Massive postglacial gene flow between European white oaks uncovered genes underlying species barriers

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ABSTRACT

Oaks are dominant forest tree species widely distributed across the Northern Hemisphere, where they constitute natural resources of economic, ecological, social and historical value. Hybridization and adaptive introgression have long been thought to be major drivers of their ecological success. Thus, the maintenance of species barriers remains a key question, given the extent of interspecific gene flow. In this study, we scanned the genomes of four European white oak species for reproductive barriers. We identified the ecological and phylogenic relationships of these species and inferred a long-term strict isolation followed by a recent and extensive postglacial contact. Then, we made use of the tremendous genetic variation among these species (31 million SNPs) to identify genomic regions for reproductive isolation. A literature-based functional annotation of the underlying genes highlighted important functions driving the reproductive isolation between these sister species. These functions were consistent with their ecological preferences and included tolerance to biotic and abiotic constraints. This study holds important implications for the renewal of European forests under global warming.

MAIN

Oaks are a diverse group of about 350 to 500 species widely distributed throughout the Northern Hemisphere [1, 2]. The variability in the number of recorded oak species highlights the challenge of delineating species limits within a genus displaying a high degree of morphological diversity, sometimes described as a “botanic horror” by taxonomists [3–5]. Genetic markers have corroborated these taxonomic concerns, particularly in European white oaks, which have been the subject of a large number of genetic surveys. Studies based on nuclear DNA markers have reported

48 unambiguously high levels of admixture between European white oak species,
49 confirming the reported taxonomic issues for oaks [6]. Several detailed empirical studies
50 based on chloroplast DNA markers have revealed an absence of private chlorotypes
51 between European white oak species, but congruent associations between chlorotypes
52 and expansion routes during the last postglacial recolonization, suggesting cytoplasmic
53 capture via recurrent hybridization and backcrossing [7, 8]. Recent advances in oak
54 genomics [9, 10] have made it possible to investigate interspecific gene flow at the whole-
55 genome scale. Indeed, Leroy et al. [11] have provided evidence suggesting that extensive
56 secondary contacts have occurred between four European white oak species, probably at
57 start of the current interglacial period. These results reconcile earlier findings of
58 contrasting species differentiation at the nuclear and organelle levels. The inferences
59 drawn are also consistent with the persistence of genomic regions impermeable to gene
60 flow due to functional reproductive barriers, corresponding to a typical case of semi-
61 isolated species [11]. However, the genetic basis of these barriers remains unknown.

62 Controlled pollination trials have provided empirical evidence for the existence of
63 strong reproductive barriers in these four European white oak species [12, 13]. Ecological
64 preferences *in situ* have also been previously reported, with tolerance to dry (*Q. petraea*)
65 or wet (*Q. robur*) sites (14), or acidic (*Q. pyrenaica*) or limy (*Q. pubescens*) soils [15] but
66 fine-grained ecological surveys do not yet exists for all these four species. The four
67 species occupy different geographic ranges (Fig 1A): extending up to Scandinavia for *Q.*
68 *petraea* and *Q. robur*; whereas the other two species are present mostly in
69 Mediterranean and sub-Mediterranean regions. However, the distribution ranges of
70 these species overlap in some areas, mostly in South-West France, but the four species
71 are rarely found together in the same stand [but see ref. 6]. The overlapping species
72 ranges in South-West France thus provide an ideal "natural laboratory" [16] for
73 investigating reproductive barriers between these European white oak species.

