information can be found on the degree of genetic relationship between the trees. Such information may be provided by molecular-genetic markers. Information on such markers may be collected in a forestry trial, not only to determine which trees are close relatives but also to determine whether the genetic variation is randomly distributed in space, as has so far been assumed (see Introduction). If this proves to be the case, spatial analysis could be used not only to distinguish genetic, spatial and non-spatial environmental components of variance, much as SAKAI and HATAKEYAMA envisaged, but also to estimate the breeding value of individual trees.

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Microsatellite DNA Profiling of Phenotypically Selected Clones of Irish Oak *(Quercus* spp.*)* and Ash (*Fraxinus excelsior* L.)

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Abstract

Oak and ash are two of the main forest species in Europe. Because of their commercial importance, genetic improvement of such species is considered important. The recent availability of microsatellite sequences for both oak (*Quercus robur, Q. petraea*) and ash (*Fraxinus excelsior*) allowed the characterization of phenotypically selected clones of oaks and ash trees of Irish origin by microsatellite DNA profiling. Oak clones were characterised at nine microsatellite loci and ash clones at 10 microsatellite loci. Allele ranges in selected clones were found to be similar to those observed in natural stands of oaks in Austria and ash in France, but the number of alleles at each locus was higher. Heterozygosity differed between Irish and Austrian oaks at several loci. Analysis of microsatellite profiling provided individual profiles for each clone. Microsatellite data analysis was performed with the software NJBAFD and the calculations of stepwise weighted genetic distance showed the genetic distances between clones. Five clones from a managed oak stand, which were probably from the same source were found to be genetically related to several other Irish sources. Two Irish origins of ash were found to be related to a French source. Microsatellite profiling also showed three bands patterns at several loci in 5 oaks and at one locus in 7 ash trees, suggesting the occurrence of triploidy or aneuploidy. In the latter case, the hypothesis of locus duplication should be checked by crossing studies.

Key words: Fraxinus excelsior, microsatellite, *Quercus petraea, Quercus robur,* single sequence repeats (SSRs).

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Introduction

Broadleaf tree species such as oaks (*Quercus robur, Q. petraea*) and ash (*Fraxinus excelsior*) represent a biological richness in forest ecosystems as well as playing an important economic role. In Europe, oaks range from Ireland in the west to the Ukraine in the east and southern Sweden in the north to southern Italy in the south. Oaks represent 9% of the total growing stock in European forests and 29% of broad-leaved forests. In Ireland, where only 8% of the land area is forested, oaks represent 2.5% of the total forest area and 26% of broad-leaved area.

Ash (*Fraxinus excelsior*) ranges from Ireland in the west to northern Iran in the east and southern Norway in the north to southern Italy in the south. Although less is known about the genetics of ash compared to oak, ash still represent a commercially important trees in many European countries and is used for the production of high value timber products. In Ireland ash accounts for 1.6% of the total forest area and 17% of broadleaf forests.

The natural coverage of both species is known to have decreased sharply during the last 4,000 years mainly due to destruction of forests for agricultural needs (PLIURA, 1999). *Fraxinus excelsior* is considered as a noble hardwood important for gene conservation in Europe (TUROK *et al.,* 1999), for which a gene conservation strategy needs to be developed in order to maintain a broad genetic variation as part of management plans for commercial forestry (ROTACH, 1999). A national survey for the selection of phenotypically superior oak and ash was carried out in Ireland by Coillte Teoranta- the Irish Forestry Board to identify superior material for further genetic improvement. Scions of selected trees were conserved as grafted clones that can be used as a source of material for vegetative propagation or for further breeding purposes.

Because native Irish oak and ash provenance experiments are too young to provide information on the levels of genetic variation in these species, alternative methods need to be considered. Forest trees improvement and breeding programmes are tending more and more to rely on molecular markers for such a characterisation and microsatellites are increasingly the favored molecular markers in such programmes (SMITH and DEVEY, 1994; KOSTIA et al., 1995; ECHT et al., 1996; VAN DE VEN and MCNICOL, 1996; PFEIFFER et al., 1997; Isagi and SUHANDONO, 1997; STEINKELLNER et al., 1997; LEFORT et al., 1999a and for a review see LEFORT et al., 1999b). In oaks, they have also been used recently for analysing pollen and seed dispersal and parentage for population structure studies (STREIFF et al., 1998; DOW and ASHLEY, 1996). Microsatellites, also called simple sequences repeated (SSRs), are short tandem repeats characterised by short motifs (1 bp to 6 bp) with a low degree of repetition (5 up to 100 repeat units) and a randomly dispersed distribution of about 10^4 to 10^5 per genome (TAUTZ, 1993). Flanking regions of the repeats are known to be highly conserved in species, but also throughout species and even genera while the number of repeat units of the repeated sequence is variable and is source of a high degree of polymorphism. This high polymorphism combined to other properties such as a co-dominant inheritance (that allows discrimination of homo- and heterozygotic states in diploid organisms), a frequent occurrence, a generally even distribution throughout the nuclear genome and a selectively neutral behaviour make them convenient markers which can be easily, quickly and reproducibly assayed. Though their main applications in plant genetics are in genome mapping, identification of individuals, clones or cultivars as well as in population analysis, microsatellites are also useful for taxonomy and parentage analysis. They also have a strong potential for use in commercial issues such as certifications of seeds, cultivars or clones. Several microsatellite loci were recently made available for oaks (*Quercus petraea* and *Q. robur*) and ash trees (*Fraxinus excelsior* L.) (STEINKELLNER et al., 1997; LEFORT et al., 1999a).

