon *et al.*, 2006a; Verheyen *et al.*, 2007; Vanhellemont *et al.*, 2010). Massive underplanting and filling of conifer stands occurred until the late fifties in Belgium, Germany and The Netherlands (Muys *et al.*, 1992). Black cherry has been planted for forestry purposes since the

1980s in Poland and Romania and local introductions were performed in Italy and the UK (Muys *et al.*, 1992). Presently, the species grows spontaneously in many European countries from France to Poland and Romania as well as from Denmark to Italy. *Prunus serotina* is spreading throughout temperate forests in north-western Europe, especially on well-drained poor soils. This tree is now considered as one of the 100 most invasive alien species in Europe (DAISIE, 2009). It competes for resources with native plant species, especially during forest regeneration and under high herbivore pressure (e.g. Verheyen *et al.*, 2007; Chabrierie *et al.*, 2008; Vanhellemont *et al.*, 2009). Invaded stands have higher levels of phosphorus, a shallower litter layer, and lower pH values than non-invaded stands (Chabrierie *et al.*, 2008). Shading out light-demanding species, especially seedlings of other tree species, it can impede natural regeneration of native tree species and induces a decrease in species richness, mainly in disturbed stands. Fruits are produced in high quantities and are well dispersed, mainly by birds. More than 50 % of the seeds are dispersed at higher distances than 50 m; the average dispersal distance being estimated to 257 m (Pairon, 2007; Chabrierie *et al.*, 2008). Despite important investment, the control of its spread remains ineffective (Vanhellemont *et al.*, 2009).

In this study, the invasion history of black cherry in Europe was inferred from genetic patterns. Both cpDNA markers and nuclear microsatellite loci were used to genotype native and invasive populations. This study was undertaken to (a) find the putative source(s) of European populations and (b) test whether invasive populations have lower genetic diversity than native populations.

MATERIALS AND METHODS

Sampling scheme

Prunus serotina Ehrh. var. *serotina* plant material was collected from 23 populations in the invasive range and from 22 populations in the native range (Table 1) for a total number of 442 and 321 trees, respectively. The collection covered nearly the entire geographical distribution of the species (Fig. 1A, B). Samples were collected in countries where the species is known to be abundant and/or of commercial value in northern America and where it is classified as invasive or naturalized in Europe. In each population, two or three leaves were sampled from 10–20 randomly chosen trees. The leaves were either dried for shipping or frozen in liquid nitrogen. DNA was extracted from 1 cm² of leaf tissue using a modified CTAB protocol (Pairon and Jacquemart, 2005).

Microsatellite and cpDNA typing

Eight nuclear microsatellite loci previously described for *P. serotina* (Table 2; Pairon *et al.*, 2008) were amplified in all sampled individuals. Polymerase chain reactions (PCR) were performed in a total volume of 15 µL using the methods previously described (Pairon *et al.*, 2006b). PCR products were separated on an ABI3100 genetic analyser (Applied Biosystems) and individuals genotyped using GENEMAPPER 3.5 (Applied Biosystems).

TABLE 1. Geographical location of the *Prunus serotina* populations sampled in this study together with their basic genetic information content at eight microsatellite loci

