

Transatlantic invasion routes and adaptive potential in North American populations of the invasive glossy buckthorn, *Frangula alnus*

Hanne De Kort^{1,2,*}, Joachim Mergeay³, Hans Jacquemyn¹ and Olivier Honnay¹

¹Plant Conservation and Population Biology, Biology Department, University of Leuven, Kasteelpark Arenberg 31, B-3001 Heverlee, Belgium, ²Station d'Ecologie Théorique et Expérimentale du CNRS, Centre National de la Recherche Scientifique, 2 Route du CNRS, FR-09200 Moulis, France and ³Research Institute for Nature and Forest, Gaverstraat 4, B-9500 Geraardsbergen, Belgium

*For correspondence. E-mail hanne.dekort@bio.kuleuven.be

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- **Background and Aims** Many invasive species severely threaten native biodiversity and ecosystem functioning. One of the most prominent questions in invasion genetics is how invasive populations can overcome genetic founder effects to establish stable populations after colonization of new habitats. High native genetic diversity and multiple introductions are expected to increase genetic diversity and adaptive potential in the invasive range. Our aim was to identify the European source populations of *Frangula alnus* (glossy buckthorn), an ornamental and highly invasive woody species that was deliberately introduced into North America at the end of the 18th century. A second aim of this study was to assess the adaptive potential as an explanation for the invasion success of this species.
- **Methods** Using a set of annotated single-nucleotide polymorphisms (SNPs) that were assigned a putative function based on sequence comparison with model species, a total of 38 native European and 21 invasive North American populations were subjected to distance-based structure and assignment analyses combined with population genomic tools. Genetic diversity at SNPs with ecologically relevant functions was considered as a proxy for adaptive potential.
- **Key Results** Patterns of invasion coincided with early modern transatlantic trading routes. Multiple introductions through transatlantic trade from a limited number of European port regions to American urban areas led to the establishment of bridgehead populations with high allelic richness and expected heterozygosity, allowing continuous secondary migration to natural areas.
- **Conclusions** Targeted eradication of the urban populations, where the highest genetic diversity and adaptive potential were observed, offers a promising strategy to arrest further invasion of native American prairies and forests.

Key words: Adaptive potential, conservation, *Frangula alnus* Mill., invasive species, ornamental, population assignment, routes of invasion.

INTRODUCTION

Because many invasive species profoundly disrupt ecosystem functioning in their introduced range, unravelling the routes and mechanisms of successful exotic species invasions is a major goal in conservation biology, evolutionary ecology and pest management (Liao *et al.*, 2008; Wardle *et al.*, 2011; Richardson *et al.*, 2014; Barrett, 2015). Whereas plant traits such as short lifespans and clonal growth are generally considered to increase plant vigour during invasions (e.g. Daehler, 2003; van Klinken *et al.*, 2013), invading tree and shrub species are being increasingly recognized for their potential to impact ecosystems. More specifically, invasive trees and shrubs compromise the survival of numerous native species by dramatically altering soil chemical characteristics, hydrology, understorey light availability, parasite abundance and herbivore food quality and quantity (Mack *et al.*, 2000; Petit *et al.*, 2004; Liao *et al.*, 2008; Richardson and Rejmánek, 2011). This places woody species, which represent more than 65 % of the plant species reported on the widely cited list of '100 of the world's worst invaders' (Lowe *et al.*, 2000), among the most damaging and vigorous invaders of terrestrial ecosystems (Petit *et al.*, 2004; Richardson and Rejmánek,

2011). Due to their intentional and often massive introductions, shrubs and trees that were historically introduced for ornamental purposes in particular now dominate global invasion patterns (Richardson and Rejmánek, 2011). Despite the associated potentially detrimental effects, the mechanisms behind the success of woody plant invasions remain largely conceptual, jeopardizing the efficacy of targeted conservation actions (Estoup and Guillemaud, 2010; Richardson *et al.*, 2014).

One of the most prominent and fundamental questions in invasion biology is how invasive populations can overcome genetic founder effects, which are thought to reduce their genetic diversity and potential to adapt to the conditions in the introduced range, to the extent that they outperform well-adapted native species (Dlugosch and Parker, 2008; Forsman, 2014; Franks and Munshi-South, 2014; Barrett, 2015; Burrell *et al.*, 2015). Although both phenotypic plasticity (Davidson *et al.*, 2011; Higgins and Richardson, 2014) and epigenetic variation (Richards *et al.*, 2012) have also been proposed to compensate for this lack of population genetic variation, many invasive species have apparently benefited from repeated introductions, increasing propagule pressure and genetic variation in the new range (Kolbe *et al.*, 2004; Dlugosch and Parker, 2008; Matesanz

et al., 2014; Shirk *et al.*, 2014; Barriball *et al.*, 2015). Repeated introductions from multiple source populations can be expected to be especially beneficial for adaptive genetic diversity in the invasive range when the source populations in the native range cover distinct environmental conditions (e.g. Lavergne and Molofsky, 2007; Lucek *et al.*, 2010; Le Roux *et al.*, 2011; Rius and Darling, 2014). The introduction of genotypes from different environments may indeed facilitate invasion through pre-adaptation, and may generate novel allelic combinations with beneficial genotype \times environment interaction patterns (Lee, 2002; Lockwood *et al.*, 2005; Lavergne and Molofsky, 2007; Rius and Darling, 2014). High genetic variation in genes underlying ecologically relevant functions, i.e. genes governing ecological responses to local biotic and abiotic conditions (e.g. Wright *et al.*, 2014), may be particularly beneficial during species invasions, as they provide the adaptive potential necessary to cope with novel environments (Holderegger *et al.*, 2006; Eizaguirre and Baltazar-Soares, 2014).

Invasions of natural areas by ornamental shrubs and trees most likely originate from planted and nursed populations in urban areas (e.g. parks and public gardens). These urban populations may serve as bridgehead populations (*sensu* Lombaert *et al.*, 2010; Estoup and Guillemaud, 2010), enabling regular secondary migration to natural areas (Donaldson *et al.*, 2014). The success rate of secondary spread can be expected to further increase if the planted populations originate from genetically distinct native sources, a scenario that is not unlikely in the ornamental plant trade, given that larger trait variation provides a wider basis for artificial selection towards cultivars. Mixing source material may prevent genetic bottlenecks in park populations, and generates genetic variation upon which selection can act after migration to natural areas. It can therefore be hypothesized that introduced park populations harbour greater genetic variation than populations invading natural areas. A related hypothesis involves the expectation that urban populations are genetically very similar to the native source populations because the planted individuals directly originate from the native populations, or at least have been cultivated using native parental lineages, and because selection is of minor importance due to the mitigation of selection-driving environmental pressures by continuous population nursing. Ornamental species therefore offer good, yet hitherto unexploited, opportunities to study the routes and processes underlying invasiveness in this large group of invaders.