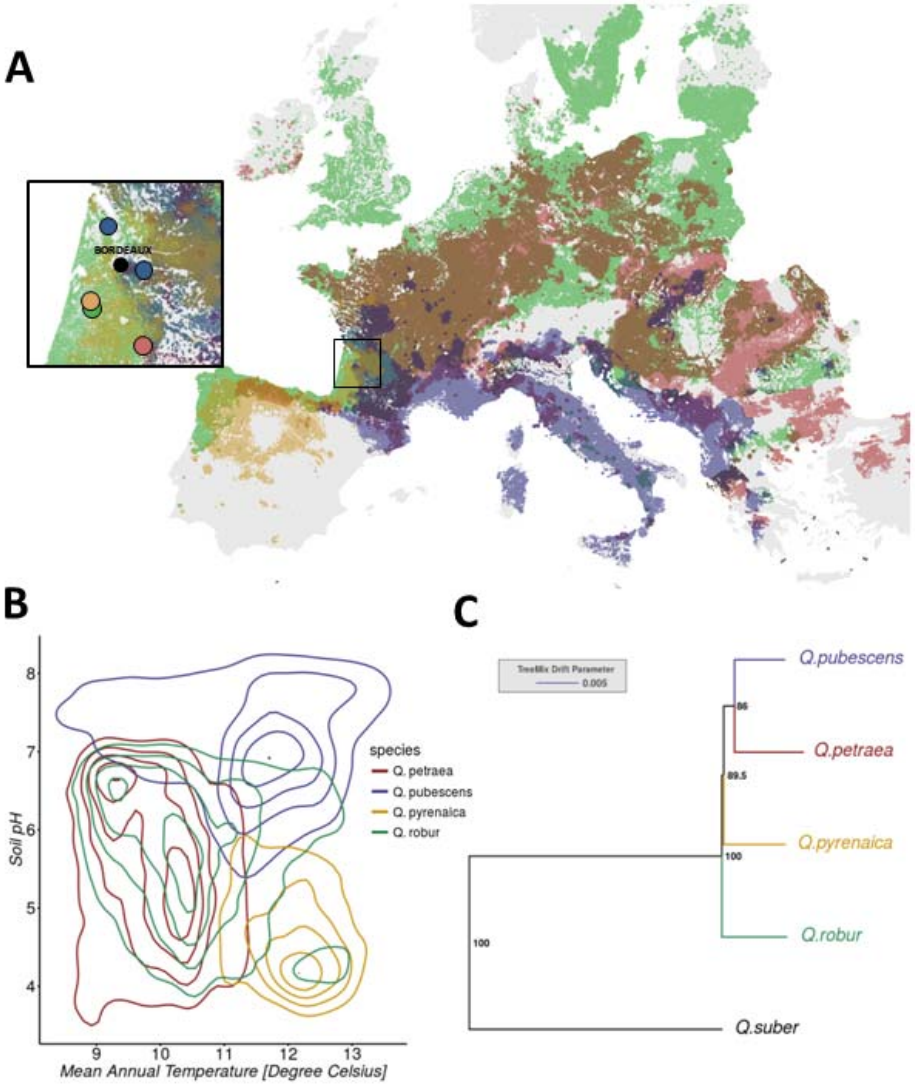

Figure 1: Continental-scale species distributions and origin of the study material (A) and, ecological (B) and phylogenic relationships of the four European white oak species under investigation (C).

Here we combined state-of-the-art methods in population genomics to explore the genomic distribution of reproductive barriers (Fig. 1): (i) we used approximate Bayesian computation (ABC) to perform ascertainment bias-free demographic inferences in order to refine estimates of the timing of secondary contacts, and (ii) scan genomes for reproductive barriers. Our findings identified important intrinsic and ecological functions driving the reproductive isolation of these four oak species including tolerance to biotic and abiotic constraints, and intrinsic mating barriers.

RESULTS

Ecological preferences of the four species

We intersected the distribution maps of the four species (Fig. 1A) with climatic and soil data derived from a large-scale floristic survey in France. Bivariate density distributions (Fig. 1B) show clear patterns of ecological preferences among the four white oak species. As expected, the two so called temperate white oaks (*Q. petraea* and *Q. robur*) are more frequently observed under cooler climates than Mediterranean and sub-Mediterranean species (*Q. pubescens* and *Q. pyrenaica*). pH of the soils segregates particularly *Q. pyrenaica* from *Q. pubescens*. Although we could not combine climatic and soil data over the whole species' ranges, univariate density distributions for both climate (Fig S1) and soil pH (Fig S2) based on continental-scale data showed similar trends.

Divergence and post-glacial secondary contact between European white oaks

A total of 31,894,340 SNPs were identified after the filtering of variants with a low minor allele frequency (MAF<0.02) in population samples for the four species (*Q. petraea, Q. robur, Q. pubescens, Q. pyrenaica)*, corresponding to one SNP every 23.2 bp,

on average. We also used genome-wide data for a *Q. suber* accession described by Leroy *et al.* [11] to root a phylogenetic tree and investigate relationships between species for 9,084,835 of the 31.9 million SNPs. The best maximum-likelihood tree suggested that *Q. robur* initially diverged from the ancestor of the other three species (Fig. 1C).

We then randomly selected 50,088 SNPs from the entire set of 31.9 million SNPs for ascertainment bias-free demographic ABC inference. We compared two models of divergence with gene flow (Fig. 2) for each of the six possible species pairs: an isolation-with-migration model assuming constant gene flow since the divergence time (T_{SPLIT}), and a model assuming secondary contact with gene flow starting at T_{SC}, a time point after divergence ($T_{SC} < T_{SPLIT}$). For all pairs, we obtained strong statistical support for the secondary contact model (>98% posterior probability, Tables 1 & S1), consistent with our previous findings based on individual data for 3,524 SNPs [11].

We generated parameter estimates under the best-fitting secondary contact model for each pair of species. Taking into account the 95% confidence intervals for each T_{SC}/T_{SPLIT} ratio (Tables 1 & S2) and the divergence time between these species (1-10 million years, [2,17], the analysis yielded quite large estimates with secondary contact occurring between 100 and 62,400 years ago, corresponding to up to 1,225 generations, assuming a generation time of 50 years [18]. Even assuming the upper bound for the divergence between these species (10mya [17]), median estimates of the timing of secondary contacts had much less variation and ranged between 2,450 and 21,760 years (up to 435 generations). These estimates are consistent with the general hypothesis of a resumption of secondary gene flow at the start of the current interglacial period.

