

Published in final edited form as:

. 2019 February ; 15(1): . doi:10.1007/s11295-018-1309-2.

## Heritability and genetic architecture of reproduction-related traits in a temperate oak species

Thomas Caignard<sup>1</sup>, Sylvain Delzon<sup>1</sup>, Catherine Bodénès<sup>1</sup>, Benjamin Dencausse<sup>1</sup>, and Antoine Kremer<sup>1</sup>

<sup>1</sup>UMR BIOGECO 1202-INRA, University of Bordeaux, 33615, Pessac, France

### Abstract

Reproduction, one of the main components of plant fitness, is highly variable in response to environmental cues, but little is known about the genetic determinism underlying reproduction-related traits in forest tree species. There is therefore an urgent need to characterize the genetic architecture of those traits if we are to predict the evolutionary trajectories of forest populations facing rapidly changing environment and mitigate their impacts. Using a full-sib family of pedunculate oak (*Quercus robur*), we investigated the within population variability of seed production and mean seed mass during four consecutive years. Reproductive traits were highly variable between trees and between years. The high narrow sense heritability and evolvability estimated underline the important genetic effect on the variability in seed production and mean seed mass. Despite a large variability over years, reproductive traits show significant genetic correlation between years. Furthermore, for the first time in forest tree species, quantitative trait loci (QTLs) associated with seed production and mean mass of a seed have been identified. While it is commonly assumed and observed that fitness-traits have low narrow sense heritabilities, our findings show that reproduction-related traits may undergo evolutionary changes under selective pressure and may be determinant for tree adaptation.

### Keywords

Tree reproduction; Seed production; Fitness; Heritability; QTLs; *Quercus robur*

## 1 Introduction

Tree fitness defines the ability of an individual to survive, grow and reproduce in an environment. Numerous studies have investigated to which extent the phenotypic variation of growth and survival in response to environmental changes was genetically or environmentally driven in forest tree species but there is a lack of studies investigating reproduction (Kang et al., 2003; Santos del Blanco et al., 2010; Santos-del-Blanco et al.,

---

**Corresponding author:** Thomas Caignard | [tcaignard@gmail.com](mailto:tcaignard@gmail.com) | +33 5 40 00 30 06.

#### Authors' contributions

T.C., A.K. conceived the idea for this work; T.C. and B.D. assembled the dataset; T.C and C.B. analyzed the data; T.C., A.K. wrote the manuscript and C.B. and S.D. revised the manuscript.

#### Data Archiving Statement

<http://mapedegree.pierroton.inra.fr/qmap/>

2012). Yet, understanding the variability in tree reproduction is essential in order to assess the process of adaptation of forest tree species in response to environmental changes (Aitken et al., 2008; Anderson, 2016). Increased reproduction enhances fitness of trees through different processes. For example, higher seed production increases the probability of dispersion by animals (Howe and Smallwood, 1982; Schupp et al., 2010; Traveset et al., 2014) and thus enhances population growth and dynamics. Long-distance dispersion generally due to scatter-hoarding, i.e. the way animals buried their seeds for latter consumption, favors regeneration because (1) seed dispersed escape density-dependent competition under the mother tree and (2) because during years of abundant seed production, buried seeds will not all be consumed by predators (Vander Wall, 2010). Furthermore, the variability in seed size within a same species also plays a role as it is correlated with the germination rate (Gómez, 2004; Walters and Reich, 2000). Thus, because of the larger amount of reserves, bigger seed are more likely to survive to infestation from insects and seedlings' early development is favored (Bonal et al., 2007; Sousa et al., 2003).

Tree reproduction is highly variable in response to environmental changes. Previous studies have shown large variations in seed production, seed size or seed germination along environmental gradients and across years (in oaks Caignard et al., 2017; Koenig et al., 2009; in pine Lopez-Toledo et al., 2017; in black spruce Sirois, 2000). Part of this variation is driven by environmental changes such as temperature and precipitation. For example, seed production in European oak species increases with a rise in temperature during the period of pollination (Caignard et al., 2017; Schermer et al. submitted) and decreases with increasing drought occurring during late summer (Bogdziewicz et al., 2017; Pérez-Ramos et al., 2010; Sanchez-Humanes and Espelta, 2011). While numerous studies have investigated the phenotypic variability of reproduction traits in response to environmental changes little is known about the genetic source of this variation.

Common garden experiments have been useful in documenting genetically based differentiation among and within populations but few have focused on the genetic variations of reproductive traits, most likely because their assessments can only be made on reproductively mature trees while other fitness related traits can be measured on juvenile traits. In comparison, the genetics of reproductive traits have been already explored for annual plants (Alonso-Blanco et al., 1999; Mitchell-Olds, 1996), plant crops (Doligez et al., 2002; Houel et al., 2015; Kadri et al., 2017) and fruit trees (Guitton et al., 2011; Sadok et al., 2013; Wu et al., 2014). For example, quantitative traits loci (QTLs) associated with seed size, seed number or size at first reproduction were detected for *Arabidopsis thaliana* (Alonso-Blanco et al., 1999) and *Vitis vinifera* (Doligez et al., 2002). In fruit trees, genetic investigations were oriented towards flowering and fruiting phenology (Dirlewanger et al., 2012; Romeu et al., 2014) and biennial fruit bearing, i.e. the yearly alternation of flowering and fruiting (Guitton et al., 2011; Sadok et al., 2013; Shalom et al., 2012). In forest trees, the phenological variation of the apical buds has been extensively investigated in recent years (Derory et al., 2010; Jermstad et al., 2001; Pelgas et al., 2011; Scotti-Saintagne et al., 2004) but very few studies have targeted the genetic variation of reproduction within population (Bilir et al., 2006; Sivacioglu et al., 2009; Tsubomura et al., 2012) and even fewer have attempted to detect QTLs underlying reproductive traits (see Ujino-Ihara et al., 2012 for male strobilus abundance). Finally, Pearse et al. (2016) strongly advocated for assessing

heritability of masting related traits, while recalling that relevant data may already exist (El Kassaby and Barclay, 1992). As the selective response to environmental changes, and so the capacity of adaptation of tree population largely depend on the genetic variations and the genetic architecture underlying fitness related traits, it is necessary to investigate more reproduction.

In this study we assessed the phenotypic and genetic variation of reproductive traits and searched for their underlying quantitative trait loci (QTL) in a full-sib family of a broadly distributed temperate oak species (*Quercus robur* L.). Our main objectives were to (i) subdivide the total phenotypic variation of reproductive traits into environmental and genetic components (ii) to dissect the genetic architecture of reproductive traits by detecting their underlying QTLs (iii) to examine the stability of QTLs expression over successive years.

## 2 Material and Methods

### 2.1 Experimental design

A full-sib family originating from a controlled cross of pedunculate oak (*Quercus robur*) was used. The male parent (A4) was selected in a park close to Arcachon in the South West of France (44°40'N, 1°11'W) and the female parent (3P) originated from the INRA forest research station of Pierroton (44°44'N, 0°46'W). The controlled cross was carried out in 1992 and provided 278 seedlings, which subsequently were vegetatively propagated. Rooted cuttings from 207 genotypes (with approximately ten clonal replicates per genotype) were planted in the field in 2000 at the INRA's experimental unit of Bourran (44°20'N, 0°24'W), located in the south west of France (Saintagne et al., 2004). Spacing of trees was 4m x 1.5m at the plantation. In 2012 a systematic thinning was carried out in the parcel, reducing the overall density by one half on average with a total amount of 1130 individuals. On average, the annual precipitation was 650.1 mm and the average temperature was 13.9°C from 2014 to 2017, i.e. during the four years of monitoring (Table 1).

Genetic linkage maps were established for both parents (3P and A4) using gene-based SNP (Bodénès et al., 2016). Using the JoinMap procedure, a subset of SNP markers evenly distributed along the 12 linkage groups (LG) was selected to reconstruct two new parental linkage maps for QTL analysis. In total, the male and female linkage maps used for QTL detection contain 341 and 345 markers, respectively.

**Monitoring reproduction**—During spring 2014, when the trees were 16 years old since they were vegetatively propagated from the ortet (and 23 since seed production), we set up nets to collect acorns at 1 m above the ground under the whole canopy of each tree (Figure S1). The nets were stretched at the individual scale every two rows within the design and the trees which were very close to one another within a given row (i.e with no trees cut between them after thinning) were not sampled for recording reproduction, as their canopies were intermingled (Figure S1). In total seed crop was assessed on 173, 331, 339 and 337 individuals (clonal replicates) in 2014, 2015, 2016 and 2017 corresponding from 117 to 170 different genotypes over the four years (Table 1). In what follows the full sib genotypes will be called clones. Trees were planted within a randomized incomplete block design comprising 8 blocks with  $47 \pm 6$  individuals per block. The number of clonal replicates per

clone per block was one and the overall mean number of replicates used for this study varied between 1.48 and 1.99 (Table 1). To minimize predation and facilitate the acorns sorting, trees were harvested twice during mid-October and mid-November by collecting all the material (litter and acorns) deposited in the nets. Acorns were sorted from the rest of the litter in the laboratory and dried at room temperature.

