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An evaluation of seed zone delineation using phenotypic and population genomic data on black alder *Alnus glutinosa*

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Summary

1. Delineation of seed zones or provenance regions to preserve local adaptation is a common practice in forestry and restoration, as locally adapted plants generally possess relatively high levels of productivity and resistance. Provenance trials typically quantify the degree of phenotypic divergence among individuals and populations raised under common conditions, which is time-consuming and potentially confounded by phenotypic plasticity and maternal effects.

2. Here, we put forward population genomics, the screening of individual genomes for the genetic signature of adaptation, as a fast and reliable strategy to evaluate seed zone delineation. To illustrate the value of this approach, we quantified the degree of genomic adaptation within and among Belgian black alder *Alnus glutinosa* provenances and compared results with traditional provenance trials. Distant European reference regions were included to validate the approaches, as larger environmental differences at a European scale are expected to result in larger adaptive responses.

3. Local provenances did not perform better than foreign provenances at the scale of Belgian seed zones, in contrast to the comparisons with the distant European regions. A significant site effect indicated that plastic responses rather than local adaptation explain phenotypic differences among seed zones. The common garden revealed little evidence for adaptation for all measured traits, both among seed zones and among distant regions.

4. The number and strength of genetic outliers was not significantly larger among Belgian seed zones than within these seed zones, but was significantly larger between Belgian seed zones and the distant European reference regions.

5. *Synthesis and applications*. The lack of adaptive divergence among Belgian seed zones supports an expansion of current provenance regions into larger seed zones. The results also show that population genomics can be an accurate and time-efficient resource to assist decisions on seed sourcing. This highlights the importance of raising awareness of the potential benefits of this novel approach among policy makers, foresters and restoration practitioners.

Key-words: adaptive differentiation, Genotyping-By-Sequencing, natural selection, outlier detection, population genomics, provenance regions

Introduction

Delineation of seed zones or provenance regions – areas within which plants can be transferred with little risk of being poorly adapted to their new location – is a common

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practice in the United States, Canada, Europe and Australia (e.g. Bower, St. Clair & Erickson 2010; Vander Mijnsbrugge, Bischoff & Smith 2010; Krauss *et al.* 2013; St Clair *et al.* 2013). A pivotal assumption is that local populations have higher fitness, that is, higher levels of survival, reproduction, productivity, disease resistance and abiotic resilience, than non-local populations (McKay *et al.* 2005; Jones 2013). Using locally adapted populations not only preserves alleles that are adapted to local conditions but also prevents the introduction of maladapted genotypes, which may cause loss of adaptive potential and population extinction, and potentially affects interactions between co-evolved species and ecosystem functioning (Kawecki & Ebert 2004; Urban *et al.* 2008; Jones 2013). This 'local-is-best' concept has given rise to the delineation of tree seed zones, which vary considerably in size, from 39 364 km² in British Columbia over 25 503 km² in Oregon and 10 150 km² in the United Kingdom, to only 4649 km² in Belgium (Ministry of Forests, Lands and Natural Resource Operations BC, Oregon Department of Forestry, UK Forestry Commission, and Research Institute for Nature and Forest Belgium, respectively).

A reliable procedure to delineate seed zones is crucial, as the underestimation of provenance sizes not only leads to redundant regulations, administration and legislation on seed sourcing, but may also increase levels of inbreeding among planted trees and decrease the potential to cope with global change (Kawecki & Ebert 2004; Broadhurst *et al.* 2008; Leimu & Fischer 2008; Sgrò, Lowe & Hoffmann 2011; Kremer *et al.* 2012; Jones 2013). Thus far, the delineation of provenances is commonly based on overall estimates of environmental homogeneity, such as climate or soil characteristics, or on phenotypic differences among populations inferred from provenance studies including common garden trials and reciprocal transplantation experiments (Vander Mijnsbrugge, Cox & Van Slycken 2004; Leimu & Fischer 2008; Bower, St. Clair & Erickson 2010; De Kort, Vandpitte & Honnay 2013; Richardson *et al.* 2013; St Clair *et al.* 2013). In contrast to field observations, provenance trials allow the extraction of the phenotypic variation that is not caused by environmental variation and thus reflects genetic differences among populations. In common garden experiments, the magnitude of genetic differentiation of quantitative phenotypes raised under common conditions (Q_{ST}, Spitze 1993) is compared to the magnitude of among-population allelic differentiation measured on a set of neutral genetic loci (F_{ST}, Wright 1951). If Q_{ST} exceeds F_{ST}, the measured phenotypic differences are larger than expected under neutrality and are therefore assumed to reflect among-population adaptive divergence (Merilä & Crnokrak 2001; Leinonen *et al.* 2008; De Kort, Vandpitte & Honnay 2013). However, environmental maternal effects and plastic responses to the common environment may affect the magnitude of Q_{ST} and therefore bias Q_{ST}–F_{ST} inferences (Cano *et al.* 2004; Kawecki & Ebert 2004; Scheepens, Frei & Stöcklin 2010). Reciprocal transplantation experiments, where higher fitness in local relative to foreign transplanted populations is interpreted as evidence of local adaptation, are less feasible to implement but allow the contribution of phenotypic plasticity induced by the environment to be assessed (Kawecki & Ebert 2004; Leimu & Fischer 2008; Hereford 2009).