A specific case of an ornamental and highly invasive woody species concerns *Frangula alnus*, an insect-pollinated shrub native to Europe, where it typically occurs in habitats with high light availability, including wetlands and gaps in moist forests (Godwin, 1943). The species was introduced as an ornamental shrub in North America in the late 18th or the early 19th century, as indicated by historical American garden literature (M'Mahon, 1806) and by herbarium records originating from New Jersey (1879) and southern Ontario (1898) (Catling and Porebski, 1994; Aiello-Lammens, 2014). The early trade in *F. alnus* corroborates these findings, as multiple nurseries, including the Biltmore nursery (North Carolina, 1907), New England Nurseries (Massachusetts, 1910) and Storrs and Harrison (Ohio, 1925) commercially offered the species prior to 1930 (D. Adams, pers. comm.). The popularity of the species in parks and gardens likely facilitated the rapid spread throughout North America, from the early 20th century onwards (Aiello-Lammens, 2014). *Frangula*

alnus is now among the most abundant and aggressive invasive shrubs in North American forests and prairie fens, where it outcompetes the native flora and inhibits tree regeneration by forming dense thickets that block the sunlight (Fiedler and Landis, 2012; Lee and Thompson, 2012). Horticultural practices and efficient seed dispersal by birds, water and occasionally small mammals (Hampe, 2004) have likely facilitated the invasion of natural habitats by *F. alnus* (Richardson and Rejmánek, 2011). The species currently occurs in at least 25 American States and Canadian Provinces, from North Carolina and Tennessee in the south to Québec in the North, and from Idaho in the west to Prince Edward Island and Nova Scotia in the east (Natural Resources Conservation Service of the United States Department of Agriculture). Despite various conservation actions taken to manage *F. alnus* in invaded areas, including herbicide applications and cambium torching, the species continues to threaten natural ecosystems (Cunard and Lee, 2009; Hamelin *et al.*, 2015).

Here, we screened an extensive dataset of 38 native European and 21 North American invasive populations of *F. alnus* using 133 annotated single-nucleotide polymorphisms (SNPs) to (1) identify the European source regions and the intercontinental invasion routes, (2) test whether genetic diversity in the invasive range is higher in urban populations than in populations invading natural areas, and (3) assess the adaptive potential and ongoing adaptive evolution in the invasive range based on putatively functional loci. These aims were tackled using two SNP subsets that were defined based on their annotations, including a putatively neutral SNP set ($n = 34$) and a putatively functional SNP set ($n = 99$). The functional SNPs in particular played a major role in our study for several reasons. First, functional genetic variation may provide the adaptive potential to invade new areas. Second, functional loci are expected to be more sensitive to environmental changes, thereby allowing the study of adaptive evolution in the invasive range. Third, due to higher levels of genetic differentiation, especially in species with high gene flow, functional SNPs are more suitable for discriminating among genetically differentiated populations compared with neutral SNPs (see also Freamo *et al.*, 2011; Gagnaire *et al.*, 2015). Although the use of loci potentially subjected to natural selection may affect the reliability of demographic inferences, the nurture of *F. alnus* shrubs in parks protects these populations from environmental stochasticity, hence largely exempting park populations, which represent the focal sites of introduction, as targets of natural selection (see also Donaldson *et al.*, 2014).

MATERIALS AND METHODS

Sampling and genotyping

Leaf samples of *Frangula alnus* were taken from 15–40 individuals originating from 38 European (native) populations and 21 North American (invasive) populations (Fig. 1, Table 1), covering a wide range of climatic conditions (Supplementary Data S1). Samples were collected from a total of ten European and seven North American regions, with regions represented by groups of geographically proximate populations (Supplementary Data S1). The North American populations were chosen near ports and cities, as well as in more natural environments. A total of 805 native and 457 invasive individuals were genotyped and analysed.

