Plastic expression of heterochrony quantitative trait loci (hQTLs) for leaf growth in the common bean (Phaseolus vulgaris)

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Summary

- Heterochrony, that is, evolutionary changes in the relative timing of developmental events and processes, has emerged as a key concept that links evolution and development. Genes associated with heterochrony encode molecular components of developmental timing mechanisms. However, our understanding of how heterochrony genes alter the expression of heterochrony in response to environmental changes remains very limited.
- We applied functional mapping to find quantitative trait loci (QTLs) responsible for growth trajectories of leaf area and leaf mass in the common bean (Phaseolus vulgaris) grown in two contrasting environments.
- We identified three major QTLs pleiotropically expressed under the two environments. Further characterization of the temporal pattern of these QTLs indicates that they are heterochrony QTLs (hQTLs) in terms of their role in influencing four heterochronic parameters: the timing of the inflection point, the timing of maximum acceleration and deceleration, and the duration of linear growth. The pattern of gene action by the hQTLs on each parameter was unique, being environmentally dependent and varying between two allometrically related leaf growth traits.
- These results provide new insights into the complexity of genetic mechanisms that control trait formation in plants and provide novel findings that will be of use in studying the evolutionary trends.

Introduction

It has been recognized that much of morphological diversity may have resulted from evolutionary shifts in the regulation of developmental timing and events, that is, a phenomenon called heterochrony (Gould, 1977; Smith, 2001, 2003; Rice, 2002; Geuten & Coenen, 2013). As a fundamental aspect of all developmental processes, heterochrony may affect the evolution of development in plants by altering the timing of key developmental events, which enables the organism to better respond to changes in developmental and environmental cues (Keyte & Smith, 2014). Several studies have identified a particular set of endogenous machineries that control heterochrony (Moss, 2007; Huijser & Schmid, 2011). For example, in Arabidopsis, two components of the MEDIATOR CYCLIN DEPENDENT KINASE 8 (CDK8) module, Mediator complex subunit 12 encoded by CENTER CITY and Mediator complex subunit 13 encoded by GRAND CENTRAL, were found to be crucial for regulating the timing of radial pattern formation during early embryogenesis (Gillmor et al., 2010, 2014). Similarly, epigenetic regulation of gene expression through HISTONE 3 LYSINE 27 (H3K27) methylation was found to play an important role in affecting the timing of seed-to-seedling and vegetative-to-reproductive transitions in plants (Bastow et al., 2004; Bouyer et al., 2011; Crevillen & Dean, 2011). Nevertheless, these studies simply focused on particular pathways that cause heterochronic changes, and did not address the entire landscape of the genetic control of heterochrony in organ development.

More recently, Sun et al. (2014) have proposed a quantitative framework for characterizing the genetic architecture of heterochrony by mapping these so-called heterochronic quantitative trait loci (hQTLs). This framework was founded on a dynamic mapping approach, called functional mapping, which integrates mathematical aspects of growth laws into a mapping setting to localize dynamic QTLs that govern the biological process of trait formation (Ma et al., 2002; Wu & Lin, 2006; He et al., 2010; Li & Wu, 2010; Zhao et al., 2012). By simultaneously modeling phenotypic measurements taken at a finite number of time-points, functional mapping can provide biologically meaningful results that can be used to interpret the function of QTLs encoded by KINASE 8 (CDK8) module, Mediator complex subunit 12 and Mediator complex subunit 13 respectively.
from a developmental perspective. Zeng et al. (2014) used functional mapping to detect and map four QTLs that control stem height and base diameter growth in juvenile seedlings of a coniferous tree, *Torreya granis*. In many other studies, functional mapping has identified QTLs for key growth traits, such as whole-plant biomass growth in soybean (*Glycine max*; Wu et al., 2011), plant height growth in rice (*Oryza sativa*; Zhao et al., 2004b), stemwood growth in Scots pine (*Pinus sylvestris*; Li et al., 2014), human height growth (Li et al., 2009) and mouse mass growth (Zhao et al., 2004a).

By further investigating whether and how these QTLs detected mediate the heterochronic pattern of trait growth, results from functional mapping can be upgraded not only to provide more useful information for designing breeding and management plans for agricultural crops, but also to predict the evolution of development (Rice, 2002, 2008).

The common bean has long served as one of the most important grain legume crops for human consumption and has also played a pivotal role in sustainable agriculture because of its ability to fix atmospheric nitrogen (Cichy et al., 2009). As the main source of photosynthetic products, leaf organs are particularly important for plant growth and production. Leaf morphological traits, which are well represented by leaf area and dry weight, are primary determinants of many physiological mechanisms, such as nutrient accumulation and water and energy exchange (Milla & Reich, 2007). In cereal crops, it was found that source leaves, particularly flag leaves, are associated with grain filling, 1000-grain weight, panicle weight and many other yield-related traits (Li et al., 1998; Quarrie et al., 2006). As a result of this, an understanding of the physiological roles of leaf area and dry weight is, therefore, of paramount relevance for improving plant growth. In the past few decades, a considerable body of studies has focused on the ecological function of leaf area and leaf dry weight across a range of environments (Yin et al., 1999; Milla & Reich, 2007), but knowledge of the underlying genetic control of these two traits is very limited. Byrne et al. (1997) used genetic mapping to detect two QTLs affecting leaf area in *Eucalyptus nitens*. Several QTLs were found to control the leaf area of single leaves located on different types of branch in *Populus* hybrids (Wu et al., 1997). In a genome-wide association study (GWAS) of the maize (*Zea mays*) nested association mapping panel, Tian et al. (2011) characterized the polygenic inheritance of leaf size traits affected by many QTLs of small effects.

