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RAPD mapping of three QTLs determining trichome formation in *Microseris* hybrid H27 (Asteraceae:Lactuceae)

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Abstract Segregation for 289 random amplified polymorphic DNA markers (RAPDs) has been determined in 106 F_2 plants of an interspecific hybrid (H27) between *Microseris douglasii* (strain B14) and *M. bigelovii* (C94). Multicelluar trichomes ("type D", specific for *Microseris*) occur on the leaf teeth of early vegetative rosettes of the B14 parent and on the leaf blades of later rosettes in both parents. Trichomes on the leaf blades appear earlier and eventually more densely in B14. Segregation for trichome appearance is quantitative and strongly transgressive in the F_2 hybrid. Cosegregation between RAPDs and trichome phenotypes combined with linkage data have revealed a main gene ("quantitative trait locus A", QTL-A) with a pleiotropic effect on all trichome characters and two unlinked additive modifiers (QTL-B, QTL-C). Alleles of both modifiers reduce the main gene effect in each parent. Their recombination explains the occurrence of plants with transgressive phenotypes in the hybrid offspring. Additional QTLs affecting trichomes are at and below the level of statistical significance.

Key words *Microseris* · RAPD · QTL · Trichomes Gene regulation

Introduction

One of the few diagnostic characters of the lactucean genus *Microseris* is a specific type of trichome that consists of a short stalk of three cells terminating in a bulbous terminal cell ("type D" trichomes of Chambers 1955). Epidermis cells anywhere on the vegetative or flowering shoot seem to be able to differentiate into type D trichomes, but for each natural strain of *Microseris*, the location and developmental timing of type D trichomes seems to be genetically regulated (Bachmann et al. 1987; Bachmann and Chambers 1990). Where they appear first on leaves of vegetative rosettes, they are restricted to the angles of the leaf teeth or to the abaxial (under) side of the leaf tip. Their appearance in these positions may be transient. Later, type D trichomes appear on the leaf lamina. These trichomes also always are present on the upper part of the scape directly under the involucral bracts (phyllaries) of the flowering head, and they may appear on the small outer whorl of phyllaries. In one single strain (C96b) of the Chilean annual, *M. pygmaea*, these trichomes also appear on the long inner phyllaries. In strain C96b, trichomes in the early vegetative rosette are restricted to the leaf tips. Van Heusden et al. (1989) have crossed strain C96b of *M. pygmaea* and a strain of *M. bigelovii* with these trichomes in the angles of the leaf teeth. They have identified a gene ("hairy inner phyllaries", *hip*) that determines with two alternative alleles the differences in trichome position and timing between the two parental strains. There must be minor modifier genes affecting the phenotype, and trichome appearance is sensitive to environmental influences, but the gene *hip* is the major regulating factor for the differentiation of epidermis cells into trichomes. Van Houten et al. (1994) have mapped *hip* relative to random amplified DNA polymorphisms (RAPDs) in an intraspecific cross of two strains of *M. pygmaea* and confirmed its overriding effect on trichome development.

We are now using an interspecific cross between strains of *M. douglasii* and *M. bigelovii* to generate a molecular marker map that can be used to investigate most of the characters that are polymorphic in the annual species of the genus. In this article we present our analysis of characters relating to type D trichomes in this cross and demonstrate the presence of a major regulating gene (presumably *hip*) located within a well-marked region of about 10 cM and two modifiers that counteract the effect of the major gene in each parental strain and explain the transgressive segregation of the trichome characters in the F_2.