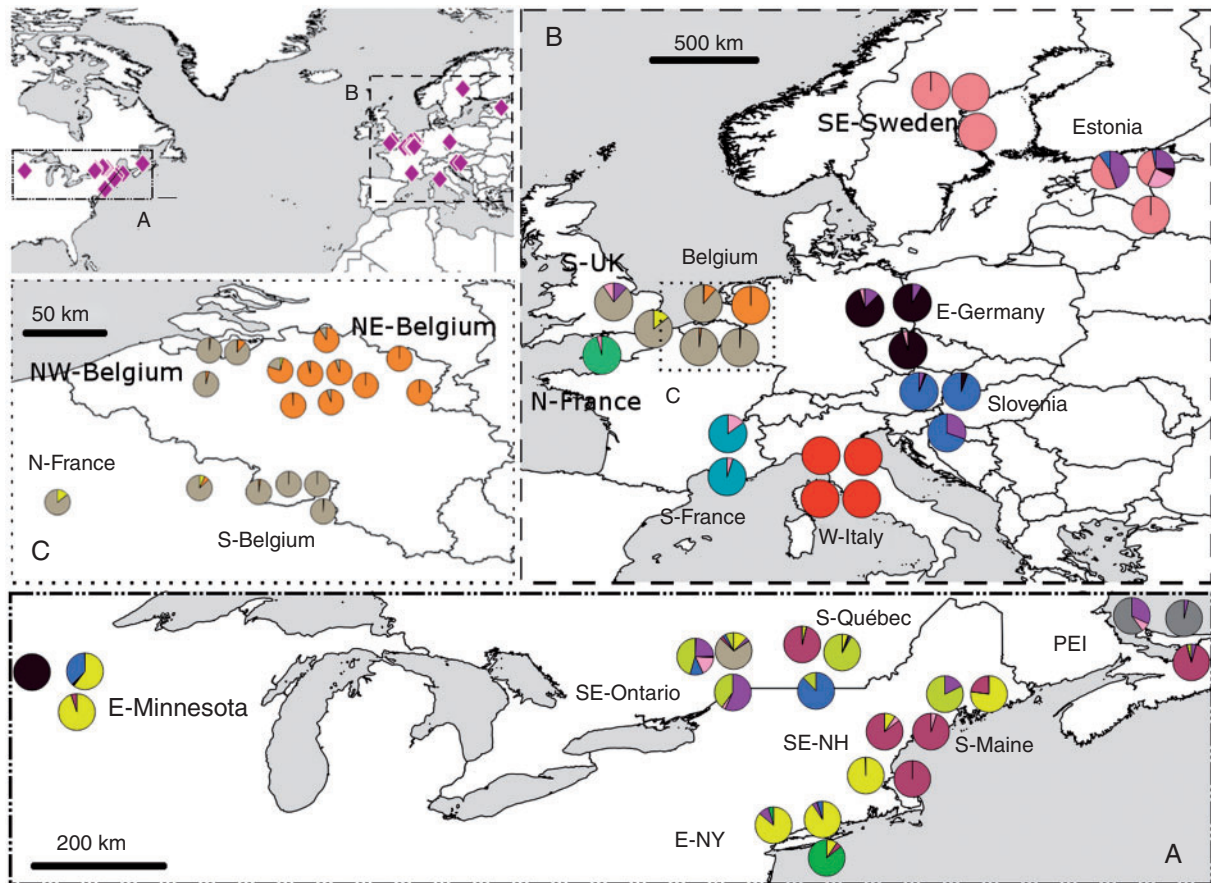


FIG. 1. Map showing geographical locations of populations in the invaded range (A) and the native range (B) of *Frangula alnus*. The symbols in the small box of panel (B) represent the three genetic clusters in this area. A more detailed picture of this box is provided in panel (C), which focuses on populations from northern France and Belgium, in Europe. Circle diagrams represent mean population probabilities of belonging to a genetic cluster, with different colours representing the 15 global genetic groups.

TABLE 1. North American populations of *Frangula alnus* along with their geographical location and genetic diversity (U_{HE} , unbiased expected heterozygosity). The prefix of a population name reflects the location in a region (e.g. E-Minnesota is eastern Minnesota). The wide rivers mentioned in the description are directly connected to sea bays and served as riverine trade routes. Urban environment refers to populations within 500 m of urban area; natural environment refers to forests or wetlands at least 10 km from the edge of a city. See Supplementary Data S1 for details of sample sizes and genetic diversity for all populations

Population	Latitude	Longitude	Description	Environment	U_{HE}
E-Minnesota-A	44.88124	-93.5936	Regional park Minnewashta	Urban	0.322
E-Minnesota-B	44.87455	-93.7130	Natural habitat	Natural	0.249
E-Minnesota-C	44.89462	-93.3791	Park near Minneapolis (10 km) – Mississippi river	Urban	0.332
E-NY-A	40.83537	-73.2207	Park near Massapequa (10 km)	Urban	0.331
E-NY-B	40.69534	-73.4525	Preserve in city of Massapequa, close to bay and ports (5 km)	Urban	0.310
E-NY-C	40.90136	-73.1225	Natural habitat	Natural	0.282
PEI-A	46.26031	-63.1450	In port city of Charlottetown	Urban	0.256
PEI-B	46.34632	-63.3443	Natural habitat	Natural	0.121
PEI-C	46.42635	-63.6757	Natural habitat	Natural	0.168
S-Maine-A	44.26612	-69.8880	Natural habitat	Natural	0.203
S-Maine-B	44.26913	-68.6838	In harbour complex, close to bays, Bucksport, Belfast, Northport,	Urban	0.320
S-Maine-C	43.79307	-70.2777	Near city of Portland (10 km)	Urban	0.255
S-Québec-A	45.41518	-72.6838	Natural habitat	Natural	0.285
S-Québec-B	45.48130	-73.4144	Park in city of Montréal – Fleuve St Laurent	Urban	0.277
S-Québec-C	45.62827	-73.7917	Near city of Montréal (5 km) – Rivière des Mille-Îles	Urban	0.326
SE-NH-A	43.23359	-70.9893	Natural habitat	Natural	0.284
SE-NH-B	43.15292	-70.9374	College woods near small city of Durham (5 km) – Oyster River	Urban	0.308
SE-NH-C	43.06078	-70.7651	In city of Portsmouth	Urban	0.291
SE-Ontario-A	45.45227	-75.6689	In city of Ottawa – Rivière des Outaouais	Urban	0.340
SE-Ontario-B	45.30689	-75.6105	Near city of Ottawa (10 km)	Urban	0.307
SE-Ontario-C	44.87091	-75.6996	Natural habitat	Natural	0.253

A total of 1262 *F. alnus* trees were genotyped for 250 SNPs that were annotated to putative functional loci. The 250 SNPs were selected from a large set of 7383 previously discovered SNPs on the basis of their putative function (for the full discovery, genotyping and annotation procedure see De Kort *et al.*, 2013). The use of annotated SNPs increases the likelihood of finding ecologically relevant allelic trends and increases the power of genetic structure and assignment analyses. Briefly, pooled paired-end sequencing of restriction-site associated DNA (RAD-PE), using the methylation-sensitive restriction enzyme PstI, was employed to find polymorphisms randomly distributed over the *F. alnus* genome. A total of eight samples distributed across a latitudinal gradient was used for this SNP discovery step, including two samples from Belgium (one from Flanders and one from Wallonia), as well as two French, two Swedish and two Italian samples. This ascertainment panel had been chosen previously to find signatures of selection on a small spatial scale as well as across a latitudinal gradient in the native range (De Kort *et al.*, 2015). The RAD-PE procedure generated 33.5 million reads, which were assembled into 56 404 contigs with a mean length of 323 (N50 = 374 bp) and with a high average sequencing depth of 39.79 (De Kort *et al.*, 2013). A total of 7383 high-quality Illumina-compatible SNPs were called at a local sequencing depth of 18–1579. The contig sequences of the 7383 SNPs were subsequently subjected to a BlastX search against all plant proteins available in the Swiss-Prot database using Blast2Go, and hits were mapped to their corresponding gene annotations using the gene ontology database and several additional database files. Using a maximum *E*-value threshold of 10^{-18} (mean *E* = 10^{-36}), a total of 250 genic SNPs was selected for genotyping using allele-specific primer extension genotyping (KASPar, LGC Genomics), of which 180 were associated with ecologically relevant gene ontology terms (i.e. putatively adaptive), such as ‘response to stress’, ‘reproduction’ and ‘response to abiotic stimulus’. Out of the 250 annotated SNPs, 133 were successfully (<10 % missing data) genotyped in both North American and European samples, of which the majority (99 SNPs) were associated with at least one ecologically relevant annotation (Supplementary Data S2). The remaining SNPs (34) were considered non-functional.