Population genomics offers an alternative approach to investigate the distribution of adaptive variation within and among provenance regions (e.g. Morin *et al.* 2004; Allendorf, Hohenlohe & Luikart 2010; Helyar *et al.* 2011; Narum *et al.* 2013). Whereas genetic drift and gene flow exert a genome-wide influence, natural selection is presumed to predominantly increase the magnitude of genetic differentiation in and near loci underlying traits under selection (outlier loci) (Lenormand 2002; Morin *et al.* 2004; Allendorf, Hohenlohe & Luikart 2010). In non-model organisms, the lack of a reference genome may complicate the characterization of truly adaptive loci. However, the use of candidate loci that are known to be adaptive or linked to adaptive loci choice may result in higher overall F_{ST} values, causing overestimated neutral expectations, and therefore potentially increases the number of false-negative outlier results (Pérez-Figueroa *et al.* 2010; Le Corre & Kremer 2012). On the other hand, aiming at thousands of anonymous markers increases the likelihood of capturing a representative part of the genome that is mainly shaped by neutral processes and simultaneously harbours detectable adaptive variation. The identification of exceptional variation at molecular markers therefore is a powerful tool to detect putatively adaptive loci in species without reference genome (Morin *et al.* 2004; Allendorf, Hohenlohe & Luikart 2010). As extraordinary genetic patterns at specific loci presumably reflect adaptive differentiation, higher numbers of outlier loci and stronger genetic differentiation at outliers most likely represent stronger adaptive responses to environmental cues. The association between the number of outliers and the magnitude of local adaptation has been demonstrated repeatedly (Strasburg *et al.* 2012), for example in brown trout *Salmo trutta* (Meier *et al.* 2011) and white spruce *Picea glauca* (Namroud *et al.* 2008). Moreover, the use of adaptive loci has been proposed as a tool to define conservation units, that is, population units identified within species to help guide management and conservation efforts, in order to preserve adaptive differentiation within species (Funk *et al.* 2012). Recent high-throughput sequencing technologies, such as Genotyping-By-Sequencing (GBS; Elshire *et al.* 2011), have enabled the possibility to genotype hundreds to thousands of genetic markers in a targeted fraction of the genome, which facilitates the screening of a comprehensive set of loci for genomic signatures of selection (Siol, Wright & Barrett 2010; Helyar *et al.* 2011; Narum *et al.* 2013). The suitability of GBS to genotype species with complex genomes, without the availability of a reference genome, has also recently been proven successful in *Pinus contorta* and *Picea glauca*

(Chen *et al*. 2013; Narum *et al*. 2013). Population genomic screens therefore provide a powerful tool to assist seed provenances delineation, unconfounded by non-genetic plastic responses.

In this study, we measured the degree of local adaptation in the context of provenance delineation in black alder *Alnus glutinosa*, a monoecious, self-incompatible and wind-pollinated tree species that naturally occurs throughout Europe (Mac Vean 1953; Meusel, Jager & Weinert 1965). Low nutrient demands, high growth rates, and the ability to stabilize riverbanks and prevent erosion, introduced *black alder* as a priority species in the reforestation of riverine ecosystems and degraded soils, which renders the species particularly suited for the evaluation of seed zones and the improvement of guidelines for provenance delineation (Claessens *et al*. 2010). Our main study area was the country of Belgium. Despite the small area of Belgium (32 545 km^2), as much as seven seed provenance zones have been delineated, which has chiefly been motivated by the characteristic high soil heterogeneity (Vander Mijnsbrugge, Cox & Van Slycken 2004). To assess the validity of the current Belgian provenance delineation, we quantified the distribution of adaptive variation among black alder populations within and among Belgian provenance regions. For reference, we also included Danish and Italian populations of black alder in our analyses. Comparisons between Belgian populations and Danish and Italian populations represent much larger environmental contrasts and are therefore expected to reflect stronger adaptive differences. Hence, these geographically distant populations provide a litmus test for the general validity of the approaches to detect patterns of local adaptation.

We integrated results of traditional common garden and reciprocal transplantation experiments with a population genomic screen of black alder individuals to answer the following questions: (i) do local provenances outperform foreign provenances in the reciprocal transplant experiments?; (ii) does phenotypic differentiation among populations exceed neutral expectations ($Q_{ST} > F_{ST}$), and is Q_{ST}–F_{ST} smaller within provenance regions than among regions?; (iii) is the number and strength of putative adaptive single nucleotide polymorphisms (SNPs) smaller within provenance regions than among regions?; and (iv) does the population genomic approach reveal the same patterns of local adaptation as the provenance trials?

