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SOM, GGV, and BF designed the experiments. HL performed all bioinformatic analysis (ORF determination), the simulation of demographic scenarios with the help of SCGM, and identified the functional role of genes under selection. KC and SOM performed all other statistical analyses. KC and SOM wrote the paper (and HL the initial draft). All authors contributed to data interpretation and revisions of the manuscript.

Key-words: F_{ST} outlier, haplotype, variance components of linkage disequilibrium, Ohta's test, gene network, budburst phenology, abiotic stress

ABSTRACT

Detecting signatures of selection in tree populations threatened by climate change is currently a major research priority. Here, we investigated the signature of local adaptation over a short spatial scale using 96 European beech (*Fagus sylvatica* L.) individuals originating from two pairs of populations on the northern and southern slopes of Mont Ventoux (south-eastern France). We performed both single and multi-locus analysis of selection based on 53 climate-related candidate genes containing 546 SNPs. F_{ST} outlier methods at the SNP level revealed a

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weak signal of selection, with three marginally significant outliers in the northern populations. At the gene-level, considering haplotypes as alleles, two additional marginally significant outliers were detected, one on each slope. To account for the uncertainty of haplotype inference, we averaged the Bayes Factors over many possible phase reconstructions. Epistatic selection offers a realistic multi-locus model of selection in natural populations. Here, we used a test suggested by Ohta based on the decomposition of the variance of linkage disequilibrium. Over all populations, 0.23% of the SNP pairs (haplotypes) showed evidence of epistatic selection, with nearly 80% of them being within genes. One of the between gene epistatic selection signals arose between an F_{ST} outlier and a non-synonymous mutation in a drought response gene. Additionally, we identified haplotypes containing selectively advantageous allele combinations which were unique to high or low-elevations and northern or southern populations. Several haplotypes contained non-synonymous mutations situated in genes with known functional importance for adaptation to climatic factors.

INTRODUCTION

Spatially variable selection plays a crucial role in shaping phenotypic variation within and among natural populations. Local adaptation occurs when populations distributed across heterogeneous environments evolve different phenotypic trait values conferring fitness advantage in their local environment (Le Corre & Kremer, 2003; Schoville *et al.* 2012).

Increasing concerns regarding climate change have renewed the interest in estimating species adaptive capacity under spatially variable selection in the hope of better understanding short-term potential for evolutionary responses (Hansen *et al.* 2012). Forest trees play a key role in this context: they cover approximately three quarters of the earth's terrestrial biomass and are often keystone species in their habitats. Furthermore, tree species are ideal case studies for

detecting selection in natural populations because they have large effective population sizes, where selection is expected to be efficient, and demonstrate weak neutral genetic differentiation even over large geographic ranges, which decreases the chance of confusing the effects of selection and population structure (Savolainen & Pyhäjärvi, 2007).

The existence of locally adapted forest tree populations has long been suggested based on several lines of evidence. First, phenotypic clines along environmental gradients are common in forest trees (typically for phenological traits) and the triggering of major adaptive traits by climatic clues is often prescribed to selection (Mikola, 1982). Second, forest geneticists have long been conducting semi-controlled transplantation experiments, so-called provenance tests, which often demonstrate strong latitudinal genetic clines for potentially adaptive traits such as bud set, cold tolerance, growth or photoperiod sensitivity (Alberto *et al.* 2013a).

Third, more recently, using genomic tools developed for forest tree species (Neale & Kremer, 2011; Kremer *et al.* 2012), several studies corroborate the evidence of adaptive genetic differentiation among tree populations along environmental gradients (Eveno *et al.* 2008; Ma *et al.* 2010; Chen *et al.* 2012; Kujala & Savolainen, 2012; Alberto *et al.* 2013b; Mosca *et al.* 2014).

The detection of molecular imprints of local adaptation, however, has been recently challenged by several authors both from methodological and biological points of view (e.g. Pritchard *et al.* 2010; Le Corre & Kremer, 2012). Among the most commonly used methods, the so-called F_{ST} outlier tests are based on the idea that loci under divergent or homogenizing selection are expected to have unusually high or low levels of differentiation between populations, respectively (e.g. Beaumont and Nichols 1996). Several recent simulation studies suggested that these F_{ST} outlier tests can have a high rate of false positives and lack

power (Excoffier *et al.* 2009; Vilas *et al.* 2012; De Mita *et al.* 2013; Lotterhos & Whitlock, 2014). From a methodological point of view, this is because, first, F_{ST} outlier tests assume a demographic model that is often far from the reality (Excoffier *et al.* 2009; Lotterhos & Whitlock, 2014), and, second, F_{ST} outlier tests are often applied to insufficient or inappropriate data (Foll & Gaggiotti 2008). For example, in the case of candidate gene data, the presence of linked loci within genes decreases the effective number of loci (Vilas *et al.* 2012). From a biological point of view, the genetic architecture of adaptive traits in natural populations is complex and probably influenced by many loci simultaneously (e.g. Mackay, 2014), thus looking for the signature of selection at individual loci is rather naive. Therefore, more realistic mechanisms of evolutionary change, such as polygenic and epistatic selection, need to be considered in selection tests (Fu & Akey, 2013); so far, however, methodological developments are lagging behind.

