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Ontogenetics of QTL: the genetic architecture of trichome density over time in *Arabidopsis thaliana*

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Abstract

Although much is known about the molecular genetic basis of trichome development in *Arabidopsis thaliana*, less is known about the underlying genetic basis of continuous variation in a trait known to be of adaptive importance: trichome density. The density of leaf trichomes is known to be a major determinant of herbivore damage in natural populations of *A. thaliana* and herbivores are a significant selective force on genetic variation for trichome density. A number of developmental changes occur during ontogeny in *A. thaliana*, including changes in trichome density. I used multiple interval mapping (MIM) analysis to identify QTL responsible for trichome density on both juvenile leaves and adult leaves in replicate, independent trials and asked whether those QTL changed with ontogeny. In both juvenile and adult leaves, I detected a single major QTL on chromosome 2 that explained much of the genetic variance. Although additional QTL were detected, there were no consistent differences in the genetic architecture of trichome density measured on juvenile and adult leaves. The finding of a single QTL of major effect for a trait of known adaptive importance suggests that genes of major effect may play an important role in adaptation.

Abbreviations: cM – centiMorgans; LOD – logarithm of the odds; MIM – multiple interval mapping; n – sample size; QTL – quantitative trait locus; RI – recombinant inbred; SE – standard error.

Introduction

The density of leaf hairs, or trichomes, is a trait of considerable ecological importance for many plants. One of the primary adaptive hypotheses commonly proposed for the presence and density of plant hairs involves their role in defense against herbivores (Levin, 1973; Johnson, 1975; Ågren and Schemske, 1994; Elle et al., 1999). For example, in natural populations of *Arabidopsis thaliana*, genotypes with higher trichome densities suffer significantly less herbivore damage than genotypes with lower trichome densities (Mauricio, 1998). Furthermore, herbivores have been shown to be a significant selective agent acting on genetic variation for trichome density in *A. thaliana* (Mauricio and Rausher, 1997).

In many plants, trichomes differ on leaves of different age (Poethig, 1997, 2000, 2003). Leaf age has long been recognized as having an important effect on plant resistance to herbivores – herbivores often have strong preferences for tissue of a particular age (Janzen, 1979; Coley 1980; Krischik and Denno, 1983; Karban and Thaler, 1999; Lawrence et al., 2003). Damage to leaves of different ages can have different effects on plant fitness (Stinchcombe, 2002). Therefore, herbivores can impose very different selective pressures on

plants depending on their pattern of feeding (Mauricio et al., 1993). Difference in trichome density on juvenile and adult leaves might mediate such selection.

The vegetative phase change from juvenile to adult rosette leaves in *A. thaliana* is well-described, particularly with respect to trichomes (Telfer et al., 1997). The distribution and density of trichomes varies during vegetative development and has been used in *A. thaliana* to distinguish the juvenile and adult rosette (Lawson and Poethig, 1995; Telfer et al., 1997). Leaves produced early in development have no trichomes on the abaxial (lower) surface and rosette leaves produced later have trichomes on both adaxial (upper) and abaxial surfaces. There are differences in the density of trichomes between juvenile and adult leaves in *A. thaliana*, although the change in trichome density between these vegetative phases occurs gradually through development (Telfer et al., 1997). In particular, total trichome number in *A. thaliana* has been reported to increase with rosette age (Martínez-Zapater et al., 1995; Payne et al., 2000).

Since the magnitude of selection on plants by herbivores may differ depending on the age of the leaves eaten and the density of trichomes on those leaves, the ability to predict the evolutionary response of the plants to that selection will depend on an understanding of the genetic architecture of the traits under selection. Our ability to predict the potential response to selection is directly predicated on knowledge of the number of genes and their effects on the expression of the phenotype (Lande, 1983; Lynch and Walsh, 1998; Barton and Keightley, 2002). Although much is known about the molecular genetics of trichome development in plants (Hülskamp and Schnittger, 1998; Hülskamp and Kirik, 2000; Szymanski et al., 2000; Walker and Marks, 2000), less is known about the genetic basis of trichome density (Larkin et al., 1996) and very little is known about whether the genetic architecture of trichome density changes with ontogeny.

There is a strong genotypic component to variation in trichome density in *A. thaliana*. Considerable among- and within-population variation for trichome density exists in natural populations of *A. thaliana* (Mauricio, 1998, 2001a). The segregation of trichome density in *A. thaliana* strongly suggests that multiple genetic factors and the environment affect the inheritance of this trait (Larkin et al., 1996; Mauricio, 1998). Trichome density is, therefore, a quantitative trait and the appropriate tool for genetic analysis is QTL (quantitative trait loci) mapping (Mackay, 2001; Mauricio, 2001b).