Materials and methods

STUDY SPECIES

Black alder *A. glutinosa* (L.) Gaertn, Betulaceae, is a widespread deciduous tree distributed across all of Europe (Meusel, Jager & Weinert 1965). It is a monoecious, self-incompatible and wind-pollinated species, and its seeds are mainly dispersed by water (Mac Vean 1953; Chambers & Elliott 1989). *Black alder* is a typical water-demanding species, generally occurring on wetlands and river sides because its leaves have no mechanism for controlling transpiration (Braun 1974; Herbst, Eschenbach & Kappen 1999). The species can fix atmospheric nitrogen in symbiotic root nodules (Bond, Fletcher & Ferguson 1954) and therefore does not set high demands on soil nutrition content. For this reason, it is often planted on moist degraded soils where it has a good potential for timber production and restoration purposes (Claessens *et al*. 2010). Black alder is also characterized by very high growth rates during the first 20 years, and the wood is very suitable for joinery, energy, fibre for paper and underwater constructions (Claessens *et al*. 2010). Moreover, the species contributes particularly to riverine ecosystems, to biodiversity by providing habitats for a specific flora and fauna both on the tree itself and in the flooded root system (Dussart 1999) and to water filtration and purification in waterlogged soils (Pinay & Labroue 1986; Schnitzler & Carbiener 1993). Finally, the root system helps to control floods and stabilize riverbanks; for example, alders form the 'alder-line-netscape' of the Netherlands (approximately 100 000 km of corridor plantations) where alders protect banks from eroding (De Boer & Oosterbaan 2005).

PLANT MATERIAL

Seeds were collected from a total of 225 trees across 15 Belgian populations (Fig. 1; Table 1). Five provenance regions (three populations per region) were sampled, including Sandy Region (S), Eastern Brabant (EB), Western Brabant (WB), Campine (C) and Ardennes (A). In addition, samples were taken from 15 mother trees (half-sib seed families) in each of three populations in a Danish region (Sjælland) and three populations in an Italian region (Tuscany). Seeds were stored on moist filter paper at 4 °C for 12 weeks to break dormancy. Approximately 100 stratified seeds per seed family were planted on potting soil in 48-cell division trays for germination in an open glasshouse. Germination success was measured as the percentage of final germination 30 days after sowing. After this early growth period, we randomly chose three seedlings from 15 half-sib families per population and transplanted them into plastic pots (18 cm diameter × 21 cm depth) filled with universal Saniflor potting soil to set-up the common garden. After 1 year of growth, leaves were collected from one seedling per seed family and dried on silica gel prior to DNA extractions. The same generation of trees was therefore used for both phenotypic and genetic analyses, resulting in very reliable comparisons between quantitative and neutral genetic divergence measures.

DNA EXTRACTION AND GENOTYPING

DNA from 182 individuals was extracted using DNeasy Plant Extraction kits (Qiagen Inc., Valencia, CA, USA), at a concentration of approximately 100 ng μL^{-1} as measured using a NANODROP 2000 spectrophotometer (Isogen Life Science, Temse, Belgium). The integrity of DNA was assessed on 1·5% agarose gels. De Novo GBS was used for constructing reduced representation libraries for the Illumina next-generation platform. The simultaneous collection of thousands to millions of short-read sequences for every individual and the improvement of sequence coverage per locus through genome complexity reduction allow accurate genotyping of hundreds to thousands of SNPs (Elshire *et al*. 2011). A *Pst*I GBS library comprising 187 tree DNA samples including five replicas and five negative controls were

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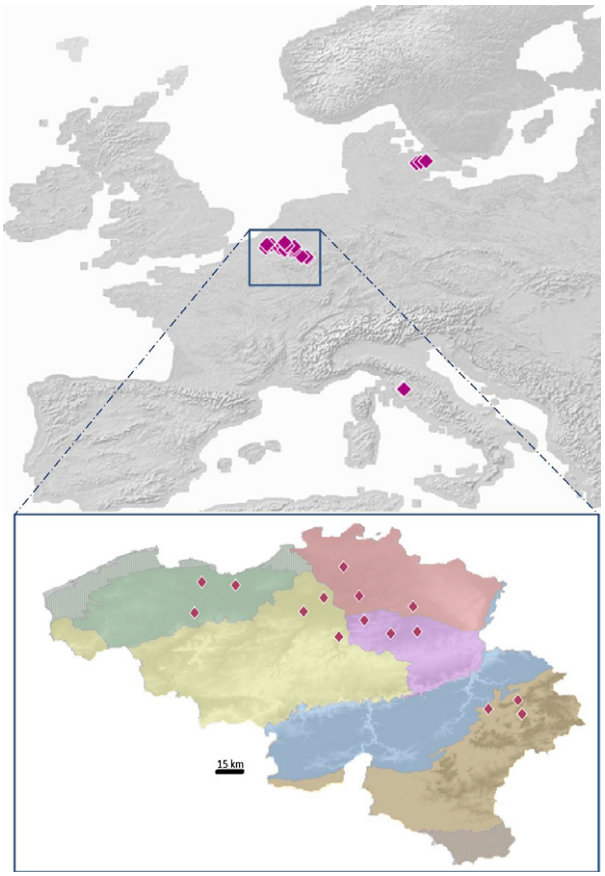