In the context of an improvement programme for these forest species, we tested molecular markers as a method for the genetic characterisation of clones of oaks and ash.

Material and Methods

Plant material

The availability of a collection of selected trees of oak species (*Quercus robur, Q. petraea*) and *Fraxinus excelsior* represents a very small percentage of the total population for these trees in Ireland. These trees displayed good height and diameter growth, a straight stem, strong apical dominance and a stem free of defects, pests and disease. Their age was estimated in the range 60 to 100 years-old. Scions from the crown of selected trees of *Quercus robur, Q. petraea* and *Fraxinus excelsior* were sampled and grafted on rootstocks and kept initially in greenhouse and later in the field. From over 100 clones of oaks, only about 30% of the grafts succeeded while out of over 100 ash clones more than 95% of the grafts were successful.

The 17 selected oaks used in this study were from eleven different Irish locations and one German provenances: Allen 44 (A44), Bramwald 6 (Bd6), Bree 9 (Be9), Donadea 33 (Da33), Dundrum 91 (Dm91), Knocktopher 71 (K71), Laragh 21(L21), Mount Bellew 25 (MB25), Mount Bellew 26 (MB26), Phoenix Park (PP), Thomastown 6 (Tn6), Tullynally 3 (Ty3), Tullynally 4 (Ty4), Tullynally 6 (Ty6), Tullynally 7 (Ty7), Tullynally 11 (Ty11). In addition a known German triploid tree of *Quercus robur* (GT) characterised as triploid by NAUJOKS et al. (1994) and confirmed by microsatellite analysis (LEFORT and DOUGLAS, 1999a) was also included. This tree expressed the superior traits as Irish selected trees and can be thus considered as a superior individual. It was included in this study as a marker for triploidy.

The 16 ash used in this study were from 7 Irish and one French location: Athenry 5 (AT5), Athenry 6 (AT6), Athenry 8 (AT8), Athenry 9 (AT9), Durrow 55 (DU55), Durrow 56 (DU56), Durrow 59 (DU59), Durrow 60 (DU60), Graigue 63 (GR63), Knocktopher 64 (KN64), Roscrea 65 (RO65), Roscrea 69 (RO69), Shillelagh 61 (SH61), Thomastown 70 (TH70), Ville 43 (VI43) and Ville 157 (VI157).

DNA extraction

Mature leaves of *Quercus spp.* and *Fraxinus excelsior* trees were harvested in September and October and DNA extraction was performed according a rapid protocol of DNA extraction developed previously for *Acer, Fraxinus, Prunus* and *Quercus spp.* and described elsewhere (LEFORT and DOUGLAS, 1999b). For the identified German triploid (GT), dry leaves, collected 2 years earlier were provided by D. EWALD (Federal Research Centre for Forestry and Forest products, Waldsieversdorf, Germany).

