Report for the Future Trees Trust

Sweet Chestnut Group

Genetic Characterisation of the Future Trees Trust's Sweet Chestnut Breeding Collection Using Microsatellite Markers

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Summary

Sweet chestnut (*Castanea sativa*) is an important timber species. The Future Trees Trust established their Sweet Chestnut Group in 1999 with the overall aim to improve sweet chestnut - growth, timber quality, resistance/tolerance to pests and diseases, and adaptability to climate change – by using conventional breeding and selection techniques to enable the provision of high quality, resilient seed and seedlings to growers.

Outstanding timber trees (plus trees) from across Britain and Ireland have been identified, propagated and established to create the breeding parental collection and seed orchards. Whilst the breeding collection of plus trees has been characterised phenotypically (i.e. their physical traits) no genetic characterisation had been undertaken.

This project was to undertake the genetic characterisation of the individual plus trees and the breeding collection using microsatellites. For the first time, the genetic diversity of the plus trees and the breeding collection would be assessed and compared to well characterised European populations and a British historic collection.

Measures of diversity for the FTT samples indicate that a genetic diversity has been captured within the FTT plus tree collection that is representative of the species across its western Eurasian distribution zone. Thus the FTT collection has captured sufficient genetic diversity required for the improvement programme without the need for the inclusion of additional sweet chestnut material from a wider geographic area. These results inform the breeding programme enabling the full potential of the collection to be realised whilst providing confidence that sufficient diversity has been captured to provide resilient, improved seed and seedlings to industry and growers.

Introduction to Sweet Chestnut and FTT's work on this species

Sweet chestnut (*Castanea sativa* Mill.) is a continental European native tree species highly valued for its wood, charcoal for metal smelting and nuts: Its distribution and populations have been highly influenced by humans over millennia, due to the species being widely utilised and dispersed (Conedera *et al.* 2004). During the last glacial period, it survived as refugia populations in parts of Portugal, Spain, Italy, Greece, Turkey and eastwards in the Caucasus before becoming distributed once again across Europe (Krebs *et al*. 2004).

It is believed to be a non-indigenous species in Britain and Ireland and is referred to as an 'honorary' native species In Britain (Rackham, 1980). However, it is unclear when chestnut was introduced and from where. In Britain, its introduction has been most commonly attributed to the Roman period (Preston et al 2002, Rackham 2006), whilst in Ireland, its introduction may have been significantly later during the medieval period.

In Britain and Ireland, sweet chestnut is grown as coppice and high forest crops. Commercial nut production is extremely limited. There are some 20,000+ hectares of sweet chestnut grown mainly in southern Britain where it is forms predominately as a coppice crop. Due to its rapid growth and highly versatile timber, it is from an economic point of view the most important British coppice species, especially in SE England where historically it has been intensively grown to provide a wide range of fencing materials and hop poles (Braden & Russell, 2001). It is now becoming increasingly important as a sustainable, biomass crop. In Ireland, sweet chestnut is a minor species with a few 100 of hectares being grown, usually as high forest, predominately in counties of southern Ireland.

Sweet chestnut is usually found growing below 300m above sea level (masl) with chestnut crops being typically grown upto150masl. It has the ability to grow on a wide range of acid (pH

4.5 to 6.5), moderately fertile, moist but free draining, soil types and does particularly well in sheltered, frost free sites. As a coppice crop it is typically grown as a pure crop with a rotation of 14 to 20 years for fencing materials. As a timber crop, it can be found as a pure crop or in mixtures with rotation times of usually 50 to 70 years. It provides valuable habitats and food sources for wildlife (Buckley & Howell, 2004), as well as the raw material for a wide range of naturally durable traditional and modern timber products which do not require the use of chemical preservatives.

Its timber is highly valued and has characteristics similar to oak with which it shares many external and internal uses (Everard & Christie, 1995). Its natural durability, even at small dimensions, and the ease with which the wood can be split make it particularly suited for cleft products. It has a more stable, lighter timber which is more easily worked than oak but lacks oak's strength and is less favoured for decorative purposes. The sapwood is narrow resulting in much less wastage. It is a good carpentry and joinery timber less prone to faults developing during the drying process and it offers more flexibility in the time of year the logs can be cut. Like oak, sweet chestnut is susceptible to timber defects such as ring and star shake.

Traditionally, sweet chestnut timber from standard trees is used in building construction, furniture, joinery and coffin boards, while coppice is used to produce small diameter logs for the production of traditional fencing stakes, cleft materials such as paling fencing and hop poles. Minor uses for coppiced chestnut include turnery, cask staves, walking sticks, charcoal and firewood. In the past, the bark was used for tanning leather. In recent years it has been fashionable for cleaved chestnut coppice to be used to produce rustic garden furniture and climbing frames for plants. The grading and structural standards data for the use of chestnut as a construction timber in the UK has enabled development of finger jointing and pioneering of modern wet glueing technology (Braden & Russell, 2001). This in turn, has created exciting possibilities for the structural utilisation of such small diameter chestnut timber as well as for the manufacture of windows and external joinery.

There are several diseases which pose significant threats to sweet chestnut. The most serious is 'Chestnut blight', caused by the fungus *Cryphonectria parasitica*, which until recently was confined to mainland Europe where it has caused considerable damage to and losses of chestnut. At present, its occurrence in England is limited to relatively few sites and with very little damage (Forestry Commission[1], 2018). Efforts are being made to eradicate the disease. Also worrying is *Phytophthora ramorum, a* fungal-like pathogen that causes extensive damage and mortality to a wide range of trees and other plants. In 2015, it was found to be sporulating on sweet chestnut in England (Forestry Commission[2], 2018). Thus for the first time, sweet chestnut was acting as a host species. Another is Ink Disease, caused by *P. cinnamomi and P. cambivora*. It has been present for many years and is typically only damaging in compacted and/or waterlogged sites where it can result in tree death.

Sweet chestnut is relatively unpalatable to deer, however browsing, particularly on young plants or coppice can be particularly damaging. By contrast, squirrels attack the species very early compared with many species, at 5-10cm stem diameter, stripping bark and ringing stems and thus are a major pest (Forestry Commission[3], 2018). The Asiatic insect pest, the Oriental chestnut gall wasp (OCGW) was discovered for the first time in the UK in a woodland in Kent, England, in June 2015 (Forestry Commission[4], 2018). Its larvae cause abnormal growths, called galls, to form on the buds, leaves and petioles. Whilst OCGW can be damaging (affecting form and growth), it typically doesn't result in plant death.

Like all trees, sweet chestnut can play an important role in the sequestration of carbon and hence helping to reduce CO2 levels and mitigate climate change. With its fast growth rates and its natural durability timber, chestnut sequesters carbon rapidly and can lock it up for decades/centuries in a range of solid timber products as well as accumulating it in tree itself and roots. It is also a species predicted to benefit significantly under climate change. These

factors plus its ability to substitute for imported tropical hardwoods and treated softwoods and its short rotation time made it a high priority forest species for the future (Russell K, 2009).

Recognising this, the Future Trees Trust (formerly the British & Irish Hardwoods Improvement Programme) established their Sweet Chestnut Group in 1999 and its work to improve sweet chestnut continues today. The overall aim is to improve sweet chestnut - growth, timber quality, resistance/tolerance to pests and diseases, and adaptability to climate change – by using conventional breeding and selection techniques to enable the provision of high quality, resilient seed and seedlings to growers. The Group's work follows the four main tasks set out below:

1. To identify, conserve and utilise key genetic resources (seed stands and outstanding trees) for the improvement chestnut in Britain and Ireland
2. To increase the quality, quantity and utility of the timber and coppice produced per hectare through genetic and silvicultural studies
3. To improve industry and public awareness and support of chestnut by promoting its merits and uses
4. To provide industry with supplies of high quality and resilient seed and seedlings from selected seed stands, and clonal seed orchards

Much progress has been made on Tasks 1, 3 & 4. Outstanding timber trees (known as plus trees) have been identified across their distribution range, propagated by grafting, planted in gene banks to conserve them and then utilised to establish clonal seed orchards which are now starting to produce quality seed of known provenance to industry. Mature selected seed stands have also been identified to provide supplementary and complementary good quality seed in the interim before large seed volumes from the orchards becomes available.

The plus trees form the breeding parental collection and thus are absolutely core to the improvement programme. It is essential that these plus trees are well characterised, both physically and genetically. This enables them to be used in an informed manner in the breeding programme which enables their full potential to be realised whilst providing confidence that sufficient diversity has been captured to provide resilient, improved seed and seedlings to industry and growers.

The plus trees have been described phenotypically (i.e. their physical characteristics) and were collected from a very wide range of geographic and climatic locations across Britain and Ireland. However, nothing was known about the genetic characterisation of the individual plus trees or the collection as a whole, nor how the genetic diversity of the collection compares to well characterised European populations.

FTT's Sweet Chestnut Breeding Collection of Plus Trees

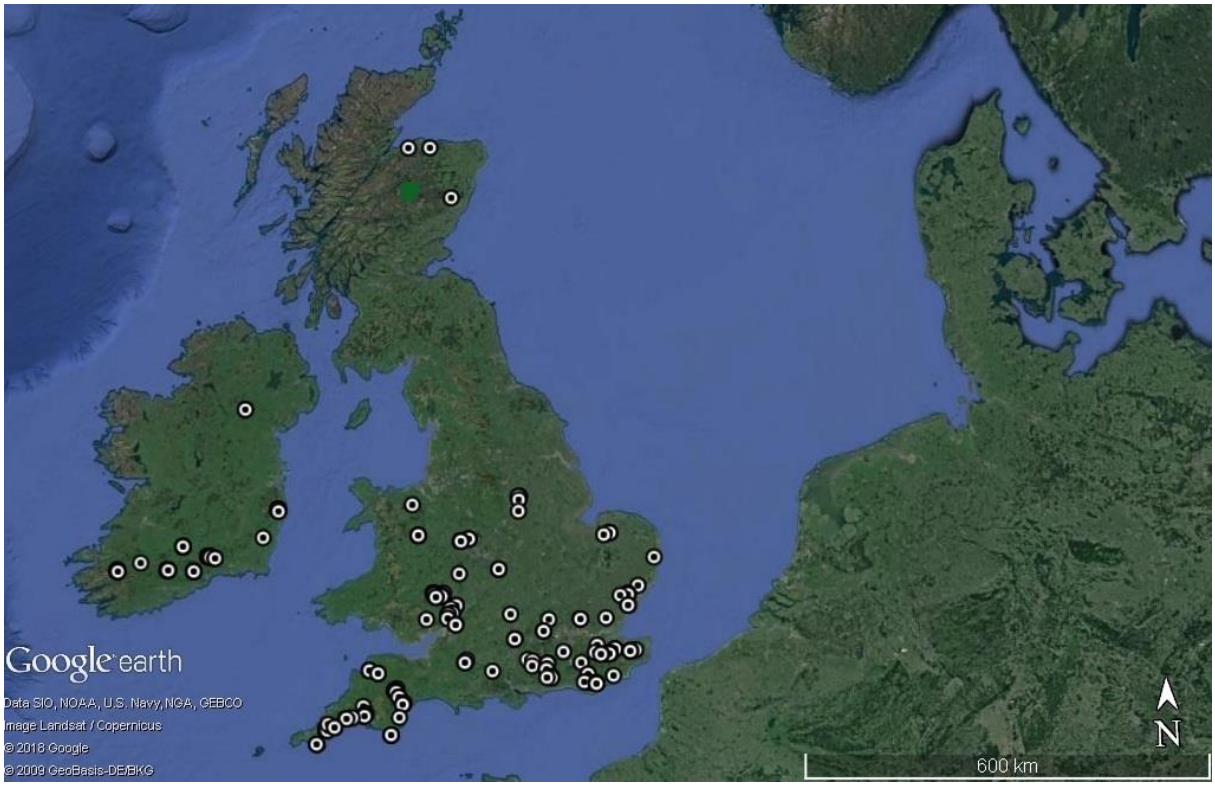
Over the course of the programme, the FTT Sweet Chestnut Group has identified a total of 206 plus trees – 150 from across Britain and 56 from Ireland for its improvement programme. In Britain, the plus tree sites were identified by private forest managers, workers and owners, public forest and NGO site managers, timber merchants, saw millers, researchers and from the Forestry Commission inventory records. Whereas in Ireland, all sites except one in private ownership, were identified from examination of Collite forest inventory data.

The plus trees were variously maiden timber trees in high forest, singled stemmed trees from coppice or coppiced trees. They were either located in long-established woodland (i.e. ancient woodland sites as wooded prior to 1600AD) or recent plantations established in the last century or so. In Britain, the plus trees were located in 108 woods/sites distributed across 73 Estates/properties of which the vast majority (142 plus trees) were located in England. Five plus trees were located on three Estates/properties in Wales and three were located in

Scotland, again on three Estates/properties. In Ireland, the plus trees were located across 20 woods/sites in Eire, all of which were owned by Coillte except for four plus trees on the Cappoquin Estate which is privately owned. A map showing the distribution of plus tree locations used in this study can be found at **Figure 1**. It includes 102 of the British sites and 19 of the 20 Irish sites.

