

Genetic and morphological variability among autochthonous *Prunus spinosa* populations in Flanders (northern part of Belgium): implications for seed sourcing

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Background and aims – Seed sourcing on a local scale is an emerging awareness among nature conservationists. Guidelines should be tuned for woody species in highly anthropogenic landscapes such as Flanders (Belgium). We investigated the genetic variation within and between eight autochthonous Flemish *Prunus spinosa* populations and assessed their potential as seed source for gene conservation and ecological restoration.

Methods and key results – All sampled sites were old hedges or wooded banks. Using AFLP, the overall gene diversity (H_t) was estimated at 0.13, with a low average between population diversity ($H_b = 0.02$) and a comparatively high average within-population diversity ($H_w = 0.11$). The genetic differentiation was remarkably variable among populations (Φ_{ST} ranging from 0.07 to 0.43). There was no clear relationship between genetic and geographic distances, nor between genetic and morphometric characteristics. Only a small but significant correlation between endocarp and leaf morphological characters was observed.

Conclusions – The variable genetic differentiation among populations points to different vegetation histories. Two populations, DEF and WES, were characterized by a dominant presence of sloe, high genetic differentiation from all other populations and low within-population diversities. No obvious morphometric variability of leaves could be related to these genetically deviating populations, however, WES showed a deviating endocarp morphology. Possibly, these two populations were originally planted or supplemented using local and much related material whereas the others may have been planted with more diverse material. The relatively high within-population diversities and moderate, although variable, between-population differentiation of the other Flemish populations point to a considerable amount of gene exchange and can justify extensive seed sourcing for the production of autochthonous planting stock. These results stress the importance of a proper evaluation of genetic diversity for seed source selection and the need for regional field-applicable guidelines.

Key words – Amplified Fragment Length Polymorphism, Flanders, genetic diversity, variable genetic differentiation, morphometrics, seed collection, sloe, *Prunus spinosa*.

INTRODUCTION

After ages of neglecting, the use of native species in forestation and landscape programmes is gaining importance all over the world. The basic underlying ecological principle is local adaptation of the natives and co-evolution of all members of the forest ecosystems. Especially in Western Europe, awareness has led to massive plantations of indigenous tree and shrub species, not only in forestry but also for

native woodland restoration and other landscape plantings such as thickets, wooded banks and hedge rows. Seed collection, storage, stratification, sowing and subsequent growth of planting stock in nurseries is a traditional and straightforward way to obtain planting material. The European directive on the marketing of forest reproductive material obligates certification of seeds and planting material for tree species, but not for shrubs (Anonymous 2000). As a commercial consequence, Western European nurseries grow planting stock

of many native shrub species starting from cheap seed lots originating from foreign sources, often low income countries in Eastern and Southern Europe. Putative consequences such as maladaptation, loss of genetic diversity, loss of adaptation and outbreeding depression in the natural populations (e.g. McKay et al. 2005, Krauss & He 2006, Laikre et al. 2010) become a growing concern and several initiatives in different European countries are worked out to promote the use of locally sourced seeds for the production of planting stock (e.g. Flanders: Vander Mijnsbrugge et al. 2005, Germany: Kleinschmit et al. 2008, BNatSchG 2010, Denmark: Kjaer et al. 2009).

Prunus spinosa L. is a widely distributed species naturally occurring in the temperate parts of Europe and Asia (Woldring 2000). This thorny shrub or small tree is commonly found in European deciduous forests, hedges and thickets along forest edges and in open farmland. *P. spinosa* is insect-pollinated and propagates vegetatively through root suckers (Guitian et al. 1993). Seed is dispersed by mammals and birds. *P. spinosa* is supposed to be an allotetraploid species ($2n = 4x = 32$; Zohary 1992). When considering seed collection for gene conservation and restoration purposes, genetic aspects are of concern. Maintenance of the genetic diversity of autochthonous *P. spinosa* populations is necessary for ongoing adaptability of the species to complex heterogeneous environments (e.g. Kleinschmit et al. 2004, Hubert & Cottrell 2007, Kramer & Havens 2009). Autochthony of woody vegetation is interpreted here as a continuation of the populations since their colonization after the last glaciation and implies local adaptation (Kleinschmit et al. 2004). Still, autochthonous populations may as well suffer from genetic distortions due to anthropogenic influences and may not always be the ideal seed source for restoration purposes. Various guidelines exist to direct practitioners in seed collection strategies (e.g. Vander Mijnsbrugge et al. 2010). These guidelines stress the importance of a significant level of genetic diversity, geographical proximity between source population and planting site, habitat-matching, life-history variables and taxonomic boundaries. In addition, the history of a population, and the landscape within which it exists, are factors influencing the genetic relationships of populations (Pautasso 2009). Moreover, while reviewing literature on seed sourcing of mostly herbaceous plant taxa for restoration purposes, Broadhurst et al. (2008) argue that it may be more important for seed to be genetically diverse and of high quality than being local. Of course, the geographic scale of 'local' is an ongoing debate as the measurement of local adaptation, tolerance and plasticity against a changing environment in woody species requires extensive and long-term research (Matyas 2007).

Anthropogenic transport and subsequent natural gene flow from the cultivated damson plum, *P. insititia* L., into the wild populations of *P. spinosa*, giving rise to the taxon *P. × fruticans* Weihe, was suggested in Germany (Körber-Grohne 1996) and Denmark (Nielsen & Olrik 2001) based on morphological studies and chromosome counts. Still, this putative hybrid is probably a rare event, as polyploidy levels differ (*P. spinosa* being $4x$, whereas domesticated plums show $6x$). A phylogenetic study of *Prunus* section *Prunus* taxa also highlighted the distinct evolutionary origins of the

different polyploid groups (Reales et al. 2010). We doubt the wide-spread occurrence of this hybrid in our study area (northern part of Belgium). Firstly, we found no continuous morphological swarm between *P. spinosa* and *P. insititia* for endocarp and leaf characters (Depypere et al. 2009). Only a morphological continuum is present between *P. spinosa* and a large fruited form of the latter described as *P. × fruticans* (Depypere et al. 2009). Secondly, *P. spinosa* and *P. × fruticans* could not be separated in an AFLP analysis, suggesting a genetically homogenous group (Depypere et al. 2009). And, there was a clear distinction between this group and *P. insititia*, leaving the latter as a separate and distinguishable taxon. These results suggested that *P. × fruticans* can be considered as a large fruited variety of *P. spinosa* and should not be treated as a taxonomic hybrid.