Polygenic adaptation in natural populations can be reached via weak allele frequency changes at multiple loci across the genome, where advantageous alleles exist at low to moderate frequencies (Pritchard *et al.* 2010; Fu & Akey, 2013). Le Corre & Kremer (2003) proposed a theoretical framework to predict polygenic adaptation, and showed that adaptive phenotypic divergence in response to polygenic selection (traditionally measured by Q_{ST}) is first achieved through the filtering of combinations of advantageous alleles at multiple loci (typically in <10 generations), while changes in allele frequencies occur later (increasing F_{ST}). Polygenic adaptation may be estimated as the covariance of allele frequencies among populations, i.e. the between-population variance component of the total linkage disequilibrium (the so-called D_{ST} in Ohta, 1982 or Z_g in Storz & Kelly, 2008). In practice, Storz & Kelly (2008) showed that the Z_g of 11 alpha-globin genes, known to contribute to differences in aerobic capacity between mice populations from low and high elevations, was significantly higher than

expected from a simulated sample of neutral genes. In another study, Ma *et al.* (2010) showed that the covariance of allelic effects was higher for photoperiodic genes than for control genes in *Populus tremula*, and that most of the observed phenotypic variation was explained by the covariance among individual locus effects and not by individual SNPs.

Methodological developments to detect epistatic selection in natural populations are also lagging behind (Fu & Akey, 2013; Hansen, 2013). Ohta (1982) proposed four statistics based on the decomposition of the variance of linkage disequilibrium (LD) that may be used to test if LD between two loci is due to drift (or linkage) or epistatic selection. She argued that if epistatic selection is responsible for LD, haplotypes with favorable combinations of alleles would become frequent in every sub-population. Thus, in comparison to the test of local adaptation based on the between population component of the variance of LD (Le Corre & Kremer, 2003; Storz & Kelly, 2008), Ohta's test can be viewed as its opposite: while the former aims at identifying haplotypes with unusually high D_{ST} values, the latter is looking for unusually low D_{ST} values. An important technical difference between the two tests is that while unusually high D_{ST} values can only be identified using a genomic control (typically using supposedly neutral loci), unusually low D_{ST} values can be identified by comparing D_{ST} to the gametic phase equilibrium of the same loci. Despite its conceptual simplicity, Ohta's test has rarely been applied to experimental data, especially to recent genetic data (but for tests on allozyme data in forest trees, see Cheng *et al.* 2001; Fernández-López & Monteagudo, 2010).

Here, we investigated the signatures of local adaptation and epistatic selection at a local scale from candidate gene data in European beech (*Fagus sylvatica*), a dominant tree species of

many lowland forests across Europe. Genetic differentiation along local elevational gradients has been previously reported in *F. sylvatica* for various traits related to climate response, including phenology of budburst (Vitasse *et al.* 2009), leaf mass per area (LMA), nitrogen content and leaf size (Bresson *et al.* 2012). Nevertheless, so far, only a few published studies attempted to detect the molecular signature of divergent selection in *F. sylvatica*. First, using AFLP genome scans along an elevational gradient, Jump *et al.* (2006) identified one outlier locus (among 241 scored) and showed that the gene frequency at this locus was correlated with mean annual temperature. Second, using again AFLP genome scans and 3 replicated pairs of mesic and dry sites, Pluess & Weber (2012) detected 13 outlier loci (among 517 scored), from which 7 changed their frequencies with local moisture availability. Three climate-related candidate genes datasets were recently developed for *F. sylvatica* (Seifert *et al.* 2012; Muller 2013; Lalagüe *et al.* 2014), but their potential has not yet been fully exploited to investigate the signature of local adaptation.

We sampled 96 individuals from two pairs of populations situated on the northern and southern slopes of Mont Ventoux (south-eastern France). This sampling design ensured sharp environmental differences across elevations at short spatial scale, favorable for the detection of recent selection (Alberto *et al.* 2013b; Körner, 2007). We used 53 candidate genes potentially involved in climate response (Lalagüe *et al.* 2014) to investigate signatures of selection both at single- and multi-locus levels. At the single-locus level, we used F_{ST} outlier methods for both SNPs and genes. Following Eveno *et al.* (2008), we used F_{ST} outlier tests for entire genes to account for non-independence among loci and we additionally proposed an averaging Bayesian model to take into account the uncertainty of haplotype inference when the haplotype phase is unknown. At the multi-locus level, we applied Ohta's test of epistatic selection, for the first time, to candidate gene data. We paid particular attention to the

functional genomic interpretation of haplotypes showing evidence for epistatic selection, and illustrated the advantage of combining different approaches and data to detect signatures of selection.

MATERIALS AND METHODS

Sampling and populations

Fagus sylvatica populations on Mont Ventoux originate from a common Holocene gene pool (Magri *et al.* 2006). In the 17th century, they went through a bottleneck, where only four remnant populations survived at high elevations (Lander *et al.* 2011). Since then, following changes in land-use, *F. sylvatica* recurrently re-colonised Mont Ventoux from the remnant populations. At microsatellite loci, a low, but significant genetic differentiation was shown between the northern and southern slopes ($F_{CT} = 0.2\%$), and among populations within each slope ($F_{SC} = 2.5\%$; Lander *et al.* 2011).