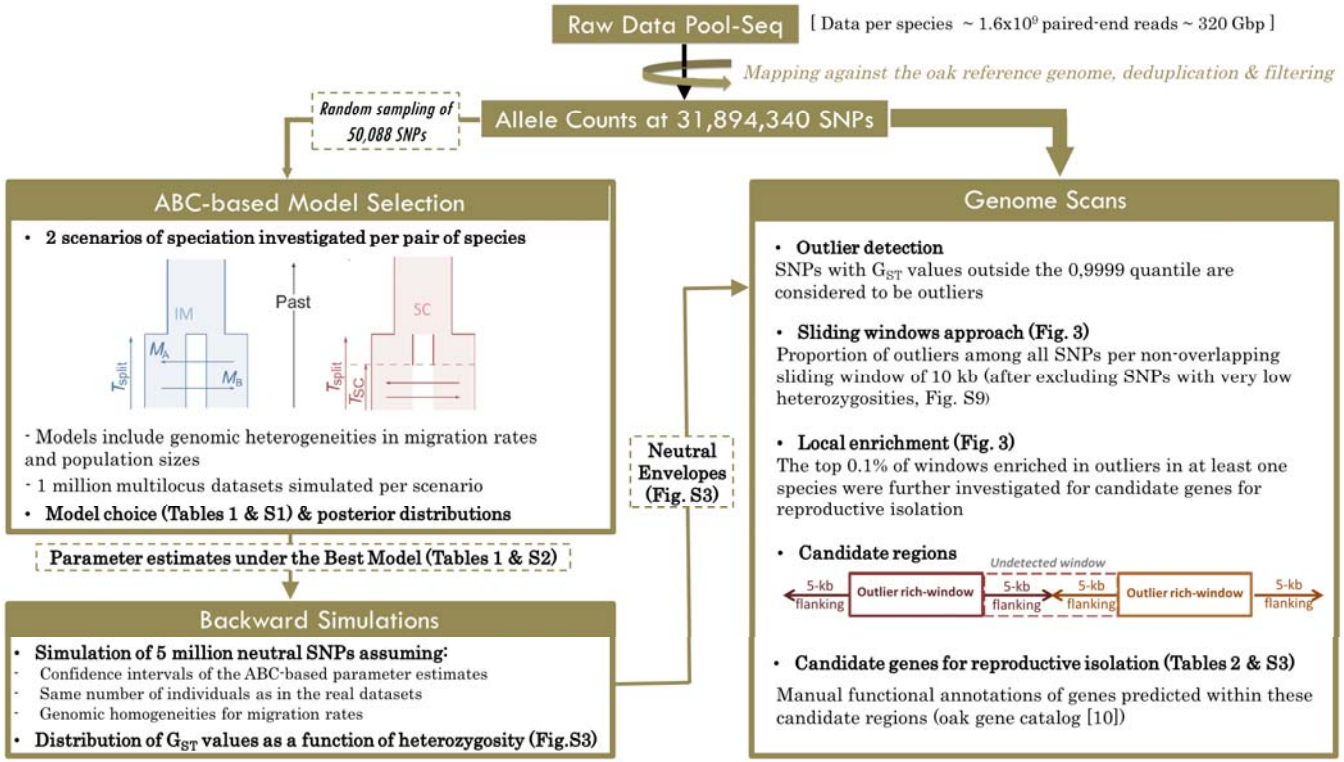

Figure 2: Workflow used to identify genes contributing to reproductive isolation between four European white oak species*. A subset of 50 thousand of the called SNPs was selected at random and used for model selection under an ABC framework and the generation of parameter estimates under the best model. Large neutral datasets were then simulated to create null envelopes for the identification of SNPs displaying significant departure from expectations under neutrality. We searched for candidate genes* in regions enriched in outliers.

134 ***Table 1: Posterior probabilities of the SC scenario and timing of secondary contacts.***
135 *Mean (bold) relative posterior probability of the secondary contact scenario and standard*
136 *deviation (round brackets). Median (bold) and 95% confidence intervals (square brackets)*
137 *for both the inferred ratio between divergence time (T_{SPLIT}) and time of the secondary*
138 *contact (T_{SC}) and the secondary contact 'expressed in number of years) after setting*
139 *T_{SPLIT} to 10 million years (the upper bound for the divergence of this species*
140 *complex,[17]). More details are given in Tables S1 & S2.*