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Materials and methods

Plant strains and hybrid pedigree

Hybrid strain H27 is derived from a cross between *Microseris douglasii* strain B14 and *M. bigelovii* C94. *M. douglasii* B14 (Chambers accession number CH4284) was collected near Parkfield, Fresno County, Calif. by K.L. Chambers on April 3, 1977. *M. bigelovii* C94 (CH-A303) was originally collected in Uplands Park, Victoria B.C., Canada, by M.C. Melburn on May 28, 1967. Both accessions are maintained as inbred strains. A head of B14 was crossed with C94 as pollen donor by J. Battjes in 1991. Emasculation of the florets is impossible, and successful hybridization has to be checked empirically. Thirty-three achenes from the B14 capitulum were sown out in the fall of 1991. There were 30 spontaneous selfs and 3 hybrids. Fertilities by spontaneous selfing for the 3 hybrids was $9.6 \pm 5.4\%$, $10.7 \pm 5.0\%$, and $8.8 \pm 4.9\%$ (average and standard deviation among capitula). An F_2 of 106 offspring from 1 of these hybrid plants (H27107) was raised in 1992/1993 together with control plants from the parental inbred strains. One-to-three-day old seedlings were planted in individual 10-cm clay pots in the greenhouse on October 15, 1992 (=day 1). The plants were raised as winter annuals under natural light, with greenhouse temperatures maintained at above 10 °C. Achenes were harvested from the F_2 plants in the spring of 1993. F_2 fertilities ranged from 0.0% to 92.9%, with an average of $23.4 \pm 19.5\%$ (standard deviation among plants). Ninety small F_3 families of 5 plants each were raised in 1993/1994. Parental controls were also raised in 1993/1994, but are not included in the data, since germination in the B14 parent was considerably delayed, while all time determinations in the hybrids are based on plants that germinated essentially simultaneously within a few days after 4 days on moist filter paper in the cold.

Scoring procedures

Vegetative rosettes were scored for the appearance of type D trichomes on the leaf teeth twice a week by visual inspection on a scale of 0 (absent), 1 (present), and 2 (dense patch). The date of the first appearance of trichomes on the leaf blade was determined by daily inspection. Trichomes on the leaf blade (not restricted to the leaf teeth or the abaxial side of the leaf tip) usually begin appearing around the time of transition to bud formation. Overall plant hairiness (density of haris on the leaf blade) was determined only in the F_3 on day 123 after planting, when three observers independently scored all plants on a scale from 0 (no leaf hairs) to 4 (plant densely hairy). Scores were added to yield a sum score between 0 and 12.

DNA isolation and amplification

Total plant DNA was isolated from 50 mg fresh leaves according to a modified protocol of Saghai-Maroof et al. (1984). The tissues were ground to a fine powder under liquid nitrogen and incubated at 65 °C in 750 µl 2 × CTAB extraction buffer (100 m*M* Tris-HCl pH 8.0, 20 m*M* EDTA pH 8.0, 1.4 *M* NaCl, 2.0% CTAB and 1.0% mercaptoethanol) for 45 min. The lysate was extracted twice with 750 µl chloroform/isoamyl alcohol (24:1). After an isopropanol precipitation the DNA pellets were dissolved in 250 µl TE followed by an ethanol precipitation with 25 µl 7.5 *M* NH_4Ac and two volumes of ethanol. The final DNA pellets were dissolved in 500 µl TE and stored at −70 °C until use. Amplifications were performed in 25 µl of reaction mixture containing 50 m*M* KCl, 10 m*M* Tris-HCl pH 9.0, 1.5 m*M* $MgCl_2$, 0.001% gelatine, 0.1% Triton X-100, 100 µ*M* of each dNTP, 0.2 µ*M* random decamer primer (Operon Technologies), 0.25 U *Taq* polymerase (HT Biotechnologies, England) and 25 ng genomic DNA. The reactions were overlaid with one drop of mineral oil. DNA amplification was performed in a MJ Research PTC-100/96 thermal cycler programmed for 3 min at 94 °C, 35 cycles of 15 s at 94 °C, 30 s at 40 °C and 1 min at 72 °C followed by 5 min at 72 °C. Amplification products were resolved by agarose gel electrophoresis (1.5%) and visualized by ethidium bromide staining.