Genetic variation within and among populations

Unbiased expected (U_{H_E}) and observed (H_O) heterozygosity were estimated for each population using GENALEX 6.5 (Peakall and Smouse, 2006). Mixed models with geographical region as a random factor were applied to compare (1) mean population genetic diversity (U_{H_E}) between urban and natural environments of the North American populations (fixed effect: ‘environment’), and (2) U_{H_E} between North American and European populations (fixed effect: ‘continent’). To compensate for differences in sample size among populations, we repeated the mixed models with allelic richness using rarefaction (HP-RARE; Kalinowski, 2005). To distinguish between the putatively functional loci ($n = 99$) and the putatively non-functional loci ($n = 34$), we added ‘marker type’ and its interaction with ‘environment’ and ‘continent’, respectively, as fixed effects to the mixed models. For each pair of populations, we computed pairwise F_{ST} values with 10 000 permutations using

ARLEQUIN 3.5 (Excoffier and Lischer, 2010). A hierarchical analysis of molecular variance (AMOVA) with 10 000 permutations as implemented in ARLEQUIN 3.5 was used to calculate genetic differentiation and its statistical significance among the two continents (F_{CT}) and among populations within continents (F_{SC}).

Population structure

Discriminant analysis of principal components (DAPC) was performed to identify general genetic structure in the data (R package adegenet; Jombart *et al.*, 2010). Due to expected stronger genetic differences at functional loci that are the target of selection compared with neutral loci with no ecological relevance, functional SNPs tend to increase the power of genetic structure analyses (Freamo *et al.*, 2011; Guichoux *et al.*, 2013). Hence, in situations where historical patterns can be inferred from functional markers while limiting confounding by divergent selection, functional markers are preferred over non-functional markers. Such situations include the assignment of individuals to their present-day populations (Freamo *et al.*, 2011) and the elucidation of the invasion history of nursed populations (this study). We therefore included all SNPs in our analyses, but provide results based on putatively non-functional and functional SNP datasets separately in Supplementary Data S3. In a first step, *K*-means clustering of pairwise genetic distances among individuals served to discriminate between groups of individuals by maximizing genetic variation among groups (*K*). The Bayesian information criterion (BIC) was used to select the model that most likely represented the true number of genetic clusters while preventing overfitting. Unlike Bayesian structure analyses (e.g. Pritchard *et al.*, 2000; Corander *et al.*, 2004), *K*-means clustering does not assume Hardy–Weinberg equilibrium and is therefore very likely to be more realistic in scenarios with recent population changes, including invasions (Kalinowski, 2011; Rodríguez-Ramilo and Wang, 2012). In a second step, DAPC was used to explore the genetic relationships among the predefined groups, involving principal components analysis (PCA) of allele frequencies to obtain uncorrelated genetic variables, and discriminant analysis to obtain discriminant axes (linear discriminants) representing the genetic group differences (see Supplementary Data S3 for details). Cross-validation (function `xvalDapc`) with a training dataset (90 % of the data) and a validation dataset (10 % of the data), replicated 30 times, served to define the optimal number of principal components in the DAPC, by minimizing the root mean squared error and maximizing predictive success (Jombart *et al.*, 2010). Two additional DAPC analyses were performed: one for the North American and one for the European samples. The resulting linear discriminants reflect the genetic structure in each of the datasets and were therefore used as conditional variables in the landscape genetic analyses to account for demographic genetic background processes.

Assignment of North-American individuals to putative source regions

To assess the European origins of the invasive populations and the amount of admixture between introduced genotypes,

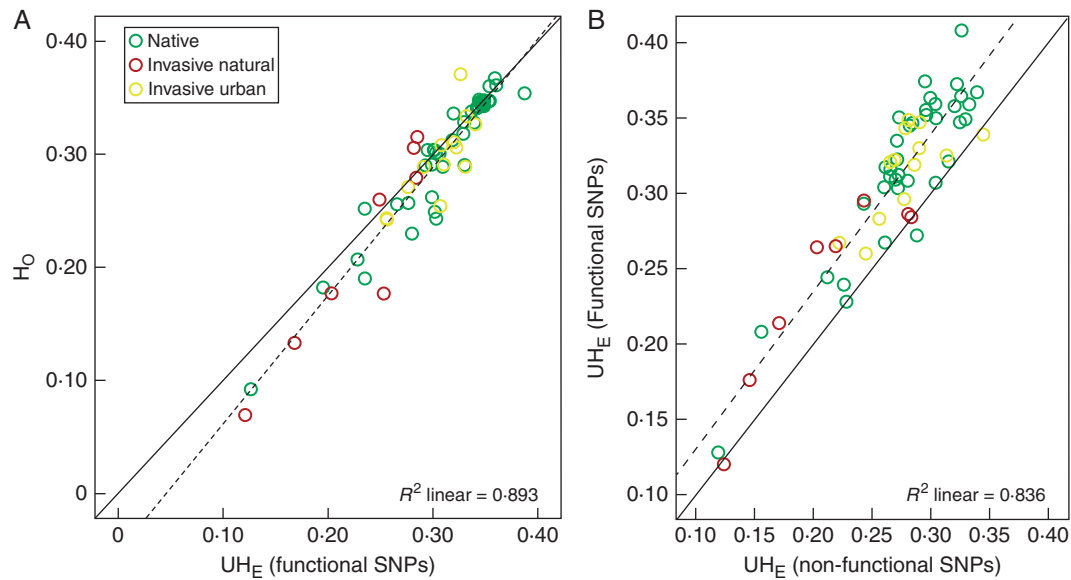


FIG. 2. Relation between observed and expected heterozygosity (A) and comparison of expected heterozygosity between putatively functional and non-functional SNPs (B) for 58 populations of *Frangula alnus* sampled in its native and invasive distribution area. The dashed line represents the relation between the x and y axis measures; the solid line reflects zero inbreeding in panel (A).

we used the assignment procedure of DAPC. First, K -means clustering and DAPC were applied to the European dataset. The resulting linear discriminants were used to estimate assignment probabilities for each of the North American individuals. More specifically, assignment probabilities were based on the scores of the North American individuals on the European discriminant function (i.e. the position of the North American individuals in the European K -dimensional space). Whereas assignment at the group level provides a broad overview of the native areas of origin based on genetic (dis)similarities among native populations, we also performed an assignment analysis at the population level to obtain higher geographical resolution. As is the case for most studies examining routes of invasion (e.g. Miller *et al.*, 2005; Clavero and Garcia-Berthou, 2006), we assume that the sampled populations are genetically and/or geographically close to the true source populations. The distance-based DAPC assignment is preferred over Bayesian assignment software such as GENECLASS 2 (Piry *et al.*, 2004) because the latter assumes Hardy–Weinberg equilibrium. This assumption may be violated due to the characteristics of invading populations and because many of our (annotated) SNPs show signs of natural selection, especially in the native range and the invaded North American natural areas.