A QTL mapping approach is likely to be a fruitful one in a completely sequenced model organism, such as *A. thaliana*. Many genetic markers are available, as are several sets of mapping populations. Genome scans for QTL have the potential to identify chromosomal segments containing genes that contribute to variation in a trait of interest (e.g., Doebley et al., 1997; Frary et al., 2000; Johanson et al., 2000).

Despite the fact that QTL mapping has been used extensively in the past decade, some caveats have been raised as to its use (Beavis, 1994, 1998; Mauricio, 2001b). In at least one study, replicate crosses were made from the same parents and QTL analyses were completed on each of the replicates – although the same QTL were detected across studies, some of the QTL detected were unique to each cross (Beavis, 1994, 1998). Environmental conditions have also been shown to play a significant role in the outcome of QTL mapping experiments (Paterson et al., 1991). Obviously, the ability to replicate QTL experiments is of paramount interest, but few studies have specifically addressed this question. In this study, we take advantage of replicate experiments to examine the repeatability of QTL studies.

In addition to providing information about the genetic basis of complex traits, genome scans for quantitative traits provide an empirical basis for testing one of the more enduring controversies in evolutionary biology: the genetic basis of adaptation. Fisher (1930) suggested that mutations of very small effect were responsible for adaptive evolution. Orr and Coyne (1992) reexamined the evidence for this Fisherian view and argued that both the theoretical and empirical basis for it were weak and that adaptive traits may well be controlled by genes of major effect. They encouraged evolutionary biologists to reexamine this research question by the genetic analysis of adaptive differences in natural populations.

In the present study, I investigate three questions addressing the genetic architecture of quantitative variation in trichome density in the plant, *A. thaliana*. First, using QTL analysis, what chromosomal segments in the *A. thaliana* genome

contribute to trichome density variation in juvenile leaves and in adult leaves? Second, do QTL for trichome density change with ontogeny? Third, how variable are QTL analyses completed on a similar trait but performed at different times and in different labs?

Materials and methods

All seeds were obtained from the Arabidopsis Biological Resource Center (ABRC, Columbus, OH, USA). I used a mapping population of 100 recombinant inbred (RI) lines (ABRC stock number CS1899) that had been generated from a cross between the "Columbia" (Col-4; ABRC stock number CS-933) and "Landsberg erecta" (Ler-0; ABRC stock number CS-20) accessions of *A. thaliana* (L.) Heynh. (Lister and Dean, 1993). Progeny from the initial cross were taken through eight generations of selfing *via* single seed descent to produce nearly homozygous lines with an estimated heterozygosity of 0.42% (Lister and Dean, 1993; Juenger et al., 2000). I constructed a linkage map using a total of 228 markers (chromosome I, 54 markers; chromosome II, 33 markers; chromosome III, 37 markers; chromosome IV, 50 markers; and chromosome V, 54 markers). The map position of each marker was estimated from the observed recombination frequencies using the Kosambi mapping function as implemented by the software MapMaker 3.0 (Lander et. al., 1987). This analysis provided unique positions for each marker and a map spanning 592 centiMorgans (cM) of the *A. thaliana* genome (99% of the 597 cM estimated size of the *A. thaliana* genome based both on the *Arabidopsis* Genome Initiative sequence map and the Lister and Dean RI genetic map; www.arabidopsis.org/servlets/mapper). The mean intermarker distance was 2.8 cM. The map did not differ in marker order from the published linkage map of *A. thaliana* (www.arabidopsis.org).

Plants were grown from seed sowed singly in an approximately $5 \times 5 \times 6$ cm plastic pot filled with a soilless mix of peat moss, perlite, pine bark and vermiculite (Fafard #3B, Agawam, MA). All replicates of each RI line were randomly assigned to an individual pot in a flat. The seeds were cold stratified at 4°C for three days and then transferred to a single growth chamber with control for both daylength (14 hours) and temperature (18°C). Five replicate plants were grown for each of the RI lines and trichome density was measured on leaves of the same age. Trichome density was estimated as the total number of trichomes within a 2.4 mm^2 area (using a micrometer in a dissecting microscope) of the upper central area of the adaxial leaf surface. In the first experiment (trial 1), I measured adult leaf trichome density on three fully expanded leaves from each replicate. In the second experiment (trial 2), I measured juvenile trichome density on the first two true leaves (the first two leaves of *A. thaliana* are initiated simultaneously) and adult trichome density on three fully expanded leaves of the same whorl. Larkin et al. (1996) counted the total number of trichomes (not density) on the first leaf of ten replicate plants from the same RI lines used here. J. C. Larkin kindly provided me with the original data from his experiment, which I have reanalyzed using this map and statistical approach.