The use of genetic markers provides a powerful approach for a first assessment of genetic structure in natural populations. Molecular markers often represent neutral genetic variation within and among populations, which does not necessarily correspond to adaptive variation (e.g. Hufford & Mazer 2003). Still, they are very useful for detecting three phenomena that either predict or reflect population genetic risks of restoration: (1) strong founder effects; (2) genetic swamping; and (3) population genetic divergence that might indicate ecotypic or epitypic variation (Hufford & Mazer 2003). Few studies have addressed genetic diversity of *P. spinosa* populations using molecular markers. Mohanty et al. (2002) describe a relatively high genetic diversity within European *P. spinosa* populations based on chloroplast DNA markers. They show a relatively low interpopulation differentiation and a weak correlation between genetic and geographic distances. In a study incorporating related species, *P. spinosa* showed a higher level of cpDNA allelic richness in comparison to *P. domestica* (Horvath et al. 2011). In a recent study of Eimert et al. (2012), autochthonous *P. spinosa* seed stocks were compared to commercially available ones using high annealing temperature random amplified polymorphic DNA (HAT-RAPD) markers. Low genetic diversity was found both in the autochthonous populations and in the conventional seedstocks, the major part of it residing within populations and a minor part among them, as is usually observed in long-living woody species (Duminil et al. 2009).

The objectives of the present study were to elucidate the levels of genetic variation within and among Flemish populations of *P. spinosa*; to look for congruencies between genetic and phenotypic characteristics and to examine the implications for the utilization of the Flemish *P. spinosa* populations as seed source for gene conservation and restoration purposes. For this, we conducted an AFLP analysis and a morphological study.

MATERIALS AND METHODS

Plant material

The taxon *P. spinosa* in this paper refers to populations as found in the field including large fruited forms. In the autumn of 2005, 139 autochthonous shrubs of *P. spinosa* were sampled at eight different locations (further in text referred to as 'populations') in three Flemish regions (table 1, fig. 1), based

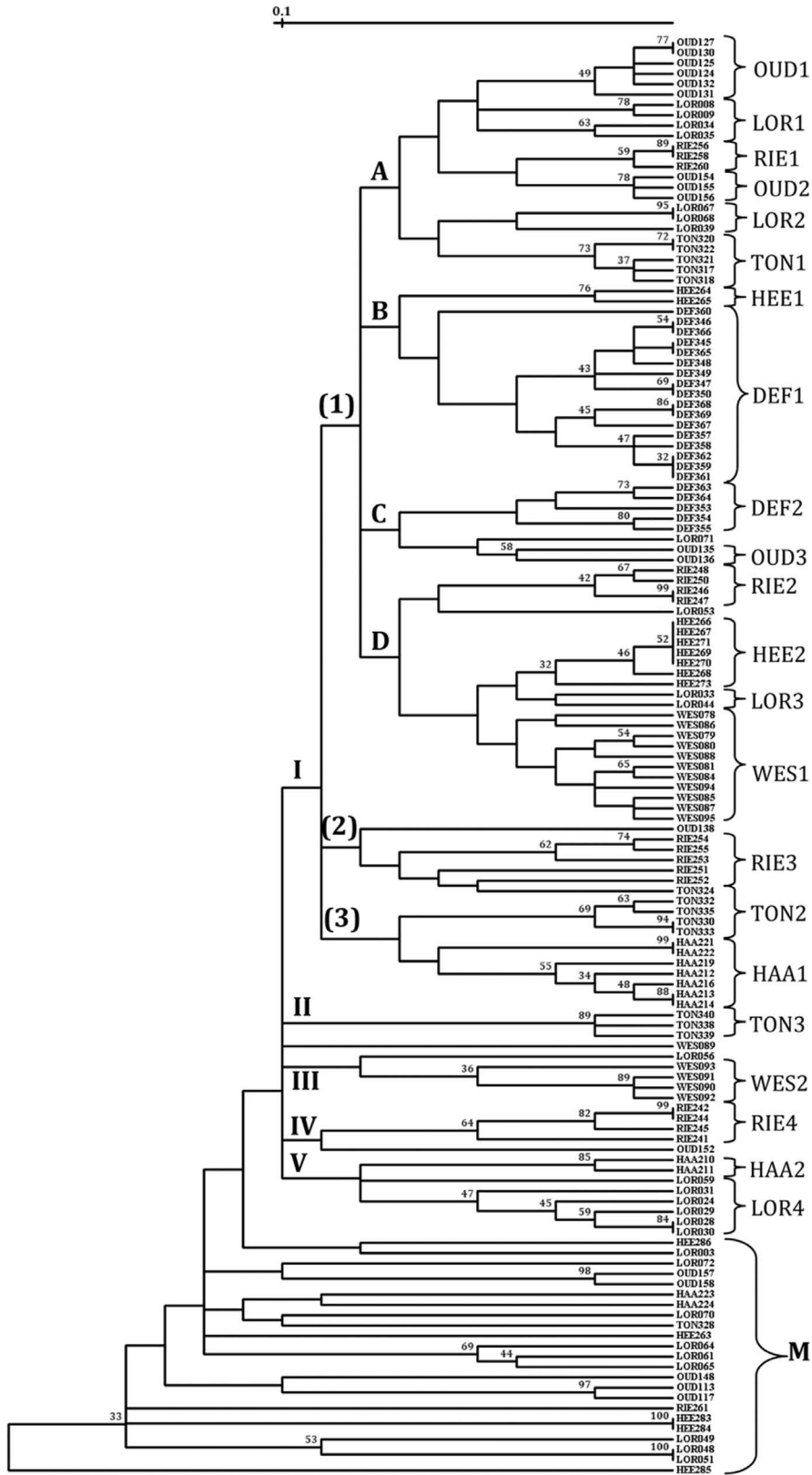


Figure 2 – UPGMA phenogram based on AFLP data, using the Nei & Li (1979) distance estimation of 139 *P. spinosa* accessions sampled across eight Flemish populations. Numbers shown at the different nodes indicate bootstrap confidence values (2000 replicates). Bootstrap values above 30% are indicated on the branches. Location abbreviations are in table 1.