Functional versus neutral allelic patterns in the invasive range

More pronounced changes in allele frequencies of functional SNPs compared with neutral SNPs when comparing urban with natural invasive populations may indicate allele frequency shifts that are due to ongoing divergent selection during the invasion process. We therefore performed canonical redundancy analyses (RDAs) as a multivariate landscape genetic tool using the R package Vegan (Oksanen *et al.*, 2008). The functional SNPs were used as response variables, with ‘environment’ as

fixed factor while controlling for ‘region’ as a conditioning variable. SNPs that contributed most to the resulting RDA axes, which represent the variation in the functional allele frequency matrix that can be explained by ‘environment’, were extracted for an annotation study (standardized SNP scores >0.6 , hereafter called ‘invasion SNPs’). The RDA was repeated for the neutral SNPs. For an annotation enrichment analysis, GO term counts were compared between the invasion SNP dataset and the total SNP dataset using the χ^2 test.

Data accessibility

The SNPs used in this study are part of a larger set of previously discovered SNPs, which has been submitted to GenBank and can be accessed using the following accession number PRJNA203129. <http://www.ncbi.nlm.nih.gov/bioproject/?term=PRJNA203129>.

RESULTS

Genetic variation within and among populations

Expected heterozygosity (U_{H_E}) of the 59 *F. alnus* populations varied between 0.121 (Prince Edward Island B) and 0.340 (Ontario A, New York A) in the North American populations, and between 0.126 (United Kingdom B) and 0.387 (Northern France C) in the European populations (Fig. 2, Supplementary Data S1). U_{H_E} (0.288 versus 0.269) and allelic richness (A_R 1.29 versus 1.27) did not differ significantly between the European and the North American populations (Table 2). SNPs with putative ecological functions rendered higher population genetic diversity than putatively non-functional SNPs (U_{H_E} 0.297 versus 0.260, respectively, and A_R 1.296 versus 1.260, respectively; Table 2). This result holds for both native and

TABLE 2. Population genetic diversity indices among environments, continents and marker types. X represents the interaction between the two main effects in each of the two models (environment \times marker interaction and continent \times marker interaction, respectively). The environment model applies to the invasive range, whereas the continent model refers to the comparison between invasive (North American) and native (European) populations. Significant effects ($P < 0.01$) are shown in bold type

	Environment		Marker		X		Continent		Marker		X	
	F_{df}	P	F_{df}	P	F_{df}	P	F_{df}	P	F_{df}	P	F_{df}	P
UH _E	$F_{32.40}$ 38.090	<0.001	$F_{31.95}$ 12.131	<0.01	$F_{31.95}$ 0.172	0.681	$F_{15.60}$ 0.627	0.440	$F_{97.60}$ 37.434	<0.001	$F_{97.60}$ 0.249	0.619
A _R	$F_{32.39}$ 42.18	<0.001	$F_{31.95}$ 12.426	<0.01	$F_{31.95}$ 0.192	0.664	$F_{15.59}$ 0.680	0.422	$F_{97.60}$ 35.607	<0.001	$F_{97.59}$ 0.231	0.632

invasive populations (non-significant interaction between ‘continent’ and ‘marker type’, Table 2). Genetic diversity (UH_E) of North American populations from urban environments was significantly higher than that of populations in natural environments (0.292 versus 0.231; Table 2, Fig. 2), as was the case for A_R (1.292 versus 1.230; Table 2). Again, SNPs with putative ecological functions rendered a significantly higher UH_E and A_R than putatively non-functional SNPs for both continents (Table 2).

Pairwise genetic differentiation (F_{ST}) between populations varied from 0.010 (between Northern Belgium A and Northern Belgium H) to 0.692 (between Prince Edward Island B and United Kingdom B) (Supplementary Data S1). Among continents, the smallest pairwise difference ($F_{ST} = 0.06$) was observed between Northern Belgium L and South-Eastern Ontario A. The hierarchical AMOVA revealed limited but significant genetic differentiation among continents ($F_{CT} = 0.016$, $P < 0.001$) and high genetic differentiation among populations within continents ($F_{SC} = 0.206$, $P < 0.001$).

Population structure

A structure analysis (DAPC) grouped all 58 populations into 15 genetic groups, represented by 14 linear discriminants (see Supplementary Data S3 for eigenvalues and individual posterior probabilities). All linear discriminants were retained to optimize group assignment. A clear-cut structure was observed in Europe, where the populations from Italy, Southern France, Slovenia and Germany formed different clusters. The island population south of the UK (S-UK-B) also clustered separately, while the population on the mainland of the UK (S-UK-A) was grouped together with the populations from Northern France, North-Western Belgium and Southern Belgium (Fig. 1). Also, the populations from Sweden and Estonia were genetically similar (Fig. 1B). North American populations showed less genetic structure, with high differentiation occurring between proximate populations (e.g. in Québec) and low differentiation between distant populations (e.g. between Minnesota and New York). Several unique clusters were identified, including one population in Minnesota, one in Québec and two on Prince Edward Island (Fig. 1A, Supplementary Data S3).

Some genetic similarities between European and North American populations could be observed (Fig. 1A). More specifically, populations from North-Western Europe (S-UK-A, NW-Belgium, N-France) clustered together with a population from SE-Ontario (beige), and Germany was genetically similar

to a population in E-Minnesota (black, Fig. 1). There also seemed to be some influence from Eastern Europe (Slovenia and Estonia) in North American populations (purple in Fig. 1).

Assignment of North American individuals to putative source regions

North American individuals were assigned to two major groups that represent genetically different clusters, i.e. a group that was formed by most Belgian populations, the French populations and the S-UK-A population (Fig. 3A, dark red, Supplementary Data S3), and a second group of populations that originated from North-Eastern Belgium (Fig. 3A, light red, Supplementary Data S3). Populations from E-Germany and Slovenia also contributed to the genetic make-up of North American populations. Some of the North American populations were relatively pure with regard to their putative genetic origin, including the populations from PEI-A (dark red), S-Maine-B and S-Maine-C (dark and light red), S-Québec-C (dark and light red), SE-NH-C (dark and light red) and SE-Ontario-A (dark and light red) (Fig. 3A). These populations occur in urban areas and also have high genetic diversity relative to the more admixed populations (Table 1).