We used the candidate gene dataset previously described in Lalagüe *et al.* (2014) for all analyses presented in this paper. The dataset comprised 96 individuals from four populations; two situated on the northern slope (high -NH- and low -NL- with 35 and 36 individuals, respectively) and two on the southern slope (high -SH- and low -SL- with 12 and 13 individuals, respectively) (see Fig. 1). Individuals from the NH, NL, and SH sites represent remnant populations, while the SL site has recently been colonized by *F. sylvatica*. Local measurements of temperature, relative humidity and precipitation at the four sampling sites over five years revealed that climatic conditions differ between high and low elevation populations both at the northern and southern slopes (Table 1). Accordingly, phenotypic and genotypic differences have been demonstrated between NH and NL populations for the date

of budburst (nine days lag, Gauzère et al., 2013; $Q_{ST} = 5\%$ for populations located less than one km apart, J. Gaüzère, pers comm).

Genetic data and functional annotation

A reduced version of the candidate gene dataset described in Lalagüe *et al.* (2014) was generated for this study, by removing four duplicate genes (see supporting information ESM1 of Lalagüe *et al.* 2014). The new dataset comprises 546 SNPs from 53 candidate genes (Table S1, Supporting information) that are potentially involved in response to abiotic stress (e.g. drought or frosts) and phenology (e.g. bud-burst). Note that the full-length sequences of these candidate genes were generally not available, but only gene fragments. We determined the open reading frames (ORF) and the intron-exon boundaries of the 53 candidate genes by comparing *F. sylvatica* Expressed Sequence Tags (ESTs) with proteins of related species available in the NCBI Reference Sequence (RefSeq) or non-redundant (nr) databases in May 2013. To perform these analyses, we used CodonCode Aligner v3.7.1 (CodonCode Corporation, <http://www.codoncode.com>) and BLASTX (Altschul *et al.* 1990) with default parameters. We also determined whether changes in SNPs located within exons were synonymous or non-synonymous. In total, 235 SNPs were found in exons, with 82 coding for non-synonymous and 153 for synonymous mutations; 262 SNPs were found in introns, 30 in 3'UTR and 1 in 5'UTR regions (Table S2, Supporting information).

Haplotype inference

We used PHASE version 2.1 (Stephens & Donnelly, 2003) to simultaneously impute the missing genotypes at each SNP, infer the phase and estimate the haplotype frequencies for each gene independently. We used the MR0 model with varying recombination rate (Li & Stephens, 2003) and probability thresholds of 0.95 for both missing alleles and phase

inference. We ran five independent Markov chains of length 10^4 , with a thinning interval of 10 and a burn-in period of 10^4 . Using PHASE the original rate of missing data of 24.6% was decreased to 12.1% in the imputed and to 12.4% in the phased datasets. Further, we used the `-s` option of PHASE to draw 10^3 samples from the posterior distribution of the haplotypes, i.e. 10^3 different realizations of the phased dataset where all missing data were replaced by a “best guess” haplotype. To calculate Ohta's LD statistics between SNPs situated in different genes, we used the EM algorithm implemented in the R package `haplo.stat` (<http://www.mayo.edu/research/labs/statistical-genetics-genetic-epidemiology/software>) to infer the haplotype phase. To summarize, we used three different versions of the dataset for further analysis (each based on 546 SNPs): imputed genotype data, phased data, and best-guess haplotype samples (10^3 realizations). For all analyses except the calculation of F_{ST} and hierarchical AMOVA, loci with more than 30% missing data were removed from the imputed and phased datasets, leaving 483 and 481 SNPs, respectively, for further analysis.

Genetic differentiation and F_{ST} outlier tests

F_{ST} (Weir & Cockerham, 1984) between populations was estimated both at the SNP and gene levels using the imputed genotype and phased data, respectively. Components of the variance in allele frequencies within and between the northern and southern population pairs were estimated using the hierarchical AMOVA implemented in Arlequin v.3.5.1.3 (Excoffier *et al.* 1992).

We used the F_{ST} outlier methods to detect selection due to climatic stress induced by elevational differences (Table 1). We tested two scenarios. First, we considered the northern and southern slopes as replicates for elevation-related climatic stress; second, we considered the four populations in a hierarchical model (i.e. two populations nested within northern and

southern slopes). Thus, the latter also accounted both for hierarchical genetic structure and for the marked differences between the two slopes themselves (Table 1). Overall, we expected to detect few F_{ST} outlier loci because gene flow is high between our sampled populations and because outlier detection methods lack power when few populations are tested (Foll & Gaggiotti, 2008). We looked for both unusually differentiated SNPs and genes.

