Computational identification of genes modulating stem height–diameter allometry

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Introduction

The allocation pattern of stem growth to height and diameter is a key characteristic of a tree that reflects its capacity to adapt to various environmental conditions (Feldpausch et al., 2011; Franco and Kelly, 1998; Niklas and Spatz, 2004; Price et al., 2007). As a key element of stem form, the allometric relationship between stem height and diameter determines the volume of the harvestable stem and carbon storage and is also thought to be associated with wood structure, for example lignin and cellulose content (Voelker et al., 2011a,b; Waghorn et al., 2007). Differences in the height–diameter relationship arise from differences in biomass allocation throughout the stem, affected by growth conditions, such as spacing, biomechanical factors, site quality and age (Feldpausch et al., 2011; Ilomaki et al., 2003; Niklas, 1995). It has been observed that the relationship between tree height and diameter varies substantially along spatial and environmental gradients and that such pronounced differences also occur at the species, population, family and genotype levels. For example, light-demanding (pioneer) species typically have a slender stem, assisting them to quickly attain or maintain position in the canopy (Harrington and DeBell, 1996; King, 1990; Poorter et al., 2003). Populations in higher altitudes that encounter frequently high winds and snows might evolve stouter stems and crowns to withstand those extreme climates than those from the same species in lower altitudes (Feldpausch et al., 2011; Hulshof et al., 2015; Scarano, 2002).

Summary

The developmental variation in stem height with respect to stem diameter is related to a broad range of ecological and evolutionary phenomena in trees, but the underlying genetic basis of this variation remains elusive. We implement a dynamic statistical model, functional mapping, to formulate a general procedure for the computational identification of quantitative trait loci (QTLs) that control stem height–diameter allometry during development. Functional mapping integrates the biological principles underlying trait formation and development into the association analysis of DNA genotype and endpoint phenotype, thus providing an incentive for understanding the mechanistic interplay between genes and development. Built on the basic tenet of functional mapping, we explore two core ecological scenarios of how stem height and stem diameter covary in response to environmental stimuli: (i) trees pioneer sunlit space by allocating more growth to stem height than diameter and (ii) trees maintain their competitive advantage through an inverse pattern. The model is equipped to characterize ‘pioneering’ QTLs (piQTLs) and ‘maintaining’ QTLs (miQTLs) which modulate these two ecological scenarios, respectively. In a practical application to a mapping population of full-sib hybrids derived from two Populus species, the model has well proven its versatility by identifying several piQTLs that promote height growth at a cost of diameter growth and several miQTLs that benefit radial growth at a cost of height growth. Judicious application of functional mapping may lead to improved strategies for studying the genetic control of the formation mechanisms underlying trade-offs among quantities of assimilates allocated to different growth parts.

Keywords: height–diameter allometry, mathematical equation, functional mapping, quantitative trait loci.
functional mapping, a dynamic model of QTL mapping using mathematical aspects of trait formation and development (Li and Wu, 2010; Ma et al., 2002; Wu and Lin, 2006; Wu et al., 2004; Li & Sillanpaa, 2015), can facilitates our understanding of the developmental genetic architecture of height–diameter relationship in forest trees. In living systems, biological traits are conferred a function to alter their phenotypes in better response to environmental or developmental signals, during which there must exist a particular set of genes that regulate or reflect such alteration (Gilchrist and Nijhout, 2001; Salazar-Ciudad and Jernvall, 2010; Wang et al., 2005). The estimation of the temporal pattern of genetic regulation allows us to assess the dynamic interplay of genes and development. This question of fundamental importance that remains unanswered from conventional genetic mapping has become tractable through functional mapping. Specific genes that act by changing their effects during development have been identified for age-specific body mass index (Das et al., 2011) and mouse body growth (Wu et al., 2004, 2005). Through extensive simulation in previous studies (Li and Sillanpaa, 2013), increased power of detecting significant genes was observed using functional mapping than single-trait analysis. This finding, from a methodological perspective, strongly argues for the immediate application of functional mapping to dynamic height–diameter allometry. In this article, we implement functional mapping to study the genetic architecture of how height and diameter covary during development and further explore its application to map and identify QTLs for this covariation by analysing a practical mapping data for a forest tree.

