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NNEXT Non-native Tree Species for European Forests - Experiences, Risks and Opportunities (FP1403)

Editorial

The last two centuries were marked by an increased use of non-native tree species in European forestry. For much of this time, the question of seed origin and genetic variability had received little attention. In many, if not in most cases, it is unknown where forest stands of introduced species come from. Practical experience and research in the field of forest genetics have shown that both provenance (the geographic origin) and genetic diversity are of utmost importance for the survival and growth performance of forest trees. Knowledge about these factors is even more crucial for introduced species, given the lack of autochthonous, locally adapted provenances. This is why the NNEXT action puts a special focus on origin identification and genetic variability of non-native forest tree species.

The development and application of molecular markers has opened avenues for studying population genetic variation in forest trees. Advances in this field have been rapid and the availability of tools for answering various questions has been increasing steadily. Nowadays, markers from the nuclear and organelle genomes of forest trees enable us, for instance, to assess their genetic diversity and to trace back the origin of forest stands and forest reproductive material. Especially the latter is of particular importance for non-native species.

With the "Technical Guidelines for Molecular Genetic Analysis in Non-Native Forest Tree Species of Europe", we aim to provide a comprehensive collection of available molecular markers for *Abies grandis*, *Cedrus* spp., *Larix* spp., *Picea sitchensis*, *Pinus strobus*, *Pinus contorta*, *Pseudotsuga menziesii*, *Ailanthus altissima*, *Quercus rubra* and *Robinia pseudoacacia*, as well as technical details for their use in a molecular genetics laboratory. Extensive literature about all analysis steps and the most important results of case studies from across the world, in summary, complete the picture. While we do not claim completeness, we intend to supply forest geneticists with a basic manual for their work with non-native tree species and to facilitate their search of suitable molecular markers for this purpose.

The „Technical Guidelines" were compiled by members of the Working Group 2-'Pathways' of the Cost Action FP 1403 NNEXT (Non-Native Tree Species for European Forests: EXperiences, Risks and OpporTunities).

May 30th, 2018

Charalambos Neophytou, Monika Konnert

Molecular markers used for genetic studies in Grand fir (*Abies grandis* (Douglas ex D.Don) Lindl.)

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1. General remarks

Among the true firs of the western United States, Grand fir is the species that is able to grow under the most diverse site conditions. Grand fir has a split distribution along the Pacific Coast from Southern British Columbia (Canada) to Northern California. In the continental interior it occurs from the Okanagan and Kootenay lakes region of British Columbia in the north to eastern Oregon, central Idaho, and western Montana in the south. Grand fir can hybridize with white fir (*Abies concolor*). A broad zone of hybridogenous grand × white fir populations occurs from northeastern Washington and Oregon southwards to northern California and eastwards to west-central Idaho (Steinhoff 1978). However, there seems to be a reproductive isolation between *Abies grandis* and the Mediterranean *Abies* species, minimizing the danger of hybridization among *Abies grandis* and the local *Abies* species when introduced to Europe (Kormutak 2004). There are no recognized varieties of grand fir, although a green coastal form and gray interior form are often assumed (Figure 1).

Grand fir grows frequently in mixed forests of coniferous and hardwood species, but also occurs in pure stands. Depending on the region, other fir species as *Abies lasciocarpa*, *Abies amabilis, Abies magnifica* and *Abies procera* grow together with *Abies grandis* in mixed stands (Howard and Aleksoff 2000, Marvin et al. 2003).

While other fir species, as for example *Abies alba* and *Abies procera*, have been extensively studied with various genetic markers, there are very few genetic studies for *Abies grandis*. It is, however, assumed that genetic markers, which have been successfully used in other fir species, can also be used for *Abies grandis*. For example, Postolache et al. (2014) have tested the transferability of *Abies alba* developed transcriptome-derived expressed sequence tags (EST)-SSR-markers to 17 congeneric taxa including one sample of *Abies grandis*. Thirteen of these markers worked also in grand fir (see also chap. 4.a). Moreover, seven nSSR-markers developed for *Abies fraseri* could successfully be transferred to *Abies grandis*, but they have not yet been used for population genetic studies (Josserand et al. 2006). However, continuing test runs and case-by-case modifications of the methodology are necessary to obtain an optimized DNA-marker-set for the grand fir.

Since there are hardly any publications on genetic investigations for *Abies grandis*, selected references on genetic studies carried out in other fir species are cited in the current technical guidelines. The citations mentioned above provide information on the analytical methodology used.

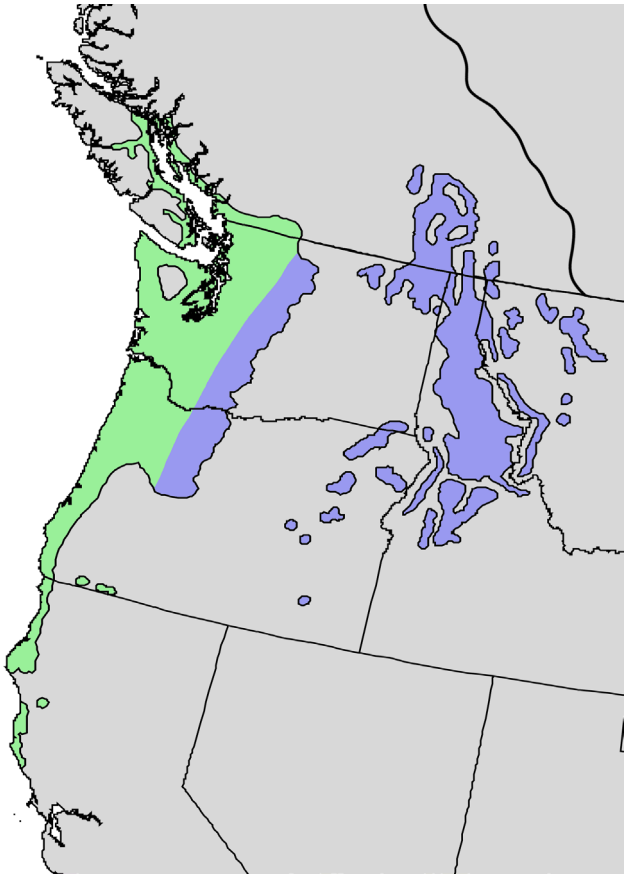

Figure 1. Native distribution range of Grand fir. The coastal form denoted with green and the interior form denoted with blue (source: USGS, USA).

2. Isozymes

Only one publication (Konnert and Ruetz 1997) dealing with isozyme analysis for *Abies grandis* is to our knowledge up to now. Konnert and Ruetz (1997) used isozyme analysis to investigate the genetic structure of eleven *Abies grandis* provenances tested in a provenance trial in Southern Germany.

The list of enzymes, number of loci and number of obtained alleles are presented in Table 1.

Table 1: List of enzymes, scored loci and number of alleles reported by Konnert and Ruetz (1997)

Enzyme system	E.C. Number	Scored loci	No. of alleles
Isocitrate dehydrogenase	1.1.1.42	IDH-A,-B	1, 4
Glutamate oxaloacetate transaminase	2.6.1.1	GOT-A,-B,-C	3, 1, 3
Leucine aminopeptidase	3.4.11.1	LAP-A,-B	3, 3
Phosphoglucose isomerase	5.3.1.9	PGI-A,-B	1, 2
Phosphoglucomutase	2.7.5.1	PGM-A	3
Diaphorase	1.6.4.3	DIA-A	2

Material for protein extraction

Proteins were extracted from dormant buds or seed endosperm (Konnert and Ruetz 1997).

Protein extraction and separation protocols

The extraction procedure and separation protocols are described in Konnert and Ruetz (1997).

Important results

- Clear differences in the genetic structure between provenances were found at the polymorphic gene loci Idh-B and Pgm-A.
- Considering all gene loci, a clear regional differentiation between the provenances from Washington/British Columbia and western Oregon was observed.
- The northern provenances had higher genetic diversity and heterozygosity.
- The provenance Post (1450 m) from central Oregon (Interior) had by far the highest genetic variability and a very specific genetic structure, and could not be assigned to any of the two regional groups.

3. Organelle markers (chloroplast (cp)DNA, mitochondrial (mt)DNA)

Different studies have used so far organelle DNA-

Table 2: PCR-RFLP- markers (cpDNA) for Grand fir

Locus	Primer sequence (F= forward, R= reverse)	Restriction Enzymes	References
rbcL1-rbcL2	F: TGTCACCAAAAACAGAGACT R: TTCCATACTTCACAAGCAGC	Hind III, VSP I, Dra I	1,2
trnS-psbC	F: GTTTGGAATCCCTCTCTCTC R: GGTCGTGACCAAGAAACCAC	Hae III, Taq I, Tru 1I	2,3
trnL-trnV	F: CTGCTTCCTAAGAGCAGCGT R: TTGACATGGTGGAAGTCATCA	Msp I,	2,4
trnK	F: AACCCGGAACTAGTCGGATG R: TCAATGGTAGAGTACTCGGC	Hpa II, Hinf I,	2,5
rpoC1	F: TCGATTGAAACGAGTACGACC R: CACTGGAGGGCCAATACCTA	Msp I, Taq I,	2,6
rpl2	F: AAAGGTCGTAATGCCAGAGGAAT R: TTCCAAGYGCAGGATAACCCCA	Msp I,	2,7
psbB	F: ATGGTTTTGCCTTGGTATCGTGTTCATAC R: CCAAAAGTRAACCAACCCCTTGGAC	Msp I	2,4
psbD	F: ATGACTGGTTACGRAGGGACCA R: CATAACCRAAGAAKGGAAAAGAATC	Cfr 131	2,4

1-Hopkins et al. 1990, 2-Kormutak et al. 2004, 3-Demesure et al. 1995, 4-Wang et al. 2000, 5-Hiratsuka et al. 1989, 6-Parducci and Szmidt 1999, 7-Graham and Olmstead 2000

markers in Abies grandis, aiming primarily at species differentiation/identification and clarification of the taxonomy of the different Abies species, e.g. as attendance of interspecific crossing experiments (e.g. Kormutak et al. 2004).

Loci and primers used

Analysis of Kormutak et al. 2004 (see Table 2)

- Restriction fragment length polymorphism (RFLP)-chloroplast marker: rbcL1-rbcL2, trnS-psbC, trnL-trnV, trnK, rpoC1, rpl2, psbB, psbD using the restriction enzymes Msp I, Cfr131, Hinf I, Tag I, Hpa III, Trn II, Hind III, VSP I, Dra I,

Analysis at ASP, Teisendorf (unpublished, see Table 3)

- mitochondrial marker Nad5.4 (Liepelt *et al.* 2002, Jaramillo-Correa et al. 2013);
- chloroplast simple sequence repeats (SSR)-marker Pt71936, Pt30141, Pt30249 (Vendramin et al. 1996).

Material for DNA-extraction

In the mentioned studies (Kormutak *et al.* 2004, ASP unpublished) DNA was extracted from fresh or silicagel dried needles.

DNA-extraction protocols

For DNA isolation at the ASP Teisendorf the CTAB-method of Dumolin et al. (1995) was used. Kormutak et al. (2014) used a slightly modified protocol of Murray and Thompson (1980) in which only 0.5 g of needles per individual were used instead of prescribed 10 g of needle material. This also reduced the amount of extracting buffer to 7 ml instead of 200 ml.

Important results

- It is possible to distinguish different groups in the genus *Abies* using the above mentioned organelle DNA markers, especially by using the mitochondrial marker NAD5.4 to identify even closely related Abies species (Jaramillo-Correa et al. 2013, Ziegenhagen et al. 2005).
- Phylogenetic relationships among *Abies* species could also be constructed by using chloroplast-markers (e.g. Kormutak at al. 2004).

4. Nuclear DNA markers (nSSRs, EST-SSRs, SNPs)

a) nSSRs (putatively neutral microsatellites) and EST-SSRs (expressed sequence tag derived microsatellites)

Ten nuclear microsatellite markers were recently used for *Abies grandis* in an internal analysis at ASP Teisendorf for seed source identification purposes and species differentiation (between *Abies alba* and *Abies grandis*). The analyses were carried out within the scope of controlling the rules of the law for forest reproductive material in Germany (Cremer 2016, internal report ASP, unpublished).

Loci and primers used

The following nuclear microsatellite markers developed for *Abies nordmanniana* and *Abies alba* were successfully transferred to *Abies grandis* (see Table 4):

- NFF3, NFF7, NFH3 from A. nordmaniana (Hansen et al. 2005). These markers are simple sequence repeats (SSRs);
- Aat01, Aat04, Aat05, Aat06, Aat10, Aat11, Aat15 from *Abies alba* (Postolache et al. 2014). These markers are expressed-sequence-tag (EST-SSRs).

Table 3: Primer information of the mtDNA markers and cpSSRs used for the genetic analysis of *Abies grandis* (Ta = annealing temperatures, Na = number of alleles scored)

Locus	Motif	Type of marker	Primer sequence (F= forward, R= reverse)	Ta (°C)	N_a	Size (bp)	References
NAD5.4	2-4 imperfect STRs, repeats 0-8	mt-intron-DNA marker (maternally inherited)	F: GGACAATGACGATCCGAGATA R: CATCCCTCCCATTGCATTAT	52	2		1,2
Pt71936	(T)22	cp-SSR-marker (paternally inherited)	F: TTCATTGGAAATACACTAGCCC R: AAAACCGTACATGAGATTCCC	55		124-148	3
Pt30141			F: CCGAAGACAACGACTGTATTTT R: CATTAAACTAGAGATACAAGGG	55			3
Pt30249			F: CCCTTGTATCTCTAGTTTAATG R: CTAGTTAGGCTTGGTCAACTAA	55			3

1-Liepelt et al. 2002, 2- Jaramillo-Correa et al. 2013, 3-Vendramin et al. 1996

Material used for extraction

DNA was extracted from silicagel dried needles (ASP Teisendorf, unpublished)

DNA-extraction protocols

DNA extraction was performed using the method of Dumolin et al. (1995).

Important results

- The ten nSSRs that could be transferred from other *Abies* species and were only used in a case study of *A. grandis* (ASP, Teisendorf 2016) are a promising tool for continuing analyses of further populations of grand fir.
- Differentiation between *A. alba* and *A. grandis* is clearly possible with the nSSRs used; species-specific-alleles/variants exist.
- The diversity (genetic diversity and heterozygosity) of a grand fir plant- and seed lot show slightly lower values when compared to those found in *A. alba* populations by using the same nSSR-markers.

b) SNPs (single-nucleotide polymorphisms)

There is no publication on SNP-analysis for *Abies grandis*.

5. References

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Table 4: Primer sequences, annealing temperatures (Ta), allele length in base pairs (bp), number of alleles scored (Na) and references for nSSR markers available for genetic analyses in *Abies grandis*. (* = nSSR; ** = EST-SSR)

Locus	Motif	Primer sequence (F= forward, R= reverse)	Ta (°C)	Size (bp)	N_a	References
NFF3*	-	F: CCAATGGGTTGTCAGAGTGTT R: GGCATTCGAGATTGCTTGAT	58	109-123	8	1
NFF7*	-	F: CCCAAACTGGAAGATTGGAC R: ATCGCCATCCATCATCAGA	57	126-156	15	1
NFH3*	-	F: TTGCCATCAAATTAAAAATGCTT R: CATCATTCTCTCTATCCCCATCA	58	95-139	22	1
Aat01**	(GCG)10	F: CCATGTCTCCGATTTCCAGT R: GGCCTAACGAAAGCAGAATC	57	96-11	4	2
Aat04**	(CAG)11	F: CCATGTATGGTGCTCCTCCT R: CCTTCATTGCAGAAAAGCAA	57	147-159	2	2
Aat05**	(GCA)7	F: AGCATCCACATTCCGTAACC R: AGTTGACCGTTGGAGAGCAG	57	192-201	3	2
Aat06**	(GCA)8	F: TTATGCGGAGCAGTTCTGTG R:TGTTGCTGGCGTACTGGTAG	57	198-207	4	2
Aat10**	(AT)12	F: GAGCACGATGAAGAGGAAGC R: AAAACCCCCACGCGGTAT	57	219-235	8	2
Aat11**	(AAC)9	F: AGCGTTGATTGGAAGCAGTC R: GAAGCATGGTGTCGTTGTTG	57	266-269	2	2
Aat15**	(AGA)8	F: AGGAGGAGGTTCAGCATGTC R: CTTGCTCTCTGACCCAGTTG	57	358	1	2

1-Hansen et al. 2005, 2-Postolache et al. 2014

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The cited publications contain information on markers and methodological aspects for genetic investigations in different Abies species.

Isozymes

<u>*Abies alba*</u>

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Abies fraseri

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Abies procera

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Molecular markers (cpDNA, mtDNA)

Abies alba

Cremer, E., Liepelt, S., Ziegenhagen, B., Hussendörfer, E. (2003): Microsatellite and isozyme markers for seed source identification in silver fir. Forest Genetics, 10(3): 165-171.

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Molecular markers (nSSR, SNP)

Abies alba

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Molecular markers used for genetic studies in *Cedrus* spp.

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1. General remarks

The Genus Cedrus includes three species native to Mediterranean mountains and one restricted to the Himalayas.

Cedrus atlantica

Atlas ceder (*Cedrus atlantica*) is highly fragmented and native to the northern African regions of Morocco and Algeria. In Morocco regions with *Cedrus atlantica* are Rif, Middle-Atlas and High-Atlas. The species optimum is the Middle-Atlas region in altitudes between 1600 and 2200 m. In Algeria the main regions are the Tell-Atlas and the Sahara-Atlas. The whole distribution range covers an area of 145.000 ha.

Cedrus brevifolia

Cedrus brevifolia is a narrow endemic island tree species of Cyprus. It is divided into five neighboring sites (geographic regimes) on the mountains of the Paphos forest. *C. brevifolia* ranges from 900-1400 m above sea level. On all five sites are growing approximately 15.800 trees with a diameter at breast height over 12 cm.

Cedrus deodara

The natural distribution range of *Cedrus deodara* is restricted from Afghanistan to the southern slopes of the western Himalayas. The optimum of *Cedrus deodora* is the high range of Kashmir from 2000-2500 m. This tree species is reaching heights from 40-50 m and diameter up to 3 m.

Cedrus libani

Cedrus libani can be divided into two main distribution areas: Turkey, with South- and Southwest-Anatolia and the geographical distinct occurrence in Lebanon and Syria. In Turkey this includes large parts of the west- and middle Taurus and the Amanos-mountain area, with around 400.000 ha. Due to heavy depletion, the occurrence of *Cedrus libani* is scattered in many small populations. Larger populations can be found mainly in the western Taurus. In Lebanon the natural distribution range has declined from 500.000 ha, to 1.700 ha for the same reasons as in Turkey.

The native distribution of the *Cedrus* ssp. is depicted in Figure 1.

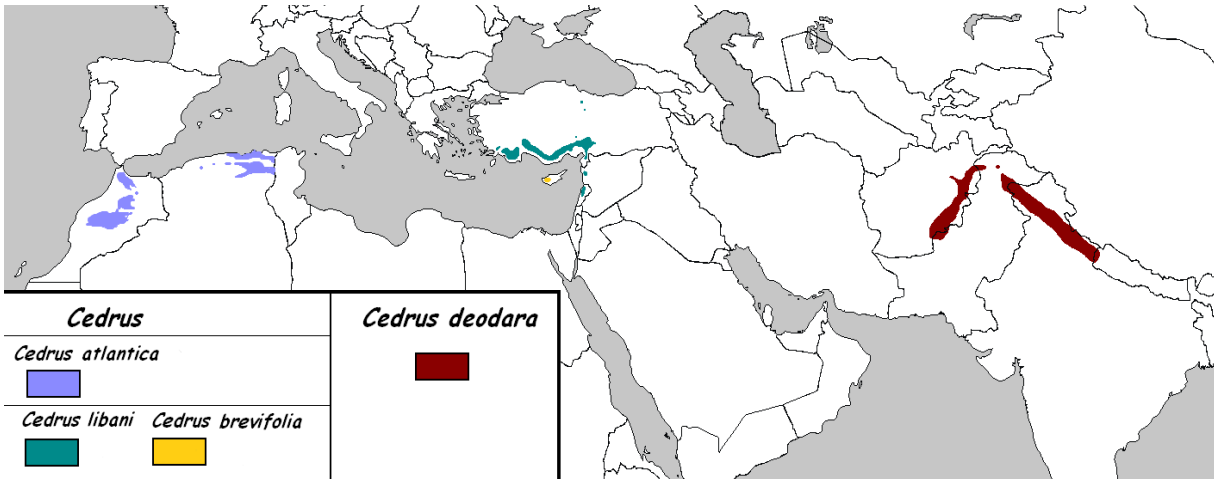

Figure 1. Native distribution range of *Cedrus* ssp. (modified after: https://commons. wikimedia.org/wiki/File:Cedrus_map.png)

2. Isozymes

First publication on isozymes for *Cedrus* species appeared in 1992 (Panetsos et al. 1992). Further on isozymes were used to determine the allozyme differentiation and phylogeny of cedar species (Bariteau et al. 1999, Scaltsoyiannes 1999, Fady et al. 2000) or to see the genetic variation in natural populations of the same species in a restricted part of the natural distribution range (Yahyaoglu et al. 1997, Gülbaba and Özkurt 2002, Kurt et al. 2008).

The list of enzymes, number of loci and references are presented in table 1. In some studies (e.g. Kurt et al. 2008) loci are indicated by numbers (e.g. IDH-1), in other studies by letters (e.g. IDH-A) (e.g. Scaltsoyiannes 1999). Given this situation it has to be considered that IDH-A in table 1 is identical with IDH-1.

Material for protein extraction

Proteins were extracted from different tissues as for example seed (megagametophytes) (e.g. Kurt et al. 2007, Panetsos et al. 1992, Fady et al. 2008), radicle from germinated seed (Scaltsoyiannes 1999) and dormant buds (Panetsos et al. 1992, Scaltsoyiannes 1999).

Protein extraction and separation protocols

Details on extraction procedures and starch gel electrophoretic analysis are given in Panetsos et al. (1992), Scaltsoyiannes (1999) and Kurt et al. (2008). In general the separation buffers and staining recipes from Fady and Conkle (1992), Conkle et al. (1982) and Cheliak and Pitel (1984) were applied.

Important results

- *Cedrus libani* in Turkey exhibits a high intrapopulation variation but a low genetic variation

Table 1: List of enzymes, scored loci and number of alleles for *Cedrus* species

Enzyme system	E.C. Number	Scored loci	No. of alleles	References
Acid phosphatase	3.1.3.2	ACP-A,-B	2, 2	1,2,6
Alcohol dehydrogenase	1.1.1.1	ADH-A	1	1
Aspartate aminotransferase	2.6.1.1	AAT-A,-C resp. GOT-A,-C	4, 3	1,2,3,7
Diaphorase	1.6.4.3	Dia-A*	4	1,4,5
Glutamate dehydrogenase	1.4.1.2	GDH-A	2	1,2
Isocitrate dehydrogenase	1.1.1.42	IDH-A	3, 5	1,2,5,6,8
Leucine aminopeptidase	3.4.11.1	LAP-A,-B	3, 4	1,4,5,8
Malate dehydrogenase	1.1.1.37	MDH-A,-B	2, 2	1,4
Menadione reductase	1.6.99.2	MNR-A,-B,-C	2, 4, 3	1
6-Phosphogluconate dehydrogenase	1.1.1.44	6PGDH-A,-B	3, 6	1,2,4,5,8
Phosphoglucose isomerase	5.3.1.9	PGI-A,-B	1, 6	1,4,5,8
Phosphoglucomutase	2.7.5.1	PGM**		9
Superoxide dismutase	1.15.1.1	SOD-A	1	1
Shikimate dehydrogenase	1.1.1.25	SKDH**		9

** corresponds to the MNR-A locus (Scaltsoyiannes 1999); ** no indication on loci resp. allele numbers*

1-Kurt et al. 2008, 2-Gülbaba and Özkurt 2002, 3-Panetsos et al. 1994, 4-Panetsos et al. 1992, 5-Scaltsoyiannes 1999, 6-Fallour et al. 2001, 7-Yahyaoglu et al. 1997, 8-Bariteau et al. 1999, 9-Fady et al. 2008

among populations (Kurt et al. 2008). In contrary, in Lebanon high differentiation and genetic drift effects were found for the same species with a combination of molecular markers and microsatellites (see also chapter 3). Populations from Turkey and Lebanon constitute two genetically isolated groups (Fady et al. 2008). For planting outside the natural range seed from Turkish populations have to be clearly preferred.

- Based on isozyme markers distinction between different *Cedrus* species is possible in great part (Panetsos et al. 1992, Scaltsoyiannes 1999). Differences are in allele frequencies but also in species specific alleles (e.g. Scaltsoyiannes 1999, Bariteau et al. 1999, Fady et al. 2000). Genetic distances and cluster analysis illustrate the clear genetic differences between the *Cedrus* species (Scaltsoyiannes 1999, Fady et al. 2008).
- For *Cedrus libani* alleles with a particular geographic pattern (e.g. 6-PGDH-B4) were found (Scaltsoyiannes 1999).
- Great variation was observed in heterozygosity levels among species with low values in *Cedrus deodara* and high values in *Cedrus brevifolia* (Scaltsoyiannes 1999).

3. Organelle DNA markers (chloroplast (cp) DNA, mitochondrial (mt)DNA)

Loci and primers used

All chloroplast microsatellites applied in *Cedrus* genetic studies were originally designed for the *Pinus thunbergii* chloroplast genome (Wakasugi et al. 1994). Nine primers out of twenty (Vendramin et al. 1996) could be amplified in different *Cedrus* taxa (Table 2). Studies with these primers refer to gene flow among different taxonomic units (Fady et al. 2003), conservation studies (Fady et al. 2008), identification of glacial refugia (Cheddadi et al. 2009) and genetic characterization of natural populations (Terrab et al. 2006, Eliades et al. 2011).

Material for DNA-extraction

In all studies DNA was extracted from fresh or frozen needles (Fady et al. 2003, 2008, Terrab et al. 2007, Cheddadi et al. 2009, Eliades et al. 2011).

Table 2: Primer sequences, annealing temperatures (Ta), allele length in base pairs (bp) and references for cpSSRs available for genetic analyses in *Cedrus* species

Locus	Primer sequence [5'-3'] F= Forward, R= Reverse	Ta (°C)	Size (bp)*	Variability	References
Pt15169	F:CTTGGATGGAATAGCAGCC R:GGAAGGGCATTAAGGTCATTA	55	118	Polymorphic among and within species	1,2,3,4,5
Pt63718	F:CACAAAAGGATTTTTTTTCAGTG R:CGACGTGAGTAAGAATGGTTG	55	93	Polymorphic among and within species	1,2,3,4,5
Pt71936	F:TTCATTGGAAATACACTAGCCC R:AAAACCGTACATGAGATTCCC	55	148	Polymorphic among and within species	1,2,3,4,5
Pt87268	F:GCCAGGGAAAATCGTAGG R:AGACGATTAGACATCCAACCC	55	165	Polymorphic among species	1,2,4
Pt26081	F:CCCGTATCCAGATATACTTCCA R:TGGTTTGATTCATTCGTTCA	55	112	monomorphic	1,2,4
Pt36480	F:TTTTGGCTTACAAAATAAAAGAGG R:AAATTCCTAAAGAAGGAAGAGCA	55	147	monomorphic	1,2,4
Pt110048	F:TAAGGGGACTAGAGCAGGCTA R:TTCGATATTGAACCTTGGACA	55	88	monomorphic	1,2,3
Pt30204	TCATAGCGGAAGATCCTCTTT CGGATTGATCCTAACCATACC	55	145	-	1,3
Pt109567	F:TATTATCGAACAACGAGAATAATCC R:TCACTGTCACTCTACAAAACCG	55	115	-	1,3

** values for Pinus thunbergii*

1-Vendramin et al. 1996, 2-Fady et al. 2003, 3-Terrab et al. 2006, 4-Fady et al. 2008, 5-Eliades et al. 2011

DNA-extraction and amplification protocols

Total DNA was extracted from ground needle tissue using:

- the DNeasy 96 Plant Kit from QIAGEN (Eliades et al. 2011, Fady et al. 2008)
- the Doyle and Doyle (1990) protocol (Fady et al. 2003)
- the CTAB miniprep protocol of Cullings (1992) (Terrab et al. 2006)

For amplification the following PCR-protocols were used:

- 95°C for 3 min followed by 25 cycles of 94°C for 1 min, 55°C for 1 min, 72°C for 1 min, 1 cycle at 72°C (Terrab et al. 2006)
- 95°C for 5 min followed by 25 cycles of 94°C for 1 min, 55°C for 1 min, 72°C for 1 min with a final extension step of 72°C for 8 min (Fady et al. 2003, 2008)

Important results

- Chloroplast DNA was found to be paternally inherited in *Cedrus*. Therefore chloroplast markers are useful to monitor male gene flow in forests through the genotyping of single trees and their open-pollinated progeny (Fady et al. 2003). It seems that no strong reproductive barrier exists between the Mediterranean *Cedrus* species (*atlantica, brevifolia* and *libani*) (Fady et al. 2003).
- *Cedrus libani* populations from Turkey and Lebanon constitute two genetically isolated groups (Fady et al. 2008), probably because they arose from two different glacial refugia. Turkish populations have high genetic variation inside populations and low differentiation between them. In Lebanon, *Cedrus* populations are highly differentiated and show sever cases of genetic drift due to strong human influence and reduction of population size. For planting outside the natural range seed from Turkish populations have to be clearly preferred. Similar results were found in the same study with isozymes (see chapter 2).
- *Cedrus atlantica* in Morocco appears to maintain a high level of haplotypic diversity, which is mostly within populations. Differences between populations are low. No clear center of genetic diversity in *Cedrus altlantica* was identified (Terrab et al. 2006). High genetic diversity is found in the High Atlas Mountains. To preserve the species under climate change it is suggested to transfer genetic material from this region to western High Atlas (Cheddadi et al. 2009).
- *Cedrus brevifolia*, a narrow endemic island tree species from Cyprus, has high genetic diversity both at the nuclear and plastid level (see also chapter 4), suggesting no drift effects or genetic bottleneck (Eliades et al. 2011).

4. Randomly amplified polymorphic DNA markers (RAPD)

Loci and primers used are listed in Table 3

Material for DNA-extraction

For RAPD-analysis DNA was extracted from needles collected from seedlings (Fady et al. 2003) or adult trees (Renau-Morata et al. 2005, Semaan and Dodd 2008) and from seed (megagametophytes) (Kayihan et al. 2006).

DNA-extraction and amplification protocols

Total DNA was extracted from needle tissue using

- the Doyle and Doyle (1990) protocol (Semaan and Dodd 2008)
- the DNeasy 96 Plant Kit from QIAGEN (Renau-Morata et al. 2005).

For the extraction of DNA from megagametophytes Kayihan et al. (2006) used a combination of DNA extraction procedure from Dellaporta et al. (1983) and Kreike (1990).

For amplification the following PCR-protocols were used:

- 45 cycles of 94°C for 20, 40 °C for 20 sec, and 72 °C for 90 sec with a final extension step at 72°C for 10 min (Semaan and Dodd 2008).
- Renau-Morata et al. (2005) performed the DNA-amplification as described in Neubauer et al. (2000): 1min denaturation at 94°C, followed by 45 cycles of 30 sec at 94°C, 30 sec annealing at 39°C and a 90-sec extension at 72°C, with a last step of an additional 7min extension at 72°C.
- Fady et al. (2003) used PCR, separation and staining protocols form Lefebvre et al. (1995).

Important results

- RAPD markers in combinations with cpSSR are a good tool to study hybridization between species.

Table 3: Primers employed and RAPD markers obtained from DNA amplification in *Cedrus* species (Ta= annealing temperature)

Locus	Sequence 5'-3'	Ta (°C)	Size (bp)	Number of bands		References
				polymorphic	monomorphic	
OPA18*	AGGTGACCGT	39	550-2100	22	2	1,2
OPA20*	GTTGCGATCC	39	630-2000	18	0	1,2
OPB7*	GGTGACGCAG	39	480-1600	18	0	1,2
OPB8*	GTCCACACGG	39	750-2250	18	0	1,2
OPB12*	CCTTGACGCA	39	500-2200	23	1	1,2
OPC2*	GTGAGGCGTC	39	400-1700	38	0	1,2
OPA7**	GAAACGGGTG	37.4	400-2500	22	0	3
OPA10**	GTGATCGCAG	37.4	400-2500	22	0	3
OP17**	GACCGCTTGT	37.4	400-2500	15	1	3
A15***	TTCCGAACCC					4
A20***	GTTGCGATCC					4
B12***	CCTTGACGCA					4
C16***	CACACTCCAG					4
E9***	CCTCACCCGA					4
P14***	CCAGCCGAAC					4
Q5***	CCGCGTCTTG					4

** Cedrus atlantica, ** Cedrus libani, *** Cedrus atlantica, libani, brevifolia*

1-Renau-Morata et al. 2005, 2-Neubauer et al. 2000, 3-Semaan and Dodd 2008, 4-Fady et al. 2003

- Intensive gene flow between *Cedrus atlantica* and *Cedrus libani* was observed in open pollinated seedlings in two plantation forests (see also AFLP) (Fady et al. 2003).
- For *Cedrus libani* from Turkey no clear geographic differentiation pattern was observed. For one geographically isolated stand based on RAPD the putative origin was traced back (Kayihan et al. 2006).
- For *Cedrus libani* from Libanon RAPDs show considerable variation within populations and low population differentiation. Inbreeding effects or genetic drift was not observed. Correlation between genetic diversity and climatic conditions (temperature, humidity) was observed (Semaan and Dodd 2008).
- Genetic diversity of *Cedrus atlantica* in Morocco analyzed using RAPD markers was high and comparable to that revealed by isozymes. No differences in genetic diversity of natural and managed populations were observed (Renau-Morata et al. 2005).

5. Nuclear DNA markers (AFLPs, nSSRs, SNPs)

a) AFLPs (Amplified Fragment Length Polymorphisms)

Loci and primers used

In both studies AFLP primers contained one selective nucleotide for pre-amplification and three selective nucleotides for amplification. Dagher-Kharrat et al. (2006) generated a total of 107 polymorphic amplification products with eleven selective AFLP primer pairs. In total 388 bands appear. Among them 107 AFLP showed clear polymorphic bands. Information on used primer combinations is given in Table 4.

Material for DNA-extraction

For AFLP-analysis DNA was extracted from frozen needles collected from seedlings (Fady et al. 2003) and megagametophytes (haploid) (Dagher-Kharrat et al. 2006).

DNA-extraction and amplification protocols

Total DNA was extracted following the Doyle and Doyle (1990) protocol (Fady et al. 2003). For megagametophytes Dagher-Kharrat et al. (2006) modified this protocol slightly by the addition of 2 % PVP mW 40.000 to the extraction buffer.

AFLP analyses were carried out using the "AFLP Analysis System I" kit from "Life Technologies". For details on the analysis procedures see Fady et al. (2003) and Dagher-Kharrat et al. (2006).

Important results

- There was no indication of strong reproductive isolating barriers between the Mediterranean *Cedrus* species (*atlantica, brevifolia* and *libani*). An intensive gene flow between *Cedrus atlantica* and *Cedrus libani* was observed in open pollinated seedlings in two plantation forests (Fady et al. 2003).
- Himalayan cedar (*Cedrus deodara*) is genetically clearly different from the Mediterranean cedars. 13 out of 25 species specific bands distinguished *Cedrus dodara* from the other *Cedrus* species (see also Table 5). Within the Mediterranean cedars *Cedrus atlantica* is different from *Cedrus libani* and *brevifolia*. The latter two species are genetically similar (Dagher-Kharrat et al. 2006).
- *Cedrus brevifolia*, a narrow endemic island tree species from Cyprus, has high genetic diversity (same results as for chloroplast markers) (Dagher-Kharrat et al. 2006).

Table 4: Primer combinations used to produce AFLP fingerprints (Dagher-Kharrat et al. 2006)

	Sequence	NNN-3′											
		I	II	III	IV	V	VI	VII	VIII	IX	X	XI	Total
*Eco*RI + NNN-3'	5'-GACTGCGTACCAATTC	ACC	ACA	ACT	AAC	ACT	ACT	AAC	ACA	ACG	ACG	ACG	
*Mse*I + NNN-3'	5'-GATGAGTCCTGAGTAA	CTT	CAA	CTA	CTT	CAC	CTT	CAT	CAG	CTC	CTA	CAG	
No of variable bands		1	2	2	2	4	3	5	23	52	63	66	223
No of fixed bands		16	24	13	11	17	11	9	28	18	9	9	165
Total band number		17	26	15	13	21	14	14	51	70	72	75	388
Level of polymorphism in %		5.9	7.7	13.2	15.4	19	21.3	35.6	45.1	74.3	87.5	88	57.4

b) nSSRs (putatively neutral microsatellites)

Loci and primers used

Chaib et al. (2006) developed primers for the amplification of six polymorphic nuclear microsatellites in Mediterranean *Cedrus* taxa. Microsatellites originated from two *Cedrus atlantica* genomic libraries enriched for TC (four markers) and TG (two markers) motifs. Eliades et al. (2011) tested the transferability and polymorphism of these primers in *Cedrus brevifolia*. Four out of the six nSSR developed for *Cedrus atlantica* showed amplification also in *Cedrus brevifolia*, namely CatITgD4, CatXITcE11, CatXITcD12, CatXIITcC6 (see Table 6). CatXIIITcA3, CatITgD4, CatXITcD12 also amplified in the Himalayan cedar (*Cedrus deodara*).

Material for DNA-extraction

In both studies DNA was extracted from needle tissues (Chaib et al. 2006, Eliades et al. 2011).

DNA-extraction and amplification protocols

Total DNA was extracted from ground needle tissue using:

- the Doyle and Doyle (1990) protocol (Chaib et al. 2006)
- the DNeasy 96 Plant Kit from QIAGEN (Eliades et al. 2011)

For amplification the following PCR-protocols were used:

<u>*Cedrus atlantica*</u> (Chaib et al. 2006)
5 min at 95 °C followed by 35 cycles of 1 min at 94 °C, 1 min at the annealing temperature (Table 6), 1 min at 72 °C and a final extension of 5 min at 72 °C.

<u>*Cedrus brevifolia*</u> (Eliades et al. 2011)

- primers CatITgD4 and CatXITcD12:
  95°C for 15 min, followed by 1 cycle at 80°C for 4 min, 30 cycles at 94°C for 1 min, Ta (annealing temperature) (53°C and 55,5°C) for 1 min, 72°C for 1 min, extension 72°C for 30 min

Table 5: Primer combinations giving species- or populations-specific markers (Dagher-Kharrat et al. 2006)

Primer combination	Number of markers for the identification of					
	C. atlantica	***C. libani***	***C. brevifolia***	***C. deodara***	***C. libani* and *C. brevifolia***	**Mediterranean cedars**
E-ACT/M-CTT	1	1 (Turkish populations)		1		1
E-ACC/M-CTT						1
E-AAC/M-CTT				2		
E-ACC/M-CAT	1	1(Lebanese populations)	1		1	
E-ACT/M-CAC	1 (Algerian and French populations)	1 (Turkish populations)	1			
E-ACT/M-CTA	1			1	1	
E-ACA/M-CAA						1
E-ACA/M-CAG				1		1
E-ACG/M-CAG					1	
E-ACG/M-CTC				3		1

Table 6: Primer sequences, annealing temperatures (Ta), allele lengths in base pairs (bp) and references for microsatellite markers available for genetic analyses in *Cedrus* species. Number of alleles refer to *Cedrus atlantica*. For Ta and Size (bp) values below refer to Cedrus atlantica, values above to *Cedrus brevifolia*.

Locus	Motif	Primer sequence [5'-3'] F= forward, R = reverse	Ta (oC)	Size (bp)	No. of alleles	References	GenBank Accession no.
CatXIIITcA3*	$(GA)_{13}(TA_{)2}(GA)_2$ $(GT)_2(GA)_2$	F: TTAGAGGGAGACAGAGAGTCACAG R: ACACGCACTCTCTTACACTCAC	59	223-225	2	1	DQ303445
CatITgD4**	$(TC)_8(AC)_6$	F: GCTTTACGCAATTCCTCCTATG R. TGAGAATTGTGAACCATTGAAAG	55 53	183-201 188-204	7	1,2	DQ303446
CatXITcE11***	$(TC)_{17}$	F: TCACGATAACTCTCACGCAGAC R: AGAGAGAAAGAAAGGGGGAGAG	57 60	189-210 **	5	1,2	DQ303447
CatXITcD12**	$(TC)_{15}$	F: TGGTTTTTCCACCTTAGTTTCC R: GGGATGGAAGGAATAAGATAGAGG	63 55,5	258-294 274-310	7	1,2	DQ303448
CatXITcE8*	$(GA)_{19}$	F: CTGTGATGTGGATGGAGAAGAG R: ACCCCATTCTTACTATCTTTACCTC	53	262-268	3	1	DQ303449
CatXIITcC6***	$(GT)_{27}(GA)_{14}$	F: GGGTTATAAGTTTTAATTATATGTGTG R: CACCACCTTGACTTCCCTTG	59 54	277-297 280-305	8	1,2	DQ303450

**amplification in C. atlantica, C. deodara; ** amplification in C. atlantica, C. deodora, C. brevifolia, ***amplification in C. atlantica, C. brevifolia*

1-Chaib et al. 2006, 2-Eliades et al. 2011

- primer CatXIITcC6:
  95°C for 5 min, followed by 1 cycle at 80°C for 4 min, 30 cycles at 94°C for 1 min, 54°C for 1 min, 72°C for 1 min, extension 72°C for 30 min
- primer CatXITcE11:
  Touch down PCR: 95°C for 15 min, followed by 6 cycles at 94°C for 1 min, 60°C for 1 min (about 1°C per cycle) and 72°C for 1 min, followed by 35 cycles at 94°C for 1 min, 54°C for 1 min and 72°C for 1 min, extension 72°C for 30 min

Important results

- The biparentally inherited codominant genetic nSSR markers should be helpful for species identification, diversity studies, parentage analysis and genome mapping (Chaib et al. 2006).
- *Cedrus brevifolia*, a narrow endemic island tree species from Cyprus, has high genetic diversity both at the nuclear and plastid level (see also chapter 2), suggesting no drift effects or genetic bottleneck (Eliades et al. 2011).

c) SNPs (single-nucleotide polymorphisms)

Loci and primers used

Restriction site associated DNA sequencing (RAD-sequencing) and mRNA sequencing were used in a recent genome mapping study of *Cedrus atlantica* conducted by Karam et al. (2015) in a panel of one single individual and three pools of three individuals each.

17,348 single nucleotide polymorphisms (SNPs) were identified in the RADseq data set and 5,714 simple sequence repeats (SSRs) in the transcriptome. Various stringent filters were applied resulting in 400 high-quality SNPs along with 192 SSRs. A subset of 282 SNPs was validated using the Fluidigm genotyping technology. The 192 in silicodetected high-quality SSRs still need to be tested for validation by sequencing. The authors consider that the high-quality molecular markers they developed constitute a valuable resource for future population genetic studies.

Detailed information on the four hundred SNPs selected within the RADseq dataset, functional annotation of the contigs where they are located and results of the Fluidigm genotyping is provided as supplementary material (Appendix S4) by Karam et al. (2015). Appendix S5 from the same publication contains information for SSRs as the pure SSR and clean flanking regions.

Important results

The high-quality molecular markers developed constitute a valuable resource for future population genetic studies.

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Molecular markers used for genetic studies in Japanese Larch (*Larix kaempferi* (Lamb.) Carr.)

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1. General remarks

Larch is one of the most abundant conifers in the northern hemisphere where it grows at both high latitudes and high elevations. It comprises around ten species distributed over North America, Europe and Asia.

Japanese larch is native on Honshu Island, where it grows at 1300 to 2900 m elevation. Faster growing than European larch at early stage, it has usually been preferred in Western Europe's oceanic areas. Presently, hybrid larch (*Larix* x *eurolepis*, hybrid between European and Japanese larches), which is more resistant to cold and drought, tends to replace Japanese larch in the reforestation of northern Europe.

Since 2009, infection of Japanese larch by the *Phytophtora ramorum* has led to a severe decline of the species in the British Isles. For this reason, the species is no more planted there.

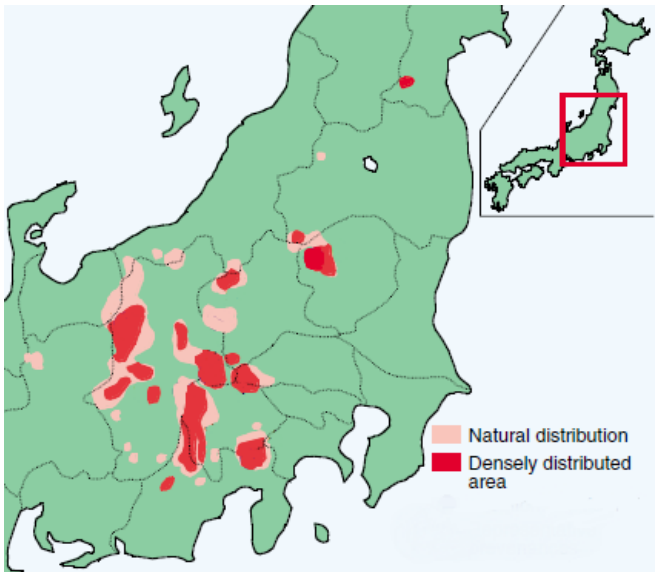

Figure 1. Natural distribution of Japanese Larch in Japan (in Hoshi 2004)

Due to literature scarcity on the use of molecular markers to implement genetic studies on *Larix kaempferi,* references in the present document are often given for related species (*Larix sibirica, Larix gmelinii, Larix decidua, Larix eurolepis*) when available.

2. Isozymes

Genetic studies on *Larix kaempferi* based on isozyme markers have been generally used to identify interspecific hybrid seeds (embryos) between *Larix kaempferi* and *Larix decidua* (e.g. Bergmann and Ruetz 1987, Häcker and Bergmann 1991, Tröber and Hasemann 2000). Isozyme patterns are very similar for the two species in the number of scored loci, but for some loci the relative position of bands differs. For both species the same analysis method can be applied. As detailed studies on genetic variation for *Larix kaempferi* are missing, references in Table 1 refer not only to *Larix kaempferi*, but also to *Larix decidua.*

Material for protein extraction (only Larix kaempferi)

Proteins were extracted from dormant buds and seeds (both endosperm and embryos) (Bergmann and Ruetz 1987, Häcker and Bergmann 1991, Tröber and Hasemann 2000).

Protein extraction and separation protocols

Isozyme extraction, separation by starch gel electrophoresis and staining of gels were carried out based on standard procedures decribed by Siciliano and Shaw (1976), Cheliak and Pitel (1985), Häcker and Bergmann (1991), Müller-Starck and Starke (1993) and Konnert and Maurer (1995).

Important results (only Larix kaempferi and hybrids)

Estimation of the proportion of hybrid seed from seed orchards consisting of *Larix kaempferi* and *Larix decidua*

clones was possible (Bergmann and Ruetz 1987, Häcker and Bergmann 1991, Tröber and Hasemann 2000) based on the loci SKDH-A and NADH-A. At these two loci the two larch species could be unambiguously distinguished by the position of bands in the zymogram and, therefore, the proportion of hybrids and selfings (individual and clonal) could be exactly determined.

Table 1: List of enzymes, scored loci and number of alleles for *Larix decidua* and *Larix kaempferi*

Enzyme system	E.C. Number	Scored loci	No. of alleles*	References
Aspartate aminotransferase	2.6.1.1	AAT-A,-B -C	3, 4, 3	1,5,9
Diaphorase	1.8.1.4	Dia		9
Esterase	3.1.1.2	EST-A,-C	2, 4	1,3,4,9
Glutamate dehydrogenase	1.4.1.2	GDH-A	3	2,3,4,5,9
Glucose-6-phospate dehydrogenase	1.1.1.49	G6PDH-A	3	2,3,4,9
Glyceraldehyde-3-phosphate-dehydrogenase	1.2.1.9	G3PDH-A,-B,	4, 2	1
Isocitrate dehydrogenase	1.1.1.42	IDH-A,-B	1, 3	1,2,3,4,5,9
Leucine aminopeptidase	3.4.11.1	LAP-A,-B	4, 4	3,4,5
Malate dehydrogenase	1.1.1.37	MDH-A,-B,-C,-D	3, 3, 4, 3	1,2,3,4
Menadione reductase	1.6.99.2	MNR-B,-C,-D	1, 4, 2	1,3,4
Phosphoglucose isomerase	5.3.1.9	PGI-A,-B	2, 3	1,5, 6
Phosphoglucomutase	2.7.5.1	PGM-A	6	1,5,9
6-Phosphogluconate dehydrogenase	1.1.1.44	6PGDH-A,-B	3, 3,	1,2,5,9
Triose-phosphate isomerase	5.3.1.1	TPI-A,-B	1, 2	1
Shikimate dehydrogenase	1.1.1.25	SKDH-A	6	1, 2,3,4,5 6,7,8
Superoxide dismutase	1.15.1.1.	SOD-A,-B	1, 3	3,4,9
Sorbitol dehydrogenase	1.1.1.14	SrDH-A	2	3,4
NADH dehydrogenase	1.6.99.3	NDH-A	2	6

**- values for Larix decidua except SKDH-A and NADH-A which refer also to Larix kaempferi*

1-Beletti et al. 1996, 2-Maier 1992, 3-Lewandowski and Mejnartowicz 1988, 4- Lewandowski and Mejnartowicz 1992, 5-Müller-Starck and Felber 2010, 6-Häcker and Bergmann 1991, 7-Bergmann and Ruetz 1987, 8-Tröber and Haasemann 2000, 9-Semerikov and Lascoux 1999

3. Organelle DNA markers (chloroplast (cp) DNA, mitochondrial (mt)DNA)

Detailed information on markers used for DNA analyses from organelle and nuclear DNA from *Larix* species is given in Heinze et al. (2012). In the present guidelines, the focus is on *Larix kaempferi*. Thus, only primers working also for this species are included.

Loci and primers used

According to Heinze et al. (2012), chloroplast DNA (cpDNA) variation has been studied based on PCR-RFLPs (Semerikov and Lascoux 2003, Acheré et al. 2004), sequence variation (Wei and Wang 2003, Gros-Louis et al. 2005) and microsatellites (Semerikov and Lascoux 2003). Mitochondrial DNA (mtDNA) variation has been studied using direct PCR, PCR-RFLPs (Semerikov and Lascoux 2003, Acheré et al. 2004, Semerikov and Polezhaeva 2007) and sequencing (Gros-Louis et al. 2005).

Acheré et al. (2004) applied PCR-RFLP markers on cpDNA (paternally inherited) and mtDNA (maternally inherited) to identify European x Japanese larch hybrids. They used universal primers (Taberlet et al. 1991, Demesure et al. 1995, Dumolin-Lapégue et al. 1997, Petit et al. 1998). For cpDNA, ten out of 22 tested primer pairs gave clear amplification products in *Larix kaempferi* and *Larix decidua*. Only these primers are introduced in Table 2. Amplification products were digested with five restriction enzymes – *Taq*I, *Hap*II, *Hha*I, *Hae*III and *Bcl*I. For mtDNA eight of the eleven tested primer pairs amplify (see also table 2).

For PCR-RFLPs, Semerikov et al. (2003, 2006) and Semerikov and Lascoux (2003) used also published universal primers (Taberlet et al. 1991, Demesure et al. 1995, Dumolin-Lapégue et al. 1997, Parducci and Szmidt 1999) to amplify cpDNA and mtDNA fragments. cpDNA amplified fragments were cut with *Alu*I, *Hae*III, *Hin*fI, *Hpa*II, *Mbo*I, *Rsa*I, *Sf*13.

Material for DNA-extraction

DNA was extracted from buds, needles or germinated seed (Semerikov and Lascoux 2003, Semerikov et al. 2003, Acheré et al. 2004, Gros-Louis et al. 2005, Wei and Wang 2003, Polezhaeva et al. 2010, San Jose-Maldia et al. 2009).

DNA-extraction and amplification protocols

Total DNA was extracted from the mentioned tissue using:

- the CTAB protocol of Devey et al. (1996) cited in Ostrowska et al. (1998) (Semerikov and Lascoux 2003)
- the CTAB protocol of Rogers and Bendich (1988) (Wei and Wang 2003)
- the QIAGEN DNeasy Kit (Acheré et al. 2004, Gros-Louis et al. 2005, Pluess 2011)
- NucleoSpin Plant II (Macherey Nagel, used in INRA lab, unpublished)

Examples for amplification protocols (PCR-RFLP)

- 94°C for 6 min followed by 35 cycles of 94°C for 45 s, 55°C for 45 s, 70°C for 3 min, 30 s (Acheré et al. 2004).
- 94°C for 5 min followed by 35 cycles of 94°C for 30 s, 65°C for 45 s (UBC460), 50°C for nad4-3/4, 55°C for nad5-1/2, atpA1-R, elongation 3 min for UBC460 and 2 min for the rest of primers at 72° C, final elongation at 72° C for 10 min (Polezhaeva et al. 2010).

Examples for amplification protocols (cpSSR):

- 94°C for 5 min followed by 35 cycles of 94°C for 30 s, 55°C for 45 s, 72°C for 30s; final elongation at 72° C for 10 min (Polezhaeva et al. 2010).
- 94°C for 5 min followed by 35 cycles of 94°C for 30 s, 55°C for 30 s, 72°C for 30s; final elongation at 72° C for 6 min (Semerikov and Lascoux 2003).

Example for amplification protocols (cpDNA sequencing)

- 94°C for 3 min followed by 35 cycles of 94°C for 1 min, 56°C for 1 min, 72°C for 1 min and 20 s; final elongation at 72° C for 10 min (Gros-Louis et al. 2005).

Example for amplification protocols (mtDNA sequencing)

- 94°C for 2 min followed by 35 cycles of 94°C for 30 s, 60°C for 30 s, 72°C for 1 min; final elongation at 72° C for 10 min (Gros-Louis et al. 2005).

Important results

- Four cpDNA (matK, trnL-intron, trnT-trnL trnL-trnF) and five mtDNA markers (cox1-1, matR-1, nad1-b/c, nad3-1 and nad5-1) were developed to distinguish unambiguously four larch species (*Larix laricina, Larix decidua, Larix kaempferi,* and *Larix sibirica*) used in intensive forestry in Western Europe or eastern North America and trace forest reproductive material (Acheré et al. 2004, Gros-Louis 2005).

- By combining the mitochondrial PCR-RFLP marker *f13* and the chloroplast PCR-RFLP marker *rbcL-TaqI*, *Larix decidua* and *Larix kaempferi* could be discriminated (Acheré et al. 2004). The two markers are sufficient to identify first-generation hybrid individuals.
- Japanese larch is found to be closely related to populations of *Larix kamtschatica* inhabiting the Kuril Islands and South Sakhalin (Polezhaeva et al. 2010).
- Despite the restricted natural distribution of Japanese larch, the mtDNA showed geographic structure (San Jose-Maldia et al. 2009).

Table 2: PCR-RFLP markers (cpDNA, mtDNA) used for *Larix kaempferi* and other larix species

Type	Amplified region	Primer sequence 5‘ – 3‘	References	Source of primer pairs
cpDNA	trnT-trnF	CATTACAAATGCGATGCTCT ATTTGAACTGGTGACACGAG	1,3	Taberlet et al. (1991)
	rpl20-trnW	T3 + TTTTCGAACTGCTAACCAACG (T3 = AATTAACCCTCACATAAGGG) T7 + ACCTACGGCATCAGGTTTTG (T7=GTAATACGACTCACTATAGGGC)	1,2	Parducci and Szmidt (1999), modified by Semerikov et al. (2006)
	trnL-trnV	CTGCTTCCTAAGAGCAGCGT TTGACATGGTGGAAGTCATCA	1	Parducci and Szmidt (1999)
	psbC-trnS	GGTCGTGACCAAGAAACCAC GGTTCGAATCCCTCTCTCTC	1, 3	Parducci and Szmidt (1999)
	psbD-16S	CCACAAAAACGAAACGGTCT ACTAACTAATCAGACGCGAGCC	1	Parducci and Szmidt (1999)
	rbcL	ATGTCACCACAAACAGAAACTAAAGCAAGTA CTTCACAAGCAGGAGCTAGTTCAGGACTCC	3	Petit et al. (1998)
	trnK	GGGTTGCCCGGGACTCGAAC CAACGGTAGAGTACTCGGCTTTTA	3	Demesure et al. (1995)
	trnK-trnQ	TAAAAGCCGAGTACTCTACCGTTG CTATTCGGAGGTTCGAATCCTTCC	3	Dumolin-Lapégue et al. (1997)
	trnQ-trnR	GGGACGGAAGGATTCGAACC ATTGCGTCCAATAGGATTTGAA	3	Dumolin-Lapégue et al. (1997)
	trnS-trnfM	GAGAGAGAGGGATTCGAACC CATAACCTTGAGGTCACGGG	3	Demesure et al. (1995)
	trnS-trnT	CGAGGGTTCGAATCCCTCTC AGAGCATCGCATTTGTAATG	3	Demesure et al. (1995)
	atpF-rps2	Primer sequence not published	3	Acheré et al. (2004)
	trnR-atpF	Primer sequence not published	3	Acheré et al. (2004)

Type	Amplified region	Primer sequence 5‘ – 3‘	References	Source of primer pairs
mtDNA	nad5-1/2	TTTTTTCGGACGTTTTCTAG TTTGGCCAAGTATCCTACAA	1	Wu et al. (1998)
	nad4-3c/4r	GGAGCTTTCCAAAGAAATAG GCCATGTTGCACTAAGTTAAC	1	Dumolin-Lapégue et al. (1997)
	F13	CTGTTGGTAACTTGGGG GCGCCTCTTTCGGAATAG	3	Acheré et al. (2004)
	UBC460	AACCTAGAGCCAACAGCAGCACCT CCCAACTTCCTCGAAAGCAGATG	4,5	Semerikov et al. (2006)
	C8	GGATCGTAGCGTGGGAACTA AGGGAACTTGTGAACGTTGG	4	Semerikov et al. (2006)
	B11	TACCCGCCTTAACCGTAAGA GACCCGTAGTTTGGCTGAGA	4	Semerikov et al. (2006)
	R11	CATCCCGTCGCTTGTTTAAT CCGGTTGGCACCTTAAATAGA		Semerikov et al. (2006)
	Cox2	TTTTCTTCCTCATTCTKATTT CCACTCTATTGTCCACTTCTA	3	Dumolin-Lapégue et al. (1997)
	nad1-2/3	GCATTACGATCTGCAGCTCA GGAGCTCGATTAGTTTCTGC	3,4	Demesure et al. (1995)
	Nad3-rps12	AATTGTCGGCCTACGAATGTG GCTCG(A=I)GTACGGTC(C=I)GTGCG	3	Wu et al. (1998)
	Nad4-1/2	CAGTGGGTTGGTCTGGTATG TCATATGGGCTACTGAGGAG	3,4	Demesure et al. (1995)
	Nad4-2/3	CTCCTCAGTAGCCCATATGA AACCAGTCCATGACTTAACA	3	Dumolin-Lapégue et al. (1997)
	Nad4-3/4	GGAGCTTTCCAAAGAAATAG GCCATGTTGCACTAAGTTAC	5	Dumolin-Lapégue et al. (1997)
	Nad4-2/4	TGTTTCCCGAAGCGACACTT GGAACACTTTGGGGTGAACA	4	Demesure et al. (1995)
	Nad5-1/2	GAAATGTTTGATGCTTCTTGGG ACCAACATTGGCATAAAAAAAGT	3,4,5	Wu et al. (1998)
	Rps14-cob	CACGGGTCGCCCTCGTTCCG GTGTGGAGGATATAGGTTGT	3	Demesure et al. (1995)
	Mh02	TTTTAGGGCCATTTGCCTGC TCTATGGACAAGAGCCCGACCT	4	Jeandroz et al. (2002)
	Mh09‘	CCATCCAGCCATGTCTCATC AGGGCTTCACATAGAGCATC	4	Jeandroz et al. (2002)
	Mh27	TGCTTTCCAATTTACCACGAG GATACGCTTTCCTGGCATAC	4	Jeandroz et al. (2002)
	Mh50	AGAATGGCAGCAACTAATAAGC ACTATGCACTTCCCTCCCTCA	4	Jeandroz et al. (2002)
	Atp1-R	GCTGGCAAATTCAACCATTT GCAATTAGGCTGGCTTTCC	5	Polezhaeva et al. (2010)

1-Semerikov and Lascoux 2003, 2-Semerikov et al. 2003, 3-Acheré et al. 2004, 4-San Jose-Maldia et al. 2009, 5-Polezhaeva et al. 2010

Table 3: Primer information for amplification of chloroplast microsatellites (cpSSRs) and variable fragments for sequencing (cpDNA and mtDNA) in genetic analysis of *Larix* species (including *Larix kaempferi*) (Ta= annealing temperature)

Locus	Type	Primer sequence [5'-3'] F= forward, R = reverse	T a (°C)	Size (bp)	Ref.	Source of primer pairs
Pt9383	cpSSR	F: AGAATAAACTGACGTAGATGCCA R: AATTTTCAATTCCTTTCTTTCTCC	48	118	1	Vendramin et al. (1996)
Pt9393		F:GACGTAGATGCTATGGGTACG R:GAGAGCGGTATGAGGGAAGA	55	135	2	Polezhaeva et al. (2010)
Pt9833		F:GACGATGGACGCTCTTTCTC R:GATCGGGCGGGATAATGTA	55	84	2	Polezhaeva et al. (2010)
Pt30		F:TCAATCCTAACCATATCAGGTG R:TCATAGCGGAAGATCCTCTTT	55	139	2	Polezhaeva et al. (2010)
Pt26081		F:CCCGTATCCAGATATACTTCCA R:TGGTTTGATTCATTCGTTCAT	55	112	1	Vendramin et al. (1996)
TrnLV		F:AAATACCACGGGCCTCCTA R:TTGACATGGTGGAAGTCATCAT	55	86	2	Polezhaeva et al. (2010)
Pt30204		TCATAGCGGAAGATCCTCTTT CGGATTGATCCTAACCATACC	55	145	1	Vendramin et al. (1996)
matK	cpDNA amplification and sequencing	F:GAACTCGTCGGATGGAGTG R:GAGAAATCTTTTTCATTACTACAGTG	56		3	Wang et al. (1999)
trnL Intron		F:CGAAATCGGTAGACGCTACG R:GGGGATAGAGGGACTTGAAC	56		3	Taberlet et al. (1991)
trnT-trnL		F:CATTACAAATGCGATGCTCT R:CGAAATCGGTAGACGCTACG	56		3	Taberlet et al. (1991)
trnL-trnF		F:CGAAATCGGTAGACGCTACG R:ATTTGAACTGGTGACACGAG	56		3	Taberlet et al. (1991)
trnT-trnF		CATTACAAATGCGATGCTCT ATTTGAACTGGTGACACGAG	48		4	Taberlet et al. (1991)
Cox1-1	mtDNA amplification and sequencing	F:TTATTATCACTTCCGGTACT R:AGCATCTGGATAATCTGG	60		3	Lu et al. (1998)
matR-1		F:CGACAGAAGCACGAAATTCC R:ACCCGACGATAACTAGCTTC	60		3	Qiu et al. (1999)
nad1-b/c		F:GCATTACGATCTGCAGCTCA R:GGAGCTCGATTAGTTTCTGC	60		3	Demesure et al. (1995)
nad3-1		F:CAGAAGTCGTTTCGATATACG R:ATTGATTCGATGTAGGCATCG	60		3	Soranzo et al. (1999)
nad5-1		F:AGTCCAATAGGGACAGCACAC R:GCTTTGATAGCTGCTTTATCTGC	60		3	Jaramillo-Correa et al. (2003)

1-Semerikov and Lascoux 2003, 2- Polezhaeva et al. (2010), 3- Gros-Louis et al. 2005, 4-Wei and Wang 2003

4. Randomly amplified polymorphic DNA (RAPD) markers

Primer used and important results

Scheepers et al. (2000) analyzed the following 11 markers that differentiated *Larix decidua* and *Larix kaempferi.* Two of these markers were mitochondrial (maternally inherited) (DeVerno et al. 1993).

- OPH-11 – 2,2 kb – 100 % presence in *Larix decidua*
- OPD-15 – 1,4 kb - 100 % presence in *Larix decidua* (mtDNA)
- OPE-17 – 0.8 kb - 100 % presence in *Larix decidua*
- OPF-05 – 2.25 kb -100 % presence in *Larix decidua*
- OPG-12 – 1.3 kb - 100 % presence in *Larix kaempferi*
- OPH-14 – 1.45 kb -100 % presence in *Larix kaempferi*
- OPC-16 – 1.38 kb - 100 % presence in *Larix kaempferi*
- OPC-06 – 0.93 kb - 100 % presence in *Larix kaempferi*
- OPR-08 – 1.2 kb - 100 % presence in *Larix kaempferi* (mtDNA)
- OPD-10 – 1.2 kb - 100 % presence in *Larix kaempferi*
- OPF-13 – 1.0 kb - 100 % presence in *Larix kaempferi*

The following four markers were sufficient to estimate the F1 hybrid (*Larix* X *eurolepis*) fraction in a seed lot: OPH-14, OPC-06, OPH-11, OPF-05.

For DNA-amplification the following PCR-protocol was used:

- 1 cycle of 3 min at 93°C, 1 min at 37°C and 2 min at 72°C, 35 cycles of 1 min at 93°C, 1 min at 37°C and 2 min at 72°C, followed by a final cycle of 10 min at 72°C.

Semerikov et al. (2003) used 4 RAPD primers to develop PCR-based mitochondrial DNA markers useful for phylogenetic studies in larch species. The following four RAPD primers produced fragments considered for further analysis:

UBC460 - 5‘-ACTGACCGGC-3‘
OPB11 - 5‘-GTAGACCCGT-3‘
OPC8 - 5‘-TGGACCGGTG-3‘
OPR11 - 5‘-GTAGCCGTCT-3‘

The RAPD fragments were cut out of a 1% agarose gel, purified using a gel extraction kit (Qiagen), cloned into pGEM-T easy plasmid (Promega) and sequenced.

Gros-Louis et al. (2005) tested 130 RAPD-primers using the following kits from Operon Biotechnologies, Alameda, CA:

- OPL–OPQ,
- OPC-6,
- OPD-10, OPD-15,
- OPE-17,
- OPF05, OPF-13,
- OPG-12,
- OPH-11, OPH-14,
- OPR-18.

For DNA-amplification the following PCR-protocol was used:

- 1 cycle of 1 min at 94°C, 20 cycles of 15 s at 94°C, 15 s at 35°C, and 1 min 30 s at 72°C followed by 25 cycles of 15 s at 94°C, 15 s at 35°C, and 1 min 30 s at 72°C, with a ramp at this extension step of 5 s per cycle, final extension of 10 min at 72°C.

Amplification products (6 µl) were separated into a 0.5% Synergel (Gordon Technologies, Mississauga, Ontario) plus 1.0% agarose gels using 0.75× Tris–phosphate–EDTA (TPE) running buffer. Amplification products were stained with ethidium bromide and visualized under UV light.

5. Nuclear DNA markers (AFLPs, nSSRs, EST-SSRs, SNPs)

a) AFLPs (Amplified Fragment Length Polymorphism)

Semerikov and Lascoux (2003) and Semerikov et al. (2003) used besides other markers the AFLP technique (Vos et al. 1995) for analyzing larch species differentiation at the nuclear level.

DNA was digested with *Eco*RI and *Mse*I. Three selective nucleotides were used in the case of the *Eco*RI primer and four for the *Mse*I primer. The *Eco*RI primer was labeled by g^{33}P-ATP.

The following primer combinations were used:

- *Eco*1+ ACG x *Mse*1+CCCA, *Mse*1+CCAC, *Mse1*+CCAG (Semerikov and Lascoux 2003)
- *Eco*1+ ACG x *Mse*1+CCTC, *Mse*1+CCCA, *Mse*1+CCAC, *Mse1*+CCAG, *Mse*1+CCTG, *Mse*1+CCAG (Semerikov et al. 2003)

Arcade et al. (2000) analysed 114 AFLPs resulting from 5 AFLP primer combinations and constructed a single-tree genetic linkage map of European and Japanese larch.

b) nSSRs (putatively neutral microsatellites) and EST-SSRs (expressed sequence tag derived microsatellites)

Loci and primers used

More then 200 nuclear microsatellites were developed for *Larix kaempferi*: 19 polymorphic simple sequence repeats (SSR) markers in Isoda and Watanabe (2006), 165 SSR marker in Chen et al. (2015, Supplementary Material), six ***expressed sequence tags*** (EST-SSRs) in Yang et al. (2011).

