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Morphological Traits, Microsatellite Fingerprinting and Genetic Relatedness of a Stand of Elite Oaks (*Q. robur* L.) at Tullynally, Ireland

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Abstract

A morphological and molecular characterisation of phenotypically elite oaks (*Quercus robur* L.) which were estimated as 220 years old was undertaken to test the utility of molecular tools to examine the genetic origin of the stand. The 11 trees shared many excellent characteristics in tree form. Quantitatively, DBH ranged from 104 cm to 126.5 cm and stem height from 10 m to 25.5 m. The molecular analysis using microsatellites for nine genetic loci was on five trees. It concluded that the trees were not closely related. This small sample showed many polymorphisms and much heterozygosity. Loci AG16 and AG 9 showed 9 and 8 different alleles respectively while loci AG1/2 and Ag15 displayed 3 and 5 alleles among the five trees. At least two trees had a three-band profile for some loci indicating potential triploidy.

The historical records of the estate refers to one elite tree in 1837 and the detail of its description suggests it may correspond to one extant today. It also suggests an active silvicultural management and the practise of coppicing with standards. Such management may have resulted in this excellent stand by conversion of a natural woodland in stages starting with coppice, leading to coppice with standards, then to high forest and ultimately to a parkland stand.

Key words: microsatellite, *Quercus,* repeated DNA, SSR.

FDC: 165.3/4; 181.6; 176.1 *Quercus;* (417).

Introduction

Oak (*Quercus* spp.) is an important species for its valuable timber and its adaptability to a range of sites. It is also valued for its longevity and beauty and as a host for a wide range of organisms.

Among our oak forests are phenotypically elite specimen trees which may be characterised as having: clean stems, a round cross section with straight butts, freedom from knots or shake and with heartwood straight down the middle. They are usually found as single specimens or widely scattered in large stands. Such trees can be utilised optimally by: using them as seed sources, or propagating them vegetatively (grafting) to create clone banks or seed orchards to produce improved seeds.

At Tullynally Castle, Castlepollard, County Westmeath Ireland, there is, unusually, a group of 11 oak trees which share many elite characteristics; they are straight and of good form in a relatively small, open parkland area of approximately 3 ha. LOUDON, (1844) described one exceptionally straight and tall tree in this estate in the 1830s and which may in fact be one of the trees included in the present study. Most trees in the group are straight with strong apical dominance and two have fastigiate – type branching. Their exceptional quality might indicate some genetic relatedness or a positive combination of good genetic material together with good silvicultural practises.

When phenotypically superior material is identified in a small area, the question of its provenance or origin usually arises. We considered the possibility that the trees in this study might be closely related due to their similarity in age and their close proximity. While there are several approaches to this question, molecular techniques have been suggested as being reliable. Indeed, such techniques have been useful to determine paternity and the genetic relatedness of individuals in a stand, especially by using the analysis of microsatellite profiles (DOW and ASHLEY, 1996).

A large part of higher eukaryote genomes consists of repetitive DNA of different kinds (WEISING *et al.,* 1995). Among them repeated sequences containing iterations of short motifs are referred to as microsatellites or simple sequence repeats (TAUTZ, 1993). Each microsatellite locus consists of tandemly repeated sequences of one up to 6 nucleotides. The regions of DNA which flank each side of the repeated sequences are highly conserved and the whole represents a conserved genetic locus.

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Although the flanking regions are conserved, there is great variability in the number of repeat units due to the mutational loss or addition of repeat units, and this accounts for a large number of distinguishable alleles and provides a basis for estimating genetic polymorphisms at the DNA level. This polymorphism can be analysed by amplification of the microsatellite loci by the polymerase chain reaction (PCR) using primers targeting the conserved flanking regions. The amplified microsatellite sequences are then separated on a sequencing gel and distinguished on the basis of their length in base pairs.

The objective of this study was to describe the important morphological traits of some elite oak trees and to demonstrate the utility of DNA analysis in determining their genetic relationships so as to allow an assessment of the contribution of genetic background and silvicultural management in producing excellent trees.

