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For quality of life

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CGN Report 1

Centre for Genetic Resources, the Netherlands (CGN)

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Report 1

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Preface

This report is the result of a joined project of the Centre for Genetic Resources, the Netherlands and the Forestry Ecology and Forest Management group of WageningenUR. The project was funded by program 436 “Genetic Resources” of the Dutch Ministry of Agriculture, Nature and Food Quality. Additional support was provided by the Forest Ecology and Forestry Management group (WUR). We like to thank Staatsbosbeheer Kootwijk (in particular A. Boonen) and Het Gelders Landschap (especially W. Lammertink & C. van der Genugten) for providing the opportunity to study oak clusters in Maanschoten and at the Wilde Kamp respectively. We like to express our gratitude to Sven de Vries (head Cluster Forest Genetics Resources, CGN). We like to thank Bert Maes for a field trip to Maanschoten and the Wilde Kamp. We also like to express our gratitude to Gert Kranenborg (Alterra) and Leo Goudzwaard (Wageningen University) for their technical assistance in the field and Jan Bovenschen for laboratory assistance (Alterra). Furthermore we like to thank Ad van Hees (Staatsbosbeheer), Rienk-Jan Bijlsma and Sandra Clerkx (Alterra) for their contribution to the discussion, Kristof Haneca (University Gent) for delivering (unpublished) results about dendrochronology and Hans Peter Koelewijn (Alterra) for statistical support.

Paul Copini

September 2005

Summary

This report is about oak clusters, which are found at many places in the Netherlands. It was thought that these oak clusters (up to 12m in diameter) are genetically identical and originated by continuous coppicing over many ages. In this report three main questions were studied: (1) are oak clusters genetically identical, (2) can leaf morphology be used to identify clonal structures of oak and (3) what is the origin of oak clusters. The study sites were situated in a drift sand area (Maanschoten) as well as in an area with pre-glacial material (Wilde Kamp). In both areas micro-satellite analyses were used to describe clones of *Quercus petraea* and *Q. robur* with sizes up to 12.4m in diameter. Some of the studied clusters were genetically identical, while others contain a mixture of genotypes. Leaf morphology analysis show that leaves of stems belonging to different genotypes are sometimes comparable in morphology. Thus leaf morphology analysis can not be used to identify clonal structures. In the drift sand area soil analyses showed that these clusters are overblown. In literature drift sand areas as Maanschoten are described as areas containing oak shrubs. Besides that, it is mentioned these oak shrubs produce new roots when the twigs are overblown (layering). Probably the harsh environment in these drift sand caused the oak grew as shrubs. It is likely that when the drift sand areas were afforestated the oak shrubs became the oak clusters as they can be found nowadays. In historical documents the Wilde Kamp is described as heath land containing oak shrubs. Also the many horizontally growing stems indicate a shrub-like past and maybe an origin in which layering is an important factor. It is likely that when sheep herding became less interesting the oak shrubs became the oak clusters as they can be found nowadays. Since in both areas no indications are found that clonal growth of oak clusters occurred because of coppicing, an alternative hypothesis is formulated: clonal growth of oak could occur because of the shrub-like growth form with many horizontally growing twigs, in combination with the ability of oak to make natural layerings of horizontally growing or overblown twigs.

Samenvatting

In dit rapport worden eikenclusters bestudeerd die in veel plaatsen in Nederland vookomen. Men vermoedde dat deze eikenclusters (tot 12m in diameter) genetisch identiek zijn en ontstaan zijn door eeuwenlang continu hakhoutbeheer. Drie onderzoeksvragen werden geformuleerd: (1) Zijn de clusters genetisch identiek? (2) Kan blad morfologie gebruikt worden om klonale structuren van eik te identificeren? (3) Hoe zijn de clusters ontstaan? Het onderzoek is zowel gedaan in een stuifzandgebied (Maanschoten) als in een gebied met een preglaciale afzetting (Wilde Kamp). De onderzochte clusters bleken genetisch identiek te zijn of een mix van genotypen te bevatten. Bladmorfologische analyses lieten zien dat bomen met verschillende genotypen soms een vergelijkbare bladmorfologie hebben. Dus bladmorfologische analyses kunnen niet gebruikt worden voor de identificatie van eikenklonen. In de stuifzandgebieden hebben bodemanalyses uitgewezen dat de clusters overstoven zijn. In historische documenten worden eikenstruiken beschreven in stuifzandgebieden zoals Maanschoten. Daarnaast wordt vermeld dat deze eikenstruiken afleggers vormen. Waarschijnlijk zorgde het extreme klimaat in de stuifzandgebieden ervoor dat de eiken in struikvorm groeiden. Het is aannemelijk dat de eikenstruiken door konden groeien toen de stuifzandgebieden werden herbebost omdat het klimaat milder werd. De Wilde Kamp wordt in historische bronnen beschreven als een heidegebied waar eikenstruiken in voorkomen. Ook de vele horizontaal groeiende stammen duiden op een ontstaan vanuit eikenstruiken waar mogelijk ook afleggers een belangrijke rol spelen in de horizontale groei. Waarschijnlijk konden de struiken toen de heidevelden niet meer begraasd werden uitgroeien tot de huidige clusters. Omdat in beide gebieden geen aanwijzingen zijn gevonden over een ontstaan uit continu hakhoutbeheer is er een nieuwe hypothese opgesteld: klonale groei van eiken kan geschieden door de struikachtige groeivorm met vele horizontale groeiende takken in combinatie met de mogelijkheid van eik om vanuit deze horizontale takken afleggers te vormen.

1. Introduction

1.1 General introduction

At the end of 2001 a lot of media attention was given to oak clusters (*Quercus petraea* and *Q. robur*). These oak clusters (see Figure 1.1, 1.2 & 4.2) were found in Belgium and in the Netherlands (Maes & Rövekamp, 1999; Rövekamp & Maes, 1998; 2002). Based on morphological similarities it was suggested that these oak clusters are genetically identical. About the origin it was concluded that the (assumed) genetically identical oak clusters were originated by continuous coppicing over many centuries. The largest clusters (circumference: >30m) were thought to originate from the age of Charlemagne or even from the iron age and are thus thought to be up to 1500 years old (Rövekamp & Maes, 2002).

Many oak species like *Q. geminata*, *Q. myrtifolia, Q. chrysolepis*, *Q. havardii* form clonal groups (=genetically identically clusters) (Guerin, 1993; Montalvo *et al.*, 1997; Mayes *et al.*, 1998; Ainsworth *et al.*, 2003). Genetically identical oak clusters originate through vegetative reproduction in which mechanisms like coppice management[1] (Bakker, *et al.*, 2001; Rackham, 2003), layering (Schaars, 1974; Boer, 1857) and grazing/ browsing (Pott & Hüppe, 1991) can be important factors. Also uprooting of trees (Koop, 1987; Rackham, 2003), fire, root suckers and overblowing (Burrichter *et al.,* 1980) or a combination of these factors can induce vegetative reproduction of oaks.

Maes & Rövekamp (1999) and Rövekamp & Maes (1998; 2002) used morphological similarities (visual detection) to identify clones. This method was used with the idea that morphological similarities resemble genetic similarities. The validity of this method has never been studied. The only research performed which made a comparison between morphology and genetics of clones is from Bakker *et al* (2001). Bakker *et al.* (2001) found that a clear separation between clones using leaf morphology was not possible and therefore concluded that leaf morphological characteristics are not suitable to identify clonal structures. Also the genetic identity of stems can not be determined by digging up the root system. Parts of the formerly connecting root system may have died. Also natural grafting between roots of neighbouring stems may occur (Graham and Bormann, 1966). Therefore Bakker *et al.* (2001) concluded that the best method for clone detection is to genotype all stems with molecular markers of which the microsatellite technique is most suitable for this goal.

Maes & Rövekamp (1999) and Rövekamp & Maes (1998; 2002) used a method to estimate the age of the trees based on the stool size as described by Pigott (1989). Tree ring research, to determine the exact age of a tree, is not possible when a tree is regularly coppiced. The reason for this is that the oldest parts of the coppice stool are decomposed and are no longer present. The radiocarbon method can neither be used to determine the exact age. This is because the oldest parts of the moot (=coppice stool of oak) are most likely already decomposed. Pigott (1989) studied the age of coppiced Lime (*Tilia cordata*) stools in England and made a theory about the size of coppice stools in relation to normal trees. According to this theory a Lime coppice stool with a diameter of 6 meter could be 1300 years old! [2] Rackham (2003) also mentioned high ages of coppiced trees; for instance an Ash *(Fraxinus excelsior)* can become 500 to 1000 years old when coppiced (Rackham 2003), based partly on dendrochronological research.

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1 Coppice management is a form of woodland management that provided both fuel and a host of raw materials. The products of oak coppicing were e.g.: laths, tan bark, shingles, break blocks & fuelwood. Normal coppice cycles are between 4 and 28 year (Rackham 2003).

2 These ages are not the age of a "tree" as it is used normally, but it is the period in which a genotype is present at a particular site.

In this study a DNA technique using microsatellites was selected to identify clones. Microsatellites - also known as simple sequence repeats (SSR) - are short tandemly repeated simple sequences like AG AG AG AG… They normally are one to six base pairs in length and are widespread in eukaryotic genomes (Ashley & Dow 1994). Although the mutation rate of microsatellites is relatively high, (Kashi *et al.*, 1997) clones of more than 1000 years old can be detected (Gil *et al.*, 2004; Reusch *et al.*, 1999). The results of the DNA analyses can be compared with the morphological analyses in order to see if clones have a similar morphology and can be identified based on morphological characteristics.

To get more insight in the origin of the oak clusters, many different sources and techniques can be used. Oliver Rackham (2003) states "*different kinds of evidence are complementary. A study based on documents, however scholarly, is bound to be weak unless related to what the land itself has to tell; but it is rash to infer the history of a wood solely from vegetation without support from earthwork or written evidence*". Therefore in this study the following four sources are used:

1. cpDNA haplotype composition

Another DNA technique (PCR-RFLP, *PCR Restriction Fragment Length Polymorphism)* can be used to analyse chloroplast DNA variation in order to get information about the phylogenetic origin. The distribution of *Q. petraea* and *Q. robur* across Europe since the last glacial is well known (Petit *et al.*, 2002). Post glacial migration routes from refugia have been reconstructed with PCR RFLP using chloroplast DNA and palynological data (Petit *et al.*, 1993, 1997; König *et al.*, 2002). König *et al.* (2002) concluded that haplotypes of the Spanish and Italian migration lineage are autochthonous in the Netherlands. Research of the haplotype composition of oak stands at the Veluwe (Buiteveld & Koelewijn submitted) showed that there are four haplotypes considered autochthonous: 1 (Italian), 10 (Spanish), 11, (Spanish), and 12 (Spanish) (haplotype nomenclature as defined by Petit *et al.*, 2002). If the oak clusters have another haplotype than mentioned above, this indicates that the clusters are not part of the autochthonous vegetation and are probably imported and therefore not considered very old. Move over, if a mixture of more than two haplotypes is found in a population this might indicate that genetic material has been (at least partly) introduced (Buiteveld *et al.,* 2005).

2. Dendrochronology

Dendrochronology (study of tree ring patterns) can help to find more information about the origin of the clusters. With the use of dendrochronology the age of stems is estimated. If the stems are not even aged, then it is not likely that these stems are originated by coppice management. Secondly, wood anatomical observations to study the growth pattern of stems belonging to a cluster can give more information about the origin of the oak clusters. Wide rings in the beginning followed by smaller rings are a characteristic for stems which have been coppiced (Spurk, 1992; Vera, 2000; Haneca *et al*, submitted). Also year ring analysis on coppice stools can give information about coppice cycles in the past (Rackham, 2003).

3. Soil analysis

As many large oak clusters are located on these drift sands, it might be that these cluster are overblown (Burrichter *et al.,* 1980). Soil analysis can exclude this.

4. Written sources

Written sources can explain for instance how oaks in the past were managed and what the oaks looked like.

1.2 Historical overview of oak management in The Netherlands

Neolithic time and Bronze Age (5000 BC - 1000 BC)

During this period human influence on forests steadily increased. There are several suggestions that oaks were favoured above other species. First, during the Neolithic time the agricultural systems started a process of soil depletion. On these poorer soil oak is better adapted than other species (Dirkx, 1998). Second, the people started to keep boar. These boars were probably fed with acorns (Ten Cate, 1972). Last but not least there are indications from England that in this period the first oak coppice systems were used (Rackham, 2003).

Iron Age and Roman era (1000BC – 500AD)

During this time the speed of human impact on the landscape seems to increase. On the poor sand soils overexploitation took place (Spek, 1998). This was probably caused by the invention of iron ploughs and by the increasing population. Besides this the Celtic field system was invented, which made "permanent" agriculture possible. These Celtic fields were fertilised with manure and sods. Cattle was kept outside these fields and grazed in the forests (Spek, 1998). Because of the overexploitation, especially in the Veluwe area, the amount of heath land increased enormously (Havinga, 1962)! New uses of oak occurred in this period; the most important one is of course iron melting which needed a lot of (oak) wood for melting. From the Veluwe area it is known that iron industry developed and devastated large parts of the forest which were unable to regenerate (Buis, 1993). During the roman occupation the deforestation took place in such an extent that most parts were now covered by agriculture (Bunnik, 1999). But old land uses like boar-keeping were still common. The largest oak coppice stools which are found at the Veluwe (Maanschoten) are thought to date from this period (Rövekamp & Maes, 2002)

Dark ages/ early medieval (500AD – 1000AD)

During this period the forest area increased once more since the ice age. The Roman Empire had collapsed and the Dutch population was reduced (Dirkx 1998). Because of the tremendous overexploitation of the foregoing period a complete recovery of the prehistoric forest was not possible because of the poorer conditions (Dirkx 1998). The forests, which were developed, were dominated by beech on the rich places and *Q. petraea* and *Q. robur* on the poor places (Pals, 1987) as for instance at the Veluwe.

