The Petite Charnie oak Intensive Study Plot (ISP)

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The main objective of the Petrite Charnie ISP is to investigate genetic and ecological processes driving evolutionary changes in an even-aged silvicultural system over successive generations, in two temperate white oak species *Quercus petraea* (sessile oak) and *Q. robur* (pedunculate oak)

The ISP was established in 1989, and is part of the Petite Charnie State Forest located in western France. The ISP is 5.19 ha in size, and comprised at the beginning of our investigation, *Q.petraea* and *Q. robur* in approximatively even proportions. The two species were distributed along a slight elevational gradient (*Q. petraea* upland and *Q. robur* lowland), which corresponds to the typical distribution when the two species cohabit. Intensive investigations were conducted during the past three decades addressing genetic diversity, spatial genetic structure, mating system, gene flow, hybridization, flowering and vegetative phenology, seed crop, phenotypic and genetic variation, and inheritance of multiple phenotypic traits. The present study entails now the next generation that resulted from natural regeneration. In 1989, when the mature trees were 90 years old, a regeneration felling was implemented leaving on the area 426 seed trees (68trees/ha). The opening of the stand facilitated seedling establishment and was followed by an additional removal cut in 1992 and 1993 leaving 298 standing trees (48 trees/ha). The final clear cut of the 298 trees was done over three successive years (1999 to 2001). Before the final cut, between 1995 and 2001, scions were collected on the 298 remaining trees and grafted in a conservation collection located in a State Nursery of Guéméné Penfao. Hence the former generation has been maintained in an *ex situ* conservation plantation. Current investigations are now conducted comparing the two generations and aiming at estimating the direction and strength of contemporary selection, due to the combined human and natural pressures. The attached document provides a detailed description of the ISP, summarizes research and results obtained during the last three decades and outlines current activities going on in the ISP.

This review assembles information about the operations and activities conducted in the ISP since the establishment of the ISP in 1989, and summarizes the main results obtained so far. More detailed information is available in the cited publications, grey literature and PhD thesis listed at the end of the document.

1. The Petite Charnie State Forest

The Petite Charnie oak ISP is part of the Petite Charnie State Forest (Forêt domaniale de la Petite Charnie) located in the western part of France (latitude: 48.086°N; longitude: 0.168°W), 35 Km west of the city of Le Mans (Figure 1). The local climate is typically atlantic, temperate and wet. Rainfall and mean temperature of the local climate are as follows:

Source of data	Period covered	Yearly rainfall	Mean yearly temperature
WorldClim	1970-2000	753 mm	10.4°C
Aurelhy	1960-1990	805 mm	10.8°C
Aurelhy	1980-2010	844 mm	11.4°C

The State Forest extends over 712 ha and is mainly composed of stands with mixed composition of broadleaves (*Q. petraea*, *Q.robur, Fagus sylvatica*), with a predominance of *Q. petraea* (Figure 2). According to the last management plan, in 1997 the forest was mainly composed of *Quercus petraea* (47% of the surface, including *Fagus sylvatica* in mixtures), *Quercus robur* (15%) and conifers (mainly *Pseudotsuga menziesii*, *Abies Nordmanniana, Pinus nigra* var *corsicana*, *Picea abies*). Further information on the forest can be found in Anonymous (1996).

The Petite Charnie forest was originally a private property belonging to the Family Duc Des Cars until 1917, when it was sold to other owners. It was bought by the State of France in May 1929, and managed as coppice-with-standards until 1957, when it was decided to convert it into high forest. Additionally conifer plantations were also established since 1957. According to historical records, it is likely that the Petite Charnie Forest was growing as high forest up to the 18th century (Dufour 1984). Indeed Dufour mentions that oak forests in the local area of the Petite Charnie were mostly growing as high forests and particularly Forêt de Grande Charnie which is nearby. She cites a publication dated 1766 by Piganiol de la Force describing the Grande Charnie Forest as a “very large high forest”. At that time, important and very active ironworks were established in the area and the Petite Charnie Forest was intensively treated as short term coppice to maintain high levels of wood production for feeding the forge industry during the 18th and 19th century (Dufour, 1984; Pesche, 1829). Switching to coppice with standards and high forest came later at the end of 19th or 20th century. Given that coppicing corresponds essentially to a clonal reproduction it is unlikely that the genetic composition of the forest was substantially modified by human actions during the recent centuries.