Material and Methods

Plant material

The site was visited and trees of *Quercus robur* L. numbered and measured in September 1997. Crown or epicormic shoots, where accessible, were collected previously and grafted on rootstocks and maintained in the greenhouse. Grafts were successful for five trees. Mature leaves from five grafted trees were harvested in September for DNA extraction.

Morphological scoring among the trees was rated 1 to 4 with 4 as the best condition for a number of traits (*Table 1*). The length of clean stem was measured from ground level to the first forking point of the main stem. Trees with high and wide buttresses were rated poorly as were trees with a bow or ridges in the stem, or with a large number of epicormic shoots. Trees in which the leading stem showed continuity with the main stem were rated as having high apical dominance. Tree diameter was measured using a diameter tape, and height using a clinometer.

Table 1. – Characteristics of high quality oaks at Tullynally Castle, Castlepollard, Co. Westmeath, Ireland. Rating scale 1 = poorest, 4 = best.

			Tree Form Rating 1-4				
Tree	Stem DBH	Stem Ht	Buttress Form	Stem Form	Epicormic Shoots	Apical Dominance	Total Rating
No	(cm)	(m)					
1	104	10.0	4	2	3	2	11
2	149	11.0	2	2	4	2	10
3	**108.5**	**18.5**	**3**	**4**	**2**	**4**	**13**
4	**102.9**	**16.5**	**3**	**3**	**1**	**3**	**10**
5	109.7	25.5	2	4	3	4	13
6	**103.7**	**22.0**	**4**	**4**	**1**	**3**	**12**
7	**106.4**	**20.5**	**4**	**2**	**3**	**2**	**11**
8	139.5	13.0	2	3	4	4	13
9	114.2	15.0	2	3	1	2	8
10	123.6	11.5	2	3	4	4	13
11	**126.5**	**20.5**	**2**	**2**	**1**	**4**	**9**

The trees shown in bold have been characterized by microsatellite analysis at nine loci.

DNA extraction

DNA extraction has been performed according a rapid protocol of DNA extraction developed in our laboratory for extracting DNA from mature leaves of *Acer, Fraxinus, Prunus* and *Quercus.*

Fresh plant material (100 mg of leaf) was ground in liquid nitrogen using a ceramic mortar and pestle to give a green powder. The powder was transferred to a new 1.5 ml polypropylene tube using a spatula. At this time, 1 ml of DNA extraction buffer [50 mM Tris-HCl pH 8.0, 20 mM EDTA pH 8.0, 0.7 M NaCl, 0.4 M LiCl, 1% w/v CTAB (hexadecyltrimethylammonium bromide), 1% w/v PVP 40, 2% w/v SDS] and 10 µl of β-mercaptoethanol (1% final concentration) was added. The DNA extraction buffer is a white emulsion. The mixture was mixed with the spatula, vortexed 5 seconds and then incubated 15 min. at 65 °C in a water-bath. After addition of the powdered leaf material and immersion in the 65 °C water bath, the mixture became clear in a few seconds, as soon as the different reagents interacted with proteins and phenolic compounds.

After incubation, 0.5 ml of chloroform/isoamylalcohol (24:1) was added to the tube, the mixture was agitated thoroughly until making an emulsion and centrifuged 1 min. to 5 min. in a microfuge at 17000 g (14000 rpm in an ALC microcentrifugette 4214 rotor A-12). As much as possible of the aqueous phase was transferred to a new 1.5 ml tube and centrifuged 1 min. at 17000 g; 0.8 ml of the supernatant was then transferred to a new tube and 0.8 ml of isopropanol (cold as an option) was added to the aqueous solution. The tube was swirled gently and a white DNA precipitate appeared. The tube was then centrifuged 1 min. at 17000 g and the supernatant was withdrawn. The DNA pellet was washed with 1 ml of 70% ethanol, centrifuged again 1 min. at 17000 g. The second ethanol wash is optional. Finally the supernatant was withdrawn and the pellets allowed to dry on the bench for 10 min. DNA pellets were resuspended in 50 µl to 100 µl of 10 mM Tris-HCl pH 8.0, 1 mM EDTA. As the solution contained RNA and DNA, the protocol was followed by an RNase digestion to remove RNAs (optional). RNA digestion was performed by adding 2 µl of RNase (0.5 mg/ml) (Boehringer Mannheim, UK) and incubating for 30 min. at 37 °C. The resulting DNA mixture could be purified through a column such as the Wizard Clean-up System (Promega Biotec, Madison, Wl, USA), but could also be directly used after RNase digestion. In cases where the samples are of a brown colour, it is recommended to purify them through a column system.