In this article, we describe the implementation of Sun et al.’s (2014) *hQTL* mapping model to identify and map specific *hQTL*s for leaf growth trajectories in a mapping population of the common bean (*Phaseolus vulgaris* L.), grown in two contrasting environments. The mapping population is composed of 177 recombinant inbred lines (RILs), derived from a Mesoamerican cultivar (Jamapa) and an Andean cultivar (Calima). This article reports the first study of its kind to attempt to elucidate the genetic architecture of the heterochrony of leaf area and dry weight growth trajectories and, more importantly, to characterize environment-dependent changes of the effects of *hQTL*s on developmental timing. The *hQTL* mapping model identified two major *hQTL*s that pleiotropically determine leaf area and mass growth of the common bean in a growing season as well as each of these two traits expressed in two distinct environments. A trait- and environment-specific *hQTL* was observed to operate under a particular environment. The identification of *hQTL*s may not only provide scientific guidance for marker-assisted selection of economically important traits in the common bean, but will also help us to address fundamental questions about the genetic mechanisms of heterochrony that drive morphological diversification and evolution.

### Materials and Methods

**Mapping population**

We obtained a mapping population composed of 177 recombinant inbred lines (RILs), derived from a Mesoamerican cultivar (Jamapa) and an Andean cultivar (Calima) of *Phaseolus vulgaris* L. These RILs and the two parents were genotyped for 513 molecular markers located on 11 linkage groups each covering a common bean chromosome (Bhakta et al., 2015).

**Experimental design and data collection**

During 2011–2012, the mapping population (including both parents) was planted at two sites with contrasting temperature regimes in southwestern Colombia: Palmira and Popayan (Table 1). At each site, the field experiment was laid out in a randomized complete block row-column design with three replicates (six for each parent) each with 35–50 plants per RIL. One plant from each replicate for each RIL was harvested weekly, starting with seedlings at approximately stage V0, which is when the primary leaves are unfurled, and ending with plants at stage R1, which is when the flowers are fully open and functional. In total, we conducted five weekly harvests. Because of differences in phenology, the first harvest was taken 15 and 18 days after planting.

| Site       | Latitude      | Longitude    | Altitude (m) | Soil type              | Growing season | Solar radiation (MJ m^{-2} d^{-1}) | Temperature (min–max) (°C) | Day length (h) |
|------------|---------------|--------------|--------------|------------------------|----------------|-----------------------------------|---------------------------|---------------|
| Palmira (PAL) | 03°29’N      | 76°81’W     | 1000         | Mollisol, aquic hapludoll | 11 Nov 2011 to Jan 2012 | 14.7                          | 19.5–28.8                  | 11.8          |
| Popayan (POP) | 02°25’N      | 76°62’W     | 1800         | Inceptisol, typic dystrandept | 23 Mar 2012 to Jun 2012 | 15.6                          | 13.7–25.5                  | 12.1          |

Weather values represent averages calculated during the growing season at each site.
at Palmira and Popayan, respectively. At each time of harvest, the first five leaves were measured independently for leaf area and leaf mass (dry weight), but only the first leaf was considered for this study because it is the one for which we had more complete data in a time series. Individual leaves were separated into petioles and laminas at node positions. Lamina area was measured with a Li-Cor® LI-3100C area meter. A mix of petiole and lamina for each leaf was then dried in a forced-air oven for 48 h at 55°C for dry weight determination. We took the mean of three replicates for each RIL at each time-point for the subsequent statistical mapping analysis.

Dissecting the growth curve

One of the most important equations for capturing age-specific change in growth is the logistic curve (Niklas, 1994; West et al., 2001), which we used to describe leaf area and dry weight growth according to the following expression:

\[
g(t) = \frac{a}{1 + be^{-rt}}
\]

(Eqn 1)

\(g(t)\), the trait value at time \(t\); \(a\), the asymptotic value of \(g\) when \(t \rightarrow \infty\); \(b\), a parameter to position the curve on the time axis; \(r\), the relative growth rate which determines the spread of the curve along the time axis. Consequently, any specific growth characteristics described by the logistic growth function (Eqn 1) can be captured by estimating its parameters \((a, b \text{ and } r)\), and these in turn can be used to determine the coordinates of biologically important benchmarks along the growth trajectory.