Figure 1. Location of the Petite Charnie State Forest and the Intensive Study Plot.

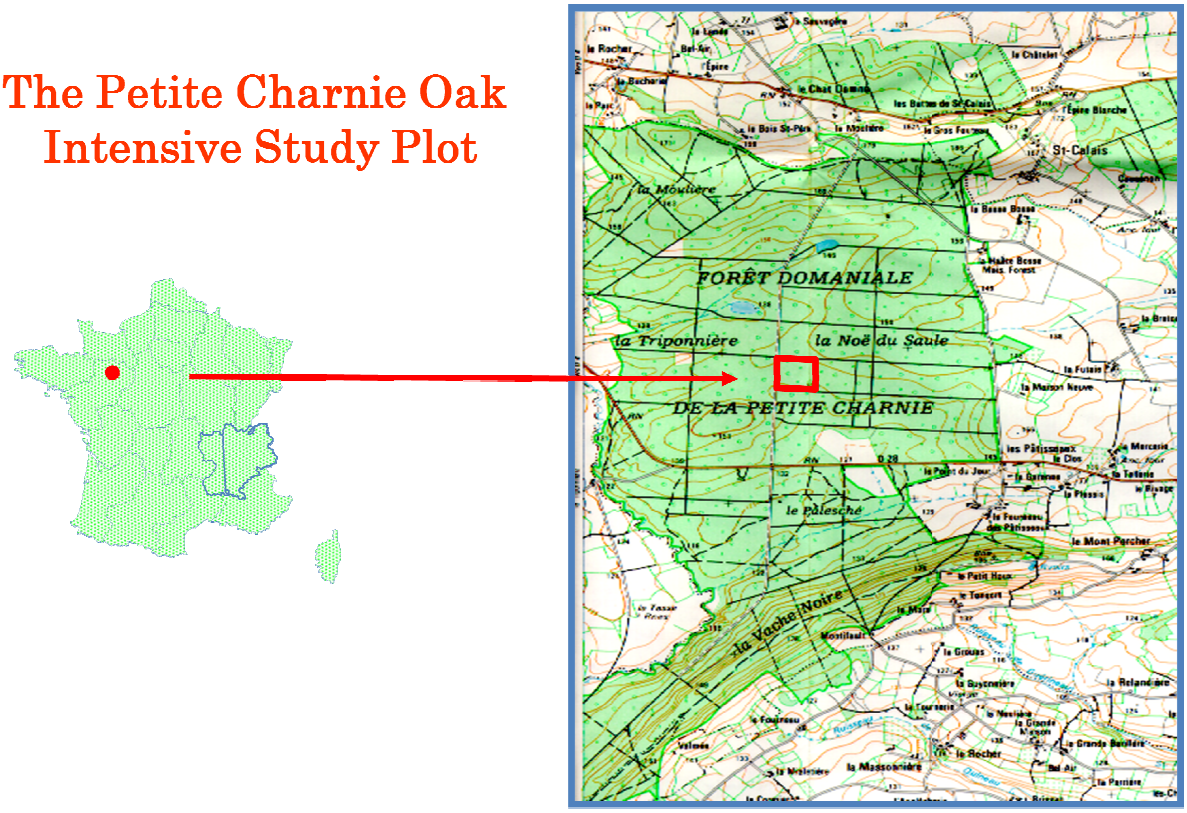
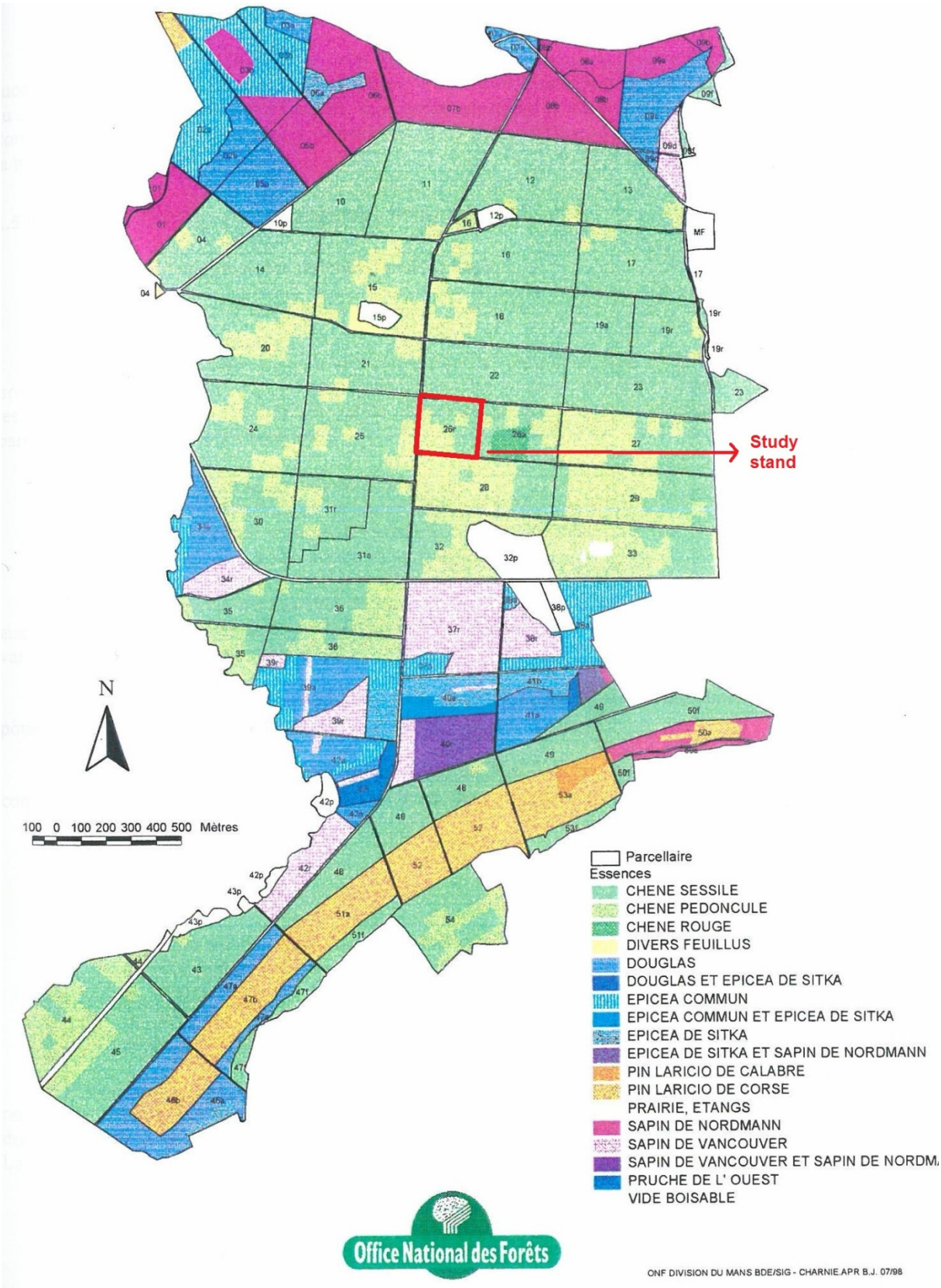


