Journal of Biogeography (J. Biogeogr.) (2016) 43, 679–690

ORIGINAL ARTICLE

Combining molecular and fossil data to infer demographic history of *Quercus cerris*: insights on European eastern glacial refugia

F. Bagnoli[1,†], Y. Tsuda[1,a†], S. Fineschi[2], P. Bruschi[3], D. Magri[4], P. Zhelev[5], L. Paule[6], M. C. Simeone[7], S. C. González-Martínez[8,9] and G. G. Vendramin[1*]

[1]*Institute of Biosciences and Bioresources, National Research Council, 50019 Sesto Fiorentino, Italy,* [2]*Institute for Sustainable Plant Protection, National Research Council, 50019 Sesto Fiorentino, Italy,* [3]*Department of Agriculture, Food and Environmental Science, University of Florence, 50144 Florence, Italy,* [4]*Department of Environmental Biology, Sapienza University of Rome, 00185 Roma, Italy,* [5]*University of Forestry, 1797 Sofia, Bulgaria,* [6]*Technical University in Zvolen, 96053 Zvolen, Slovakia,* [7]*Agriculture, Forest, Nature and Energy Department (DAFNE), University of Tuscia, 01100 Viterbo, Italy,* [8]*UMR1202 BIOGECO, INRA, 33610 Cestas, France,* [9]*UMR 1202 BIOGECO, University of Bordeaux, 33400 Talence, France*

*Correspondence: Giovanni G. Vendramin, Institute of Biosciences and Bioresources, National Research Council, Via Madonna del Piano 10, 50019 Sesto Fiorentino (FI), Italy. E-mail: giovanni.vendramin@ibbr.cnr.it

[a]Present address: Sugadaira Montane Research Center, University of Tsukuba 1278-294 Sugadairakogen, Ueda, Nagano 386-2204, Japan.

[†]Contributed equally.

ABSTRACT

Aim Phylogeographical studies of Eastern Mediterranean species are rare. We aim to fill a gap in the current understanding of the role of Eastern Mediterranean glacial refugia, and their connections with other refugia across Europe. To this end, we studied the genetic diversity distribution and genetic structure of the modern population of *Quercus cerris* in relation to its Quaternary demographic history and to more ancient events.

Location Mediterranean Basin; Italian, Balkan, Anatolian peninsulas.

Methods A total of 192 populations were genotyped with six polymorphic chloroplast microsatellites, and the genetic diversity and differentiation of the populations were evaluated. The geographical structure of genetic variation was analysed with a Bayesian clustering method using BAPS 5.2. The demographic history of *Q. cerris* was explored by an approximate Bayesian computation procedure using DIYABC 2.0. To reconstruct the past distribution of *Q. cerris*, we also considered the chronology and geographical distribution of fossil records.

Results Thirty-five haplotypes were found, three of which (together) were found in 71.82% of individuals. Bayesian analysis resulted in three genetically and geographically distinct clusters: a Western group, a Central group, and an Eastern group. The approximate Bayesian computation analysis, together with fossil data, showed a possible bottleneck leading to the divergence of the Eastern and Central populations in the Early Pleistocene (Gelasian). The split into two groups of populations in the Italian and Balkan Peninsulas, respectively, was probably caused by a marked population contraction during a glacial phase of the Middle Pleistocene.

Main conclusions This study provides information on the potential role of Eastern Europe and the Near East as refugia and as a source for ancient westward range expansions in the Mediterranean region. Our study covers a remarkable gap in European oak phylogeography, showing a putative eastern origin of *Q. cerris* and the presence of large amounts of genetic diversity in this region.

Keywords

approximate Bayesian computation, BAPS, cpSSR, Mediterranean basin, palaeobotany, phylogeography, Pleistocene, *Quercus cerris*

INTRODUCTION

Understanding how past evolutionary processes shaped the genetic diversity distribution of plant species is important to enable predictions of ecosystem dynamics under today's impending climate change (Alberto *et al.*, 2013). This is particularly relevant for our ability to forecast the response of European forest ecosystems from temperate continental and Mediterranean regions to predicted climate changes, such as an increase in global mean precipitation, or increased dryness

doi:10.1111/jbi.12673

Journal of Biogeography

in some regions, such as the Mediterranean Basin (IPCC, 2007). In the Mediterranean Basin, biodiversity is influenced by complex patterns associated with the heterogeneous historical biogeography and landscape complexity (Blondel *et al.*, 2010). Despite the numerous recent investigations into the richness of genetic diversity in Mediterranean plants over substantial parts of their ranges, the genetic structure of many species and the role of key palaeobiogeographical regions, particularly in the eastern part of the Basin, are still far from being understood (Médail & Diadema, 2009).

The current patterns of genetic diversity found in the European flora reflect a clear influence of Quaternary climate oscillations. Studies accumulated during the past two decades in Europe inferred these patterns assuming a contraction/expansion model (Provan & Bennett, 2008). It is worth noting that, in the southern European regions, the disappearance of both genotypes and populations was minimized due to less severe climate conditions during the glacial periods (Nieto Feliner, 2011). In the case of European temperate tree species, the accepted hypothesis assumes the existence of main refugial areas in the three southern peninsulas (Iberia, Italy and the Balkans), from which the recolonization of the rest of Europe took place (Bennett *et al.*, 1991). This continues to be the prevailing hypothesis, despite an increasing volume of evidence suggesting that middle- to high-latitude refugia may have also contributed to post-glacial recolonization (Gavin *et al.*, 2014).

A novel approach based on the analysis of the spatial distribution of analogous and non-analogous climate conditions between the Last Glacial Maximum (LGM) and the present age, quantified and mapped potential source and sink areas for species recolonization in Europe. This method identified south-eastern Europe as the region bearing the highest post-glacial sink potential, in particular the Balkan Peninsula and Turkey, which likely acted as both source and sink areas for species range shifts since LGM (Ohlemüller *et al.*, 2012). Despite the importance of Anatolia and the Balkan Peninsula as refuge areas and recognized sources of genetic diversity for the European flora, these regions have not been thoroughly investigated yet. This knowledge gap needs to be filled because in the Mediterranean area there is an east–west gradient in genetic diversity for many plant and animal species, in addition to a complex demographic history related to latitudinal displacements (Conord *et al.*, 2012). The east–west gradient in genetic diversity may be the result of processes acting at different time-scales, including the legacy of a Neogene Palaeotropical flora that extended along the edge of the Tethyan Ocean (Chen *et al.*, 2014), the contraction of formerly continuous ranges in relation to Quaternary oscillations, and the successive westward or eastward waves of colonization fostered by east–west climate gradients (Nieto Feliner, 2014).

In addition to the generally accepted LGM southern refugia hypothesis, Magri *et al.* (2006), examining genetic and palaeocological data of European beech (*Fagus sylvatica*), proposed that older temporal scales and repeated ice ages in the Quaternary should also be considered when discussing factors affecting modern phylogeographical structure in tree species. For example, González-Martínez *et al.* (2010) found patterns of genetic diversity and genetic structure in *Taxus baccata* that reflect processes at different spatial and temporal scales, including ancient ones. In addition, genetic resequencing studies on European tree species detected severe bottlenecks predating the LGM in pine, spruce and aspen (Heuertz *et al.*, 2006; Pyhäjärvi *et al.*, 2007; Ingvarsson, 2008). Finally, the southern European regions did not represent homogeneous and continuous refugial areas during the ice ages. On the contrary, those areas were characterized by a set of different ecological and climatic conditions that embraced many glacial refugia, resulting in the so-called 'refugia-within-refugia' scenario (Gómez & Lunt, 2007). Increasing evidence from multiple species within the southern European regions supports this hypothesis (e.g. López de Heredia *et al.*, 2007).

All these studies suggest that it is important to consider not only post-LGM episodes but also the demographic history of species in the Quaternary and/or the Neogene, and ask for more detailed spatial analysis within known southern refugia, in order to explain the current genetic diversity of Europe's forest species.

This research investigates the modern genetic structure of *Quercus cerris* and its Quaternary demographic history, considering east–west patterns of genetic diversity in the Mediterranean region and the role of ancient events. Moreover, our case study aims to provide new and relevant insight into the role of Eastern Mediterranean refugia on the current populations, as well as on their connection to refugia across Europe. *Q. cerris* has a central-eastern Mediterranean distribution, centred on the Italian and Balkan peninsulas, from the western Alps to the Black Sea, Anatolia and Lebanon (Gellini & Grossoni, 1997). It co-occurs with other oaks, some of which are suggested to hybridize with *Q. cerris* (e.g. *Q. suber* Camus, 1938–39). Our study combines palaeobotanical information with data on the current distribution of *Q. cerris* genetic variation, using large-scale chloroplast SSR analyses. Recent advances in population demographic analysis provide a flexible methodology to infer past demographic history (Bertorelle *et al.*, 2010). In our study, palaeoecological data and demographic inference using approximate Bayesian computation (ABC) were combined to obtain insights into the origin, migration routes, and persistence of this important species in glacial refugia. These findings increase the biogeographical knowledge of poorly studied regions in the Balkans and Anatolia. Moreover, we provide indications for core diversification areas, contact zones, and gene flow patterns between these regions and the rest of Europe.