Microsatellite PCR and microsatellite analysis

We used flanking primers designed for microsatellite regions described by STEINKELLNER *et al.* (1997). The loci that we analysed for this study are presented in *table 2.* PCR conditions were found different than the original conditions developed by STEINKELLNER *et al.* (1997); the main reason for this fact could be the different method of DNA extraction.

PCR conditions were the following: 50 µl reactions included 75 mM Tris-HCl pH 9.0, 50 mM KCl, 2 mM $MgCl_2$, 62.5 µM dNTPs each (Biofinex, Praroman, Switzerland), 0.2 µM to 1 µM forward primer, 0.2 µM to 1 µM reverse primer, 1.25 u to 1.5 u AmpliTaq polymerase (Perkin Elmer, Foster City, Ca, USA) and 5 ng to 50 ng DNA template. Hot-start PCR technique was performed because it was known to give better amplification of pure PCR products. Mineral oil was replaced in all PCR reactions by a home-purified pharmacy parafffin wax (LEFORT and DOUGLAS, 1997). Following an initial denaturation of 5 min. at 96 °C, 28 to 35 cycles [94 °C for 1 min., AT°C (AT = annealing temperature) for 1 min., 72 °C for 30 s] were performed, terminated by an 8 min. final extension at 72 °C. PCR products were checked on a 2% w/v agarose gel in 1xTBE and then analysed on a CastAway precast 6% polyacrylamide 7M urea sequencing gel (Stratagene Cloning Systems, La Jolla, Ca, USA). PCR samples (5 µl) were mixed (1:1) with a sequencing gel loading buffer (95% (v/v) formamide, 0.05% (v/v) bromophenol blue, 0.05% (w/v) xylene cyanol, 10 mM NaOH). Samples were then denatured for 6 min. at 96 °C and placed on a water-ice bath prior to loading. Gels were run 1 hour to 1.5 hour at 1600 V in a CastAway Sequencing System (Stratagene

Table 2. – Microsatellite loci, primers and annealing temperatures used for microsatellite fingerprinting of elite Irish oaks.

Locus [1]	EMBL Accession Number [1]	Forward primer [1] (5'-3')	Reverse primer [1] (5'-3')	Annealing Tempearture AT
ssrQpZAG1/2	X84080	tcctccgctcactcaccatt	aaacctccaccaaaacattc	50
ssrQpZAG1/5	X84081	gcttgagagttgagatttgt	gcaacacccttaactacca	57
ssrQpZAG9	X98753	gcaattacaggcctaggctgg	gtctggacctagccctcatg	49-50
ssrQpZAG15	X98758	cgatttgataatgacactatgg	catcgactcattgttaagcac	49-50
ssrQpZAG16	X84082	cttcactggcttttcctcct	tgaagcccttgtcaacatgc	59
ssrQpZAG58	X98757	ctgcaagattcggacaagcaa	tctttttcctaatctcacctg	49-50
ssrQpZAG104	X98761	atagggagtgaggactgaatg	gatggtacagtagcaacattc	49-50
ssrQpZAG108	X98762	ctagccacaattcaggaagag	cctcttttgtgaatgaccaag	49-50
ssrQpZAG110	X98763	ggaggcttccttcaacctact	gatctcttgtgtgctgtattt	49-50

[1]) STEINKELLNER *et al.,* 1997.