**Results**

**Mathematical modelling of height–diameter allometry**

Through stem analysis, we obtained the annual growth data of stem height (H) and diameter (D) at stem base in a full-sib family of *Populus* during the first 24 growing seasons. The developmental pattern of H-D allometry was plotted in Figure 1, where two parents, I-69 and I-45, were observed to display a dramatic difference in the H-D allometry over development. The trunk of small-sized I-45 is consistently more slender in ontogeny than that of large-sized I-69, and the progeny derived from these two parents produce remarkable segregation in both stem height and diameter and their developmental relationship. Considerable variation in the H-D relationship trajectory among progeny reveals possible involvement of specific genes that control this trait. By comparing a series of commonly used H-D equations given in Huang et al. (1992), we finally found that equations (1a) and (1b) are the most parsimonious ones that best fit H-D relationships in polar hybrids. Thus, by estimating power coefficients ($a_{pi}$, $b_{pi}$) and ($a_{mi}$, $b_{mi}$) from these equations, we can illustrate how stem height scales with stem diameter and how stem diameter scales with stem height, respectively.

**QTLs for height–diameter allometry**

By integrating equations (1a) and (1b) into functional mapping, we test how a QTL controls allometric scaling of height and diameter during ontogeny. The integration of each equation can reveal a different view of ecological significance underlying the genetic control of height–diameter allometry. Equation (1a) describes an ecological process of how stem diameter invests competitive environment. Given the same growth of stem diameter, a taller height has a better capacity to increase the exposure of leaves to light, increase the shading of competitors and elevate reproductive or dispersal organs than a shorter height. Significant genes that control the change in height growth with diameter growth detected by equation (1a) are called ‘pioneering QTLs’ (or piQTLs), because they may play a pioneering role in activating trees to capture spatial resources for the optimal fitness of the future.

Equation (1b) specifies different ecological processes in which spatial advantage can be maintained by investing radial growth. Among those trees of the same height, stout ones can preserve the potential of growth towards next stages of competition than slender ones. Those significant genes that determine the increasing amount of radial growth resulting from height growth detected by equation (1b) are named ‘maintaining QTLs’ (or miQTLs) in terms of their role in maintaining the spatial advantage of trees in a competing environment. The identification of piQTLs and miQTLs helps to interpret the mechanistic basis of H-D allometry in response to environmental perturbations.

**PiQTLs: How height growth scales with diameter growth**

Functional mapping based on equation (1a) leads to the detection of 16 significant piQTLs responsible for the way how stem height growth is determined by the growth of stem diameter (Table 1; Figure 2a). These piQTLs are distributed on different regions, one on chromosome 6, 9, 11 and 16, two on chromosome 5 and five on chromosome 8 and 14, respectively. As shown in Figure S1, genotypic differences in H-D curves by equation (1a) have remarkable goodness of fit to the raw data. Different genotypes at each piQTL detected vary in the pattern of how height scales with diameter, and also different piQTLs exemplify different patterns of allometric scaling. Figure 3a illustrates how different piQTLs affect the change in stem height growth as a function of stem diameter. It seems that both additive and dominant effects have been at work, following a similar pattern of determining height–diameter allometry. Although the direction of genetic effects may be different, all piQTLs display an increasing influence on the height–diameter relationship during the early stage of growth. When trees achieve a certain size, say 11 cm in stem diameter, genetic effects decrease with increasing tree size.

Based on estimated genotype-specific mathematical parameters $a_{pi}$ and $b_{pi}$ from equation (1a) (Table S1), we can draw the profile of stem height and diameter growth. Genotype CC at piQTL 5/4226091 is taller than genotypes CT and TT when they achieve the same diameter at a late stage of growth (Figure 4). However, in early stages, genotype CC and CT are similar in height–diameter allometry, taller than genotype TT of the same diameter. These suggest that this piQTL exerts a time-varying effect on stem form during ontogeny. Similar phenomena can be observed for the other piQTLs.

