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Full Paper

High-density linkage mapping and distribution of segregation distortion regions in the oak genome

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Abstract

We developed the densest single-nucleotide polymorphism (SNP)-based linkage genetic map to date for the genus *Quercus*. An 8k gene-based SNP array was used to genotype more than 1,000 full-sibs from two intraspecific and two interspecific full-sib families of *Quercus petraea* and *Quercus robur*. A high degree of collinearity was observed between the eight parental maps of the two species. A composite map was then established with 4,261 SNP markers spanning 742 cM over the 12 linkage groups (LGs) of the oak genome. Nine genomic regions from six LGs displayed highly significant distortions of segregation. Two main hypotheses concerning the mechanisms underlying segregation distortion are discussed: genetic load vs. reproductive barriers. Our findings suggest a predominance of pre-zygotic to post-zygotic barriers.

Key words: high-density linkage map, SNP, segregation distortion, reproductive barriers, *Quercus*

1. Introduction

The genus *Quercus* is a major forest tree genus with 300–600 species spread throughout the world (<http://www.mobot.org/MOBOT/research/APweb/orders/fagalesweb.htm#Fagales>). The European white oak species complex—including *Quercus robur* (Qr), pedunculate oak and *Quercus petraea* (Qp), sessile oak, in particular—is an exceptional model for studies of the genetics of speciation in sympatry.^{1–4} These two main oak species have similar distribution areas, but display morphological differences for many traits, including leaf shape, acorn peduncle length, and hairiness.⁵ They also have different ecophysiological requirements: Qr tolerates waterlogging and light exposure more effectively than Qp,⁶ whereas Qp has a higher water use efficiency and is more shade-tolerant, consistent with its role as a post-pioneer species.^{7–9} These two sympatric species are interfertile, forming natural hybrids in mixed stands and displaying only partial reproductive isolation (RI).^{3,10}

Low-density genetic linkage maps in oaks have been constructed with various genotyping techniques: random amplified polymorphic DNA, amplified fragment length polymorphism and simple sequence repeat (SSR).^{11–13} Progress has recently been made, with the development of a single-nucleotide polymorphism (SNP) assay for Qr and Qp.¹⁴ Using this resource, we were able in this present study to construct high-density gene-based maps with a large number of SNP markers, providing a detailed scan of segregation distortion (SD) within the genome. SD, corresponding to deviation from the expected Mendelian proportion of individuals in a given genotypic class within a segregating population, has been widely documented in plant species, within the framework of genetic mapping studies, e.g. in barley,¹⁵ clementine,¹⁶ eucalyptus,¹⁷ maize,¹⁸ rice,¹⁹ monkeyflower,²⁰ poplar²¹ and tomato.²² The detection of SD depends heavily on the type of cross. In most cases, SD is stronger and, therefore, easier to detect, in interspecific crosses than in intraspecific crosses.²³ Some of these studies showed that distortion spreads over linked markers (and

even over large genetic distances in some cases), creating SD regions (SDR). SD may result from three underlying causes. Gametic incompatibility (one of the many types of prezygotic barrier)²⁴ and/or reduced hybrid viability (one of the many forms of post-zygotic selection)^{25,26} may be involved. Indeed, various developmental processes in the pollen, embryo and seedling offer opportunities for differential selection at the gamete (pollen tube competition, pollen tube growth) and/or zygote (embryo competition, ovule abortion) level. The accumulation of deleterious mutations is an alternative mechanism that may give rise to SD. Genetic load has often been advocated as a source of SD, particularly in trees, which form large populations likely to have accumulated large number of deleterious mutations.^{27,28} SD may also arise due to asymmetric allelic inheritance in heterozygotes (i.e. meiotic drive),²⁹ but this process is not considered here as it relates to hybrid fertility rather than hybrid viability.

In this context, the first objective of this study was to establish a high-density gene-based genetic map of oak by genotyping more than 1,000 full-sibs from two intraspecific and two interspecific full-sib families of *Q. petraea* and *Q. robur*. A single composite map was constructed by merging the eight parental maps. This map includes 4,261 gene-based markers and is the densest linkage map ever produced for oak. The second objective of our study was to carry out a detailed analysis of the SDRs based on the patterns of SD observed for intraspecific and interspecific crosses. The SDRs identified on the eight linkage maps were compared to investigate the causes of the observed SDRs that span 6 of the 12 chromosomes. More precisely, we describe the extent of SD along linkage groups (LGs) and depict the distribution of SDRs, compare the consistency of SDRs across different genetic backgrounds and draw inferences about the potential sources of the widespread SD in the oak genome.