Fig. 1. Topographical map of the sampling locations of the 15 Belgian populations (three populations per provenance region), and the Danish and Italian populations. The Belgian provenance regions include Sandy Region (green), Western Brabant (yellow), Eastern Brabant (purple), Campine (red), Low Maas Plateau (blue), Ardennes (brown), Lotharingen (grey).

prepared according to Elshire *et al.* (2011). *Alnus glutinosa* has high levels of intra-population genetic diversity (Cox *et al.* 2011); therefore, the six-base cutter *Pst*I (CTGCAG), which does not cut frequently in the genome, was selected to call heterozygotes more reliably with higher sequence coverage. Briefly, individual DNA samples were digested with the restriction enzyme, adapters were ligated, and ligation products were pooled and purified as described previously (Elshire *et al.* 2011). To minimize the risk of misidentifying samples as a result of adapter or sequence synthesis error, all pairwise combinations of barcodes differed by a minimum of three mutational steps. Genomic fragments were subsequently amplified in a 50 μL volume containing 2 μL pooled DNA fragments, 1x Tag Master Mix, and 25 pmol of the forward and backward primer (Elshire *et al.* 2011). The resulting library was diluted and sequenced on the Illumina HiSeq 2000 at the Cornell University Genomics Core Laboratory.

Raw DNA sequences were analysed with the Universal Network Enabled Analysis Kit pipeline, implemented in TASSEL v3.0. Briefly, the raw Illumina DNA sequence data (100-bp qseq files) were trimmed to remove barcodes and low-quality edges to 64-bp sequences. Sequences were then aligned to each other, to identify unique sequences (sequence tags) and to generate clusters of related sequences. For each cluster, a network was generated, in which sequence tags were organized according to mutational relationship (Elshire *et al.* 2011). A single base-pair mismatch was allowed among cluster members. Networks were then filtered to retain SNPs originating from reciprocal tag pairs (see Lu *et al.* 2013). To reduce the impact of sequencing errors, the error tolerance rate parameter was set to 0.03. The resulting genotypes were then filtered using default parameters. Final filtering of the data set was done to eliminate individuals with >90% missing data and SNPs with more than 20% missing data.

PROVENANCE TRIAL I: RECIPROCAL TRANSPLANT EXPERIMENTS

After 5 months of growth in an open glasshouse, 25 seedlings from Eastern Brabant, Western Brabant and Campine were transplanted to a site in each of these three provenance regions, resulting in a total of 225 seedlings (75 seedlings per transplant site with 0·5-m spacing between the plants). In each transplant site, the seedlings were randomly distributed across two fenced plots to account for local environmental differences. In addition, reciprocal transplant plots were setup in Tuscany (Italy), Sjælland (Denmark) and Eastern Brabant (Belgium) as a reference.

Table 1. Sampling details of the 15 Belgian populations. N_{GEN} refers to the number of successfully genotyped individuals. N_{PHE} refers to the number of seed families used in the common garden

Population	Provenance region	Latitude	Longitude	N_{GEN}	N_{PHE}
De Pinte	Sandy Region	50·983930	3·629963	13	15
Evergem	Sandy Region	51·165980	3·681092	14	14
Moerbeke	Sandy Region	51·145547	3·918045	5	15
Zemst	Western Brabant	50·992488	4·402188	15	16
Sint-Katelijne-Waver	Western Brabant	51·071254	4·543055	7	14
Bertem	Western Brabant	50·845358	4·648771	1	15
Kortenaken	Eastern Brabant	50·863720	5·016410	10	15
Holsbeek	Eastern Brabant	50·939812	4·830925	14	15
Nieuwerkerken	Eastern Brabant	50·873416	5·204984	14	14
Zoersel	Campine	51·253481	4·683878	15	15
Beringen	Campine	51·021084	5·175844	14	15
Heist-op-den-Berg	Campine	51·084241	4·793124	15	15
Spa	Ardennes	50·472583	5·916774	14	14
Sprimont	Ardennes	50·494566	5·589459	14	15
Stavelot	Ardennes	50·395210	5·947033	17	16

Twenty-five seedlings per region were also transplanted to each site, with two plots per site. The data from the Italian site were excluded from the analyses because the majority of the plants in Tuscany died due to extreme drought in the summer of 2012. After 6 months, the surrounding vegetation in the plots was cut once to assure successful establishment. Interspecific competition was allowed after this period to simulate natural conditions. Height and diameter at stem basis were measured at the start of the experiment and after 2 years to measure growth and diameter increment. However, because most of the seedling tips were removed by herbivores at the end of the second growing season, growth was excluded from our analyses. Survival was measured once after 2 years. A general linear model with plot (nested within site), provenance, site and provenance*site as fixed effects was used to assess whether diameter increment was significantly higher for local provenances than for foreign provenances in each site. A generalized linear model with the same fixed effects was applied for survival.

