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ORIGINAL PAPER

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Detection of quantitative trait loci controlling bud burst and height growth in *Quercus robur* L.

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Abstract Genetic variation of bud burst and early growth components was estimated in a full-sib family of *Quercus robur* L. comprising 278 offspring. The full sibs were vegetatively propagated, and phenotypic assessments were made in three field tests. This two-generation pedigree was also used to construct a genetic linkage map (12 linkage groups, 128 markers) and locate quantitative trait loci (QTLs) controlling bud burst and growth components. In each field test, the date of bud burst extended over a period of 20 days from the earliest to the latest clone. Bud burst exhibited higher heritability (0.15–0.51) than growth components (0.04–0.23) and also higher correlations across field tests. Over the three tests there were 32 independent detected QTLs ($P \leq 5\%$ at the chromosome level) controlling bud burst, which likely represent at least 12 unique genes or chromosomal regions controlling this trait. QTLs explained from 3% to 11% of the variance of the clonal means. The number of QTLs controlling height growth components was lower and varied between two and four. However the contribution of each QTL to the variance of the clonal mean was higher (from 4% to 19%). These results indicate that the genetic architecture of two important fitness-related traits are quite different. On the one hand, bud burst is controlled by several QTLs with rather low to moderate effects, but contributing to a high genetic (additive) variance. On the other hand, height growth depends on fewer QTLs with moderate to strong effects, resulting in lower heritabilities of the trait.

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Introduction

Environmental variations induced by global change may profoundly affect the distribution and composition of the European forest (Jarvis 1998; Walker et al. 1999). Similar changes have occurred recurrently during longer time scales in the past, midst the succession of glacial and interglacial periods. It is known that these changes have resulted either in the extinction of species (Birks 1986) or genetic diversification of their remaining populations. In contrast to past climatic changes, futures changes are expected to occur at much faster rates, and extant forest tree populations will have to adapt to the environmental modifications within a short period of time. As predicted by quantitative genetics, the response of populations to selection depends on the level of genetic variation. Whether long-lived trees will be able to adapt to these changes depends on the level of diversity existing within and between populations for adaptive traits. In this paper we attempt to decipher the sources of genetic variation of bud burst and height growth in *Quercus robur* L., a widely distributed forest tree species in Europe. These two traits will most likely be affected by climatic changes, as they are important components of the fitness of trees (Saxe et al. 2001). Bud burst and growth contribute to the viability and fecundity of trees. Late bud-bursting trees escape the detrimental effects of late frost on vegetative and reproductive buds. Rapid early growth is an essential step to reach dominance in natural regeneration of forest trees when competition for light and resources is strong. Different models predicting the timing of bud burst for trees are now widely used to predict the consequences of global warming on tree phenology (Hänninen 1990; Kramer 1995; Chuine et al. 1999). The predicted increase of temperature due to global change will hasten bud

burst in the season and increase the exposure of trees to frost. As outlined in recent reviews, ongoing changes in temperature have already contributed to a 16-day shift in bud flushing in Mediterranean deciduous species (Penuelas and Filella 2001) as compared to 50 years ago. Surveys conducted in temperate European phenological gardens also indicate that leaf unfolding has receded 6 days over the last 30 years (Menzel and Fabian 1999). However in species with high chilling requirements, higher temperatures in autumn can delay bud burst the following spring (Heide 2003). Earlier investigations on the extent and distribution of genetic variation of bud burst and early growth in *Quercus* have shown that these traits are extremely variable between provenances (Kleinschmit and Svolba 1994; Ducousso et al. 1996; Stephan et al. 1994). Within populations, variation has been less studied in oaks. In other trees, bud burst has shown from moderate to high heritability values (up to 94% in poplar Frewen et al. 2000; Howe et al. 2000; between 0.44 and 0.95 in Douglas fir, reviewed in Jermstad et al. 2001). Recently, quantitative trait loci (QTL) detection has indicated that bud burst in Scots pine and poplar was determined by a few loci with major effects (Hurme et al. 2000, Bradshaw and Stettler 1995; Frewen et al. 2000). On the contrary, in Douglas fir, phenological traits (bud burst, growth cessation), were controlled by a few number of QTLs (5–10) of modest effect (5–10%) (Jermstad et al. 2001, 2003). Height growth has also been extensively studied in provenance tests. Large between-provenance variations were observed in both *Q. robur* and *Q. petraea* (Kleinschmit and Svolba 1994). In other tree species heritability values are rather moderate and lower than those for bud burst (see Kremer 1994 for a review), with a few exceptions where large values were observed [in *Salix* (Tsarouhas et al. 2002) and in *Populus* (Wu and Stettler 1994; Bradshaw and Stettler 1995)]. Recent studies in *Pinus* and *Salix* have shown that height growth was controlled by few genes with major effects (Kaya et al. 1999; Lerceteau et al. 2000; Tsarouhas et al. 2002).

The objectives of this study were twofold: (1) to estimate the level of genetic variation of bud burst and early growth in a full-sib cross of *Q. robur* and (2) to dissect the genetic architecture of these two traits by estimating the number, effects and location of the QTLs controlling these traits in different environments. Because vegetative propagation was used to replicate the mapping pedigree in different environments, the phenotypic variation could be subdivided in genetic and environmental components, and the stability of QTLs across environments could be tested.