**MiQTLs: How diameter growth scales with height growth**

Functional mapping based on equation (1b) identified 11 significant miQTLs that determine the allometric scaling of stem diameter with stem height (Table 1; Figure 2b), of which one is distributed on chromosome 7, 8, 9 and 16, two distributed on chromosome 14 and five distributed on chromosome 5. It can be seen that the detected miQTLs can well fit the raw data of stem diameter scaling with height (Figure S2). Genetic effects on radial growth, including additive and dominant effects, at all miQTLs identified increase consistently with stem growth (Figure 3b), but there is substantial difference in the trajectory of additive and
dominant effects at the same miQTL. For example, the additive curve of miQTL 5/4220981 on chromosome 5 is convex with height growth, whereas its dominant curve is concave. Estimated parameters ($a_{d_{0...h}}$ and $b_{d_{0...h}}$) from equation (1b) (Table S2) were used to draw the profiles of tree stem for individual genotypes. With the same stem height, genotype CC at miQTL 5/4220981 is stouter than genotype CT, which is stouter than genotype TT (Figure 5). This shows that this miQTL determines the capacity of a tree to maintain its competitive advantage in a crowd stand. Differences in stem profiles between different genotypes can also be seen at the other miQTLs.

It is interesting to note that there are four piQTLs each that also act as a miQTL; that is, these QTLs simultaneously govern the allometric scaling of stem height with stem diameter and the allometric scaling of stem diameter with stem height. These shared QTLs are located on chromosome 8 (8/10163499), chromosome 9 (9/8371476) and chromosome 14 (14/763510 and 14/17660801). As these two aspects of allometry present different biological processes, the QTLs detected for both types are thought to be pleiotropic. However, their effects on the two aspects of H-D allometry were found to display different significance levels, suggesting the existence of QTL–development interactions. It is interesting to see that miQTL 5/4226091 is very close to miQTL 5/4220981 in a similar region of chromosome 5, suggesting that their linkage may be a cause of a tree’s pioneering and maintaining capacity for growth resource.

Functional annotation of QTLs

A critical issue about results from genetic mapping is how to interpret the biological function of significant QTLs detected. Among the piQTLs detected to affect the developmental allometry of height with diameter by functional mapping, more than half (9/16) are located near candidate genes for cell metabolism, growth and differentiation (Table 1). For example, piQTL 8/10163499 is in the proximity of genes that encode ribosomal protein S24e family, and piQTL 14/791796 is located in the gene family that encodes P-loop containing nucleoside triphosphate hydrolases superfamily protein. Six of the 11 miQTLs are related to candidate of known function (Table 1). For example, miQTL 5/4804826 is near the gene that encodes CBS/octicosapeptide/Phox/Bemp1 (PB1) domain-containing protein. The biological function of those QTLs whose annotations are not available remains to be validated.

Discussion

Tree height for a given diameter may vary strikingly among species (King, 1996) and across biogeographical regions (Feldpausch et al., 2011; Hulshof et al., 2015). Such variation could hold important implications for carbon storage potential of forests, but their underlying genetic control mechanisms has been long a puzzle. Currently, emerging genomewide association studies are equipped with a capacity to unravel a complete resolution of the genetic architecture of complex traits by identifying individual genes involved in phenotypic variation, their number, locations, actions and interactions, and pleiotropy (Wang et al., 2005). Given that any trait undergoes a dynamic process, the integration of developmental processes that give rise to a final phenotype into association studies has become essential, in order to better chart a genotype–phenotype map (Salazar-Ciudad and Marin-Riera, 2013). The basic principle and procedure for such integration have been recorded in functional mapping (Wu and Lin, 2006) or functional genomewide association studies (FGWAS) (Das et al., 2011). By utilizing mathematical aspects of trait formation and development, these approaches have been shown to be both statistically powerful and biologically relevant (Li and Sillanpää, 2016).

This study presents the application of functional mapping to unveil the genetic architecture of stem height–diameter allometry by integrating biologically meaningful equations of this phenomenon (Henry and Aarssen, 1999; Huang et al., 1992; Hulshof et al., 2015). This new application can not only characterize how individual genes affect dynamic variation in stem apical and radial growth, but also explore how these two growth processes are related by pleiotropic genes or different genes in a strong linkage. In nature, stem height–diameter relationship is a predictor of
Table 1  A list of piQTLs for the allometry of tree height with stem diameter and miQTLs for the allometry of stem diameter with tree height in a full-sib family of interspecific hybrids