Assignment at the level of populations confirmed the genetic contribution of two main sources to the invasive populations: all populations from the western part of Belgium (NW-Belgium and S-Belgium) and E-Germany (Supplementary Data S3, Fig. 3B). On the other hand, populations from Northern France, which is geographically very close to North-Western Belgium, could not be identified as native source populations (Supplementary Data S3, Fig. 3B). Populations from Southern Europe (S-France and W-Italy) and Northern Europe (SW-Sweden and Estonia) were generally not associated with the invasion of North America (Fig. 3). Some American regions, including S-Québec, S-Maine and SE-NH, seem to have been almost entirely colonized by genotypes originating from the western part of Belgium (Fig. 3A, B). Also, many individuals from E-NY show high similarity with the populations from the western part of Belgium. E-Minnesota, PEI and SE-Ontario are genetically more mixed and have some influence from E-Germany (Fig. 3).

These assignment results were very similar to those based on the neutral and functional SNPs separately (Supplementary Data S3). More specifically, using only the neutral SNPs in the assignment analysis, which resulted in a total of 15 European groups, revealed that 44.0 % of the North American genotypes

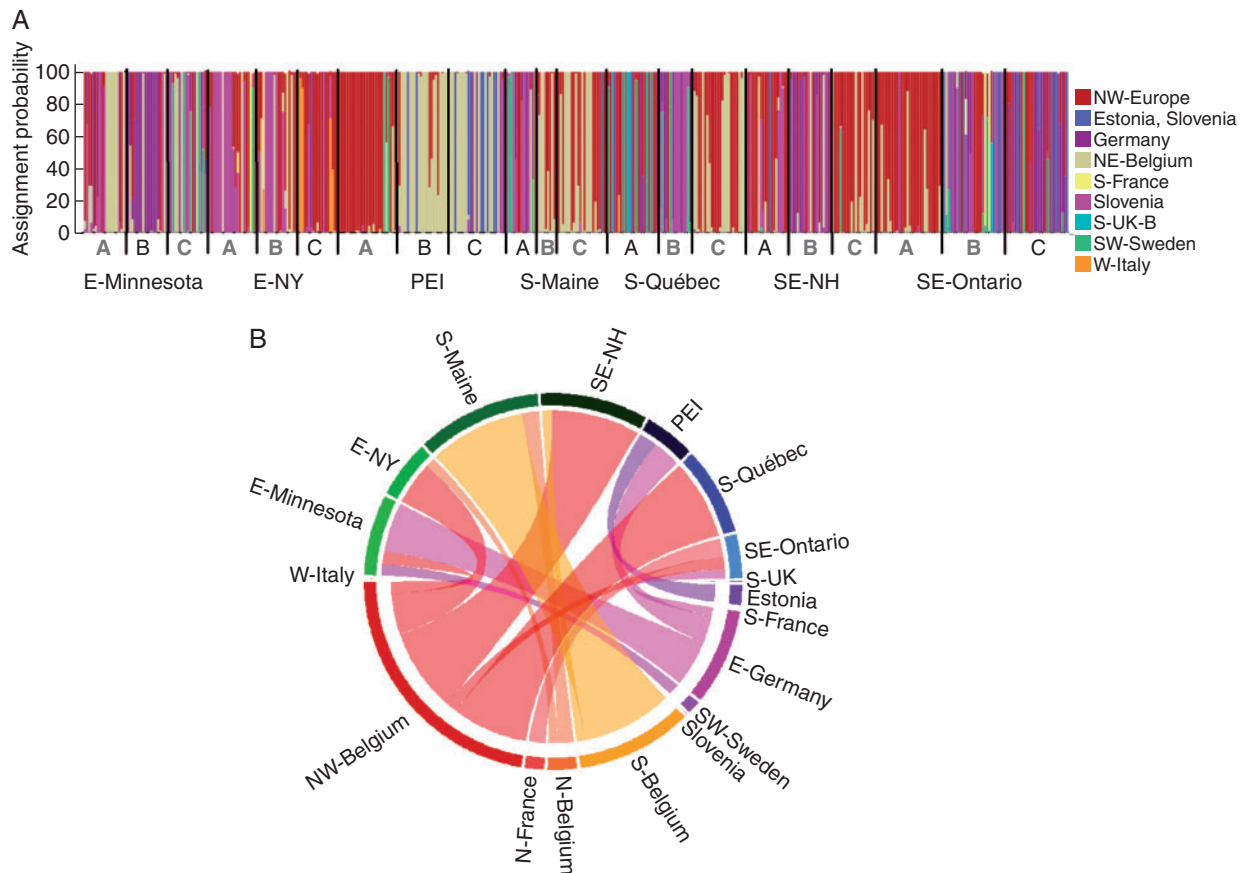


FIG. 3. Results of the DAPC assignment analyses assigning (A) individuals and (B) populations of *Frangula alnus* in their invasive range to populations in their native range. (A) Posterior probabilities of North American individuals assigned to the nine European groups (identified by the European DAPC), as obtained by the DAPC assignment. Individuals are labelled with their corresponding population. Urban population labels are grey and bold. (B) Circos plot presenting mean assignment probabilities of invasive American populations (green and blue) to native European populations (orange and purple). Wider links reflect higher probabilities. Only probabilities higher than 5 % are shown, to emphasize important patterns. Populations are coloured according to their geographical region (Supplementary Data S3).

were assigned to a group consisting of the populations from N-Belgium, with a mean assignment probability (AP) of 92 %. An additional 20.4 % were assigned to a group formed by individuals from Belgium and N-France (mean AP = 90 %). Slovenia and Germany each contributed 7 % to the assignment of North American individuals, with a mean AP of 88 and 96 %, respectively (Supplementary Data S3). The assignment analysis based on the functional SNPs only, which resulted in a total of 11 European groups, showed that most North American genotypes were assigned to three groups involving Belgian populations, with a total of 46.5 % assignments (mean AP = 0.93 %), followed by two groups comprising Slovenian and German populations (28 % assignments with a mean AP of 98 %).