PROVENANCE TRIAL II: COMMON GARDEN EXPERIMENT

Plants were arranged in a common garden using a random block design of three blocks, each block containing a replicate of the same seed family. A total of 15 populations × 15 seed families × 3 seedlings (one seedling per block) = 675 seedlings were screened during 3 years. Plant height and stem diameter at the basis were measured after 2 and 3 years to calculate growth and diameter. Leaf length and width of the third leaf (starting from the top of the seedling) were measured at the end of the second growing season. Leaf fall (when a leaf was discoloured completely or had fallen) was screened twice a week after the second growing season.

Linear-mixed models were used to estimate pairwise quantitative genetic variation (Q_{ST}, Spitze 1993) from phenotypic variance components, with population as random effect while accounting for maternal effects (germination success as a covariate) and variation among blocks (block as fixed effect). Germination success is in general significantly correlated with seed mass and was therefore included as a covariate to account for potential environmental maternal effects on the measured seedling traits (Meyer & Carlson 2001; Easton & Kleindorfer 2008; Bischoff & Müller-Schärer 2010). Q_{ST} was estimated as $\sigma^2_{BP}/(\sigma^2_{BP} + 2\sigma^2_{WP})$, where σ^2_{BP} and σ^2_{WP} are the additive genetic variances among and within populations resp. σ^2_{WP} was estimated as four times the observed variance among half-sib families within populations (σ^2_f) (Lynch & Walsh 1998).

Pairwise neutral F_{ST} (Wright 1951) values were calculated using 10 000 permutations (ARLEQUIN 3.5; Excoffier & Lischer 2010) to estimate neutral genetic differentiation among pairs of populations. To improve the neutrality of the pairwise F_{ST} values, we first applied a global outlier test using BAYESCAN 2.1 and removed significant outliers from the data set. Q_{ST}–F_{ST} larger than zero implies adaptive differentiation as more phenotypic variation is observed than expected under neutrality (Merilä & Crnokrak 2001; Leinonen *et al.* 2008). Q_{ST}–F_{ST} values were calculated for each pair of populations and subsequently compared between the following groups (fixed effects) using a linear-mixed model: within Belgian provenance regions (group P), between adjacent Belgian provenance regions (group A), between non-adjacent Belgian provenance regions (group NA) and between Belgian provenance regions and an Italian/Danish region (group ID). Two random effects were included in the model to account for dependency among data points (for example, the Q_{ST}–F_{ST} of Spa-Moerbeke and Spa-Beringen are not independent). For each pair of population comparisons, the first population belonged to random factor 1 and the second population to random factor 2 (see Table S1, Supporting information). Hence, all pairs containing a specific population are treated as dependent data points, both within and between groups.

GENOMIC OUTLIER DETECTION TO VALIDATE BELGIAN PROVENANCE REGIONS

Observed and expected heterozygosity were calculated using GENALEX 6.4 (Peakall & Smouse 2006). To identify putative loci under selection and corresponding F_{ST} values among each pair of populations, we used the Bayesian outlier detection approach implemented in BAYESCAN 2.1. Simulations demonstrated this method to be less prone to false-positive and false-negative results compared to FDIST2 and ARLEQUIN (Narum & Hess 2011). The Bayesian outlier method assumes an island demographic model in which F_{ST} coefficients are decomposed into a population-specific and a locus-specific component (Foll & Gaggiotti 2008). For a given locus, selection is assumed when a locus-specific component is required to explain the observed pattern of diversity. Log_{10} values of the posterior odds (PO) >0·5 and 1·5 are usually taken as 'substantial' and 'very strong' evidence for selection, respectively (Jeffreys 1961; Foll & Gaggiotti 2008). However, because there is less power to detect outlier loci when examining pairs of populations (Beaumont 2005; Pariset *et al.* 2009; Fischer *et al.* 2011), minimum Log_{10} (PO) values of 0·0 were adopted for the pairwise comparisons so as to improve the detection of loci under weak selection and to minimize false negatives. Analogous to the Q_{ST}–F_{ST} analysis, a linear-mixed model (SPSS) with two random factors to account for non-independency among data points was used to statistically compare the number of pairwise outliers between group P, A, NA and ID. To take the strength of the outliers into account, a similar pairwise analysis was performed using the outlier F_{ST} values. The F_{ST} values that were associated with the outliers reflect both neutral and adaptive processes; therefore, we subtracted the average pairwise F_{ST} across all neutral loci to retain the adaptive component. The resulting outlier-specific adaptive F_{ST} values were summed for each population pair. The total adaptive F_{ST} per population pair (total outlier strength) therefore includes both the number of outliers and the strength of each outlier.