two additional selective nucleotides, was carried out with four primer combinations (*EcoRI*-ACT/*MseI*-CTG, *EcoRI*-ATC/*MseI*-CAT, *EcoRI*-ACT/*MseI*-CAG, *EcoRI*-ATC/*MseI*-CAC). After amplification, the DNA fragments were separated on a Nen IR² genetic analyzer (Licor). The automatically generated TIFF-files were analyzed in SAGAmx version 3.0 (Licor). Around 10% of the samples were duplicated for the verification of reproducibility of the banding patterns. In total, 95 (37 monomorphic and 58 polymorphic) markers between 66bp and 784bp were scored. Monomorphic bands were kept in the analysis to avoid an underestimation of the overall similarity between the samples. The scoring results were transformed into a matrix with values 1 (present) or 0 (absent).

AFLP-based data analysis

Cluster analysis of the *P. spinosa* samples was performed using the unweighted pair group method with arithmetic mean (UPGMA) and the Nei & Li distance estimation in TreeCon version 1.3b (Vandepeer & De Wachter 1994). Principal co-ordinate analysis (PCoA) was performed using Genalex version 6 (Peakall & Smouse 2006). A Bayesian inference of the genetic structure was performed with Bayesian Analysis of Population Structure (BAPS, version 4.14, Corander et al. 2004) using an independent model and mixture analysis. Five replicates of all K-values between four and 10 were tested and the optimal number of clusters was deduced from the obtained log (marginal likelihood) values.

Allele-frequency based analyses of genetic diversity were performed using AFLPsurv version 1.0 (Vekemans et al. 2002). Allelic frequencies at AFLP loci were estimated from the binary presence-absence matrix using the Bayesian method with non-uniform prior distribution of allele frequencies, as described by Zhivotovsky (1999) for diploid species. As an allotetraploid, *P. spinosa* can be treated as a diploid species. Hardy-Weinberg genotypic proportions were assumed.

As a measure of genetic differentiation, the overall and pairwise Φ_{ST} values were derived from the genetic distances among populations using an AMOVA in Genalex v6 and their significance was determined using the Monte Carlo procedure (999 permutations). Φ_{ST} is an analogue for F_{ST} used for dominant markers.

Isolation-by-distance among the Flemish populations was checked by performing a Mantel test between Nei's pairwise genetic distances and pairwise geographical distances in Genalex. Geographical distances were derived from the latitude/longitude values of the different sampling locations.

Endocarp and leaf morphometrics

Five representative leaves that were insect and damage free were separated from the twigs for each individual. Endocarps and leaves were digitized and dimensions and shape characteristics were analyzed with TomatoAnalyzer (Brewer et al. 2006). For visualization and calculation methods of the listed characteristics, we refer to Brewer et al. (2006) and Depypere et al. (2007). No endocarps were available for the population from Ukraine. Leaf material was missing for all individuals from LOR, WES, and Ukraine; for ten of four-

teen individuals from HEE and for two of eighteen individuals from OUD. For each individual and for each character, means were calculated from the five measured endocarps and leaves. These endocarp and leaf data were explored in box-plots and PCA was applied for a multivariate analysis in S-plus 6.2 Professional (Insightful Corp.).

RESULTS

Cluster, principal co-ordinate and Bayesian analysis

In an UPGMA analysis using the Nei distance measure (fig. 2) or in a PCoA analysis (data not shown) of the *P. spinosa* individuals, sampled across eight Flemish populations, no clear geographic clusters could be observed. Still, individuals originating from the same location tended to cluster together in more or less distinguishable groups. Despite this tendency of co-occurrence, several distinct groups with samples from one location were spread out over the UPGMA phenogram, e.g., groups of at least two individuals originating from LOR could be found in different clusters (fig. 2): in group I we found LOR1 and LOR2 in subgroup A and LOR3 in subgroup D, whereas LOR4 resided in group V. Several individuals were located in a mixed group at the base of the phenogram (group M in fig. 2). Similarly the samples from RIE were found in subgroups of group I [RIE1 in A and RIE2 in D, both in subgroup (1), with RIE3 in subgroup (2)] and group IV (RIE4). At the population level, a PCoA analysis clearly showed the discrimination of WES (fig. 3). The three axes together explained 71.1% of the total variation.

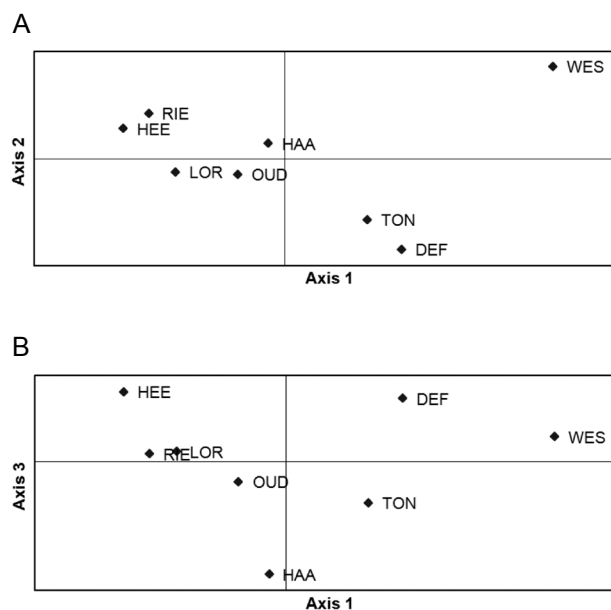


Figure 3 – Principal co-ordinate plots based on the Jaccard similarity coefficient of genetic similarity between eight Flemish *P. spinosa* populations. The first (28.67%), second (25.02%), and third (17.44%) axis together account for 71.13% of the variation (eigenvalues are 0.009, 0.008 and 0.005, respectively). A, plot of the first two principal co-ordinates; B, plot of principal co-ordinates 1 and 3. Population abbreviations are in table 1.