Pop ID	Location	Country (state)	Latitude	Longitude	<i>N</i>	<i>N_A</i>	<i>N_{PA}</i> [†]	<i>A</i>	<i>H_O</i>	<i>H</i>	<i>F_{IS}</i>
Native range											
CA	Guénette	Canada (Quebec)	46.50 °N	-75.25 °W	15	6.63	4 (0)	5.92	0.75	0.74	-0.02
IN	Bloomington	USA (Indiana)	39.19 °N	-86.51 °W	15	8.00	4 (1)	6.75	0.62	0.70	0.12
IA1	Ames	USA (Iowa)	42.04 °N	-93.68 °W	15	8.25	4 (0)	7.17	0.83	0.81	-0.02
IA2	New Sharon	USA (Iowa)	41.58 °N	-92.50 °W	15	6.88	6 (1)	5.92	0.60	0.70	0.14***
MO	Springfield	USA (Missouri)	37.17 °N	-93.33 °W	15	8.00	13 (2)	6.93	0.69	0.75	0.08
NC	Otto	USA (North Carolina)	35.03 °N	-83.48 °W	10	6.50	4 (0)	6.31	0.67	0.76	0.12
NE1	Lincoln	USA (Nebraska)	40.81 °N	-96.64 °W	14	7.50	16 (2)	6.64	0.66	0.73	0.09
NE2	Omaha	USA (Nebraska)	41.26 °N	-96.01 °W	15	8.25	14 (1)	7.15	0.75	0.78	0.03
OH1	Ashtabula	USA (Ohio)	41.89 °N	-80.79 °W	15	7.50	4 (0)	6.58	0.72	0.74	0.03
OH2	Delaware	USA (Ohio)	40.37 °N	-83.05 °W	14	8.50	14 (1)	7.27	0.73	0.79	0.07
OH3	Ashville	USA (Ohio)	39.67 °N	-82.93 °W	14	7.75	8 (1)	6.72	0.74	0.73	-0.01
OK	Broken Bow	USA (Oklahoma)	33.97 °N	-94.54 °W	13	6.13	13 (3)	5.61	0.64	0.63	-0.02
PE1	Behrend	USA (Pennsylvania)	42.11 °N	-79.98 °W	15	7.38	2 (0)	6.38	0.74	0.76	0.02
PE7	Denton Hill	USA (Pennsylvania)	41.77 °N	-77.82 °W	15	8.50	12 (0)	7.45	0.82	0.81	0
PE2	Buckaloons	USA (Pennsylvania)	41.80 °N	-79.25 °W	15	9.38	11 (2)	7.83	0.74	0.76	0.03
PE3	Chapman Dam	USA (Pennsylvania)	41.75 °N	-79.17 °W	15	7.75	10 (0)	6.66	0.71	0.79	0.1
PE4	Tionesta	USA (Pennsylvania)	41.71 °N	-78.94 °W	15	8.25	8 (0)	7.27	0.84	0.80	-0.05
NY	Pawling	USA (Pennsylvania)	41.58 °N	-73.61 °W	15	7.88	3 (0)	6.74	0.77	0.78	0
SC	Charleston	USA (South Carolina)	32.75 °N	-79.91 °W	15	7.13	12 (1)	6.32	0.68	0.78	0.13*
WI1	Saukville	USA (Wisconsin)	43.39 °N	-88.02 °W	15	8.13	6 (1)	6.83	0.71	0.74	0.05
WI2	Madison	USA (Wisconsin)	43.04 °N	-89.44 °W	15	8.63	14 (2)	7.31	0.77	0.77	0.01
WV	Spruce Knob	USA (West Virginia)	38.71 °N	-79.54 °W	14	7.38	5 (0)	6.70	0.82	0.79	-0.04
Mean						7.74	-	6.75	0.72	0.76	0.03
Invasive range											
BE	Lagland	Belgium	49.40 °N	05.46 °E	20	7.13	0 (1)	5.69	0.72	0.72	0.007
DA1	Copenhagen	Denmark	55.95 °N	12.27 °E	20	6.88	0	5.83	0.74	0.75	0.006
DA2	Tisvilde	Denmark	56.05 °N	12.08 °E	14	7.00	0	6.28	0.79	0.74	-0.069
DA3	Aarhus	Denmark	56.18 °N	9.65 °E	14	6.38	0	5.82	0.71	0.73	0.04
FR1	Fontainebleau	France	48.38 °N	2.75 °E	20	4.63	0 (1)	4.14	0.57	0.59	0.035
FR2	Alsace	France	48.72 °N	7.68 °E	16	6.50	0	5.65	0.66	0.72	0.09
FR3	Compiègne	France	49.37 °N	2.88 °E	20	7.25	0 (1)	6.08	0.74	0.74	-0.004
FR4	Bordeaux	France	44.13 °N	0.90 °W	20	6.38	0 (2)	5.29	0.71	0.70	-0.023
GE1	Potsdam S	Germany	52.73 °N	12.30 °E	20	7.5	0 (1)	5.46	0.76	0.75	0.008
GE2	Potsdam T	Germany	52.47 °N	13.22 °E	20	6.38	0	6.16	0.71	0.72	-0.003
GE3	Neuwig	Germany	50.43 °N	7.63 °E	20	7.13	0	5.94	0.72	0.72	0.008
GE4	Mannheim N	Germany	49.65 °N	8.53 °E	20	7.25	0	5.95	0.69	0.74	0.068
GE5	Karlsruhe	Germany	48.97 °N	8.37 °E	20	7.13	0	5.87	0.73	0.70	-0.042
GE6	Saarbrücken	Germany	49.30 °N	7.23 °E	19	6.75	0	5.78	0.68	0.74	0.08
GE7	Cuxhaven	Germany	53.82 °N	8.63 °E	20	6.63	0	5.67	0.69	0.74	0.07
HO1	Arnhem	The Netherlands	52.00 °N	5.83 °E	20	7.25	0	5.82	0.73	0.71	-0.014
HO2	Amsterdam	The Netherlands	52.35 °N	4.55 °E	19	6.63	0	5.54	0.63	0.71	0.123
IT1	Ticino	Italy	45.52 °N	8.72 °E	20	4.50	0	4.08	0.66	0.64	-0.018
IT2	Udine	Italy	46.12 °N	13.17 °E	20	3.13	0	2.98	0.60	0.55	-0.088
PO1	Poznan	Poland	52.17 °N	17.15 °E	20	5.38	0	4.76	0.67	0.68	0.015
PO2	Wroclaw	Poland	51.37 °N	16.55 °E	20	5.00	0	4.49	0.69	0.61	-0.119
UK1	Shakelford	UK	51.18 °N	0.65 °W	20	4.63	0	3.91	0.59	0.59	0.006
UK2	Bagshot	UK	51.38 °N	0.73 °W	20	5.50	0 (2)	5.03	0.76	0.75	-0.011
Mean						6.36	-	5.31	0.69	0.70	0.01