141

Pair	**Post. Probability SC**	**T_{SC}/T_{SPLIT} estimates**	**T_{SC} (years ago)**
Q. robur – Q. petraea	**0.98883** (±0.01089)	**1.487 x 10^{-3}** [0.43-4.05] x 10^{-3}	**14,870** [4,300-40,500]
Q. robur – Q. pyrenaica	**0.99249** (±0.01152)	**1.197 x 10^{-3}** [0.31-4.95} x 10^{-3}	**11,970** [3,100-40,500]
Q. robur – Q. pubescens	**0.98719** (±0.01342)	**0.245 x 10^{-3}** [0.09-0.72} x 10^{-3}	**2,450** [900-7,200]
Q. pubescens – Q. petraea	**0.98549** (±0.02231)	**2.176 x 10^{-3}** [0.77-6.24] x 10^{-3}	**21,760** [7,700-62,400]
Q. pubescens – Q. pyrenaica	**0.99087** (±0.01152)	**0.865 x 10^{-3}** [0.32-2.16] x 10^{-3}	**8,650** [3,200-21,600]
Q. pyrenaica – Q. petraea	**0.99378** (±0.00697)	**0.383 x 10^{-3}** [0.10-1.14] x 10^{-3}	**3,830** [1,000-11,400]

142

143 Narrow regions of non-neutral evolution

144 We then took advantage of these demographic inferences to perform
145 differentiation outlier tests. We performed extensive backward simulations (5,000,000
146 independent SNPs) under the best inferred scenario to generate null distributions for
147 each pairwise comparison (Fig. S3, see also *Online Results*). The most outlier-enriched
148 windows were retained for the identification of candidate s genes underlying species
149 barriers. We identified 281 windows containing the highest proportion of outliers (top
150 0.1% of window enriched in outliers for at least one pair of species). We then analyzed
151 the clustering of these outlier-enriched windows. We defined a candidate genomic region
152 by merging close windows, *e.g.* two contiguous sequences of two outlier-enriched
153 windows, with possible interruption by a single undetected window (Fig. 2). The 281
154 windows were distributed over 215 candidate genomic regions, distributed over all
155 chromosomes (blue lines, Fig. 3).

156 We listed all the *Quercus* genes located within or flanking these 215 genomic
157 regions. We identified 227 genes distributed over 133 of the 215 regions, with very few
158 candidates per region (mean: 1.71±1.76 genes per region, 1.49 ±0.82 genes after
159 excluding 5 regions with chloroplast-like DNA signatures). We subdivided genes into
160 three major functional categories, and particularly focused on 32 candidate genes based
161 on quality and sharpness of the annotations in terms of physiological function (Table 2,
162 see also Sup Info). The first category comprises genes underlying the ecological
163 preferences of the four species: tolerance of water deficit, cold tolerance, adaptation to
164 alkaline soils. The second includes genes involved in biotic interactions, such as immune
165 responses, resistance to biotic stresses, and mycorrhization. The third gatherss to genes
166 probably contributing to intrinsic barriers, and includes genes with functions related to
167 flowering time, pollen recognition, pollen growth and embryo development.

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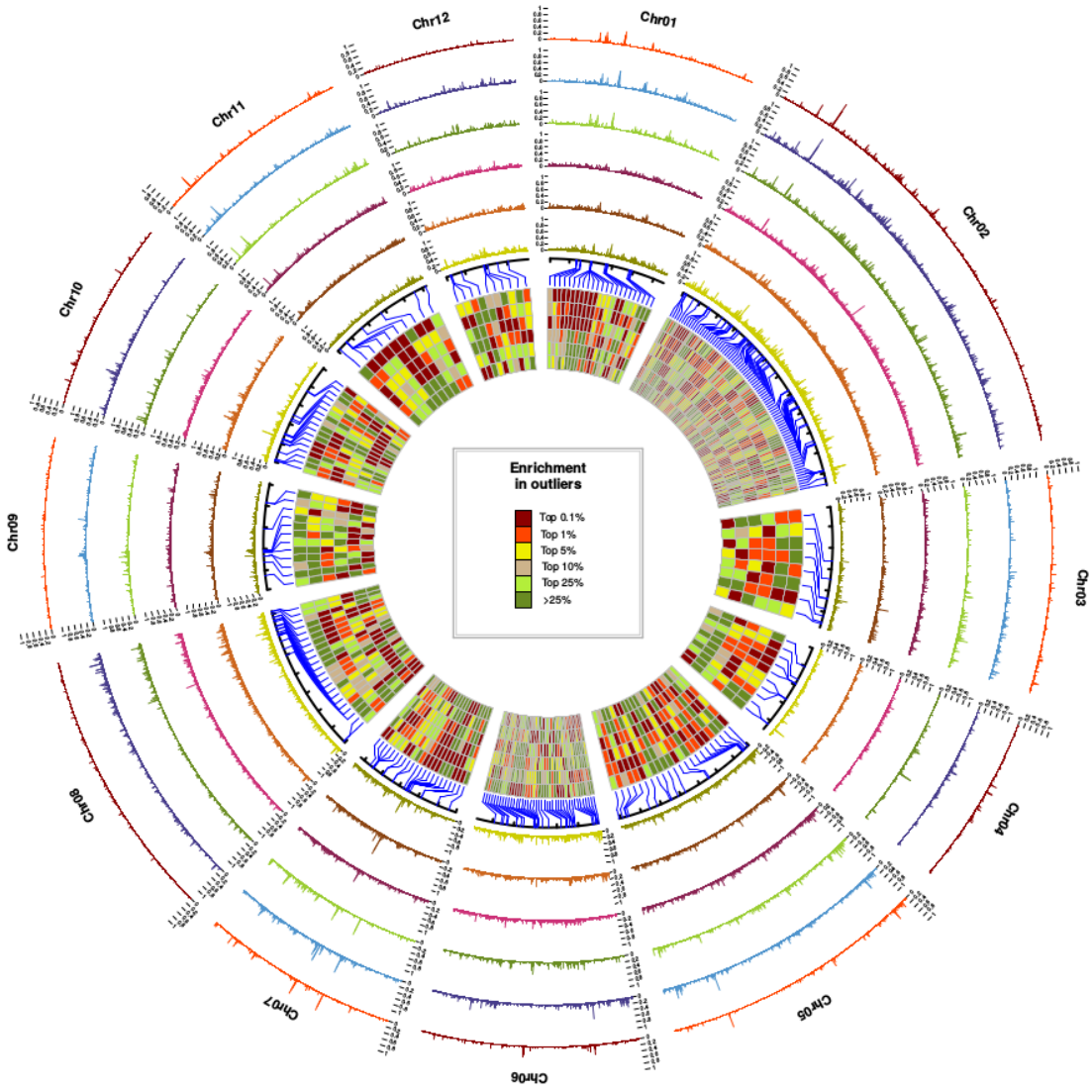