Table 2 – Distribution of the individuals of each population over the seven BAPS clusters (I – VII).

Population abbreviations are in table 1. UKR, Ukraine outgroup.

	Cluster						
	I	II	III	IV	V	VI	VII
DEF	0	0	0	5	0	17	0
HAA	2	0	0	7	2	0	0
HEE	2	7	0	3	0	0	2
LOR	14	4	1	0	5	0	3
OUD	2	0	7	2	4	0	3
RIE	5	4	0	0	8	0	0
TON	7	0	0	7	0	0	0
WES	5	11	0	0	0	0	0
UKR	1	1	0	0	0	0	6

As expected, the Ukraine outgroup deviated clearly from all Flemish populations in a combined PCoA (electronic appendix 1). BAPS analysis resulted in seven clusters and the log (marginal likelihood) of the optimal partition was -2718.01. The predicted BAPS clusters were very similar to those obtained in the UPGMA analysis. Table 2 shows the distribution of the individuals within each population over the seven clusters. Cluster I contained individuals of each population except for DEF. In contrast, cluster VI was private for DEF. The populations of LOR and OUD were spread over five different clusters, while DEF, TON, and WES were restricted to two clusters.

There was no sign of isolation-by-distance at the Flemish scale as there was no significant correlation between geographic and genetic distances of the populations (Mantel $r = 0.0077$, $p = 0.314$).

Population genetic diversity

As cluster analysis indicated the presence of putative clonal individuals (fig. 2), a second dataset was created by excluding 22 individuals with an identical AFLP pattern (table 1). The genetic diversity of the *P. spinosa* shrubs was studied in both datasets. Based on the original dataset including all samples, the overall gene diversity (H_j) was estimated at

0.13, with the within-population diversity (H_w) at 0.11 (SE 0.008) and the between population diversity (H_b) at 0.02 (SE 0.0016). Levels of genetic diversity within-populations (= expected heterozygosity, H_j , table 1) varied: the populations of DEF (0.07) and WES (0.09) had the lowest H_j values, while OUD had the highest within-population diversity (0.14). There was no correlation between sample size and H_j (Pearson correlation coefficient $r = -0.34$, $p = 0.37$). The overall Φ_{ST} was 0.213 and highly significant ($p < 0.001$). In the pairwise comparisons of the Flemish populations, the highest genetic differentiation was observed between DEF and WES ($\Phi_{ST} = 0.428$). Both populations were almost as highly differentiated from the other Flemish populations as the Ukrainian outgroup (table 3). When excluding putative clones, the results only marginally changed. H_b dropped to 0.01 and the Φ_{ST} values were a bit lower (see electronic appendix 2). When the two deviating populations DEF and WES were removed, the overall Φ_{ST} diminished to 0.16.

Endocarp and leaf morphometrics

A PCA analysis was performed on the endocarp morphology data (fig. 4). WES endocarps clearly deviated from the other studied populations. They showed a low SL (were not elongated), had low values for X100ST.SW (were relatively thick) and high values for Circular (were more rounded) in comparison to the others. Endocarps of DEF and OUD also tended to have lower values for SL and X100ST.SW (fig. 4 and electronic appendix 3). Leaf morphological characters were variable (electronic appendix 4). HEE was the only population displaying deviating characters: it had wide leaves (low LL/LW value, electronic appendix 4). There was a small but significant relationship between endocarp and leaf morphology (electronic appendix 5, linear regression with R^2 of 0.12, $p = 0.001$): rounded endocarps correlated with relatively wider leaves whereas elongated endocarps correlated with relatively narrow leaves.

DISCUSSION

Genetic diversity of plant populations in anthropogenic landscapes is the result of various interacting evolutionary forces (mutation, genetic drift, selection, migration, and

Table 3 – Pairwise genetic differentiation (Φ_{ST} values) between populations in total dataset.

Φ_{ST} values are shown below the diagonal, p-values, based on 999 permutations, above the diagonal. Population abbreviations are in table 1.

	DEF	HAA	HEE	LOR	OUD	RIE	TON	WES	UKR
DEF	-	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
HAA	0.369	-	0.001	0.001	0.001	0.001	0.005	0.001	0.001
HEE	0.362	0.236	-	0.001	0.001	0.001	0.001	0.001	0.001
LOR	0.253	0.183	0.125	-	0.002	0.002	0.001	0.001	0.001
OUD	0.287	0.179	0.179	0.078	-	0.001	0.001	0.001	0.001
RIE	0.371	0.214	0.111	0.068	0.115	-	0.001	0.001	0.001
TON	0.251	0.124	0.158	0.131	0.114	0.152	-	0.001	0.001
WES	0.428	0.341	0.281	0.237	0.248	0.282	0.247	-	0.001
UKR	0.461	0.365	0.327	0.258	0.227	0.349	0.331	0.369	-