Materials and methods

Mapping pedigree and field tests

The mapping population was a full-sib family (F_1) of *Q. robur*, obtained from the cross between two pedunculate oaks, comprising 278 offspring. The female parent (accession 3P) is from the Forestry Research Station of Pierroton (latitude 44.44°N, longitude 0.46°W) and the male parent (accession A4) originates from Arcachon (latitude 44.40°N, longitude 1.11°W). The 278 seedlings were raised in a seedbed in the nursery until age 3, and they were then transplanted in a clonal bank as stool beds. The spacing between the stool beds was 1.5×1.5 m. The stool beds were vegetatively propagated each year, starting in 1997. Stump sprouts were harvested every

Table 1 Description of the field tests used for the detection of quantitative trait loci (QTLs)

	Field test 1	Field test 2	Field test 3
Type of test	Stool bed	Clonal test	Clonal test
Location	Pierroton (44.44°N, 0.41°W)	Bourran (44.20°N, 0.24°W)	Bourran (44.20°N, 0.24°W)
Date of installation	Spring 1995	Fall 1998	Spring 2000
Number of offspring (clones)	278	174	207
Number of cuttings		1,080	2,196
Mean number of cuttings/clone		6.2	10.6
Number of blocks	No experimental design	36	183
Number of clones/block		30	12
Spacing	1.5×1.5 m	1.5×4 m	1.5×4 m
Traits assessed	ND[a], S	ND, S	ND, S, H, HI, LF, NF
Growing season of assessment	Eighth since germination	Fourth season since vegetative propagation	Fifth season since vegetative propagation for ND and S Fourth season since vegetative propagation for growth traits (H, HI, LF, NF)

[a] *ND* Number of days to reach stage 3, *S* stage of bud burst reached at the midperiod of observation, *H* total height, *NF* number of flushes, *HI* height increment during one growing season, *LF* mean length of a flush (= HI/NF)

spring on the stool beds and were rooted in a greenhouse (as described in more detail in Saintagne et al. 2004). In addition to the assessments made in the nursery on the ortets (field test 1), data were recorded in two additional field tests that were established with vegetative propagules of the stool beds (Table 1).

The two additional field tests were installed in two different years (1997 and 1999). The success of vegetative propagation was uneven; hence the total number of clones installed (174 in field test 1 and 207 in field test 2) differed from the original number available (278). The cuttings were raised in the nursery during the first two seasons (in a greenhouse during season 1 and in a nursery during season 2). They were then transplanted in the field on the INRA Domain of Bourran located in the south-west of France (latitude 44.20°N, longitude 0.24°W). Each experimental plantation was designed with incomplete blocks with random composition of clones per block and one vegetative copy per clone per block (Table 1). Field tests 2 and 3 were installed on the same ecological site and were actually adjacent to each other.

Phenotypic measurements

Bud burst

Bud burst was monitored in each of the three field tests every 2 days once the first tree started to flush (day 0) until the last tree had flushed. The time elapsed between these extremes varied between 1 and 21 days. At each observation day, the stage of development of the apical bud was recorded using a grading system varying between score 1 (scales turning yellowish) and score 5 (fully expanded leaves). After the observation period, the distribution of the scores over the whole period was analysed, and for each plant the following records were used for the detection of QTL:

- Number of days (ND) since day 0 until the plant reached stage 3 (bud breaking and leaves becoming visible).
- Score (S) of bud burst at the midperiod of observations. The midperiod corresponded to the day when the distribution of the flushing scores was the most variable over the trees. The assessments were then made with the same methodology in the three experiments (1999 in field test 1, 2000 in field test 2 and 2002 in field test 3).

Growth traits

In addition to bud burst we also assessed several growth components in field test 3. Oaks are known to exhibit recurrent flushes during a growing season. Hence the following traits were assessed at the end of 2001 (end of third growing season since cutting propagation): the number of flushes (NF), the total height increment during the third growing season (HI), the mean flush length (LF = HI/NF) and the total height at the end of the third growing season (H).

For all traits assessed the additional number to initials of the traits indicates the field tests where the assessments were made (for example S3 means flushing score in field test 3).

Statistical analysis

Estimation of variance components in the full sibs pedigree

All data were analysed using analysis of variance (ANOVA) with the OPEP Software (Baradat and Labbé 1995). The distribution of the phenotypic values was tested by the Box and Cox method (Box and Cox 1964). Bud burst for the three years (either ND or S) in field tests 1, 2 and 3; total height (H3) and the number of flushes (NF3) at the end of the third growing season in field test 3 exhibited a continuous distribution. However, the height increment (HI3), and the mean length of a flush (LF3) exhibited a skewed distribution and were further analysed after making a square root transformation. Prior to any further statistical analysis, all data were adjusted to block effects by using the following mixed model:

$$\begin{aligned} Y_{ij} &= \mu + C_i + b_j + \varepsilon_{ij} \\ Z_{ij} &= Y_{ij} - b_j, \end{aligned} \quad (1)$$

where Y_{ij} is the observed value of the trait on cutting j of clone i, μ the overall mean, C_i the random clone effect of clone i, b_j the fixed block effect of block j and ϵ_{ij} the residual effect due to cutting j, and Z_{ij} is the adjusted value to the block effect.

Because vegetative propagation was used to propagate the full sibs of the mapping pedigree in field tests 2 and 3, the subdivision of the phenotypic value Z_{ij} (adjusted for block effects) of a cutting could be made as follows within each field test:

$$Z_{ij} = \mu + C_j + Cu_{ij}, \quad (2)$$

where C_i is the effect of clone i and Cu_{ij} is the effect of cutting j within clone i.

Furthermore a combined analysis over the two field tests was done to test for clone × test interaction.

$$Z_{ijk} = \mu + C_i + t_j + (Ct)_{ij} + \varepsilon_{ijk}, \quad (3)$$

where t_j is the effect of field test j and $(Ct)ij$ the interaction between the clone i and the field test j, and ϵ_{ijk} is the residual effect.

In a set of full-sib families, the total genotypic variance (V_G) is the sum of within (σ_w^2) and between (σ_b^2) full-sib family variances

$$V_G = \sigma_w^2 + \sigma_b^2$$

$V_G = V_A + V_D$, where V_D and V_A are the dominance and additive variance. And where the variance between full-sib families is:

$$\sigma_b^2 = \frac{1}{2}V_A + \frac{1}{4}V_D.$$

In what follows we assume that the environmental variance is absorbed by ${\sigma_{Cu}}^2$ (the variance among clonal replicates), and that there are no persistent environmental differences associated to the propagated clones. Given that the trees vegetatively propagated were full sibs, the clonal variance ${\sigma_C}^2$, is equal to ${\sigma_w}^2$, and the expectation of the clonal variance ${\sigma_C}^2$ in this case, is:

$$\sigma_C^2 = \frac{1}{2}V_A + \frac{3}{4}V_D.$$

As dominance variance is generally lower than the additive variance in forest trees (Cornelius 1994), we consider two extreme values for the dominance variance in order to estimate additive variances and narrow sense (h^2) heritabilities [$V_D = 0$ and $V_D = (1/2)V_A$].