2. Materials and methods

2.1. Plant material and DNA extraction

Six parental trees, three *Q. robur* (3P, A4, 11P) and three *Q. petraea* (QS28, QS21, QS29), were used to generate four full-sib families (F1) (Fig. 1):

- One *Q. robur* intraspecific family (3P × A4) referred to as P1.
- One *Q. petraea* intraspecific family (QS28f × QS21) referred to as P4.
- Two interspecific families (11P1 × QS28m, 11P2 × QS29) referred to as P2 and P3.

Crosses are denoted 'female parent × male parent' below. The same parental tree of *Q. petraea* (QS28) was used as the male parent in one cross (QS28m) and the female parent (QS28f) in another cross. Furthermore, the *Q. robur* parent 11P was used as a female parent in two different crosses and is therefore named 11P1 and 11P2.

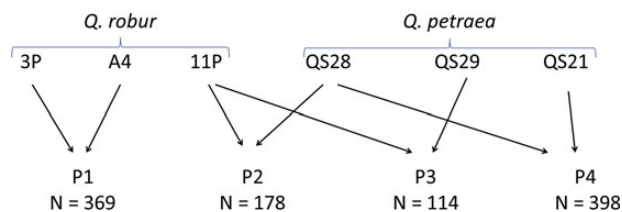


Figure 1. Description of the four pedigrees (3P, A4, 11P, QS28, QS29 and QS21 referred to the name of the parents used for the controlled crosses. P1, P2, P3 and P4 correspond to the name of the pedigree; *n* represents the number of offspring in each pedigree).

Quercus robur had to be used as the female parent in all interspecific crosses, due to the strong asymmetry of hybridization success^{3,30} between these two species. The sizes of the progenies of the full-sib crosses varied from 398 (QS28f × QS21) to 114 (11P2 × QS29) individuals. Intraspecific and interspecific controlled crosses were implemented over the years since 1993. The offspring were installed in stool beds at the nursery of the INRA Forestry Research Station Pierroton (latitude 44.44°N, longitude 0.46°W) in South West of France, before subsequent vegetative propagation of the offspring. Bud and leaf material was collected on the stool beds and DNA was extracted as described by Bodénès *et al.*¹³

2.2. SNP genotyping

SNP genotyping was carried out on the four mapping populations (1,059 samples in total), with the Illumina[®] Infinium iSelect Custom Genotyping Array (Illumina Inc., San Diego, CA, USA), according to the manufacturer's standard protocol, using 200 ng of genomic DNA per sample. The selected SNPs correspond to two different data sets, the first one was obtained from the resequencing of six trees used as parents of the four controlled crosses and the second one was obtained from the oak Unigene established by Ueno *et al.*³¹ In total, 7,913 SNPs were selected for array design. We deliberately selected a limited number of markers (from 1 to 3) per contig, to ensure a broad distribution of SNPs across the genome.¹⁴

2.3. Linkage map construction

For each cross, two data sets were generated, each containing the meiotic segregation information from one of the parents. Markers segregating in a 1:1 ratio (testcross configuration) were mapped with JoinMap 4.0 (Kyazma, Wageningen, NL).³² For each population, the minimum logarithm of odds (LOD) threshold for grouping was determined by identifying grouping tree branches with stable marker numbers over increasing consecutive LOD values for a total number of groups of 12 which corresponds to the number of chromosome. The minimum LOD threshold for most of the linkage maps was 5 and increased to 6 for P4m map or 7 for P2m map. The two parental linkage maps for each mapping population were constructed with the maximum likelihood (ML) algorithm, with a minimum LOD of 5 and the default parameters (recombination frequency of 0.4 and a maximum threshold value of 1 for the jump). Recombination frequencies were converted to map distances in cM with the Kosambi's mapping function. The maps shown were plotted with MapChart 2.0³³ or using an R script. LG numbering was based on SSR markers, as in the study by Bodénès *et al.*¹³ The cosegregation of SSRs and SNPs (data not shown) made it possible to identify homologous LGs unambiguously.