MATERIALS AND METHODS

Plant material, DNA extraction and cpSSR genotyping

Leaf samples of *Q. cerris* were collected from 192 populations (1,175 individuals) throughout the whole natural species range (see Table S1 in Appendix S3 in Supporting Information,

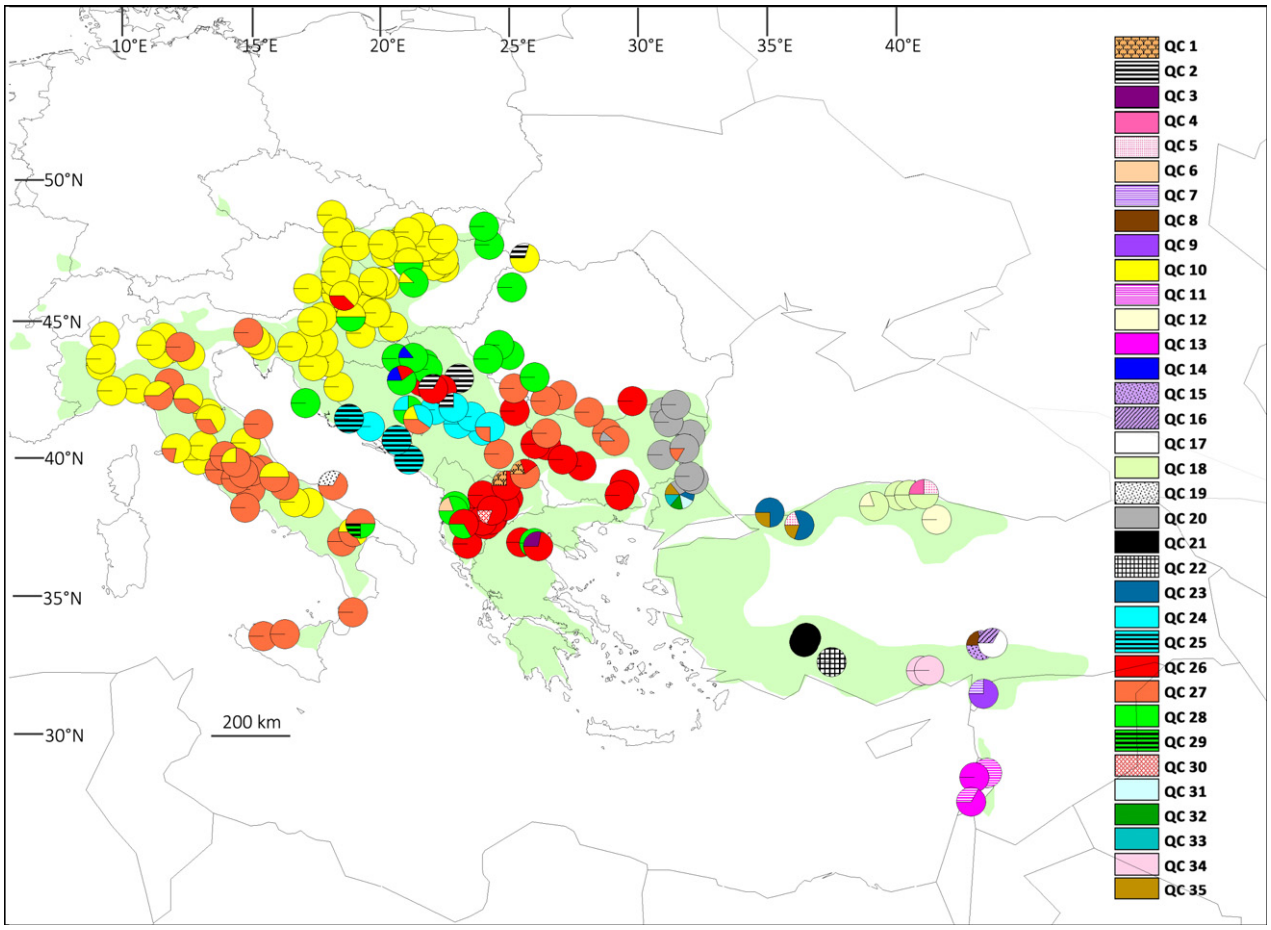

Figure 1 Geographical distribution of *Quercus cerris* cpDNA haplotypes. Haplotypes are defined by numbers (on the right). In green *Q. cerris* distribution range.

Fig. 1). Fresh leaves were stored at –80 °C prior to DNA extraction. Total DNA was extracted using Invitek Invisorb Spin Plant Mini Kit (following the manufacturers' instructions), from approximately 100 mg of fresh leaves, ground in a Retsch MM200 grinding mill. Six polymorphic chloroplast microsatellite (cpSSR) markers were used: ccmp6 (Weising & Gardner, 1999), cmcs1, cmcs6, cmcs7, cmcs8 and cmcs9 (Sebastiani *et al.*, 2004). Polymerase chain reaction details and sequencing are described in Appendix S1. To test whether differences in SSR allele lengths were due to simple changes in the number of repeats or to mutations in the flanking regions (i.e. size homoplasy) the amplified cpSSR fragments of at least two individuals for each haplotype were directly sequenced from both ends using an automatic sequencer MegaBACE (GE Healthcare), (methods are given in Appendix S1).

To rule out the possibility that oak species other than *Q. cerris* had been sampled in Anatolia, at least two individuals for each haplotype found in this region were sequenced for the *trnH-psbA* region. This region represents a rapidly evolving intergenic spacer currently considered the most informative for fast species-level identification in oaks (Simeone *et al.*, 2013). Details are provided in Appendix S1.

Genetic diversity and population structure

Gene diversity (h), number of haplotypes and number of population-specific (i.e. private or endemic) haplotypes were determined for each population using GenAlEx 6.5 (Peakall & Smouse, 2006, 2012). A haplotype frequency map was constructed using MAPINFO PRO 12.5 (MapInfo Corporation, New York, NY, USA). Population genetic differentiation was evaluated by analysis of molecular variance (AMOVA) (Excoffier *et al.*, 1992) using GenAlEx 6.5, G_{ST} for unordered (Pons & Petit, 1996) and R_{ST} for ordered (Slatkin, 1995) alleles using SPAGEDI 1.4 g (Hardy & Vekemans, 2002). Permutation (1,000) of individuals among populations was used to test for deviation of G_{ST} or R_{ST} from zero. The impact of mutations on among-population differentiation [test for $R_{ST} > R_{ST}$ (permuted)] was assessed using SPAGEDI by 1,000 permutations of pairwise haplotype distances among pairs of haplotypes to evaluate whether distribution of haplotypes showed phylogeographical structure, according to Pons & Petit (1996). Moreover, G'_{ST} (Hedrick, 2005), a standardized G_{ST} estimate, was calculated using haplotype frequencies obtained by GenAlEx 6.5. The overall geographical structure of genetic variation was analysed with a group

clustering Bayesian method as implemented in BAPS 5.2 (Corander & Marttinen, 2006), see details in Appendix S1.