$$\text{If } V_D = 0, \text{ then } V_A = 2\sigma_C^2 \text{ and } h^2 = \frac{2\sigma_C^2}{2\sigma_C^2 + \sigma_{Cu}^2}$$

$$\text{If } V_D = 1/2\, V_A, \text{ then } V_A = \tfrac{8}{7}\sigma_C^2 \text{ and } h^2 = \frac{\frac{8}{7}\sigma_C^2}{\frac{12}{7}\sigma_C^2 + \sigma_{Cu}^2}.$$

In addition we also calculated the repeatability of the clonal means (R):

$$R = \frac{\sigma_C^2}{\sigma_C^2 + \frac{\sigma_{Cu}^2}{n_0}},$$

where n_0 is an adjusted mean number of cuttings per each clone (Snedecor and Cochran 1980)

$$n_0 = \frac{1}{(n_c - 1)}\left[N - \frac{\sum_{i=1}^{n_c} n_i^2}{N}\right],$$

with N being the total number of cuttings in the test, n_c the number of clones, and n_i the number of cuttings for clone i. n_0 is close but smaller than the arithmetic mean of the number of cuttings per clone.

R should not be misinterpreted with heritability of clonal means, as the clonal variance is not equal to the genotypic variance, but only to a fraction of the genotypic variance [${\sigma_C}^2 = (1/2)V_A + (3/4)V_D$].

Construction of a genetic map

A map was constructed for each parent of the cross following the so-called double-pseudo-testcross mapping strategy (Grattapaglia and Sederoff 1994) as previously described in Saintagne et al. (2004). The data originated from 128 markers recorded on each of the 278 full sibs (34 microsatellites, 1 SCARs, 84 AFLPs, 9 RAPDs) and was approximately evenly distributed throughout the genome. The two maps shared 19 microsatellites. The size of the map was slightly different from the one presented in Saintagne et al. (2004), because the data set was since completed for missing genotypes. The female framework map comprised 75 markers and covered a distance of 902 cM, with an average spacing between markers of 14 cM. In comparison to the reference map, this framework map covered about 76% of the pedunculate estimated genome length (Barreneche et al. 1998, 1,192 cM). The male framework map comprised 72 markers over a total distance of 933 cM, covering 76% of the genome (estimated total length is 1,235 cM), with an average marker spacing of 15.5 cM. Charts of linkage maps and QTL were made using the MapChart software, version 2.0 (Voorrips 2001).

QTL detection

Composite interval mapping was used to identify putative QTLs and to estimate their phenotypic effects (CIM, Zeng 1994; Jansen and Stam 1994). Calculations were made with the Multiqtl software (Britvin et al. 2001; http://esti.haifa.ac.il/~poptheor, no LOD normalization and no missing data restoration). The standard deviation for each QTL position was estimated by bootstrap (Visscher et al. 1996) with 1,000 resamplings. Empirical statistical significance thresholds for declaring the presence of a QTL were determined by permutations of the data set (Churchill and Doerge 1994). Two theoretical critical thresholds were considered for the detection of a QTL: the first corresponding to a type I error of 5% at the chromosome level and the second corresponding to a genome-wise type I error of 5%. Because the permutations tests were calculated at the chromosome level, we further computed the corresponding type I error rate at the whole-genome level, by proceeding as follows. The type I error at the marker level (α_m) can be written as a function of the type I error at the genome level (α_g) as:

$$\alpha_m = 1 - (1 - \alpha_g)^{1/M},$$

where M is the total number of markers on the map.

Similarly, if α_m is the type I error at the marker level, the type I error α_{chr} at a chromosome comprising m markers is:

$$\alpha_{chr} = 1 - (1 - \alpha_m)^m.$$

Hence the relationship between type I error rate at the genome level (α_g) and type I error rate at the chromosome level (α_{chr}) can be written as:

$$\alpha_{chr} = 1 - \left\{1 - \left[1 - \left(1 - \alpha_g\right)^{1/M}\right]\right\}^m.$$

The QTL detection was done separately for each parental map in both experiments.

The following ANOVA model was used to subdivide the variance components of the clonal means in the QTL detection:

$$\bar{Z}_{ij} = \mu + M_i + C_{ij}, \quad (4)$$

where $\bar{Z}_{ij}$ is the mean value of clone ij, M_j is the effect of the marker j and C_{ij} is the effect of the clone i within marker class j.

If $\sigma^2_{\bar{Z}}$ is the variance of the clonal means ($\sigma^2_{\bar{Z}} = \sigma^2_C + \sigma^2_{Cu}/n_0$), and if P is the proportion of $\sigma^2_{\bar{Z}}$ explained by the QTL, then the proportion Q of the clonal variance explained by the QTL is Q with $Q = P/R$, with R being the repeatability of the clonal mean.

Hence we can derive the proportion T of the genetic (additive) variance explained by the QTL in the two cases where $V_D = 0$ and $V_D = (1/2)V_A$.

When $V_D = 0$, recalling that $V_A = 2{\sigma_C}^2$, then $T = Q/2$. And when $V_D = (1/2)V_A$, recalling that $V_A = (8/7){\sigma_C}^2$ then $T = 7Q/8$.

Finally, we can compute the proportion of the phenotypic variance explained by the QTL (PV) with $PV = T \times h^2$.

Co-localisation of QTLs detected in two different field tests

To test whether the overlap of QTLs in two different experiments was due to chance, we calculated the probability of co-occurrences of QTLs in the same genomic region in the two tests under the null hypothesis of random distribution of QTLs.

To do so, the genome was subdivided in $N1$ intervals. An interval corresponded to the mean distance between the position of the highest LOD score from the composite interval mapping (P1) and the position of the mean value for maximum LOD score after bootstrap analysis (P2). If $N3$ is the number of QTLs in the experiment exhibiting the largest number of QTLs, $N4$ the number of QTLs in the second experiment, and $N2$ the number of QTLs shared by the two experiments, then the probability, P, of having $N2$ intervals in common between the two experiments is given by (Lin et al. 1995):

$$p = \frac{C_{N3}^{N2} C_{N1-N3}^{N4-N2}}{C_{N1}^{N4}}.$$

On average the distance between P1 and P2 was 6.7 cM (Table 4). The length of the female map was 902 cM, and the number of intervals which can be compared was 135. The number of intervals in the male map was 139 (933 cM).