There are three physiologically important benchmarks on the growth curve: the point of maximum acceleration, the inflection point, and the point of maximum deceleration, with coordinates denoted \(P_a\), \(P_i\) and \(P_d\), respectively. The inflection point \(P_i\) marks the point at which the relative growth rate reaches its maximum. The coordinates of \(P_i\) are obtained by calculating the second derivative of the growth equation, as

\[
(t_i, g_i) = \left(\frac{\log b}{r}, \frac{a}{2}\right)
\]

(Eqn 2)

The growth curve is divided into two phases separated at \(P_i\), the exponential growth (from time \(t = 0\) to \(P_i\)) and the asymptotic growth (from \(P_i\) to infinite time).

The points \(P_a\) and \(P_d\) mark the timing of maximum acceleration and maximum deceleration of growth, which are the first and second inflection points of the growth rate curve, respectively. These two points partition the growth curve into three phases, the exponential growth phase (from time \(t = 0\) to \(P_a\)), the linear growth phase (from \(P_a\) to \(P_d\)) and the ageing phase (from \(P_d\) onwards). By calculating the third derivative of the growth Eqn 1 with respect to time, the coordinates of \(P_a\) and \(P_d\) can be obtained as:

\[
(t_a, g_a) = \left(\frac{\log b}{r}, \frac{a(3 - \sqrt{3})}{6}\right)
\]

(Eqn 3)

\[
(t_d, g_d) = \left(\frac{\log b(2 + \sqrt{3})}{r}, \frac{a(3 + \sqrt{3})}{6}\right)
\]

(Eqn 4)

The duration of linear growth can be calculated as

\[
\Delta T = t_d - t_a
\]

(Eqn 5)

According to Sun et al. (2014), four parameters, that is, the timing of the inflection point (Eqn 2), the timing of maximum acceleration of growth (Eqn 3), the timing of maximum deceleration of growth (Eqn 4), and the duration of linear growth (Eqn 5), are defined as the heterochronous parameters of growth processes. These heterochronous parameters are determined by the growth process, and define the growth trajectory of the organ under study (Smith, 2001; Rice, 2002).

Modeling genetic variation in the growth curve through functional mapping

If specific QTLs exist to affect the dynamic change of a trait, the growth parameters that specify the change should be different among QTL genotypes. Functional mapping based on a mixture model-based likelihood can be used to estimate QTL genotype-specific parameters (Ma et al., 2002). For a particular trait, leaf area or leaf dry weight, this study contains two environments of study because it is the one for which we had more complete data in a time series. Individual leaves were separated into petioles and laminas at node positions. Lamina area was measured with a Li-Cor® LI-3100C area meter. A mix of petiole and lamina for each leaf was then dried in a forced-air oven for 48 h at 55°C for dry weight determination. We took the mean of three replicates for each RIL at each time-point for the subsequent statistical mapping analysis.

\[
(L | \Phi | y, y') = \prod_{i=1}^{n} \left[ \omega_{1i}f_1(y_i; y_i') + \omega_{2i}f_2(y_i; y_i') \right]
\]

(Eqn 6)

where \(\Phi\) represents the unknown parameters which include: the QTL position, the time-dependent effects of different QTL genotypes, and the time-dependent residual variances and correlations. \((y_i, y_i') = (y_i(1), f_1(y_i), \ldots, y_i(5), f_2(y_i', y_i'))\) is the phenotypic vector of RIL \(i\) measured at five time-points grown in Palmira (denoted by \(y\)) and Popayan (denoted by \(y'\), respectively; \(\omega_{1i}\) and \(\omega_{2i}\) are the conditional probabilities of QTL genotypes \(QQ\) (coded by 1) and \(qq\) (coded by 2), respectively, given the marker genotype of RIL \(i\) and \(f_1(y_i, y_i')\) and \(f_2(y_i, y_i')\) are a multivariate normal distribution with time-dependent mean vector for genotype \(QQ\) and \(qq\).

\[
[u_i, u'_{i}] = [(u_1(1), u_1'(1)), \ldots, (u_i(5), u_i'(5))] \text{ for } QQ
\]

\[
[u_i, u'_{i}] = [(u_2(1), u_2'(1)), \ldots, (u_i(5), u_i'(5))] \text{ for } qq
\]

(Eqn 7)

where \(u\) and \(u'\) denote the means for Palmira and Popayan, respectively, and \((10 \times 10)\)-dimensional longitudinal covariance matrix, expressed as

\[
\sum = \left( \begin{array}{c|c} \Sigma_1 & \Sigma_{12} \\ \hline \Sigma_{12} & \Sigma_2 \end{array} \right)
\]

(Eqn 8)

We assume that \(\Sigma_1\) and \(\Sigma_2\), the covariance matrices for Palmira and Popayan, respectively, have stationary structure, which can
be modeled by the first-order autoregressive (AR(1)) approach (Ma et al., 2002). The covariance matrix between Palmira and Popayan, \( \Sigma_{12} = \Sigma_{21} \), can be modeled as a separable structure (Mitchell et al., 2005). It should be noted that, because of their consistency at two sites, time-points have been normalized as Eqs 1 to 5 in the joint model (Eqn 6).