Microsatellite PCR

For *Quercus spp.,* we used microsatellite primers for PCR amplification designed by STEINKELLNER et al. (1997) and described in *table 1a*. For *Fraxinus excelsior,* we used microsatellite primers that we designed previously (LEFORT et al., 1999a) and which are described in *table 1b*. PCR reactions were made up to 50 µl final volume including 75 mM Tris-Hcl pH 9.0, 50 mM KCl, 1.3 to 2.5 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 Taq polymerase (Biotools, Madrid, Spain) and 5 ng to 50 ng DNA template. Following an initial denaturation of 5 min at 96°C, 2835 cycles [94°C for 1 min, AT°C (AT = annealing temperature, given for each locus in *tables 1a* and *1b*) for 30 s, 72°C for 1 min) were performed, terminated by an 8 min final extension at 72°C. PCR products were checked on a 2% agarose gel in 1xTBE and then analysed on a CastAway precast 6% polyacrylamid 7 M urea sequencing gels (Stratagene Cloning Systems, La Jolla, Ca, USA). PCR samples (5 µl) were mixed (1:1) with a sequencing gel loading buffer (95% formamide, 0.05% bromophenol blue, 0.05% 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 for 1 to 1.5 hours at 1600 V in a CastAway Sequencing System (Stratagene Cloning Systems) and then submitted to silver staining. The silver staining protocol is a modified protocol developed to provide a high contrast coloration and a clear background. CastAway gel trays were thoroughly washed prior to each use with a commercial bleaching agent (containing sodium hypochloride and hydrogen peroxide, such as Domestos), rinsed thoroughly with ultra-pure water and dried with paper wipes. CastAway gels stuck to the smaller plate were incubated 20 minutes in reagent 1 (1 liter 10% acetic acid in ultra-pure water) on a shaking tray. Reagent 1 was then removed and saved for later use. Gels were then washed twice for 2 minutes with ultra-pure water. Water was withdrawn and reagent 2 (2 g silver nitrate, 1.5 ml 40% formaldehyde (AnalR, BDH Laboratory Supplies) in 1 liter ultra-pure water was added for 30 minutes, with the tray being covered by aluminium foil to protect the silver staining solution from light. The gel plate was then removed from the silver staining solution and the back of the plate was washed with ultra-pure water. The gel plate was rinsed for 5 seconds in water, which was then discarded and reagent 3 [freshly prepared before adding, 30 g sodium carbonate (anhydrous, Merck), 200 (l sodium thiosulfate 10 mg/ml (Sigma, stock solution stored at 4°C), 1.5 ml 40% formaldehyde in 1 liter ultra-pure water] was poured into the tray. Once development was achieved, reagent 2 was discarded and reagent 1 that was saved earlier was added to the tray for 2 minutes followed by a final bath of 2 minutes in ultra-pure water. Gels were then dried for 30 minutes in the CastAway System gel dryer. *Figure 1* shows an example of silver staining of microsatellite profile.

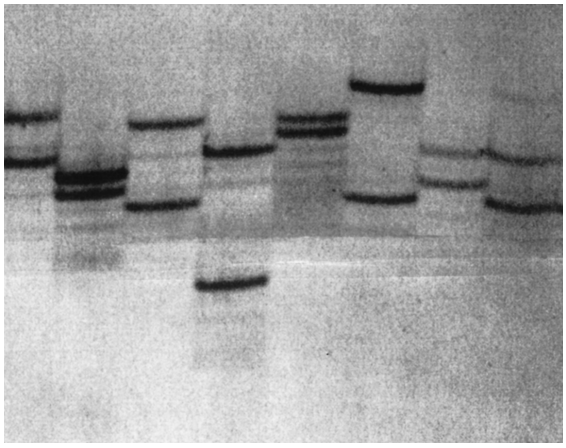

Figure 1. – Detail of a silver- stained sequencing gel (8 oaks DNA samples amplified at locus AG1/5)

Table 1a. – Microsatellite loci, amplification primers and condtions for *Quercus spp.* (STEINKELLNER et al., 1997).

Locus	Forward (5'-3')	Reverse (5'-3')	AT°C	[$MgCl_2$] mM	[Amp. Primers] µM
ssrQpZAG1/2	tcctccgctcactcaccatt	aaacctccaccaaaacattc	50	2	1
ssrQpZAG1/5	gcttgagagttgagatttgt	gcaacacccttaactacca	57	2	1
ssrQpZAG9	gcaattacaggcctaggctgg	gtctggacctagccctcatg	50	2	1
ssrQpZAG15	cgatttgataatgacactatgg	catcgactcattgttaagcac	50	2	1
ssrQpZAG16	cttcactggcttttcctcct	tgaagcccttgtcaacatgc	59	2	1
ssrQpZAG58	ctgcaagattcggacaagcaa	tctttttcctaatctcacctg	50	2	1
ssrQpZAG104	atagggagtgaggactgaatg	gatggtacagtagcaacattc	50	2	1
ssrQpZAG108	ctagccacaattcaggaagag	cctcttttgtgaatgaccaag	50	2	1
ssrQpZAG110	ggaggcttccttcaacctact	gatctcttgtgtgctgtattt	50	2	1

Table 1b. – Microsatellite loci, amplifcation primers and conditions for *Fraxinus excelsior* (LEFORT et al., 1999).

Locus	Forward (5'-3')	Reverse (5'-3')	AT°C	[$MgCl_2$] mM	[Amp. Primers] µM
FEMSATL1	agcagcatttatgaatgttc	atcaactgaagatgacgacg	60	1.3 – 1.5	0.5
FEMSATL2	tctttatcatcaaaaaataa	tacaaggtgatatcacttct	50	2.5 – 3	1
FEMSATL4	ttcatgcttctccgtgtctc	gctgtttcaggcgtaatgtg	52	2.5	0.5
FEMSATL5	ggattgagattcaatttgca	tccgagtgatgcctactcta	54	2	1
FEMSATL8	tgtagctcaggattggcaat	agcgttgtccttaactttt	52	2.5	0.5
FEMSATL10	ttgagcaacatgtaattatg	aaatatccggtgcttgtgta	51	2.5	0.4
FEMSATL 11	gatagcactatgaacacagc	tagttctactacttcaagaa	52	2	0.5
FEMSATL 12	tttttggaacccttgatttt	gatggacgggcattcttaat	52	2	0.5
FEMSATL 16	tttaacagttaactcccttc	caacatacagctactaatca	52	2 – 2.5	0.5
FEMSATL 19	ctgttcaatcaaagatctca	tgctcgcatatgtgcagata	52	2 – 2.5	0.5