Results

Subdivision of the phenotypic variation

Clonal effects were significant for all the characters as indicated by the ANOVA results (data not shown). There was an important variation of the flushing dates among the trees in the three field tests. The whole duration of bud burst within the full-sib family ranged over 21 days on average from the earliest to the latest flushing trees (Table 2). The wide range of variation for bud burst was also illustrated by the values of the phenotypic coefficient of variation that generally exceeded 30% (Table 2). A major component of the variation among trees was due to clonal variation as shown by the strong repeatability values (Table 2). On aggregated data over the test 2 and 3, the repeatability of clonal means S and ND reached, respectively, 0.81, and 0.82. An illustration of the clonal effect and the within-clonal variation is given for S in field test 3 (Fig. 1). At the midperiod of observation (11 days) some plants were still fully dormant, whereas others exhibited already elongating leaves. Within a given field test, heritability of the two measurements used to assess bud burst (ND and S) reached similar values. However there were important differences between the two field tests, with lower repeatability and heritability in field test 2 than 3. These differences might be related to stronger transplantation effects in test 2, where assessments were made only one year after plantation, whereas in test 3 measurements were made 2 years after plantation. On average, growth traits (H3, NF3, HI3, LF3) exhibited higher phenotypic variation and lower heritability values than phenological characters. Among the different traits, composite traits (H3 or HI3) were more repeatable and heritable than growth components (LF3 or NF3).

Table 2 Partition of bud burst and growth traits variation in the mapping pedigree. CV_P Coefficient of phenotypic variation, R repeatability of the clonal mean

Trait[a]	Mean	Min.	Max.	CV_P	R	Heritability	
						$V_D = 0$	$V_D = (1/2)V_A$
ND1	13.6	1	21	0.16			
ND2	13.8	1	20	22.90	0.50	0.25	0.15
ND3	10.0	1	21	45.80	0.84	0.52	0.32
S1	2.6	1	4	0.32			
S2	2.7	0	5	36.77	0.56	0.31	0.18
S3	3.0	1	5	31.32	0.83	0.51	0.31
H3	62.2 cm	7 cm	156 cm	35.59	0.58	0.23	0.14
HI3	24.4 cm	0.3 cm	125 cm	74.87	0.54	0.21	0.12
LF3	9.5 cm	0.2 cm	54 cm	61.27	0.45	0.16	0.09
NF3	2.4	1	7	39.78	0.26	0.07	0.04

[a]Additional numbers attached to the trait initials indicate the field test in which the trait was assessed, e.g. H3 means total height in field test 3

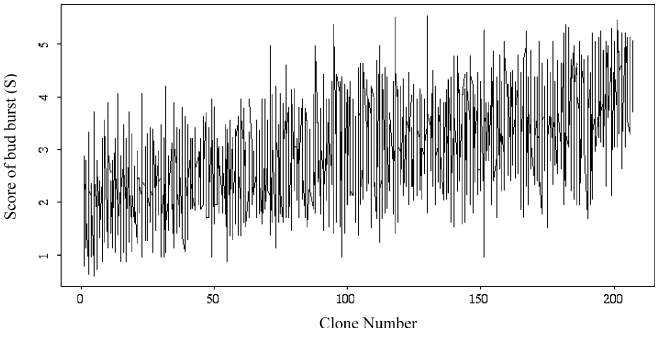

Fig. 1 Inter and intra-clone variability of bud burst at the midperiod of observations (11 days) in field test 3. Each *bar* indicates the variation of the flushing scores (*y*-axis) for different ramets of a clone (full sib)

Interaction clone × test

The ND and S showed significant clone × test interaction between the two field tests, using the two-way ANOVA (model 2, results not shown). However interaction effects did not profoundly modify the ranking of clones between the different tests as confirmed by correlation analysis among clone means across the three tests (Table 3). Correlations were highly significant for the same traits measured in the different tests, ranging between 0.41 and 0.61 for ND and between 0.36 and 0.57 for S. Clone mean values of test 2 were less correlated with the values in test 1 (0.41 for ND and 0.36 for S) than with values in test 3 (0.42 for ND and 0.46 for S). Correlations were the highest between test 1 and test 3 (0.61 for ND and 0.57 for S), although the two fields tests were located on different ecological sites separated by more then 150 km. Again transplantation effects that were more pronounced in test 2 than test 3 may explain these discrepancies.

Correlation among traits

Within a given test ND and S were always strongly correlated with the correlation coefficient among clonal means ranging from −0.84 to −0.98 (Table 3). The four growth components measured in test 3 also exhibited significant correlations among themselves: clones that grew more also tended to have more and longer flushes per season (Table 3). As a general trend there was a positive significant correlation between lateness of flushing and growth performance, whatever the growth component or the phenological trait assessed.

QTL of phenological traits

There was a great similarity in the number, effects and contributions of QTLs for ND and S, as was actually expected, given the high correlations between these traits (Table 3). Hence for the sake of clarity, we only presented the results concerning ND (Table 4; Fig. 2). In total over the three field tests, 32 QTLs were detected for ND that were significant at 5% at the chromosome level, from which 19 were also significant at 5% at the genome level. Among these 32 QTLs, nine were detected in field test 1, eight in field test 2 and the remaining 15 in field test 3. Among the eight QTLs detected in the field test 2, only one was significant at the genome level. These results confirmed the earlier observations that genetic variation represented a smaller proportion of the total phenotypic variation in field test 2. Because the detection was separately done within each parent and within each field test, several of the 32 QTLs may probably correspond to the same gene. Overall there are at least 12 different unique QTLs involved in bud flush, as there is at least one QTL per linkage group (LG) across the two parents and the three sites (Fig. 2). All LGs except LG1, LG8 and LG12 displayed QTLs detected in at least two field tests, and five LGs showed QTLs detected in all three sites (Fig. 2). Due to the large confidence interval of the QTL position, the identity of QTLs detected in different sites and located on the same LGs remained, however, questionable. The contribution of each QTL to the phenotypic variance of the clonal means was low to moderate, varying between 3.1% and 10.7% (Table 4).

