Mapping epigenetic quantitative trait loci (QTL) altering a developmental trajectory

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Abstract: Genetic variation in a quantitative trait that changes with age is important to both evolutionary biologists and breeders. A traditional analysis of the dynamics of genetic variation is based on the genetic variance–covariance matrix among different ages estimated from a quantitative genetic model. Such an analysis, however, cannot reveal the mechanistic basis of the genetic variation for a growth trait during ontogeny. Age-specific genetic variance at time t conditional on the causal genetic effect at time $t-1$ implies the generation of episodes of new genetic variation arising during the interval $t-1$ to t. In the present paper, the conditional genetic variance estimated from Zhu's (1995) conditional model was partitioned into its underlying individual quantitative trait loci (QTL) using molecular markers in an F_2 progeny of poplars (*Populus trichocarpa* and *Populus deltoides*). These QTL, defined as epigenetic QTL, govern the alterations of growth trajectory in a population. Three epigenetic QTL were detected to contribute significantly to variation in growth trajectory during the period from the establishment year to the subsequent year in the field. It is suggested that the activation and expression of epigenetic QTL are influenced by the developmental status of trees and the environment in which they are grown.

Key words: epigenetic modification, development, marker, poplar, QTL.

Résumé : La variation génétique d'un caractère quantitatif qui change avec l'âge est d'intérêt tant pour les biologistes étudiant l'évolution que pour les sélectionneurs. Une analyse traditionnelle de la dynamique de la variation génétique s'appuie sur une matrice de variance–covariance génétique pour les différents groupes d'âge, tel qu'estimé sur la base d'un modèle de génétique quantitative. Une telle analyse ne peut pas cependant révéler les mécanismes sous-tendant la variation génétique pour un caractère au cours du développement. Une variance génétique, spécifique d'un âge, au temps t et qui est conditionnée par l'effet génétique au temps $t-1$ implique une série de nouvelles variances génétiques survenant dans l'intervalle $t-1$ à t. Dans ce travail, la variance génétique conditionnelle estimée à l'aide du modèle conditionnel de Zhu (1995) a été décomposée en composantes loci de caractère quantitatif (QTL) individuelles à l'aide de marqueurs moléculaires au sein d'une population F_2 des peupliers *Populus trichocarpa* et *Populus deltoides*. Ces QTL, définis comme des QTL épigénétiques, gouvernent les changements au niveau de la courbe de croissance au sein d'une population. Trois QTL épigénétiques, contribuant de manière significative à la variation de la courbe de croissance lors de la période séparant l'année d'établissement de l'année qui suit, ont été détectés. Les auteurs suggèrent que l'activation et l'expression de QTL épigénétiques sont influencées par le statut ontogénique des arbres et par l'environnement dans lequel ceux-ci se trouvent.

Mots clés : modification épigénétique, développement, marqueur, peuplier, QTL.

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Introduction

An individual's phenotype changes with age. A trait that changes with age can be represented as a trajectory (Alberch et al. 1979; Atchley 1987; Kirkpatrick and Lofsvold 1989). The growth trajectory of an individual is the consequence of interactions among different genes, cells, tissues, organs, and the environments in which the individual is grown. For example, when more nutrients are supplied, a growing plant may turn off genes for the growth of lateral roots to allocate more carbon for foliage, branch, and stem growth, resulting in the change of growth trajectory for the above-ground component (Zhang and Forde 1998; Wu et al. 2000). Because these interactions, which extrinsically regulate the expression of genetic material, display heritable changes (Vogl et al. 1993), they have been recognized as playing a central

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role in the growth, development, and evolution of morphological structures. Understanding their origins and the underlying control mechanisms has therefore become an important subject of current developmental and evolutionary genetic studies (Atchley and Hall 1991; Cowley and Atchley 1992; Atchley et al. 1994, 1997).