Cloning Systems) and then submitted to silver staining (STREIFF and LEFORT, 1997).

After a final wash (5 min.) in ultra-pure water, they were dried in a CastAway System Gel Dryer.

Stained gels were subsequently photographed using an image analysis system (Imagestore 5000, UVP, UK).

Results

Morphological analysis

Shoot samples were obtained from nine trees and all were identified by leaf morphology as *Q. robur.* The total heights for trees 3 and 5 were estimated as 34.5 m and 26.5 m respectively. The other characteristics of each tree are given below in *table 1.*

Tree 2 had the greatest DBH and was the only tree with downward arching branch-ends with crown shoots which were accessible at ground level.

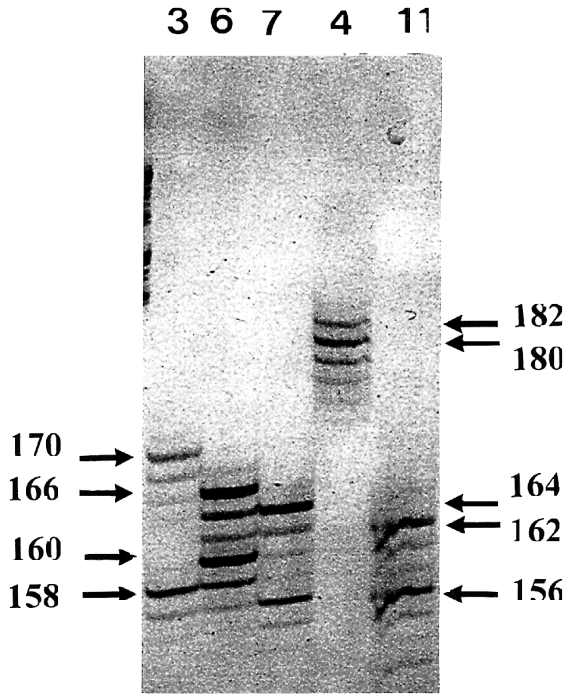

Figure 1. – Microsatellite profiles for locus AG16. The microsatellite allele is the major band. The length (bp) of some sequences are marked in the figure and all allele length are given in *table 3.*

Trees 8 and 10 were notable in having the greatest diameter combined with the best rating for tree form, while trees 5, 6 and 7 had trunk lengths over 20 m combined with high ratings for tree form. It was notable that trees 3 and 6 were almost fastigiate in nature with ascending crown branches.

The age of several trees was computed as 220 years using the method of WHITE (1994) for mature trees.

Molecular analysis

Figure 1 shows the DNA amplification pattern for trees No. 3, 4, 6, 7 and 11, obtained by using the amplifying primers for microsatellite locus AG16. Each band represents a different length in base pairs of microsatellite DNA for this locus, thus each band is an allele. The real alleles are only the darkest bands. Other bands which appear are "stutter or shadow bands" and are caused by (among others) the addition of a nucleotide to the 3' end of the amplification product by the DNA polymerase or by a slipped strand mispairing leading to deletion of repeat units during the PCR (SMITH et al., 1995; LITT et al., 1993). Stutter bands are not scored but represent a helpful ladder for allele length determination.

Two strong bands were evident for each of the trees analysed in *figure 1* and each tree was represented by alleles of different molecular weight indicating that each was heterozygous and that this locus (AG 16) was polymorphic. A total of 9 different alleles were recorded and only trees 7 and 11 had one allele (156 bp) in common. A summary of the microsatellite profiles at all loci for all trees is presented in *table 3.*

For locus AG104 in the same 5 trees, tree No. 3 and 7 shared the same alleles, each with a DNA length of 232 bp and 234 bp. Tree No. 11 had one allele in common with tree 6. Tree No. 4 has no common alleles with any of the others *(Table 3).*