When a combined analysis was conducted over the two test sites established with cuttings (tests 2 and 3), 13 different QTLs were detected in male and female maps (with three on the same male and female LG), from which 11 (except QTL in LG8 and LG11) were common to the separate analysis conducted within each test. In addition to the one QTL model, we conducted the two QTL model to test whether more than one QTL per chromosome were involved in bud burst. The two QTL model never did show any significant result, whether conducted on each test separately or on the combined data over the two tests 2 and 3 (results not shown).

In order to test if the overlapping of QTLs detected in different field tests were due to chance, the probability of co-occurrences of QTLs in the same genomic region under the null hypothesis of random distribution was computed. According to our hypotheses two QTLs shared the same location if their positions occupied the same interval. As stated earlier, the whole genome was

Table 3 Pearson and genetic (*in parentheses*) correlations between phenological and growth traits. Pearson correlations were calculated with clonal means for the field test 2 and 3 and single tree data for field test 1. Genetic correlations were calculated on traits measured on the same trees (type A correlations, Burdon 1977)

	ND1	S1	ND2	S2	ND3	S3	H3	HI3	LF3
ND1									
S1	**−0.84**								
ND2	**0.41**	**−0.35**							
S2	**−0.41**	**0.36**	**−0.94 (−0.99)**						
ND3	**0.61**	**−0.59**	**0.42**	**−0.47**					
S3	**−0.61**	**0.57**	**−0.4**	**0.46**	**−0.98 (−1)**				
H3	**0.23**	**−0.22**	0.11	−0.08	**0.29 (0.32)**	**−0.32 (−0.38)**			
HI3	**0.2**	**−0.21**	−0.06	0.01	**0.46 (0.49)**	**−0.47 (−0.50)**	**0.51 (0.50)**		
LF3	**0.15**	**−0.16**	−0.15	0.09	**0.4 (0.45)**	**−0.41 (−0.46)**	**0.44 (0.45)**	**0.91 (0.995)**	
NF3	**0.19**	**−0.2**	0.12	−0.16	**0.36 (0.43)**	**−0.36 (−0.48)**	**0.40 (0.52)**	**0.72 (0.83)**	**0.39 (0.72)**

Significant correlations ($P \leq 0.001$) are indicated in *boldface*

Table 4 QTL data. *LG* Linkage group (*m* male, *f* female), *N* number of individuals genotyped for the flanking markers, *LOD* highest LOD score in the interval, α_{chr} probability for the null hypothesis of no QTL at the chromosome level obtained after 1,000 permutations, *P1* position of the highest LOD score, *P2* position of the mean value for maximum LOD of the 1,000 bootstrap samples, *CI* QTL confidence interval (95%) based on the 1,000 bootstrap samples, *P* percentage of the clonal mean variance explained by the QTL, *T* percentage of the genetic (additive) variance explained by the QTL, *PV* percentage of the phenotypic variance explained by the QTL

Trait	LG	*N*	LOD	α_{chr}	P1	P2	CI	*P*	$V_D = 0$		$V_D = 1/2\ V_A$	
									T	PV	*T*	PV
Phenological traits												
ND1	2f	191	3.6	0.001[b]	16.7	16.7	18.5	5.8				
ND1	4f	189	3.3	0.002[b]	24.5	34.5	51.2	5.3				
ND1	5m	196	1.8	0.044[a]	22.4	39	65.5	3.6				
ND1	6m	216	4.8	0.001[b]	51.5	49.1	15	10.7				
ND1	7f	162	4.4	0.001[b]	72.5	69.8	27.6	7.1				
ND1	9f	207	3.3	0.001[b]	48.1	33.7	36.3	5				
ND1	10f	220	6.1	0.001[b]	19.4	24.3	31.9	8.7				
ND1	10m	192	1.8	0.027[a]	13.8	24.8	34.3	3.6				
ND1	11f	207	4.2	0.001[b]	72.5	64.5	31.6	6.8				
ND2	2f	148	3.6	0.001[b]	50.8	48	20	9.5	9.6	2.4	16.7	2.5
ND2	3m	157	2	0.013[a]	22.1	21.3	10.9	5.7	5.7	1.4	10	1.5
ND2	4f	156	2.5	0.010[a]	71.2	68.4	35.9	5.8	5.8	1.5	10.2	1.5
ND2	7f	98	2.1	0.023[a]	79.8	64.2	50.2	8.2	8.3	2.1	14.4	2.2
ND2	9f	154	1.7	0.042[a]	51.8	34.5	41.6	4.3	4.3	1.1	7.6	1.1
ND2	9m	129	2.2	0.010[a]	0	13.7	47.2	6.8	6.8	1.7	12	1.8
ND2	10m	89	2	0.016[a]	0	4.1	24.1	9.7	9.8	2.5	17.1	2.6
ND2	11f	155	1.8	0.032[a]	17.7	24.3	36.8	4	4	1	7	1.1
ND3	1m	156	8.3	0.001[b]	0	1.4	5.8	10.1	6	3.1	10.5	3.4
ND3	2f	181	6	0.001[b]	43.7	42.8	20.6	10	5.9	3.1	10.4	3.3
ND3	2m	154	2.4	0.011[a]	76.9	60.3	40.4	3.2	1.9	1	3.3	1.1
ND3	3m	159	1.9	0.018[a]	40.1	35.8	25.7	3.1	1.8	1	3.2	1
ND3	4m	168	5.7	0.001[b]	38.4	34.4	28	8.7	5.2	2.7	9.1	2.9
ND3	4f	185	2	0.037[a]	27.7	36.7	51	3.9	2.3	1.2	4.1	1.3
ND3	5m	174	4	0.001[b]	13.5	14.9	20.2	4.8	2.9	1.5	5	1.6
ND3	6m	192	5	0.001[b]	50.2	49.3	11.9	5.2	3.1	1.6	5.4	1.7
ND3	7f	122	3.5	0.001[b]	81.9	73.2	32.3	10.2	6.1	3.1	10.6	3.4
ND3	9f	122	2.2	0.015[a]	15.9	20.6	28.8	6.1	3.6	1.9	6.3	2
ND3	9m	185	5	0.001[b]	33.1	30.9	25.9	5.7	3.4	1.8	5.9	1.9
ND3	10f	193	4.1	0.001[b]	27.7	22.9	20.2	7.2	4.3	2.2	7.5	2.4
ND3	10m	172	6.4	0.001[b]	34.8	28.4	19	6.2	3.7	1.9	6.5	2.1
ND3	11m	178	8.9	0.001[b]	47.7	49.4	16.2	9.6	5.7	3	10	3.2
ND3	12f	179	2.7	0.003[b]	25.2	26.8	13.2	5.3	3.2	1.6	5.5	1.8
Growth components												
H3	5m	164	2.5	0.004[b]	53.4	48.9	28.4	6.7	5.8	1.3	10.1	1.4
H3	6m	188	3.2	0.001[b]	28.3	29	13.4	6.4	5.5	1.3	9.7	1.3
H3	10m	170	7.5	0.001[b]	18.4	19.1	12.8	17.3	14.9	3.5	26.1	3.6
H3	11f	172	3.5	0.001[b]	40	36.3	30.8	9.1	7.8	1.8	13.7	1.9
HI3	3m	191	5.1	0.001[b]	22.1	27	19.3	9.8	9.5	1.8	16.7	1.8
HI3	10f	178	3.9	0.001[b]	12.2	16.1	26.5	9.5	9.2	1.7	16.2	1.8
HI3	10m	170	8.2	0.001[b]	15.5	16.5	9.6	18.7	18.2	3.4	31.8	3.5
LF3	3m	191	5.5	0.001[b]	21.3	24.6	18.2	10.7	11.6	1.8	20.3	1.9
LF3	10m	170	7	0.001[b]	15.8	17.3	11.3	16.4	17.8	2.8	31.2	2.9
LF3	10f	170	2	0.014[a]	5.1	18.3	42.1	5.9	6.4	1	11.2	1
NF3	7f	166	1.8	0.032[a]	30.7	35.9	32.5	4.1	7.8	0.6	13.6	0.6
NF3	10f	178	6.1	0.001[b]	12.2	16.1	17	13.7	25.9	1.9	45.3	1.9
NF3	10m	170	3.1	0.001[b]	17.9	23.7	22.9	8.9	16.8	1.2	29.5	1.2
NF3	11f	193	2.5	0.008[b]	51.1	44.4	25.1	5.1	9.6	0.7	16.9	0.7
Mean								10.2	11.9	1.8	20.9	1.8