Demographic history inferred by ABC

To trace the demographic history of *Q. cerris*, an ABC procedure (Beaumont *et al.*, 2002) implemented in DIYABC 2.0 (Cornuet *et al.*, 2014) was performed, using the three clusters identified by Bayesian analysis of population structure (BAPS) (see Results, Fig. 2): Western Group (WG; green dots in Fig. 2), Central Group (CG; blue dots) and Eastern Group (EG; red dots). DIYABC implementation of ABC does not consider gene flow (only admixture); however, our case study showed high genetic differentiation and low haplotype sharing among the geographical groups used for demographic inference (see Results) and, thus, no serious bias is expected. Two sets of preliminary simulations were used to produce final competing scenarios (see Appendix S1), then five relatively simple demographic scenarios (Fig. 3) were examined. Details of each competing scenario, mutation rate,

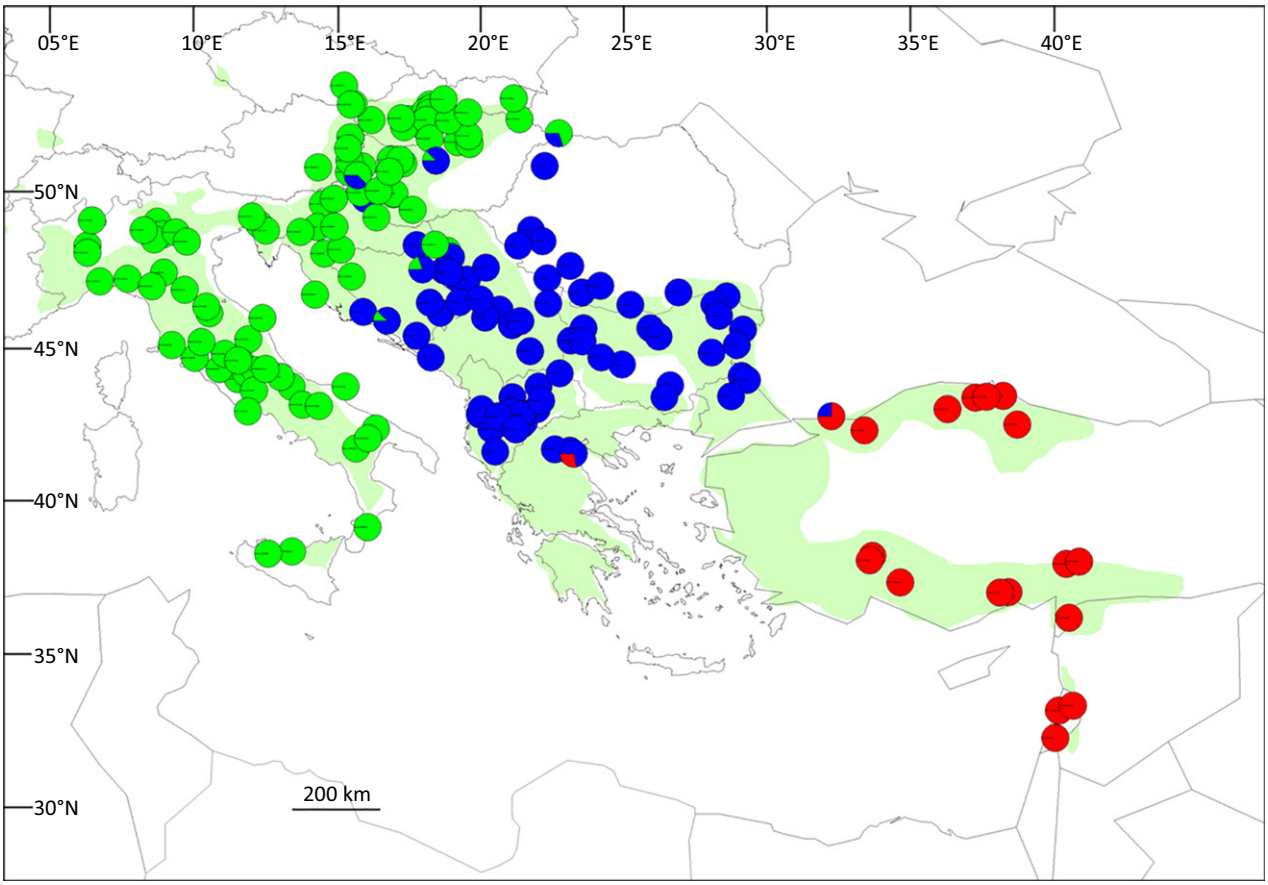

Figure 2 Results of the spatial Bayesian cluster analysis, BAPS, showing genetically homogeneous population groups in *Quercus cerris*. Western Group (WG) = green; Central Group (CG) = blue; Eastern Group (EG) = red. In green *Q. cerris* distribution range.

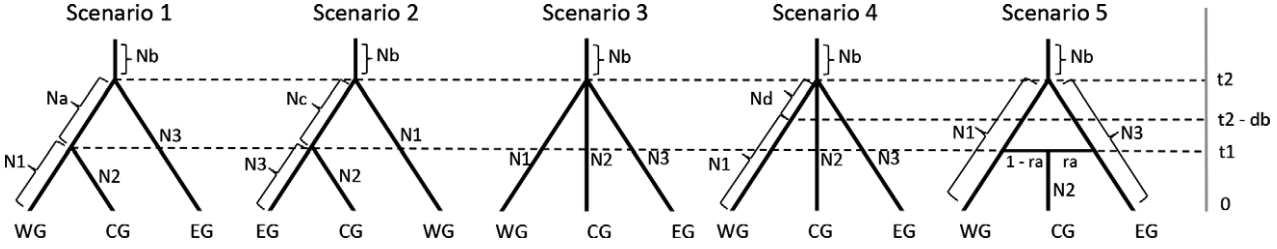

Figure 3 Demographic models compared in approximate Bayesian computation. WG = Western group; CG = Central group; EG = Eastern group; t# = divergence time-scaled by generation time; N# = effective population size; ra = admixture rate; db = duration of bottleneck.

summary statistics, and model checking are given in Appendix S1. In all scenarios, t# refers to time-scale (scaled by generation time) and N# refers to effective population size of the corresponding populations (i.e. WG, CG, EG, ancestral population 'b', and intermediate populations 'a', 'c' and 'd') during each time period (i.e. $0-t_1$ and t_1-t_2) (Fig. 3). Finally, because genetic diversity was the lowest in WG, all scenarios assumed that this group split from CG or EG at t_1 or t_2.

Fossil data

Fossil data were obtained through a bibliographic search. Different fossil types have been considered to reconstruct the past distribution of *Q. cerris*. Based on pollen data, the presence of *Q. cerris* may only be detected in areas where the distributions of *Q. cerris* and *Q. suber* do not overlap, as the morphology and ornamentation of the pollen grains of the two species are indistinguishable (van Benthem *et al.*, 1984). Bark has some distinctive anatomical characters (Şen *et al.*, 2011), but it is seldomly found as a fossil. Wood of *Quercus* section *Cerris* is very similar to that of the white oak group, although it can be distinguished by some features (Akkemik & Yaman, 2012). Several species of *Quercus* Section *Cerris*, including *Q. cerris*, *Q. ithaburensis* subsp. *macrolepis*, *Q. brantii*, *Q. libani* and *Q. trojana*, currently grow in Asia Minor and cannot be distinguished by fossil wood (Emery-Barbier & Thiébault, 2005). Fossil leaves and cupules are the best evidence for the local presence of *Q. cerris* in an area, although identification of leaf specimens without acorns may be controversial because of reported high variation in leaf morphology (Uslu & Bakiş, 2014). Going back in time, the attribution of fossil leaves to *Q. cerris* may be problematic and so it is also often limited to *Quercus* Section *Cerris*.

RESULTS

Haplotype sequencing

Allele sequencing for each cpSSR revealed no sequence differences in the microsatellite flanking regions, except for cmcs9 (see details in Appendix S2).

Genetic diversity and population structure

The cpSSR loci ccmp6, cmcs1, cmcs6, cmcs7, cmcs8 and cmcs9 (after correction for variation in the flanking regions, see Appendix S2) were polymorphic in *Q. cerris*, and displayed 4, 2, 11, 5, 6 and 6 allele size variants respectively. They combined into 35 haplotypes (see Table S2 in Appendix S3 and Fig. 1), resulting in a total haplotypic diversity of $h = 0.795$ and a mean gene diversity over populations of $h = 0.109$. Most populations (80.2%) were fixed for one haplotype, and only 38 populations out of 192 (19.8%) showed two or three haplotypes (35 and 3 respectively). A total of 16 private haplotypes were identified, 68.7% of which were detected in Turkey. Analysis of sequence variation at the *trnH-psbA* marker assigned all Turkish haplotypes to *Q. cerris*, although genetic distance from sister species *Q. trojana* and *Q. ithaburensis* was very low (data not shown). Genetic differentiation, as evaluated by G_{ST} and R_{ST}, was very high, 0.8807 and 0.8034, respectively, and significantly different from zero ($P < 0.05$). A phylogeographical structure was not observed (i.e. R_{ST} was not significantly larger than the permuted value).

BAPS resulted in three genetically distinct clusters with a well-defined geographical pattern, and which, from here on, are referred to as Western group (WG, green dots in Fig. 2), Central group (CG, blue dots) and Eastern group (EG, red dots). Each cluster distinguished a well-defined part of the natural range of *Q. cerris*. In particular, EG was located only in Turkey and Lebanon, with the exception of one population in North Greece. WG extended from Italy to the northern countries of the Balkan Peninsula, and CG included all remaining Balkan populations and the westernmost population from Turkey. Eastern group had the highest genetic diversity ($h = 0.922$; SE 0.010) compared to CG ($h = 0.788$; SE 0.012) and WG ($h = 0.459$; SE 0.017). G_{ST} among groups was 0.4364 and $R_{ST} = 0.3881$, both significantly different from zero ($P < 0.05$). The standardized G_{ST} (G'_{ST}) (Hedrick, 2005), which avoids biases due to high intra-population genetic variation, was 0.955 and pairwise-G'_{ST} ranged from 0.901 (WG *versus* CG) to 1.000 (WG *versus* EG), revealing extremely high population differentiation among the three BAPS-defined groups. Furthermore, AMOVA analysis showed that a high proportion of the total genetic variation (33% $P < 0.001$) was explained by differences among the three groups.