N is the number of individuals sampled per population, *N_A* is the mean number of alleles per locus, *A* is the mean allelic richness per locus (based on *n* = 10) and *N_{PA}* is the number of private alleles. Estimates of observed heterozygosity (*H_O*), gene diversity (*H*) and inbreeding coefficient (*F_{IS}*) are also given. * 0.01 < *P* < 0.05, *** *P* < 0.001

[†] For the private alleles, the first number is the number of alleles private to a given range (invasive or native) in the considered population, while the number in parenthesis is the number of alleles that are private to the considered population in its range.

Five cpDNA loci previously described for *P. serotina* (Petitpierre *et al.*, 2009) were used on a subsample of ten randomly chosen individuals per population (i.e. 230 individuals in the invasive range and 220 in the native range). These markers are derived from non-coding plastid regions and correspond either to length variation in polyT/A stretches (ccmp5, *trnT-trnL*-poly-T and *trnT-trnL*-poly-A) or to restriction fragment length polymorphisms due to a single nucleotidic substitution (*trnD-trnT-TasI* and *trnS-trnG-TasI*).

Data analyses

Analysis of nuclear microsatellite data. For each microsatellite locus, the total number of alleles, Nei's unbiased estimate of gene diversity (*H_S*; Nei, 1987), and the inbreeding coefficient (*F_{IS}*) were separately estimated for invasive and native populations using FSTAT 2.9 (Goudet, 1995). To characterize further the genetic structure within loci, Wright's fixation index (*F_{ST}*) and its analogue based on allele size (*R_{ST}*;