Late medieval till 20th century (1000AD – 1900AD)

During the middle ages the Dutch population started to grow again in such an extend that in the year 1300 almost all high forest had disappeared (Buis, 1985). After this period the use of forest products increased. Especially oaks were used for different (new) products like fuel wood, boar feeding, but also for timber production (Buis, 1985). Also a lot of timber was needed for shipbuilding. The tan bark industry also needed a lot of oak bark for tannins. Coppice systems were a common forest type in the Netherlands. Between the 12th and the 13th century commons were created (see Buis, 1985 for complete description). These commons had a strict regulation on land management. Unfortunately these commons were unable to stop forest destruction. At the end of this period only 1% of the land area consisted of forest (Spek, 1998). During the industrial revolution (1750-1850) artificial fertilisers and tannins were invented.

Twentieth century till present (1900AD – 2005AD)

During the 20th century a huge increase of forested area occurred. This was mainly done by the State Forest Service, which was founded in 1899 with the purpose to carry out afforestations. A lot of the plant material was imported from other regions. One of the most important changes concerning use of wood happened in this period. Because of the invention of artificial fertiliser and tannins the slow growing oaks were no longer important for their bark. Therefore most of all oak coppice land was replaced by pine plantations or by exotic species as for instance Douglas fir. Fifty years ago still 10,311 ha of oak coppice could be found in the Netherlands (Anonymous, 1966)

while 20 years ago 7085 ha of oak coppice could be found of which 5,626 ha is degraded (Anonymous, 1985). So because of the imported trees and planting material and because of new uses of wood, autochthonous oak populations in the Netherlands became increasingly rare. Only very recently the attitude of foresters changed towards more natural forestry. Multi-functionality became important, which means besides wood production also nature and tourism are important in forest. Following the UNCED conference of Rio de Janeiro in 1992 and the Ministerial Conference on the Protection of Forest, the protection of genetic diversity is increasingly becoming a priority objective for national and international environmental policies. This means that not only indigenous species are worth to be protected but that populations of autochthonous origin are even more important. Research (Maes, 1993; Maes *et al.*, 1996; Rövekamp & Maes, 1998, 2002) indicated that there are still some autochthonous oak populations left in The Netherlands, mostly comprising oak coppice. But this is only a few percent of the 60,000 ha (Dam *et al.*, 1996) of oak forests which are present in the Netherlands nowadays.

1.3 Objective and research questions

Very old oaks might be located in The Netherlands but there are still a lot of uncertainties about the genetic identity and about the origin of oak clusters. Because knowledge about the origin is a prerequisite to determine the age, no special attention is given to age determinations. The following objective and research questions are formulated:

The objective of this study is:

The determination of genotypic variation of oak clusters and conditional means of clonal propagation and regeneration.

The research questions are:

1. Are the single oak stems that form clusters genetically identical?
2. Are morphological characteristics suitable for identifying clonal structures of oak?
3. What is the origin of the oak clusters?
    - Does the cpDNA haplotype composition confirm that these oak clusters might belong to the autochthonous vegetation?
    - Do the oak clusters result from oak stems overblown by drift sand?
    - Do the stems that constitute a cluster establish at the same year?
    - How are areas, which contain oak clusters nowadays, described in historical documents?

Figure 1.1. Photograph of cluster 10 (plot 3) at the Wilde Kamp.

Figure 1.2. Photograph of cluster 5 (plot 1) Maanschoten.

2. Methodology

2.1 Study areas

In the Netherlands large clusters of oak are found at different locations. For instance in the province of Brabant: Loonse en Drunense Duinen (Maes *et al.*, 1996) and in the province of Gelderland: Maanschoten, Kootwijkseveld, Wilde Kamp (Rövekamp & Maes, 2002) and many other places. In this study Maanschoten and the Wilde Kamp are selected. Maanschoten was chosen because this area contains the largest known oak clusters (circumference: >30m) located on drift sand, which are thought to be genetically identical (Rövekamp & Maes, 2002). The Wilde Kamp was selected because this area contains the largest known oak clusters (7m in diameter) on pre-glacial material, which are thought to be of clonal origin (Rövekamp & Maes, 2002).

Maanschoten

This area is located in the centre of the Netherlands in close proximity to the village of Kootwijk. This area contains the largest known oak clusters with a diameter up to 11 meters. The age of the largest clusters is thought to be at least 1500 years old (Rövekamp & Maes, 2002)! The clusters are clearly recognisable because they are situated on the top of small hills. In the 19th and 20th century Maanschoten has been afforested with pine *(Pinus sylvestris).* Between the oak clusters crowberry *(Empetrum nigrum)* and juniper (*Juniperus communis)* are present. The area is owned by "Staatsbosbeheer" (State forest Service).
In this study area one plot of 90*120m was selected. In this plot the positions, species and DBH of 245 oak stems , belonging to eight clusters and all individual oak trees were recorded (DBH > 5 cm). Afterwards a digital map (see Figure 3.1) was made using ArcView® GIS (Environmental Systems Research Institute Inc., Redland, USA).

Wilde Kamp

This area is located to the south of the village of Garderen. In this area an embankment is present with coppice. In the transition area between this coppice woodland and the heath area, many oak clusters can be found. The largest clusters have a perimeter of 25 meter and are thought to be living there since the middle Ages (Rövekamp & Maes, 2002)! The area is owned by "Het Gelders Landschap". In this area five oak clusters were selected. Two clusters are also studied by the ROB (Rijks instituut voor Oudheidkundig Bodemonderzoek) for e.g. soil and pollen analysis (Spek *et al.*, in preparation). Another cluster was selected because this cluster seems larger than the surrounding clusters. In this study area in total 5 plots of 15 * 15 meters each (no clusters larger than this size are found in the Netherlands) were selected. Every plot contained one of the clusters plus surrounding trees. The positions, species and DBH of all oak stems (DBH > 5) were recorded in every plot (in total 126 stems). Afterwards a map (see Figure 3.2) was produced as mentioned in the paragraph about Maanschoten.

2.2 Genotype identification

Sampling

Two leaves of each living stem were collected and stored in the freezer for DNA isolation. In total 287 stems were sampled (176 in Maanschoten and 111 at the Wilde Kamp).

DNA extractions

Total genomic DNA was extracted from dry-frozen leaves using the Puregene kit (Puregene®, Gentra Systems, Minneapolis, USA).

Microsatellites analysis

All stems were genotyped using six microsatellite loci (see Table 2.1). The first four loci were also used by Bakker (2001) to detect clones in oak stands. PCR amplification was performed using the protocol of Streiff *et al.* (1998). Electrophoresis and visualisation of amplification products was performed on polyacrylamide gels using a LiCor 4200 DNA analyser according to Streiff *et al.* (1998). Samples with known allele length were used as a reference.

After microsatellite analysis the samples were placed in groups sharing the same allele lengths. Using the program API-calc.1.0 (Ayres *et al.* 2004) the probability that two individuals, drawn at random, from a population will have the same genotype at multiple loci was estimated. This probability for a natural Dutch oak population based on the first four loci of Table 2.2 is $3x10^{-7}$. With the addition of two variable loci (SsrQrZ**AG11** & SsrQrZ**AG20)** the chance is even smaller. Therefore it was concluded that all identical genotypes belong to one single clone.

Table 2.1. Overview of microsatellites.

Locus	Developed for	Repeat type	References
SsrQpZ**AG104**	*Q. petraea*	$(AG)_{16}AT(GA)_3$	Steinkellner *et al.*, 1997
SsrQpZ**AG9**	*Q. petraea*	$(AG)_{12}$	Steinkellner *et al.*, 1997
SsrQpZ**AG1/5**	*Q. petraea*	$(GT)_{16}(GA)_9$	Steinkellner *et al.*, 1997
MSQ13	*Q. macrocarpa*	$(GA)_{14}$	Dow *et al.*, 1995
SsrQrZ**AG11**	*Quercus robur*	$(TC)_{22}$	Kampfer *et al.*, 1998
SsrQrZ**AG20**	*Quercus robur*	$(TC)_{18}$	Kampfer *et al.*, 1998

2.3 Morphology

Sampling

Rövekamp & Maes (2002) use morphological characteristics, such as leaf morphology, bark morphology and growth form to identify clones. Since bark morphology is age dependent and because of the difficulty finding parameters to describe growth form, this study uses leaf and fruit morphology. Bakker (2001) used five leaves in her analysis for trying to identify clones based on morphology, but without success. For this reason a pilot-study was implemented to find out how many leaves and acorns should be collected to make a distinction between genotypes, using a method proposed by Eckblad (1991). The average difference of the hereunder mentioned characteristics, between three genotypes, was 10% and 3% for leaf and acorn morphology respectively. Because of this it was concluded that on average 20 leaves and 25 acorns are sufficient to make a distinction between genotypes. In total three

stems of every cluster were included in this analysis (=39 stems belonging to 18 genotypes). Leaves (20 per stem) were collected at 7m height from different places of the crown and 25 acorns of three stems, of which the genotypes were studied in paragraph 2.2, belonging to thirteen clusters, were collected. The leaf material was collected during August and September 2004. The acorns were collected in October.

Leaf morphology

In total fourteen leaf characteristics were scored to describe leaf morphology (see Figure 2.1). These characteristics were derived from Rushton (1978, 1983), Kissling (1979) and Bruschi *et al.* (2003) and are commonly used in oak research e.g. Coart *et al.* (2002), Bakker *et al.* (2001) and Kremer *et al.* (2002).

Five dimensional characters were used: lamina length (LL), petiole length (PL), lobe width LW; sinus width (SW) and length of lamina at largest with (WP). WP and LW were measured at the tip of the widest lobe (see Figure 2.1).

Two counted variables were used: Number of lobes (NL); this is the total number of lobes including both sides except the terminal lobe. Note that only lobes with a clear axillary vein will be included. Number of intercalary veins (NV), an intercalary vein was a secondary vein irrigating a sinus and extending at least halfway from midrib to the base of the sinus.

Two categorical variables were included: Abaxial laminar pubescence PU was scored on a scale from 1 to 6 (Kissling, 1980). Basal shape of the lamina (BS) was scored according to Figure 2.1b.

Five transformed variables were taken into account. Lamina shape or obversity (OB): OB = 100 ×WP /LL, Lobe depth ratio (LDR): LDR = 100 × (LW –SW)/LW, Petiole ratio (PR): PR = 100 ×PL / (LL + PL), Percentage venation (PV): PV = 100 ×NV /NL and Lobe width ratio (LWR): LWR = 100 ×LW /LL

Fruit morphology

A total of four characteristics derived of Rushton (1978, 1983) were used to describe the acorn morphology.

Three dimensional characters were used: Peduncle length (PeL) measured from the point of attachment to the first acorn, acorn length (AL) and acorn breadth (AB). The width was measured using the mean of two measurements under an angle of 90 degrees.

One transformed variable was included: acorn shape (AC) = AL/AB

Data analysis

Two types of analysis were performed with NTSYS-pc 2.02k software (Applied Biostatistics Inc., Setauket, New York, USA). Firstly a principal component analysis (PCA) was performed based on standardised data and a correlation matrix. Secondly a cluster analysis was performed using standardised data, Euclidean distance and UPGMA clustering.