Genome scans for QTL were done using the multiple interval mapping (MIM) procedure described by Kao and Zeng (1997), Kao et al. (1999) and Zeng et al. (1999) and implemented by the software package, QTL Cartographer, version 2.0 (Basten et al., 1994, 2004). Like other QTL approaches, this procedure tests the hypothesis that an interval flanked by two adjacent markers contains a QTL affecting the trait. Multiple interval mapping statistically accounts for the effects of additional segregating QTL outside the tested interval by using multiple marker intervals rather than individual markers. The procedure can specifically condition the statistical model on all putative QTL identified rather than markers alone. Kao et al. (1999) have shown that MIM tends to more powerful and precise in detecting QTL as compared to such techniques as interval mapping (Lander and Botstein, 1989) or composite interval mapping (Zeng, 1993, 1994).

The MIM procedure tests each parameter (putative QTL) in an initial model for significance using a backward elimination procedure and those parameters that do not lead to a significant improvement in fit are dropped (Basten et al., 2004). For the refinement of QTL position, for each QTL, the position is moved within the QTL interval from one end to the other and an information criterion is calculated for each position (Basten et al., 2004). The information criterion is a

function that gives an indication of how good the model fits the data and that depends upon the likelihood ratio and the number of parameters in the model. The function is

$$I(L_k, k, n) = -2(\ln(L_k) - kc(n)/2),$$

where L_k is the likelihood for a k parameter model, $c(n)$ is a penalty function and ln is the natural log (Basten et al., 2004). For a model with k QTL, MIM searches for k + 1st QTL over all intervals that do not presently have a QTL in them. For each of these intervals, the program walks along the interval and calculates the information criterion for the presence of a QTL. The MIM protocol keeps track of the minimum information criterion (equivalent to the maximum likelihood) within each interval. When all intervals have been tested, the minimum over all intervals is determined and compared to the information criterion of the k QTL model. If $I(L_k, k, n) - I(L_{k+1}, k + 1, n)$ is greater than the threshold, the QTL at that site is retained in the model. The process repeats until no new QTL are retained (Basten et al., 2004).

I began analysis in the MIM module of QTL Cartographer using the MIM default parameters to search for an initial model. I used a walking speed of 1 cM and an initial penalty function, $c(n)$, equal to the $\ln(n) = 4.6$, with a threshold value of 0.0. After this initial run of the analysis, I iteratively reran the model in phases. In the first phase, QTL were located. In the second phase, the positions of those QTL were refined. In the third phase, I searched for additional QTLs. In order to obtain a more conservative estimate of additional QTL, I doubled the penalty function to 2 $\ln(n)$ in this phase of the analysis. In the final phase, I tested for significance of all the QTLs. I calculated conservative confidence intervals (CI) around each QTL by estimating a drop of approximately two LOD scores around the likelihood peak (van Ooijen, 1992; Juenger et al., 2000). The markers located closest to these likelihood cutoffs were considered the two LOD CI flanking markers (Juenger et al., 2000). For some QTL of small effect, I could not detect a drop off of two LOD scores. In those cases, the confidence interval effectively extends across the entire linkage group.

The MIM procedure also estimates such quantitative genetic parameters as variance components, heritabilities, and additive effects. I used the estimates of phenotypic variance, genetic variance, additive effect, and percentage of variance explained that were directly calculated by QTL Cartographer for each trait and QTL. I calculated the coefficient of genetic variation, CV_G, as $(\sqrt{V_G}/\bar{x})$ in order to facilitate comparisons of evolvability between trials (Houle, 1992). The biological interpretation of the additive (or average) effect of an allele is the difference between the mean genotypic value of individuals carrying at least one copy of that allele and the mean genotypic value of a random individual from the entire population. Statistically, the additive effect is a least squares regression coefficient of genotypic value on the gene content (Lynch and Walsh, 1998). The expected population mean value of the additive effect is zero. In these RI lines, a positive additive effect indicates the action of the "Columbia" allele and a negative additive effect indicates the effect of the "Landsberg" allele. In other words, the "Columbia" allele acts to increase trichome density and the "Landsberg" allele decreases trichome density.