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170 **Fig. 3:** ***Local density in outliers per non-overlapping 10 kb sliding window.*** *From outside*
171 *to inside, the species pairs are Q. robur/Q. petraea, Q. pyrenaica/Q. petraea, Q.*
172 *pubescens/Q. petraea, Q. robur/Q. pubescens, Q. robur/Q. pyrenaica and Q. pubescens/Q.*
173 *pyrenaica. Detailed patterns are accessible from:*
174 *https://github.com/ThibaultLeroyFr/GenomeScansByABC/. Each rectangle in the inner*
175 *circle represents the level of enrichment in outliers with He ≤ 0.2 for each pair of species*
176 *at a given position in the genome, assuming the same order of pairs. These rectangles*
177 *correspond to the 281 most outlier-enriched windows found in at least one of the six*
178 *pairs (top 0.1%).*

Table 2: Genomic positions, gene names, annotations for 32 candidate genes. All other annotations are available in Table S3 (see FileS1 for details, including references). Species patterns were determined from the analysis of the most outlier-enriched windows between pairs, as detailed in Table S4.

chr.	*positions (regions)*	*Candidate gene*	*Gene name*	*Gene functions*	*#Paralogs*	*Pattern*
Intrinsic barriers						
Flowering						
Chr08	62493250-62513250	Qrob_P0412860.2	Two-component response regulator-like PRR73	photoperiodic flowering response, circadian clock	1	Complex
Chr08	62673250-62693250	Qrob_P0749650.2	Floral homeotic protein APETALA 2 | Transcr. factor RAP2-7	Delay transition to flowering time, biotic/abiotic stresses	0	Complex
Chr07	44592072-44632072	Qrob_P0088630.2	Transcr. activator DEMETER (DME) / protein ROS1-like	Transcriptional activator required for floral development	1	Complex
Pollen development (Cycloartenol synthases)						
Chr06	28660694-28680694	Qrob_P0684330.2	Cycloartenol synthase 2	Sterol or triterpenoid synthesis, pollen development	13	Complex
Chr06	28690694-28710694	Qrob_P0684400.2	Cycloartenol synthase 2	Sterol or triterpenoid synthesis, pollen development	0	Shared
Chr06	28770694-28810694	Qrob_P0684360.2	Cycloartenol synthase 2	Sterol or triterpenoid synthesis, pollen development	0	Shared
Pollen recognition & seed germination (G-type lectin S-receptor-like Serine/threonine kinase genes)						
Chr03	26118565-26138565	Qrob_P0538230.2	G-type lectin S-receptor-like STK At2g19130	Putatively involved in recognition of pollen	0	Complex
Chr05	4001785-4021785	Qrob_P0641650.2	G-type lectin S-receptor-like STK At1g11300	Putatively involved in recognition of pollen	190	Complex
Chr06	1453464-1473464	Qrob_P0430150.2	G-type lectin S-receptor-like STK LECRK	Regulates expression of immunity genes & seed germination	36	**Q. pyrenaica-specific**
Chr06	1513464-1563464	Qrob_P0430190.2	G-type lectin S-receptor-like STK LECRK	Regulates expression of immunity genes & seed germination	36	**Q. pyrenaica-specific**
Chr08	1749735-1769735	Qrob_P0138480.2	G-type lectin S-receptor-like STK LECRK	Regulates expression of immunity genes & seed germination	36	**Q. pyrenaica-specific**
Embryo development & organogenesis						
Chr12	30514806-30534806	Qrob_P0436820.2	Transcriptional corepressor LEUNIG-like isoform	Leaf, flower (gynoecium) and embryo development	5	**All except Q. pub/Q. pet**
Chr02	31611918-31651918	Qrob_P0297580.2	CHD3-type chromatin-remodeling factor PICKLE	Repressor of LEC1, activator of embryo development	0	Complex
Chr06	43006752-43026752	Qrob_P0309300.2	Probable N-acetyltransferase HLS1-like	Auxin-responsive gene expression, shoot organogenesis	1	**All except Q. rob/Q. pet**
Chr08	49805801-49825801	Qrob_P0248780.2	Receptor-like protein 12 (RLP12)	Meristem maintenance control, organogenesis	0	Complex
Photoreceptor & UV-B tolerance						
Chr02	37788250-37808250	Qrob_P0338870.2	Ultraviolet-B receptor UVR8	Photoreceptor/response to UV, circadian clock, stomata	2	Shared
Chr03	34667252-34687252	Qrob_P0500290.2	DNA mismatch repair protein MSH2	UV-B-induced DNA damage response pathway	0	Shared
Chr06	11400887-11420887	Qrob_P0577750.2	Transcription factor MYB12	Positive regulator of flavonoid biosynthesis, UV-B tolerance	0	**All except Q. rob/Q. pet**