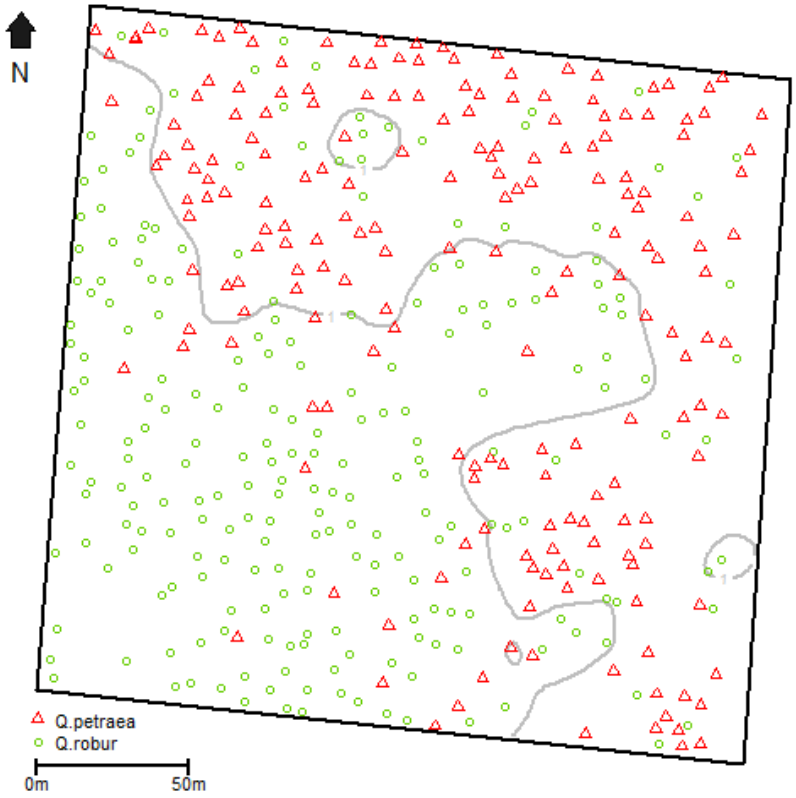
Figure 2. Species composition of the Petite Charnie State Forest.