### Mapping height–diameter allometry

\[ H(t) = H_0 + e^{aD + bH} \]

| No. | piQTL Chr. Position Type Alleles | P-value | Biological function |
|-----|-------------------------------|---------|---------------------|
| 1   | 5/4220981 5 4220981 | C/T | 5.90 × 10^{-5} | Intergenic |
| 2   | 5/24780848 5 24780848 | G/A | 2.76 × 10^{-4} | Intergenic, POPTR_0005s26940.1 LSD1-like 1 AT5G62830.1 |
| 3   | 6/17682099 6 17682099 | G/T | 6.00 × 10^{-5} | Intergenic |
| 4   | 8/10141590 8 10141590 | T/C | 2.10 × 10^{-4} | Intergenic, POPTR_0008s15160.1 AT5G43150.1 |
| 5   | 8/10163499 8 10163499 | G/T | 4.90 × 10^{-5} | Intergenic, POPTR_0008s15190.1 Ribosomal protein S24 family AT5G28060.1 |
| 6   | 8/10214577 8 10214577 | C/A | 2.41 × 10^{-4} | Intergenic |
| 7   | 8/10214969 8 10214969 | A/G | 2.10 × 10^{-4} | Intergenic |
| 8   | 5/24783080 5 24783080 | G/A | 2.76 × 10^{-4} | Intergenic, POPTR_0005s26940.1 LSD1-like 1 AT5G62830.1 |
| 9   | 6/17682099 6 17682099 | G/T | 6.00 × 10^{-5} | Intergenic |
| 10  | 11/6458021 11 6458021 | G/A | 2.30 × 10^{-5} | Intergenic |
| 11  | 14/139257 14 139257 | T/G | 1.25 × 10^{-4} | Intergenic |
| 12  | 14/763510 14 763510 | G/T | 2.89 × 10^{-4} | Intergenic |
| 13  | 14/791796 14 791796 | C/T | 2.92 × 10^{-4} | Intergenic |
| 14  | 14/807942 14 807942 | T/A | 2.59 × 10^{-4} | Intergenic |
| 15  | 14/17660801 14 17660801 | A/G | 2.00 × 10^{-4} | Intergenic |
| 16  | 16/11358187 16 11358187 | C/G | 2.00 × 10^{-4} | Intergenic |

### Mapping stem diameter–height allometry

\[ D(t) = D_0 + e^{aH + bD} \]

| No. | miQTL Chr. Position Type Alleles | P-value | Function |
|-----|-------------------------------|---------|----------|
| 1   | 5/4220981 5 4220981 | T/C | 2.56 × 10^{-4} | Intergenic, POPTR_0005s06350.1 Disease resistance protein (TIR-NBS class), putative AT5G16990.2 |
| 2   | 5/2421532 5 2421532 | G/C | 2.68 × 10^{-4} | Intergenic |
| 3   | 5/2422282 5 2422282 | C/T | 2.13 × 10^{-4} | Intergenic |
| 4   | 5/2426091 5 2426091 | C/T | 9.90 × 10^{-5} | Intergenic |
| 5   | 5/4804826 5 4804826 | G/C | 2.62 × 10^{-4} | Intergenic |
| 6   | 7/13898377 7 13898377 | T/C | 3.05 × 10^{-4} | Intergenic, POPTR_0005s07150.1 CBS/secticosapentapeptide/Phox/ Bemp1 (PB1) domain-containing protein AT3G52950.1 |
| 7   | 8/10163499 8 10163499 | G/T | 2.69 × 10^{-4} | Intergenic, POPTR_0008s15190.1 Ribosomal protein S24 family AT5G28060.1 |
| 8   | 9/8371476 9 8371476 | A/T | 2.41 × 10^{-4} | Intergenic, POPTR_0009s09470.1 Protein phosphatase 2C family AT4G33920.1 |
| 9   | 14/763510 14 763510 | G/T | 3.14 × 10^{-4} | Intergenic |

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species’ geographical distribution and ecosystem functioning (Feldpausch et al., 2011; Hulshof et al., 2015). Better height growth for a given diameter enables trees to acquire an advantage of competing for sunlight (Moles et al., 2009). Thus, any genes that modulate the change in stem height as a function of diameter are likely to participate in the pioneering process of space occupation. By analysing a GWAS data of poplar hybrids grown in a field trial, functional mapping shows it unique power to identify and map these so-called pioneering QTLs ($piQTLs$). We have found 16 such $piQTLs$ that mediate the allometric change in stem height with stem diameter, some of which have been validated in terms of their biological function.