[a]Probability corresponding to a 5% chromosome-wise-type error
[b]Probability corresponding to a 5% genome-wide-type error

split into intervals of 6.7 cM, which corresponded to the mean distance separating P1 (the observed position of the QTL) and P2 (the mean bootstrap position of the QTL). Following these assumptions there were six co-localisations between QTLs detected in two different sites on LGs 2F, 4F, 9F, 10F, 6 M, 10 M and one co-localisation on LG7F between QTLs detected in the three sites (Fig. 2). There were more frequent co-localisations between field tests 1 and 3 (six co-localisations) than with other pairs of tests. Using the exact test of Lin et al. (1995) (see 'Materials and methods'), the probability of occurrence of these co-localisations by chance

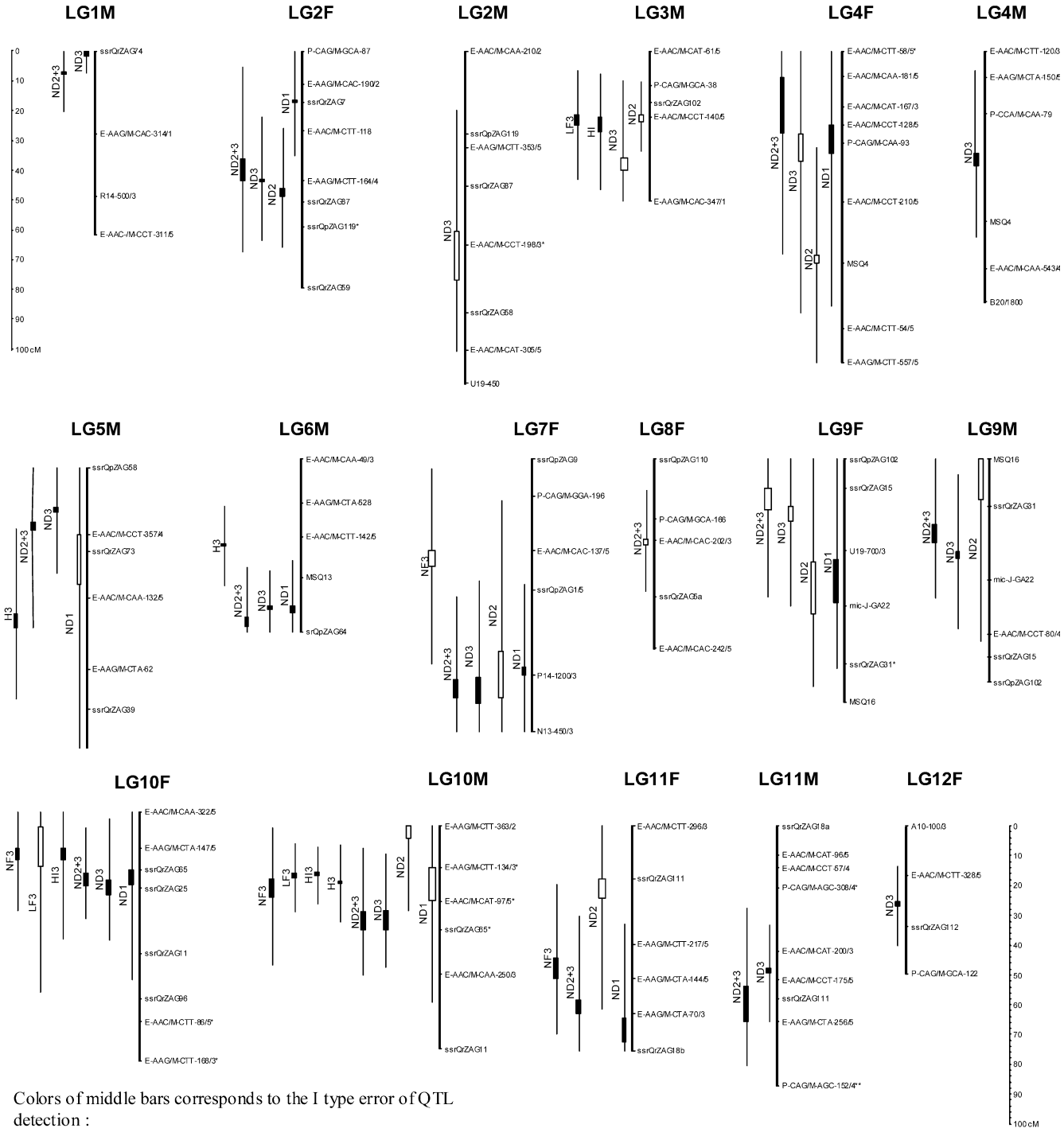

Fig. 2 Genetic map of *Quercus robur* showing the locations of quantitative trait loci QTLs) for bud burst and height growth components. *ND* Number of days until the plant reached stage 3, *NF* number of flushes, *LF* mean flush length, *HI* total height increment, *H* total height. The *subscripts* following the trait (*1*, *2*, *3*, *2+3*) correspond to the field test where assessments were made (2+3 means combined analysis over tests 2 and 3). Each QTL is delineated by the position of the highest LOD score (P1 in Table 4) and the bootstrap mean value of the highest LOD score (P2 in Table 4). Confidence intervals of their position based on 1,000 bootstrap samples are indicated as *lines*

Table 5 Statistical significance of co-localisation between QTLs involved in the bud burst detected in two different field tests. *N1* Total number of intervals in the map, *N3* number of QTLs in the field test (between the two that are compared) exhibiting the largest number of QTLs, *N4* number of QTLs in the second experiment, *N2* number of QTLs shared by the two experiments

Map	Field-test comparison	LGs	N1	N3	N4	N2	*P*-value[a]
Female	1 and 2	7, 9	135	6	5	2	0.015
Female	1 and 3	4, 7, 10	135	6	6	3	0.001
Female	2 and 3	2, 7	135	6	5	2	0.015
Male	1 and 3	5, 6, 10	139	9	3	3	0.0001
Female	1 and 2 + 3	7, 10, 11	135	6	6	3	0.001
Male	1 and 2 + 3	5, 6, 10	139	7	3	3	8×10^{-5}

[a] *P*-value is the probability for observing N2 intervals in common between the two experiments by chance

was lower then 2% (Table 5). When the comparison was made between QTLs detected in field test 1 and QTLs detected in the combined analysis of tests 2 and 3, the exact test was also extremely low, suggesting that the observed co-localisations are not due to chance.

QTLs of growth traits

Between two and four QTLs were detected for each growth component, explaining from 4.1% to 18.7% of the clonal mean variance and from 5.5% to 45.3% of the additive genetic variance, depending on the level of the dominance variance (Table 4).

Their position on the map (Fig. 2) suggested that one QTL was common to all four growth traits. On LG10, a QTL was detected at position 19 cM for H3 (male map), at position 16 cM for HI3 (male and female map), at position 17 cM (female map) and 18 cM (male map) for LF3 and at position 16 cM (female map) and 24 cM (male map) for NF (Table 4; Fig. 2). Given the mean confidence interval (18 cM), it is highly likely that these QTLs corresponded to the same gene. There were examples suggesting common QTLs for other growth traits: there was one QTL on LG11 at position 36 cM for H3, and at position 44 cM for NF and one QTL at position 25 cM on LG 3 for LF3 and HI3.

Discussion

Genetic variation and heritability

Because vegetative reproduction was used to propagate the full sibs of the mapping pedigree, we were able to estimate the components of the genetic variance in *Q. robur*, assuming that dominance was low to moderate. Heritability values were higher for bud burst than for growth components (Table 2).