Then, the total amount of seed produced  $N_{tot}$ , the total mass of seeds produced  $M_{tot}$  in g and the mean mass of one seed  $M_a$  in g were assessed. Along the last three years of measurement (2015, 2016 and 2017) we also estimated the coefficient of variation of each individual  $CV_i$  and the mean coefficient of synchrony  $r_i$  (Buonaccorsi et al., 2003). To estimate  $r_i$ , we assessed a correlation matrix between individuals using Pearson coefficient, and estimate the average coefficient for each of them. As the number of trees assessed in 2014 was much lower due to technical constraints related to the stretching of the nets, data of 2014 were not used in this analysis. In addition, diameter at breast height ( $D$  in mm) and height ( $H$  in m) of each tree were measured in 2014 and the ratio between reproduction and growth ( $N_{tot}/D$ ) were calculated for each year in order to normalize the total seed production relative to the size of the tree.

## 2.2 Statistical analysis

We used a univariate linear mixed effect model to assess the genetic parameter of reproductive and growth traits for the four years of measurement:

$$Y_{ijk} = \mu + b_i + C_j + \varepsilon_{ijk} \quad [1]$$

where  $Y_{ijk}$  denotes the observed phenotypic value of clone replicates  $k$  of clone  $j$  in block  $i$ ,  $\mu$  the overall mean,  $b_i$  the fixed effect associated with block  $i$ ,  $C_j$  the random effect associated with clone  $j$  (genetic effect) and  $\varepsilon_{ijk}$  the residuals. In addition to assess the multi-annual variability we used an additional univariate linear mixed model for each trait:

$$Y_{ijkl} = \mu + b_{ij} + t_l + C_j + \varepsilon_{ijkl} \quad [2]$$

where  $Y_{ijkl}$  is the observed phenotypic value of clone  $j$  in block  $i$  and year  $l$  and  $t_l$  the fixed effect associated to year  $l$ . For both models, the reproductive traits  $M_{tot}$ ,  $N_{tot}$  and the ratio  $N_{tot}/D$  were log transformed. Best linear unbiased predictions (BLUPs) of random effects were estimated for each trait and within each year from the two linear mixed effect models. Year-Year correlations were estimated for phenotypic and BLUP values of  $\log(N_{tot} + 1)$ ,  $\log(M_{tot} + 1)$ ,  $\log(N_{tot}/D + 1)$  and  $M_a$  using Pearson coefficient, thus leading to values close to phenotypic and genotypic correlations. In addition, the differences between years for the same traits were tested using an analysis of variance (ANOVA). Finally, genetic correlations were also estimated between reproductive and growth traits, using the BLUPs estimated from the multi-annual model for reproductive traits and the BLUPs estimated in 2014 for growth traits.

Variances of random clone effects were used to estimate the genetic parameters of each trait. Following Scotti-Saintagne et al. (2004) we assume that the environmental effect was absorbed by the variance among replicates (cuttings) of a same genotype (clone). Considering that the cuttings were full sibs, the clonal variance estimated ( $\sigma_c^2$ ) was equal to the within full-sib family ( $\sigma_w^2$ ), where:

$$\sigma_c^2 = \frac{1}{2}V_A + \frac{3}{4}V_D \quad [3]$$

Here,  $V_A$  is the additive genetic variance and  $V_D$  is the dominance variance. To estimate the narrow sense heritability ( $h^2$ ) of each trait, which represents the proportion of phenotypic variance that can be attributed to  $V_A$ , we considered two cases encompassing the likely range of the dominance variance ( $V_D=0$  and  $V_D = \frac{1}{2}V_A$ ), as  $V_D$  is generally lower than  $V_A$  in forest trees (Cornelius 1994). Thus, we estimated  $h^2$  using:

$$(i)h_0^2 = \frac{2\sigma_C^2}{2\sigma_C^2 + \sigma_\epsilon^2} \text{ and } (ii)h_{1/2}^2 = \frac{\frac{8}{7}\sigma_C^2}{\frac{12}{7}\sigma_C^2 + \sigma_\epsilon^2} \quad [4]$$

Confidence intervals of narrow sense heritability values were estimated using the method of Visscher and Godard (2014) applied to a single full sib family, assuming that the true values of heritability and phenotypic variance corresponded to the estimated values.

In addition, we also estimated the repeatability, expressing the proportion of the phenotypic variance of clonal means due to the clone effect, using:

$$R = \frac{\sigma_C^2}{\sigma_C^2 + \frac{\sigma_\epsilon^2}{n_0}} \quad [4]$$

where ( $\sigma_\epsilon^2$ ) is the environmental here residual variance and  $n_0$  is the number of cuttings per clone.

Finally, we also estimated the evolvability (Hansen et al., 2011) for the two cases previously described for the estimation of  $h^2$ , using:

$$(i)e_0 = \frac{2\sigma_C^2}{m^2} \text{ and } (ii)e_{1/2} = \frac{\frac{8}{7}\sigma_C^2}{m^2} \quad [6]$$

were  $m$  is the mean of the trait. For  $N_{\text{tot}}$ ,  $M_{\text{tot}}$  and  $N_{\text{tot}}/D$ , we estimated the evolvabilities of the non-transformed data since as reported by Hansen et al. (2011), the additive variance of the log-transformed data is approximately equal to the evolvability of the non-transformed scale. Evolvability is a mean standardized measure of the additive variation, in comparison to heritability which is a variance standardized measure of additive variation. It was shown earlier (Hansen et al., 2011) that the latter might respond more to the environmental variance (which is on the denominator of heritability) than to the additive variance (which is on the numerator). To reduce the noise created by the environmental variance on heritability, Hansen et al. (2011) recommended to estimate evolvability as well.

The analyses were performed in R Studio version 1.0.153 (R core team 2014). All the linear mixed effect models were fitted by the restricted maximum likelihood (REML) method in the lme4 R package (Bates et al., 2014; Bolker et al., 2009).

### 2.3 QTL detection

Quantitative trait locus (QTL) mapping was performed using Haley-Knott regression (Haley and Knott, 1992) in R/qtl package (Broman et al., 2003) with 1cM step using the BLUP values. QTLs were selected using a stepwise model selection approach (Manichaikul et al., 2009) based on a significant threshold at 5% error rate made from 1000 permutations. The 95 % confidence interval was calculated for each selected QTL using Bayesian methods (Manichaikul et al., 2006). The effect of each QTL and the percentage of phenotypic variance explained (PEV) were also estimated. In addition, we also used a two QTL model when the results of one QTL model suggested the likely presence of a second QTL on the same linkage group, LG<sub>3</sub> (Figure S3). The *scantwo* function on R/QTL was used in this case (Figure S4).

## 3 Results

### 3.1 Phenotypic and genetic variation of reproductive traits

Large phenotypic variation was observed for seed production and size over the four years (Figure 1). Mean annual number of acorns per tree ( $N_{\text{tot}}$ ) varied between 27.8 and 226.6 seeds while  $N_{\text{tot}}/D$  varied between 0.54 and 2.34 seeds per mm and  $M_{\text{tot}}$  between 16.1 and 385.9 gramme. Similar results can be observed when comparing seed production of a given tree over years (Inter-annual coefficient of variation for  $N_{\text{tot}}$  amounts to  $1.05 \pm 0.29$ , for the four years and  $1.07 \pm 0.35$  for the last three years). On average the production of seeds was higher in 2014 and 2017 (Figure 1a, b and d) and the mean mass of one seed was higher in 2014 (Figure 1c) with an average mass of 2.5 g. Furthermore, within a given year we observed a large inter-individual variability for all reproductive traits (Figure 1). The large differences between marginal and conditional coefficient of determination ( $R^2_{\text{m}}$  and  $R^2_{\text{c}}$ ) observed for most of the annual and multi-annual traits (trait over the four years of monitoring, see model [2]), show that the clone random effect of the linear mixed effect model explains most of the variability predicted by model 1 for one-year traits and by model 2 for multi-annual traits (Table 2). Clonal repeatability varied between 0.22 for  $M_{\text{a}}$  to 0.70 for  $N_{\text{tot}}$  and  $N_{\text{tot}}/D$ , suggesting that the between clonal variance was almost as large as the within clonal variance. One can notice that the difference between  $R^2_{\text{m}}$  and  $R^2_{\text{c}}$ , estimated

from the multi-annual model of the mean mass of one acorn ( $M_a$ ), is lower than for the other traits ( $R^2_m = 0.05$  and  $R^2_c = 0.1$ ). This discrepancy is most likely due to the large differences of mean acorn mass between years already pointed out. The narrow sense heritabilities ( $h^2_0$  and  $h^2_{1/2}$ ) estimated for each year were moderate and similar over time for every reproductive trait monitored (Table 2). For each year, the highest heritabilities were observed for  $N_{tot}$  and  $N_{tot}/D$  and the lowest heritabilities were estimated for  $M_a$ , in most cases the heritabilities estimated for growth traits (D and H) were lower than for reproductive traits. In comparison, very high evolvabilities were observed for  $N_{tot}$  and  $M_{tot}$ , ranging from 0.37 to 1.24 but there were more moderate for  $N_{tot}/D$ . The evolvabilities estimated were much lower for  $M_a$  (ranging from 0.0008 and 0.04), H, D nevertheless there are still relatively high compared to the other estimated values in the literature except for H (Hansen et al., 2011). Heritability values of the masting related traits (coefficient of variation  $CV_i$  and coefficient of synchrony  $r_i$ ) were of the same magnitude than values of the reproduction related traits (Table 2).