2. The oak ISP in compartment 26

The ISP occupies part of compartment 26 of the Petite Charnie State Forest and is situated in the centre of the forest (Figure 1 and 2). The ISP covers 5.19 ha (approximate square of 230 m x 226 m) and comprised at the beginning of the genetic investigations *Q.petraea* and *Q. robur* in approximatively even proportions (Figure 3) and a few ash trees present in the wet part of the stand (Bacilieri *et al.*, 1995). At the start of the investigations, the stand was under even aged management and later tree ring analysis indicated that the stand was established in 1900. The ISP follows a slight ascending slope oriented from southwest to the northeast with a difference of 14 meters in elevation (mean elevation of the stand is 140 m absl). Soil conditions vary from a humid clay soil with a superficial water table (pseudogley) in the southwest corner up to dry silt and sandy soil in the upper northeast corner. The two species are distributed along this elevational gradient which corresponds to the typical oak distribution when the two species cohabit (Figure 3).

Figure 3. Map of the ISP and spatial distribution of the trees.



3. The early years 1989-1993

3.1. Silvicultural operations

During the winter 1988-1989, when the mature trees were about 90 years old, a regeneration felling was implemented leaving on the area 426 seed trees (68 trees/ha). This is the start of the genetic investigations. Natural seeding was implemented using standard silvicultural methods in even aged high oak forests. These methods consist in the opening of the stand by a regeneration felling (or a seed cut) followed by successive additional removal cuts aiming at enhancing seed crop and seedling establishment. A final cut is practiced when the seedling coverage is complete and evenly distributed. Depending on the occurrences of seed crops, the whole regeneration period –from the seedcut to the final cut- may last from 8 to 15 years (Jarret, 2004).

3.2. Technical field operations

Geomapping of all standing trees after the seed cut (426 trees) was conducted in October 1989.

A survey of soil conditions was made in May 1991 on soil cores collected on systematically distributed sampled points along a grid system (50 m square). 8 additional cores were collected along the diagonal from the southwest to the northeast. In each sampling point, soil cores were extracted to determine texture at different depths, and the depth of the pseudogley horizon. Soil texture analysis were done at the INRA Laboratory at Arras (Bacilieri Roberto 1994, p 17)

Botanical survey. In July 1992, a floristic survey was conducted within 34 plots systematically distributed throughout the study stand. The sampling included 8 survey plots located along the main diagonal from Southwest to the Northeast which was orthogonal to the slight slope in the study stand (Gaillard Véronique, 1992). Each survey plot consisted of a circular area of 64 m^2.

3.3. Material collection

In summer 1989, collection of 5 to 7 leaves from the upper part of the crown, storage of the leaves in an herbarium for leaf morphology analysis (Bacilieri Roberto, 1994, p.15)

In winter 1990 and 1991 collection of buds harvested in the upper part of the crown for extraction of isozymes of all the 426 adult trees (Bacilieri Roberto, 1994 p.16)

Collection of 46 open pollinated progenies in the fall of 1989 (on 31 *Q. petraea* trees and 15 *Q. robur* trees) and 15 open pollinated progenies of *Q.petraea* only in 1992. This is the material used for the mating system analysis using isozymes (Bacilieri *et al*., 1996a). The seed were sown and isozymes were extracted from roots of the germinated seedlings (destructive sampling)

Collection of an additional 21 open pollinated progenies of *Q. petraea* and 16 open pollinated progenies of *Q. robur* in the fall of 1989. This sample was used for the estimation of hybridization rates using RAPD markers (Bacilieri *et al*., 1996b). Germinated seed were transferred in the nursery for subsequent DNA extraction on leaves.