Concerning bud burst, heritability estimates were of the same magnitude, whether bud burst was assessed by the score or by the number of days elapsed since the first tree flushed. In addition, these estimates were rather large in comparison to other traits routinely assessed in forest trees (Cornelius 1994; Kremer 1994). Strong genetic control of bud burst was also found in poplar, where the repeatability varied from 94% (Frewen et al. 2000) to 98% (Bradshaw and Stettler 1995) and in *Betula pubescens* (Billington and Pelham 1991). Our results were also similar to those reported in a recent review on genetic variation of bud flush (Howe et al. 2003). In this review, narrow-sense heritabilities varied between 0.25 and 0.87 among seven species, whereas in our case they varied between 0.15 and 0.52, depending on the trait, the test and the level of dominance. However heritability and repeatability values exhibited important variations between field tests 2 and 3. These differences may be related to the after effects of transplantation that increase environmental variation shortly after plantation. Repeatability and heritability were two times lower in field test 1, where assessments were made the first year following plantation, whereas in field test 2 assessments were made 2 years after plantations. There were also differences in the experimental design between the field tests, as the number of clones was lower in field test 2 (Table 1). If early- or late-flushing trees were omitted in field test 2 due to these constraints, then the genetic variation of bud burst may have been reduced, as suggested by the lower repeatability values. To sum up, our study confirmed the wide range of variation of the date bud burst in oaks, and that an important part of this variation was due to genetic effects. Within one single full-sib family, the date of bud burst can vary over 3 weeks (see Table 2). Interestingly the same range (up to 25 days) was observed repeatedly over 7 years in a population of *Q. robur* (Crawley and Akhteruzzaman 1988), raising the question of the existence and maintenance of such a large genetic variation within populations. As in other tree species, between-population variation has also been reported in oaks, although the latitudinal clinal trend was in most cases different to the one observed in conifers (Kleinschmit and Svolba 1994; Ducousso et al. 1996; Stephan et al. 1994). The extensive within-population variation can not solely be driven by the climatic (temperature) selective factors (Hunter and Lechowicz 1992). The development of numerous defoliating insects feeding on European oaks (Skuhravy et al. 1998) requires phenological coincidence with oak bud development (Hunter 1990; Ivashov et al. 2002). Synchrony between the host and the insect can also vary for specific stages of bud development—some insects (*Operophtera brumata*, the winter moth; *Tortrix viridana*, the oak leaf roller moth) feed on juvenile developing leaves, whereas others (*Lymantria dispar*, the gypsy moth; *haumetopea processionnea*, the oakprocessionary moth) feed on aged leaves. Insects with different phenological requirements represent contrasting selective pressures that may act in opposing directions, contributing to maintain both early- and late-flushing trees in the same population. In addition the high intraspecific phenological variation in *Q. robur* may be seen as a mechanism for protection at the population level against

defoliating insects. When phenological variation is large, there is less opportunity for colonisation of insect herbivores (Tikkanen and Julkunen-Tiitto 2003).