### 3.2 Phenotypic and genetic correlations between traits over time

Phenotypic and genotypic correlations between traits over years were positive. For all six pairwise year-year combinations the phenotypic and genotypic correlations were significant for each trait. The coefficients of Pearson (Figure 2 and 3) were, in most cases, higher for the estimated BLUP values than for the phenotypic values with one exception for  $M_a$  (2015-2017), for which the phenotypic correlation ( $r = 0.33$ ) was higher than the estimated genotypic value ( $r = 0.31$ ). In average, the year-year genotypic and phenotypic correlations were much lower for  $M_a$  than for the three other traits. In addition, the highest  $r$  values for the genotypic and phenotypic correlations were observed between 2014 and 2017 for  $\log(N_{tot}+1)$ ,  $\log(M_{tot}+1)$  and  $\log(N_{tot}/D + 1)$ , while for  $M_a$ , the highest phenotypic correlation was observed between 2014 and 2016 and the highest genotypic correlation between 2016 and 2017. The genotypic correlations of reproductive traits with growth traits (H and D) were positive for  $M_{tot}$ ,  $N_{tot}$  and nearer 0 for  $M_a$  and  $N_{tot}/D$  (Figure S2).

### 3.3 QTL detection (Table 2)

Annual and multi-annual models were used to detect the QTLs of reproductive traits. We detected significant QTLs for all traits related to reproduction and growth but not for  $CV_i$  neither for  $r_i$ . For  $M_a$  we detected significant QTLs on both maps (Male and Female), 1 QTL on the female linkage group 1 (LG<sub>1</sub>F) for 2016, 2017 and for the multi-annual model, and 2 QTLs on LG<sub>7</sub>M (male linkage group 7) for 2016 on LG<sub>11</sub>M for the multi-annual model (Table 3, Figure S5). The QTLs detected on LG<sub>1</sub>F were, for the three cases, located at 26 cM and the percentage of phenotypic explained variance (PEV) was of the same amount over the years: 9.80 % in 2016, 8.08 % in 2017 and 12.81 % over the four years, but the confidence intervals in 2016 and 2017 were much larger. For traits related to seed production ( $M_{tot}$ ,  $N_{tot}$  and  $N_{tot}/D$ ) we detected significant QTLs at the same location on LG<sub>3</sub>F in 2015, 2016, and 2017 and across the three years with the multi-annual model. The positions were very similar through time and between traits. The highest PEVs on this linkage group were observed for  $N_{tot}$ , with 10.94% in 2015, 15.01% in 2016, 17.51 % in 2017 and 16.47 % for across the four years. In addition, on LG<sub>11</sub>M, we also detected highly significant QTLs for the same traits than previously in 2014, 2016, and 2017 and across all years. The highest

PEVs observed were for  $N_{\text{tot}}/D$  with respectively 15.38%, 17.54%, 12.32% and 14.11% in 2014, 2016, and 2017 and across all years. Another QTL was detected on the male map for traits related to seed production: for  $N_{\text{tot}}$  in 2014 and  $N_{\text{tot}}/D$  in 2014 on LG<sub>5</sub>M. For both cases the position estimated on the linkage group was 65.03 cM and the PEV amounted to 10.11 % and 13.84 % respectively. Finally we also detected a significant QTL for  $N_{\text{tot}}/D$  in 2015 on LG<sub>12</sub>F but the PEV estimated was relatively low (7.69 %) compared to the other QTLs.

## 4 Discussion

Reproduction in forest tree species is highly variable between and within populations (Haymes and Fox, 2012; Kang et al., 2003; Pérez-Ramos et al., 2014). Although few studies have shown and quantified the genetic contribution to the between population variation of reproductive traits (Santos-del-Blanco et al., 2012), our study is one of the first to investigate the genetic variation within a single population. Using a full-sib family of *Quercus robur*, this study highlights a large variability of reproductive traits at the within population level, and underlines the important contribution of genetic effects. Furthermore, we detected for the first time in forest tree species quantitative trait loci (QTLs) associated with seed production and the mean mass of a seed. Despite a large phenotypic variability over years, the genetic contribution to reproduction was highly correlated between years.

### 4.1 Large genetic variation of reproductive traits

The repeated assessment of reproduction related traits over four years resulted in estimates of large phenotypic variability for  $N_{\text{tot}}$ ,  $M_{\text{tot}}$  and  $N_{\text{tot}}/D$  and to a lesser extent seed size ( $M_a$ ), as well as for masting related traits ( $CV_i$  and  $r_i$ ). In addition, the estimated heritability and evolvability values suggested significant and substantial genetic contributions to the phenotypic variability. It is tempting to compare our values of heritability and evolvability to other reported values in trees; however, the scarce published tree studies reported broad sense heritabilities ( $H^2$ ), which are larger than narrow sense heritabilities. Nevertheless, our results suggest slightly larger genetic (additive) variance of reproductive traits in oaks than in other species. For example Sivacioglu et al. (2009) reported  $H^2$  values ranging from 0.18 to 0.38 for cone production in *Pinus sylvestris* while  $H^2$  was found lower, around 0.15 in *Pinus pinea* (Mutke et al., 2005). These values are similar to the  $H^2$  estimated for female and male inflorescences in pines and spruces (Bilir et al., 2006; Nikkanen and Ruotsalainen, 2000; Sivacioglu et al., 2009). For example  $H^2$  values estimated for female and male flowering were around 0.38, in *Picea abies* (Nikkanen and Ruotsalainen, 2000) and 0.12 in *Pinus sylvestris* (Bilir et al., 2006). Compared to other traits commonly assessed in forest trees and especially in oak, narrow sense heritability values ( $h^2$ ) assessed here on reproduction traits are slightly lower than for phenological traits (Baliuckas and Pliura, 2004; Alberto et al., 2011; Firmat et al., 2017), about the same magnitude than wood density and wood anatomical related traits (Nepveu, 1982, 1984; Mather et al., 1993; Savill et al., 1993), and higher than growth traits (see our results, Jensen et al., 1997; Bogdan, et al. 2004,2017; Barzdajn, 2008). Therefore, our results suggest that reproduction traits in trees may undergo evolutionary changes if they are targets of selection under ongoing environmental changes.



Over the four years of monitoring, seed production was highly variable and synchronized between trees. This phenomenon, also known as masting or mast-seeding, is characteristic of several forest tree species including oak species (Koenig et al., 1996, 1994). The coefficient of variation (CV<sub>i</sub>) and the coefficient of synchrony ( $r_i$ ) estimated for each individual are common measures of the ability to express multi-annual variability of seed production and synchrony among trees, respectively (Buonaccorsi et al., 2003). The moderate heritability estimated for both CV<sub>i</sub> ( $h^2_0 = 0.49$  and  $h^2_{1/2} = 0.30$ ) and  $r_i$  ( $h^2_0 = 0.40$  and  $h^2_{1/2} = 0.24$ ) suggests that the variation of both measures, estimated from 2015 to 2017, were partly due to genetic effects. While the period of monitoring was relatively short in our study, these results suggest that masting-related traits might be heritable. To our knowledge, no study has attempted so far to study the genetic determinism of masting while it is commonly assumed that it is an adaptive response to the selection pressure by predators (Kelly and Sork, 2002). Our results should however be considered as very preliminary, as long-term longitudinal monitoring would be necessary to estimate the genetic contribution of masting related traits.

Despite the large multi-annual variability and the significant sensitivity of reproduction to environmental changes, the genetic contribution was highly correlated over time. Thus, our results showed that most prolific trees were the same during the four years of monitoring, regardless of the overall level of seed crop within each year. For every trait assessed, heritabilities estimated using the multi-annual model was lower than the heritabilities estimated within single year. These differences may be explained by the increase of the residual variation over years due to the changes of biotic and abiotic conditions over time. Evidence for the inter-annual variation of the residual variance is also suggested by the changes of single year heritabilities over years. Similar trends were also observed in other species; for example broad sense heritability ( $H^2$ ) of female and male inflorescence production in *Picea abies* (Nikkanen and Ruotsalainen, 2000) and cone production in *Pinus sylvestris* (Kroon et al., 2009) changed substantially over years. Despite variation of the overall mean and variance of reproductive traits over years, phenotypic and genetic correlations between years were quite high suggesting that monitoring of reproduction for genetic evaluation of clones can be limited to a very few numbers of years, if masting is not foreseen as an objective of the study.

While it is commonly assumed and observed that fitness-traits have low narrow sense heritabilities (Hoffmann et al., 2016; Price and Schluter, 1991), we obtained relatively high values for reproductive traits which are known to be main components of tree fitness. Merilä and Sheldon, (1999) have shown that contrary to what was admitted before, the low heritability of fitness traits was mostly explained by high residual variances including the environmental and dominance variance. Admittedly we assumed in our estimation of narrow sense heritability that dominance (on a broader scale non-additive) variance was lower than additive variance in trees based on earlier reported values (White et al., 2007). Our assumption of low  $V_D$  may account for the discrepancy between observed values and predicted values based on evolutionary theory of fitness related traits. However, the underestimation of the residual variance would have a lesser impact on evolvability which is a standardization of the additive variance on the mean of the trait. And evolvability values still suggest that there is large additive variation existing in this oak clonal trial. Indeed, with a few exceptions our reported values of evolvability are in the upper half of all reported

values of various species in the meta-analysis of Hansen et al. (2011). An alternative interpretation of the moderate values of genetic variation we found for reproductive traits is that four years assessments of reproduction at a still young stage of oak trees might not provide a relevant proxy of fitness. Hence the traits we measured might not have undergone so far sufficient selection pressures to the point to erode the genetic variation of the trait. Finally, the evolutionary implications of our results are still questionable, as estimates of heritability in our study were done in an experimental design where micro-environmental variation is minimized. These estimates should be compared in the future with *in situ* estimates, which can now be obtained by retrieving realized genetic relatedness among trees using large numbers of genetic markers (Lesur et al., 2018; Vinkhuysen et al., 2013).