28 microsatellite markers were amplified in cross-species transferability tests for *Larix kaempferi* (6 SSR marker in Khasa et al. 2000, 13 SSR marker in Wagner et al. 2012 and 9 SSR marker in Zhang et al. 2015) (Table 4). Wagner et al. (2012) designed multiplexes for Larch SSRs. Gros-Louis et al. (2005) tested the transferability of EST-SSRs developed by Perry and Bosquet (1998) to *Larix* species, among them also *Larix kaempferi.*

Table 4: Primer sequences, annealing temperatures (T_a), allele length in base pairs (bp), number of alleles scored (N_a) and references for nSSR markers available for genetic analyses in *Larix kaempferi* (* = EST-SSR developed by Perry and Bousquet 1998).

Locus	Motif	Primer sequence		Size (bp)	T_a	N_A	Ref.	Genebank accession number
		Forward	Reverse					
bcLK033	(TC)14	M13-GGAAATGTAGAGATGAGCAATAA	AGGTGCGGTAGTACAAAGTGA	197–251	63-53	9	1	AB234185
bcLK056	$(AG)_{20}$	M13-ATGGGCTAAGGTATGTTTTACG	TTGCCAACATCTATACCAAGTCT	174–200	63-53	12	1	AB234186
bcLK066	$(TG)_{12}$	M13-GCAACCGACAATGATTACATAG	CCTAAAACTGAACTTTGCTCAAT	155–172	63-53	5	1	AB234187
bcLK093a	$(AG)_{17}$	M13-TTCCCCGATGTATATTCACCT	TGACCGTGGTATTTGGATGTA	136–176	63-53	17	1	AB234188
bcLK187	$(AG)_{13}$	M13-AGGACGGAGAGAGTCATTCTG	AACCCTAGTGATTTTAAGGAGAGA	160–186	63-53	12	1	AB234189
bcLK189	$(AG)_{17}AT(AG)_6$	M13-ACCATACGCATACCCAATAGA	AGTTTTCCTTTCCCACACAAT	122–196	63-53	12	1,4, 5	AB234190
bcLK194	$(AG)_{17}$	M13-AAGAGCAAGAATGGGAGTAAG	CATCCAATATCTCCTCTATAAACC	116–136	63-53	7	1	AB234191
bcLK211	$(CT)_{16}$	M13-CCATTCTCCATAGGTTCATTG	ATGCTCCTTACTAAGTCAGATACAC	207–232	63-53	12	1,4, 5	AB234192

Locus	Motif	Primer sequence		Size (bp)	T_a	N_A	Ref.	Genebank accession number
		Forward	Reverse					
bcLK224	$(AG)_{17}$	M13-GGAGAGGCCACTACTATTATTAC	ATGCGTTCCTTCATTCCTCT	152–168	63-53	9	1	AB234193
bcLK225	$(GA)_{20}$	M13-CGTGGTTCCCATCCTCTAAA	TGGCAGCTAAAGGATTAAAGAA	180–213	63-53	12		AB234194
bcLK228	$(AG)_{18}$	M13-CCCTAACCCTAGAATCCAATAA	GAGGAAGGCGACAAGTCATT	183–234	63-53	17	1,4, 5	AB234195
bcLK229	$(GA)_{21}$	M13-ATGCCCAAAAACGAAAAAGT	TTTGCACTGCCAGATTCAGA	108–134	63-53	12	1,4	AB234196
bcLK232	$(AG)_{19}$	M13-TGTTGCTGGGTTGTTGTTAGA	GGGTAATAGTTCCAGTCTTTG	142–178	63-53	10	1	AB234197
bcLK235	$(TC)_9(AC)_2AG(AC)_{14}$	M13-TTCACTTGTGATCCTAGAGTTA-GA	AACCCCTAACCATATAATATCCA	177–220	63-53	9	1,4	AB234198
bcLK241	$(AG)_{12}$	M13-TGAGGTTAGGAGCATCTCGT	GTCCTTCATCGCCTCTTCTT	164–176	63-53	5	1	AB234199
bcLK253	$(AG)_{17}$	M13-AACACCATAGTGCAATGTGC	TCCTCTTGTTGATGCCACTT	217–243	63-53	14	1,4	AB234200
bcLK258	$(TC)_{29}TT(TC)_8$	M13-AAGGTGCTCGTATAATCTCTGG	AGAGTGCCTTCGATCATCAT	107–179	63-53	26	1	AB234201
bcLK260	$(TG)_{14}(AG)_9$	M13-CTCCATAAGGGGCATCACAT	TGGGCTCAAGTTTGGACATTA	115–126	63-53	5	1,4	AB234202
bcLK263	$(TC)_{20}$	M13-CGATTGGTATAGTGGTCATTGT	CCATCATACCTTCTTGAAGAG	205–255	63-53	23	1,4, 5	AB234203
LAReSSR12*	$(ATT)_4(TGT)_4(GTGGCA)_4$	ATTATTGCCCTCTGAGTTTG	ATTCACCCCAATCCCATC	131	56	4	2	JG745369
LAReSSR14*	$(TCAGGC)_5$	ACATTGAGCAGATGACCCAC	ATGCGGAGGTTGAGTTGG	146	56	3	2	AB251473
LAReSSR19*	$(CAT)_4$	CCGAAATGAAGTCCGTGAG	GCAGCAGCAAGTCCTAAAT	140	55	2	2	JG745370
LAReSSR27*	$(AGTCC)_4(GTCCA)_6$	GGCTGAGGTTGCGAAAGA	CAATTACATAAGTGGGACGAGA	142	56	4	2	JG745371
LAReSSR72*	$(AT)_6$	ATGGCTGTGGAAGCGAATA	AAGGGATCACGAACTGAACTGG	168	60	4	2	JG745368
LAReSSR85*	$(TAC)_4$	TTTCGTATGGTCAAGTTCTG	TGTCTATCCCAAGTCAGTCAT	172	52	3	2	JG771979
Sb14*	-	TACTTCGAGTGTCTCTCATTG	GCTGTCAGAGTTTGTAACATC	-	55	1	7	
Sb34*	-	TATCCATCGCCTGCTTCTCAC	TGTAGTCAGTCCGAATGTACC	-	55	1	7	
Sb41*	-	GCTGAGGGGAAGGATTGATAC	GCTTCGACAGGCATATTACAG	-	55	1	7	
Sb46*	-	GGCTGTCAATACAAGTCATTC	TCACGTTGTTATTGTTGTCAC	-	55	1	7	
Sb51*	-	TGAAACAGACTTCTCGTACTG	TTCTTACGTAGCTGCTCTAAC	-	55	1	7	
Sb60*	-	TGGGAGAATGACTAGATTGTG	AAGCCTTGACAATAGTAAGTG	-	55	1	7	
Sb62*	-	GTATTACCCAGCTCAAGTTCC	ACAGTACGCCGCAGACAAATG	-	55	1	7	
UAKLla1	$(TCT)_4$	ATCTCCTTCATCGTCCAC	CCCCAACTAATACCTAATCTAC	175–178		1	3	X54464
UAKLly2	$(CA)_5$	CGAAAGCGAAGAGAGTATCG	GTTCCCAAGGAGAAACCCTA	250–276		1	3	LLY2 (EL)
UAKLLy7	$(TG)_8$	GATTACATCGTGGGTAGGAC	AAGTGATTTGGTGTGGTGAC	182–190		2	3	LLY7 (EL)
UAKLly10a	$(CA)_5AA(CA)_7$	TGGTCGGATTGAGTGAAG	ACCCATCCCATGATAGGAG	274–330		2	3	LLY10 (EL)
UAKLly13	$(AT)_5(GT)_{20}(GA)_6(A)_7$	TCTGTTTACCATCCATAAATC	CCACAACCCATTTTAATATC	154–186		1	3	LLY13 (EL)

Locus	Motif	Primer sequence		Size (bp)	T_a	N_A	Ref.	Genebank accession number
		Forward	Reverse					
UAKLly6	$(GT)_{17}$	AGTTGTACTGTGTGTTGGTC	CTGCCTCAACCACTTCTTC	214–264		1	3,4	LLY6 (EL)
lardec012611(Ld31)	$(AC)_{18}$	TTGAACTAGGGAGATCCGGC	AATAAAATAGCATTCCATGTGTAGC	104–147		8	5	-
lardec022835(Ld50)	$(CA)_{18}$	GAAGGCGACTTTACATGCCC	TCCATCTTTATGTCTCTTCCATGC	157–205		12	5	-
lardec023929(Ld42)	$(TG)_{14}$	TCGTATGCATTGTCCAAATTTCC	TCCAAGTGAGGTCACACGAG	167–191		6	5	-
lardec025807(Ld101)	$(AC)_{12}$	ACACCAAGGACTCTCTGACTAC	GGTGATTCCAGAAGCAGGTG	179–215		7	5	-
lardec023228(Ld56)	$(AC)_{16}$	AGCCATCGTGGTTCTTCTTTG	CTTGTAACTGTGCACCCACC	219–247		9	5	-
Lg01	$(AGC)_{4}$	CAGTGGTGTCCGTGGTGTA	GACCTCCTCCACACCTAAT	141–160	51.3	3	6	XP_006375-910.1
Lg02	$(AGG)_{4}$	CTCTGTGACCAAGAAACCAA	CATGAAGACGAAGAATGCACT	120–140	51.8	2	6	XP_002306-980.2
Lg06	$(AGA)_{5}$	CAAGGATGGAGCAGACGAT	AGCCTCGCACTTTGACAGA	135–150	50.4	2	6	-
Lg14	$(TC)_{6}$	GGGGATTGCAGAGTAGAAA	AAACAGCCATCGAAATGAG	140–150	49.5	1	6	XP_002319-953.1
Lg25	$(AAG)_{4}$	GTGAGAGGTCAAACCCCAA	AGAAGAGTCTGGTCCACGCT	105–125	53.8	2	6	XP_003608-708.1
Lg32	$(AT)_{6}$	CTCTGTCGCACCAGCATTG	TTGTCTTCCGGTATTCACA	105–115	47.5	1	6	XP_002307-364.1
Lg36	$(GA)_{5}$	TGCCCATCCTCTTTGTTTA	AGCACCTGATTCCACATTCT	175–190	50.6	1	6	-
Lg37	$(CT)_{6}$	ACAATGGCTTCCTTCAACA	TATGAGGTGGTTAGGGAGA	175–190	47.3	1	6	XP_002299-125.2
Lg41	$(AGA)_{4}$	ACTTCCACTAAGGTTGACA	ATCCACTGCCTTCTGGTCAT	147–180	49.4	3	6	XP_002313-280.1
LARKeSSRH002	$(AGC)_{6}$	AGGAGGCGGTTCAGTTCAG	GACCTCCTGGGATTTGGATT	117-156	56	7	8	KP863070
LARKeSSRH008	(ACTGGGC)4	GAGATGTACACAGTCCGCCC	CCTGTTCGGATCCACAGAAT	400-414	56	3	8	KP863071
LARKeSSRH028	(AAAATGTGAC)2	TGCCCATTTGAATCCTTAACA	TCGTTGTAGAAGAATGGGGC	198	56	1	8	KP863072
LARKeSSRH029	(AAAGGACCTC)2	TGGAGTTGCACACTACGAGG	GTGATCGGGAGTTCATCGAC	263	56	1	8	KP863073
LARKeSSRH034	(AAACTCTTC)3	AACACACCTGGCCCTGTAAG	GCGCTGTATTGTATTGATAAGGC	93-111	56	3	8	KP863074
LARKeSSRH042	(AAATAG)3	GGACACTTTTCTGCTTCCCA	CAGGTGGCAGAGTACCCACT	334-346	56	2	8	KP863075
LARKeSSRH045	(AAATATATAT)2	CGCCACCTTCCCTATTTACA	CCCCAACCCTAAGACACAGA	274	56	1	8	KP863076
LARKeSSRH046	(AAAATTCTTT)2	ATGTTTTTGGGTTTTGGAGC	CAGGTTTATAGCTTTGGTTTGGA	154-174	56	3	8	KP863077
LARKeSSRH052	(AATG)6	AGGGATGGTTGCTGTGGTAG	CATTTCTCCGAGTGGGTTGT	333-349	56	3	8	KP863078
LARKeSSRH057	(AT)11	GGACGTCTTAAGCATGCCA	AAAGTTCGAAGTGAAGCGGA	110-130	56	7	8	KP863079

Locus	Motif	Primer sequence		Size (bp)	T_a	N_A	Ref.	Genebank accession number
		Forward	Reverse					
LARKeSSRH094	(ACATAGTAGG)2	CTGATGGCACATAGCTGCAC	CTTGACAAAGGAGCCAAAGC	253-265	56	4	8	KP863080
LARKeSSRH106	(AGCAT)4	AGCAGCTGTTGTTGTTGTGG	TGCAAATCGTCTTCACAAGC	247-257	56	2	8	KP863081
LARKeSSRH122	(ACCCTC)6	TGCTTCCGCAGATATAGCCT	CTAAGTTTGTGCGCCGAGAT	240-264	56	5	8	KP863082
LARKeSSRH125	(AT)11	TCTCCCAACCACCCAAGTTA	TCAGGTTCTGGGTTTGGTTC	237-251	56	6	8	KP863083
LARKeSSRH128	(AAATTGGCCT)2	TGGCCAATTTTGAGTTCAAGT	AGAGGTCTCGTAACGGCAGA	239-249	56	2	8	KP863084
LARKeSSRH131	(AGATG)5	GAAGATCACAACAAAGGGCG	TGTCCAGGCAACTGAAACAG	287	56	0	8	KP863085
LARKeSSRH136	(AACCAACCAG)2	GGGACGTACTGAGACCGTGT	TCATTAACTGGGCATGTGGA	373-397	56	2	8	KP863086
LARKeSSRH137	(AAATAAGC)2	ATACATATTCCTTCCGGCCC	TTGGAAAGACTCCAGGATGG	170	56	1	8	KP863087
LARKeSSRH140	(AAAGCC)3	GGAGTAGTGCATATGGGCGT	TATGCTTTTTCCCAGCCAAC	294-336	56	6	8	KP863088
LARKeSSRH147	(AGC)8	AAATGAAGAACCCGAACACG	AGCTCTCGATTCATGGCTGT	181-193	56	2	8	KP863089
LARKeSSRH149	(ACGGCACTCC)2	CAAGGAGAACTGAAGGCTGG	TTTCTCGTCAACTGAGGGCT	251-291	56	3	8	KP863090
LARKeSSRH168	(AGCAGG)5	ACTTCAGTATCACCCGCCAC	CGATCTTTCGGCTCTTATCG	145-169	56	5	8	KP863091
LARKeSSRH177	(AAATAGCTTC)2	TGGCTTTTGCAACAAGTGAC	GGCCATCCTCTGTCATGATT	394-414	56	3	8	KP863092
LARKeSSRH179	(AAAGAAGTTC)2	AACACCAAAGTTGCTGGGAC	GGCTGAGGATTATGATCGGA	335	56	1	8	KP863093
LARKeSSRH180	(AAAGATACC)2	ACATCCTCCCCTTGGTCTCT	CTTGCTCCTGGCGAAGTAAC	169-178	56	2	8	KP863094
LARKeSSRH182	(AAACCC)3	CTGATCAGGGTGAGATGGGT	GCTGCTGTTGTTGTTGCTGT	314	56	1	8	KP863095
LARKeSSRH187	(AACAGC)5	AGATTTGGAAGCAGCAGGAA	AAGTTGTTCAGCCCATCTCG	123-141	56	3	8	KP863096
LARKeSSRH189	(ACTGGC)6	GTAAGGAGGAGGATTGCGGT	AGTTCATCCTTCTGGCTGGA	255-273	56	4	8	KP863097
LARKeSSRH191	(AACCCTCCC)2	TTGAATTCGTCCTGGGTCTC	GTCTGAACGACGAAGAAGGC	145-163	56	3	8	KP863098
LARKeSSRH197	(AAACGGACGG)2	TTAGCAAAAGTCTTCGCCGT	ACGAAACTACGCGGATGAAC	327-337	56	2	8	KP863099
LARKeSSRH206	(AACAATAATT)2	TGCAGTTCGTGTTGCTAACC	CCACCTGGCGAAGTATTGAT	312-362	56	3	8	KP863100
LAREeSSRH217	(ACGCCC)3	ATCCCAAGAACCGATATCCC	TGACCGATTTTCTCTCGCTT	418-436	56	3	8	KP863101
LARKeSSRH221	(AGCATC)3	AGATTCGGTTTTCATGGACG	GCAAGCGAGAGAAAGCAGTT	376-394	56	4	8	KP863102
LARKeSSRH224	(AACGTCC)3	GCTGCCCAGGTGAAGAATAC	TCCCAATTTCACAATCATAGGAG	177-184	56	2	8	KP863103
LARKeSSRH233	(ATCCCC)4	AGGGGCAGGCTTAATCACTT	GATTCGAAGAAAATTGCCCA	444-456	56	3	8	KP863104
LARKeSSRH236	(AGC)8	GAATGCCATTGGAACAGCTT	TGCCTGTGCTCGTTCATAAG	300-321	56	6	8	KP863105
LARKeSSRH239	(AATCCAGTG)2	AATAGTTTGGGGAACCGACC	CCCTGGTTCTATTGACGCAT	333-342	56	2	8	KP863106
LARKeSSRH251	(AACAGC)3	GTTGTTCAGCCCATTTCGAT	AGATTTGGAAGCAGCAGGAA	125-143	56	3	8	KP863107
LARKeSSRH253	(AGGATC)3	AACGGGGTTATCAAGCACTG	ATGCGTTTCATTCGATCCTC	342-366	56	2	8	KP863108
LARKeSSRH256	(AGCCCC)4	TATCCGGCACCCCTGTAATA	GGTTTGATGGGAAACTGCAT	113-125	56	3	8	KP863109
LARKeSSRH264	(AGATGG)3	CCGACGCTATTCCCAACTAA	CTTGGAAGGCTATGGCTACG	96-132	56	6	8	KP863110
LARKeSSRH274	(AGCCC)5	CGGACGAATAGATCCCAGAA	ATGAGGCAGGGTCGTGTTAG	252-272	56	5	8	KP863111

Locus	Motif	Primer sequence		Size (bp)	T_a	N_A	Ref.	Genebank accession number
		Forward	Reverse					
LARKeSSRH276	(AACCGG)3	GAACCAAACCCAGAACCTGA	CTGGGGATATAAATGGGGCT	154-189	56	5	8	KP863112
LARKeSSRH279	(AATCGATGC)2	AATTCAGGGGACATTGCTTG	TTTCTGGGTCTCAGGAATGG	160-187	56	4	8	KP863113
LARKeSSRH283	(AAAGATGAC)2	TCTAGCCATGTGCATTGTCC	ATTCTGTGTTTTGTCGCACG	331-367	56	4	8	KP863114
LARKeSSRH299	(AAGGAG)3	CGATCTTTCGGCTCTTATCG	ACTTCAGTATCACCCGCCAC	149-173	56	5	8	KP863115
LARKeSSRH301	(AATGGC)4	CCAAGGAAACCAGTGCATTT	CATTGGTTGAGGTGGAGGAG	256-280	56	4	8	KP863116
LARKeSSRH309	(ACCTCC)3	AATGGGCTCTCAATGCAATC	AGGTGACAAATGGGACCAAG	466	56	1	8	KP863117
LARKeSSRH339	(AGC)7	AATTCGTTGGCCTTCAGATG	CGATCTCGGGCATTATGAGT	316-319	56	2	8	KP863118
LAREeSSRHL003	(AAGAT)4	TGTGGTCATTGGTGGACATT	GAGTCCCACATTTGCAGGTT	304-324	56	5	8	KP863119
LAREeSSRHL004	(AACCTC)7	AGATGAGCTCCTGTTGGGAA	TTGCTTTGCAGCTTACCAGA	200-224	56	5	8	KP863120
LAREeSSRHL006	(AAT)10	TGCGTTCTGTGTGTCTCTCC	GGGTAGGCCTGAAGAAGGCT	99-117	56	4	8	KP863121
LAREeSSRHL007	(ACAGC)5	GGACGAGACCAATCCAAACT	CAAAAGCCGGGAGAAATGTA	238-278	56	4	8	KP863122
LAREeSSRHL009	(AGGATG)4	GGTCTTAGTCACAGCCCAGC	TTCGATCCTTCTGAATTGGC	151-175	56	6	8	KP863123
LAREeSSRHL021	(AACAGTCTAG)2	GGTCACATGGGAATGAGCTT	TGACTTGTTATTTCTGAATTTTGGA	160-170	56	2	8	KP863124
LAREeSSRHL034	(AAG)7	CCTTCCGTTGCAATCTTCAT	CTTTCCACACTGCCAAACCT	92-116	56	8	8	KP863125
LAREeSSRHL042	(ACGTCC)3	GAATCTGAGAGCTCCGGGTA	ATCCATGTTTTTGCCTCGAC	87-117	56	6	8	KP863126
LAREeSSRHL046	(AAGCTGTGTC)2	ATCCAACTGGATCCATCAGC	CCGGATAAAGTCCAGCAAGA	380	56	1	8	KP863127
LAREeSSRHL062	(AATGCATACT)2	CGGATCTCCTCCTGAATGAA	GTTGAGCTGTCGGATCACAA	222-242	56	3	8	KP863128
LAREeSSRHL079	(ATCCCC)3	GATTCGAAGAAAATTGCCCA	TACCCGTTTCCATTTCCATC	172-202	56	5	8	KP863129
LAREeSSRHL083	(AAAATCAAG)2	CCAAACCTCAACAACAGCAA	GTGCTGCGGATGAGTACAGA	142	56	1	8	KP863130
LAREeSSRHL085	(AACATTG)2	TTTGGCAGTTTTGACAGTCG	CGAGCCATTTGTGTCTTTGA	123-141	56	4	8	KP863131
LAREeSSRHL101	(AGGCGG)4	ATCAAGATCGCCGGTGTTAC	GATTGCCAAAGCCAATGC	232-250	56	4	8	KP863132
LAREeSSRHL104	(ATC)8	CGGATACGGCAAATTTTCAA	CCTTTGTCTTGGTGCTGGAT	283-313	56	7	8	KP863133
LAREeSSRHL114	(AGC)7	AGGAGGCGGTTCAGTTCAG	CAACGCCAGATTAGGAGAGC	187-232	56	7	8	KP863134
LAREeSSRHL120	(AAAG)8	GAAAAGGGTGGAAATGCAAA	GGCACTACCTAACCAAAGTAGGA	133-145	56	3	8	KP863135
LAREeSSRHL129	(AAAAGCATC)2	ATCTTCCCCTGCTGTTTGTG	GGGAGCGTTGAATGGATAGA	254	56	0	8	KP863136
LAREeSSRHL137	(AGC)7	GAGGATTGTGCACACCTTGA	ATGGGTTTGACAGCGGATAA	100-112	56	4	8	KP863137
LAREeSSRHL138	(AATCATCAT)3	AAGGAGTGGGTTTATTGGGG	AGGTGATGATGATGATGTACAATG	243-252	56	2	8	KP863138
LAREeSSRHL159	(AGAGCC)5	CACAGACCTCATGACGATGG	TTCTGATTCTGCCTCTGGCT	224-236	56	3	8	KP863139
LAREeSSRHL161	(AGATGG)5	CGTTTCCAAAATGCCTCAGT	ACACCCAGGGAAGCTCCTAT	302-332	56	6	8	KP863140
LAREeSSRHL162	(AC)10	GGGTCACGTCTACGAGGTTT	GCTAGGACTGCCACTGGATT	85-119	56	9	8	KP863141
LAREeSSRHL163	(AAGGCC)3	AATGGAAGCGTGAGGACATC	TGGTTAAGGGCAACCAAAAG	263-287	56	4	8	KP863142
LAREeSSRHL165	(AAAGGATGAT)2	TATCCTCCTGCACCATCCTC	TCCTCAGTTGCCCTTTGTTT	382	56	1	8	KP863143

Locus	Motif	Primer sequence		Size (bp)	T_a	N_A	Ref.	Genebank accession number
		Forward	Reverse					
LAREeSSRHL166	(AAACCCT)3	CTCAAGAGGTATCAAGCGGC	TAAGGGCTAAGTGGGTGCTC	207	56	1	8	KP863144
LAREeSSRHL215	(AGGCGG)3	TAAATACGCACAAGCCCACA	GGAGGAGCAAATGGATCAAA	203-209	56	2	8	KP863145
LAREeSSRHL217	(ACTCATATG)2	AATCCAACAGAAGGCCAAGA	GCCGGCAAATAGGTTGATATT	204-249	56	3	8	KP863146
LAREeSSRHL246	(AAT)9	TGGATGGTAAGAACGCACAG	ACTTTTACCCGTTGTGGTCG	188-218	56	5	8	KP863147
LAREeSSRHL272	(AATATATAT)2	GCAACAACATCGAACAGCAA	TGTTTATAGGCCAAGCCACC	354-372	56	3	8	KP863148
LAREeSSRHL275	(AAACAAAT)2	CTACCTAAGTCGGCCCACAA	TATCCTCGGAAACCATGAGG	354-362	56	2	8	KP863149
LAREeSSRHL283	(AATGGCAGAC)2	CCATTCCCAAACTAAAACGC	GATGATGAGGCCCTTCAAAA	188-198	56	2	8	KP863150
LAREeSSRHL299	(AAACCCTACG)2	GAACAGCATACAATGGGGCT	CGTCGCGAACAAGAATGATA	446-456	56	2	8	KP863151
LAREeSSRHL308	(AAACATTT)2	TGCATTGTCTTGCTGCCTAT	ATTGCACTGAATGCACAAGC	286	56	1	8	KP863152
LAREeSSRHL346	(ACCAGC)4	TAGAAAGGGCAAAGGCACTG	GGTGCATTTCTCTCCACTCC	99-111	56	3	8	KP863153
LAREeSSRHL357	(AACAGC)3	AGGTCCAGCCATTGATGAAG	TCAATGCAATCCTGGGGTAT	162-168	56	2	8	KP863154
LAREeSSRHL358	(AATAATTCTC)2	CTCCCACCTTACCACGAAAG	TGTGTTAGCATTCCGTGCTC	135-145	56	2	8	KP863155
LAREeSSRHL361	(ACTC)5	GTATGCTGCCAAAGGTGGTT	CATTTCCGGGCTTGTATTTG	275-311	56	3	8	KP863156
LAREeSSRHL366	(ACGGAT)3	TCGTATCTGGATCTCGGGTT	AAAGAGGCAAGCGGTACTCA	244-256	56	3	8	KP863157
LAREeSSRHL372	(AAACCCG)3	GATTCGGAAATGCGGAAATA	AGTTCAAAAATTGGGCGTTG	114-128	56	3	8	KP863158
LAREeSSRHL374	(AC)4	AGTTGAACCAACCCTCATCG	CTGTGGGGTGGAGATCCTTA	246-268	56	9	8	KP863159
LAREeSSRHL380	(AACGGC)3	GGCTGGTACATTTCAGGCAT	AGCCTCTCCTCCTCCTCAAC	184-202	56	3	8	KP863160
LAREeSSRHL391	(ACTGGC)4	AGCGTATGAATTGGTCCAGG	ACGAAGATAGCTCGAACGGA	224-230	56	2	8	KP863161
LAREeSSRHL392	(AAACAAACAG)2	GCGGTCAGGCTTTATCTCAG	ACCTGATGACCACGGGATAG	306-324	56	4	8	KP863162
LAREeSSRHL393	(CCG)8	GCCAGAACCACCGTTAAAAG	AGAGGCGATTATGGGAGCTT	296-302	56	3	8	KP863163
LAREeSSRHL394	(AAAGGC)4	GGGGAGGTGTTTGACAGAGA	AATCAACCGTTGGGAATGAG	255-261	56	2	8	KP863164
LAREeSSRHL395	(ACCAGG)5	TTTGCTTTAAGCTGGGCAGT	CAAAGCTTTCCGAAGGGAAT	272-308	56	6	8	KP863165
LAREeSSRHL396	(AAGAGC)5	CTTTTGCCCTTTTCCTTTCC	TTGTGGGTGTCGTTCACAAT	308-332	56	5	8	KP863166
LAREeSSRHL397	(AT)14	CAATGATCGAACTGTGGTTCA	GCTCATCTTCAACTTCATGTGG	223-273	56	6	8	KP863167
LAREeSSRHL398	(AGCCTG)4	AGTCGGGGATGAAATCTGTG	TGTTTCTTTGCGCATACACC	293-299	56	2	8	KP863168
LAREeSSRHL399	(AAAATC)5	CTTTGTGTTGCGGGATTCTC	TTCCTTTTCCCCTTGGTCTT	275-305	56	4	8	KP863169
LAREeSSRHL400	(AGGCGG)5	GAGGACCTCCTGGCTTTGAT	TTAGAGCTGTGTTGCGCTGT	272-326	56	4	8	KP863170
LAREeSSRHL401	(ACCGCC)3	AGCAGAATAACGAGCCGAAG	CCCGCCACTACTCTGCTTAG	302-320	56	2	8	KP863171
LAREeSSRHL402	(AC)13	CACATATCTGTGTGTGCCTGTG	TTAGGTTGCCAAAACTGCAA	242-270	56	9	8	KP863172
LAREeSSRHL403	(AT)11	TCCATATTGCATAACGCTCCT	GCTCCTTCATGTTGTAAGCAAA	286-290	56	3	8	KP863173
LARKeSSRHL404	(AAGCCC)4	TCTTGTGACATTCGCCTCTG	TCGATGGTGATCTTCACCTG	299	56	1	8	KP863174
LAREeSSRQ001	(CA)10	GCAAACTCATGTAGACTCGCC	CATTGGTGGAACATTGCTTG	182-210	56	6	8	JR170819

Locus	Motif	Primer sequence		Size (bp)	T_a	N_A	Ref.	Genebank accession number
		Forward	Reverse					
LAREeSSRQ005	(GA)8	TTCCCTATTCTCATCCACGG	GTCGCCAGTAAATGGCCTTA	246-252	56	4	8	JR171181
LAREeSSRQ006	(AT)6	CCAAGAAGACCAAAACATCAGA	TCTGTCCCTGTTCACAACCA	131-175	56	6	8	JR171219
LAREeSSRQ010	(TC)7	CCCAGAATGCAATACGGACT	TTCCCAAGGAAAATCTGGTG	216, 222	56	2	8	JR171974
LAREeSSRQ017	(CAG)5	CCACCTCAAATCTTCTCCCA	CCTGCATATGAGTCTGCTGC	127-139	56	5	8	JR173000
LAREeSSRQ020	(AG)6	TGATCCGCTTAAGGTAACCAA	TTGTGAGTGTTTGTGTCGCA	219-231	56	3	8	JR173379
LAREeSSRQ032	(TTG)6	CCCCCTGCACACCATTTT	CAAGAATGCCGATACCGAAT	152-170	56	2	8	JR175164
LAREeSSRQ035	(AT)7	CCTCGAACACTCACTAAACTTGC	ATGCCTCCTTGTGCATTCTT	108-118	56	5	8	JR175381
LAREeSSRQ036	(TGC)6	TACTTCCCTGTGCTGGGTTT	GAAAAAGACTCCCAAAGGGG	207-219	56	6	8	JR175557
LAREeSSRQ048	(GAA)5	TGAAGAAGAAGCGGAAGAGG	AGGCTATACGCTTCCTGCAA	434-461	56	2	8	JR176325
LAREeSSRQ051	(TA)8G(TA)6	CGACTCAGCCACCTCGTAAT	ATTGCCAGAACCCCTTTTCT	234-268	56	13	8	JR176852
LAREeSSRQ053	(AT)6	TGTCGCCTTCACTCTGTGAG	ATCAATGCGGTGAAGATTCC	167-181	56	6	8	JR177135
LAREeSSRQ066	(CA)14	GCTCTTGTTGAGCCACCTTC	ATGGTTTGGATGCACATGAA	142-156	56	3	8	JR178582
LAREeSSRQ067	(TC)8	ATCTCCTTGGAATGTGTGCC	GGGGCGATTACCCTAAATGT	221-233	56	6	8	JR178682
LAREeSSRQ070	(TA)6	GCTCCTCTTGCACAGTCTCC	TGCTCCATTTGTGGGTGTTA	164-198	56	12	8	JR178932
LAREeSSRQ074	(AT)8	GTATGAAGAGCACCCCAAGG	GCAAATAGTTGCAAGGCATGT	124-146	56	11	8	JR179414
LAREeSSRQ104	(CA)7	ATCACTGCTCATGAGTCGCA	GTATGCGTTTGCGTGTGTGT	205-233	56	6	8	JR183015
LAREeSSRQ113	(AC)10	TCCAATGGAGGACGTAAAGG	TCATGCATCATAACATTGAATAACA	184-204	56	8	8	JR184160
LAREeSSRQ114	(CA)7	GAAACGGATATGGGAATGGA	TTGATGAATGGTAATCTGACCTATG	129-147	56	6	8	JR185111
LAREeSSRQ115	(CTG)6	AATTAAATGCGCTCACCTCG	GCAGATAACGCAGCCTTCTT	317-332	56	3	8	JR185400
LAREeSSRQ120	(ACTCT)5	ATTCCCCATTTCACGAAGC	TACTCCGAGAGGAGGCAGAA	110-115	56	2	8	JR186302
LAREeSSRQ125	(AT)10	AAGGGAAAATAAAGCCCTCG	TGCTCTCAGGTTGCAATGAG	100-144	56	11	8	JR186594
LAREeSSRQ127	(ATG)5	GGTTTCCATTACAACTCAAGGG	GGATTCAGCTTCGCTTTCAC	371-377	56	3	8	JR186781
LAREeSSRQ137	(TG)7	GTGCCTTGTGGGTTGTCTTT	AAGAGTTGCCACCCATAAGC	272-278	56	3	8	JR188117
LAREeSSRQ141	(TC)9	CACACATGCAAAGCAAACAA	TGTGTGTGAATGTGAGAGGGA	133-141	56	5	8	JR188688
LAREeSSRQ183	(CTC)7	TGTTTGACGGTGACTGAAGG	TAGAGGAGCAGCGAGAGGAG	126-141	56	4	8	JR193542
LAREeSSRQ187	(TG)8	TGAGGATTCTTTCCCAATGC	CATTGGATCCCAAAGGGTAG	178-190	56	5	8	JR193964
LAREeSSRQ195	(AGA)5	GCAGATTGTAGAAGGGCTGC	CATCGCCTTTCTCACACAGA	220-223	56	2	8	JR194843
LAREeSSRQ206	(GTT)5	GCAGACCCATTTTCGTGATT	CGCATCTCAGAGGGAGAGAG	446-470	56	5	8	JR139531
LAREeSSRQ209	(GGA)5	CCACGGAGTTTGGACTGAAT	CTAAACAGAGCCCAAGCGTC	182-188	56	3	8	JR139801
LAREeSSRQ210	(TA)9	GTTCGATTTTGGCCCATCTA	GATCAATTTTGGTTCGTTGTCA	165-191	56	8	8	JR139804
LAREeSSRQ213	(TTC)5	TTTTGCTTTGTGAATGTGGC	TGGGATCCTGAGGGACTATG	300	56	1	8	JR140280
LAREeSSRQ216	(AT)8	ATTTCTGCGGCAAAGAGTTG	AGAGAGGAAGGACTTTCGGC	374-406	56	3	8	JR140886

Locus	Motif	Primer sequence		Size (bp)	T_a	N_A	Ref.	Genebank accession number
		Forward	Reverse					
LAREeSSRQ218	(AT)6	AATTAGTGGTGCTTCGGTGG	TGGCACTTCTTGTAAATAAAATCAA	245-281	56	4	8	JR140959
LAREeSSRQ235	(CAGCAA)5	CACCATAAGCAACAGCGAAA	GTGCCGATGGATGTCTTTCT	183-195	56	3	8	JR143407
LAREeSSRQ243	(AT)7	TTCGTGTACAGCGTTCAAGC	TCCGGAATATCGTCACAACA	149-185	56	13	8	JR144253
LAREeSSRQ247	(CT)8	CTACGAGAGGCTCGATACGC	CTTCAGTCTGGAGCTGACCC	428-466	56	8	8	JR144913
LAREeSSRQ257	(ATC)5	TCTGCATCCTAGTGCTGTGG	CCCCTGGATCTTCTGAAACA	116-131	56	4	8	JR146140
LAREeSSRQ285	(GAG)5	CCGAGACATGATGCTGAGAA	TATTTGCAGAAGCCCAAACC	162-192	56	7	8	JR149637
LAREeSSRQ299	(CTG)5	AAACCAATGAAAATGCCTGC	TCCCCAGCCAACTCTCATAC	431-485	56	2	8	JR151216
LAREeSSRQ316	(TC)7	AGCTCTCTGTGCTTTCTCGC	GGAAAGAGCAATTCAGCAGG	194-206	56	2	8	JR153273
LAREeSSRQ322	(CAG)5	AGGCGTCTGAGCTACCAAAA	CGACGACACCCAATACCTTT	426-459	56	3	8	JR153722
LAREeSSRQ330	(TC)6	CAGGAAGTTGGGCAGCTTAG	GGTCTTGGCCTTGTTGTTGT	255-267	56	3	8	JR154204
LAREeSSRQ352	(GCA)5	CCACCTCAAATCTTCTCCCA	AGATGGAATACTGTTGGCGG	249-288	56	5	8	JR155690
LAREeSSRQ364	(AT)7	GATGAAATTGCGGAAAGCAT	ACTGGGCAATGTCCAAACTC	280-306	56	11	8	JR157274
LAREeSSRQ375	(TC)6	AGTGGCAGTCAGCATCTCCT	AGAAGATTTTGCAGAGGGCA	212-230	56	3	8	JR158646
LAREeSSRQ377	(TCATCC)5tcagtctca gtt(TCAGTC)5	TCATCATCCTCCTCGTCCTC	AAGATTCAGTGGATGGCGAC	178-208	56	6	8	JR158866
LAREeSSRQ382	(CAG)5	TGGTTCAACTTCTCTCGCCT	GGAATGTGAACGAAGACGGT	299	56	1	8	JR159113
LAREeSSRQ386	(GA)13	TCCATCTTTATTTGGCAGGC	CCATCAGAGATGGGAGTGCT	128-148	56	9	8	JR159815
LAREeSSRQ393	(AG)6	CCTTGTGAAGGGCACAGTTT	ATGAGGTCTGTGAGGGGTTG	371	56	1	8	JR160488
LAREeSSRQ397	(GA)9	TCTGAATCAATGTATCATGTATCGAA	CTGTCAGTCATGCTGCGTTT	132-154	56	10	8	JR161052
LAREeSSRQ399	(AAG)5	AGACTCCTGTTGGAAAGGCA	AGACTCCTGTTGGAAAGGCA	254-263	56	2	8	JR161168
LAREeSSRQ403	(CAT)8	ACACAACATGCTACGATGCC	GCTTCTAGGCGTTCAACGAG	216-246	56	9	8	JR161642
LAREeSSRQ406	(AG)6	TGCATTCTGTTAATGCCCAA	TGTTGATGAGCAATGACCGT	361-385	56	4	8	JR161926
LAREeSSRQ408	(GACTG)7	CAAGCATTCTTCCCCAAAAA	TAAGTCCAGTCCAGTCCGGT	144-180	56	6	8	JR162009
LAREeSSRQ409	(AT)9	AAAATTTCATCCTCGAACACTCA	TGGACAATGTTCCATGCAGT	181-199	56	8	8	JR162187
LAREeSSRQ430	(CGG)5(CTG)2TTGA (TGC)6tgttgatgctgatg g(TGC)8	TTTTGGTCCGATCAGGAGTC	CAACTTTTGGGTTGGGAGAA	286-313	56	9	8	JR166454
LAREeSSRQ439	(AAT)5	TCTCGCTCGGCTTCTACATT	GAGATTCTGCTGCTTCCCTG	258	56	1	8	JR168298
LAREeSSRQ444	(TGC)6	GAACGTTCAAACTGCACACG	TTGAGTTCATTGGCTGCAAG	406-415	56	2	8	JR168664
LAREeSSRQ449	(AT)8	CCCTTAGCCTCTTTTTGAGGA	ACCATCGAACGTGTCAACAA	272-298	56	5	8	JR169475

1-Isoda and Watanabe 2006, M13(–21) -tail was attached to the 5′ end of forward primer of each locus, 2-Yang et al. 2011, 3-Khasa et al. 2000, 4-Pluess 2011, 5-Wagner et al. 2012, 6-Zhang et al. 2015, 7- Gros-Louis et al. 2005, 8-Chen et al. 2015

Material for extraction

Plant tissue used for extraction included seed, buds, leaves (in general frozen needles), e.g. Isoda and Watanabe (2006), Wagner et al. (2012), Nishimura and Setoguchi (2011), Gros-Louis et al. (2005), Chen et al. (2015), phloem (Wagner et al. (2012) and cambium (Khasa et al. 2000).

DNA-extraction and amplification protocols

Total DNA was extracted from the mentioned tissue using:

- NucleoSpin Plant II (Macherey Nagel, used in INRA lab)
- the QIAGEN DNeasy Plant Mini Kit (Gros-Louis et al. 2005, Pluess 2011, Zhang et al. 2015)
- a CTAB protocol after Shiraishi and Watanabe (1995) (Isoda and Watanabe (2006) and after Doyle and Doyle (1990) (Yang et al. 2011, Chen at al. 2015)

Examples for amplification protocols (nSSR)

- 94°C for 1 min followed by 10 cycles of 94°C for 30 s, 63-53^0 C (-1^0 C at each cycle) for 45 s, followed by 25 cycles of 94°C for 30 s, 53^0 C for 30 s, 72°C for 1 min; final elongation at 72^o C for 10 min (Isoda and Watanabe 2006).
- 94°C for 4 min followed by 30 cycles of 94°C for 45 s, 56^0 C for 45 s, 72°C for 45 s; final elongation at 72^o C for 7 min (Chen et al. 2015).

Examples for amplification protocols (EST-SSR):

- 94°C for 3 min followed by 40 cycles of 94°C for 30 s, Ta (Table 4) for 45 ,s 72°C for 1 min; final elongation at 72°C for min (Yang et al. 2011).
- 95°C for 5 min followed by 25 cycles of 95°C for 30 s, Ta (Table 4) for 45 s, 72°C for 60s; final elongation at 72^o C for 20 min (Zhang et al. 2015).
- 94°C for 4 min followed by 40 cycles of 95°C for 30 s, 55°C for 30 s, 72°C for 1 min; final elongation at 72^o C for 10 min (Gros-Louis et al. 2005).

SSR – Multiplexing (Wagner et al. 2012)

- Multiplex 1 – Ld31, bcLK211, Ld30, bcLK228, Ld50, bcLK189, bcLK253
- Multiplex 2 – Ld58, Ld45, Ld42, bcLK263, Ld101, Ld56
- Amplification protocol for multiplexes 1 and 2:
- 95°C for 15 min followed by 35 (multiplex 1)/30 (multiplex 2) cycles of 94°C for 30 s, T_a (Table 4) 56°C for 1 min, 72°C for 1 min; final elongation at 60°C for 30 min

Important results

- New EST-SSR markers were developed for Larix *kaempferi* (Gros-Louis et al. 2005, Yang et al. 2015, Zang et al. 2015). The markers are transferable also to other *Larix species.*
- New SSR markers were developed and identified as highly polymorphic in *Larix kaempferi*. Most of them could be amplified in related *Larix* species (*Larix olgensis, Larix gmelinii, Larix principi-rupprechtii*) (165 nSSR between them 145 polymorphic developed by Chen et al. 2015, 20 primer pairs between them 19 polymorphic developed by Isoda and Watanabe 2006).
- In a *Larix kaempferi* Danish seed orchards, SSR markers were used to evaluate the selfing rate, the paternal contribution to the progenies and the pollution rate from external larch sources (Hansen 2008).
- A 34% introgression rate by spontaneous hybridization between *L. kaempferi* and *L. laricina* was observed in Québec (Canada), suggesting to take into consideration the proximity of this exotic species in the management of natural genetic resources (Meirmans et al. 2014).

c) SNPs (single-nucleotide polymorphisms)

- Gros-Louis et al. (2005) used SNP in a study aiming at distinguishing larch species (*Larix decidua, Larix sibirica, Larix kaempferi* and *Larix laricina*). The results were the identification of three gene loci (Sb14, Sb48, Sb51) with fixed interspecific polymorphisms implicating 17 SNPs and 2 indels.
- Li et al. (2014) identified many single-nucleotide polymorphisms (SNPs) in a genome-wide marker development for *Larix kaempferi*. Among these SNPs, 364227 (78.6%) were determined from transcripts with annotation information, and they were distributed in 32453 known genes.

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7. Selected references referring to genetic investigations on related *Larix* species

Isozymes

Larix decidua

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DNA-markers

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Larix decidua

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Larix gmelinii

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Molecular markers used for genetic studies in Lodgepole pine (*Pinus contorta* (Dougl. ex. Loud.))

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1. General remarks

Lodgepole pine is widely distributed in Western North America, from Mexico to Alaska and from the Pacific eastwards to South Dakota. It is ecologically and economically important, occurring as a dominant tree species from costal to subalpine forests.
It is divided into four morphologically distinct subspecies (ssp. *latifolia, contorta, murrayana* and *bolanderi*) (Critchfield 1957). Each subspecies grows in a separate part of the species' range and is hypothesized to be adapted to local climate and environmental conditions (Ying and Liang 1994, Rehfeldt et al. 1999, 2001). The geographic distributions of the subspecies do not overlap except for the subspecies *contorta* and *bolanderi* in California.

High gene flow among subspecies and various divisions of population structure has been deduced from population genetic analysis (Wheeler and Guries 1982a, Fazekas and Yeh 2006, Bisbing 2013). In general, no correlation between genetic structure and differences among subspecies were observed while there was discontinuity between coastal and montane stands and a north-south differentiation along the coast and the Rockies revealed by isozymes, RAPDs, mtDNA and cpDNA SSRs (reviewed in Jaramillo-Correa et al. 2009 but see Bisbing 2013 for an example where such correlation was observed).

2. Isozymes

Isozymes were used in single-locus (Wheeler and Guries 1982a) and multi-locus (Yang and Yeh 1993) genetic diversity studies as well to compare six quantitative traits and isozyme variation in five populations (Yang et al. 1996). Yang and Yeh (1995) conducted a range-wide isozyme variation study with 21 loci (14 enzyme systems) in 66 populations from the three subspecies to study the gene flow patterns and the genetic structure among the three subspecies *contorta*, *latifolia* and *murrayana*.

Material for protein extraction

Proteins were extracted from seed (megagametophytes) (Wheeler and Guries 1982 a,b).

Protein extraction and separation protocols

Sample preparation, extraction and separation protocols are described in Guries and Ledig (1978) and O'Malley et al. (1979, 1980). Staining and gel-buffer recipes were described by Shaw and Prasad (1970), Harris and Hopkinson (1976) and Siciliano and Shaw (1976).

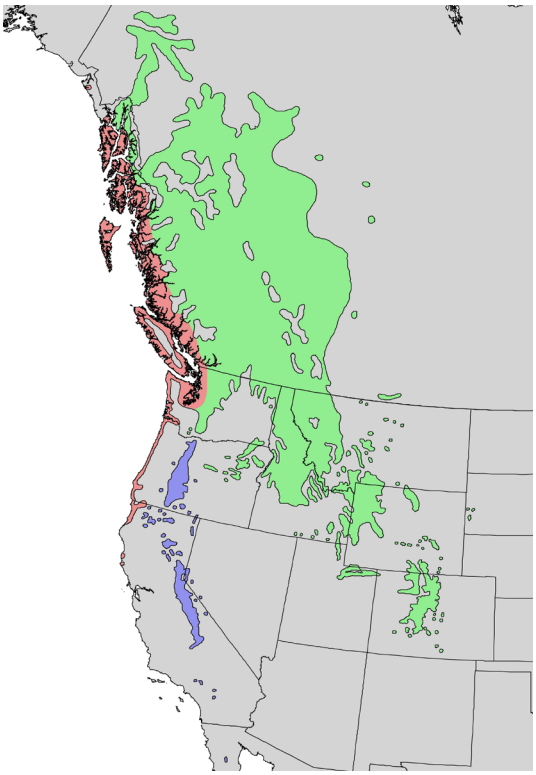

Figure 1. Native distribution range of Lodgepole pine. Subspecies latifolia denoted with green, subspecies contorta denoted with red and subspecies murrayana marked with blue (Source: USGS, USA)

Table 1: List of enzymes, scored loci and number of alleles for Lodgepole pine

Enzyme system	E.C. Number	Scored loci	References
Aspartate aminotransferase	2.6.1.1	AAT-1,-2	1,2,3
Acid phosphatase	3.1.3.2	ACP-1	1
Aconitase	4.2.1.3	ACO-1	1,2,3
Alcohol dehydrogenase	1.1.1.1	ADH-1,-2,-3	1,2,3
Alcaline phosphatases	3.1.3.1	APH	2,3
Adenylate kinase	2.7.4.3	AK-1	1
Aldolase	4.1.2.13	ALD-1,-2	1
alpha-Galactosidase	3.2.1.22	alpha-GAL-1,-2	1
Diaphorase	1.6.2.2	DIA-1,-2,-3,-4	1,2,3
Hexosdiphosphatase	3.1.3.11	HDP-1	1
Fumarase	4.2.1.1	FUM-1	1
4-Methylumbelliferyl acetate	3.1.1.56	4-MUA-1	1
4-Methylumbelliferyl butyrate	3.1.1.56	4-MUB-1	1
Glycerate dehydrogenase	1.1.1.29	GLD-1	1
Glutamate dehydrogenase	1.4.1.2	GDH-1	1,2,3
Alanine aminotransferase	2.6.1.2	ALAT-1,-2	1
Glucose-6-phosphate dehydrogenase	1.1.1.49	G6PD-1	1,2,3
6-Phosphogluconate dehydrogenase	1.1.1.44	6PGD-1,-2	1,2,3
Isocitrate dehydrogenase	1.1.1.42	IDH-1	1,2,3
Malate dehydrogenase	1.1.1.37	MDH-1,-2,-4,-5	1,2,3
Malic enzyme	1.1.1.40	ME-1	1,2,3
Mannosephosphate isomerase	5.3.1.8	MPI-1	1
Phosphoglucose isomerase	5.3.1.9	PGI-1, -2	1,2,3
Phosphoglucomutase	2.7.5.1	PGM-1	1,2,3
Leucyl-tyrosine peptidase	3.4.13.11	PEPLT-1,-2,-3	1
Valyl-leucyl peptidase	3.4.13.11	PEPVL-4	1
Sorbitol dehydrogenase	1.1.1.14	SDH-1	1

1-Wheeler and Guries 1982a, 2-Yang and Yeh 1993, 3-Yang and Yeh 1995

Important results

- Yang and Yeh (1993, 1995) observed that loci Pep-1 and Pep-2 were monomorphic in all populations, while the remaining 19 loci were polymorphic.
- The mean number of polymorphic loci and the mean heterozygosity over 19 polymorphic loci were, respectively, 13 and 0.194 in ssp. *latifolia*, 12 and 0.196 in ssp. *murrayana*, and 12 and 0.180 in ssp. *contorta* (Yang and Yeh 1993).
- Yang and Yeh (1995) observed the lack of isolation by distance in spp. latifolia but a slightly signifcant pattern of isolation by distance in spp. *contort*a. They observed an extensive gene flow among populations in each subspecies.
- Low differentiation between populations (6%) as well as between subspecies (3%) was detected.

2. Organelle DNA markers (chloroplast (cp) DNA, mitochondrial (mt)DNA)

Loci and primers used are listed in Table 1

Material for DNA extraction

DNA was extracted from buds (Marshall et al. 2002) and needles (Godbout et al. 2005, 2008).

DNA-extraction and amplification protocols

Total DNA was extracted from 40 mg of ground needle tissue using the DNeasy Plant Mini Kit (QIAGEN) (Godbout et al. 2005, 2008).
Total genomic DNA extraction from buds followed the standard CTAB (cetyl trimethylammonium bromide) procedures (Doyle and Doyle 1987) in Marshall et al. (2002).

For amplification the following PCR-protocols were used:

- cpSSR (Stoehr and Newton 2002)
  95°C for 3 min followed by 25 cycles of 94°C for 30 s, 55°C for 30 s, 72°C for 1 min.
- mtDNA-marker (Godbout et al. 2008)
  94°C for 2 min followed by 35 cycles of 94°C for 30 s, 69°C for 15 s, and 72°C for 1 min, followed by a final extension step at 72°C for 5 min

Important results

- High levels of cpDNA variation were obtained; in 69 orchard parents genotypes, 46 multilocus haplotypes were detected. The number of alleles per locus ranged from two to seven with gene diversity levels of 0.44–0.72. cpSSR loci 8/87, 10FR, 96FR, L2T1 in I1A2 evolve primarily by a stepwise mutation model (Stoehr and Newton 2002). Coalescent simulations point to a mutation rate of about 10^{-3} for chloroplast microsatellites underlying the stepwise mutation model (Marshall et al. 2002).
- mtDNA analysis revealed no correlation between genetic structure and differences among subspecies. However, three major genetic clusters were identified (Godbout et al. 2008).

Table 2: Primer information of the organelle DNA-markers used for DNA sequencing in the genetic analysis of Lodgepole pine

Locus	Primer sequence		T_a	Type	Ref.
	Forward	Reverse			
G2R1	AGATCGGGACAATGTATGCC	TGTCCTATCCATTAGACGAT	55	cpSSR	1,2
8/87	ACTGCAAGGAACAGTAGAAC	CGGAACGTTTTCTGATGCAC	55		
10FR	CAGAAGCCCAAGCTTATGGC	CGGATTGATCCTAACCATAC	55		
96FR	TTTCGGGCTCCACTGTTATC	CGTACTCAATTTGTTACTAC	55		
L2T1	ACCAATTCCGCCATATCCCC	CTAGGGGAGGATAATAACATTGC	55		
I1A2	TTCAAGTCCAGGATAGCCCA	CTACCAACTGAGCTATATCC	55		
nad7 intron 1	GAGGGACAACCCTGGAATACT	AAGGCCTCTCCATTTCCAAT	69	mtDNA	3,4

1-Stoehr and Newton 2002, 2-Marshall et al. 2002, 3-Godbout et al. 2005, 4-Godbout et al. 2008, T_a = annealing temperature

3. Nuclear DNA markers (nSSRs, EST-SSRs, SNPs)

a) nSSRs (putatively neutral microsatellites), EST-SSRs (expressed sequence tag derived microsatellites

Loci and primers used

In the following table Pico, LOP and PtTX2164 markers are EST-SSRs, the others are nSSRs.

Table 3: Microsatellite markers available for genetic analyses in Lodgepole pine (T_a = annealing temperature)

Locus	Motif	Repeat Length (bp)	Primer sequence		T_a	Ref.
			Forward	Reverse		
Pico_1	CTT	18	ATGCGGTTTAACAAGCACTAC	GGATTGTCACCTGGACTAGAG	57	1
Pico_3	TGC	15	CTTACACCCAGGATTCCAAC	AAAGATGAAGGAGGACTGGAC	57	1
Pico_4	CAT	21	ACACTGGGCTACAAAATTCAC	TTCCTTGCTCTTTTTATCAGC	57	1
Pico_5	GCA	15	TTCTTCAATCATGGCTCTGTC	GTTGTCTTCTGCTCCCATTC	58	1
Pico_6	CCT	18	GACTCCTCCTCTCTCTCAAGG	CAACATACAGCAGCAACAGAG	57	1
Pico_7	GCA	15	TCGCAAACCCTAATCAGAAC	CTGATATTGAGGCTGCTGTG	58	1
Pico_8	GAA	18	ACTCGATCCTGTTCCCTTG	GGGTAGATTCCCCACAAATAC	58	1
Pico_10	GCA	15	CCAAATCCAATACAGCAAGAG	GTTGTCCAGTTTGACCTGATG	57	1
Pico_11	CAG	18	AATTGAGGTGATTTTGACTGG	TCCTCATCTCCACTATCTTCG	57	1
Pico_13	GGC	15	GGTCGTGGAGGAGGTAGAG	CGCACTTGTAACAGCTTCC	57	1
Pico_14	CAG	15	GTCAACAAGGACAACGGATAG	TTGTACCCATTAGAGCCTGTG	57	1
Pico_16	AGC	15	TCCTGGGAAGTGTGTTATTTG	GGATTGTGTGATGGGTAAAAG	58	1
Pico_17	CCA	15	ACAATAGTGGAGGGAATGGAC	ATTACTAGCTCTGCGGTTGC	58	1
Pico_18	GGT	15	AGTGACGAACAGTCAATGAGG	CACCTCCGTAACCACCAC	58	1
Pico_19	AT	18	AACCTGAAACAAACCCCTAAC	TTCCTCCCCTCTCTATTTGTC	57	1
Pico_20	CAG	18	GTACTGGGGTCTTCAGCCTAC	GTGCCTCTGTCTCACTCCTC	58	1
Pico_23	GCA	15	CTTCAGAAAACTGGTGGTTTG	TTGGTGATGATGTTGTCTCTG	57	1
Pico_25	GCA	15	TCGCAAACCCTAATCAGAAC	CTGATATTGAGGCTGCTGTG	58	1
Pico_26	CTG	18	CATTTGGTGAGACATGGTTG	ATTGCGAGAATTTACCAGTTG	57	1
Pico_27	GAG	18	CGGAAGAAACATGGTAATGAG	GCATACCCAGTGCAAATAAAC	57	1
Pico_28	GCA	21	GACCATCCAGTTCCAGTTATG	CAGATAGCCATTTCTGATTCG	57	1
Pico_31	CCT	18	CTGCTGCTGCTCTTACCC	CAACAGCGGGACTATTAGAAG	57	1
Pico_32	CAA	15	CTTACCCCTTTCTAGCTCCAG	CATAGGAGGAGGAGGAGGAG	57	1
Pico_33	TAA	15	CCAAGTTGAAACAGTGCAAG	AAAACTTCTTCTCGGCATTTC	57	1
Pico_34	CTT	15	CAGCACATCATTATCAGGAGAG	TAGTGCCTGTCTTTGATGGTC	57	1
Pico_38	GGA	15	TCTGTTCTATAAGCGCGTTG	CTCCTCTCCCTCCCACTC	57	1
Pico_39	GAA	15	GATTTACAGACCCCCTTCATC	ATAACGAACACCCACACAATC	57	1
Pico_44	TCT	18	GGATGGTGTCTTTGGACATAC	GTGGAAAGCATGGATGTAAAG	57	1
Pico_45	ATT	18	TATTGAAAACGGCAGAGTAGC	GACAAATAAATGCTCGACCTG	57	1
Pico_46	TA	22	CAAAATGAATGATGAGGATGG	TTTTAGAATTGGCAAGTGTCG	58	1

Locus	Motif	Repeat Length (bp)	Primer sequence		T_a	Ref.
			Forward	Reverse		
Pico_49	TTA	15	ATCCATGCTACACCAATCAAC	CAAAGGAATACAACATTCATGG	57	1
Pico_50	CAA	15	CCTTAGTTCTGTCCCGTTAGG	ACGCAGTTGGCTAGTAGAGTG	58	1
Pico_51	TC	20	AGTGCAAGCAATGAAATAAGG	TGTTTGAGGGGTGAGATAGAG	57	1
Pico_52	CCT	18	TGGAACTTTCTACTTCCTACCG	AGAGTGGTCAAACATACCTGTG	58	1
Pico_53	AT	20	ATCACTCTCTCCCCTCTTCTG	TTTAGGGCGTTTAGTTGAGTG	58	1
Pico_57	GAA	18	GTTTCTTCCTCCCCTTCTTC	GAGGCTTGATGGATGATTTC	57	1
Pico_60	TG	22	GTTTGCACCTTGAGAGATTTG	AGAAGCAGAGAGGAAGAGGAG	57	1
Pico_61	AACA	20	AATATCCCCAGGTGAAAAATC	GGGTTTCCTTTCTCTTCTTTG	57	1
Pico_62	CA	20	GAAGTGGCATTTGATGTGG	ATTGGAGCCATTGTTAGGG	57	1
Pico_63	TC	20	TCATCACTTGGGGTTAAGATG	GAGGACAACAGGGGAAGATAG	58	1
Pico_66	CTT	18	CCTGCACTAGAGGACTCACAC	GTTGGAATTGTCAAAAGATGG	58	1
Pico_69	TC	22	TCTCTCATCGTCTCTGTCTCC	AGTAGTGTGTGTGTGTGTGTGTG	57	1
Pico_70	CT	20	TTTAGGCAAGGTGTTGAATTG	GAGGGCGAGAGAGAGAGAG	58	1
Pico_74	CT	20	AACTCCACCAATCAACACTTC	GGAAGGAGACAGGAGAGAGAG	57	1
Pico_75	TCC	18	TCAATTTCATGTTTTCAAGTGTC	AGAGAGTGAGAGGAAGGAAGG	57	1
Pico_77	TGA	18	GTGCTTGTGGTTGGATATTTG	AAGGAAGTTGGAAGACCGTAG	58	1
Pico_81	TG	20	TTGACAGATTCATGGTAATATGG	TACAAGGGGAAGAGAGAGAGG	57	1
Pico_84	CCT	18	ATCCCATCCACAACATTGAC	AGGTGGTGTAGGAGGAGGTAG	58	1
Pico_85	CT	18	CTCTCTCTCATTCCCCCTATC	GAAAGGGTTAGAAAATGCAAAG	57	1
Pico_86	TC	18	TCCCCACTCCCTCTTTTC	GCAGGGGAGGTAGGAGAG	58	1
Pico_90	CT	20	TAAGGCAAAGGTGTTGAATTG	AGGAGGAGAGAGAGAGAGAGAAG	58	1
Pico_93	AC	18	GGATAGTTTAAGGCGGTATCC	GAGAGGGGAACCTTGAAAAC	57	1
Pico_95	TG	18	GACCTGGCTGTCACTCAATAC	CACTTTGACTTGCTCTCTTGC	57	1
Pico_98	CT	114	CCATTTCGATTATTTCCTTG	AGAGAGAGAGAGAGAGAGAGAGG	55	1
Pico_104	ATT	42	CCTGATCAAGCCTTCAAATAC	GATGTTGAAAGATATCCCATTG	56	1
Pico_105	CT	36	TCAATGTAAGGATAATGGTTTTG	GAGAGAGAGAGAGAGAGGAGGAG	56	1
Pico_106	CT	46	TGTATTATTATGATGGAATGTGG	AGAGAGGAGAGAGAGAGAGAGG	54	1
Pico_108	TC	80	CCTCCTAAACATATGTCATGC	AGAGAGAGAGAGAGAGAGAGGAG	55	1
Pico_118	TC	42	CTTTGGAATTGTCAAGAACG	GAGAGAGAGAGAGAGGAGGAG	56	1
Pico_120	ATCT	32	TGTCAGCACCGACAACAGTC	GGGCACAGCACAGCACAG	61	1
Pico_129	TTTA	36	ACCGTACATGAGATTTTCACC	CAACGAGAATTATCCAATTTATC	57	1

Locus	Motif	Repeat Length (bp)	Primer sequence		T_a	Ref.
			Forward	Reverse		
Pico_130	TC	54	CCCTCCAACTCCAACTCC	ACGAGAGAGGAGAGAGAGAGAG	58	1
Pico_135	AC	34	CTTACGATCCCATCAGACAAC	TGCATGAAATGCATGAATAAG	57	1
Pico_136	CT	82	GCTGATGTGCTATGGATATTG	AGAGAGAGAGAGAGAGAGAGGAG	56	1
Pico_138	TG	34	GAAGTGGTGCCTCTATGTTTG	ATGCAAATGGAAGAACTTGTG	57	1
Pico_139	CT	44	TTTCATCCTTTCATAGCTTGG	AGAGAGAGAGAGGAGGAGGAG	57	1
Pico_140	CT	38	AATCACCCTGCCAACATAC	AGAGAGTAGGAAGGAAGAAGAAG	56	1
Pico_141	CT	128	CCCAAACATACACACACTCTC	GAGGAAGGGAAGAAGGAG	56	1
Pico_143	AC	70	CCTATTTGTTAAGCCTTTTGG	GTACGTCGTCGTCTCTCTCTC	56	1
Pico_144	CT	50	TCCTCTTTATCCGTTTCTTTTC	AGAGGAGAGGAGAGGAGGAG	57	1
Pico_147	CT	42	TCTAATTCCCTCCCTTTGTTC	GAGGAGGAGGAGGAGGAG	57	1
Pico_152	TC	42	CCTCAACCCCTAACTACCTC	AGGAGGAGGAGAGAGAGAGAG	55	1
Pico_154	TG	40	AGTCTCAAAATGGACAAGTCG	ACCTAACATAACCGCAATCAC	57	1
Pico_161	CT	48	CTGTCACTCAAAAGCTTCCTC	AGAGACGAGAGAGAGAGAGAGAG	57	1
Pico_164	CT	44	ATTAGGGACCAATAACAGAGC	GACGTAGAGAGAGAGAGAGAGAG	55	1
Pico_165	CT	62	AATGTTTATGATGATCCCTCAAG	AGGAGAGGAGGAGGAGAGG	57	1
Pico_170	CT	40	AAGTTCCCTCCCCCAATC	AGAGACGAGAGAGGAGAGGAG	59	1
Pico_174	TG	40	AAATTCGATCAACTCTCTAGTCG	TTAATGCATACATTCCTACACG	57	1
Pico_175	CT	38	ATGCCATTTCGATTATTTTCC	AGAGAGAGAGAGGAGGAGGAG	58	1
Pico_176	AC	36	TCTTAAGATCATGCCCTTTC	AAACTTACTTGTATAGGCGAGAG	55	1
Pico_179	ATG	33	TCACGAAAGACCTTGAAAGAC	CCAAGAAAGACAAGGAGTCAC	57	1
Pico_182	CT	38	GTGAATCGACTGCTTAATGTG	GAGGAGGAGGAGGAGGAG	56	1
Pico_183	TTA	33	TAACTCAAACCTGGAGCAATC	CACTATTCCGTAGCCAGAAAG	57	1
Pico_185	CT	100	GAATTTACTAAAGTGTCCTGCTC	AGAGAGAGAGAGAGAGAGAGACG	54	1
Pico_189	CT	38	CAATGTAAGGATAATGGTTTTTG	AGAGGAGGAGGAGGAGGAC	56	1
Pico_192	TG	32	AGAATCCTATTGTAGCTGCTTG	GTGGAAGAATGAGAGAGATAAAG	56	1
APC3	GA	33	AGTGCTTCAAGAAAATCTAAGT	TTGTAACCTTTTATGAGTTCAG	60	2
APC9	TA_{16}-GA_{21}		TGAATGAGAAGTCGTGTAAG	GGAATAAGACAGGTTCAGAT	61	2
APC11	AT_{10}-AG_{21}		TCCCTTTAGATAGTTCATGG	GATATTGTCTTCGCTGATAG	57	2
APC13	AG	29	TCAAGCCTAGTCAGTGTTAAG	CCAAGAAAACTCTAAGTGAGC	60	2
APC15	AG	7	AATTCTTCAAAAGTTCAAT	TTATGTTTCTGTTGTTTTA	49	2
LOP1	TA	10	GGCTAATGGCCGGCCAGTGCT	GCGATTACAGGGTTGCAGCCT	55	3

Locus	Motif	Repeat Length (bp)	Primer sequence		T_a	Ref.
			Forward	Reverse		
LOP5	TA	33	AGCCGTAAAAGCTATCTTGTG	GGCATACTTACATTTTAATAA	45	3
LOP8	CTT	6	TATCCACCAGAAGGGCATC	CGGGAGCTTTAATGATCTTGA	50	3
LOP9	GCC	6	GGATTCTCGTTGTGGCTGG	TTGCCTTTGCACATAATATCT	55	3
LOP11	$TA_2T(AT)_{12}$		CCAGAAGGCTATAGTACAC	CAACAATACAAGTAGCAATAC	45	3
PtTX2146	$(GCT)_4GCC(GCT)_7GCC(GCT)_8$		CCTGGGGATTTGGATTGGGTATTTG	ATATTTTCCTTGCCCCTTCCAGACA	55	4
PtTX2123	AGC	8	GAAGAACCCACAAACACAAG	GGGCAAGAATTCAATGATAA	55	4
PtTX2128	GAC	8	TGGATAATCCTTTCAGTC	TCTCGGATTCTCTTACAG	55	4
PtTX3011	$(GAA)_5(A)_6(GAA)_3(GAT)_{15}$		AATTTGGGTGTATTTTTCTTAGA	AAAAGTTGAAGGAGTTGGTGATC	55	4
PtTX3025	CAA	10	CACGCTGTATAATAACAATCTA	TTCTATATTCGCTTTTAGTTTC	59	4
PtTX3029	$(GCT)_5(GCT)_8(GCT)_5$		CTTGTTGCTGCTTCTGC	AACAAAATAATATAAATGCTCTGC	61	4
PtTX3030	$(TA)_4(GGT)_{10}$		AATGAAAGGCAAGTGTCG	GAGATGCAAGATAAAGGAAGTT	59	4
PtTX3034	$(GT)_{10}(GA)_{13}$		TCAAAATGCAAAAGACG	ATTAGGACTGGGGATGAT	55	4
PtTX3049	TG	16	GAAGTGATAATGGCATAGCAAAAT	CAGACCCGTGAAAGTAATAAACAT	55	4
PtTX3052	$(ATC)_8(ATC)_4$		CCTCACTAGGAGGCTACGGAAGAG	AAAGACTCCTTGATGTTGTGAACA	55	4
PtTX3107	CAT	14	AAACAAGCCCACATCGTCAATC	TCCCCTGGATCTGAGGA	55	4
PtTX3127	CAA	10	ACCCTTACTTTCAGAAGAGGATA	AATTGGGGTTCAACTATTCTATTA	55	4
PtTX4046	$(TA)_3(TG)_{13}$		AATGTATATTGGCAACCCTATCA	ACTATGGAACATTGGGAAACC	55	4
PtTX4054	GA	21	TGCATTCACCTTGGAGTT	TAGGAGATAATATAAAATGTT	55	4
PtTX4056	GA	17	TTAAGGCCAGTTCCAATACAAAAT	GAGCCCAACAACTAAAACAATGAG	65	4
PtTX4058	$(CA)_3(GA)_{20}$		AAGTGTTGGGAGAAAAATGTAAT	CTCCTTCTGTCCCTATCCTCT	55	4
PtTX4139	CT	21	TGGCATGCTAGGAAGAAGA	TTGTATGTTGCCTGTGGAGA	59	4

1-Lesser et al. 2012, 2-Hicks et al. 1998, 3-Liewlaksaneeyanawin et al. 2004, 4-Auckland et al. 2002 (crosschecked for usefulness in lodgepole pine by Liewlaksaneeyanawin et al. 2004 and primer sequences copied from Cullingham et al. 2011, Elsik et al. 2000, Elsik & Williams 2001, Echt and Josserand 2018, Furlan et al. 2007 and Zhou et al. 2002);

Material for DNA-extraction

DNA was extracted from needles (Hicks et al. 1998, Lesser et al. 2012).