Field surveys to identify the individual plus trees *in situ* and collect graftwood (dormant, one-year old shoots) were undertaken between 2000 and 2014. Data relating to the tree (tree height (m), stem diameter (cm) at 130cm from ground, stem quality traits, timber length (m), branching habit, ranking of tree quality, tree description including estimation of tree age if planting date not known, grid reference, presence of seed) and site characteristics (stand type; aspect; altitude; slope; drainage; and vegetation type) were taken for each plus tree. The data was compiled into an Excel file to create the FTT Sweet Chestnut Plus Tree Database which is held by FTT and the Forest Research. Grid references were converted subsequently to WGS84 Latitude/Longitude for this project. Selected outline information for the Plus Trees is provided in **Table 1**.

Figure 1: Distribution map of British and Irish plus tree sites



Once graftwood was collected, the plus trees were then propagated by grafting onto seedling rootstocks of sweet chestnut. The British trees were propagated initially by East Malling Research, Kent and the Irish trees by Coillte nursery, Kilmacurragh, County Wicklow in Ireland. Grafted plants and graftwood were then exchanged between the countries so that as far as possible, a near full set of grafted plus trees was secured in gene banks (archive collections) in Britain (Shenmore, Herefordshire) and Ireland (Kilmacarragh and Mucklagh nurseries, County Wicklow). These gene banks then provided grafting material for the propagation of seed orchards as well as being a readily available resource for further research and characterisation.

British Historic Collection of Sweet Chestnut

Some 430 veteran and notable sweet chestnut trees, pollards and stools form the British Historic Collection. This collection has been assembled as part of a PhD study examining the origin and cultural significance of sweet chestnut in Britain, using genetic, palaeoenvironmental and historical evidence. The study trees were distributed across 132 sites in England and 14 sites in Wales; Scotland was not included in this survey. The historical context of the sites and the trees has been researched, unlike the FTT plus tree collection. It is on the basis of this historical information that the trees were selected for sampling. Details of the British Historic Collection are not included in this report as they will be reported elsewhere following further work (Jarman *et al*. unpublished results).

Genetic characterisation using microsatellites

Microsatellites are short sequences of DNA consisting of multiple repetitions of a set of base pairs. They can be used as genetic markers when individuals differ in the number of repetitions. In this project they were used to:

1) Provide individual genetic DNA 'fingerprints' for the FTT plus trees analysed.

2) Characterise of the genetic diversity of the FTT plus tree collection and to determine whether the FTT collection sufficient genetic diversity required for an improvement programme.

3) Provide an understanding of the genepools to which FTT plus tree collection and the British Historic Collection belong to and their relationships and thus context with each other and Western European continental populations analysed in previous studies.

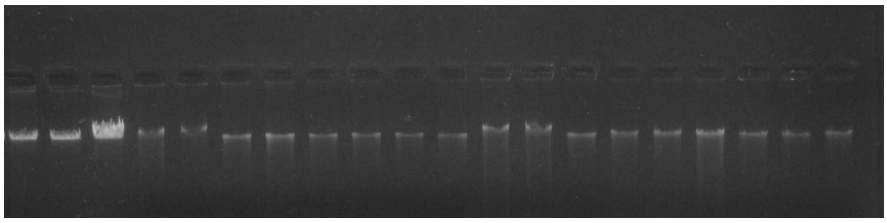
Collection of leaf samples for DNA analysis

During the growing season of 2016, leaf samples were collected from 192 plus trees available *ex situ* variously from the Shenmore gene bank, seed orchard at Torry Hill Estate, Kent or in propagation at East Malling Research, Kent. In contrast, 413 leaf samples were collected *in situ* from the trees forming the Historic collection. From each tree, two leaf samples (one for the DNA analysis and one spare sample) were collected by placing four, 9mm diameter leaf discs into individually labelled Eppendorf tubes. These were placed initially on ice then frozen at -18C until dispatched in dry ice to the IBAF laboratories in Porano, Italy where they remained frozen until DNA was extracted.

DNA extraction and microsatellite analysis

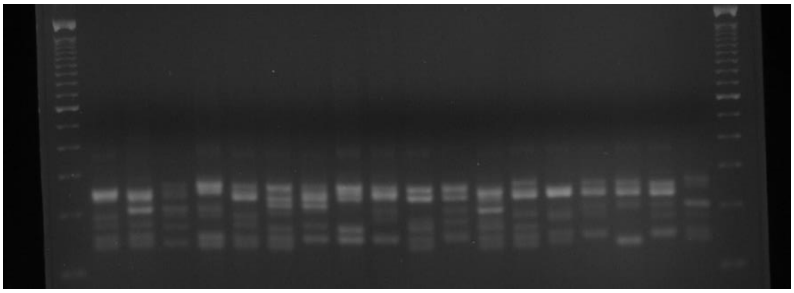
For each tree, total genomic DNA was isolated from 60-50 mg of leaf tissue using the DNeasy 96 Plant Kit (Qiagen) according the manufacturer's instructions. The DNA quality and quantity were checked with agarose gel (1%) electrophoresis (Figure 2) and spectrophotometer measurement. Extraction was repeated if necessary to provide DNA of high quality.

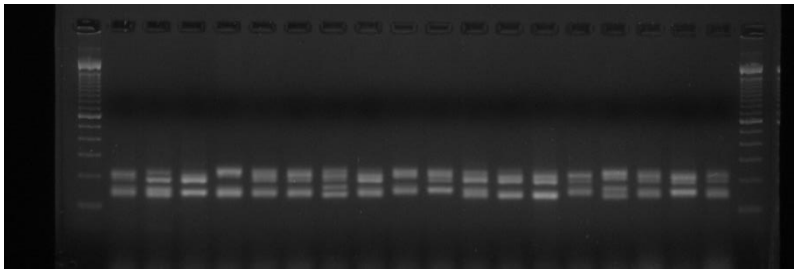
Figure 2: Total genomic DNA



A set of eight, independent, polymorphic microsatellite markers (CsCAT1, CsCAT2, CsCAT3, CsCAT6, CsCAT14, CsCAT16, EMCs25, EMCs38) developed for sweet chestnut (Buck *et al*., 2003, Marinoni *et al*., 2003) was selected and used for the analysis. Multiplex Polymerase Chain Reaction were carried out on a GeneAmp 2700 Thermal Cycler (Applied Biosystems, Foster City, USA). Two multiplex-PCR reactions each with four microsatellites were set up: MIX1 (CsCAT3, CsCAT6, CsCAT16, EMCs38) and MIX2 (CsCAT1, CsCAT2, CsCAT14, EMCs25). The reactions were performed in 15 µl total volume containing 20 ng of genomic DNA following the Qiagen Type- it kit protocol. Cycling parameters were as follows: 15 min at 95 °C; 30 cycles for 30 s at 94 °C, 90 sec at 57 and 1 min at 72 °C; and a final step of 30 min at 72 °C. The efficiency of amplifications was tested on agarose gel (2%) electrophoresis (Figures 3A and 3B).

Figures 3A and 3B: Gel agarose 2% Multiplex PCR





Amplification products (1 µL) were added to 10µL formamide and 0.3 µL LIZ and denaturated at 95 °C for 5 min. The samples were run on ABI Prism 3130 Avant DNA sequencer with a set of known control samples to enable comparison with other datasets. The resulting raw data were analysed with GeneMapper software (Life Techonogies) Figure 4.

Table 1: Outline information for the British and Irish Plus Trees
Note: Plus trees highlighted in grey were not available for DNA analysis.

DNA Sample ID	FRM Accession Number	Tree ID	Woodland/Site	County	Region	Seed Zone	Site type	Total height (m)	DBH (cm)	Quality Ranking	Altitude (m)
FTT 01	csa403022	C. s. Aconbury Hill	Aconbury Hill	Herefordshire	WM	403	PN	33	120	A	220
FTT 02	csa305001	C. s. Arrallas Drive	Arrallas Drive	Cornwall	SW	305	PN	26	65	B-	80
FTT 03	csa405001	C. s. Bergholt A	Spooner's Wood	Suffolk	EE	405	PN	22	94	B-	30
FTT 04	csa405002	C. s. Bergholt B	Warren Wood	Suffolk	EE	405	PN	24	60	B+	30
FTT 05	csa403001	C. s. Big Wood A	Big Wood	Herefordshire	WM	403	AW	16	38	B+	155
FTT 06	csa403002	C. s. Big Wood B	Big Wood	Herefordshire	WM	403	AW	25	36	B	115
FTT 07	csa305030	C. s. Black Forest	Black Forest	Devon	SW	305	PN	21	34	A	50
FTT 08	csa404001	C. s. Blakeney Walk	Blakeney Walk	Gloucestershire	SW	404	AW	28	43	A	140
FTT 09	csa305002	C. s. Boconnoc A	Roughparks Plantation	Cornwall	SW	305	PN	20	44	B-	110
FTT 10	csa305003	C. s. Boconnoc B	Obelisk Plantation	Cornwall	SW	305	PN	24	86	A	120
FTT 11	csa405003	C. s. Boxted Hall	Little Wood	Essex	EE	405	AW	20	68	B-	30
FTT 12	csa403003	C. s. Brampton Hill A	Brampton Hill	Herefordshire	WM	403	AW	22	53	A-	169
FTT 13	csa403023	C. s. Brampton Hill B	Brampton Hill	Herefordshire	WM	403	AW	26	50	A-	160
FTT 14	csa404002	C. s. Brierley	Brierley	Gloucestershire	SW	404	AW	18	48	B	200
FTT 15	csa403004	C. s. Brockhampton A	Brockhampton Park	Worcestershire	WM	403	PN	28	129	B+	205
FTT 16	csa403005	C. s. Brockhampton B	Yeld Wood	Worcestershire	WM	403	AW	32	111	B-	100
FTT 17	csa304001	C. s. Bryngwyn Wood A	Bryngwyn Wood	Powys	MW	304	PN	28	70	A-	130
FTT 18	csa304002	C. s. Bryngwyn Wood B	Bryngwyn Wood	Powys	MW	304	PN	32	58	A	133
FTT 19	csa304003	C. s. Bryngwyn Wood C	Bryngwyn Wood	Powys	MW	304	PN	32+	61	A	160
FTT 20	csa402001	C. s. Burnt Stump Park	Burnt Stump Park	Nottinghamshire	EM	402	PN	26	83	B+	120
FTT 21	csa405004	C. s. Bus Stop Coppice	Bus Stop Coppice	East Sussex	SE	405	AW	15	21	A-	35
FTT 22	csa402002	C. s. Carburton	Carburton	Nottinghamshire	EM	402	PN	27	57	B-	72

FTT 23	csa404003	C. s. Clanna A	Clanna	Gloucestershire	SW	404	PN	26	43	B	90
FTT 24	csa404004	C. s. Clanna B	Clanna	Gloucestershire	SW	404	AW	26	38	B-	80
FTT 25	csa404005	C. s. Clements End A	Clements End	Gloucestershire	SW	404	AW	29	53	A	150
FTT 26	csa404006	C. s. Clements End B	Clements End	Gloucestershire	SW	404	AW	32	45	A	145
FTT 27	csa402003	C. s. Clumber	Clumber	Nottinghamshire	EM	402	PN	20	44	B+	80
FTT 28	csa201001	C. s. Cluny Hill	Cluny Hill	Moray	SCO	201	PN	18	40	C	50
FTT 29	csa305004	C. s. Cockington A	Hellinghay Wood	Devon	SW	305	PN	26	129	A-	75
FTT 30	csa405005	C. s. Cockney Hill Wood	Cockney Hill Wood	East Sussex	SE	405	AW	12	31	B+	35
FTT 31	csa304004	C. s. Coed Orllwyn	Coed Orllwyn	Denbighshire	NWA	304	PN	26	55	A-	110
FTT 32	csa305005	C. s. Cotehele A	Cotehele Wood	Cornwall	SW	305	AW	27	80	B	40
FTT 33	csa305006	C. s. Cotehele B	Cotehele Mill	Cornwall	SW	305	AW	25	120	B-	50
FTT 34	csa405046	C. s. Coxheath Wood	Coxheath Wood	Kent	SE	405	PN	3.5	3	A	110
FTT 35	csa402004	C. s. Cuckney Hay Wood	Cuckney Hay Wood	Nottinghamshire	EM	402	AW	24	53	B-	70
FTT 36	csa404012	C. s. Daniel's Wood	Daniel's Wood	Gloucestershire	SW	404	AW	25	80	A-	40
FTT 37	csa406001	C. s. Dodnash	Dodnash Woods	Suffolk	EE	406	AW	18	32	A	55
FTT 38	csa305007	C. s. Downes A	Hookway	Devon	SW	305	PN	19	41	A	90
FTT 39	csa305008	C. s. Downes B	Hookway	Devon	SW	305	PN	18	46	A-	85
FTT 40	csa305009	C. s. Endsleigh House A	The Dell	Devon	SW	305	AW	25	95	B+	70
FTT 41	csa305010	C. s. Endsleigh House B	The Dell	Devon	SW	305	AW	25	112	B	100
FTT 42	csa405006	C. s. Firth Wood	Firth Wood	Buckinghamshire	SE	405	PN	28	77	B-	110
FTT 43	csa405048	C. s. Frith Wood A	Frith Wood	West Sussex	SE	405	AW	-	110	A+	70
FTT 44	csa405049	C. s. Frith Wood B	Frith Wood	West Sussex	SE	405	AW	25	87	B-	65
FTT 45	csa405007	C. s. Gastons Copse	Gastons Copse	Hampshire	SE	405	PN	25	86	B+	100
FTT 46	csa201002	C. s. Gordon Castle	Gordon Castle	Moray	SCO	201	PK	16	137	C-	40
FTT 47	csa403006	C. s. Greenfield Wood	Greenfield Wood	Worcestershire	WM	403	PK	17	58	B	175
FTT 48	csa405047	C. s. Greybury Wood	Greybury Wood	Kent	SE	405	AW	22	21	B+	110