Demographic history inferred by ABC

In ABC models, the highest posterior probability was found for scenario 1 (Fig. 3; 0.7316, 95% CIs, confidence intervals: 0.7141–0.7491), for which posterior probability was significantly higher than that for scenario 2 (0.0036), scenario 3 (0.0197), scenario 4 (0.0237), and scenario 5 (0.2213) (see Table S3 in Appendix S3). This result suggested that the divergence of EG from a hypothetical ancestral population occurred before the divergence of WG and CG, as also indicated by genetic differentiation estimates (see above). The median values of effective population sizes for this scenario were 10,500, 180,000, 249,000, 39,600 for N1 (WG), N2 (CG), N3 (EG), Na (the branch conducting to WG between t_1 and t_2), and 44,700 for Nb (ancestral population) respectively (Table 1). The posterior parameters showed that the effective population size of the ancestral population was 5.5 times lower than that of EG, suggesting an expansion event at time t_2. At time t_1, a bottleneck and an expansion event gave rise to WG and CG respectively. The median values of the divergence times, t_1 and t_2, were 8,700 (95% CIs: 1,360–25,400) and 55,800 (95% CIs:

Table 1 Parameter estimates for the best demographic scenario based on approximate Bayesian computation.

				Quantiles			
Parameter	Mean	Median	Mode	2.5%	5%	95%	97.5%
N1	1.22×10^4	1.05×10^4	9.10×10^3	2.55×10^3	3.36×10^3	2.71×10^4	3.17×10^4
N2	1.79×10^5	1.80×10^5	1.77×10^5	7.53×10^4	8.89×10^4	2.65×10^5	2.75×10^5
N3	2.39×10^5	2.49×10^5	3.00×10^5	1.28×10^5	1.49×10^5	2.96×10^5	2.98×10^5
t_1	1.05×10^4	8.70×10^3	3.44×10^3	1.36×10^3	1.86×10^3	2.54×10^4	2.76×10^4
Na	4.40×10^4	3.96×10^4	2.27×10^4	7.82×10^3	1.03×10^4	9.09×10^4	9.51×10^4
t_2	5.67×10^4	5.58×10^4	5.54×10^4	1.70×10^4	2.09×10^4	9.41×10^4	9.70×10^4
Nb	4.64×10^4	4.47×10^4	2.86×10^4	2.34×10^3	4.43×10^3	9.39×10^4	9.67×10^4
μmic	2.25×10^{-5}	1.78×10^{-5}	1.11×10^{-5}	5.43×10^{-6}	6.80×10^{-6}	5.70×10^{-5}	7.26×10^{-5}
pmic	2.10×10^{-1}	2.13×10^{-1}	3.00×10^{-1}	1.10×10^{-1}	1.17×10^{-1}	2.93×10^{-1}	2.99×10^{-1}
snimic	1.16×10^{-6}	2.59×10^{-7}	1.00×10^{-8}	1.14×10^{-8}	1.36×10^{-8}	5.77×10^{-6}	7.33×10^{-6}

N1 = WG effective population size; N2 = CG effective population size; N3 = EG effective population size; Na = WG intermediate effective population size; Nb = effective population size of the ancestral population; times are considered from present (0) backwards in time; t_1 = divergence of CG from WG; t_2 = divergence of WG from EG; μmic = mean mutation rate of microsatellites; pmic = mean parameter of geometric distribution (GSM, Generalized Stepwise Mutation Model); snimic = individual locus SNI (Single Nucleotide Insertion/deletion) rate.

17,000–94,100) generations ago respectively (Table 1). Assuming 30 years as generation time, divergence times scaled to 0.261 Ma (95% CIs: 0.0408–0.762) and 1.674 Ma (95% CIs: 0.510–2.823) for t_1 and t_2 respectively. Considering a longer generation time of 50 years, these values increased to 0.435 Ma (95% CIs: 0.068–1.270) and 2.790 Ma (95% CIs: 0.850–4.705). All observed summary statistics were not significantly different from the simulated values (see Table S6 in Appendix S3), based on parameter values drawn from the posterior distributions for scenario 1, suggesting, together with the principal components analysis comparison implemented in DIYABC (see Fig. S1 in Appendix S3), a high goodness-of-fit of observed data to scenario 1. Type I error rate was 41.0%, and the average type II error rate was 10%.

Fossil data

Fossil leaves and fruits of *Quercus* Sect. *Cerris* were found in several mid-Miocene to Pliocene deposits in Germany, Poland, Slovakia, Alsace, Georgia, Moldavia, Greece and Abkashia (cf. Song *et al.*, 2000 for a review; Teodoridis *et al.*, 2009; Worobiec *et al.*, 2012; Velitzelos *et al.*, 2014). They cannot be attributed to *Q. cerris* yet, as they belong to various fossil taxa in the Section, including *Q. cerresaecarpa* Kol., *Q. sapperi* (Menzel) Mai ex Hummel, *Q. microcerrisaecarpa* Kol. (fossil fruits), *Q. gigas* Goepp. emend. Walther & Zastawniak, and *Q. czeczottiae* Hummel and *Q. pseudocastanea* Goepp. (fossil leaves).

No Pliocene or Pleistocene fossils are available from Turkey that can demonstrate the local presence of *Q. cerris*, but fossil leaves from an Early Pleistocene deposit in Armenia (Ollivier *et al.*, 2010) suggest that the distribution of the Anatolian populations of *Q. cerris* could have been more extensive in the past than at present. Moreover, a long pollen record from the Black Sea, documenting the history of vegetation in Anatolia from 6 Ma to present (Site 380; Biltekin *et al.*, 2015), shows that approximately 6 to 3.37 Ma deciduous oaks were very abundant within a forest-dominated vegetation with meso-megathermic taxa (e.g. Taxodiaceae) (Fig. 4). Between 3.37 Ma and ca. 1.76 Ma, there was a clear reduction in oaks (including *Q. cerris*), culminating with their near-disappearance around 2.62 Ma, in correspondence with the earliest glacial cooling in the Northern Hemisphere. After 1.76 Ma deciduous oaks are found again in significant amounts; their frequencies fluctuate following the climatic cycles, although since 1.22 Ma, there has been a supremacy of steppe vegetation over mesophilous forests during both glacial and interglacial periods (Biltekin *et al.*, 2015).

Starting from the Pliocene, in Greece and south-east Albania extinct taxa with *Quercus* foliage co-occur with species markedly similar to modern western Eurasian species of *Quercus* section *Cerris* (*Q. cerris* and *Q. castaneifolia*) (Velitzelos *et al.*, 2014). In Bulgaria, macrofossils of *Q. cerris* have been reported from Pliocene deposits (Bozukov & Tsenov, 2012). In various sites of the Balkan Peninsula, pollen records of Middle (Fletcher *et al.*, 2013) to Late Pleistocene and Holocene age (Panagiotopoulos *et al.*, 2014; Tonkov *et al.*, 2014) support the local persistence of *Q. cerris* through the Pleistocene glacial-interglacial cycles, as also documented for undifferentiated deciduous oaks (Tzedakis *et al.*, 2006).

In Italy, macrofossils of Miocene and Pliocene age (Kovar-Eder *et al.*, 2006) are barely compatible with modern *Q. cerris* (E. Martinetto, personal communication), while leaves attributed to *Q. cerris* are reported from Early and Middle Pleistocene deposits (Martinetto & Sami, 2001; Martinetto *et al.*, 2014). In addition, pollen data from various sites of Early Pleistocene age support the presence of *Q. cerris* since at least 2 Ma (Fusco, 2007; Magri *et al.*, 2010; Sadori *et al.*, 2010).