2.4. Composite map construction

We constructed a composite map from the eight parental maps, with the LPmerge software developed by Endelman and Plomion,³⁴ which is available as an R package <https://cran.r-project.org/web/packages/LPmerge/index.html>. This approach is based on the integration of linkage map data rather than observed recombination between markers, with linear programming used to minimize the mean absolute error between the composite map and the linkage map for each population as efficiently as possible. For assessment of the goodness-of-fit for the composite map, LPmerge computes a root-mean-square error (RMSE) per LG by comparing the position (in cM) of all markers on the composite map with that on the component maps (http://w3.pierroton.inra.fr/cgi-bin/cmap/viewer?ref_map_set_acc=51&ref_map_accs=-1). We calculated this metric for different maximum interval

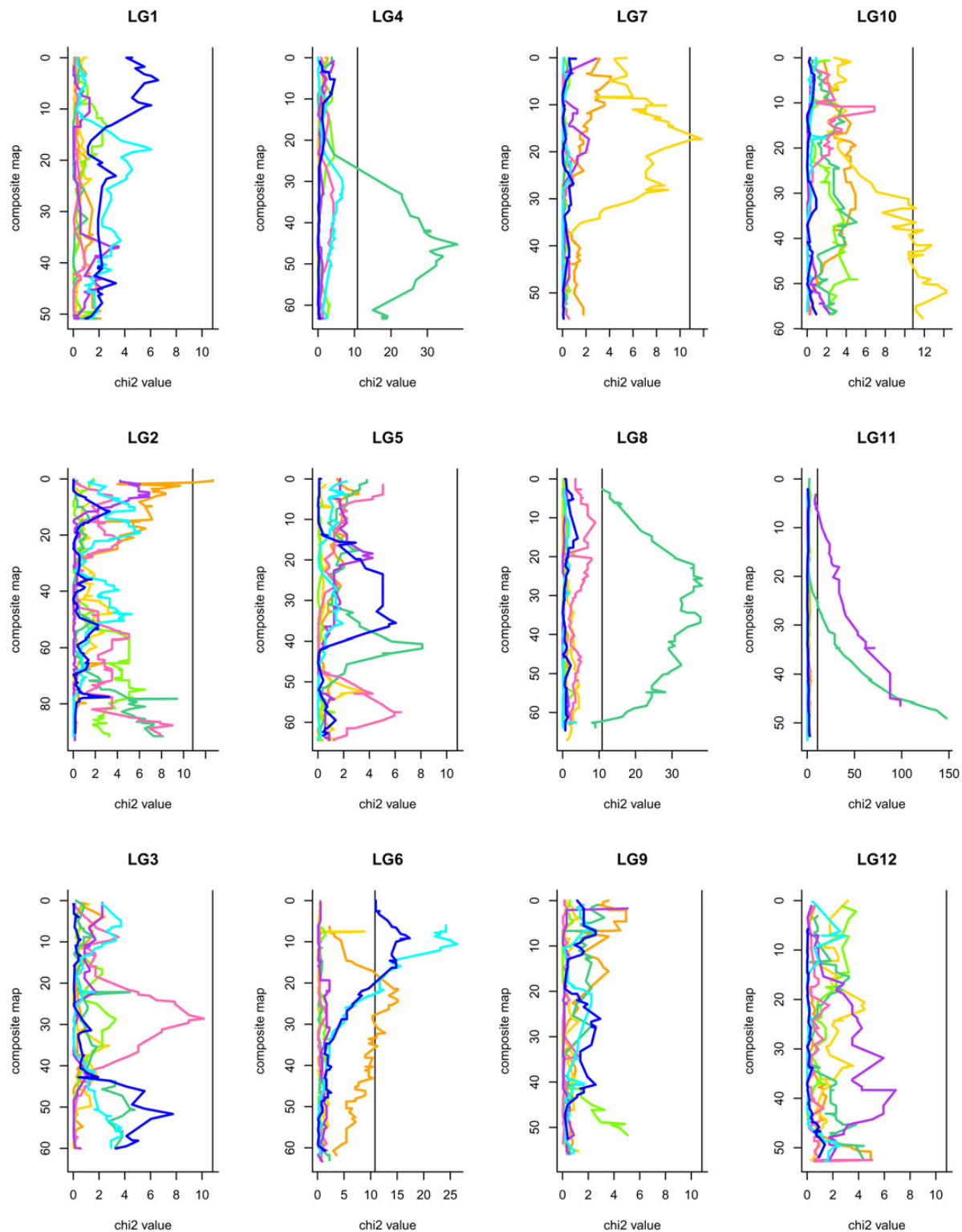


Figure 2. Distribution of SD for the 12 LGs for the 8 parental maps, $\alpha = 0.001$. The vertical line corresponds to a type I error of 0.001. The x-axis corresponds to the χ^2 value and the y-axis corresponds to the map length of the LG in cM. The black vertical line corresponds to the χ^2 threshold value, orange line corresponds to P1f, yellow line: P1m, light green: P2f, dark green: P2m, pink: P3f, violet: P3m, light blue: P4f and dark blue: P4m.

sizes (k in the algorithm), ranging from 1 to 8. The value of k minimizing the mean RMSE per LG was selected for construction of the composite map (Supplementary File S1). Intercross markers (segregating in

a 1:2:1 ratio) were added as accessory markers in a second step taking the rate of recombination between these loci and the closest linked test-cross framework marker (FM) into account.