Results

PROVENANCE TRIAL I: RECIPROCAL TRANSPLANT EXPERIMENTS

No significant interaction between provenance and site was observed for survival and diameter increment for the Belgian seed zones ($P > 0{\cdot}05$; Table 2; Fig. S1, Supporting information). Local provenances therefore did not perform better than foreign provenances. A significant site effect on diameter increment was observed with higher values for all provenances in Western Brabant. The transplant experiment involving populations from Sjælland

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Table 2. Wald chi-square statistics of the generalized linear model with survival as response variable and *F*-statistics of the general linear model with diameter increment as response variable

	Transplant Belgium		Transplant Denmark	
	Survival	Diameter increment	Survival	Diameter increment
Population	$W_2 = 0{\cdot}001$	$F_2 = 0{\cdot}420$	$W_1 = 2{\cdot}278$	$F_1 = 12{\cdot}288$**
Population*Site	$W_4 = 4{\cdot}454$	$F_4 = 2{\cdot}032$	$W_1 = 0{\cdot}002$	$F_1 = 15{\cdot}393$**
Site	$W_2 = 0{\cdot}141$	$F_2 = 31{\cdot}502$**	$W_1 = 6{\cdot}601$*	$F_1 = 106{\cdot}173$**
Plot (site)	$W_3 = 17{\cdot}194$**	$F_3 = 8{\cdot}685$**	$W_2 = 3{\cdot}179$	$F_2 = 1{\cdot}057$

Significance is shown by *$P < 0{\cdot}05$ and **$P < 0{\cdot}01$.

(Denmark) revealed a significant interaction between provenance and site for diameter increment, with local provenances clearly outperforming foreign provenances ($P < 0{\cdot}01$; Table 2; Fig. S1, Supporting information). A significant site effect was observed for both diameter increment and survival ($P < 0{\cdot}05$; Table 2; Fig. S1, Supporting information).

PROVENANCE TRIAL II: COMMON GARDEN EXPERIMENT

Pairwise Q_{ST}–F_{ST} values were not significantly lower within provenance regions than (i) between adjacent provenance regions and (ii) between non-adjacent provenance regions ($P < 0{\cdot}05$; Fig. S2; Table S2, Supporting information). A paired *t*-test with 10 000 bootstrap samples showed that pairwise Q_{ST}–F_{ST} values for growth ($0{\cdot}024 \pm 0{\cdot}004$; $P < 0{\cdot}01$), diameter ($0{\cdot}004 \pm 0{\cdot}002$; $P < 0{\cdot}01$), leaf width ($0{\cdot}007 \pm 0{\cdot}002$; $P < 0{\cdot}05$), leaf length ($0{\cdot}003 \pm 0{\cdot}001$; $P > 0{\cdot}05$) and leaf fall ($0{\cdot}001 \pm 0{\cdot}001$; $P > 0{\cdot}05$) were slightly different from zero among Belgian populations, indicating limited adaptation within Belgium. Contrasting adaptive genomic results, Q_{ST}–F_{ST} values did not increase in comparisons between the Belgian and the Danish/Italian reference populations (Fig. S2, Table S2, Supporting information).

GENOMIC OUTLIER DETECTION TO VALIDATE BELGIAN PROVENANCE REGIONS

A total of 1990 SNPs were screened among the 21 populations. Neutral genetic differentiation (pairwise F_{ST}) varied from $0{\cdot}017 \pm 0{\cdot}013$ between Belgian and Danish/Italian populations to $0{\cdot}004 \pm 0{\cdot}004$ within Belgium (Table S3, Supporting information). Both the number of outliers and the total outlier strength were significantly higher between the Belgian provenance regions and the Danish and Italian reference regions than (i) within Belgian provenance regions, (ii) between adjacent Belgian provenance regions and (iii) between non-adjacent Belgian provenance regions, matching the expectations of the pairwise outlier analyses ($P < 0{\cdot}01$; Figs 2 and 3; Table S2, Supporting information). The number of outliers and the total outlier strength were not significantly smaller within

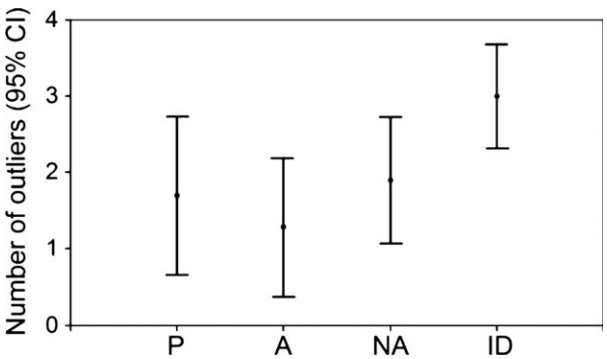

Fig. 2. Error plot of number of outliers within Belgian provenance regions (group P), between adjacent Belgian provenance regions (group A), between non-adjacent Belgian provenance regions (group NA) and between Belgian provenance regions and a Danish/Italian reference region (group ID). Confidence intervals were estimated using a linear-mixed model, accounting for dependency among data points.