3.4. Tree monitoring

Male and female **flowering phenology** of trees was monitored every three days in spring 1990, every 14 days in spring 1991 and every 7 days in spring 1992 (Bacilieri Roberto, 1994 p15, p32)

Seed production was planned to be assessed in the fall of 1989, 1990, 1991 and 1992 using seed traps. Monitoring failed in 1990 due to an early fall of the seed prior to the setting up of the traps. No traps were installed in 1991 because the crop was too poor. In his thesis Bacilieri (1994 p. 48) reported that seed crop was poor in both years. Hence data were only obtained for 1989 and 1992 (Mailait Laurent, 1993). Bacilieri mentions again that seed crop in *Q. robur* was extremely poor in 1992 (Bacilieri Roberto, 1994 p 48)

3.5. Research and main results

Leaf morphology variation and species assignment based on leaf morphology (Bacilieri *et al*., 1996a; Kleinschmit Joerg, 1995; Kleinschmit *et al.,* 1995; Kremer *et al*. 2002). Based on Factorial Discriminant Analysis analysis, Bacilieri concluded that the stand was composed of 190 sessile, 217 pedunculate and 12 "intermediate" trees (Bacilieri Roberto, 1994 p 18, p 31).

Species differences for female flowering periods. Male flowering occurs slightly earlier than female flowering in both species. In spring 1990 overall (across all trees) male flowering period in *Q. robur* extended from April 4th to may 11th, and female flowering from April 10th to May 12th. In *Quercus petraea,* these periods were respectfully : April 5Th to May 11th for male flowering and April 10th to May 13th for the female flowering (Expert Frédéric, 1991; Bacilieri Roberto 1994, p18 figure3; Bacilieri *et al.*, 1995a)

Species genetic divergence and differentiation. Minor differentiation between *Q. petraea* and *robur* using either isozymes (Bacilieri *et al*., 1996a), RAPD (Moreau *et al.*,1994 a&b; Kleinschmit, 1995; Kleinschmit *et al.,* 1995), SCARs (Bodénès et *al*.,1997) Microsatellites (Mariette *et al*., 2002) and AFLPs (Mariette *et al*., 2002).

Spatial genetic structure based on 7 isozyme loci. There is more spatial genetic structure in *Q. petraea* than *Q. robur* (Bacilieri *et al*., 1994a)

Spatial structure for flowering time based on flowering time recorded in spring 1990**.** In both species, neighboring trees tend to flower synchronously (Bacilieri *et al*., 1994a)

Mating system, hybridization rates analysed with allozymes (7 loci). The two species are mainly outcrossing. There is asymmetric mating: *Q. petraea* trees tend to be preferentially mated by more "*petraea*" trees. Interspecific matings with *Q. petraea* as male parent are more frequent than vice versa (Bacilieri, 1994b, 1996a)

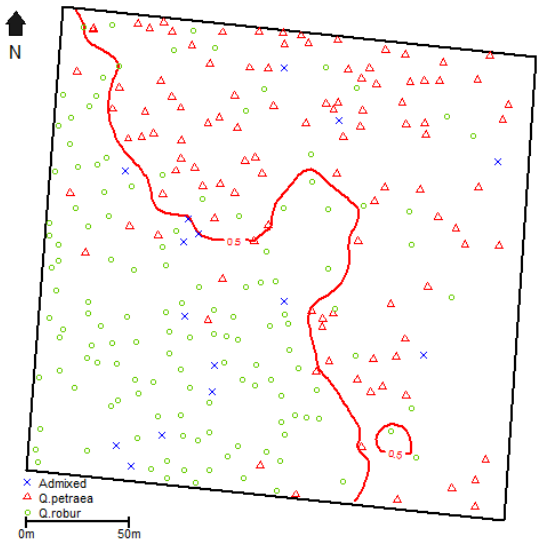
Seed production. Monitoring of seed crops using acorn traps in 1989 and 1992 indicated a higher seed production *in Q. petraea* than in *Q. robur*, in both years (Mailait Laurent, 1993)

4. The second period 1993-1997. Removal cuts

4.1. Silvicultural operations

The opening of the stand in 1989 facilitated seedling establishment and was followed by an additional removal cut in 1992 and 1993 leaving 298 standing trees (48 trees/ha). Actually the removal cut was made in two successive session: first reduction from 426 to 355 and then from 355 to 298. Figure 4 indicates the distribution of the remaining trees following the removal cut (to be compared with Figure 3).