After trees obtain a spatial advantage, how to maintain this advantage through strengthening mechanical support (McMahon, 1973; Niklas, 1993) and water transport efficiency (Bullock, 2000) will quickly become their next task in order to maximize the fitness of trees. More rapid stem radial growth for a given stem height can facilitate the maintenance of competing advantage. Therefore, any genes that are associated with the change in stem diameter growth with stem height may play an important role in modulating the investment of energy in structural support. A total of 11 such so-called maintaining QTLs ($miQTLs$) have been identified by GWAS from the poplar GWAS experiment. Unlike $piQTLs$ that operate mostly before the emergence of spatial competition, these $miQTLs$ may be activated after competition sets in the stand. However, $piQTLs$ and $miQTLs$ are not exclusive from each other because many of them pleiotropically affect both the allometric scaling of stem height with stem diameter and the allometric scaling of stem diameter with stem height, although QTL–development interactions are also identified.

In general, the expected sizes of genetic effects on a complex trait are small and, also, the expected number of false-positive findings can be large so that large sample sizes and replication samples are needed (Wang et al., 2005). However, unlike other small-sized and well-funded organisms, it is practically challenging to obtain large sample sizes for forest trees. Forest trees have many desirable properties which can compensate for a small sample size. Through intra- or interspecific cross, they can generate a full-sib family with homogeneous genetic background, requiring no extra samples needed to remove the influence of population structure and other covariates that leads to spurious associations as seen in human genomewide association studies (Manolio, 2010). The study material of forest trees can be grown in a uniform environment and allow destructive sampling for precise phenotyping, which increases the heritability of a complex trait and, therefore, the power of gene identification, by reducing environmental noises and measurement errors. These advantages, plus repeated measurement of the same phenotype at a series of time points, can augment the amount of informativeness involved in trait variation for forest tree association studies.

This article describes and demonstrates a study of using functional mapping to map growth allometry. The idea behind the study deserves a widespread application to more comprehensive experiments. For example, our current study was established in a single environment, but this is obviously inadequate to

![Figure 2](image-url)
study the genetic control of how height–diameter allometry responds to changing environment. However, possible vegetative propagation of trees allows the study population to be replicated in time and space, enabling the dissection of genetic and environmental contributions to developmental variation (Wu, 1996). In particular, since height–diameter allometry is sensitive to density, field studies laid with multiple sets of spacing using clonal replicates allow the identification of how piQTLs or miQTLs alter their expression in response to the change in environmental conditions like sunlight and resources.

Association studies based on a simple regression approach may be limited for statistical inference about the genetic basis of quantitative traits. By simultaneously analysing all possible SNPs, this issue can be resolved from a variable selection model, such as LASSO, allowing a subset of predictors to be identified from genetic data in which the number of variables is far larger than the number of samples (Li et al., 2011; Mutshinda and Sillanpaa, 2010). More recently, Li et al. (2015) has incorporated variable selection into fGWA$^2$, allowing geneticists to chart a global picture of genetic control over developmental processes of any complex traits.

**Experimental procedures**

**Mapping design**

We generated a mapping population composed of full-sibs derived from interspecific cross between female clone I-69 of cottonwood (Populus deltoides) introduced from the United State...
and male clone I-45 of P. ×euramerica, a hybrid between P. deltoides and P. nigra originally from Europe. In 1987, this family containing 450 hybrid trees was planted with ramets in a uniform site in terms of soil physical properties, moisture and nutrient contents at Zhangji Forest Farm, Xuzhou, Jiangsu. After growing in the field for 24 years, part of the family (64 hybrids and two parents, I-69 and I-45) was harvested for stem analysis, from which annual growth for stem height and diameter was measured.

The harvested trees were genotyped at SNPs for genomewide mapping. Genotyping was performed on the Applied Biosystems™ QuantStudio™ 12K Flex Real-Time PCR System. SNP genotypes were obtained after stringent quality-control filters. To the end, a total of 156 362 segregating SNPs were obtained. Of these, 94 591 and 61 771 belongs to the testcross and intercross markers, respectively. In a full-sib family derived from two heterozygous tree parents, there are two possible marker types that are segregating with different patterns: (i) testcross

![Figure 4](image1.png) Developmental allometry of how stem height scales with stem diameter, modulated by πQTL 5/4226091 with three different genotypes CC, CT and TT. Red lines show the differences of stem height growth among three genotypes at five representative ages.