FTT 49	csa305031	C. s. Hall A	Hawkridge Wood	Devon	SW	305	AW	20	43	A-	30
FTT 50	csa305032	C. s. Hall B	Hawkridge Wood	Devon	SW	305	AW	26	73	B	55
FTT 51	csa404013	C. s. Hawker's Grove	Hawker's Grove	Gloucestershire	SW	404	PN	24	51	A	70
FTT 52	csa405008	C. s. Hermitage A	States Hill Wood	Berkshire	SE	405	AW	29	62	A-	120
FTT 53	csa405009	C. s. Hermitage B	States Hill Wood	Berkshire	SE	405	PN	22	53	B-	80
FTT 54	csa305038	C. s. Homers Down Plantation A	Homers Down Plantation	Cornwall	SW	305	PN	22	71	B-	60
FTT 55	csa305039	C. s. Homers Down Plantation B	Homers Down Plantation	Cornwall	SW	305	PN	25	70	B	70
FTT 56	csa403007	C. s. Hopleys Wood	Hopleys Wood	Herefordshire	WM	403	AW	25	86	B-	110
FTT 57	csa405010	C. s. Howletts A	Howletts	Kent	SE	405	PN	25	69	B	30
FTT 58	csa405011	C. s. Howletts B	Howletts	Kent	SE	405	PN	27	80	B	30
FTT 59	csa405012	C. s. Issac Newton	Isaac Newton	East Sussex	SE	405	PN	20	45	B-	30
FTT 60	csa405050	C. s. Kilsham Copse	Kilsham Copse	West Sussex	SE	405	PN	1.8	-	A	25
FTT 61	csa404007	C. s. Lake Plantation	Lake Plantation	Wiltshire	SW	404	PN	26	58	A-	60
FTT 62	csa405013	C. s. Larkey Valley L35	Larkey Valley Wood	Kent	SE	405	AW	-	-	-	80
FTT 63	csa405014	C. s. Larkey Valley L36	Larkey Valley Wood	Kent	SE	405	AW	25.6	45.2	-	80
FTT 64	csa405015	C. s. Larkey Valley L40	Larkey Valley Wood	Kent	SE	405	AW	26.6	38.2	-	80
FTT 65	csa405016	C. s. Leeds 1	Warren Wood	Kent	SE	405	PN	23	69	B	100
FTT 66	csa405017	C. s. Leeds 2	Warren Wood	Kent	SE	405	PN	28	64	B+	100
FTT 67	csa403008	C. s. Lizard Wood 1	Lizard Wood	Shropshire	WM	403	PN	17	42	B+	155
FTT 68	csa403009	C. s. Lizard Wood 2	Lizard Wood	Shropshire	WM	403	PN	18	60	A	133
FTT 69	csa403025	C. s. Lizard Wood 3	Lizard Wood	Shropshire	WM	403	PN	18	63	B	244
FTT 70	csa305011	C. s. Lodge Wood	Lodge Wood	Cornwall	SW	305	AW	26	47	B-	110
FTT 71	csa305012	C. s. Long Plantation A	Long Plantation	Devon	SW	305	AW	32	69	B+	100
FTT 72	csa305013	C. s. Long Plantation B	Long Plantation	Devon	SW	305	AW	30	55	B+	100
FTT 73	csa305014	C. s. Long Plantation C	Long Plantation	Devon	SW	305	AW	30	60	A	100

FTT 74	csa404008	C. s. Longleat	Cannimore	Wiltshire	SW	404	PN	21	69	B+	50
FTT 75	csa405018	C. s. Luxon Wood	Luxon Wood	Kent	SE	405	AW	25	78	A-	150
FTT 76	csa406002	C. s. Martlesham Plantation	Martlesham Plantation	Suffolk	EE	406	PN	18	50	B-	30
FTT 77	csa405019	C. s. Mill Wood	Mill Wood	East Sussex	SE	405	AW	19	53	C+	110
FTT 78	csa405020	C. s. Mintching Wood A	Mintching Wood	Kent	SE	405	AW	14	16	A+	100
FTT 79	csa405021	C. s. Mintching Wood B	Mintching Wood	Kent	SE	405	AW	13	14	B	62
FTT 80	csa405022	C. s. Nashdom A	Rose Hill	Buckinghamshire	SE	405	AW	26	53	B	50
FTT 81	csa405023	C. s. Nashdom B	Wymers Wood	Buckinghamshire	SE	405	AW	27	89	B	110
FTT 82	csa405024	C. s. Nower Wood	Nower Wood	Surrey	SE	405	AW	25		B+	130
FTT 83	csa405025	C. s. Nutkins Wood	Nutkins Wood	East Sussex	SE	305	AW	23	83	C	50
FTT 84	csa305015	C. s. Obelisk A	Obelisk Wood	Cornwall	SW	305	AW	26	68	B	60
FTT 85	csa305016	C. s. Obelisk B	Obelisk Wood	Cornwall	SW	305	AW	30	57	B-	60
FTT 86	csa305033	C. s. Pencalenick	Hut Plantation	Cornwall	SW	305	PN	26	135	B-	5
FTT 87	csa305017	C. s. Pentillie A	Mount Ararat	Cornwall	SW	305	PN	28	75	A-	150
FTT 88	csa305018	C. s. Pentillie B	Mount Ararat	Cornwall	SW	305	PN	25	63	B+	80
FTT 89	csa404014	C. s. Petridges A	Petridges	Wiltshire	SW	404	PN	24	58	A-	185
FTT 90	csa404015	C. s. Petridges B	Petridges	Wiltshire	SW	404	PN	25	61	B+	180
FTT 91	csa403010	C. s. Plantation A	Plantation	Gloucestershire	SW	403	PN	25	57	B+	150
FTT 92	csa403011	C. s. Plantation B	Plantation	Gloucestershire	SW	403	PN	25	54	A	150
FTT 93	csa303001	C. s. Pontypool Park	Plantation	Torfaen	SWA	303	PK	28	68	B+	50
FTT 94	csa305034	C. s. Powderham A	Powderham Old Plantation	Devon	SW	305	PN	26	141	A-	20
FTT 95	csa305035	C. s. Powderham B	Kenton Common	Devon	SW	305	PN	25	77	B	50
FTT 96	csa305036	C. s. Powderham C	Kenton Common	Devon	SW	305	PN	24	53	A-	55
FTT 97	csa403012	C. s. Rough Hill Wood	Rough Hill Wood	Herefordshire	WM	403	PN	23	42	A	110
FTT 98	csa406003	C. s. Scarles Grove	Scarles Grove	Suffolk	EE	406	AW	22	50	B	26
FTT 99	csa405026	C. s. Scrub Wood 1	Scrub Wood	Kent	SE	405	AW	3	2	B	110
FTT 100	csa405027	C. s. Scrub Wood 2	Scrub Wood	Kent	SE	405	AW	3	2	B	110

FTT 101	csa405051	C. s. Sellings Wood	Sellings Wood	West Sussex	SE	405	AW	22	38	B+	75
FTT 102	csa403013	C. s. Skippet Wood A	Skippet Wood	Herefordshire	WM	403	PN	28	87	B+	180
FTT 103	csa403014	C. s. Skippet Wood B	Held Wood	Herefordshire	WM	403	AW	26	86	A	210
FTT 104	csa403024	C. s. Skippet Wood C	Held Wood	Herefordshire	WM	403	AW	29	97	B+	200
FTT 105	csa202001	C. s. Smithy Wood	Smithy Wood	Aberdeenshire	SCO	202	PN	20	58	B-	200
FTT 106	csa405028	C. s. Snipe Pond Shaw Wood	Snipe Pond Shaw	East Sussex	SE	405	AW	18	21	B+	20
FTT 107	csa406004	C. s. Soigne chestnut	Soigne Wood	Norfolk	EE	406	PN	18	37	A	60
FTT 108	csa404009	C. s. Staple-edge Wood A	Staple-edge Wood	Gloucestershire	SW	404	AW	21	36	A-	190
FTT 109	csa404010	C. s. Staple-edge Wood B	Staple-edge Wood	Gloucestershire	SW	404	AW	23	38	A	170
FTT 110	csa405029	C. s. Stopham Estate A	Stopham Park	West Sussex	SE	405	PN	20	75	B	50
FTT 111	csa405030	C. s. Stopham Estate B	Fittleworth Wood	West Sussex	SE	405	AW	2.5	n/a	A	60
FTT 112	csa403015	C. s. Stretton Hall A	Stretton Wood	Staffordshire	WM	403	PN	24	52	B	120
FTT 113	csa403016	C. s. Stretton Hall B	Stretton Wood	Staffordshire	WM	403	PN	26	42	A	120
FTT 114	csa405031	C. s. Swanton Valley	Swanton Valley	Kent	SE	405	AW	27	84	A-	130
FTT 115	csa305019	C. s. Tapeley Park A	Tapeley Park	Devon	SW	305	PN	25	129	A-	50
FTT 116	csa305020	C. s. Tapeley Park B	Tapeley Park	Devon	SW	305	PN	23	100	B+	35
FTT 117	csa403017	C. s. Thick Thorn Wood A	Thick Thorn Wood	Warwickshire	WM	403	AW	25	152	B-	87
FTT 118	csa403018	C. s. Thick Thorn Wood B	Thick Thorn Wood	Warwickshire	WM	403	AW	24	99	B+	78
FTT 119	csa405032	C. s. Thorrington A	Thorrington Wood	Essex	EE	405	AW	19	40	B	15
FTT 120	csa405033	C. s. Thorrington B	Thorrington Wood	Essex	EE	405	AW	23	45	B+	15
FTT 121	csa405034	C. s. Thrift Wood	Thrift Wood	Essex	EE	405	AW	15	44	B-	50
FTT 122	csa403019	C. s. Timberline A	Timberline Wood	Herefordshire	WM	403	PN	12	50	A	170
FTT 123	csa403020	C. s. Timberline B	Timberline Wood	Herefordshire	WM	403	PN	32	51	B	170
FTT 124	csa405035	C. s. Tolt	The Tolt	Surrey	SE	405	PN	25	47	B+	110
FTT 125	csa305021	C. s. Tregothnan	Simmons's Plantation	Cornwall	SW	305	PN	22	48	B+	40
FTT 126	csa305022	C. s. Trelowarren A	The Warren	Cornwall	SW	305	AW	23	33	B	30
FTT 127	csa305023	C. s. Trelowarren B	The Warren	Cornwall	SW	305	AW	22	35	A-	20
FTT 128	csa305024	C. s. Trew Wood A	Trew Wood	Devon	SW	305	PN	30	64	A+	100