## 4.2 Genetic architecture

Our QTL detection was implemented in a trial prone to sampling biases known as the Beavis effect (Beavis 1998). Typically, when the sample size (number of clones in our experiment) is less than 100, then the statistical power to detect QTLs of small effects are low, and their effects are inflated. Because of our limited sample size, our results should be interpreted with caution. We have likely only detected QTLs with major effects, and their effects may also have been overestimated. Thus, the refined genetic architecture (number of QTLs and distribution of their effects) needs still further investigations based on the distribution of allelic effects as was done by Hall et al. 2016

Despite statistical limitations for exploring the genetic architecture, our results have important biological implications regarding potential genomic regions containing genes that contribute to the variation of reproduction traits. Indeed, for seed production, significant QTLs on LG<sub>3</sub>F and LG<sub>11</sub>M were repeatedly detected over years, explaining from 8.21 to 18.40 and 10.70 to 17.54 percent of the phenotypic variance, respectively. The inferred position on the linkage groups were almost identical between years. Although we found a significant QTL on LG<sub>11</sub>M for M<sub>a</sub> with the multi-annual model, the other QTLs identified were not co-localized with the QTLs identified for the seed production related traits, even with the QTLs detected for M<sub>tot</sub>. These genomic regions will be targets for association studies in natural populations to reduce their range within the genome and narrow down the search of candidate genes within the genome of *Quercus robur* that was recently sequenced (Plomion et al., 2016, 2018). As very few studies have attempted to dissect the genetic architecture of tree reproduction, it is difficult to compare our results with earlier reported studies. While Ujino-Ihara et al. (2012) undertook detection of QTLs associated with the production of male strobili in *Cryptomeria japonica*, to our knowledge, seed production and seed size have never been investigated in forest tree species. In fruit trees, numerous studies investigated the genetic determinism of biennial fruit bearing i.e. the irregular fruit production of a tree over consecutive years. Guitton et al. (2011) found that the QTLs associated with biennial bearing co-localized with genomic regions containing genes involved in floral development (floral integration gene, meristem identity gene and gibberellin oxidase gene). These results illustrate how our results could further lead to the identification of candidate genes by refining genomic regions containing the 3 major QTLs that we detected.

Finally, it is worthwhile checking whether the QTLs of reproduction related traits may colocalize with QTLs of other important traits particularly growth and phenology that were investigated earlier on the same QTL mapping pedigree (Scotti-Saintagne, 2004; Derory et al. 2010). While, the likely positive correlation between tree size and non-normalized seed production ( $N_{tot}$ ) may lead to detect similar QTLs for  $N_{tot}$  and growth traits, the cross comparison of our results with QTLs of growth resulted in only one notable co-localization. LG<sub>5</sub>M for the total tree height in 2001 and for  $N_{tot}$  and  $N_{tot}/D$  in 2014. Due to the low sample size in 2014 and the absence of repetition for the QTL on LG<sub>5</sub>M, the co-localization of QTLs of seed production and growth on this region is weak. Apart from this region, no co-localization was observed between growth and reproduction. Potential co-localization may witness negative pleiotropic effects that may support the negative mechanistic trade-off between growth and reproduction that has often been reported in the literature (Camarero et al., 2010; Drobyshev et al., 2010; Han et al., 2008; Ishihara and Kikuzawa, 2009; Koenig and Knops, 1998; Monks and Kelly, 2006; Silvertown and Dodd, 1999). The lack of co-localization of QTLs in our study may thus be in line with the more recent hypothesis by Knops et al. (2007) that the negative correlation observed might not be causal, or that the trade-off may only be driven by environmental effects. Furthermore, the positive or non-genetic correlation observed between growth and reproductive traits (Figure S2), confirmed the likely absence of a trade-off between both. Furthermore, the positive or non-genetic correlation observed between growth and reproductive traits (Figure S2), confirmed the likely absence of a trade-off between both. The cross comparison of our results with QTLs of phenology (bud burst) resulted in only one notable co-localization on one linkage group (LG<sub>11</sub>M), which would support genetic correlation with leaf phenology, seed production and seed size if the gene effects at the co-localized QTLs are strong.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

## Acknowledgments

This research was supported by the European Research Council through the Advanced Grant Project TREEPEACE (#FP7-339728). We thank the experimental units of Bourran (UE 0393 INRA, Domaine de la Tour de Rance 47320 Bourran, France) and Toulence (UE 0393 INRA, Domaine des Jarres 33210 Toulence, France) for technical support. We thank Jérôme Bartholomé for his assistance in the QTL analysis. TC received a PhD grant from TREEPEACE and the Initiative of Excellence program (IdEX-03-02) of Bordeaux University. BIOGECO is supported by a grant overseen by the French National Research Agency (ANR) as part of the “Investissements d’Avenir” through the Cluster of Excellence COTE (ANR-10-LABEX45).

## References

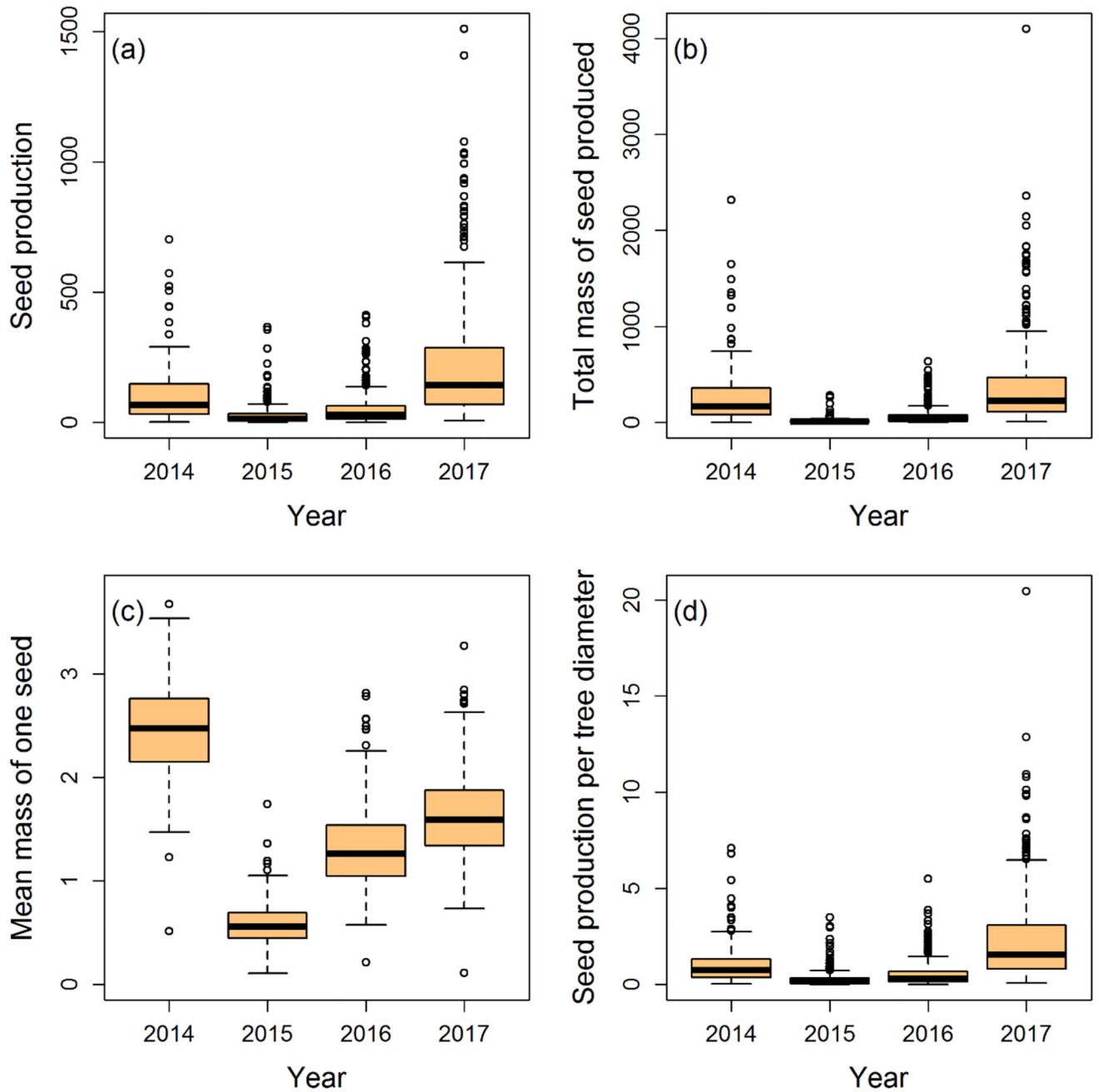
- Aitken SN, Yeaman S, Holliday JA, Wang T, Curtis-McLane S. Adaptation, migration or extirpation: climate change outcomes for tree populations: Climate change outcomes for tree populations. *Evol Appl.* 2008; 1:95–111. [PubMed: 25567494]
- Alberto F, Bouffier L, Louvet JM, Delzon S, Kremer A. Adaptive responses for seed and leaf phenology in natural populations of sessile oak along altitudinal gradients. *Journal of evolutionary Biology.* 2011; 24(7):1442–1454. [PubMed: 21507119]
- Alonso-Blanco C, Vries HB, Hanhart CJ, Koornneef M. Natural allelic variation at seed size loci in relation to other life history traits of *Arabidopsis thaliana*. *Proc Natl Acad Sci.* 1999; 96:4710–4717. [PubMed: 10200327]