Heritability for growth components was moderate and varied from 7% for the number of flushes to 23% for the total height. The repeatability was higher and varied from 26% to 58%. In other broadleaves, recent data have indicated higher repeatability values. In *Salix* for example, repeatability for height growth ranged from 57% to 86% (Tsarouhas et al. 2002) and in poplar from 66% to 76% (Wu and Stettler 1994). In forest trees in general, genetic variation of height growth is extremely variable across studies and species as shown by reviews on the subject (Kremer 1994; Cornelius 1994) due to the composite structure of the trait.

QTL detection

The number of QTLs detected at the chromosome level for the date of bud burst varied between 8 and 15, depending the field test used. There was at least one QTL per LG, suggesting that the number of QTLs for this trait may be larger, as more QTLs can be suspected on several LGs. The contribution of a QTL to the variance of the clonal mean for bud burst varied between 3.1% and 10.7%, which represented from 1.8% to 16.7% of the additive genetic variance (1% to 4% of the phenotypic variance). Results from QTL detection in other forest species show a slightly different picture, with fewer QTLs detected in conifer or broadleaved species. The number of QTLs contributing to terminal bud flush in Douglas fir varied between years and testing sites (Jermstad et al. 2001). From one and five QTLs were found for a given year in a given site, explaining between 2% and 12% of the phenotypic variance. These results were confirmed in a more recent experiment conducted in controlled environments. Cuttings of 408 full-sib clones belonging to a three-generation pedigree were raised in six different treatments. Seven QTL were detected on six LGs and were present in five of the six treatments (Jermstad et al. 2003). In Scots pine four QTLs were detected with coefficients of determination ranging from 3% to 13% (Hurme et al. 2000). In poplar contrasting results were obtained in different studies. Frewen et al. (2000) found six QTLs explaining from 5% to 16.6% of the clonal mean variance, whereas Bradshaw and Stettler (1995) found five QTLs, and each contributed from 29% to 52% of the phenotypic variance. In *Salix*, where a multisite experiment was conducted (two outdoor plantations and one indoor), six QTLs were detected—four in the outdoor plantations and two in the indoor replicate test (Tsahouras et al. 2003). In another member of the Fagaceae family (*Castanea sativa*), 13 QTLs were detected (Casasoli et al. 2004). These comparisons should, however, be taken with caution, as the number of full-sibs-type of experiments (use or not of vegetative propagation) and threshold *P*-value for detecting QTLs varied markedly among the different studies. In our study, QTLs for bud burst were detected in three different tests, allowing comparisons across tests. In addition to the assessments made in the nursery on the ortets (field test 1), the offspring of the mapping population were vegetatively propagated, and two replicated field tests were successively installed. Among the 12 LGs carrying non-ambiguous QTLs, nine comprised QTLs present in at least two field tests and five in all three sites. Even if QTLs present on the same chromosome may actually be different, their position and confidence interval suggest that most of them were most likely the same. These results indicated that in oak, bud burst is predominantly controlled by 'general' QTLs that are expressed under different environmental conditions as suggested also by the correlation among the different tests (Fig. 2). Although comparisons of the number of QTL across species should be made with caution, our results suggest that bud flush in oaks is controlled by more QTLs than in other species, with the exception of *Castanea*. As stressed earlier, bud flush in oaks can respond to contrasting selective forces (abiotic and biotic), leading to a composite architecture of the trait and hence, increasing the number of potential QTLs contributing to bud burst. A higher number of QTLs may also be a peculiar feature of the Fagaceae family. *Q. robur* (pedunculate oak) and *C.sativa* (European chestnut) exhibit a high number of QTLs for bud flush (our study and Casasoli et al. 2004), show strong phylogenetic relationship (Manos et al. 2001) and their genetic maps are collinear (Barreneche et al. 2004).

The genetic architecture of height growth was different to that of bud burst. The number of QTLs per growth component was lower (from two to four), and each contributed to a larger proportion of the clonal mean variance (from 4% to 19%). Interestingly each component was controlled by a QTL located in the same region on LG10. Similar results were obtained in the *Pinus taeda*, where two QTLs were detected which explained from 4% to 11% of the phenotypic variance (Kaya et al. 1999) and in *P. silvestris*, three QTLs were detected with also moderate effects (11–13%).

Some QTLs of bud burst and height growth were located in the same regions on LGs 3M, 10M and 10F, contributing to the significant correlation that was observed between the two traits. The positive correlation between growth traits and bud burst indicated that trees flushing late tended to have longer shoots. An opposite trend was observed in older oak trees by Kleinschmit and Svolba (1994) and in Aspen clones, where correlations between growth traits and growth initiation were negative (−0.66, Yu et al. 2001). Opposite trends at different ages may be due to developmental behaviour. However at the juvenile stage, association of late flushing and rapid growth would increase the fitness of trees that would have to face late frost and strong competition for light. Our results also showed that the results (heritability, repeatability, number of QTLs) were quite similar regardless the method used for assessing bud burst (either N or SD). Hence we recommend for future

experimental work to concentrate on SD, as the assessment of SD is less costly in time and effort.

To conclude, this study showed that the genetic architecture of two important traits related to the fitness of trees may have different genetic architecture. Bud burst, despite its large genetic variance, is controlled by many QTLs with rather moderate to low effects, whereas juvenile height growth with lower heritability is controlled by fewer QTLs with moderate effects.

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