Results

The "Columbia" and "Landsberg" accessions of *A. thaliana* differ significantly in their trichomes densities for both adult (Figure 1) and juvenile leaves. The average trichome density on adult leaves from a sample of the Col-4 accession was

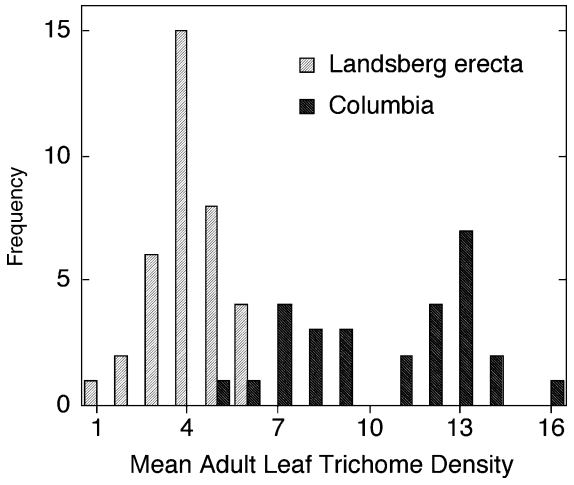

Figure 1. Frequency distribution of trichome density measured on adult leaves of the "Columbia" and "Landsberg" accessions of *A. thaliana.*

10.6 ($n = 28$ individuals; 140 leaves examined; SE = 0.6) but only 4.2 on the Ler-0 accession ($n = 36$ individuals; 180 leaves examined; SE = 0.2). The difference between these 2 accessions for juvenile leaf trichome number was of even greater magnitude. Larkin et al. (1996) reported that the mean number of trichomes per juvenile leaf in the "Columbia" accession was 30.5 ($n = 50$ individuals; 50 leaves examined; SE = 0.9) and 8.9 for the "Landsberg" accession ($n = 50$ individuals; 50 leaves examined; SE = 0.3).

Measurements of trichome density on the set of RI lines showed that juvenile leaves had double the trichome density compared to the adult leaves (Table 1). The mean trichome density on juvenile leaves in trial 2 was 18.8 while the mean trichome density on adult leaves from the two trials was 9.3 (Table 1).

The environment had a significant influence on trichome density, although that effect was more pronounced for trichome density on adult leaves than for juvenile leaves (Table 1). The environmental variance can be estimated from subtracting the genetic variance from the phenotypic variance (since $V_P = V_G + V_E$). An alternative expression of this phenomenon can be seen by comparing the proportion of the total phenotypic variance explained by the among RI line variance (the genetic variance). This "heritability" and coefficient of genetic variation were higher for juvenile leaf trichome density than for adult leaf estimates (Table 1).

The significant differences in trichome densities among the RI lines and between juvenile and adult leaves allowed me to correlate those traits with specific segments of the *A. thaliana* genome using QTL mapping techniques. The most striking result from these four analyses was that a single QTL of major effect was detected for trichome density on both juvenile and adult leaves (Table 2; Figure 2). That QTL, located on chromosome 2, was localized to an interval between 6 and 23 cM in size (depending on the trial) and explained 68.1–70.% of the variance in juvenile leaf trichome density and 28.4–27.6% of the variance in adult leaf trichome density. Comparing across the two trials for each leaf age, the magnitude of the additive effect and the variance explained for this major QTL were similar (Table 2). The additive effect of the "Columbia" allele of this QTL was uniformly positive (Table 2), meaning that the substitution of the "Columbia" allele for the "Landsberg" allele would result in a significant increase in the trichome density of that individual. An additional QTL of major effect, explaining 13.6% of the variance, was found for adult trichome density (Table 2), but only in 1 trial. This QTL has a negative additive effect and is located on chromosome 1 in a region of approximately 19 cM in size.