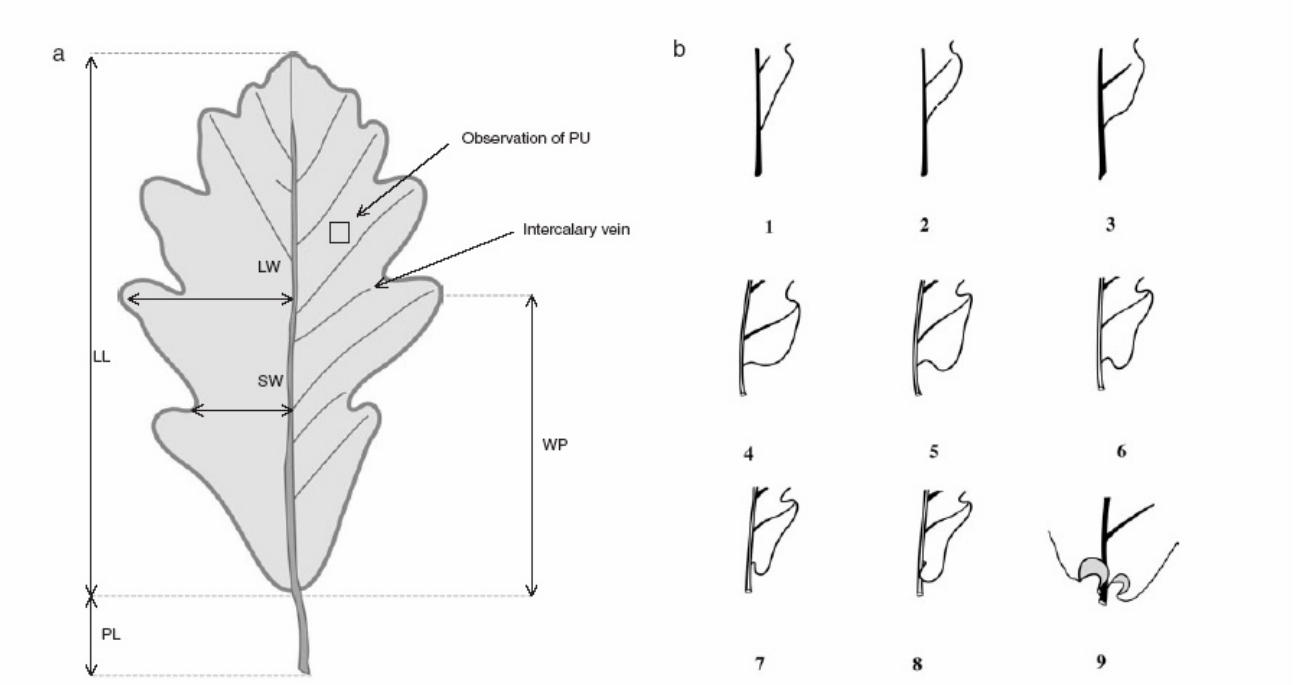

Figure 2.1. Description of leaf morphological characteristics Source: Kremer et al. (2002).

2.4 Origin of oak clusters

cp DNA haplotype composition

Sampling

Based on the result of the first research question, 1 DNA sample per genotype was selected (in total 49 samples).

Haplotype determination

The haplotypes in the plots were identified using PCR RFLP in combination with chloroplast-DNA primers according to the method of Dumolin *et al.* (1995; 1997) and Petit *et al.* (2002). Three cpDNA fragments were used, each in combination with a restriction enzyme: *trn*D/*trn*T with *Taq*I, *trn*C/ *trn*D met *Taq*1 (Demesure *et al.*, 1995) and *trn*T/*trn*F with *Alu*I (Taberlet *et al.*, 1991). The haplotypes were coded according to Petit *et al.* (2002).

Soil profile analysis

Sampling

With a hand soil drill with a maximum drill depth of 2.3m three different locations in Maanschoten (plot 1: see Figure 3.5) were sampled on the hills as well as in between the hills. No soil samples were taken from the Wilde Kamp because these soils were studied already by Spek *et al.* (in preparation).

Soil profile description

The soil profiles were described according to Locher & Bakker (1990).

Age determination

Sampling

With the use of an increment corer two samples of cluster 1 (5 stems), 4 (5 stems) and 9 (6 stems) were taken per tree in opposite directions. One sample per stem was taken of cluster 2 (10 stems), 6 (10 stems). All stems of plot 3 (cluster 10 and surrounding trees: 24 stems) and plot 6 (cluster 13 and surrounding trees: 14 stems). The samples were taken at 40 cm above the stem base, towards the centre. In total 74 stems were sampled (Maanschoten: 30 stems, Wilde Kamp: 44 stems).

Age estimation

To estimate the age of the separate stems belonging to a cluster, the rings on the cores were counted. To make the tree rings clearly visible the surface of the core was cut with a Stanley knife. When taking the cores the pith (=centre of the tree) of the stems was not always hit. The number of missing rings till the pith was estimated taking into account the course and ring width of the tree rings near that pith that are present on the core. To come up with correct age estimation it would be essential to also correct for missing rings due to sampling height of 40 cm. However, because no information is available about height growth of oak in the Wilde Kamp and in Maanschoten, it was not possible to apply this correction.

Historical description

Cadastral maps of 1832 were investigated to find out what the land use was in the research areas. Besides that a literature research was carried out to find historical information about the areas containing our plots nowadays. Also some old inhabitants of Garderen and managers were interviewed.

3. Results

3.1 Genotype identification

In total 13 clusters (287 stems) of *Q. robur* and *Q. petraea* in Maanschoten (8 clusters) and the Wilde Kamp (5 clusters) were genetically analysed. In total 7 clusters (clusters: 2, 3, 4, 5, 9, 10, 12) are genetically identical, 5 clusters contain a mixture of two genotypes (clusters: 1, 6, 7, 8, 13) and one cluster contains 8 genetically different stems (cluster 11) see figures 3.1, 3.2 and Table 3.1). The number of different alleles per microsatellite locus varied between 11 and 25. The probability that two individuals drawn at random will have the same genotype, assuming e.g. absence of mutation and random mating is 7.35×10^{-10}

The 8 clusters (237 stems) and 8 individual trees in plot 1 (Maanschoten) belong to Quercus *petraea* (2 clusters/ 31 stems), *Quercus robur* (6 clusters/ 145 stems) and to 69 unidentified dead stems. In total the examined stems belong to 19 unique genotypes: 17 *Quercus robur* genotypes and 2 *Quercus petraea* genotypes (see Figure 3.1). The maximum diameter between two stems of the same clone in Maanschoten varied between 1.4m (Cluster 6/ genotype 13) and 12.4m (cluster 1/ genotype 1). The number of stems of a clone varied between 2 (cluster 7/ genotype 15) and 33 (cluster 5/ genotype 8). The number of genotypes per clusters was 1 (cluster 2, 3, 4, 5) or 2 (cluster 1, 6, 7, 8). See also Table 3.1.

At the Wilde Kamp five clusters (105 stems) and 21 individual trees in 5 plots were studied. In total these plots included 113 stems that belong to *Quercus robur* and 13 to dead stems. These stems (excluding dead stems) belong to 30 unique genotypes (see Figure 3.2). The maximum diameter between two stems of the same clone at the Wilde Kamp varied between 0.9m (cluster 13/ genotype 49) and 8.9m (cluster 9/ genotype 20). The number of stems belonging to a clone varied between 4 (cluster 13/ genotype 49) and 27 (cluster 9/ genotype 20). The number of genotypes per cluster varied between 1 (cluster 9, 10, 12) and 8 (cluster 11). Table 3.1 shows information about all clusters: Genotype numbers per cluster, number of stems belonging to a genotype, number of dead stems within clusters, average DBH stems belonging to a genotype, maximum distance between stems of same genotype, average age stems belonging to a genotype and number of stems used for age estimation of stems belonging to a genotype.

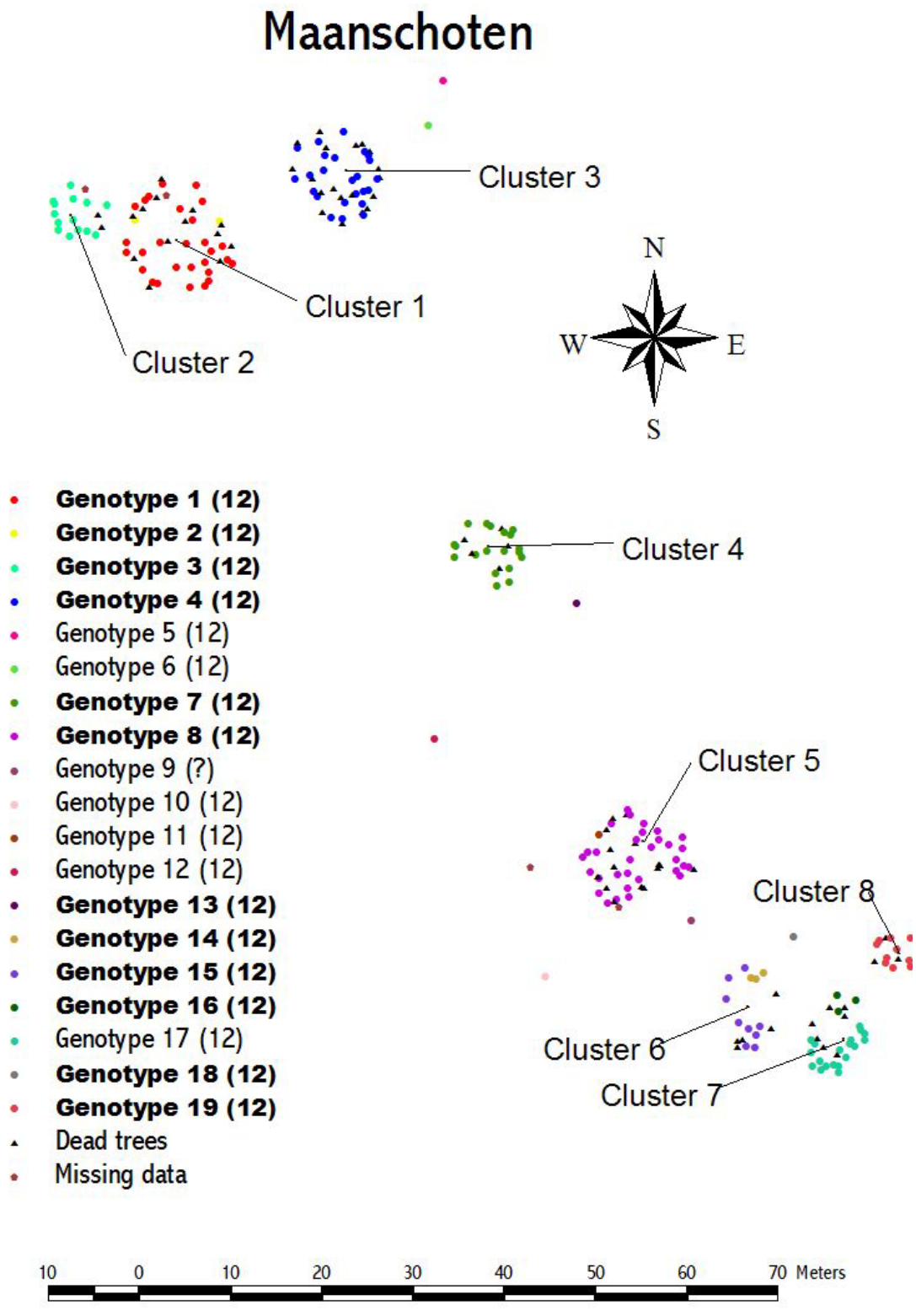

Figure 3.1. *Spatial distribution of genotypes in Maanschoten. In plot 1, 19 genotypes belonging to 8 clusters and 8 individual trees are present. In the legend the genotypes belonging to the clusters are given in bold. Within brackets the cpDNA haplotype number of a genotype is given. Genotype 7 and 18 belong to Q. petraea the other genotypes to Q. robur.*

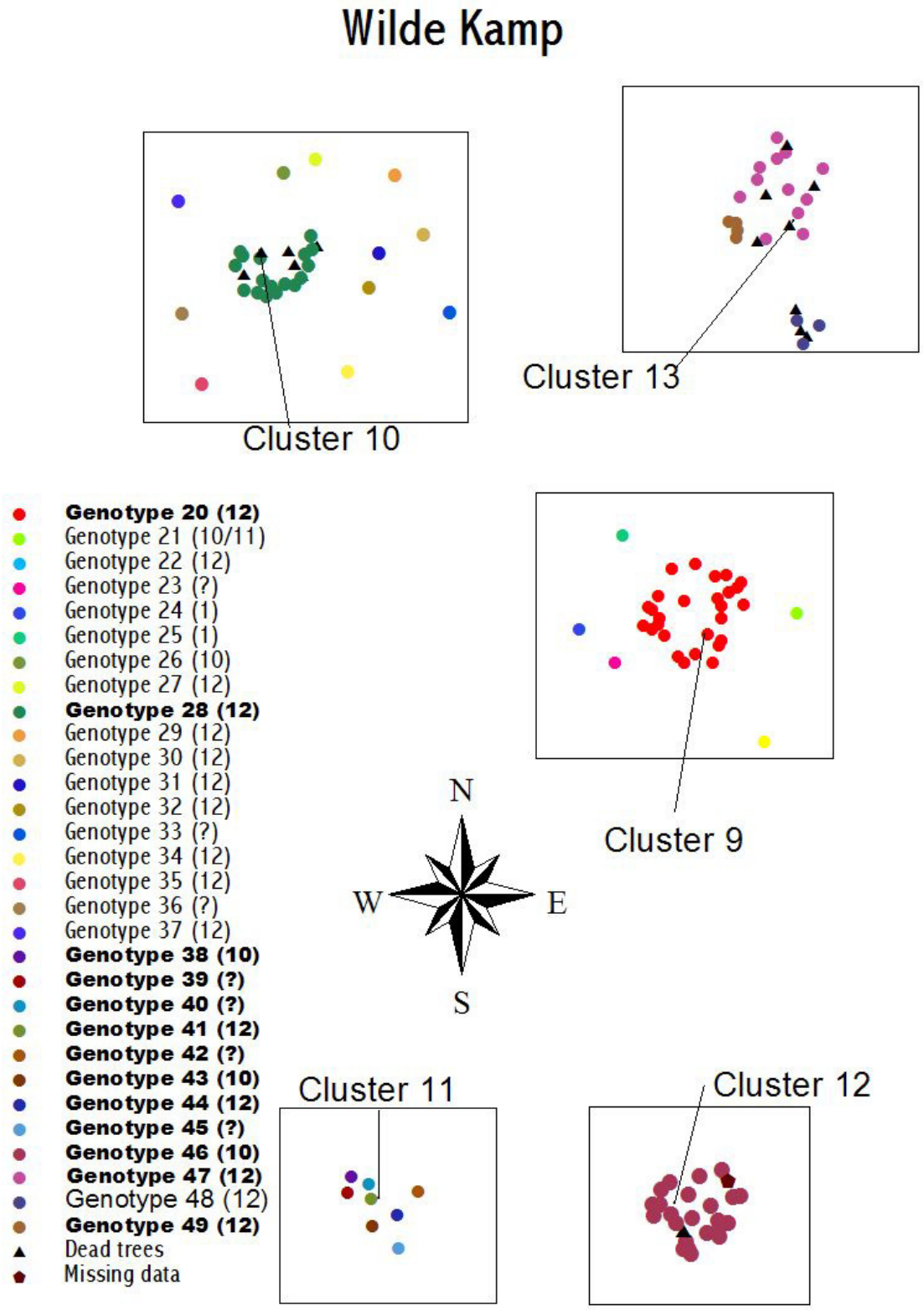

Figure 3.2. *Spatial distribution of genotypes at the Wilde Kamp. In the 5 plots (Plot 2-6 containing cluster 9-13 respectively) at the Wilde Kamp 30 genotypes are present. Note that only the relative position between the clusters is given. The genotypes belonging to the clusters are shown in bold (legend). Within brackets the cpDNA haplotype number of a genotype is given. All genotypes belong to Q. robur.*