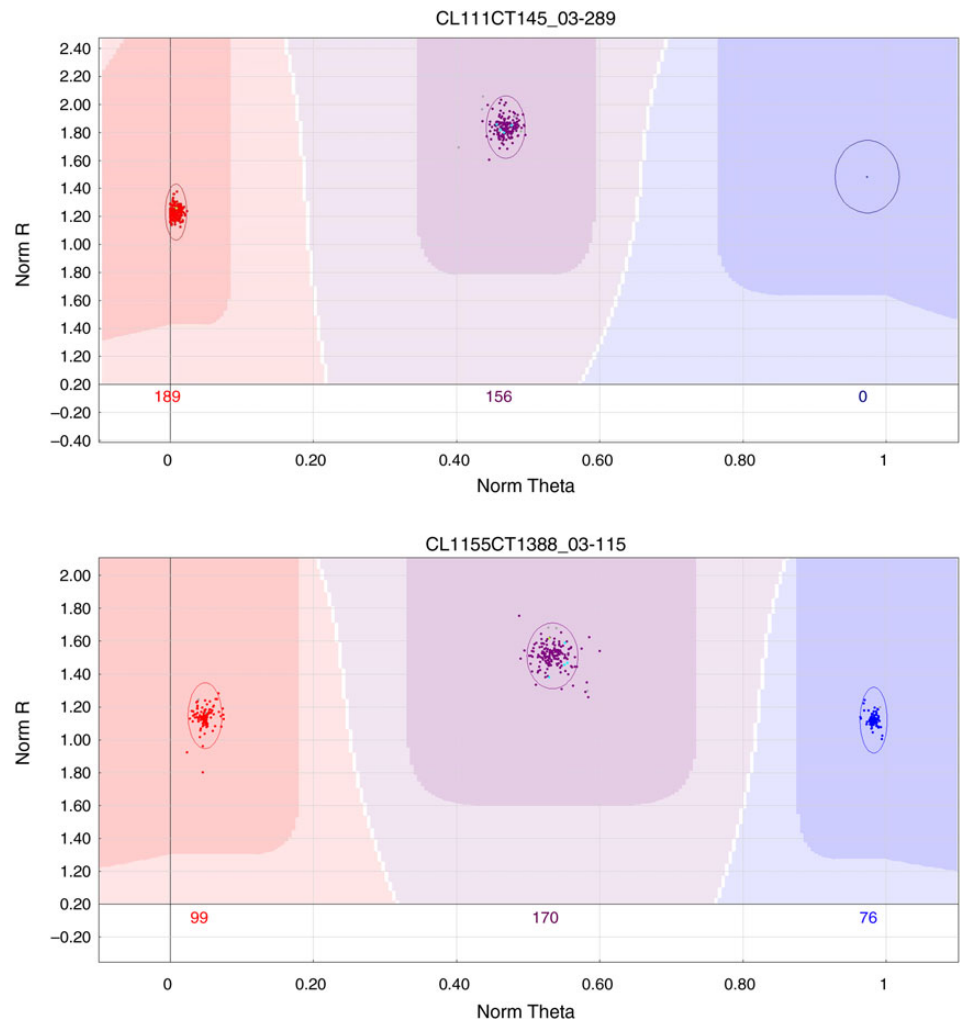


Figure 3. (a) AA×AB configuration [one parent is homozygous, the other, heterozygous, two classes of genotypes are observed among the progeny, AA (in red, left part) and AB (violet part, middle part)]. (b) AB×AB configuration [both parents are heterozygous, three classes of genotypes are observed among the progeny, AA (in red, left part) AB (in violet, middle part) and BB (in blue, right part)]. This figure is available in black and white in print and in colour at *DNA Research* online.