FTT 129	csa305025	C. s. Trew Wood B	Trew Wood	Devon	SW	305	PN	27	63	A-	100
FTT 130	csa405036	C. s. Twenty Acre Wood A	Twenty Acre Wood	East Sussex	SE	405	AW	28	75	A-	25
FTT 131	csa405045	C. s. Twenty Acre Wood B	Twenty Acre Wood	East Sussex	SE	405	AW	30	40	B+	25
FTT 132	csa405037	C. s. Walks Copse	Walks Copse	Surrey	SE	405	AW	15	12	B-	150
FTT 133	csa405038	C. s. Warren Plantation A	Warren Plantation	Essex	EE	405	AW	20	29	B	80
FTT 134	csa405039	C. s. Warren Plantation B	Warren Plantation	Essex	EE	405	AW	28	69	B-	80
FTT 135	csa406005	C. s. Weasenham A	Green Park	Norfolk	EE	406	PN	23	78	A	15
FTT 136	csa406006	C. s. Weasenham B	Great Wood	Norfolk	EE	406	PN	25	105	B-	15
FTT 137	csa402005	C. s. Welbeck	Hatfield Plantation	Nottinghamshire	EM	402	PN	19	50	B+	100
FTT 138	csa305026	C. s. West Prawle Wood A	West Prawle Wood	Devon	SW	305	AW	20	42	B+	50
FTT 139	csa305027	C. s. West Prawle Wood B	West Prawle Wood	Devon	SW	305	AW	22	31.5	B	45
FTT 140	csa305037	C. s. West Prawle Wood C	West Prawle Wood	Devon	SW	305	AW	24	65	A-	35
FTT 141	csa405040	C. s. Weston Common	Weston Common	Hampshire	SE	405	PN	25	54	B	180
FTT 142	csa305028	C. s. Whorley Pin Plantation A	Whorley Pin Plantation	Devon	SW	305	PN	24	36	B+	126
FTT 143	csa305029	C. s. Whorley Pin Plantation B	Whorley Pin Plantation	Devon	SW	305	PN	26	48	B	103
FTT 144	csa405041	C. s. Wickhill Hanger	Wickhill Hanger	Hampshire	SE	405	AW	-	-	B	160
FTT 145	csa405042	C. s. Wilderness Wood 1	Wilderness Wood	East Sussex	SE	405	AW	18	28	B	110
FTT 146	csa405043	C. s. Wilderness Wood 2	Wilderness Wood	East Sussex	SE	405	AW	20	28	B-	120
FTT 147	csa402006	C. s. Windmill Plantation	Windmill Plantation	Nottinghamshire	EM	402	PN	23	41	B+	120
FTT 148	csa403021	C. s. Woodbury Hill	Woodbury Hill	Worcestershire	WM	403	PN	17	31	B	250
FTT 149	csa405044	C. s. Wyatt Wood	Wyatt Wood	East Sussex	SE	405	AW	13	27	B	100
FTT 150	csa404011	C. s. Wytham Wood	Wytham Wood	Oxfordshire	SE	404	AW	25	59	B+	150
FTT 151	csaIRE001	C. s. Ireland Clone 01	Inch Wood	Kerry	IRE	Ireland	PN	23	46.5	B+	45
FTT 152	csaIRE002	C. s. Ireland Clone 02	Inch Wood	Kerry	IRE	Ireland	PN	23.5	33.3	B -	40
FTT 153	csaIRE003	C. s. Ireland Clone 03	Flesk Wood	Kerry	IRE	Ireland	PN	19.5	36.8	C+	40
FTT 154	csaIRE004	C. s. Ireland Clone 04	Flesk Wood	Kerry	IRE	Ireland	PN	20.5	40.6	B+	40
FTT 155	csaIRE005	C. s. Ireland Clone 05	Flesk Wood	Kerry	IRE	Ireland	PN	25	46.5	B+	40
FTT 156	csaIRE006	C. s. Ireland Clone 06	Flesk Wood	Kerry	IRE	Ireland	PN	25	37.4	B+	50

FTT 157	csaIRE007	C. s. Ireland Clone 07	Flesk Wood	Kerry	IRE	Ireland	PN	26	54.3	A	40
FTT 158	csaIRE008	C. s. Ireland Clone 08	Flesk Wood	Kerry	IRE	Ireland	PN	25.5	39	B	45
FTT 159	csaIRE009	C. s. Ireland Clone 09	High Wood	Kerry	IRE	Ireland	PN	25.5	44.8	B+	95
FTT 160	csaIRE010	C. s. Ireland Clone 10	Island Wood	Cork	IRE	Ireland		23	36.2	B	135
FTT 161	csaIRE011	C. s. Ireland Clone 11	Island Wood	Cork	IRE	Ireland	AW	24.5	39	A	135
FTT 162	csaIRE012	C. s. Ireland Clone 12	Johnson Wood	Cork	IRE	Ireland	PN	21	44.1	B	85
FTT 163	csaIRE013	C. s. Ireland Clone 13	Johnson Wood	Cork	IRE	Ireland	PN	24	45.8	A	90
FTT 164	csaIRE014	C. s. Ireland Clone 14	Johnson Wood	Cork	IRE	Ireland	PN	20	37.7	B+	90
FTT 165	csaIRE015	C. s. Ireland Clone 15	Priest Wood	Cork	IRE	Ireland	PN	25.5	42.2	B -	90
FTT 166	csaIRE016	C. s. Ireland Clone 16	Priest Wood	Cork	IRE	Ireland	PN	25	38.4	B	90
FTT 167	csaIRE017	C. s. Ireland Clone 17	Priest Wood	Cork	IRE	Ireland	PN	26.5	54.5	A -	85
FTT 168	csaIRE018	C. s. Ireland Clone 18	Philip's Wood	Cork	IRE	Ireland	AW	25	37.5	B+	70
FTT 169	csaIRE019	C. s. Ireland Clone 19	Philip's Wood	Cork	IRE	Ireland	AW	23.5	79.8	B	120
FTT 170	csaIRE020	C. s. Ireland Clone 20	Philip's Wood	Cork	IRE	Ireland		25	54.5	B	120
FTT 171	csaIRE021	C. s. Ireland Clone 21	Philip's Wood	Cork	IRE	Ireland	AW	26	50.9	B	125
FTT 172	csaIRE022	C. s. Ireland Clone 22	Philip's Wood	Cork	IRE	Ireland		23	44.1	B +	125
FTT 173	csaIRE023	C. s. Ireland Clone 23	Upper Glen	Waterford	IRE	Ireland	PN	25	40.4	B	35
FTT 174	csaIRE024	C. s. Ireland Clone 24	Upper Glen	Waterford	IRE	Ireland	PN	25	40.5	A -	35
FTT 175	csaIRE025	C. s. Ireland Clone 25	Coolnamuck	Tipperary	IRE	Ireland	PN	20.5	25.4	B +	110
FTT 176	csaIRE026	C. s. Ireland Clone 26	Coolnamuck	Tipperary	IRE	Ireland	PN	19	24.1	B	110
FTT 177	csaIRE027	C. s. Ireland Clone 27	Camolin Park	Wexford	IRE	Ireland	AW	25	49.1	B+	100
FTT 178	csaIRE028	C. s. Ireland Clone 28	Camolin Park	Wexford	IRE	Ireland	AW	26	37.8	B-	100
FTT 179	csaIRE029	C. s. Ireland Clone 29	Ballymanus	Wicklow	IRE	Ireland	PN	25	32.1	B	125
FTT 180	csaIRE030	C. s. Ireland Clone 30	Glenealy	Wicklow	IRE	Ireland	PN	26	39	B +	140
FTT 181	csaIRE031	C. s. Ireland Clone 31	Glenealy	Wicklow	IRE	Ireland	PN	29	38.1	B	140
FTT 182	csaIRE032	C. s. Ireland Clone 32	Glanmore	Wicklow	IRE	Ireland	PN	25.5	64	A -	130
FTT 183	csaIRE033	C. s. Ireland Clone 33	Kilshane	Tipperary	IRE	Ireland	PN	26.5	37.1	B	120
FTT 184	csaIRE034	C. s. Ireland Clone 34	Kilshane	Tipperary	IRE	Ireland	AW	25.5	35.2	A -	120
FTT 185	csaIRE035	C. s. Ireland Clone 35	Kilshane	Tipperary	IRE	Ireland	AW	25	46.5	B +	120

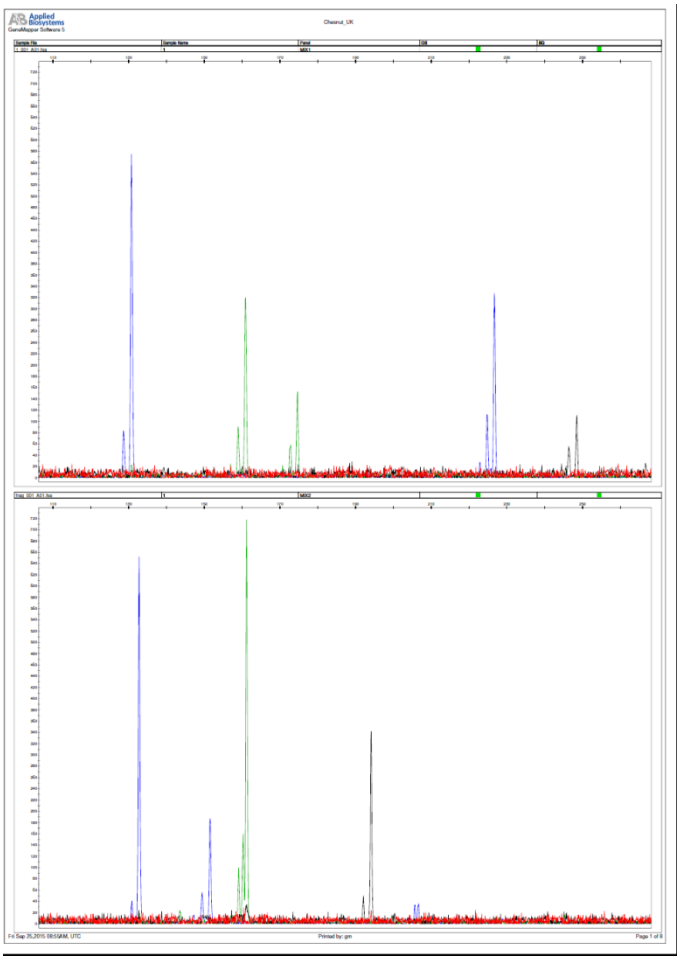
FTT 186	csaIRE036	C. s. Ireland Clone 36	Kilshane	Tipperary	IRE	Ireland	PN	26	37.5	B +	120
FTT 187	csaIRE037	C. s. Ireland Clone 37	Kilshane	Tipperary	IRE	Ireland	PN	26	41.2	B -	120
FTT 188	csaIRE038	C. s. Ireland Clone 38	Cappoquin House	Waterford	IRE	Ireland	PN	25	49.6	B +	50
FTT 189	csaIRE039	C. s. Ireland Clone 39	Cappoquin House	Waterford	IRE	Ireland	PN	27	49.9	B +	50
FTT 190	csaIRE040	C. s. Ireland Clone 40	Cappoquin House	Waterford	IRE	Ireland	PN	25	52.3	B	50
FTT 191	csaIRE041	C. s. Ireland Clone 41	Cappoquin House	Waterford	IRE	Ireland	PN	25	45.5	B -	50
FTT 192	csaIRE042	C. s. Ireland Clone 42	Upper Glen	Waterford	IRE	Ireland	PN	22	43.8	B	90
FTT 193	csaIRE043	C. s. Ireland Clone 43	Upper Glen	Waterford	IRE	Ireland	PN	22	48.8	B +	90
FTT 194	csaIRE044	C. s. Ireland Clone 44	Upper Glen	Waterford	IRE	Ireland	PN	22.5	47.5	B +	90
FTT 195	csaIRE045	C. s. Ireland Clone 45	Gurteen	Waterford	IRE	Ireland	PN	23	51.3	B +	45
FTT 196	csaIRE046	C. s. Ireland Clone 46	Gurteen	Waterford	IRE	Ireland	PN	22	48.2	B	45
FTT 197	csaIRE047	C. s. Ireland Clone 47	Glen	Waterford	IRE	Ireland	PN	27	43.7	A	60
FTT 198	csaIRE048	C. s. Ireland Clone 48	Glen	Waterford	IRE	Ireland	PN	27	38.7	B +	60
FTT 199	csaIRE049	C. s. Ireland Clone 49	Glen	Waterford	IRE	Ireland	PN	27	51	A -	60
FTT 200	csaIRE050	C. s. Ireland Clone 50	Glen	Waterford	IRE	Ireland	PN	26	43.9	A -	60
FTT 201	csaIRE051	C. s. Ireland Clone 51	Glen	Waterford	IRE	Ireland	PN	25	48.3	B +	60
FTT 202	csaIRE052	C. s. Ireland Clone 52	Glen	Waterford	IRE	Ireland	PN	27	45.7	B +	60
FTT 203	csaIRE053	C. s. Ireland Clone 53	Glen	Waterford	IRE	Ireland	PN	25	41.9	A -	60
FTT 204	csaIRE054	C. s. Ireland Clone 54	Thorney Paddock	Monaghan	IRE	Ireland	PN	20	42.2	B+	100
FTT 205	csaIRE055	C. s. Ireland Clone 55	Shepherds	Monaghan	IRE	Ireland	PN	21	37.4	B -	90
FTT 206	csaIRE056	C. s. Ireland Clone 56	Shepherds	Monaghan	IRE	Ireland	PN	19.5	30.8	B	90

KEY

FRM	Forest Reproductive Material	
Region	EE	East England
	EM	East Midlands
	SE	South East England
	SW	South West England
	WM	West Midlands
	NW	North Midlands

	NWA	North Wales
	SWA	South Wales
	IRE	Ireland
	SCO	Scotland
Site Type	AW	Ancient woodland
	PN	Plantation woodland
Quality Ranking	A+ to C-	Exceptional timber quality tree (A+) going down by graded steps to just above average for species

Figure 4: Multiplex analysis using the sequencer ABI-PRISM 3130



Data Analysis

The statistical analysis was undertaken in two stages initially: Firstly all samples as a single group then the samples grouped by their geographic origin (England and Ireland) or collection sites. Null allele frequencies were estimated for each locus and population using the FreeNa software (Chapuis & Estoup, 2007). A set of measures of intra population genetic statistics were calculated using the software GeneAlEx 6.5 (Peakall & Smouse, 2005). Observed (Na) and effective (Ne) number of alleles, observed (Ho) and expected (He) heterozygosis (Nei 1973) and unbiased estimate of mean expected heterozygosis (UHe) were computed. The diversity index of Shannon (I) and the inbreeding coefficient (Fis) for the population over all loci were also calculated. The allelic richness was calculated by the statistical method of rarefaction implemented by the software HP-rare 1.1 (Kalinowski, 2005). The genotypes were analysed to identify any clonal individuals. The genetic distance (GD) among individuals and the Nei genetic distance among groups of individuals were calculated with the software GeneAlex the matrix obtained was used to perform the Principal Coordinates Analysis. The Neighbor Joining Cluster analysis was realized by using the program Darwin ver.6 (Perrier & Jacquemoud-Collet, 2006).