DNA-extraction and amplification protocols

Total genomic DNA extraction followed the standard CTAB (cetyl trimethylammonium bromide) procedures (Doyle and Doyle 1987) in Lesser et al. (2012).

For amplification the following PCR-protocols were used (Lesser et al. 2012):

- 94 °C for 5 min, followed by 32 cycles of 94 °C for 1 min, 45 °C for 1 min and 72 °C for 1 min, followed by a final extension step of 72 °C for 3 min.

Important results

- All listed markers are polymorphic, exhibit good amplification and clean patterns (no multiple bands). Listed LOP and PtTX markers did not exhibit null alleles in the original publications.
- In her thesis, Bisbing (2013) identified three genetic clusters that coincided with three of the four subspecies (*contorta*, *latifolia*, and *murrayana*) using markers 69FR, PICO1, PICO4, PICO7, PICO77, PICO104, PICO109, PICO138, PICO154 and PICO179. This supports treatment of these three subspecies as separate taxonomic units. Subspecies *bolanderi* populations were assigned

b) SNPs (single nucleotide polymorphisms)

Loci and primers used are listed in Table 4.

Table 4: Primers for amplification of SNP loci used in Cullingham et al. (2013)

Locus	SNP polymorphism	Null	Primer sequence F=Forward, R= Reverse	T_a	Annotation
C26372-P562	G/C	8	F:GAGCAGCCTCTGCTAGTGAA R:ACAAAGAACTAGCTCACTTGTAC	60	Calcium-dependent lipid binding family protein
C35213-P325	C/T	1	F:GCCAAGGGACCACACGCTCT R:CCTTGACTTGCTAATTGTGATGGCA	65	Eukaryotic aspartyl protease family protein
C39371-P429	A/G	41	F:CACTTGCTGTTGGGTGGCTGT R:GCCCAGCAGGATTAATGAACTCA	65	Protein of unknown function (DUF3353)
C54523-P103	A/T	1	F:AGAACTTTTGTACACCTGACAAACT R:GCGAGGCATCTATCCATAGCTCA	60	Translation protein SH3-like family
C55350-P439	C/T	6	F:AGAGCTAAAGGAGTACAATTGTGCA R:TCAGAGGACTCACTTGGTTCA	60	Chaperone protein dnaJ-related
C55378-P723	T/G	2	F:GAACGTGGTGGCTGTGGCAA R:GTGCAGCTGGACAGTACAAGAAA	65	Transcription factor jumonji domain-containing protein
C55401-P415	T/G	0	F:TGACACTAATATCAGCAATGTGGCA R:TGGCGCACTTTTCTGACCCA	60	Transcribed locus
C63961-P710	C/T	1	F:CGCTCATCAGTGGCTCTTCTGGT R:GTGGACGATTCTCCTGGCGCT	65	
C64907-P190	A/C	0	F:AGGTACCGCTCCAATTATTGTGT R:GTCGGATGATTGCACCTCTA	60	Thioredoxin superfamily protein
C66807-P512	C/T	1	F:TAAAACTTCTAGTCACGCTG R:TAGCCATCTCTATCATGACA	60	Beta-amylase/glycosyl hydrolase family 14
C84852-P331	A/T	17	F:ACCTAATGCAATCCCTTCACCTCC R:GGACTCTGAACATGACAGGTCCACA	65	CRAL/ TRIO domain/Sep14p-like phosphatidylinositol transfer protein
C85320-P102	C/G	11	F:TGAGCGAACAAACACTTAGGGT R:CCATTGCCCTGTGACTCCGT	65	DEK domain-containing chromatin associated protein
C85407-P1002	C/G	16	F:ACGCTTTCTAGATACAGCATG R:TTTATTTTATATTCACTCACGTCTT	60	Embryo defective 2737

Null = missing data/locus, T_a = annealing temperature used for resequencing

Material for DNA-extraction

DNA was extracted from megagametophytes, seedlings, needles (Cullingham et al. 2013).

DNA-extraction and amplification protocol

SEQUENOM® iPLEX® Gold technology.

Amplification protocol: (Culingham et al. 2013):

- 94 °C for 2 min, followed by 35 cycles of 94 °C for 30 s, Ta (50–60 °C) for 45 s, and 72 °C for 60 s, and a final extension at 72 °C for 30 min.

Important results

Using a panel of 7-14 SNPs Cullingham et al. (2013) could differentiate between *P. contorta*, *P. banksiana* and their interspecific hybrids, with minor decreases of assignment accuracy in the second- and third-generation backcrosses.

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Molecular markers used for genetic studies in Eastern white pine (*Pinus strobus* L.)

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1. General remarks

Eastern white pine (*Pinus strobus* L.) is naturally distributed in eastern North America, spanning from Newfoundland to northern Georgia and westward to Manitoba and Minnesota (Figure 1). The species is characterized by a vast phenotypic and genetic variation, resulting from its wide and disjunct distribution in a broad spectrum of environmental niches. Eastern white pine is an ecologically important species and a high-valued one as timber resource. The species is among the most extensively planted American trees, due to its use for the Christmans tree industry, for reforestation and landscaping.

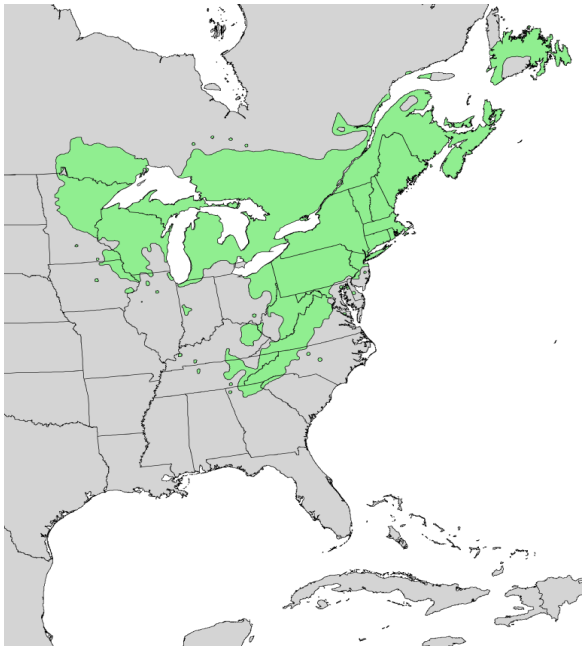

Figure 1. Natural distribution range of Pinus strobus. (Adopted from: http://www. usgs.gov/, Based on: Little 1971).

It grows in cool and humid regions of eastern North America, where the average July temperature ranges from 18 to 23° C, the annual precipitation from 510 mm to 2030 mm and the growing season from 90 to 180 days. The species can withstand a frost depth of above 178 cm, an average annual snowfall from 13 cm to more than 254 cm and it can grow on various soils, ranging from sandy ones (where it is a strong competitor) to sandy loams, slit-loams, clay soils and poorly to very poorly drained soils (Wendel 1980, Mader 1985, Wendel and Smith 1990). The species forms pure and mixed forests and can function as a pioneer species, but also as a physiographic climax species on dry and sandy soils, as a long-lived successional species and thus, as a component of the climax forests throughout its range.

Eastern white pine may hybridize with other native and exotic to North America pines such as: *Pinus monticola, Pinus peuce, Pinus griffithii, Pinus parviflora, Pinus flexilis* and *Pinus ayacahuite.*

2. Isozymes

Isozyme studies focused mainly on the study of the genetic variation present among and within populations of the species. Populations could be under different management practices (Epperson and Chung 2001), or natural populations declining for over a century (Rajora et al. 1997) or under harvesting effect (Buchert et al. 1997). For enzymes encoded by multiple loci, the loci were numbered from anodal to cathodal direction, as described in Buchert et al. 1997 (Epperson and Chung 2001).

Material for isozyme extraction

- Haploid megagametophytes (Rajora et al. 1997, Chagala 1996, Buchert et al. 1997)
- Needles (Epperson and Chung 2001, Chagala 1996, Buchert et al. 1997, Myers et al. 2007)

Table 1: List of enzymes, E.C. number, scored loci and relative references for eastern white pine.

Enzyme system	E.C. Number	Scored loci	Number of alleles per locus	References
Acid phosphatase	3.1.3.2	APH-1,-2,-3,-4	5,2,1,2	1,3,4
Adenylate kinase	2.7.4.3	AK-1,-2	3,2	3,4,6
Alcohol dehydrogenase	1.1.1.1	ADH-1,-2,-3	4,4,2	4,5
Aldolase	4.1.2.13	ALD-1,-2-,3,-4	2,2,3,2	3,4
Aconitase	4.2.1.3	ACO-1,-2	*,3	3,6
Aspartate aminotransferase *or* Glutamate oxaloacetate transaminase	2.6.1.1	AAT(GOT)-1,-2,-3	2,3,3	1,2,3,4,5,6
Diaphorase	1.6.4.3	DIA-1,-2,-3,-4, 5	2,2,1,2,2	1,3,4,5
Fumarase	4.2.1.2	FUM-1	2	1,6
Formate dehydrogenase	1.2.1.2	FDH	n.a.	5
Fluorescent Esterase	3.1.1.1	FE	n.a.	5
Glutamate dehydrogenase	1.4.1.2	GDH-1,-2,-3.-4.-5	3,3,3,2,2	4,5
Glucose-6-phosphate-dehydrogenase	1.1.1.49	G6PDH-1	2	6
Hexokinase	2.7.1.1.	Hex-1	2	6
Isocitrate dehydroenase	1.1.1.42	IDH-1,-2	5,3	1,3,4,5
Leucine aminopeptidase	3.4.11.1	LAP-1,-2,-3	4,4,3,	1,3,4,5
Malate dehydrogenase	1.1.1.37	MDH-1,-2.-3,-4	2,,4,4,3	1,2,3,4,5,6
Malic enzyme	1.1.1.82	ME-1	3	1,4
Menadione reductase	1.6.99.2	MNR-1,-2,-3	3,3,3	4
Phosphoglucose isomerase	5.3.1.9	PGI-1,-2,-3	6,5,4,	1,2,4,5,6
Peroxidase	1.11.1	PER-1,-2,-3	4,3,3	3
Phosphoglucomutase	2.7.5.1	PGM-1,-2	3,4	1,3,4,5,6
Shikimate dehydrogenase	1.1.1.25	SDH-1,-2	4,4	1,3,4
Triose-phosphate isomerase	5.3.1.1	TPI-1,-2	n.a.	2,5
6-Phosphogluconate dehydrogenase	1.1.1.44	6-PGDH-1,-2,-3, -4	4,3,2,2	1,3,4,5

**-unclear patterns*

1-Rajora et al. 1997, 2-Epperson and Chung 2001, 3-Chagala 1996, 4-Buchert et al. 1997, 5-Myers et al. 2007 6-Beaulieu and Simon 1994

Important results

Epperson and Chung (2001) reported the structured distribution of genotypes in an old growth population, following an isolation-by-distance pattern, and a random distribution in a logged population, which they attributed to the logging effect. Rajora et al. (1997) reported that the genetic variation among populations from Ontario and Newfoundland was only 6% and the rest of the variation was harbored within populations, due to the extensive gene flow among the populations. The above authors reported no decline of genetic diversity in the declining in size, for almost a centrury, populations of the species. Buchert et al. (1997) reported on the genetic diversity between pre- and postharvest gene pools of virgin, old growth forest stands. Genetic erosion after harvesting was detected. The authors concluded that gene frequency changes occur in the progeny stands, following harvesting, and they suggest that silvicultural practices need to ensure that the gene pools of remaining old-growth stands have been reconstituted in the regenerated stands. Myers et al. (2007) reported that the allozyme-based spatial genetic structure analysis across the landscape of an isolated island in Lake Michigan revealed the existence of significant spatial genetic structure, which suggests that gene flow via seed dispersal was rather limited across the island.

3. Organelle DNA markers (chloroplast (cp)DNA)

Loci and primers used

Cloutier et al. (2003) used four cpSSRs together with eight putatively neutral nuclear microsatellites (nSSRs) to examine the somatic stability of the microsatellite loci

within 12 individual genets of Eastern white pine, while Myers et al. (2007) used cpSSRs and allozymes to study the spatial genetic structure across the landscape of an island that is isolated in Lake Michigan, USA. Zinck and Rajora (2016) applied cpSSR markers to investigate post-glacial phylogeography and evolution of the species in North America. All primers used in the aforementioned studies had been initially developed for amplification of cpSSR-loci in other *Pinus* species (Cato and Richardson 1996, Vendramin et al. 1996).

Material for DNA-extraction

- Plant tissue (Cloutier et al. 2003)
- Needles (Myers et al. 2007, Zinck and Rajora 2016)

DNA-extraction protocols

- Cato and Richardson (1996), as well as Zinck and Rajora (2016) reported DNA extraction by using a modified CTAB protocol (Doyle and Doyle 1987).
- DNA isolation system "AutoGen 850a" (AutoGen, Inc.) using plant protocol "Plant tissue DNA (system 4) is reported by Myers et al. (2007).

Important results

Cloutier et al. (2003) reported that there was no within-individual variation among the cpSSR loci they tested in the studied material. Myers et al. (2007) reported that no evidence of spatial genetic structure was found in cpDNA SSR data across an island population isolated in Lake Michigan, USA. It was shown that pollen flow has been sufficient to maintain genetic diversity and prevent differentiation across an isolated island landscape over several thousand years of isolation. Zinck and Rajora (2016) observed a broad consensus between nuclear and chloroplast genetic markers in their phylogeographic study. Results support one single glacial refugium in the mid-Atlantic plain in eastern North America. From there, *Pinus strobus* spread and colonized its current native range mainly through two major migration routes during the Holocene: one route gave rise to populations at the western margin of the species' range in Minnesota and Ontario; the other one gave rise to central-eastern populations.

Table 2: Chloroplast microsatellite (cpSSR) markers (chloroplast DNA).

Locus	Repeat motif	Primer sequences F=Forward, R=Reverse	Ref.	Source of primer pairs
cpSSR 1	$(A)_n(G)_n$	F:CAACAGAAGCCCAAGCTTATGG R:TGTATTGTATGCGGAATCAACTGG	1	Cato and Richardson (1996)
cpSSR 5	$(T)_n$	F:TCCAGGATAGCCCAGCTG R:TATATCCCCCGTACTTGGACC	1	Cato and Richardson (1996)
Pt63718	$(T)_n$	F:CACAAAAGGATTTTTTTTCAGTG R:CGACGTGAGTAAGAATGGTTG	1	Vendramin et al. (1996)
Pt71936	$(T)_n$	F:TTCATTGGAAATACACTAGCCC R:AAAACCGTACATGAGATTCCC	1	Vendramin et al. (1996)
Pt9383	-	F:AGAATAAACTGACGTAGATGCCA R:AATTTTCAATTCCTTTCTTTCTCC	2*	Vendramin et al. (1996)
Pt15169	-	F:CTTGGATGGAATAGCAGCC R:GGAAGGGCATTAAGGTCATTA	2	Vendramin et al. (1996)
Pt26081	-	F:CCCGTATCCAGATATACTTCCA R:TGGTTTGATTCATTCGTTCAT	3	Vendramin et al. (1996)
Pt30204	-	F:TCATAGCGGAAGATCCTCTTT R:CGGATTGATCCTAACCATACC	2	Vendramin et al. (1996)
Pt36480	-	F:TTTTGGCTTACAAAATAAAAGAGG R:AAATTCCTAAAGAAGGAAGAGCA	2*	Vendramin et al. (1996)
Pt63718	-	F:CACAAAAGGATTTTTTTTCAGTG R:CGACGTGAGTAAGAATGGTTG	2,3	Vendramin et al. (1996)
Pt71936	-	F:TTCATTGGAAATACACTAGCCC R:AAAACCGTACATGAGATTCCC	2*,3	Vendramin et al. (1996)

*1–Cloutier et al. 2003, 2-Myers et al 2007, 3-Zinck and Rajora 2016, *-no polymorphism found in this study*

4. Nuclear DNA markers (RAPDs, ISSRs, nSSRs, SNPs)

a) RAPDs (Randomly amplified polymorphic DNA markers) and ISSRs (Inter-simple sequence repeat polymorphisms)

Molecular markers such as Random Amplification of Polymorphic DNA (RAPD) and Inter-Simple Sequence Repeats (ISSR) were used by Mehes et al. (2007) to study the variation among *P. strobus* populations in Canada, while Nkongolo et al. (2012) used ISSR genetic markers to assess the genetic distance among populations of the species growing in stressed areas of Northern Ontario, Canada (Tables 3, 4).

Material for DNA-extraction

- Seedling tissue (Mehes et al. 2007)
- Needle tissue (Nkongolo et al. 2012)

DNA-extraction protocols

- The method reported by Nkongolo (1999) was used for DNA extraction by Mehes et al. (2007).
- Nkonglolo et al. (2012) used also the method of Nkongolo (1999) with some modifications that involved addition of PVP (polyvinylpyrrolidone) and β-mercaptoethanol to the CTAB extraction buffer.

Table 3: RAPD markers applied in DNA amplification in *Pinus strobus*

Primer Identification	Nucleotide sequence (5'-3')	Fragment size range (bp)	Ref.
9	ACGACGTAGG	n.a.	1
10	CCGCGGTTC	n.a.	1
11	CCGGCTGGAA	n.a.	1
12	GAGGGCCTGA	n.a.	1
13	GCTCCCCCAC	360–1500	1
14	CGATGGCTTT	n.a.	1
15	TAGCCCGCTT	n.a.	1
16	GTAGACGAGC	480–1500	1
17	GTGCGTCCTC	250–1500	1
18	GTTCTCGTGT	n.a.	1
19	AACACACGAG	n.a.	1
23	CCCGCCTTC	280–3000	1
63	TTCCCCGCCC	450–2200	1
146	ATGTGTTGCG	350–2000	1
184	CAAACGGCAC	220–2400	1
E1	CCGCCCAAAC	380–2000	1
E2	GTGGTCCGCA	280–1800	1
E3	GTGGCCGCGC	400–1500	1
E4	GAGGCGCTGC	250–900	1
E5	CGCCCCCAGT	400–1800	1
E6	CGTCGCCCAT	420–1800	1
E7	CACGGCGAGT	260–1800	1
E8	GGGTAACGCC	220–2100	1
E9	GTGATCGCAG	300–2000	1
E10	CAGCACCCAC	350–3000	1
E12	TTATCGCCCC	600–900	1
E18	GGACTGCAGA	200–1800	1
OPA 1	CAGGCCCTTC	500–2000	1
OPA 2	TGCCGAGCTG	250–2000	1

Primer Identification	Nucleotide sequence (5'-3')	Fragment size range (bp)	Ref.
OPA 3	AGTCAGCCAC	220–2000	1
OPA 4	AATCGGGCTG	250–2500	1
OPA 5	AGGGGTCTTG	400–2500	1
OPA 6	GGTCCCTGAC	300–3000	1
OPA 7	CAAACGGGTG	400–2000	1
OPA 8	GTGACGTAGG	400–3000	1
OPA 11	CAATCGCCGT	200–2000	1
OPA 12	TCGGCGATAG	n.a	1
OPA 14	TCGGCGATAG	500–1600	1
OPA 15	TTCCGAACCC	300–850	1
OPA 16	AGCCAGCGAA	350–2000	1
OPA 17	GACCGCTTGT	300–1600	1
OPA 18	AGGTGACCGT	450–1600	1
OPA 19	CAAACGTCGG	400–3000	1
OPA 20	GTTGCGATCC	400–2000	1
OPB 1	GTTTCGCTCC	500–1600	1
OPB 7	GGTGACGCAG	500–900	1
UBC 186	GTGCGTCGCT	380–2000	1
UBC 197	TCCCCGTTCC	680–1500	1

1–Mehes et al. 2007

Table 4: ISSR markers applied in DNA amplification in *Pinus strobus*

Primer Identification	Nucleotide sequence (5'-3')	Fragment size range (bp)	References
17898B	CACACACACACAGT	300–3000	1
17899A	CACACACACACAAG	300–1300	1
Echt 1	AATAATAATCG	n.a.	1
Echt 1 (2)	GATAGATATG	n.a.	1
Echt 2	ATCATCATCCG	400–3000	1
Echt3	AACAACAACGC	n.a.	1
Echt 5	AGACAGACGC	350–2000	1,2
Echt 6	ACTCACTCGC	500–2000	1
Echt 7	ACAGACAGCG	300–2000	1
HB 12	CACCACCACGC	320–1300	1
HB 13	GAGGAGGAGGC	300–650	1,2
HB15	GTGGTGGTGGC	320–1800	1,2
UBC 809	AGAGAGAGAGAGAGAGG	300–700	1
UBC 823	TCTCTCTCTCTCTCTCC	500–700	1
UBC 825	ACACACACACACACACT	180–580	1,2
UBC 827	ACACACACACACACACG	500–1600	1
UBC 829	TGTGTGTGTGTGTGTGC	500–650	1
UBC 834	AGAGAGAGAGAGAGAGYT	220–1300	1
UBC 841	GAAGGAGAGAGAGAGAYC	340–1800	1,2
UBC 849	GTGTGTGTGTGTGTGTYA	400–1300	1
ISSR 1	AGAGAGAGAGAGAGAGRG	n.a.	2
ISSR 5	ACGACGACGACGAC	n.a.	2
ISSR9	GATCGATCGATCGC	n.a.	2
17899A	CACACACACACAAG	n.a.	2
17898B	CACACACACACAGT	n.a.	2

1–Mehes et al. 2007, 2-Nkongolo et al. 2012

Important results

Following the Mehes et al. (2007) results, the RAPD primers 23, 63, 184, E6, E12, OPA 2,3,4,11,18, and UBC186 yielded the best amplification results, as well as the ISSR primers 17898B, 17899A, Echt 5, HB 15, UBC 834 and 841. The study revealed that the level of genetic variation among the *P. strobus* populations was higher than that among the *P. monticola* ones. The results also indicated that the *P. strobus* populations were not as closely related as the populations of *P. monticola*. Nkongolo et al. (2012) reported a low differentiation among the *Pinus strobus* populations (planted and natural) they studied. They report that the percentage of polymorphic loci within each *P. strobus* population varied between 22% and 36%, while the planted populations found to have similar or even significantly higher genetic variation from the natural ones that grew on the same site.

b) nSSRs (putatively neutral microsatellites)

Loci and primers used

Development of SSR markers for Eastern white pine was mainly done by Echt et al. (1996, 1999) and Echt and Nelson (1997) (Table 5).

Table 5: Primer sequences, annealing temperatures (T_a), allele lengths in base pairs (bp) and references for nSSRs available for genetic analyses in *Pinus strobus*

Locus	Motif	Primer sequences		T_a	Size [bp]	N_A	References
		Forward	Reverse				
RPS1b	$(AC)_{10}$	GCCCACTATTCAAGATGTCA	GATGTTAGCAGAAACATGAGG	55	191-217*	13	1,2,3,5,6,7,8,9
RPS2	$(AC)_{15}$	CATGGTGTTGGTCATTGTTCCA	TGGAGGCTATCACGTATGCACC	55	145-173*	13	1,2,3,5,6,7,8,9
RPS6	$(AC)_{14}$	TTTTCTAATCAGTGTGCGCTACA	CACCGCTGCCCTATTTTACA	55	162	5	1,2,3,4,7,9
RPS12	$(AC)_{17}$	TCAATGTGGAGATGGTGATT	ACTTCTGACCTAACCAGAAACC	57	149-229*	36	1,2,3,4,5,6,8,9
RPS18	$(AC)_{14}$	TTTCTAATCAGTGTGCGCTACAT	CACCGCTGCCCTATTTTACA	54	160	4	1,2,3
RPS20	$(AC)_{16}(AT)_6$	ACTTCCCCACAGGTTAACACA	AACAAGATAGGCGGGATTCA	54	100-180*	26	1,2,3,4,5,6,8,9
RPS25b	$(AC)_{17}AG(AT)_9$	CACATATGGCAGAACACACA	GATCGTCGCACTATCGAAC	55	81-163*	10	1,2,3,5,6,8,9
RPS34b	$(AC)_{14}$	CAGTGTTCTCTTATCACAGCG	GCACTATAATGAAATAGCGCA	55	141-171*	12	1,2,3,5,8,9
RPS39	$(AC)_{17}$	GCCAGCTCCAACCAGAATC	GGCTCGCTGACCCAATAA	57	158-188*	11	1,2,3,4,5,7,8,9
RPS50	$(AC)_{17}$	CCCAGAAATCTGTTTTAGAGC	ACACATGAAATGTCAGAATGC	50	152-192*	18	1,2,3,4,5,6,7,8,9
RPS60	$(AC)_{19}(AT)_7$	ACGATAATGGCGGTGAGAACAA	CCACCTGTCCTTCGTACATCCA	57	269	17	1,3,4,5
RPS84	$(CT)_{10}(AC)_{11}$	CCTTTGGTCATTGTATTTTTGGAC	CTTCCTTTTCCTTCTTGCTCCAC	52	147	5	1,2,7
RPS90	$(AC)_{23}$	ACCCATTGTGGTGTGTTTGTG	CCTCCGACCATAAACCTTAATG	55	164	5	1,2,3
RPS118b	$(AC)_{23}$	CATTGTGGTGTGTTTGTGAA	CCACCTCCGACCATAAAC	52	124-168*	23	1,2,3,4,5,6,8,9
RPS119	$(AC)_{10}(AT)_5$	TTGTGAGAAGATACTTCCTCCA	CCTTGTCTTCTAAAAAACACTTTT	55	203-205*	2	1,3,5,8,9
RPS124	$(AC)_{12}$	AGAGTTCTCACTTCACAATAGGTG	ATTTCACACAATTTTGAGTGTTT	56	149	4	1,2,3
RPS127	$(AC)_{10}(AT)_5$	ACTTCCTCCAAGTTACTATTGTCA	CCTTGTCTTCTAAAAAACACTITT	55	191-195*	3	1,2,3,5,7,8,9
RPS150	$(GAG)_4$	TCCATCAGTGAGCAGTGG	CACTTGGGCTTCCTCTTC	52	248	1	1,2,3
RPS160	$(ACAG)_3AGGC(AGAC)_3$	ACTAAGAACTCTCCCTCTCACC	TCATTGTTCCCCAAATCAT	55	246	1	1,2,3
RPS3	$(AC)_{19}$	AATGAAGGACAGTTGGGATGAT	TGCTTCCTTCTCATGTTCTCC	n.a	n.a.	n.a.	2
RPS61	$(AC)_{12}$	TCCATTTCCATCCTTCTTCG	ACGCAACTACCCAGAAGCAA	n.a	n.a.	n.a.	2
RPS105	$(AC)_{17}(AT)_3$	TGGACATCCTAGTCGGAACC	AAAATCATTTCTGTATCAGAACAA	n.a	n.a.	n.a.	2
RPS152	$(ANAC)_6(N)_{12}(CAGA)_3$	AAGGGTTTCATTTTGAGAGG	AAATGGCAATGGGAAATG	n.a	n.a.	n.a.	2
Pschi1	GenBank accession No. U57409	CGTGTTCGATGTTGTGGTCAGCAAAGAG	ATTTGGCTACCATGACCAACGT	n.a.	253	n.a.	3

1-Echt et al. 1996, 2-Echt et al. 1999, 3-Echt and Nelson 1997, 4-Cloutier et al. 2003, 5-Rajora et al. 2000, 6-Mehes et al. 2009, 7-Marquard and Epperson 2004, 8-Chhatre and Rajora 2014, 9–Zink and Rajora 2016 (reported in Chattre and Rajora, 2014)*

Material for DNA-extraction

- Haploid megagametophytes (Echt et al. 1996, Echt and Nelson 1997, Cloutier et al. 2003)
- Terminal buds (Echt et al. 1999, Marquardt and Epperson 2004)
- Needles (Rajora et al. 2000, Marquardt and Epperson 2004, Myers et al. 2007, Zinck and Rajora 2016)
- Seedling tissue (Mehes et al. 2009)

DNA-extraction protocols

- Genomic DNA was extracted by using the DNeasy plant mini-kit (Quiagen, Valencia, CA, USA) (Cloutier et al. 2003, Marquardt and Epperson 2004). Details on the amplification can be found in Marquardt and Epperson (2004) and Cloutier et al. (2003).
- Mehes et al. (2009) used the DNA extraction method described by Nkongolo (1999) with some modifications.
- Echt and Nelson (1997) reported the following protocol for DNA extraction from megagametophytes: "Each megagametophyte was homogenized in 100 mM sodium acetate, 50 mM sodium chloride, 50 mM EDTA, 1.4% SDS,0.5% polyvinyl pyrolidone and 60 mM cysteine, pH 5.3. The homogenate was incubated at 55°C for 30 min, followed by the addition of a 0.5 vol of 3.5M potassium acetate, pH 5.3. The chilled mixture was cleared by centrifugation, transferred to a fresh tube, and the DNA precipitated in isopropanol. The DNA was re-suspended in 10 mM Tris-Cl, 1 mM EDTA, pH 8, treated with RNase, and extracted twice using Strataclean resin (Stratagene Cloning Systems). For SSR analysis only, 10 ng of each DNA template were transferred into 96-well, V-bottom plates, then dried in a food dehydrator at 50°C, and stored at -20°C until used for PCR".
- Zinck and Rajora (2016) used a modified CTAB extraction protocol according to Doyle and Doyle (1987).

Important results

Results reported by Echt et al. (1996) indicate the potential for substantial genetic gains in terms of timber production and wood quality, as well as stress-tolerance, following breeding programs that rely on efficient artificial selection. Rajora et al. (2000) studied the impact of harvesting on the genetic diversity of two old-growth stands of the species in Canada and the subsequent loss of genetic variation. They reported reduction in total and mean number of alleles by nearly 26%, after a reduction of tree density of about 75%, while 18-21% of the low frequency and 76-92% of the rare alleles were lost from the studied stands. Mehes et al. (2009) studied the potential impact of forest fragmentation on population inbreeding and found that inbreeding levels in the studied populations were low, despite the geographic isolation and the small stand size, concluding that gene flow was high and population differentiation was low for the studied fragmented sites. Marquardt and Epperson (2004) reported results on the genetic diversity of old growth and second growth populations growing in Michigan, USA. They found high genetic diversity within the populations and low inbreeding, and lower spatial structuring in the second growth population due to logging. The presence of higher number of rare alleles in the old growth populations and higher mutation rates were also reported. Chhatre and Rajora (2014) when studying the diversity of marginal versus core populations of the species in Canada found that the central populations had significantly higher allelic and genotypic diversity. The marginal populations were genetically divergent from the central ones, and signatures of natural selection were detected at three loci in the marginal populations; two loci showing divergent selection with directional change in allele frequencies and one balancing selection. Zinck and Rajora (2016) found a high and significant genetic differentiation among 33 populations in the native range (F_{ST} = 0.104) and a south to north trend of declining genetic diversity. The latter is consistent with repeated founder effects during post-glacial recolonization. Regarding migration routes, results from nuclear microsatellites are in agreement with findings based on cpSSRs (see above).

c) SNPs (single nucleotide polymorphisms)

Loci and primers used

Nadeau et al. (2015) used Single Nucleotide Polymorphisms (SNPs) to characterize the patterns of genetic diversity and population structure across the ranges of two species; namely *Pinus strobus* (133 populations) and *Pinus monticola* (61 populations). In the Tables that follow (Table 6a – SNPs for *P. strobus* and Table 6b – SNPs designed for other species but tested in the above study) SNPs are reported that were used for the analysis of *P. strobus* populations.

Table 6a: Description of Successful SNPs (Sequenom iPlex Gold technology) for *Pinus strobus* as reported by Nadeau et al. 2015.

SNP	C*	Type	pos	Amplicon	Seq. set**	Forward amplification primer	Reverse amplification primer	Extension probe
G-008_S-038	I	G/A	490	GQ0015.BR_K18	C.G.W.F.	ACGTTGGATGGCAAGGATAGTCACATGCTG	ACGTTGGATGTGCATGGAACTTACACCCAG	GGATATTGCTGAACATT
G-020_S-039	I	C/T	280	GQ0026.BR_B03	C.G.W.F.	ACGTTGGATGACAGCCTATGGCAACGTTCG	ACGTTGGATGATGTACCAGGTGAGCTACGG	ctccCCGTGGCGAAGTTCCCCAG
G-023_S-040	I	G/A	575	GQ0045.B3_E18	C.G.W.F.	ACGTTGGATGTCTGTTGTTGACACTGTCCC	ACGTTGGATGCCTACCACCATAGAGTTCTG	TGACACTGTCCCTATTGA
M-001	I	A/T	269	0_13978_01	WHISP	ACGTTGGATGTGGTACTGGATCAGTGAAGC	ACGTTGGATGGTTGACAAGGTTAAAACCAC	ccccggGAAGCTCAATCAAATTTTGT
M-002	I	T/A	288	0_13978_01	WHISP	ACGTTGGATGGTTGACAAGGTTAAAACCAC	ACGTTGGATGTGGTACTGGATCAGTGAAGC	gGACAAAAGGGGCCAAAA
M-003	I	T/C	142	0_14837_01	WHISP	ACGTTGGATGCAGTGTACTGGCAATTCCTG	ACGTTGGATGAGCACCTTTGCTTCCAGATG	ggggATTCCTGTTTGATTTAGATATCG
M-006	I	T/A	47	0_18261_01	WHISP	ACGTTGGATGTTGCATGGAACTGCACAGAC	ACGTTGGATGCCATCCAATCCTTCATCTTC	CATTTCGGCAGTTTCC
M-007	I	C/T	174	0_18267_01	WHISP	ACGTTGGATGGATCGTCAATGCCCGGAAC	ACGTTGGATGAGGTCCATAACTGCTCTGTG	tggcTGCTTCCCGTGACCGG
M-008	I	C/T	306	0_18267_01	WHISP	ACGTTGGATGCTATCGTTTAGCAGCTCCTG	ACGTTGGATGAGGAAGTGGCATCATGGGATTCGAAG	ggtGAAGAGGCGGAGATCA
M-010	I	A/G	133	0_3073_01	WHISP	ACGTTGGATGGAACAAAATGCTAGCCCCTC	ACGTTGGATGAGGAAGTGGAACCCCCTTTC	cAGGAGCAATTCCCTCC
M-011	I	G/T	241	0_3073_01	WHISP	ACGTTGGATGCCTCTTTCTTCATGCCCAAC	ACGTTGGATGGACCAATCAGATGAACCCAA	gggGAGAATTCACCCATTGTTGA
M-012	I	T/C	120	0_3192_01	WHISP	ACGTTGGATGGACTCTTATAGACTACGTTG	ACGTTGGATGGCCCCAAGACTAGGAAAAAG	ccctACTACGTTGATATTTATTCATCT
M-013	I	C/A	370	0_3192_01	WHISP	ACGTTGGATGGTCACTCTTCTACAAAATCCC	ACGTTGGATGCATGGATAGTGGAAAGGTGG	gtaaAAAATCCCTCATTGGAGGCAAT
M-014	I	G/A	119	0_4756_01	WHISP	ACGTTGGATGTAAGCCCTATTCTCCACCAC	ACGTTGGATGTTTTATGATCCGGAGGCGAG	GACAAGTAAGTCGGCTT
M-015	I	C/A	224	0_8683_01	WHISP	ACGTTGGATGATCGGATGTCTACAGCTTCG	ACGTTGGATGAGCAAGGTTGATTTCCCCTC	gtcgtGGTCGTTCTGGTAGAG
M-016	I	G/A	371	0_8683_01	WHISP	ACGTTGGATGTCTGTCATCCCCTTCTGACG	ACGTTGGATGTAGTGGATCCGCAACTAGAG	gctgccACAGCACTTACCATGG
M-017	I	A/G	244	0_8844_01	WHISP	ACGTTGGATGCTTGGAGGAGGACTAACATC	ACGTTGGATGAAGCAAGCAACCATTACAAG	cTTTCAACTATGTTTTAGTTTACC

SNP	C*	Type	pos	Amplicon	Seq. set**	Forward amplification primer	Reverse amplification primer	Extension probe
M-018	I	T/C	99	0_9462_01	WHISP	ACGTTGGATGAATGGTGC-ATTGCCACTTCC	ACGTTGGATGGGATTGAA-GGTCGTGTCATC	taaCACTGATATCTGAA-CTATTCAA
M-025	I	T/C	93	CL1536Contig1_03	WHISP	ACGTTGGATGCACAAACAT-GCACATCCTCC	ACGTTGGATGTGTCTCTGG-GAGCATCATTG	ggCATCCTCCCCTAACAC
M-026	I	G/T	180	CL1694Contig1_02	WHISP	ACGTTGGATGTGGTTGACAA-CTATCCAAGG	ACGTTGGATGGTTGGAATGT-CAGCAGTGTGGTGGTTGTG	ACAGCATCAATTGCATCAT-TA
M-027	I	T/A	260	CL1694Contig1_02	WHISP	ACGTTGGATGCCAAAGGATT-CAATGACTGG	ACGTTGGATGCCTTGGA-TAGTTGTCAACC	ATTGCAAAACCAAAAAC-CAATATA
M-028	I	C/A	150	0_18267_01	WHISP	ACGTTGGATGTTAAGGGCAA-CACTGGTTGG	ACGTTGGATGGCAGCC-GATCGTCAATGCC	ctACGGGAAGCATCCATAT
M-029	I	T/C	253	CL1806Contig1_01	WHISP	ACGTTGGATGTCACGTTC-CATGGCTCGTC	ACGTTGGATGTTAGGGACAC-TGCTGCCATC	ccccTCCTGCACGTTACGG-GCCAC
M-030	I	T/A	30	CL1905Contig1_03	WHISP	ACGTTGGATGCTAAAGGTG-GACAAGGTAAC	ACGTTGGATGAGATTAAGA-CACCCAACAGG	ccccgTCTTGTGTGCCA-GAAAACA
M-031	I	C/T	328	CL2332Contig1_01	WHISP	ACGTTGGATGTTGCTCT-GAAGGTATGTTCC	ACGTTGGATGCAGCAATCAC-CTGAATTGAG	cTGTTCCTATAATATCTAAT-GAGACAAA
M-032	I	C/T	281	CL3007Contig1_02	WHISP	ACGTTGGATGGACAGGAA-CATCCCGAAATG	ACGTTGGATGAAAGCCCATAG-ACAGAACCG	CATCCCGAAATGAAGTTAG-TAC
M-034	I	G/A	169	CL3097Contig1_01	WHISP	ACGTTGGATGCTCTCTTAC-TTCAAAACTGC	ACGTTGGATGTCCTTGTC-CAAAGCTAAGTC	GAAATCTCACCTGGAGAG-TA
O-021_ Q-024	I	A/G	436	2_7852_01	WHISP	ACGTTGGATGTGGTGAA-CATGTGCATGCTG	ACGTTGGATGTGGATCCATT-TAGACTTCCC	cccccctAGATCTTGCTCAGCAA
T-016	I	A/G	257	2_3720_01	C.G.G.P.	ACGTTGGATGCCAAACCA-GTTTCTGCGAAC	ACGTTGGATGAGACGATTT-GCTGCTGCTGG	AATCGAAGCAGAAAAAA
T-019	I	A/T	362	2_4724_01	C.G.G.P.	ACGTTGGATGAGCAGCATAT-GCTGCTGCTT	ACGTTGGATGATATC-CAAGCAGTTGCCCAG	tcCCATCCATTGTGTATACAT
T-026	I	C/T	187	CL4023Contig1_01	C.G.G.P.	ACGTTGGATGGCTACAAGAT-GAAGATGGGC	ACGTTGGATGGCTACACCAAT-GCATTTCAC	cgaggAGTGCTGGGTAATATT
T-028	I	C/T	316	2_9280_01	C.G.G.P.	ACGTTGGATGACGAGAGCA-GATTGAAAGGG	ACGTTGGATGACAATGCAC-TGGATCTTGGC	GACATAGATTACATTTT-GAACG
T-029	I	G/A	526	CL866Contig1_01	C.G.G.P.	ACGTTGGATGATAGGTCCATC-TGTTGTAGC	ACGTTGGATGTCTCTGGTAA-CTCACCATGC	TACATATAGCAGGCTACC
T-031	I	G/C	197	0_2433_01	WHISP	ACGTTGGATGGAAACAATAAA-CCGTTGGAG	ACGTTGGATGCCTGTTT-GATTCCTCACAGC	ggggagggACTTCAATAATATT-GAAACT

SNP	C*	Type	pos	Amplicon	Seq. set**	Forward amplification primer	Reverse amplification primer	Extension probe
G-001	III	G/C	1354	GQ0015.B3.r_B10	C.G.W.F.	ACGTTGGATGACTTCCTTGC-CTGTGTAGTC	ACGTTGGATGTGGGTGCC-CATTTTGATGAG	aggTCCTTGCCTGTGTAGT-CCTGCGTGA
G-002	III	C/T	234	GQ0254.B7_N02	C.G.W.F.	ACGTTGGATGGAAATGCAGC-CCAAAGGAAG	ACGTTGGATGGCCTTG-TAAAGTATGCGACC	aggaaATTGCTGCGGAA-GATGTTCCAAT
G-003	III	G/T	802	GQ0047.B3_H11	C.G.W.F.	ACGTTGGATGGCAGAAAGC-TGTGCTTATAC	ACGTTGGATGTTGGATGTT-GACGCACAGCTGAAGTTG	TGCTTATACATTATGACTT-TAAAA
G-004	III	T/C	245	GQ0033.TB_H23	C.G.W.F.	ACGTTGGATGTCTCT-CAGCAGGGTTTGTTC	ACGTTGGATGTTTGTCG-TAGCGGACACAAC	aAGGGTTTGTTCTTTAG-AATTAGC
G-005	III	C/T	577	GQ0011.B3.r_G02	C.G.W.F.	ACGTTGGATGCTGGCAAACT-CTTGCGAAAC	ACGTTGGATGAAAGGACA-GAACAGCCAAGG	GCTCAGTTTCGTGCAAGGT
G-009	III	G/T	126	GQ00410.B3_B06	C.G.W.F.	ACGTTGGATGAAAATCTCTT-GACAACCGAC	ACGTTGGATGGCCTTC-TTTGGGTTTAATGG	tctctagaTTGACAACCGACT-GAATTA
G-010	III	G/A	313	GQ0045.B3_N12	C.G.W.F.	ACGTTGGATGCAGAG-GAAGGTGCATTATCA	ACGTTGGATGACGTCCT-GATGTTCAGCTTG	tgCACAATAATATGGTGAA-TACCTT
G-011	III	G/A	151	GQ0025.BR_C19	C.G.W.F.	ACGTTGGATGTCTCCAGTTC-GAGACTCTGC	ACGTTGGATGTGAAAGCC-TACCATGTCCAG	gcGACTCTGCCCCGAGGGC-CGGCAT
G-012	III	C/T	175	GQ0045.B3_N12	C.G.W.F.	ACGTTGGATGGGATGATCC-TTGGGAGTATG	ACGTTGGATGCAAAGTACAAA-CAGGAACAC	TGTCTACTGTATTTAT-GAAAG
G-014	III	A/G	184	GQ0081.BR.1_D09	C.G.W.F.	ACGTTGGATGATGAGGGC-TGTGAAAAGTGG	ACGTTGGATGTCTAGTG-TAATTGCCGTGCC	gggcAAGGAAGGTAGTGTT-GACATG
G-015	III	G/A	161	GQ0048.TB_H08	C.G.W.F.	ACGTTGGATGGCTAGAATCA-GAGATCAGCA	ACGTTGGATGTACATTCAGT-CATTCTCAGC	ccacATTGCAACATTTCTGT-CTT
G-017	III	G/T	250	GQ00410.B3_G18	C.G.W.F.	ACGTTGGATGACACATGAA-CAGCCTGGAAC	ACGTTGGATGGGGTTTGAAC-CTGCGACTTG	ccgTCCTCTACGCCCTCGT
G-019	III	C/G	582	GQ00612.B3_J14	C.G.W.F.	ACGTTGGATGCCCTCTACATC-CACATAGAC	ACGTTGGATGCTAGCAATCC-GATGATACCC	TGACAAACACACCAGG
G-021	III	T/C	609	GQ0033.TB_H23	C.G.W.F.	ACGTTGGATGCAGATATTCC-TGGCTGGAAC	ACGTTGGATGGGTATATGCAA-CGGTACCTG	atggcggAAAAATTGCGTGAC-TTTGC
G-022	III	G/A	258	GQ0032.TB_I23	C.G.W.F.	ACGTTGGATGTCCGCCATTT-TAGTCGCAAC	ACGTTGGATGGAACCGG-TAAAGACTCTGTG	CGCAACCCACTTCCAGA
G-025	III	T/C	255	GQ0042.BR_E10	C.G.W.F.	ACGTTGGATGTTGAAGTTCT-CTGTGCTCGG	ACGTTGGATGGTAGACTTGC-CGGTTTTCAG	gggaGAGCAACGGTTCACAG
G-026	III	C/T	213	GQ0206.B3_C13	C.G.W.F.	ACGTTGGATGATGCCCGA-GAGCTTTTCGTC	ACGTTGGATGCACTCCTAC-TTCGAAGAAGC	ctcgTTTCGTCACCAACATTG

SNP	C*	Type	pos	Amplicon	Seq. set**	Forward amplification primer	Reverse amplification primer	Extension probe
G-027	III	A/G	220	GQ0073.TB_E24	C.G.W.F.	ACGTTGGATGACAGGGAACT-GAACTTCACC	ACGTTGGATGGTCCTGCC-TGTGGAAAATTC	ggGGAGCAACAGATCGTA
G-028	III	C/A	115	GQ0162.B3.r_L01	C.G.W.F.	ACGTTGGATGCATCTGAGCA-GACCTGCAAG	ACGTTGGATGCCGAAGCT-CGCTGGTAATAA	TTGGCAGGCAGAATCT
G-029	III	C/A	521	GQ0044.B3.r_N02	C.G.W.F.	ACGTTGGATGTCTCTAACC-CAACCCAAACC	ACGTTGGATGAAGATTTCCTT-GCCTCTCCC	cccGAAACCCACTTTTGC-TTGT
G-030	III	A/G	100	GQ0026.B3.r_O24	C.G.W.F.	ACGTTGGATGACTATTC-TGGTTGTGATGGG	ACGTTGGATGCAGTAATTCC-CTCCTCTTGG	GATGGGGTCATGAAGT
G-031	III	C/A	432	GQ0011.BR_F15	C.G.W.F.	ACGTTGGATGGCTGATGCA-GAAATACAAGG	ACGTTGGATGACGGATGAGA-CATCGTACTG	taccTCACAAAGAGCCCCAT
G-033	III	A/G	357	GQ0132.B3_K05	C.G.W.F.	ACGTTGGATGCCATTCTGGG-TAGTTTCCTG	ACGTTGGATGTTTGCTTGAC-GAGGAAGCTG	cggagaGTAGTTTCCTGAAGC-TATGGC
G-034	III	C/A	200	AGP6	GenBank	ACGTTGGATGTTTAGTGGTG-GGAGGAGGAG	ACGTTGGATGTCGCTCCCAC-GAAACCGACT	gtttGTGGGAGCAACGGCGG-GCGC
G-035	III	C/A	518	IFG8612	GenBank	ACGTTGGATGGCCAAACTGT-CAACATTGTG	ACGTTGGATGTCCTGTG-GAAAGGATCCTAC	ACTGTATGATCCTTTTGTG-TAG
G-036	III	C/A	320	IFG8612	GenBank	ACGTTGGATGAAGCTGAGTC-TAGGCCATTG	ACGTTGGATGCTTCCACTG-GAGATCCATTC	ggtGAAGGATGGAACAATTT-CC
M-004	III	T/C	259	0_1688_02	WHISP	ACGTTGGATGATTCAACAGC-TTGGTAGAAG	ACGTTGGATGGAATTGCTCT-TACATTTGG	ggAGAATAGCATATTGAT-TAACTGA
M-009	II	G/T	275	0_2433_01	WHISP	ACGTTGGATGGAAACAATAAA-CCGTTGGAG	ACGTTGGATGCCTGTTT-GATTCCTCACAGC	TGGAGTTAAAAAATTATAA-CATAAACACA
M-019	III	G/C	297	2_2799_03	WHISP	ACGTTGGATGCAGAAA-TAGCAATTAGGCCC	ACGTTGGATGTTCCCATTT-TACTGGGAAG	ATGTTATACAAAATTGAAAA-GAGA
M-021	II	A/G	137	2_7189_01	WHISP	ACGTTGGATGAACGTTGCTC-CCTGGATAAC	ACGTTGGATGTCCGCTATA-CATTGACACGC	GCTGGGCGACGGGAGT
M-022	II	A/G	55	2_7852_01	WHISP	ACGTTGGATGTGCTTCAGC-TATGGATACAC	ACGTTGGATGAGGAAACAGG-GCTATAGGAG	TCAGCTATGGATACACT-GATTTTATT
N-002	II	C/G	242	0_11270_01	WHISP	ACGTTGGATGTAGAGAGACT-CCTTGAAGGG	ACGTTGGATGAACTCCTCTT-CAGCAGTGTC	GAGTCGAAGCCTTCCTT
N-003	III	C/T	221	0_11649_03	WHISP	ACGTTGGATGAGTGCTCATG-GATCTCGAAC	ACGTTGGATGCAGACTGGCC-GAACACAAAG	gCATGGATCTCGAACCCG-GAACCATGGA
N-004	III	A/G	71	0_11649_03	WHISP	ACGTTGGATGTGGAGCAAA-GTTCTGGGAAG	ACGTTGGATGTATCCTCTC-CAACTGAAGGC	AAAGTTCTGGGAAGTTA-TATGTGACGA

SNP	C*	Type	pos	Amplicon	Seq. set**	Forward amplification primer	Reverse amplification primer	Extension probe
N-005	II	A/G	166	0_12156_02	WHISP	ACGTTGGATGCAAGAGACA-GATTCAGGAGG	ACGTTGGATGAAATGG-GCATTGGGTTGTCG	tAGACAGATTCAGGAG-GAGTTGG
N-006	II	G/T	98	0_12216_02	WHISP	ACGTTGGATGTGTTTGCCTA-CAGCATGGAC	ACGTTGGATGACCAGGGTGT-GCTTATCTTC	caAAAGTACTCATGATTGAA
N-007	II	A/G	320	0_12745_01	WHISP	ACGTTGGATGTGGACTAG-GATTAAGCAGCC	ACGTTGGATGACCAAACTCC-CCATGGTAAC	CCATTCCGTCTCCTCT
N-008	II	G/C	214	0_12978_02	WHISP	ACGTTGGATGTTGTGTAG-GATGCATCGCTG	ACGTTGGATGGGATTACTC-TTTGTTTGGGC	ccaaacaCATTATATGATA-CACAATGG
N-010	II	G/A	166	0_13957_02	WHISP	ACGTTGGATGCAAGCGCA-GAAGCTACAAAG	ACGTTGGATGGGAACCCAC-CATTGACATTG	aGCAGAAGCTACAAAG-TAGCAG
N-011	II	A/G	285	0_13957_02	WHISP	ACGTTGGATGAAGAAGCTT-GCCCCTGTAAC	ACGTTGGATGAATGGCC-CACAAGTGGGTAG	TGCCCCTGTAACTTT-TAGGGTATATTTACA
N-012	II	A/T	161	0_14221_01	WHISP	ACGTTGGATGAGTGAGAC-TGTTCTTTTGGG	ACGTTGGATGAAGGCATGTC-TAGAGTCCTG	gTTCATGTCATA-TAAGTGTTTGTTG
N-013	II	C/T	468	0_14221_01	WHISP	ACGTTGGATGCGGCCT-GCAATTGAAGAATC	ACGTTGGATGGCTGTT-GCATTTAATGTGTG	CGGCCTGCAATTGAA-GAATCAAATCC
N-015	II	C/T	164	0_15187_01	WHISP	ACGTTGGATGATGCCAA-CATCACCATCAGG	ACGTTGGATGATTCCCTGAA-CCAGTACCAC	ATCACCATCAGGTGGAAC
N-016	III	C/T	312	0_15762_01	WHISP	ACGTTGGATGTCTTCTGAAC-CACTGATGGC	ACGTTGGATGTGCGTC-CAATCTTCGGAATG	aCAGTCTATTGATGGCTA
N-017	II	T/C	276	0_15991_01	WHISP	ACGTTGGATGATGC-CAAGCAGCAGGTTTTG	ACGTTGGATGTTGTG-TATCACAAGGTGTGC	AAGCAGCAGGTTTTGAAA-TAGCCT
N-018	III	C/A	139	0_16619_01	WHISP	ACGTTGGATGATTGCTAGC-TTCCCTACCTC	ACGTTGGATGGGAAGAGA-TAGATGGTATTC	ATCTGACACAAATCATT-CACTC
N-019	III	C/A	422	0_16619_01	WHISP	ACGTTGGATGATGCAGAGA-TAAAGCTCGTG	ACGTTGGATGCCACCATCATT-GCTCACAAC	cggggTAAAGCTCGTGAGAAA-CAACGT
N-020	II	C/T	235	0_16889_02	WHISP	ACGTTGGATGGAAGATGGTT-CTCTACCAGG	ACGTTGGATGCCCTCA-GAAAGCATATGTCC	CACACACTAGTGCCTT
N-021	II	T/C	270	0_17938_01	WHISP	ACGTTGGATGTACACTTGTT-GGAGAGCGTG	ACGTTGGATGGAAGTACAC-GAGGATCCATC	gGGAGAGCGTGGTGTGAG
N-022	II	G/T	275	0_18261_01	WHISP	ACGTTGGATGAGTGTGAAGC-CGAAGATGAG	ACGTTGGATGTCTTCAGCAA-CACCAACACG	gGAAGATGAGATTGGCCA
N-023	II	G/A	80	0_2576_02	WHISP	ACGTTGGATGCAAAATTGAGA-GAAATGCGG	ACGTTGGATGCAGCTCGAC-TGGTATCATTG	ggcTCGGAAAAGAAACAGA

SNP	C*	Type	pos	Amplicon	Seq. set**	Forward amplification primer	Reverse amplification primer	Extension probe
N-024	II	C/T	363	0_3073_01	WHISP	ACGTTGGATGTTTTCTC-TGGGTGGTTTGGG	ACGTTGGATGGTAATTAG-GCACAGGAGGAG	GTTTGGGAAGAAGAAAA
N-028	II	G/T	146	0_4032_02	WHISP	ACGTTGGATGTGACTCTGTT-CTGGACACTC	ACGTTGGATGTGCGCTATT-GCAAGTGTGTC	ccaggCTTCAGTTGATGGTTG
N-029	II	T/C	221	0_6047_02	WHISP	ACGTTGGATGGGGAGGGA-CATCGGCTCTG	ACGTTGGATGCCCTGGAAT-TACAAAGTCCG	GAGGGACATCGGCTCT-GATCATA
N-030	II	G/T	126	0_6448_02	WHISP	ACGTTGGATGACACCCT-TAAGCAGCTTACC	ACGTTGGATGTTTTCTCTC-CAGCTACCTCC	cagATTCTTCTTTCATTT-GAGTC
N-032	II	G/A	352	0_6878_01	WHISP	ACGTTGGATGCTGGATAA-TAATCCTCCCGC	ACGTTGGATGGTGCAACTC-TTCGGAAACTC	aTGGATAATAATCCTCC-CGCAACTGTA
N-033	II	C/A	59	0_7001_01	WHISP	ACGTTGGATGAACAGGCTGT-GCACCATTTC	ACGTTGGATGATAGGAGCT-GCAGGTTCATC	ccgggggcaTTTAATTGAAGAC-CGAG
N-034	II	C/A	94	0_7001_01	WHISP	ACGTTGGATGAACAGGCTGT-GCACCATTTC	ACGTTGGATGTTGGTTT-CATTTCTACAGCC	cccttgACCTGCAGCTCCTATT
N-035	II	C/G	367	0_771_01	WHISP	ACGTTGGATGTGTGCCT-CAATCTTCTCTGG	ACGTTGGATGTGTGTCTCAA-CAGAGAGATG	CTCAATCTTCTCTGGGC-TAATTCTAC
N-036	II	G/A	198	0_846_01	WHISP	ACGTTGGATGTGCTGCTAC-TTCTTTCTGC	ACGTTGGATGTGATGGT-GCAGCTCATTGTG	TAGCCTTTGAACTTCC-TTTTT
N-037	II	A/G	108	0_9408_01	WHISP	ACGTTGGATGCTGATGTAG-AAGCTGCTTCC	ACGTTGGATGTTCAGAGGCT-CTTCTCTACC	GCTGTCATTGAGAAAA
N-038	II	T/C	193	2_2501_01	WHISP	ACGTTGGATGGTGGGAATTC-CATCTGCATC	ACGTTGGATGCTATTCACA-TAGCACTGAGG	aAAAAGCTCTCCTCTACATC
N-039	III	G/A	158	2_3726_02	WHISP	ACGTTGGATGACCAGC-TTTTTGCTCAGCAG	ACGTTGGATGGGGAATGC-CTATTGGAAAAG	GTTGGGAACTTAATGT-CAAA
N-040	III	G/T	243	2_4107_01	WHISP	ACGTTGGATGGCACAGGT-CACAGAAATGAG	ACGTTGGATGTAACGTC-CAGTGAGAGAAGG	cATCTCAAAATCATAATAG-AGCTG
O-001	III	G/A	326	0_15762_01	WHISP	ACGTTGGATGTGCGTC-CAATCTTCGGAATG	ACGTTGGATGTCTTCTGAAC-CACTGATGGC	gggAATCTTCGGAATGAGTG-GCTTTTTT
O-002	II	C/T	152	0_8844_01	WHISP	ACGTTGGATGAGCAGGCAAC-TTTGATCCAG	ACGTTGGATGGATGTTAGTC-CTCCTCCAAG	tGGCATATGGTATGAACATC-CT
O-003	II	G/A	289	2_2952_01	WHISP	ACGTTGGATGAACGGTCG-TACTCTATTTGC	ACGTTGGATGTTCTCTTAA-CAAAAGCTGCC	CACTCAACTTCCGCAG
O-004	II	G/A	317	2_2952_01	WHISP	ACGTTGGATGAACGGTCG-TACTCTATTTGC	ACGTTGGATGTAACAAAAGCT-GCCACCACC	cctgGGGTATTACAAAACC-CAG

SNP	C*	Type	pos	Amplicon	Seq. set**	Forward amplification primer	Reverse amplification primer	Extension probe
O-005	II	G/A	118	2_3465_01	WHISP	ACGTTGGATGAGGTCGC-CATCATGATCATC	ACGTTGGATGGGGACGATAT-GCATATGGAG	cCATCTACACTTGAACT-GAAACATCTC
O-008	II	C/A	155	2_3852_01	WHISP	ACGTTGGATGCCCTGTAGTT-GCTGCAAATG	ACGTTGGATGTGATACAAC-CAACCAGTGTC	cCTTGTAATACTTCTTAG-GATATTGCTCGA
O-009	II	C/T	181	2_3867_02	WHISP	ACGTTGGATGTCC-CGTGTTGTTGACTCTCTC	ACGTTGGATGTCTAA-CAGCACCACAACCAC	GAGGTTGTGATGAATCT-GATGA
O-010	II	C/T	166	2_5483_02	WHISP	ACGTTGGATGCATG-GAATGGTGGCCATTTC	ACGTTGGATGCGACGCTTAC-CTTGTACAAC	tgAAATTGTTACTT-TAAGCGCAC
O-011	III	C/A	264	2_5668_01	WHISP	ACGTTGGATGGAAGAG-GATGTTAAATGGTG	ACGTTGGATGAGGACAGATC-TTCCAATGGC	AATGGTGTACCTTCCTTG
O-012	II	G/A	133	2_5724_02	WHISP	ACGTTGGATGCCGTAGGAA-GAGCATCTATG	ACGTTGGATGGCCACTT-GACGTCAATTTGG	GTATGACACTCAAATATGCT
O-013	II	G/T	267	2_6052_01	WHISP	ACGTTGGATGGAATC-CACAGCATATCCACC	ACGTTGGATGTGTGGGAT-TACGATGAGGTG	ATATCCACCTTTGTGG-GCATGGCCCGCTA
O-014	II	A/G	255	CL1698Contig1_01	WHISP	ACGTTGGATGTTACAGGGCT-CTCCTTTACG	ACGTTGGATGTCCAAAA-CACGCCATGAAGG	TGTACAGGTACAAGTAGCA-GAAAACT
O-015	II	C/T	121	2_6491_01	WHISP	ACGTTGGATGTT-CGCAGCATGTTGAATCAG	ACGTTGGATGAGGTTCCTC-CATTATGCTCC	TCGGCCTTCAGAACTTCAC
O-016	III	T/C	288	2_6731_01	WHISP	ACGTTGGATGGGGTT-GATTTGGAGTCCATC	ACGTTGGATGAATAAA-CAGTGTCCCTCTGG	GTTTGGAAGCTTTCAGAG
O-017	II	T/C	379	2_684_01	WHISP	ACGTTGGATGAGCTCAA-CAAGCAGAAGTGG	ACGTTGGATGGTGAGAGA-GAACAGTGATGG	ggGCAGAAGTGGATCAA-TAGCCTCAGC
O-019	II	C/A	100	2_7213_02	WHISP	ACGTTGGATGGGGAGGTAC-TACATAACCCT	ACGTTGGATGGTGTACAC-TAGTCTCATTAGG	gCCTCTTCAATGCGGAC
O-020	II	G/A	32	2_7532_01	WHISP	ACGTTGGATGGCCCCTGA-TAATGAAAGTCG	ACGTTGGATGTGGCATCTGC-CAGTTGTATC	cgCTAGATGATCTATGGGG-TAGA
O-022	II	T/C	442	2_7852_01	WHISP	ACGTTGGATGTGGATCCATT-TAGACTTCCC	ACGTTGGATGTGGTGAA-CATGTGCATGCTG	tcCTTCCCTTGTAGTAAAA-TACA
O-024	II	C/A	173	2_8627_01	WHISP	ACGTTGGATGCTCGCATG-GAGCAATTAGAG	ACGTTGGATGTTCATAAGG-GCGGTTGTCAC	ccacGCAATTAGAGCAATT-GCAT
O-025	II	G/T	120	2_9466_01	WHISP	ACGTTGGATG-GCATCACAGCATTTCGTGAAC	ACGTTGGATGCAGGGCAT-TATGTCTTCTGG	cgAGAGTATCACGAA-CAATCAGGAA
O-026	II	G/A	121	2_9542_01	WHISP	ACGTTGGATGTGCAAACTC-TAAGAGCATGG	ACGTTGGATGGAGACGGT-TAACTGTTCGTG	AGAGCATGGTCAATGT

SNP	C*	Type	pos	Amplicon	Seq. set**	Forward amplification primer	Reverse amplification primer	Extension probe
O-027	II	C/T	33	2_9665_01	WHISP	ACGTTGGATGTCAGGC-CACACAAGGAAAAC	ACGTTGGATGAAGCTGTTT-CATTCAGGCGG	aaggCACAAGGAAAACTG-GATG
O-028	II	T/C	128	CL1367Contig1_03	WHISP	ACGTTGGATGATTGATGTC-CAGGCATTGGG	ACGTTGGATGTTCTGCTGTA-CAAGTCGAGG	cTGGGGAAAACCTGTTACC-CC
O-029	III	T/A	115	CL1588Contig1_04	WHISP	ACGTTGGATGACCTGTAGGC-TTAAATCTGG	ACGTTGGATGTATGACCTG-GCAGAAGGTCG	CAACATTTCCAAGAAGAC-TTTCTAT
O-030	III	A/T	158	CL1646Contig1_01	WHISP	ACGTTGGATGATGCCATGGG TGTTTGCTAC	ACGTTGGATGTTTGTT-GCATGGTTTGCAGGG	AGGGAGGGAACTTTAATAC
O-032	III	A/G	347	2_3726_02	WHISP	ACGTTGGATGGTTGTGAT-TAAGAGACGAGG	ACGTTGGATGACAAGAGT-GAAGTCCACACC	ggGGAATGTTCAAAGTC-TGGGTTTG
O-033	II	C/T	44	CL1692Contig1_05	WHISP	ACGTTGGATGATTTGATGGTG GCGAATAGC	ACGTTGGATGGATCGTGACA-GACACTGCAT	tgtCGAATAGCAAACTTGCA
O-034	II	G/A	136	CL1767Contig1_02	WHISP	ACGTTGGATGCGAGCGCAC AACCTGTTAAA	ACGTTGGATGACCAGCGGA-TAGGCAATACC	agTACGGTCCCCATAAATAA-CAGGTCTT
O-035	II	C/A	259	CL1852Contig1_01	WHISP	ACGTTGGATGGGTTTTGT-GAGGGATAGTGC	ACGTTGGATGTTCCC-CAAGCATCTGCAAAG	ccTGCATCACAATTTTTCT-TATATTAG
O-036	II	C/A	107	CL1905Contig1_03	WHISP	ACGTTGGATGGAGAAATGCC-TTGCTTGTGG	ACGTTGGATGCCAG-GCAAGTTTACCCAATC	aCCATTGAAAAGGT-CATTATTTTTCA
O-037	III	C/T	160	CL206Contig1_03	WHISP	ACGTTGGATGGGTCATAAT-GCCCATATTCG	ACGTTGGATGGGCATTGAAC-CCATCATTAC	cccAGCTAAATATTTAAAAAA-GAGCTG
O-038	II	C/T	275	CL3097Contig1_01	WHISP	ACGTTGGATGGCAGTTTT-GAAGTAAGAGAG	ACGTTGGATGTTGGCACAAG-GAATTCAAGC	GTGGTATAAAATGTTC-TTTGTTAA
O-039	II	C/T	218	CL3770Contig1_01	WHISP	ACGTTGGATGAGTATGAGGT-CTCTGGATCG	ACGTTGGATGCCATATCCTC-TTCTTGAGGC	gATTGACGCCCTTGAAG
O-040	III	G/C	289	CL3795Contig1_01	WHISP	ACGTTGGATGCCAGTGCA-TAAAGAACGGAG	ACGTTGGATGCCTTGATTGC-TGAAGGAGAG	ccCAAGACAAACCTTCT-CAAT
T-001	II	G/A	295	UMN_5867_01	C.G.G.P.	ACGTTGGATGCCCATTTCC-TACAAGGCAAC	ACGTTGGATGCAATTT-GAGGTGCCCAAGTG	gTCCTACAAGGCAACTTT-GCGGCAACCA
T-002	II	C/T	238	0_13552_02	C.G.G.P.	ACGTTGGATGGAGTCAATCC-CTTCTCATTC	ACGTTGGATGATGAAAACGG-GCTCGTAAGG	acATCCCTTCTCATTCATC-TAAAAATG
T-003	II	C/A	141	2_10059_02	C.G.G.P.	ACGTTGGATGGTCTAGGTTC-CTCCTCAATG	ACGTTGGATGTGCAGGTTGT-GATGCTTTTC	gggTGCGTATAGAATCTTAGA
T-005	III	T/C	149	CL3116Contig1_03	C.G.G.P.	ACGTTGGATGGGGC-CACAAAGTGAAGATTAG	ACGTTGGATGTTGGTT TTGGTTTTAGCCCC	gtAGGGTGAAAACA-TAAATATTCT