Analysis of microsatellite data

Calculations of Dsw (stepwise weighted genetic distance) was performed with the programme NJBAFD (N. TAKEZAKI, National Institute of Genetics, Mishima Shizuoka, Japan, personal communication; TAKEZAKI and NEI, 1996) and processed with the programme TREEVIEW 3.0 included in the package available from ftp://ftp.bio.indiana.edu/molbio/evolve/njbafd. This software was convenient to treat tri-allelic data since it was possible to consider a clone as a population.

Results and Discussion

Tables 2 and *3* show the microsatellite profiles for the oaks analysed at nine microsatellite loci and ash trees analysed at 10 loci. Five oaks displayed a three-band pattern from one to three loci. The hypothesis of triploidy or aneuploidy for these trees have been investigated and results presented and discussed elsewhere (LEFORT and DOUGLAS, 1999a). Surprisingly seven ash also clearly showed a three-band pattern at one locus (Femsatl 19) out of the ten loci analysed. This could be explained by a possible aneuploidy or duplication at this locus. Microsatellite analyses of crosses of these trees should be performed in order to confirm this hypothesis.

Microsatellite data were also used for calculating distance matrix tables with the programme NJBAD (TAKEZAKI and NEI, 1996). These tables were converted in unrooted trees and are presented in *figure 4* and *5*. The distance used was the stepwise weighed genetic distance of GOLDSTEIN (1995). As seen in the tables and the figures, the microsatellite profiling experiments resulted in single profiles for each analysed tree. Five oaks from the same stand (Ty, Tullynally) were found to be very different from each other which would suggest that they were not related (*Fig. 4*). The fact that the five trees were similar to other Irish oak sources supports the hypothesis of a human management in this particular stand as was discussed previously (LEFORT et al., 1998). In ash, tree AT5 and RO69 were found to be similar to the 2 French clones VI43 and VI153. In the oaks, the German triploid oak (GT) used as a marker was very similar to the Irish oaks.

Distributions of allelic frequencies at all loci are shown in *figure 2* for oaks and *figure 3* for ash trees.

In oaks and ash trees, at each locus, at least one main alleles but sometimes 2 main alleles could be easily identified and should theoretically represent the more ancient allele from which the other alleles were derived either by addition or deletion of a number of repeat units (CHARLESWORTH et al., 1994). In ash trees, at loci Femsatl 2 and Femsatl 16, one main allele accounted respectively for 70% and 69% of all alleles, whereas 5 alleles were found at Femsatl 2 and 4 at Femsatl 16 (*Table 5*).

Table 2. – Microsatellite profiles in base pairs given at 9 loci for 17 clones of oak.

	Loci								
Trees	AG 1/2	AG 1/5	AG 9	AG 15	AG 16	AG 58	AG 104	AG 108	AG 110
A44	97 : 97	178 : 172	188 : 186	106 : 106	170 : 155	164 : 164	218 : 216	227 : 227	206 : 200
Bd6	101 : 101	164 : 164	198 : 188	112 : 110	180 : 156	178 : 176	204 : 200	225 : 211	234 : 216
Be9	97 : 97	176 : 169	198 : 186	158 : 118	165 : 150	192 : 172	234 : 196	233 : 213	208 : 206
Da33	97 : 97	172 : 169	198 : 192	118 : 114 : 112	170 : 160	182 : 164	234 : 206	221 : 213	208 : 206
Dm91	99 : 97	172 : 169	194 : 188	112 : 108	176 : 158 : 155	192 : 174 : 158	206 : 204	267 : 267	218 : 212 : 208
GT	99 : 97	172 : 169	204 : 188 : 186	118 : 110 : 106	174 : 170 : 155	180 : 176	206 : 202	235 : 233 : 213	208 : 206
K79	101 : 97	172 : 162	186 : 186	112 : 108	158 : 150	192 : 178	242 : 242	233 : 211	214 : 194
L21	101 : 97	174 : 174	192 : 188	126 : 106	159 : 156	188 : 154	220 : 214	235 : 233	226 : 210
PP	101 : 97	171 : 169	188 : 186	146 : 114 : 112	180 : 178 : 162	176 : 174	220 : 218	243 : 211	208 : 208
MB25	99 : 97	172 : 164	188 : 188	112 : 110	155 : 155	164 : 164	220 : 214	233 : 211	208 : 206
MB26	99 : 99	164 : 164	194 : 186	114 : 112	167 : 158	164 : 164	242 : 234	219 : 219	208 : 208
Tn6	117 : 115	169 : 169	192 : 188	118 : 108	158 : 154	164 : 162	206 : 200	227 : 225	210 : 208
Ty3	97 : 97	176 : 170	198 : 188	118 : 112	170 : 158	192 : 154	234 : 232	233 : 211	220 : 206
Ty4	97 : 97	172 : 170	200 : 192 : 186	114 : 112 : 110	182 : 180	156 : 156	200 : 186	237 : 225	208 : 208
Ty6	101 : 101	172 : 170	196 : 192	144 : 110	166 : 160	196 : 164	220 : 204	223 : 211	234 : 210
Ty7	99 : 97	174 : 169	196 : 186	114 : 112	164 : 156	164 : 158	234 : 232	223 : 217	208 : 208
Ty11	101 : 97	172 : 169	200 : 192 : 186	114 : 112 : 110	162 : 156	172 : 172	242 : 206	223 : 211	208 : 194