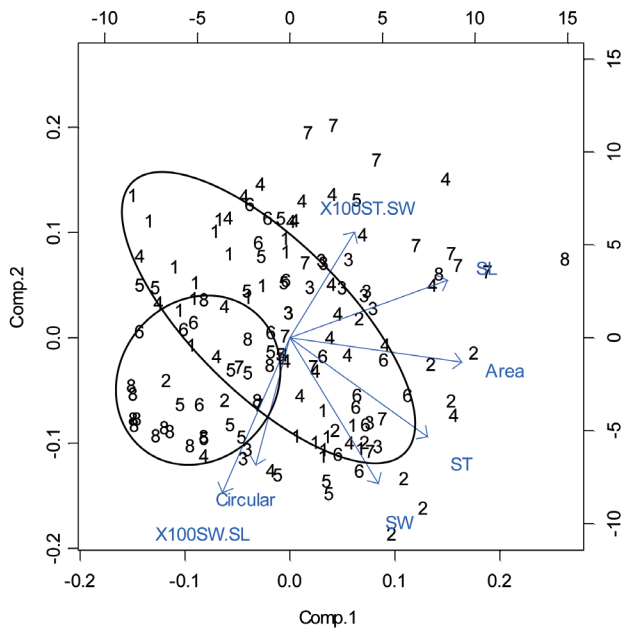


Figure 4 – Principal component biplot based on endocarp morphometrics of eight Flemish *P. spinosa* populations. Axis 1 accounts for 42,9% and axis 2 for 40,4% of the variation. Locations: 1, DEF; 2, HAA; 3, HEE; 4, LOR; 5, OUD; 6, RIE; 7, TON; 8, WES. Location abbreviations are in table 1. Endocarp characters: SW, width; ST, thickness; SL, length; Area, area; Circular, shape circular; X100ST.SW, 100*(thickness/width); X100SW.SL, 100*(width/length). Ellipses indicate the two populations DEF and WES that show a low within genetic diversity.

mating-system) and human interferences. The present study assessed the level of genetic variation within and between Flemish populations of *P. spinosa* to evaluate their potential for the purpose of seed sourcing aimed at the production of forest reproductive material used in both forestry and landscape plantings. One of the intentions of the usage of locally sourced plant material is to preserve the regional genetic constitution. The question can be raised whether local populations show genetic and/or morphological differentiation among each other. This may influence decisions on the demarcation of regions of provenance.

The overall gene diversity of the Flemish *P. spinosa* populations comprised of a low average between population diversity ($H_b = 0.02$, 15%) and a comparatively high within-population diversity ($H_w = 0.11$, 85%). The discrepancy among the between and within-population diversity is consistent with results for other woody species based on allozyme and DNA marker analyses as e.g. reviewed by Hamrick & Godt (1996), Petit & Hampe (2006), or Duminil et al. (2009). In this type of reviews tree species are compared to herbaceous species, but shrubs are not included or not treated as a separate group. Genetic studies dealing with trees far exceed the ones on shrubs, as trees represent an economic value. The average within-population gene diversity that we observed was low in comparison to the values noted in several wind pollinated tree species in Flanders using AFLP markers ($H_w = 0.30$ for *Carpinus betulus* in Coart et al. 2005, $H_w = 0.29$ for *Quercus petraea*, $H_w = 0.28$ for *Quercus robur* in Co-

art et al. 2002). Although insect pollination in *P. spinosa* may hamper gene flow through pollen dispersal in comparison to wind pollinated species, seeds are easily dispersed over longer distances by birds. Unfortunately, no data for shrubs or insect pollinated tree species are available for our study area. Similar results were obtained by Eimert et al. (2012) on autochthonous *P. spinosa* populations in Germany applying HAT-RAPD markers. Gene diversity (H_f) varied between 0.12 and 0.13 for the different studied populations. But, only 5.8% of the total genetic diversity could be attributed to between population variation. This lower value in comparison to our study is remarkable, as their study area is much larger than Flanders. But, our dataset included two populations with low intrapopulation diversity (see further) which affected the interpopulation diversity results (Φ_{ST} decreases from 0.213 to 0.16). In addition, it should be noted that Eimert et al. (2012) studied seedlings whereas we looked at the mother shrubs and levels of genetic diversity are dependent on ontogenetic stage (i.e. seedlings or mature individuals, e.g. Nurtjahjaningsih et al. 2007) and also on the sample sizes which were small in our study.

The overall differentiation between the Flemish *P. spinosa* populations was estimated at 0.213. But, cluster analysis and PCoA of individual samples showed ample biologically interpretable clustering, except for the fact that several shrubs from the same population are most probably much related in origin and clonality may be involved. In a German study, clonality in *P. spinosa* has been demonstrated (Leinemann et al. 2010). Exclusion of putative clonal individuals from our AFLP dataset influenced slightly numerical output (table 3 versus electronic appendix 2) but did not affect the general results of the analysis. There seems to be little correlation between geographic and genetic distances between the shrub populations. These results are consistent with Eimert et al. (2012). Still, the pairwise genetic differentiation in our study varies to a considerable extent and this deviates from Eimert et al. (2012) where pairwise genetic differentiation between autochthonous *P. spinosa* populations is low in all cases, even when compared to commercially available seedstocks originating from Hungary. They suggest that the genetic diversity, which is evenly distributed among the populations, and the low interpopulation differentiation can be ascribed to both natural and anthropogenic influences. Firstly, *P. spinosa* is a very common species in Germany (no barriers to gene flow) and its polyploidy may enhance plasticity, reducing the necessity for selection and subsequent differentiation. Secondly, the wide-spread historic usage of *P. spinosa* for human consumption and more recently the large-scale plantings all over Germany with commercial seedstock may have further diminished population differentiation. Both arguments also hold true for the Flemish situation of *P. spinosa* populations. The deviating results might be related to the fact that our dataset includes populations with low genetic diversity. As discussed below, these may have originated as a result of former customs of hedge row creation among farmers. It is possible that Flanders harbours a higher relative concentration of such hedge rows in comparison to Germany because of the historical higher population density. Also remarkable is the absence of a clear population differentiation between autochthonous populations and

Hungarian seed stock in the study of Eimert et al. (2012) whereas our Ukraine outgroup clearly differentiated from all studied Flemish populations. In a phylogeographical study, Mohanty et al. (2002) distinguished at a European level the populations from northern Europe, displaying a low number of cpDNA haplotypes, from those in southern countries with a higher number of haplotypes. Possibly, the Hungarian seed lot in the study of Eimert et al. (2012) represent closer the northern European populations correlating with the low population differentiation between the seed lot and German populations, whereas our Ukraine outgroup may exemplify the populations of more southern Europe, correlating with the observed differentiation from the Flemish populations in this analysis (Hungary nor Ukraine are present in the study of Mohanty et al. 2002). Alternatively, this discrepancy can be by chance.