The QTL analysis revealed several other QTL, but most of them were of minor effect (explaining less than 10% of the variance) (Table 2; Figure 2). In most cases, it was impossible to accurately estimate a confidence interval for these minor QTL: effectively, the confidence interval extends over the entire linkage group. Despite this, in two cases the best estimates for the region associated with a minor QTL for juvenile trichome density did co-localize (Figure 2). In the first case, at approximately 48 cM on chromosome 3 (Table 2) I identified a QTL for juvenile leaf trichome density in both trial 2 and in my re-analysis of the Larkin et al. (1996) data. That QTL explained a similar amount of the variation and had a similar additive effect in the two trials (Table 2). The other case of co-localization also involved juvenile leaf trichome density and was found between position 10.9 and 18.3 cM on chromosome 4 in both trial 2

Table 1. Quantitative genetic parameters for trichome density measured in the RI lines

Trichome density measured on	$\bar{x} \pm (SE)$	Phenotypic variance (V_P)	Genetic variance (V_G)	V_G/V_P	CV_G
Juvenile leaves (Trial 2)	18.8 (0.8)	57.68	44.31	0.77	0.35
Juvenile leaves (Larkin)	20.2 (0.9)	81.64	69.60	0.85	0.41
Adult leaves (Trial 2)	11.5 (0.4)	12.40	7.24	0.58	0.23
Adult leaves (Trial 1)	7.1 (0.4)	12.50	5.44	0.44	0.33

Table 2. Trichome density QTL identified using multiple interval mapping analysis

Trichome density measured on	Linkage group	Position (cM)	2-LOD confidence interval (cM)	Nearest marker	2-LOD confidence interval markers	Additive effect	% variance explained
Juvenile leaves (Trial 2)	2	46.03	41–49	er	GPA1 – mi54	+6.52	68.1
Juvenile leaves (Trial 2)	3	49.61	NE	mi178	NE	+1.08	2.5
Juvenile leaves (Trial 2)	4	10.90	NE	mi390	NE	+1.89	6.3
Juvenile leaves (Larkin)	2	46.04	43 –49	er	er – mi54	+7.72	70.5
Juvenile leaves (Larkin)	3	47.70	NE	mi178	NE	+1.57	3.3
Juvenile leaves (Larkin)	4	18.30	6 –27	app	g3843 – HY4	+2.30	5.8
Juvenile leaves (Larkin)	4	55.30	23 –113	m226	mi167 – ve031	−1.57	1.6
Juvenile leaves (Larkin)	5	60.20	NE	mi125	NE	−1.45	4.0
Adult leaves (Trial 2)	1	83.55	NE	mi72	NE	+0.93	6.5
Adult leaves (Trial 2)	1	150.10	138–157	g17311	PAB5 – pAtT32CX	−1.28	13.6
Adult leaves (Trial 2)	2	53.93	45–57	m220	er – ve096	+1.84	28.4
Adult leaves (Trial 2)	4	78.70	33–113	O6455	pCITf3 – ve031	+1.08	9.9
Adult leaves (Trial 1)	2	40.95	35–58	GPA1	O802F – mi277	+1.93	27.6
Adult leaves (Trial 1)	3	67.00	NE	g4117	NE	+0.94	8.4
Adult leaves (Trial 1)	4	115.61	NE	g3713	NE	−0.85	7.6

NE = not estimable.

and in my re-analysis of the Larkin et al. (1996) data (Table 2; Figure 2). Again, that minor QTL explained a similar amount of variation and had a similar additive effect in the two trials (Table 2).

The four different trials did yield different results for the remaining minor QTL (Table 2; Figure 2). Given the inability of the analysis to accurately localize those QTL, it is impossible to conclude that, for example, 4 of the 5 QTL identified on chromosome 4 or the 3 QTL on chromosome 3 are different (Figure 2). Clearly, two independent QTL were detected on chromosome 4 in the Larkin et al. (1996) trial (Figure 2). In another case, the QTL for adult leaf trichome density located on chromosome 4, has an additive effect that differs in sign in the two trials, suggesting that these are, in fact, different QTL (Table 2).

Discussion

Although much is known about the molecular genetic basis of trichome development in *A. thaliana*, less is known about the underlying genetic basis of continuous variation in trichome density: a trait known to be of adaptive importance. The density of leaf trichomes is a major determinant of herbivore damage in natural populations of *A. thaliana* (Mauricio, 1998). Herbivores have been shown to be a significant selective force on genetic variation for trichome density in natural populations of *A. thaliana* (Mauricio and Rausher, 1997).