Table 3. – Microsatellite profiles at 10 microsatellite loci of 16 ash trees.

	Loci									
Trees	Femsatl1	Femsatl2	Femsatl4	Femsatl5	Femsatl8	Femsatl10	Femsatl11	Femsatl12	Femsatl16	Femsatl19
AT5	190 : 180	194 : 174	192 : 172	181 : 183	160 : 146	216 : 182	198 : 198	200 : 198	200 : 200	194 : 190
AT6	182 : 176	224 : 174	192 : 172	117 : 115	160 : 154	246 : 212	184 : 184	186 : 180	188 : 186	206 : 174
AT8	182 : 180	174 : 174	198 : 172	109 : 107	160 : 150	252 : 204	184 : 184	186 : 180	186 : 180	198 : 198
AT9	184 : 182	208 : 208	172 : 166	117 : 115	162 : 162	252 : 230	192 : 192	262 : 194	186 : 186	210 : 186
DU55	182 : 176	174 : 174	176 : 176	114 : 112	162 : 152	216 : 216	198 : 198	200 : 194	200 : 186	206 : 194 : 176
DU56	182 : 176	174 : 174	166 : 166	117 : 115	152 : 152	250 : 174	198 : 198	200 : 200	186 : 186	196 : 186 : 176
DU59	190 : 190	174 : 174	176 : 172	117 : 115	184 : 184	252 : 252	184 : 184	186 : 186	200 : 186	198 : 186 : 176
DU60	184 : 182	174 : 174	176 : 166	155 : 117	184 : 138	252 : 182	184 : 184	186 : 186	186 : 180	198 : 196 : 176
GR63	182 : 182	174 : 174	178 : 166	109 : 107	184 : 142	184 : 174	208 : 208	208 : 208	200 : 186	206 : 190 : 176
KN64	176 : 170	174 : 174	184 : 166	109 : 107	162 : 160	230 : 216	184 : 184	186 : 186	186 : 186	210 : 188 : 176
RO65	212 : 180	174 : 174	192 : 176	107 : 107	146 : 146	252 : 174	196 : 196	186 : 186	186 : 186	186 : 186
RO69	188 : 188	224 : 174	184 : 176	179 : 179	164 : 156	216 : 216	182 : 180	180 : 180	186 : 180	194 : 190
SH61	212 : 202	174 : 174	166 : 166	117 : 115	162 : 146	248 : 212	200 : 196	186 : 180	186 : 186	204 : 186 : 176
TH70	188 : 180	224 : 174	166 : 164	121 : 121	176 : 164	216 : 182	196 : 188	186 : 186	186 : 186	206 : 198
VI43	NA	224 : 174	228 : 228	181 : 181	156 : 156	252 : 182	226 : 226	188 : 182	186 : 180	214 : 194
VI157	188 : 188	178 : 174	172 : 166	177 : 177	188 : 146	230 : 182	194 : 194	198 : 198	186 : 186	198 : 186