It is suggested that development resulting from interactions between genes and their products is a sequential and hierarchical process that involves cascades of extrinsically acting cause-and-effect relationships (Atchley et al. 1994). This nature of development implies that an event at a certain time can have a significant consequence on subsequent phenotypes later in ontogeny, and that new variation may occur through the actions and interactions of many genes that act differentially during development. Recently, considerable attention has been paid to develop quantitative genetic models for detecting the genetic variation of a trait caused by newly activated genes at a particular time interval (Atchley et al. 1994; Zhu 1995). These models assume that the underlying genetic control of a quantitative trait may change significantly during ontogeny (Atchley 1984, 1987; Riska et al. 1984; Riska and Atchley 1985). The conditional genetic model proposed by Zhu (1995) has been used to observe new genetic variation for body weight and tail length arising at specific intervals during the ontogeny of mice (Atchley and Zhu 1997). The combination of molecular mapping and the conditional genetic model has led to the identification of several quantitative trait loci (QTL) that give rise to new genetic variation for plant height in rice during different periods (Yan et al. 1998). The QTL detected from the conditional genetic model are suggested to be epigenetically activated during development, with the extent depending on the metabolic status of an organism and its coupling with the surrounding environment in previous stages. These QTL (namely, those activated by an epigenetic event) can be defined as epigenetic QTL and have the potential to alter the developmental trajectory of a growth trait. It should be noted that age-specific QTL have been mapped in a few species, e.g., mice (*Mus musculus*) (Cheverud et al. 1996), *Drosophila* (Nuzhdin et al. 1997), rice (*Oryza sativa*) (W. Wu et al. 1999) and poplar (*Populus*) (R. Wu et al. 1999). However, the QTL identified in these experiments are confounded by the QTL that are expressed in current and previous stages and, thereby, cannot explain epigenetic variation at a developmental stage.

According to the theory of developmental genetics, the gene expression of an epigenetic QTL is contingent upon the environment in which an organism is reared. The development of a complex trait is the adaptation of an organism to particular environments and represents the impact of genetic and environmental interactions. In contemporary evolutionary biology, a major challenge is to understand how genetic and developmental factors interact with the environments to determine the evolution of a morphological trait (Brakefield et al. 1996; Pigliucci 1998). From an evolutionary perspective, identifying the strength and regulation of epigenetic QTL × environment interactions can offer general insights into the genetic and developmental bases of morphological evolution in a population.

In this paper, we report on the detection of the epigenetic QTL of growth traits in a forest tree using the conditional developmental genetic model and molecular markers. We also examine the ways in which interactions between epigenetic QTL and environments are analyzed. The future developments of the statistical methodologies for mapping epigenetic QTL are discussed.

Materials and methods

The plant material used is an F_2 progeny derived from two different but evolutionarily related forest tree species, *Populus trichocarpa* and *Populus deltoides*. A female *P. trichocarpa* clone (93-968), native to western Washington, was crossed with a male *P. deltoides* clone (ILL-129) from central Illinois to generate F_1 family 53. Two F_1 hybrids, 53-246 and 53-242, were further crossed to produce F_2 family 331, with 90 members. All F_2 seedlings were raised in the greenhouse, then transplanted to a nursery site at Farm 5 of the Washington State University Research and Extension Center in Puyallup, Washington. A genetic map based on the F_2 progeny has been constructed using 343 restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), and sequence tagged site (STS) markers (Bradshaw et al. 1994).

In spring 1991, a replicated trial was established next to the nursery site at Farm 5 with 20-cm unrooted cuttings from the F_2 progeny and their F_1 parents and grandparents. Two years later (1993), stecklings (rooted cuttings) of the same materials were planted in two other environments, one east of the Cascades in Boardman, Oreg., the other west of the Cascades in the lower Columbia River Valley near Clatskanie, Oreg. All three trials were laid out in a randomized complete-block design with three replicates and two-tree plots and surrounded by two border rows, with a spacing of 2 × 2 m at Puyallup and 1.5 × 3.0 m at Boardman and Clatskanie. Height and basal diameter were measured for each tree at the end of each growing season and were further used to calculate volume growth (height × diameter^2). We focus on the data of the first two years because there is a remarkable transition in root growth and development between the establishment year and subsequent growth in the field (Wu and Stettler 1994, 1997). It is likely that epigenetic QTL play a role in affecting this transition.