Low within-population genetic diversity and consequences for seed collection

Following the estimates of the within-population diversity the Flemish populations DEF and WES are characterized by low within-population genetic diversities and both consist of only two BAPS clusters. Morphologically, WES endocarps clearly deviate from the other studied populations, but those of DEF do not, excluding a possible link between the low within-population diversity and an easy identifiable morphological character. DEF and WES are autochthonous following the statement that in older days local farmers did not buy planting stock but produced it themselves starting from local seed or plant sources in the neighbourhood. They may have been planted as hedge rows at the end of the 19th or first half of 20th century. Because of absence of management, DEF now looks like a wooded bank. *P. spinosa* is clearly the dominant species present. Local farmers may have experimented with *P. spinosa* as a substitute for *Crataegus* to create dense hedges. The low within and high between population diversity can result from collection of seeds on one or a limited number of autochthonous mother plants in nearby scrubs or forests for the local growth of planting stock. The genetic result can be compared to what is known as a founder effect: successive foundation events that occur during colonization yield a strong genetic differentiation and low within-population diversity, especially in populations far from refuges (e.g. Austerlitz et al. 1997). Although other populations such as HAA and LOR may have known a similar origin as old hedge, these may have been planted with genetically more diverse material, sourced on a wider group of mother plants and/or may have been supplemented, in the course of time, spontaneously (dispersal by birds) or anthropogenic (farmers that closed occasional gaps in their hedges).

A concern in the delineation of seed zones for restoration purposes is to keep genetically differentiated populations separate (Vander Mijnsbrugge et al. 2010). In the absence of information on the geographical scale of local adaptation of the sampled populations, and in the absence of a clear correlation between pair-wise population differentiation (table 3) and geographical distance, a deduction of recommendations for an overall seed zone delineation in Flanders remains speculative. A pan-European consensus on how seed zones

should be delineated is unavailable and the official regions of provenances in different European countries tend to follow the size of the country: small countries have small ones and vice versa. Still, our analyses demonstrate genetic differentiation between both the DEF and WES populations and the other populations studied, even in the absence of a geographic correlation, indicating evolutionary divergence and/or human interference. The latter is highly probable as the Flemish landscape has known a severe degree of deforestation, fragmentation and intensive forest use. Seed collection in these populations for restoration purposes is not recommended because of the low within-population gene diversities. And, the absence of any correlation between morphological characters and genetic diversity prevents easy field identification of genetically depauperate populations. Old hedges and wooded banks with a dominant presence of the species are easy and thus tempting to collect seeds from. But, following our results, seed should be collected preferentially from different sources (taking into account the general guide lines for seed sourcing) and be mixed. In this way, negative impacts of putative seed sources with a low within-population genetic diversity can be counteracted. Although our study area is small in terms of geography, it exemplifies strongly anthropogenically affected landscapes. Therefore our results may apply for similar regions in North Western Europe. Forest edges with autochthonous *P. spinosa* were not present in our study as in these locations *P. spinosa* is far less abundant and collection would be less rewarding in terms of cost-efficiency. Our result can be suggested to hold true for other species farmers used to plant in hedges such as *Crataegus monogyna*. As this is the most typical and dominant species in many relicts of old hedge rows in anthropogenic landscapes of North Western Europe, the chance of low within-population diversities may similarly occur.

SUPPLEMENTARY DATA

Supplementary data are available in pdf format at *Plant Ecology and Evolution*, Supplementary Data Site (<http://www.ingentaconnect.com/content/botbel/plecevo/supp-data>), and consists of the following: (1) principal co-ordinate plot including the Ukraine outgroup; (2) pairwise genetic differentiation (Φ_{ST} values) between populations in the dataset excluding putative clones; (3) box and whisker plots of endocarp morphometric characteristics; (4) box and whisker plots of leaf morphometric characteristics; (5) scatter plot of a leaf and an endocarp morphometric characteristics.

ACKNOWLEDGMENTS

Research funded by a PhD grant of the Institute for the Promotion of Innovation through science and Technology in Flanders (IWT-Vlaanderen). We are grateful to Stefaan Moreels and Leen Verschaeve for their assistance during sampling, Tine Paessens, Joel Dockx, Leen Verschaeve, David Halfmaerten, Sabrina Neyrinck, An Van Breusegem, and Tina Kyndtfor guidance with DNA isolation and/or AFLP analysis, Bert Maes for sampling in Ukraine and for help with plant identification.