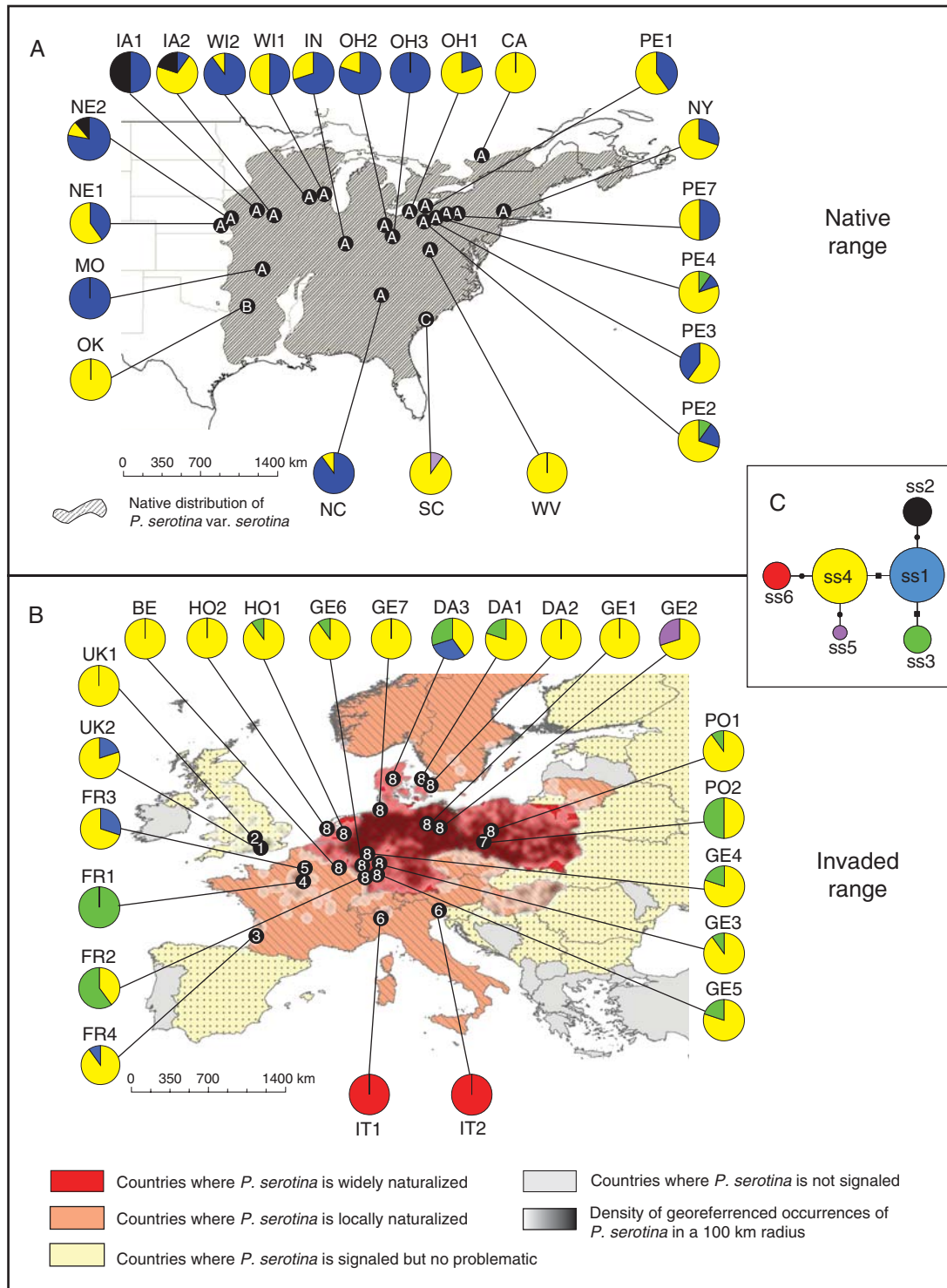


FIG. 1. Geographical distribution of nuclear and chloroplast genetic variation in *Prunus serotina*. (A) Native range with sampling locations. The hatched area indicates the natural occurrence of *P. serotina* var. *serotina* in the wild. Dots on the map represent the three genetic clusters defined by BAPS based on nuclear microsatellite data. These dots are labelled from A to C according to the cluster they belong to. Pie charts represent the relative proportions of cpDNA haplotypes in a population, as indicated in (C). (B) Invasive range with sampling locations. Dots on the map represent the eight genetic clusters defined by BAPS based on the nuclear microsatellite data. The dots are numbered from one to eight according to the cluster they belong to. (C) Median-joining network of cpDNA haplotypes. The size of the circles represents the relative frequency of the haplotypes. The biggest size corresponds to haplotypes with a frequency > 10 %, the intermediate size corresponds to haplotypes with a frequency between 1 and 10 % and the smallest size corresponds to haplotypes with a frequency < 1 %. Steps between haplotypes are either insertion/deletion (i.e. length variation – dots) or nucleotide substitutions without length variation (squares).

TABLE 2. Genetic diversity detected at eight microsatellite loci in invasive and native *Prunus serotina*

Locus	Allele size range		Total no. of alleles		H_S		F_{IS}		F_{ST}			F'_{ST}		
	Invasive	Native	Invasive ($n = 442$)	Native ($n = 321$)	Invasive	Native	Invasive	Native	Invasive	Native	Whole dataset	Invasive	Native	Whole dataset
UDP96-005	82–88	82–96	4	7	0.57	0.60	0.025 ^{ns}	0.09 ^{ns}	0.06*	0.09*	0.09*	0.16*	0.24*	0.24*
UDP96-025	109–137	108–137	13	18	0.78	0.84	–0.008 ^{ns}	0.07*	0.09*	0.03*	0.08*	0.41*	0.20*	0.42*
UDP96-405	109–123	109–123	6	8	0.51	0.63	0.119 ^{ns}	0.01 ^{ns}	0.15*	0.09*	0.14*	0.32*	0.26*	0.32*
UCD-CH14	130–168	124–182	19	29	0.81	0.90	–0.002 ^{ns}	0.02 ^{ns}	0.10*	0.04*	0.09*	0.56*	0.47*	0.60*
PccGA34	135–175	131–175	19	24	0.80	0.89	–0.030 ^{ns}	0.01 ^{ns}	0.08*	0.05*	0.08*	0.38*	0.45*	0.53*
Pchpgms2	127–159	125–163	11	18	0.69	0.55	0.007 ^{ns}	–0.02 ^{ns}	0.09*	0.13*	0.12*	0.30*	0.29*	0.32*
Pchpgms3-1	168–198	166–198	11	12	0.65	0.79	–0.012 ^{ns}	0.05 ^{ns}	0.10*	0.05*	0.10*	0.30*	0.23*	0.33*
Pchpgms3-2	200–238	200–238	15	18	0.80	0.86	0.011 ^{ns}	0.08 ^{ns}	0.07*	0.05*	0.06*	0.35*	0.32*	0.37*
Mean					0.70	0.76	0.01 ^{ns}	0.03 ^{ns}	0.09*	0.06*	0.09*	0.35*	0.31*	0.39*

The Nei's gene diversity per locus (H_S) and Wright's inbreeding coefficient (F_{IS}) were averaged over the native and invasive populations. The Wright's fixation index (F_{ST}) and an analogue unconstrained by gene diversity (F'_{ST}) were averaged over the native and invasive populations or the whole dataset. ^{ns}, not significant; *, $P < 0.001$.