A quantitative developmental genetic model was proposed by Zhu (1995) to estimate the net genetic effect and variance at time t, conditional on the net genetic effect and variance at time $t - 1$. The conditional genetic effect and variance estimated at time t imply the new effects of genes that are independent of the causal genetic effects at time $t - 1$ (Atchley and Zhu 1997). This conditional model is used to estimate the genetic effect and variance of a quantitative trait contributed by an individual QTL that is epigenetically activated at the time interval $t - 1$ to t. The model is extended to map epigenetic QTL that exert a pleiotropic effect on two different traits of genetic correlation. In all the analyses, a putative epigenetic QTL is assumed at known marker loci and, therefore, a traditional analysis of variance may provide approximate estimates for the conditional genetic parameters at the QTL level. An advantage of the analysis of variance is that the phenotypic data are collected from clonal replicates for each genotype, whose use can provide more accurate estimates for QTL parameters (see Knapp et al. 1990). Unfortu-

Table 1. Variance components (in percentages) estimated from the analysis of variance of epigenetic-QTL mapping for volume growth of the first two years in the F_2 progeny of *Populus* planted in different environments (Puyallup, Wash., Clatskanie, Oreg., and Boardman, Oreg.).

QTL	Marker	Year	*R*	*A*	*D*	*A*×*E*	*D*×*E*	*A*×*R*	*D*×*R*	Error effect
B	G12_15	1	8[a]	12[a]	6[ns]	9[b]	13[a]	6[ns]	5[ns]	41
		2/1	5[a]	8[a]	12[a]	11[a]	14[a]	12[a]	11[a]	27
		2	nd	nd	nd	nd	nd	nd	nd	nd
C	P1290	1	8[a]	8[a]	12[a]	9[b]	10[b]	8[b]	7[c]	38
		2/1	nd	nd	nd	nd	nd	nd	nd	nd
		2	6[b]	11[a]	17[a]	8[b]	10[b]	7[c]	5[ns]	36
E	P1340	1	nd	nd	nd	nd	nd	nd	nd	nd
		2/1	5[a]	9[a]	14[a]	8[b]	13[a]	10[b]	8[c]	27
		2	6[b]	14[a]	27[a]	5[ns]	4[ns]	5[ns]	5[ns]	34
J	P1296	1	8[a]	9[a]	18[a]	8[b]	11[a]	7[c]	10[b]	29
		2/1	nd	nd	nd	nd	nd	nd	nd	nd
		2	6[b]	8[b]	21[a]	9[a]	11[a]	8[b]	9[b]	28
O	A18_05	1	nd	nd	nd	nd	nd	nd	nd	nd
		2/1	5[a]	11[a]	17[a]	9[b]	7[c]	8[b]	6[c]	37
		2	6[b]	9[a]	14[a]	10[b]	11[a]	8[b]	12[a]	30

Note: The F_2 progeny planted in Puyallup, Wash. includes a set (55) of the 90 members. QTL are indicated by linkage groups reported in Bradshaw et al. (1994). 2/1 indicates the volume growth of the second year conditional on the same trait of the first year. *R*, replicate effect; *A*, additive effect; *D*, dominant effect; *E*, environment effect; ns, not significant; nd, not detected.

[a]Significant at $P < 0.001$.
[b]Significant at $P < 0.01$.
[c]Significant at $P < 0.05$.

nately, only the analysis of variance can treat clonal replicates among current mapping methods.

Assuming that all effects except environment are random (Wu and Stettler 1997), the analysis of variance can provide estimates of variance components caused by the conditional replicate effect, the additive and dominant genetic effects of an epigenetic QTL, and its interaction effects with the environments and replicates. The epigenetic QTL detected across the three environments were further assessed within each environment. In all QTL analyses, additive and dominant effects cannot be separated when dominant RAPD markers are used.

Results

A linkage map constructed by Bradshaw et al. (1994) was used to map QTL affecting volume growth during the first two years in the field (Table 1). Epigenetic QTL whose expression is activated during the transition from year 1 to 2 were mapped based on the conditional transformation of volume growth. Three QTL on linkage groups B, C, and J were found to affect volume growth during the establishment year. Of them, QTL on the latter two linkage groups continued to affect total volume growth in the second year. Two additional QTL on linkage groups E and O were detected to determine the second year's total growth. All the QTL for volume growth during both years displayed significant additive and dominant effects. In most cases, dominant genetic variance accounted for a larger percentage of the total phenotypic variance than additive genetic variance. Significant QTL × environment interactions were observed for volume growth in both years.