Functional mapping models the time-dependent genotypic values (Eqn 7) determined by a growth equation (Eqn 1). At two different sites, we will use a different set of growth parameters, that is, \((a_1, b_1, r_1)\) for genotype \(QQ\) and \((a_2, b_2, r_2)\) for genotype \(qq\) in Palmira and \((a_3, b_3, r_3)\) for genotype \(QQ\) and \((a_4, b_4, r_4)\) for genotype \(qq\) in Popayan. Functional mapping has been implemented with the EM algorithm to estimate these parameters.

Sun et al. (2014) proposed a procedure to test and estimate the effect of the \(h\) QTLs detected on heterochrony. Here, we list

**Fig. 1** Growth trajectories for leaf area and mass from stage V0 to R (in days) for recombinant inbred lines (RILs) of the common bean (\textit{Phaseolus vulgaris}) derived from a Mesoamerican cultivar (Jamapa) and an Andean cultivar (Calima), planted at two sites, Palmira and Popayan. The red line is the fit of the growth equation (Eqn 1) to the mean curves of all RILs, whereas the green lines are the observed data for the two parents.

**Fig. 2** The profile of the log-likelihood ratios that test the existence of quantitative trait loci (QTLs) for leaf area growth (red, outer circle) and leaf mass growth (blue, inner circle) of the common bean (\textit{Phaseolus vulgaris}) grown at two sites across the 11 chromosomes. The genomic position corresponding to the peak of the curve is the maximum likelihood estimate of QTL locations. The map distances (in centiMorgans) between two markers are calculated using the Haldane mapping function. The broken lines indicate genome-wide critical thresholds to declare the existence of a QTL obtained from 1000 permutation tests. The gray regions indicate the location at which significant QTLs reside. Two QTL regions for leaf area on chromosome 6 are separated by over 60 cM so that they are considered to harbor two independent QTLs. [Correction added after online publication 27 March 2015: figure and text altered to reflect updated nomenclature.]
several key hypothesis tests of how an \( h \)QTL affects the heterochronic parameters and how it interacts with the environment:

1. the effect of \( h \)QTL on a heterochronic parameter within the environment;
2. the pleiotropic effect of \( h \)QTL on a heterochronic parameter of two environments;
3. the effect of the \( h \)QTL–environment interaction on a heterochronic parameter.

### Table 2

The maximum likelihood estimates (MLEs) of growth parameters (\( a \), \( b \) and \( r \)) and standard errors of the estimates for three quantitative trait loci (QTLs) found to affect leaf area and leaf mass growth in the common bean (\( Phaseolus vulgaris \)) grown at two different sites, Palmira and Popayan.

|       | Leaf area                        | Leaf mass                          |
|-------|----------------------------------|------------------------------------|
|       | Palmira                          | Popayan                            |
|       |                                  |                                    |
| LeafG1|                                  |                                    |
| QQ    |                                  |                                    |
| \( a \) | \(100.23 \pm 1.04 \)              | \(66.08 \pm 0.84 \)               |
| \( b \) | \(132.71 \pm 20.27 \)             | \(54.13 \pm 8.26 \)               |
| \( r \) | \(3.51 \pm 0.11 \)                | \(2.61 \pm 0.08 \)                |
| qq    |                                  |                                    |
| \( a \) | \(73.11 \pm 1.10 \)               | \(48.47 \pm 0.72 \)               |
| \( b \) | \(66.31 \pm 20.47 \)              | \(44.70 \pm 7.91 \)               |
| \( r \) | \(3.28 \pm 0.23 \)                | \(2.64 \pm 0.11 \)                |
| LeafG2|                                  |                                    |
| QQ    |                                  |                                    |
| \( a \) | \(95.54 \pm 1.18 \)               | \(65.35 \pm 0.97 \)               |
| \( b \) | \(107.13 \pm 11.66 \)             | \(45.52 \pm 3.09 \)               |
| \( r \) | \(3.38 \pm 0.08 \)                | \(2.38 \pm 0.05 \)                |
| qq    |                                  |                                    |
| \( a \) | \(82.33 \pm 1.03 \)               | \(52.88 \pm 0.67 \)               |
| \( b \) | \(101.31 \pm 10.57 \)             | \(46.80 \pm 3.69 \)               |
| \( r \) | \(3.48 \pm 0.10 \)                | \(2.75 \pm 0.06 \)                |
| LeafG3|                                  |                                    |
| QQ    |                                  |                                    |
| \( a \) | \(95.14 \pm 1.36 \)               | \(64.26 \pm 1.11 \)               |
| \( b \) | \(129.06 \pm 10.27 \)             | \(47.07 \pm 2.94 \)               |
| \( r \) | \(3.54 \pm 0.08 \)                | \(2.44 \pm 0.04 \)                |
| qq    |                                  |                                    |
| \( a \) | \(81.86 \pm 1.41 \)               | \(51.99 \pm 0.67 \)               |
| \( b \) | \(94.78 \pm 9.13 \)               | \(50.56 \pm 4.36 \)               |
| \( r \) | \(3.40 \pm 0.09 \)                | \(2.78 \pm 0.06 \)                |

[Correction added after online publication 27 March 2015: the marker names have been corrected.]
In this study, we considered two different but allometrically related traits, leaf area and leaf mass. Functional mapping has been extended to map QTLs that contribute to the developmental correlation between different traits (Zhao et al., 2005). We used functional mapping to test how an hQTL affects pleiotropically a heterochronic parameter of the two traits.