In addition, the FTT samples were also analysed together with samples from the British Historic Collection and 18 populations from western Europe (Portugal (2), Spain (6), France (2), Italy (4), Slovakia (2), Hungary (1) and Romania (1)) to provide an indication on genetic similarity of the UK population with the European germplasm. A Bayesian approach implemented by the software STRUCTURE v.2.3.4 (Pritchard *et al*., 2000) was used. This method attempts to reveal the populations structure by placing individuals in *K* number of

clusters. STRUCTURE was run with the option of including prior information on the spatial location of populations and using the admixture model on the whole dataset and the correlated allele frequencies (Falush *et al.*, 2007). According to the different sampling locations the number of tested clusters (*K*) was from 1 to the putative number of provenance plus 2. Six independent runs were performed for each *K* value, with a burn in period of 10000 steps followed by 105 MCMC replicates. To identify the number of clusters (*K*) that best explained the data, the rate of change on L(*K*) (*ΔK*) between successive *K* values was calculated according to Evanno *et al*., (2005) using STRUCTURE HARVESTER software (Earl & vonHoldt, 2012). The six runs for each simulation were averaged using CLUMPP 1.1.2 software (Jakobsson & Rosenberg, 2007) and represented graphically with DISTRUCT (Rosenberg 2004).

Results

FTT Plus Trees

Of the 192 plus trees for which leaf samples were provided, DNA was successfully extracted and analysed for 189. Multi-locus matches analysis revealed that all samples had a single genotype, i.e. were unique individuals, except for three plus trees: FTT 41, 128 & 129. For these three samples, DNA extraction and PCR analysis was repeated. The result of the three samples sharing one genotype was confirmed and thus they form a clonal group. The microsatellite genotype (genetic fingerprint) for each of the 189 FTT plus trees analysed is provided in **Table 2**. Note that FTT 128 was used as the representative for this group in analyses looking at population structure.

Tables 3 and 4 report the intra population genetic diversity measures considering each locus for all samples and all samples. Analysis of the FTT samples gave a total of 98 alleles for the eight microsatellites. The locus CsCAT3, CsCAT6 and EMCs38 are the most polymorphic displaying a higher number of alleles and higher values of genetic diversity indices (I and He). **Table 4** genetic diversity considering all the samples analysed. The values of I and He are comparable to those observed in the other European populations (Mattioni *et al*., 2017).

Table 3: Genetic diversity for each locus considering all samples

Locus	N	Na	Ne	I	Ho	He	uHe	Fis
CsCAT1	189	9	2.25	1.31	0.598	0.556	0.558	-0.075
CsCAT2	189	13	4.42	1.92	0.741	0.774	0.776	0.043
CsCAT3	189	27	6.16	2.39	0.783	0.838	0.840	0.065
CsCAT6	189	14	7.60	2.19	0.868	0.869	0.871	0.001
CsCAT14	189	6	2.69	1.23	0.635	0.628	0.630	-0.011
CsCAT16	189	9	3.72	1.58	0.704	0.731	0.733	0.037
Cmcs25	189	5	2.33	1.15	0.413	0.571	0.573	0.278
Emcs38	189	15	8.98	2.37	0.926	0.889	0.891	-0.042

N = samples number, Na = observed number of alleles, Ne = effective number of alleles, I = Shannon diversity index, Ho = Observed heterozygosity, He = Expected heterozygosity, uHe = unbiased expected heterozygosity, Fis = inbreeding coefficient.

Table 4: Genetic diversity considering all loci and all samples analysed

	N	Na	Ne	I	Ho	He	uHe	Fis
Mean	189	12.250	4.770	1.770	0.708	0.732	0.734	0.037
SE		2.469	0.902	0.182	0.057	0.047	0.047	0.038

N = samples number, Na = observed number of alleles, Ne = effective number of alleles, I = Shannon diversity index, Ho = Observed heterozygosity, He = Expected heterozygosity, uHe = unbiased expected heterozygosity, Fis = inbreeding coefficient.

An analysis to compare FTT British samples with FTT Irish samples was also undertaken. The values of genetic diversity are provided in **Table 5**. To enable consideration of the differences in the sample sizes, the allelic richness was calculated with the rarefaction method and unbiased heterozygosity. Similar values of genetic diversity were observed in both the FTT British and Irish sample groups. The allelic richness calculated with the rarefaction method was also is similar for both geographical areas.

Table 5: Genetic diversity considering the geographic origin – Britain and Ireland.

GB	**N**	**Na**	**Ne**	**I**	**Ar**	**Ho**	**He**	**uHe**	**Fis**
Mean	141	12.000	4.749	1.773	10.21	0.724	0.738	0.740	0.022
SE		2.390	0.858	0.176		0.054	0.044	0.044	0.036
Ire	**N**	**Na**	**Ne**	**I**	**Ar**	**Ho**	**He**	**uHe**	**Fis**
Mean	48	10.000	4.431	1.669	10.01	0.661	0.700	0.708	0.026
SE		1.637	0.867	0.191		0.069	0.057	0.058	0.052

N = samples number, Na = observed number of alleles, Ne = effective number of alleles, I = Shannon diversity index, Ar = Allelic richness, Ho = Observed heterozygosity, He = Expected heterozygosity, uHe = unbiased expected heterozygosity, Fis = inbreeding coefficient.

The genetic distance amongst individuals and the Nei genetic distance amongst groups of individuals was low and unclear separation based on geographic origin were observed as shown by the dendrogram below (**Figure 5**) and the PCoA analysis (**Figure 6**). Thus the British and Irish FTT samples do not form separate populations.

Table 2: Genotypes for the 189 plus trees analysed with eight microsatellites

Note that FTT41, 128 & 129 have the same genotype

DNA Plus Tree ID	FRM accession number	Microsatellite and loci scores															
		CsCAT1		CsCAT2		CsCAT3		CsCAT6		CsCAT14		CsCAT16		EMCs25		EMCs38	
FTT 01	csa403022	192	221	210	232	224	255	161	161	131	131	140	154	140	150	241	261
FTT 02	csa305001	192	192	216	232	267	270	166	177	131	139	143	143	160	160	243	263
FTT 03	csa405001	192	192	195	216	224	253	179	182	131	139	140	143	140	140	255	257
FTT 04	csa405002	192	192	210	210	224	224	179	182	131	150	128	140	140	150	230	241
FTT 05	csa403001	192	192	210	225	226	252	161	161	131	148	128	128	140	140	239	257
FTT 06	csa403002	174	192	210	210	224	226	177	196	131	160	128	130	140	140	230	257
FTT 07	csa305030	192	192	210	210	224	236	177	194	131	139	124	140	160	160	243	261
FTT 08	csa404001	203	215	195	229	224	226	164	179	139	150	128	140	140	140	230	247
FTT 09	csa305002	192	192	195	210	224	234	161	190	131	160	140	140	140	140	263	263
FTT 10	csa305003	192	192	210	229	224	235	172	177	131	131	130	140	140	150	241	261
FTT 11	csa405003	192	192	216	225	224	235	161	196	139	150	128	140	140	150	241	257
FTT 12	csa403003	192	192	210	232	224	224	161	172	131	139	140	143	140	140	230	257
FTT 13	csa403023	192	192	210	232	224	224	161	177	131	131	138	143	140	150	241	261
FTT 14	csa404002	192	215	216	232	224	261	177	182	131	160	140	140	140	140	247	257
FTT 17	csa304001	206	215	216	232	267	270	177	180	131	139	140	143	140	150	241	257
FTT 18	csa304002	192	215	210	212	234	270	177	196	131	139	143	143	140	140	255	257
FTT 19	csa304003	192	221	232	232	267	270	179	190	139	139	128	143	140	140	255	257
FTT 20	csa402001	192	192	206	210	226	226	161	177	131	131	138	138	140	140	257	263
FTT 21	csa405004	192	192	210	232	195	195	190	194	131	139	128	140	140	150	250	261
FTT 22	csa402002	192	192	214	234	224	257	161	194	139	139	140	140	140	150	241	255
FTT 23	csa404003	192	215	210	214	208	224	177	182	131	150	128	128	160	160	243	255
FTT 24	csa404004	192	192	210	210	224	224	166	182	131	150	128	140	140	140	247	257
FTT 25	csa404005	174	215	212	232	234	234	161	179	131	139	140	143	140	140	230	250
FTT 26	csa404006	192	192	210	210	226	234	166	177	131	131	140	143	140	150	241	255
FTT 28	csa201001	192	203	210	210	226	257	161	182	139	160	140	143	160	160	241	243
FTT 29	csa305004	192	217	216	232	195	259	161	177	148	160	140	140	140	150	261	263
FTT 30	csa405005	192	213	210	216	224	224	161	194	131	148	128	140	150	150	247	250
FTT 31	csa304004	192	221	210	216	246	255	182	182	131	148	128	143	160	160	243	261
FTT 32	csa305005	192	192	216	232	252	253	161	161	131	139	138	143	160	160	230	243
FTT 33	csa305006	174	192	210	216	226	226	161	177	131	139	138	143	140	160	239	243
FTT 34	csa405046	192	215	210	210	226	267	175	194	131	139	130	140	140	147	241	250
FTT 36	csa404012	192	192	212	212	224	224	177	177	131	139	138	140	140	140	255	257
FTT 37	csa406001	192	206	210	210	224	226	161	190	131	139	140	140	140	140	239	261
FTT 38	csa305007	192	217	210	214	252	253	172	177	131	139	140	140	140	150	239	241
FTT 39	csa305008	192	192	210	210	224	224	177	182	131	131	128	140	147	160	237	243
FTT 40	csa305009	192	192	210	210	224	226	182	182	131	139	128	140	140	150	241	257
FTT 41	csa305010	192	213	210	225	224	242	172	194	131	139	140	140	140	150	241	255
FTT 42	csa405006	192	192	210	210	224	257	179	196	131	148	128	130	140	150	241	261
FTT 43	csa405048	203	215	216	218	195	224	164	177	139	139	128	128	140	160	243	255
FTT 44	csa405049	192	221	210	210	208	224	161	194	131	139	138	140	140	140	230	247
FTT 45	csa405007	206	206	214	218	195	259	172	196	131	139	124	140	140	140	239	257
FTT 46	csa201002	192	192	210	214	226	235	161	182	131	131	128	128	140	140	239	257
FTT 48	csa405047	192	215	214	220	224	226	172	177	131	139	140	140	140	150	241	250