We have also detected epigenetic QTL for growth that are expressed only in the second year and are independent of the genetic effect in the first year (Table 1). One QTL on linkage group B affecting the first year's growth appeared to be responsible for tree-growth increment in the second year. Given its non-significant effect on total growth in year 2, however, this QTL may display inverse directions of gene action between the first- and second-year increments. Unlike this QTL, two QTL on linkage groups E and O were newly activated during the second year's growth. Their involvement leads to compositions of underlying QTL for volume growth differing between year 2 and year 1. In all cases, epigenetic QTL were found to significantly interact with replicates (microenvironments) and sites (macroenvironments).

The epigenetic QTL detected across different environments were further analyzed within each environment (Table 2). Such analyses can reveal possible relationships between the expression of epigenetic QTL and the environment. All the three epigenetic QTL were expressed in a trial at Puyallup, Wash., where trees were planted with unrooted cuttings. Two QTL on linkage groups E and O were activated in the Boardman trial with higher environmental heterogeneity, as shown by a larger replicate effect (Table 2), as compared with one QTL on linkage group O in the Clatskanie trial with less heterogeneity.

The conditional genetic model for multiple traits was used to estimate the effect of the epigenetic QTL of pleiotropic effect on stem height and diameter growth during the second year separately for each site. At Boardman, an epigenetic QTL on linkage group O was detected to have significant pleiotropic effects on second-year stem height and diameter

Table 2. Variance components (%) estimated from the analysis of variance of epigenetic-QTL mapping for volume growth of the first two years for the F_2 progeny of *Populus* in each of the three environments.

Epigenetic QTL	Marker	Source	Environment		
			Puyallup	Clatskanie	Boardman
B	G12_15	*R*	6^b	—	—
		A	7^b	—	—
		D	8^b	—	—
		A×*R*	4^{ns}	—	—
		D×*R*	7^b	—	—
		Error	68	—	—
E	P1340	R	6^b	—	10^a
		A	10^a	—	10^a
		D	14^a	—	17^a
		A×R	13^a	—	10^a
		D×R	14^a	—	12^a
		Error	43	—	41
O	A18_05	R	6^b	4^{ns}	10^a
		A	6^b	6^c	11^a
		D	6^b	7^b	13^a
		A×R	9^a	5^{ns}	9^b
		D×R	10^a	10^b	11^a
		Error	63	68	46

Note: The F_2 progeny planted in Puyallup, Wash. includes a set (55) of the 90 members. QTL are indicated by linkage groups reported in Bradshaw et al. (1994). 2/1 indicates the volume growth of the second year conditional on the same trait of the first year. *R*, replicate effect; *A*, additive effect; *D*, dominant effect; *E*, environment effect; ns, not significant; nd, not detected.

[a] Significant at $P < 0.001$.
[b] Significant at $P < 0.01$.
[c] Significant at $P < 0.05$.

increments. The genetic correlation between these two increments owing to this epigenetic QTL was 0.45, compared with the genetic correlation of 0.80 owing to all genetic effects. At Puyallup and Clatskanie, no epigenetic QTL were found to exert a pleiotropic effect on stem height and diameter growth.

Discussion

Development of complex morphological structures is generally controlled by many genes whose expression is often age-specific during ontogeny (Atchley et al. 1994). Atchley et al. (1997) initiated a selection experiment in mice to explore how selection operating at different points during ontogeny affects subsequently formed morphological structures. The experiments of Atchley and colleagues (1997) and those by others (Kirkpatrick and Lofsvold 1989; Kirkpatrick et al. 1990, 1994) elegantly demonstrate that genes are expressed selectively at different growth stages. As a result of such differentiated expression, the genetic model based on final characters cannot fully reflect the reality of morphological evolution (Atchley and Hall 1991). Thus, to better understand the developmental and genetic basis of complex phenotypes, the identification of genes that are activated in and responsible for a particular developmental stage becomes essential. Molecular markers, along with well-developed statistical methods, provide unique power to identify epigenetically activated genes during ontogeny.