**Results**

**Leaf growth trajectories**

By plotting leaf area and dry weight growth as a function of time at the two planting sites for each RIL and the parents (Fig. 1), we identified considerable genotypic variation in the time trajectories of both traits. In general, leaves were larger and heavier in Palmira than in Popayan, perhaps because the former was warmer than the latter. The patterns of growth at the two sites were similar from V1 (first trifoliate unfurled) to R1 (anthesis) stages, but started to diverge after R1. At Palmira, leaves of the parent Calima were slightly larger, but strikingly heavier than those of the parent Jamapa, whereas the parent Calima presented consistently larger values than the parent Jamapa for both leaf traits at Popayan.

Leaf area and mass growth trajectories were fitted by a logistic growth equation (Eqn 1), respectively, for each site. Results of fitness using a nonlinear least-squares approach indicate that the mean growth of all RILs can be well described by the growth equation for both traits at both sites ($P < 0.001$; Fig. 1). The shapes of fitted growth trajectories were markedly divergent for the same trait between different sites and also varied between the two traits at the same site. While the environment-dependent difference may result from the response of plants to changing environment, the trait-typical discrepancy implies the use of different developmental machineries for two allometrically related traits.
Detection of growth QTLs and test of h QTLs

The joint model of functional mapping (Eqn 2) by combining information from two sites was used to scan over 11 chromosomes for QTLs that control leaf growth. Three significant QTLs were identified for leaf growth trajectories, which we named LeafG1, located between markers DiM 7-7 and DiM 7-8 on chromosome 7, LeafG2, located between DiM 6-15 and Bng088 on chromosome 6, and LeafG3, located between Bng183 and DiM 6-25 on chromosome 6 (Fig. 2) [Correction added after online publication 27 March 2015: the chromosome number was updated in this sentence following a change in nomenclature]. LeafG1 and LeafG2 exert a pleiotropic effect on two different traits and also display an environmental pleiotropy on the same trait expressed in different environments. LeafG3 is only responsible for leaf area growth, although it affects pleiotropically this trait at both sites.

Table 2 lists the estimates of the growth equation parameters (Eqn 1) for each of the two alleles from each detected QTL and at each of the two sites; also listed is the standard error of each.
It was seen that two genotypes at each QTL detected differed from each other in a manner depending on the type of leaf traits and the environment where the plants were grown (Figs 3–5), as a result of different temporal patterns of genetic effects triggered by a QTL. For the same trait, all QTLs were expressed more rapidly over time at Palmira than at Popayan. The alleles derived from the parent Calima (denoted as \(QQ\)) at all QTLs detected increased leaf area and mass growth at both sites, as compared with those from the parent Jamapa (denoted as \(qq\)). We used Sun et al.’s *h*QTL mapping model to test whether the QTLs detected govern heterochronic parameters of growth processes, that is, the timing of the inflection point, the timing of maximum acceleration, the timing of maximum deceleration, and the duration of linear growth. All the QTLs detected were found to be heterochronic in nature, and thus can be called *h*QTLs, because they are responsible for at least one heterochronic parameter for leaf trait growth at one or two sites (Table 3). *LeafG1* affected all four heterochronic parameters of leaf area growth when the plants were grown at Palmira, but it only controlled the timing of maximum acceleration for the same trait at Popayan. This environment-dependent *h*QTL did not influence any heterochronic parameter for leaf mass growth at both sites. All this suggests that the expression of an *h*QTL can be sensitive to environmental change and may also vary depending on the type of trait.

*LeafG2* showed a different pattern of environment-dependent genetic effects on heterochrony (Fig. 4; Table 3). Heterochronic parameters for both leaf traits were more likely to be affected by...
this hQTL when the plants were grown at Popayan than at Palmira. LeafG2 had no effect on the heterochrony of leaf mass growth at Palmira. As a QTL specifically for leaf area growth, the heterochronous nature of LeafG3 was also environment dependent. It affected the timing of the inflection point, the timing of maximum acceleration and the timing of maximum deceleration for leaf mass growth only when the plants were grown at Popayan (Fig. 5; Table 3).