![Figure 5](image2.png) Developmental allometry of how stem diameter scales with stem height, modulated by mQTL 5/4220981 with three different genotypes TT, TC and CC. Genotype CC is much thicker than the other two (TT, TC) per the same amount of stem height growth.
markers at which one parent is heterozygous but the other is homozygous, and (ii) intercross markers at which both parents are heterozygous (Lu et al., 2004; Maliepaard et al., 1997; Tong et al., 2011; Wu et al., 2002).

We assume that all progeny are planted in a randomized design in a field trial, measured longitudinally for both stem height and diameter growth at a multitude of ages during ontogeny. From this experiment, we obtain both marker and dynamic phenotypic data (with T time points) whose structure is schematically shown in Table 2, where we will test whether any of these markers (denoted as 1, ..., p) is significantly associated with stem growth phenotype. Traditional approaches test the significance of association between marker genotypes and phenotypes measured at a static time. Functional mapping breaks through the limitation of traditional approaches by associating marker genotypes with longitudinal phenotypes across a series of time points. This treatment allows the characterization of how a QTL governs the dynamic change in phenotypic traits. Here, we show that functional mapping can be extended to map dynamic trait–trait relationships during ontogeny, which can shed light on the pleiotropic control mechanisms of trait correlations.

Functional mapping for H-D allometry

Tremendous efforts have been made to quantify the allometric relationship of stem height and diameter by deriving robust mathematical equations (Henry and Aarssen, 1999; Huang et al., 1992; Hulshof et al., 2015). One example of describing how stem height and diameter covary for popular species is expressed as

\[ H(t) = H_0 + e^{a_{\text{h-d}}t} \]  
\[ D(t) = 1 - \ln(H(t) - H_0) - a_{\text{h-d}}b_{\text{d-h}} \]

where \( H(t) \) and \( D(t) \) are the stem height and diameter of a given tree at time \( t \), \( H_0 \) is the stem height in the establishment year; and \( a_{\text{h-d}} \) and \( b_{\text{h-d}} \) are two different power coefficients of stem height scaling with stem diameter in forest trees, whereas \( a_{\text{d-h}} \) and \( b_{\text{d-h}} \) are those of stem diameter scaling with height.

Although equations (1a) and (1b) are mathematically interchangeable in describing the H-D relationship, they differ in the direction of allometric scaling. Equation (1a) describes how stem height increases in response to the increase in stem diameter through parameters \( a_{\text{h-d}} \) and \( b_{\text{h-d}} \), whereas equation (1b) specifies the impact of stem height on radial growth during ontogeny through parameters \( a_{\text{d-h}} \) and \( b_{\text{d-h}} \). As will be shown below, \( (a_{\text{h-d}}, b_{\text{h-d}}) \) and \( (a_{\text{d-h}}, b_{\text{d-h}}) \) are two different sets of parameters, if the genotypic value of a QTL as a response at the left side of equation (1a) or (1b) is predicted by the phenotypic value as a dependent variable at the right side.

The integration of functional mapping into a QTL mapping study of H-D allometry can be made through a multiplicative likelihood of different marker genotypes (Das et al., 2011). As all progeny are from a single full-sib family, they share the same mutual relationship based on quantitative genetic theory. Let \( y_i = (y_i(1), ..., y_i(T)) \) and \( z_i = (z_i(1), ..., z_i(T)) \) denote stem height and diameter growth over a series of \( T \) time points, respectively. Their likelihoods are formulated as

\[ L(y) = \prod_{j=1}^{n} f(y_j; \mu_j^y, \Sigma_j^y), \]
\[ L(z) = \prod_{j=1}^{n} f(z_j; \mu_j^z, \Sigma_j^z), \]

where \( j \) stands for the \( j \)th genotype of a SNP \( \{j = 1, ..., J\} \), \( n_j \) is the observation of the \( j \)th genotype, and \( f(y_j; \mu_j^y, \Sigma_j^y) \) and \( f(z_j; \mu_j^z, \Sigma_j^z) \) are multivariate normal distributions of stem height and diameter, respectively, with expected mean vector of genotype \( j \) for progeny over \( T \) time points, expressed as

\[ \mu_j^y = \left( \mu_j^y(1), ..., \mu_j^y(T) \right) \]
\[ \mu_j^z = \left( \mu_j^z(1), ..., \mu_j^z(T) \right) \]

and \( (T \times T) \) longitudinal matrix composed of time-dependent variances and covariances expressed as