Michalakis and Excoffier, 1996) were computed using SPAGEDi 1.2 (Hardy and Vekemans, 2002). To detect whether F_{ST} and R_{ST} were significantly different from 0, SPAGEDi was also used to perform a 10 000-permutations test on alleles. F_{ST} values cannot be higher than the homozygosity level, $1 - H_S$ (Hedrick, 2005). F'_{ST} , an estimator unconstrained by genetic diversity, was therefore calculated as $F_{ST}/1 - H_S$. 10 000 randomizations tests were performed to assess the differences of genetic diversity and differentiation between the native and invaded range. The partitioning of the gene diversity at different geographic scales (i.e. within population, among populations and between invasive and native ranges) was also measured using an AMOVA with ARLEQUIN 3.1.1 (Excoffier *et al.*, 2005).

To characterize the genetic diversity at the population level over the eight loci, the observed heterozygosity (H_O) was calculated with GENALEX 6.0 (Peakall and Smouse, 2006), as well as the allelic richness (A), Nei's unbiased estimate of gene diversity (H_S ; Nei, 1987) and the inbreeding coefficient (F_{IS}) with FSTAT. Significance of the inbreeding coefficient was assessed with FSTAT using permutations. Hardy–Weinberg equilibrium was tested using GENEPOP 3.4 (Rousset, 2008). The number of private alleles was computed for each population in the native range.

Introduced populations are often subject to founder effects that result in reduced genetic diversity (i.e. a bottleneck). To assess the decrease in diversity from native to invasive populations, three different methods were used. First, the values of allelic richness and gene diversity averaged over the whole invasive and native ranges were compared, postulating that a genetic bottleneck should decrease the number of alleles present in the invasive range. Differences in A and H_S were assessed using a paired t -test in the SAS software version 8.2 (SAS Institute, 1999). Secondly, BOTTLENECK 1.2 (Cornuet and Luikart, 1996) and the Wilcoxon test for heterozygote excess were used under the two-phase model and the stepwise mutation model. The Wilcoxon test is preferable when few loci are used (Cornuet and Luikart, 1996; Cornuet *et al.*, 1999). Thirdly, the allele frequencies were plotted for each invasive population based on the method described in Luikart *et al.* (1998). This method postulates that recent bottlenecks should cause a decrease in rare alleles, creating a characteristic mode-shift distortion in the distribution of allele frequencies.

The population genetic structure at the geographic level is shaped by different forces such as genetic drift and the possibility of inter-population gene exchanges. The characterization of genetic relationships between populations can be informative about their histories. The relatedness among populations was first assessed by computing F -statistics (Weir and Cockerham, 1984) and Nei's standard genetic distance (D ; Nei, 1972) between pairs of invasive populations. A significance test of 10 000 permutations of the estimates of F_{ST} and D obtained was performed using GENETIX 4.052 (Belkhir *et al.*, 2000). The genetic structure in the invasive range was further described, together with that of the native range, by using the Bayesian clustering method implemented in BAPS 4.14 (Corander and Martinen, 2006). BAPS uses an analytical integration strategy combined with stochastic search methods to infer the number of clusters K and was run using both

genotype and sample group information (group mode). The maximum number of populations (prior information) was set to 40.

The genetic clusters defined by BAPS in the native range were then used as reference populations in GENECLASS2 (Piry *et al.*, 2004) to assign individuals from invasive populations. The frequency-based method of Paetkau *et al.* (1995) and Monte-Carlo resampling was used to compute assignment probabilities with the simulation algorithm of Piry *et al.* (2004). To gain insight into the source of the clusters defined by BAPS in the invasive range, the proportion of individuals assigned to the different clusters of the native range was calculated within each cluster of the invasive range.

Analysis of cpDNA data. To characterize the genetic diversity of the chloroplast genome, the allelic richness (A), cpDNA haplotype diversity within populations (H_S), total cpDNA haplotype diversity (H_T) and the level of genetic differentiation (F_{ST}) within both ranges were calculated using FSTAT. Ten thousand randomizations tests were performed to assess the differences of genetic diversity and differentiation between the native and invaded range. Relationships among cpDNA haplotypes were visualized by constructing a statistical median-joining network implemented in NETWORK 4.112 (Bandelt *et al.*, 1999).

Haplotypes were then plotted on maps to illustrate their geographical distribution and the putative introduction sources. Their percentage distribution was also estimated within both ranges.