An examination of the profiles at locus AG1/2, showed that trees 3, 7, 4 and 11 shared the same allele of 97 bp. There was one strong band present for trees No. 3 and No. 4 indicating that they were homozygous for this allele: however trees No. 7 and No. 11 were heterozygous while tree No. 6 was homozygous for the allele of 110 bp. The microsatellite profiles for all 9 loci are summarised in *table 3.*

An examination of locus AG9 showed the presence of 3 alleles in common for trees No. 4 and No. 11 and these are shown in *figure 2.* A three band pattern was scored when each band was clearly resolved from the others. In addition, these two trees also showed a similar 3 alleles profile for locus AG15. Furthermore tree No. 11 showed three alleles at locus AG1/5 while tree No. 4 showed two *(Table 3).*

Table 3. – Genetic polymorphisms obtained for 9 microsatellite loci with 5 elite oak trees.

Microsatellite locus	Microsatellite length in base pairs	Estimated number of alleles per microsatellite				
		Tree Number				
		3	6	7	4	11
Locus AG 1/2	97	2	0	1	2	1
	99	0	0	1	0	0
	101	0	2	0	0	1
Locus AG 1/5	169	0	0	1	0	1
	170	1	1	0	1	0
	171	0	1	0	0	0
	172	0	0	0	1	1
	174	0	0	1	0	0
	175	1	0	0	0	0
	176	0	0	0	0	1
Locus AG 9	186	0	0	1	1	1
	188	1	0	0	0	0
	192	0	1	0	1	1
	196	0	1	1	0	0
	198	1	0	0	0	0
	200	0	0	0	1	1
Locus AG 15	110	0	1	0	1	1
	112	1	0	1	1	1
	114	0	0	1	1	1
	118	1	0	0	0	0
	144	0	1	0	0	0
Locus AG 16	156	0	0	1	0	1
	158	1	0	0	0	0
	160	0	1	0	0	0
	162	0	0	0	0	1
	164	0	0	1	0	0
	166	0	1	0	0	0
	170	1	0	0	0	0
	180	0	0	0	1	0
	182	0	0	0	1	0
Locus AG 58	154	1	0	0	0	0
	156	0	0	0	2	0
	160	0	0	1	0	0
	163	0	1	0	0	0
	166	0	0	1	0	0
	171	0	0	0	0	2
	192	1	0	0	0	0
	196	0	1	0	0	0
Locus AG 104	186	0	0	0	1	0
	200	0	0	0	1	0
	206	0	1	0	0	1
	220	0	1	0	0	0
	232	1	0	1	0	0
	234	1	0	1	0	0
	240	0	0	0	0	1
Locus AG 108	211	1	1	0	0	1
	217	0	0	1	0	0
	223	0	1	0	0	1
	225	0	0	0	1	0
	233	1	0	1	0	0
	237	0	0	0	1	0
Locus AG 110	194	0	0	0	0	1
	206	1	0	0	0	0
	208	0	0	2	2	1
	210	0	1	0	0	0
	220	1	0	0	0	0
	234	0	1	0	0	0

1 = 1 allele at the microsatellite indicated.
2 = 2 alleles at the microsatellite indicated (homozygous).

It can be deduced from the microsatellite profiles that the five trees analysed do not share a common mother or father. For example, analysis of AG1/5 suggest that trees No. 3, No. 6 and No. 4 may be related since they have one allele of 170 bp in common. However, analysis of locus AG16 shows that each is polymorphic *(Table 3).* Similarly, for trees 7 and 11, that share a common allele of 156 bp at locus AG 16 but for locus AG104 no alleles are in common.

As shown in *table 3*, the number of alleles differed greatly from one locus to another, ranging from 3 alleles for AG1/2 up to 9 alleles for AG16 and this, with a small sample of just five trees. Locus AG15 displayed only 5 different alleles whereas locus AG58 displayed 8. AG16 has been shown to be a very polymorphic locus displaying 20 alleles within a sample of 17 elite Irish oaks from 10 different provenances (unpublished).