FTT 49	csa305031	174	192	222	232	195	224	182	196	131	148	128	140	140	150	241	257
FTT 50	csa305032	192	217	216	232	224	224	161	182	139	139	128	140	140	140	239	257
FTT 51	csa404013	174	192	212	212	226	229	182	194	131	160	128	143	140	150	241	257
FTT 52	csa405008	192	215	210	216	224	257	170	177	131	131	140	140	140	160	243	255
FTT 53	csa405009	192	217	210	216	189	242	177	194	139	150	130	140	140	140	230	259
FTT 54	csa305038	192	192	210	210	224	226	182	182	131	131	138	140	140	140	247	257
FTT 55	csa305039	192	192	212	216	224	224	177	182	139	139	124	130	140	140	257	261
FTT 56	csa403007	192	192	210	212	195	195	164	182	182	131	140	140	140	150	241	261
FTT 58	csa405011	192	192	210	210	224	224	166	166	131	131	128	140	140	150	241	261
FTT 59	csa405012	192	215	210	210	234	253	177	182	131	139	128	140	140	150	239	241
FTT 60	csa405050	174	192	210	210	208	234	179	194	131	139	140	140	140	140	247	250
FTT 61	csa404007	192	206	206	232	195	195	194	196	131	160	128	140	140	150	239	261
FTT 62	csa405013	192	192	210	216	235	252	161	179	131	131	128	140	140	150	241	257
FTT 63	csa405014	192	192	210	210	235	255	172	172	139	148	128	140	140	160	243	257
FTT 64	csa405015	192	192	216	232	195	195	172	182	131	131	124	140	160	160	241	241
FTT 65	csa405016	192	215	210	210	224	236	179	182	131	139	140	140	140	150	239	241
FTT 66	csa405017	215	221	210	210	224	224	161	166	131	139	140	140	140	160	239	243
FTT 67	csa403008	192	192	210	210	224	235	161	182	131	131	128	138	140	150	239	241
FTT 68	csa403009	221	221	210	210	224	242	172	172	131	148	124	140	160	160	243	247
FTT 69	csa403025	192	192	212	232	195	195	161	177	131	139	124	140	140	140	239	250
FTT 70	csa305011	192	221	216	232	252	265	164	190	131	139	140	143	140	140	230	255
FTT 71	csa305012	192	192	212	232	252	267	164	177	131	139	130	140	140	140	255	257
FTT 72	csa305013	192	215	216	232	224	224	177	182	139	139	140	143	140	140	250	257
FTT 73	csa305014	192	206	214	232	224	236	166	194	131	150	128	128	157	157	241	270
FTT 74	csa404008	174	221	210	212	224	257	166	177	139	139	128	140	140	147	237	257
FTT 75	csa405018	192	221	210	210	224	235	164	182	131	131	140	140	140	150	239	241
FTT 76	csa406002	192	192	210	232	224	226	161	177	131	148	128	140	140	140	230	257
FTT 77	csa405019	192	221	210	214	226	234	172	182	131	131	128	143	140	140	230	239
FTT 78	csa405020	174	192	210	234	235	259	177	177	131	139	128	143	160	160	241	243
FTT 79	csa405021	174	192	210	232	235	259	177	177	131	139	128	143	160	160	241	243
FTT 80	csa405022	192	213	210	212	195	235	161	172	150	150	128	128	157	157	237	270
FTT 81	csa405023	192	192	206	210	235	255	161	182	131	139	128	143	140	150	241	263
FTT 82	csa405024	192	192	195	214	195	224	179	182	139	139	128	128	140	140	247	250
FTT 84	csa305015	192	215	232	232	261	270	177	182	160	160	128	128	157	157	241	270
FTT 85	csa305016	192	215	212	232	246	267	166	194	139	139	128	143	140	140	257	261
FTT 86	csa305033	192	215	210	216	224	226	161	196	131	131	128	140	140	150	241	263
FTT 87	csa305017	174	192	210	234	224	235	166	182	131	131	124	140	140	150	241	250
FTT 88	csa305018	215	221	210	232	224	226	166	190	131	139	128	140	140	150	241	257
FTT 89	csa404014	192	192	210	212	224	224	177	182	131	131	130	140	140	157	230	233
FTT 90	csa404015	213	221	214	222	234	235	161	161	131	131	128	130	140	140	257	263
FTT 91	csa403010	192	221	225	232	224	250	161	172	150	160	128	140	140	140	257	261
FTT 92	csa403011	192	213	210	212	195	242	194	194	131	131	138	143	140	160	257	259
FTT 93	csa303001	215	217	210	234	226	235	166	172	131	160	128	138	140	140	239	250
FTT 94	csa305034	192	192	210	210	224	224	180	182	131	148	128	140	140	147	237	257
FTT 95	csa305035	192	192	210	222	224	226	161	177	131	131	128	130	140	140	230	239
FTT 96	csa305036	192	215	210	214	224	267	177	182	131	139	124	138	140	157	241	257
FTT 97	csa403012	192	192	210	212	224	252	161	177	131	139	128	140	140	147	241	243
FTT 98	csa406003	192	203	206	210	224	226	161	177	131	131	128	138	147	160	237	241
FTT 99	csa405026	192	215	210	210	224	252	161	172	131	131	128	140	150	150	241	247

FTT 100	csa405027	174	213	210	212	242	270	177	184	131	131	130	143	160	160	243	255
FTT 101	csa405051	192	215	212	234	226	226	182	194	139	160	128	143	140	140	255	257
FTT 102	csa403013	192	192	206	210	224	224	172	182	131	148	140	145	140	150	241	255
FTT 104	csa403024	192	213	214	222	226	226	161	182	131	131	140	140	140	140	257	257
FTT 105	csa202001	192	192	218	232	224	270	177	190	139	160	140	140	140	140	255	257
FTT 106	csa405028	206	217	210	229	252	253	161	182	131	139	140	143	160	160	243	255
FTT 107	csa406004	192	215	206	210	224	226	161	194	131	131	128	138	147	147	237	237
FTT 109	csa404010	192	206	214	216	224	242	179	190	131	150	124	124	140	140	257	259
FTT 110	csa405029	174	192	210	229	228	235	182	182	131	148	128	138	150	150	241	241
FTT 111	csa405030	174	192	212	216	253	267	177	177	131	139	143	143	140	140	250	261
FTT 112	csa403015	192	203	210	216	224	226	161	194	131	139	128	140	140	140	255	257
FTT 113	csa403016	192	203	210	214	224	226	161	194	131	160	128	140	140	140	255	257
FTT 114	csa405031	192	203	212	225	234	235	182	196	148	150	138	140	140	150	241	255
FTT 115	csa305019	192	203	214	214	224	226	161	182	131	160	138	140	140	140	257	261
FTT 116	csa305020	192	221	210	232	224	224	161	190	139	139	143	154	140	140	261	261
FTT 117	csa403017	192	192	210	225	195	224	166	182	131	131	128	128	157	157	241	268
FTT 118	csa403018	192	192	212	216	224	226	166	182	131	150	128	140	140	157	257	270
FTT 119	csa405032	192	213	195	210	224	228	161	194	131	139	140	143	147	150	241	241
FTT 120	csa405033	192	192	232	232	250	252	172	177	131	131	140	143	140	160	243	255
FTT 121	csa405034	192	221	206	214	195	224	177	177	139	160	140	143	140	140	239	259
FTT 122	csa403019	192	192	195	210	224	235	166	182	131	148	140	140	140	140	247	257
FTT 123	csa403020	192	192	210	220	224	224	161	182	131	131	128	140	147	150	241	255
FTT 124	csa405035	192	221	210	225	195	224	161	179	131	139	128	140	140	160	243	257
FTT 125	csa305021	192	206	210	216	223	224	161	182	131	139	140	140	140	150	241	250
FTT 126	csa305022	192	192	210	212	224	226	166	166	131	139	128	138	157	160	243	270
FTT 127	csa305023	192	221	206	210	226	226	182	190	131	131	140	140	140	140	257	261
FTT 128	csa305024	192	213	210	225	224	242	172	194	131	139	140	140	140	150	241	255
FTT 129	csa305025	192	213	210	225	224	242	172	194	131	139	140	140	140	150	241	255
FTT 130	csa405036	192	215	210	218	224	234	161	172	131	148	140	140	140	140	230	263
FTT 131	csa405045	215	221	210	216	253	261	161	196	139	139	128	143	140	140	250	255
FTT 132	csa405037	192	192	210	212	224	252	161	161	139	148	140	143	140	140	230	241
FTT 133	csa405038	192	206	232	232	224	236	172	194	131	160	128	140	150	157	241	255
FTT 134	csa405039	192	206	232	232	224	236	172	194	131	160	128	140	157	157	241	255
FTT 135	csa406005	192	221	212	232	226	250	161	179	131	131	124	143	140	147	239	241
FTT 136	csa406006	192	219	210	210	224	224	182	196	131	150	128	140	140	140	239	261
FTT 137	csa402005	192	192	216	232	195	224	166	194	139	160	140	140	140	140	239	255
FTT 138	csa305026	192	192	210	216	235	243	161	182	131	148	124	138	140	140	230	255
FTT 139	csa305027	192	192	210	222	226	234	161	177	131	131	140	140	147	150	241	255
FTT 140	csa305037	192	213	210	225	224	226	179	179	148	148	128	130	140	147	241	250
FTT 141	csa405040	192	217	216	216	195	226	182	196	131	131	124	128	140	140	261	263
FTT 142	csa305028	192	215	212	232	226	235	177	177	131	148	124	140	140	140	230	257
FTT 143	csa305029	192	215	212	216	224	253	177	179	131	139	138	143	140	140	255	257
FTT 144	csa405041	192	203	210	222	226	257	166	182	131	139	130	140	140	140	239	250
FTT 145	csa405042	192	215	216	218	224	270	166	179	131	139	140	143	140	140	257	263
FTT 146	csa405043	192	192	210	232	224	248	177	179	131	139	124	130	140	160	243	255
FTT 147	csa402006	192	215	210	214	224	224	161	180	131	139	128	140	140	157	239	270
FTT 148	csa403021	192	203	216	218	195	224	177	179	131	148	128	130	140	140	239	259
FTT 149	csa405044	192	213	212	214	244	253	172	172	131	148	140	145	140	147	237	250
FTT 150	csa404011	215	221	195	210	226	235	166	194	139	160	140	140	140	150	241	261

FTT 151	csaIRE001	192	192	210	210	226	261	182	196	131	131	140	140	150	150	241	261
FTT 152	csaIRE002	192	192	195	206	224	257	161	182	131	160	140	140	157	160	243	255
FTT 153	csaIRE003	192	203	210	234	226	236	161	172	131	131	128	140	140	140	259	261
FTT 154	csaIRE004	192	213	210	210	224	235	161	172	131	131	128	128	140	140	257	261
FTT 155	csaIRE005	192	192	216	232	224	267	177	182	131	131	124	128	140	140	257	257
FTT 157	csaIRE007	192	192	210	216	242	246	182	196	131	139	128	140	147	147	241	241
FTT 158	csaIRE008	192	192	210	212	226	235	161	166	131	131	138	140	140	140	250	257
FTT 159	csaIRE009	192	221	210	214	224	224	177	182	131	131	140	143	140	150	239	241
FTT 161	csaIRE011	192	221	195	210	229	255	166	194	139	148	140	140	140	140	257	257
FTT 162	csaIRE012	192	203	216	216	261	264	177	177	131	148	130	140	140	140	230	239
FTT 163	csaIRE013	192	192	210	216	226	226	177	194	131	131	140	145	140	157	259	261
FTT 164	csaIRE014	192	192	210	216	224	226	182	194	131	139	140	140	140	157	257	270
FTT 165	csaIRE015	192	213	212	232	226	235	161	175	131	148	128	138	140	140	230	257
FTT 166	csaIRE016	192	203	210	212	226	226	177	194	131	148	124	138	140	140	230	261
FTT 167	csaIRE017	192	221	216	216	226	255	177	194	139	148	140	140	140	140	230	255
FTT 168	csaIRE018	192	192	214	232	195	228	177	179	148	148	138	140	140	140	255	257
FTT 169	csaIRE019	192	192	195	210	226	235	177	190	131	148	124	140	140	150	230	241
FTT 171	csaIRE021	192	192	212	212	228	252	177	182	139	148	124	138	140	140	257	261
FTT 173	csaIRE023	192	213	210	210	235	257	182	194	131	148	140	143	140	140	239	261
FTT 174	csaIRE024	192	213	210	222	224	255	166	194	131	131	130	143	140	140	239	255
FTT 175	csaIRE025	215	221	195	212	226	228	177	194	139	139	140	140	140	157	250	270
FTT 176	csaIRE026	192	221	212	216	224	236	161	194	139	160	140	143	140	140	247	263
FTT 177	csaIRE027	192	213	210	212	226	235	172	177	131	131	128	145	140	147	230	261
FTT 179	csaIRE029	192	192	210	212	224	226	179	194	131	131	140	143	160	160	243	261
FTT 180	csaIRE030	192	215	212	216	224	253	175	179	131	139	140	140	140	150	257	261
FTT 181	csaIRE031	192	213	229	232	195	195	172	190	131	148	140	140	140	140	230	239
FTT 182	csaIRE032	192	221	210	212	234	252	172	182	131	139	128	130	140	140	250	257
FTT 183	csaIRE033	192	213	210	210	224	267	161	194	131	139	140	140	140	150	257	261
FTT 184	csaIRE034	192	192	210	210	224	259	166	177	131	131	140	140	160	160	243	261
FTT 185	csaIRE035	192	192	210	210	224	224	166	177	131	131	140	140	160	160	243	261
FTT 186	csaIRE036	192	192	210	210	224	226	161	166	131	139	128	140	140	140	247	257
FTT 187	csaIRE037	192	192	210	210	226	259	166	182	131	131	140	140	160	160	243	261
FTT 188	csaIRE038	192	192	229	232	224	224	161	161	131	131	128	140	140	150	241	257
FTT 189	csaIRE039	192	215	212	214	224	236	172	182	131	150	128	140	140	140	239	255
FTT 190	csaIRE040	192	192	212	214	224	224	161	166	131	150	128	140	140	140	239	255
FTT 191	csaIRE041	192	206	214	234	236	236	161	172	131	150	128	138	150	150	241	255
FTT 192	csaIRE042	192	221	210	232	224	259	161	177	131	148	128	140	140	157	239	270
FTT 193	csaIRE043	192	203	210	210	226	253	161	166	131	139	124	140	140	140	257	257
FTT 194	csaIRE044	192	203	195	210	226	226	166	182	131	131	124	132	157	160	243	270
FTT 195	csaIRE045	203	217	222	232	226	226	166	177	131	131	140	140	140	157	230	230
FTT 196	csaIRE046	192	192	210	212	223	252	177	194	131	139	128	140	140	147	241	263
FTT 197	csaIRE047	192	192	210	210	226	226	166	180	148	148	124	128	140	140	257	261
FTT 198	csaIRE048	192	192	210	222	224	235	177	194	131	131	128	138	140	150	241	257
FTT 200	csaIRE050	192	192	210	210	224	226	166	180	131	131	124	138	140	140	257	261
FTT 202	csaIRE052	192	192	210	218	195	234	161	177	131	160	128	128	140	147	230	237
FTT 203	csaIRE053	192	192	210	210	195	252	172	179	131	131	143	143	140	140	239	239
FTT 205	csaIRE055	192	219	210	212	224	253	194	194	131	148	140	145	140	140	261	263
FTT 206	csaIRE056	192	219	210	212	224	234	177	180	139	148	145	145	140	140	257	257

Figure 5: Dendrogram showing genetic distance using unweighted Neighbour Joining Method. The Irish FTT samples are in red.