At the SNP level, we used the F_{ST} outlier methods of Beaumont & Nichols (1996) and Foll & Gaggiotti (2008) implemented in Arlequin v.3.5.1.3 and BayeScan 2.1, respectively. We used the imputed genotype data, and removed loci with a minor allele frequency (MAF) below 0.05 (leaving 307 SNPs to analyze). In the Beaumont & Nichols (1996) approach, the expected neutral F_{ST} distribution was obtained by simulating 10^5 SNPs under a hierarchical island model (with 10 groups and 10 demes per group). The target F_{ST} was estimated from all SNPs, assuming that the majority of them were neutral. The p -values obtained from Arlequin were corrected for multiple testing using the False Discovery Rate (FDR) method of the function “*p.adjust*” in R (package *stats*; R Development Team 2011). Further, we investigated the sensitivity of the Beaumont & Nichols (1996) method to the demography assumed by the null model. We used information from the well-documented demographic history of European beech on Mont Ventoux (Lander *et al.* 2011) to simulate more realistic alternative null models using *ms* (Hudson, 2002; Appendix S1, Supporting information). In the Foll & Gaggiotti (2008) method, we used Bayes Factors to evaluate the evidence for selection, based on Jeffreys' scale of evidence. BayeScan was run with 20 pilot runs of 5,000 iterations and then a burn-in of 50,000 iterations followed by 50,000 iterations (thinning interval of 10). Since the probability of being under selection is higher for a SNP situated in a candidate gene than for a random SNP, we decreased the prior odds for the null model from 10 (default) to 2 (M. Foll, pers. comm.). Further, we investigated the sensitivity of BayeScan

to the MAF level (Appendix S2, Supporting information). Note that there was no hierarchical model implemented in BayeScan when this study was done.

We also performed an F_{ST} outlier test at the level of genes following Eveno *et al.* (2008), where each gene was considered as a multi-allelic locus. In contrast to Eveno (2008), the haplotype phase was unknown, so we had to rely on estimates from PHASE. We only used the Foll & Gaggiotti (2008) approach. BayeScan was run for each of the 10^3 different best-guess haplotype samples, and the median log₁₀ Bayes Factor was used to summarize the overall evidence for selection.

Epistatic selection

We used Ohta's test (1982) to detect epistatic selection, which is based on the decomposition of the variance of linkage disequilibrium (LD) within a subdivided population into within (D_{IS}^2) and between population (D_{ST}^2) components. When epistatic selection is responsible for LD, haplotypes with favorable combinations of alleles are expected to increase in all populations, i.e. $D_{ST}^2 < D_{IS}^2$. In contrast, if the observed LD is a consequence of genetic drift and limited migration between sub-populations, the expected variance of LD within sub-populations should be smaller than the variance in the expected frequencies of different gametes. Ohta defined two further variance components: $D'_{IS}{}^2$ is the variance of the correlation of genes of the two loci of one gamete in a sub-population relative to that of the total population and $D'_{ST}{}^2$ is the variance of LD of the total population. According to Ohta (1982), epistatic selection is responsible for LD if $D_{ST}^2 < D_{IS}^2$ and $D'_{IS}{}^2 < D'_{ST}{}^2$. Here, $D'_{IS}{}^2$ was obtained as $D_{IT}^2 - D_{ST}^2$ (equation 16 in Ohta 1982), where D_{IT}^2 depends only on the haplotype frequencies of the sub-populations.

First, we performed Ohta's test for all four populations combined to identify SNP pairs (haplotypes) that systematically carry favorable combinations of alleles in any environments (i.e. any exposition and elevation). These SNP pairs thus show a global evidence of epistatic selection. Second, we applied Ohta's test only to the two northern or two southern populations, and to the two high or two low elevation populations. Haplotypes with selectively favored allele combinations that were unique to northern and southern populations, or to low or high elevations were interpreted as climate-specific signatures of epistatic selection. In both cases, we used the intron/exon status and the synonymous or non-synonymous state of the SNPs located in exons to screen Ohta's test results for loci with a potential functional role.

Among the 481 SNPs of the phased dataset, 115,440 pairwise comparisons were possible. However, we did not perform Ohta's test if (i) a population had only missing data, (ii) a locus was not polymorphic at least in one of the populations, (iii) more than half of the individuals had missing gametes in at least one of the populations. As a result, between 63% and 76% of the pairwise comparisons between SNPs were removed depending on the scenario tested (Table 2). Further, some complete genes were lost (i.e. all of their SNPs were excluded).

Since our sample sizes were relatively small and Ohta's test strongly relies on phase reconstruction (i.e. the haplotype frequencies), we used the 10^3 different best-guess haplotype samples to test the robustness of Ohta's test to the haplotype inference. In these analyses, although there were no missing data (all missing data were replaced by a best guess that varies from one dataset to another), we nevertheless only tested SNP pairs that were present in the original dataset. We accepted an epistatic interaction as “stable” if it passed Ohta's test in at least 95% of the different best-guess haplotype samples.