### Table 2 Marker and phenotypic data structure of a full-sib family derived from two parents \( P_1 \) and \( P_2 \). Height and diameter covary over age in equation (1a) or (1b).  

| No. | Marker | Parent | Progeny | Longitudinal phenotype | Height | Diameter |
|-----|--------|--------|---------|------------------------|--------|----------|
|     |        |        |         | \( y_1(1), y_1(T) \) | \( z_1(1), z_1(T) \) |          |
|     |        |        | 1       | \( y_1(1), y_1(T) \) | \( z_1(1), z_1(T) \) |          |
|     |        |        | 2       | \( y_1(1), y_1(T) \) | \( z_1(1), z_1(T) \) |          |
|     |        |        | 3       | \( y_1(1), y_1(T) \) | \( z_1(1), z_1(T) \) |          |
|     |        |        | 4       | \( y_1(1), y_1(T) \) | \( z_1(1), z_1(T) \) |          |
|     |        |        | 5       | \( y_1(1), y_1(T) \) | \( z_1(1), z_1(T) \) |          |
|     |        |        | 6       | \( y_1(1), y_1(T) \) | \( z_1(1), z_1(T) \) |          |
|     |        |        | 7       | \( y_1(1), y_1(T) \) | \( z_1(1), z_1(T) \) |          |
|     |        |        | 8       | \( y_1(1), y_1(T) \) | \( z_1(1), z_1(T) \) |          |
|     |        |        | \( n \) | \( y_1(1), y_1(T) \) | \( z_1(1), z_1(T) \) |          |

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growth equation (1a) or (1b), which is expressed as

\[
\Sigma_g^T = \begin{pmatrix} \sigma_g^2(1) & \cdots & \sigma_g(n,1) \\ \vdots & \ddots & \vdots \\ \sigma_g(n,1) & \cdots & \sigma_g^2(T) \end{pmatrix}
\]

(4a)

\[
\Sigma_D^T = \begin{pmatrix} \sigma_D^2(1) & \cdots & \sigma_D(n,1) \\ \vdots & \ddots & \vdots \\ \sigma_D(n,1) & \cdots & \sigma_D^2(T) \end{pmatrix}
\]

(4b)

Functional mapping models the mean vector (2a) and (2b) by a biologically meaningful mathematical equation, such as allometry growth equation (1a) or (1b), which is expressed as

\[
\mu_j^H(t) = \begin{pmatrix} \mu_{h,j}(1) \\ \vdots \\ \mu_{h,j}(T) \end{pmatrix} = \begin{pmatrix} H_{0,j} + e^{a_{h,j} \cdot d - \frac{1}{2} \sigma_{h,j}^2} \\ \vdots \\ H_{0,j} + e^{a_{h,j} \cdot d - \frac{1}{2} \sigma_{h,j}^2} \end{pmatrix}
\]

(5a)

\[
\mu_j^D(t) = \begin{pmatrix} \mu_{d,j}(1) \\ \vdots \\ \mu_{d,j}(T) \end{pmatrix} = \begin{pmatrix} 1 - \frac{\ln(y(1) - H_0) - a_{d,j} \cdot b_{d,j} \cdot D_{d,j}}{\ln(y(T) - H_0) - a_{d,j} \cdot b_{d,j} \cdot D_{d,j}} \\ \vdots \\ 1 - \frac{\ln(y(T) - H_0) - a_{d,j} \cdot b_{d,j} \cdot D_{d,j}}{\ln(y(T) - H_0) - a_{d,j} \cdot b_{d,j} \cdot D_{d,j}} \end{pmatrix}
\]

(5b)

where power parameters \(a_{h,j} \cdot D_{h,j}\) and \(a_{d,j} \cdot b_{d,j} \cdot D_{d,j}\) are defined for genotype \(j\). Equations (5a) or (5b) indicates that the genotypic value of one growth trait at a time is modelled by the phenotypic value of its allometrically related trait. This can be more generally expressed as

\[
\mu_j^H(t) = h(a_{h,j} \cdot b_{h,j} \cdot d; \mu_j^H(t) + e_j^H(t))
\]