SNP	C*	Type	pos	Amplicon	Seq. set**	Forward amplification primer	Reverse amplification primer	Extension probe
T-006	II	G/T	110	CL3539Contig1_01	C.G.G.P.	ACGTTGGATGATGTCTCCT-CAAACACAGGC	ACGTTGGATGGTGAATAA-CAGGGAGAAGGC	ccctcctaAAACACAGGCAAG-TACC
T-008	II	T/C	339	UMN_1590_01	C.G.G.P.	ACGTTGGATGTATCTTA-CACAGCGCCGTTC	ACGTTGGATGTGGATCATG-GCTTTGTGATG	gaACAGTTGTGCCTCCA-GATGTCAC
T-009	III	G/A	271	2_4281_02	C.G.G.P.	ACGTTGGATGATTGCCAC-GAAATTTGCCCG	ACGTTGGATGCCTTCCGG-GAAAAAAATGAG	cGCTTTCGAATTTGACTG-TAAAA
T-013	III	A/T	190	CL3602Contig1_03	C.G.G.P.	ACGTTGGATGTCCATC-TTATTTGCAGTCCC	ACGTTGGATGCAATATACAC-CGGACTTGGC	caTGCAGTCCCTGCTTTT
T-014	III	C/T	82	CL3602Contig1_03	C.G.G.P.	ACGTTGGATGATC-CAAGTTTGGCCAAGTCC	ACGTTGGATGTAGCTTTCT-CGGGATCACTG	ccCAACTCGGCTTCCTTT-GAAAATCAG
T-015	II	G/A	299	2_3720_01	C.G.G.P.	ACGTTGGATGTGCTTCGATT-TACTGTTCGC	ACGTTGGATGCGCG-GAGCAATTTCAGCATT	caGCAGAAACTGGTTTGG
T-017	II	C/A	240	CL866Contig1_01	C.G.G.P.	ACGTTGGATGTCTACCCCAAA-CATCCCAAG	ACGTTGGATGAAAAACCAGT-GCTGTCCGTG	aggGGATAGAGTAAATGTTC-CTAT
T-018	III	C/T	177	UMN_927_01	C.G.G.P.	ACGTTGGATGAGAAACTACAT-GCCCTGCTC	ACGTTGGATGGACATTG-TAGCTGGTGATGG	ggCAGGAAGAGAGCATTC
T-021	III	C/T	376	CL1029Contig1_01	C.G.G.P.	ACGTTGGATGAAGACCTACT-CGTGATTGCC	ACGTTGGATGCTGCAGCAT-TAGCCATCAAG	ctgATTGCCTTTTTGTT-GATGT
T-022	II	G/T	147	CL1430Contig1_06	C.G.G.P.	ACGTTGGATGGCGACATGAT-GCTTTTTTGC	ACGTTGGATGTGCTTGAGG-GCATCCTTGTG	cccaTTTTGCATGTATATAT-GCTTGTTAT
T-023	II	G/C	333	CL1430Contig1_06	C.G.G.P.	ACGTTGGATGGCCCCAAT CACGGTATGTTC	ACGTTGGATGGTTCC-CAAATTCAACCTGAC	ccaCTTTTTGTGAGCATTTT
T-027	II	T/A	186	CL4023Contig1_01	C.G.G.P.	ACGTTGGATGGCTACACCAAT-GCATTTCAC	ACGTTGGATGGCTACAAGAT-GAAGATGGGC	TTGATGTCTTATCTCTGCA
T-032	III	T/C	139	0_1688_02	WHISP	ACGTTGGATGCTAGACAACC-TTCTTCATTCC	ACGTTGGATGATGGAGAATCT-CAGTGTTGC	gGACACAGATAAACATACCC-CGTA
T-033	III	C/T	525	0_1688_02	WHISP	ACGTTGGATGCGAAAA-CAGGTGGCTTTTGG	ACGTTGGATGATATTG-GCAGCCTTCAGGTC	cagggg CAGGTGGC-TTTTGGTGCAGCT
T-034	II	C/A	235	0_350_01	WHISP	ACGTTGGATGTGTGGAGG-GAAATATTGCAG	ACGTTGGATGCTCTAGT-TATGTTTGGGCTTG	tTTACAGAAAGAAAA-CAAAAATTACC
T-035	II	C/G	188	2_3591_03	WHISP	ACGTTGGATGAGCAGCAATAA-CTCAACCCC	ACGTTGGATGCTCCAA-GATTTGGAAGCCTG	cACCCCATAAAAATTTTCAG-TAA
T-036	II	T/C	57	2_8852_01	WHISP	ACGTTGGATGAAAGCAACT-GCACAACCACC	ACGTTGGATGGGTTTGTCAA-GATAGTGGAG	aCACCCCATCCAGCTCCAGT

*C= Category (According to Nadeau et al. 2015 " I: 'orthologous genes' between P. strobus and P. monticola,, II: 'SNPs of orthologous genes' occurring within putative orthologus genesbut having different SNP positions among species; III: 'single-species SNPS' with no detected or successful SNP in the corresponding ortholog of P.monticola", **Seq. set: sequence set (C.G.G.P.= candidate gene for growth and phenology, C.G.W.F.= candidate gene for wood formation, WHISP= White Pine Resequencing Project) (Nadeau et al. 2015)

Table 6b: Primer pairs designed for the closely related *Pinus lambertiana, Pinus taeda Pinus pinaster, Pseudotsuga menziesii* were also tested by PCR for amplification of SNP loci (Nadeau et al. 2015) (ID=Primer ID, Ampl.=amplicon, T_a=annealing temperature, Sel. SNP=selected SNP, SNP gen.=SNP genotype)

ID	Gene ID	Primer forward	Primer reverse	Amp.	T_a (°C)	Sel. SNP	SNP gen.	Primer source
55	0_10240_01	TGACGGTCTACATAGG	CTCCCATTTTCTTCCC	Y	53	Y	Y	Eckert et *al.* (2010b)
56	0_13552_02	AAACCCTGCGATGGTTG	ACTTGAATCCTTCTGGTG	Y	55	Y	Y	Jermstad *et al.* (2010)
14	0_18132_01	GGGCATTACTTCTTTCAC	CAATACATCGAGGAGG	Y	55	Y	Y	Jermstad *et al.* (2010)
85	2_10059_02	GGAAAACAAAGGATAACAGGAG	CCAGTTCGACGTGTAAAG	Y	55	Y	Y	Jermstad *et al.* (2010)
15	2_3720_01	AATCTCGGCTCCCTTTC	AAAAGCTCAAGGCGTG	Y	55	Y	Y	Jermstad *et al.* (2010)
11	2_4281_02	ACAAAGTGTGCAGCATC	CTCAACTACCCATCATTCTC	Y	58	Y	Y	Jermstad *et al.* (2010)
12	2_4724_01	TGTTCTCTGGATCGGAAAGGG	AAACTGGTAGATTTTGGGCTGGCAAGGG	Y	55	Y	Y	Jermstad *et al.* (2010)
17	2_9280_01	TGTTGGGCACTAAGAGG	AAGTTGGAGATCAAGTTGAGG	Y	55	Y	Y	Jermstad *et al.* (2010)
52	2_9480_01	GCTGCTCATCTTATTTGTC	GTAACATTCACCCAGG	Y	53	Y	Y	Jermstad *et al.* (2010)
8	CL1029Contig1_01	CTCCTAACAATTCCACATC	GTACTGCGAGCACAATTTCC	Y	55	Y	Y	Jermstad *et al.* (2010)
45	CL1430Contig1_06	CATCTCTTCACAGTCATC	TTCTTTGGCTATCAGGCTC	Y	55	Y	Y	Jermstad *et al.* (2010)
78	CL3116Contig1_03	CAACTTTCCGAATTTCTTCC	CAGGTTACTTTTCACAGG	Y	55	Y	Y	Jermstad *et al.* (2010)
46	CL3539Contig1_01	GATGAGATGGTGAAGATG	GTGGAGGAGTGAATATTG	Y	55	Y	Y	Jermstad *et al.* (2010)
84	CL3602Contig1_03	AGCAAGTCCAACAAGC	CTTCTTTTTCCACCTTTCC	Y	56	Y	Y	Jermstad *et al.* (2010)
1	CL4023Contig1_01	GAAGATGTTAGATTGATAGGTGTGG	TAAGGAAGCTGTGCTCTGG	Y	60	Y	Y	Jermstad *et al.* (2010)
3	CL4138Contig1_01	ATAACAACCACATCCAAACC	TGCAAGCAGCCCAAAAAGAAAAAG	Y	58	Y	Y	Jermstad *et al.* (2010)
60	CL4470Contig1_01	CCTCATCTACCCATATTAC	GATCCAGACAGACATGCAG	Y	55	Y	Y	Jermstad *et al.* (2010)
4	CL866Contig1_01	GCTGTAACTGTAGGTTTTGATG	TGCAATGGATGGAGGAC	Y	55	Y	Y	Jermstad *et al.* (2010)
61	UMN_1142_01	TTGGGGGGATTGAGTAG	GAAAAACTGTAGGTGAATGCACAAG	Y	53	Y	Y	Jermstad *et al.* (2010)
86	UMN_1590_01	CGATGCCTTTTTTAAGTCAG	CGAGAATAGGATTTTCAGGAAG	Y	60,8	Y	Y	Jermstad *et al.* (2010)
9	UMN_2399_01	CGTCTGGAATGTGAAGAAGTATTG	TTACTAGGGTTTCTAGGGTTTG	Y	55	Y	Y	Jermstad *et al.* (2010)
62	UMN_4361_01	CCTTCTATTTGAATCCCTTG	CATAGTAACAGCCTACAG	Y	55	Y	Y	Jermstad *et al.* (2010)
47	UMN_5867_01	GGATGTAGTTGAGTGG	TCTGGACCCCTTCATTG	Y	53	Y	Y	Jermstad *et al.* (2010)
63	UMN_927_01	GCAATGAGGGATTGAATTAC	TTGGAAGAATACAAGGCAGG	Y	55	Y	Y	Jermstad *et al.* (2010)
48	0_10049_02	CTAGCCATGTGAAATCC	TCTCATACCCATCTCC	Y	53	Y	N	Jermstad *et al.* (2010)
25	0_12021_01	GCACAATAGATGGAGAGCAAAC	CGCCTACATCATCCTAACATTACAGAAC	Y	55	Y	N	Jermstad *et al.* (2010)
40	0_382_01	TTTAGGTCCTCCTGCTG	TATGAGAATCGAGAAAGACTGGATG	Y	58	Y	N	Jermstad *et al.* (2010)
65	0_8850_01	TGATTCAGGAATAGCGACATGAAC	TTGGCAAGGCAATTTTGAAGCATGGG	Y	60	Y	N	Jermstad *et al.* (2010)
26	0_8850_02	GAGCCATATCAGTCAG	CAAAAATGCCGAATCCC	Y	55	Y	N	Jermstad *et al.* (2010)
75	2_1818_01	CCCGAATCCAAACAGAAC	GACCTCCCAGACCATTATTCC	Y	55	Y	N	Jermstad *et al.* (2010)
27	2_1850_01	TACGGTGGAGTAGAGGGATG	TGGTATGACACTGCTGGAAATAATTGG	Y	55	Y	N	Jermstad *et al.* (2010)

ID	Gene ID	Primer forward	Primer reverse	Amp.	T_a (°C)	Sel. SNP	SNP gen.	Primer source
49	2_2015_02	CCTCAATCAAGCATCC	CATCGCCTCTTCAAAC	Y	53	Y	N	Jermstad *et al.* (2010)
41	2_4892_01	CAACAATCATTCCAGGAG	AACAACAACAACAGCAGCAAC	Y	58	Y	N	Jermstad *et al.* (2010)
66	2_9930_01	CCCTAACATCATCAACAACATCATC	ACAACATCTCCACAAGCTCAAC	Y	55	Y	N	Jermstad *et al.* (2010)
28	CL1019Contig1_01	CAGGCTTGAAGAATCTTAGCAAAAC	GGGGATTCAAATATGCTGG	Y	55	Y	N	Jermstad *et al.* (2010)
68	CL1530Contig1_04	GGGGAGATAAGAAAAAGAAGAAGAG	CGGAAGAACAAGTTTAACAGCAG	Y	55	Y	N	Jermstad *et al.* (2010)
69	CL1933Contig1_06	GTGGAAGAGAGAAACTTG	GAAGAGGGAATGAGATG	Y	53	Y	N	Jermstad *et al.* (2010)
29	CL2117Contig1_03	ATGCAGCAACTCCAAC	GGATTTTGAGGAGAGTAAAG	Y	53	Y	N	Jermstad *et al.* (2010)
82	CL304Contig1_01	TGACGGTGAACAGGAAG	CGGGGAAATACGAGATGAAG	Y	58	Y	N	Jermstad *et al.* (2010)
31	CL3162Contig1_02	GTGTGATTTCCATTGCC	GCTTGAAGAATTGAGAAACC	Y	56	Y	N	Jermstad *et al.* (2010)
32	CL3363Contig1_04	CAACACCCAACTTTCTTC	GCATTTGCAGAACGAG	Y	53	Y	N	Jermstad *et al.* (2010)
44	CL4284Contig1_01	CAGAAATGGTTGGCAG	AATGTCACAGTGGTGG	Y	53	Y	N	Jermstad *et al.* (2010)
51	CL4552Contig1_01	GTCTATGTTCTCTTCTGG	CATGTTAGATACTCAAGAGG	Y	56	Y	N	Jermstad *et al.* (2010)
13	CL594Contig1_06	GGAATTGGATATTGAGGG	TGTGTGGTATTGGGTC	Y	53	Y	N	Jermstad *et al.* (2010)
74	CL708Contig1_02	TACCAGCAGAATAAGCAAG	ATTGAGATTATGATCCACCCAC	Y	55	Y	N	Jermstad *et al.* (2010)
83	CL730Contig1_04	CTGGTGCTTGGTCGTAAAAAAATTC	GCTATCAATGCTAATAACAACGGTGGTC	Y	58	Y	N	Jermstad *et al.* (2010)
88	UMN_6899_04	CGGCACGAGGATGAATTTCAAGAGAAAG	TTTGCGTAGCGGGGATAGAG	Y	55	Y	N	Jermstad *et al.* (2010)
57	0_12517_01	CACATGCTCTTGATGAGG	TTGGTGCTATGGGCTTTGG	Y	55	N	N	Jermstad *et al.* (2010)
58	0_18830_01	ACACTGCTTTCACCCTTCC	TACAGTTCACCATTCCGCACCC	Y	62,6	N	N	Jermstad *et al.* (2010)
33	0_2643_01	GGACAAATCCCTTTGAAC	AGCTTCAATGGCTGCTG	Y	53	N	N	Jermstad *et al.* (2010)
87	0_3046_01	TATCTTGGGCAGTGGTC	CGCCTCTTCTTATTCATC	Y	54	N	N	Jermstad *et al.* (2010)
79	0_439_02	GGATTCTTATGAGAAACTGG	TTTTTGTGTGGCTGCGG	Y	60	N	N	Jermstad *et al.* (2010)
59	0_6566_02	GCTCAAAAGAGGGGACTTTATTTAC	CATGCTAACAGATTACTCAC	Y	60,8	N	N	Jermstad *et al.* (2010)
38	2_1405_01	CATTTTGCAGAGGCAAG	TGCGTAAGGCAGAACAG	Y	58	N	N	Jermstad *et al.* (2010)
16	2_5996_01	AAGCCAGGAGTGAACATAAG	ATTTATTTCATAAGAACAGCCCCCGAG	Y	55	N	N	Jermstad *et al.* (2010)
34	2_6413_01	TCTTCATCCATAGCTCCATGTC	GAACAGCACTGGAACTGGCAATAC	Y	62,6	N	N	Jermstad *et al.* (2010)
37	CL1104Contig1_03	GGTTGTGTTCTCCTTC	AGTGCCAGCAATCAGTG	Y	56	N	N	Jermstad *et al.* (2010)
35	CL1920Contig1_01	TGGCAACTTTGTTGGGG	TATGGGGTGAGAAGAGG	Y	53	N	N	Jermstad *et al.* (2010)
18	CL382Contig1_06	GATATGGAGTATGGGG	GGATGGGAAGATTTCAC	Y	53	N	N	Jermstad *et al.* (2010)
2	CL4354Contig1_01	AGAGATTCCCCATCCAC	TTACAGACCTTTTTGACTACTTCCC	Y	60,8	N	N	Jermstad *et al.* (2010)
19	CL813Contig1_03	CAGACAGAGTCATTAATCTCTCAG	TGAAATTATTGTGGATGGGG	Y	58	N	N	Jermstad *et al.* (2010)
36	CL814Contig1_06	GACTAAGATATGGCTGAGG	GAAGGATATTATAGGGCTTTTGAGG	Y	60,8	N	N	Jermstad *et al.* (2010)
20	CL91Contig1_01	CCAGAAATAAGTAATCCTCAAGCC	AAATCAGACTTTAGAGAGCC	Y	58	N	N	Jermstad *et al.* (2010)

ID	Gene ID	Primer forward	Primer reverse	Amp.	T_a (°C)	Sel. SNP	SNP gen.	Primer source
77	CN637306.1	CTAAACAATGGGAAGGG	ATCTCGTTGTCCGTTC	Y	53	N	N	Eckert *et al.* (2009)
39	dhn2	CTGCAGAGACTGTGCCTGAGC	CCAGGGAGCTTTTCCTTGATCT	Y	55	N	N	Eveno *et al.*(2008)
21	UMN_3055_01	CATCTGGTTTCTCTGG	CTGTGCTTCAAATCTGTC	Y	55	N	N	Jermstad *et al.* (2010)
5	UMN_6852_02	TTCCTCCCCTTCATTC	CAACTGCTTCAAATACGG	Y	53	N	N	Jermstad *et al.* (2010)
22	0_1320_01	TGTCGGTCGGATTCTAC	CTGCCTGAGAAACTTG	N	n.a.	N	N	Eckert *et al.* (2010a)
23	0_16015_01	GCATTCTAGCTGTGTTC	CTATTGTTTTGTTGCCCC	N	n.a	N	N	Jermstad *et al.* (2010)
10	0_17419_03	GCTATTCAACGACGAGG	GGGCATAGCAAGTCAAG	N	n.a	N	N	Jermstad *et al.* (2010)
76	0_5488_01	AGTGTTTTGGTCGAGGAATACAGTGGG	CGGCGTTGTGTCGTGTGCTGATATAG	N	n.a	N	N	Eckert *et al.* (2010a)
64	0_9082_01	CAGTTGCATATCGAGAAG	TGCTCTGTTTCAGTCC	N	n.a	N	N	Jermstad *et al.* (2010)
6	2_3141_01	GTTTTCATATTTGGCTGCTC	GTGCTCTCAGTTAGAAG	N	n.a	N	N	Jermstad *et al.* (2010)
7	2_6618_01	ATGACTGCCGCAAAGTAACCACCC	CAAGTTCCTATTGATGCTCTTTTCC	N	n.a	N	N	Jermstad *et al.* (2010)
42	2_7961_01	CCCAAGCTAAGGAAAGGCCT	CCTGGCCGTTTTTCATCATG	N	n.a	N	N	Jermstad *et al.* (2010)
54	4cl-Pta	TCTGGCTCCTGCGGAACAGT	AGGAACGACTGCTGCGTCAG	N	n.a	N	N	Grivet *et al.* (2011)
67	CL1045Contig1_03	GTTGGCATCACAGTATG	GTGGAAAATGGGTTGG	N	n.a	N	N	Jermstad *et al.* (2010)
43	CL1061Contig1_03	TTCGGAAGATGCGACAG	TGGATGGAGGTGGAAAG	N	n.a	N	N	Jermstad *et al.* (2010)
80	CL1205Contig1_04	AACTCGGTGTTTTCGG	ATCTTGGTCTCAGGGTC	N	n.a	N	N	Jermstad *et al.* (2010)
70	CL1437Contig1_03	GGAGGAAAGAAGAAAGG	TGTGGTGGGCTTGTAAC	N	n.a	N	N	Jermstad *et al.* (2010)
30	CL1888Contig1_02	GTCCATATCATTGTCAACC	ATGTGTCCACCATTGC	N	n.a	N	N	Jermstad *et al.* (2010)
71	CL1888Contig1_03	GGAAGAGGTATGATGG	GTCAGCTATTGATGTTGAG	N	n.a	N	N	Jermstad *et al.* (2010)
50	CL2108Contig1_02	GCCATAAAGGATGACCAG	TTGGGGGACAAGGATTC	N	n.a	N	N	Jermstad *et al.* (2010)
72	CL2166Contig1_01	AGTCTCTCTCCTTGTG	CTGTCTTCCTCTATATGTTC	N	n.a	N	N	Jermstad *et al.* (2010)
81	CL2172Contig1_09	GATTTGCTGGAGATGATG	TGAGGAGAATAGGGTG	N	n.a	N	N	Jermstad *et al.* (2010)
73	CL3949Contig1	GCGTAATGGTAAAGGG	GGTCAAGTTGTAGAGG	N	n.a	N	N	Eckert *et al.* (2010b)
53	CL4737Contig1_03	GATTGGGGGACTTATG	AAAGATCGAGAGCAGG	N	n.a	N	N	Jermstad *et al.* (2010)
95	CL533Contig2_04	CAAAATGGTGGGAAGAG	AAAGGAATGGGTGCGAG	N	n.a	N	N	Jermstad *et al.* (2010)
96	dhn1	GAAGAAAGGGTCGAAGGACAA	GTGCTTTCCATCACCAGG	N	n.a	N	N	Eveno *et al.* (2008)
94	LEA-EMB11	CTCCGGGTATACAGCTCGC	GACTTTCTTGAAAGAAGCTTCTGC	N	n.a	N	N	Eckert *et al.* (2009)
89	UMN_1037_01	CATCTTCCATTTCCCC	TTGCTGCAACTTCCAC	N	n.a	N	N	Jermstad *et al.* (2010)
92	UMN_2415_03	TGTATTCGTTTTCCAGG	ACCCACGAAAAATCAAACAG	N	n.a	N	N	Jermstad *et al.* (2010)
90	UMN_4156_02	CAGTTTCATCTCCACTACCTTTTGTC	GTAATACGTGTGTGTCTGGCGTCGTTC	N	n.a	N	N	Jermstad *et al.* (2010)
91	UMN_5272_01	AGACTGTTGAGAGAGCC	TATGCACCATCTTGACAAAATTGCC	N	n.a	N	N	Jermstad *et al.* (2010)
93	UMN_L309Contig1_03	GCGATTTCGGGTATGTTCTATG	AGTGGACTTTCTGGCTG	N	n.a	N	N	Jermstad *et al.* (2010)

Material for DNA-extraction

- Haploid megagametophytes (Nadeau et al. 2015)
- Vegetative buds (Nadeau et al. 2015)
- Needles (Nadeau et al. 2015)

DNA-extraction protocols

Nadeau et al. (2015) reported that 'DNA was extracted from buds or current-year needles using the Nucleospin 96 Plant II kit (Macherey-Nagel, Bethlehem, Pennsylvania, USA) with the following modifications made to the manufacturer's protocol: (1) cell lysis using buffer PL2 at 65° C for 2h (instead of 30 min); and (2) elution with an in-house 0.01 mM Tris-HCl pH 8.0 buffer'.

Amplification Protocol

As reported by Nadeau et al. (2015) 'for PCR amplification of the candidate genes, about 15 ng of *P. strobus* megagametophyte DNA was added to a 30 μL reaction volume containing 1×PCR buffer, 1.66 mM MgCl 2, 0.133 mM of each dNTP, 0.133 μM of each primer, 1U of Platinum Taq polymerase (Invitrogen, Burlington, Ontario, Canada). DNA regions were amplified using a PTC200 Thermal Cycler (MJ Research, Waltham, Massachusetts, USA) according to the following protocol: an initial 3 min step at 94° C; 35 cycles of 1 min at 94° C, 45 s at the annealing temperature, 3 min at 70° C; and a final 10 min step at 72° C'.

Important results

Nadeau et al. (2015) investigated the influence of two physiographic landscapes on the structure of populations and the post glacial colonization of two white pine species growing at contrasting habitats; *P. monticola*, growing in high mountainous regions of western North America, and *P. strobus* occurring in much less mountainous areas of eastern North America. They studied 133 *Pinus strobus* populations and 61 *Pinus monticola* ones, by using a big number of Single Nucleotide Polymorphisms (SNPs). They interpreted the steep latitudinal decrease in genetic diversity for *Pinus monticola* as a possible result of the postglacial colonization that involved long-distance dispersal evens, while the lack of patterns in diversity of *P. strobus* was attributed to recolonization through a gradually advancing front or frequent long-distance dispersal events. For both species, two distinct northern and southern genetic groups were identified that most probably originated from two different glacial lineages. The smaller groups detected by the authors mentioned above were characterized as remnants of cryptic refugia.

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Molecular markers used for genetic studies in Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco)

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1. General remarks

Douglas-fir (*P. menziesii* (Mirb.) Franco) has one of the widest natural ranges of any tree species and the largest south-to-north distribution of any commercial conifer in North America, extending from 19°N in Mexico to 55°N in Western Canada. In Western Oregon and Washington it grows from sea level to 1700 m (Hermann and Lavender 1990). Within this large geographic area, contrasting climatic conditions are found. Douglas-fir populations seem to have a great adaptive potential and are generally regarded as being adapted to the different environments (St. Clair et al. 2005, St. Clair 2006, Gould et al. 2012). Winter temperatures and frost dates are of great importance for the adaptation of Douglas-fir, whereas summer drought is less important (St. Clair et al. 2005). Douglas-fir is a wind-dispersed and wind-pollinated species with high gene flow and outcrossing rates exceeding 90% (e.g. El-Kassaby et al. 1981, Shaw and Allard 1982, Neale and Adams 1985, Yeh and Morgan 1987).

Two taxonomic varieties are recognized: the coastal variety (*P. menziesii* var. *menziesii* or *viridis*) found along the North American Pacific Coast and the interior variety (*P. menziesii* var. *glauca*) found inland, in the mountains from British Columbia to Central Mexico (Lavender and Hermann 2014). There is no reproductive barrier between them (Gugger et al. 2010, Wei et al. 2011, van Loo et al. 2015). In Europe, a third variety, *caesia,* is widely recognized as an intermediate type between the coastal (*viridis*) and the interior (*glauca*) variety (Schober 1954, Aas 2008).

The varieties differ in a number of important traits: The coastal variety grows faster and gets considerably taller than the interior variety, which tends to be more shade tolerant and more cold-hardy (Lavender and Hermann 2014). Within the coastal variety trees from the coastal areas are less cold hardy than trees from the western slope of the Cascades (e.g. Aitken et al. 1996). Variation in bud-burst, emergence and growth is strongly related to elevation and cool-season temperatures (e.g. St.Clair et al. 2005). Besides the high phenotypic diversity, Douglas-fir shows high genetic variation.

Here, we provide a review of available laboratory techniques that can be used to investigate the genetic diversity and structure in natural and artificial Douglas-fir populations and to trace back the origin of European Douglas-fir plantations.

2. Isozymes

Since the beginning of 1970s, isozymes have been used for genetic analysis on Douglas-fir. Studies with isozymes focused on:

- investigation of the genetic structure in natural

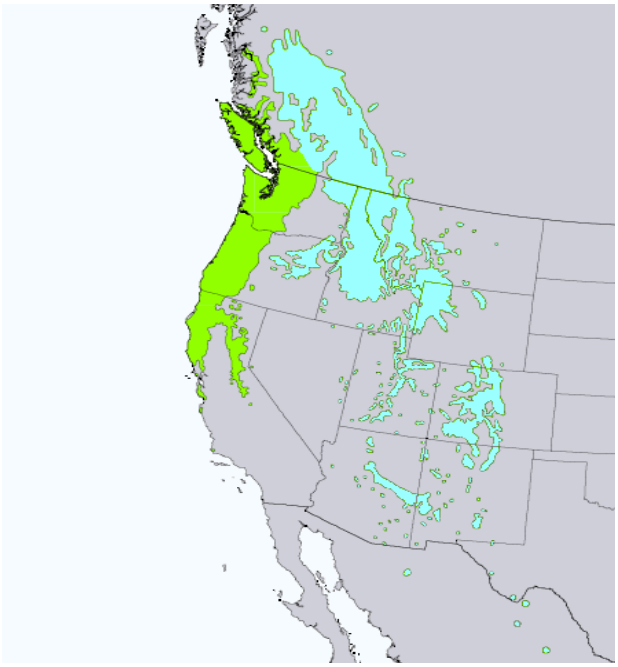

Figure 1. Native distribution range of Douglas-fir (green colour denotes var. *menziesii*, blue colour denotes var. *glauca)* (source: USGS, USA)

populations (e.g. Yang 1974, Neale 1984, El-Kassaby and Ritland 1996a,b, Krutovsky et al. 2009);
- investigation of the genetic structure in planted stands in Europe (Prat and Arnal 1994, Stauffer and Adams 1993, Konnert and Ruetz 2006);
- study of the mating system in Douglas-fir stands (Neale and Adams 1985) and seed orchards (e.g. Shaw and Allard 1982) including determination of effective pollen dispersal distance (e.g. El-Kassaby and Davidson 1991, Prat 1994) and pollen contamination rates (Adams et al. 1997);
- delineation of Douglas-fir breeding zones (Westfall and Conkle 1992, Merkle and Adams 1987);
- differentiation of Douglas-fir varieties (Yeh and O'Malley 1980, Klumpp 1999, Leinemann 1996, Leinemann and Maurer 1999, Konnert and Ruetz 2006, Konnert and Fussi 2012, Fussi et al. 2013);
- genetic differences between resistant and susceptible forms of interior Douglas-firs to western spruce budworm (Chen et al. 2001);
- the influence of forest management on the genetic structure of stands (Adams 1998, Neale 1984);
- tracing back the origin of artificial stands in Europe (Klumpp 1999, Konnert and Ruetz 2006, Prat and Burczyk 1998, Fontes et al. 2003).

Material for protein extraction

Proteins were extracted from the following tissue types:
- seed tissue (megagametophytes, embryo, radicles) (e.g. Adams et al. 1990, El-Kassaby et al. 1981, 1982, El-Kassaby and Davidson 1990, 1991, Fussi et al. 2013, Klumpp 1999, Konnert and Ruetz 2006, Krutovskii et al. 2009, Li and Adams 1989, Merkle and Adams 1987, 1988, Prat and Arnal 1994, Stauffer and Adams 1993, Yeh and O'Malley 1980);

Table 1: List of enzymes, scored loci and number of alleles for genetic studies in Douglas-fir (in the table only references are included where the respective enzymes were described for the first time and/or information on interpretation of zymogrames is given)

Enzyme system	E.C. Number	Scored loci	No. of alleles	Reference
Aconitase	4.2.1.3	ACO-A,-B	3,5	1,3,4,5,6
Aspartate aminotransferase or Glutamate oxaloacetate transaminase	2.6.1.1	AAT(GOT)-A,-B,-C	3,6,6	1,2,3,4,5,6
Acid phosphatase	3.1.3.2.	APH-B	4	6
Alcohol dehydrogenase	1.1.1.1	ADH-A	3	4
Aldolase	4.1.2.13	ALD-A	3	4
Catalase	1.11.1.6	CAT-A	6	1,5
Diaphorase	1.6.2.2	DIA-A,-B	2,5	1,3,4,5,6
Esterase	3.1.1.1	EST-A	4	4,6
Fluorescent esterase	3.1.1.2	FEST-A,-B	4,3	1,3,5
Glucose-6-phosphat dehydrogenase	1.1.1.49	G6PDH-A	7	1,3,4,5,6
Glutamate dehydrogenase	1.4.1.2	GDH-A	4	1,3,4,5
Glycerate dehydrogenase	1.1.1.29	GLYD-A	4	1,5
Isocitrate dehydrogenase	1.1.1.42	IDH-A	9	1,2,3,4,5,6
Leucine aminopeptidase	3.4.11.1	LAP-A,-B	7,6	1,2,3,5
Malate dehydrogenase	1.1.1.37	MDH-A,-B,-C,-D	4,4,6,5.	1,3,4,5,6
Malic enzyme	1.1.1.40	ME-A,-B	2,3	4,6
Peptidase	3.4.13.1	PEP-B,-C	3,1	1,4
Phosphoglucomutase	2.7.5.1	PGM-A,-B	6,7	1,3,4,5,6
Phosphoglucose isomerase	5.3.1.9	PGI-A,-B	3,5	1,3,4,5,6
Phosphomanose isomerase	5.3.1.8	PMI	3	5
Shikimate dehydrogenase	1.1.1.25	SKDH-A	5	1,2,3
Superoxide dismutase	1.15.1.1	SOD-A	6	1,4,5,6
6-Phosphogluconate dehydrogenase	1.1.1.44	6PGDH-A,-B	6, 4.	1,2,3,4,5,6

1-Adams et al. 1990, 2-Klumpp 1999, 3-Moran and Adams 1989, 4-Yeh and O'Malley 1980, 5–Krutovsky et al. 2009, 6-El-Kassaby et al. 1982

- buds (e.g. Adams et al. 1990, Fussi et al. 2013, Klumpp 1999, Konnert and Ruetz 2006, Leinemann 1996, Leinemann and Maurer 1999, Moran and Adams 1989);
- needle tissue (Neale et al. 1984);
- bark (inner cambium) (Copes 1978).

Protein extraction and separation protocols

Details on extraction procedures are given in nearly all references cited below. For electrophoretic procedures and staining recipes, see the following references: Adams and Joly (1980), Adams et al. (1990), Cheliak and Pitel (1984), Conkle et al. (1982), Davis (1981), El-Kassaby et al. (1982), Konnert (2004), Merkle and Adams (1988), Mitton et al. (1979), Neale et al. (1984), Shaw and Prasad (1970), Yeh and O'Malley (1980).

Important results

- Coastal and interior Douglas-fir can be clearly differentiated based on allozymes. Besides the high genetic distances between populations from the two varieties (Li and Adams 1989), the assignment to the coastal or the inland type is possible on the basis of the frequency of specific "marker-alleles", namely 6-PGDH-A3 and A6, PGM-A4 and A6 and LAP-A2 (Yeh and O'Malley 1980, Merkle and Adams 1987, Li and Adams 1989). The allele 6-PGDH-A3 is more common (frequency > 90 %) in the coastal populations than in the interior ones. On the contrary, the allele 6-PGDH-A6 has larger frequencies (> 60 %) in the interior Douglas-fir populations. The allele PGM-A4 has a frequency of over 80 % in the coastal type. In the interior, type PGM-A4 declines in favour of PGM-A6. These findings can be used to assign artificial European Douglas-fir stands to a specific variety – coastal or interior - by analysing the genetic structure at the loci 6PGDH-A and PGM-A (Stauffer and Adams 1993, Leinemann 1996, Klumpp 1999, Leinemann and Maurer 1999, Konnert and Ruetz 2006, Konnert and Fussi 2012, Fussi et al. 2013, Milenkova et al. 2018), but also to a specific region within a variety (Klumpp 1999, Fussi et al. 2013).
- In its natural range, Douglas-fir has an enormous amount of genetic diversity but more than 95% of it resides within populations rather than between them (Yeh and O'Malley 1980, Merkle and Adams 1987, Li and Adams 1989, Moran and Adams 1989, El-Kassaby and Ritland 1996a). Artificial populations of Douglas-fir in Europe do not appear strongly different in genetic diversity from the natural ones as long as a sufficient number of trees are involved in the original seed collection (Prat and Arnal 1994, Konnert and Ruetz 2006, Stauffer and Adams 1993, Prat and Burczyk 1998).
- The two varieties of Douglas-fir, var. *glauca* and var. *menziesii* are completely interfertile. The natural regeneration in mixed stands with both Douglas varieties shows a specific genetic composition, which indicates that hybridization between varieties has taken place (Konnert and Fussi 2012).
- As long as the number of parental trees is not too small, forest management has no substantial influence on the genetic structure of the next generation in naturally regenerated stands (Adams et al. 1998, Neale 1985).
- Based on isozyme analysis, the following characteristics of the mating system have been determined: high gene flow (El-Kassaby and Ritland 1986a) and outcrossing rates exceeding 90% (e.g. El-Kassaby et al. 1981, Shaw and Allard 1982, Neale and Adams 1985, Yeh and Morgan 1987). The effective pollen dispersal distance was estimated to be around 30 m. Within 30 m, mating success is only weakly related to distance (Erickson and Adams 1989). The reproductive phenology affects significantly the mating system and induces a larger part of crossing between individuals of the same phenological (early, intermediate, late flowering) class (El-Kassaby and Ritland 1986b). Crossing between related trees might represent a major part of mating system in natural populations of Douglas-fir. This could also explain the observed fixation index, which is often higher in progenies than in old stands (Prat and Arnal 1994).
- Early selection and breeding of this highly polymorphic species do not reduce genetic variation significantly if the number of clones is not too low (El-Kassaby and Ritland 1996b).
- Allozymes have been succesfully used to estimate pollen contamination in seed orchards (El-Kassaby and Ritland 1986a,b).
- Chen et al. (2001) studied genetic variation of 25 protein loci in interior Douglas-fir trees that were phenotypically resistant or susceptible to defoliation by the western spruce budworm (*Choristoneura occidentalis* Freeman). They found frequency differences in the most common alleles, as well as differences in the proportion of homozygotes for different allozyme loci (e.g. FEST-1, ACO-1, 6PGDH-1). Chen et al. (2001) suggest that the phenotypic differences in resistance of interior Douglas-fir to *Ch. occidentalis* defoliation are partly caused by genetic differences among trees.

3. Organelle DNA markers (chloroplast (cp)DNA, mitochondrial (mt)DNA)

Loci and primers used

CpDNA and mtDNA (RFLP, PCR-RFLP, direct sequencing studies) have been carried out to study phylogeography of Douglas-fir (Gugger et al. 2010, 2011, Wei et al. 2011) to evaluate pollen contamination and natural selfing in a Douglas-fir seed orchard (Stoehr et al. 1998) and to study the gene flow and genetic variability in natural populations of Douglas-fir (Ponoy 1993, Nelson et al. 2003).

Hipkins et al. (1995) analysed a length-mutation hotspot in the chloroplast genome of Douglas-fir, whereas Tsai (1989) used restriction mapping and DNA sequencing to characterize dispersed repetitive DNA in the chloroplast genome of Douglas-fir. Also chloroplast microsatellites (cpSSR), originally designed for *Pinaceae* by Vendramin et al. (1996) and Provan et al. (1999), have been applied in Douglas-fir genetic studies. Using cpSSRs, Viard et al. (2001) studied the genetic variation in adult natural populations of Douglas-fir from British Columbia in comparison with biparentally inherited markers (isoenzymes, RAPDs), whereas Angelier et al. (2011) investigated the genetic quality of natural regeneration in a mixed stand with Douglas fir, Japanese larch and white fir.

Ponoy et al (1993) digested the cpDNA with the following six restriction enzymes: *BamHI, EcoRI, EcoRV, HindIII, SstI, XbaI* and used 24 enzyme-probe combinations to evaluate the genetic variation of cpDNA. Primers used for PCR-RFLP and DNA-sequencing are introduced in Table 2.

Material for DNA-extraction

DNA was extracted from needle or bud tissue (Tsai 1989, Stoehr et al. 1998, Nelson et al. 2003, Gugger et al. 2010, 2011, Wei et al. 2011), embryos (Stoehr et al. 1998), whole seed (for cpDNA) or megagametophytes (for mtDNA) (Wei et al. 2011, Ponoy 1993).

DNA-extraction and amplification protocols

Total DNA has been extracted:

- from needles using the NucleoSpin^{R} 96 Plant II (Macherey.Nagel) kit (Wei et al. 2011) and the DNeasy Plant Mini Kit (Qiagen) (Guger et al. 2010);
- from seed tissue using DNeasy Plant Mini Kit

Table 2: Primer information of the cpDNA-markers and mtDNA-markers used for PCR- RFLP analysis and DNA sequencing in Douglas-fir

Type	Locus	Method used/ restriction enzymes	Primer sequences 5‘ – 3‘	Ref.	Source of primer pairs
cpDNA	trnD-GUC trnS-GCU	*PCR_RFLP/Eco*RI	F:TCTAGAAAGGCACTGGCTATCGATC R:TGATAATTCTAGGCTTTCTAGTTCA	1	Hipkins et al. 1995
	trnD-GUC trnY-GUA	*PCR_RFLP/Eco*RI	F:TCTAGAAAGGCACTGGCTATCGATC R:ATGCCTACGCTGGTTCAAA	1	Hipkins et al. 1995
	trnE-UUC trnS-GCU	*PCR-RFLP/Eco*RI	F:TGCCTCCTTGAAAGAGAGATGTCC R:TGATAATTCTAGGCTTTCTAGTTCA	1	Hipkins et al. 1995
	trnD-trnE	*PCR-RFLP/Taq*I	F:CTAAATATAAATCTATTGG R:AAGTATCAATTCATATGG	2	Stoehr et al. 1998
	PM12L-PM12R	*PCR-RFLP/Hind, BslI, Tsp451*	F:CAGGGCGGTACTCTAACCAA R:AGATCACGTGCGTGTGAAAA	3	Nelson et al. 2003
	rps7-trnL	DNA sequencing	F:GGTTATTAGGGGCATCTCG R:CGTGTCTACCATTTCACCATC	4,5	Gugger et al. 2010
	rps15-psaC	DNA sequencing	F:GGTATCCGTGGGCTAAAAAC R:CAATACATCTGTGGGACAAGC	4,5	Gugger et al. 2010
	trnfM-trnS	DNA sequencing	F:CATAACCTTGAGGTCACGGG R:GAGAGAGAGGGATTCGAACC	6	Shaw et al. 2005
mtDNA	19S rDNA V7	DNA sequencing	F:GAGCCAAGGAGGCAGATTG R:ATCCTTGGTCTGATGCTTCG	4,5	Gugger et al. 2010
	nad7-1	DNA sequencing	F:ACCTAACAGAACGCACAAGG R:TTCCAACCAAGAATTGATCC	4,5,6	Gugger et al. 2010

1–Hipkins et al. 1995, 2-Stoehr et al. 1998, 3-Nelson et al. 2003, 4-Gugger et al. 2010, 5- Gugger et al. 2011, 6-Wei et al. 2011

(Qiagen) (Wei et al. 2011) and the CTAB procedure following Doyle and Doyle (1987) (Stoehr et al. 1998);
- from buds using the protocol of Guillemaut and Maréchal-Drouard (1992), (Nelson et al. 2003), and the CTAB procedure following Doyle and Doyle (1987) (Stoehr et al. 1998).

Examples for amplification protocols (PCR-RFLP)
- 36 cycles of 20 s at 94°C, 20 s at 52°C (cpDNA) or 56°C (mtDNA) and 1 min 30 s at 72°C followed by a final extension step of 6 min at 72°C (Wei et al. 2011).
- 94°C for 2 min followed by 35 cycles each at 94°C for 1 min, 55°C for 1 min, 70°C for 2 min and a final extension step at 72^0C for 15 min (Gugger et al. 2011).
- 95°C for 2 min, followed by 30 cycles of 94°C for 30 s, 48°C for 30 s, 72°C for 1 min, and a final extension step at 72°C for 5 min (Stoehr et al. 1998).

Important results

- By analysing range-wide genetic variation of maternally inherited mtDNA and paternally inherited cpDNA, detailed insights into the evolutionary history of Douglas-fir (ice-age refugia, colonization routes, introgression zones, migration rates) have been obtained (Gugger et al. 2010, 2011, Wei et al. 2011). For example, Gugger et al. (2010, 2011) found that the Rocky Mountain (interior variety) Douglas-fir survived in three or even more glacial refugia. In addition, the postglacial colonization of Canada from refugia of both varieties resulted in a wide inter-varietal hybrid zone, formed mainly by pollen exchange and chloroplast DNA introgression.
- Wei et al. (2013) identified 2 mitotypes and 42 chlorotypes. Based on cpDNA, three separated lineages were discovered, which correspond to the Pacific Coast, the Rocky Mountain and Mexican populations. The Mexican lineage is characterized by low genetic diversity and high genetic differentiation. The authors proposed that the Mexican populations originated by southward migration from a refugium of the Rocky Mountain lineage.
- Nelson et al. (2003) suggested that the gene flow from pollen dispersal between coastal and interior Douglas-fir is restricted as no identical cpDNA haplotypes were found in studied trees of both varieties. Out of 31 distinct haplotypes identified in a total of 58 trees, 15 haplotypes were observed in coastal trees while 16 haplotypes were detected in trees from the interior of British Columbia. Gene diversity was in general high, however slightly higher in the coastal sample (0.95) than in the interior one (0.88).
- Similar results were reported by Ponoy (1993) for six British-Columbian Douglas-fir populations. Using six restriction enzymes (*BamHI, EcoRI, EcoRV,*

Table 3: Primer information of the chloroplast microsatellite markers used in genetic analysis of Douglas-fir (T_a= annealing temperature)

Locus	Motif	Primer sequences 5‘ – 3‘	Size (bp)	T_a (°C)	Ref.	Source of primer pairs
Pt26081	$(T)_{14}$	F:CCCGTATCCAGATATACTTCCA R:TGGTTTGATTCATTCGTTCAT	125-126[1)] 99-105[2)] 102-110[3)]	60	1,2,3,4	Vendramin et al. 1996
Pt30204	$(A)_{12}(G)_{10}$	F:TCATAGCGGAAGATCCTCTTT R:CGGATTGATCCTAACCATACC	142-145	50	3	Vendramin et al. 1996
Pt36480	$(T)_{11}$	F:TTTTGGCTTACAAAATAAAAGAGG R:AAATTCCTAAAGAAGGAAGAGCA	142-147	50	3	Vendramin et al. 1996
Pt63718	$(T)_{10}$	F:CACAAAAGGATTTTTTTTCAGTG R:CGACGTGAGTAAGAATGGTTG	110-111[1)] 90-93[2)]	60	1,2,4	Vendramin et al. 1996
Pt71936	$(T)_{16}$	F:TTCATTGGAAATACACTAGCCC R:AAAACCGTACATGAGATTCCC	166-167[1)] 148-151[2)] 143-148[3)]	60	1,2,3,4	Vendramin et al. 1996
Pt87314	$(T)_{16}$	F:TATATCCCCCGTACTTGGACC R:TCCAGGATAGCCCAGCTG	98-99	50	3	Provan et al. 1999
Pt110048	$(T)_{10}$	F:TAAGGGGACTAGAGCAGGCTA R:TTCGATATTGAACCTTGGACA	86	60	1,4	Vendramin et al. 1996

1-Viard et al. 2001, 2-Gugger et al. 2011, 3-Valadon et al. 2011, 4-Angelier et al. 2011

HindIII, SstI, XbaI) in four cpDNA fragments altogether, 16 haplotypes were detected. 5 haplotypes were found in coastal, 7 in interior Douglas-fir populations. Populations from the transition zone, with 11 haplotypes, show the highest polymorphism rate.

- Angelier et al. (2011) and Viard et al. (2001) found that the universal cpDNA microsatellites Pt26081, Pt36480, Pt71936, Pt63718 show clear patterns, low polymorphism within Douglas-fir populations and no differentiation among populations. Primer Pt36480 was monomorphic for Douglas-fir.
- In a mixed stand with Douglas-fir, Japanese larch and white fir in France, pollen flow from outside the stand was low. The stand was isolated from other Douglas-fir stands. Genetic differentiation of succesfull pollen clouds between Douglas-fir mother trees was significantly lower than for Japanese larch and white fir (Angelier 2011). Genetic differentiation between age classes (adults, natural regeneration) of this stand was low.
- cpDNA markers proved to be very useful in assessing seed orchard mating dynamics and orchard management efficacies for Douglas-fir (Stoehr et al. 1998). In a clonal seed orchard from British Columbia they found the pollen contamination to be of 40%. Natural selfing in six individual clones ranged from 0 to 19% with an average of 6%. Supplemental mass pollination efficacy was estimated to be 55%, ranging from 39 to 73%, depending on the maternal clone and flowering phenology.
- Hipkins et al. (1995) identified the source of variation in cpDNA within the genus *Pseudotsuga* as a partially duplicated and an intact trnY-GUA gene. The sequenced Douglas-fir individuals differed from each other only in a single tandem repeat unit, whereas from the Japanese Douglas-fir (*Pseudotsuga japonica*) they differed in approx. 34 repeat units. RFPLs revealed a 1 kb variation in length among different *Pseudotsuga* sp. and 200 bp among *Pseudotsuga menziesii* individuals from different geographical regions.

4. **Randomly amplified polymorphic DNA markers (RAPD)**

RAPD markers were used to study the inter-varietal and intra-varietal differentiation in natural populations of Douglas-fir (Aagard et al. 1995, 1997, 1998a,b) and for genetic linkage mapping studies (Carlsson et al. 1991, Krutovskii et al. 1998).

Loci and primers used

Loci and primers used are listed in Table 4. For further markers, see Jermstad et al. (1994), where a list of 80 *c*ommercially prepared primers (Operon Technologies) for amplification of RAPD loci are listed. Among them, 29 display Mendelian inheritance. Krutovskii et al. (1998) used 96 10-bp random amplified polymorphic RADP primers. These have been also commercially prepared (Operon Technologies Inc. and the Biotechnology Laboratory of the University of British Columbia).

Material for DNA-extraction

Total genomic DNA has been extracted from megagametophytes (Krutovskii et al. 1998), embryos (Aagaard et al. 1995, 1997, 1998a,b), needles and buds (Carlson et al. 1991).

DNA-extraction and amplification protocols

DNA has been extracted with a modified CTAB protocol of Wagner et al. (1987) in which the DNA was further purified by four phenol:chloroform:isoamyl alcohol (25:24:1) extractions and a final ethanol precipitation (Aagard et al. 1995, Carlsson et al. 1991, Tsumura et al. 1996, Krutovskii et al. 1998). A special protocol for extraction of mitochondrial DNA is given in Aagard (1998b). For extraction of DNA from buds, a protocol can be found in Carlsson et al. (1991).

Examples for amplification protocols (RAPD)

- 7 min denaturation at 94°C followed by a total of 45 cycles of 1 min denaturing at 94°C, 1 min annealing at 35°C and 2 min extension at 72°C, with a final extension step of 72°C for 10 min (Carlson et al. 1991).
- 45 cycles of 93°C for 1 min, 37°C for 1 min, and 72°C for 2 min (Aagard et al. 1995, 1997).

Important results

- From 41 fragments amplified by RAPD markers, 29 showed variability between and within the coastal, north and south interior varieties. 10 bands proved to be variety specific (Aagard et al. 1995).
- RAPD-based linkage maps for Douglas-fir have been constructed by Krutovskii et al. (1998).
- Aagard et al. (1998b) developed a method of screening RAPD markers for the presence of organelle DNA products using enriched organelle DNA. Based on this method, Aagard et al. (1998b)

Table 4: Primers employed and RAPD markers obtained from DNA amplification in Douglas-fir (T_a = annealing temperature)

Primer name	Sequence 5` - 3`	T_a (°C)	Total no. of RAPD bands	Ref.
UBC 111	AGTAGACGGG	37	6	2
UBC 114	TGACCGAGAC	37	6	2
UBC 197	TCCCCGTFCC	37	14	2
UBC 234	TCCACGGACG	37	9	2,3
UBC 264	TCCACCGAGC	37	6	2
UBC 266*	CCACTCACCG	37	n.i.	1
UBC 268*	AGGCCGCTTA	37	n.i.	1
UBC 275*	CCGGGCAAGC	37	n.i.	1
UBC 285	GGGCGCCTAG	37	6	2
UBC 300	GGCTAGGGCG	37	n.i.	1
UBC 304	AGTCCTCGCC	37	9	2
UBC 323	GACATCTCGC	37	13	2
UBC 327	ATACGGCGTC	37	5	2
UBC 328	ATGGCCTTAC	37	5	2,3
UBC 330	GGTGGTTTCC	37	5	2,3
UBC 336	GCCACGGAGA	37	11	2,3
UBC 337	TCCCGAACCG	37	10	2
UBC 341	CTGGGGCCGT	37	15	2
UBC 372	CCCACTGACG	37	13	2
UBC 387	CGCTGTCGCC	37	12	2
UBC 409*	TAGGCGGCGG	37	n.i.	1
UBC 411	GAGGCCCGTT	37	10	2,3
UBC 414	AAGGCACCAG	37	n.i.	1
UBC 419*	TACGTGCCCG	37	n.i.	1
UBC 428*	GGCTGCGGTA	37	8	1,2,3
UBC 438*	AGACGGCCGG	37	n.i.	1
UBC 446	GCCAGCGTTC	37	n.i.	1
UBC 460	ACTGACCGGC	37	14	2,3
UBC 467	AGCACGGGCA	37	12	2
UBC 497	GCATAGTGCG	37	10	2
UBC 504	ACCGTGCGTC	37	13	2,3
UBC 530	AATAACCGCC	37	3	2
UBC 570	GGCCGCTAAT	37	9	2,3
OP-J1	CCCGGCATAA	37	13	2
FCPI	GCTTACCACC	35	n.i.	4
FCP3	CCATTCACCG	35	n.i.	4
FCPA3:	TATCGCACGCTA	35	n.i.	4
PRCI:	CGCATCCGA	35	n.i.	4

*1-Aagaard et al. 1995, 2-Aagaard 1997, 3-Aagaard et al. 1998a,b, 4-Carlson et al. 1991,source of primers Williams et al. 1990; * - maternal inheritance, mitochondrial origin, n.i. – not indicated*

found that RAPD markers of mitochondrial origin in Douglas-fir show lower genetic diversity than RAPD markers of nuclear origin, but differentiation is much higher for mitochondrial RAPD at both the population and varietal levels.

- A similar level of genetic diversity and differentiation was estimated for Douglas fir populations using RAPD markers of nuclear origin (36 loci) and isozymes (20 loci) by Aagard et al. (1998a).

5. Nuclear DNA markers (ISSR, nSSR, SNPs)

a) ISSRs (inter-simple sequence repeat polymorphisms)

Inter-simple sequence repeat (ISSR) technique allows studying polymorphisms located between microsatellite sequences. The microsatellite sequences serve as primers in a PCR to generate multilocus markers (Zietkiewicz et al. 1994).

Loci and primers used

Tsumura et al. (1996) examined 96 ISSR primers, from which 22 gave clear banding patterns for Douglas-fir. Out of these, 19 primers showed polymorphism for coastal Douglas-fir and 16 primers for interior Douglas-fir (Table 5).

Material for DNA-extraction

DNA was extracted from megagametophytes (Tsumura et al. 1996).

DNA-extraction and amplification protocol

DNA was extracted with a modified CTAB protocol of Wagner et al. (1987) in which the DNA was further purified by four phenol:chloroform:isoamyl alcohol (25:24:1) extractions and a final ethanol precipitation (Tsumura et al. 1996).

Table 5: List of 22 ISSR markers and primer sequences (T_a-annealing temperature, N_A-number of alleles, R-purine, Y-pyrimidine) after Tsumura et al. (1996).

Primers	Sequence (5′-3′)	Size (bp)		T_a (°C)	No of loci within varieties*
		Coastal variety	Interior variety		
857	$(AC)_8$YG	440/480/500/680	870	52	6
855	$(AC)_8$YT	290/650	880	52	4
856	$(AC)_8$YA	No amplification	340/700	52	
808	$(AG)_8$C	600	1000	52	7
809	$(AG)_8$G	480/580	No amplification	52	4
807	$(AG)_8$T	650/1050	460	52	
836	$(AG)_8$YA	400	No amplification	52	
834	$(AG)_8$YT	260	560	52	
835	$(AG)_8$YC	No amplification	230	52	
817	$(CA)_8$A	650/850	450	52	3
846	$(CA)_8$RT	600	340	52	2
844	$(CT)_8$RC	480/850	480/850	52	3
845	$(CT)_8$RG	500	No amplification	52	2
812	$(GA)_8$A	520	No amplification	52	10
811	$(GA)_8$C	280	680	52	4
810	$(GA)_8$T	520/850	No amplification	52	
841	$(GA)_8$YC	520	No amplification	52	
842	$(GA)_8$YG	400/600/700	220	52	
840	$(GA)_8$YT	440/650	440/800	52	
850	$(GT)_8$YC	270/440/520	310	52	6
851	$(GT)_8$YG	130/260/290	260	52	3
849	$(GT)_8$YA	No amplification	800	52	

For amplification, the following PCR-protocol was used:

- 95°C for 7 min followed by 45 cycles of 94°C for 30 s, 52°C for 45 s, 72°C for 2 min, final 7 min extension at 72°C.

PCR products were analyzed on 2% agarose gels in 1 x TBE buffer, then stained with ethidium bromide, and scored for band presence or absence.

Important results (all results from Tsumura et al. 1996)

- Coastal variety and interior variety show differences in genetic variability at ISSR loci. The average number of loci per primer was 1.8 for coastal Douglas-fir and 1.12 for interior Douglas-fir, whereas the proportion of polymorphic primers was 92% to 87%, respectively. The coastal variety shows higher heterozygosity than the interior one, with 35 heterozygote loci from 19 primers when compared to 19 heterozygote loci from 16 primers.

b) nSSRs (putatively neutral microsatellites)

Loci and primers used

Amarasinghe and Carlson (2002) developed 50 nuclear microsatellite (nSSR) markers for Douglas-fir, among which 48 loci showed polymorphism with a mean of 7.5 alleles per locus. Mendelian inheritance and genetic variability was confirmed by analyzing 24 unrelated Douglas-fir trees. In addition, 22 nSSR markers were developed by Slavov et al. (2004). From this set, 15 proved to be a valuable tool in genetic identification and parentage analysis (see Table 6). These markers were used in different studies on genetic variability of Douglas-fir populations (e.g. Krutovsky et al. 2009, Fussi et al. 2013), to trace back the origin of European Douglas-fir stands (Fussi et al. 2013, Eckhart et al. 2017, Hintsteiner et al. 2018) and to analyse the mating system in Douglas-fir seed orchards (Slavov et al. 2005, Sk Lai et al. 2010, Kess and El-Kassaby 2015, Korecký and El-Kassaby 2016).

Table 6: Primer sequences, annealing temperatures (T_a), allele lengths (bp) and number of alleles for 72 nSSR markers available for genetic analyses in Douglas-fir

Locus	Motif	Primer sequences		T_a	Size (bp)	No. of alleles	References
		Forward	Reverse				
BCPsmAC1		TGCAGCCCGATCTAACAATA	TTGTGAGGGAGTTGGACAAG	56	151–183	9	1
BCPsmAC2		GATCAGGACGGCACACGC	GTCGGAGATTGGCACGAGG	60	99–130	5	1
BCPsmAC3		CAACAACTACCATGAACCACA	GACAAAGGCACGCAAACC	53	123–202	10	1
BCPsmAC4		AGAACACAAAGATTTATCCT	ACTTGTGTATAGCGCAT	43	113–158	6	1
BCPsmAC5		TCTAAGCTTGACCACCCTGG	CTCGACCTATTTATATGGACA	50	125–176	11	1
BCPsmAC6		TGCGTGTTCCGTTCTCTTC	GCGGCCAGCTTCATATCC	56	109–124	6	1
BCPsmAC7		TCCGTCTTCCCAGCTAAATC	CGGCCAGCTTCATATCCAAG	53	126–148	3	1
BCPsmAC8		AACACACACAATAGAGGCACA	GCTGAGTGAGCTGGAGAATG	50	79–146	13	1
BCPsmAC9		GATCCGTTCTGAGTGCCG	TCCGTACCAACGCCAAAG	56	103–122	5	1
BCPsmAC10		CCAGCTGACAACTAGGAATG	CCGTATGTACTAATCTCCGA	50	88–148	7	1
BCPsmAG1		ACACTCGTGCTCTGGTTCTG	CTTCTCTTTCTCTATATTGCC	50	149–153	3	1
BCPsmAG2		GATCTTAATGTTCATGCAAGG	GAGATTTAGGGAGAGCGC	50	96–140	14	1
BCPsmAG3		ATGTATCTCTCCCCCCTGCA	GGGGAGAGATAAATGAGAAAGGG	56	81–96	4	1

Locus	Motif	Primer sequences		T_a	Size (bp)	No. of alleles	References
		Forward	Reverse				
BCPsmAG4		ATATCGAATTCCTGCAGCC	GTGCGAGGGAGGGAAAT	50	174–208	9	1
BCPsmAG5		ACCACCATATCTCTGTTCCC	GATCAGGAATGAGCAAAGCTA	50	77–113	13	1
BCPsmAG6		TCTCCCTACCACTTACTCCG	GGTGTTAGCAGTAAGGGGATAC	53	157–209	9	1
BCPsmAG7		CCGCGATCATATTTCCAC	TAAGCTGTGCAATGCGGA	53	123	1	1
BCPsmAG8		ACATGCTCATTCCCATTCTCC	TAGAGGGAGGGACGAAGGG	56	133–145	12	1
BCPsmAG9		GATCCACTCTCTATCCCTCCAC	GAGAGCAAAGCAGAATGGTG	53	98–125	5	1
BCPsmAG10		CCTCTCTATACCTAGCCCTAAAC	ATCAGGAATGAGCAAAGCTA	50	100–152	14	1
BCPsmAG11		TCCATCCTTCACCCCCAA	GAGAGATTTGAGGTTAAGGGTTG	53	80–198	17	1
BCPsmAG12		TTAATGCCTACTAACCACAAATC	GTATCGATGCTTAGCTATAGGG	50	191–245	15	1
BCPsmAG13		ATTTGTAAACTACATCCCACTACC	TGGGTAATGGTTTAGGGAAC	50	116–148	8	1
BCPsmAG14		TTAATGCCTACTAACCACAAATC	GTATCGATGCTTAGCTATAGGG	50	191–224	10	1
BCPsmAG15		CCTCAATCTACTTATGTACACC	AGAGGGATATTGGAGGTTAGG	50	99–116	6	1
BCPsmAG16		CCCTCCCTATCCATGCTTC	AGATGGTTTCAAAGACAGAGGG	53	73, 81	2	1
BCPsmAG17		CATCAGTGGTCTTGAGCATG	TGAGTGAGAAAGATTGATATGGG	53	233–292	8	1
BCPsmAG18		TTGTCTCGTGTACAAACTCAGC	CAGAATGGGAGAACACGATG	53	152–202	8	1
BCPsmAG19		CTTCCTCTCCACTTTCCACC	AGGTGATGAGAAAGGGTTGG	53	167–187	4	1
BCPsmAG20		ACCCCCGAACCGTTACTAC	CATAGAGAGGGGGCATATCAA	53	108–160	17	1
BCPsmAG21		GATCCTCCACCTATGCCTAAC	AGAGGGGTTTAGGGACAGAA	50	121–188	4	1
BCPsmAG22		TATCACTATAAATCCCCAATCCTAA	TCGAGTGGGTTTTAGTGGTTT	53	103–139	8	1
BCPsmAG23		TCACTCTAAACCACTAACACCCA	GTTAGAGATTAGGGGGAAG-TATCG	53	103–137	11	1
BCPsmAG24		CTCTCTGAACTCCTAACCTCCAA	CCTTGAATCTTTCTCTCTCTA-AAAC	56	117, 133	2	1
BCPsmAG25		ATGTACACCCCAAGCCCATA	TGGGAATTAGGGGTTAGAGAAA	53	105–130	3	1
BCPsmAG26		ACCTCTAACCCCTCAACCAC	GGAGAAGTAGGGGAGTGTGA	53	228–264	9	1
BCPsmAG27		CCTTCCTCTCCACTTTCAACC	ACGGGGAGGGAGGGTAAC	56	84–144	5	1
BCPsmAG28		TATCTCGGCTACCACATGGC	CATTGCTGGCTGAATTGACA	56	98–157	3	1
BCPsmAG29		TCAGATATATAAACTAAGAGAATG-GGG	CACTCTAAATGCCTAACCACCA	53	137, 142	2	1
BCPsmAG30		GAGATTGAGTGTTAGGTGTTTAG-GG	CTCCCTCTCTTTCCCTCCAT	53	97–118	5	1
BCPsmAG31		GTACCCCACGCCTCACC	CAAACAAGAGTAGGGGGTGC	50	138	1	1

Locus	Motif	Primer sequences		T_a	Size (bp)	No. of alleles	References
		Forward	Reverse				
BCPsmAG32		CAATTCCAAGGCTCTCTTTG	TATGGAACAGAGACGAAGCA	50	88–130	4	1
BCPsmAG33		CCCGTCATCCTACCAATTT	GGGGTATGTGGTATGGGTAA	50	191–222	6	1
BCPsmAG34		CCTGCTGGCTGTTTCTGT	ACGCGCATGTCTGTGTG	50	74, 83	2	1
BCPsmAG35		TAAGAAGTATTCAATAGCTATC-CATC	TCCTCACACTCTCAAGTTCG	50	75, 87	2	1
BCPsmAG36		CCTTATGCCCCTCTATTTACG	TAGTCGAGGAGTGGGTGTTT	50	172–216	3	1
BCPsmAG37		CACCTACCCCTAACATCACC	GGGGATTGAGTACATGGTGT	50	185–217	12	1
BCPsmAG38		TGTACTTGTATATCCTTTCTAT-CAACA	GCAAGCCAATAACTGGAATAA	50	100–132	11	1
BCPsmAG39		CTTGTAGTTGGAATTTATATACG	GGTGTAAATGAACAACAAGC	50	77–124	20	1
BCPsmAG40		GATCTTCTCGCAGTCGTCC	GTGCGAGGGAGGGAAAT	50	78–123	3	1
PmOSU_1C3	$(TC)_{25}(AC)_{32}$ $(TC)_4$	CTCCCCTCCAGATTTTACTC	TGGCGTAACAAATAAGAGAAA	57	166–232	28	2,3,9,10,11,12
PmOSU_1F9	$(AG)_{34}$	CCTCATGCATTGGACACTC	GGATTCTTGAGCAGGTAGG	55	201–319	33	2,3,5,9,11,12
PmOSU_2C2	$(AC)_{32}..(CT)_4$	TAAATCCGCAGCTCATAGAATC	GGGTGGTGGCTAGGGAAAC	60	142–200	12	2,3,5,8,9,11,12
PmOSU_2C3	$(TC)_{24}(AC)_{18}$	AAAGACAACATTATGAAAGG	GTAATGGTTCGAAAAATAATG	50	163–251	25	2,3
PmOSU_2D4	$(AT)_4..(TG)_{18}(AG)_{26}$	TTATTGCACATGAGTATTATGA	CAGATGTTGTTTTTTATACCAC	50	108–194	30	2,5,8, 9,11,12
PmOSU_2D6	$(AC)_5..(AC)_4..(GC)_8(AC)_{13}..(AC)_7$	GGAAAATATACATCTCACGAC	AAGCATGCGTACTAGGTG	54	162–264	30	2,9,11,12
PmOSU_2D9	$(TC)_{32}(AC)_{15}$	TCGATTTACGCTTTTTTCTC	TGTTTATCCCCAGTCTCAAG	57	125–181	8	2,9,11,12
PmOSU_2G12	$(AC)_{11}..(AC)_{19}..(GCAC)_5..(GCAC)_4(AC)_7..(AC)_6$	CAAGGACTCATATGGGAAA	AACATCAGTAATAACCTTTT	51	244–310	16	2,3,4,5,6,7,8,9, 10,11,12
PmOSU_3B2	$(TG)_{22}(CG)_7$	CTTTGGAGTTCTTAATATAG	GATAATAGCACCCCACCATA	49	88–176	27	2,3,4,6,7,9, 10,11,12
PmOSU_3B9	$(CG)_6(CA)_6..(AC)_6..(AC)_5..(AC)_6$	TGTGTAAAAATGTCTAATCC	ACTACTATTCGAGGTTTTCT	47	119–223	25	2,5,8,9,11,12
PmOSU_3D5	$(TG)_{16}(AG)_{26}$	GGCATCCTATTTTTCATTTT	GTGATTACCTAACTTGTGC	50	125–193	19	2,5,8,9,11,12
PmOSU_3F1	$(TG)_6..(TG)_7(AG)_{27}..(AC)_4$	GACTAGATCATCGCAACTT	GGTATTCTTATGGTTTTTAT	50	144–246	20	2,3,4,5,8,9, 10,11,12
PmOSU_3G9	$(TG)_{12}(AG)_{28}$	ATTCCTTTTGAGACCTACTT	CTTCAAAAATTCCTACAACA	51	110–192	22	2,3,4,5,6,7,8,10
PmOSU_4A7	$(TG)_5..(TG)_5..(CG)_7(TG)_4..(TG)_{29}..(ATC)_5$	TTGTAAAAATTCCCATGTAT	AAGTGGGGGAGTGTGTAAT	48	196–340	30	2,3,4,6,7,9, 10,11,12
PmOSU_4G2	$(AT)_6..(AG)_{29}$	ATTTTTTGTATTGTGCTTG	TGGATATATTTGCATTTTAC	48	138–168	16	2,3,8

Locus	Motif	Primer sequences		T_a	Size (bp)	No. of alleles	References
		Forward	Reverse				
PmOSU_2B6f	$(TG)_{19}(AG)_{31}..(AT)_4$	TTGTTGGGTATAATTTTCA	TAATAAAATAGCTCTAACCC	49	134–346	28	2
PmOSU_2G4f	$(TC)_{24}(AC)_{29}..(AC)_1$	ATGCATTCTTGAAAGTAAA	ATAATATGCAAGTGAATCCC	51	180–272	19	2
PmOSU_3E3f	$(TG)_5..(TG)_4..(TG)_{15}(AG)_{28}$	TGCTTCAATTTCATATCTA	TAACATTTCAATCTATTCAC	48	126–266	31	2
PmOSU_3H4f	$(GC)_9(AC)_{22}$	TTTGCCGTCACATTTTTATTG	GCATCTTTCAGGCATAGTCT	55	170-256	25	2
PmOSU_4E9f	$(AC)_{36}$	GTTGGTTGTGTATATTCAGTTT	GCCTCTTCTTGGTTTGGT	54	120–218	24	2
PmOSU_5A8f	$(TG)_{11}..(TG)_{10}$	CATTTTTGGATTCTGGTTTTG	ATGCCTCAAGCTATGTAATC	54	166–190	7	2,9,11,12
PmOSU_783f	$(AT)_5..(AT)_5$	GAGCTGATGCCTTGAAGACT	CAAGTCAGTTCACAATTCCT	57	205–303	15	2

1-Amarasinghe and Carlson 2002, 2-Slavov et al. 2004, 3-Slavov et al. 2005, 4-Krutovsky et al. 2009, 5-Sk Lai et al. 2010, 6-Konnert and Fussi 2012, 7-Fussi et al. 2013, 8-Korecký and El-Kassaby 2016, 9-van Loo et al. 2015, 10-Neophytou et al. 2016, 11-Eckhart et al. 2017, 12-Hintsteiner et al. 2018

Material for DNA-extraction

DNA has been extracted from needles (Neophytou et al. 2016, Fussi et al. 2013, Slavov et al. 2004, 2005, van Loo et al. 2015, Eckhart et al. 2017, Hintsteiner et al. 2018), buds (Slavov et al. 2005, Sk Lai et al. 2010, Korecký and El-Kassaby (2016), seed (embryos, megagamethophytes) (Krutovskii et al. 2009, Sk Lai et al. 2010) and cambium (Neophytou et al. 2016, van Loo et al. 2015, Eckhart et al. 2017, Hintsteiner et al. 2018).