- Anderson JT. Plant fitness in a rapidly changing world. *New Phytol.* 2016; 210:81–87. [PubMed: 26445400]
- Barzdajn W. Comparison of provenance, family and individual heritability of growth traits in pedunculate oak (*Quercus robur* L.) in the family-provenance trial in the Milicz Forest District. *Sylwan.* 2008; 144(12):57–67. (in Polish).
- Bates D, Maechler M, Bolker B, Walker S, et al. lme4: Linear mixed-effects models using Eigen and S4. R Package Version. 2014; 1(7):1–23.
- Beavis WD. QTL analyses: power, precision, and accuracy. *Molecular dissection of complex traits.* 1998:145–162.
- Bilir N, Prescher F, Ayan S, Lindgren D. Growth characters and number of strobili in clonal seed orchards of *Pinus sylvestris*. *Euphytica.* 2006; 152:1–9.
- Bodénès C, Chancerel E, Ehrenmann F, Kremer A, Plomion C. High-density linkage mapping and distribution of segregation distortion regions in the oak genome. *DNA Res.* 2016; 23:115–124. [PubMed: 27013549]
- Bogdan S, Katicic-Trupcevic, Kajab D. Genetic variation in growth traits in a *Quercus robur* L. open-pollinated progeny test of the Slavonian provenance. *Silvae Genetica.* 2004; 53:198–201.
- Bogdan S, Ivankovic M, Temunovic M, Moric M, Franjic J, Bogdan IK. Adaptive genetic variability and differentiation of Croatian and Austrian *Quercus robur* L. populations at a drought prone field trial. *Annals of Forest Research.* 2017; 60:33–46.
- Bogdziewicz M, Fernández-Martínez M, Bonal R, Belmonte J, Espelta JM. The Moran effect and environmental vetoes: phenological synchrony and drought drive seed production in a Mediterranean oak. *Proc R Soc B.* 2017; 284 20171784.
- Bolker BM, Brooks ME, Clark CJ, Geange SW, Poulsen JR, Stevens MHH, White J-SS. Generalized linear mixed models: a practical guide for ecology and evolution. *Trends Ecol Evol.* 2009; 24:127–135. [PubMed: 19185386]
- Bonal R, Muñoz A, Díaz M. Satiation of predispersal seed predators: the importance of considering both plant and seed levels. *Evol Ecol.* 2007; 21:367–380.
- Broman KW, Wu H, Sen , Churchill GA. R/qtl: QTL mapping in experimental crosses. *Bioinformatics.* 2003; 19:889–890. [PubMed: 12724300]
- Buonaccorsi JP, Elkinton J, Koenig W, Duncan RP, Kelly D, Sork V. Measuring mast seeding behavior: relationships among population variation, individual variation and synchrony. *J Theor Biol.* 2003; 224:107–114. [PubMed: 12900208]
- Caignard T, Kremer A, Firmat C, Nicolas M, Venner S, Delzon S. Increasing spring temperatures favor oak seed production in temperate areas. *Sci Rep.* 2017; 7 8555.
- Camarero JJ, Albuixech J, López-Lozano R, Casterad MA, Montserrat-Martí G. An increase in canopy cover leads to masting in *Quercus ilex*. *Trees.* 2010; 24:909–918.
- Cornelius J. Heritabilities and additive genetic coefficients of variation in forest trees. *Can J For Res.* 1994; 24:372–379.
- Derory J, Scotti-Saintagne C, Bertocchi E, Dantec LL, Graignic N, Jauffres A, Casasoli M, Chancerel E, Bodénès C, Alberto F, Kremer A. Contrasting relationships between the diversity of candidate genes and variation of bud burst in natural and segregating populations of European oaks. *Heredity.* 2010; 104:438. [PubMed: 19812610]
- Dirlwanger E, Quero-García J, Dantec LL, Lambert P, Ruiz D, Dondini L, Illa E, Quilot-Turion B, Audergon J-M, Tartarini S, Letourmy P, et al. Comparison of the genetic determinism of two key phenological traits, flowering and maturity dates, in three *Prunus* species: peach, apricot and sweet cherry. *Heredity.* 2012; 109:280. [PubMed: 22828898]
- Doligez A, Bouquet A, Danglot Y, Lahogue F, Riaz S, Meredith C, Edwards K, This P. Genetic mapping of grapevine (*Vitis vinifera*) applied to the detection of QTLs for seedlessness and berry weight. *Theor Appl Genet.* 2002; 105:780–795. [PubMed: 12582493]
- Drobyshev I, Övergaard R, Saygin I, Niklasson M, Hickler T, Karlsson M, Sykes MT. Masting behaviour and dendrochronology of European beech (*Fagus sylvatica* L.) in southern Sweden. *For Ecol Manag.* 2010; 259:2160–2171.
- El-Kassaby YA, Barclay HJ. Cost of reproduction in Douglas-fir. *Can J Bot.* 1992; 70:1429–1432.

- Firmat C, Delzon S, Louvet JM, Parmentier J, Kremer A. Evolutionary dynamics of the leaf phenological cycle in an oak metapopulation along an elevation gradient. *Journal of Evolutionary Biology*. 2017; 30:2116–2131. [PubMed: 28977711]
- Gómez JM. Bigger is not always better: conflicting selective pressures on seed size in *Quercus ilex*. *Evolution*. 2004; 58:71–80. [PubMed: 15058720]
- Guitton B, Kelner J-J, Velasco R, Gardiner SE, Chagné D, Costes E. Genetic control of biennial bearing in apple. *J Exp Bot*. 2011; 63:131–149. [PubMed: 21963613]
- Haley CS, Knott SA. A simple regression method for mapping quantitative trait loci in line crosses using flanking markers. *Heredity*. 1992; 69:315. [PubMed: 16718932]
- Hall D, Hallingbäck HR, Wu HX. Estimation of number and size of QTL effects in forest tree traits. *Tree Genet Genomes*. 2016; 12:110.
- Han Q, Kabeya D, Iio A, Kakubari Y. Masting in *Fagus crenata* and its influence on the nitrogen content and dry mass of winter buds. *Tree Physiol*. 2008; 28:1269–1276. [PubMed: 18519258]
- Hansen TF, Pélabon C, Houle D. Heritability is not Evolvability. *Evol Biol*. 2011; 38:258–277.
- Haymes KL, Fox GA. Variation among individuals in cone production in *Pinus palustris* (Pinaceae). *Am J Bot*. 2012; 99:640–645. [PubMed: 22434774]
- Hoffmann AA, Merilä J, Kristensen TN. Heritability and evolvability of fitness and nonfitness traits: Lessons from livestock. *Evolution*. 2016; 70:1770–1779. [PubMed: 27346243]
- Houel C, Chatbanyong R, Doligez A, Rienh M, Foria S, Luchaire N, Roux C, Adivèze A, Lopez G, Farnos M, Pellegrino A, et al. Identification of stable QTLs for vegetative and reproductive traits in the microvine (*Vitis vinifera* L.) using the 18 K Infinium chip. *BMC Plant Biol*. 2015; 15:205. [PubMed: 26283631]
- Howe H, Smallwood J. Ecology of Seed Dispersal. *Annual review of ecology and systematics*. 1982; 13(1):201–228.
- Ishihara MI, Kikuzawa K. Annual and spatial variation in shoot demography associated with masting in *Betula grossa*: comparison between mature trees and saplings. *Ann Bot*. 2009; 104:1195–1205. [PubMed: 19734164]
- Jensen JS, Wellendorf H, Jager K, De Vries SMG, Jensen V. Analysis of a 17-year old Dutch open-pollinated progeny trial with *Quercus robur* (L.). *Forest Genetics*. 1997; 4(3):139–147.
- Jermstad KD, Bassoni DL, Jech KS, Wheeler NC, Neale DB. Mapping of quantitative trait loci controlling adaptive traits in coastal Douglas-fir. I. Timing of vegetative bud flush. *Theor Appl Genet*. 2001; 102:1142–1151.
- Kadri A, Julier B, Laouar M, Ben C, Badri M, Chedded J, Mouhouche B, Gentzbittel L, Abdelguerfi A. Genetic determinism of reproductive fitness traits under drought stress in the model legume *Medicago truncatula*. *Acta Physiol Plant*. 2017; 39:227.
- Kang K-S, Bila AD, Harju AM, Lindgren D. Estimation of fertility variation in forest tree populations. *Forestry*. 2003; 76:329–344.
- Kelly D, Sork VL. Mast seeding in perennial plants: why, how, where? *Annu Rev Ecol Syst*. 2002:427–447.
- Knops JM, Koenig WD, Carmen WJ. Negative correlation does not imply a tradeoff between growth and reproduction in California oaks. *Proc Natl Acad Sci*. 2007; 104:16982–16985. [PubMed: 17940035]
- Koenig WD, Knops JM, Carmen WJ, Stanback MT, Mumme RL. Acorn production by oaks in central coastal California: influence of weather at three levels. *Can J For Res*. 1996; 26:1677–1683.
- Koenig WD, Knops JM. Scale of mast-seeding and tree-ring growth. *Nature*. 1998; 396:225–226.
- Koenig WD, Knops JM, Dickinson JL, Zuckerman B. Latitudinal decrease in acorn size in bur oak (*Quercus macrocarpa*) is due to environmental constraints, not avian dispersal. *Botany*. 2009; 87:349–356.
- Koenig WD, Mumme RL, Carmen WJ, Stanback MT. Acorn production by oaks in central coastal California: variation within and among years. *Ecology*. 1994:99–109.
- Kroon J, Wennström U, Prescher F, Lindgren D, Mullin TJ. Estimation of Clonal Variation in Seed Cone Production Over Time in a Scots pine (*Pinus sylvestris* L.) Seed Orchard. *Silvae Genetica*. 2009; 58(1–6):53–62.