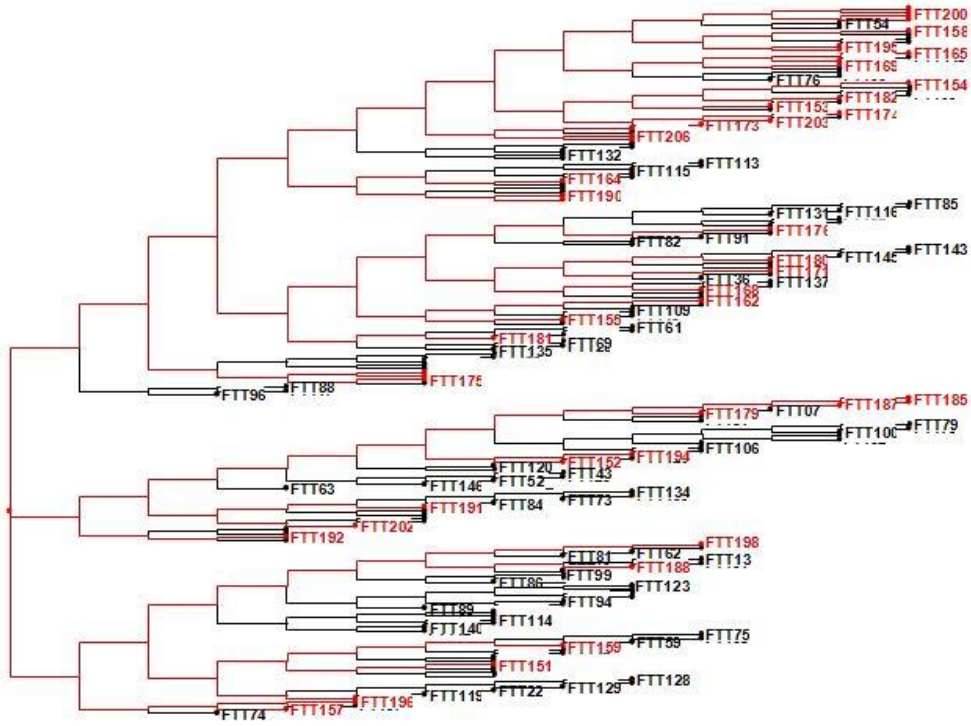
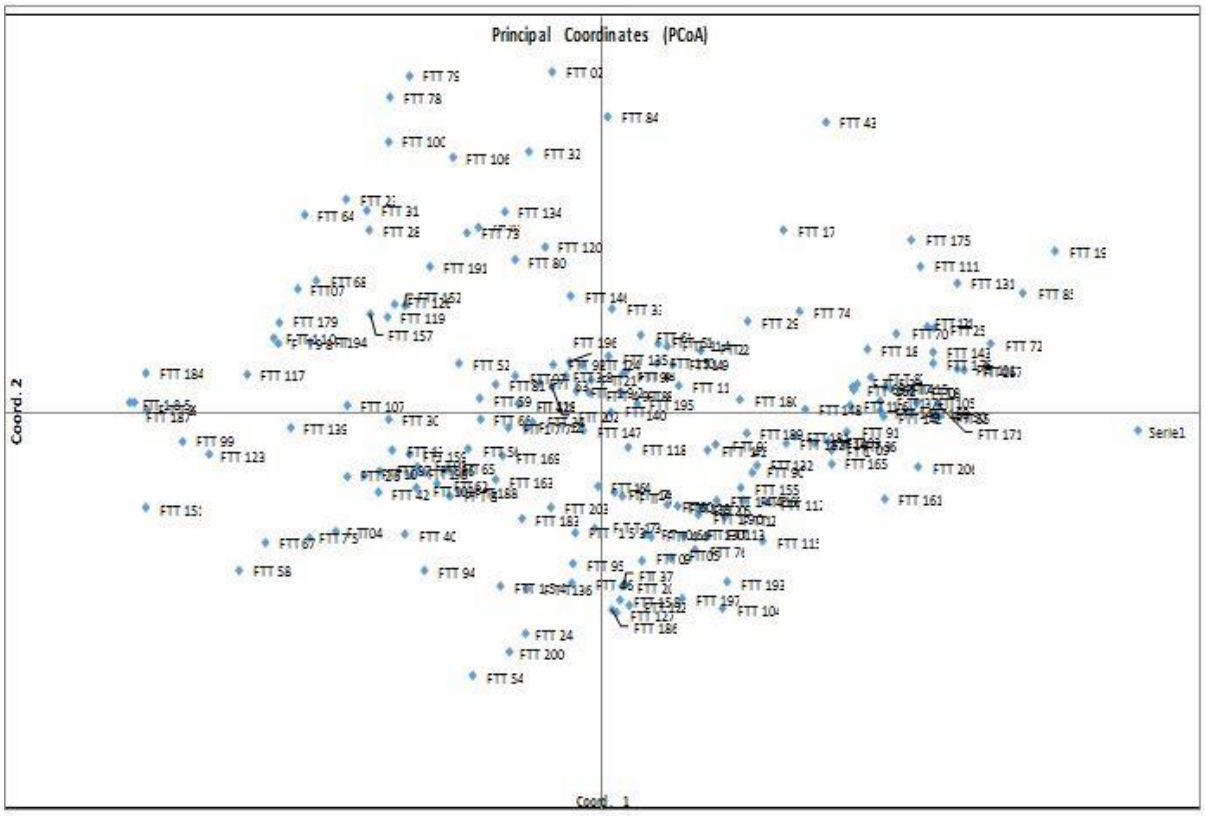


Figure 6: Principal Coordinates Analysis based on the genetic distance matrix



FTT Plus Trees and British Historic Collection

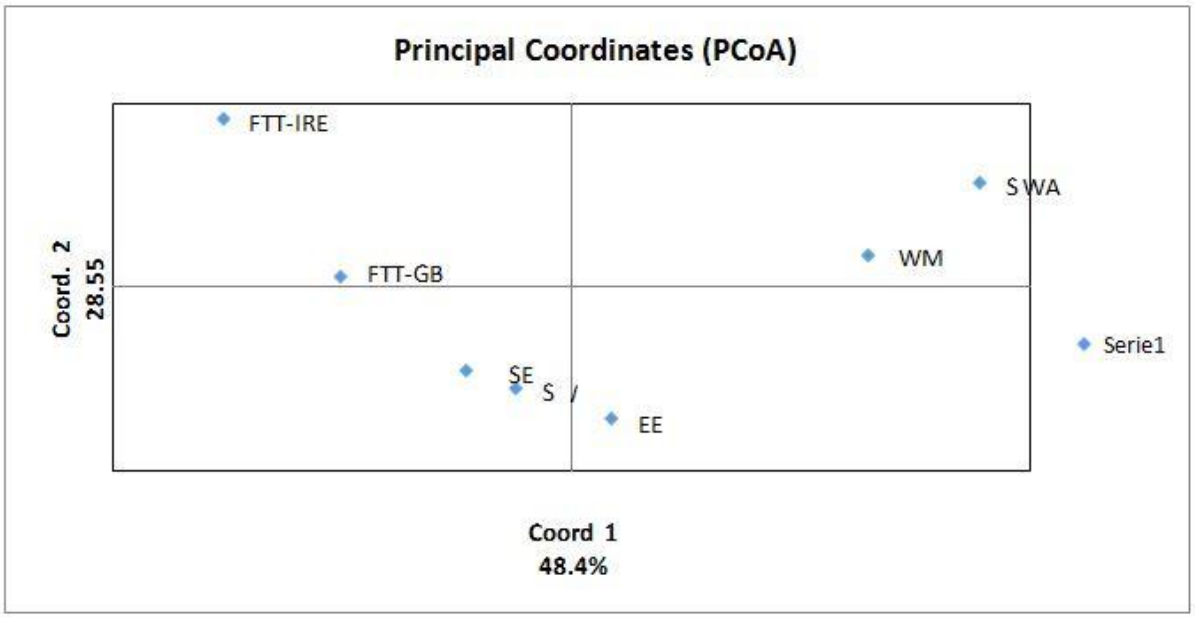
When the FTT sample groups were run against groups of British Historic Collection representing different geographic regions of England, again a low genetic distance was observed amongst the groups (**Table 7**). The corresponding PCoA analysis indicated a higher genetic distance among the Irish FTT group and the other groups from England and south Wales.

Table 7: Genetic distance matrix based on Nei genetic diversity of British Heritage Collection grouped by geographic regions and the FTT sample groups.

	EE	SE	SW	SWA	WM	FTT-GB	FTT-IRE
EE	0.000						
SE	0.006	0.000					
SW	0.013	0.005	0.000				
SWA	0.024	0.029	0.033	0.000			
WM	0.024	0.015	0.010	0.000	0.000		
FTT-GB	0.025	0.007	0.011	0.036	0.022	0.000	
FTT-IRE	0.037	0.022	0.030	0.043	0.039	0.017	0.000

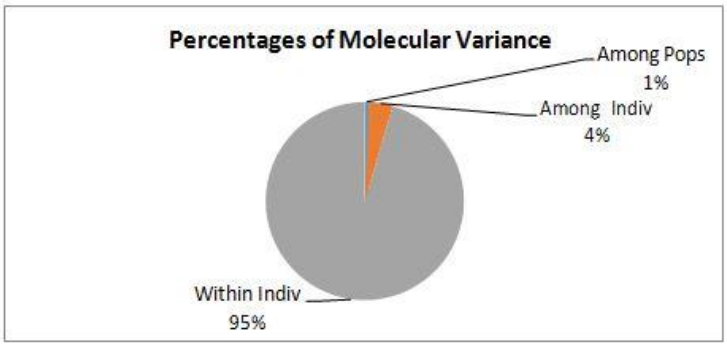
SE = South East England, EE = East England, SW = South West England, SWA = South Wales, WM = West Midlands, FTT-GB = FTT Great Britain, FTT-IRE = FTT Ireland.

Figure 7: Principal Coordinates Analysis based on Nei genetic diversity of FTT Plus Trees and British Historic Collection grouped by country and region respectively.



The AMOVA analysis (**Figure 8 and Table 8**) confirms the low genetic variability observed. The majority of variability was found within individuals, while the percentage of molecular variance among populations was very low (1%).

Figure 8: Molecular variation (AMOVA) of FTT Plus Trees and British Historic Collection.



The F-statistics below provide difference measures (Fst, Fis and Fit) which related to the amounts of heterozygosity at various levels of population structure. Fst value (the effect of subpopulations compared to the total population) at 0.006 is very low. The Fis value is comparable to values seen in the earlier analyses.

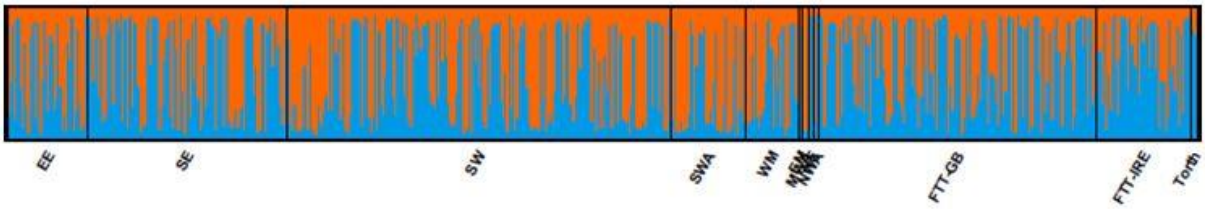
Table 8: Summary AMOVA and F-statistics values for FTT and British Historic Collection samples

Source	df	SS	MS	Est. Var.	%
Among Populations	6	35.183	5.864	0.018	1%
Among Individuals	583	1777.641	3,049	0.116	4%
Within Individuals	590	1662.500	2.818	2.818	95%
Total	1179	3475.324		2.952	100%

F-Statistics	Value	P(rand >= data)
Fst	0.006	0.001
Fis	0.039	0.001
Fit	0.045	0.001

The Structure analysis which considers genepools, highlights the low genetic diversity observed. The highest Delta K value is equal to 2 indicating a most probable grouping of the populations in two clusters (genepools). The high degree of admixture of the two clusters observed, shown by orange and blue, for all the different geographical locations indicates the homogeneity of the germplasm. Each sample (represented by a vertical line in Figure 9) shows a combination of orange and blue, but the proportion of each, thus contribution from each genepool to the individual sample, varies.

Figure 9: Structure analysis providing graphical representation of the two clusters.



SE = South East England, EE = East England, SW = South West England, SWA = South Wales, WM = West Midlands, FTT-GB = FTT Great Britain, FTT-IRE = FTT Ireland, Torth = Tortworth chestnut.