Table 1. Number of polymorphic (P), monomorphic (M) and failed (NA) SNPs in the four pedigrees, P1, P2, P3 and P4

	P1	P2	P3	P4	All pedigrees
P	3,515	3,734	3,399	3,418	5,726
M	2,012	2,120	2,488	2,222	637
NA	1,483	1,156	1,123	1,370	647

2.5. SD analysis

We tested each marker for significant deviation from the expected Mendelian genotype frequencies (χ^2 with 1 degree of freedom for codominant markers, $\alpha = 0.05$ calculated with JoinMap software) to detect SD. Assuming that each LG corresponds to one chromosome ($n = 12$ in *Quercus*) and that each chromosome contains at least two independent regions (the mean length of LGs was 66 cM, this paper), we expected there to be at least 24 independent genomic regions. A threshold of at least $0.05/24 \approx 0.002$ would therefore be required to obtain a genome wide error rate of $\alpha = 0.05$. However, we applied a more stringent threshold ($\alpha = 0.001$) and only considered distorted regions with more than three tightly linked distorted loci, to decrease the false-

positive rate and to ensure that only biologically meaningful SDRs were detected. Markers displaying SD were conserved and integrated into the map. We investigated the patterns of distortion on the eight parental maps, by plotting the χ^2 value of each marker along the 12 LG of the composite map obtained with the LPmerge programme (Fig. 2).

3. Results

3.1. SNP genotyping

The 7,913 SNPs were submitted to Illumina for Oligo Pool All (OPA) design for use in the Infinium assay. From the initial set of 7,913 SNPs, 903 (11.4%) did not pass Illumina production quality control and were eliminated. The genotyping data, across the four mapping populations, for the 7,010 SNPs retained were analyzed with Illumina GenomeStudio software, which clusters and calls data automatically, making it possible to visualize the data directly.¹⁴ For each SNP, the representation of the genotyping data included three main clusters, corresponding to the AA homozygote, the AB heterozygote and the BB homozygote. We obtained four different segregating configurations in the F1 mapping populations: AB×AA (heterozygous in the female parent), AA×AB (heterozygous in the male parent) (Fig. 3a),