- Lesur I, Alexandre H, Boury C, Chancerel E, Plomion C, Kremer A. Development of Target Sequence Capture and Estimation of Genomic Relatedness in a Mixed Oak Stand. *Frontiers in plant science*. 2018; 9
- Lopez-Toledo L, Heredia-Hernández M, Castellanos-Acuña D, Blanco-García A, Saénz-Romero C. Reproductive investment of *Pinus pseudostrobus* along an altitudinal gradient in Western Mexico: implications of climate change. *New Forests*. 2017; 48(6):867–881.
- Manichaikul A, Dupuis J, Sen S, Broman KW. Poor Performance of Bootstrap Confidence Intervals for the Location of a Quantitative Trait Locus. *Genetics*. 2006; 174:481–489. [PubMed: 16783000]
- Manichaikul A, Moon JY, Sen S, Yandell BS, Broman KW. A Model Selection Approach for the Identification of Quantitative Trait Loci in Experimental Crosses, Allowing Epistasis. *Genetics*. 2009; 181:1077–1086. [PubMed: 19104078]
- Mather RA, Kanowski PJ, Savill PS. Genetic determinism of vessel area in oak (*Quercus robur* L. and *Q. petraea* Liebl): a characteristic related to the occurrence of stem sakes. *Annals of Forest Sciences*. 1993; 50(Suppl.1):395s–398s.
- Merilä J, Sheldon BC. Lifetime Reproductive Success and Heritability in Nature. *Am Nat*. 2000; 155:301–310. [PubMed: 10718727]
- Merilä J, Sheldon BC. Genetic architecture of fitness and nonfitness traits: empirical patterns and development of ideas. *Heredity*. 1999; 83:103–109. [PubMed: 10469197]
- Mitchell-Olds T. Genetic Constraints on Life-History Evolution: Quantitative-Trait Loci Influencing Growth and Flowering in *Arabidopsis thaliana*. *Evolution*. 1996; 50:140–145. [PubMed: 28568847]
- Monks A, Kelly D. Testing the resource-matching hypothesis in the mast seeding tree *Nothofagus truncata* (Fagaceae). *Austral Ecol*. 2006; 31:366–375.
- Moran EV, Clark JS. Causes and consequences of unequal seedling production in forest trees: a case study in red oaks. *Ecology*. 2012; 93:1082–1094. [PubMed: 22764494]
- Mutke S, Gordo J, Gil L. Cone Yield Characterization of a Stone Pine (*Pinus pinea* L.) clone bank. *Silvae Genetica*. 2005; 54(1–6):189–197.
- Nepveu G. Genotypic determination of the anatomical structure of wood in *Quercus robur*. *Silvae Genetica*. 1984a; 33(2–3):91–95.
- Nepveu G. Hereditary control of density and retractability of wood from 3 oak species (*Quercus petraea*, *Quercus robur* and *Quercus rubra*). *Silvae Genetica*. 1984b; 33(4–5):110–115.
- Nikkanen T, Ruotsalainen S. Variation in flowering abundance and its impact on the genetic diversity of the seed crop in a Norway spruce seed orchard. *Silva Fenn*. 2000; 34(3):205–222.
- Pearse IS, Koenig WD, Kelly D. Mechanisms of mast seeding: resources, weather, cues, and selection. *New Phytologist*. 2016; 212(3):546–562. [PubMed: 27477130]
- Pelgas B, Bousquet J, Meirmans PG, Ritland K, Isabel N. QTL mapping in white spruce: gene maps and genomic regions underlying adaptive traits across pedigrees, years and environments. *BMC Genomics*. 2011; 12:145. [PubMed: 21392393]
- Pérez-Ramos IM, Aponte C, García LV, Padilla-Díaz CM, Marañón T. Why Is Seed Production So Variable among Individuals? A Ten-Year Study with Oaks Reveals the Importance of Soil Environment. *PLoS ONE*. 2014; 9:e115371. [PubMed: 25532130]
- Pérez-Ramos IM, Ourcival JM, Limousin JM, Rambal S. Mast seeding under increasing drought: results from a long-term data set and from a rainfall exclusion experiment. *Ecology*. 2010; 91:3057–3068. [PubMed: 21058565]
- Plomion C, Aury JM, Amselem J, Alaeitabar T, Barbe V, Belser C, Berges H, Bodénès C, Boudet N, Boury C, Canaguier A, et al. Decoding the oak genome: public release of sequence data, assembly, annotation and publication strategies. *Molecular Ecology Resources*. 2016; 16:254–265. [PubMed: 25944057]
- Price T, Schluter D. On the Low Heritability of Life-History Traits. *Evolution*. 1991; 45:853–861. [PubMed: 28564058]
- Romeu JF, Monforte AJ, Sánchez G, Granell A, García-Brunton J, Badenes ML, Ríos G. Quantitative trait loci affecting reproductive phenology in peach. *BMC Plant Biol*. 2014; 14:52. [PubMed: 24559033]