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RESULTS

Genetic differentiation

The average genetic differentiation measured by F_{ST} on the northern and southern slopes, respectively, was 0.017 and 0.013 at the SNP level and 0.015 and 0.012 at the gene level (Fig. S1, Supporting information). The hierarchical AMOVA confirmed the existence of a significant genetic differentiation among populations within the northern and southern slopes ($F_{SC} = 0.02$ at the SNP level and $F_{SC} = 0.015$ at the gene level), and between the slopes at the gene level ($F_{CT} = 0.006$), but not at the SNP level ($F_{CT} = -0.001$) (Table S3, Supporting information). Gene specific F_{ST} showed a mild variation between genes, with 14 and 4 genes showing a significant differentiation on the northern and southern slopes, respectively (Fig. S1).

F_{ST} outlier tests

The hierarchical model of Arlequin detected two outlier SNPs: one at position 450 in gene 88_1 ($F_{ST}=0.30$, q-value<0.0001) and the other at position 787 in gene 23_1 ($F_{ST}=0.27$, q-value<0.0001). When we tested for outlier SNPs using different demographic models accounting for the recent *F. sylvatica* population expansion, we found that the constant size island model was always the most conservative (Appendix S1). When accounting for more realistic demographic scenarios, additional outlier SNPs were revealed; notably, the strongest evidence was found for the SNP at position 328 in gene 142. No outliers were detected using Arlequin when testing the northern and southern slopes separately. Using BayeScan, in agreement with Arlequin, we found evidence for divergent selection for the (position 787) SNP in gene 23_1 and marginal evidence for the (position 328) SNP in gene 142, in the northern populations (Fig. 2A). We found that BayeScan was little sensitive to the MAF

criteria (Appendix S2): (i) the same two outlier loci were confirmed across the three MAF criteria tested and (ii) the greater the MAF was, the stronger the evidence was. None of the SNP-level outliers coded for non-synonymous mutations or were located in 3'UTR regions.

BayeScan at gene level displayed an extreme sensitivity to phase reconstruction (Fig. 2C-F): the Bayes Factor varied from nearly 0 to over 20 depending on the phase reconstruction. Using the median log₁₀ Bayes Factor as a summary over the 10³ different best-guess haplotype samples, gene 134_2_2 showed a weak signal of homogenizing selection in the northern populations (Fig. 2B and E) and gene 58 a weak signal of divergent selection in the southern populations (Fig. 2E and F). Further, the two genes that contained an outlier SNP also showed a weak signal of selection at the gene level: gene 23_1 revealed a weak signal of homogenizing selection (Fig. 2C) and gene 142 a weak signal of divergent selection (Fig. 2D).

Epistatic selection

The two conditions of Ohta's test were satisfied for less than 0.5 % percent of the SNP pairs (Table 2). The condition $D_{ST}^2 < D_{IS}^2$ was satisfied more than two times more frequently than $D'_{IS}^2 < D'_{ST}^2$, which may be because the variance components that depend only on the allele frequencies (D_{ST}^2) can be estimated with more confidence. Across the 10³ different estimates of the haplotype frequencies, Ohta's test performed consistently: overall, between 63 (south) and 90 (all populations) percent of the SNP pairs that initially passed the test in the phased dataset showed consistent evidence in over 95% of the best-guess haplotype samples (Table 2).

We found evidence for epistatic selection in 0.23 % of all SNP pairs across the four populations (Table 2, column « All »). Nearly 80% of them represented SNP pairs within the same gene, from 15 unique genes. Some of them involved SNPs coding for non-synonymous mutations or SNPs in 3'UTR regions (genes 20, 39, 68, 110, 52_1, Table S4, Supporting information). Two of the three between-gene interactions were found between genes of similar function, i.e. between the two catalase 3 coding genes (91_2 and 98_1) and between the two adenosyl-homocysteinase 2 coding genes (52_1 and 52_2) (Table S5, Supporting information). Only one of the between-gene epistatic selection signals arose between two SNPs situated in genes with different annotations: between an indel in gene 142 (Fig. S2, Supporting information) and a non-synonymous mutation in gene 39 (Table S4).

When considering pairs of populations in the northern and southern slopes, or at high or low elevations, the highest number of pairs with evidence of epistatic selection arose in the southern populations (0.41%) and the lowest number of pairs (0.24%) in the low elevation populations (Table 2, Fig. 3 and 4, Fig. S3, Supporting information). Most epistatic interactions also arose between SNPs within genes, with the lowest number of within-gene interactions detected in the southern populations (48%, Table 2). Most importantly, however, most epistatic interactions that were unique to one of the population pairs were observed between different genes (Table 2). Four unique between-gene epistatic interactions were present in the northern populations: one between genes 52_2 and 98_1, and a small network involving genes 142, 61_2, and 68 (Fig. 4). Notably, gene 68 was connected to each of the other genes via two non-synonymous coding SNPs (Table S4), while gene 61_2 had only synonymous mutations (Table S2). Both genes have important functional roles related to stress response: 61_2 is a member of the heat shock protein 70 family and 68 catalyzes glycolysis (Table S5). The epistatic interaction between genes 142 and 61_2 was also

confirmed in the high-elevation populations (Fig. 3 and 4). One unique within-gene epistatic interaction in gene 50 was observed in the southern and low-elevation populations, including a SNP from a 3'UTR region (Table S4). Gene 50 is a major transcription factor involved in response to abiotic stress and has been shown to respond to cold temperatures (Table S5).