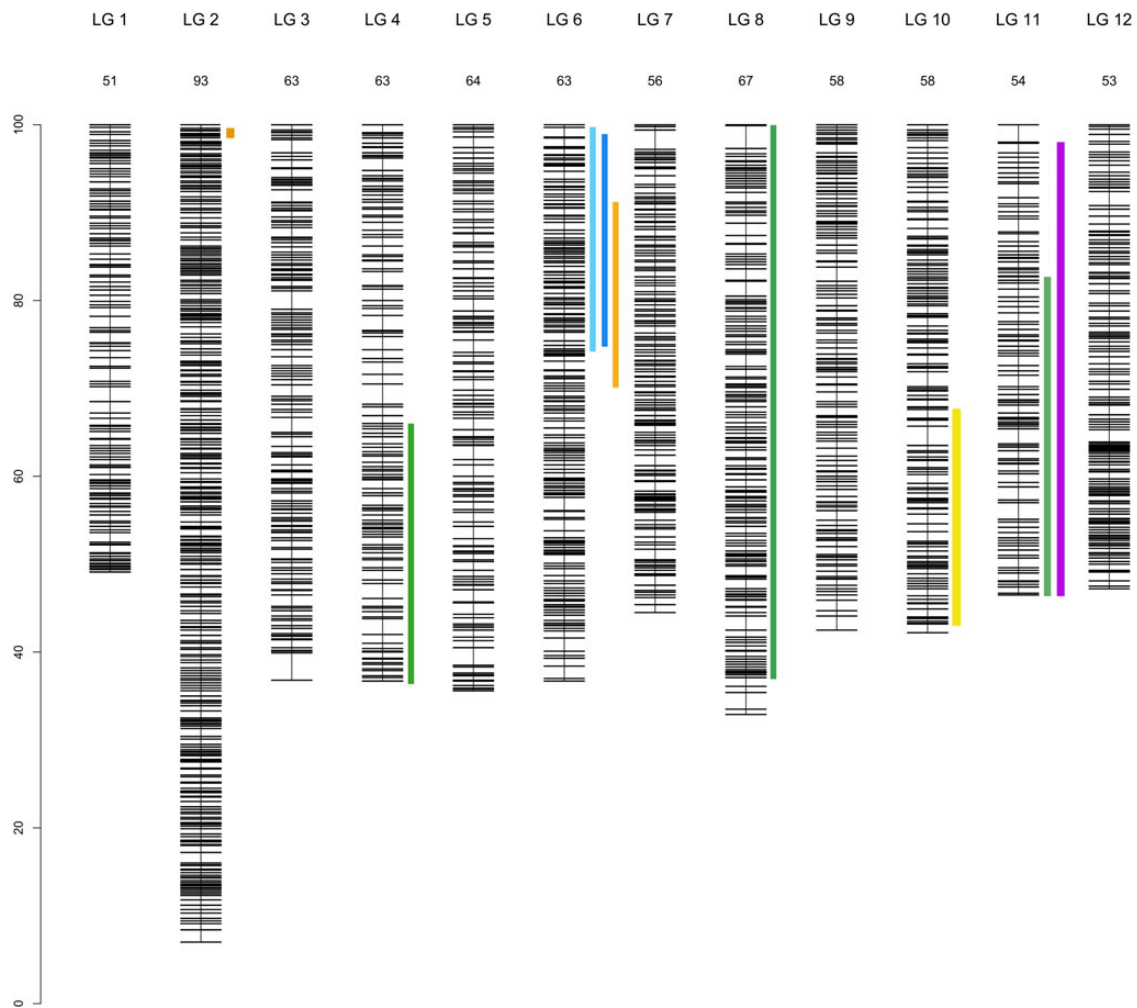


Figure 4. Composite linkage map for the 12 LGs and localization of the 9 segregation distorted regions (SDRs). The SDRs are represented by a vertical colour trait along the LG2, in orange: P1f, LG4, in dark green: P2m, LG6, left to right, in light blue: P4f, dark blue: P4m, orange: P1f, LG8, in dark green: P2m, LG10, in yellow: P1m, LG11, in dark green: P2m, in violet: P3m). The LG size in cM is given in the LG name. This figure is available in black and white in print and in colour at *DNA Research* online.

Table 4. Number of loci displaying SD for the eight parental maps, $\alpha = 0.001$

Pedigree name	Cross type	Name of the parents	Mapping population size	SNP genotyped	Distorted loci $\alpha = 0.001$	LG (number of distorted loci)	Percentage of distorted SNPs
P1	Intraspecific	P1f	324	1,170	38	LG2 (11); LG6 (27)	3
3PxA4	F1	P1m		1,136	25	LG7 (4); LG10 (23)	2
P2	Interspecific	P2f	178	1,229	0		0
11P×QS28	F1	P2m		1,205	177	LG4 (34); LG8 (95); LG11 (48)	15
P3	Interspecific	P3f	114	1,421	0		0
11P×QS29	F1	P3m		905	47	LG11 (47)	5
P4	Intraspecific	P4f	350	1,393	38	LG6 (38)	3
QS28×QS21	F1	P4m		889	34	LG6 (34)	4

LG for linkage group.

4. Discussion

4.1. Construction of a high-density gene-based linkage map and application in genetic studies in oak and beyond

We report here the development and validation of the first high-throughput Illumina SNP genotyping assay for oaks, with a success

rate of 88.6%, i.e. 7,010 of the 7,913 SNPs were successfully genotyped. Overall, 82% of the SNPs successfully genotyped were polymorphic in at least one of the four pedigrees and 63% were mapped as FM on the parental genetic maps.

The use of multiple segregating populations of diverse genetic backgrounds made it possible to map a larger number of markers and to achieve greater genome coverage. This has already been

Table 5. Number and coverage of SDRs over the LGs, $\alpha = 0.001$

Parental genotype	LG	Number of distorted SNP loci	Proportion of distorted loci (%)	SDR (in cM)	LG size (in cM)	Proportion of distorted LG (%)
P1f	LG2	11	6	1	89	1
	LG6	28	28	10	52	20
P1m	LG10	23	28	24	59	40
P2f	—	—	—	—	—	—
P2m	LG4	34	61	31	70	44
	LG8	95	91	55	58	96
	LG11	48	64	34	57	60
P3f	—	—	—	—	—	—
P3m	LG11	47	87	34	38	88
P4f	LG6	38	30	17	52	32
P4m	LG6	34	33	15	71	21

illustrated in watermelon, for example, in which the genotyping of four mapping populations increased the proportion of mapped loci from the most polymorphic pedigree by 29%.³⁶ In this study, the gain in terms of the number of newly segregating test-cross markers from the pedigree with the largest number of SNPs (2,472 in P2) with respect to the other three pedigrees was remarkably high, reaching +1,756 SNPs (i.e. +41% newly mapped markers).

The development of array-based genotyping technologies has made it possible to generate high-density linkage maps rapidly, but the integration of independent maps containing thousands of loci remains a real challenge.^{37–39} Two main approaches have been developed for the construction of combined maps. The first involves pooling genotypic data and minimizing the sum of recombination frequencies, as proposed in the ML method implemented in JoinMap (e.g. as used in triticale by Alheit *et al.*³⁷ and in sunflower by Bowers *et al.*).³⁸ However, this method is time-consuming and may be not appropriate when thousands of markers are used, due to the computational time required. The second approach involves the direct integration of independent linkage maps, as proposed in MergeMap^{40,41} and LPmerge³⁴ and recently used in pine,^{39,42} barley⁴³ and wheat.⁴⁴ Using this second approach, we merged eight linkage maps each containing 889–1,421 markers, to obtain a composite map including 4,261 loci corresponding to 4,239 different contigs of the oak UniGene database⁴⁵ and covering 742 cM on the 12 LGs. This map is considerably denser than a previously published linkage map based on 397 EST and genomic SSRs.¹³