Figure 4 Distribution of trees after the removal cuts.



4.2. Technical field operations

Conservation of the trees. All adult trees remaining after the removal cut (298) were vegetatively reproduced by grafting. Grafting operations started at the nursery in INRA Pierroton, and were subsequently transferred to State Nursery of Guéméné Penfao. Survival of grafts was low due to the local poor soil conditions in Pierroton. Ultimately all remaining grafting operations were conducted at the nursery of Guéméné Penfao. The grafts made at Pierroton were finally transferred to Guéméné Penfao. All grafting operations were repeated over years and lasted from 1995 to 2001. A conservation graft orchard was established at the State Nursery of Guéméné Penfao. Despite repeated grafting there were some losses and ultimately (in 2015) only 265 of the 298 trees were still present in the conservation orchard.

A progeny test comprising 51 open pollinated progenies (23 *Q. petraea* and 28 *Q. robur*) was planted in compartment 37 of the Petite Charnie Forest about 2 Kms south of compartment 26. Seeds were collected in October 1995, sown in the nursery of Guéméné Penfao, and seedlings were transplanted in March 1998 in compartment 37.

4.3. Material collection

Collection of 13 open pollinated progenies (7 *Q. petraea* and 6 *Q. robur*) (in total 984 acorns) in the fall of 1994. All collections were made from the canopy, except for 2 trees, where collections were made from the ground. This is the material that was used by Streiff *et al* (1996) and by Gerber *et al.* (2014) for the pollen dispersal analysis. The mother trees are located along the diagonal from the southwest to the northeast.

Collection of 51 open pollinated progenies (23 *Q. petraea* and 28 *Q. robur*) between September 26 and October 18 1995. This is the material that was ultimately transferred in the progeny test in compartment 37. The seeds were sown in the State Nursery of Guéméné Penfao.

Collection of leaves and buds from 177 seedlings distributed in the central part of the ISP in 1997. This material was used for parentage analysis in (Schibler Lorène, 1999) and Gerber *et al.* (2014).

4.4. Tree monitoring

4.5. Research and main results

Confirmation of the stronger **spatial genetic structure of *Q. petraea* than *Q. robur*** (Streiff *et al.* , 1998 a & b). The confirmation came by the use of microsatellites in comparison to earlier results obtained with isozymes.

First results on **pollen dispersion inferred from paternity analysis**. More than 65 % of the matings involved male parents coming from outside the ISP in both species. No clear difference in pollen dispersion distance between the two species (Streiff *et al.* , 1999 a & b)

5. The regeneration phase 1998-2001. Removal cut

5.1. Silvicultural operations

The **final cut** was implemented over 3 successive years (December 1998 to March 2001). The spread of the operations over three years facilitated the in depth assessments of many phenotypic traits, before the cut of the trees. The removal cut was achieved with the assistance of the staff of INRA Nancy and of the logging company. Stem sections of each tree were collected and transported to Nancy for tree ring analysis and further wood analysis.

Number of trees cut in December 1998 and January 1999 : 112

Number of trees cut in January and February 2000 : 126

Number of trees cut in March 2001 : 60

5.2. Technical field operations

Right after the felling of the trees during the final cut, the stumps were all labeled with aluminium tags.

The ISP was extended in 2001 by including trees that came from the surrounding compartments. These trees were about the same age. The extension comprised 270 trees. Hence the total number of adult trees amounted to 298 + 270 = 568. All additional 270 trees were labeled and geomapped (Figure 6). The extension was decided in order to capture more male parents that contributed to the regeneration in the original ISP. Unfortunately, when the extension was put in place, mature trees of the original ISP were already removed after the final cut. Finally research investigations were limited within the extension.