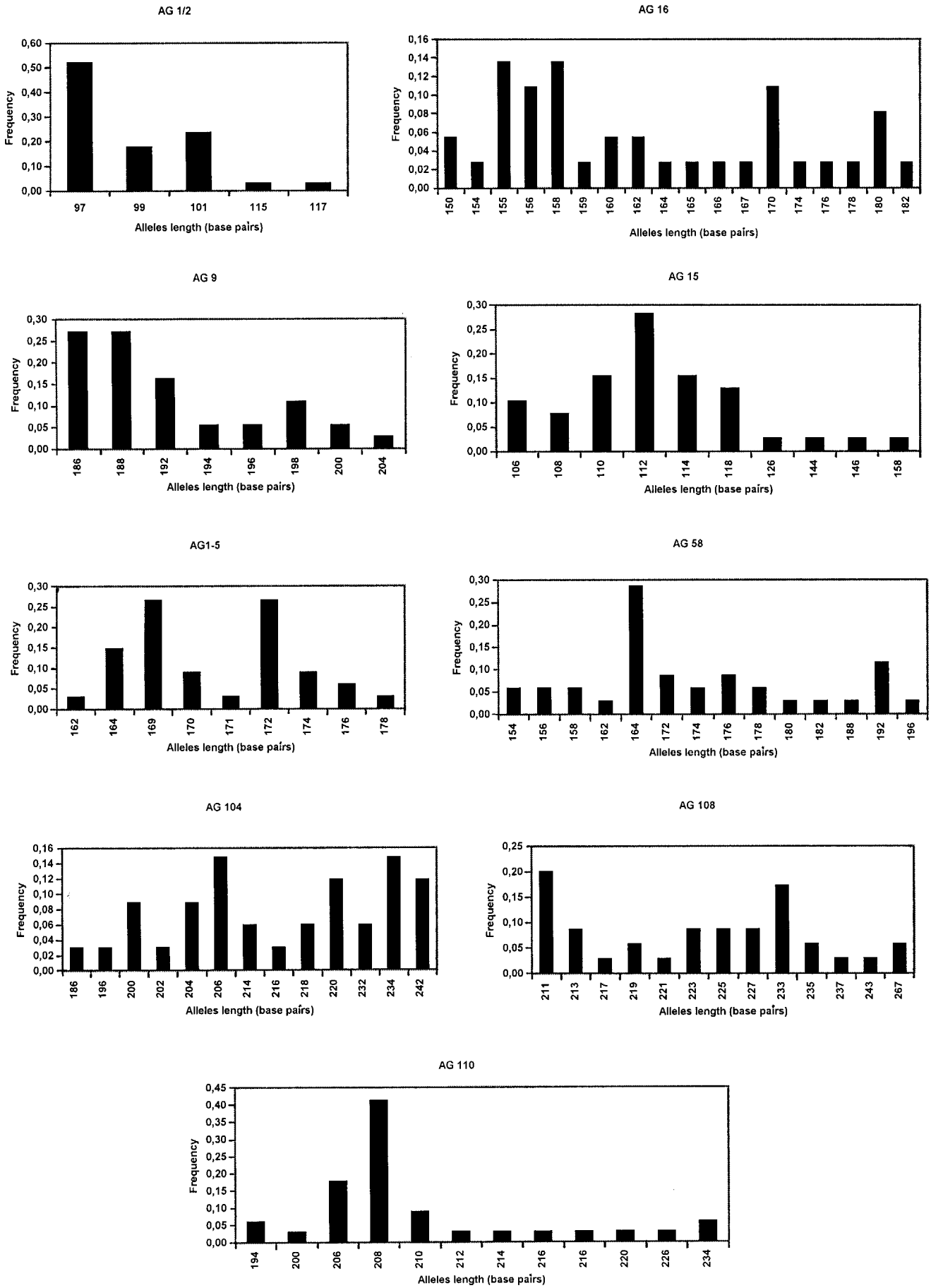

Figure 2. – Relative allele frequencies at nine microsatellite loci in 17 oaks.