Environmental impact on the allelic expression of h QTL

We also tested how the environment affects the expression of hQTL alleles by using Sun et al.’s (2014) model. The two LeafG1 alleles, derived from Calima and Jamapa, displayed similar environmental responses for all heterochronous parameters, except for the timing of maximum acceleration (Fig. 6; Table 3). Compared with cooler Popayan, warmer Palmira could accelerate the occurrence of the inflection point and maximum deceleration and shorten the duration of linear growth for leaf area growth in LeafG1 genotypes. However, the inverse pattern was found for leaf mass growth. In some cases, LeafG1 displayed a significant effect of the QTL–environment interaction on heterochrony for two leaf traits (Table 3).

Like LeafG1, genotypes of LeafG2 were not affected for the timing of maximum acceleration by the environment (Fig. 7). Also, this hQTL’s genotypes were accelerated in the timing of development by warmer Palmira which shortened the duration of linear growth for leaf area. It is interesting to see that the environment did not affect the heterochrony of leaf mass growth for the genotype QQ at LeafG2 with alleles from Calima, but did so for genotype qq with alleles from Jamapa. QTL–environment interactions were quite common in heterochrony for LeafG2.

At LeafG3, Jamapa allelic genotype qq was not responsive to the environment in four heterochronous parameters for leaf area growth, but genotype QQ composed of Calima alleles displayed a significant difference in most parameters between the two environments (Fig. 8; Table 3). The pattern of environmental influence on leaf area growth for genotype QQ was similar to those of the other two hQTLs. Also, QTL–environment interactions for LeafG3 were observed.

Discussion

There is a rich body of evidence indicating that leaf area is positively correlated with growth and productivity in annual crops (Li et al., 1998; Vos et al., 2005; Quarrie et al., 2006) and perennial trees (Wu & Stettler, 1994; Byrne et al., 1997). The reason leaf area and leaf mass play a pivotal role in plant growth and productivity is that the leaf is the organ where the majority of light interception and carbon fixation takes place. Genetic mapping has been used to identify specific QTLs that control leaf area and its relationship to grain yield in rice (Li et al., 1998) and barley (Hordeum vulgare, Yin et al., 1999) and stemwood growth in poplar (Wu et al., 1997) and eucalyptus (Byrne et al., 1997). All these findings have provided important information to enhance the molecular breeding of yield traits through marker-assisted selection of leaf area.

While many studies in the literature have focused on the identification of QTLs for leaf traits measured at a single time-point, the current study in common bean attempted to map leaf QTLs from a dynamic perspective, producing several novel findings. First, we used functional mapping to detect QTLs that control leaf growth traits and to characterize their temporal pattern of gene action. The QTLs we detected increased leaf area and leaf mass. Second, this study revealed the intriguing complexity of some QTL-by-environment interactions, a phenomenon of widespread occurrence in biological systems (El-Soda et al., 2014). Although the three QTLs were found to affect leaf area expansion at both sites, the environment dictated what dynamic aspects of the QTL would be effective. For instance, the LeafG1 Calima allele had a significant effect on the timing of the inflection point, maximum acceleration, maximum deceleration, and duration of linear growth in Palmira, but it only had a significant effect on the timing of maximum acceleration in Popayan. QTL–by-environment interactions of different complexities were also detected at the other QTLs. Third, we investigated two leaf traits, leaf area and leaf mass. Functional mapping allowed us to obtain a picture of pleiotropic control over these two allometrically related but developmentally different traits. LeafG1 and LeafG2 are...
strong pleiotropic QTLs that affect both leaf area and leaf mass, whereas LeafG3 only affects leaf area growth. All QTLs are environmentally pleiotropic by triggering their effect on the same leaf traits expressed in different environments. Environmental pleiotropy may play an important role in the production of phenotypic plasticity and in adaptive evolution (Szamecz et al., 2014). Finally, the most important and significant contribution of this study is the use of a modified model of functional mapping (Sun et al., 2014) to characterize the dynamic mechanism of leaf growth and development by dissecting it into its heterochronic components.

Our study is the first of its kind to map specific QTLs for heterochrony, designated hQTL by Sun et al. (2014). We found three leaf growth hQTLs in the common bean with a characteristic heterochronic nature, which was manifested through significant alterations in the timing of several key growth and developmental events. These included the timing of maximum growth rate, the timing of maximum acceleration, the timing of maximum deceleration, and the duration of linear growth. Furthermore, our analysis revealed diverse and complex genotype-by-environment interactions for each of the hQTLs. The timing of the four heterochronous characters controlled by each of the hQTLs was not affected uniformly by the environmental sites. These results showed that the hQTL functional mapping model can reveal more intricate details of environmental responses of dynamic traits such as leaf growth, or any other similar trait. These results also show the dynamic complexity of growth and developmental mechanisms plant can use for adaptation to different environments (Geuten & Coenen, 2013). Heterochrony genes control precisely timed switches for developmental transitions, and provide in this way a time dimension to developmental regulation (Moss, 2007; Geuten & Coenen, 2013). Some heterochrony genes may possess homologs in other species. Thus, determining the identity of hQTLs detected in this study may have applications beyond the common bean.