In summary, fossil and pollen record evidence suggest the long-term continued presence of *Q. cerris* in the Anatolian,

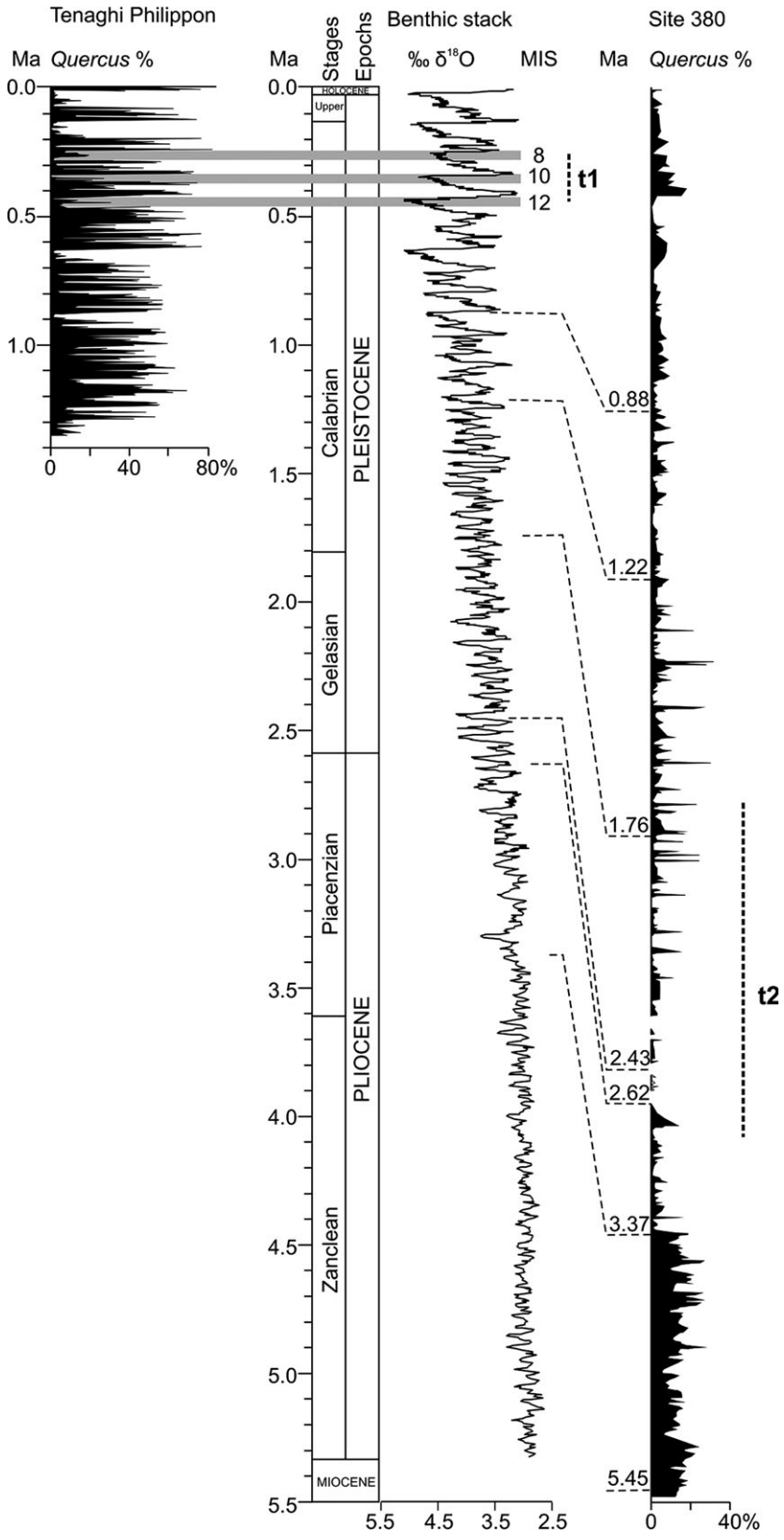

Figure 4 The pollen records of deciduous oaks from Tenaghi Philippon (Tzedakis *et al.*, 2006) and site 380 (Biltekin *et al.*, 2015) are correlated with the oxygen isotope curve by Lisiecki & Raymo (2005). t_1 and t_2 are the divergence times suggested by DIYABC best demographic model (scenario 1).

Balkan, and Italian peninsulas. *Q. cerris* populations in these regions probably increased in both number and size during the Pleistocene interglacial phases and diminished – without ever disappearing – during the glacial periods, similarly to other deciduous oaks (Fig. 4) (Tzedakis *et al.*, 2006; Magri & Palombo, 2013; Biltekin *et al.*, 2015).

DISCUSSION

Chloroplast DNA (CpDNA) phylogeography and introgression with *Q. suber*

Quercus cerris showed a high degree of chloroplast variation, similar to that detected in other European oak species (Petit *et al.*, 2002), although not equally distributed throughout its range, with much higher levels of genetic diversity found in the easternmost populations. A recent meta-analysis on a high number of plant and animal species demonstrated the overall increase in genetic diversity eastward in the Mediterranean basin (Conord *et al.*, 2012). The role of longitude in shaping genetic diversity is particularly interesting because in many species longitudinal variation affects transitions between discrete sets of genotypes, while latitudinal patterns typically involve refugial diversity (Rokas *et al.*, 2003). This is clearly evidenced in the distribution of *Q. cerris*' private haplotypes, which have been detected mainly (about 69%) in the Anatolian peninsula. Haplotype sharing, involving QC10 and QC27, between the Italian and the Balkan regions was also observed. In particular, the presence of haplotype QC27 in the Balkans suggests possible contacts between Balkan and Italian populations of *Q. cerris* through land bridges across the Adriatic Sea. Geological studies provide evidence that the Apulian Platform connected twice, during the Early Oligocene and then again during the latest Miocene, to the Balkan mainland by a trans-Adriatic land bridge, approximately where the Tremiti islands are today (Patacca *et al.*, 2008). In white oaks (Section *Quercus*), the presence of the same haplotype in the Balkan and Italian Peninsulas supports this idea (Fineschi *et al.*, 2002). The clear genetic relationship between southern Italian and Balkan populations of *Fagus sylvatica* L. (Vettori *et al.*, 2004), was also attributed to an amphi-Adriatic connection.

The *Q. cerris* distribution overlaps that of *Q. suber* in the Italian Peninsula and, interestingly, we found that they share some haplotypes in this region (haplotypes QC10 and QC27 in *Q. cerris* correspond to haplotypes QS2 and QS1 in *Q. suber*, described in Magri *et al.*, 2007), as shown in the haplotype network in Fig. S2 in Appendix S3. Sharing of haplotypes may result from species introgression (Petit, 2004), as reported for other oak species (Dodd & Afzal-Rafii, 2004; Eaton *et al.*, 2015). Repeated asymmetrical reproductive events between species with weak reproductive barriers can lead to morphologically (and genetically) unambiguous individuals of a species with plastid signatures of another (Petit & Excoffier, 2009). It has been suggested that this process is triggered (or favoured) by environmental changes, disturbance of local communities and historical range fluctuations (Excoffier *et al.*, 2009; Lagache *et al.*, 2013). As the pollen of *Q. cerris* and *Q. suber* is morphologically identical (van Benthem *et al.*, 1984), it is not possible to define the age of their coexistence from pollen records. However, the ABC analysis suggested that WG (including the Italian Peninsula), where the shared haplotypes QC10 and QC27 are found, split from CG (i.e. most of the Balkan Peninsula) at relatively recent times, even when considering generation time uncertainty and the wide 95% CIs obtained for time divergence estimates (see below). Thus, haplotype sharing between *Q. suber* and *Q. cerris* in Pleistocene glacial refugia, as suggested by other oak species (see below), seems a reasonable hypothesis.

At the intra-generic level, a wide sharing of identical haplotypes has been repeatedly documented across sympatric oak taxa (Okaura *et al.*, 2007; Simeone *et al.*, 2009, 2013). With few exceptions, a stronger correlation between plastid differentiation and geographical distribution rather than taxonomic identity has been detected, which is compatible with the well-known, extensive hybridization ability of oaks and its persistence in shared glacial refugia (Petit *et al.*, 2002). However, the possibility of shared ancestral polymorphism across related oak species cannot be fully ruled out (Denk & Grimm, 2010). Interestingly, interspecific genetic exchange similar to that suggested for *Q. cerris* and *Q. suber* in our study has been found between *Q. suber* and *Q. ilex* sympatric populations in the Western Mediterranean (Belahbib *et al.*, 2001; López de Heredia *et al.*, 2007). Additional studies in *Q. cerris*, *Q. suber* and *Q. ilex* using nuclear markers would be necessary to better evaluate the impact of introgression and hybridization on demographic history of Mediterranean oak species and to obtain deeper understanding of oak speciation in the Mediterranean region.