Nineteen unique between-gene epistatic selection signals were observed in the southern populations, and four of them were also observed in the low-elevation populations (Fig. 3 and 4). Thirty SNP pairs in these interactions contained non-synonymous mutations or were located within regulatory regions of genes 39, 52_1, 68, 80, and 155_3 (Table S4). The presence of gene 80 in the gene networks of the southern and low-elevation populations is of particular significance because it regulates stomatal closure (a key trait involved in response to drought) and has been suggested to play a role in dormancy (Table S5). Further, two of the budburst candidate genes (genes 148_1 and 145_2, Table S5) were also present in the gene networks of the southern and low-elevation populations (only gene 148_1 in the latter).

DISCUSSION

Several recent studies have attempted to detect signatures of selection from candidate gene data in forest trees. This study is based on recently developed candidate genes (Lalagüe *et al.*, 2014) for an ecologically and economically important species, European beech, for which genomic resources are still scarce (see also Müller, 2013; Seifert, 2011). Signatures of selection were investigated at a short geographical scale, where pronounced environmental differences may impose a strong selection pressure. We combined classic F_{ST} outlier methods with a multi-locus approach and detected signatures of directional, homogenizing and

epistatic selection. Loci and genes found under selection often had well-documented functional roles.

F_{ST} outliers

Despite the small spatial scale investigated and the fact that only four populations were sampled, we nonetheless detected a few F_{ST} outliers, which suggests that the environmental differences that exist between sampling sites influence the adaptive strategies of *F. sylvatica* on Mont Ventoux and that selection can be efficient even when populations are under strong gene flow (Audigeos *et al.* 2013). Two of the outlier SNPs (in genes 23_1 ad 142) detected in the northern populations are a signature of selection imposed by elevational differences, while the third outlier SNP (in gene 88_1) most likely is a signature of selection imposed by differences between the northern and southern slopes, irrespectively of elevation. Two of the outlier SNPs were detected using a hierarchical model, which also made it possible to detect the imprint of selection along environmental gradients in other forest tree populations (e.g. Alberto *et al.*, 2013b; Mosca *et al.*, 2014).

Candidate gene data are composed of blocks of often tightly linked SNPs. As a result, the assumption of independence between loci imposed by genome scan methods is violated, which potentially leads to elevated rates of false positives (Vilas *et al.* 2012). While the proportion of linked SNP pairs are negligible when using many genes with few SNPs per gene (e.g. in Alberto *et al.* 2013b, 105 genes with 2-3 SNPs per gene), our dataset contained 9 SNPs per gene, on average. Following the ideas of Foll & Gaggiotti (2008) and Eveno *et al.* (2008), we applied BayeScan at the gene level (using haplotypes as alleles). Our study highlighted the extreme sensitivity of outlier tests to phase reconstruction: some extremely

high Bayes factors were observed in some realizations of the phased dataset (i.e. in some of the posterior samples from PHASE). To avoid over-interpretation, we proposed a model averaging approach, using the median F_{ST} and Bayes factors over several BayeScan runs, and thus averaging over the uncertainty inherent to the estimation of haplotype frequencies.

F_{ST} outlier tests based on haplotypes appear promising, not only because detecting selection using multiallelic markers may increase statistical power (Foll & Gaggiotti, 2008), but also because such tests can enhance our understanding of how selection operates. Notably, at the SNP level we only detected directional selection, whereas at the gene level, several genes showed unusually low F_{ST} values, thus balancing selection signatures (Fig. 2B). Although none of the genes had a Bayes Factor over 3, the gene 134_2_2 deserves particular attention as Bayes Factors were over 20 in some phase reconstructions (Fig. 2E). Gene 134_2_2 codes for a metallothionein 2a protein that has a ubiquitous role in stress response in plants (Table S5). Interestingly, gene 23_1, which had one SNP under directional selection, showed a weak signal for balancing selection at the gene level (Fig. 2C). These results suggest that there is more power to detect balancing selection at the gene than at the SNP level, consistently with the success of haplotype based methods for detecting selection (e.g. Fariello et al., 2013). However, the possibility that such balancing selection is, at least partly, an artifact, cannot be excluded. Even though the uncertainty of the phase reconstruction was taken into consideration, we always replaced missing data with the most common haplotype, thus generating haplotypes that are frequent in all populations, which may induce a (true or false) signal of balancing selection. Note that when applying Ohta's method, we do not replace missing data with a best guess haplotype.

Epistatic selection

Selection on epistatic deviations has long been considered negligible due to its transitory nature: the elevated frequency of co-occurrence of beneficial allele combinations (i.e. haplotypes) is expected to be continuously broken down by recombination (Griffing 1960). However, past studies ignored that this statistical effect of selection (visible through the build-up of LD) may be maintained because most genes do not act independently, but as members of complex gene interaction networks (so-called functional epistasis; Lehner 2011, Hansen 2013, Mackay, 2014). Consequently, on the one hand, it is important to realize that Ohta's test can principally capture the signature of recent selection. In fact, Le Corre & Kremer (2003) and Storz and Kelly (2008) also argued that recent adaptation can be detected with the between population component of LD (i.e. with Ohta's D_{ST}). However, on the other hand, if Ohta's test is applied to a set of loci that are either directly or indirectly connected (e.g. play a role in the same metabolic network), the chances of detecting “older” epistatic selection may be increased. This advantage does not apply to single-locus methods of detection of local adaptation using Ohta's D_{ST} (as e.g. in Ma et al. 2010).