In the present study, I investigated three questions related to understanding the genetic architecture of quantitative variation in trichome density in *A. thaliana*. The first and second questions focused on identifying QTL responsible for trichome density on juvenile leaves and adult leaves and asked whether those QTL changed with ontogeny. A considerable literature has demonstrated that a number of developmental changes occur during vegetative phase change in *A. thaliana* (e.g., Telfer et al., 1997), including changes in trichomes. I found dramatic differences in the mean trichome density in both juvenile and adult leaves between two parental lines of *A. thaliana* that

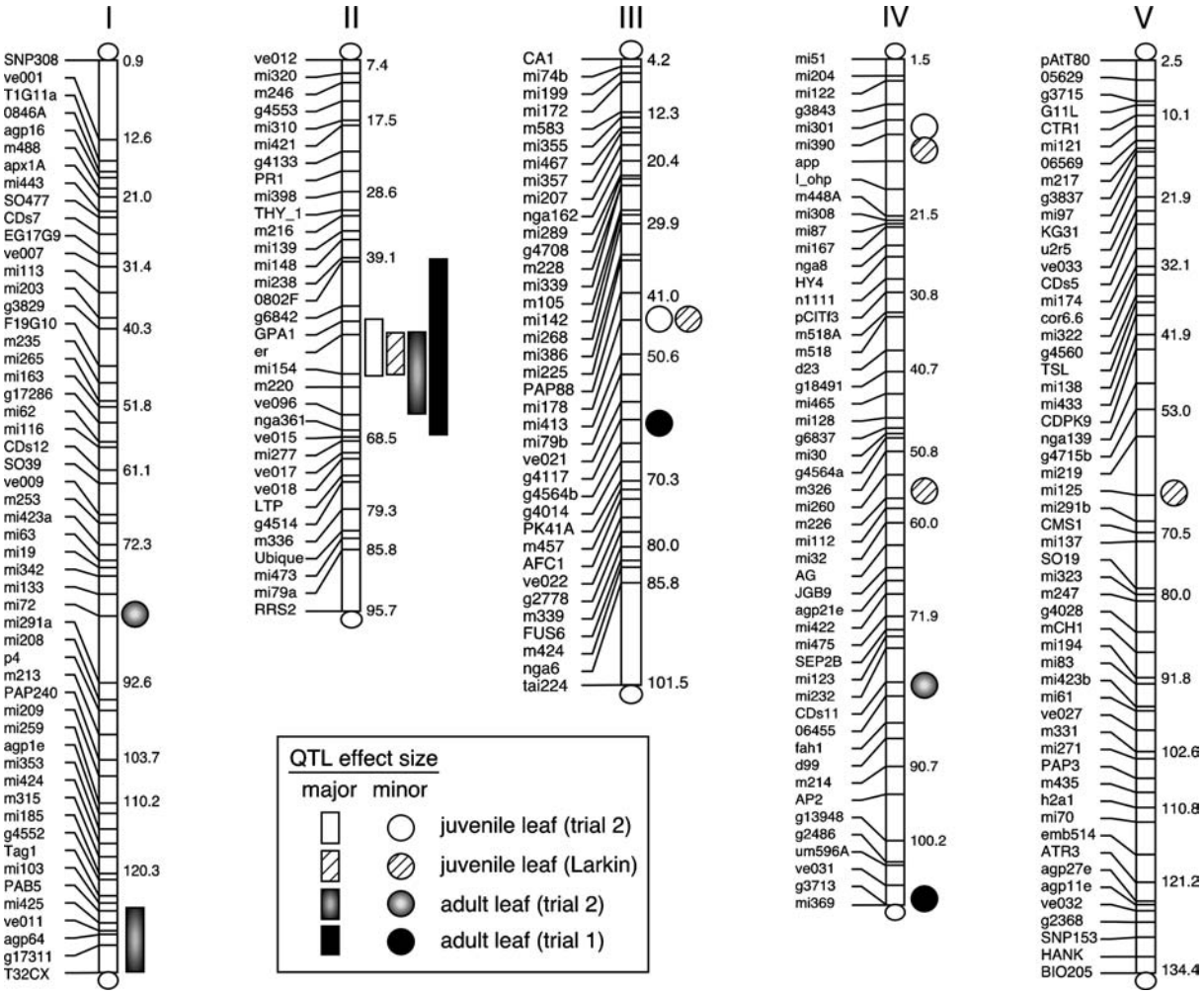

Figure 2. The five chromosomes of *A.thaliana* showing all QTL identified using multiple interval mapping. Markers used in the analysis are listed to the left of the chromosome and genetic distances in Kosambi centiMorgans are listed to the right. QTLs of major effect (explaining >10% of the variance) are identified by bars. The length of the bar spans the markers included in the 2-LOD confidence interval. Minor QTL are indicated by circles to the right of the marker identified as being linked to the QTL. The analysis was unable to establish confidence intervals for most minor QTL and the entire linkage group on which the minor QTL is located should be considered as the confidence interval. The shading of the bar/circle indicates the trait and experimental trial from which the data were obtained.

allowed for mapping of QTL. In addition, I found that trichome density differed between juvenile and adult leaves with juvenile leaves tending to have higher trichome densities than adult leaves. On the surface, this finding contradicts the results of Martínez-Zapater et al. (1995) and Payne et al. (2000) who found that trichome number increased with age. Because they measured total trichome number and I measured trichome density (number of trichomes per unit area), our measures are not directly comparable.