Data analysis

Molecular marker maps were constructed with the help of the computer program JoinMap 1.3 (Stam 1993). To detect linkage, a threshold LOD score of 3.7 was used. For determination of the marker orders a Map LOD of 0.5 was used. Recombination distances were converted to centiMorgans (cM) by the mapping function of Kosambi (1944). With the addition of more markers, the relative map positions of most markers have meanwhile become stabilized. At present, 197 of 289 polymorphic markers can be assigned to 17 linkage groups of 3 or more linked markers. Details on the molecular marker map will be published elsewhere. The available linkage data were used here to determine if markers cosegregating with phenotypic differences represent independent quantitative trait loci (QTLs). QTL detection was performed with the help of MAPMAKER/QTL 1.1 (Lander et al. 1987). The threshold LOD score was set at 2.7. Alternatively, cosegregation between quantitative character segregations and individual markers was determined by sorting the data in a spreadsheet program and performing *t*-tests on marker scores (0 or 1) associated with the lowest and highest quantitative character values. The significance of the potential QTLs revealed by these methods was determined by using them to analyze phenotype segregations as illustrated below.

Results

In strain B14, trichomes appear first in the angles of the leaf teeth in vegetative rosettes. Strain C94 and the three F_1 hybrids have no hairs on the leaf teeth. Trichomes appear on the leaf blades in strain C94 much later than in B14. In 1992, leaf hairs in B14 appeared on average 76.86 ± 4.19 days after planting (standard deviation, $n = 64$ plants); in C94, 188.70 ± 1.38 days after planting ($n = 26$). Leaf hairs in the three F_1 hybrids appeared simultaneously with those of the B14 maternal controls (74.67 ± 0.58 days, $n = 3$). One year later, 10 parental controls each were raised together with the hybrid F_2. Here the average times for the parents were 84 days for B14 and 103 days for C94. The large time difference between the parental strains therefore is heritable, but subject to considerable plastic variation from year to year. The average time for the first hairs on the leaf blade in the F_2 was 70.4 days, i.e. even earlier than in the early parent, with a range of 43–139; this strongly transgressive segregation was repeated in the F_3 (average 105 days, range 53–200; late parent, C94, 115 days). Average and maximum scores varied from year to year. This plasticity affects the estimate of heritabilities of the two characters by parent/offspring regression.

Figure 1 shows that F_2 plants without hairs on the leaf teeth can have offspring with such hairs and that even F_2 plants with stronger than average expression of the character can have offspring without it. Linear regression (not shown in Fig. 1) yields an intercept of 0.8544 ± 0.4864 (not significant), a slope of 0.8811 ± 0.1055 and a coefficient of determination, $R^2 = 0.4412$. Recalculation without an intercept yields a slope of 1.0041 ± 0.0804 and an R^2 of 0.4212.

Figure 2 shows the parent/offspring relationship for the date of appearance of hairs on the leaf blade. The wide F_3 segregation in offspring of a group of F_2 plants that formed hairs simultaneously around day 50 sug-

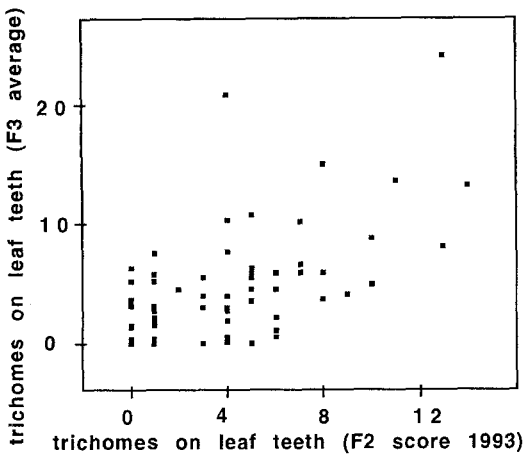

Fig. 1 Appearance of trichomes on the leaf teeth of young rosettes of 92 F_2 plants and 5 F_3 offpsring of each of these. The values are sums of twice-weekly scores of trichomes and range from 0 (absent) to 2 (strong expression)