Ecological barriers - abiotic stresses						
Nramp metal transporters						
Chr09	7739510-7759510	Qrob_P0191830.2	Metal transporter Nramp5	Manganese and cadmium uptake	8	**Q. pubescens-specific**
Chr06	38764748-38784748	Qrob_P0097150.2	Metal transporter Nramp6	Involved in iron ion homeostasis	1	**All except Q. rob/Q.pet**
Dehydration/lateral root growth						
Chr08	68418235-68443235	Qrob_P0457680.2	Protein DEHYDRATION-INDUCED 19-like	Stress-induced sensor, interacting with CPK11 & S-Rnase	0	**All except Q. rob/Q.pet**
Chr02	27061274-27081274	Qrob_P0304800.2	Transcription factor WER	Controls cell fate specification, e.g. hairy roots or stomata	0	**All except Q. rob/Q.pet**
Chr02	28240136-28260136	Qrob_P0299670.2	Root Primordium Defective 1 (RPD1)	Lateral root morphogenesis; active cell proligeration	0	Complex
Chr02	36392234-36442234	Qrob_P0422470.2	Alkaline/neutral invertase CINV2	Regulator of root growth, sucrose catabolism	7	Shared
Chr09	19506093-19526093	Qrob_P0418880.2	1-aminocyclopropane-1-carboxylate synthase	Ethylene biosynthetic process, lateral root formation	4	**All except Q. rob/Q.pet**
Chr04	19182244-19222244	Qrob_P0652510.2	Phosphatidylinositol-3-phosphatase myotubularin-1 (or 2)	Role in soil-water-deficit stress	2	Shared
Chr02	96008743-96028743	Qrob_P0387640.2	Protein WVD2-like 6	Organ stockiness (periph. root cap, trichomes & leafs)	0	**All except Q. rob/Q.pet**
Freezing/cold adaptation						
Chr09	18536093-18556093	Qrob_P0768740.2	Dehydration-responsive element-binding protein 1	Key role in freezing tolerance and cold acclimation	6	Complex
Chr02	112153196-112173196	Qrob_P0339800.2	B3 domain-containing transcription factor VRN1	Vernalization responsiveness, repressor of FLC	3	**All except Q. pub/Q.pet**
Ecological barriers - biotic stresses						
Pathogen resistance/mycorrhization						
Chr10	12310682-12340682	Qrob_P0070130.2	Transportin MOS14	Plant immunity (splicing of resistant genes)	0	**Q. pyrenaica-specific**
Chr01	26763631-26783631	Qrob_P0648170.2	Ubiquitin carboxyl-terminal hydrolase 12-like	Protein deubiquitination, regulator of disease resistance	3	Complex
Chr05	3053435-3073435	Qrob_P0622110.2	Nodulation-signaling pathway 1 (NSP1)	Nodulation & mycorrhization	0	Complex

183 Species-specific ecological and non-ecological reproductive barriers

184 Unlike studies aiming at interpreting every region enriched in outliers, our
185 objective was rather to focus on genes displaying distinct patterns among pairs of
186 species. This is especially important since these situations are unexpected to arise via
187 background selection (see *Online Results* for details). After excluding genes with a
188 "shared" pattern, several different situations were observed (Table S4): (i) 9 regions
189 enriched in outliers for all but one pair of species (including 7 regions for all pairs except
190 *Q. robur* – *Q. petraea* and 2 regions for all pairs except *Q. pubescens* – *Q. pyrenaica*
191 pairs), (ii) 5 regions specific to all pairs sharing the same species (4 for *Q. pyrenaica* and
192 1 for *Q. pubescens*) and (iii) 11 regions with more complex patterns.