In agreement with the cpDNA haplotype distribution discussed above, BAPS analysis and genetic differentiation indices reflected strong spatial genetic structure in *Q. cerris*, involving three distinct genetic clusters along a westward longitudinal gradient. The highest genetic differentiation was observed in Turkey, where 68.7% of private haplotypes were identified. This region has a complex geological history that influenced the biogeography of many plant and animal taxa. The Anatolian Peninsula contains many barriers to gene flow, such as mountain chains and the Central Anatolian Lake System, whose formation can be traced back to the Neogene (Bilgin, 2011; and references therein). The presence of these geo-morphological barriers may explain the very high level of genetic differentiation and haplotype endemism in this region. Furthermore, these eastern populations may not only represent a current hotspot of genetic diversity, but also the origin of populations now occurring in Europe (see also the discussion of ABC results below). A similar westward pattern was observed in the gallwasp *Andricus quercustozae* (Rokas *et al.*, 2003), an obligate parasite whose sexual generation develops in galls induced on oaks of the section *Cerris*, and whose distribution is strictly dependent on the hosts. Together with other gallwasps, *A. quercustozae* diverged in Asia Minor and expanded westwards prior to the Pleistocene glaciations.

Demographic history

A hierarchical population split model (scenario 1) was found to be the most likely explanation for the observed data using

ABC, suggesting successive historical divergences of *Q. cerris* populations. However, this result has to be interpreted with caution given different sources of uncertainty in ABC inference, such as generation time, assumed models and wide confidential intervals (Tsuda *et al.*, 2015). Indeed, estimation of generation time is still controversial in forest tree species, because of their longevity, overlapping generations, variable age of first flowering, and forest replacement dynamics (Petit & Hampe, 2006). On the basis of field observations (P. Grossoni, personal communication), we considered a conservative generation time of 30–50 years for *Q. cerris*. Using these generation times, scenario 1 suggests that WG (Italy and northern Balkan Peninsula) diverged at t_1 from CG (mainly the Balkan Peninsula) 0.261 Ma (generation time of 30 years) to 0.435 Ma (generation time of 50 years). Although t_1 was inferred with wide 95% CIs, lower limits are still 0.0408 Ma (generation time of 30 years) or 0.068 Ma (generation time of 50 years), and the divergence time clearly predates the LGM regardless of the generation time assumed. Therefore, the impact of the LGM on the demographic history of *Q. cerris* would have been limited even when the species' European distribution was reduced to glacial refugia in the Italian and Balkan Peninsulas, as previously reported in other European oaks (Petit *et al.*, 2002).

The Italian and Balkan populations (WG and CG) diverged from the Eastern populations (EG, mainly consisting of Turkish and Lebanese populations) at t_2, which was estimated 1.674–2.790 Ma assuming a generation time of 30 or 50 years respectively. The divergence point at t_2 also had wide 95% CIs ranging from 0.510 Ma at lower limit (generation time of 30 years) to 4.705 Ma at upper limit (generation time of 50 years). Thus, despite divergence time uncertainty, these results still suggest that the ancient divergence of WG and CG from EG probably predated the Quaternary. These findings, together with the higher richness of private haplotypes and higher haplotypic diversity suggest a possible origin of *Q. cerris* in the Anatolian Peninsula and/or close to the Middle East region.

The fossil data, although limited due to species identification issues, are consistent with the demographic history of *Q. cerris* suggested by the genetic data. In particular, the possible bottleneck that led to the divergence of the Eastern and Central populations (see Results) is also reflected in the pollen record of Site 380 in the Black Sea region, which suggests that around 2.620 Ma a considerable reduction in deciduous oak populations (including *Q. cerris*) occurred in Anatolia and the eastern Balkan Peninsula, followed by a very slow recovery (Fig. 4). Vigorous oak populations are found only after 2 Ma. This historical bottleneck, which corresponds to the most important oak distribution reduction in the region during the last 6 Ma, is in excellent accordance with the hierarchical population split model that suggested divergence times between 2.790 and 1.674 Ma (t_2). After that time, corresponding to the Gelasian, *Q. cerris* is well documented by fossils in both the Italian and Balkan Peninsulas, where it persisted up to the Present. Since *c.* 0.900 Ma, increased amplitude and duration of the glacial-interglacial cycles provoked range reductions of different magnitude, depending on the severity of climate conditions (Tzedakis *et al.*, 2006; Magri & Palombo, 2013). The long pollen record from Tenaghi Philippon in Greece (Tzedakis *et al.*, 2006) indicates that the most extreme tree population contractions in the region, in decreasing order, occurred during the glacial maxima of marine isotope stages (MIS) 12, 16, 6, 2, 10 and 8 (Fig. 4). These stages were also the time periods when bottlenecks and genetic divergence were most likely to occur. Three of these glacial maxima, including MIS 12 (ca. 430 ka), MIS 10 (ca. 340 ka) and MIS 8 (ca. 260 ka), chronologically correspond to the divergence time t_1 suggested by scenario 1, within the range 0.435–0.261 Ma (Fig. 4), and support a Middle Pleistocene age for the split of a former continuous population of *Q. cerris* into two populations in the Italian and Balkan Peninsulas respectively.

After the genetic divergence of the Western and Central populations, later forest expansions may have led to the diffusion of haplotype QC10 into Slovenia and neighbouring countries. Extensive land exposure in the northern Adriatic, caused by low sea level during the glacial periods, may have acted as a land bridge for early-interglacial population migrations. The amphi-Adriatic distribution of haplotype QC27, supported by similar evidence in other taxa (see above), highlights the possibility that land bridges were also formed at much lower latitudes, in the southern Adriatic Sea. If so, the demographic history of *Q. cerris* populations would suggest a southern Adriatic land bridge with a more recent age than the divergence of the Western and Central populations.

The current lack of knowledge on genetic diversity and population structure of European species with an eastern distribution has resulted in underestimation of the role of eastern refugia for European plant biodiversity. Our extensive research of the modern genetic structure of *Q. cerris* covered a remarkable gap in European oak phylogeography, showing a putative eastern origin of the species and the presence of large amounts of genetic diversity in this region.

ACKNOWLEDGEMENTS

We warmly thank several co-workers and friends who made it possible to realize such a comprehensive collection of *Q. cerris* material throughout its distribution range. Without their help, we would not have managed to complete this research: Dalibor Ballian (Bosnia and Herzegovina), Robert Brus (Slovenia), Vasilije Isajev and Vladislava Galović (Serbia), Davorin Kajba and Želimir Borzan (Croatia), Juraj Piecka, Dušan Gömöry, Erika Gömöryová, Karol Ujházy, Margaréta Tullová (Slovakia), Jaromír Koblížek, Václav Buriánek (Czech Republic), Csaba Mátyás (Hungary), Raphael Klumpp (Austria), Hajri Haska (Albania), Tolga Ok, Sezgin Ayan, Hakan Şevik and Emrah Cicek (Turkey), Flaviu Popescu and Dragoş Postolache (Romania), Athanassios Rubos (Greece), Kiril Sotirovski and Jane Acevski (Macedonia).

REFERENCES

Akkemik, Ü. & Yaman, B. (2012) *Wood anatomy of eastern Mediterranean species*. Kessel Publishing House, Remagen-Oberwinter, Germany.

Alberto, F.J., Aitken, S.N., Alía, R., González-Martínez, S.C., Hänninen, H., Kremer, A., Lefèvre, F., Lenormand, T., Yeaman, S., Whetten, R. & Savolainen, O. (2013) Potential for evolutionary responses to climate change – evidence from tree populations. *Global Change Biology*, **19**, 1645–1661.

Beaumont, M.A., Zhang, W. & Balding, D.J. (2002) Approximate Bayesian computation in population genetics. *Genetics*, **162**, 2025–2035.

Belahbib, N., Pemonge, M.H., Ouassou, A., Sbay, H., Kremer, A. & Petit, R.J. (2001) Frequent cytoplasmic exchanges between oak species that are not closely related: *Quercus suber* and *Q. ilex* in Morocco. *Molecular Ecology*, **10**, 2003–2012.

Bennett, K.D., Tzedakis, P.C. & Willis, K.J. (1991) Quaternary refugia of north European tree species. *Journal of Biogeography*, **18**, 103–115.

Bertorelle, G., Benazzo, A. & Mona, S. (2010) ABC as a flexible framework to estimate demography over space and time: some cons, many pros. *Molecular Ecology*, **19**, 2609–2625.

Bilgin, R. (2011) Back to the suture: the distribution of intraspecific genetic diversity in and around Anatolia. *International Journal of Molecular Sciences*, **12**, 4080–4103.

Biltekin, D., Popescu, S.M., Suc, J.P., Quézel, P., Jiménez-Moreno, G., Yavuz, N. & Çağatay, M.N. (2015) Anatolia: a long-time plant refuge area documented by pollen records over the last 23 million years. *Review of Palaeobotany and Palynology*, **215**, 1–22.