Table 3.1. Overview data clusters

Cluster nr.	**1**		**2**	**3**	**4**	**5**	**6**		**7**		**8**		**9**	**10**	**11**	**12**	**13**	
Cluster located in plot nr.:	1		1	1	1	1	1		1		1		2	3	4	5	6	
Genotype nr. per cluster	1	2	3	4	7	8	13	14	15	16	18	19	20	28	38-45	46	47	49
Number of stems belonging to genotype	28	2	14	24	19	33	3	9	2	19	11	1	27	17	1 per gen.	22	12	4
Number of dead stems within cluster	13		2	21	5	15	5		7		3		0	6	0	1	5	
Average diameter (DBH) of genotype (cm ± SD)	22.5 ± 4.4	23.6 ± 2.3	22.2 ± 6	19 ± 5.4	24.3 ± 8.8	21.7 ± 5.4	29.9 ± 6.1	24.2 ± 7.6	23.7 ± 0.2	27.5 ± 6.9	18.9 ± 6.8	41.6	21.2 ± 7.9	17.14 ± 4.0	n.d.	20.4 ±7.8	20.2 ± 9.6	19.5 ± 7.5
Max. distance between stems of genotype in m.	12.4	9.3	6.12	10.4	7.8	11.7	1.4	8.8	6.8	2.2	4.9	n.d.	8.9	5.5	n.d.	3.8	5.1	0.9
Average age of stems of genotype (yr ± SD)	93.3 ± 1..2	96	93.2 ± 1.9	n.d.	95.3 ± 1.7	n.d.	92 ± 1.4	91.8 ± 1.6	n.d.	n.d.	n.d.	n.d.	72.0 ± 4.3	81.8 ± 1.0	n.d.	n.d.	75.8 ± 7.9	58.0 ± 14.9
Number of stems in age estimation of genotype	3	1	6	n.d.	4	n.d.	2	6	n.d.	n.d.	n.d.	n.d.	6	12	n.d.	n.d.	4	3

3.2 Morphology

Principal component analysis (PCA) of 14 leaf morphology characteristics of 39 stems (18 genotypes) resulted in separation between the two species but did not reveal a unique clustering of stems of the same genotypes (see Figure 3.3). The first axis is responsible for the separation of the two species. All 14 morphological characteristics show high loadings except for LWR.

The same results were obtained using a cluster analysis (see Figure 3.4). Ten clones are clustered together while genotype 3 and 16 are separated by other genotypes. The first two branches split the group in *Q. petraea* and *Q. robur* as is indicated with the arrow.

Also fruit morphology was included in this study. Since there were only enough acorns found in two clusters (3 genotypes), acorn morphology was not included in the PCA and cluster analysis.

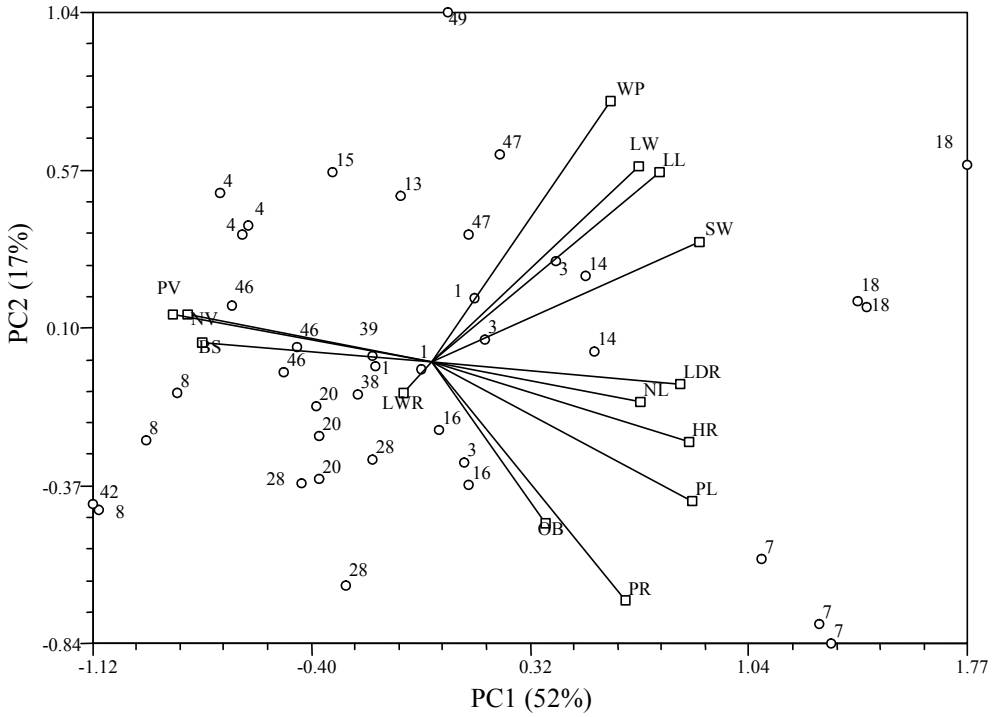

Figure 3.3. Scatter plot of principal coordinates of 39 oak stems belonging to 18 genotypes in combination with the eigenvectors of 14 leaf morphological characteristics. The genotypes are numbered and are the same as described in paragraph 3.1. The first and second dimensions explain respectively 52% and 17% of the variance. Genotype 7 + 18 belongs to Q. petraea while the other genotypes belong to Q. robur.

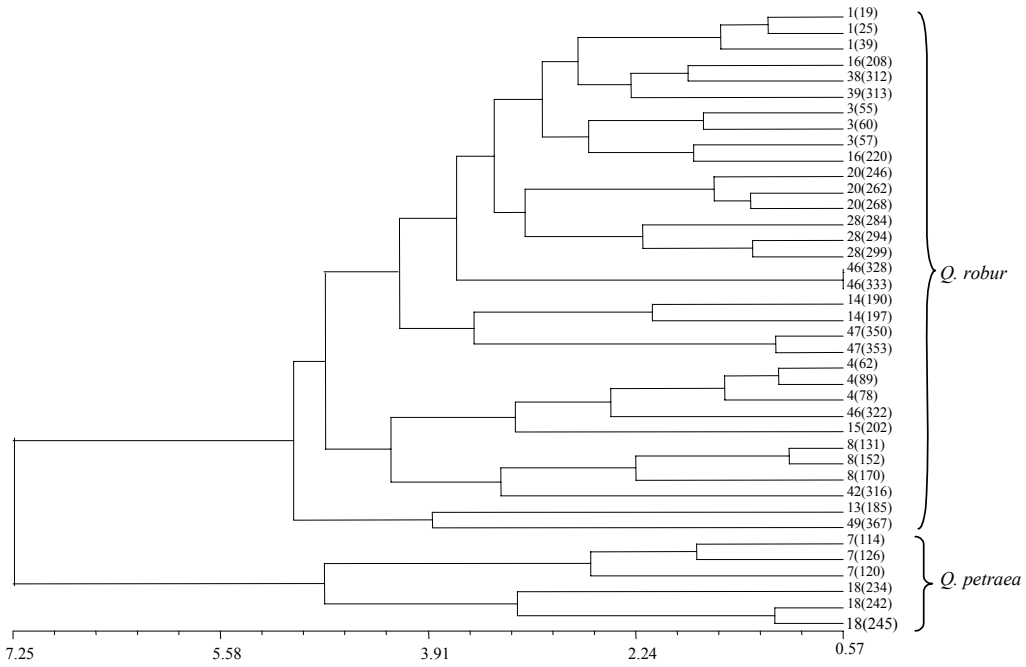

Figure 3.4. Phenogram of the 39 studied oak stems, belonging to 18 genotypes, based on 14 leaf morphological characteristics. The genotypes are the same as described in paragraph 4.1. The stem numbers are given within parenthesis. The two different species are indicated by braces. Genotypes 7 + 18 belong to Q. petraea while the other genotypes belong to Q. robur.

3.3 Origin of oak clusters

Haplotype composition

In Maanschoten all 19 investigated genotypes have the Spanish haplotype 12 (see Figure 3.1). At the Wilde Kamp the haplotypes 1, 10, 12 were found (see Figure 3.2). The clusters in these plots consisted of haplotype 10, 12 or both. Haplotype 1 was found in the surrounding trees.

Soil profile

In Maanschoten three soil profiles were described (see annex I), which were sampled at different places; as well as in the clusters as in between the clusters (see Figure 3.5). All of them contain buried profiles. In profile 1 and 3 second A layers were found at a depth of 220 and 185cm respectively. In profile 2 a buried B mix layer was found 55 cm under the surface. In sample 2 and 3 the overblown profile contained a red soil layer (see annex I) while the new profile contains only yellow, grey or brown sand layers. The texture of the sand varied between medium fine sand and fine sand with sometimes gravel (<5mm).

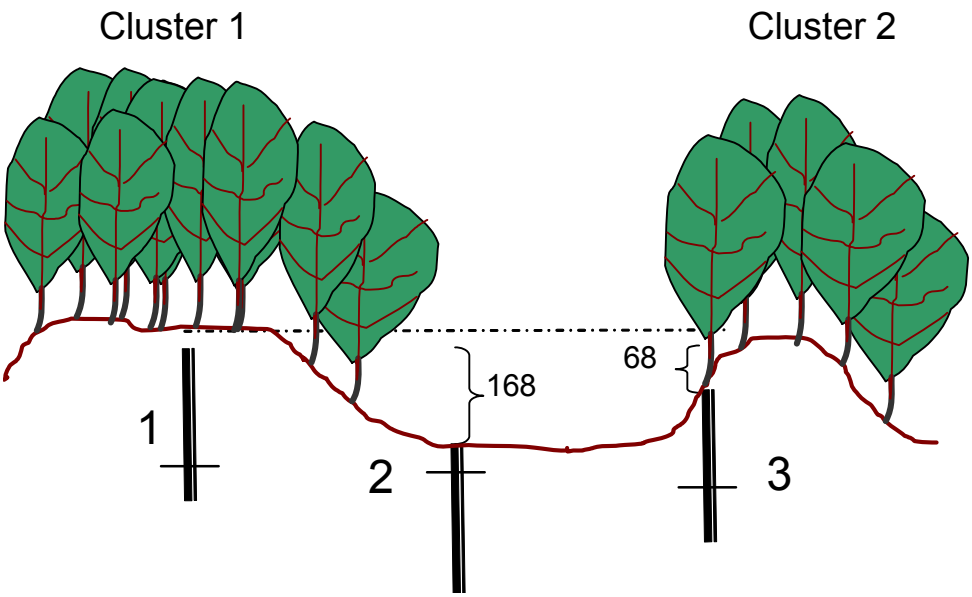

Figure 3.5. Positions of soil profile 1-3. The difference in height between profile 1 and 2 is 168 cm and between profile 1 and 3 68 cm. The depths of the buried profiles are indicated with the horizontal lines.

Age estimation

In Figure 3.6 – 3.12 the age of the stems is shown in combination with the spatial structure of the sampled stems. Note that in Figure 3.6 – 3.9 (Maanschoten) only clusters are shown in comparison to Figure 3.10 -3.12 (Wilde Kamp) where plots (cluster and surrounding trees) are shown.

Five stems of cluster 1 (Figure 3.6) and 4 (Figure 3.8) were sampled and six stems of cluster 9 (plot 2: Figure 3.10). All stems of cluster 2 (Figure 3.7), cluster 6 (Figure 3.9), plot 3 (Figure 3.11) and plot 6 (Figure 3.12) were tried to be sampled. Unfortunately, because of frost damage, this was not always successful.