Figure 5 Map of the extension of the ISP

Map of the French I.F.S. : National Forest of "La Petite Charnie" compartiment 26

5.3. Material collection

When the trees were felled during the final cut, a log of 1.5 meter long from the base of the tree was cut and transported to Nancy (Qualité des Bois, Gérard Nepveu) for further wood and growth (tree ring) analysis. A detailed protocol of all the assessments made is attached

At INRA Nancy, a 5 cm thick disk was cut and shipped to INRA Montpellier (UMR Sciences Pour L'Oenologie, Jean Louis Puech) for later analysis of wood volatile compounds (papers by Prida *et al*. 2006 & 2007)

5.4. Tree monitoring

In 2000, the remaining trees (185 tree), were individually photographed before being cut.

A deep phenotyping of the stem and canopy of each tree was implemented. Numerous traits were assessed just before felling, and wood traits were later also assessed in Nancy on the the collected stem sections.

5.5. Research and main results

Spatial genetic structure: a strong spatial genetic structure was depicted for *Q. robur* when the extension of ISP was taken into account (Schehr Catherine, 2003) in comparison to earlier results limited to the original ISP.

Seed dispersal : Seed immigration in the ISP (original ISP) amounted to 34% for oaks that are generally considered to have limited seed dispersal (Gerber *et al.*, 2014)

Species differentiation for wood volatile compounds: *Q. petraea* is richer in eugenol and whisky lactone than *Q. robur*. However there is large variation of whisky lactone within Quercus petraea within the stand and some spatial structure (Prida *et al.*, 2006 & 2007)

6. The next generation since 2001 and ongoing

6.1. Silvicultural operations

After the final cut, cleaning operation was implemented in the regeneration recurrently after . The mechanical clearing operation removed all trees along linear strips evenly spaced every 9 meters (Figure 6). The width of linear cleaned strips was 3 meters

Figure 6 Aerial view of the ISP after the cleaning of the stand. The linear strips can easily be seen.

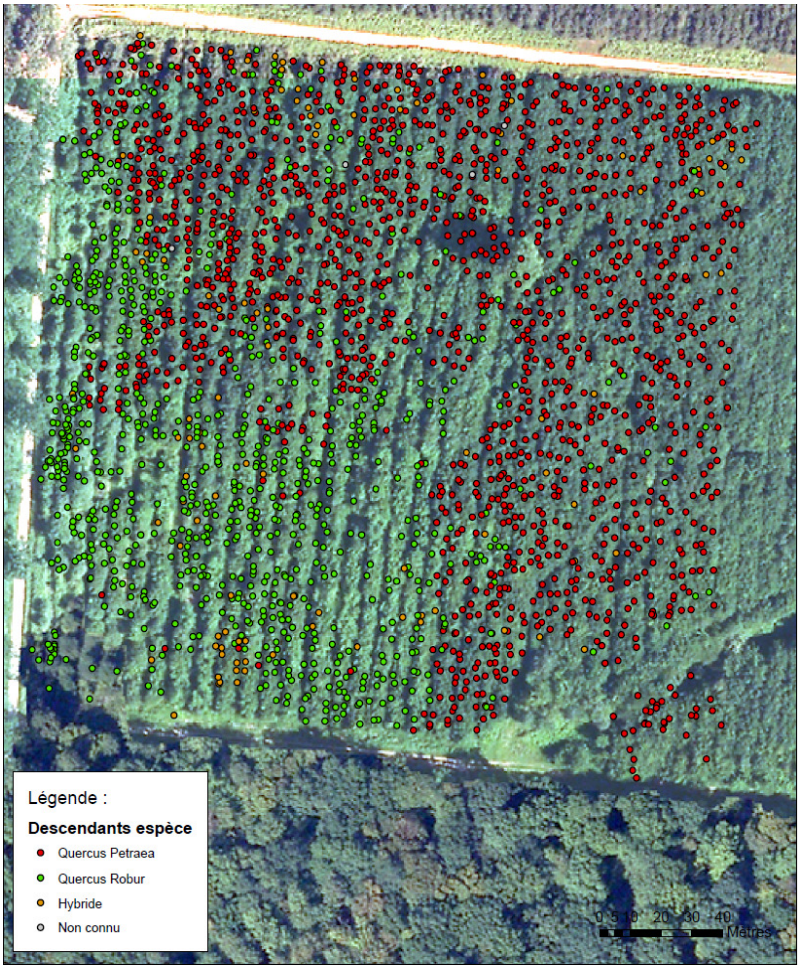
6.2. Technical operations

A sampling of the study material representative of the new generation was implemented in summer 2014. A systematic sampling of 2510 saplings was made in the regeneration, corresponding to the selection of 1 seedling every 3 to 6 meters along the linear strips. All samplings were labeled, and were signed with a green paint mark (Figure 8)