FTT Plus trees, British Historic Collection and European Populations

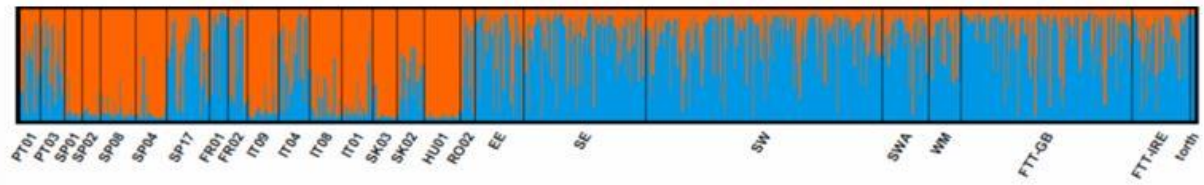
To help clarify the origin of the British & Irish chestnut samples, the Structure analysis was performed with a suite of 18 populations from seven continental European countries: Portugal, Spain, France, Italy, Slovakia, Romania and Hungary (Table 9 and Figure 10). The European populations were selected on the basis of results obtained previously by Mattioni *et al.,* 2017.

Table 9: European populations selected for Structure analysis

Country	Region	Sampling site	ID	Long.	Lat.	N
Portugal	Bragança District	Bragança	PT01	-7.2465	41.2440	17
Portugal	Guarda District	Guarda	PT03	-7.2890	40.7030	20
Spain	Andalucia	Bubión	SP01	-3.3522	36.9461	14
Spain	Cataluña	Castanyet	SP02	2.6300	41.8900	15
Spain	Andalucia	Gaucín	SP04	-5.3094	36.5389	26
Spain	Andalucia	Güejar Sierra	SP05	-3.4306	37.1494	14
Spain	Asturias	Mieres	SP08	-5.7639	43.2169	29
Spain	Andalucia	Trasierra	SP17	-4.8467	37.9194	35
France	Aquitania	Dordogne	FR01	1.0390	44.6871	15
France	Midi-Pyrénées	Aveyron	FR02	2.9175	44.0678	16
Italy	Sicilia	Madonie	IT01	14.0900	37.8300	26
Italy	Marche	Mt. Laga	IT04	13.4100	42.7300	26
Italy	Piemonte	V. Pellice	IT08	7.1400	44.8000	26
Italy	Friuli	V. del Natisone	IT09	13.5600	46.1200	26
Slovakia	Nitra	Jelenec	SK02	18.2221	48.4116	22
Slovakia	Bratislava	Častá	SK03	17.3582	48.4029	20
Hungary	Pest	Nagymasros	HU01	18.9483	47.7924	30
Romania	Baia Sprie	Maramures	RO02	23.6650	47.6640	11

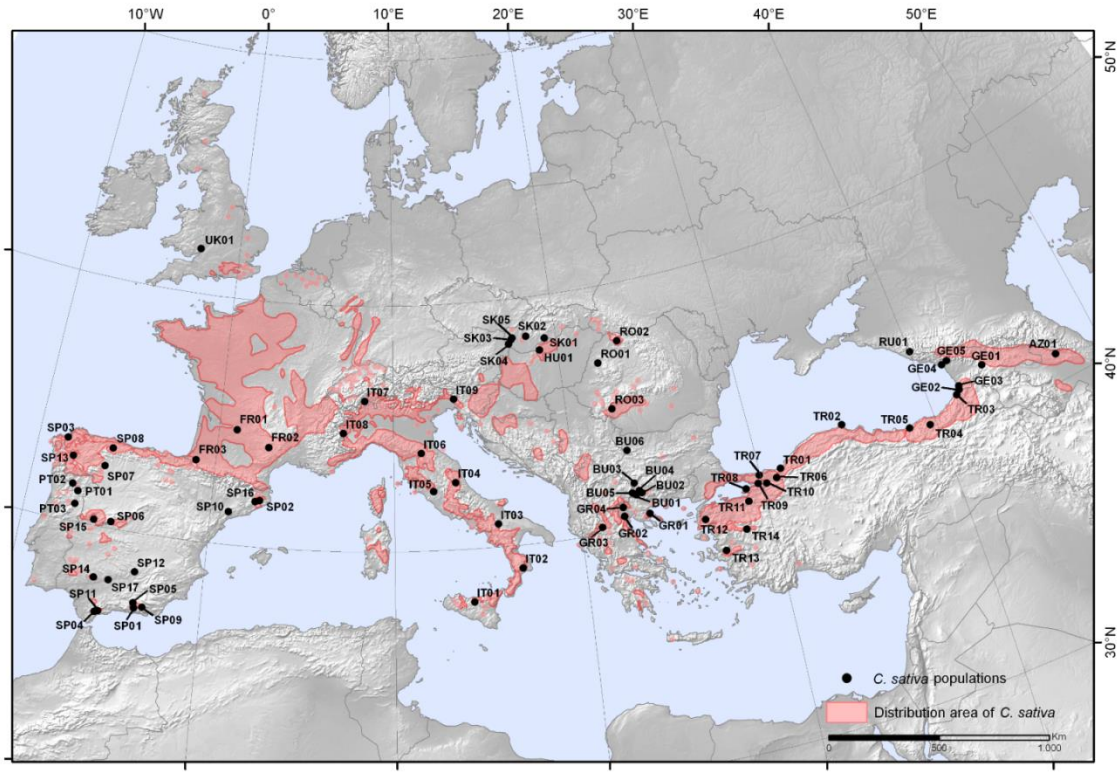
Again, the highest Delta K value is equal to 2 indicating a most probable grouping of the populations in two clusters (genepools). The majority of the British and Irish samples are included in group 2 (blue) showing a higher genetic similarity with Portuguese populations PT01 & PT03, Spanish population SP17, French populations FR01 & FR02 and the Italian population IT04.

Figure 11: Structure analysis providing graphical representation of the most probable grouping of the populations in two clusters (blue and orange).



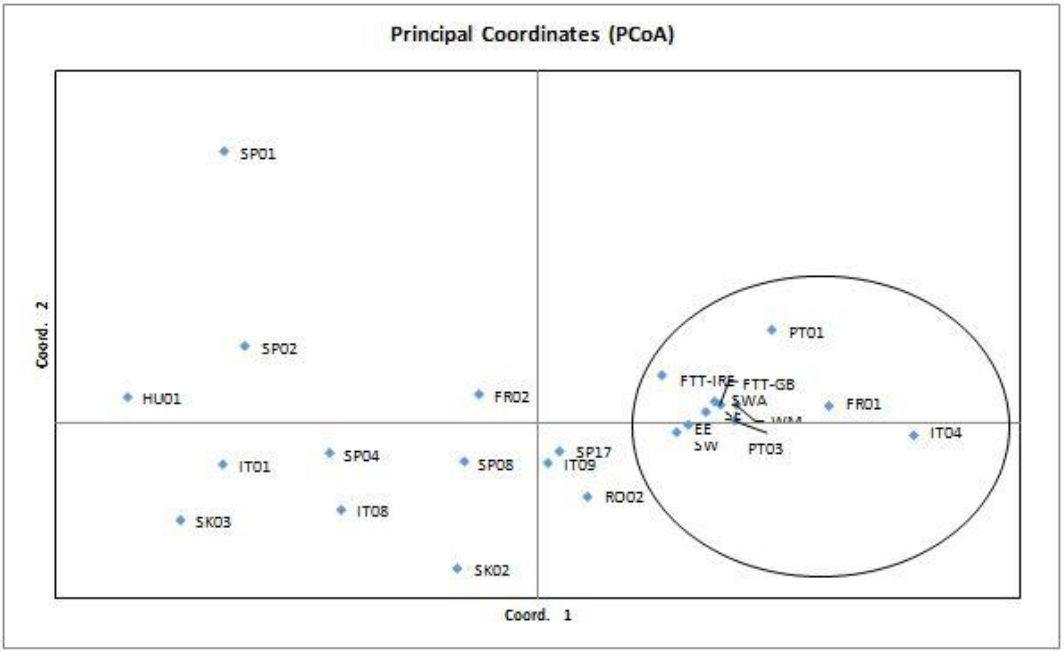
SE = South East England, EE = East England, SW = South West England, SWA = South Wales, WM = West Midlands, FTT-GB = FTT Great Britain, FTT-IRE = FTT Ireland, Torth = Tortworth chestnut. See Table 9 for the European population codes.

Figure 10: Map showing the distribution of European chestnut populations (taken from Mattioni *et al*., 2017).



The Structure results were confirmed by the PCoA analysis based on Nei genetic distance (**Figure 12**) which shows populations which belonged to group 2 (blue) congregating together – see the ringed group.

Figure 12: Principle Coordinates Analysis based on Nei genetic diversity for the FTT Plus Trees, British Historic Collection and selected European populations



The molecular variance amongst populations is 7% whilst the variance within individuals is 92% (**Figure 13** and **Table 10**). The higher variance amongst populations is to be expected given the higher number and greater geographic range of populations included in the analysis.

Figure 13: Molecular variation (AMOVA) considering the FTT, British historic and European populations

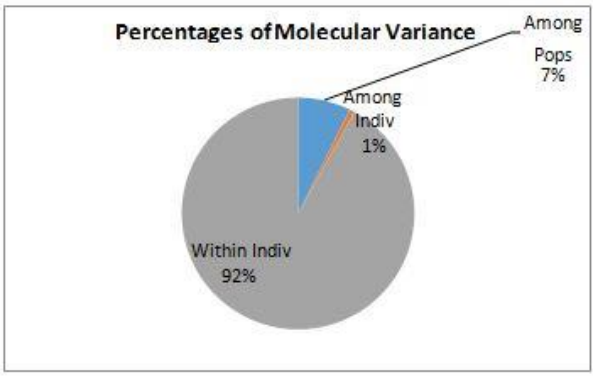


Table 10: Summary AMOVA and F-statistics values considering the FTT, British heritage and European populations

Source	df	SS	MS	Est. Var.	%
Among Populations	23	297.723	12.944	0.145	7%
Among Individuals	941	1739.132	1.848	0.014	1%
Within Individuals	965	1756.500	1.820	1.182	92%
Total	1929	3793.354		1.979	100%

F-Statistics	Value	P(rand >= data)
Fst	0.073	0.001
Fis	0.008	0.114
Fit	0.080	0.01

The results described above are provisional and subject to further development of the datasets following comparisons with a wider set of British historic trees and additional continental European populations. The final output will form a peer reviewed publication which is currently in preparation.

Conclusions and Implications

All the samples analysed gave good and reproducible amplification. The eight microsatellite loci were polymorphic in all samples except for three Plus Trees: FTT 41, 128 & 129. These three plus trees, two from one site and another from a geographically distant site, are accepted as a clonal group rather than the result of the microsatellite markers not have sufficient discriminating power. The set of microsatellites used in this study are known to be highly discriminating as they were selected on the basis of their wide range of polymorphism and are spread across the genome to optimise the capture the genetic information. They have been used extensively in several European studies and are well characterised.

Individual genetic DNA 'fingerprints' for 189 FTT plus trees have been obtained. These can be used to provide genotypes for the individual plus trees which aids their registration for Forest Reproductive Materials purposes. The 'fingerprints' would also help to resolve potential propagation mistakes and identify maternal and paternal contributions to seed lots/seedlings arising from the FTT improvement programme. In addition, the identification of a clonal group (FTT 41, 128 & 129) can be used to ensure that only one representative of the clonal group is included in future seed orchards to prevent over-representation of a genotype.

The characterisation of the genetic diversity of FTT plus tree collection has been undertaken. Based on the number of samples analysed and considering their geographical location it is possible to draw the following conclusions in terms of the genetic diversity of FTT plus tree collection.

1) The values of genetic diversity measured are comparable to those observed in the other European populations.

2) No genetic structure was observed in the FTT samples, i.e. the samples from Ireland (FTT-IRE) are genetically similar to the British samples (FTT-GB). However, the Irish trees appear to show slightly higher genetic distance from the British and Historic trees.

3) The FTT samples are also genetically similar to the British Historic Collection of sweet chestnut from a range of different England and Wales sites. The results of AMOVA indicate that the majority of the genetic variation is at an individual level (95%) rather than within or between populations.

4) Analysis of the population's genetic structure highlights the similarity of the British and Irish germplasm to some Portuguese, Spanish, French and Italian populations in that they share the same probable genepools. All consist of two 'genepools' which are well represented by the British and Irish FTT plus trees, and have a high degree of overlap.

Thus measures of diversity for the FTT samples indicate that a genetic diversity has been captured within the FTT plus tree collection that is representative of the species across its western Eurasian distribution zone. This confirms that the FTT collection has captured sufficient genetic diversity required for an improvement programme and enables work to proceed with confidence without the need for the inclusion of additional sweet chestnut material from a wider geographic area. This is fortunate, as bringing in new material from continental Europe would be very difficult now given the rise in pests and diseases, and the corresponding restrictions on the movement of plant material.

The genetic characterisation has provided unique information for individual FTT plus trees and confirms that the breeding collection captures levels of diversity similar to other European populations. A great result for the improvement programme.

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