Despite these ontogenetic differences, the most striking result of this study is that there were no consistent differences in the genetic architecture of trichome density measured on juvenile and adult leaves. In all cases, a single QTL on chromosome 2 explained much of the genetic variance. In juvenile leaves, this QTL explained approximately 70% of the variation. In adult leaves, the proportion of genetic variation explained was approximately 28%, although that is twice the variance of any other single QTL identified. A

QTL on chromosome 2 is clearly a major determinant of trichome density variation in both juvenile and adult leaves.

The QTL on chromosome 2 maps in the same rough location as another QTL first identified by Larkin et al. (1996) in juvenile leaves, which they called the *RTN* locus. Larkin et al. (1996) were able to specifically localize *RTN* to the interval on chromosome 2 between the *er* and the *m220* markers. Larkin et al. (1996) observed that the difference in trichome density between the "Columbia" and "Landsberg" parents was related to the duration of trichome development in the leaf primordia. In "Landsberg", trichome development ceases when the leaf primordia are about 500 μm long while in "Columbia", trichome production continues until even after the leaf primordia reach 700 μm in length (Larkin et al., 1996).

A number of QTL of minor effect seemed to be detected in leaves of all ages. Since I was generally unable to establish a confidence interval smaller than the entire length of the chromosome for minor QTL, the QTLs detected on chromosomes 3 and 4 are possibly located in the same region. Those minor QTL were identified from both juvenile and adult leaves.

Although the chromosome 2 QTL was the most significant QTL identified, there were some differences in the QTL detected for leaves of different ages. A major QTL, explaining almost 14% of the genetic variation for trichome density on adult leaves, was detected on the end of chromosome 1. This QTL was not detected in either of the trials on juvenile leaves. However, the inability to detect that same QTL in the adult leaves in trial 1 suggests that the identification of that QTL be considered tentative. Similarly, a minor QTL unique to juvenile leaf trichome density was detected on chromosome 5, but was not found in the juvenile leaf trial 2.

There were clear differences in the contribution of the environment to phenotypic variation in trichome density on leaves of different ages. The heritability of juvenile leaf trichome density was very high. In contrast, the heritability for adult leaf trichome density was much lower. This is not surprising considering the development of trichomes. Because trichome development ceases before the leaves are fully developed, a number of sources of environmental variation can be introduced in the time it takes for the leaves to fully develop and age.

Much is known of the molecular genetic basis of trichome development in *A. thaliana* since plant developmental biologists use trichomes as a model system for understanding pattern formation (Marks, 1997; Hülskamp and Schnittger, 1998; Hülskamp and Kirik, 2000; Szymanski et al., 2000). At least 24 distinct loci are required for normal trichome development and expression (Hülskamp et al., 1994; Marks, 1997). Seven loci, *GL1* (Marks and Feldmann, 1989; Herman and Marks, 1989; Larkin et al., 1993, 1994, 1999; Esch et al., 1994; Schnittger et al., 1998; Szymanski and Marks, 1998), *GL3* (Payne et al., 2000), *TTG* (Larkin et al., 1994, 1999), *GL2* (Rerie et al., 1994; Szymanski et al., 1998a), *TRY* (Schnittger et al., 1998; Szymanski and Marks, 1998), *CPC* (Wada et al., 1997) and *COT1* (Szymanski et al., 1998b) have been described that may play a role in the regulation of trichome density (Szymanski et al., 2000). The mutant alleles identified for *TTG* completely eliminate leaf trichomes, as do most of the alleles for *GL1*. However, at least one mutant allele of *GL1* (*gl1-2*) produces a plant with lower trichome density compared to the wild-type allele (Esch et al., 1994). The mutant alleles identified at the *GL3* locus produces plants with reduced trichome density (Payne et al., 2000). Mutant alleles identified at the four other loci have normal trichome densities, but have been functionally shown to play a role in trichome initiation.