Ohta's test (1982) has been relatively little used in the past (but see e.g. for forest trees by Cheng et al. 2001; Fernández-López & Monteagudo, 2010), and, more strikingly, most studies found no signal of epistatic selection (but see Black et al. 2008). We argue that we were able to detect epistatic selection because conditions were extremely favorable for the test. First, it has been estimated that *F. sylvatica* populations re-colonized Mont Ventoux about five generations ago (Lander et al. 2011), thus we were studying a population of recent origin, exposed to sharp environmental differences only in the past few generations. Second, our genetic data comprise functionally related candidate genes (Lalagüe et al. 2014, Tables S1 and S5), which could have favored the build-up and maintenance of LD due to epistatic

selection. The frequency of between- and within-gene epistatic interactions seems to further corroborate this idea. Although we found that most epistatic selection signals arose between SNPs within-genes, those unique to northern, southern, high-, or low-elevation populations arose principally between SNPs from different genes. Many known examples of within-gene epistasis involve mutations that act in a multiplicative manner (Lehner 2011), thus it seems plausible that they have been advantageous in all environments. In contrast, most systematically mapped epistatic interactions that involve mutations between different genes bring new functionality that may only be advantageous in a particular environment (Lehner 2011).

*Evidences of recent selection in *F. sylvatica* on Mont Ventoux*

By combining F_{ST} outlier methods with a multi-locus test of epistatic selection, we identified several *F. sylvatica* candidate genes that may have been under recent, climate-induced selection. A remarkable difference was observed between the northern (and high-elevation) and southern (and low-elevation) populations in all results that may be explained by recent population history. While the northern populations had a relatively high overall F_{ST} (Fig. S1), possessed most of the detected F_{ST} outliers and only a few unique epistatic interactions, the southern populations had a low overall F_{ST} (Fig. S1), no F_{ST} outliers, but many unique between gene epistatic interactions. These results suggest that conditions for divergent selection have been more favorable in northern populations. Further, one of the F_{ST} outliers, gene 142 (Fig. 2A and D, Appendix S1), also played a central role in the epistatic interaction network of northern populations (Fig. 4). Results of Ohta's test from the southern populations have to be considered with caution because the sample size to estimate haplotype frequencies is small in the southern populations (25 diploids). However, comfortably, the numerous epistatic interactions detected were confirmed over many different possible phase

reconstructions, and involved well-documented bud-burst candidate genes (Table S5). Further, the SL population experienced the most recent population expansion of all four populations (Lander et al. 2011), generating the most favorable conditions for Ohta's test. Additionally, the SL population may have been re-colonized both from the eastern and western remnant populations of Mont Ventoux, thus potentially generating favorable allele combinations. Finally, and overall, most loci detected to be under selection were involved in the “reactive oxygen species” (ROS) stress response (Table S5), which opens new perspectives for understanding the functional roles of these loci.

Sampling strategies for future studies

Our study illustrates that different selection mechanisms may act simultaneously in natural populations, such as directional, homogenizing, and epistatic selection. Detecting different types of selection, however, may require different sampling strategies. Most studies aimed at detecting local adaptations with F_{ST} outlier methods or allele frequency clines, generally prefer sampling many populations along an environmental gradient at the expense of having rather few samples per populations (10-15 individuals). In contrast, for Ohta's (1982) test, one has to estimate haplotype frequencies, which requires more individuals than estimating allele frequencies. We suggest that for efficiently combining F_{ST} outlier/clinal methods with Ohta's (1982) test, an appropriate compromise would be to sample many populations along an environmental gradient with few individuals, but sampling more individuals at the extremes for a test of epistatic selection or other haplotype based methods.

Conclusions

Two different ideas seem to dominate the current methodological developments for detecting selection in natural populations: first, integrating environmental and ecological data into population and landscape genomics tools (Schoville *et al.* 2012), and, second, shifting from single locus to more realistic multi-locus models of evolution, such as polygenic and epistatic selection (e.g. Pritchard *et al.* 2010; Le Corre & Kremer, 2012; Fu & Akey, 2013; Pannell & Fields, 2014). This study illustrates that combining F_{ST} outlier methods and Ohta's test can be fruitful for gaining a deeper understanding of the mechanisms driving selection. Testing for epistatic selection is particularly relevant to candidate gene data, because one can make use of the, often neglected, functional genomic information inherent to this type of data. Finally, we recommend testing for polygenic and epistatic selection using candidate gene data from haploid tissue (easily accessible from conifer megagametophytes), hence removing the high uncertainty relative to haplotype inference in diploid organisms.