Functional versus neutral allelic patterns in the invasive range

A small but significant amount of variation in functional allele frequencies could be explained by ‘environment’, as revealed by redundancy analyses ($_{adj}R^2 = 0.049$, $P = 0.014$). A total of ten SNPs contributed strongly to this pattern (SNP scores >0.6) and showed strong differences in allele

frequencies between urban and natural environments (Fig. 4). The opposite pattern was observed for neutral SNP frequencies, for which no variation could be explained by ‘environment’ ($_{adj}R^2 = 0.000$, $P = 0.588$).

The enrichment analysis revealed a strong overrepresentation of SNPs that were annotated with the gene ontology term ‘Reproduction’ in the invasion SNP dataset compared with the complete dataset (Table 3, Supplementary Data S2). ‘Growth’ and ‘Flower development’ also tended to be enriched in the invasion SNP dataset, but these patterns were only marginally significant (Table 3).

DISCUSSION

Comparison of genetic diversity within and among invasive and native populations showed that genetic diversity in urban invasive populations near North American port cities was comparable to that in native populations. Individuals of urban American populations were also consistently assigned to specific European regions, and can therefore be considered bridgehead populations connecting invasive populations outside urban areas to European source populations. The resulting putative

patterns of invasion correspond well with late 18th to early 19th century transatlantic trading routes, and suggest that mainly one international European region, located in north-western Europe, played a substantial role in the introduction of *F. alnus* into America. High allelic richness and heterozygosity at SNPs with ecologically relevant putative functions suggest considerable potential to cope with the environmental conditions experienced during the invasion of North America. Correspondingly, we found evidence for divergent selection at SNPs potentially involved in reproduction, a life-history trait that has often been associated with invasion success.

Transatlantic patterns of invasion

The introduction of *F. alnus* into America probably occurred in the late 18th and early 19th centuries (Catling and Porebski, 1994; Aiello-Lammens, 2014), a period dominated by maritime transatlantic trade. Moreover, the 18th century was characterized by abundant plant exploration and trade, associated with increased prosperity, landscaping and leisure time in the cities (Reichard and White, 2001; Chambers, 2008). We identified genetic associations between several invasive American populations and native north-western European populations of *F. alnus*. Urban American populations, especially, gave clear-cut assignment results (Fig. 3A), which corroborates our

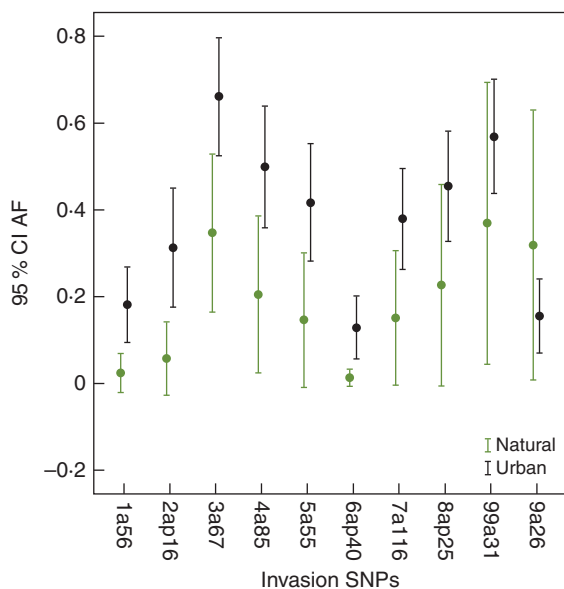


FIG. 4. Error plot showing 95 % confidence intervals (CI) associated with the allele frequencies (AF) of the ten invasion SNPs, contrasting urban versus natural populations in the invasive range.

expectation that park populations may be excellent candidates for unravelling historical patterns of ornamental invasion by woody species. North-western Europe seems to have played a dominant role in the invasion of multiple North American regions, comprising the urban populations SE-New Hampshire C, S-Maine B and C, S-Québec C, SE-Ontario A and Prince Edward Island-A (Fig. 3A). The populations from north-western Belgium, especially, seem to have contributed to these invasion patterns (Fig. 3B), suggesting that the closest port (Bruges-Sluis, ~30 km from the corresponding populations) played a key role in the distribution of genetic material of *F. alnus* throughout North America. Samples collected from near Antwerp or nearby French ports did not contribute to North American populations, indicating that Bruges was the main shipping point of *F. alnus* seeds, rather than Antwerp or Calais. Following seed transport across the Atlantic Ocean towards New York, Maine, New Hampshire and Prince Edward Island, the large Saint Lawrence River (connecting the Atlantic Ocean with Québec) and the Ottawa (or Grand) River (connecting Québec with Ontario) probably allowed seed transport into port regions of Québec and Ontario.

Individuals from the urban populations of New York (E-NY-A and -B) were rather diverse with respect to their putative origin. Based on genetic similarities between European groups and American genotypes, both Slovenian and Belgian populations may have contributed to the genetic composition of the New York populations. The more complex invasion history of *F. alnus* in New York can be attributed to the historical role of plant trade in New York. Indeed, New York played an important role in the early trade of ornamental plants, as the first commercial American nursery to conduct international trade was started in 1737 in Flushing, New York (Reichard and White, 2001). Seeds from Slovenia (or proximity) may have been transported along a historical trade route between Venice (~ 200 km from the Slovenian populations, and likewise Slovenia was under Habsburg rule at the beginning of the 19th century) and New York. Finally, the complex genetic make-up of the populations in Minnesota and the lack of historical records on *F. alnus* occurring in that state may suggest that the invasion of more inland areas occurred relatively recently through land and/or water transport from nurseries established throughout the continent. The genetic similarity between some North American populations (New York and Minnesota) and the Eastern German populations may be the consequence of genetic mixture between Belgian and Slovenian populations, creating intermediate genotypes resembling those of Germany.

Our results do not exclude additional trading routes, unsampled source regions or alternative nearby ports (e.g. Boston in the USA, Trieste in Italy and Ostend in Belgium) that could have played a role in the early modern transport of

TABLE 3. Gene ontology counts of ecologically relevant annotations associated with the ten invasion SNPs (showing allele frequency shifts between urban and natural populations) in comparison with the complete SNP dataset. The enrichment analysis was χ^2 -tested (* $P < 0.1$; ** $P < 0.01$)

	Reproduction	Growth	Flower development	Response to abiotic stimulus	Response to biotic stimulus	Response to stress
Invasive SNPs	7/10 (70 %)**	3/10 (30 %)*	3/10 (30 %) (*)	3/10 (30 %)	2/10 (20 %)	3/10 (30 %)
All SNPs	27/133 (20.3 %)	15/133 (11.3 %)	16/133 (12 %)	36/133 (27.5 %)	25/133 (18.8 %)	40/133 (30.5 %)

F. alnus. Similarly, we cannot deny a potential role for nurseries in seed collection and transport prior to shipping. Nevertheless, given the lack of mechanized land transport in the 18th century, seed and seedling collections close to inter-continental ports were the most cost-efficient way to transport plant material overseas.