REFERENCES

- Anonymous (2000) Council Directive 1999/105/EC of 22 December 1999 on the marketing of forest reproductive material. Official Journal of the European Communities, 15 January 2000. L11: 17–40.
- Austerlitz F., JungMuller B., Godelle B., Gouyon P.H. (1997) Evolution of coalescence times, genetic diversity and structure during colonization. *Theoretical Population Biology* 51:148–164. <http://dx.doi.org/10.1006/tpbi.1997.1302>
- BNatSchG (Gesetz über Naturschutz und Landschaftspflege) (2010) Bundesnaturschutzgesetz vom 29.07.2009, in Kraft getreten am 01.03.2010. BGBl I: 2542.
- Brewer M.T., Lang L.X., Fujimura K., Dujmovic N., Gray S., van der Knaap E. (2006) Development of a controlled vocabulary and software application to analyze fruit shape variation in tomato and other plant species. *Plant Physiology* 141: 15–25. <http://dx.doi.org/10.1104/pp.106.077867>
- Broadhurst L.M., Lowe A., Coates D.J., Cunningham S.A., McDonald M., Vesk P.A., Yates C. (2008) Seed supply for broadscale restoration: maximizing evolutionary potential. *Evolutionary Applications* 1: 587–597. <http://dx.doi.org/10.1111/j.1752-4571.2008.00045.x>
- Coart E., Lamote V., De Loose M., Van Bockstaele E., Lootens P., Roldan-Ruiz I. (2002) AFLP markers demonstrate local genetic differentiation between two indigenous oak species [*Quercus robur* L. and *Quercus petraea* (Matt.) Liebl] in Flemish populations. *Theoretical and Applied Genetics* 105: 431–439. <http://dx.doi.org/10.1007/s00122-002-0920-6>
- Coart E., Van Glabeke S., Petit R.J., Van Bockstaele E., Roldan-Ruiz I. (2005) Range wide versus local patterns of genetic diversity in hornbeam (*Carpinus betulus* L.). *Conservation Genetics* 6: 259–273. <http://dx.doi.org/10.1007/s10592-004-7833-7>
- Corander J., Waldmann P., Marttinen P., Sillanpää M.J. (2004) BAPS 2: enhanced possibilities for the analysis of genetic population structure. *Bioinformatics* 20: 2363–2369. <http://dx.doi.org/10.1093/bioinformatics/bth250>
- Depypere L., Chaerle P., Breynne P., Vander Mijnsbrugge K., Goetghebeur P. (2009) A combined morphometric and AFLP based diversity study challenges the taxonomy of the European members of the complex *Prunus* L. section *Prunus*. *Plant Systematics and Evolution* 279: 219–231. <http://dx.doi.org/10.1007/s00606-009-0158-8>
- Depypere L., Chaerle P., Vander Mijnsbrugge K., Goetghebeur P. (2007) Stony endocarp dimension and shape variation in *Prunus* section *Prunus*. *Annals of Botany* 100: 1585–1597. <http://dx.doi.org/10.1093/aob/mcm260>
- Duminil J., Hardy O.J., Petit R.J. (2009) Plant traits correlated with generation time directly affect inbreeding depression and mating system and indirectly genetic structure. *BMC Evolutionary Biology*, 2009, 9: 177. <http://dx.doi.org/10.1186/1471-2148-9-177>
- Eimert K., Rückert F.-E., Schröder M.-B. (2012) Genetic diversity within and between seedstock populations of several German autochthonous provenances and conventionally propagated nursery material of blackthorn (*Prunus spinosa* L.). *Plant Systematics and Evolution* 298: 609–618. <http://dx.doi.org/10.1007/s00606-011-0570-8>
- Guitian J., Guitian P., Sanchez J.M. (1993) Reproductive biology of 2 *Prunus* species (Rosaceae) in the Northwest Iberian Peninsula. *Plant Systematics and Evolution* 185: 153–165. <http://dx.doi.org/10.1007/BF00937654>
- Hamrick J.L., Godt M.J.W. (1996) Effects of life history traits on genetic diversity in plant species. *Philosophical Transactions of the Royal Society of London Series B, Biological Sciences* 351: 1291–1298. <http://dx.doi.org/10.1098/rstb.1996.0112>
- Horvarth A., Balsemin E., Barbot J.-C., Christmann H., Manzano G., Reynet P., Laigret F., Mariette S. (2011) Phenotypic variability and genetic structure in plum (*P. domestica* L.), cherry plum (*P. cerasifera* Ehrh.) and sloe (*P. spinosa* L.). *Scientia Horticulturae* 129: 283–293. <http://dx.doi.org/10.1016/j.scienta.2011.03.049>
- Hubert J., Cottrell J. (2007) The role of forest genetic resources in helping British forests respond to climate change. *Forestry Commission Information Note* 86:1–12.
- Hufford K.M., Mazer S.J. (2003) Plant ecotypes: genetic differentiation in the age of ecological restoration. *Trends in Ecology & Evolution* 18: 147–155. [http://dx.doi.org/10.1016/S0169-5347\(03\)00002-8](http://dx.doi.org/10.1016/S0169-5347(03)00002-8)
- Kjær E.D., Hansen L.N., Graudal L., Olrik D.C., Ditlevsen B., Jensen V., Jensen J.S. (2009) The Danish program for domestication of native woody species. In: Kafé I.S. (ed.) Abstracts from the workshop: Genetic conservation and management of sparsely distributed trees and bushes, held 15–17 September 2008, Sorø, Denmark. *Forest and Landscape Working Papers* n° 36-2009. Hørsholm, University of Copenhagen.
- Kleinschmit J.R.G., Kownatzki D., Gegorius H.R. (2004) Adaptational characteristics of autochthonous populations, consequences for provenance delineation. *Forest Ecology and Management* 197: 213–224. <http://dx.doi.org/10.1016/j.foreco.2004.05.037>
- Kleinschmit J.R.G., Leinemann L., Hosius B. (2008) Gene conservation through seed orchards, a case study of *Prunus spinosa* L. In: Lindgren D. (ed.) *Seed Orchards: Proceedings from a Conference at Umeå, Sweden, 26–28 September 2007*: 115–125. Available from <http://www-genfys.slu.se/staff/dagl/Umea07/ZProcFinalFeb08.pdf> [accessed 1 Feb. 2013]
- Körber-Grohne U. (1996) Pflaumen, Kirschkirschen, Schlehen: Heutige Pflanzen und ihre Geschichte seit der Frühzeit. Udelgard Körber-Grohne. Konrad Theiss Verlag GmbH, Stuttgart.
- Kramer A.T., Havens K. (2009) Plant conservation genetics in a changing world. *Trends in Plant Science* 14: 599–607. <http://dx.doi.org/10.1016/j.tplants.2009.08.005>
- Krauss S.L., He T.H. (2006) Rapid genetic identification of local provenance seed collection zones for ecological restoration and biodiversity conservation. *Journal for Nature Conservation* 14: 190–199. <http://dx.doi.org/10.1016/j.jnc.2006.05.002>
- Laike L., Schwartz M.K., Waples R.S., Ryman N. (2010) Compromising genetic diversity in the wild: unmonitored large-scale release of plants and animals. *Trends in Ecology & Evolution* 25: 520–529. <http://dx.doi.org/10.1016/j.tree.2010.06.013>
- Leinemann L., Steiner W., Hosius B., Kleinschmit J. (2010) Klonale Reproduktion in naturnahen Vorkommen der Schlehe (*Prunus spinosa* L.). Clonal reproduction in near nature populations of black thorn (*Prunus spinosa* L.). *Forstarchiv* 81: 165–169. <http://dx.doi.org/10.4432/0300-4112-81-165>
- Maes N. (1993) Genetische kwaliteit inheemse bomen en struiken. Deelproject: Randvoorwaarden en knelpunten bij behoud en toepassing van inheems genenmateriaal. IBN-rapport 20, IKC-NBLF, IBN-DLO, Wageningen.
- Matyas C. (2007) What do field trials tell about the future use of forest reproductive material? In: Koskela J., Buck A., Teissier du Cros E. (eds) *Climate change and forest genetic diversity: implications for sustainable forest management in Europe*: 53–69. Rome, Bioversity International.
- Mckay J.K., Christian C.E., Harrison S., Rice K.J. (2005) “How local is local?” - A review of practical and conceptual issues in