The stems of the four investigated clusters in Maanschoten (1, 2, 4, and 6) have ages between 86 and 96 years with an average of 93 year. The clusters at the Wilde Kamp are more variable in age. The six stems of cluster 9 (Figure 3.10) are estimated at ages between the 63 and 73 year while the stems of cluster 10 (Figure 3.11) are all about 84 years old. The individual trees surrounding cluster 10 are all younger. The stems of cluster 13 (Figure 3.12) belong to 2 genotypes. Genotype 47 has the age of around 80 years. The genotype 49 (4 stems) consists of stems with the age of 50 till 75 years old. In Table 3.1 the average age in combination with the standard deviation is shown.

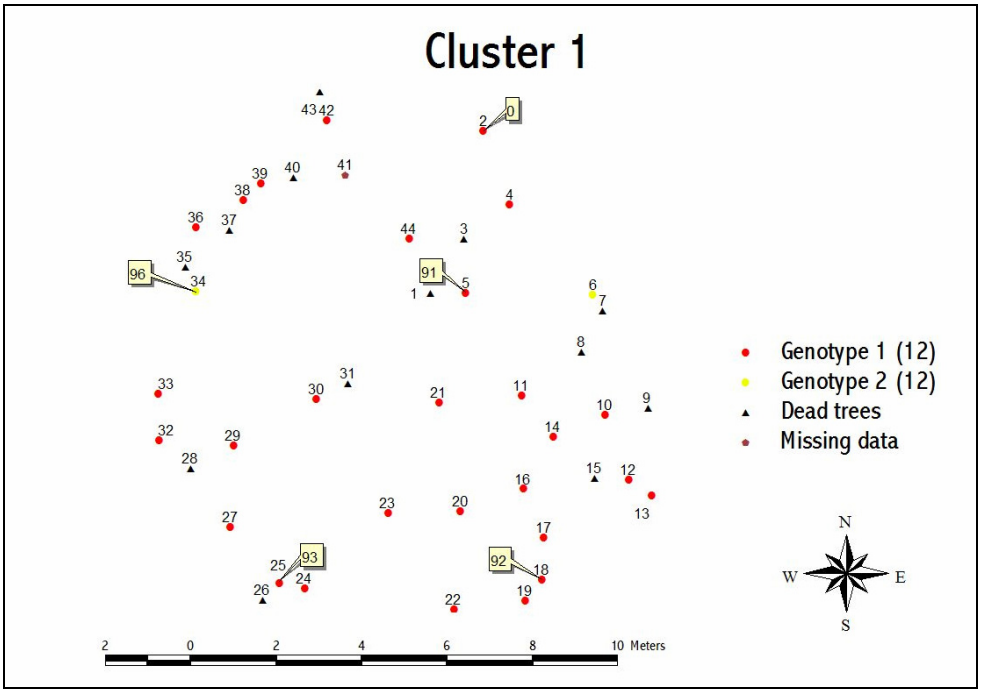

Figure 3.6. *Ages (flagged) in combination with genotypes and haplotypes of cluster 1 (plot 1: Maanschoten). Age 0 means not successfully determined. Genotypes are indicated with different colours. Between brackets the haplotypes are shown. The stems are numbered from 1 to 43. Location cluster centre in State Survey Co-ordinates (SSC): 179700, 467847.68.*

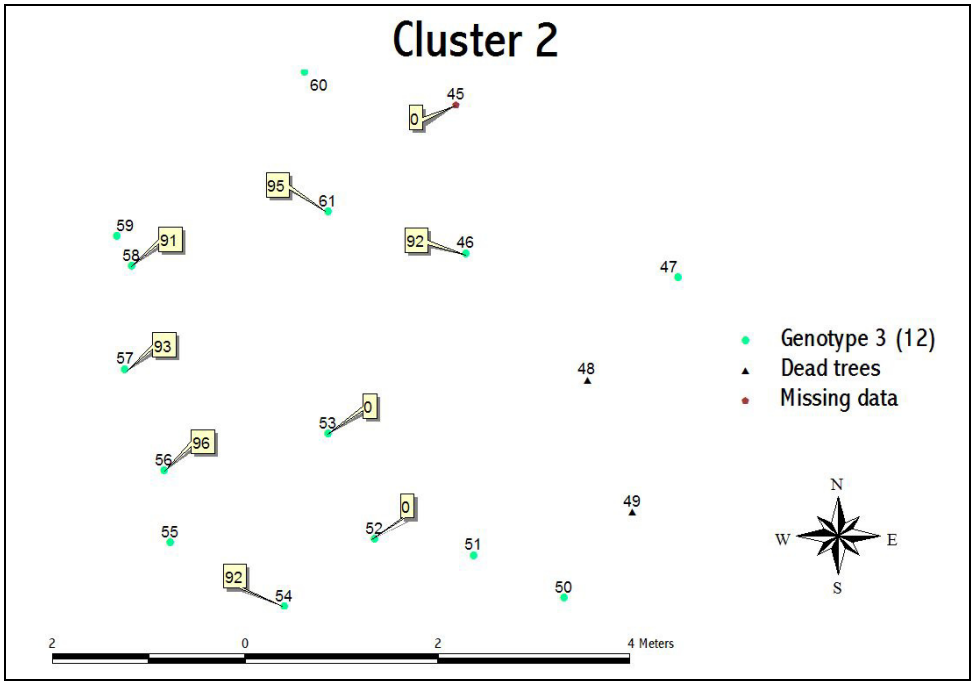

Figure 3.7. *Ages (flagged) in combination with genotypes and haplotypes of cluster 2 (plot 1: Maanschoten). Age 0 means not successfully determined. The genotype is indicated with colour. Between brackets the haplotype is shown. The stems are numbered from 45 to 61. Location cluster centre in SSC: 179786.77, 467848.50.*

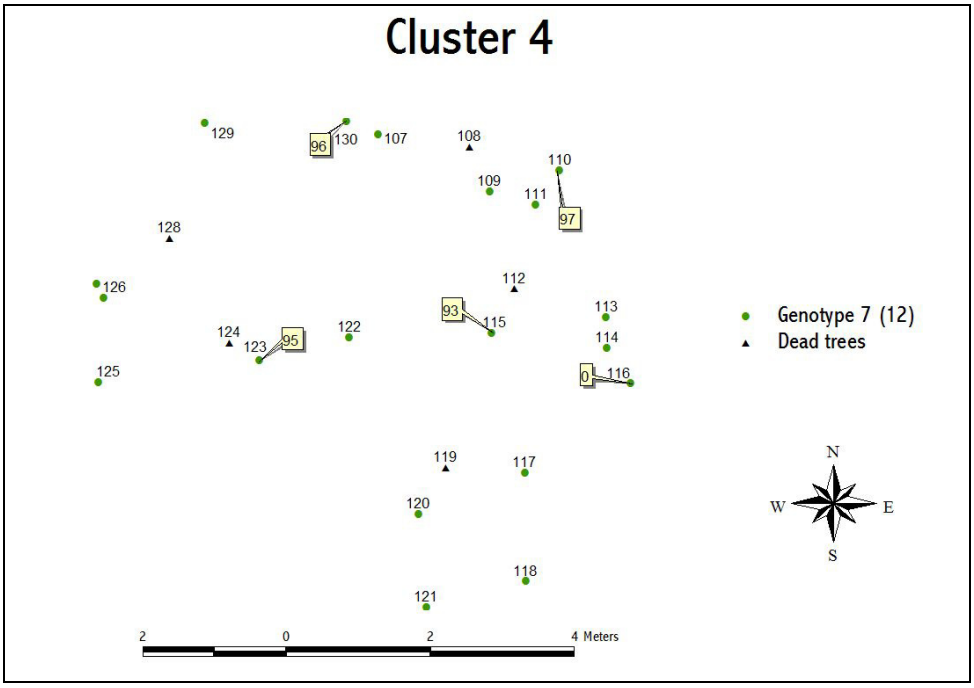

Figure 3.8. *Ages (flagged) in combination with genotypes and haplotypes of cluster 4 (plot 1: Maanschoten). Age 0 means not successfully determined. The genotype is indicated with colour. Between brackets the haplotype is shown. The stems are numbered from 107 to 130. Location cluster centre in SSC: 179832.34, 467811.27.*

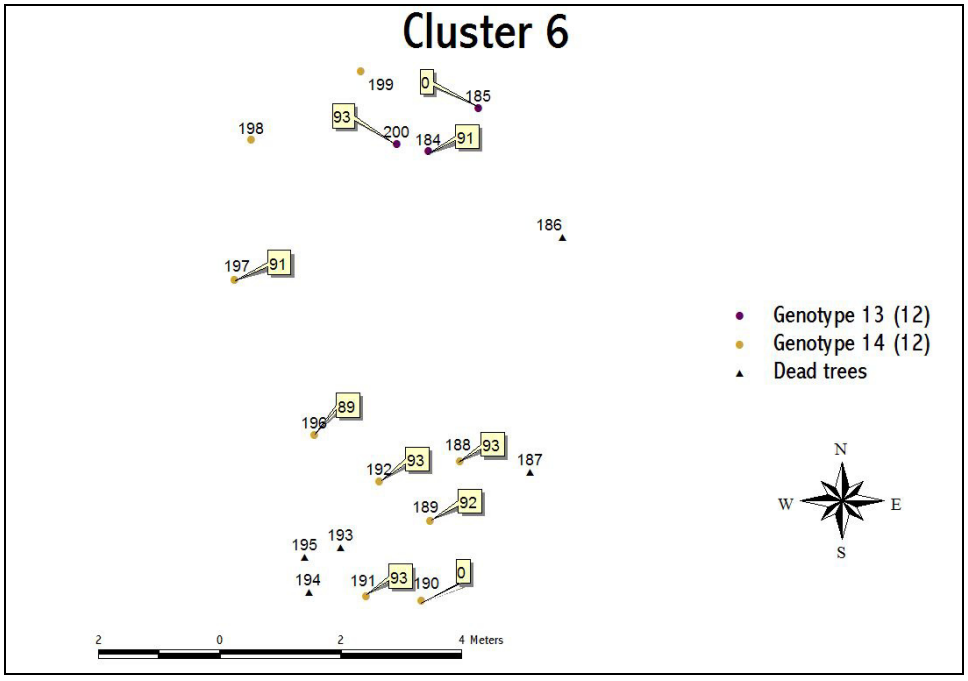

Figure 3.9. *Ages (flagged) in combination with genotypes and haplotypes of cluster 6 (plot 1: Maanschoten). Age 0 means not successfully determined. Genotypes are indicated with different colours. Between brackets the haplotypes are shown. Stems are numbered from 184 to 200. Location cluster centre in SSC: 179861.43, 467761.58.*

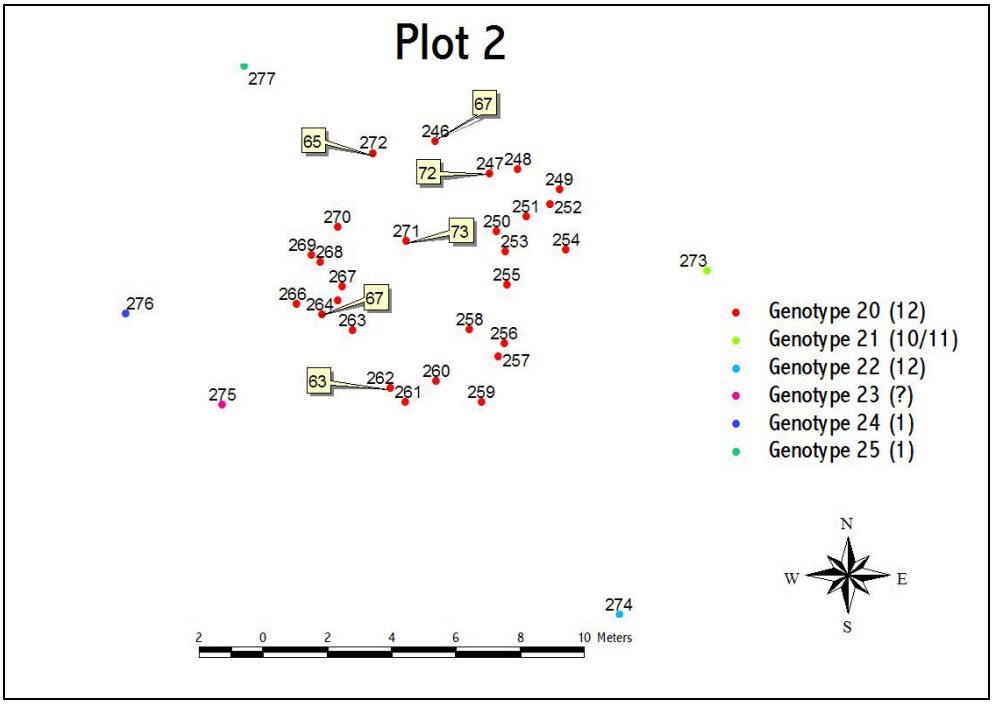

Figure 3.10. Ages (flagged) in combination with genotypes and haplotypes of plot 2 including cluster 9 and individual trees (Wilde Kamp). Genotypes are indicated with different colours. Between brackets the haplotypes are shown. Stems are numbered from 246 to 277. Location plot centre in SSC: 177463, 470566.

Other observations

Maanschoten

In Maanschoten sometimes stems with a wide base were found.