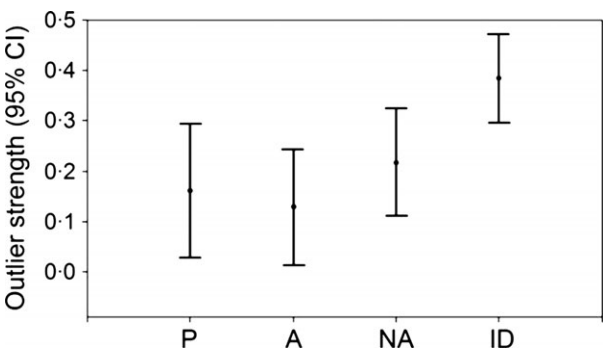

Fig. 3. Error plot of the total outlier strength (total pairwise adaptive F_{ST} of outliers) within Belgian provenance regions (group P), between adjacent Belgian provenance regions (group A), between non-adjacent Belgian provenance regions (group NA) and between Belgian provenance regions and a Danish/Italian reference region (group ID). Confidence intervals were estimated using linear mixed model, accounting for dependency among data points.

Belgian provenance regions than (i) between adjacent Belgian provenance regions and (ii) between non-adjacent Belgian provenance regions ($P > 0{\cdot}05$; Figs 2 and 3; Table S2, Supporting information). Overall, the number of

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outliers (1·4 ± 0·1; 0·1%) and the total outlier strength (0·178 ± 0·026) among pairs of Belgian populations was low, indicating little adaptive differentiation within Belgium.

Discussion

Our results show an overall congruence with regard to patterns indicative of local adaptation within Belgium, using population genomics and conventional provenance trials (a common garden and a reciprocal transplant experiment). In all cases, we found very limited support for adaptation at the scale of Belgian provenance regions. Our findings therefore do not validate current Belgian seed zone delineation, but rather support an expansion beyond the national borders. Interestingly, the population genomics approach matched our expectations, as corroborated by the inclusion of distant European reference regions.

Provenance*site effects of reciprocal transplant experiments reflect local adaptation if local provenances show higher fitness than foreign provenances and if no maternal effects assumed (Kawecki & Ebert 2004; Leimu & Fischer 2008; Hereford 2009). In agreement with the adaptive genomic results, no fitness advantages were observed for local provenances in Belgian seed zones, indicating little evidence for adaptive differentiation among seed zones (Table 2). The presence of a significant site effect for diameter increment suggests that phenotypic plasticity in response to the environments of the transplant sites rather than local adaptation explains phenotypic differences among Belgian seed zones. Although survival was low for Danish seedlings in all sites, higher diameter increments were observed for the Belgian and Danish seedlings in their home sites. This finding suggests higher competitive ability for local provenances at a larger geographical scale, which strongly affects long-term survival and reproduction (Dodd *et al.* 2005; Liancourt, Callaway & Michalet 2005). The ability to include naturally occurring interspecific competition is an important advantage of reciprocal transplant experiments compared to greenhouse experiments, as competition may considerably affect responses to selection (Grant & Grant 2006; Sambatti & Rice 2006).

If quantitative genetic divergence (Q_{ST}) exceeds neutral genetic divergence (F_{ST}), phenotypic differences among populations are assumed to partially result from positive natural selection (Merilä & Crnokrak 2001; Leinonen *et al.* 2008; De Kort, Vandpitte & Honnay 2013). In our study, Q_{ST}–F_{ST} was on average smaller than 0·04 for all traits, both within Belgium and at the larger scale (Fig. S2, Supporting information). This low Q_{ST}–F_{ST} is in contrast to the results of most other Q_{ST}–F_{ST} studies conducted on tree species, which often reveal values exceeding 0·1 (e.g. Yang, Yeh & Yanchuk 1996; González-Martínez, Alía & Gil 2002; but see Navarro *et al.* 2005; Waldmann, García-Gil & Sillanpää 2005; Alberto *et al.* 2011; Keller *et al.* 2011). Although common gardens offer the advantage of assessing the quantitative genetic nature of phenotypic traits, plastic responses to the common garden conditions as well as maternal effects may considerably bias Q_{ST}–F_{ST} results (Cano *et al.* 2004; Gienapp *et al.* 2008). Here, a very long winter with temperatures below zero up to the second week of April 2012 most probably postponed bud burst in all populations, assimilating growing seasons and subsequent growth. The combination of the resulting low variation in phenotypic traits with high neutral genetic differentiation (F_{ST}; Table S3, Supporting information) between Belgian and Danish/Italian populations probably resulted in underestimated Q_{ST}–F_{ST} estimations. Phenotypic plasticity thus potentially impairs the efficacy of common garden trials to infer patterns of genetic adaptation, unless (i) traits are measured in multiple generations and in multiple, strategically located gardens and (ii) stringent experimental control for maternal effects is performed.