Heterochrony is regarded as an exceedingly important process by which development can be modified to engender evolutionary changes (Smith, 2003; Keyte & Smith, 2014). In fact, heterochrony has been studied within explicit phylogenetic contexts as a concept linking evolution and development for over a century. The past decade has seen a tremendous increase in interest in the study of heterochrony, from the whole organism to the organ, cell, molecule, and gene level (Rice, 2002; Carleton et al., 2008; Wu et al., 2009; Moda et al., 2013). Our study focusing on mapping, under different environments, heterochrony genes controlling leaf growth and development provides fuel for efforts aiming to link fundamental questions of morphological diversity and phenotypic evolution in plants with the mechanistic pathways underlying these questions.

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References

Bastow R, Mylne JS, Lister C, Lippman Z, Martienssen RA, Dean C. 2004. Vernalization requires epigenetic silencing of FLC by histone methylation. Nature 427: 164–167.

Blakta M, Jones V, Vallejo CE. 2015. Punctuated distribution of recombination hotspots and demarcation of pericentromeric regions in Phasolus vulgaris L. PloS ONE 10: e0116822.

Bouyer D, Roudier F, Heese M, Andersen ED, Gey D, Nowack MK, Goodrich J, Renou J-P, Grini PE, Colon V et al. 2011. Polycomb repressive complex 2 controls the embryo-to-seedling phase transition. PloS Genetics 7: e1002014.

Byrne M, Murrell JC, Owen JV, Kriedemann P, Williams ER, Moran GF. 1997. Identification and mode of action of quantitative trait loci affecting seedling height and leaf area in Eucalyptus nitens. TAG. Theoretical and Applied Genetics 94: 674–681.

Carleton KL, Spady TC, Streelman JT, Kidd MR, McFarland WN, Loew ER. 2008. Visual sensitivities tuned by heterochronic shifts in opsin gene expression. BMC Biology 6: 22.

Cichy KA, Caldas GV, Snapp SS, Blair MW. 2009. QTL analysis of seed iron, zinc, and phosphorus levels in an Andean bean population. Crop Science 49: 1742–1750.

Crevels P, Dean C. 2011. Regulation of the floral repressor gene FLC: the complexity of transcription in a chromatin context. Current Opinions in Plant Biology 14: 38–44.

El-Soda M, Maloletti M, Zwaan BJ, Koornneef M, Aarts MG. 2014. Identification and mode of action of quantitative trait loci affecting trends in scots pine wood traits. Trees: 207: 1751–1759.

Geuten K, Coenen H. 2013. Heterochronous genes in plant evolution and development. Frontiers in Plant Science 4: 381.

Gillmor CS, Silva-Ortega CO, Willmann MR, Buendia-Morneil M, Poethig RS. 2014. The Arabidopsis Mediator Cdk8 module genes CCT (MED12) and Cct (MED13) are global regulators of developmental phase transitions. Development 141: 4580–4589.

Gould SJ. 1977. Ontogeny and phylogeny. Cambridge, MA, USA: Harvard University Press.

He QL, Berg A, Li Y, Vallejos CE, Wu R. 2010. Modeling genes for plant structure, development and evolution: functional mapping meets plant ontology. Trends in Genetics 26: 39–46.

Huysper P, Schmid M. 2011. The control of developmental phase transitions in plants. Development 138: 4117–4129.

Keyte AL, Smith KK. 2014. Heterochrony and developmental timing mechanisms: changing ontogenies in evolution. Seminars in Cell & Developmental Biology 34: 99–107.

Li N, Das K, Wu R. 2009. Functional mapping of human growth trajectories. Journal of Theoretical Biology 261: 33–42.

Li Y, Wu R. 2010. Functional mapping of growth and development. Biological Reviews 85: 207–216.

Li Z, Hallingback HR, Abrahamsson S, Fries A, Anderson Gull B, Sillanpää MJ, Garcia-Gil MR. 2014. Functional multi-locus QTL mapping of temporal trends in Scots pine wood traits. G3 (Bethesda) 4: 2365–2379.

Li Z, Pinson SRM, Stansel JW, Paterson AH. 1999. Genetic dissection of the source-sink relationship affecting fecundity and yield in rice (Oryza sativa L.). Molecular Breeding 4: 419–426.

Ma CX, Casella G, Wu R. 2002. Functional mapping of quantitative trait loci underlying the character process: a theoretical framework. Genetics 161: 1751–1762.

Milla R, Reich PB. 2007. The scaling of leaf area and mass: the cost of light interception increases with leaf size. Proceedings of the Royal Society of London. Series B: Biological Sciences 274: 2109–2115.
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Mitchell MW, Genton MG, Gumpertz ML. 2005. Testing for separability of space-time covariances. *Environmetrics* 16: 819–831.

Moda LM, Vieira J, Guimarães Freire AC, Bonatti V, Bomtorin AD, Barchuk AR, Simões ZL. 2013. Nutritionaly driven differential gene expression leads to heterochronic brain development in honeybee castes. *PLoS ONE* 8: e64815.