Blondel, J., Aronson, J., Bodiou, J.Y. & Boeuf, G. (2010) *The Mediterranean region: biological diversity in space and time*, 2nd edn. Oxford University Press, New York.

Bozukov, V.S. & Tsenov, B.V. (2012) Catalogue of the Cenozoic plants of Bulgaria (Eocene to Pliocene) Addendum and Corrigendum. *Phytologia Balcanica*, **18**, 237–261.

Camus, A. (1938–39) *Les chênes: Monographie du genre Quercus, tome 2, Genre Quercus, sous-genre Euquercus (sections Lepidobalanus et Macrobalanus) Texte*. Lechevalier, Paris.

Chen, C., Qi, Z.C., Xu, X.H., Comes, H.P., Koch, M.A., Jin, X.J., Fu, C.X. & Qiu, Y.X. (2014) Understanding the formation of Mediterranean–African–Asian disjunctions: evidence for Miocene climate-driven vicariance and recent long-distance dispersal in the Tertiary relict *Smilax aspera* (Smilacaceae). *New Phytologist*, **204**, 243–255.

Conord, C., Gurevitch, J. & Fady, B. (2012) Large-scale longitudinal gradients of genetic diversity: a meta-analysis across six phyla in the Mediterranean basin. *Ecology and Evolution*, **2**, 2595–2609.

Corander, J. & Marttinen, P. (2006) Bayesian identification of admixture events using multilocus molecular markers. *Molecular Ecology*, **15**, 2833–2843.

Cornuet, J.M., Pudlo, P., Veyssier, J., Dehne-Garcia, A., Gautier, M., Leblois, R., Marin, J.M. & Estoup, A. (2014) DIYABC v2.0: a software to make approximate Bayesian computation inferences about population history using single nucleotide polymorphism DNA sequence and microsatellite data. *Bioinformatics*, **30**, 1187–1189.

Denk, T. & Grimm, G.W. (2010) The oaks of western Eurasia: traditional classifications and evidence from two nuclear markers. *Taxon*, **59**, 351–366.

Dodd, R.S. & Afzal-Rafii, Z. (2004) Selection and dispersal in a multispecies oak hybrid zone. *Evolution*, **58**, 261–269.

Eaton, D.A.R., Gonzalez-Rodriguez, A., Hipp, A.L. & Cavender-Bares, J. (2015) Introgression obscures and reveals historical relationships among the American live oaks. BioRxiv, doi:10.1101/016238.

Emery-Barbier, A. & Thiébault, S. (2005) Preliminary conclusions on the Late Glacial vegetation in south-west Anatolia (Turkey): the complementary nature of palynological and anthracological approaches. *Journal of Archaeological Science*, **32**, 1232–1251.

Excoffier, L., Smouse, P.E. & Quattro, J.M. (1992) Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics*, **131**, 479–491.

Excoffier, L., Foll, M. & Petit, R.J. (2009) Genetic consequences of range expansions. *Annual Review of Ecology Evolution and Systematics*, **4**, 481–501.

Fineschi, S., Taurchini, D., Grossoni, P., Petit, R.J. & Vendramin, G.G. (2002) Chloroplast DNA variation of white oaks in Italy. *Forest Ecology and Management*, **156**, 103–114.

Fletcher, W.J., Mueller, U.C., Koutsodendris, A., Christanis, K. & Pross, J. (2013) A centennial-scale record of vegetation and climate variability from 312 to 240 ka (Marine Isotope Stages 9c-a, 8 and 7e) from Tenaghi Philippon, NE Greece. *Quaternary Science Reviews*, **78**, 108–125.

Fusco, F. (2007) Vegetation response to early Pleistocene climatic cycles in the Lamone valley (Northern Apennines, Italy). *Review of Palaeobotany and Palynology*, **145**, 1–23.

Gavin, D.G., Fitzpatrick, M.C., Gugger, P.F. *et al.* (2014) Climate refugia: joint inference from fossil records, species distribution models and phylogeography. *New Phytologist*, **204**, 37–54.

Gellini, R. & Grossoni, P. (1997) *Botanica Forestale, Vol. II: Angiosperme*. CEDAM, Padova.

Gómez, A. & Lunt, D.H. (2007) Refugia within refugia: patterns of phylogeographic concordance in the Iberian Peninsula. *Phylogeography of Southern European Refugia* (ed. by S. Weiss and N. Ferrand), pp. 155–188. Springer Verlag, Dordrecht.

González-Martínez, S.C., Dubreuil, M., Riba, M., Vendramin, G.G., Sebastiani, F. & Mayol, M. (2010) Spatial genetic structure of *Taxus baccata* L. in the western Mediterranean Basin: past and present limits to gene movement over a broad geographic scale. *Molecular Phylogenetics and Evolution*, **55**, 805–815.

Hardy, O.J. & Vekemans, X. (2002) SPAGeDi: a versatile computer program to analyse spatial genetic structure at the individual or population levels. *Molecular Ecology Notes*, **2**, 618–620.

Hedrick, P.W. (2005) A standardized genetic differentiation measure. *Evolution*, **59**, 1633–1638.

Heuertz, M., De Paoli, E., Källman, T., Larsson, H., Jurman, I., Morgante, M., Lascoux, M. & Gyllenstrand, N. (2006) Multilocus patterns of nucleotide diversity, linkage disequilibrium and demographic history of Norway Spruce (*Picea abies* (L.) Karst.). *Genetics*, **174**, 2095–2105.

Ingvarsson, P.K. (2008) Multilocus patterns of nucleotide polymorphism and the demographic history of *Populus tremula*. *Genetics*, **180**, 329–340.

IPCC (2007) *Climate change 2007: the physical science basis*. Cambridge University Press, Cambridge.

Kovar-Eder, J., Kvaček, Z., Martinetto, E. & Roiron, P. (2006) Late Miocene to Early Pliocene vegetation of southern Europe (7-4 Ma) as reflected in the megafossil plant record. *Palaeogeography, Palaeoclimatology, Palaeoecology*, **238**, 321–339.

Lagache, L., Klein, E.K., Guichoux, E. & Petit, R.J. (2013) Fine-scale environmental control of hybridization in oaks. *Molecular Ecology*, **22**, 423–436.

Lisiecki, L.E. & Raymo, M.E. (2005) A Pliocene-Pleistocene stack of 57 globally distributed benthic δ18O records. *Paleoceanography*, **20**, PA1003.

López de Heredia, U.L., Carrion, J.S., Jimenez, P., Collada, C. & Gil, L. (2007) Molecular and palaeoecological evidence for multiple glacial refugia for evergreen oaks on the Iberian Peninsula. *Journal of Biogeography*, **34**, 1505–1517.

Magri, D. & Palombo, M.R. (2013) Early to Middle Pleistocene dynamics of plant and mammal communities in South West Europe. *Quaternary International*, **288**, 63–72.

Magri, D., Vendramin, G.G., Comps, B., Dupanloup, I., Geburek, T., Gömöry, D., Latałowa, M., Litt, T., Paule, L., Roure, J.M., Tantau, I., Van Der Knaap, W.O., Petit, R.J. & De Beaulieu, J.-L. (2006) A new scenario for the Quaternary history of European beech populations: palaeobotanical evidence and genetic consequences. *New Phytologist*, **171**, 199–221.

Magri, D., Fineschi, S., Bellarosa, R., Buonamici, A., Sebastiani, F., Schirone, B., Simeone, M.C. & Vendramin, G.G. (2007) The distribution of *Quercus suber* chloroplast haplotypes matches the palaogeographical history of the western Mediterranean. *Molecular Ecology*, **16**, 5259–5266.

Magri, D., Di Rita, F. & Palombo, M. (2010) An Early Pleistocene interglacial record from an intermontane basin of Central Italy (Scoppito, L'Aquila). *Quaternary International*, **225**, 106–113.

Martinetto, E. & Sami, M. (2001) Paleoflora delle "sabbie gialle" mediopleistoceniche di Oriolo, presso Faenza (Ravenna). *Quaderno di Studi e Notizie di Storia Naturale Della Romagna*, **14**, 1–28.

Martinetto, E., Bertini, A., Basilici, G., Baldanza, A., Bizzarri, R., Cherin, M., Gentili, S. & Pontini, M.R. (2014) The plant record of the Dunarobba and Pietrafitta sites in the Plio-Pleistocene palaeoenvironmental context of Central Italy. *Alpine and Mediterranean Quaternary*, **27**, 29–72.

Médail, F. & Diadema, K. (2009) Glacial refugia influence plant diversity patterns in the Mediterranean Basin. *Journal of Biogeography*, **36**, 1333–1345.