Framework maps were constructed exclusively from markers with a testcross configuration. All intercross markers were excluded from construction of the framework map because they provide little information about linkage.³⁵ We added markers segregating in the intercross configuration at a later stage, as accessory markers. The eight parental maps displayed remarkably high degrees of collinearity and no chromosomal rearrangements were observed, providing support for our approach of merging maps to construct a unified composite map for the genus *Quercus*, including both species maps. In the framework of the oak genome sequencing project,⁴⁶ this genomic resource will be crucial for the assembly of genome scaffolds into chromosomal pseudomolecules. High-density sequence-based linkage maps have been used to anchor and orient scaffolds in many plant species, including *Cicer arietinum* (chickpea),⁴⁷ *Rubus* (raspberry)⁴⁸ and *Eucalyptus*.⁴⁹ The oak composite linkage map also provides a framework for genome-wide analysis at the centimorgan scale, for genomic scans of species/population divergence,⁵⁰ studies of the evolutionary relationships between related species,^{16,51} the detection of recombination hot and cold spots,⁵² studies

of the extent of long-distance linkage disequilibrium and genetic diversity,^{39,53} the detection and positional characterization of QTLs through co-localization with gene-based markers (e.g.^{54–56}), and the identification of chromosomal rearrangements.⁵⁷ This unified linkage map for the genus *Quercus* will also be useful for analyses of synteny and collinearity within the Fagaceae at a much higher resolution than previously reported (e.g. between *Quercus* and *Castanea*),^{13,14,58} and for extending such analyses to other Eurosids, providing insight into genome evolution⁵⁹ and a framework for the transfer of genetic information between species.

4.2. Patterns of SD identify gametic incompatibility as a major RI barrier in oak

Deviation from the ratios expected for Mendelian inheritance reveal disturbances in the transmission of genetic information from one generation to the next, generating interesting hypotheses about the mechanisms underlying RI for exploration in further studies. Overall, our results revealed that SD was widespread in the oak genome. Regardless of the species or cross, half of the 12 LGs displayed SD, for 6–91% of markers, depending on the LG. We observed large differences in SD values between intra- and interspecific crosses, and between male and female parents.

These SDRs may result from chromosome loss or rearrangements, genetic load or pre- or post-zygotic selection, and interpretation may differ between intra- and interspecific crosses.

Peculiar life history traits of trees may raise their genetic load and result in substantial SD. Oaks, like most forest trees species, form very large populations, and generally outcross through wind pollination, resulting in high levels of gene flow.^{60,61} These characteristics lead to the accumulation of deleterious mutations and the build-up of a large genetic load.²⁷ The accumulation of recessive deleterious mutations in fitness genes (pollen fertility, anther receptivity, seed fertility) or loci closely linked to fitness genes decreases the viability of plants with homozygous at these loci.⁶² Genetic load may be partially purged at early embryonic stages, by the death or sterility of hybrids carrying homozygous recessive deleterious mutations. Genetic load varies considerably between plant species and may be a major source of SDRs.¹⁷ For the intraspecific pedigree P4, we observed SDRs on LG6 that were conserved between the two parental maps. This could indicate the presence of lethal or sublethal genes compromising seedling survival in both parental genotypes.⁴⁸

Gametic incompatibility and/or reduced hybrid viability can also contribute to SD. In this study, the proportion of loci displaying SD

differed between the eight linkage maps (0–14.5%). The frequency of SD was higher on three LGs: LG6 (28% of loci displaying SD distributed over 16 cM, for three parents), LG 8 (26.5%, 55 cM, one parent) and LG11 (26.5%, 33 cM, two parents). In several previous studies (eucalyptus,⁶³ rice¹⁹ and monkeyflower),²⁰ clusters of distorted loci were found to extend over all or most of the LG. In rice, for example, distortion gradually decreases with increasing distance from the markers displaying the highest levels of SD, located at or near previously reported gametophytic gene loci or sterility loci. In our study, 79% of the loci presenting SD were of paternal origin whatever the type of cross (intra- or inter-specific). LG11 in the cross with QS29 as the male parent (P3m) provides an extreme example, with an SDR encompassing 88% of the SNPs on this LG, as previously reported in a study with far fewer EST–SSR markers and a much smaller number of offspring.¹³ These figures may reflect strong pollen incompatibilities between the parents of the different crosses, as previously reported by Abadie *et al.*⁶⁴ Indeed, the results obtained for one genotype (QS28), used as either the male or the female parent in controlled crosses, strongly support the observed trend: the number of markers displaying SD was five times higher when this genotype was used as the male parent (P2m) than when it was used as the female parent (P4f). A confounding effect of the type of cross may also have contributed to the observed pattern (see next paragraph) because QS28 was used as the male in the interspecific cross and as the female in the intraspecific cross. Male gametophytic selection has been identified as the phenomenon most frequently causing skewed segregation, due to selective influences of the gynoecium, resulting in genetic incompatibility.¹⁹