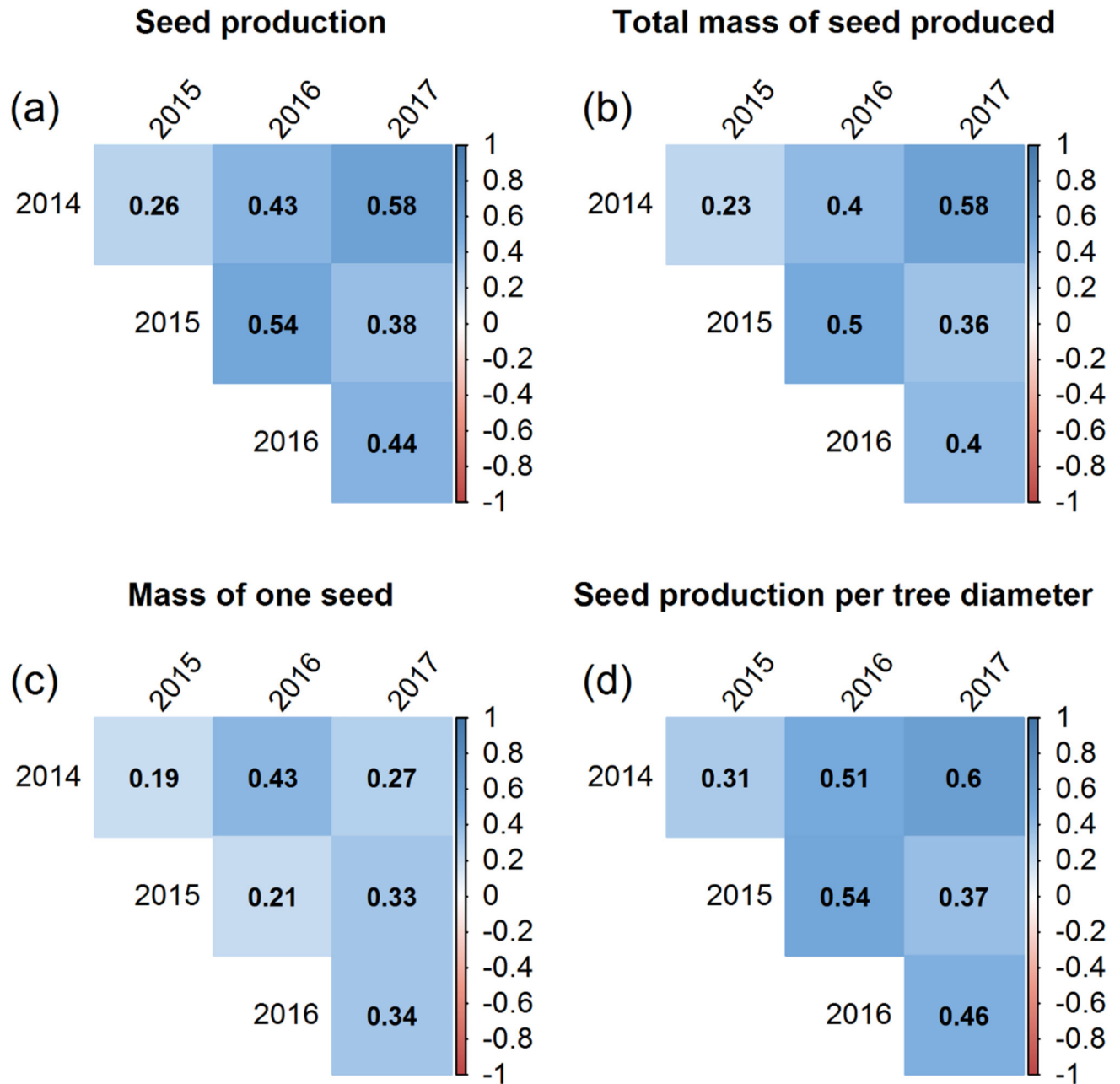
- Sadok IB, Celton J-M, Essalouh L, Aabidine AZE, Garcia G, Martinez S, Grati-Kamoun N, Rebai A, Costes E, Khadari B. QTL Mapping of Flowering and Fruiting Traits in Olive. PLOS ONE. 2013; 8:e62831. [PubMed: 23690957]
- Saintagne C, Bodénès C, Barreneche T, Pot D, Plomion C, Kremer A. Distribution of genomic regions differentiating oak species assessed by QTL detection. Heredity. 2004; 92:20–30. [PubMed: 14508500]
- Sanchez-Humanes B, Espelta JM. Increased drought reduces acorn production in *Quercus ilex* coppices: thinning mitigates this effect but only in the short term. Forestry. 2011; 84:73–82.
- Santos del Blanco L, Zas R, Notivol Paño E, Chambel MR, Majada J, Climent J. Variation of early reproductive allocation in multi-site genetic trials of Maritime pine and Aleppo pine. Variación en asignación reproductiva temprana en ensayos multi-localidad de pino carrasco y pino negra. Forest Systems. 2010; 19:381–392.
- Santos-del-Blanco L, Climent J, González-Martínez SC, Pannell JR. Genetic differentiation for size at first reproduction through male versus female functions in the widespread Mediterranean tree *Pinus pinaster*. Ann Bot. 2012; 110:1449–1460. [PubMed: 23002272]
- Savill PS, Kanowski PJ, Gourlay ID, Jarvis AR. Genetic and intra tree variation in the number of sapwood rings in *Quercus robur* and *Q. petraea*. Silvae Genetica. 1993; 42:371–375.
- Schupp EW, Jordano P, Gómez JM. Seed dispersal effectiveness revisited: a conceptual review. New Phytol. 2010; 188:333–353. [PubMed: 20673283]
- Scotti-Saintagne C, Bodénès C, Barreneche T, Bertocchi E, Plomion C, Kremer A. Detection of quantitative trait loci controlling bud burst and height growth in *Quercus robur* L. Theor Appl Genet. 2004; 109:1648–1659. [PubMed: 15490107]
- Shalom L, Samuels S, Zur N, Shlizerman L, Zemach H, Weissberg M, Ophir R, Blumwald E, Sadka A. Alternate Bearing in Citrus: Changes in the Expression of Flowering Control Genes and in Global Gene Expression in ON- versus OFF-Crop Trees. PLOS ONE. 2012; 7:e46930. [PubMed: 23071667]
- Silvertown J, Dodd M. The Demographic Cost of Reproduction and Its Consequences in Balsam Fir (*Abies balsamea*). Am Nat. 1999; 154:321–332. [PubMed: 10506547]
- Sirois L. Spatiotemporal variation in black spruce cone and seed crops along a boreal forest - tree line transect. Can J For Res. 2000; 30:900–909.
- Sivacioglu A, Ayan S, Çelik D. Clonal variation in growth, flowering and cone production in a seed orchard of Scots pine (*Pinus sylvestris* L.) in Turkey. African Journal of Biotechnology. 2009; 8(17)
- Sousa WP, Kennedy PG, Mitchell BJ. Propagule size and predispersal damage by insects affect establishment and early growth of mangrove seedlings. Oecologia. 2003; 135:564–575. [PubMed: 12684857]
- Traveset A, Heleno R, Nogales M. The ecology of seed dispersal. Seeds: the ecology of regeneration in plant communities. 2014; 3:62–93.
- Tsubomura M, Fukatsu E, Nakada R, Fukuda Y. Inheritance of male flower production in *Cryptomeria japonica* (sugi) estimated from analysis of a diallel mating test. Ann For Sci. 2012; 69:867–875.
- Ujino-Ihara T, Iwata H, Taguchi Y, Tsumura Y. Identification of QTLs associated with male strobilus abundance in *Cryptomeria japonica*. Tree Genet Genomes. 2012; 8:1319–1329.
- Vander Wall SB. How plants manipulate the scatter-hoarding behaviour of seed-dispersing animals. Philosophical Transactions of the Royal Society of London B: Biological Sciences. 2010; 365(1542):989–997. [PubMed: 20156821]
- Visscher PM, Goddard ME. A General Unified Framework To Assess the Sampling Variance of Heritability Estimates Using Pedigree or Marker-Based Relationships. Genetics. 2014; 199(1): 223–232. [PubMed: 25361897]
- Walters MB, Reich PB. Seed size, nitrogen supply, and growth rate affect tree seedling survival in deep shade. Ecology. 2000; 81:1887–1901.
- Wu J, Li L-T, Li M, Khan MA, Li X-G, Chen H, Yin H, Zhang S-L. High-density genetic linkage map construction and identification of fruit-related QTLs in pear using SNP and SSR markers. J Exp Bot. 2014; 65:5771–5781. [PubMed: 25129128]



**Figure 1.**

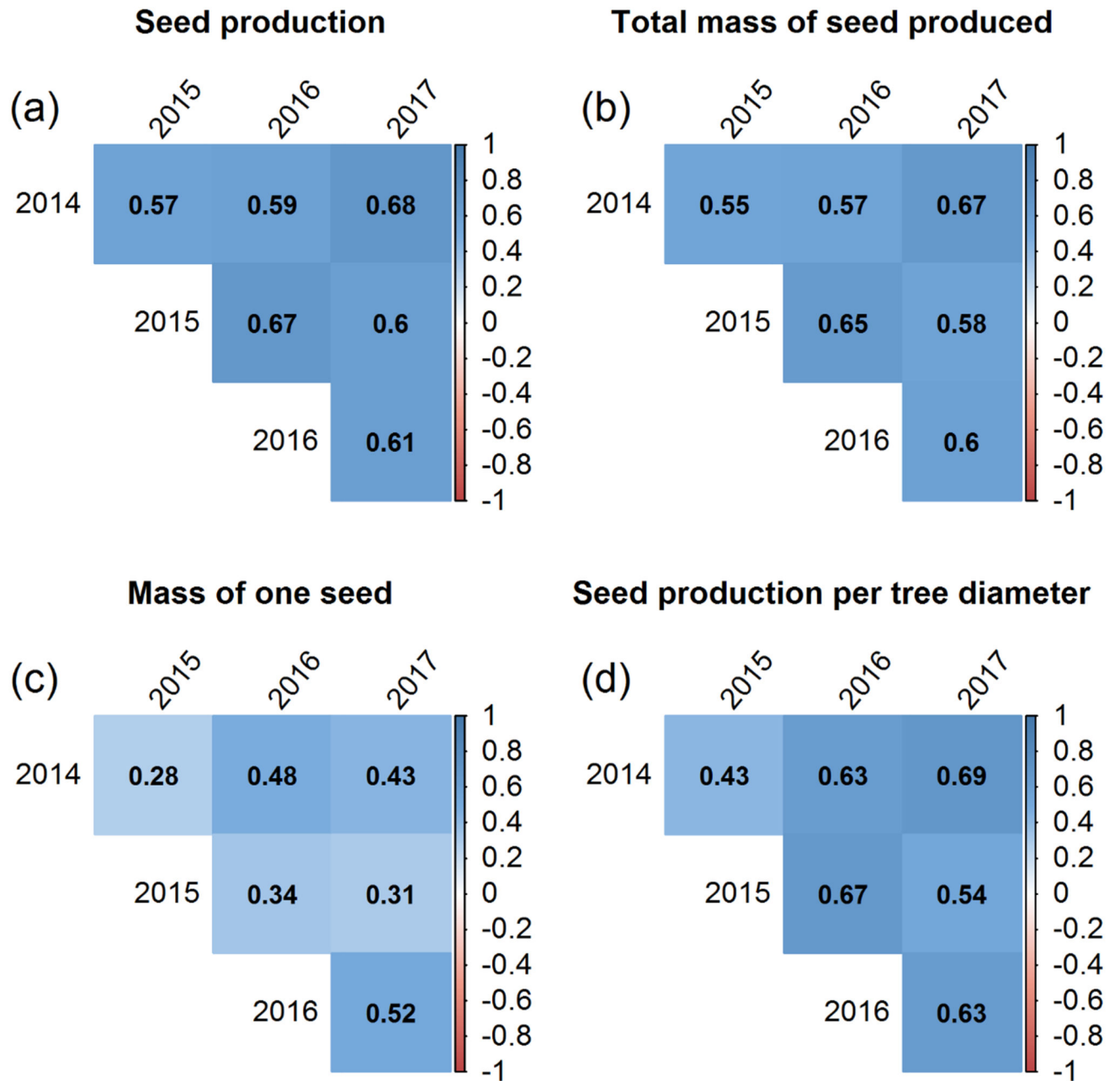
Boxplots of the 4 phenotypic traits represented for each year. Seed production, total mass of seed produced (g) and seed production per tree diameter ( $\text{seed} \cdot \text{mm}^{-1}$ ) were log transformed. The mean mass of one seed was assessed in grammes.





**Figure 2.**

Correlation between years, estimated for the phenotypic values of each trait using the coefficient of Pearson. Phenotypic values were log transformed for seed production (a), total mass of seed produced (b) and seed production per tree diameter (d).



**Figure 3.**

Correlation between years, estimated for the genetic values (BLUPs) of each trait using the coefficient of Pearson. Genetic values of seed production (a), total mass of seed produced (b) and seed production per tree diameter (d) were estimated on the log-transformed phenotypic values.