DNA-extraction and amplification protocols

Total DNA has been extracted from the mentioned tissue using:

- the commercial DNeasy Plant Maxi DNA-extraction Kit (QIAGEN) (Slavov et al. 2004, 2005) and DNeasy 96 plant kit (Qiagen) (Neophytou et al. 2016);
- the OMEGA E.Z.N.A Plant DNA Kit (OMEGA Bio-Tek, Inc., Norcross, Georgia, USA) (van Loo et al. 2015, Eckhart et al. 2017, Hintsteiner et al. 2018);
- the CTAB extraction protocol of Doyle and Doyle (1990) (Sk Lai et al. 2010), Korecký and El-Kassaby 2016);
- the ATMAB extraction protocol of Dumolin et al. (1995) (Fussi et al. 2013).

Examples for amplification protocols

In most studies, the following PCR conditions described by Slavov et al. (2004) have been used:

- seven cycles of touchdown PCR: 95 °C for 30 s, empirically determined optimal annealing temperature (T_a)+7 °C for 30 s, then 72 °C for 45 s. The T_a was decreased by 1 °C for each of the six subsequent touchdown cycles. Following touchdown PCR, the program continued with 32 cycles of 95°C for 30 s, T_a °C for 30 s, 72 °C for 45 s, and a final extension of 72 °C for 20 min.

Important results

- Nuclear SSRs are useful tools for genetic studies in Douglas-fir. Especially the nSSRs developed by Slavov (2004) have been applied in several studies (e.g. Krutovsky et al. 2009, Sk Lai et al. 2010, Fussi et al. 2013, Neophytou et al. 2016, van Loo et al. 2015, Korecký and El-Kassaby 2016, Hintsteiner et al. 2018).
- Based on nSSRs, coastal and interior Douglas-fir can be clearly differentiated from each other (Fussi

et al. 2013, van Loo et al. 2015, Neophytou et al. 2016). Following Fussi et al. (2013), the nSSR loci PmOSU_3B2 and PmOSU_4A7 are indicated to distinguish between the two varieties.

- Intra-varietal variation (division into different clusters within a variety) and hybridization patterns were described and related to the phylogeographic history (van Loo et al. 2015). Results were used to trace back the origin of numerous Douglas-fir stands in Europe (Austria and Germany) (Fussi et al. 2013, Eckhart et al. 2017, Hintsteiner et al. 2018).
- European seedlings have a lower genetic diversity than the American seedlings and native populations (Konnert and Ruetz 2006, Eckhart et al. 2017).
- Weak genetic differentiation among populations and high within population differentiation for coastal Douglas-fir, already observed for allozyme loci, has been confirmed by nSSRs. Genetic distance has been positively and significantly correlated with geographic distance (Krutovsky et al. 2009).
- nSSR markers are also powerful tools for a) studying the pollination dynamics in seed orchard, b) characterizing seed lots and c) improving the design and management of Douglas-fir seed orchard (Slavov et al. 2005, Sk Lai et al. 2010, Kess and El-Kassaby 2015, Korecký and El-Kassaby 2016). For example, Korecký and El-Kassaby (2016) found that in the coastal Douglas-fir seed orchard 80% of parental gametes were produced by 52% of the parents, 13% of paternal gametes resulted from pollen contamination and 12% of the seed were the product of selfing. Slavov et al. (2005) discovered that levels of pollen contamination in a seed orchard in Oregon varied substantially among clones. These levels were higher in clones with early female receptivity (mean = 55.5%) than in those with mid- and low-receptivity.
- Neophytou et al. (2016) assessed the link between genetic and phenotypic variation in the height growth. Statistical evidence was provided for a genetic component in the height growth.

c) SNPs (single-nucleotide polymorphisms)

Loci and primers used

Krutovsky and Neale (2005) studied single-nucleotide polymorphisms (SNPs) and linkage disequilibrium in 15 cold-hardiness- and 3 wood quality-related candidate genes in Douglas-fir. Their study, which primarily aimed to select SNPs for further association mapping, shed light on SNP frequency, haplotype and nucleotide diversities of studied candidate genes. Four years later Eckert et al. (2009a) analysed diversity and divergence for a set of 121 cold-hardiness candidate genes (classified by 933 SNPs) in coastal Douglas-fir trees. These represented 24 unrelated Douglas-fir individuals from six regions located across Washington and Oregon. In their following study, Eckert et al. (2009b) used a candidate gene-based approach to search for genetic associations between 384 SNPs from 117 candidate genes and 21 cold-hardiness related traits within natural populations (700 unrelated families) of coastal Douglas-fir from western Oregon and Washington. The SNPs were selected from the already existing sets of SNPs represented by 400 SNPs and 933 SNPs from Krutovsky and Neale (2005) and from Eckert et al. (2009a), respectively. A list of 154 candidate genes with SNPs used by Krutovsky and Neale (2005) and Eckert et al. (2009a,b) is presented in Table 7.

Applying transcriptome sequencing on cDNA from Douglas-fir seedlings included in a drought stress experiment Müller et al. (2012) identified about 1,000 candidate genes related to drought stress with a total number of 187,653 SNPs. They established a catalogue of putative unique transcripts (PUTs) and a large SNP database for Douglas-fir. Using targeted sequence capture, Müller et al. (2015) re-sequenced 72 trees of both coastal and interior variety in order to characterize genetic diversity in coding regions and to identify genes involved in local adaptation. To facilitate genomic selection in Douglas-fir breeding programs Howe et al. (2013) combined high-throughput sequencing technologies (454 pyrosequencing and Illumina-sequencing-by-synthesis) to sequence the transcriptomes of diverse tissues of Douglas-fir genotypes.

Table 7: Primers for amplification of 154 SNP loci used in Douglas-fir; Amplicon length (bp) = base pairs, T_a = annealing temperature

Candidate gene	Total SNPs	Primer sequences		Amplicon length (bp)	T_a	Gene product	Ref.
		Forward primer (5'-3')	Reverse primer (5'-3')				
EF1A	14	CTTTTGTGCCTATCTCTGGTTT	TAATATTAATTCCAAGTTTTCTCAATC	1072	55	Translation elongation factor-1, a-subunit	1,3
TBE	58			2954		Thiazole biosynthetic enzyme	1,3
amplicon 1		AAGGCAAGAAGAAGAAGAATGG	CATCGACACCAGGGCCCA		55		1,3
amplicon 2		TTGTTCACCTCGACTATCATG	GCCGTGAGTGAGGAGTTAGTGTG		55		1,3
amplicon 3		CGAATGAAAATATTGCCCTCAAG	GCCGTGAGTGAGGAGTTAGTGTG		55		1,3
amplicon 4		CGAATGAAAATATTGCCCTCAAGT	TCCGCTACTCCATTGCAAAAC		55		1,3
F3H1	14	TTCAAGAGCGCGGACCATCG	CGGTGTGAAAGCTTTATTGCTAC	365	60	Flavanone-3-hydroxylase	1,3
F3H2	14	AAAGTGGTGATAAACTTCTATCC	GTATAAACGTGATTGCAGAGG	647	55	Flavanone-3-hydroxylase	1,3
forming-like	3	AAAAGGGATGAAAATGCAAGGAA	GAAGATTCGTCATCAAGACTATCAAC	337	55	Formin-like protein AHF1	1,3
AT1:	93			2578		a-tubulin	1,3
amplicon 1		GGTGGTATTGCAGGAGGCG	GACAGTCGGCTCCAAATCTAC		60		1,3
amplicon 2		TTGAGGGTTTTGTTCTTACTAGCC	CACTTTTAAGAGGGCGAACAGAC		60		1,3
amplicon 3		ACATACACAAATCTGAACCGACT	AACATCATGCCCAAACTTTC		55		1,3
LEA2	18	TGATTGATATCTGGTTTTCGAG	GTCAGAAACCTTAACAGCCA	504	55	Late embryogenesis abundant type 2 dehydrinlike protein	1,3
MT-like	20	ATTTCCCGCACGTTTGTATCAG	AAGAGGAAGGCCGGCATTACA	579	60	Metallothioneinlike protein	1,3
60S-RPL31a	21	AATGGTGGAGAAGGGAAAGAG	AAATGCTATCCAGATACTATCGC	609	60	60S ribosomal protein L31a	1,3
LEA-EMB11	33	CTCCGGGTATACAGCTCGC	GACTTTCTTGAAAGAAGCTTCTGC	545	60	Late embryogenesis abundant EMB11-like protein	1,3
40S-RPS3a	12	TGAAAAAGCTACTTCCTCAATTTATC	AACAGCATGGTTCATGTCCC	500	55	40S ribosomal protein S3a	1,3
PolyUBQ	17	CCCTTAGTTATTTTCCAGTCTCAA	TTGCATAACGGTTACCGAAG	898	55	Polyubiquitin	1,3
ERD15-like	14	GTTACAGATATCTCCAGGCTCGG	TTCTCACAGTGCTTCGCTTCAAA	646	60	Early response to dehydration protein	1,3
ABA-WDS	9	GTCTGGCGAGAATCGTCACC	GCAGCCATGGATCCGAGCTC	344	60	Abscisic acid, water deficit stress and ripeninginducible protein	1,3
LP3-like	16	AACATCGTCGTCACCTCTTC	TCTCGTGGTGCTCGTGGAA	481	60	Water deficitinducible protein	1,3
4CL1	8	GATAAATTGGCCGATCTGCAGC	GAAAAATTAAACGACTGGGGGC	628	65	4-coumarate: CoA ligase 1	1,3
4CL2	10	GATAAATTGGCCGATCTGCAAA	GAAAAATTAAACGACGACTGGGTC	629	60	4-coumarate: CoA ligase 2	1,3

Candidate gene	Total SNPs	Primer sequences		Amplicon length (bp)	T_a	Gene product	Ref.
		Forward primer (5'-3')	Reverse primer (5'-3')				
APX:	26			867		Ascorbate peroxidase	1,3
amplicon 1		GACAAGGATATTGTGGCGCT	ATGAATATCAAGTTGCATTTGACC		55		1,3
amplicon 2		GACAAGGATATTGTGGCGCT	AACACAGCAGAGATGTGAGAGC		60		1,3
amplicon 3		CACCTGAAGCTCTCTGAACT	AACACAGCAGAGATGTGAGAGC		60		1,3
CD028057.1	20	AACGTCTTACGGCTGCTGAG	GGCTTGACCAATACCACAGAA	686	57	calcium-dependent protein kinase	2,3
CN634517.1	23	AATCAGGAGTGCCAAGACCG	GCCTCTCTTTGATTTTATTCTCCCA	777	55	lumenal binding protein	2,3
		GGACAAGCACCAGAGCACGA	CGAGGGAAGTCCAAATCTGAAGTA		55		
CN634677.1	23	AACACGCTCTCGTATTGCC	GCTTTTCCAGTCAGAATC	288	60	LRR receptor-like protein kinase	2,3
CN634994.1		Not published				ADP-ribosylation factor	3
CN635137.1	21	CAAGGGAATGAACTGAAGTTGGTG	CAAATGGCTCCCAAACACTGA	738	55	aquaporin	2
CN635490.1	23	TCAACACATACCCATAGTCGCAC	GGTTGGGTGTGTGTAAATATGTC	448	55	rare cold inducible protein	2,3
CN635596.1	23	GTTTTTTTCAGGGCGACGG	AGTAAAAGGATTTGTAACAGCCACCAG	311	60	phosphate-responsive protein	2,3
CN635661.1	20	AAGAAGGGCAGAAAGAGCAGCAC	TGCTCTCTCTGCCCGTTCCCTTG	254	55	auxilin-related protein	2
CN635674.1	19	CTTCATAGGCGTTTTGTC	GCTTCAGGTTTTACTGG	163	60	pentatricopeptide (PPR) containing protein	2,3
CN635691.1	23	GAGAAAATTCGCCTTCAG	CATCGTCATCAATGTTACC	635	60	homeodomain protein (HB2)	2,3
CN636014.1	24	TTGTCTCTTGGATTGGAAACTGC	TTCATCCTCAGCGTTGTATTTCTCT	675	55	heat shock protein 70 kDa	2,3
		TGAAGGTGAAAGAGCAAGGA	ACCAGAAGCTCCACCAAAAG		55		
CN636043.1	22	TATGACTTTGGGGGTTACTGCTATG	ATTGACGAGAACACTGAGGGGA	1176	55	cysteine protease pseudotzain	2
CN636093.1	22	GAGCCAGCACAGCGAAACAT	ACTATGATGTGGAAAGCAAAACC	353	55	calmodulin	2
CN636134.1		Not published				CBL-interacting protein kinase	3
CN636149.1	18	TCCAAACAACCTTCTCGCAGTATT	ACGACGTAACATTCAAGGTAGCA	341	55	cinnamyl alcohol dehydrogenase	2,3
CN636303.1	23	CCCCCATCCCAAAAGTTAAT	CAAACTTATGGCGACTTCAC	650	55	actin depolymerizing factor	2,3
CN636471.1	23	GCGTGGTCTCTCAGGTGGC	CCTTTCCGATGTATTCACCCG	433	55	phenylalanine ammonia-lyase	2,3
CN636492.1	24	GGAGAAGGATTTGTCAGTACGGGA	CTCAAATTCAACAGCACATCGTC	406	55	phosphoethanolamine methyltransferase	2
CN636784.1	24	GGTTGAGACCAGATGGGAAA	TTTCCTTGTCTGGGATCCTG	803	55	S-adenosylmethionine synthetase	2,3
		TCCCTGAGGTGCTGTTCTCT	AGGGCTCTGCTGTTCAATGT		55		
CN636795.1	23	ACAGATTCGGGTGGTTCAGCAT	ACGAAGATACAAATGGAGCAGC	676	55	xyloglucan:xyloglucosyl transferase	2,3
CN636901.1	23	CCTGGTTCAGGATTCGGTCAA	GCGTGTGTTTATGATTTTTATGATGTG	569	55	alanine aminotransferase	2
CN636999.1	23	AGGATTTGCTTTCACTGGAGAGA	CATTCACTCCTCCCTTGCCA	357	55	BURP domain-containing protein	2
CN637166.1	16	CCAGAAGATGAAGAATCC	TGAAGCCCTGTAAAACTCC	366	60	phloem protein	2

Candidate gene	Total SNPs	Primer sequences		Amplicon length (bp)	T_a	Gene product	Ref.
		Forward primer (5'-3')	Reverse primer (5'-3')				
CN637226.1	20	AATCGGGTCATTAGCC	CGTTGTTACAGTCGTC	188	60	prephenate dehydratase family protein	2
CN637244.1	22	AAATGCCGACGAGATGCTGC	GCCGCATACAGCTTGGTTTTA	366	55	cysteine protease inhibitor	2,3
CN637306.1	23	CTAAACAATGGGAAGGG	ATCTCGTTGTCCGTTC	670	60	MYB-like transcription factor	2,3
CN637339.1	22	AGGATGGAGATGGCAAC	CTGAACTGAAGACGAG	533	60	unknown hypothetical protein	2,3
CN637473.1		Not published				protein kinase domain containing protein	3
CN637587.1		Not published				glycosyl hydrolase family protein	3
CN637910.1	23	GCACCGTCACATAAATTTTC	TTCATCAAGTCGCAGCC	303	60	ABC family protein	2,3
CN637944.1	20	ATGTCTGGTCCGTGTTG	TCCCATCTTTCTCCTC	237	60	bet v I domain containing protein	2,3
CN638015.1	21	TCCAATCTACTCAAGGCGTCCA	CAGTCTTAGCAGCGAAATAACAACA	395	55	unknown hypothetical protein	2
CN638070.1	12	GTTTCTCTTGTTCCTCCTC	GCATCATATACTTCTTCACATAGCC	185	60	acid phosphatase class B family protein	2
CN638310.1		Not published				chloroplastic copper/ zinc-superoxide dismutase	3
CN638367.1	23	CTTCCTGAGCAAACCCTGAGC	GCATAAAGTGTCGGAAGGAAAGGAT	889	55	ATP-dependent RNA helicase-like protein	2,3
CN638381.1	16	TTGAGGAAAACCAGATGAAGCAGT	CTGCTACCAAAGTCAAACATAATCAATACA	959	57	iron-inhibited ABC transporter	2,3
CN638489.1	15	ACCAATTTCTGCCCTCC	GATAAAGTCTGCCCAACC	456	60	alpha-expansin	2,3
CN638545.1	23	TTCCGCTCCTCGTTCCTCACAT	GCAAAACTACTGACAGCAAAAACAA	512	55	trans-cinnamate 4-hydroxylase	2,3
CN638556.1	23	ATGGGGTGAGCGTTATCTGG	CAACTCTCAGTCATATCAGG	380	60	transcription regulation protein	2,3
CN638735.1	23	CCTTTTACTGTGTTGTGATTCC	GAAGAAGAGATAGACACC	596	60	cellulose synthase-like A1	2
CN639074.1	24	AGTGCCCGAACCTCTGTCTGT	AAGCCATTACATCCAAAGCGT	547	57	S-adenosylmethionine synthetase	2,3
CN639087.1	23	AGACAGAAAAGCAGTTCAAG	ATAACCCAAGTCACCAAAATCACC	370	60	LRR receptor-like protein kinase	2,3
CN639130.1	21	CTCATTGAGAGATGCAAAG	GGATTCACAGTAACATTTGG	117	60	chloroplast heat shock protein 70 kDa	2
CN639211.1		Not published				eukaryotic initiation factor 4A	3
CN639236.1	20	TTCTCCATTGATAACCGCCAG	ATAGTAACTCCGTCTTTGCCGCCGT	308	55	guanine nucleotide-binding beta subunit protein	2,3
CN639311.1	22	CTATGGCATCTGTTGG	GCTGAAGGATTTGCTC	812	60	replication protein	2
CN639346.1	17	CAACTGAGCAACTCCGACTGATT	CCACTTCGGCATCGCAGAGC	189	55	MADS-box transcription factor	2
CN639480.1	23	ATCATTCGCATGAGGG	GGGTTTCTTCATCATAACAG	422	60	2-hydroxyacid dehydrongenase	2,3

Candidate gene	Total SNPs	Primer sequences		Amplicon length (bp)	T_a	Gene product	Ref.
		Forward primer (5'-3')	Reverse primer (5'-3')				
CN639782.1		Not published				serine/threonine protein kinase	3
CN640010.1		Not published				eukaryotic initiation factor-5	3
CN640037.1	22	CTGCTGCAATCAAACC	TCAACCAAACCAATCCC	458	60	tau class glutathione S-transferase	2
CN640110.1	23	ATTGTCTCACTCTGCGG	CGAATTTCAATATGCGTCTC	364	60	galacturonosyltransferase	2
CN640155.1	17	GAAGATAAGGGCAGAG	CGAATCAACCATTCATCAC	318	60	bicoid-interacting 3 domain containing protein	2,3
CN640247.1	24	TATTTACGCTTTCTCCCGAT	AATTCTTCCCGACAGCGACG	754	55	chalcone synthase	2
CN640289.1	23	AGCCGATTTTGAGAAGATTGGAGAG	TATGCTATTCCTATGGTCACTTATCGG	228	55	serine hydroxymethyltransferase	2
CN640361.1	22	AATCCAATGTTACTTCTGCTGCTTC	ATCTCCTTGCTAGTCCGCTGTCTC	757	60	zinc-finger (C2H2 type) family protein	2,3
CN640419.1	22	TGGAGAAGGTGGAGAAGAAA	TTCCAGTGCCATTGTCCTCT	618	55	heat shock protein 70 kDa	2
CN640485.1	23	CACATTTATCCCCTCTC	TTGGCAATTCTCAACTGTAGTC	528	60	HNH endonuclease domain containing protein	2,3
CN640493.1	16	A T G T T C T C C A C C A G T C T C - CCCAAATG	GGTTGTAAGAATCCTGAGAGTCCA	247	55	nuclear transport factor	2
CN640521.1	23	AGCGGATGTCAGATTG	GGGTAGACTTTGTAGG	418	60	DNA-binding bromodomain-containing protein	2,3
CN640694.1		Not published				heat shock cognate protein 70 kDa	3
CN641217.1		Not published				somatic embryogenesis receptor-like kinase	3
CN640670.1	23	ATATTGGGAATTGGTACGG	TTCTGGTGAGGATGTGG	197	60	GH3 auxin-responsive promotor family protein	2
CN640738.1	21	ACATCCAGGTTGCCATTGCG	CGCAGAAGGCCGCTACATTT	542	55	anthocyanidin reductase	2
CN641116.1	23	TTCTCGCACCTTTTCCC	GCCATAATGACATCCCC	739	60	carboxy-terminal kinesin	2
CN641171.1	22	AACTCAGGTATATGTGGATGTAAGGG	CCGAGGAACAGTGGAGAGAG	470	55	cinnamoyl CoA reductase	2
CN641226.1	23	GTGAGATCACCAATATTCAG	CTCCAAACATTGAAGGAATC	375	60	LRR receptor-like protein kinase	2,3
ES418315.1	22	GCAGCGGTGGTGAAAGAGACT	CAGATGGGAAGGAAGACGAGCA	426	55	flavenoid 3-hydroxylase	2,3
ES418915.1	23	AGCCATTGCCTACTATTCTGTG	CTTCTTGCATCCATATTCTCCCTG	401	55	cellulose synthase-like protein	2
ES419198.1	23	TGGTGTTGTGTCATCAGTCCCTCA	GTCATACACAAGCCATAGCGATTAC	368	55	LIM domain protein	2,3
ES419223.1a	22	CCCAAGTTCAAGTTTATTTCATCATC	AAATGAAGGATACAAACAAACTGCTC	445	55	phytosulfokine precursor	2,3
ES419242.1	16	ATTGCATGCCCAGGAACTACAG	AGTCTTCGGCTCCTTCATCTAAA	831	57	response regulator protein	2,3
ES419657.1	23	AAGCATTCCGGGTTTTCGAC	TATCACCCAGGACATTCAAAAAGTTAT	377	55	calmodulin	2,3
ES419739.1		Not published				proline-rich protein	3

Candidate gene	Total SNPs	Primer sequences		Amplicon length (bp)	T_a	Gene product	Ref.
		Forward primer (5'-3')	Reverse primer (5'-3')				
ES420071.1		Not published				desaturase-like protein	3
ES420171.1	23	CAAGAGCACATCCGACGCAA	ATTCCTCGGTCCATCCTTCTCT	235	55	cold regulated plasma membrane protein	2
ES420250.1	23	GTGGTGTCAGGTGGAAGGACAGT	CATCAAAGAGAAAATACAGTCGGC	884	55	dehydrin-like protein	2,3
ES420560.1		Not published				HVA22F like protein	3
ES420603.1	23	GAAGGAAGAAGAGGAAGGCGAGA	TCCGACGCATCTTACACATTATTA	425	55	dehydrin-like protein	2,3
ES420757.1	19	CCAATGCGGCGTGTGTTCAGTA	TTTTACAAGTTCAGCAAGACCACA	648	55	unknown hypothetical protein	2,3
ES420771.1	20	CATTGGTATGGGGTGCGTGTA	TTCCAAGCAATGGCTGTTCAC	292	55	anaphase promoting complex/cyclsome protein	2,3
ES420802.1		Not published				MADS-box transcription factor	3
ES420862.1	23	AAAGACAGAGGTGAAAGAGATTGC	AACATCTCAAAGTATTCATCATCTCAA	679	55	late embryo abundance (LEA) protein	2,3
ES421219.1	22	GGCGTTGGGAGAGGATTTTTC	AATCTTCCTCTTCTAATGCCACCA	499	55	UDP-glucosyltransferase family protein	2,3
ES421311.1	21	ATCCTTAGTCCGACATTTTGCTGCT	AAGGCACTACACAATACAAACGACAC	729	55	unknown hypothetical protein	2,3
ES421603.1	23	CGACCTCCTCCATCTTGCTG	CGACCGTGTTGTGGATTCTC	528	55	heat shock protein 90 kDa	2,3
		TCAAGCAAGAAGACAATGGAAATA-AAT	GTCCACATAAATCCAAAATAAAACAGT		57		
ES421877.1	19	ATTCCATCGAGTTGCTACGCCAG	CCAACATAATAGGTCTCCATCAACG	550	55	ccr4-NOT transcription complex protein	2
ES422367.1	16	GACAATGCTGAGAAGGGCGA	ATACAAATCGCGGCTACCAAAAT	1038	55	ferritin	2,3
ES424016.1	21	TGTCAACCAGCGGGGATACTCA	TCTTTTGTTTCGTCACCTCCC	397	55	glutathione S-transferase	2,3
ES425204.1		Not published				2-phospho-D-glycerate hydroxylase	3
ES428620.1	17	ATAGACTCCACCAATCCGCT	TTGAATTCGCCAGATAGAGC	686	55	14-3-3 protein	2,3
Pm_CL135Contig1	8	CTCACTAACACCATACC	GAGCATTTAACAACTTTGGG	769	60	cysteine proteinase	2,3
Pm_CL1400Contig1	23	TGCGTCACTCTAATGCC	ATCTGTCAAAGTAGAGAACACC	378	60	alpha-L-arabinofuranosidase/ beta-D-xylosidase	2,3
Pm_CL150Contig1	16	CACTCTTTCTTTCAGGTTAC	CAAATCCTTCATTGTCTCCTTC	356	60	phloem protein	2
Pm_CL1692Contig1	22	CTTCAGGGTTAAAGAATGG	TGCGGAGAAGATGATG	358	60	zinc-finger containing protein	2,3

Candidate gene	Total SNPs	Primer sequences		Amplicon length (bp)	T_a	Gene product	Ref.
		Forward primer (5'-3')	Reverse primer (5'-3')				
Pm_CL1811Contig1	16	GTTTGTCGCATTGATGG	GTTTGTCGCATTGATGG	672	60	Swi2/Snf2-related chromatin remodeling ATPase	2,3
Pm_CL1814Contig1	23	TAATGCCTCAGCCATCC	GCAGTAAGCAACAGTATTACAG	368	60	tetraspanin	2,3
Pm_CL1868Contig1	21	GTCTCCTGACAAATCC	GTAGACCGTAGAATCC	398	60	actin depolymerizing factor	2,3
Pm_CL1982Contig1	23	CTGAAAAAGTCGGAATTTGG	GCTAATAACATCAAATGCGG	271	60	peptide transporter	2
Pm_CL1994Contig1	23	TGTGGGTGGAGATATG GAGATACAACAGTATTTTCAACAGGGG	CCAAGTGGAATATGGAATGAAG AAGTGTCATGCAATAATTATCACCCC	831	60	caffeate O-methyltransferase	2,3
Pm_CL1997Contig1	21	GATTAATGACGCTGTCTG	CCCCTCCATACAAAATAC	711	60	sucrose synthase	2,3
Pm_CL1Contig2	16	CTGGTTCTCTTTGGTTC	TGCCATTCATTGCGTC	631	60	glycine-rich RNA-binding protein	2
Pm_CL2089Contig1	23	GTTGTGAAAGACTTCCC	CACCCCCACTAAAATC	400	60	putative formide amidohydrolase	2,3
Pm_CL2133Contig1	23	GCCAACACTCTATTTTTTGC	CTCAACATCGTTTTCAACC	334	60	mitochondrial transcription termination factor	2,3
Pm_CL214Contig1	22	CCAACAATGTGAAATCCAG	GCCTACGAAACAACAAGAAATG	360	60	beta-tubulin	2
Pm_CL2282Contig1	23	AAATCCTGCCCTTGTC	AAATCTCTTCCTCCTCC	351	60	unknown hypothetical protein	2
Pm_CL234Contig1	18	GAAGTGGAAGTGGAAGATAG	ACATTCATGTCCTCCTTAGCTG	753	60	RAB GTPase	2,3
Pm_CL618Contig1	23	CAGCTTATCATCTGTCTC	TTGTCACTTGCCCACTC	348	60	tropinone reductase	2
Pm_CL61Contig1	21	AACCTGCTGAAAACCC	TCTTCGGTCAAGTCGTC	392	60	cyclosporin A-binding protein	2,3
Pm_CL73Contig1	23	GATTGCCAGCAGATTCAACC	AATAAGTAAGAATGATACAGCCAGCCC	462	60	glycosyl hydrolase family protein	2,3
Pm_CL783Contig1	22	AAAGCTCTCCAGAGTCCCAACC	GTCGAAAAAGAAAACACCATATTCCCC	406	60	SOUL heme-binding family protein	2,3
Pm_CL795Contig1	23	GGACATTCATACACGG	TAGACTTTGGGATGTTAGG	417	60	WD-40 repeat family protein	2,3
Pm_CL855Contig1	23	ACATTCCCAAGAACGCC	CACTTCAAATGTCTGCC	394	60	flavonoid 3-hydroxylase	2,3

Candidate gene	Total SNPs	Primer sequences		Amplicon length (bp)	T_a	Gene product	Ref.
		Forward primer (5'-3')	Reverse primer (5'-3')				
Pm_CL908Contig1	18	CAAACTCTGGACAACAC	CGGAACTCTGTATATCTC	501	60	GRAM-containing/ABA-responsive protein	2
Pm_CL919Contig1	22	ATCAACAGTGGCTGAC	CAGTATATGATCCAAATGGG	738	60	HVA22-like protein	2,3
Pm_CL922Contig1	22	CAACTACATTTTCGTCGG	GGATGGCTACAATCTTC	433	60	thaumatin-like protein	2,3
Pm_CL939Contig1	21	CAGACCAGAGGAAAAC	GGAAGTGGAGTCATAAAG	437	60	aluminum-induced protein	2,3
Pm_CL969Contig1	23	GGAGGATGATACTGAAG	CAGATTTCCATAGCATAGAC	404	60	cell division cycle protein	2,3
Pm_CL988Contig1	23	TTATCAGGCTTCGGCTTCGG	TCTGAAGGTGGACTCTGAC	555	60	thioredoxin-like protein	2
sM13Df243	19	AGTCCGTAAACGGGCTTTCT	CGTGGAAACCGAGGAAGTAG	286	55	arabinogalactan 4	2,3
sSPcDFD005F06506	21	GAAGGTACAGTATAGGAATG	AAGTCTTTTGCTGGGG	507	60	regulator of chromosome condensation protein	2,3
sSPcDFD015C12212	23	CTCTAAAGACTTCTCTCTG	TTACCTGCCACCCATTC	152	60	phospholipase D	2
sSPcDFD024D11311	23	CATCAATCTACAGTCCTCC	TCGAATCAAATCGAATCCAGCC	388	60	polcalcin	2,3
sSPcDFD040B03103	22	GCGGGAGAAAAATTAAAGG	GGAGAAGGAAAGAATGG	378	60	MADS-box transcription factor	2,3
sSPcDFE002A03003	12	CATTAACCCAACAAACCAC	TCATACAGATGGAGGAG	501	60	ACC oxidase	2,3
sSPcDFE025C06206	13	CAACTCCAATGTGCTTC	CCAATACAAGTGGCTTC	456	60	purple acid phosphatase	2,3
sSPcDFE028B10110	22	GAGATTTGAGATGTGTTGAG	CTGCTTTGAGATGAAGG	431	60	beta-amylase	2,3
sSPcDFE038D06306	23	TTACAGTTGGTTAGGGTTTC	AATTGGGGTTTCAATCAAGGCTTC	386	60	calcium binding protein with EF-hand motif	2,3
sSPcDFE044F10510	23	GTCCTTGAAAACCTTCC	CATGATCGTTGACAGCC	353	60	mitochondrial substrate carrier family protein	2,3
sSPcDFE049B06106	21	CAACCATAACAGCAACAC	GCCACGATTTTCACAAG	358	60	auxin-responsive family protein	2,3
sSPcDFE049E11411	20	CAACACCTGCAAAACTC	GGCATCTATGTACTGGTTAATC	407	60	pentatricopeptide (PPR) containing protein	2,3

Candidate gene	Total SNPs	Primer sequences		Amplicon length (bp)	T_a	Gene product	Ref.
		Forward primer (5'-3')	Reverse primer (5'-3')				
sSP-cDFF014F08508	13	TGACGTGAAGGAACTG	CTGAGAATGTGGATTTGG	499	60	hypothetical water stress induced protein	2,3
sSP-cDFF015H05705	23	CAGCAGCATTAAAGATGG	GGATGGAGAGTGTTTGG	286	60	cytochrome P450 family protein	2,3
sSP-cDFF044H10710	16	CTGTGTTTTAGGTCTCTC	TCGCAATGGCTGTTAG	674	60	auxin:hydrogen symporter/trans-porter	2,3
U22458.1	23	CAATTTTGGTCCACTCCAAG	AATCCCAAAAACTTGCATCA	706	57	phytochrome B	2
Z49715.1a	20	AGGCAACTGAAACGACCAAGG	TAAAACCAGCCAAGCCAAACAA	984	57	late embryogenesis abundant (LEA) protein	2,3
		CGACGATGAAGAGGAGGGAG	TAATCAAAACCACACAACACGCAC		57		

1-Krutovsky and Neale 2005, 2-Eckert et al. 2009a, 3-Eckert et al. 2009b

Material for DNA-extraction

The cited studies (Krutovsky and Neale 2005, Eckert et al. 2009a,b) used haploid seed megagametophytes for DNA extraction.

DNA-extraction protocols

DNA was isolated using commercial DNeasy kits (QIAGEN, Valencia, CA) such as DNeasy plant mini kit (Krutovsky and Neale 2005), or DNeasy 96 plant kit (Eckert et al. 2009b).

Material and protocols for RNA-extraction and analysis

Detailed information on the material used, RNA isolation, preparation of cDNA libraries as well as the background and technical procedure of SNP-development and analysis are given in all previously mentioned publications and in Müller et al. (2012), Howe et al. (2013) and Müller et al. (2015).

Important results

- The large SNP database for Douglas-fir and the large number of putative unique transcripts (PUTs) are useful resources for the further characterization of the genome and transcriptome of Douglas-fir, for the analysis of genetic variation using genotyping or re-sequencing methods and for breeding applications (Eckert 2009b, Müller at al. 2012, Müller 2015, Howe et al. 2013).
- Howe et al. (2013) developed a reference transcriptome for Douglas-fir and identified 278,979 unique SNPs across both varieties. This set of SNPs was deposited in the dbSNP database with submitted SNP ID numbers (ss#) ranging from 523,746,501 to 524,245,331. 183,380 SNPs were detected in more than one dataset (sort of plant material). 151,014 SNPs were detected in 17,361 isogroups in both the coastal and interior datasets. On average, the shared SNPs represented 74% of all coastal SNPs and 67% of all interior SNPs. 8067 SNPs were validated on 260 trees using an Illumina Infinium SNP genotyping array. Out of these SNPs, 5847 (72.5%) were called successfully and were polymorphic.
- Müller et al. (2015) found 79,910 SNPs, whose genotypes were called in all individuals. In their data, genetic differentiation between interior and coastal provenances as well as little differentiation

between coastal provenances was confirmed. 58 high-confidence candidate genes for directional selection with a broad functional diversity were identified. Genes involved in drought tolerance showed a significantly higher genetic differentiation between interior and coastal Douglas-fir suggesting a different evolution despite a low level of polymorphism.

- The analysed single-nucleotide polymorphisms (SNPs) refer to candidate genes for:
  1. *cold hardiness* (Krutovsky and Neale 2005 – 15 cold hardiness related genes; Eckert et al. 2009a – 121 cold hardiness candidate genes in coastal Douglas-fir),
  2. *drought resistance* (Müller et al. 2012, Müller et al. 2015) - about 1,000 candidate genes related to drought stress with a total number of 187,653 single nucleotide polymorphisms (SNPs),
  3. *wood quality* (Krutovsky and Neale (2005) – 3 wood quality-related genes).

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Molecular markers used for genetic studies in Sitka spruce (*Picea sitchensis* (Bong.) Carr.)

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1. General remarks

The native range of Sitka spruce (*Picea sitchensis* (Bong.) Carr.) occupies a narrow band along the northwest coast of North America, from Kodiak Island and Prince William Sound (61°00 N), in the western Gulf of Alaska to California, Mendocino County (latitude 39°20 N). In total, its north to south natural distribution is approximately 2.900 km, whereas the west to east extension is nowhere more than 400 km from the Pacific Ocean (Figure 1). The species reaches elevation up to 700 m a.s.l. The range of Sitka spruce is dependent on abundant moisture content during the growing season and its maximum development occurs when summer precipitation is high and there is no pronounced summer drought (Roche and Haddock, 1987).

The species was firstly described in 1787 by the Scottish botanist Archibald Menzies, who recorded it on the shores of Puget Sound. In Europe, it was first introduced into Great Britain in 1831 by the Horticultural Society of London from seed collected by David Douglas, who named it *Pinus menziesii* (Mitchell 1978). The north part of Europe, especially Ireland, United Kingdom, Norway, France and Denmark was the main destination for Sitka spruce (Mason and Perks 2011). Sitka spruce is also grown in Iceland and southern Sweden and several stands have been established in New Zealand (Peterson et al. 1997), too. Hybridization with white spruce (*Picea glauca*) is frequent and well explored (e.g. Bennuah 2004; Hamilton et al. 2012; 2013).

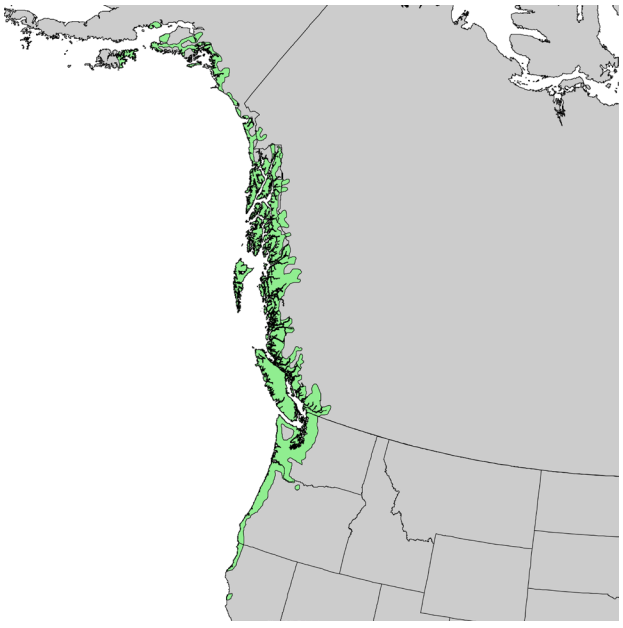

Figure 1. Native distribution range of Sitka spruce (source: USGS, USA)

Most of the genetic markers used for investigation on Sitka spruce had been developed for Norway spruce and white spruce and later transferred to Sitka spruce. The present overview on genetic markers used for genetic investigations in Sitka spruce might give better insight into marker efficiency and lead researchers to easier choice of the right marker depending on research goals.

2. Isozymes

First publications on isozymes for Sitka spruce appeared in the second half of the 1970th. Further on, isozymes were used to study:

- the genetic variation pattern of natural stands (Yeh and El-Kassaby, 1980) and to compare it with gene diversity of other species (Douglas fir, lodgepole pine) from the same region (Yeh, 1979);
- to differentiate Sitka spruce, white spruce and their hybrids and to analyse introgression aspects (Copes and Beckwith 1977, Yeh and Arnott, 1986);
- to describe diversity and outcrossing rates in Sitka spruce seed orchards (Cottrell and White 1995, Chaisurisri et al. 1994) in comparison with natural populations as well (Chaisurisri and El-Kassaby 1994).

The list of enzymes, number of loci and maximum number of alleles per locus found in the cited studies is presented in Table 1.

Table 1: List of enzymes, scored loci and number of alleles for Sitka spruce

Enzyme	E.C. Number	Scored loci	No. of alleles*	Reference
Aspartate aminotransferase	2.6.1.1	AAT-1,-2 resp. GOT-1,-2	3, 3	1,2,3, 4,5
Aconitase	4.2.1.3	ACO-1	3	1,3,4,
Aldolase	4.1.2.13	ALD-1	1	3,4
Alcohol dehydrogenase	1.1.1.1	ADH-1	3	5
Diaphorase	1.6.2.3	DIA-1,-2,-3	3, 2, 2	3,4,5
Esterase	3.1.1.2	EST-1	4	1,4,5
Glutamate dehydrogenase	1.4.1.3	GDH-1	3, 3	1,2,3,4,5
Glucose-6-phosphate dehydrogenase	1.1.1.49	G6PDH-1	3	1,3,4,5
Isocitrate dehydrogenase	1.1.1.42	IDH-1	3	1,3,4,5
Malate dehydrogenase	1.1.1.37	MDH-1,-2,-3	4, 3, 4	1,3,4,5
Malic enzyme	1.1.1.39	ME-1	2	3,4
Peptidase	3.4.14.5	PEP-1,-2,-3	1, 1, 1	3,4
Phosphoglucose isomerase	5.3.1.9	PGI-1,-2	4, 3	1,2,3,4,5
Phosphoglucomutase	2.7.5.1	PGM-1,-2	3, 5	1,2,3,4,5
6-Phosphogluconate dehydrogenase	1.1.1.44	6PGDH-1,-2,-3	3, 4, 3	1,3,4,5

**the highest number of alleles reported in at least one reference is given*

1- Chaisurisri and El-Kassaby 1993, 1994, 2–Cottrell and White 1995, 3-Yeh 1979, 4-Yeh and El-Kassaby 1980, 5-Yeh and Arnott 1986,6-Chaisurisri et al. 1993.

Material for protein extraction

Proteins were extracted from dormant germ buds (Copes and Beckwith, 1977) and megagametophytes (Yeh, 1979; Yeh and Kassaby, 1980; Chaisurisri and El-Kassaby 1993, 1994; Yeh and Arnot, 1986).

Protein extraction and separation protocols

Isozyme extraction, separation by starch gel electrophoresis and staining of gels were carried out by procedures described in detail by Yeh and O'Malley (1980) (protocol developed first for Douglas fir) and Yeh and Layton (1979) (protocol developed first for lodgepole pine).

Important results:

- Some isozymes (e.g. GDH, 6PGDH) seem to be useful to distinguish between Sitka spruce and white spruce. Therefore, they can be used for taxonomic classification of spruce seed lots collected in areas where hybridization between the two species occurs (Copes and Beckwith 1977, Yeh and Arnott, 1986).
- For populations in the natural range only little differentiation at isozyme loci has been found. The genetic diversity within populations is high (92%). Only 8 % of the gene diversity was attributed to differentiation between populations (Yeh and El-Kassaby, 1980).
- Significant differences in gene diversity and heterozygosity estimates were found between a seed orchard in British Columbia and natural populations. The seed orchard population showed a significantly higher number of alleles per locus and percentage of polymorphic loci (Chaisurisri and El-Kassaby 1994).
- In seed orchards outcrossing rates and pollen allelic frequencies differ between trees. Minor differences in pollen allelic frequencies were observed in the upper vs. the lower crown, whereas outcrossing rate of the upper crown exceeds that of the lower crown (Chaisurisri et al. 1994).
- For a Sitka spruce seed orchard in Scotland, high estimates of outcrossing rates were found in a year with intensive flowering. The attempt to identify all clones by means of four enzyme systems failed. Due to the comparatively low degree of polymorphism of the enzymes GOT, PGM, PGI and GDH used, only 42 % of the clones could be identified (Cotrell and White 1995).

3. Organelle DNA markers (chloroplast (cp) DNA, mitochondrial (mt)DNA)

Loci and primers used

Studies dealing with genetic analysis of organelle DNA markers in Sitka spruce are rare. As mitochondrial DNA is maternally inherited, whereas chloroplast DNA paternally, organelle DNA markers are suitable to analyze the introgression between coastal Sitka spruce (*Picea sitchensis*) and interior spruce species (*Picea glauca* and *Picea engelmanii*) (Szmidt et al. 1988, Sutton et al. 1991, Hamilton and Aitken 2013). Organelle markers have been also used in a phylogenetic study of the genus *Picea*, which includes also *Picea sitchensis* (Ran et al. 2006). Besides nuclear SSRs, Hamilton and Aitken (2013) applied one chloroplast PCR-RFLP marker and one mitochondrial marker to study the mechanisms involved in tree migration and adaptation in response to past environmental changes.

Coombe et al. (2016) published the first Sitka spruce chloroplast genome assembled exclusively from *Picea sitchensis* genomic libraries prepared using the 10X Genomics protocol.

Szmidt et al. (1988) and Sutton et al. (1991) analyzed restriction fragment length polymorphisms (RFLP) from the chloroplast DNA (cpDNA). According to Szmidt (1988), cpDNA was digested with four restriction endonucleases – *Bam-H*I, *Bcl*-I, *Kpn*-I and *Sac*-I – each of which recognizes a specific six base pair nucleotide sequence for cleavage. Sutton et al. (1991) digested mtDNA with *Bam-H*I before cloning. In the aforementioned papers, morphologically pure trees of white spruce (*Picea glauca*), western white spruce (*Picea glauca* var. *albertiana*) and Engelmann spruce (*Picea engelmannii*) were also analyzed in addition to Sitka spruce (*Picea sitchensis*).

Hamilton and Aitken (2013) amplified one universal cpDNA locus and one mtDNA locus and sequenced the products to find polymorphisms. Ran et al. (2006) also sequenced the maternally inherited mitochondrial nad5 intron and two paternally inherited chloroplast regions (see Table 2).

Material for DNA-extraction

DNA was extracted from fresh buds (Hamilton and Aitken 2013) or needles from adult trees (Szmidt et al. 1988, Sutton et al. 1991, Coombe et al. 2016, Ran et al. 2006) and seedlings four months after germination (Szmidt et al. 1988).

DNA-extraction and amplification protocols

Total DNA was extracted from ground, frozen needle tissue using the Doyle and Doyle (1990) CTAB protocol (Ran et al. 2006, Hamilton and Aitken 2013) and the method of Wagner et al. (1987) (Sutton at al. 1991).

For the extraction of chloroplast DNA from frozen needles, Szmidt et al. (1988) applied a method described by Szmidt et al. (1986), which represents a modified version of White's (1986) extraction protocol.

Amplification of the two cpDNA regions and the nad5 region of mtDNA was done using the following PCR-protocol:

- one cycle of 4 min at 70 °C, 4 cycles of 2 min at 94 °C, 20 s at 55 °C (trnC-trnD) or 50 °C (trnT-trnF and nad5), and 2 min at 72 °C, followed by 36 cycles of 20 s at 94 °C, 20 s at 55 °C (trnC-trnD) or 50 °C (trnT-trnF and nad5), and 2 min at 72 °C, with a final extension step for 10 min at 72 °C (Ran et al. 2006, Hamilton and Aitken 2013).

Table 2: Primer information of the chloroplast microsatellite markers used in genetic analysis of Sitka spruce (T_a= annealing temperature)

Type	Locus	Primer sequence [5'-3'] F= Forward, R= Reverse	T_a (°C)	Size (bp)	Ref.	Source of primer pairs
cpDNA sequencing	trnT-trnF	F:CATTACAAATGCGATGCTCT R:ATTTGAACTGGTGACACGAG	50	500	1,2	Taberlet et al. (1991)
	trnC-trnD	F:CCAGTTCGAATCCGGGTGTC R:GGGATTGTAGCTCAATTGGT	55	2324	1	Demesure et al. (1995)
mtDNA sequencing	nad5a.1	F:CGCATATGGGTAGCAAGAGGGC R:GAGGTTTCCCATCACACGGCTCACC	50	500	1,2	Wang et al. 2000, Jaramillo-Correa and Bousquet, (2003)

1-Ran et al. 2006, 2-Hamilton and Aitken 2013

Important results

- Coombe et al (2016) found out that the chloroplast genome is 124,049 base pair long. It shares high sequence similarity with the related white spruce and Norway spruce chloroplast genomes.
- The cpDNA restriction patterns generated by *Bam-HI, Bcl*-I from individual trees of Sitka spruce, white spruce and Engelmann spruce were species-specific. Based on this finding, Szmidt et al. (1988) used cpDNA restriction polymorphism successfully for classifying seed lots in regions where hybridization between the two species occurs.
- Based on sequencing of two chloroplast and one mitochondrial fragment, Ran et al. (2006) found out that *Picea sitchensis* were basal to other North American spruces that were further divided into three clusters in the cpDNA phylogeny.
- According to Hamilton and Aitken (2013) only two chloroplast haplotypes were observed within the ~500-bp region Trnf-TrnL region among 255 individuals sequenced. One haplotype was fixed in the reference Haida Gwaii (Queen Charlotte Island) Sitka spruce population and another one in the reference Fort Nelson white spruce population. These haplotypes were distinguished by single-nucleotide base-pair mutations at five sites across the sequenced region (at 87, 188, 193, 268, and 285 bp) and a single base-pair deletion 339 bp from the 5' end of the sequenced region.
- Within the ~500-bp region sequenced of the nad5a gene only two haplotypes were observed. "A four-nucleotide tandem base repeat (CTTGACTTG) at 276 base pairs from the 5' end of the sequenced nad5a region distinguishes white spruce; Sitka spruce mitotypes lack the repeat. The white spruce mitotype was found only in the 11 individuals sequenced within the reference white spruce population. The Sitka spruce mitotype was fixed in all other populations, including the hybrid and reference Sitka spruce populations" (Hamilton and Aitken 2013).

4. Nuclear DNA markers (nSSR, EST-SSR, SNP)

a) nSSRs (putatively neutral microsatellites), EST-SSRs (expressed sequence tag derived microsatellites)

Loci and primers used

The first nuclear microsatellite markers for Sitka spruce were developed by van de Van and McNicol (1996). They used nSSRs to screen 58 Sitka spruce clones. Later on A'Hara and Cottrell (2004, 2007, 2009) developed additional microsatellite markers for this species as tools for genetic characterization of Sitka spruce populations and distinction from other related spruce species (e.g. white spruce).

Due to frequent introgression between *Picea* species, the problem of species identification and characterization of introgression zones is of high importance. Therefore, the search for species specific markers, among them highly polymorphic microsatellites, is central in *Picea sitchensis* research. Besides development of new markers, the transferability of already developed nSSR markers from other species to Sitka spruce was tested by many research teams (Hodgetts et al. 2001; Rungis et al. 2004, Bennuah, et al. 2004, Bérubé et al, 2009; Ralph et al. 2008). For example, Hodgetts et al. (2001) developed 13 nSSRs for *Picea glauca,* 10 of which he amplified successfully in *Picea sitchensis*. Similarly, from eight primer pairs developed by Rajora et al. (2001) for *Picea glauca*, five amplified also in *Picea sitchensis*. Rungis et al. (2004) developed 25 polymorphic EST-SSR markers which could be amplified in three spruce species namely Sitka spruce (*Picea sitchensis*), white spruce (*Picea glauca*) and black spruce or interior spruce (*Picea glauca* × *Picea engelmannii*).

EST-SSR-primers developed for black spruce by Perry and Bousquet (1998b) were successfully used to reveal sequence tag polymorphisms in Sitka spruce and to characterise the genetic diversity of natural populations (Bennuah et al. 2004, Gapare et al. 2005).

Table 3: Primer sequences, annealing temperatures (T_a), allele length in base pairs (bp), number of alleles scored (N_a) and references for microsatellite markers (both nSSRs and EST-SSRs) available for genetic analyses in Sitka spruce.

Locus	Motif	Primer sequence		T_a (^{0}C)	Size (bp)	N_A	Ref.	Genebank accession number
		Forward	Reverse					
SSgata3	(TA)9(RA)16(GATA)14	CTGTGTACTTTTTCATGGCC	CTTTGTATCAAACTCCCCCT	52 resp54	346	6	1	-
SStg3a	$(TG)_{27}$	TCAAGCTCTCCAACCCAGA	TGTCGAGTTTGACTTGTACCA	52 resp 54	136	15	1	-
SStg4	$(TA)_4(TG)_{11}(TA(TG)_3$	CTCACCTCCGGTTTCCATTA	CATTGTCCCCCACCATTTAC	52 resp 54	207	13	1	-
SStg4a	$(TA)_6TA(TG)_2TC(TG)_2$	ACAATGTCAGGCATCGCTTA	GTCCCTTCCCCTTTACAATG	52 resp 54	122	4	1	-
SStg4c	$(TG)_8$	TAACCCCGAGGTACTCAACC	ATTCGGTTAACTTGTTCGGC	52 resp 54	139	3	1	-
SScac4	$(CAC)_2CAA(CAC)_3C$-$CAA(C)_4(A)_4$	TTGGGGAGTAGTTAAAGTAACGAA	AATGCGAAACCAGTTCAGG	52 resp 54	119	-	1	-
SStg3	$(TA)_6TC(TG)_{22}(TATG)_5TA$-$TAAATA(TG)_8TT(TG)_2(TAT$-$G)_5TATAAATA(TG)_8$	TTCACATGCACCCCTTTTTA	TCGACTTACAATACACACAACATTC	52 resp 54	225	-	1	-
SS01	$(AT)_9$	M13**-AATGCGTTGGAAACGTGAC	AATCCACGATGGGTGAAGAG	55	No amplif.	-	2	CK440148
SS02	$(AT)_{12}$	M13**-TTTGTCATGTGGGTCAGTCC	CATTCAAGGATGGTGTGCAA	55	No amplif.	-	2	CK436660
SS03	$(TA)_8$	M13**-TACACAACGCAGACTGAGCA	CGGAATGAAGATCGCAGTTT	55	No amplif.	-	2	CK438590
SS04	$(TA)_{24}$	M13**-CCGAGGTTGCCTTATGTTGT	TGGATCAAGTTCAGGTGCAA	55	No amplif.	-	2	CK443896
SS05	$(AT)_{10}$	M13**-ATGAAGGCAGGGAAACAGAA	CCTGGGATTTCAAGTTGAGG	55	Com-plex pattern	-	2	CK440928
SS06	$(TA)_{21}$	M13**-TTCATTTTGAGCTGCATGGT	TTTCCAAGTGCCTACCACCT	55	Com-plex pattern	-	2	CK442714
SS07	$(TA)_9$	M13**-CCAGCTCAGGTGAATCCTTC	TGGAGATGGTCCGACTTAGG	55	Com-plex pattern	-	2	CK444999

Locus	Motif	Primer sequence		T_a (^{0}C)	Size (bp)	N_A	Ref.	Genebank accession number
		Forward	Reverse					
SS08	$(AT)_7(AC)_3$	M13**-CACATCATGAAGAGCGCATT	TCCAGTACGAGCAGCGTAAA	55	156–158	2	2	CK437916
SS09	$(TA)_7$	M13**-CCAACTCTGACTGTGGAGCA	AGCAACATGTGAGCGATTCA	55	176–220	17	2	CK442075
SS10	$(AT)_7$	M13**-GGAAACACAAACCACGGAGT	TCACCGCCGATTAGACATACT	55	220–228	4	2	CK442920
SS12	$(T)_{14}ATTTGCG(TGGCG)_4$	M13**-CTTGATTTTTGGCGATCGTT	ACGTGTGAACCGGAGGAGAT	55	200–250	19	2	CK438258
SS13	$(TA)_7$	M13**-ACTCATAGCGTCACGGGAAC	TGAATCTCCACCTCCTCTGG	55	250–260	7	2	CK435966
SS14	$(AT)_{22}$	M13**-GCCAGCATGAAGACACGTTA	CCCTCAAATGAAGGAATTTGC	55	230–238	5	2	CK444248
SS15	$(GA)_9A(AG)_8$	M13**-GGAATAAAATGGCAGGTGGA	GCCTGCAGTAGTTGGCAGA	57	202–224	13	2	CK438739
SS16	$(TA)_8T(TA)_5$	M13**-GCAGCACTGGCAACATTCTA	ACGGAGACAAATCGCTTGTT	55	306–322	9	2	CK439419
SS17	$(AT)_{11}$	M13**-CCGCTTTCACGGGTTTAATA	GAGGTGGGAGGGTTTTTCTC	55	170–244	18	2	CK437301
SS18	$(A)_{19}(TA)_6$	M13*-GTGCCGTGAACCACATTAAC	GCGTACATGAAAACCTGCAT	55	248-282	-	3	40766244
SS19	$(AT)_6$	M13*-GAGAAAACCTGGCCTCTA-GAAA	GGGACCATCTCCTAGACACG	55	218-226	-	3	40767122
SS20	$(AT)_6$	M13*-TAACCGATGCCTCTCACAGC	TGATGCATCCATCCTTGTTG	55	196-212	-	3	40775728
SS21	$(A)_8(CA)_5(TA)_{10}$	M13*-TGCTGTGCCTGCCTATACAT	TGCATTACAAGCACCTCAGA	55	180-226	-	3	40779303
SS22	$(AT)_5(CT)(AT)(AT)_3$	M13*-CACTCCAAGCAAGAATCTCCA	GACATCTCAGGCGATTCCAT	55	246-250	-	3	49016032
SS23	$(AT)_7(T)_5$	M13*-GCTCCTTCAGATCAGCGAAC	AGAGGCAAGTTCAGGGGATT	55	196-210	-	3	49017151
SS24	$(TA)_4(A)_3(TA)_4$	M13*-CCTTAACCCCTACTGTCAAT-GC	CGGCTTGTGGGAGTAACAAG	55	258-282	-	3	49024553
SS25	$(AT)_{13}$	M13*-AACATCCACCCATTCAAAGC	GTGCATGCGAATCCCTAAAC	55	218-264	-	3	49037755

Locus	Motif	Primer sequence		T_a (^{0}C)	Size (bp)	N_A	Ref.	Genebank accession number
		Forward	Reverse					
SS26	$(TA)_8$	M13*-AGGTTGGAAACCCTGTGATG	ATTCGGATTGCGGTTCTGTA	55	198-218	-	3	49041358
SS27	$(TA)_{10}$	M13*-TGACGTTATTAATGGCGTTTG	TTTGAGGGGAGATCTTGTGG	55	176-204	-	3	49041823
SS28	$(TA)_{17}$	M13*-GCTGAGGCAATGATGTCAAC	GACATCTGTTCATTGCTCTACTTG	55	256-284	-	3	49043234
SS29	$(TA)_{12}$	M13*-CAAGGCACATGCTTCTGTCA	AAGATGGCCTTTGCGTCATA	55	254-286	-	3	49043836
SS30	$(TA)_9$	M13*-TGAGTTTCCCCAAACTCTATT-CC	GGTGTTGTTTAAATGATTTGGAAG	55	270-294	-	3	49044204
SS31	$(TA)_{11}$	M13*-CGGAGTCTTGGTGGAACATT	GGAGCAATCCCTCTTCTTCC	55	144-190	-	3	49045444
SS32	$(TA)_4(A)(TA)_9(CA)_4(TA)_6$	M13*-CTGAAGCAAACACGACAAGC	CCACATGCCTGCACTATCAT	55	242-262	-	3	49045765
SS33	$(TA)_8$	M13*-ACCTCAAGGGGCTACACTGA	TAACGAGCCTGGTGTGTTTG	55	188-194	-	3	49046524
SS34	$(TA)_3(TG)_3(TC)(TA)_4$	M13*-TTGCCCTGAGTAGGGTACAA	AGCATTTGCTTGTTTCGTGA	55	260-262	-	3	49047398
SS35	$(TA)_8$	M13*-TCCAATCCAAAACCGAAAAC	GGCCTGTGTCTTTTCCATGT	55	262-268	-	3	49047601
SS36	$(GA)_8$	M13*-GTGTTCGAATCCCAGGAAGA	TGCCCTGTGCGATGTTATAG	55	232-238	-	3	49047619
SS37	$(TA)_5$	M13*-GGCCCCAAGATTGAAGAAGT	TTTGGAATGCAATGCAGTTTAC	55	224-226	-	3	49047962
SS38	$(GGA)_6$	M13*-GAACGCAAGATGGGACACTT	TGACCGTCTCTGTCCTTGTC	55	206-221	-	3	49048259
SS39	$(TA)_{10}$	M13*-CTGTCCATCATGAACCCTGA	CGAAGGAAAGGAATTCCACA	55	224-258	-	3	49048405
SS40	$(CT)_6$	M13*-TCCCTCCTATACGGGAAATGT	GCGTACGTTAGTCGATTGGAA	55	202-204	-	3	49048492
SS41	$(TA)_6(AA)_3(TA)_4$	M13*-GCCAGAATGGTTTTCACGAG	ATATCCTTGGGAGCGACCTT	55	274-280	-	3	49048842
SS42	$(TA)_6$	M13*-TCGACCCTCTAACGTCTTGC	TGAGAATCATCCCGAACTCC	55	230-248	-	3	49049101

Locus	Motif	Primer sequence		T_a (^{0}C)	Size (bp)	N_A	Ref.	Genebank accession number
		Forward	Reverse					
SS43	$(TA)_{16}$	M13*-ACAATGAGGATGAGGGCTTG	CCCCTTTCGAACTGTGTCAT	55	266-298	-	3	49049495
SS44	$(A)_{10}$	M13*-CAAACGGAAGTCGAACCATT	ATTTGTTTTCGGGTCTGCTG	55	222-226	-	3	49049559
SS45	$(AT)_{8}$	M13*-AACACACGAGGGGATTGAAC	ACCTGGCTTTCGGTATTGTG	55	216-236	-	3	49135131
SS46	$(AT)_{13}$	M13*-GACAGGCACCCAGAACTGAT	CTGGAATTTACAATGCACAGGA	55	232-288	-	3	49137541
SS47	$(AT)_{14}$	M13*-AGGCTCACAGCTCCGTCTTA	CCCAAGTGCCCTTGAATATG	55	242-302	-	3	69437955
SS48	$(AT)_{8}$	M13*-TCAAATCCAATCACGTACAACA	CAACTTGAAGCCCCACTTGT	55	256-278	-	3	70256787
SS49	$(CT)_{16}$	M13*-CAGTGCACGTCCAAACAAGT	TGGCTGTTTGCTCATATTGC	55	266-292	-	3	70258880
SS50	$(AT)_{10}$	M13*-TCAGAAGACGCAAACAATCCT	TTCTTAGGAGGCAGCGGTTA	55	164-178	-	3	70260028
SS51	$(TA)_{11}$	M13*-CAAAACACAACTTGCCCACA	TGGAACACTTGGAACTCCATT	55	176-220	-	3	70285403
SS52	$(TA)_{10}$	M13*-AAGAGTCGCTCAAGGGCATA	CATCTGAGAGGTCGCCATTT	55	196-218	-	3	70297371
SS53	$(TA)_{8}$	M13*-CCGACGGTAGTTCCTTTCAA	CCGGTGCTAAATCCTTGTGT	55	198-218	-	3	70318999
SS54	$(GA)_{10}$	M13*-AAGCATGATCAGTGGATAGCA	AATTGAGGCAATTGGTGAGG	55	220-240	-	3	70346839
SS55	$(TA)_{12}$	M13*-GAATTTAATGGCAACCATAC-CC	TGTTTTGACTCGGATCTCTCTC	55	160-178	-	3	70346839
SS56	$(TA)_{8}$	M13*-GAAAATCGCCGAAAGATCAC	GCCAGCATTCACTTGACAGA	55	248-264	-	3	70350346
SS57	$(TA)_{16}$	M13*-CATCGTACAGCCAATCTCCA	GAGGTCGTCCCGACAGTAGT	55	126-140	-	3	70355804
SS58	$(GC)_{5}(CA)_{15}(AT)_{4}$	GATTGTAGGACGCACCGACT	GACATGGACAAACTTCCTTGG	55	210–240	14	4	FJ147466
SS59	$(CA)_{12}(AT)_{3}$	TTCTGGAGATTTCTCATTTGTG	CTTGGGTTCTATCCCCTTG	55	220–240	9	4	FJ147467

Locus	Motif	Primer sequence		T_a (^{0}C)	Size (bp)	N_A	Ref.	Genebank accession number
		Forward	Reverse					
SS60	$(CA)_{20}(AT)_6$	CGGTTAAACCCACCGTAAT	ACCACGCACAATACAAAATG	55	180–220	14	4	FJ147468
SS61	$(CA)_{12}$	TGGATTCCATGTCATACAAAC	TTCGATCTGACCCCATAAC	55	140–180	8	4	FJ147469
SS62	$(CA)_{16}$	AAAACCCCAGACCTCCATG	CCGCGCCATATAACACATT	55	160–200	8	4	FJ147470
SS63	$(CA)_{30}$	CCACTTTCACAAATAATCTCCA	ATGCTTTGGACAAGGTCAA	55	200–240	19	4	FJ147471
SS64	$(CA)_{18}GC(CA)_9(AT)_8$	TGCACGTAGTTAGTGCTCATC	CATGCAGAAGCAACATTTAAC	55	200–220	15	4	FJ147472
SS65	$(CA)_{23}GCGT(GC)_8$	AATTTGGGTCTCTCTACTCTTG	ATTAGCCTCCCCACCTAG	55	230–250	19	4	FJ147473
SS66	$(CA)_6TA(CA)_8$	GTGTCAGGGGGGGAGTAT	GTTTGCGAGACTATTTGAAGC	55	280–310	12	4	FJ147474
SS67	$(CA)_{14}(TA)_4$	ATAGGACCGCACACACATAC	CCTCCAAGTAGGGGTAAATC	55	310–330	8	4	FJ147475
SS68	$(CA)_{36}(AT)_5$	GAAACGTGGACTAACGTCTTC	AGATTGACCCATACGATACTCA	55	260–320	15	4	FJ147476
SS69	$(CA)_{28}(CG)_7$	TCCACACATGGATAGTGTAA	GAGGTGGCAGATTTTACTC	55	200–310	21	4	FJ147477
SS70	$(CA)_{13} + (CA)_{16} + (CG)_6(CA)_{37}$	TGTGAGAGGAGCCATTTCTAGT	CCAAGGGCTACCTCAAGTG	55	140–160	6	4	FJ147478
SS71	$(GA)_{16}$	AACCCAGCAAAAGTTCCA	TAAAGCCCGAGCATAAGC	55	150–200	15	4	FJ147479
SS72	$(GA)_3AA(GA)_{14}$	AAGGAAAAACCCAATGTGG	GTGGTGGGACAATTTTGTG	55	140–190	7	4	FJ147480
SS73	$(GA)_{30}$	CCAAGATACTCCAGGAGGAT	GCGAAGAAGAAGATGTTGG	55	230–270	16	4	FJ147481
SS74	$(GA)_{29}$	ATTGTCGGAGGGCATTTAC	GTGTCCAGGGAAGAGATGA	55	180–240	9	4	FJ147482
SS75	$(GA)_{18}$	CCTTGGACTTAAAGAATGATCC	CCATGCACTTGTGCTTATTC	55	180–210	9	4	FJ147483
SS76	$(GA)_{35}$	AACACTGCCAAGTGTCAATCC	GGTAGTCCCGACATGCTGAT	55	230–280	18	4	FJ147484