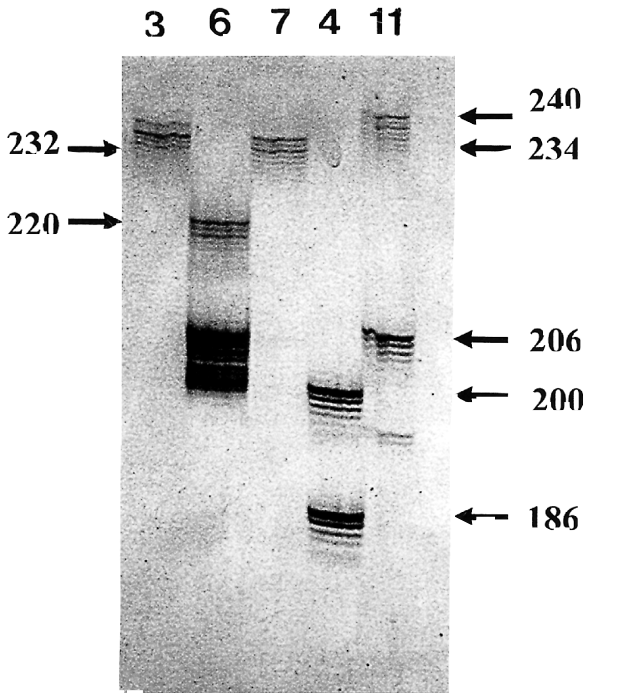

Figure 2. – Microsatellite profile for locus AG104. The microsatellite allele is the major band. The length (bp) of some sequences are marked in the figure and all allele length are given in *table 3.*

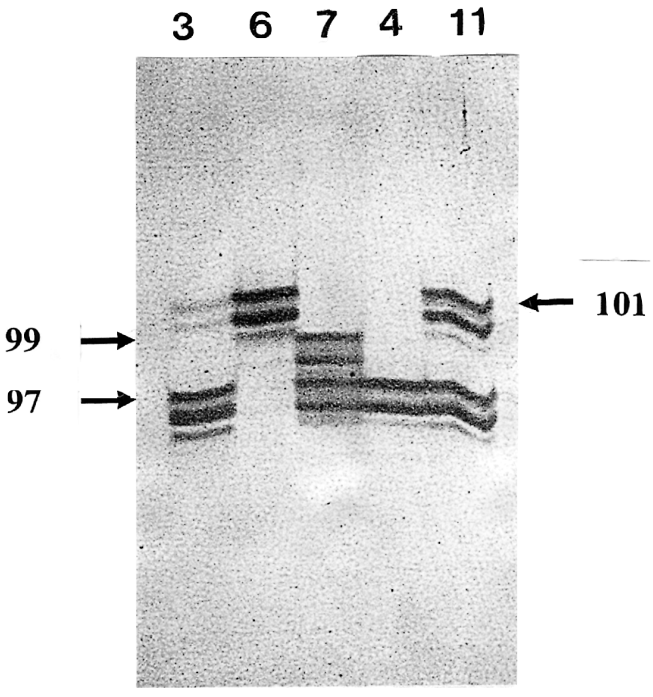

Figure 3. – Microsatellite profile for locus AG1/2. The microsatellite allele is the major band. The length (bp) of some sequences are marked in the figure and all allele length are given in *table 3.*