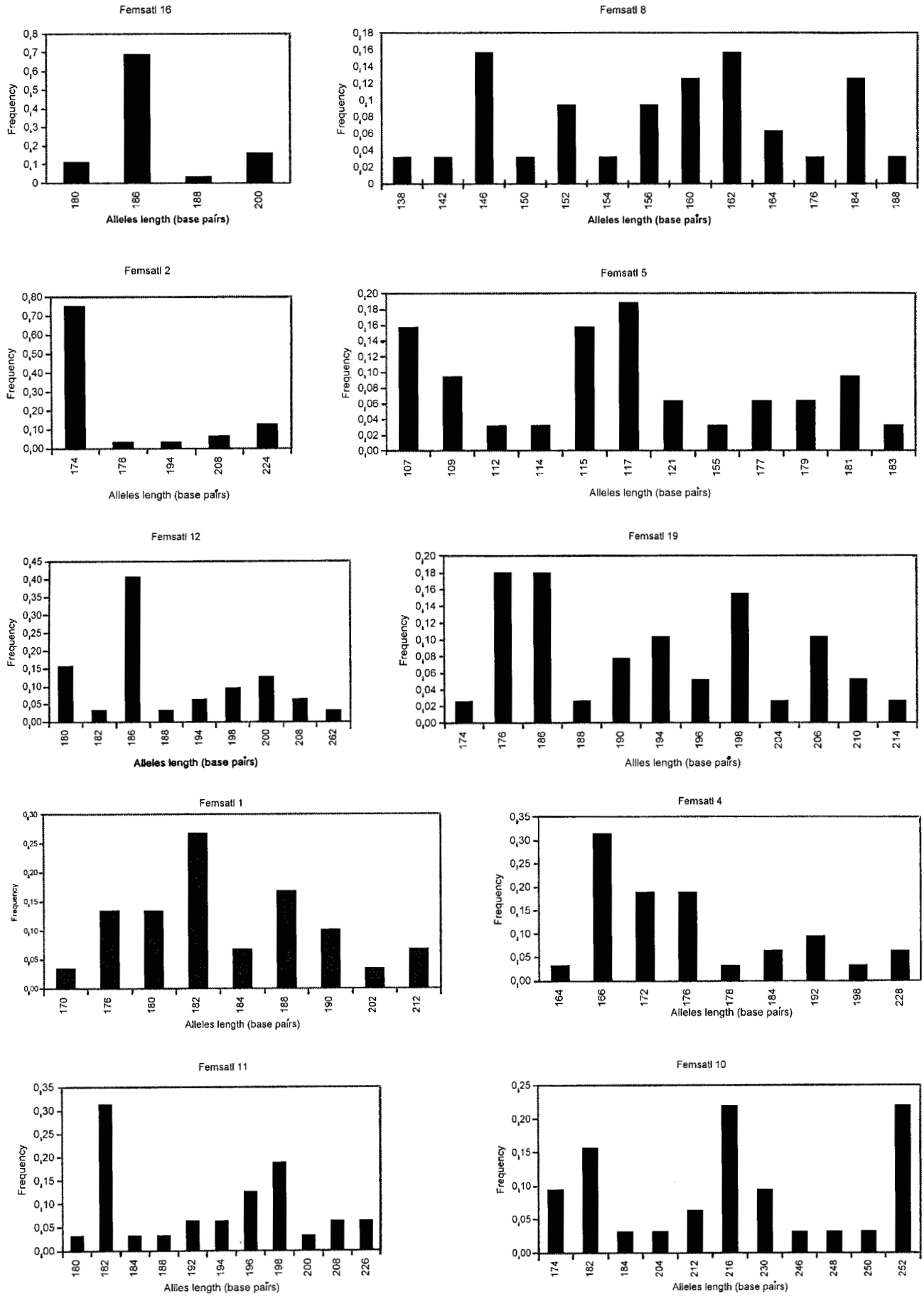

Figure 3. – Relative allele frequencies at 10 microsatellite loci in 16 ash trees.

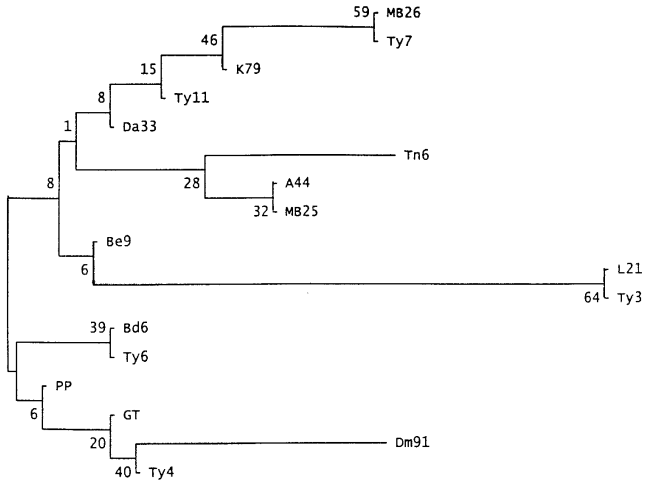

Figure 4. – Dendrogramme showing the genetic relationship between 17 elite oaks.

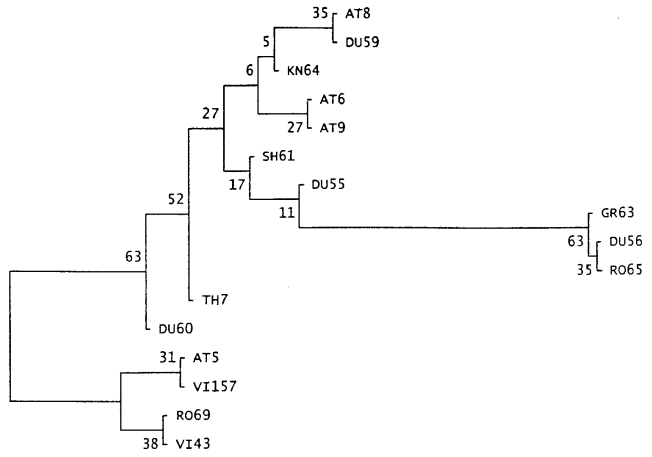

Figure 5. – Dendrogramme showing the genetic relationship between 16 elite ash trees.

A main allele with such a high frequency combined to a small number of alleles resulted in a low heterozygocity at these loci.

As shown in *table 2* and *4,* polymorphism in oaks was found to vary from 5 alleles at locus AG1/2 to 19 alleles to locus AG16. This was high considering the small number of tested

Table 4. – Comparison between Austrian oaks [1] STEINKELLNER, H. et al. 1997) and Irish oaks.