Wilde Kamp

During the field work it was striking that many stems at the Wilde Kamp were horizontally connected with each other (see Figure 3.12). Also stem number 259 and 260 (plot 2) of cluster 9 were horizontally connected. Besides that no wide stem bases were found at the Wilde Kamp. In the stems of cluster 13 and 9 a remarkable growth pattern was found. In the first few years of cluster 13, starting from the pith, the oaks grow around 1 mm per year followed by a period of 20 years in which the growth was very low (see Figure 3.11). After the period of 20 years the growth starts increasing again. The beginning of the growth depression is dated around 1920 and the end around 1948. In cluster 9 the growth depression starts around 1938 and ends around 1948. Also wound tissue was found in one of the wood samples.

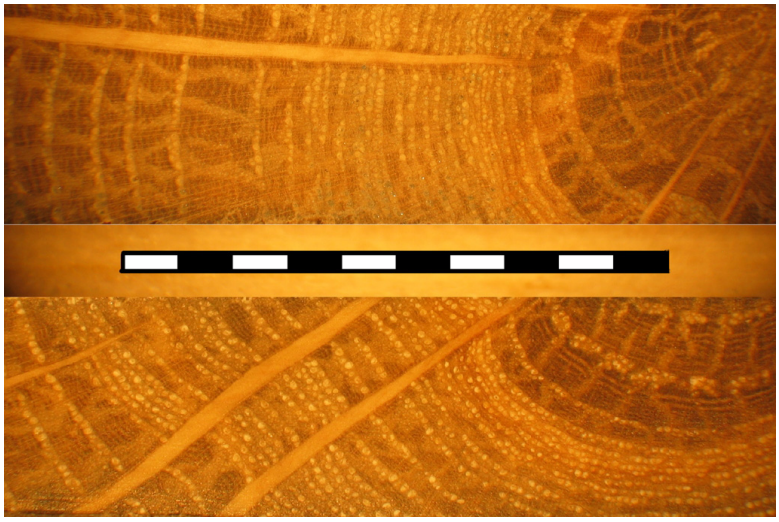

Figure 3.11. Two wood samples of two stems of cluster 13. The scale bar represents 1 cm.

Figure 3.12. Two horizontally growing stems (indicating layering) are shown at the left picture. The right picture shows the horizontally growing stems at the background of the left picture. Further research must confirm if natural layering is key factor in clonal propagation of oak clusters.
Photos: Leo Goudzwaard (WUR).

Maanschoten

In the cadastral map of 1832 the location of plot 1 in Maanschoten was described as heath land (Stichting Werkgroep Kadastrale Atlas Gelderland, unpublished). Tesch *et al.* (1926) shows different photographs of the drift sand areas surrounding Kootwijk. Figure 3.14 & 3.15 shows two pictures taken in close proximity of Maanschoten. At the horizon some oak shrubs are visible. The environment is harsh; only some mosses are growing in the sand in 1909 and in 1926 besides mosses some grasses are growing in the sand area. In the minutes of a meeting of the "Nederlandse Heidemij" in 1920 the area around Kootwijk is described as a desert (Hesselink, 1920).

Wilde Kamp

The different plots at the Wilde Kamp are described in the cadastral map of 1832 as heath land class 2 (Stichting Werkgroep Kadastrale Atlas Gelderland, unpublished). Moerman (1940) described the Wilde Kamp as an area, which is surrounded by a wall and a ditch containing oak coppice (see Figure 3.13) of probably more than 100 years old. The surrounding area, in which the large oak clusters are located, was property of the common and is described as heath land with oak-shrubs. Old inhabitants of the village Garderen could tell that in their youth the oak clusters were grazed by sheep. In Figure 3.13 an area containing heather and shrubs can be found. This is the location of plot 2.

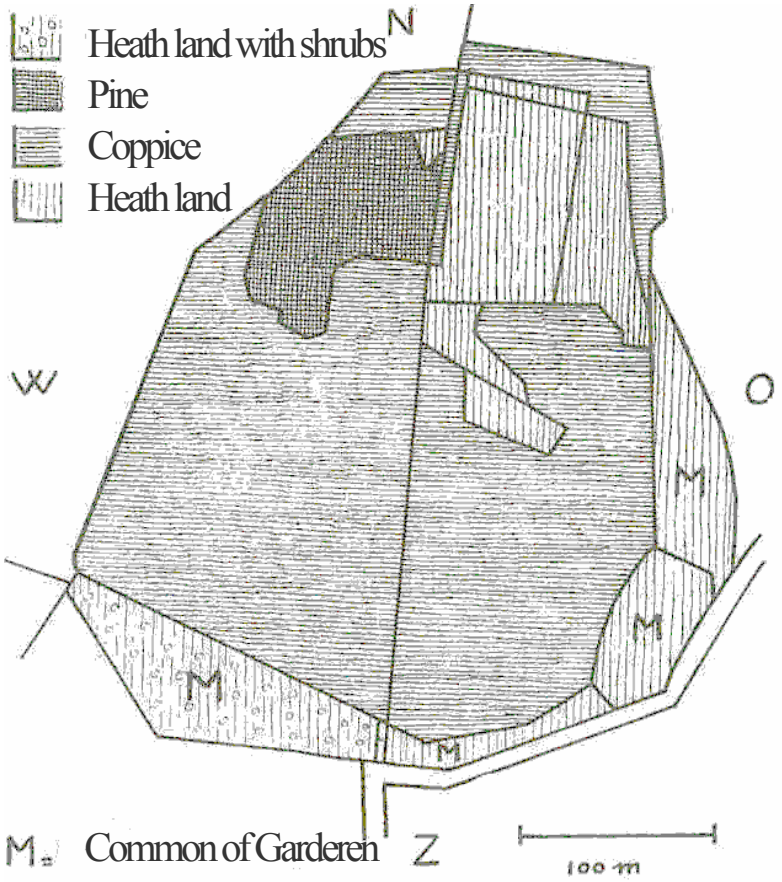

Figure 3.13. Map of the Wilde Kamp based on the cadastral map of 1888. Source: Moerman (1940).

Figure 3.13. Photo taken in close proximity of Maanschoten (source Tesch et al., 1926). The picture shows a photo taken just in the south of Maanschoten in 1911. The vegetation consists of mosses (Politrichum piliferum) and some oak shrubs at the horizon.

Figure 3.14. Photo taken in close proximity of Maanschoten (source Tesch et al., 1926). The picture shows the same location but than in 1926. The vegetation shows more grasses and the same oak shrubs at the horizon. Source: Tesch et al. (1926).

4 Discussion

4.1 Genotype identification

Based on six microsatellite loci we were able to describe the number, size and spatial distribution of clones of *Q. robur* and *Q. petraea* in two populations. Our results show that in Maanschoten and in the Wilde Kamp, oak clusters can be genetically identical but also can contain a mixture of genotypes. The shape of the clusters in this study is in common circular, while Bakker (2001) found more linear clones at the Stompert. Maybe the form of the clones depends on the openness of the area; circular clones as in this study, could be found in open areas while linear clones could be found in closed high forest as for instance in the plot of Bakker (2001) at the Stompert. The size of the clones is larger than observed by Rackham (2003) and Bakker *et al.* (2001). The clones of cluster 1 $(12.41m)^3$ with a circumference of 36.6m and cluster 4 $(7.77m)^3$ with a circumference of 22.3m in Maanschoten are, according to our research, the largest of *Q. robur* and *Q. petraea*, that are described in literature based on DNA research.

4.2 Morphology

PCA and cluster analysis of 14 leaf morphology characteristics of 39 stems (20 leaves per stem) belonging to 13 clusters of *Quercus robur* (11) and *Quercus petraea* (2) did not reveal a clear cut separation between genotypes. Although variation in morphology of stems belonging to a clone is low, this is substantial as compared with variation among stems of different genotypes. Due to environmental heterogeneity the leaf morphology characteristics can vary between stems belonging to the same clone (Whitham, 1981). Also somatic mutations in meristemic tissue can cause variation in morphology (Antolin & Strobeck, 1985). Stems of *Quercus robur* and *Quercus petraea* could be distinguished based on cluster analysis.

Leaf morphology analysis could not be used to identify clonal structures of oak, since no clear separation between the clones could be obtained. Although in this study more leaf material per stem was used than done by Bakker *et al.*, (2001), comparable results were obtained. Phenology was not used in this study and might be important to identify clones. But also flushing of leaves at the same moment can not give the guarantee that these stems have the same genotype. Visual detection in the field can neither be advised since clusters can contain different genotypes. The best way to identify clones is using DNA analysis of all stems.

4.3 Origin of oak clusters in Maanschoten

cpDNA haplotype composition

In Maanschoten all successfully investigated stems (18 genotypes), including both clusters and individual trees, have the Spanish haplotype 12. This haplotype occurs naturally at the Veluwe (Buiteveld *et al.*, 2005). Based on this knowledge there are no indications that these clusters could not belong to the autochthonous vegetation.

Soil profiles

All three investigated soil profiles contained burrowed profiles. Also samples (not described) taken from other places in plot 1 show overblown profiles. These buried profiles indicated that there was an old profile (with red sand) which was overblown (see Figure 4.1). The buried Bmix layer found in sample 2 possibly indicates soil management practises.

3. This is the maximum distance between two stems of the same genotype

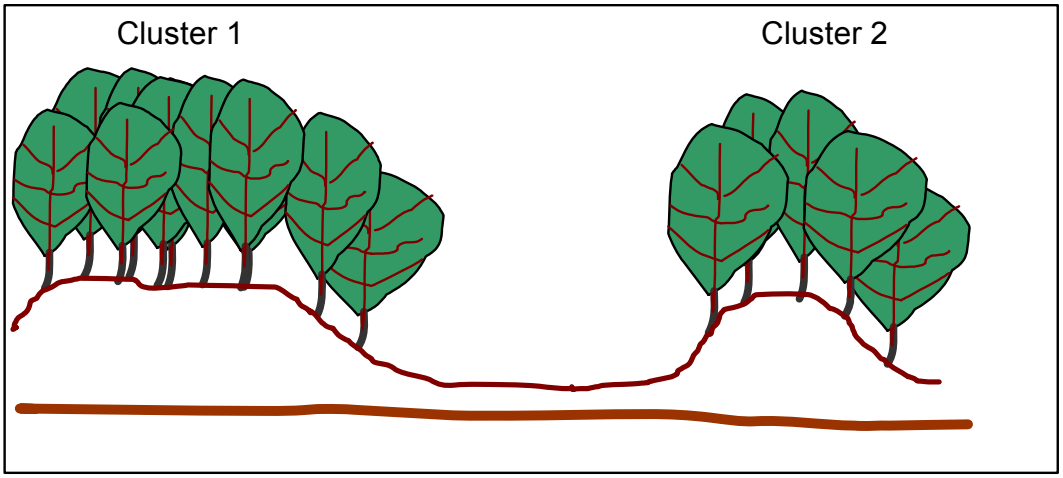

Figure 4.1. Buried soil profile and current soil profile with the oak clusters.

Tesch *et al.* (1926) found red soils beneath oak shrubs surrounding Kootwijk. He concluded that the oak shrubs (see Figure 4.2) all have roots in overblown red soil profile. Moerman (1947) also found red soil layers in Maanschoten. He states that the red soil layer is not drift sand, he also mentioned that under the red soil layer drift sand is never present. Also Stoutjesdijk (1959) found red soils or old heath soils beneath oak shrubs at the Veluwe. According to Hissink (1915) the soil is red because of iron (FE_2O_3). According to Faveljee (1950) FE_2O_3 is formed under alkaline conditions. He indicates that these conditions could occur during wood burning. According to Moerman (1947) the soil is red coloured because of ash which was used as a fertiliser in primitive agricultural systems. About the origin of the hills beneath the oak shrubs Stoutjesdijk (1959) writes: *"A characteristic feature of the landscape formed by the resting inland dunes are solitary dome-shaped hills covered with oak shrub. The base of the oak trunks is covered with sand. As the sand is fixed by the numerous trunks it is less easily borne away by the wind, and this explains why these hills may have very steep slopes and differ therefore very markedly in shape from the grass-covered hills. The hills are traversed at their base by the old heath soil or by a layer of red sand. The development of these hills will have proceeded in this way: on a rest of heather podsol Aeolian sand is deposited, and when the latter is sufficiently stabalized, it is colonized by oak".*

Stem age

The average age of all investigated stems in Maanschoten is 93 years (86-96 years). Differences in the age estimations within a cluster could occur because of the structure of the dunes. In the middle of the clusters the dunes are highest. This can indicate that the sample size of 40 cm above ground level can be actually more then 2 meters above the roots while at the outside it looks like 40 cm above the root system. So depending on the height growth several rings can be excluded from the age determination. In some clusters wide stem bases were found (see below), showing that it is more likely that the stems have been cut. The even aged stems of about 93 years old could confirm this.

Other observations

In Maanschoten some clusters contain stems with a wide stem base. A wide stem basis indicates that a stem has been cut once.

Historical description

Maanschoten is described as heath land in the cadastral map of 1832. In such areas shrub-like oaks (see Figure 4.2) were present (Bijlsma, 2004; Clerkx en Bijlsma, 2003; Schelling, 1955). Tesch *et al.* (1926) describes oaks shrubs in drift san areas surrounding Kootwijk: "*For those who visit Kootwijk, it is always a miracle, how oaks can grow considerably at these poor inland dunes. I solved this mystery by digging up such shrubs. It seems that we are dealing with oaks located at red soils, which had the strength to grow above the sand again after they were overblown. These oaks formed new roots in the drift sand and besides they still have roots in the red soil layer*".