193 Among the nine regions with an "all-versus-one-pair" relationship, seven excluded
194 the *Q. robur*/*Q. petraea* pair and the other two excluded the *Q. pubescens*/*Q. pyrenaica*
195 pair. Four of the seven genes for which the *Q. robur*/*Q. petraea* pair was excluded are
196 known to be involved in drought tolerance or in lateral root growth (Table 2). This
197 pattern is consistent with the higher drought tolerance of *Q. pyrenaica* and *Q. pubescens*
198 compared to *Q. petraea* or *Q. robur* [19]. Reciprocally, we observed an "all vs. one"
199 pattern (undetected for the *Q. robur*/*Q. petraea* pair) for a VRN1 gene involved in
200 responsiveness to vernalization and known to play a key role in cold acclimation in many
201 plant species [20]. Overall, the genomic variation of these nine genes parallels the
202 Northern-Southern distribution of the studied species, suggesting that the underlying
203 barriers are driven by climate preferences (Fig 1 A, B).

204 Among the five genes with branch-specific patterns, *i.e.* found in all pairs
205 containing a given species, four have *Q. pyrenaica*-specific patterns and concerned genes
206 involved in plant immunity, including three encoding G-type lectin S-receptor-like
207 serine/threonine kinases (LECRKs) and one encoding a transportin (MOS14). *Q.*
208 *pyrenaica* is extremely sensitive to oak powdery mildew, a pathogen that was introduced

209 into Europe at the start of the 20th century [21]. Soon after the first detection of the
210 fungus in Europe, high mortality rates were reported for *Q. pyrenaica* in the humid
211 warm-temperate forests of Southwestern and Western France [21 and references
212 therein]. *Q. pyrenaica*-specific alleles at several genes involved in immunity may,
213 therefore, be the signature of the high susceptibility of *Q. pyrenaica* to biotic stresses in
214 moist environments. Additionally, we identified a *Q. pubescens*-specific pattern for a
215 gene encoding a metal transporter (Nramp5) involved in the assimilation of manganese
216 and cadmium in rice and barley [22]. Manganese assimilation is known to be essential
217 for many plant functions, but manganese availability in the soil tends to decrease with
218 increasing pH, and becomes limiting beyond a soil pH of 6.5. This gene probably signs
219 the greater ecological preference of *Q. pubescens* for lime-rich soils (Fig 1B) in
220 comparison to the other three species.

221 Among genes with complex patterns, we identified many candidate genes for
222 intrinsic premating and postmating barriers. We identified several genes involved in the
223 timing of flowering, including APETALA2 and PRR73. APETALA2 is a key transcription
224 factor for the establishment of the floral meristem [23]. Similarly, PRR73 contributes to
225 flowering time variation in barley and wheat [24 and references therein]. Several
226 species-specific genes may also be involved in mating barriers, including previously
227 described ecological genes with pleiotropic effects, such as *VRN1*. In addition to its
228 primary role in vernalization, VRN1 is involved in the repression of *FLC* (itself a known
229 repressor of flowering) in *Arabidopsis,* through a vernalization-independent floral
230 pathway [20]. Several of the candidate genes for intrinsic barriers identified are involved
231 in pollen or embryo development, suggesting that both premating and postmating
232 intrinsic barriers operate in oaks. Three of these genes encode cycloartenol synthases
233 known to be essential for pollen development in *Arabidopsis* [25].

234

DISCUSSION

The increasing availability of genomic resources for phylogenetically related species has the potential to greatly improve our understanding of their evolutionary trajectories and the molecular basis of their reproductive isolation as shown here for European temperate oaks. Our demographic reconstruction supports long periods of isolation between these oak species for most of their history leading to the gradual loss of shared alleles and the accumulation of reproductive barriers. We further found evidence of a systematic shift in their trajectories that occurred towards the beginning of the Holocene. More precisely, this shift took place while the oak species were migrating northwards as the climate became warmer, and resulting in their encountering in central Europe. In line with our previous conclusions [11], our inferences cannot however exclude that a few secondary contact periods had already taken place earlier. Still, the mixture of different species and populations in central Europe occurring during the Holocene was so massive that private (or near private alleles) were redistributed among interfertile species. Indeed, current levels of interspecific differentiation are extremely low along almost all the genome (mean interchromosomal 10-kb estimates of F_{ST} below 0.08 for all pairs), at a level compatible with many reports of within-species population structure in the literature [26]. However, at some narrow regions distributed throughout the genome, interspecific differentiation reaches extremely high levels (10-kb estimates of F_{ST} above 0.8). These peaks most likely correspond to narrow regions where selection counteracted the homogenizing effect of gene flow, thus leading to the present-day highly heterogeneous landscape of differentiation.