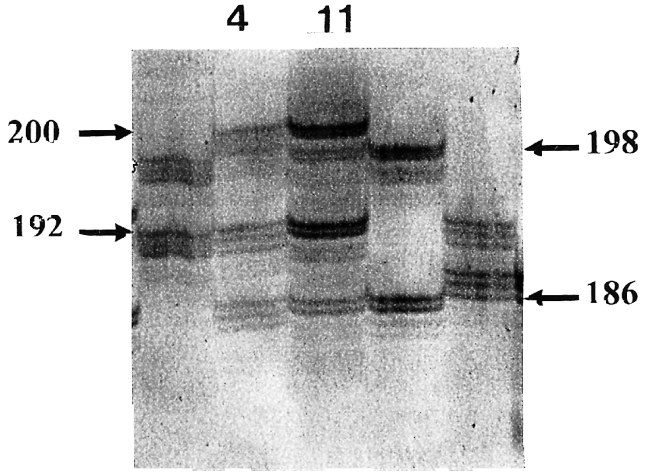

Figure 4. – Microsatellite profile of trees No. 4 and No. 11 for locus AG9 showed a 3-band pattern. The microsatellite allele is the major band. The length (bp) of some sequences are marked in the figure and all allele length are given in *table 3*.

Discussion

Analysis of microsatellite profiles is a useful and accurate tool to confirm genetic relatedness to the level of siblings in tree populations (DOW and ASHLEY, 1996). However, molecular markers such as isozymes or RAPDs were not very useful for discriminating between provenances or even species since this genus seems to be highly hetreozygous and has not yet undergone intense specialisation (KLEINSCHMIDT, 1993; KREMER and PETIT, 1993).

We have shown that none of the five trees analysed could be genetically related as siblings or half siblings, using two very polymorphic loci such as AG16 (*Fig. 1*) and AG104 (*Table 3*). A similar conclusion could be deduced by examining other loci which displayed a low or moderate polymorphism. The trees displayed a high degree of heterozygosity and this is typical of oaks which have been shown by recent analyses of molecular markers to disperse pollen over great distances (DOW *et al.,*1995; DOW and ASHLEY, 1996).

Some trees displayed a 3-band pattern for some loci. Tree No. 4 showed a 3-band pattern at loci AG9 and AG15 and tree No. 11 at loci AG1/5, AG9 and AG15. A three-band pattern could result from the presence of three copies of the haploid genome provided the tree was heterozygous at every locus i.e. triploidy. Triploids probably arise from unreduced female or male gametes. If the 2 n female gamete was heterozygous at any given locus and fertilised by pollen carrying a third allele, a three-band pattern will appear as the microsatellite profile. Triploids would give a two-band pattern if, for example, the 2 n female gamete was homozygous and the pollen carried a different allele. Indeed, triploids could give a one-band pattern if each gamete carried the same allele. It is not possible to assay the dosage effect of two microsatellite alleles which co-migrate to the same point on the gel since PCR is not quite quantitative. The three-band pattern for some loci shown with trees No. 4 and No. 11 may indicate that these trees are triploids. It could also be due to the presence of one (or more) extra-chromosomes (aneuploidy) or a duplication of one region of the genome. Previous studies provide evidence for triploidy and aneuploidy in oaks. JOHNSSON (1946) found three triploids among 726 oaks (0.0041%) in Sweden. In another study, isozyme analysis and size distribution of stomata indicated triploidy in one tree among 400 oaks (0.0025%) in Germany and cytological analysis showed the most frequently counted number of chromosomes as 33 to 35 (NAUJOKS *et al.,* 1995). BUTORINA (1993) presented a cytogenetic study of two *Q. robur* trees showing that 3 n cells were predominant with occasional diploid, hypoaneuploid and hyperaneuploid cells. Although such trees were considered triploids, they were mixoploids *stricto sensu.*

In addition, OHRI and AHUJA (1990) reported a few cases of aneuploidy in karyotyping *Q. petraea, Q. robur* and *Q. rubra* by Giemsa C-banding and the presence of B-chromosomes (JONES, 1981). The hypothesis of triploidy in two trees which gave a three-band pattern for some loci (trees No. 4 and No. 11) will be further tested by measurement of stomatal size and DNA quantification by flow cytometry (FAVRE and BROWN, 1996).

If these analysis confirm triploidy among our sample of five elite trees, it may point to a higher frequency of tripioids among elite trees than previously believed. Indeed, the two triploids studied by BUTORINA (1993) were noticed because they were unusually large. The 90 years old triploid among 400 trees analysed by NAUJOKS *et al.* (1995) was fertile and had all the characteristics of an elite tree, having been described as "27 m high with an excellent straight stem form and a diameter of 42 cm at 1.30 m. Its crown started at 18.5 m".