Zhu (1995) proposed a quantitative developmental genetic model to detect new effects of genes activated at a particular interval by estimating the genetic effects at time t conditional upon the genetic effects at time $t-1$. Phenotypic data measured at time t, after being transformed using Zhu's conditional genetic model, excluded the influences of the causal genetic and environmental effects at time $t-1$. Thus, the conditional genetic effects estimated are suggested to only derive from new genes that are independent of the causal genetic effects in earlier stages. These new genes that are particular for a developmental stage, but whose activation is obviously dependent on both the developmental status of an organism in previous stages and the external environment in which the organism is reared, can be detected by associating the transformed phenotypes with molecular markers. Because of their extrinsic nature, these marker-associated genes detected under the conditional genetic model may be defined as epigenetic QTL, which are the major cause of altering the developmental trajectory of a morphological trait during ontogeny.

The ideas described above were employed to map epigenetic QTL in an F_2 progeny of *Populus* in which three QTL were identified for stem volume growth during the second year in the field. One of the three QTL is likely the same as that for the first year but with altered expression patterns, whereas the other two are likely activated as a result of either the particular developmental status of trees in the first year or a new environment in the second year. Similar studies were also performed in rice by Yan et al. (1998), who mapped epigenetic QTL for plant-height growth on a 10-day interval basis. The information about epigenetic QTL is especially useful for formulating an efficient early-selection strategy for forest trees, which are long-lived organisms. After epigenetic QTL responsible for growth increment at a particular time interval (for example, 5–20 years) are detected, marker-assisted selection incorporating these QTL can be expected to increase the efficiency of breeding for growth performance at age 20 years based on indirect selection at age 5 years.

It is found that epigenetic QTL display significant interactions with growth sites and replicates, suggesting that the expression of epigenetic QTL is critically dependent on the environment. More heterogeneous environments, like Boardman, Oreg. in our example, may induce more epigenetic QTL than a less heterogeneous environment, like Clatskanie, Oreg. A trial established at Puyallup using unrooted cuttings exhibited more epigenetic QTL than the trials at Boardman and Clatskanie, which used rooted cuttings. This may also suggest that the expression of epigenetic QTL is controlled by a plant's resource status. Also, the extent to which an epigenetic QTL exerts pleiotropic effects on different traits, such as stem height and diameter in our study, is contingent upon the environment in which plants are grown. However, a more precise assessment about the relationship between the expression of epigenetic QTL and the environment and developmental status requires a further investigation into older trees.

Although some epigenetic QTL were observed in our experiment, the results should be explained with caution be-

cause the sample size (90) is modest. Small sample sizes may cause two problems: (*i*) the effects of QTL detected may be overestimated, and (*ii*) the QTL of little effect may not be detected (Beavis 1994). However, these two problems can be more or less compensated for by the use of clonal replicates that can provide more precise estimates for phenotypic values. If the epigenetic QTL detected are biologically real, a marker-assisted selection program can be mediated to alter growth trajectories at a particular stage and achieve maximum final growth. Comparatively, conventional marker-assisted selection incorporating QTL for final growth is less efficient at making an effective early selection.

There are two major areas in mapping epigenetic QTL that remain to be explored. First, the mapping method used in this study is a simple analysis of variance, which has many disadvantages in estimating QTL effects and locations. The incorporation of the conditional genetic model and more advanced QTL-mapping methods (e.g., Zeng 1993, 1994; Jiang and Zeng 1995; Kao and Zeng 1997; Korol et al. 1998; Kao et al. 1999) into a joint framework helps to increase the precision and power of QTL mapping. Second, besides the conditional genetic model, many other developmental models have been proposed to understand the genetic structure of growth traits during ontogeny (Kirkpatrick and Heckman 1989; Kirkpatrick and Lofsvold 1990; Kirkpatrick et al. 1994; Meyer and Hill 1997; Pletcher and Geyer 1999; Pletcher et al. 1999). By decomposing the covariance function into its eigenvalues and eigenfunctions, these models can identify potential evolutionary changes in a population's mean-growth trajectory for which there is genetic variation. Therefore, identifying the epigenetically activated QTL that contribute substantially to the eigenvalues and eigenfunctions of molecular markers is of primary interest for projecting the evolution of the population's mean-growth trajectory. The theoretical analyses and resolution in these two areas, identified above, will be discussed in our later publications.

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