Locus	Motif	Primer sequence		T_a (^{0}C)	Size (bp)	N_A	Ref.	Genebank accession number
		Forward	Reverse					
SS77	$(GA)_{17}$	GGTAACGCCCAACTATTGAAC	CACCCACAGGTCAGAAAGTC	55	220–250	8	4	FJ147485
SS78	$(GA)_{22}$	GTGCCAGAGAGAGAAAGTGAA	GGACAGCGTAATATGTGAGTTG	55	260–290	9	4	FJ147486
SS79	$(GA)_{13}$	ACTGGAGGTCTCAGTGTTGA		55	250–280	10	4	FJ147487
UAPgCT3	CT_{15}	TTGAAAAAGAGGTTAGGAAGGGA	TTCTTAAAGAAGCAGGGCATTG	60	220–232		5	-
UAPgAC/AT6	AC_{10}/AT_7	GTTTGGAGAGATAGAGATTGTAC	TTTTTGACGGCTGGAAACTTC	60	114		5	-
UAPsTG25	TG_{27}	TCAAGCTCTCCAACCCAGAT	TGTCGAGTTTGACTTGTACCAA	62	96–104		5	-
UAPgTG64	TG_{16}	AATTTCCTTCCTCTATGTCGAC	CAATATGATGTGTAATTCCTTCC	56	104–106		5	-
UAPgGAT64	GAT_8	TGTTAAATAAGGAAGGAATTACAC	CACTTACCCTCTTCAGGTCC	57	102		5	-
UAPgCA91	CA_{20}	TCTGTTCTTCATACGTCTCAC	GGAAATTGGCACTCTGTATTC	60	108–126		5	-
UAPgAG105	AG_{11}	CAACTACCTTGAGCCAATCA	GTCCGGCATTATTGATCATT	56	159–161	3	5,11	-
UAPgCT144	CT_{18}	CACTCGATCACTTTCTCATC	CAAGATAGTAATGGTGAGGC	58	132–164		5	-
UAPgAG150A	AG_{19}	ACCAATGCTTTTACCAAACG	TTGATTGCAAGTGATGGTTG	54	153–157	2	5,11	-
UAPgAG150B	AG_{19}	As above	As above	54	126–130	5	5,11, 12	-
UAPgCT189 A	CT_{23}	TGCACTCCTTGCGGAAATTCTTC	GTTGTTGACTAAGGTTGAAGGGG	65	134–148		5	-
UAPgCT189B	CT_{23}	As above	As above	65	114–116		5	-
PGL6	$(AG)_4$	TACTTCAGGACTTCAGGATTCAGGG	TTTGCAAAGGCCTAAAGACCGTTGG	60↓54	116-118	2	6	-
PGL7	$(AG)_{38}$	TCACTATTTATTTCCCAAATGCTC-GTA	TCTCCNCAAGAAATCCNCCCTC	60↓54	104-170	22	6	-
PGL12	$(AAG)_2$ $(AG)_3$ $G4(AG)_9$	CCATCTCAAAATATTTAATTGTCCA-GT	TCATATCTGCATGCAAAGTCTGAAC	60↓54	250-222	No amp-lif.	6	

Locus	Motif	Primer sequence		T_a (^{0}C)	Size (bp)	N_A	Ref.	Genebank accession number
		Forward	Reverse					
PGL13	$(AG)_{21}$	AAAAATAGTTTATATTTTC-TTTATTACTC	TATAAATCATTTTTCTTATGTTGTG	50	110-160	19	6	-
PGL14	$(AG)_{20}$	AAAAATGATTTATATCTTCTTATT-GTCT	GNGTCATAAACGCCCATCAATAG	50	136-180	18	6,11	-
PGL15	$(CT)_4N16(CT)_{11}$	CATACTCTCACACCCACACCCTCTC	CAAGAACAGAAGAGAGGTCAA-GATTG	60↓54	176-248	15	6	-
SPAGC1	$(TC)_5TT(TC)_{10}$	TTCACCTTAGCCGAGAACC	CACTGGAGATCTTCGTTCTGA	53	104–150	21	7,11, 12	-
SPAGG3	$(GA)_{24}$	CTCCAACATTCCCATGTAGC	AGCATGTTGTCCCATATAGACC	53	105–137	18	7,11, 12	-
SPL3AG1A4	$(GA)_{21}$	CATACTCAATGCACCTAGATATGC	AAGCAAATGAAAGCTCCTTGT	53	85–105	2	7,11	-
SPL3AG1H4	$(GA)_{20}$	GGAAAGGAGGAGGACAAGAG	TAAGGATCGAGTCTCTCACTCC	53	127–161	5	7,11	-
PAAC17	$(AC)_{36}$	GAAACAAAAATTATTACGCG	ATGCCCTCCTAATGAATG	53	132–148	5	8,11	AJ131107
PAAC19	$(CT)_{23}CAA(TG)_{12}$	ATGGGCTCAAGGATGAATG	AACTCCAAACGATTGATTTCC	53	155–173	6	8,11	AJ131108
PAAC23	$(GT)_{14}$	TGTGGCCCCACTTACTAATATCAG	CGGGCATTGGTTTACAAGAGTTGC	53	266–276	3	8,11	AJ131109
EAC6A06	$(AC)_{20}$	AATTAAGGGGTAATGTGCCAC	AATGATGTTAAAGCAATATGTCTTG	53	95–141	8	9,11	AJ292706
EAC6B03	$(AC)_{25}$	GAAGGTTATAATATTCAGTGAAGG	TAATGCTTATCAATGAGGTTG	53	-	-	9,11	AJ292712
EAC7C11	$(AC)_{19}$	AACTCTATAAAATAACGCACCTCG	CCAAAACAAAGGAAGGATGTT	53	105–137	11	9,11	AJ292730
EAC7H07	$(CA)_{23}(CAT)_{10}$	GGTTCAAACCTCCCACCTAC	ACCAACTAAGCCACAAGTGC	53	125–139	5	9,11	AJ292739
EATC3C05	$TAT(CAT)_{10}(AT)_{20}$	TTAGTGGACGTTCATCATCATC	TCACAATCACTTTTTTAGTCGC	53	249–267	6	10,11	AJ296736
WS0011. P12**	$(AGGA)_{32}$	CGATAAGATGGCTCCTCAAA	GGAGGCTGAAAAGTGGTTACA	53	291–295	3	11	CN480892
WS0015.I04**	$(AT)_{29}$	CACCCTTTAACCAAGCAAGC	GGTCTACATGTTTATCACCAACGA	53	179–229	8	11	CN480893
WS0016. O09**	$(AT)_9$	CTTTGGGGGCTAGCAAGTTT	ATTCGGGCTTCATAGCACAA	53	390–406	9	11	CN480894

Locus	Motif	Primer sequence		T_a (^{0}C)	Size (bp)	N_A	Ref.	Genebank accession number
		Forward	Reverse					
WS0019. M09**	$(AT)_{20}$	TTTCAAATCGGAGTGCATTG	GGAGATCGTGGTAACCCAAA	53	236–312	3	11	CN480895
WS0019. F22**	$(AT)_{13}$	AAGCGTTTCTCATTTTCTTGG	GGGCCCAGAACTAACAATGA	53	352–366	6	11	CN480896
WS0022. B15**	$(AG)_{12}$	TTTGTAGGTGCTGCAGAGATG	TGGCTTTTTATTCCAGCAAGA	53	183–203	4	11	CN480899
WS0023. B03**	$(AT)_{10}$	AGCAGCTGGGGTCAAAGTT	AAAGAAAGCATGCATATGACTCAG	53	174–218	9	11	CN480900
WS0023. B12**	$(TA)_{22}$	GATGAGTGGAATTGGGAGAGA	AAAGTCAATTTTTCATGGCTTCA	53	160–188	7	11	CN480901
WS0032. M17**	$(ATT)_{6}$	GCTTGACACCTGAAAATTACATTAG	AAGGCAAGAGGGATCGTAAA	53	278–308	8	11	CN480906
WS0033. A18**	$(TA)_{26}$	GGCTGCTCTCTTATCCGTTTT	TGGCTCTCATCCAGAAAAGAA	53	145–149	3	11	CN480907
WS0035. A01**	$(AT)_{11}$	GGGCGAAATATGTCGATTTT	TCATCCCTGCATTGTCTCG	53	148–150	2	11	CN480908
WS0046. M11**	$(AAG)_{6}$	CACTAGGGCATTGGGAAGAA	ATGAGAGGCTGGGGTATGAA	53	287–287	1	11	CN480891
WS0053. K16**	$(AT)_{13}$	ACATATCATGGTTGCGATGC	CCACAGCCCCTAAAATGTGA	53	201–217	5	11	CN480898
WS0061. C21**	$(CTTT)_{5}$	TTTTTAGCCTCATGGACGTT	GGTTAAACGGACGCTGAAAG	53	259–279	3	11	CN480886
WS0061. K02**	$(AT)_{9}$	TCAAGAATCAGCTCCGCTTT	GGCGCAGATACGTTGACAT	53	209–217	4	11,12	CN480887
WS0071.J15**	$(AT)_{22}$	TTTTTAACCATGGGAATTGG	GGATCGAAGGGATGTCAAGA	53	205–247	11	11	CN480902
WS0073. H08**	$(AT)_{14}$	TGCTCTCTTATTCGGGCTTC	AAGAACAAGGCTTCCCAATG	53	188–218	12	11,12	CN480903
WS0079. H08**	$(GCAG)_{6}$	GGGATGCCTGGGTAAATAAAA	TTTTGCATTTGCTTTGATATGTG	53	252–256	2	11	CN480904
WS0082. E23**	$(TA)_{11}$	CAGGTCAAATCCTTCCTTCC	GAAGAAAATGCTGGCTTTCG	53	239–247	4	11	CN480909
WS0082. O23**	$(TA)_{15}$	AGTGACAGTTGTCTTAGCACATCA	AAGGTTTCCGATCGCATCTA	53	214–224	6	11	CN480910

Locus	Motif	Primer sequence		T_a (^{0}C)	Size (bp)	N_A	Ref.	Genebank accession number
		Forward	Reverse					
WS0092. A19**	$(AC)_9$	TGTGGTTTTCTGCTTGGAAA	CCCATTTTGACTTTGAAATAAGC	53	215–223	5	11	CN480888
WS0092. M15**	$(TCC)_6$	GATGTTGCAGGCATTCAGAG	GCACCAGCATCGATTGACTA	53	212–218	3	11	CN480889
WS0092. H13**	$(GCT)_8$	CCACGATGTCGTTGAAAGAA	TTTCAGTCTTCCTGCATTCG	53	220–226	3	11	CN480890
WS00111. K13**	$(AT)_9$	GACTGAAGATGCCGATATGC	GGCCATATCATCTCAAAATAAAGAA	53	215–225	3	11	CN480897
WS00716. F13**	$(GA)_{10}$	TCAAGTAATGGACAAACGATACA	TTTCCAATAGAATGGTGGATTT	53	281–307	7	11	CN480905
2	$(TC)_{25}$	TTTGGACTCTTTTTAATGAGATTG	ACAGACAATGTGACAATATAGTG	53	172–216	9	11	-
44	$(AG)_n$	TTACACTTCAGAGAGAGAGAGA	GGCCCACATCAACCCTTACC	53	107–129	6	11	-
BCPsmAC1		TGCAGCCCGATCTAACAATA	TTGTGAGGGAGTTGGACAAG	56	151–183		13	AF409142
BCPsmAC6		TGCGTGTTCCGTTCTCTTC	GCGGCCAGCTTCATATCC	56	109–124		13	AF409147
BCPsmAG1		ACACTCGTGCTCTGGTTCTG	CTTCTCTTTCTCTATATTGCC	50	149–153		13	AF409152
BCPsmAG2		GATCTTAATGTTCATGCAAGG	GAGATTTAGGGAGAGCGC	50	96–140		13	AF409153
BCPsmAG10		CCTCTCTATACCTAGCCCTAAAC	ATCAGGAATGAGCAAAGCTA	50	100–152		13	AF409161
BCPsmAG12		TTAATGCCTACTAACCACAAATC	GTATCGATGCTTAGCTATAGGG	50	191–245		13	AF409163
BCPsmAG13		ATTTGTAAACTACATCCCACTACC	TGGGTAATGGTTTAGGGAAC	50	116–148		13	AF409164
BCPsmAG14		TTAATGCCTACTAACCACAAATC	GTATCGATGCTTAGCTATAGGG	50	191–224		13	AF409165
BCPsmAG15		CCTCAATCTACTTATGTACACC	AGAGGGATATTGGAGGTTAGG	50	99–116		13	AF409166
BCPsmAG17		CATCAGTGGTCTTGAGCATG	TGAGTGAGAAAGATTGATATGGG	53	233–292		13	AF409168

Locus	Motif	Primer sequence		T_a (^0C)	Size (bp)	N_A	Ref.	Genebank accession number
		Forward	Reverse					
BCPsmAG18		TTGTCTCGTGTACAAACTCAGC	CAGAATGGGAGAACACGATG	53	152–202		13	AF409169
BCPsmAG19		CTTCCTCTCCACTTTCCACC	AGGTGATGAGAAAGGGTTGG	53	167–187		13	AF409170
BCPsmAG21		GATCCTCCACCTATGCCTAAC	AGAGGGGTTTAGGGACAGAA	50	121–188		13	AF409172
BCPsmAG25		ATGTACACCCCAAGCCCATA	TGGGAATTAGGGGTTAGAGAAA	53	105–130		13	AF409176
BCPsmAG26		ACCTCTAACCCCTCAACCAC	GGAGAAGTAGGGGAGTGTGA	53	228–264		13	AF409177
BCPsmAG27		CCTTCCTCTCCACTTTCAACC	ACGGGGAGGGAGGGTAAC	56	84–144		13	AF409178
BCPsmAG29		TCAGATATATAAACTAAGAGAATGG-GG	CACTCTAAATGCCTAACCACCA	53	137, 142		13	AF409180
BCPsmAG30		GAGATTGAGTGTTAGGTGTTTAG-GG	CTCCCTCTCTTTCCCTCCAT	53	97–118		13	AF409181
Sb16**		GATTCCACACAAAACCAAGCG	CAAAGTATACCCCTTGAACAC	55	1050	6	14,15 ,16	Y09971
Sb17**		GAGGGATGAATATGGTCTACG	AATAACGCCAAATGCCTCCAC	55	640	5	14,15 ,16	-
Sb21**		CAGATCAGGCACGCATTGTTG	GTCCATCAGGGCTCATGTTTG	55	471-474	2	14,15 ,16	X69930
Sb29**		AGCGGCATTGAACAGAGTAAC	AATGGAAATGAAGGCAGACTC	55	553-580	2	14,15 ,16	X74755
Sb32**		TGCTGTCTACACTGCTCAATG	CAGAAGCCTGAGGATGTTACC	55	760	4	14,15 ,16	X62303
Sb49**		AGGTCCTCCAAAAGTTCTGTG	GCCTCATGTTCCCAAAGTCTC	55	323	2	14,15 ,16	Z72532
Sb60**		TGGGAGAATGACTAGATTGTG	AAGCCTTGACAATAGTAAGTG	55	378	2	14,15 ,16	-
Sb62**		GTATTACCCAGCTCAAGTTCC	ACAGTACGCCGCAGACAAATG	55	681-706	4	14,15 ,16	X78167

**M13* = AGGGTTTTCCCAGTCACGACGTT ; **EST-SSR-markers*

1-van de Ven and McNicol 1997, 2-A'Hara and Cottrell 2004, 3-A'Hara and Cottrell 2007, 4-A'Hara and Cottrell 2009, 5-Hodgetts et al. 2001, 6-Rajora et al. 2001, 7-Pfeiffer et al. 1997, 8-Scotti et al. 2000, 9-Scotti et al. 2002a, 10- Scotti et al. 2002b; 11-Rungis et al. 2004, 12- Mimura and Aitken 2007a, 2007b, 13-Amarasinghe and Carlson 2002, 14-Perry and Bousquet 1998a, 15 -Bennuah et al. 2004, 16- Gapare et al. 2005

Material for extraction

DNA was extracted from:

- needles (fresh and frozen) (van de Ven and McNicol 1997, Hodgetts et al. 2001, Rajora et al. 2001, Bennuah et al. 2004, Gapare et al. 2005, Gapare and Aitken 2005, Mimura and Aitken 2007a, 2007b);
- embryos (van de Ven and McNicol 1997) and germinants (Rungis et al. 2004, Mimura and Aitken 2007a, 2007b);
- megagametophyte from the seed (van de Ven and McNicol 1997, Hodgetts et al. 2001, Mimura and Aitken 2007a, 2007b).

DNA-extraction and amplification protocols

DNA was extracted from the above mentioned tissues using:

- the DNeasy 96 Plant Kit from QIAGEN (A'Hara and Cottrell 2004, 2007, 2009);
- the Doyle and Doyle (1990) CTAB protocol (Gapare et al. 2005, Gapare and Aitken 2005, Bennuah et al. 2004, Rungis et al. 2004);
- a modified protocol of Murray and Thompson (1980) which is described in detail in Hodgetts et al. (2001).

Examples of amplification protocols

- Initial denaturation step of 95°C for 2 min; 30 cycles of 95°C for 20 s, 53°C for 20 s, and 72°C for 30 s; followed by a final extension step of 72°C for 3 min (Rungis et al. 2004).
- Initial denaturation step of 94 °C for 3 min, followed by 10 cycles of 94 °C for 30 s, 57 °C for 1 min and 72 °C for 30 s. This was followed by 28 cycles of 94 °C for 30 s, 55 °C for 1 min then 72 °C for 30 s. A final elongation step at 72 °C for 6 min was then carried out (A'Hara and Cottrell 2007, 2009).
- Initial denaturation step at 94°C for 5 min was followed by 25 cycles consisting of 94°C (denaturation) for 30 s, the appropriate annealing temperatures (see Table 3) for 30 s and a final elongation step at 72°C for 30s (Hodgetts et al. (2001).

Important results

- A considerable number of highly polymorphic nSSR markers are available for genetic analysis of Sitka spruce (see Table 3). A part of them were developed especially for Sitka spruce; others were transferred from related species.
- nSSRs were successfully applied to species and clone identification, hybridization studies, analysis of genetic diversity along the natural distribution range and in introgression zones.
- Comparison of central and peripheral Sitka spruce populations within the natural distribution range shows pronounced genetic differences. While core populations of Sitka spruce have little within-population genetic structure, peripheral populations are strongly spatially structured at distances up to 500 m. Higher allelic richness and gene diversity was observed in central populations in comparison with peripheral ones. Also, in the central and continuous populations, more private alleles were identified. Gene diversity was lowest in isolated peripheral populations. Selfing rate increased from 7.3% in central populations to as high as 35.2% in a northern, isolated population from Kodiak Island (Mimura and Aitken 2007a, 2007b). Inbreeding is higher in peripheral populations. In core populations, the number of migrants is significantly higher (Gapare et al. 2005, Gapare and Aitken 2005, Gapare et al. 2008).
- Given the above findings, different sampling protocols are needed for central and peripheral populations. Because of stronger within-population spatial genetic structure, a higher number of samples is needed in peripheral areas (indication: 180 samples from at least 324 ha) (Gapare et al. 2008).
- Following Rajora et al. (2001), the closely related spruce species *Picea sitchensis* and *Picea glauca* can be distinguished by the PGL12 SSR marker. PGL12 amplifies only in *Picea glauca*, while in *Picea sitchensis* no amplification products were observed.
- Bennuah et al. (2004) developed a useful hybrid index for classifying individual families and populations in introgressed populations between Sitka and white spruce based on EST-SSR markers developed by Perry and Bosquet (1998a, 1998b). Results led to the conclusion that in the middle of the introgression zone no pure individuals of one species are present.
- In the introgression zone, little differentiation between populations was found. Hamilton and Aitken (2013) attribute this to widespread gene flow between the two species.

b) SNPs (single-nucleotide polymorphisms)

Holliday et al. (2010) investigated single-nucleotide polymorphisms (SNPs) in Sitka spruce using an Illumina

GoldenGate genotyping array. These SNPs were developed from re-sequencing of coding genes that were chosen partially on the basis of gene expression differences during autumn cold acclimation (Holliday et al 2008) and partially on the basis of functional information from model species (e.g., *Arabidopsis thaliana, Populus trichocarpa*). In addition, ~100 SNPs were genotyped from randomly chosen genes to serve as a set of neutral markers. Among 768 SNPs designed for the array, 339 gave high quality genotypes and were polymorphic, which is similar to contemporaneous studies in other conifers. These SNPs were used to investigate demographic history and local adaptation. For the latter, numerous genotype-phenotype relationships were uncovered, demonstrating the utility of SNP markers for understanding adaptation (Holliday et al. 2010). This same SNP panel served as a basis for studies related to introgression between Sitka spruce and white spruce (Hamilton et al. 2012; Hamilton et al. 2013), and between Sitka spruce and Engelmann spruce (De La Torre et al. 2015).

Pavy et al. (2013) designed two high-density SNP arrays relying on the Infinium iSelect technology (Illumina) for use in white spruce (*Picea glauca*), one with 7338 segregating SNPs representative of 2814 genes of various molecular functional classes, the other one with 9559 segregating SNPs representative of 9543 genes. 22.4% of these SNPs were segregating in Sitka spruce, too.

These studies demonstrate the efficiency and quality of array-based genotyping, and we expect such methods to continue to be useful. At the same time, the field is moving toward next generation sequence based genotyping. Of particular utility for conifers such as Sitka spruce, with their large genomes, is genotyping by sequencing (GBS) and especially sequence capture. For example, Suren et al. (2016) showed that sequence capture can be successfully used in both interior spruce (*Picea glauca x engelmanii*) and lodgepole pine (*Pinus contorta*), and while we are not aware of related efforts in Sitka spruce, it should be feasible to use the same capture baits in this species (based on Suren et al's successful capture and sequencing of DNA from several congeners).

Material for extraction

For DNA extraction, needles were used (Holliday et al. 2010).

DNA-extraction methods

DNA extraction was performed using a modified CTAB protocol (Doyle and Doyle, 1990) (Holliday et al. 2010).

Important results

- According to Holliday et al. (2010), SNPs reveal three clusters for Sitka spruce:
  1. Populations in California, Oregon, and British Columbia;
  2. Populations in Alaska;
  3. Populations from Kodiak Island.
- Genotype-phenotype associations for cold hardiness and bud set timing were found in 28 of the candidate genes described above. Interestingly, co-variance between tests of selective neutrality and latitudinal population origin suggest that postglacial history has impacted variation across the range of Sitka spruce, and suggests that caution is warranted in genotype-environment tests, as spurious relationships are likely if population structure is not effectively controlled (Holliday et al. 2010).
- The high number of SNPs developed can be useful in studies of genetic association, population genetics, genomic prediction and genome linkage mapping (Pavy et al. 2013).

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Molecular markers used for genetic studies in tree of heaven (*Ailanthus altissima* (Mill.) Swingle)

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1. General remarks

The genus *Ailanthus* consists of 5-15 species (depending on the authors and cited Flora), which have a wide distribution ranging from Asia to north Oceania (Engler 1931, Nooteboom 1962, eFloras 2008).

Ailanthus altissima (Mill.) Swingle (tree of heaven), with its largest native distribution in China, was in the middle of 18th century mistakenly introduced to France and later to England when the seed sent from China was believed to be that of Chinese lacquer tree (Burch and Zedaker 2003). The tree of heaven soon became a popular planted species in Europe because of its attractive foliage, rapid growth, timber qualities and as forage for *Samia cynthia* (Drurvy), a species of silk-producing caterpillar (Huemer and Rabitsch 2002).

Three varieties of *A. altissima* have been described. Two of them (*Ailanthus altissima* var. *altissima* and var. *sutchuensis* (Dode) Rehd. & Wilson) are distributed in China and North Vietnam (see Figure 1), whereas the third variety, var. *tanakae* (Hayata) Kanehira et Sasaki, was reported for Taiwan (eFloras 2008).

The tree of heaven is a diploid, dioecious species (Kowarik and Säumel 2007), with reported 80 and 64 chromosomes (Slavik 1997). It reproduces both sexually and asexually. Asexual reproduction takes place by vegetative sprouting from stumps or root portions (Hu 1979) forming clones smaller than 50 meters (Kowarik and Säumel 2007, Chuman et al. 2015). Flowering occurs rather late in spring (June). Seeds ripen in large crowded clusters from September to October of the same year and may persist on the tree through the following winter (Little 1974, Hu 1979). Not only can individual *Ailanthus* produce >1 million seeds per year, but a significant relationship exists between seed production and tree diameter (Wickert et al. 2017, Martin and Canham 2010). In addition, it can already produce seed as young as four years of age. Seedlings grow quickly in full sunlight and average a meter of growth in height per year for at least the first 4 years (Adamik and Brauns 1957).

Prolific fruiting, ready germination, adaptability to infertile sites and rapid growth rate make *A. altissima* a noxious weed in many countries where it has been introduced (Feret 1985, Shah 1997). This species is often invasive in its introduced range, where it can colonize native plant communities and greatly reduce their species richness (Kowarik 1995, Knapp and Canham 2000, Merriam 2003, Wickert et al. 2017).

2. Isozymes

Only one publication - Feret and Bryant (1974) - dealing with isozyme analysis is known for *Ailanthus altissima.* Fifteen peroxidase isozymes were investigated for their presence/absence in five Chinese- (native range) and five American- seed sources (introduced range). Isoenzymes

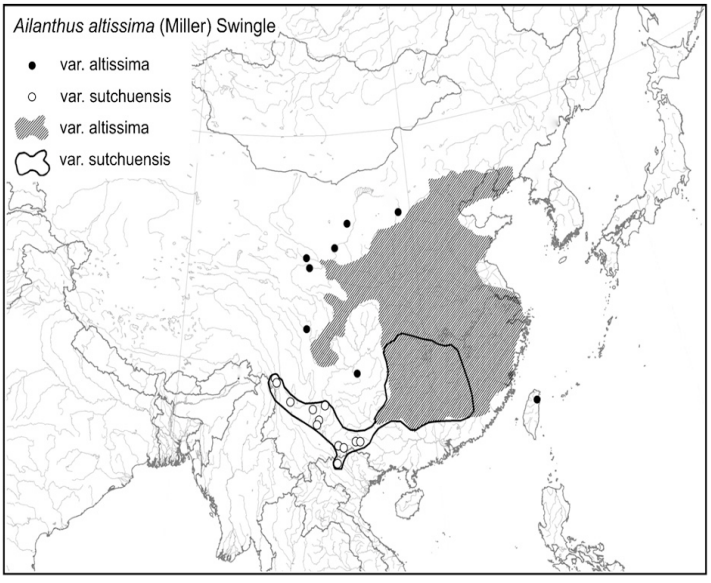

Figure 1. Native distribution range of tree of heaven (source: Kowarik and Sämuel 2007)

were numbered from 1 to 15. Authors didn´t present the names of isozymes (including E.C. numbers), nor scored loci or alleles. They refer only to presence-absence of peroxidase isozymes 1 to 15.

Material for protein extraction

Enzymes were extracted from leaf material (Feret and Bryant 1974).

Protein extraction and separation protocols

For isozyme separation electrophoresis on polyacrylamide disc gels was used. Methodological details are published in Feret (1970).

Important results

- No unique isozymes exist for the North American and Chinese seed sources.
- The studied isozymes were similar in frequency in both ranges. According to the authors, this indicates that the gene pool of *Ailanthus* in North America is not necessarily depauperate and probably is nearly as diverse as native populations with regard to "allelic variability".
- From the practical view, authors (Feret and Bryant 1974, Feret 1985) assumed that these results probably means that if you want to improve the species in North America, you might not gain much by importing additional genotypes from China.

3. Organelle markers (chloroplast (cp)DNA)

Four studies have so far used chloroplast DNA-markers in *Ailanthus altissima*: Liao et al. (2014), Chuman et al. (2015), Kurokochi et al. (2013, 2015).

Loci and primers used (see Table 1)

Analysis of Liao et al (2014)
- 3 Plastid DNA markers psbA-trnH, trnD-trnT, trnL-trnF

Analysis of Kurokochi et al. (2013)
- 4 Plastid DNA markers trnL5´F - 3´trnL R, 3´trnG-5´trnG2 G, matKM - A6_inner, ycf6-R - E2_inner
- 3 Plastid DNA markers psbA-trnH, trnD-trnT, trnL-trnF

Table 1: Primer information of the chloroplast DNA-markers used for DNA sequencing in *Ailanthus altissima*

Locus	Primer sequence F= Forward, R= Reverse	kind of marker	References
psbA-trnH	F:GTTATGCATGAACGTAATGCTC (psbAF) R:CGCGCATGGTGGATTCACAAATC (trnHR)	Plastid m.* sequencing	1,9
trnD-trnT	F:ACCAATTGAACTACAATCCC (trnD^{GUC}F) R:CTACCACTGAGTTAAAAGGG (trnT^{GGU}R)		2,9
trnL-trnF	F:ATTTGAACTGGTGACACGAG (trnF^{GAA}=TabF) R:GGTTCAAGTCCCTCTATCCC (trnL^{UAA}F=TabE)		3,9
trnL5´F-3´trnL R	F:CGAAATCGGTAGACGCTACG (TabC) R:GGGGATAGAGGGACTTGAAC (TabD)		3,6
3´trnG-5´trnG2 G	F:GTAGCGGGAATCGAACCCGCATC R:GCGGGTATAGTTTAGTGGTAAAA		4,6
matKM-A6_inner	F:TCGACTTTCTGGGCTATC R:CGTGCTTGCATTTTTCATTGC		5,6
ycf6-R-E2_inner	F:GCCCAAGCRAGACTTACTATATCCAT R:CGAAACAGCTGGGGTTTCTG		4,6
Aacp01	F:CGCTTATCCTTCATCCCTTTT R:GGTGCAGAGACTCAATGGAGG		7,8
Aacp02	F:CGACAACCCAATCTGTAGTTC R:CGATCAGATTATGGAGTGAATG		7,8
Aacp03	F:GACACCCTTGATGAAAGACT R:GGGCAACAAAAAAACGAATAGGTC		7,8

**maternally inherited, 1-Sang et al. 1997, 2-Demesure et al. 1995, 3-Taberlet et al. 1991, 4-Shaw et al. 2005, 5-Tate and Simpson 2003, 6-Kurokochi et al. 2013, 7-Kurokochi et al. 2015, 8-Chuman et al. 2015, 9-Liao et al 2014*

Analysis of Kurokochi et al. (2015) and Chuman et al. (2015)

- 3 Plastid DNA markers Aacp01, Aacp02, Aacp03

Material for DNA-extraction

DNA was extracted from leaves dried in silica (Kurokochi et al. 2013, Liao et al. 2014, Kurokochi et al. 2015, Chuman et al. 2015).

DNA-extraction protocols

Total genomic DNA extraction followed the method of Kurokochi et al. (2013) or Doyle and Doyle (1987).

Important results

- By sequencing of 4 non-coding plastid regions in 449 *A. altissima* trees sampled in 64 non-native Japanese populations and four native Chinese populations, six haplotypes were identified. Three plastid haplotypes were observed in Japan, whereas four were detected in Chinese populations. Most *A. altissima* trees in Japan harbored two different haplotypes. These two haplotypes were not genetically similar. Analysis of molecular variation showed some genetic differentiation among populations. Twenty-two Japanese populations contained two haplotypes within each population, whereas the other 42 Japanese populations were composed of only one haplotype indicating that Japanese populations may have originated from limited number of seed introductions (Kurokochi et al. 2013).
- Biogeographic history (refugia and patterns of migration during past climatic changes) in native range, in China, was revealed by sequencing three cpDNA markers and 440 individuals. Identification and geographical distribution of twelve haplotypes led to the hypothesis that multiple glacial refugia existed in mainland China during the Quaternary oscillations, out of which Ailanthus spread by three main dispersal routes (Liao et al. 2014).
- For results of Kurokochi et al. (2015) and Chuman et al. (2015) see paragraph 4 a) where analyses of cpDNA were combined with nSSRs.

4. Nuclear DNA markers (nSSRs, SNPs)

a) nSSRs (putatively neutral microsatellites)

Dallas et al. (2005) developed nine $(CT)_n$ nuclear microsatellite markers using European *Ailanthus* trees collected across Mediterranean region (Corsica, Crete, Lesbos, Mallorca, Menorca, and Sardinia). Microsatellites were developed using a combination of published methods based on enrichment (Koblízková et al. 1998, Gardner et al. 1999, Hamilton et al. 1999, see the full protocol at www.abdn.ac.uk/ ~nhi571). These simple sequence repeats (SSRs) were further used together with plastid markers by Kurokochi et al. (2015) and Chuman et al. (2015). Only five nSSRs were used in a study of American *Ailanthus* by Aldrich et al. (2010).

Material for DNA-extraction

Genomic DNA was extracted from silica-gel-dried leaf tissue (Dallas et al. 2005, Aldrich et al. 2010, Chuman et al. 2015).

DNA-isolation protocols

Protocols for DNA-isolation are published in Dallas et al. (2005). Aldrich et al (2010) extracted DNA from leaf tissue using the DNeasy Plant Minikit (Qiagen).

Important results

- Developed nSSRs are useful for genetic analyses of tree of heaven and its close relatives as they were polymorphic in each of three studied varieties of tree of heaven (*A. altissima* var. erythrocarpa, *A. altissima* var. sutchuensis, *A. altissima* var. tanakai) and two other Ailanthus species (*A. giraldii, A. vilmariniana*) (Dallas et al. 2005).
- In the USA, where this species was introduced from both sides of the continent, small but significant genetic differences were found between populations with little correspondence between geographic and genetic distance (Aldrich et al. 2010). These conclusions are consistent with a model of multiple introductions followed by high rates of genetic exchange between cities and regions.
- In Japan, the mechanism of range expansion was estimated by 9 nSSRs and 3 plastid markers (see the paragraph on plastid markers for detail) in 35 patches located within three sites (Chuman et al. 2015). Analyses with markers showed limited asexual reproduction up to 45 meters. Most related genotypes were detected within the same patch with an extent of nonrandom spatial genetic structure up to 2 km indicating natural regeneration from seeds.
- In Japan, also genetic structure of planted and naturalized populations was estimated by

Table 2: Primer information of nSSR-markers used for genetic analysis of *Ailanthus altissima* (N_a = number of alleles scored)

Locus	Motif	Primer sequence [5'-3']^a F= Forward, R= Reverse	Size (bp)	N_a	Ref.	GenBank Accession no.
Aa22	$(CT)_{20}$	F:CTGGTATCTGAATTTGAGCAGTAGC R:GAACAAATTAATCCCAAGTGAAGC	171–211	11	1,4	AY750965
Aa68	$(CT)_{24}ATCT$ $(AT)_9$	F:AACTTGATTAGTTTATATTAGGCGTGAC R:AAGTCCGATTGAAATTACAAGTCC	206–233	12	1	AY750966
Aa69	$(CT)_{15}$ $(CA)_{17}$	F:CATGGAAGCCTCTTGGAAAC R:TGAAGCAAATATGTGAAACAACC	152–214	8	1,4	AY750967
Aa75	$(CT)_{25}$	F:CTCTTGCATCTGAAATAGTGAACG R:GTTTGTTTTGGCTAAATGCTATTACC	93–138	10	1,4	AY750968
Aa76	$(CT)_{24}$	F:AAGCAAAGTCAAGGCCAGAC R:CCATTTCACCCACCTTCTTC	141–194	8	1,4	AY750969
Aa79	$(CT)_{14}CC$ $(CT)_{14}$	F:TGCTGCCAATGTCAGTGATG R:TTCACACAAAGAAACCCATGTC	122–172	12	1	AY750970
Aa80	$(CT)_{26}$	F:GAAGAAATGAATTGACAGTTGACC R:ATTTACACTAGGGCTACCAACACC	167–225	7	1	AY750971
Aa82	$(CT)_{29}$	F:CAACATTCCGTGATTCACACTC R:CTTGCACGCTTCAGTGAAAG	109–170	7	1,4	AY750972
Aa92	$(CT)_{16}CC$ $(CT)_8(AT)_{11}$	F:CTTGAACAGAAACAAATGCAAAG R:GAAATTGTTTAAATGCCACTACCTG	157–195	11	1,2,3	AY750973

T_a = annealing temperature, in touch down PCR first 60°C – 49°C (decreasing by 0.5 per cycle), then 50°C.*

1- Dallas et al. 2005, 2-Kurokochi et al. 2015, 3-Chuman et al. 2015, 4-Aldrich et al. 2010.

combination of 9 nSSRs and 3 plastid markers (see the paragraph on plastid markers for details) (Kurokochi et al. 2015). There was no obvious genetic differentiation between planted and naturalized populations. Nevertheless, two main plastid haplotypes were recognized within trees allowing to separate studied individuals into two groups. Within each haplotype group most trees were strictly assigned to one cluster indicating two distinct provenances. An admixture between the two lineages has occurred, but remained limited.

b) SNPs (single-nucleotide polymorphisms)

No publications on SNP analysis for *Ailanthus altissima* exist.

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Molecular markers used for genetic studies in Northern red oak (*Quercus rubra* L.)

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1. General remarks

Northern red oak (*Quercus rubra* L.), also known as common red oak, eastern red oak, gray oak or mountain red oak, is widespread in the eastern part of North America. Northern red oak is the only native oak extending northeast to Nova Scotia (Figure 1). It grows from Cape Breton Island, Nova Scotia, Prince Edward Island, New Brunswick, and the Gaspé Peninsula of Quebec, to Ontario, in Canada; from southern Minnesota to eastern Nebraska and Oklahoma; east to Arkansas, southern Alabama, Georgia, and North Carolina. Outliers are found in Louisiana and Mississippi and almost the entire USA is a potential planting range (Gilman and Watson 1994).

Northern red oak grows on a variety of soils and topography. Pure stands are the most common plant community. Moderate to fast growing, this tree is one of the most important lumber species of red oak and is an easily transplanted, popular shade tree with good form and dense foliage.

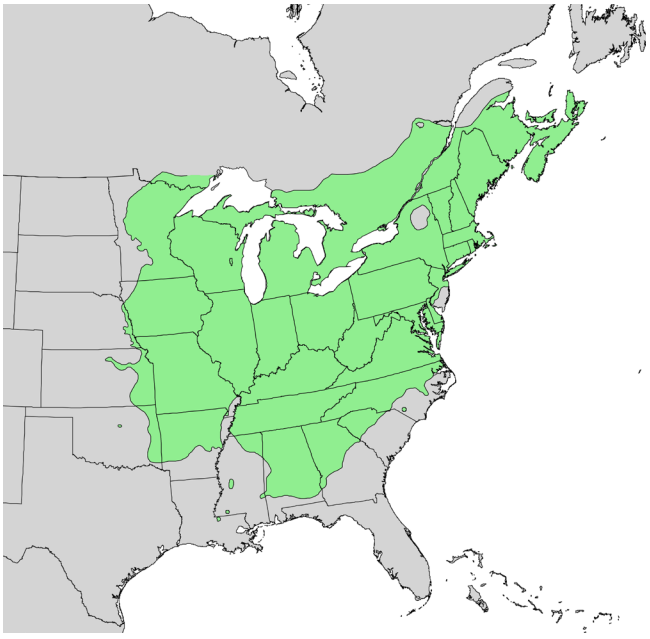

Figure 1. Native distribution range of northern red oak (source: USGS, USA)

According to Sander (1965), in the area where northern red oak grows, mean annual precipitation varies from about 760 mm in the Northwest to about 2030 mm in the South. Annual snowfall ranges from a trace in southern Alabama to 254 cm or more in the Northern States and Canada. Mean annual temperature is about 4° C in the northern part of the range and 16° C in the extreme southern part. The frost-free period averages 100 days in the North and 220 days in the South (Sander 1965).

Mature northern red oaks are usually from 20 to 30 m tall and 61 to 91 cm in dbh (diameter at breast height) in undisturbed stands on good sites. Forest-grown trees develop a tall, straight columnar bole and large crowns. Open grown trees tend to have short boles and spreading crowns (Sander 1965).

Northern red oak hybridizes with other species in the subgenus *Erythrobalanus* and the following hybrids have been named: *Quercus x columnaris* Laughlin (*Q. palustris x rubra); Q. x fernaldii* Trel. *(Q. ilicifolia x rubra); Q. x heterophylla* Michx. f. *(Q. phellos x rubra); Q. x hawkinsiae* Sudw. *(Q. velutina x rubra); Q. x riparia* Laughlin *(Q. shumardii x rubra); and Q. x runcinata (A. DC.)* Engelm. *(Q. imbricaria x rubra).* It also hybridizes with blackjack oak *(Q. marilandica)* and with northern pin oak *(Q. ellipsoidalis)* (Little 1979).

Up to date, several molecular markers have been developed (e.g. Aldrich et al. 2002, Magni et al. 2005, Sullivan et al. 2013, Konar et al. 2017) and used to investigate phylogeographic patterns (Birchenko et al. 2009, Magni et al. 2005) and population genetic variation at the local (Aldrich et al. 2003b), regional (Gerwein and Kesseli 2006) and range-wide scale (Daubree and Kremer 1993, Sork et al. 1993, Borkowski et al. 2017, Merceron et al.

2017). A lack of pronounced large-scale phylogeographic structure revealed by chloroplast DNA markers could be due to the species' biogeographic history (putative recolonization from one single glacial population) or due to low resolution of the universal markers used (Magni et al. 2005).

For long time, only isozyme studies from the 90s had provided some range-wide data from indigenous populations (Daubree and Kremer 1993, Sork et al. 1993). Such data are particularly useful in order to trace the origin of introduced populations. Recently, genetic structure across the whole natural range of northern red oak was studied based on microsatellites (Borkowski et al. 2017) and SNPs (Merceron et al. 2017). In addition, new chloroplast DNA markers may increase the resolution of population genetic analyses (Alexander and Woeste 2014, Borkowski et al. 2014). These studies may open up new perspectives in traceability of introduced populations and forest reproductive material of northern red oak.

Here, we provide a review of available laboratory techniques which can be used to investigate the origin of northern red oak, as well as its genetic diversity and differentiation.

2. Isozymes

In the period from 1980 to 1995, investigation of isozymes took place in order to:

- determine species identity due to hybridization among the *Quercus* species (Tobolski 1978, Manos and Fairbrothers 1987, Hokanson et al. 1993);
- investigate genetic diversity among natural and planted populations (Manos and Fairbrothers 1987, Schwarzmann and Gerhold 1991, Daubree and Kremer 1993, Hokanson et al. 1993, Sork et al. 1993).

A summary of the isozyme systems, scored loci and number of alleles found in the cited studies is presented in Table 1.

Material for protein extraction

Proteins were extracted from mature leaves (Manos and Fairbrothers 1987, Sork et al. 1993, Jones et al. 2006), young leaves (Hokanson et al. 1993, Daubree and Kremer 1993), buds (Tobolski 1978, Hokanson et al. 1993, Daubree and Kremer 1993), acorns (Tobolski 1978, Hokanson et al. 1993, Daubree and Kremer 1993) and embryos (Schwarzmann and Gerhold 1991).

Table 1: List of enzymes, scored loci, number of alleles for northern red oak *(Quercus rubra)*

Enzyme system	E.C. Number	Scored loci	No. of alleles*	References
Alcohol dehydrogenase	1.1.1.1	ADH-1	5	6,7
Acid phosphatase	3.1.3.2	ACP-1,-2	3, 2	7
Fluorescent esterase	3.1.1.1	FEST-1,-2	n.a.; 2	5
Glutamate dehydrogenase	1.4.1.2	GDH-1	3	1
Glutamate oxalacetate transaminase	2.6.1.1	GOT-1	1	2,7
Isocitrate dehydrogenase	1.1.1.42	IDH-1,-2,-3	5, 4	1,2,4,5,6
Leucine aminopeptidase	3.4.11.1	LAP-1,-2	4, 2	1,2,4,5,6,7
Malate dehydrogenase	1.1.1.37	MDH-1,-2,-3	5, 3	1,2,3,4,6
Malic enzyme	1.1.1.40	ME-1	3	6
Menadione reductase	1.6.5.2	MNR-1	2	6
Phosphoglucose isomerase	5.3.1.9	PGI-1,-2	1, 8	1,2,3,4,5,6
Peroxidase	1.11.1	PER-1,-2	4, 3	1,5
Phosphoglucomutase	2.7.5.1	PGM-1,-2	5, 6	1,2,5,6
Shikimate dehydrogenase	1.1.1.25	SKDH -1	n.a.;6	1,3,4,5,6
Triose-phosphate isomerase	5.3.1.1	TPI-1	n.a.	5
6-Phosphogluconate dehydrogenase	1.1.1.44	6-PGDH-1,-2	3, 5	1,3,4,5,6

**only the highest number of alleles reported in at least one reference is given; 1–Manos and Fairbrothers 1987, 2–Schwarzmann and Gerhold 1991, 3-Daubree and Kremer 1993, 4-Hokanson et al. 1993, 5–Sork et al. 1993, 6– Jones et al. 2006, 7-Tobolski 1978.*

Protein extraction and separation protocols

Separation protocols are described in: Manos and Fairbrothers (1987). Tobolski (1978) followed the separation protocols described by Scandalios (1969).

Important results

- The similarity in isozyme patterns was high among *Quercus* species. Most isozyme bands were found to be common to two or more oak species (Tobolski 1978).
- The diversity among natural populations of red oak was low (Manos and Fairbrothers 1987).
- Schwarzmann and Gerhold (1991) confirmed little differentiation of isozyme gene frequencies among northern red oak populations in Pennsylvania.
- Most of the genetic variation which was observed for isozyme loci was found to reside within populations.
- Pronounced genetic differentiation of European populations in comparison to native ones was found for loci PGI and PGM. Frequency of rare alleles was increased in European populations resulting in a higher genetic diversity (mean number of alleles). This was interpreted as a possible result of weaker selective pressure on northern red oak regeneration in Europe (Daubree and Kremer 1993).

3. Organelle DNA markers (chloroplast (cp) DNA, mitochondrial (mt)DNA)

Loci and primers used

Magni et al. (2005) applied PCR-RFLP markers in order to study phylogeography of northern red oak across its natural range. They used universal primers (Dumolin-Lapègue et al. 1997, Taberlet et al. 1991) to amplify 13 chloroplast DNA (cpDNA) fragments and two different restriction enzymes (*Hinf*I and *Taq*I) independently to digest the PCR products. Among them, only 5 primer pairs combined with *Taq*I provided informative polymorphisms (Table 2).

Further cpDNA studies based on PCR-RFLPs were carried out by Romero-Severson et al. (2003) and Birchenko et al. (2009). These authors amplified three universal cpDNA loci (Demesure et al. 1995) and digested the fragments with a mix of seven restriction enzymes *Bam*HI, *Eco*RI, *Alu*I, *Hha*I, *Msp*I, *Hae*III and *Rsa*I using compatible buffers (Table 2).

Material for DNA-extraction

Plant tissue used for extraction included leaves (Romero-Severson et al. 2003, Magni et al. 2005), buds (Magni et al. 2005), twigs or cambium (inner bark; Birchenko et

Table 2: PCR-RFLP markers (chloroplast DNA) for northern red oak

Locus	Primer sequences F= Forward, R= Reverse	Restriction enzymes	No. of polymorphic bands	References
CD (trnC/trnD)	F:CCAGTTCAAATCTGGGTGTC R:GGGATTGTAGTTCAATTGGT	*Taq*I	2	3,4,6
DT (trnD/trnT)	F:ACCAATTGAACTACAATCCC R:CTACCACTGAGTTAAAAGGG		1	3,4,6
VL (trnV/rbcL)	F:CGAACCGTAGACCTTCTCGG R:GCTTTAGTCTCTGTTTGT		1	3,4,6
trnH/psbA	F:ACTGCCTTGATCCACTTGGC R:CGAAGCTCCATCTACAAATGG		1	3,4,6
trnS/trnG	F:GCCGCTTTAGTCCACTCAGC R:GAACGAATCACACTTTTACCAC		1	3,4,6
CD (trnC/trnD)	F:CCAGTTCAAATCTGGGTGTC R:GGGATTGTAGTTCAATTGGT	*Bam*HI, *Eco*RI, *Alu*I, *Hha*I, *Msp*I, *Hae*III and *Rsa*I	2	1,2,5
FV (trnF/ trnV)	F:CTCGTGTCACCAGTTCAAAT R:CCGAGAAGGTCTACGGTTCG		3	1,2,5
TC (trnT/psbC)	F:GCCCTTTTAACTCAGTGGTA R:GAGCTTGAGAAGCTTCTGGT		2	1,2,5

1–Birchenko et al. 2009, 2-Demesure et al. 1995, 3-Dumolin-Lapègue et al. 1997, 4-Magni et al. 2005, 5-Romero-Severson et al. 2003, 6-Taberlet et al. 1991

al. 2009).

DNA-isolation protocols

For DNA isolation, Magni et al. (2005) used an ATMAB (Acryltrimethylammonium bromide) protocol following Dumolin et al. (1995). Romero-Severson et al. (2003), as well as Birchenko et al. (2009) used a commercial DNA-extraction kit (Dneasy, Qiagen).

Important results

- In the study of Magni et al. (2005), twelve different haplotypes (A through G) were described. One of them, haplotype E was present in 75 % of the trees throughout the distribution range, whereas all other haplotypes were rare (< 8 %) and displayed only local distribution. For these reasons, the diagnostic power of this marker set for traceability of introduced populations and forest reproductive material is limited.
- Romero-Severson et al. (2003) detected five different haplotypes (I through V) in the State of Indiana (USA) by analyzing a limited number of individuals and populations. Using the same marker set, Birchenko et al. (2009) found four of these haplotypes in the northwestern part of the native range. Haplotypic diversity was found to decline poleward. Towards the centre of the range, high admixture and no spatial patterns were observed. Given this high admixture, the utility of this marker set to trace the origin of introduced northern red oak might be limited, too.

4. Nuclear DNA markers (nSSR, EST-SSR, SNP)

a) nSSRs (putatively neutral microsatellites), EST-SSRs (expressed sequence tag derived microsatellites)

Loci and primers used

Development of SSR markers for red oak was done by Aldrich et al. (2002; 2003a). In addition, transferability of nSSR (genomic) and EST-SSR-loci (derived from expressed sequence tags) from pedunculate oak (*Q. robur*; initial primer notes by Steinkellner et al. 1997 and Durand et al. 2010) into northern red oak was tested by Sullivan et al. (2013). Moreover, Gerwein and Kesseli (2006) successfully used loci initially developed for *Quercus myrsinifolia* (Isagi and Suhadono 1997), *Quercus robur* (Steinkellner et al. 1997) and *Quercus macrocarpa* (Dow et al. 1995) in their study with northern red oak. Finally, Konar et al. (2017) used 116 published or newly developed microsatellites in their genetic mapping study in *Q. rubra*. A list of 169 microsatellite loci (nSSRs and EST-SSRs) available for genotyping of northern red oak, as well as studies where these were used is presented in Table 3.

Table 3: Primer sequences, annealing temperatures (T_a), allele lengths in base pairs (bp) and references for microsatellite markers available for genetic analyses in northern red oak

Locus	Motif	Primer sequences		T_a	Size (bp)	References
		Forward	Reverse			
quru-GA-0A03	$(GA)_{17}$	ATTTTATATTAGCATAAGGGTG	GGCTTCACATTGAGAACGTTG	50	*	2,3,4
quru-GA-0C03	$(GA)_{18}$	TGTTGTTGTCGCCATT	CAGTGGCATTTGTTCACGAA	45	*	2
quru-GA-0C21	$(GA)_{23}$	CACCGTGATTTTATTGCCCAACA	CGGCGGACTTGCATTAAC	42	*	2,14
quru-GA-0i21	$(GA)_{16}$	ATATGGTCCCGATTAATTC	GGGCAACATTCAAATGTATCTA	50	*	2,3,4
quru-GA-1D09	$(GA)_{20}$	AGTGGGATGGGGATTCATAATA	CTCCGTGTCGCTCCGTTGTT	50	*	2,3,14
quru-GA-1H14	$(GA)_{22}$	GCTTGGGCTTGTTCCTACT	CAACACTTCTCATGGATTAGAGA	50	*	2,3
quru-GA-1i06	$(GA)_{23}$	CAAGCTTCCACTGAGTCGTCGGT	CTCTCGCTTTGATTTCACTCCCA	58	*	2
quru-GA-1i15	$(GA)_{23}$	CAGCCTCATCGATTACCCCAAAC	GGTCGCTGAGGGGGAAAG	50	*	2
quru-GA-1J11	$(GA)_{20}$	AGTTTGGGTCAAATACCTCC	AGATAATCCTATGATTGGTCGAG	50	*	2,19,14
quru-GA-1L05	$(GA)_{23}$	AAGATGCATGGTATTGTAGCAGG	GCTTGTTGCGGTAGGTTA	50	*	2
quru-GA-1M17	$(GA)_{19}$	GTTTGTGCTTGCTGGGAGG	TTCTTCTTAGCTTCCCAACTGAA	53	*	2
quru-GA-1M18	$(GA)_{23}$	ACCACTGTTGCCGACCTCCACCC	CTCTTCTTGCCGCTATTGACCC	57	*	2,14
quru-GA-2G07	$(GA)_{23}$	GCCAACAAATTTAACTATCCAT	TAACTGGGCTAGATAATCAG	50	*	2,3,4
quru-GA-2H14	$(GA)_{18}$	ATTACGCGAGCGTGCAGT	GTGCTCCACGAATGCTCTAGCCA	58	*	2,3,4
quru-GA-2H18	$(GA)_{22}$	CACTTCAAATGCATCCCCCAAA	GGAGGATGTAGGGGCTTCCAGTT	54	*	2
quru-GA-2N03	$(GA)_{22}$	CCAAGCGCAGCCCATCACTAAC	TGGCGCTCACTCCGAGAT	53	*	2,14
quru-GA-0A01	$(GA)_{11}$	CTCTCGCTCTGCACGTGACTCA	TTTGATTGATATAATTGATCGCT	50	123-129	1,3,4
quru-GA-0C11	$(GA)_{15}$	ATACCCAGCTCCCATGACCA	TCCCCAAATTCAGGTAGTGT	53	204-222	1,5,9,13,14,15,16,17,18,19
quru-GA-0C19	$(GA)_{18}$	TTAGCTTTTACGCAGTGTCG	CGGCTTCGGTTTCGTC	50	218-242	1,3,4,19
quru-GA-0E09	$(GA)_{16}$	TGCCATCCCTATACACAACCA	CCTCCATCACAAAGTTGCC	53	186-230	1,9,15,16,17,19
quru-GA-0I01	$(GA)_{15}$	GGGCTATCAAGTAAGTGCTTAAC	ACGCCATCCCTATAACACA	53	196-218	1,14
quru-GA-0M05	$(GA)_{16}$	CTACAAGTTACATGCCCAATCA	CTTTGCGCAGGTCCATTAC	53	184-215	1
quru-GA-0M07	$(GA)_{19}$	TTTAGCATCACATTTCCGTT	TTTTGTGTCATCCGGTATTA	45	185-209	1,3,4,14
quru-GA-1C06	$(GA)_{29}$	CAAATAAATATTGTGGGGTTCA	GGAGGGGATCCGGAAAA	50	234-262	1,3,4,
quru-GA-1C08	$(GA)_{29}$	TCCCAATCGATGTTTGATAAGG	GGGCTCTTGAGAGGATGTAGG	50	257-296	1,3,4,
quru-GA-1F02	$(GA)_{15}$	CCAATCCACCCTTCCAAGTTCC	TGGTTGTTTTGCTTTATTCAGCC	50	166-184	1,3,4,14,19
quru-GA-1F07	$(GA)_{22}$	CCGGTCAAAGAAGTTATCAGA	GGGTGGATTGGGTTTCTACCTA	58	306-348	1,3,5,9,14,15, 16,17,19
quru-GA-1G13	$(GA)_{14}$	AAAACTCACACAGCCGATTACTA	GATTCCATTGTCAACTGCGAAGA	50	177-193	1,3,4,14
quru-GA-2F05	$(GA)_{21}$	CCGCTTCGTGACGATTATTC	GAGGTTTGGAGGAGAGATCATTCT	53	94-322	1

Locus	Motif	Primer sequences		T_a	Size (bp)	References
		Forward	Reverse			
quru-GA-2M04	$(GA)_{20}$	GGAGAGGACGGGATGCC	TACTATGTCAGCCGGATG	56	182-220	1
quru-CA-3D15	$(CA)_{15}$	GGTGGTGGCAGATACACTGG	GACTCAGACAACCAACTTCAGG	62[a]	208–238[a]	5,9,13,15,16,17,18,22
quru-GA-1P10	$(TG)_{12}GCC(TG)_3$	ATTTCTGATGCAGGGTGTCG	TAGGCCAAGGACCAGAGACC	62[a]	237–269[a]	5,9,13,14,15,16,17,18,22
quru-CA-2P24	$(CA)_{14}$	GCAAGAGATCACACACAAACTAGC	CTTTGGGTTCACCAAACAGC	62 [a]	130–176[a]	5,9,13,14,15,16,17,18,22
quru-CA-3A05	$(CA)_{12}(CT)_2$	AACGTGACCTCTCTCACAGC	AGTGCTGGAGTGCTCATGG	62[a]	137–162[a]	5,9,13,15,14,16,17,18,22
quru-CA-1P15	-	-	-	-	-	5
quru-CA-2B16	-	-	-	-	-	5
quru-CA-2O24	-	CCACCACCACCATAACAACG	TGACCATCACAATGAATACACG	57[b]	146–148[b]	5,14
quru-CA-2F23	-	TGGACTGGACTTGACTCACC	CAAATCCGAGCTTTCACACTC	63[b]	171–179[b]	5,14
quru-CA-1C16	-	GCTAACGGGACCACTGTACC	GGGTTCCAACATTGAAGTCG	56	245–255	14
quru-CA-1F10	-	TCTTCACACACCATCACAAGC	ATATGTGGCAGCCATGTTGG	64	328–336	14
quru-CA-1H06	-	GCAATGGCCAATACCATGC	ATGCCTATTCCGCTCTTTCC	59	269–271	14
quru-CA-1J14	-	ATGCTGGCAAACTGCTCTG	GGGATGGATGAAATGTGGTC	62	166–186	14
quru-CA-1J20	-	ACATAGGGCTTGGTGACCTG	TTGCTGGGGAAGTGAAAGAG	63	324–329	14
quru-CA-1P18	-	CAAACCCAGTCTCAGTCAAGC	CTTTGCTGCCAAAGAAGACC	63	203–221	14
quru-CA-2F03	-	TTCCCACTGTGAAGGACCTC	ACCCGTTGCCCTTCCTATTC	64	215–221	14
quru-CA-2I11	-	CTTGAGCAGTGCAGAAGTAACC	TGAATTCGTTTGTCGTGAGG	53	207–215	14
quru-CA-2J23	-	TGGGCATCAGTTGCAGTATG	TGCACCAAATTGAGACATTC	54	149–157	14
quru-CA-2K20	-	AATCCTCACTCTTTGCTTTGC	GGGTTGTGATCGATTCTTGG	61	294–314	14
quru-CA-2N12	-	CCTTGAACGTGTGTTGATGC	CCACCACTCTTGTGTTCTATATCC	63	220–240	14
quru-CA-3A23	-	AGCCCAAATATCGCTGACAC	TGGAACGAAGAAAGGGAGTG	63	142–148	14
quru-CA-3B24	-	CCAGCTATACGACCCAAACC	TTTCGAACTGTTTCCCTTCC	56	218–236	14
quru-CA-3C05A	-	ACGCCCTAACTAACCCTTGC	AGTGCACATGATAGCGATGC	55	164–240	14
quru-CA-3C14	-	GGCTTCCACTCATTGTGTCC	TGCCATGTCTACCTTTCTGC	63	225–237	14
quru-CA-3F17	-	TTCACCATCCTAATTCCTAGCC	ATGCAACAACAACCCAAACC	61	352–366	14
quru-CA-3G22	-	AGTTTCCAATTGTGCCTTCG	GAAGCCCACATAGAAACATTCAG	63	157–161	14
quru-CA-3H01	-	CATCTCATCTGTATTGGAAGAGC	AACAGCTAGGTGGATTGATCG	55	129–161	14
quru-CA-3J12	-	GCCATGTATGCTAGGCTTGG	GGTGTTAGGCTCCATAAACTGC	63	233–243	14
quru-CA-3K16	-	CCAAATGCCACCTTGATCC	ACCGAATTGTTGATGTTTCC	64	126–130	14
quru-CA-3O12	-	CACTTGCTCTCCTTTCATTTCC	GAGAGGTATGATGGGTGTAGTGG	64	222–226	14

Locus	Motif	Primer sequences		T_a	Size (bp)	References
		Forward	Reverse			
ssrQpZAG9	$(AG)_{12}$	GCAATTACAGGCTAGGCTGG	GTCTGGACCTAGCCCTCATG		182–210	3,4,21
ssrQpZAG9	-	GCAATTACAGGCTAGGCTGG	TACTTGGCGGGATATCACA [d]	50[e]	n.a.	10,21
ssrQpZAG15	$(AG)_{23}$	CGATTTGATAATGACACTATGG	CATCGACTCATTGTTAAGCAC	54[a]	103–144[a]	5,9,15,16,17,21
MSQ4	$(AG)_n$	TCTCCTCTCCCCATAAACAGG	GTTCCTCTATCCAATCAGTAGTGAG	50[e]	n.a.	7,10
QM50	$(CCT)_3(CCG)(CCA)(CCT)_2+(CCA)_7$ [c]	CCCGATTTCCCTTCCCTGCT	GAGTGCATCGGGTCGGAGGC [d]	50[e]	n.a.	10,12
QM58	$(CAA)_{11}$ [c]	GGTCAGTGTATTTTGTTGGT	AAATGTATTTTGCTTGCTCA	50[e]	n.a.	10,12
FIR004	$(CT)_{18}$	TCTCTCTCAGGGCAGCTTCT	AACCAAACTCAGATCCAGATTCA	59[a]	122–186[a]	6,8,9,15,16,17,18,22
FIR008	-	AATCAGCCGTGAGTTTCTCG	ACCGAAATCGAAGAGGGAGT	56	148-162[b]	8,14
FIR013	$(CAG)_5$	CGGGGAGGTTGATGAGTATT	AACACTGTCACCCCCATAGC	56[a]	133–144[a]	6,8,13,16,18
FIR021	-	CTCATCATCGGAGGTGGAGT	TTTGAGAAGAGGCGAGTCGT	60	185-188[b]	8,14
FIR024	$(CCT)_6$	CGCTTCTCCTCATCCTCAAG	CTCAAAAGGCACGATTCTCC	59[a]	214–229[a]	6,8,16,22
FIR026	-	CTTCATGCACCAATTCCTCA	GGCCATGTATGTGTGCAAAA	60[b]	190-191[b]	8,14
FIR027	-	GCTCGAGGAGAAGAAGCTCA	GGGACTTTCCACTTAGCGATT	60[b]	197–211[b]	8,14
FIR028	$(TC)_8$	GGAAGAGTGTTCGGAAAGCA	CCAGCTCCTCCACAATAGCA	56[a]	201–237[a]	6,8,16
FIR030	$(AG)_7$	GGACATATTTATCTAGGAGACGAG-GT	ATGTCCCATAGCACAGAGCA	57[a]	157–183[a]	6,8,13,14,16,22
FIR031	$(TC)_7$	ACGAGTCCAACGGAAGTTGT	CACAACTTCACAAGGCAAGG	59[a]	135–182[a]	6,8,16,22
FIR035	$(AT)_6$	GCTAAGGTTCCGTGTTCCAA	GGCCAGCAACTAAACCAAGA	56[a]	146–152[a]	6,8,16
FIR039	$(CT)_7$	GAGCCTCTTTCATCGCTCAC	TCAACACCCCAAAACTCCAT	59[a]	111–132[a]	6,8,13,16,22
FIR043	$(TC)_9$	TTCTCCATTTCACACGCTTC	ACGACATCGTTTTGGAGCTT	56[a]	114–146[a]	6,8,16
FIR048	$(CT)_9$	TGCACCAAAATTGGAGGATG	TTGATGCAAGGTGCAGTTTC	56[a]	187–219[a]	6,8,9,13,15,16,17,22
FIR048	-	GAGTGCAAAGATTGACAATAAAGC[g]	ACTGGCAGCTTATTGGGTTG[g]	60	267–281[b]	(8),14
FIR051	-	TGCTGGTGCAAATATTGGTG[g]	GGCCTGTGTGTAAGCAAACC[g]	58	197-199	8,14
FIR053	$(GTG)_7$	AGTTTCCCCACATTTGTTGC	TACCATGCACCAAGCAATTC	59[a]	136–150[a]	6,8,13,14,16,22
FIR065	-	GTAACATCCTCATTCCCATGC[g]	TCACAGAAGGAACCACAGGTC[g]	60[b]	207-210[b]	8,14
FIR089	$(GA)_6$	AGCGACTAACCCAACTTCCA	GCGGATTCGATAGCATTTTT	56[a]	159–181[a]	6,8,13,16
FIR095	-	TCCCACTTTCCCTCTCACAC[g]	TGGAATATGGAAGTGGGTTC[g]	54	161–163[b]	8,14
FIR104	$(GGT)_7$	TTAACTCGGTTTGCGACTCA	AGCACGTGACTCGACCTGTA	59[a]	203–224[a]	6,8,13,14,16,22
FIR110 L1[f]	$(AG)_{12}$	ACTTGCTCGCTTCAACCTTC	ATTCCTCCTCATCAGGCTCA	56	166–200	14,15,22

Locus	Motif	Primer sequences		T_a	Size (bp)	References
		Forward	Reverse			
FIR110 L2[f]	$(AG)_{12}$	ACTTGCTCGCTTCAACCTTC	ATTCCTCCTCATCAGGCTCA	56	180–236	14,15,22
GOT004	$(TG)_{12}$	GGGCATATTGATCGCTTAGG	TGAGCATTCATACATTCCATGAT	59[a]	264–294[a]	6,8,9,14,15,16,17,18,22
GOT009	$(TC)_{7}$	CACCTCACTAAGCAACCTGTCA	TTTTGGAGGCGGAGATAATG	56[a]	225–249[a]	6,8,9,13,15,16,17,18,22
GOT009	-	CAAACTACACCTCACTAAGCAACC[g]	CGAGTCAATATCGGTGATGG[g]	60[b]	154-162	(8),14
GOT011	$(TC)_{11}$	CCCCACCGTCTACTCTCAAA	GCGTTCACCACGTCCATAAT	56[a]	162–212[a]	6 8,16
GOT021	$(AT)_{13}$	AGAAAGTTCCAGGGAAAGCA	CTTCGTCCCCAGTTGAATGT	59[a]	95–101[a]	6,8,9,13,15,16,17,18,22
GOT037	$(CT)_{11}$	CCATCCTTTTCATTCTTTCCA	TGTTGTTGTTGCTGTTGTCG	57[a]	239–265[a]	6,8,16,22
GOT040	$(GA)_{11}$	AAGGCACTCGTCGCTTTCTA	ACCGATTTGAAGCTCGAGAA	59[a]	234–252[a]	6,8,13,14,16,22
GOT047	$(A)_{10}(CT)_{12}$	AACCCAAACCCAAACCTTTC	TGGTGAATTCGAGGTGTTGA	56[a]	250–268[a]	6,8,16
GOT063	-	TGACAAATGTTGGGAAACCA	AACCAGAGGGCTGGTAACCT	57[b]	230–236[b]	8,14
GOT066	-	AGTGAAGAAGACCTTGCTATGC[g]	AGAGCTGATGCCAATCTTCG[g]	60[b]	198–212[b]	(8),14
GOT067	-	TGCAAGGCTACAAACTAGACG[g]	ATCTGGGTTAGCAGCAGCAG[g]	62[b]	156–165[b]	8,14
PIE002	-	CTCCTCCATTTCCCAATTCA	TCGCTTGTGGTACATCTTGG	56[b]	157–159[b]	8,14
PIE020	-	CGGCTTACCGTTCATACAGC[g]	GCTAGCGACTTGGTGAAACC[g]	60[b]	333–341[b]	(8),14
PIE027	-	CTCGCTTCTCAAACTGAAACC[g]	AAGATTGATGGGAGGATTCG[g]	57[b]	168–172[b]	(8),14
PIE028	-	GAAAGCCAATTCACTGAGATCC[g]	GGGATAGGCTTGGAAGAAGG[g]	60[b]	138–155[b]	(8),14
PIE039	$(CTT)_{8}$	CCTCACCCTCTGCGGTCT	CAGAAAGGGCTGCAAAGC	59[a]	157–178[a]	68,16
PIE039	-	TCACCCTCTGCGGTCTATCT[g]	AGCCAATGAAGAATGGGTTG[g]	60[b]	146–152[b]	(8),14
PIE040	$(TTC)_{8}$	G T G A G A G A G A G A G A G A - CAAAGAAGAAAAA[h]	AAATTCTCCGCCACATTGAG[h]	59[a]	155–174[a]	6,8,9,13,15,16,17,18
PIE099	$(TC)_{9}$	GGCTACCGACTACTACCACTTC	CGGTGGACCCAATATGTAAC	56[a]	179–209[a]	6,8,9,13,14,15,16,17,18,22
PIE100	-	GCCAGAAATCCATTTCTCCAAC	TTGTATTTTCCGGTGTGGTC	56	-	8,14
PIE101	$(AT)_{13}$	GCGACAGTCACAATTAAGCTAC	CACCCAATTTTCAATCTGTG	56[a]	139–173[a]	6,8,13,14,16
PIE111	-	TGCTAATCTTGAACGGAAGC	CAACCTCTCCAACACATTCC		-	8,14
PIE125	$(GGAAGC)_{3}$	AATACAAATCGCAGGAGGTG	CTAACCCATCGTTCATGGAG	57[a]	146–162[a]	6,8,16,22
PIE126	-	ACCGAAAGAAAAGCAGTGAG	TAGTCGCGGAAGAAAGAGAG	56	243–245	8,14
PIE164	-	TCAAGCGGTTTCCAATTCC[g]	GTGCAGATCTAGACGACATTCC[g]	58[b]	308–346[b]	(8),14
PIE176	-	GACATAAAGATGGGCATGG	GGCAGCATCTCCCTAATGTT	58	163–172	8,14
PIE183	-	TGGAGAGGGACATAAAGATGG[g]	TCCCTAATGTTGGTGATGTCC[g]	60[b]	162–171[b]	(8),14
PIE200	$(CAA)_{5}$	ACAACATGTGCCAAAACTGC	TCGATGATGTGGTTGTTGATG	56[a]	107–119[a]	6,8,16
PIE228	-	TGGAGGAGCACTGCATATTG	CACTGTGGCTGGAGCATCTA	60	234–240	8,14