GPS coordinates were recorded for each sampled saplings. GPS coordinates of the adult trees of the former generation were retrospectively assessed using data of the earlier geo mapping operations conducted in 1989 (see paragraph 3.1). These operations were facilitated by the persistence of multiple stumps –and their labels- of trees of the former generation. The 260 adult trees and 2510 saplings were genotyped with 82 SMPs, and parentage analysis was subsequently conducted to assign parents to the saplings, based on the SNP data

Parentage analysis was ultimately implemented to assign paternity to the progenies installed in the nearby progeny test (paragraph 4.2) (Guichoux Erwan, 2011; Lagache Lelia 2012) using genotyping data of 12 microsatellites.

Figure 7 Sampling of saplings in the natural regeneration



6.3. Material collection

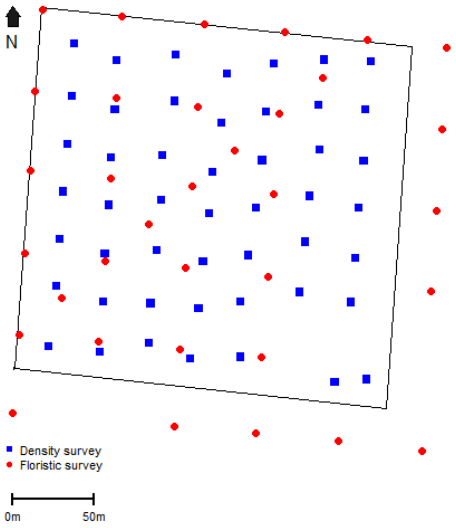
Buds and leaves were harvested in 2009 on all still living trees in the progeny test at that age (3213 trees). This is the progeny test established in parcel 37. Similarly buds and leaves harvested on grafts of adult trees of Petite Charnie planted in Guéméné (260 adult trees). This material was used for DNA extraction and parentage assignment of the 3213 open pollinated progeny raised in parcel 37.

In summer 2014, during the sampling of the saplings, leaves were collected and put in bags with silica gel for further DNA extraction and genotyping.

6.4. Tree monitoring

In July 2016 a demographic survey was conducted to assess sapling densities to calculate census estimates. The survey was based on a systematic sampling of 49 square survey plots distributed according to a grid system throughout the study stand. The area of each plot was on average 25 m^2 and all oak saplings present in a given plot were counted without species assignment. A given plot was usually inserted between the linear cleaning strips (see paragraph 6.1). Mean sapling density of *Q. petraea* was higher than in *Q. robur* (8516 saplings/ha *vs* 6209 saplings/ha). These differences were not significant (Wilcoxon rank sum test, p=0.43) because of the extreme variation in sapling densities among inventory plots in both species (from 285 to 30 330 in *Q. petraea* *vs* 327 to 15 490 in *Q. robur*) (see detailed data of the sapling densities)

Figure 8 Distribution of the survey plots for the demographic survey(Blue dots)



6.5. Research and main results

Species genetic assignment: design of microsatellites -plexes and SNP chips that allow species resoltuion in the *Q. petraea* and *Q. robur* complexes (Guichoux et al., 2011, 2012)

Hybridization dynamics: Hybridization is highly dependent on the demographic context, i.e., sopecies composition of the neighborhood (Lepais *et al.*,2009; Lagache *et al.,* 2013a). Premating barriers are stronger in *Q. petraea* than in *Q. robur* (Lagache *et al*. 2014)

Species occupancy: Ecological, genetic and demographic monitoring over two generations showed that is a clear expansion of *Q. petraea* at the expense of *Q. robur* (Truffaut *et al*., 2016).

Recruitment: Reproductive success –assessed by the presence of saplings in 2015- was inferred by parentage analysis and was higher in *Q.petraea* than in *Q.robur* (Truffaut *et al*., 2016).

7. References

7.1 Articles in journals (articles that use data recorded in the ISP)

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