Population genomics is increasingly being used as a tool to detect signs of adaptation among populations (Morin *et al.* 2004; Allendorf, Hohenlohe & Luikart 2010; Helyar *et al.* 2011; Narum *et al.* 2013) and may also facilitate a rapid and efficient evaluation of seed provenance delineations. Here, the number and strength of outlier SNPs was not significantly different within and among Belgian seed zones (Figs 2 and 3), implying that current Belgian seed zones not truly reflect adaptive divergence. The recent application of population genomics to evaluate seed zones for *Frangula alnus* (De Kort *et al.*, in press), an insect pollinated tree with a shorter life span, rendered results that are very consistent with the patterns we found in *A. glutinosa*. An expansion of the seed zones beyond the Belgian scale may therefore suit a number of species with different life histories. The increase in the number and strength of outliers in comparisons between Danish, Italian and Belgian populations (Figs 2 and 3) confirms the robustness of the population genomic approach, as environmental conditions (especially climate and seasonal day length variation) differ considerably between Denmark, Belgium and Italy. Stronger adaptive differentiation was therefore expected among Danish, Italian and Belgian populations than among populations within Belgium. Hence, although genome scans may miss the signal of selection on polygenic phenotypes via subtle allele frequencies shifts at many loci, our findings highlight the value of the presented population genomics procedure in evaluating adaptive patterns within and among seed zones.

The correspondence between the population genomic results and climatic differences among regions suggests the utility of environmental data to efficiently define seed zones. However, environmental differences not necessarily result in adaptive differences, as other factors, including the amount of gene flow among populations and non-adaptive phenotypic plasticity, may affect responses to environmental cues (Kawecki & Ebert 2004; Grether 2005; McKay *et al.* 2005; Ghalambor *et al.* 2007). Genetic variation at

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molecular markers exceeding neutral variation from gene flow and genetic drift is not affected by plastic phenotypic responses and is therefore expected to more accurately reflect adaptive genetic differentiation among populations and regions than environmental variables. On the other hand, outliers can be difficult to find in the case of weak divergent selection and high neutral population genetic differentiation. Pairwise outlier detection approaches may therefore have relatively low power to detect adaptive loci (Narum & Hess 2011; Le Corre & Kremer 2012a,b). Nevertheless, within Belgium, neutral differentiation (pairwise neutral F_{ST}) was very low and therefore not expected to considerably underestimate the number of outliers. Higher numbers of false negatives can be expected when considering the comparisons between Belgian provenance regions and the Italian and Danish region due to higher population differentiation, but this would make our results conservative.

Taken together, we proposed a novel strategy to assist the evaluation of seed zones, applicable to any species. In contrast to traditional provenance trials, our population genomics procedure allows to rapidly assess patterns of adaptive differentiation among seed zones. Moreover, the genomics approach we put forward is not flawed by non-genetic plastic responses to environmental cues or by maternal effects and is therefore complementary to traditional reciprocal provenance trials. A reliable way to delineate seed zones that truly reflect local adaptation is crucial, as inadequate seed transfer guidelines may negatively impact restoration success through the potentially detrimental effects of decreased genetic variation on plant fitness (Reed & Frankham 2003; Leimu *et al.* 2006); especially, when small and/or fragmented populations are involved, the delineation of too small provenances may decrease levels of genetic diversity and therefore reduce plant performance (Young *et al.* 1996; Reed & Frankham 2003; Vranckx *et al.* 2012). Moreover, a loss of adaptive genetic variation may decrease the capacity to cope with climate change (Jump & Peñuelas 2005; McKay *et al.* 2005; Barrett & Schluter 2008; Hoffmann & Sgrò2011). The presented time-effective population genomics approach should therefore assist foresters and land managers with the selection of guidelines on seed sourcing and delineations of provenance regions.

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Data accessibility

Raw SNP data and phenotypic data are accessible in Dryad using the following http://datadryad.org/resource/doi:10.5061/dryad.rg82f (De Kort *et al.*, 2014).

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Supporting Information

Additional Supporting Information may be found in the online version of this article.

Fig. S1. Reaction norms for survival and diameter increment.

Fig. S2. Error plots of pairwise Q_{ST}–F_{ST} comparisons for phenotypic traits among groups P, A, NA and ID.

Table S1. Assignment of data points (number of outliers, outlier strength and Q_{ST}–F_{ST} of population pairs) to the two random factors included in the mixed model of seed zone evaluation.

Table S2. Differences in Q_{ST}–F_{ST}, number of outliers and total outlier strength between groups P, A, NA and ID.

Table S3. F_{ST} among all pairs of populations and corresponding *P*-values.

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