Origin

We assume that the oak clusters in Maanschoten are comparable to the oak shrubs as described and photographed by Tesch *et al.* (1926) and Stoutjesdijk (1959), Our soil analysis shows that a lot of sand has overblown these oaks and that they are located at red soils. Tesch *et al.* (1926) and Ouden *et al.* (in preparation) show that branches of oak shrubs which are growing horizontally at the surface can form roots (layerings). Especially in drift sands were the branches are buried regularly this mechanism can play an important role in the horizontal growth of oak clusters.

The reason why the oaks grow shrub-like could be because of the natural adaptation to the harsh environment on these drift sand in combination with the poor soils. Stoutjesdijk (1959) shows that at the Veluwe in drift sands the temperature can rise to 50 °C on the plains to 60 °C on south exposed areas, while at the same night the temperature drops to 0 °C. Also sand transportation caused damage to leaves and twigs. When the State Forest Service started the afforestation in 1897 the environment became less harsh and the shrubs could grow more in height. This might be the reason why nowadays large oak shrubs are not present anymore.

Figure 4.2. Photograph of oak shrubs surrounding Kootwijk. In the front planted pines can be found (Tesch et al., 1926).

Figure 4.3. Photograph of cluster 1 (left) and cluster 2 (right) in plot 1 (Maanschoten) shows the situation of 2004.

4.4 Origin of oak clusters at the Wilde Kamp

cpDNA haplotype composition

At the Wilde Kamp 3 chloroplast haplotypes (1 Italian, 10 Spanish and 12 Spanish) were found. This is in congruence with research on the cpDNA haplotype composition of the Veluwe (Buiteveld *&* Koelewijn, submitted) and previous research of the Wilde Kamp (Buiteveld *et al.*, 2005). The five clusters at the Wilde Kamp all have haplotype 10 or 12. The surrounding trees have haplotype 1, 10 and 12. These haplotypes occur naturally on the Veluwe. Based on this knowledge there are no indications that these clusters could not belong to the autochthonous vegetation. However a mixture of more than two haplotypes within a stand provides evidence that genetic material has been introduced in this area (at least partly) (Buiteveld *et al.*, 2005).

Stem age

The age distribution shows that the stems of cluster 10 and of the large clone in cluster 13 have probably originated around 1920. Cluster 9 probably originated ten years later. The precise date of origin of the studied stems can not be given, because it was not possible to estimate the period in which the oaks reached 40cm (sample height). The sampled individual trees surrounding the clusters are younger; this indicates that the clusters were situated in a more open area compared to the present day situation.

Other observations

In most of the stems of cluster 13 (plot 6) and cluster 9 (plot2) an abrupt and long lasting growth depression occurred which indicates the presence of a chronicle stress factor. In one sample wound tissue was found close to

the pith. These observations could suggest that at least these (or some) of the trees in the oak clusters were affected by grazing over many years. This would fit the observations of Van der Waals-Nachenius (1978) (see below). In a new research project the relation between growth pattern and origin is studied. The many horizontally growing stems indicate a shrub like origin. Ouden *et al.* (in preparation) show that branches of grazed oak shrubs which are growing horizontally at the surface can form natural layerings. Also the stems which are connected horizontally between tree number 259 and 260 of cluster 9 could be explained in this way. The process in which horizontal stems form new roots (layering) could easily explain the large distances between genetically identical stems.

Historical description

In the cadastral map of 1832 this area is described as heath land class 2 (Stichting Werkgroep Kadastrale Atlas Gelderland, unpublished). Class 2 indicates the marginal profits obtained in these areas. It is known that such heather areas were intensively grazed and also that these areas were burned (Buis, 1985). Here many shrub-like oaks were present (Bijlsma, 2004; Clerkx en Bijlsma, 2003). Also Moerman (1940) described the locations of the plots as a heath land area containing oak shrubs. Waals-Nachenius (1974) describes oaks shrubs for the surrounding area Boeschoten. "*The whole area was covered by heather and oak shrubs and some individual pines. The oak shrubs were grazed by sheep and did not come above the heather. How old are these oaks? But how well they developed after they were not longer grazed by sheep. They became nice erratic trees*". Also Van der Waals (oral communication) could tell the clusters surrounding his house in Boeschoten were stocky shrubs in his youth, which could easily be overlooked.

Origin

We assume that the oak clusters at the Wilde Kamp are originated from oak shrubs which formed natural layerings based at observations and the historical description of the Wilde Kamp described above. Further research must confirm if the stems originated by layering.

4.5 Age of oak clusters

This study does not support the hypothesis that the clusters in Maanschoten and at the Wilde Kamp are up to 1500 years old based on the study of Pigott (1989). The clusters do not always originate from one individual; this is a prerequisite for using the size of the clusters as an indication for age estimation. Besides that the clusters, also the ones which consist of one clone, probably did not originate by continuous coppicing, which is a prerequisite for using the method of Pigott (1989).

5. Conclusions

Clusters of oak can be genetically identical, but can also contain a mixture of genotypes. The largest clone of *Quercus robur* (cluster 9) has a maximum diameter of 8.9m and a circumference of 25.8m at the Wilde Kamp. The largest clone of *Q. robur* (cluster 1) in Maanschoten has a maximum diameter of 12.41m and a circumference of 36.6m. Note that this clone mingled with another clone as cluster 1 contains more than one genotype. The largest *Quercus petraea* clone (cluster 4) has a maximum diameter of 7.77m and a circumference of 22.3m. These clones are the largest clones described in literature based on DNA research!

Leaf morphology analyses are not suitable to identify clonal structures of oak.

Oak clusters in Maanschoten are over blown by drift sand. Historical documents describe over blown oak shrubs in drift sand areas, which form natural layerings. A shrub-like origin of oak clusters in Maanschoten seems likely.

Oak clusters at the Wilde Kamp are described in historical documents as oak shrubs located in heath land. The stems of the investigated clusters show long lasting growth depressions indicating a grazing history. A shrub-like origin of oak clusters at the Wilde Kamp seems likely.

In both areas no indications are found that the clonal growth of oak clusters occurred because of continuous coppicing. An alternative hypothesis to he clonal growth of oak clusters is that clonal growth could occur because of the shrub-like growth form in combination with the ability of oak to make natural layerings of horizontally growing or over blown twigs. This subsequently means that the age estimations of clusters up to 1500 years old based on the study of Pigott (1989) could not be supported since a continuous coppice origin is a prerequisite for using this method.

References

Ainsworth, E.A., P. J. Tranel, B.G. Drake & S.P. Long. 2003.
The clonal structure of *Quercus geminata* revealed by conserved microsatellite loci. *Mol. Ecol.* 12 (2): 527-532.

Anonymous. 1966.
De Nederlandse bosstatistiek. Deel 9 Nederland 1952-1963. Utrecht; De Haan: 51 P.

Anonymous. 1985.
De Nederlandse bosstatistiek. Deel 1 de oppervlakte bos 1980-1983. 's-Gravenhage; Staatsuitgeverij: 83 p.

Antolin, M.F. & C. Strobeck. 1985.
The population genetics of somatic mutations in plants. *Am. Nat.* 126: 52-62.

APG (Angiosperm Phylogeny Group). 2003.
An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG II. TBot. J. Linn. Soc. 141 (4): 399-436.

Ashley, M.V. & B.D. Dow. 1994.
The use of microsatellite analysis in population biology: Background, methods and potential applications. In Molecular ecology and evolution: approaches and applications Author(s): Schierwater, B., B. Streit & G. P. Wagner. Basel; Birkhaeuser: 622 p.

Ayres K.L. & A.D.J. Overall. 2004.
API-CALC 1.0: a computer program for calculating the average probability of identity allowing for substructure, inbreeding and the presence of close relatives. *Mol. Ecol Notes* (4) 315-318.

Bakker, E.G., B.C. Van Dam, H.J. van Eck & E. Jacobsen. 2001.
The description of clones of *Quercus robur* L. and *Q. petraea* (Matt.) Liebl. with microsatellites and AFLP in an ancient woodland. *Plant Biology* 3 (6): 616-621.

Boer, R.W. 1857.
Bijdragen tot de kennis der houtteelt. Zwolle Tjeenk Willink

Bijlsma, R.-J. 2004.
Struikbos (kreupelhout en struwelen) op de Veluwe: 1832 versus 2003. In: Bouwer, K., J. van Laar & F. Scholten (red) Het bos in 1832. De betekenis van de eerste kadastrale gegevens. Bijdragen aan de studiedag op 25 maart 2004 te Ellecom. pp. 17-29. Stichting Boskaart Nederland 1832. Zuidwolde; Stichting Boskaart Nederland: 70 p.

Bossema, I. 1979.
Jays and oaks: an eco-ethological study of symbiosis. PhD thesis. Leiden; Brill: 117 p.

Bruschi P, G.G. Vendramin, F. Bussotti, & P. Grossoni. 2003.
Morphological and molecular diversity among Italian populations of *Quercus petraea* (Fagaceae)**.** *Ann. Bot.* 91 (6): 707-716.

Buis, J. 1985.
Historia Forestis Nederlandse bosgeschiedenis. In A. A. G. Bijdragen deel 26 & 27. PhD thesis LH. Wageningen: 1060p.

Buis, J. 1993.
Holland houtland: een geschiedenis van het Nederlandse bos. Amsterdam; Prometheus: 243 p.

Buiteveld, J, M.C. Boerwinkel, J. Bovenschen, K.G. Kranenborg & S.M.G. de Vries. 2005.
Chloroplast DNA haplotype samenstelling van eikenopstanden (categorie 'van bekende origine') van de Rassenlijst van Bomen. Alterra-rapport 1169, 40 pp.

Bunnik, F.P.M. 1999.
Vegetationsgeschichte der Lössbörden zwischen Rhein und Maas : von der Bronzezeit bis in die frühe Neuzeit. PhD thesis UU, Utrecht: 194p.

Burchel, P. & J. Huss. 1997.
Grundriss des Waldbaus: ein Leitfaden fuer Studium und Praxis. Berlin; Parey: 487 p.

Burrichter, E., R. Pott, T. Raus & R. Wittig. 1980.
Die Hudelandschaft Borkener Paradis im Emstal bei Meppen. Abhandelungen aus dem Landesmuseum für Naturkunde zu Münster in Westfalen 42, Heft 4. Muenster ; Westfaelische Vereinsdrueckerei: 69p.

Cate, C.L. ten. 1972.
Wan god mast gift... : Bilder aus der Geschichte der Schweinezucht im Walde. Wageningen; Pudoc: 300 p.

Clerkx, A.P.P. M. & R.-J. Bijlsma. 2003.
Veluwse heide blijkt open boslandschap na ecologische interpretatie van het kadastrale archief van 1832. *De Levende Natuur* 104: 148-155.

Coart, E, V, Lamote M. de Loose, E. Van Bockstaele, P. Lootens & I. Roldan-Ruiz. 2002.
AFLP markers demonstrate local genetic differentiation between two indigenous oak species *Quercus robur L.* and *Quercus petraea* (Matt.) Liebl in Flemish populations. *Theor. Appl. Genet.* 105 (2-3): 431-439.

Cook, E. R.. 1985.
A time series analysis approach to tree ring standardization. PhD thesis, University of Arizona, Tucson: Ariz: 171 pp.

Dam, B.C. van, A. Oosterbaan & S.M.G. de Vries. 1996.
Nieuw onderzoek aan Nederlandse eiken. Behoud van inheemse bomen. *Nederlands bosbouw tijdschrift* 68 (5):190-193.

Demesure, B., N. Sodzi & R.J. Petit. 1995.
A set of universal primers for amplification of polymorphic non-coding regions of mitochondrial and chloroplast DNA in plants. *Mol. Ecol.* 4: 129-131.

Dirkx, G.H.P. 1998.
Wood pasture in Dutch common woodlands and the deforestation of the Dutch landscape In Kirby, K. J. & C.Watkins. The ecological history of European forests: selected papers given at a international conference held at the University of Nottingham in Sept. 1996. Wallingford; CAB International: 373 p.

Dow, B.D., M.V. Ashley, & H.F. Howe. 1995.
Characterization of highly variable $(GA/CT)_n$ microsatellites in the bur oak, *Quercus macrocarpa. Theor. Appl. Genet.* 91: 137-141.

Dumolin, S., B. Demesure & R. J. Petit. 1995.
Inheritance of chloroplast and mitochondrial genomes in pedunculate oak investigated with an efficient PCR method *Theor. Appl. Genet.* 91 (8): 1253-1256.

Dumolin, S., B. Demesure, S. Fineschi, V. Le Corre & R.J. Petit. 1997
Phylogeographic structure of white oaks throughout the European continent. *Genetics* 146: 1475-1487.

Eckblad, J.W. 1991.
How many samples should be taken? *Bioscience 41*(5): 346-348.

Faveljee, J.C. 1950.
Over het voorkomen van ijzeroxiden in de bodem. *Tijdschrift van het Aardrijkskundig Genootschap* 67: 684.