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DATA ACCESSIBILITY

The raw, imputed and phased genotype datasets (546 SNPs) have been archived in DRYAD : [doi:10.5061/dryad.dg4hq](https://doi.org/10.5061/dryad.dg4hq).

FIGURE LEGENDS

Fig. 1 Map of the four sampling sites situated on the northern and southern slopes on Mont Ventoux, France.

Fig. 2 F_{ST} outlier SNPs and genes detected with Bayescan. **A** outlier detection at the level of SNPs from 52 candidate genes and two pairs of populations situated at the northern and southern slopes of Mont Ventoux. **B** outlier detection at the level of candidate genes using the

phased dataset, where each gene is treated as a multiallelic locus, with the inferred haplotypes as alleles. Median F_{ST} and median Bayes Factors were calculated from 10^3 independent BayeScan runs each on a different realization of best-guess haplotype sample. **C-F** F_{ST} and Bayes Factors in the 10^3 best-guess haplotype samples at the four genes that were outliers either at the SNP (C-D) or gene level (E-F).

Fig. 3. Heatmap of evidence for epistatic selection between candidate genes from **A:** the northern (upper triangle) and southern (lower triangle) slope, and **B:** high (upper triangle) and low (lower triangle) elevational sites of Mont Ventoux. Colors indicate the number of SNP pairs within genes that passed Ohta's test for epistatic selection (i.e. $D_{ST}^2 < D_{IS}^2$ and $D'_{IS}^2 < D'_{ST}^2$). The redder the color of the cell is the more SNP pairs are under epistatic selection between a pair of genes. The diagonal shows the within gene epistatic effects averaged over the northern and southern (A) or higher and lower (B) populations. White cells indicate missing data (i.e. Ohta's test was not performed). See Figure S1 for a heatmap between all individual SNP pairs.

Fig. 4 Networks of genes constructed based on evidence from Ohta's test for the northern, southern, higher, and lower population pairs. Only between-gene gene epistatic interactions are shown. Black edges indicate interactions that were present in all four populations, while red edges indicate those specific to the population pairs.

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article.

Appendix S1 Sensitivity analyses of F_{ST} outlier tests to population demography.

Appendix S2 Sensitivity analyses of F_{ST} outlier tests to the minor allelic frequency (MAF) criterion.

Table S1 Annotations and GeneBank accession numbers of the 53 studied candidate genes.

Table S2 Summary of the open reading frames (ORF) and the intron-exon boundaries analysis for each of the 53 *Fagus sylvatica* candidate genes.

Table S3 Global AMOVA tests of genetic differentiation at *Fagus sylvatica* candidate genes at the SNP and gene levels.

Table S4 SNP pairs showing evidence of epistatic selection in at least one of the population pairs or across all populations (All) and involving at least one SNP coding for a non-synonymous mutation or situated in a 3'UTR region.

Table S5 Functional interpretation of loci showing evidence of selection (directional, balancing or epistatic).

Fig. S1 Distribution of F_{ST} -values at 53 candidate genes and 446 SNPs.

Fig. S2 The sequences of all different haplotypes of gene 142 and their frequencies in the different populations.

Fig. S3 Heatmap of evidence for epistatic selection between SNPs of *F. sylvatica* candidate gene fragments. A: between populations on the northern (upper triangle) and southern (lower triangle) slopes. B: A: between populations at High (upper triangle) and Low (lower triangle) elevations.

TABLES

Table 1 Climate of the four sampling sites as *in situ* measures averaged from 2007 to 2013.

Climatic data include the mean (Tmean), maximum (Tmax) and minimum (Tmin) yearly temperature and the mean (RHmean%) and minimum (RHmin%) relative humidity.

	Elevation	Tmean	Tmax	Tmin	RHmean	RHmax	RHmin
Population	(m)	(°C)	(°C)	(°C)	(%)	(%)	(%)
North Low	995	9.8	13.4	6.8	74	86.4	58.6
North						87.5	
High	1340	7.3	10.3	4.6	75.5		59.8
South Low	895	10.8	16.1	6.6	69.6	85.1	52.3
South						86.5	
High	1517	6.5	10.5	3.3	73.1		56.9

Table 2 Summary of Ohta's test results. The upper part shows the number of realized pairwise comparisons, and the number of SNP pairs that passed one or the two conditions of the test. In the lower part, “Confirmed” stands for the number of SNP pairs where the test results were confirmed in at least 95% of the datasets sampled from the posterior distributions of the haplotypes generated by PHASE.

Pairs of populations	North	South	High	Low	All
Ohta's test (number of pairs)					
Number of realized pairwise comparisons	79,955	76,794	72,998	87,834	85,036
$D^2_{ST} < D^2_{IS}$	455	814	603	758	457
$D^{2'}_{IS} < D^{2'}_{ST}$	299	424	290	372	227
$D^2_{ST} < D^2_{IS}$ and $D^{2'}_{IS} < D^{2'}_{ST}$	287	410	278	333	222
Number of confirmed pairs	228	318	184	211	199
Confirmed SNP pairs within-genes (percent of pairs)					
Overall	68	48	72	77	79
Unique to the sub-populations	26	12	16	58	NA