Nieto Feliner, G. (2011) Southern European glacial refugia: a tale of tales. *Taxon*, **60**, 365–372.

Nieto Feliner, G. (2014) Patterns and processes in plant phylogeography in the Mediterranean Basin. A review. *Perspectives in Plant Ecology, Evolution and Systematics*, **16**, 265–278.

Ohlemüller, R., Huntley, B., Normand, S. & Svenning, J.C. (2012) Potential source and sink locations for climate-driven species range shifts in Europe since the Last Glacial Maximum. *Global Ecology and Biogeography*, **21**, 152–163.

Okaura, T., Duc Quang, N., Ubukata, M. & Harada, K. (2007) Phylogenetic structure and late Quaternary population history of the Japanese oak *Q. mongolica* var. *crispula*, and related species revealed by chloroplast DNA variation. *Genes and Genetic Systems*, **82**, 465–477.

Ollivier, V., Nahapetyan, S., Roiron, P., Gabrielyan, I., Gasparyan, B., Chataigner, C., Joannin, S., Cornée, J.J., Guillou, H., Scaillet, S., Munch, P. & Krijgsman, W. (2010) Quaternary volcano-lacustrine patterns and paleobotanical data in South Armenia. *Quaternary International*, **223**, 312–326.

Panagiotopoulos, K., Böhm, A., Leng, M.J., Wagner, B. & Schäbitz, F. (2014) Climate variability over the last 92 ka in SW Balkans from analysis of sediments from Lake Prespa. *Climate of the Past*, **10**, 643–660.

Patacca, E., Scandone, P. & Mazza, P. (2008) Oligocene migration path for Apulia macromammals, the Central-Adriatic bridge. *Bollettino Della Società Geolologica Italiana*, **127**, 337–355.

Peakall, R. & Smouse, P.E. (2006) GenAlEx 6: genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes*, **6**, 288–295.

Peakall, R. & Smouse, P.E. (2012) GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research-an update. *Bioinformatics*, **28**, 2537–2539.

Petit, R.J. (2004) Biological invasions at the gene level. *Diversity and Distributions*, **10**, 159–165.

Petit, R.J. & Excoffier, L. (2009) Gene flow and species delimitation. *Trends in Ecology and Evolution*, **24**, 386–393.

Petit, R.J. & Hampe, A. (2006) Some evolutionary consequences of being a tree. *Annual Review of Ecology, Evolution and Systematics*, **37**, 187–214.

Petit, R.J., Csaikl, U.M., Bordacs, S. *et al.* (2002) Chloroplast DNA variation in European white oaks: phylogeography and patterns of diversity based on data from over 2600 populations. *Forest Ecology and Management*, **156**, 5–26.

Pons, O. & Petit, R. (1996) Measuring and testing genetic differentiation with ordered versus unordered alleles. *Genetics*, **144**, 1237–1245.

Provan, J. & Bennett, K.D. (2008) Phylogeographic insights into cryptic glacial refugia. *Trends in Ecology and Evolution*, **23**, 564–571.

Pyhäjärvi, T., García-Gil, M.R., Knürr, T., Mikkonen, M., Wachowiak, W. & Savolainen, O. (2007) Demographic history has influenced nucleotide diversity in European *Pinus sylvestris* populations. *Genetics*, **177**, 1713–1724.

Rokas, A., Atkinson, R.J., Webster, L.M.I., Csóka, G. & Stone, G.N. (2003) Out of Anatolia: longitudinal gradients in genetic diversity support an eastern origin for a circum-Mediterranean oak gallwasp *Andricus quercustozae*. *Molecular Ecology*, **12**, 2153–2174.

Sadori, L., Giardini, M., Chiarini, E., Mattei, M., Papasodaro, F. & Porreca, M. (2010) Pollen and macrofossil analyses of Pliocene lacustrine sediments (Salto river valley, Central Italy). *Quaternary International*, **225**, 44–57.

Sebastiani, F., Carnevale, S. & Vendramin, G.G. (2004) A new set of mono- and dinucleotide chloroplast microsatellites in Fagaceae. *Molecular Ecology Notes*, **4**, 259–261.

Şen, A., Quilhó, T. & Pereira, H. (2011) Bark anatomy of *Quercus cerris* L. var. *cerris* from Turkey. *Turkish Journal of Botany*, **35**, 45–55.

Simeone, M.C., Papini, A., Vessella, F., Bellarosa, R., Spada, F. & Schirone, B. (2009) Multiple genome relationships and a complex evolutionary history in the eastern range of *Quercus suber* L. (Fagaceae), implied by nuclear and chloroplast DNA variation. *Caryologia*, **62**, 236–252.

Simeone, M.C., Piredda, R., Papini, A., Vessella, F. & Schirone, B. (2013) Application of plastid and nuclear markers to DNA barcoding of Euro-Mediterranean oaks (*Quercus*, Fagaceae): problems, prospects and phylogenetic implications. *Botanical Journal of the Linnean Society*, **172**, 478–499.

Slatkin, M. (1995) A measure of population subdivision based on microsatellite allele frequencies. *Genetics*, **139**, 457–462.

Song, Y., Krajewska, K. & Wang, Y.F. (2000) The first occurrence of the *Quercus* section *Cerris* Spach fruits in the Miocene of China. *Acta Palaeobotanica*, **40**, 153–163.

Teodoridis, V., Kvaček, Z. & Uhl, D. (2009) Pliocene palaeoenvironment and correlation of the Sessenheim-Auenheim floristic complex (Alsace, France). *Palaeodiversity*, **2**, 1–17.

Tonkov, S., Lazarova, M., Bozilova, E., Ivanov, D. & Snowball, I. (2014) A 30,000-year pollen record from Mire Kupena, Western Rhodopes Mountains (south Bulgaria). *Review of Palaeobotany and Palynology*, **209**, 41–51.

Tsuda, Y., Nakao, K., Ide, Y. & Tsumura, Y. (2015) The population demography of *Betula maximowicziana*, a cool-temperate tree species in Japan, in relation to the last glacial period: its admixture-like genetic structure is the result of simple population splitting not admixing. *Molecular Ecology*, **24**, 1403–1418.

Tzedakis, P.C., Hooghiemstra, H. & Pälike, H. (2006) The last 1.35 million years at Tenaghi Philippon: revised chronostratigraphy and long-term vegetation trends. *Quaternary Science Reviews*, **25**, 3416–3430.

Uslu, E. & Bakiş, Y. (2014) Morphometric analyses of the leaf variation within *Quercus* L. Sect. *Cerris* Loudon in Turkey. *Dendrobiology*, **71**, 109–117.

van Benthem, F., Clarke, G.C.S. & Punt, W. (1984) Fagaceae. *The Northwest European Pollen Flora, IV* (ed. by W. Punt and G.C.S. Clarke), pp. 87–110. Elsevier, Amsterdam.

Velitzelos, D., Bouchal, J.M. & Denk, T. (2014) Review of the Cenozoic floras and vegetation of Greece. *Review of Palaeobotany and Palynology*, **204**, 56–117.

Vettori, C., Vendramin, G.G., Anzidei, M., Pastorelli, R., Paffetti, D. & Giannini, R. (2004) Geographic distribution of chloroplast variation in Italian populations of beech (*Fagus sylvatica* L.). *Theoretical & Applied Genetics*, **109**, 1–9.

Weising, K. & Gardner, R.C. (1999) A set of conserved PCR primers for the analysis of simple sequence repeat polymorphisms in chloroplast genomes of dicotyledonous angiosperms. *Genome*, **42**, 9–19.

Worobiec, G., Worobiec, E. & Szynkiewicz, A. (2012) Plant assemblage from the Upper Miocene deposits of the Bełchatów Lignite Mine (Central Poland). *Acta Palaeobotanica*, **52**, 369–413.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Appendix S1 Materials and Methods details.
Appendix S2 Results of haplotype sequencing.
Appendix S3 Supplementary tables and figures.

BIOSKETCH

The authors belong to a research consortium dealing with population genetics of forest tree species, with particular emphasis to the study of genetic structure at different temporal and spatial scales. They have been involved in projects studying the range-wide phylogeography of several forest tree species in Europe and the Mediterranean Basin.

Author contributions: F.B., S.F. and G.G.V. designed the study; F.B., S.F., P.B., P.Z., L.P., M.C.S. and G.G.V. organized the sampling; F.B., S.F. and M.C.S. performed the molecular laboratory analysis; F.B., Y.T. and G.G.V. performed the statistical analysis, with advices by S.C.G.M.; D.M. compiled the palaeobotanical section; F.B. led the writing; Y.T., S.C.G.M., D.M. and G.G.V. contributed to the writing; all authors revised and approved the manuscript.

Editor: Stephen Cavers