(6a)

\[
\mu_j^D(t) = d(a_{d,j} \cdot b_{d,j} \cdot D; \mu_j^D(t) + e_j^D(t))
\]

(6b)

where \(\mu_j^H(t)\) and \(\mu_j^D(t)\) are the genotypic value of stem height and diameter at time \(t\), respectively, which is a function of the phenotypic value of its allometric trait in form \(h\) or \(d\), respectively, and \(e_j^H(t)\) and \(e_j^D(t)\) are the residual errors of these two traits at time \(t\), with a longitudinal covariance matrix (4a) or (4b). Because \(e_j^H(t)\) and \(e_j^D(t)\) have different structures, parameters \(a_{h,j} \cdot b_{h,j} \cdot d\) and \(a_{d,j} \cdot b_{d,j} \cdot D\) estimated from functions \(h\) and \(d\) should be different even though these two functions are interchangeable.

Functional mapping also models the covariance matrices (4a) and (4b) by a parsimonious and flexible approach, such as an autoregressive (Ma et al., 2004), antedependence (Zhao et al., 2005), autoregressive moving average (Li et al., 2010) or nonparametric and semiparametric approaches (Das et al., 2011). The first-order autoregressive (AR(1)) approach is computationally efficient, but needs the stationarity assumption. Compared with this approach, the others are more flexible, but may be computationally more expensive. By analysing the raw data, it was observed that diameter-dependent variation in height (Figure 1a) and height-dependent variation in diameter (Figure 1b) are quite stationary over the dependent variable. Thus, the computationally efficient AR(1) approach was used to model the covariance structure of matrices (4a) and (4b).

Functional mapping has been implemented with several statistical algorithms for obtaining the maximum likelihood estimates (MLEs) of genotype-specific growth parameters and the parameters that model the covariance structure. These algorithms include the EM algorithm and some optimization techniques, like the simplex algorithm (Ma et al., 2002; Zhao et al., 2005).

Testing pioneering QTLs or maintaining QTLs

To test whether a particular SNP is associated with height–diameter growth allometry, we just need to test whether a set of power parameters \(a_{h,j} \cdot b_{h,j} \cdot D\) or \(a_{d,j} \cdot b_{d,j} \cdot D\) differs jointly among genotypes. This can be done, respectively, by formulating the following two sets of hypotheses:

\[
H_0 : (a_{h,j} \cdot b_{h,j} \cdot D) \equiv (a_{h,1} \cdot b_{h,1} \cdot D), \text{for all } j = 1, \ldots, J
\]

(7a)

\[
H_1 : \text{At least one of the equalities in the } H_0 \text{ does not hold}
\]

(7b)

After the MLEs of the unknown parameters under the \(H_0\) and \(H_1\) for each test are obtained, the log-likelihood ratio (LR) is calculated. By comparing it with the critical threshold of a chi-square distribution, the \(P\)-value reflecting the significance of the SNP considered is then obtained. The approach for adjusting for multiple comparisons, such as Bonferroni, to take into account all SNPs was used to obtain a genomewide threshold. The SNPs whose significance level is beyond the adjusted criterion are regarded as QTLs. The significance tests based on hypotheses (7a) and (7b) produce ecologically different interpretations. Hypothesis (7a) intends to find a QTL that modulates how height growth increases per diameter growth, a process critically determining a tree’s capacity to pioneer growth space. Such a QTL is called the ‘pioneering’ QTL or piQTLs. On the other hand, hypothesis (7b) can detect the so-called maintaining QTL or miQTLs that regulates the increase of stem girth per height growth, which is related to the capacity of a tree to maintain growth space.

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Supporting information

Additional Supporting Information may be found online in the supporting information tab for this article:

**Figure S1** Genotypic curves (in different colors) at \( pi \)QTL that modulate the allometry of tree height with stem diameter.

**Figure S2** Genotypic curves (in different colors) at \( mi \)QTL that modulate the allometry of tree height with stem diameter.

**Table S1** MLEs of parameters for \( pi \)QTLs that model the allometry of tree height with stem diameter by equations (1a).

**Table S2** MLEs of parameters for \( mi \)QTLs that model the allometry of tree height with stem diameter by equations (1b).