**Table 1**

Description of the experimental design for reproductive and growth traits

	<b>Reproduction</b>				<b>Growth</b>
	<b>2014</b>	<b>2015</b>	<b>2016</b>	<b>2017</b>	<b>2014</b>
Total number of genotypes	117	170	170	170	206
Total number of individuals (clonal replicates)	173	331	339	337	1130
Mean number of clonal replicates per genotype	1.48	1.95	1.99	1.98	5.46
Maximum amount of clonal replicates per genotype	4	6	6	6	10
Yearly averaged temperature (°C)	14.2	14.0	13.6	13.7	14.2
Yearly sum of precipitation (mm)	792.5	511.5	707.5	589.0	792.5

**Table 2**

Estimation of the marginal and conditional coefficient of determination ( $R^2_m$  and  $R^2_c$ ), the mean clone repeatability ( $R$ ), the narrow sense heritabilities ( $h^2_0$  and  $h^2_{1/2}$ ) and the evolvabilities ( $e_0$  and  $e_{1/2}$ ) for each trait and each year monitored (from 2014 to 2017)). The interval of confidence of the narrow sense heritabilities were estimated at 95% using the method from Visscher and Goddard (2014).

Trait	Year	$n_0$	$R^2_m$	$R^2_c$	$R$	$h^2_0$	$h^2_{1/2}$	$e_0$	$e_{1/2}$
$N_{tot}$	2014	1.48	0.02	0.43	0.52	0.59 [0.23, 0.95]	0.37 [-0.03, 0.77]	1.02	0.58
$N_{tot}$	2015	1.95	0.07	0.48	0.61	0.62 [0.32, 0.92]	0.39 [0.05, 0.72]	1.24	0.71
$N_{tot}$	2016	2.00	0.04	0.33	0.46	0.46 [0.13, 0.78]	0.28 [-0.06, 0.62]	0.76	0.43
$N_{tot}$	2017	1.98	0.02	0.54	0.69	0.69 [0.41, 0.97]	0.44 [0.11, 0.77]	1.03	0.58
$N_{tot}$	Multi-Annual	2.00	0.13	0.35	0.70	0.40 [0.07, 0.73]	0.38 [0.04, 0.71]	0.89	0.51
$M_{tot}$	2014	1.48	0.01	0.41	0.50	0.58 [0.21, 0.95]	0.36 [-0.04, 0.76]	1.16	0.66
$M_{tot}$	2015	1.94	0.07	0.47	0.60	0.60 [0.30, 0.90]	0.38 [0.04, 0.71]	1.09	0.62
$M_{tot}$	2016	2.00	0.07	0.32	0.43	0.43 [0.10, 0.76]	0.26 [-0.08, 0.60]	0.78	0.44
$M_{tot}$	2017	1.98	0.04	0.54	0.69	0.69 [0.41, 0.97]	0.44 [0.11, 0.77]	1.16	0.67
$M_{tot}$	Multi-Annual	2.00	0.13	0.23	0.49	0.22 [-0.11, 0.55]	0.13 [-0.15, 0.41]	0.65	0.37
$M_a$	2014	1.48	0.01	0.31	0.39	0.46 [0.07, 0.85]	0.28 [-0.12, 0.68]	0.0211	0.0121
$M_a$	2015	1.88	0.05	0.21	0.27	0.28 [-0.06, 0.62]	0.17 [-0.14, 0.48]	0.0402	0.0230
$M_a$	2016	1.93	0.07	0.19	0.22	0.22 [-0.11, 0.55]	0.13 [-0.15, 0.41]	0.0210	0.0120
$M_a$	2017	1.98	0.21	0.42	0.42	0.42 [0.09, 0.75]	0.26 [-0.08, 0.60]	0.0278	0.0159
$M_a$	Multi-Annual	1.93	0.05	0.1	0.28	0.10 [-0.15, 0.35]	0.06 [-0.12, 0.24]	0.0014	0.0008
$N_{tot}/D$	2014	1.48	0.02	0.43	0.52	0.59 [0.22, 0.96]	0.37 [-0.03, 0.78]	0.15	0.08
$N_{tot}/D$	2015	1.95	0.04	0.5	0.64	0.65 [0.36, 0.94]	0.41 [0.07, 0.74]	0.05	0.03
$N_{tot}/D$	2016	2.00	0.08	0.46	0.58	0.58 [0.28, 0.88]	0.36 [0.02, 0.70]	0.08	0.04
$N_{tot}/D$	2017	1.98	0.02	0.54	0.70	0.70 [0.42, 0.98]	0.44 [0.11, 0.77]	0.34	0.19
$N_{tot}/D$	Multi-Annual	2.00	0.16	0.36	0.68	0.38 [0.04, 0.71]	0.23 [-0.10, 0.56]	0.11	0.06
$CV_1$	Multi-Annual	1.93	0.14	0.42	0.48	0.49 [0.17, 0.81]	0.30 [-0.04, 0.64]	0.0593	0.0339

Trait	Year	n0	R <sup>2</sup> m	R <sup>2</sup> c	R	h <sup>2</sup> <sub>0</sub>	h <sup>2</sup> <sub>1/2</sub>	e <sub>0</sub>	e <sub>1/2</sub>
r <sub>i</sub>	Multi-Annual	1.98	0.07	0.3	0.4	0.40 [0.07, 0.73]	0.24 [-0.10, 0.58]	0.0332	0.0190
D	2014	5.46	0	0.15	0.48	0.25 [-0.06, 0.56]	0.15 [-0.13, 0.43]	0.017	0.0098
H	2014	5.46	0	0.23	0.62	0.37 [0.06, 0.68]	0.22 [-0.09, 0.53]	7.76e <sup>-5</sup>	4.44e <sup>-5</sup>

*n*<sub>0</sub>: mean number of clonal replicates per genotype; *Multi-annual*: trait value over the for years;  $h^2_0$  and  $h^2_{1/2}$ : narrow sense heritabilities assuming dominance variance = 0 and dominance variance = 1/2 of the additive variance;  $e_0$  and  $e_{1/2}$ : evolvabilities assuming dominance variance = 0 and dominance variance = 1/2 additive variance

**Table 3**

Significant QTLs detected for each trait and their related statistics.

Trait	Year	Parent	n	LG	Position	LOD	BCI	PEV	p-value
$M_s$	2016	Female	168	1	25	3.76	8.85 – 38.00	9.8	<0.001
	2016	Male	168	7	50.08	2.99	36.00 – 58.87	7.86	<0.001
	2017	Female	169	1	26.47	3.11	12.00 – 48.00	8.07	<0.001
	Multi-Annual	Female	170	1	26.00	5.06	20.81 – 38.00	12.81	<0.001
	Multi-Annual	Male	170	11	34.29	3.24	25.49 – 52.00	8.39	<0.001
	2014	Male	117	11	54.01	2.97	15.00 – 58.73	11.03	<0.001
$M_{tot}$	2015	Female	169	3	32.56	3.29	7 – 37.24	8.53	<0.001
	2016	Female	169	3	33.8	5.86	13.24 – 36.00	14.75	<0.001
	2016	Male	169	11	23.6	6.34	10.72 – 34.29	15.87	<0.001
	2017	Female	170	3	28.00	6.78	24.00 – 33.00	16.77	<0.001
	2017	Male	170	11	23.6	5.12	14.00 – 54.01	12.96	<0.001
	Multi-Annual	Female	170	3	31.00	5.85	12.93 – 34.44	14.65	<0.001
$N_{tot}$	2014	Male	117	5	65.03	2.7	45.00 – 72.24	10.11	<0.001
	2014	Male	117	11	54.01	3.02	13 – 58.73	11.24	<0.001
	2015	Female	170	3	32.56	4.28	8.86 – 36.62	10.94	<0.001
	2016	Female	170	3	31	6	13.24 – 35.05	15.01	<0.001
	2016	Male	170	11	23	5.81	10.00 – 33.00	14.56	<0.001
	2017	Female	170	3	29.00	7.76	25.00 – 32.00	17.51	<0.001
Multi-Annual	Female	170	3	31.63	6.64	13.24 – 34.75	16.47	<0.001	
	Male	170	11	23.00	4.73	10.00 – 52.00	12.04	<0.001	

Trait	Year	Parent	n	LG	Position	LOD	BCI	PEV	p.value
	2014	Male	117	5	65.03	3.78	56.78 – 72.24	13.84	<0.001
	2014	Male	117	11	22	4.24	13.00 – 56.00	15.38	<0.001
	2015	Female	170	3	32.6	3.24	4.00 – 39.13	8.41	<0.001
	2015	Female	170	12	19	2.95	10.00 – 30.69	7.69	<0.001
	2016	Female	170	3	31	5.51	16.00 – 36.62	13.86	<0.001
	2016	Male	170	11	10.72	7.12	10.00 – 32.00	17.54	<0.001
<i>N<sub>tot</sub>/D</i>	2017	Female	170	3	30.00	7.50	25.19 – 33.00	18.40	<0.001
	2017	Male	170	11	23.6	4.85	10.00 – 54.01	12.32	<0.001
	Multi-Annual	Female	170	2	30.98	3.23	16.58 – 41.00	8.10	<0.001
	Multi-Annual	Female	170	3	32.00	3.28	17.00 – 40.00	8.21	<0.001
	Multi-Annual	Male	170	11	22.00	5.56	10.72 – 33.00	13.54	<0.001

Year: year of monitoring; *Multi-annual*: trait value over the four years; *Parent*: Female or male genetic map; *n*: number of clonal replicates per genotype; *LG*: Linkage group; *Position*: Position of the QTL on LG in cM; *BCI*: Confidence interval of the position at 95% indicated in cM; *PEV*: Percentage of phenotypic explained variance by a QTL; p.value: significance level.