Locus	Motif	Primer sequences		T_a	Size (bp)	References
		Forward	Reverse			
PIE236	-	TCAATCCCATCTTCCTGCTC	TCTATGCGATTCCGCTTACC	60	204–207	8,14
PIE260	$(AG)_9$	TTCCTTACTCCTTTCCCACTTC	TGGCTGTTCCAAATCTTCAA	56[a]	136–171[a]	6,8,16
POR003	$(CT)_8$	CTCGCTCTCCTCCCCTAATC	AGCTTTGATCGAGTCCGAAA	59[a]	91–115[a]	6,8,13,16,22
POR006	-	CTATGGCTGTCACCATGTCG	AGCACCCAAAAACCCTAAGC	60	124–126	8,14
POR016	$(GGT)_6$	AGCAACAGCAGAGCCAAAAT	CAGCGGCTTTGAGGTAATTC	59[a]	110–126[a]	6,8,13,16,22
POR023	-	TCGACTTCATCCCCATCTTC	CATACGGACCACTTGCCTCT	60	142–161	8,14
VIT023	$(ATA)_6$	AATGCGAACGACATGAACAA	CTCTCGTCGGAGACTCAACC	56[a]	115–118[a]	6,8,16
VIT057	$(AACTCG)_3$	TCAGCAAAATCCCAACTTTGT	ACACTTCGCTGTTCCTCGAT	57[a]	128–153[a]	6,8,13,16,22
VIT01L1	$(CAT)_9$	AATTCAAACCCAGCCAACTG	TCCTCTGGATGCTCCATCA	56[a]	108–112[a]	6,8,16
VIT086	$(CAG)_5$	AAGAACACCCATTTCCACCA	TAAAATCCATTTGCCGGTTC	56[a]	184–207[a]	6,8,16
VIT107	$(TA)_{13}$	TGATCACAGATTGGAGCTTAACA	CCCCCACTTAGGAAAGAAGC	59[a]	124–142[a]	6,8,13,16,22
VIT142	-	ATAAGCCTGCAGACCCAGAA	AGTGACTGCAACCACGACAG	57	144–147	8,14
WAG004	$(TTC)_7$	AAAGCAATTCAACTGGGACG	ACGACACCGTTTGTTCCTTC	56[a]	250–300[a]	6,8,16
WAG016	-	CCCATGACCTCATCCTCAAC	GTGAGCCGTATAATCGGACG	60	114–127	8,14
WAG018	$(GT)_7$	GGTTCCGATTTGTTTGAGTCC	TACAAAACCCAAGCTCCCTG	56[a]	132–151[a]	6,8,13,16
WAG023	-	GGTAGCTTGCACGAAGTTGG[g]	CTTGCAGCTCCCTGGTAGTC[g]	60	354–360	8,14
WAG065	-	TCAGCACCTGTGAACATTCC	GTCCCTCCCTTTCAGAGTCC	60	268–280	8,14
REO_010	-	GCAGCTACAGTAGTAACCAATCCA	CTCATCTTCCTCAGGAGTCCA	57	153–163[b]	14
REO_031	-	ATCCCAGGCTCTCTCTCTGTC	AATACACCAACGCAACGAAG	56	249–250[b]	14
REO_035	-	GGGTAGAGAAGTTGCGGTGA	CAAGAGGCAGAGCCAGTTTC	63	133–135[b]	14
REO_051	-	AAGCTCGGGATCAGAGACTG	ACAAGCGAGAGTCTGGGAGA	60	163–172[b]	14
REO_081	-	CGCCATGAATGAATGGAAT	CGCCATGAATGAATGGAAT	60	276–287[b]	14
REO_083	-	GGAGCTCTCCTGTGCTCATT	GATCCCTTCCTCACCACAGA	63	154–164[b]	14
REO_084	-	CTAATGACCAGGGTCAAAGC	GCTGGGTTGAACAATTTGG	63	191–202[b]	14
REO_085	-	TTCAGGGTTTGTGTTTCTCG	CCAGCGAAATCCCAATTACC	60	221–223[b]	14
REO_087	-	CAACACAACAACATGCTCTCC	GCTGATCGAGCTTATCATCAAC	63	160–166[b]	14
REO_111	-	CCATGACCGATATTTCTGGATAG	AGTGTGAGACCCGTTGTTCC	63	151–153[b]	14
REO_114	-	AAACGATAGGTCGTTTACTCCTT	TTTGTGGGTTGAGAAGAGCA	60	149–155[b]	14
REO_180	-	AAAGTCTGAACCCGTGGATG	CCTAGAGGCGAGTCCAAATG	63	207–211[b]	14
REO_259	-	TTTGGCAGCTTAGAGGTACATT	TCTTGCTTCAATCTGATGTCG	63	231–239[b]	14
REO_263	-	GAGGAAGGTTGTGTCTGAAGC	CATCTCCATATTGGTCACCATC	63	206–208[b]	14

Locus	Motif	Primer sequences		T_a	Size (bp)	References
		Forward	Reverse			
REO_264	-	GTCCAAATATGGTGAGCTTGG	CCCACTTAGCAACAGTGTATGG	63	166–183[b]	14
REO_269	-	GGCCTCCATTTAAAGATTTCC	CATATGGCTCATACCATTGTCC	60	233–246[b]	14
REO_288	-	AAACAAACCAGACCCATCTCTC	ACAAGGAGCTCCAAGTCGAA	63	201–213[b]	14
REO_291	-	GGAGAAGAACAAGGACTCTCCTC	GGCAAGTCCCTTCAGTTGAG	63	170–178[b]	14
REO_306	-	TCCTGTAACTCATCGTTGTCAC	GCTCAAGGAGACCACAACAG	62	186–215[b]	14
REO_314	-	CCGCAATGCGAAGAACAA	GCCCAGATCCAACACTCACT	57	206–221[b]	14
REO_339	-	AGATATCTGGCATGGAGAAGC	CAGATCATCATGCTGCTAACG	60	102–116[b]	14
REO_366	-	GTGGACGGTGGTTCTTTAGG	TCACCTAACATTATGGCAGCAG	63	221–224[b]	14
REO_368	-	CCACTAAGCCTAATTGGTGGTG	TCCAACCACTGCAAGCAC	62	223–232[b]	14
REO_371	-	GACTCATAGCGACTCATTACTGG	TCACCTAACATTATGGCAGCAG	60	159–168[b]	14
REO_376	-	TCGACCAATCAGGTACTCAGG	AGGCTAATCCATCCACTCTATG	63	186–199[b]	14
REO_379	-	CTTATTGCACAAACTCCAACG	TTCATTGCGATGAGTGAAGG	62	162–165[b]	14
REO_380	-	CCAGCTTTCCACAGATCCAC	GTTTCTAGAGGGTGTTAGGCTTT	63	136–139[b]	14
REO_390	-	TCCATCATCGCCTCTTTAGC	AAAGGTTTGGATGTGTGTGG	62	223–229[b]	14
REO_408	-	GAACGCTCATCTTGTGTTGC	CTCAGTCCAAGGACTTGATCG	63	216–222[b]	14
REO_416	-	CAGCCCTATGATCTCCAAGC	CGTACACGTTCAAAGCAACG	63	165–171[b]	14
REO_433	-	CCCAAATCTGAGAAACAACC	TGACTGTGCCGTTGCTAACC	57	206–233[b]	14
REO_443	-	ATGTCCATCGAATCGTCTCC	AGACGAGGACATGGAAGACC	60	158–162[b]	14
REO_455	-	CTTGGTCGGATTCAAGAAACTC	GGCTCTCAAACTCCAGCTTC	56	169–176[b]	14
REO_457	-	TTGCAAGAGCAGATTTGGAC	TCTCTTTCTTCCTCTGCTACTCG	56	213–223[b]	14
REO_486	-	GCCAGAGTTTCAATCACAAGAA	GAAAGCCCTTCTTCCAAGGT	60	144–150[b]	14
REO_489	-	GAGACAACTAGCACGGTCTCC	TCAGGAAGAAGGTCAGAGAGC	54	188–205[b]	14
REO_498	-	CACACCCCAGTAAGGTCCAT	GTCTTATATCAAGCAGCTGCAAA	60	223–225[b]	14
REO_523	-	CGACTTCTTCGTATTCGATGTT	CTCTACCAATGGCGAGGAAG	56	156–159[b]	14
Cm_3815[e]	-	TCCCTTTCTTCCTGATCTTCC	GCTGGTGATTTGCTGTTTCC	-	188–196[b]	14

** Not presented in papers for northern red oak, [a] Annealing temperatures and allele lengths according to Lind-Riehl et al. (2014), [b] Values according to Konar et al. (2017) based on two mapping parents, [c] Repeat motif in Quercus myrsinifolia according to Isagi and Suhandono (1997), [d] Reverse primers redesigned by Gerwein and Kesseli (2006) in order to be specific for northern red oak, [e] Annealing temperature applied by Gerwein and Kesseli (2006), [f] This primer pair amplifies two loci: FIR110 L1 and FIR110 L2, [g] Primer redesigned for Q. rubra by Konar et al. (2017), [h] Primer sequence according to Lind-Riehl et al. (2014)*

1-Aldrich et al. 2002, 2-Aldrich et al. 2003a, 3-Aldrich et al. 2003b, 4-Aldrich et al. 2005, 5-Borkowski et al. 2017, 6-Collins et al. 2015, 7-Dow et al. 1995, 8-Durand et al. 2010, 9-Gailing et al. 2012, 10-Gerwein and Kesseli 2006,11-Hoban et al. 2009, 12-Isagi and Suhandono 1997, 13-Khodwekar and Gailing 2017, 14-Konar et al. 2017, 15-Lind and Gailing 2013, 16-Lind-Riehl et al. 2014, 17-Lind-Riehl and Gailing 2015, 18-Lind-Riehl and Gailing 2017, 19-Moran et al. 2012, 20-Murray and Pitas 1996, 21-Steinkellner et al. 1997, 22-Sullivan et al. 2013

Material for DNA-extraction

Leaves were the most common tissue used for extraction (Aldrich et al. 2002, Collins et al. 2015, Gailing et al. 2012, Gerwein and Kesseli 2006, Khodwekar and Gailing 2017, Lind and Gailing 2013, Lind-Riehl et al. 2014, 2015, 2017, Moran et al. 2012, Sullivan et al. 2013) followed by cambium from the base of trunk (Aldrich et al. 2003a, 2003b, 2005). Khodwekar and Gailing (2017) additionally extracted DNA from embryos (from acorns) in order to carry out a paternity analysis. In one case, tissue used for extraction was not defined (Borkowski et al. 2017).

DNA-extraction protocols

In most studies, a commercial DNA-extraction kit (DNeasy 96, Qiagen) was used (Aldrich et al. 2003a, Collins et al. 2015, Gailing et al. 2012, Lind and Gailing 2013, Lind-Riehl et al. 2014, 2015, 2017, Sullivan et al. 2013). Some authors extracted DNA using a CTAB-protocol (Borkowski et al. 2017, Gerwein et al. 2006, Moran et al. 2012). Borkowski et al. (2017) refers to Hoban et al. (2009) for modifications to the CTAB-protocol used. Finally, Aldrich et al. (2002) refer to Murray and Pitas (1996) for the octanol-chlorophorm based protocol used for DNA-isolation in their study. Aldrich et al. (2003b, 2005) cite Aldrich et al. (2002) for DNA-extraction.

Important results

- Differences between northern red oak and other oak species, as well as hybridization were investigated by Aldrich et al. (2003b), Moran et al. (2012), Sullivan et al. (2013) and Lind-Riehl and Gailing (2015).
- Until recently, microsatellite-based genetic studies within northern red oak were of regional or local scope (e.g. Aldrich et al. 2003b, Gerwein and Kesseli 2006). Aldirch et al. (2005) used a panel of 14 loci to screen for diversity in 10 adult *Q. rubra* from two old-growth stand at the Davis-Purdue Research Forest in east-central Indiana. They found slight but significant differentiation among stands but also weak isolation by distance within large stands. In total, 105 alleles were detected with a mean of 7,5 alleles per locus (range, 4-13 alleles). Gailing et al. (2012) found that genetic distance at the 15 microsatellite markers developed by Durand et al. (2010) for *Q. robur*, seven simple sequence repeat (SSR) markers developed for *Q. rubra* (Aldrich et al. 2002, Sullivan et al. 2013) and the *Q. robur* microsatellite QpZAG15 (Steinkellner et al. 1997) was not correlated with geographic distance.
- Lind and Gailing (2013) identified a small but significant differentiation between managed and unmanaged populations of *Quercus rubra.* Lind-Riehl et al. (2014) showed that locus FIR013 displayed a high differentiation between *Q. rubra* and *Q. ellipsoidalis*, which might be due to divergent selection (outlier approach). This marker is located within a CONSTANS-like gene which is involved in photoperiodic control of growth, which might pose a prezygotic barrier between these two species (Collins et al. 2015). In subsequent publications, Khodwekar and Gailing (2017) and Lind-Riehl and Gailing (2017) suggest adaptive introgression between the two species in sympatric populations based on results from allelic frequencies and sequences of locus FIR013.
- In a recent study, Borkowski et al. (2017) investigated genetic differentiation across the native range of northern red oak. By performing a Bayesian cluster analysis (STRUCTURE-method; Pritchard et al. 2000; Falush et al. 2003) based on genotypic data from 10 unlinked microsatellites, they found a well-defined genetic cluster including populations from the area around the Lake Superior in the northwestern part of the species' native range. They also detected a cluster with regional distribution in the northeastern part and another one covering most of the species' range which is spread across southern and central areas. There was a continuous gradient between those two clusters. Finally, a fourth cluster with a local occurrence in only one population in Massachusetts was identified.

b) SNPs (single-nucleotide polymorphisms)

Loci and primers used

Restriction site associated DNA sequencing (RAD-sequencing) was used in a recent genome mapping study conducted by Konar et al. (2017) using a full-sib family in *Q. rubra*. In total, 78 725 SNPs were called by applying RAD-sequencing. Various quality filters (e.g. for missing data and deviation from the Hardy-Weinberg Equilibrium) were applied resulting in 1413 SNPs along with 116 SSRs available for mapping. A list of all SNP-loci finally used for mapping (849 in total), along with sequence reads where the SNP occurred, position in linkage groups and distances in centimorgans is provided as supplementary material by Konar et al. (2017).

In a following study, Merceron et al. (2017) published

their results about range-wide variation of northern red oak at SNP-loci using Sequenom® technology. A total of 1410 bi-allelic SNPs already described in Konar et al. (2017) were used to design 3 multiplexes with a total of 115 SNP markers (40, 40 and 35, respectively). After removal of monomorphic or non-amplifiable loci, 80 SNPs were usable for population genetic analysis. However, among them 69 were included in the final population genetic analysis after filtering for missing values.

The 115 primer sequences from Merceron et al. (2017) including primer tags are presented in Table 4. These were organized in 3 multiplexes based on Sequenom® methodology (Table 4). Initial PCR reaction produced amplicons of length between 72 and 85 bp. For more details about the Sequenom method the reader is referred to Bradić et al. (2011).

Table 4: Primers for amplification of SNP loci used in Merceron et al. (2017); Amplicon length (bp) = base pairs, T_m = extend primer melting temperature (calculated)

Locus	SNP polymorphism	Primer sequences		Amplicon length (bp)	T_m	Multiplex
		Secondary amplification primer (including secondary tag)	Primary amplification primer (including primary tag)			
loc322451_43	A/G	ACGTTGGATGATCGACTCAATGCACTCTCC	ACGTTGGATGGTTGTGCACCTCACTACTTG	81	46	1
loc171946_28	G/A	ACGTTGGATGCTGTACTATTGTATCTGGAGC	ACGTTGGATGCGGTTTCGTCTTCTCTACGG	78	47,9	1
loc219809_27	C/T	ACGTTGGATGTGAAGCACGGGCTCCTAAAG	ACGTTGGATGCAATCTAGCACCTCCCTTAC	84	46,4	1
loc175265_52	C/T	ACGTTGGATGATTTTCGCGCACACACGCAC	ACGTTGGATGTTCCTCTTCTCTTTCCTTTC	83	45,1	1
loc301815_40	C/T	ACGTTGGATGTCATACTCAAAGTTGCCCCC	ACGTTGGATGTTATAATTGCTAGCAGACG	79	52,9	1
loc308441_30	G/A	ACGTTGGATGATAGGCCCTAGGCAGGCAAT	ACGTTGGATGCATTCAGCACTAAGCAAAAGC	80	48,4	1
loc76894_51	C/T	ACGTTGGATGGGCTCTGGATAATGGACAAG	ACGTTGGATGAATATGTGTTGGCAGTTGGG	81	50,3	1
loc131778_34	G/C	ACGTTGGATGCACACGTTTGAGAGTATTGC	ACGTTGGATGCTGTGACCATATCAGGCTTC	85	52,1	1
loc229042_42	C/T	ACGTTGGATGGCATGCTGGTTGAAATTGG	ACGTTGGATGGTCCCGCAAGCTCTATCCTA	82	45,5	1
loc209821_59	C/A	ACGTTGGATGCGTTCAACATTTCCTCTCTC	ACGTTGGATGTGGTGGGTTTCAAGTGAGAA	84	46,8	1
loc108606_28	C/A	ACGTTGGATGCGATTCTAGGGTTCGATGAC	ACGTTGGATGGAGTGTAATCCCCGTGAAAG	74	49,8	1
loc236389_31	C/G	ACGTTGGATGTTGTATTTCTAGTTGACCC	ACGTTGGATGGCTACTACAAAACAATGCAAG	84	48,6	1

Locus	SNP polymorphism	Primer sequences		Amplicon length (bp)	T_m	Multiplex
		Secondary amplification primer (including secondary tag)	Primary amplification primer (including primary tag)			
loc297569_33	C/T	ACGTTGGATGCTTCTTTCCCTTCCTTTCAG	ACGTTGGATGTATTGTTCTAAACCCATAC	75	49,8	1
loc303419_31	A/G	ACGTTGGATGTAGCGACAATGACCCTTCAC	ACGTTGGATGGGTTAGCACCATGGTTATAG	84	53,5	1
loc4799_58	A/T	ACGTTGGATGGCGATTCTTCCTCTTTGCTC	ACGTTGGATGACTCTAGATGAACCCGAAAG	83	49,5	1
loc303854_27	C/T	ACGTTGGATGATGTAGCCGAGGATCCTAAA	ACGTTGGATGTTAGACTTATGCTCGGCAAG	79	45,3	1
loc62609_37	T/C	ACGTTGGATGGTTCGATGTGCGATATCGCC	ACGTTGGATGAGTCAGTAATAACTTACCCC	76	58,5	1
loc91944_32	A/T	ACGTTGGATGTTCAAAAAAATTGCCTCAG	ACGTTGGATGAAAAAGTGCTTGCAGTCTGG	78	48,8	1
loc177517_28	T/A	ACGTTGGATGAGAAACCGGACCATACAAAG	ACGTTGGATGGTTCTACCTATCTTGAATC	75	48,5	1
loc15453_54	G/C	ACGTTGGATGATGGTACGAAAGATAGGGAC	ACGTTGGATGGTTCGCTAAATTTTTATGGGC	75	47	1
loc323446_46	G/T	ACGTTGGATGCGGATCAGTATGAGTTCAAT	ACGTTGGATGGATAGTTGAACAAAATTTTC	85	49,6	1
loc14800_56	T/A	ACGTTGGATGCCTGTTACTGGAGTTTGCCC	ACGTTGGATGGTATACAGAAGAAACAATC	84	57,8	1
loc251988_52	G/A	ACGTTGGATGGCCCTTCGAAAACAGGTTC	ACGTTGGATGCTCCGCAAACAAACAGAAATC	84	48,1	1
loc265253_31	G/A	ACGTTGGATGGGAGCTGATCTCCATCAAAG	ACGTTGGATGTCCACTTTACATTATGTAG	83	57,2	1
loc121720_62	A/T	ACGTTGGATGGGAACGGTATCATACTCATC	ACGTTGGATGATCGGCATATCCTCCAAA	84	51	1
loc166327_54	T/A	ACGTTGGATGGCGATGAGGTCAAATTACAAG	ACGTTGGATGATATCTCCGCCCACGGCTA	85	55,7	1
loc212883_33	T/C	ACGTTGGATGCCTTGTCTAGCTTTCACTCT	ACGTTGGATGCTCCGAAGCATATTGATTTC	85	50,8	1
loc199417_54	G/A	ACGTTGGATGCCGATCTAAGTTTTCAAGTGC	ACGTTGGATGATAGCAACGCCAATAGAGCC	83	50,9	1
loc311295_42	T/C	ACGTTGGATGCACAAACACAGTGAAAGTAG	ACGTTGGATGGATTAGGCTTAGTTGAGCTG	76	47,3	1
loc304430_40	C/T	ACGTTGGATGAGAGGAGCTCCAAGAAAGTC	ACGTTGGATGGCCACTCTGATTCCCTATTC	77	52,2	1
loc59111_28	T/C	ACGTTGGATGCAGTTACCGGTCCTTCATTG	ACGTTGGATGTGTGGTTTCCTTGTTGCCC	76	57,5	1
loc8795_29	C/T	ACGTTGGATGCATTCAAGATAAGGGGTCAA	ACGTTGGATGTCTGGCGCGTATATGAATCC	80	52	1
loc88073_33	C/T	ACGTTGGATGAGTAGAAGTTACCTTGATGG	ACGTTGGATGGCTCTTCCGATCTAATGGTA	85	49,9	1
loc343655_48	A/G	ACGTTGGATGTTTACTACCTGAACAAAGGG	ACGTTGGATGGAGAAGCTTGGAGATTTTAC	84	56,2	1
loc162429_32	C/T	ACGTTGGATGGACTTATTGTTGAGTTTGCC	ACGTTGGATGACTGAAGAGGCGAAATCGTG	84	55,9	1
loc233149_56	T/C	ACGTTGGATGCCATGATAGGATCAAGGTG	ACGTTGGATGTAGTTCACAAAAATGGCAC	82	48,4	1
loc8473_32	G/A	ACGTTGGATGAGTTCTCTTGCAGCTTCTTG	ACGTTGGATGTGTTGCAGTGAAAGTTTTGG	83	57,7	1
loc92819_57	A/G	ACGTTGGATGCAATGCTAGATAGTCAGCAAG	ACGTTGGATGCTAATTTCTGGTGAATCATCC	85	55,4	1
loc281428_28	A/G	ACGTTGGATGAAATTTACCAGGAGCAGCAA	ACGTTGGATGGTATCATGCACTTATTGAG	78	54,9	1
loc58391_57	G/A	ACGTTGGATGGGTAAAATGGTCGTATTTG	ACGTTGGATGAAAGATATGCAGGAGTGAGG	75	52,3	1
loc8803_25	C/T	ACGTTGGATGCATGATCTCGACATATGC	ACGTTGGATGCATCTGAAGAGCGTCTTCTG	72	48,9	2
loc47977_32	C/T	ACGTTGGATGACTGTGAACCTGAAGGACAA	ACGTTGGATGCTGAAAGTAGATGATATAC	83	47,2	2
loc65241_27	G/A	ACGTTGGATGGATAAGGCAATCACTGAGGG	ACGTTGGATGGGATCAACCTGGAATACCC	73	45,4	2

Locus	SNP polymorphism	Primer sequences		Amplicon length (bp)	T_m	Multiplex
		Secondary amplification primer (including secondary tag)	Primary amplification primer (including primary tag)			
loc210357_32	C/T	ACGTTGGATGTCAATAACCACCATCCAATC	ACGTTGGATGTTCCTAATTTGGGTTGATGG	81	46,6	2
loc94091_29	G/A	ACGTTGGATGGAGTTCTACGCCATTATTCC	ACGTTGGATGTTTGAATGAGATAGGGCTAC	75	45,1	2
loc337077_42	T/A	ACGTTGGATGGGTCTACCTAGTTGATCTTG	ACGTTGGATGAATGGTAAAGGGGTCACTGC	81	45,7	2
loc272405_41	C/T	ACGTTGGATGCCTTGGCCTTGGTTGAAAAC	ACGTTGGATGACCCGCTACTTCACAGTATG	81	46,9	2
loc31392_41	C/T	ACGTTGGATGTGTCATCCCCACTGCAACAC	ACGTTGGATGTCCGATCTAAAGAGTTGCCC	85	53,5	2
loc242705_34	C/T	ACGTTGGATGCAATCTCTAAGTCTCATTCG	ACGTTGGATGTCATTACCCAGGAATTGGGC	77	45,8	2
loc50525_27	G/T	ACGTTGGATGCGCTCTGCCACATTTACATC	ACGTTGGATGGAACTACCAAGTGTGCCTAC	76	47,9	2
loc271178_45	C/A	ACGTTGGATGATGTAATCTTGACTTGTGC	ACGTTGGATGGTCATCTATGGTTATTTTCTC	84	50,5	2
loc164433_38	G/T	ACGTTGGATGGGGTGGGTTTGCAATTTGAG	ACGTTGGATGGTGCTCTTCCGATCTAAATTG	84	49,3	2
loc27548_53	T/C	ACGTTGGATGATACACGTACGAGAGTCTTC	ACGTTGGATGGCTTAGTTATGTAAGTTTGTC	84	49,8	2
loc208444_30	G/T	ACGTTGGATGGAGATGGCATAAATCTCTCC	ACGTTGGATGGCCCCCAATATGGCATCTAA	81	47,6	2
loc327671_26	T/A	ACGTTGGATGTCATGTCTCAGATGAAGGTC	ACGTTGGATGAAAGAAAATTCGGAGAGAG	78	45,9	2
loc163756_30	C/T	ACGTTGGATGGGTTTCCTTTGTAGGCATGT	ACGTTGGATGAGACGGAATGCGATGTACAC	83	47,2	2
loc179815_34	C/T	ACGTTGGATGGTGTCCAACCGAACTTACTC	ACGTTGGATGAAGTGAGGAAGAACTCCTCG	85	49,1	2
loc67049_48	A/T	ACGTTGGATGGAAACCAATAGTCACACTTG	ACGTTGGATGGTAATTGTCAAATGGAGTGG	81	47,7	2
loc312561_61	C/T	ACGTTGGATGTGGAACTCGAGTCACAAGTC	ACGTTGGATGTACCATTTACAGTCAATAT	83	46,2	2
loc181647_35	C/T	ACGTTGGATGCAGAGTAGAAGTTATCAATG	ACGTTGGATGGTATGCATCTTTGTTGCTACG	81	45,6	2
loc123590_45	A/T	ACGTTGGATGAGACAGCACTGTTGTTCTTC	ACGTTGGATGCAACTGGATATGCTGGAAAC	85	50,2	2
loc23843_25	C/T	ACGTTGGATGCACACCCAAGAAACAGAT	ACGTTGGATGCCGAGGAAGATTTCCCACAT	84	49,2	2
loc2734_36	T/C	ACGTTGGATGCTGTGCAAGATAAATAATCG	ACGTTGGATGGGACATTTGGATTGCATCTG	85	47,6	2
loc180863_46	T/C	ACGTTGGATGGAGACTGTAGTTGCGGTGAC	ACGTTGGATGATGGAGGAAGACAACGAGAG	81	57,8	2
loc190405_32	C/T	ACGTTGGATGAAAAACCCATGGTCACCATT	ACGTTGGATGGATGAATAATATACCATAGC	77	51	2
loc349268_52	T/C	ACGTTGGATGGACATGGCAGTAGATTCACC	ACGTTGGATGGCTTGCAGGAAGACTAAATG	77	51,3	2
loc279671_50	A/G	ACGTTGGATGAGCTGAAGCTGATCATGCCC	ACGTTGGATGTCAGAAGGAGATCTAAAGC	85	55,8	2
loc156030_31	T/C	ACGTTGGATGACCCCTTTGTTCTGTTTCGG	ACGTTGGATGCAAGAACACTAAAATAGTCC	80	53,1	2
loc125771_55	C/G	ACGTTGGATGATCATCACCTAGATTGGTGC	ACGTTGGATGCGGTGGAAAAGAAGAAAGAG	84	55,9	2
loc109258_47	C/G	ACGTTGGATGCATAGCTTTTTTACTTATGG	ACGTTGGATGCTGTCCCTTCCATCACCAAC	85	46,7	2
loc105193_34	A/G	ACGTTGGATGTACTTCTTGCCATGACTACC	ACGTTGGATGATAGTGTCATTGTTTGGTGC	76	54,8	2
loc266349_36	C/A	ACGTTGGATGCTTGCCAGGATTATTGCTAT	ACGTTGGATGTGTTGTCAACTTGTGGTGCG	85	49,3	2
loc346733_35	T/C	ACGTTGGATGGGAAGCATTGTAAAAGAGTG	ACGTTGGATGGCCAGTGTTTTTTATCTAATC	82	49,6	2
loc307943_50	G/T	ACGTTGGATGCCTCATTCGAAATAACAATC	ACGTTGGATGGGATGATAGAATCATCAAG	81	50,1	2

Locus	SNP polymorphism	Primer sequences		Amplicon length (bp)	T_m	Multiplex
		Secondary amplification primer (including secondary tag)	Primary amplification primer (including primary tag)			
loc106220_34	C/A	ACGTTGGATGGGTACCAATTCACAATGTATC	ACGTTGGATGCTAAAAACTCAACAGGAGTC	83	48,4	2
loc253441_26	A/G	ACGTTGGATGATAGAAACCTTGAGTTGGC	ACGTTGGATGGGATTCTCAGGTTCTATCTC	84	56,5	2
loc61770_37	T/A	ACGTTGGATGATCCCAACCGTGAAAGCAAC	ACGTTGGATGTTGCTGTGCTGATAAGCTCG	80	64	2
loc284407_26	G/A	ACGTTGGATGGTGCTCTTCCGATCTAATTC	ACGTTGGATGGGCGCAAAAGACCGGCTA	81	56,4	2
loc74953_55	T/A	ACGTTGGATGTTGCAAGTTGCGAAGACCCC	ACGTTGGATGACAAGGTTGTTCTCCGCTTC	81	57	2
loc337556_30	A/G	ACGTTGGATGATTCAAGTAATTTAGTCAAG	ACGTTGGATGGGAATATGGGCAATTTGGAC	80	47,5	2
loc285513_52	T/C	ACGTTGGATGAGGAACGCTAAATGGAAATC	ACGTTGGATGTTGGGACTCTATGAACTAGG	81	47,3	3
loc94103_27	A/T	ACGTTGGATGCTCTTTTTATGCTTAGCTGG	ACGTTGGATGCACGTATGTAGTCCAAGCAC	82	46,9	3
loc245418_28	G/A	ACGTTGGATGCTCAGGAGTTCAACATGGAC	ACGTTGGATGTGATTGTCCTAAGGAGCTTG	83	46	3
loc96520_45	C/T	ACGTTGGATGTCTTTTGGAAAGCATGATG	ACGTTGGATGGCTCTTCCGATCTAAAATAA	84	45,5	3
loc84229_35	A/G	ACGTTGGATGGGATTAGATTGGCTTGATTC	ACGTTGGATGCTCGACTAATGAGTATTCGAC	82	46,9	3
loc313330_43	C/A	ACGTTGGATGGTGTTTTACTCTGTGCTGTG	ACGTTGGATGGCTCGAAACTTGACATGAAG	81	50,5	3
loc59447_43	A/T	ACGTTGGATGTTAGAATTGCATGTCAACGC	ACGTTGGATGAGTATCAAGGCATGATGTCC	77	46,8	3
loc290581_33	C/A	ACGTTGGATGCCTGAAGTTCACCAGATCAC	ACGTTGGATGCAAAGGAGTACCACATACTAC	84	47,4	3
loc90608_37	C/T	ACGTTGGATGGTTCTCATCACGGGATGGTC	ACGTTGGATGTTGATGAGGCCCTAGGTTTC	84	48,8	3
loc102297_26	G/A	ACGTTGGATGGGCTATGTGAGTTGGTGTTG	ACGTTGGATGGTAAATTGCTCTTTGCAGA	79	45,5	3
loc8473_36	T/A	ACGTTGGATGTCGAGTTCTCTTGCAGCTTC	ACGTTGGATGGTGAAAGTTTTGGTTCATGGG	79	46,8	3
loc14800_45	G/A	ACGTTGGATGCTGGAGTTTGCCCATGAGAC	ACGTTGGATGGTGAAGTTGTATACAGAAG	85	47,4	3
loc153814_35	G/A	ACGTTGGATGTAAGGGTTCAAGCTCTGAGG	ACGTTGGATGATCCAGCGACTCAACATAAG	81	49	3
loc16945_32	A/G	ACGTTGGATGCAGACCCAGCTAGGATTCAG	ACGTTGGATGGGAAGGAAACAAACAGAGAG	77	46,2	3
loc315022_60	T/C	ACGTTGGATGTGCTTATAAGATGCCACAT	ACGTTGGATGCCAAGTTACCCAACTTGGAG	78	49,2	3
loc1120_57	A/G	ACGTTGGATGCAGAAAAATATCACTAGCGAG	ACGTTGGATGGCTTACATTTACATAATTTGG	78	46,5	3
loc20330_29	G/A	ACGTTGGATGAGCCTGTGGAGCAATCATAC	ACGTTGGATGCTTTTCAATTACCCTGTGG	75	49,7	3
loc115494_31	T/C	ACGTTGGATGTCACTCACTACTCACCTCAC	ACGTTGGATGGCGCTATATGTGTCTTCAAT	82	49,3	3
loc9437_38	A/G	ACGTTGGATGAGTCTCCTATTGGAACAATC	ACGTTGGATGGTACATTTCCATGATAAAGC	82	45,7	3
loc297402_41	A/T	ACGTTGGATGCATTGTTTAGCTCATTTTTTG	ACGTTGGATGACTTGGACTCATTTTTTTG	83	46,2	3
loc340528_28	C/T	ACGTTGGATGCTTTGGAATATGTTTGAGC	ACGTTGGATGGGACTAGATAAATGGTTGTC	81	50,4	3
loc104520_31	G/A	ACGTTGGATGCTACGACAAAAGAGTAGTCA	ACGTTGGATGGGTTTATGTGGCATTCTACC	85	45,9	3
loc329754_37	G/A	ACGTTGGATGGTAATTTATAGTTCCATTAG	ACGTTGGATGCTACATAAATACTTTATAGGC	82	46	3
loc179595_40	T/A	ACGTTGGATGGGCTGTATCAAAAAGAGACC	ACGTTGGATGATGTGATCTATATCATTTGG	83	48	3
loc160886_48	C/T	ACGTTGGATGCCCCAGATAAGACAAATAAAC	ACGTTGGATGTGGGGTTTGACTCTTTGAAG	81	47,4	3

Locus	SNP polymorphism	Primer sequences		Ampli-con length (bp)	T_m	Multiplex
		Secondary amplification primer (including secondary tag)	Primary amplification primer (including primary tag)			
loc275678_33	C/T	ACGTTGGATGCGTTTACAAATGAACTTATG	ACGTTGGATGCTCTTCCGATCTAATAGGAAT	85	47,6	3
loc102883_47	G/A	ACGTTGGATGTTCCCTCCCAACTTGGATTG	ACGTTGGATGTCTCCTCAGTTACTCTGGTG	82	53,4	3
loc90813_34	A/T	ACGTTGGATGTCCCAAGCAGTCAAGACAGG	ACGTTGGATGCTCCATTCTTCTTGTTGGGC	80	55,5	3
loc160366_41	A/G	ACGTTGGATGCCATATAAAACATTGGCAC	ACGTTGGATGCTCCTTGATGATTGAATAC	80	51,5	3
loc1165_46	G/T	ACGTTGGATGTTTCCTGTGTTGCAAAATGGC	ACGTTGGATGTTTGCAACCTCAATTTGAC	80	51,3	3
loc135792_49	C/A	ACGTTGGATGCATCCTCCAATTTTTCGTAAC	ACGTTGGATGCATTCTTGCTTGTGGACTTC	85	47	3
loc270924_56	C/G	ACGTTGGATGTCCCAATATAGAGTTCACAC	ACGTTGGATGTGTCGTGGAATCTTGGTTAG	83	55,3	3
loc255757_57	C/A	ACGTTGGATGTTTTTCTTCTTGATTTTTTTT	ACGTTGGATGTGCGTGGCACTCGTTGCTCA	77	47,1	3
loc268461_35	A/T	ACGTTGGATGTTGGCAGAAGTCAAGATAG	ACGTTGGATGCCTTTAGCCTCTTCAAGAAC	85	47,1	3
loc340647_27	G/A	ACGTTGGATGCCATGCCAAATAACAGGCTC	ACGTTGGATGACTTATGGGATTGAAAGGA	78	51,2	3

Material for DNA-extraction

Both cited studies (Konar et al. 2017, Merceron et al. 2017) used both leaves and buds for DNA extraction.

DNA-extraction protocols

Konar et al. (2017) isolated DNA following a CTAB protocol described in Hoban et al. (2009) from the parental trees, whereas they used the commercial kit DNeasy (Qiagen) for DNA extraction from seedlings. Merceron et al. (2017) used a commercial DNA extraction kit (HTS 96 kit, STRATEC Molecular GmbH).

Important results

The population genetic analysis of Merceron et al. (2017) included both native provenances and introduced populations from Europe, which were represented in a provenance-progeny test. The population genetic analysis revealed three ancestral clusters in the native range with a predominant latitudinal differentiation which was rather gradual. In Europe, most introduced populations show an affinity to clusters dominating the northern part of the native range. Moreover, results suggest recent admixture among different origins after species' introduction to Europe.

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Molecular markers used for genetic studies in black locust (*Robinia pseudoacacia* L.)

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1. General remarks

Black locust (*Robinia pseudoacacia* L.) has its origin in eastern North America. Other known names for this tree species are false acacia or robinia. Its native range consists of two distinct areas and several outlying populations. The larger of these two distribution areas coincides with the Appalachian Mountains; the smaller one is located in the states Arkansas, Missouri and Oklahoma (Little 1976; see Figure 1). The precise extent of the native range, however, is not accurately known as the black locust has been widely planted and has become naturalized throughout North America. At the present, it is spread in every state of the contiguous USA and also in British Columbia, Québec, Newfoundland and Labrador in Canada (map of the naturalized / invaded area in the N. America can be found at http://plants.usda.gov/core/profile?symbol=ROPS), and many areas with temperate and Mediterranean climate across the world (Schütt 1994).

Black locust grows on a wide variety of soils; from acidic to base-rich, from nutrient-poor to nutrient-rich and from moist to dry. However, it avoids compacted, not well-aerated soils (Huntley 1990). Due to its nitrogen-fixation ability, it has been widely planted outside its native range for soil amelioration and for restoration of disturbed sites (Huntley 1990, Schütt 1994). It is fast growing and its wood is hard and durable. However, wood quality is often reduced by its crooked stem form (Hanover et al. 1991, Schütt 1994). Other uses include energy fuel production (Rédei & Veperdi 2009) and bee honey production (Schütt 1994).

Mature trees can reach a height from 15 to 30 m and a diameter at breast height (dbh) of 60 cm at the age of 40. Forest-grown trees develop a taller and straighter stem (Schütt 1994). In natural populations, clonal structures are present due to the black locust‘s ability to reproduce also asexually by root suckering (Chang et al. 1998).

In total, the genus *Robinia* is represented by ca. 20 species (depending on the cited Flora; Schütt 1994), which are native to North America and northern Mexico. *Robinia pseudoacacia* is the only species to produce white flowers. In the native range, natural hybrids have been described between the black locust and the following three *Robinia* species:

1. Clammy locust, *R. viscosa* Vent. Their hybrid: *R.* x *ambigua* Poir;
2. New Mexico locust, *R. neomexicana* Gray. Their hybrid: *R.* x *hodltii* Beissn;
3. Kelsey locust, *R. kelseyi* Cowell ex Hutch. Their hybrid: *R.* x *slavinii* Rehder (Isely and Peabody 1984, Isely 1998).

In Europe, no reports on spontaneous hybridization are available. However, hybrids are available for cultivation (e.g. Royal Horticultural Society 2018). Many cultivars

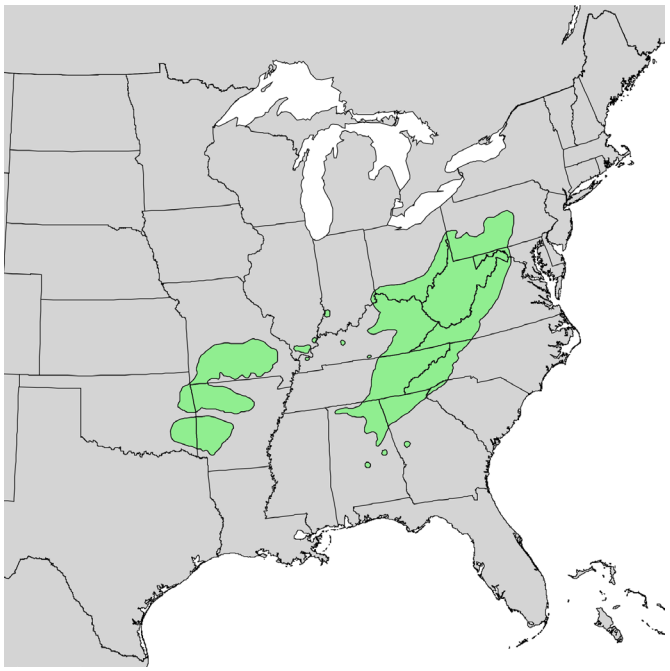

Figure 1. Native distribution range of black locust (source: USGS, USA)

do exist: they vary in crown and stem form, growth rate, growth habit (upright vs. prostrate), leaf shape, thorniness, flowering characteristics and phenology.

Until now, several different marker systems have been developed (e.g. nuclear microsatellites; Lian and Hogetsu 2002, Mishima et al. 2009) and used for population genetic studies in native and introduced populations. Molecular genetic studies have focused on population genetic variation at the local (Chang et al. 1998) and range-wide scale (Surles et al. 1989, Houser 2014), as well as on clonal structures (Chang et al. 1998). The species' phylogeography in its native range has not been studied in detail. Chloroplast DNA markers have been developed (Kimura et al. 2013) but not widely used in native populations. Use of these markers in range-wide studies within the natural distribution could enable tracing the origin of introduced populations. In addition, recently developed nuclear DNA markers (e.g. SNPs; Verdu et al. 2016) may increase the performance of population genetic studies.

Here, we provide a review of existing laboratory techniques which can be used to investigate the origin of black locust, as well as its genetic diversity and differentiation.

2. Isozymes

Earlier studies based on isozymes focused on:

- Investigation of genetic diversity within and between populations (Gu et al. 2010);
- study of clonal structures (Chang et al. 1998, Liesebach et al. 2004);
- spatial (range-wide) patterns of genetic variation (Surles et al. 1989).

Material for protein extraction

Proteins were extracted from leaves (Chang et al. 1998, Gu et al. 2010, Liesebach et al. 2004, Surles et al. 1989) or root tissue (Surles et al. 1989).

Protein extraction and separation protocols

The extraction procedure and separation protocols are described in Liesebach et al. (2004) and Surles et al. (1989).

Important results

- In an early research paper, Surles et al. (1989) described range-wide patterns of genetic variation. They investigated seedlings of 23 seed sources

Table 1: List of enzymes, scored loci and number of alleles for black locust

Enzyme system	E.C. Number	Scored loci	No. of alleles	References
Amylase	3.2.1.1	AMY-1,-2	5, 3	2,3
Adenylate kinase	2.7.4.3	AK-1	4	4
Aldolase	4.1.2.13	ALD-1	3	4
Alpha-galactosidase	3.2.1.22	α-GAL-1	3	4
Colorimetric esterase	3.1.1.	CE-1,-2	3, 3	4
Diaphorase	1.6.2.2	DIA-1,-2,-3	3, 3, 2	4
Fluorescent esterase	3.1.1.56	FEST-1,-2,-3	4, 4, 4	1,2,3,4
Fructose 1-6 phosphate	3.1.3.11	Fl-6-1,-2,-3	4 ,3, 2	4
Glutamate dehydrogenase	1.4.1.2	GDH-1	3	4
Glutamate oxaloacetate transaminase	2.6.1.1	GOT-1,-2	3, 4	4
Isocitrate dehydrogenase	1.1.1.42	IDH-1	4	1,3,4
Leucine aminopeptidase	3.4.11.1	LAP-1,-3	4, 5	1,3,4
Malate dehydrogenase	1.1.1.37	MDH-1,-2,-3	4, 3, 4	1,2,3,4
Peroxidase	1.11.1	PER-1,-2,-3,-4	4, 3, 4, 3	4
Phosphoglucose isomerase	5.3.1.9	PGI-1	2	4
Phosphoglucomutase	2.7.5.1	PGM-1,-2,-3	3, 4, 3	1,4
6-Phosphogluconate dehydrogenase	1.1.1.44	6-PGDH-1,-2,-3,-4	3, 3, 3, 3	2,3,4
Shikimate dehydrogenase	1.1.1.25	SKDH-1,-2	3, 3	1,2,3,4
Triose-phosphate isomerase	5.3.1.1	TPI-1,-2,-3,-4	2, 1, 3, 3	4

1-Chang et al. 1998, 2- Gu et al. 2010, 3- Liesebach et al. 2004, 4-Surles et al. 1989

(populations) within the native range and scored 40 loci across 18 allozyme systems. Most of the genetic diversity (88 %) resided within seed sources. The most differentiated sources were located on disjunct sites of marginal populations in Georgia and the Ozark Mountains of Arkansas and Missouri, and in Pennsylvania. No patterns of geographic differentiation were determined, which the authors attributed to widespread plantings from different (even European) seed sources.

- In their study, Chang et al. (1998) investigated fine-scale population structure and clonality in two populations of black locust in North Carolina, within the species' native range. They showed that native populations maintain very high levels of genetic diversity, however no noticeable geographic patterns could be recognized. Out of 200 and 420 plants analysed within each population, 13 and 15 unique genotypes were distinguished, respectively. These genotypes represented one clone each, represented by several ramets. The largest clones (genets) in both study sites covered more than 100m x 100m.
- Liesebach et al. (2004) compared genetic diversity between progenies of two native North American populations of black locust and 16 introduced European populations collected in Germany, Hungary and Slovakia. All seedlings were assayed for 11 enzyme systems. In Europe, Hungarian populations had high within population genetic variation (diversity) and low among population genetic variation. Conversely, in Germany one progeny had low within population genetic variation and there was high differentiation between populations. Slovak populations were similar to Hungarian with regard to the within population diversity. In the two populations from the USA, genetic diversity was similar to German stands. The authors attributed the contrasting patterns of diversity and differentiation found in Hungary and Germany to differences in management of black locust in Europe.
- Finally, a more recent study (Gu et al. 2010) described genetic diversity of 19 black locust populations distributed in China and evaluated them using seven allozyme systems. Most of the genetic variation resided within populations. Moreover, a significant pattern of isolation by distance was found.

3. Organelle DNA markers (chloroplast (cp) DNA, mitochondrial (mt)DNA)

Loci and primers used

Liesebach and Schneck (2012) applied PCR-RFLP techniques to investigate polymorphisms in a set of populations analyzed previously with isozymes. Mostly using universal primers, they amplified five chloroplast DNA (cpDNA) loci by means of PCR and digested the amplicons with eight restriction enzymes (Table 2).

In their technical report, Kimura et al. (2013) developed a set of five polymorphic chloroplast microsatellites (cpSSRs) for genetic analyses in black locust. Locus description, primer sequences and annealing temperatures are presented in Table 3.

Material for DNA-extraction

In both cited studies, leaves were used for DNA extraction (Liesebach and Schneck 2012, Kimura et al. 2013).

Table 2: PCR-RFLP markers (cpDNA) for black locust (Liesebach and Schneck 2012)

Locus	Primer sequences F= Forward, R= Reverse	Restriction enzymes	No. of polymorphic bands
DT (trnD/trnT)	F:ACCAATTGAACTACAATCCC R:CTACCACTGAGTTAAAAGGG	BamHI, DraI, HinfI, MboI, MspI, RsaI, SspI, TaqI	2
HK (trnH/trnK)	F:ACGGGAATTGAACCCGCGCA R:CCGACTAGTTCCGGGTTCGA		5
K1K2 (trnK/trnK)	F:GGGTTGCCCGGGACTCGAAC R:CAACGGTAGAGTACTCGGCTTTTA		3
BB (psbB/petB)	F:CAGAAGCTTGGTCTAAAATTCC R:GRTCCCAAGGGAARGAATAACCAGT		3
ED (trnE/psbD)	F:GTCCCGACGTAACCAGTCAT R:TGAACCACTAGACGATGGGG		2

Table 3: List of markers and primer sequences (T_a-annealing temperature, N_A-number of alleles scored) (Kimura et al. 2013)

Locus	Motif	Primer sequences		Size (bp)	T_a (°C)	N_A
		Forward	Reverse			
Ropscp03	A_{10}	GGATCTTTCTGAATTCCGTAG	CAGCAAATCAATCATTTCCTG	155-164	54	6
Ropscp04	T_5AT_{10}	TACGATCTTGTAGTAATTTCC	ACTACTCTCCTTTCATCAAAG	99-113	54	3
Ropscp06	$T_{10}AT_5$	CCATGGTATTTGATTTACCAA	TCAAGGTCGAGAGTGAATTTC	137-140	54	4
Ropscp07	T_{10}	AGTACCGGACCAATTATTTGG	TTAACCGATCAACTTGCTTTG	171-176	54	6
Ropscp08	A_{11}	GAACACGAGTCGAGGCTATC	GATACCATGGATTCTGGTATC	186-201	54	8

DNA-extraction protocols

For DNA isolation, the cetyltrimethyl ammonium bromide, the (CTAB) protocol was used in both cited studies (Liesebach and Schneck 2012 according to Dumolin et al. 1995; Kimura et al. 2013 according to Lian et al. 2003).

Important results

Liesebach and Schneck (2012) used PCR-RFLP techniques to study polymorphisms in a set of European populations. They additionally analysed four American populations from the native range with the identical markers. They found eleven cpDNA haplotypes belonging to two clearly separated groups of related haplotypes. The four US populations from the native range (representing the four US States: Illinois, Tennessee, Virginia and Georgia) displayed a low average number of haplotypes per population and were significantly differentiated from each other. In Europe, no spatial distribution pattern was found. Genetic variation within populations was higher than in the native range, whereas the opposite was observed for the genetic variation among populations. This result was attributed to repeated seed introductions from arbitrarily selected sources.

4. Nuclear DNA markers (AFLPs, nSSRs, ISSRs, SNPs)

a) AFLPs (Amplified Fragment Length Polymorphism)

Loci and primers used

In Huo et al. (2009), 10 populations of black locust collected in China were analyzed by AFLP using 10 primer combinations. Primer combination, total number of bands, number of polymorphic markers, and polymorphic rate are presented in Table 4.

Material for DNA-extraction

Leaves were used for DNA extraction (Huo et al. 2009).

DNA-extraction protocols

Huo et al. (2009) used a CTAB-protocol for DNA extraction. Authors provide all details for modification of the Vos et al. (1995) protocol.

Important results

Huo et al. (2009) used 10 AFLP primer pairs to detect genetic diversity of 10 black locust populations originated

Table 4: Primer combinations, number of bands and degree of polymorphism

Primer combination	Total bands	Polymorphic bands	Polymorphism %
E-GGA / M-TAT	91	46	50.0
E-GGA / M-CTC	87	37	42.5
E-CGG / M-GGA	77	38	49.4
E-CGG / M-CAG	83	31	37.3
E-AGC / M-TAT	88	39	44.3
E-AGC / M-CAC	38	17	44.7
E-AGC / M-CTT	78	37	47.4
E-AAG / M-CAA	97	44	45.4
E-ACA / M-CAA	74	44	59.5
E-AAC / M-CAC	39	19	48.7

from China. Genetic diversity within populations (66.10%) was higher than among populations (33.90%). The UPGMA cluster analysis resulted in three major groups, where the most individuals of the same population clustered together. Furthermore, no significant correlation between the genetic diversity parameters (*D*, I_N, *P*, *Ne*, *H*, and *I*) and geographic (longitude, latitude) and climatic factors (annual mean temperature, and annual mean precipitation) was found.

b) nSSRs (putatively neutral microsatellites)

Loci and primers used

Lian and Hogetsu (2002) developed seven and Mishima et al. (2009) 11 nSSRs in black locust which have been used for population genetic studies. Primer sequences, repeat motifs and allele length range are presented in Table 5.

Table 5: Primer sequences, allele length in base pairs (bp), annealing temperatures (T_a) and references for nSSRs available for genetic analyses in black locust.

Locus	Repeat motif	Primer sequences F= Forward, R= Reverse	Size (bp)	T_a	References
Rops02	$(AC)_{13}(AT)_4$	F:CAGAACTGTGGAGAATAATTCTGAACCG R:CGCCATCTGTTAGTTTGTTGC	107-138	60	1, 2
Rops04	$(AC)_{10}$	F:GTCTAATTTCACTTTTCTCACGAG R:GGACACCACCRAAATTCTACC	105-110	56	2
Rops05	$(AC)_2GC(AC)_7$	F:TGGTGATTAAGTCGCAAGGTG R:GTTGTGACTTGTACGTAAGTC	120-138	56	2
Rops06	$(GT)_3ACA(GT)_{11}$	F:CTAAGGAGGTGCTGACCCTC R:TTAATCTGTGATGGGACACTG	117-144	56	2
Rops08	$(CA)_8TA(CA)_3$	F:TTCTGAGGAAGGGTTCCGTGG R:GTTAAAGCAACAGGCACATGG	191-205	56	1, 2
Rops09	$(TA)_6A_4(TA)_2(TG)$	F:CTCCAGGTCACTCGATTGAGG R:TTTCTCATTTGATACGACCCC	89-150	56	2
Rops10	$T_{12}AAT_4$	F:AACTTTTTCCGTATAGGGGTC R:GAGTTTTACACTTGGTCAAACC	182-187	56	2
RP035	$(TC)_{15}$	F:GGAGTGGAATGCATGCTCTCATG R:TCCAAATGGAAACTCCCTTGAAACAGC	89-112	63-53*	1, 3
RP102	$(GA)_{12}$	F:CCAAATCTCAAAATGTGCTAAGTAGC R:ACTTGGGCTATGGTATTGCA	205-211	63-53*	3
RP106	$(GT)_9$	F:AAACTGAATTATATCCCTTTACGGC R:GCATATATCCACCAGATACCCG	143-154	63-53*	1, 3
RP109	$(AG)_{17}$	F:GAGGAATCACAAAACCGTTTGG R:TGGGATTTGAGAGAGTGGTGGTG	136-160	63-53*	1, 3
RP150	$(TC)_3TT(TC)_{12}$	F:TCGTTGGATCAACATGCATGG R:ACAGAACCCTAACCCTAGCA	199-217	63-53*	1, 3
RP206	$(GT)_9$	F:GCCAAATCCCATTAGATCACAGTTGA R:AGAAGTTAGACTTACGTGCTGC	222-246	63-53*	1, 3
RP200	$(AG)_{23}$	F:GGTTTCTTTGTTCACCTGCTCTGG R:ACCTACGTGTCCACGGCTCT	160-198	63-53*	1, 3
RP032	$(TG)_{13}$	F:GCATATTGCATATGCGCTTGTG R:TCCCTGAAGCTCATAACTGTCATGTG	109-135	63-53*	3
RP211	$(TC)_7A(AC)_8$	F:TGTAATCCATGTAGTTGACCCCAC R:TGATTACTCTGCATGCATGTG	196-201	63-53*	3
RP165	$(TG)_8$	F:TTAGATGTTGCAAGTGCTGAGG R:ACAATGCCTCAATGCAGC	146-159	63-53*	3
RP01B	$(CT)_{16}$	F:ACCAATTAGGTAACGTCAGC R:TGTTCACTGACAAAGCTG	172-192	63-53*	3

**touch down PCR*

1-Houser (2014), 2-Lian and Hogetsu (2002), 3-Mishima et al. (2009)

Material for DNA-extraction

Leaves have been used for DNA extraction (Lian and Hogetsu 2002, Houser 2014).

DNA-isolation protocols

Lian and Hogetsu (2002) used a CTAB-protocol for DNA extraction, whereas Mishima et al. (2009) and Houser (2014) used a commercial extraction kit (DNEasy, Qiagen). All papers provide details on: (i) chemical concentrations, (ii) PCR programs and (iii) allele scoring methods.

Important results

Houser (2014) compared the genetic diversity between tree regions: throughout the native Appalachian region and in two invaded/naturalized regions in the Northeast and Midwest regions of the US using the microsatellites developed by Lian and Hogetsu (2002) and Mishima et al. (2009). Several genets with multiple ramets were identified in the study populations. In a total of 369 sampled trees, only 142 unique genotypes were found. Genetic differentiation among regions was low (1-3%) and no obvious genetic structure was obtained by using clustering methods. The highest genetic diversity was found in the native Appalachian region.

c) EST-SSRs (expressed sequence tag derived microsatellites)

Loci and primers used

In a recent study, Guo et al. (2017) developed a new set of 45 EST-derived microsatellite loci for population genetic analyses in black locust. Primer sequences, repeat motifs and allele length range are presented in Table 6.

Material for DNA-extraction

Leaves were used for DNA extraction (Guo et al. 2017).

DNA-extraction protocols

A commercial kit (Tiangen, Beijing, China) was used in the study of Guo et al. (2017).
Details on: (i) chemical concentrations, (ii) PCR programs and (iii) allele scoring methods are provided in Guo et al. (2017).

Important results

Due to their high polymorphism, EST-SSRs developed by Guo et al. (2017) may provide a valuable tool for investigating genetic diversity and population structure of black locust, constructing a DNA fingerprint database and performing quantitative trait locus mapping.

d) ISSRs (inter-simple sequence repeats)

Guo et al. (2006) applied 32 inter-simple sequence repeat (ISSR) markers to 41 micropropagated plants of *Robinia ambiqua* var. *idahoensis* and their donor plant, from which they have been propagated by tissue culture system. *Robinia ambiqua* is known for its tolerance to drought and pests and doesn't possess roots characteristics as aggressive as *R. pseudoacacia*, one of its parental species. The authors assessed the occurrence and the extent of genomic changes in the *in vitro* micropropagated plants in order to study genomic stability / instability of the developed micropropagation protocol.

Loci and primers used

Loci and primers used are listed in Table 7.

Material for DNA-extraction

For DNA extraction, young leaves were used.

DNA-isolation protocols

Total genomic DNA was extracted using a modified CTAB method of Kidwell and Osborn (1992) and purified by phenol extraction.

Important results

Low genomic variation within the micropropagated plants (only 10.6 % of the bands were polymorphic) was observed. The occurrence of genomic changes and their possible effect on morphological and physiological traits have to be, however, taken into consideration when the developed *in vitro* protocol will be further commercially used for micropropagation of the hybrid *Robinia ambiqua*.

e) SNPs (single-nucleotide polymorphisms)

Loci and primers used

Restriction site associated DNA sequencing (RAD-sequencing) was used by Verdu et al. (2016) to develop 377 SNP-markers. These were organized in 12 multiplexes based on Sequenom ® technology. For more details about the Sequenom method the reader is referred to Bradić et al. (2011). Thus far, no case studies using these markers have been published.