The highest differentiated SNPs contributing to reproductive barriers mostly set apart Southern (*Q. pyrenaica* & *Q. pubescens*) from Northern species (*Q. robur* & *Q. petraea*). While this observation is inconsistent with the inferred phylogeny (ref. 11; Fig. 1C), it however coincides with the climatic preferences of the four species (Fig 1 A,B). We

261 also found genetic support for *Q. pubescens* preference for alkaline soils. When
262 comparing the genomic footprints of differentiation between *Q. pyrenaica* and the other
263 three species, we found genes involved in plant immunity, in line with previous reports
264 for higher mortality rates in this species due to pathogens [21]. Finally our results also
265 show that peaks of differentiation never reach fixation, even in regions exhibiting the
266 strongest reproductive barriers. The possibility for a very low permeability to gene flow
267 at these barriers therefore calls for more research about the maintenance and evolution
268 of reproductive isolation between European white oaks.

269 Overall, our results suggest that key selective abiotic and biotic factors triggered
270 by post glacial environmental changes have molded the extant landscape of species
271 reproductive barriers in European temperate oak species. We anticipate that these
272 drivers will operate during ongoing climate changes as Mediterranean oak species (*Q.*
273 *pyrenaica* & *Q. pubescens*) are migrating northwards getting in contact in more
274 Northern latitudes with local temperate species (*Q. petraea* and *Q. robur*).

275

276 # METHODS

277 ***Ecological niche of the four species***

278 *French data*

279 We delineated the extant ecological niche of the four oak species in France (Fig
280 S4) by using their distribution maps based on the National Forest Inventory and climatic
281 data extracted from the Chelsa data base [27]. In addition to the climatic data we added
282 pH values of the soil. Proxies of pH values were derived from floristic data the National
283 Forest inventory floristic plots installed since 2005. Floristic composition of these
284 inventory plots was compared to existing database to calculate proxys of pH values [28].
285 We intersected the distribution maps with the climatic rasters (30" resolution) and

calculated a 2D density plot of species presence in the climatic (mean temperature and precipitation) and pH domain using the R package "ggplot2" v. 2.2.1 [29] under R v. 3.2.2

European data

European distributions maps were constructed based on presence data of species made available by the European atlas of forest tree species [30]. Climatic data are based on the Chelsa database [27] and soil pH were derived from JCR data [31]. Since both data origin from different geographical sites, we only computed univariate density distributions using ggplot2, after using a similar procedure than for the French data.

Sampling and sequencing

We sampled populations of the four *Quercus* species in stands of natural origin located in South-West France. We sampled 13 *Q. petraea* trees in Laveyron (Landes, France), and 20 *Q. robur* and 20 *Q. pyrenaica* trees from the Landes EVOLTREE "Intensive Study Site" (ISS). We also sampled 18 *Q. pubescens* trees from two sites in Gironde: 12 in Branne and 6 in Blaignan (Gironde, France) (see Table S5 for details). *Online Methods* contains detailed information on the methodologies used from DNA extraction to sequencing. Overall, between 1,617,465,418 and 1,813,403,677 reads per pool were retained for analysis, corresponding to 313 Gb (425X) to 356 Gb (483X) of raw data. Raw data have been deposited in the Sequence Read Archive (SRA): ERP105626.

Mapping and calling

All reads were then mapped against the v2.3 oak haplome assembly [10], with bowtie2 v. 2.1.0 [31], using standard parameters for the "sensitive end-to-end" mode. PCR duplicates were removed with Picard v. 1.106

311 (http://broadinstitute.github.io/picard/). Samtools v.1.1 [32] and Popoolation2 v. 1.201
312 [33] were then used to call biallelic SNPs with at least 10 alternate alleles and a depth
313 between 50 and 2000X at each position. To ensure a reasonably low rate of false positives
314 due to Illumina sequencing errors, all SNPs with a MAF lower than 0.02 were discarded.
315 We obtained allele counts for a total of 31,894,340 SNPs. Sliding-window F_{ST} were
316 calculated from allele frequencies with the popoolation2 bioinformatics software suite
317 [33].

318

319 **Demographic inferences & genome scans**

320 We used a strategy combining ABC [11, 26] and backward simulations to scan genomes
321 for reproductive barriers (Fig. 2). The ABC approach explicitly takes into account
322 confounding effects of barriers to gene flow [34] and linked selection [26, 35] on
323 demographic inferences. This was done by modeling among loci variation in (i) effective
324 migration rate to take into account barriers to migration [36] and (ii) effective population
325 size to take into account linked selection [37]. *Online Methods* contains detailed
326 information on the methodologies used for demographic inferences and genome scans.

327

328 **Functional annotations**

329 For all genes found within regions enriched in outliers, we conducted BlastP
330 searches in both the SwissProt and nr protein databases. Only BlastP results with e-
331 values lower than 10e-5 were considered for protein function annotation. After
332 identification of the protein function by BlastP analyses, extensive manual literature
333 searches were performed. We also reported information from a previous identification of
334 orthologous and paralogous genes in 16 plant species, including *Q. robur,* performed with
335 OrthoMCL (see ref. 10, for details).

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