RESULTS

Genetic diversity

Within microsatellite loci. All eight nuclear microsatellite loci were polymorphic in each population, and a total number of 134 alleles was observed on the whole sample (Table 2). Ninety-eight alleles were observed in invasive populations, while all of them were detected in native populations (Table 2). Thirty-six alleles (27 %) were private to the native range. Overall gene diversity (H_S) was also higher in the native range at the majority of loci (Table 2). F_{IS} ranged from -0.02 to 0.09 and a significant deviation from Hardy–Weinberg equilibrium was found at locus UDP96-025 but only in the native range. F_{ST} showed an overall differentiation of 0.06 in the native range and 0.09 in the invasive range. R_{ST} values were not significantly different from F_{ST} and were thus not shown. F'_{ST} values also showed that the overall differentiation in the native range (0.31) was lower than in the invasive range (0.35 ; Table 2) but this difference was not significant. The AMOVA analysis indicated that genetic diversity was mainly partitioned within populations ($89 \pm 2.9\%$), then among populations ($8 \pm 0.3\%$), and then between the native and invasive ranges ($3 \pm 0.1\%$).

Chloroplast DNA haplotypes. Considering allelic combinations at the five cpDNA loci, six haplotypes were detected in the whole dataset. Both native and invasive ranges shared four haplotypes (Table 3). Haplotype ss2 was private to the native range and only found in the western part of the United States (Fig. 1A). On the other hand, haplotype ss6

TABLE 3. Compared occurrences of the six cpDNA haplotypes (with their frequency given in parenthesis) in both native and invasive ranges of *Prunus serotina*

Haplotype no.	Native range	Invasive range
ss1	96 (43.8 %)	9 (3.9 %)
ss2	8 (3.7 %)	–
ss3	2 (0.9 %)	34 (14.8 %)
ss4	112 (51.1 %)	164 (71.3 %)
ss5	1 (0.5 %)	3 (1.3 %)
ss6	–	20 (8.7 %)

was private to the invasive range and exclusively found in northern Italy (Fig. 1B). Haplotypes ss1 and ss4 were by far the most abundant (Table 3), and displayed a central position in the median-joining network (Fig. 1C). This network also suggests that ss2 and ss3 have derived from ss1 while ss5 and ss6 have derived from ss4. The level of genetic differentiation measured by F_{ST} averaged 0.40 and 0.52 in native and invasive populations (Table 4), respectively, but these values were not significantly different ($P = 0.49$).

Sources of invasive populations

Nuclear microsatellite data. BAPS in group mode found three clusters in the native range (Fig. 1A) and eight clusters in the invasive range (Fig. 1B). The three clusters found in the native range only showed that one cluster (A) was formed by all populations except OK and SC, which accounted for the two remaining groups (B and C, respectively). The genetic structure was more pronounced in the invasive range and the output of the program suggested that the two populations from the United Kingdom (UK1-UK2), populations FR1, FR3 and FR4 (France), and population PO2 (Poland) were all distinct genetic clusters. The two populations from Italy represented another independent cluster, and the last cluster was formed by the remaining populations (i.e. populations from Denmark, Germany, The Netherlands and Belgium and populations FR2 and PO1). When the individual genotypes from the invasive range were used to assign each individual to one of the three clusters of the native range, very few trees were assigned to clusters B and C, and the vast majority was assigned to the main cluster (A) in the native range. When the clusters found in the invasive range were reconstructed and the proportion of individuals within each cluster that were

TABLE 4. Chloroplast DNA diversity in native and invasive *Prunus serotina* populations

Range	N	N_A	A	H_S	H_T	F_{ST}
Native	219	5	1.953	0.333	0.547	0.402*
Invasive	230	5	1.651	0.225	0.461	0.519*

Haplotypes were defined by the combination of alleles at five loci. Number of individuals (N), number of haplotypes (N_A), mean allelic richness per population (A), haplotype diversity within populations (H_S), total haplotype diversity (H_T) and the level of genetic differentiation (F_{ST}) are given for each range.

* $P < 0.001$.

TABLE 5. Proportion of individuals belonging to the eight clusters from the invasive range (Fig. 1B) assigned to each of the three clusters defined in the native range (Fig. 1A)

Clusters in invasive range	Clusters in native range		
	A (293)	B (13)	C (15)
1 (20)	0.65	0.00	0.15
2 (20)	0.95	0.00	0.05
3 (20)	0.95	0.00	0.05
4 (20)	0.00	0.50	0.00
5 (20)	0.95	0.00	0.15
6 (40)	0.83	0.00	0.25
7 (20)	0.95	0.05	0.30
8 (282)	0.90	0.00	0.20

The number of individuals within each cluster (for both native and invasive ranges) is given in parenthesis. Note that individuals can be assigned to more than one cluster.

assigned to the three main clusters of the native range were looked at, it was noted that 83–95 % of the individuals within clusters 2, 3, 5, 6, 7 and 8 were assigned to the main cluster (A) in the native range (Table 5). Clusters 1 and 4, comprising populations UK2 and FR1, respectively, showed a relatively low proportion of individuals assigned to the main cluster in the native range (65 % and 0 %, respectively), and even to any of the two other native clusters (0–15 % and 0–50 %, respectively).