Gil, L., P. Fuentes-Utrilla, Á. Soto, M. Teresa Cervera & C. Collada. 2004.
English elm is a 2,000-year-old Roman clone. ***N****ature* 431: 1053.

Gomez, J.M., D. Garcia, & R. Zamora. 2003.
Impact of vertebrate acorn- and seedling- predators on a Mediterranean *Quercus pyrenaica* forest. *Forest. Ecol. Manag.* 180: 125-134.

Grissino-Mayer, H.D. 2001.
Evaluating crossdating accuracy: A manual and tutorial for the computer program COFECHA. *Tree-Ring Research* 57(2): 205-221.

Guerin, D.N. 1993.
Oak dome clonal structure and fire ecology in a florida longleaf pine dominated community. *B. Torrey Bot Club* 120 (2): 107-114.

Havinga, A.J. 1962.
A palynological investigation of soil profiles developed in cover sand. PHd thesis LH, Wageningen: 93 p.

Hesselink, E. 1920.
Een en ander over de Veluwse zandverstuivingen, in het bijzonder over die in de omgeving van Kootwijk. Tijdschrift Nederlandse Heidemij 32: 327-335.

Kampfer, S., C. Lexer, J. Glossl & H. Steinkellner. 1998.
Characterization of (GA)n microsatellite loci from *Quercus robur. Hereditas:* 129 (2): 183-186.

Kashi, Y., D. King & M. Soller. 1997.
Simple sequence repeats as a source of quantitative genetic variation. *Trends Genet.* 13: 74-78.

Kissling, P. 1980.
Clef de détermination des chênes médioeurpéens (Quercus L.) *Ber. Schweis. Bot. Ges.* 90 (1-2): 29-44.

König A. O., B. Ziegenhagen, B. C. van Dam, U. M. Csaikl, E. Coart, B. Degen, K. Burg, S. M. G. de Vries & R. J. Petit. 2002.
Chloroplast DNA variation of oaks in western Central Europe and genetic consequences of human influences. *Forest Ecol. Manag.* 156 (1-3): 147-166.

Koop, H. 1987.
Vegetative reproduction of trees in some european natural forests. *Vegetatio* 72 (2): 103-110.

Kremer, A., J.L. Dupouey, J.D. Deans, J. Cottrell, U. Csaikl, R. Finkeldey, S. Espinel, J. Jensen, J. Kleinschmit, B. van Dam, A. Ducousso, I. Forrest, U.L. de Heredia, A.J. Lowe, M. Tutkova, R.C. Munro, S. Steinhoff & V. Badeau. 2002.
Leaf morphological differentiation between *Quercus robur* and *Quercus petraea* is stable across western European mixed oak stands. *Ann. For. Sci.* 59 (7): 777-787.

Locher, W.P., H. de Bakker. 1990.
Bodemkunde van Nederland. Den Bosch; Malmberg: 439p.

Kremer A. & P. Menozzi. 2000.
Genetic processes promoting genetic diversity in oak and beech forest in Turok, J., A. Kremer, T. Geburek, L. Paule & E. Lipman (eds.). Third EUFORGEN meeting on social broadleaves. Bulgaria; IPGRI, Borovets: 31-43.

Maes, N. C. M. 1993.
Genetische kwaliteit van inheemse bomen en struiken. Deelproject: Randvoorwaarden en knelpunten bij behoud en toepassing van inheems genenmateriaal. IKC-NBLF en IBN-DLO, Rapport nr. 020, Wageningen: 86p.

Maes, N.C.M. & C.J.A. Rövekamp. 1999. Oorspronkelijk inheemse bomen en struiken in Vlaanderen: een onderzoek naar autochtone genenbronnen in de Regionale Landschappen West-Vlaamse Heuvels en Vlaamse Ardennen en de Houtvesterijen Hechtel en Bree.

Maes, N.C.M., C.J.A. Rövekamp & R. van Loon. 1996.
Inventarisatie van autochtone bomen en struiken in West- en Midden-Brabant. Dienst LBL. Tilburg.

Mayes, S.G., M.A. McGinley & C.R. Werth. 1998.
Clonal population structure and genetic variation in sand-shinnery oak, Quercus havardii (Fagaceae) *Am. J. Bot.* 85 (11): 1609-1617.

Moerman, J.D. 1940.
De Wilde Kamp bij Garderen. *De Levende Natuur* 44: 307-313.

Moerman, J.D. 1947.
Rood zand en praehistorische bewoning. *Tijdschrift van het Aardrijkskundig Genootschap*. 44: 537-680

Montalvo, A.M., S.G. Conard, M. Thompson Conkle, & P.D. Hodgskiss. 1997.
Population structure, genetic diversity, and clone formation in *Quercus chrysolepis* (Fagaceae). *Am. J. Bot.* 84: 1553-1564.

Oosterbaan, A. & G.J. Nabuurs. 1991.
Relationships between oak decline and groundwater class in The Netherlands. *Plant Soil.* 136: 87-93.

Pals, J.P. 1987. Reconstruction of landscape and plant husbandry. In: Groenman - van Waateringe, W, L.H. van Wijngaarden - Bakker. Farm life in a Carolingian village : a model based on botanical and zoological data from an excavated site. Assen; Van Gorcum: 129 p.

Petit, R.J., U.M. Csaikl, S. Bordacs, K. Burg, E. Coart, J. Cottrell, B. van Dam, J.D. Deans, S. Dumolin-Lapegue, S. Fineschi, R. Finkeldey, A. Gillies, I. Glaz, P.G. Goicoechea, J.S. Jensen, A.O. Konig, A.J. Lowe, S.F. Madsen, G. Matyas, R.C. Munro, M. Olalde, M.H. Pemonge, F. Popescu, D. Slade, H. Tabbener, D. Taurchini, S.G.M. de Vries, B. Ziegenhagen & A. Kremer. 2002.
Chloroplast DNA variation in European white oaks phylogeography and patterns of diversity based on data from over 2600 populations. *Forest Ecol Manag* 156: 5-26.

Petit R.J., A. Kremer & D.B. Wagner. 1993.
Geographic structure of chloroplast DNA polymorphisms in European oaks. *Theor. Appl. Genet.* 87 (1-2): 122-128.

Petit R.J., E. Pineau, B. Demesure, R. Bacilieri, A. Ducousso & A. Kremer. 1997.
Chloroplast DNA footprints of postglacial recolonization by oaks. *Proc. Nat. Acad Sci (USA)*: 94 (18): 9996-10001.

Pigott, C.D. 1989.
Factors controlling the distribution of *Tilia-cordata* mill at the northern limits of its geographical range. 4 estimated ages of the trees. *New Phytol.* 112 (1): 117-121.

Pott, R. & J. Hüppe. 1991.
Die Hudelandschaften Nordwestdeutschlands. Abhandelungen aus dem Landesmuseum für Naturkunde zu Münster in Westfalen 53, Heft 1/2 Muenster; Westfaelisches Museum fuer Naturkunde: 313p.

Prins, H.A.H., N.C.M. Maes & M.J.T.M. Smit. 1993.
De wintereik in Nederland: verspreiding ecologie en toekomstmogelijkheden van de wintereik in het Nederlandse bos. Wageningen; Stichting Kritisch Bosbeheer: 106 p

Rackham, O. 2003. Ancient woodland. Its history, vegetation and uses in England. London; Castlepoint Press: 584 p.

Reusch, T.B.H., C. Bostrom, W.T. Stam & J.L. Olsen. 1999.
An ancient eelgrass clone in the Baltic. *Marine Ecol. Progr. Series* 183: 301-304.

Rövekamp, C.J.A. (redactie) & N.C.M. Maes. 1998.
Oorspronkelijk inheemse bomen en struiken in Vlaanderen: een onderzoek naar autochtone genenbronnen in de Ecologische Impulsgebieden. Brussel; Ministerie van de Vlaamse Gemeenschap, Afdeling Bos & Groen.

Rövekamp, C.J.A. (redactie) & N.C.M. Maes. 2002.
Inheemse bomen & struiken op de Veluwe, Autochtone genenbronnen en oude bosplaatsen. Provincie Gelderland: 106 p.

Rushton, B.S. 1978.
Quercus robur L. and *Quercus petraea* (Matt.) Liebl.: a multivariate approach to the hybrid problem, Data acquisition, analysis and interpretation. *Watsonia* 12: 81-101.

Ruhston, B.S. 1983
An analysis of variation of leaf characters in *Q. robur* L. and *Q. petraea* (Matt.) Liebl. population samples from Northern Ireland. *Irish Forestry* 40 (2): 52-57.

Schaars, A.H.G. 1974.
De bosbouw van het "Entel" in de tweede helft van de achttiende eeuw. Zutphen; Walburg Publication: 141 p.

Schelling, J. 1955.
Stuifzandgronden = Inland-dune sand soils. Uitvoerige verslagen Bosbouwproefstation T.N.O.. Wageningen; Dorschkamp: 58 p.

Schwarz, O. 1964.
Quercus. In: Tutin T. G., V. H. Heywood, N. A. Burges, D. H. Valentine, S. M. Walters & D. A. Webb (eds). Flora Europea. Vol 1. London; Cambridge University Press: 61-64.

Spek, T. 1998.
Interactions between humans and woodland in prehistoric and medieval Drenthe (The Netherlands): an interdiciplinary approach. In: The ecological history of European forests: selected papers given at a international conference held at the University of Nottingham in Sept. 1996. Wallingford; CAB International: 373 p.

Spurk, M. 1992.
Dendrochronologische Untersuchungen an mittelalterlichen Eichenhölzern aus den Grabungen der Altstadt von Konstanz. PhD thesis, Stuttgart-Hohenheim: 108 p.

Steinkellner, H., S. Fluch, E. Turetschek, C. Lexer, R. Streiff, A. Kremer, K. Burg, & J. Glössl. 1997.
Identification and characterization of $(GA/CT)_n$-microsatellite loci from *Quercus petraea. Plant. Mol. Biol.* 33: 1093-1096.

Stoutjesdijk, P. 1959.
Heaths and inland dunes of the Veluwe. A study of some of the relations excisting between soil, vegetation and microclimate. *Wentia* 2: 1-96.

Streiff, R., T. Labbe, R. Bacilieri, H. Steinkellner, J. Glössl, & A. Kremer. 1998.
Within-population genetic structure in *Quercus robur* L. and *Quercus petraea* (Matt.) Liebl. assessed with isozymes and microsatellites. *Mol. Ecol.* 7: 317-328.

Taberlet, P., L. Gielly, G. Pautou & J. Bouvet. 1991.
Universal primers for amplification of three non-coding regions of chloroplast DNA. *Plant Mol. Biol.* 17: 1105-1109

Tattersall, F.H., D.W. Macdonald, B.J. Hart, W.J., Manley & R.E. Feber. 2001.
Habitat use by woodmice (Apodemus sylvaticus) in changeable arable landscape. *J. Zoology* 255: 487-494.

Tesch, P., E. Hesselink & J. Valckenier Suringar. 1926.
De zandverstuivingen bij Kootwijk in woord en beeld. Utrecht; Staatsboschbeheer.

Veldhorst, A.D.M. 1965.
Uit de gechiedenis van het Nationale Park "De hoge Veluwe". *Boor en Spade* 14: 117-137.

Waals-Nachenius, C.E. van der. 1974.
De geschiedenis van het bos van Boeschoten. *Nederlands bosbouw tijdschrift* 46: 44-46

Whitham, T.G. 1981.
Individual trees as heterogeneous environments: adaptations to herbivory or epigenetic noise. In: Denno, R.F. & R.F. Dingle (eds.). Insect life history patterns: habitat and geographic variation. New York: Springer-Verlag: 9-27.

Appendix I.
Soil profile descriptions

Soil profile 1

3 - 6	Of	-
0 - 3	Oh	-
0 - -8	AE	Dark greyish layer (fine sand)
-8 - -11	B1	Dark brown layer (fine sand)
-11 - -20	B2	Light brown layer (fine sand with gravel <5mm)
-20 - -40	B3	Light brown layer, transition layer to C (fine sand)
-40 - -220	C	Drifts and with humus spots (fine sand)
-221- -231	A	Very dark greyish layer with remnants of wood

Soil profile 2

5 - 9	Of	-
0 - 5	Oh	-
0 - -1	A	Thin dark A horizon (medium fine sand with some gravel <5mm)
-1 - -6	E	Grey sand (medium fine sand with some gravel <5mm)
-6 - -15	B1	Dark reddish, brownish layer (medium fine sand with some gravel <5mm)
-15 - -55	B2	Light brownish sand with spots of humus (medium fine sand with some gravel <5mm)
-55 - -110	Bmix	Dark reddish, brownish sand with humus spots (medium fine sand with some gravel <5mm)
-110 - -230	C	Layer with humus spots and greyish spots (fine sand without gravel)

Soil profile 3

1 - 2	Of	-
0 - 1	Oh	-
0 - -4	AE	Dark horizon with humus (fine sand)
-4 - -20	B1	Dark brown (fine sand)
-20 - -33	B2	Brown sand layer (fine sand)
-33 - -185	B3	Light brown layer with humus spots (fine sand)
-185 - -190	A	Dark brown (medium fine till fine sand)
-190 - -206	B1	Brown sand layer (medium fine sand)
-206 - -230	B2	Reddish sand layer (medium fine sand)