- the genetics of restoration. *Restoration Ecology* 13: 432–440. <http://dx.doi.org/10.1111/j.1526-100X.2005.00058.x>
- Mohanty A., Martin J.P., Aguinagalde I. (2002) Population genetic analysis of European *Prunus spinosa* (Rosaceae) using chloroplast DNA markers. *American Journal of Botany* 89: 1223–1228. <http://dx.doi.org/10.3732/ajb.89.8.1223>
- Nielsen J., Olrik D.C. (2001) A morphometric analysis of *Prunus spinosa*, *P. domestica* ssp. *insititia*, and their putative hybrids in Denmark. *Nordic Journal of Botany* 21: 349–363. <http://dx.doi.org/10.1111/j.1756-1051.2001.tb00778.x>
- Nurtjahjaningsih I., Saito Y., Tsuda Y. (2007) Genetic diversity of parental and offspring populations in a *Pinus merkusii* seedling seed orchard detected by microsatellite markers. *Bulletin of the Tokyo University Forests* 118: 1–14.
- Pautasso M. (2009) Geographical genetics and the conservation of forest trees. Perspectives in Plant Ecology Evolution and Systematics 11: 157–189. <http://dx.doi.org/10.1016/j.ppees.2009.01.003>
- Peakall R., Smouse P.E. (2006) GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes* 6: 288–295. <http://dx.doi.org/10.1111/j.1471-8286.2005.01155.x>
- Petit R.J., Hampe A. (2006) Some evolutionary consequences of being a tree. *Annual Review of Ecology Evolution and Systematics* 37: 187–214. <http://dx.doi.org/10.1146/annurev.ecolsys.37.091305.110215>
- Reales A., Sargent D., Tobutt K., Rivera D. (2010) Phylogenetics of Eurasian plums, *Prunus* L. section *Prunus* (Rosaceae), according to coding and non-coding chloroplast DNA sequences. *Tree Genetics & Genomes* 6: 37–45. <http://dx.doi.org/10.1007/s11295-009-0226-9>
- Vandeppeer Y., De Wachter R. (1994) Treecon for Windows - A software package for the construction and drawing of evolutionary trees for the Microsoft Windows environment. *Computer Applications in the Biosciences* 10: 569–570. <http://dx.doi.org/10.1093/bioinformatics/10.5.569>
- Vander Mijnsbrugge K., Bischoff A., Smith B. (2010) A question of origin: Where and how to collect seed for ecological restoration. *Basic and Applied Ecology* 11: 300–311. <http://dx.doi.org/10.1016/j.baae.2009.09.002>
- Vander Mijnsbrugge K., Cox K., Van Slycken J. (2005) Conservation approaches for autochthonous woody plants in Flanders. *Silvae Genetica* 54: 197–205.
- Vekemans X., Beauwens T., Lemaire M., Roldan-Ruiz I. (2002) Data from amplified fragment length polymorphism (AFLP) markers show indication of size homoplasy and of a relationship between degree of homoplasy and fragment size. *Molecular Ecology* 11: 139–151. <http://dx.doi.org/10.1046/j.0962-1083.2001.01415.x>
- Vos P., Hogers R., Bleeker M., Reijans M., Vandeele T., Hornes M., Frijters A., Pot J., Peleman J., Kuiper M., Zabeau M. (1995) AFLP - A new technique for DNA-fingerprinting. *Nucleic Acids Research* 23: 4407–4414. <http://dx.doi.org/10.1093/nar/23.21.4407>
- Woldring H. (2000) On the origin of plums: a study of sloe, damson, cherry plum, domestic plums and their intermediates. *Palaeohistoria Acta et Communicationes Instituti Bio-Archaeologici Universitatis Groninganae* (1997/1998) 39/40: 535–562.
- Zhivotovsky L.A. (1999) Estimating population structure in diploids with multilocus dominant DNA markers. *Molecular Ecology* 8: 907–913. <http://dx.doi.org/10.1046/j.1365-294x.1999.00620.x>
- Zohary D. (1992) Is the European Plum, *Prunus domestica* L., a *P. cerasifera* Ehrh × *P. spinosa* L. allo-polyloid? *Euphytica* 60: 75–77. <http://dx.doi.org/10.1007/BF00022260>

Manuscript received 6 Jun. 2012; accepted in revised version 1 Feb. 2013.

Communicating Editor: Myriam Heuertz.