Moss EG. 2007. Heterochronic genes and the nature of developmental time. *Current Biology* 17: R425–R434.

Niklas KJ. 1994. *Plant allometry: the scaling of form and process*. Chicago, IL, USA: University of Chicago Press.

Quarrie SA, Quarrie SP, Radojevic R, Rancic D, Kaminska A, Barnes JD, Szamecz B, Boross G, Kalapis D, Kovar V, Sun L, Ye M, Hao H, Wang N, Wang Y, Cheng T, Zhang Q, Wu R. 2014. The role of heterochrony in primate brain evolution. In: Minugh-Purvis N, McNamara KJ, eds. *Human evolution through developmental change*. Baltimore, MD, USA: Johns Hopkins University Press, 154–172.

Rice SH. 2002. Dissecting a wheat QTL for yield present in a range of environments: from the QTL to candidate genes. *Journal of Experimental Botany* 57: 2627–2637.

Rice SH. 2008. Theoretical approaches to the evolution of development and genetic architecture. *Annals of the New York Academy of Sciences* 1133: 67–86.

Smith KK. 2001. Heterochrony revisited: the evolution of developmental sequences. *Biological Journal of the Linnean Society* 73: 169–186.

Smith KK. 2003. Time's arrow: heterochrony and the evolution of development. *International Journal of Developmental Biology* 47: 613–621.

Sun L, Ye M, Hao H, Wang N, Wang Y, Cheng T, Zhang Q, Wu R. 2014. A model framework for identifying genes that guide the evolution of heterochrony. *Molecular Biology and Evolution* 31: 2238–2247.

Szamecz B, Boross G, Kalapiz D, Kovacs K, Fekete G, Farkas Z, Lazár V, Hrtiany M, Kemmeren P, Groot Koerkamp MJ et al. 2014. The genomic landscape of compensatory evolution. *PLoS Biology* 12: e1001935.

Tian F, Bradbury PJ, Brown PJ, Hung H, Sun Q, Flint-Garcia S, Rocheford TR, McMullen MD, Holland JB, Buckler ES. 2011. Genome-wide association study of leaf architecture in the maize nested association mapping population. *Nature Genetics* 43: 159–162.

Vos J, Van Der Putten PEL, Birch CJ. 2005. Effect of nitrogen supply on leaf appearance, leaf growth, leaf nitrogen economy and photosynthetic capacity in maize (*Zea mays* L.). *Field Crops Research* 93: 64–73.

West GB, Brown JH, Enquist BJ. 2001. A general model for ontogenetic growth. *Nature* 413: 628–631.

Wu G, Park MY, Conway SR, Wang JW, Weigel D, Poethig RS. 2009. The sequential action of miR156 and miR172 regulates developmental timing in *Arabidopsis*. *Cell* 138: 750–759.

Wu R, Bradshaw HD, Stettler RF. 1997. Molecular genetics of growth and development in *Populus* V. Mapping quantitative trait loci affecting leaf variation. *American Journal of Botany* 84: 143–153.

Wu R, Cao JG, Huang ZW, Wang Z, Gai JY, Vallejos CE. 2011. Systems mapping: how to improve the genetic mapping of complex traits through design principles of biological systems. *BMC Systems Biology* 5: 84.

Wu R, Lin M. 2006. Functional mapping – how to map and study the genetic architecture of dynamic complex traits. *Nature Reviews Genetics* 7: 229–237.

Wu R, Stettler RF. 1994. Quantitative genetics of growth, development in *Populus*. I. A three-generation comparison of tree architecture during the first two years of growth. *TAG: Theoretical and Applied Genetics* 89: 1046–1054.

Yin X, Kropff MJ, Stam P. 1999. The role of ecophysiologics models in QTL analysis: the example of specific leaf area in barley. *Hereditas* 82: 415–421.

Zeng Y, Ye S, Wu S, Hou W, Wu R, Dai W, Chang J. 2014. Genetic linkage map construction and QTL identification of juvenile growth traits in *Torreya grandis*. *BMC Genetics* 15(Suppl 1): S2.

Zhao W, Hou W, Littell RC, Wu R. 2005. Structured antedependence models for functional mapping of multivariate longitudinal quantitative traits. *Statistical Methods in Molecular Genetics and Biology* 4.

Zhao W, Ma C-X, Cheverud JM, Wu R. 2004a. A unifying statistical model for QTL mapping of genotype × sex interaction for developmental trajectories. *Physiological Genomics* 19: 218–227.

Zhao W, Zhu J, Gallo-Meagher M, Wu R. 2004b. A unified statistical model for functional mapping of environment-dependent genetic expression and genotype × environment interactions for ontogenetic development. *Genetics* 168: 1751–1762.

Zhao XY, Tong CF, Pang XM, Wang Z, Du F, Guo YQ, Wu R. 2012. Functional mapping of ontogeny in flowering plants. *Briefings in Bioinformatics* 13: 317–328.