The development of this high quality stand at Tullynally was probably assisted by silvicultural practises and the historical record of the estate suggests that the owners showed much interest in their woodlands.

One outstanding specimen was measured in 1836 or 1837 and a description of it is given in a publication in 1844, however the first edition of this book is 1838 (LOUDON, 1844). This describes one tree in the estate as "A *Q. pedunculata* is 80 ft. high, with a trunk perfectly clear from knots or branches for 31 ft.; girthing 12 ft. at 1 ft. from the ground and 6 ft. at 31 ft., just below the swelling of the branches. The trunk is perfectly straight, and the tree which is in a healthy and growing state is about 96 years old." (LOUDON, 1844). This tree may well be tree No. 1 of the present study since it is the only tree with first branches at 10.0 m (33 feet) and is situated closest to the driveway which followed the same route as today (PAKENHAM, pers. commun.). Subsequently, in 1861 a report by the forester FRASER was commissioned and it gives the recommendation for oaks that "the best trees should be selected as standards, pruned and treated accordingly" (PAKENHAM, 1998). This suggests that the estate woodlands may have been managed as a coppice with standards i.e. a two storey forest with a coppice underwood comprising a scattering of trees (standards) to be grown for timber size. Such a management strategy was commonplace and was the legally required way of management in the time of HENRY VIII (EVANS, 1984). Oak was and still is the most common species grown in this way and planted stock is rare in such situations. Standards exhibit large open crowns with a rapid growth in stem diameter similar to that under free growth conditions, but they often develop vigorous epicormic branches (EVANS, 1984). The straightness of the trees at Tullynally and the absence of epicormic branches on the main trunk even in trees which have an inherent high capacity for epicormic shoot production *(Table 2)*, strongly suggest that the stand may have been converted from coppice and were pruned regularly.

Our study shows that there is no close relationship between the trees at the genetic level and that their good form was the result of the capacity of good genotypes to respond to good silvicultural practises which seem to have been applied throughout the life of the stand in relation to thinning and pruning.

It also suggests that triploidy and mixploidy should be investigated by cytogenetic studies in elite phenotypes, especially when microsatellite profiling raises such a possibility. Microsatellite fingerprinting is also shown to be a potential tool for cytogenetics.

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MENDELian Inheritance and Tissue Expression of RAPD-markers in *Picea abies* (L.) KARST.

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Abstract

Due to a need to identify DNA-markers well suited for genome mapping in Norway spruce, an investigation was carried out with RAPD markers. The plant material – controlled crosses – made it possible to study MENDELian inheritance of the applied RAPD markers in the following tissues: Needles and buds (2n) as well as megagametophytes (n) of parent individuals, germinating embryos, young seedlings as well as 15 year old progenies of the progeny generation (2n). Prior to the MENDELian study, the RAPD assay from DNA extraction through PCR to agarose gel electrophoresis was optimized and an extensive primer screening revealed the available level of polymorphism. The RAPD markers behaved as a rule as reproducible dominant markers and was expressed in all tested tissues, haploid as well as diploid. Occasional occuring RAPD fragments in the haploid megagametophytes, not present in either of the parents, can in mapping projects where megagametophytes are the mapping population be discarded by including a reference sample of the diploid female parent.

Key words: Picea abies, RAPD, tissue expression, MENDELian segregation, repeatability, primer-screening.

FDC: 165.3; 165.41; 174.7 *Picea abies.*

Introduction

Dealing with identification of DNA-markers suitable for genome mapping in *Picea abies* (L.) KARST., a research programme has been initiated in Denmark. This programme serves as a part of a larger project concerning localizing of quantitative trait loci (QTL) expressed later in the development of coniferous trees.

Initial point to clarify is the type of methodology to select, which again causes a wide range of questions to be answered before deciding which markers to apply.