Chloroplastic DNA data. cpDNA haplotype distribution gave more information as to which regions in the native range may have acted as source(s) for invasive populations (Table 3). This distribution was indeed quite different among the two ranges, and in the invasive area, a much lower proportion of the ss1 haplotype was detected, whereas ss3 and ss4 were overrepresented. The vast majority of populations located to the east of the Appalachian Mountains showed a predominant proportion of ss4, as observed in the invasive range, whereas most populations located to the west to the Appalachian Mountains showed a predominant proportion of ss1. Interestingly, ss3 was found in half of the European populations and only detected in two populations of the Allegheny plateau in the USA (PE2 and PE4; Fig. 1A). Finally, ss2 was not observed in the invasive range and was only found in the western part of the native range.

Partition of genetic diversity within native and invasive ranges

Within the native range. The 36 alleles that were private to the native range were relatively well distributed among the native populations (Table 1). Populations PE1 and NY (Pennsylvania) displayed, respectively, only two and three alleles that were not found in the invasive range whereas a maximum of 16 private alleles were found within population NE1 (Nebraska). The number of alleles per locus (N_A) was 7.74 in average, ranging from 6.13 to 9.38 per population. The mean allelic richness (A) ranged from 5.61 to 7.83 with a mean of 6.75. Gene diversity (H_S) ranged from 0.63 to 0.81 with a mean of 0.76 whereas mean observed heterozygosity (H_O) ranged from 0.60 to 0.84 among populations with a

mean of 0.72. The inbreeding coefficient (F_{IS}) varied widely among populations, ranging from -0.05 to 0.14 with a mean of 0.03. Significant deviations from Hardy–Weinberg genotypic proportions associated with positive F_{IS} values were found for populations IA2 and SC.

Within the invasive range. The mean allelic richness (A) was significantly lower in the invasive range (5.31) than in the native range (6.75) ($t = 6.79$, $P < 0.0001$) as expected by the higher number of alleles found in the native range (Table 1). This also resulted in a significantly lower gene diversity in the invasive range ($t = 3.75$, $P = 0.0005$; Table 1). The number of alleles per population from the invasive range varied more widely than in the native range. Populations FR1 (France), IT1, IT2 (Italy), PO1, PO2 (Poland), UK1 (United Kingdom) displayed the lowest amount of genetic diversity. Gene diversity (H_S) varied from 0.55 to 0.75 whereas mean observed heterozygosity (H_O) ranged from 0.57 to 0.79 among populations. None of the populations showed significant departure from Hardy–Weinberg proportions and inbreeding coefficients (F_{IS}) ranged from -0.12 to 0.07, with a mean of 0.01.

Even though some populations were identified as having a low number of alleles and a low gene diversity, only five populations at the margins of the invasive range (IT1, IT2, PO2, DA1 and UK2) were significantly detected as bottlenecked based on the Wilcoxon test under the two-phase model, and only two populations (IT2 and UK2) under the stepwise mutation model. In addition, the graphical method allowed two recently bottlenecked populations (PO1 and PO2) to be detected, with a marked distortion in the distribution of allele frequencies.

A maximum of two cpDNA haplotypes was found per population in the invasive range, whereas some populations of the native range exhibit three haplotypes (Fig. 1A and B). Allelic richness was significantly lower in the invasive range ($A = 1.65$) than in the native range ($A = 1.95$) (permutation test, $P = 0.04$; Table 4). The haplotype diversity within populations (H_S) and the total haplotype diversity (H_T) were lower in the invasive than in the native range (Table 4), and this difference was significant for H_T (permutation tests, $P = 0.02$) but not for H_S (permutation tests, $P = 0.054$).

DISCUSSION

The combined use of cpDNA and nuclear microsatellite loci aimed at comparing the genetic diversity of *P. serotina* var. *serotina* populations between native and invasive ranges and detecting the putative sources of invasive populations. Previous studies have demonstrated the importance of extensive sampling in both ranges (e.g. Novak and Mack, 1993; Eckert et al., 1996; Meekins et al., 2001; Okada et al., 2009) and the usefulness of combining markers with two different modes of inheritance (Williams et al., 2005) to achieve these goals, but as far as is known, the present study is one of the first attempts to answer these questions for a long-lived species.