Five of these loci have been genetically mapped (www.arabidopsis.org). The *GL1* locus has been definitively located on chromosome 3 between positions 48 and 49 cM. *GL1* appears on the sequence-based map as well as on genetic maps. The positions of *GL3*, *GL2*, *TTG* and *CPC* are less well localized (only listed on the classical map). *GL3* has been mapped to chromosome 5 at 53 cM. *GL2* is located on the bottom of chromosome 1. *TTG* is located on chromosome 5 at 28 cM. *CPC* has been mapped to chromosome 2 at 63 cM. Neither *TRY* nor *COT1* have been mapped. Given the positions, it is possible that *GL1* is the QTL I identified on chromosome 3 for both juvenile and adult trichome density. The QTL I identified for adult leaf trichome density on chromosome 1 may co-localize with *GL2*. Finally, the juvenile leaf trichome density QTL identified on chromosome 5 may co-localize with either *GL3* or *TTG*. The QTL located on chromosome 4 do not correspond to any known trichome density loci.

Obviously, given the resolution of QTL mapping, any attempt to identify a candidate gene from these data are preliminary and should be considered only as hypotheses for further investigation. Even in model organisms, the ability to move from QTL to gene is not trivial. In this study, the tightest confidence intervals around any major QTL extended between 6 and 23 cM. Even in the best of QTL studies, many QTL are defined by markers more than 10 cM apart. For example, the mean confidence interval around floral trait QTL in *A. thaliana* reported by Juenger et al. (2000) was 10.9 cM (range: 4–23). In *A. thaliana*, the estimated genetic map is 597 cM and the physical size is approximately 125 Megabases (Kaul et al., 2000). On average, there are 213 Kilobases of DNA and approximately 50 genes per cM in *A. thaliana* (Copenhaver et al., 1998). Thus, in a typical 10 cM interval, there are possibly 500 genes. Even if a genome project has identified each of the genes in that interval, proving that any particular gene is responsible for variation in a trait of interest is labor-intensive.

This study has relevance for the debate on the genetic basis of complex adaptive traits (Orr and Coyne 1992). Again, trichome density is known to be of significant adaptive value in natural populations of *A. thaliana* (Mauricio 1998; Mauricio and Rausher, 1997). Quantitative genetic studies of trichome density in *A. thaliana* support the hypothesis that this is a quantitative trait (Larkin et al., 1996). Fisher (1930) argued that many mutations of very small effect were responsible for adaptive evolution. Orr and Coyne (1992) argued that Fisher may have been premature in rejecting the hypothesis that genes of major phenotypic effect played a role in adaptation. My finding of a single QTL of major effect for a trait of known adaptive importance suggests that genes of major effect may play an important role in adaptation.

It has been argued that QTL of large phenotypic effect seen in studies of this kind are an artifact of the strong directional selection often used to create the phenotypically divergent parental lines that are used for mapping (Lande, 1983). Strong selection can fix alleles that normally segregate in the base population. In addition, artificial selection may create repeated bottlenecks through which only a sample of segregating alleles pass. Thus, fewer QTL will be able to be detected and the QTL that are eventually detected may explain an inflated portion of the phenotypic variance. As the parental lines used in this cross were not actively selected, at least not with respect to differences in trichome density, this criticism likely does not apply in this case.

The third question investigated in this study involved the variability in QTL analyses completed on a similar trait but performed at different times and in different labs. There has been some concern expressed in the literature about the repeatability of QTL studies (Mauricio, 2001b). Beavis (1994, 1998) summarized the results of a number of QTL mapping experiments on yield and height of maize, including replicate studies of the same crosses. Although the same QTL were detected across studies, some of the QTL detected were unique to each cross. Even the replicate studies did not detect the same QTL. In this paper, I measured adult trichome density on leaves from the same cross, but in independent experiments. I measured juvenile leaf trichome density and Larkin et al., (1996) measured the total number of trichomes on juvenile leaves. By and large, the similarities across the paired studies outweighed any differences. The means and heritabilities of both adult traits and both juvenile traits were very similar, even though the measures of juvenile leaf trichomes were distinctly different. And, both pairs of studies identified the same major QTL on chromosome 2. Certainly, there were differences detected within the paired trials. But, in all but one case (the QTL for adult leaf trichome density detected on chromosome 1 in only one trial) those involved QTL of minor effect.

A final caveat is that the QTL mapping approach is strictly limited to detecting the genetic variation segregating in the particular cross used. The cross I used in these experiments represents only a sample of the naturally segregating variation found in natural populations of *A. thaliana*. In order to better understand the nature of quantitative genetic variation, it would be extremely valuable to repeat these kinds of QTL studies using a much wider sample of parental accessions collected from natural populations.

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