High genetic diversity in urban bridgehead populations

Because all North American populations near ports harboured relatively high genetic diversity and because genetic diversity was higher in populations inhabiting urban areas than in populations occurring in natural areas, transport of seeds or plant material along the suggested trade routes has most likely facilitated the invasion of the species in North America. The high genetic diversity in urban areas near ports indicates that genetic founder effects have been circumvented. Strong founder effects during ornamental plant invasion can indeed be avoided if the shipped seeds harbour most of the alleles of the source population, and this relatively rich gene pool is used for cultivation and subsequent plantation (Tracy *et al.*, 2011; Donaldson *et al.*, 2014). It remains unclear, however, whether the introductions involved single large seed-shipping events or multiple small seed-trade episodes from the same European area for each of the trade routes.

The observation that the genetic diversity at putatively functional SNPs did not reduce as a result of founder effects suggests considerable adaptive potential to novel environmental conditions (Lavergne and Molofsky 2007; Vilas *et al.*, 2015). The preservation of diversity and allelic richness for ecologically relevant genes, even in the presence of limited neutral genetic diversity (e.g. Dlugosch and Parker, 2008), may result from an interplay between the rich genetic architecture underlying complex adaptive traits and balancing selection for adaptive diversity (Mojica *et al.*, 2012; Des Marais *et al.*, 2013). More specifically, a rich genetic architecture can involve multiple alternative genetic and phenotypic effects, including dominance, epistasis, pleiotropy and genotype \times environment interactions. These processes can in turn balance genetic diversity underlying quantitative fitness traits, thereby counteracting loss of the genetic diversity of genes governing adaptive phenotypic diversity during invasions of natural areas (Mojica *et al.*, 2012; Alonso-Blanco and Méndez-Vigo, 2014; El-Soda *et al.*, 2014).

Evolution in the invasive range

A total of ten SNPs were found to show consistent allelic shifts between urban and natural areas of the invasive range, suggesting divergent selective processes acting on traits that may promote invasion success. Correspondingly, seven of these SNPs were assigned a role in reproduction, one of the most influential life-history traits of plants in governing evolutionary responses to environmental change (Barrett, 2011). The low density of conspecifics during range shifts in the invasive range may have facilitated the evolution of traits increasing reproduction (Phillips *et al.*, 2010; Parker *et al.*, 2013; Colautti and Lau, 2015). Adaptive shifts towards more reproductive genotypes may have strong consequences for native species' communities, stressing the need for management actions aiming to reduce

gene flow from nurtured park populations into invasive natural populations.

SNP-set selection

Because post-introduction selection in the populations that invaded natural areas could have altered the allele frequencies, we compared assignment results between the functional genetic dataset (which contains SNPs that can affect fitness and are therefore targets of selection) and the non-functional genetic dataset (with little ecological or evolutionary relevance). Although only 34 SNPs were considered non-functional, the corresponding assignment analysis led to conclusions that were very similar to those involving the functional SNP dataset (Supplementary Data S3), i.e. a dominant role for north-western Europe (NW-Belgium in particular), followed by some influence from Slovenia and Germany in New York and Minnesota.

Pooled SNP sequencing, often performed prior to individual-based SNP genotyping, is an increasingly popular technique in non-model species lacking genomic resources, as it allows the identification of (tens of) thousands of SNPs within typical research funding budgets (e.g. Rellstab *et al.*, 2013; Vandepitte *et al.*, 2013; Silva-Junior *et al.*, 2015). However, especially if the SNPs are identified in a limited sample but applied in a larger geographical context, population genomic analyses following pooled SNP sequencing are prone to ascertainment bias, which refers to systematic bias towards common genetic variants in the ascertainment panel relative to other samples. This bias affects downstream genomic analyses that are based on allele frequencies, including estimates of demographic parameters and genetic diversity (Rosenblum and Novembre, 2007; Bradbury *et al.*, 2011). In this study, a geographically variable yet restricted ascertainment panel was used for SNP discovery, potentially affecting our results to some extent. Nonetheless, the impact of ascertainment bias on population structure and assignment analyses has been found to be limited (e.g. Haasl and Payseur, 2010; Bradbury *et al.*, 2011), probably because genetically similar populations experience similar ascertainment bias, rendering allele frequencies comparable with respect to population assignments. Moreover, the presence of SNPs showing high population differentiation (locus-specific $F_{ST} > 0.05$) has been shown to increase the power of population assignments in the presence of ascertainment bias to a level that peers with sampling designs free from ascertainment bias (Berry *et al.*, 2004; Bradbury *et al.*, 2011). Here, all SNPs showed $F_{ST} > 0.05$ and the average locus-specific F_{ST} was 0.247 ± 0.010 . Hence, although our sampling design represents a restricted ascertainment panel, we suspect limited impact of ascertainment bias on the assignment results.

CONCLUSIONS

The distance-based DAPC analyses in combination with population genomic approaches revealed the presence of genetically diverse bridgehead populations established during the early modern transatlantic introduction of *F. alnus* in North American parks and gardens. Our results suggest multiple routes of invasion from one, possibly two, region(s) in continental Europe, potentially involving distinct transatlantic

trading routes. Moreover, the higher genetic diversity of populations in the urban North American areas, especially for putatively functional SNPs, indicates that these populations may serve as permanent sources of adaptive genetic material for the more rural and genetically impoverished populations. Improved containment or eradication of the genetically diverse bridgehead populations may reduce the establishment of new populations in the invasive range and, importantly, also hamper the exchange of alleles with populations from natural habitats, thereby reducing their genetic diversity and adaptive potential. Future research with a focus on functional genomic studies must confirm the role of the putatively functional SNPs in the invasion processes of *F. alnus*.

SUPPLEMENTARY DATA

Supplementary data are available online at www.aob.oxfordjournals.org and consist of the following. Data S1: climatic and genetic population description. Data S2: gene ontology of SNPs. Data S3: DAPC.

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