Interestingly, 62% of the loci displaying SD were derived from inter-specific crosses and were only detected in the male LGs. SD is frequently observed in interspecific crosses, and has been attributed to biological factors, such as pollen–pistil incompatibility, hybrid viability, sterility due to gametophytic competition, negative epistatic interactions between alleles or positive introgression.^{17,21} Pre-reproductive barriers play a major role in the directionality of introgression: genetically based pollen discrimination is a major barrier, as it greatly increases assortative mating within species and the parental species fidelity of hybrids.⁶⁵ Alternatively, natural selection may play a key role by acting against unfit genetic combinations. Lepais and Gerber⁶⁶ observed lower levels of mating success for interspecific crosses compared with intraspecific crosses or backcross mating events, in a mixed stand of four European white oak species (including Qr and Qp). They clearly showed that the different species contributed unequally to reproduction success through differences in pollen efficacy. Most pure-bred plants reproduce preferentially with conspecific individuals. These findings were confirmed by another independent hybridization study conducted in a mixed stand of four European white oak species, Qr, Qp, *Quercus pubescens* and *Quercus frainetto*.¹ This previous study highlighted the importance of selection against hybrids, resulting in the maintenance of four distinct parental gene pools in sympatry. Based on these observations, we suggest that gametic incompatibility could lead to SD in chromosomal regions on either the female or the male map, whereas reduced hybrid variability would be likely to cause SD in the corresponding regions of both parental maps. Most of the observed SDRs were identified in only one parent, generally the male. Thus, a large fraction of the SDRs were sex-specific, suggesting that gametic selection plays an important role in shaping SDRs in oak.

5. Conclusion

Our study demonstrates the relevance of Illumina technology for SNP genotyping for multipedigree studies in oaks. The high rate of

successful SNP development for the six different parental genotypes used in the four controlled crosses provided us with a very large set of mappable SNPs, which was essential for comparative mapping and construction of the oak high-density composite linkage map.

We established a high-density composite linkage map based on more than 4,261 SNP loci suitable for use as a reference gene-based map for the genus *Quercus* and for the Fagaceae in general. Genomic resources are available for very few species within the Fagaceae family and the map developed here could be useful for studies of species from the same genus or for related genera belonging to this family.

Finally, we identified regions of SD potentially related to RI or genetic load. Further studies assessing seed abortion and the viability of young hybrid seedlings during juvenile development are required to shed light on the causal mechanisms underlying RI. Additional investigations of the co-localization between SDRs, QTLs for adaptive traits^{67,68} and species divergence hot spots should also be carried out. The recent availability of a whole-genome sequence for *Quercus*⁴⁶ will finally help to identify genes located in and underlying SDRs and the genes involved in RI.

Authors' contributions

C.B. performed the genetic analysis, E.C. extracted the DNA, E.C. and C.B. carried out the genotyping analysis, F.E. updated the databases, C.B. and C.P. wrote the manuscript. C.B., C.P. and A.K. conceived and designed the project. All authors read and approved the final manuscript.

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Supplementary Data

Supplementary Data are available at www.dnaresearch.oxfordjournals.org.

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Web portal

Quercus portal:

<https://w3.pierroton.inra.fr/QuercusPortal/>

CMap Comparative Map Viewer for the composite LGs:

[http://w3.pierroton.inra.fr/cgi-bin/cmap/viewer?](http://w3.pierroton.inra.fr/cgi-bin/cmap/viewer?ref_map_set_acc=51&ref_map_accs=-1)

[ref_map_set_acc=51&ref_map_accs=-1](http://w3.pierroton.inra.fr/cgi-bin/cmap/viewer?ref_map_set_acc=51&ref_map_accs=-1)

CMap Comparative Map Viewer for different parental LGs:

http://w3.pierroton.inra.fr/cgi-bin/cmap/map_details?ref_map_set_acc=47;ref_map_accs=1251;comparative_maps=1%3dmap_acc%3d1287;highlight=%22s_1BHPHQ_1326%22

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