Locus[1]	Tested trees/ found alleles		Alleles length (bp)		Observed heterozygocity	
	Dublin	Vienna[1]	Dublin	Vienna[1]	Dublin	Vienna[1]
AG 1/2	17/5	27/7	97-117	95-117	0.53	0.90
AG 1/5	17/9	33/7	162-178	160-190	0.76	0.91
AG 9	17/8	28/11	186-204	182-210	0.88	0.89
AG 15	17/10	45/11	106-158	108-152	0.94	0.80
AG 16	17/19	34/9	150-182	164-199	0.94	0.81
AG 58	17/14	35/12	154-196	150-210	0.76	0.42
AG 104	17/13	25/9	186-242	176-196	0.94	0.77
AG 108	17/13	44/10	211-267	213-237	0.82	0.54
AG 110	17/12	40/7	194-234	206-262	0.76	0.92

individuals but not surprising if we consider that these 17 trees represent 11 different and distant sources. Such results suggest a large genetic diversity in oaks in Ireland.

Comparison between previously published characterization data of oaks with the same loci (STEINKELLNER *et al.,* 1997) shows a few differences (*Table 4*). In the Irish oaks, the number of alleles per locus was, in general, higher except at locus AG9 where 8 alleles were found in the Irish lot as compared to 11 in the Austrian sample. This can be due to the greater heterogeneity of the material studied. The alleles length range was, in general, quite similar. In 6 loci out of nine, the alleles length range was narrower in the Irish lot than in the Austrian. The heterozygosity at each locus varied from 0.53 to 0.94 and was significantly different at some loci when compared to the one observed in the normal population of oaks. For example it was 0.53 at locus AG1/2 in Irish selected oaks and 0.9 in the Austrian oaks. On the other hand it was 0.82 at locus AG108 in Irish oaks while it as only 0.54 in Austrian oaks. A possible explanation could be that Irish oaks were derived from a small ancestral population. Despite the differences in sample sizes and type of studied material, selected clones in this study and the natural stands in STEINKELLNER's work, no marked differences was found in alleles length ranges between Irish and Austrian provenances (see *Table 2*), except for one allele of 267 bp at locus AG 108 which was out of the 211 bp to 243 bp range close to the 213 bp to 237 bp range observed in the Austrian oaks. The tree harbouring this allele was homozygous for this allele. In general, the allele length range was identical or almost identical in all loci to the ones observed in the Austrian oaks. The large number of provenances of the Irish clones selected for their superior phenotype could possibly explain the higher

Table 5. – Characterization of microsatellite loci from *Fraxinus excelsior.* The type of the repeat unit of the cloned allele, the number of alleles per locus, the size range of the found alleles in base pairs (bp), the observed heterozygocity, the main observed alleles and their frequencies are summarized.

Locus	Tested trees/ found alleles	Alleles lenght (bp)	Observed heterozygosity	Main allele(s)	Frequency of the main alleles(s)
FEMSATL 1	15/10	170-212	0.73	182	0.27
FEMSATL 2	16/5	174-224	0.375	174	0.7
FEMSATL 4	16/9	164-228	0.75	166	0.31
FEMSATL 5	16/12	107-183	0.75	115; 117	0.18 each
FEMSATL 8	16/13	138-188	0.69	162; 146	0.16 each
FEMSATL 10	16/11	174-252	0.81	216, 252	0.22 each
FEMSATL 11	16/11	180-226	0.19	184	0.31
FEMSATL 12	16/9	180-262	0.44	186	0.34
FEMSATL 16	16/4	180-200	0.5	186	0.69
FEMSATL 19	16/12	174-214	0.88	176, 186, 198	0.18 each 0.15

heterozygocity observed in the Irish material. Only locus AG16 displayed a much higher number of alleles compared to the Austrian data. Although the alleles sizing was, in our hands, less precise because relatively calculated to molecular markers migrated along the sample and with the help of stutter bands (Rf method), compared to the more precise sizing method used by the Austrian team (automated sequencer), the obtained results still stick to the same alleles range than initially found by STEINKELLNER et al. (1997). It is however known that reading differences between silver stained sequencing gels and automated sequencing gels using fluorescent labels can account for one base less up to 4 bases more at the same locus.

Microsatellite profiling proved to be a very efficient method in order to characterise genetically a collection of phenotypically selected oaks and ash clones. Such a small number of such elite trees displayed in each species a high polymorphism. This observation confirms the validity of a procedure of identifying elite clones in each species for further propagation and breeding. Such a technique additionally provides a very useful tool for assaying heterozygosity at several loci, which is important information in tree breeding to avoid inbreeding and its consequences in planting programmes (ZIEHE and HATTEMER, 1998). Such results and methodology seem to be suitable for monitoring breeding experiments for genetic improvement of these two economically important forest tree species. Profiling of more trees at nuclear and chloroplast microsatellite could provide tree specific alleles or precise identification of provenances for instance.

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