Table 6: Primer sequences, allele length in base pairs (bp), annealing temperatures (T_a) and number of alleles for EST-SSRs available for genetic analyses in black locust (Guo et al. 2017)

Locus	Repeat motif	Primer sequences		Size (bp)	T_a	N_A
		Forward	Reverse			
Rp-01	$(TGTGAA)_4$	TGCAGAAAGAGAAAGCAGAGG	CCGAACCCTTTCTGGTTAGTC	140	58	6
Rp-02	$(GAAT)_4$	GCTGCGTTTAATTTTGTCAGG	TCAATCCATCAAAGAGGAAACA	170	58	6
Rp-03	$(CTC)_7$	GTGAGAAGTGGTTAGGGTTTT	TCAAGATCACCAACGTACAA	186	55	4
Rp-04	$(AATGGT)_4$	CTCGTGATGATGGTGTTGATG	AATGGTCCAAACAACACGAAG	146	58	10
Rp-05	$(TCTGGC)_3$	CCTTGCACATTTATCCCAGAA	CGACCTCGATCTTTTCTTGTG	158	57	6
Rp-06	$(TGAGTT)_4$	TGGACAAAACATCATCGTGTG	CTCTCTTCTTTCTGCCCCTCA	147	59	5
Rp-07	$(CT)_{10}$	TTTTTCTCCCAACGAAACAAA	TGATGTGTTGTACGGAGGTGA	144	56	5
Rp-08	$(AAAAT)_4$	TCAGGTGCATAAGCTCATTACTTC	GGTTGTCAGATGAAATGCACA	152	56	3
Rp-09	$(CTTT)_5$	CGTTTAGAAGCTGAGGCAGAA	TGAGATATCTTAGTGCAGGAGCA	153	58	6
Rp-10	$(CCTTT)_4$	GGCATGTGGCTATGAAGATGT	TCAGTGGGACTTGGTTTCTTG	154	57	2
Rp-11	$(AG)_{10}$	GAAGCTATCACCGCAAATGAA	GTCGAAGTGCGTCCTAGATCA	150	57	8
Rp-12	$(AGCAGA)_4$	AAGAGTCATCACGGAGACCAA	GGAGTCCAATTAAGTGCGAGA	150	56	4
Rp-13	$(CTCTTC)_4$	CATTTCCGATTTCCAATTCCT	GCCGAGGACTCGGTAGAAGT	151	56	4
Rp-14	$(TGCAAC)_4$	TTAGCACGAACCTGGTTATGG	CACTTCATTTGGTTCCTTGAGA	151	56	3
Rp-15	$(TCAC)_5$	TTAACTAATGCGGCGAGAAGA	GAGAGGAAGTGTGCGAAACAA	119	56	9
Rp-16	$(TTCAGT)_4$	TATGAGACAGTGTTGGTTGGT	CGTGCCAGAAGAGTATAACAG	175	56	2
Rp-17	$(AACCA)_4$	GTAAGTCTGCAAAGAAGACCA	GCTTTTCACCTATCAACTCAA	150	56	3
Rp-18	$(GGTCAG)_4$	GGATGAACTTTGGCAATCCTT	AATTTGTTGGGAATGCTGTTG	158	55	6
Rp-19	$(AGGCTG)_4$	CAGGAGTGGCAGCATTAGTGT	CACAACAAGCACATTTTGCAC	123	56	4
Rp-20	$(GCAGCT)_3$	TTTCTTGGCTTGCTTTTGCTA	TCTTGGATACGCAAGGTTGTC	145	56	3
Rp-21	$(CCA)_7$	TATGATCACGTCCCCTAATGC	AAGTGGAAAGAAATGGGATGG	146	57	10
Rp-22	$(AGGGTT)_4$	GGTAAGGTGAAGGAGGTGGAG	AGCTTGGTCTCCTAGGTCGTC	150	56	4
Rp-23	$(AGAAGT)_4$	GGAGGAGCAACCATCTGTGTA	CTCCCTCTTCATCCTCACCTC	146	56	2
Rp-24	$(AATA)_4$	TGCACATATTTGCCTGGTTTA	AAAATGAGCATGACACAACCA	160	56	2
Rp-25	$(AAAG)_5$	CGGCAACAAGTTGAGAAGAAC	GGCTCACAAACCAACCTATGA	139	56	2
Rp-26	$(ATGATA)_4$	GCTGCAAGCAAAGGATCTTAC	CCTCATCATCCTCGTCATCAT	139	56	3
Rp-27	$(TGAGTT)_4$	TGGACAAAACATCATCGTGTG	CTCTCTTCTTTCTGCCCCTCA	147	57	2
Rp-28	$(CAG)_7$	CTTGGTCTAGAAAGTCCTGCT	GGTCATCAAGGTTAGTTGGAT	151	56	5
Rp-29	$(GATC)_4$	CCTGATGATCAAAACGACGAC	GGAGGTGACCCCTCTTATCCT	148	56	4
Rp-30	$(GCT)_8$	TTGAACCAAAACTGGAAGAGC	GCACCGTACAGTTACCCTATCC	151	56	5
Rp-31	$(ATT)_7$	GACCCCATTTTTCTCAAGGAC	TTGGATAAGTCGGTGAAGGTG	140	56	3

Locus	Repeat motif	Primer sequences		Size (bp)	T_a	N_A
		Forward	Reverse			
Rp-32	$(GTG)_7$	CCACGTGGTTCTTCAAACATT	CAACAACAACCCACAAACACA	163	57	4
Rp-33	$(ATC)_7$	CAAACAGTCTCATGGAAATGGA	GGGTTGGTATTGTTGGGAAAT	141	56	2
Rp-34	$(TGGTGA)_4$	AGGATATTAGCCAAGTCCATC	AGTAACCATCACCACAATCAC	164	57	3
Rp-35	$(CACAC)_4$	TCAGACGTGGTAGAGCAGTGTT	ATTTGTTTTTGGGGGAGATTG	152	58	3
Rp-36	$(GAATC)_5$	CGTTTCAGCCATTGATTTTGT	GATCATCACCGTCCACCTTC	141	57	4
Rp-37	$(GAACGA)_4$	TGTCGTCATTTTATTTTACCC	CTCACCCTTTTTATTTCCATT	152	56	6
Rp-38	$(TC)_{10}$	TCCATTCCCTGGTTTCTTCTT	AGCACAATTTCCTCAGTGCAG	150	56	10
Rp-39	$(AAGAGG)_3$	TTAAAGAATGTTCCGTTCAGA	GAGAAGATAGCCTCCTAGCTG	152	56	4
Rp-40	$(TAA)_8$	TCATTGGACATCCCTCCATAA	GGCTCGACATGGTTGATTTT	139	56	2
Rp-41	$(CCA)_7$	AACTCACCCAATTGCACACTC	GAGCAAGAGCTAAAGCAGCAA	143	56	4
Rp-42	$(AATC)_4$	CTTCGCAATCCTCACTCTTTG	CTTACCCAGAAGCCAACAATG	169	55	2
Rp-43	$(CAAAAT)_4$	CAAAGCAGAGAGAATGTATGG	ATCCCTTGCTCCTTGTAATAG	155	57	4
Rp-44	$(ATCA)_5$	TATCTGGGAGAATCGAGAGCA	CCACCATGGTTGTCCTTCTAA	145	57	2
Rp-45	$(TTC)_7$	GGGTTGAGGAAGAGAGGAGAA	AAAAATCGAATCGTGTTGGTG	156	57	3

Table 7: Primer sequences, allele length in base pairs (bp), annealing temperatures (T_a) and number of alleles for ISSRs available for genetic analyses in black locust (Guo et al. 2017)

Locus	Sequence (5′-3′)	Size (bp)	T_a (°C)	N_A
W1	$(AG)_8G$	200–1000	58	8
W3	$(AC)_8C$	100–1100	58	7
W6	$(GA)_8YC$	100–1300	58	8
W7	$GGG(TGGGG)_2TG$	300–2500	55	7
W8	$(CT)_8G$	300–1500	55	6
W9	$(CA)_8G$	200–600	55	7
W11	$(TC)_8A$	400–1500	55	8
W12	$(TC)_8C$	300–1800	55	9
W14	$(AC)_8T$	100–1200	55	9
W18	$(CA)_8RC$	100–900	55	7
W19	$(CA)_8RG$	100–1100	55	9
W20	$(GT)_8YA$	400–1300	55	5
W22	$(TC)_8RT$	800–1300	52	3
W23	$(AC)_8YT$	400–1500	58	5
W24	$(AC)_8YA$	100–1500	58	8
W25	$(AC)_8YG$	300–1600	58	8
W26	$(TG)_8RC$	300–900	58	6
W27	$(TG)_8RA$	700–900	55	2
W28	$(ATG)_6$	400–1500	55	6
W29	$(GACA)_4$	300–1200	55	4
W30	$(GGAGA)_3$	400–2000	58	11
4	$BDB(CA)_6$	400–1200	58	9
15	$CCC(GT)_6$	300–900	58	8
20	$CCAG(TGG)_3TG$	300–1800	52	9
22	$SSWN(GACA)_3$	200–1200	52	9
31	$(AG)_8T$	100–900	55	6
32	$(AG)_8C$	200–1400	58	6
33	$(GA)_8T$	100–1000	55	9
34	$(GA)_8C$	100–800	52	7
36	$(AG)_8YT$	100–1400	52	8
37	$(AG)_8YC$	200–1300	52	6
39	$(CTTCA)_3$	200–1400	52	6

Table 8: Primers for amplification of SNP loci used in Verdu et al. (2016); bp = base pairs, T_m = extend primer melting temperature (calculated), M = Multiplex

Locus	SNP polymorphism	Primer sequences		Amplicon length (bp)	T_m	M
		Secondary amplification primer (including secondary tag)	Primary amplification primer (including primary tag)			
2605_2	T/C	ACGTTGGATGACCGATACTAATTGTGGCCC	ACGTTGGATGGTTCAAATGATAGAGTTGG	112	48,7	1
12234_2	T/C	ACGTTGGATGGAACGAGGTTCACGTGTTTG	ACGTTGGATGAACTGCTATATTTATTGTAG	89	45,8	1
11547_2	G/A	ACGTTGGATGTGAAGATCATGGAGTGTAGC	ACGTTGGATGCTGGTCCCTTTCAACAAAAC	82	48,3	1
4364	T/C	ACGTTGGATGGGTTATCGTTACACTGACCA	ACGTTGGATGCTCCATGTGAATCTGCTTTC	104	45,1	1
3454_2	G/T	ACGTTGGATGTTGAGTGAGCCTTGCTTTCC	ACGTTGGATGTTGCAAGGACCAAGCCCATC	100	46,8	1
12601_1	A/G	ACGTTGGATGGCTACTTTGGTTTGAGCAAC	ACGTTGGATGTGTGGGTGACAGAGGTAATG	101	45	1
1581_1	A/G	ACGTTGGATGTCAGAGCTCAGTACTCCAAG	ACGTTGGATGGGTCTGAAATATCGAAATTG	89	45,5	1
5430_2	C/A	ACGTTGGATGTTTCACTTCCTCTCAAAGGG	ACGTTGGATGCACACTTTGCTATCGGCAAT	97	46	1
6127_5	T/C	ACGTTGGATGTACAAGCTTCCTTGAGCTCC	ACGTTGGATGAAACTTTGGAAGTGAACCC	111	46,1	1
5536_1	G/A	ACGTTGGATGTGACAATTGATGCTTGACT	ACGTTGGATGAATATTGCTTTGTGTGGGAC	91	48,6	1
2192	G/A	ACGTTGGATGTCAAAATGTGAAGACTCTGC	ACGTTGGATGTGATGACGAGAACATTAGGG	93	45,9	1
1269	C/T	ACGTTGGATGATTATTTCCTGACGAATTG	ACGTTGGATGTGCCTGCTGCTTCAGTTGTC	102	45,5	1
11211_1	A/T	ACGTTGGATGGTAACCTGACTGCTAACAAG	ACGTTGGATGGAATGCCAATCAGTGATCTC	84	46,3	1
5346	G/A	ACGTTGGATGCCAGATAACGTTCTCCATCC	ACGTTGGATGGTATTTATTAGACCATGTG	81	46,4	1
2658_2	G/A	ACGTTGGATGAAGATTTGGTAATTCGTGG	ACGTTGGATGGGTAGTATTTACATTACCTG	98	46,9	1
1582_3	A/T	ACGTTGGATGATTCCAGTTCTTCATGTCA	ACGTTGGATGCCTCTGCCTGTAAAATGAAC	97	45,7	1
301_2	G/A	ACGTTGGATGGTTGGTAATTCAGGTCGAGC	ACGTTGGATGGCACTGCATGTAACAAAATC	99	46,6	1
114	G/C	ACGTTGGATGGAAGAAACCGTTTGAAACAAC	ACGTTGGATGCTAACATGCTTCTTCTTGACC	103	45	1
2285_2	A/T	ACGTTGGATGTTACATTGAGTATGTGCAG	ACGTTGGATGGGTTGTTTCCAATCACGTGT	100	47,4	1
2355_1	C/A	ACGTTGGATGCCAACAGATCTCCATGTCTC	ACGTTGGATGGTGGAATCGAGAATGGAAAC	98	47,9	1
12533_1	C/T	ACGTTGGATGCCCACTCTAGTTCAAGGTTC	ACGTTGGATGCAATTGAATGTTGTTTGGG	87	47,9	1
13463_2	T/C	ACGTTGGATGGGCTCCATTTGTACCTAGAG	ACGTTGGATGGACTAAGCTCCTGGTGTTTC	98	45,5	1
4974_2	T/C	ACGTTGGATGGCTAACCATCATCACATCTC	ACGTTGGATGCCAATGACTCTTTTCTTGCC	101	46,2	1
4760	A/G	ACGTTGGATGAATTTGAGACTTATGCTTC	ACGTTGGATGACACCATCCGCTTTTGACAC	81	45,4	1
576_2	C/A	ACGTTGGATGTCTAGCAGCAACATTGATCG	ACGTTGGATGGCTATTGTTAGTTGCACTCC	87	45,7	1
2082	A/G	ACGTTGGATGTTGTTCTCTCAATAATTTTG	ACGTTGGATGACATTCAAGTGCAACAGTGG	94	45,4	1
38_2	G/A	ACGTTGGATGAGGCTCAAAATGACATCCTT	ACGTTGGATGCATTAGGTTATCTACTCTGG	99	45,7	1
6912_3	C/A	ACGTTGGATGCCAACTTCTCATTGTAAAA	ACGTTGGATGTGCTGGGGTACCAATGGTCA	82	45,4	1
5256_2	C/A	ACGTTGGATGTGTTTATTGATATGACTTTG	ACGTTGGATGCCAAATATGGCAAAGGAAAGG	93	45,4	1
4421_1	C/T	ACGTTGGATGTCGTGTGGATACCGATAGAG	ACGTTGGATGGCCTTTGATGATCTTGTGAAG	100	47,2	1
4756_2	A/T	ACGTTGGATGGAAAGTGTAAATCTAATCAG	ACGTTGGATGGATCGATTGTTAGTCTTCCC	86	45,2	1

Locus	SNP polymorphism	Primer sequences		Amplicon length (bp)	T_m	M
		Secondary amplification primer (including secondary tag)	Primary amplification primer (including primary tag)			
2365_1	C/T	ACGTTGGATGCATCTATATATACAAATTCAC	ACGTTGGATGATAGGGATGCTAATTAGTA	81	46,4	1
1568_3	G/A	ACGTTGGATGGTGTGTTGATCAAAATTCCTC	ACGTTGGATGCCCTCTCATTATCCATTTCC	97	46,3	1
2398_2	C/A	ACGTTGGATGAAGTTCCATATATTCCAGGG	ACGTTGGATGGCGAATGTCACTGCAGTATC	94	46,3	1
6216_2	T/A	ACGTTGGATGGGCACATGATATGATTCTAC	ACGTTGGATGGATTTGTGAGAAAGGGCCAG	85	45,1	1
8087_3	A/G	ACGTTGGATGGTGGACTGGTTTGACACCAT	ACGTTGGATGACAATCAATTTATCCCCACC	91	55,3	2
1617_2	T/C	ACGTTGGATGTGAGAATCCCTTAGTTGTCC	ACGTTGGATGTTTGGTTAGGTTACCAAAG	80	45,3	2
6560_1	A/G	ACGTTGGATGGTATTCTCCACCACCATCTG	ACGTTGGATGGAAATTTGTCTTGATGGTGG	96	45,7	2
5610_2	G/A	ACGTTGGATGGAAGATCCGGACTGCCAT	ACGTTGGATGCGATTTCAATCCAGCTAGAC	91	48,5	2
8804_2	T/C	ACGTTGGATGGGCACCCAATAGTTCGCAAG	ACGTTGGATGTCAAACTCCGTAGGTGTGT	81	46,6	2
1877_2	A/G	ACGTTGGATGCCATGGAGTCAAATGCACAC	ACGTTGGATGTAAACAAATCGTGAGACAT	99	47,5	2
6912_2	A/G	ACGTTGGATGTGCTGGGGTACCAATGGTCA	ACGTTGGATGCCAACTTCTCATTGTAAAA	82	53,6	2
2498_4	T/A	ACGTTGGATGGGACAAACCTACATGTTACAC	ACGTTGGATGAGTCTATGGACCATTGCACC	90	46,6	2
12146	T/C	ACGTTGGATGGCTCTCAAGTTTTGACATAG	ACGTTGGATGTTACATGCTACAAAAGTCG	99	46,1	2
10425	C/A	ACGTTGGATGATACCTAGACTCACGATGCC	ACGTTGGATGCCATGTTACTCAAATTGTGC	84	45,5	2
2947_2	G/A	ACGTTGGATGCAATGGTTATAATTCCTCAC	ACGTTGGATGAAAACATGCAGGATTCTTC	87	45,1	2
2843_2	T/C	ACGTTGGATGGGAATGATGGTGTTTCAATG	ACGTTGGATGGGAAAGGATTCAGTTGCAAG	83	45,9	2
5070_2	C/T	ACGTTGGATGGAGAAGAAAGGAACAAAGAG	ACGTTGGATGAACCACCTATGGGATGATGC	84	46,3	2
4874_1	T/C	ACGTTGGATGGCCATGGATTAGACTAAAAC	ACGTTGGATGAGATGTTGCATTCGCATTTG	102	45,7	2
10644_1	C/G	ACGTTGGATGCGACATTCACAGTTTCAAAGG	ACGTTGGATGGGAATAAGATGATATGGTGTC	97	51	2
596_1	C/T	ACGTTGGATGGAGAACACAAGATTGTGGAC	ACGTTGGATGGACAGTTTATTGGAAGTGTTG	98	45,2	2
1754_3	G/T	ACGTTGGATGGTGAAGCATTGATAGATTGGG	ACGTTGGATGACCTTATCTCTTCTGTAGGC	107	45,8	2
11211_2	G/A	ACGTTGGATGGAATGCCAATCAGTGATCTC	ACGTTGGATGGTAACCTGACTGCTAACAAG	84	46,8	2
8110_1	A/G	ACGTTGGATGCTTTCTTTATCATCAATCG	ACGTTGGATGGTCACAACAAAGGGGGTG	95	48,6	2
6786	G/A	ACGTTGGATGGAACTAAGAAATCCAGTCCAC	ACGTTGGATGTCATGAGAACCTAGTGCGTG	89	46,3	2
12623_3	C/A	ACGTTGGATGAACATGGCTTATACTTTAC	ACGTTGGATGGTCATAGGTGGCCTATTTAG	97	45,8	2
38_1	C/T	ACGTTGGATGCATTAGGTTATCTACTCTGG	ACGTTGGATGGGCTCAAAATGACATCCTTTC	98	46,2	2
6480_2	G/A	ACGTTGGATGCTTGAGATGACTTGAGAAAC	ACGTTGGATGAGGGCATATGTTTGTCTGGG	103	47	2
2389_3	G/A	ACGTTGGATGTGAATGTTGATTGGGTTTG	ACGTTGGATGCCCACTCTAGTTCAAGGTTC	83	45,6	2
6127_1	A/G	ACGTTGGATGAAACTTTGGAAGTGAACC	ACGTTGGATGTACAAGCTTCCTTGAGCTCC	111	45,8	2
1072_2	C/T	ACGTTGGATGCCTATGTTTATTGCAAGTGG	ACGTTGGATGCATTATCCACCAGGATCCAT	95	45,6	2
3777	A/G	ACGTTGGATGAGCCTTCAAAAATGAAGACC	ACGTTGGATGATTGTAGTACCTCGGGAATC	82	45,2	2
12492	G/C	ACGTTGGATGATTGACAGGAGGTTCAGGTG	ACGTTGGATGATCTGCAGCACCGCATATTG	100	50,6	2

Locus	SNP polymorphism	Primer sequences		Amplicon length (bp)	T_m	M
		Secondary amplification primer (including secondary tag)	Primary amplification primer (including primary tag)			
4259_1	A/G	ACGTTGGATGATAAAGGAGGCATTGGAAGC	ACGTTGGATGTGCCGTGGTGGGTTTTAT	82	51,5	2
3393	G/A	ACGTTGGATGGGGTGAATAGTACAATTTCCG	ACGTTGGATGGAGAGAATGATCCGCCAATG	96	47,7	2
823_6	G/A	ACGTTGGATGTAATTGTTCTAATGGTGAC	ACGTTGGATGTGTTTTGAACCTATGCTACC	103	47,8	2
4260_1	G/T	ACGTTGGATGCTTCAATATTACGTTTTTAGG	ACGTTGGATGGGCAAATTGGCAATTTGAAAC	81	45,1	2
4421_2	G/C	ACGTTGGATGGTAGCCTTTGATGATCTT	ACGTTGGATGTCGTGTGGATACCGATAGAG	103	51,3	2
4912	G/A	ACGTTGGATGCACAGTCTGATAAATCAAATC	ACGTTGGATGTGCAACCTCATCAATGGCTC	111	45,6	2
425_1	A/G	ACGTTGGATGAAGATAATATGTATTCTCAG	ACGTTGGATGACAACCATTGTCTTGTTTA	94	46,2	2
1348_3	C/A	ACGTTGGATGGGAATGGACCTCTTTGACAG	ACGTTGGATGTTGTTCCTTCTGTATGTGTG	94	46,7	2
3733_2	C/A	ACGTTGGATGGAGAGACACGTGTCCATTAC	ACGTTGGATGACTCCTTCTCGCAGGAACCA	90	47,4	3
11753_1	G/T	ACGTTGGATGGCATATGGTCACTGCAATGG	ACGTTGGATGACACATGATAGGTCTGAATG	82	47,4	3
8287	C/T	ACGTTGGATGGTTTCCAAGTTAGCCTTTCC	ACGTTGGATGGGCAAATCAGATGGCTATGG	98	49,1	3
2558_2	A/G	ACGTTGGATGATAATAATTGGCTACCGCCT	ACGTTGGATGACGTTCTCGAAATTTGGTTC	100	45,3	3
4590_3	T/C	ACGTTGGATGAAGAGAAGAACACGTCCAG	ACGTTGGATGGCCTTTTTGCATTTCCTCCC	103	47,4	3
4908_1	C/A	ACGTTGGATGGATTTTTATACTGAAAAGGG	ACGTTGGATGCCATTTTGTTGTGGAGTTTC	85	46,7	3
4178_2	C/T	ACGTTGGATGTGACACATTTATTTGGAGG	ACGTTGGATGGAGGATGAAAATAACATGC	92	45,5	3
1754_1	C/T	ACGTTGGATGTACCTTATCTCTTCTGTAGG	ACGTTGGATGGTGAAGCATTGATAGATTGGG	108	46	3
3503_2	G/A	ACGTTGGATGTCATTTTACCTTACCCATG	ACGTTGGATGGGACCTGTGAAAGGTCAAAT	109	45,1	3
3260_1	A/T	ACGTTGGATGGCATTTTATCAGGTTTTCC	ACGTTGGATGTGCAGCTGCAGAATAAACTC	97	46,8	3
5386	A/T	ACGTTGGATGCAGACAGTTGATGCATCAGC	ACGTTGGATGGGGTGGTTTACTTTTATTAGC	100	46,7	3
5152_2	G/T	ACGTTGGATGGGAAGAATCCCTTCCATCTC	ACGTTGGATGCTTGGTCACTTTCATGATCC	99	47,9	3
3259_1	C/T	ACGTTGGATGGACTCAACTCACGCATTAGC	ACGTTGGATGAAGATTGCATGTTATCACG	106	45,1	3
1348_2	C/T	ACGTTGGATGCATGTTCTTTTGTTCCTTCTG	ACGTTGGATGGGAATGGACCTCTTTGACAG	103	47	3
5536_2	C/T	ACGTTGGATGTTGCTTTGTGTGGGACTATC	ACGTTGGATGGATTTTTTTCTTAGGGTAG	108	46,1	3
5286_1	C/A	ACGTTGGATGACTCCGAGGTAATAGACGTG	ACGTTGGATGGTTTGTGTGGCGTTCATGAG	100	46,5	3
8119_2	A/G	ACGTTGGATGTCGTCTTGACAATGTTCTTG	ACGTTGGATGATGTACAATATCCATGCAG	101	46,1	3
576_1	C/G	ACGTTGGATGACTGCTATTGTTAGTTGCAC	ACGTTGGATGTCTAGCAGCAACATTGATCG	90	49,8	3
2843_1	C/A	ACGTTGGATGTCACTAACTGAAAGGAAAGG	ACGTTGGATGGGAATGATGGTGTTTCAATG	96	49	3
2909	A/T	ACGTTGGATGGAGCAATTTTGATTGTTTG	ACGTTGGATGCTGATGAAGGAAGTTGTGAC	90	47	3
11492	G/A	ACGTTGGATGGCTCTTGACCGTATGGAATA	ACGTTGGATGGTTCTAAATGGATGCATGTG	100	51,9	3
5979	A/T	ACGTTGGATGGATATTCAAGGAGTTCCACAG	ACGTTGGATGGTTATGGAAGGTCATATCAC	84	47,6	3
1345_1	G/T	ACGTTGGATGAAATACTTGTTTTGGAGATA	ACGTTGGATGGATATCATTCCACGGTTTC	80	46,8	3
1569	T/A	ACGTTGGATGGCTTACCTGATATGATGATG	ACGTTGGATGCTCAATATGTTGAATGAAG	101	46,3	3

Locus	SNP polymorphism	Primer sequences		Amplicon length (bp)	T_m	M
		Secondary amplification primer (including secondary tag)	Primary amplification primer (including primary tag)			
4974_1	G/T	ACGTTGGATGCCAATGACTCTTTTCTTGCC	ACGTTGGATGGCTAACCATCATCACATCTC	101	46,7	3
3149_1	G/T	ACGTTGGATGATTGCGATGACAAATTCGTG	ACGTTGGATGCCTCAGCTTCATTAGCATTAC	90	46,1	3
2766	G/A	ACGTTGGATGCTTGTAGTTTGGTAAACAC	ACGTTGGATGCTTCTCCTTTCTCCCATTTC	100	45,5	3
9285_4	G/A	ACGTTGGATGTCGTTTTGTATTATTGTTTG	ACGTTGGATGGGACTAGACTAATTCTCTAAC	98	45,3	3
596_3	G/A	ACGTTGGATGAGACAGTTTATTGGAAGTG	ACGTTGGATGGAGAACACAAGATTGTGGAC	99	46,9	3
11797_1	C/A	ACGTTGGATGCACCTAGGGTTTTGGAGTTC	ACGTTGGATGCACTATACTCAACTTAGGTG	100	49,3	3
1582_4	G/A	ACGTTGGATGCCTCTGCCTGTAAAATGAAC	ACGTTGGATGCCAGTTCTTCATGTCAAAAC	94	47,1	3
10496_1	C/T	ACGTTGGATGTTTATCCTTTCAACAACTC	ACGTTGGATGGATGTGATTGGCGTGGAACC	88	48,4	3
3348	A/T	ACGTTGGATGGGTAGCAATCTTGGTTCGAC	ACGTTGGATGTTAGGAGGAAGGGACTCAAC	83	45,5	3
5761_1	A/G	ACGTTGGATGTTTCTTTCAAATCGGCAAT	ACGTTGGATGTTTGCCTAGGAGACGGACAG	113	46	3
3369_1	C/T	ACGTTGGATGTGTAACTTTGGTTAGATTG	ACGTTGGATGATGATTAGAATATACACAAC	80	46,8	3
3884	T/A	ACGTTGGATGCTGATGATCATGAGATGCAC	ACGTTGGATGCTAATGTTGAAAGACCATCC	101	45,1	3
5667	A/T	ACGTTGGATGTGCCTTACGCCTAGTCAATC	ACGTTGGATGTGCCGACCCAAAATCTAGTG	100	46,4	3
6782	C/T	ACGTTGGATGTTTTCAAAGATAATTCTTGC	ACGTTGGATGTTCAAGTTCCTCTCTGCGTC	101	45,6	3
1849	C/A	ACGTTGGATGGACTAATCATATAATTATAAC	ACGTTGGATGTTGAGGGAACTTGATAGTAG	108	45,4	3
637_1	T/C	ACGTTGGATGCATCAGACAACATGCAAAA	ACGTTGGATGTAGTGATTCAATAAGGAAG	88	49,1	4
4949_2	C/T	ACGTTGGATGGTGGGATAGGAAAAATCACC	ACGTTGGATGCTGATGAGTTAGCTATGATT	92	45,7	4
1957	A/G	ACGTTGGATGGGTCTTCTGATGACCTGTTC	ACGTTGGATGAGGATACGCAGGAATATCCC	85	46,3	4
2143_3	G/T	ACGTTGGATGAGGGAAGAACCCCAAAGAAC	ACGTTGGATGTTTCTTTCTCACATCACAC	100	48,9	4
4590_1	A/G	ACGTTGGATGATTCCCGCGAAGCCTTTTTG	ACGTTGGATGGAGAAGAACACGTCCAGAAG	112	46,3	4
2659_1	A/G	ACGTTGGATGGTGTATATGTTGAAGGGAGC	ACGTTGGATGGATGGAAAACTTTTCTTAGC	92	45,2	4
44_2	T/C	ACGTTGGATGCTTCAACATGGTATAAGAGC	ACGTTGGATGTTTAGGGAACTTGACAGCAG	94	46,2	4
2498_3	C/T	ACGTTGGATGACTTAGTCTATGGACCATT	ACGTTGGATGGGACAAACCTACATGTTACAC	94	48,9	4
3223	G/A	ACGTTGGATGTTTCTGCAGATGAGAGAGCG	ACGTTGGATGCACCAACCTTCTTGACTTTC	90	46,4	4
236	G/T	ACGTTGGATGGGTTTAGTTCATATGGTTGG	ACGTTGGATGGTATGAAGTTACAGTTGAC	84	47,2	4
2133_2	T/C	ACGTTGGATGTGATGCATGTTTGTCTTGTG	ACGTTGGATGGGGTTGTCTAGCCTAAAAG	86	45,1	4
5602_2	A/T	ACGTTGGATGCACAACAAAGGGCACTGC	ACGTTGGATGCTAATGCTTTATGTAGGCTG	81	45,7	4
565_1	T/A	ACGTTGGATGGGTACTTGGCAGAGTTGCAG	ACGTTGGATGCTTGGGATATCTACAGAGAAG	97	47,1	4
6267	G/A	ACGTTGGATGTGTACTCTCCCCTTCTTGAC	ACGTTGGATGCCACAAAACCTCATCTTTCC	107	49,5	4
6470_1	T/C	ACGTTGGATGAGACCTAAGCCTAGCTCTAC	ACGTTGGATGATAATTATAAAGAACGGTAC	86	48,7	4
728_2	G/T	ACGTTGGATGTGTGCATAGCAGAGGCAAAG	ACGTTGGATGGTAGCATCGCACAGACAGG	81	48,4	4
5426_2	C/T	ACGTTGGATGGTTATTGAGTTGCTTGAGTG	ACGTTGGATGTCTTGTAAATTGTGTATTTC	95	49,8	4

Locus	SNP polymorphism	Primer sequences		Amplicon length (bp)	T_m	M
		Secondary amplification primer (including secondary tag)	Primary amplification primer (including primary tag)			
2186_1	C/T	ACGTTGGATGAGTGTCACTCACTCTTGGTC	ACGTTGGATGACCAATTGAATGTTGTTTG	96	46,9	4
13157	G/A	ACGTTGGATGGGGCAGTCAACTGTTTTTCC	ACGTTGGATGCTGGTTCTGAGAACATATTC	93	45,3	4
6268	A/T	ACGTTGGATGTCACTGCTTATTGAGACACC	ACGTTGGATGATGTGAAGCAAGGCACCTGA	107	47,3	4
6670_2	A/G	ACGTTGGATGTGGAAAGGTTACTTCACTCC	ACGTTGGATGCTTTGTTTCTCACTGACACC	97	45,1	4
4790_3	T/A	ACGTTGGATGTGCATGGGTTCATAAAAGGG	ACGTTGGATGTTTCAATTTGTAAAATCAAAG	89	46,8	4
1664_2	C/A	ACGTTGGATGGATGGGACTTGTAATTGTTTG	ACGTTGGATGTTACAATATATAGTTGTATTC	99	45,4	4
41	A/T	ACGTTGGATGCCAAGTTTGAGAGTGATTGTG	ACGTTGGATGCAAGCTAATTTAGCTCACC	106	45,5	4
2569_1	A/G	ACGTTGGATGTTTTGTGGTACATCGTTTC	ACGTTGGATGTTAGATCTGGGAGAGGGATG	112	45,1	4
6125	G/A	ACGTTGGATGTGGTTATCAGACTCAGAT	ACGTTGGATGTGTGAATTTCTGACCTACTG	100	45,1	4
13463_1	A/G	ACGTTGGATGGACTAAGCTCCTGGTGTTTC	ACGTTGGATGGGCTCCATTTGTACCTAGAG	98	47	4
12623_5	C/A	ACGTTGGATGGTCATAGGTGGCCTATTTAG	ACGTTGGATGCATGGCTTATACTTTACTAGG	95	46,3	4
3923_1	A/T	ACGTTGGATGCATGGCACCAAAGTAGTGTA	ACGTTGGATGCGCTAACCTTTTCTCTTTGG	96	49,3	4
1792	T/C	ACGTTGGATGGAAGAAAAATACATATTCAC	ACGTTGGATGCACTCTCAACCTTCCTTGTC	90	45,4	4
3260_2	G/A	ACGTTGGATGTGCAGCTGCAGAATAAACTC	ACGTTGGATGTATCAGGTTTTCCTGCTTTG	91	45,1	4
4418_1	T/A	ACGTTGGATGCAAATGTGAATCATGATGACC	ACGTTGGATGAATTTCTTCAAGAGGGAGAG	84	50,9	4
4041	A/T	ACGTTGGATGTTAGTGCATACTGGTATCTG	ACGTTGGATGGCTACATTTTATGATGAGGG	87	46,8	4
3454_1	C/T	ACGTTGGATGTGATTGCAAGGACCAAGCCC	ACGTTGGATGTTGAGTGAGCCTTGCTTTCC	103	45,5	5
12721_2	C/T	ACGTTGGATGCTAGCATCTTTACTTGCATC	ACGTTGGATGGAACTTGCCAGAGATAGAC	97	46,7	5
3495	C/T	ACGTTGGATGAATTTCAAGCACCAAGTCTG	ACGTTGGATGGTTAGGATAGGAAAAATAC	110	46,9	5
3596_3	A/T	ACGTTGGATGGGTACCAATGGTCACTTGGC	ACGTTGGATGACCTCAGTCACCAACTTCTC	86	54,1	5
5610_1	G/C	ACGTTGGATGAGATCCGGACTGCCATGAAG	ACGTTGGATGCGATTTCAATCCAGCTAGAC	89	45,1	5
1568_2	A/G	ACGTTGGATGCCCTCTCATTATCCATTTCC	ACGTTGGATGGTGTGTTGATCAAAATTCCTC	97	45,2	5
11349_1	C/G	ACGTTGGATGTCAACATATATGTAACTGC	ACGTTGGATGGAATATGAGGGATGGAAGCG	80	49,7	5
842_1	A/T	ACGTTGGATGCCGAAACTGCAGAGATTGTC	ACGTTGGATGCTACGACTGACTTTATGCTG	97	45,4	5
2937_1	G/T	ACGTTGGATGGAGGACATAAGTGGTGATGG	ACGTTGGATGTCTCTCTACCAATATTGCTG	99	45,1	5
2768_1	C/T	ACGTTGGATGCCCCTAACTAGAGATATTGC	ACGTTGGATGGTCGTTTACTTGATATTGC	102	45,2	5
7249	G/T	ACGTTGGATGGCGAGGTAAATCTGAAACCG	ACGTTGGATGAATTCAATCCCTCTGCAT	81	47	5
3617_2	C/T	ACGTTGGATGGACATCAACGTCCTTATGGG	ACGTTGGATGCCCTTGCTATATTCCGAACC	92	48,2	5
1262_2	C/T	ACGTTGGATGCACCTTATGGCATTTGACCC	ACGTTGGATGCATCTTTATATTTTATCTTTTTG	98	49,7	5
1091_2	A/G	ACGTTGGATGCTTGAGAAAGCTCAGCAATG	ACGTTGGATGTTCCATAGCTCAGTCGCTTC	83	51,6	5
10621	T/C	ACGTTGGATGGTTTGTCATCCTGGCATAGC	ACGTTGGATGAACGAATGGTTTTTGACTC	92	45,6	5
4178_1	C/A	ACGTTGGATGGAGGATGAAAATAACATGC	ACGTTGGATGGAGAATTTGACACATTTATT	99	45,1	5

Locus	SNP polymorphism	Primer sequences		Amplicon length (bp)	T_m	M
		Secondary amplification primer (including secondary tag)	Primary amplification primer (including primary tag)			
3753_1	A/T	ACGTTGGATGATCCTAATCTTCTCCCCACC	ACGTTGGATGCTTTCAGCACCTTATATTG	97	46	5
12870	G/A	ACGTTGGATGGATCCACATAATCAACTCCTG	ACGTTGGATGACACATCGTTTCTCCAGACC	100	45,3	5
4446	G/A	ACGTTGGATGTGAGAGGATCATCACGGTTG	ACGTTGGATGAACCTGGTTATGACACCAA	95	48,1	5
11246_1	A/T	ACGTTGGATGTTCCTAGAAATCTCTTCGTC	ACGTTGGATGGAGAGAAATCGAGCAAGCAG	96	45,2	5
5110_3	G/T	ACGTTGGATGTGGTTTGCTTGCATGGTTGG	ACGTTGGATGGTCCATTGAGCCGTGCTAAG	87	45,6	5
4260_2	G/C	ACGTTGGATGTGGCAAATTGGCAATTTGA	ACGTTGGATGCTTCAATATTACGTTTTTAGG	82	48,9	5
6778_1	A/G	ACGTTGGATGCTACCGATTTATGATCGATCC	ACGTTGGATGGGTCAAGTATATGAGACAA	90	46,2	5
5588	C/T	ACGTTGGATGGTGTGATAACAATGTTTGTGC	ACGTTGGATGGAATCTAAACCTTAGAACTAC	88	45,4	5
44_1	C/A	ACGTTGGATGTTTAGGGAACTTGACAGCAG	ACGTTGGATGCTTCAACATGGTATAAGAGC	94	46,2	5
3269	A/G	ACGTTGGATGCCTCCATTCGTTCCACTTTG	ACGTTGGATGCATATACTCAAAGCACCAG	96	45,1	5
2659_2	G/A	ACGTTGGATGTTATGATGGAAAACTTTTCT	ACGTTGGATGGTGTATATGTTGAAGGGAGC	96	47,6	5
814_4	T/C	ACGTTGGATGGAATCAATTGTAAAATGCAAG	ACGTTGGATGTTCATTGATTCCCTTGTTC	89	47,4	5
3308_4	G/A	ACGTTGGATGGTTGCATAATGATTATTGAG	ACGTTGGATGCTTTTTTCTAGCTCATTTAC	86	47,6	5
4710_3	G/T	ACGTTGGATGTGTAGTTGGGAATGACATGA	ACGTTGGATGAATCTGTTTGTTGCAGCTTC	98	46	5
1485_1	G/T	ACGTTGGATGTTACAACTACACTCGTGAGG	ACGTTGGATGCATTATTCATGAAGTGCTCTC	81	48,5	5
6387	T/A	ACGTTGGATGCTTGAGCTTCATAAAGAACTC	ACGTTGGATGTCACTGAGTATACTGTGACC	97	48,8	5
820	T/C	ACGTTGGATGCTTCTAAATAATTGTTCTTG	ACGTTGGATGGATTGGATTGGAGAATCTA	101	45,4	5
3069_1	G/C	ACGTTGGATGTTCTGGAGAGGATGGTGTTG	ACGTTGGATGTGGAGCTATAGTTTCTGTAG	93	45,6	5
5152_1	A/G	ACGTTGGATGCTTGGTCACTTTCATGATCC	ACGTTGGATGGGAAGAATCCCTTCCATCTC	99	45,1	5
4935_2	T/A	ACGTTGGATGATCCATATTCGTAAATTTTC	ACGTTGGATGCAGGCTTTGATTTTCATCGC	100	45,6	5
6913	G/T	ACGTTGGATGAGGAGCACTTGGATCCCTG	ACGTTGGATGTGAGTATGCATATGCTGCGG	90	49,7	6
2035_2	C/T	ACGTTGGATGGCTGCTTTTCCCTGCATTTT	ACGTTGGATGTGAATAAATCATGAGAACC	106	47,1	6
3738_1	C/T	ACGTTGGATGTGGATCGGACGGTGGATATG	ACGTTGGATGGGAGATTAGGGTTAGGGTTC	88	45,3	6
5892	G/T	ACGTTGGATGACTTTGGTCCTGGTGAATGG	ACGTTGGATGGATACATACAAGTAATGAC	89	45,8	6
4709	G/T	ACGTTGGATGGATCCTGCAGCAGGCAATT	ACGTTGGATGGACATGAATCACACTCTAGC	101	45,6	6
7099_1	C/T	ACGTTGGATGGAATCAAGTTGTGAATGTGGG	ACGTTGGATGGGACTTCTGAGTTTGCTATG	112	45,6	6
6017	T/C	ACGTTGGATGGGTAGAGTAGCTAATGCCAC	ACGTTGGATGAAAGTGGCCAAGTACTACTC	92	46,2	6
3500_1	C/A	ACGTTGGATGGCAATGAATGCTGAATCG	ACGTTGGATGGGTCAAACAACCAGGTGTAG	87	45,2	6
802_2	G/A	ACGTTGGATGAAGCAGATAGGTAGTTCAT	ACGTTGGATGGTCATTTTCCATTTTTGGT	86	45,4	6
10343_2	G/A	ACGTTGGATGAGCTAAAAGGGTCACTCCAG	ACGTTGGATGACCTCCAACAATGATAAAGG	92	45,8	6
5877	G/T	ACGTTGGATGGAGTGGTGATAGAACTATCC	ACGTTGGATGAACAATAAAATATACCAGAG	88	46,4	6
6052_1	G/T	ACGTTGGATGAGAAGGTTCGGTCATTTGGG	ACGTTGGATGGTTGTGTTTGACTAAAATTTG	105	50,5	6

Locus	SNP polymorphism	Primer sequences		Amplicon length (bp)	T_m	M
		Secondary amplification primer (including secondary tag)	Primary amplification primer (including primary tag)			
9741	T/C	ACGTTGGATGCTCGATATACATCCATCCAA	ACGTTGGATGTTTCTGAGTTTCAGTAAAG	89	45	6
6192_2	C/T	ACGTTGGATGGATTGTATGAGGAGAGAGAG	ACGTTGGATGTGCAAGAGACACGCACAAAC	100	48,1	6
12990	A/T	ACGTTGGATGACAGTTGTTTCAGATCTGTC	ACGTTGGATGCCATGTGATTTCACTTGATAC	96	45,2	6
5685	C/T	ACGTTGGATGGATGAATGTTGTGAAATGC	ACGTTGGATGCTCAATCCCACTCCTTTTTG	100	46,5	6
6216_1	G/T	ACGTTGGATGGATTTGTGAGAAAGGGCCAG	ACGTTGGATGGGCACATGATATGATTCTAC	85	49	6
8087_4	G/A	ACGTTGGATGACAATCAATTTATCCCCACC	ACGTTGGATGGTGGACTGGTTTGACACCAT	91	46,7	6
11547_1	C/A	ACGTTGGATGAATATCTGGTCCCTTTCAAC	ACGTTGGATGGATCATGGAGTGTAGCAAGG	83	45,9	6
9992_1	C/T	ACGTTGGATGCACCAAACTATCCTCATAC	ACGTTGGATGCTAAAATTCAATTTACGAGCC	88	46,1	6
2542	A/G	ACGTTGGATGGGGTTTCCATGTTCTCACTC	ACGTTGGATGAAAGTGTCAATTCACAATC	98	46,9	6
13193_1	C/T	ACGTTGGATGCAAAGCTCTGATTGCAATGG	ACGTTGGATGGAGAAGGCACTGCTTCTATG	104	46,2	6
7994_2	C/T	ACGTTGGATGGCTGTGTCTAGCGGTACATC	ACGTTGGATGTGATCCTAGCTTGCTTACGG	85	46,3	6
1801	C/T	ACGTTGGATGTGGTTTGATCTAAGCACTAC	ACGTTGGATGGGATTTATGGTCCAATGCAG	100	45,7	6
5523_3	T/C	ACGTTGGATGGTCTACCTACAGAACAATTCC	ACGTTGGATGCTTATTCTTAGAACATGAC	100	45,5	6
1623_1	C/T	ACGTTGGATGGGAGTGATGATGAAAAAGTGG	ACGTTGGATGGACACATTTTTTTTCTTGATG	104	45,8	6
4188_1	G/T	ACGTTGGATGCTTGTTTTGTGTTTTCTTC	ACGTTGGATGATGGAGTCATGGACACCTTC	96	49,3	6
3790_1	C/T	ACGTTGGATGGCTAAATACAACTTCAATGAC	ACGTTGGATGGATCCAATGTGAAGTAAAGG	96	49	6
1772	C/T	ACGTTGGATGTACTCTTGAAAGTGATTGCG	ACGTTGGATGGAGAACATACTCGTGGATAC	88	47,7	6
9161_1	C/A	ACGTTGGATGGCTTACTTGTCTAACTGATTC	ACGTTGGATGGATTCTATTCGAAGGCATGG	99	46,3	6
6244_2	C/T	ACGTTGGATGCTTGCATCATTTACTAAGCAG	ACGTTGGATGGCCGTGTTAGTTGGATAAGG	97	46,3	6
842_2	C/A	ACGTTGGATGCTACGACTGACTTTATGCTG	ACGTTGGATGCCGAAACTGCAGAGATTGTC	97	45,2	6
10757_2	A/G	ACGTTGGATGAGTGAAAATTTTACAGGTG	ACGTTGGATGCCCACATATGATAGGCTCAC	80	45,8	6
6132_1	G/A	ACGTTGGATGTTTGCAAATTTCGACCTTGG	ACGTTGGATGTGAAAGAATTATAGAAATGG	91	45,6	7
4154	A/G	ACGTTGGATGCATCATCACCGTAGACATGG	ACGTTGGATGTAAAGGAGAGGCAACCACAG	90	45,6	7
4398_1	G/T	ACGTTGGATGTGGAGCAAGCCATTATTTCC	ACGTTGGATGGTCAACTCAACCATGCATCC	95	46,2	7
4188_3	G/A	ACGTTGGATGATGGAGTCATGGACACCTTC	ACGTTGGATGGTGTTTTCTTCTTTAGGATTC	88	45,7	7
9161_2	T/C	ACGTTGGATGCTTCGATTCTATTCGAAGGC	ACGTTGGATGGATTCATTCTTATAGGAGGC	87	47,5	7
2143_2	C/G	ACGTTGGATGTTTCTTTCTCACATCACAC	ACGTTGGATGAGGGAAGAACCCCAAAGAAC	100	46,2	7
3148	G/A	ACGTTGGATGAAGTTAGACAAGGCCATGAC	ACGTTGGATGTTTCAGAGGCTGAACCAACC	99	46,5	7
13079_2	A/G	ACGTTGGATGAATGCAACCAATGCCCACAG	ACGTTGGATGAGCTGCTGTTCTGTATAATG	81	49,2	7
4906_2	G/A	ACGTTGGATGAACCACGATCTACAAAGAGG	ACGTTGGATGGTAAGAAGAAAGAAGATACC	97	45,8	7
3149_2	G/C	ACGTTGGATGCCTCAGCTTCATTAGCATTAC	ACGTTGGATGATTGCGATGACAAATTCGTG	90	45,9	7
5116	C/T	ACGTTGGATGTCACTTCCATACTGATGCTC	ACGTTGGATGTAACAAACGGTACCCCTCTG	86	51,1	7

Locus	SNP polymorphism	Primer sequences		Amplicon length (bp)	T_m	M
		Secondary amplification primer (including secondary tag)	Primary amplification primer (including primary tag)			
5162_2	G/T	ACGTTGGATGTGGGTTTGATTCGAACTTGG	ACGTTGGATGAAGTGTCACCCACTCTAGTT	80	45,9	7
4238	C/A	ACGTTGGATGTACAGGGTACTGCTTCATCC	ACGTTGGATGCAATATGGGCACGAGCATAC	105	46,6	7
301_1	C/G	ACGTTGGATGTTTTGTTGCACTGCATGTA	ACGTTGGATGGTTGGTAATTCAGGTCGAGC	106	48,3	7
11797_2	A/G	ACGTTGGATGCACCTAGGGTTTTGGAGTTC	ACGTTGGATGACACTATACTCAACTTAGG	101	47,2	7
1945	C/A	ACGTTGGATGCTAATGGTACATTTTGTTGG	ACGTTGGATGTATGGTGAGTTATAAGGTCG	89	48,6	7
4178_3	G/A	ACGTTGGATGGAACTAGAGGATGAAAATAAC	ACGTTGGATGTGACACATTTATTTGGAGG	98	46,6	7
2708	G/T	ACGTTGGATGCCCAAAAGACGCACAATCTC	ACGTTGGATGGCCCCATTAGCCTAGATGAT	99	47,8	7
9285_3	C/A	ACGTTGGATGAGGACTAGACTAATTCTC	ACGTTGGATGTGTTTGTTTGGATATTAGC	85	46,2	7
4590_2	G/T	ACGTTGGATGGAGAAGAACACGTCCAGAAG	ACGTTGGATGGCCTTTTTGCATTTCCTCCC	101	46,6	7
12075	T/C	ACGTTGGATGGAGAGTCCTTTAGCAGGAAC	ACGTTGGATGAAAATAATAATTTCACGTCG	103	47,3	7
3596_4	G/T	ACGTTGGATGACCTCAGTCACCAACTTCTC	ACGTTGGATGGGTACCAATGGTCACTTGGC	86	48,1	7
6053	A/G	ACGTTGGATGATGTGCCTCTAGAACTTGAC	ACGTTGGATGCATCTCAAAAGTGTACAAG	87	47,7	7
3961_2	T/C	ACGTTGGATGGTTGTCTTCAATAGTTACC	ACGTTGGATGCTTATAGTTTGAGTCAATC	96	45,7	7
1581_2	C/A	ACGTTGGATGAATTTGGTCTGAAATATCG	ACGTTGGATGTCAGAGCTCAGTACTCCAAG	94	48,1	7
141	G/T	ACGTTGGATGCCACTGCATAGACCATTTAG	ACGTTGGATGTGTCCATAGTCCTTTTTTC	105	50	7
2298_3	A/G	ACGTTGGATGTTACTTACACAAGAAATTTG	ACGTTGGATGTTTGCTAGTAGCCGTTTTGC	88	47	7
8794_2	G/T	ACGTTGGATGCATGATTTGTGATATATGTGC	ACGTTGGATGGGATAAAGTGTAAGGATAACC	103	49,1	7
2775_1	C/T	ACGTTGGATGAATCGTATGAATTTTGTTAC	ACGTTGGATGGATTCTTATATAAGGGTGCG	98	45,5	7
3668_2	T/A	ACGTTGGATGACTGCATTCATTCTCTGCTC	ACGTTGGATGCTAGAGAGTGTGCTGAAGGG	91	48,6	8
20_2	G/T	ACGTTGGATGGTTTGAAATTTGAAGAGGTGC	ACGTTGGATGTGTGCATATGATTCCTACAG	83	45,3	8
4159_1	A/T	ACGTTGGATGTCATGGAAGCAAGAAGCCAC	ACGTTGGATGTGCATATTCATGCTGGGAG	82	47,4	8
5761_2	A/T	ACGTTGGATGTCAAATCGGCAATAAAGAC	ACGTTGGATGTTTGCCTAGGAGACGGACAG	107	45,6	8
2298_4	G/C	ACGTTGGATGATCTTTTTGCTAGTAGCCG	ACGTTGGATGCACAAGAAATTTGTACACG	86	46,4	8
6704_2	A/G	ACGTTGGATGCTAATAGCCAGCTCTGGTCC	ACGTTGGATGGACATCTTTACAAGAGGTT	101	46,7	8
4935_1	A/G	ACGTTGGATGCAGGCTTTGATTTTCATCGC	ACGTTGGATGATCCATATTCGTAAATTTTC	100	45,8	8
7797_2	T/A	ACGTTGGATGTGTACCAAAACTGTGGAGAC	ACGTTGGATGCTAAAATGCACAATGAAAC	90	45,5	8
10843_1	C/T	ACGTTGGATGACTGTGCTTCATGATTACCG	ACGTTGGATGAAAAAAAAATGAAATGGGATA	81	45,7	8
5774	C/A	ACGTTGGATGTGGATCACAATGAGGTCCAG	ACGTTGGATGAGGCTCAATTACTATCCTCC	89	50,9	8
4568_4	C/A	ACGTTGGATGAGGTTCAATTTCCCTTTTCC	ACGTTGGATGGTTGATTGGTCATCAATTGAA	86	46,3	8
11168_1	C/T	ACGTTGGATGCAAGTTCCACACTTCCTTTG	ACGTTGGATGGCAATAACTAGAGGTTGAT	100	47,5	8
5837_2	T/C	ACGTTGGATGATAAGCTTCACGACCTGGAG	ACGTTGGATGTGACGATCCCTCCAAACAAG	99	47,2	8
3846	T/A	ACGTTGGATGCTCCGGTACAGTGCTTTTAC	ACGTTGGATGAGGAGTGAAAATCACTGACC	101	46,8	8

Locus	SNP polymorphism	Primer sequences		Amplicon length (bp)	T_m	M
		Secondary amplification primer (including secondary tag)	Primary amplification primer (including primary tag)			
1072_3	T/C	ACGTTGGATGCATTATCCACCAGGATCCAT	ACGTTGGATGCCTATGTTTATTGCAAGTGG	95	47,3	8
15795_1	C/A	ACGTTGGATGTTGCCCACTATTATTTCCAC	ACGTTGGATGGCTACTTCTGTTGCTGAGAC	104	45,4	8
5720_3	C/T	ACGTTGGATGGAAATCCCACCTGAGCTCAA	ACGTTGGATGAGAATGATGTACTTACAGC	80	53,2	8
449	G/T	ACGTTGGATGGATATGTTACAAATTGAGAC	ACGTTGGATGGCCCATGTAATAAGGTCAAC	99	45,1	8
13633	G/T	ACGTTGGATGCATATATATTGGAGAAGTGGC	ACGTTGGATGATATGGATGTTACACGTCAC	84	49,9	8
3313_1	C/T	ACGTTGGATGTAGACATGTGATCCATCAGC	ACGTTGGATGTTTTTACACATTTTTGAAGC	104	48,8	8
2367_2	A/G	ACGTTGGATGCCATAGGGTATCAATTCTTCC	ACGTTGGATGCCTACACTTCATCCTATAAAC	100	46,1	8
7333	G/A	ACGTTGGATGATTGCAAAGAGCAGAGCCAG	ACGTTGGATGCATAAAGATTAGCAATGCTAC	108	46	8
10757_3	T/C	ACGTTGGATGAAACCCACATATGATAGGC	ACGTTGGATGAGTGAAAATTTTACAGGTG	83	50,9	8
2775_2	G/A	ACGTTGGATGGATTCTTATATAAGGGTGCG	ACGTTGGATGAATCGTATGAATTTTGTTAC	98	46,8	8
9599	G/T	ACGTTGGATGCTTGTCTTTGAGGTATTCGG	ACGTTGGATGCCGAAAAAAAGAACAAGGAAG	99	47,3	8
2978_1	C/T	ACGTTGGATGACGCGATCGATCTGATTCTG	ACGTTGGATGTCACCTCCGTCGTATGATTC	91	48,3	8
2838	T/A	ACGTTGGATGCGTTGCTACCATGACAAAAC	ACGTTGGATGCTCCAAAAACGTACGTCAAC	101	47	8
3503_1	A/G	ACGTTGGATGGGACCTGTGAAAGGTCAAAT	ACGTTGGATGCATTTTACCTTACCCATGGC	108	45,8	8
1694	T/C	ACGTTGGATGCCTTGAAGTTTAGATTTTTC	ACGTTGGATGCATGCAAAAATGACTAAAC	89	46	8
4790_2	A/T	ACGTTGGATGTATTTTTCAATTTGTAAAATCA	ACGTTGGATGTGCATGGGTTCATAAAAGGG	93	45,9	8
3070	A/G	ACGTTGGATGTTAGAGGTTGGGCAATAGGG	ACGTTGGATGAGATGTGTTCAAGGTAAAGG	95	45,9	8
6778_2	G/A	ACGTTGGATGGGTCAAGTATATGAGACAA	ACGTTGGATGCTACCGATTTATGATCGATCC	90	45,4	8
3369_2	T/C	ACGTTGGATGATGATTAGAATATACACAAC	ACGTTGGATGTGTAACTTTGGTTAGATTG	80	47,4	8
5426_3	T/C	ACGTTGGATGTCTTGTAAATTGTGTATTTC	ACGTTGGATGGTTATTGAGTTGCTTGAGTG	95	45,6	8
5725_1	G/A	ACGTTGGATGTCCCTCTCTCAATCAAACAT	ACGTTGGATGTTGGTGTATAGTTGTGAACC	98	46,6	8
5325_3	A/G	ACGTTGGATGACTGTGAAGAAAAGCCATCC	ACGTTGGATGAAGCCCCCAGACCCACTTG	82	45,1	9
2791_1	A/T	ACGTTGGATGCTTGTTTGGTCCAATCTTAC	ACGTTGGATGCTATAATCACGGTAACATAC	82	45,4	9
12149	C/A	ACGTTGGATGTCAATATCCTCTTCCGGCTC	ACGTTGGATGTTTGCAAAGAAGTCCGTCAG	92	48,3	9
3518	T/A	ACGTTGGATGCAGGTGATATTGCTAGCTGG	ACGTTGGATGTGTGTATAGGAACTAGGAAG	92	47,2	9
6139	G/C	ACGTTGGATGTGAGCGATAATGACATGGCG	ACGTTGGATGAAAGGATGGATCTTTCGGGC	91	46,9	9
4168_2	A/G	ACGTTGGATGCAGGTTCTATATATGCACAC	ACGTTGGATGTTATGGGATATACATGAGT	86	49,7	9
1597_5	G/C	ACGTTGGATGTGGCTGGATGAGGTGAATTG	ACGTTGGATGCTCATCATATATATATGTGT	88	45,1	9
9519_3	A/G	ACGTTGGATGTTGGCGTACCTGATTCAATG	ACGTTGGATGTGGTTCTGGCAGCATCTCT	91	55,1	9
835_1	T/C	ACGTTGGATGTGTCCTTGGCACTGGCAGTA	ACGTTGGATGTCCTTGCCTCGGTATATTGG	96	46,3	9
6560_2	T/C	ACGTTGGATGCTGTTTTCAAGAAATTTGTC	ACGTTGGATGTAGGACTTTGATTGCCCTGC	82	45,8	9
6885	T/A	ACGTTGGATGTGGTCAAACTTCATTGTCCT	ACGTTGGATGCTGAGGAAAATAGAGCAGTC	80	46,5	9

Locus	SNP polymorphism	Primer sequences		Amplicon length (bp)	T_m	M
		Secondary amplification primer (including secondary tag)	Primary amplification primer (including primary tag)			
4233	G/T	ACGTTGGATGTCCCACACTCAATAGAACCC	ACGTTGGATGTGCTAGCCATGGATAGAGAC	113	46,6	9
5656_1	G/A	ACGTTGGATGTCCCTTGGTAGAACAACATC	ACGTTGGATGAATACCAACCATGCACCCTT	115	46,4	9
4059_4	G/A	ACGTTGGATGTGCTTTACACTTCCAAACAG	ACGTTGGATGATGTGAATGATTTGAAACC	80	47,7	9
302_1	C/T	ACGTTGGATGTGTCCAAATCTTGACCCAGG	ACGTTGGATGCCTCCATTGTTACTCCTACC	92	53,5	9
6573_2	T/A	ACGTTGGATGCGTTCCAATTCAGAGACCAA	ACGTTGGATGTCACAAAATCGTGGATAAG	85	49,5	9
3977_1	C/T	ACGTTGGATGCCTATTGCAAATGAACAATG	ACGTTGGATGACAGTCACTATTCATTCAG	110	45,4	9
5310	G/A	ACGTTGGATGTATCCTTAGATCTCTTCAC	ACGTTGGATGAGAAAGGTAGCATGGGTCAG	82	46,2	9
2105	C/A	ACGTTGGATGAACAGTGCTTACAATTCTCG	ACGTTGGATGTTGCACGGACAACAGCTATG	100	51,1	9
12075_2	C/A	ACGTTGGATGTAGCAGGAACAAAATCTGAC	ACGTTGGATGAATAATAATTTCACGTCGAT	91	45,9	9
2136_2	C/T	ACGTTGGATGTACGGATCAATTCACTCTC	ACGTTGGATGACTCAGTACCACTTATATT	85	45,9	9
997_2	G/T	ACGTTGGATGCCACACATCACAAATATATG	ACGTTGGATGATATAATGGGGTGCCTTTCC	99	46,1	9
1962	G/T	ACGTTGGATGCTCTCTAGCAAAACGCCTTG	ACGTTGGATGCCTTGCAATATTGTTGGTTC	100	52,8	9
1942_1	A/G	ACGTTGGATGCTCTTACCCTTCTTCCTATG	ACGTTGGATGAAGAACAGGTAGAGCGGGTG	85	45,4	9
12246_1	A/G	ACGTTGGATGTTCACTTTTTGTCACATTG	ACGTTGGATGTCAAGAAGTGTGAGAGAGGG	109	49,6	9
11500	G/A	ACGTTGGATGTATGAGACCAAATAAGCTG	ACGTTGGATGCAGTTCTATTCTTCCATGGG	80	47,4	9
6974	A/T	ACGTTGGATGCCTAATTCCAAGGATTTCTC	ACGTTGGATGCAAAAAGAAGAGATTCTCGG	103	46,2	9
3576	G/A	ACGTTGGATGTTTCTCCCTAGACTATTTGG	ACGTTGGATGAAATTGTTTTCATACATCTC	89	45,6	9
1792_1	C/T	ACGTTGGATGATGTTCACTCTCAACCTTCC	ACGTTGGATGCATGAAGAAAAATACATATTC	98	46	10
4021	A/T	ACGTTGGATGTCTGCCATAACTCCTTCCAC	ACGTTGGATGTATCCTTCAGGAAGAGGTGG	101	46,4	10
6475	C/T	ACGTTGGATGTTTTGTAGTGGACACCTATC	ACGTTGGATGACTCGTTCAAAAGGCATCGG	98	49,4	10
2978_2	C/A	ACGTTGGATGTCACCTCCGTCGTATGATTC	ACGTTGGATGACGCGATCGATCTGATTCTG	91	53,6	10
10496_4	T/A	ACGTTGGATGTCTCAAGATATTTGTTATAG	ACGTTGGATGTCAACAACTCATTTCATGC	100	48,2	10
2287_1	C/T	ACGTTGGATGGTACTTCTGTAAAATGCCTAC	ACGTTGGATGTAGCTGAAGAAATTCAAAC	98	45,8	10
2589	G/C	ACGTTGGATGAGCAACTCCAATATGCTCCG	ACGTTGGATGGAGTTGAAGATCACTGTAGG	80	46,2	10
1066	G/T	ACGTTGGATGCTGCGGATGTTGACGCAATG	ACGTTGGATGCCCGCGCTTGGTTGAATTTC	95	46,4	10
5356	A/T	ACGTTGGATGAATCTTGCGTCGTGACTCAG	ACGTTGGATGAGACTACATATTCCAGGAGG	82	45,9	10
15795_3	G/T	ACGTTGGATGAGGCAATTTGCTACTTCTG	ACGTTGGATGGTTGTTGTTATTAGCATTCCC	80	46,5	10
242_2	T/A	ACGTTGGATGCATATTACACATCACAGCAG	ACGTTGGATGATGAATTGATGTGTGGCACC	81	46,5	10
5229	C/T	ACGTTGGATGAACTATCTTTTCAGTGTGC	ACGTTGGATGTTCACCATCATGCAAGGCAG	110	45,4	10
4632	C/T	ACGTTGGATGAGTTGGGTTGGGCCACAGGA	ACGTTGGATGTCTTTAGGCCCGACCATAA	93	46,5	10
6041	C/T	ACGTTGGATGCGTCCTTCACACTTCAACAC	ACGTTGGATGGATTTCAAATTTGGTTCAGC	93	47	10
13225	C/A	ACGTTGGATGAAAGACAGAGACCCAGATCC	ACGTTGGATGATGATGGTAGAACGTTCCCG	93	46,3	10

Locus	SNP polymorphism	Primer sequences		Amplicon length (bp)	T_m	M
		Secondary amplification primer (including secondary tag)	Primary amplification primer (including primary tag)			
2791_2	G/A	ACGTTGGATGCTATAATCACGGTAACATAC	ACGTTGGATGCTTGTTTGGTCCAATCTTAC	82	45,4	10
3244_1	A/T	ACGTTGGATGCTGTGTATTATGTTATGTGGC	ACGTTGGATGCTTTTGCTAGAAGGTATTC	86	46,8	10
6132_3	G/A	ACGTTGGATGTTTGCAAATTTCGACCTTG	ACGTTGGATGGAAAGAATTATAGAAATGGC	90	45,9	10
7447	G/T	ACGTTGGATGTCTCCTTCCATCAAAAGCTG	ACGTTGGATGGAAAATTTTGTTACATGTGC	106	47,3	10
11291	T/A	ACGTTGGATGGCACTTTGATCTAGATTTGG	ACGTTGGATGTGTCAAAGTATATTGGGAGG	97	47	10
13175_4	G/T	ACGTTGGATGGAGGCCATCACTAATAACAAC	ACGTTGGATGTGCATGGTGTGCCACTAATC	97	46,2	10
8221_2	T/A	ACGTTGGATGCTCATAAGATAATTGATCATC	ACGTTGGATGCCTCAATGTTATCAAGGTTGG	87	46,8	10
141_2	T/C	ACGTTGGATGCCACTGCATAGACCATTTAG	ACGTTGGATGTGTCCATAGTCCTTTTTTC	105	46,5	10
12680_1	C/T	ACGTTGGATGGGTCTATGTAGTCTTTAGGTG	ACGTTGGATGCAAGGGACAATTTTATACAGC	84	47,8	10
233	T/C	ACGTTGGATGGATATTTTCCTGTTATTGTGC	ACGTTGGATGTTGTGGCTTCCAACAATGGG	86	48,8	10
2583	C/T	ACGTTGGATGGTCCCAATGTTTGGTTGCAC	ACGTTGGATGGTGCTATGTATAATAGACGG	92	47,3	10
6076	C/T	ACGTTGGATGTTCCTGAAATAAAATTGAAG	ACGTTGGATGCCCTTGATGCAATTACCAAAG	103	45,1	10
6059_2	G/T	ACGTTGGATGCTTTCAAATAATCCCATGGAC	ACGTTGGATGAAGGGCTAGCAAACATTTTC	84	48,5	10
5656_2	C/T	ACGTTGGATGATACCAACCATGCACCCTTC	ACGTTGGATGTCCCTTGGTAGAACAACATC	114	45,8	10
4901	G/A	ACGTTGGATGGTGTCTTGTCAAGAACTTTTC	ACGTTGGATGCTTTCAGTACTTTTGAAAGA	108	48,1	10
9519_2	C/T	ACGTTGGATGTTGGCGTACCTGATTCAAT	ACGTTGGATGGTTCTGGCAGCATCTCTTTG	89	46,7	10
1969	G/T	ACGTTGGATGGGAAGATTGGGATCGATCAC	ACGTTGGATGGATAAGGCCAAAGTTAGTTG	99	45,5	11
5761_4	G/A	ACGTTGGATGTCTTTGCCTAGGAGACGGAC	ACGTTGGATGTCAAATCGGCAATAAAGAC	109	45,2	11
5892_2	G/A	ACGTTGGATGGATACATACAAGTAATGACAC	ACGTTGGATGGAATTTGAACTTTGGTCCTG	97	47,5	11
18070_5	G/T	ACGTTGGATGAGATGGTACTTGGGTTTGGG	ACGTTGGATGAACAAATTCATGAGCTCAC	107	47,9	11
5607_1	C/T	ACGTTGGATGTCACTGTTTTGCACTTCGGC	ACGTTGGATGGTTGCCTCTCCAAGATATCA	104	45,9	11
11127_1	T/A	ACGTTGGATGGTAACCTCAAACCTGAAACC	ACGTTGGATGCTTTTGCCTGTAAGCAAGTG	95	47,3	11
12105_2	T/C	ACGTTGGATGTTACCTAACTCGACATACAC	ACGTTGGATGACCCTAACTAAAAAGGTAA	105	46,4	11
6627	G/A	ACGTTGGATGTGGTGAAAGATGCTTGTTGG	ACGTTGGATGCTACTTACGGAGACACATGC	81	47,1	11
3855	C/T	ACGTTGGATGCAATCTCAAGCTGCTCATTC	ACGTTGGATGGTCCCATTGTGTGTATTAGTC	99	52,2	11
13287_1	C/T	ACGTTGGATGGATCTGGGATGACCGTAA	ACGTTGGATGCTTTTATATAGCGCTCTTC	88	46,8	11
3977_2	G/A	ACGTTGGATGACAGTCACTATTCATTCAG	ACGTTGGATGCCTATTGCAAATGAACAATG	110	48,4	11
6742	T/C	ACGTTGGATGTCTCCCATATCCCCTTTCTC	ACGTTGGATGGGAAACTTCTTGTTCGTAT	104	45,1	11
5792	T/C	ACGTTGGATGCACTTATTTTTGTTCCCTCCC	ACGTTGGATGAAAAAGTAAACACATTGTTG	85	47,1	11
11906_1	A/G	ACGTTGGATGCGATTTTTTCATTCCGCAAC	ACGTTGGATGTGTGCCCAACACGGAATCAC	100	49,7	11
3478	G/A	ACGTTGGATGTGAGTGTGTGCATGTTTAGG	ACGTTGGATGCACTTCCTTCATAGTAGAAC	105	47,8	11
3293	G/T	ACGTTGGATGGAAGATAATCACATAAAACC	ACGTTGGATGATACCTTGTTATCGGCGAAG	95	45,5	11

Locus	SNP polymorphism	Primer sequences		Amplicon length (bp)	T_m	M
		Secondary amplification primer (including secondary tag)	Primary amplification primer (including primary tag)			
8737	G/A	ACGTTGGATGTCTTACTACCACTAGACTCG	ACGTTGGATGGCTTTGAGATAGATGTCTACG	104	45,6	11
414_2	G/A	ACGTTGGATGGTAACAATTAGCCAGAAAG	ACGTTGGATGGGTCCAAAGTGTTTCAAACG	97	45,8	11
526_2	A/G	ACGTTGGATGGCATACTGCATCCAAATCTC	ACGTTGGATGCTTGCCATGCATATCATAAG	97	45,6	11
2937_3	G/T	ACGTTGGATGTCTCTCTACCAATATTGCTG	ACGTTGGATGGAGGACATAAGTGGTGATGG	99	45,6	11
7325	A/G	ACGTTGGATGGGGACAACAAGGATTGAACG	ACGTTGGATGGGGATAGTTGGTTCGTGAC	93	45,6	11
13070_3	C/T	ACGTTGGATGGGGTCATCACTTTTTCCTCC	ACGTTGGATGGGATAGAGAAAATAACACTC	96	47	11
6215	C/A	ACGTTGGATGAACTGATGGCCCAATAGTGC	ACGTTGGATGGTACATGTTACTCAAGCACG	99	51,4	11
1782_2	G/A	ACGTTGGATGGTCAACGTGTGTTTGATGTC	ACGTTGGATGCGTTTACTTGTCTCTATCATC	93	47,8	11
3605_3	T/A	ACGTTGGATGGTTGTGATTGTCCAACTAGG	ACGTTGGATGATGTTAGGAGGATCCTTATG	104	46,4	11
5836_1	A/G	ACGTTGGATGACAAATAAAAATGTGGATTG	ACGTTGGATGCAGAAAATAAGCCTCCCACC	94	47	11
1597_3	G/T	ACGTTGGATGCTCATCATATATATATGTGT	ACGTTGGATGTGGCTGGATGAGGTGAATTG	88	45,6	11
4709_2	G/T	ACGTTGGATGGCAAGACATGAATCACACTC	ACGTTGGATGTGCAGCAGGCAATTGGCATT	100	45,8	12
1782_1	C/A	ACGTTGGATGGTCAACGTGTGTTTGATGTC	ACGTTGGATGTCGTTTACTTGTCTCTATC	94	45,3	12
2999_1	C/G	ACGTTGGATGGCTAACATCCCAAACACATTC	ACGTTGGATGCTGTCAATGCTAGACTTGTTG	100	46	12
6589	T/C	ACGTTGGATGCATTTTAGGATGGTTTGCTC	ACGTTGGATGATTGAAGTAAAACAAGTCC	85	46	12
6756_2	G/A	ACGTTGGATGGATGACTACCTTTTTTGCAG	ACGTTGGATGTGAGAGGTATTAGCAGCAGG	104	47,8	12
2819_2	A/G	ACGTTGGATGCCTATACTGTTACATCATGTG	ACGTTGGATGGTTGATATCGATAATATATAC	103	46,4	12
772	T/C	ACGTTGGATGCTATTGTTGGCGAAGAGAAG	ACGTTGGATGGCTGCCATGCAAAGAGATAG	83	47	12
2128	G/A	ACGTTGGATGGCTATTCACCTACTGTTGGC	ACGTTGGATGTTGACAGCAACTTCTTGCCC	92	46,2	12
4780_2	C/T	ACGTTGGATGTGCCAACTAGGAAAAGTCCA	ACGTTGGATGGATTCATTTTTATTTCAATCT	97	45,6	12
1371	G/A	ACGTTGGATGCCGAAATTTCTTATTGCGT	ACGTTGGATGGAAATTGCAGAATGTAATCAC	94	47,6	12
2192_2	C/T	ACGTTGGATGGAACATTAGGGATGAAGGAA	ACGTTGGATGTCAAAATGTGAAGACTCTGC	84	47,1	12
414_1	C/G	ACGTTGGATGGGTCCAAAGTGTTTCAAACG	ACGTTGGATGGTAACAATTAGCCAGAAAG	97	45,9	12
4993_2	T/C	ACGTTGGATGTATCCATTTCATGCCATGGT	ACGTTGGATGGTGGTTTTCATACAAGTGGC	91	46,1	12
5950_2	G/A	ACGTTGGATGCACATTGCCATTTTATTATT	ACGTTGGATGGCCTAGCCATTCTTTTTTCTC	104	45	12
3344_3	G/A	ACGTTGGATGCCAGAAAATCTATTTGTAG	ACGTTGGATGCAGGTGGCATTCCAAATT	96	45	12

Material for DNA-extraction

Seedlings were grown in a growth chamber and leaves were collected and used for DNA isolation (Verdu et al. 2016).

DNA-extraction protocols

A commercial DNA extraction kit was used for DNA isolation (Dneasy, Qiagen) (Verdu et al. 2016).

Important results

The study was rather methodological and focused on the effects of paralogy on marker quality. Putative paralogy was shown to inflate the observed diversity values. Moreover, a rather low (x4) threshold of sequencing depth resulted in better SNP validation (Verdu et al. 2016). Taking these factors into account, the authors developed a set of 377 SNPs ready to use in population genetic analyses.

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