Sources of invasive P. serotina

The presence of five cpDNA haplotypes in Europe suggests there were multiple introductions of individuals. The

distribution of haplotypes in the native range can be used to identify putative sources of invasive populations (e.g. Besnard *et al.*, 2007; Hufbauer and Sforza, 2008). First, frequencies of haplotypes ss1 and ss4 in both ranges and the distribution of haplotype ss2 all suggest that invasive *P. serotina* trees mainly originated from the eastern United States. Haplotype ss4 is very frequent in the invasive range (Table 3) and mainly distributed to the east of the Appalachian Mountains whereas ss1 is almost absent in Europe and very frequent in the western part of the native range (Fig. 1A). Moreover, ss2 is absent in Europe and only found in the north-western states of Indiana and Nebraska, further suggesting that such populations should not be considered as putative source regions. Secondly, ss3 is overrepresented in many invasive populations in Europe suggesting that the Allegheny plateau was an important source of introduction as this haplotype is only found in native populations PE2 and PE4 (where ss4 and ss1 are also present). *Prunus serotina* is harvested for timber only in the Allegheny plateau (Hough, 1965; Marquis, 1990) and it is not surprising that this area was an important source for introductions to Europe.

No strong geographic differentiation based on nuclear loci in the native range was detected. Only two populations in the southern native range (SC and OK) were distinguished from other populations (Fig. 1A). Their geographic isolation in the native range probably explains their high genetic differentiation from the main native cluster A. Human movement of the species for forestry purposes and high seed dispersal in the native range may in part be responsible for the low level of genetic differentiation within the large cluster A. Assignment tests of invasive individuals were not very informative as they only supported the assertion that cluster A was probably involved in most introductions to Europe. Nevertheless, the low number of native-range private alleles in some populations from the Allegheny plateau (e.g. PE1 and NY) again suggests, as cpDNA haplotypes, that this area within cluster A was the main source of introductions to Europe.

Genetic structure and low reduction of gene diversity in the invaded range

The overall genetic structure displayed by microsatellite loci in the present study was more pronounced in the invasive range than in the native range. Populations from Denmark, Germany, The Netherlands and Belgium had higher genetic diversity and lower differentiation among populations, while some populations from the United Kingdom, France and Italy had low allelic richness, low gene diversity and high genetic differentiation among populations. These two different patterns were supported by the output of BAPS as populations from Denmark, Germany, The Netherlands and Belgium were clustered together (cluster 8) whereas the other populations belonged to different separated clusters.

A reduction in the overall number of alleles, allelic richness and heterozygosity was found at microsatellite loci in the invasive range. Most introductions result in a temporary high reduction in the effective size of populations, and a reduction in diversity within invasive populations should therefore often be observed (Dlugosch and Parker, 2008). This reduction was particularly evident at microsatellite loci as these markers

contain many rare alleles, and are therefore more sensitive to founder effects (Spencer *et al.*, 2000). Despite the observed reduction in allelic diversity, strong evidence of population bottlenecks was only observed in some populations at the margins of the invaded range that probably correspond to recent introductions (PO2, DA1, IT1, IT2 and UK2). Indeed, the tests performed look at recent bottleneck events by searching for the allele depletion that should be generated by a sudden decrease of reproducing individuals in a newly formed population. Because allele depletion is a complex function that depends on several parameters, including the time since a population bottleneck (Cornuet and Luikart, 1996; Prentis *et al.*, 2008; Bucharova and van Kleunen, 2009), populations that had gone through a bottleneck several generations ago may not display allelic depletion any more (Dlugosch and Parker, 2008). This points to the importance of the temporal nature of introductions in genetic diversity studies as the particular time of study might affect the ability to detect past bottlenecks (Dlugosch and Parker, 2008).

Despite the reduction in genetic diversity in some marginally distributed populations, the invasive range in general possessed high levels of diversity (73 % of allelic richness), with an overall high gene diversity with microsatellite markers and the same number of cpDNA haplotypes in both ranges. The low reduction in genetic diversity can be attributed to multiple introductions with large numbers of individuals and the relatively long time since introduction (Bucharova and van Kleunen, 2009).

The initial propagule size and the occurrence of gene flow between established populations seem in the present case to be important factors explaining the genetic patterns observed in the invasive range. All species are likely to experience periods when founding populations are isolated from other conspecific populations, but this relative isolation cannot always be recorded, especially when natural gene flow has been rapidly restored among expanding populations, multiple introductions are geographically close to one another and/or human-mediated seed exchanges occur among introduced populations (Suarez and Tsutsui, 2008). Interestingly, genetically less diverse populations from France, Italy, Poland and England (FR1, IT1–IT2, PO1–PO2, UK1–UK2) are all situated on the margins of the species range in Europe and in countries where no records of massive plantations for forestry purposes are known (Starfinger, 1997). Populations from The Netherlands, Belgium, Germany and Denmark were more diverse and historical records support that propagule number was more important, with several massive introductions of the species for different purposes during the 19th century (Muys *et al.*, 1992; Starfinger, 1997). The relatively high genetic diversity in populations from north-western Europe probably contributed to the adaptive success and the invasive behaviour of *P. serotina*.

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