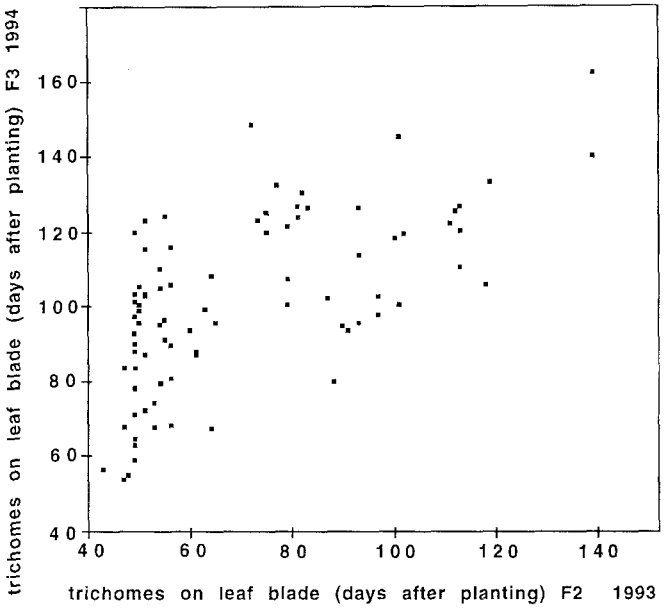

Fig. 2 First appearance of trichomes on the leaf blade (days from October 1) in 92 F_2 plants and 5 F_3 offspring of each of these determined by daily inspection

gests that an environmental factor distorts the parent/offspring proportionality. Linear regression (not shown in Fig. 2) consequently yields a high and significant intercept of 53.3 ± 6.9 days and a slope of 0.76147 ± 0.0925, $R^2 = 0.4030$.

Both characters, therefore, have a strong heritable component but are subject to enviromental modification. The genetic component in the combined F_2 and F_3 data for these characters was enhanced by determining their deviations from the mean along the regression lines. Based on a regression curve $y = a + bx$, where x and y are the F_2 and average F_3 phenotypic values, we calculated a normalized value as

$$x' = (x - x_{\text{average}}) \times \cos b + (y - y_{\text{average}}) \times \sin b.$$

RAPD markers co-segregating with leaf hair characters were found by various methods, including the Mapmaker/QTL program applied to the incomplete map and single-marker searches. All searches based on the three trichome characters showed strong correlations with a group of linked markers covering about 20 cM (Fig. 3). The highest signals were obtained with three amplification products from the B14 parent (OPA-8.04, OPW-6.05, OPD-13.01) flanked by two loci with amplification products from the C94 parent (OPX-3.08 and OPW-7.05). Using either of the two C94 flanking markers and the consensus or nearest of the three B14 markers, we have inferred codominant genotypes for "upper" and "lower" parts of the marked region. Linear regression of quantitative phenotypes against genotype (0, 1 or 2 alleles from C94) shows that F_2 genotypes on the "upper" (OPX-3.08/OPA-8.04) side of the marked region explain more of the phenotypic variance than F_2 genotypes on the "lower" (OPD-13.01/OPW-7.05) side. The variance explained (R^2) for the normalized score for trichomes on the leaf teeth is 34.5% for "lower" and 42.0% for "upper" markers; for normalized first appearance of trichomes on the leaf blade, R^2 values are 22.9% and 44.4%; for hairiness of the F_3 plants, 21.0% and 36.0%. We conclude that a QTL strongly affecting the appearance of type D trichomes ("QTL-trichomes A"; QTL-A) is situated in a region of about 10 cM marked by OPX-3.08 and OPA-8.04.

The F_2 segregation patterns of the three characters associated with QTL-A are shown in Fig. 4. In plants homozygous for the C94 allele for QTL-A few leaf hairs appear relatively late, while plants homozygous for the

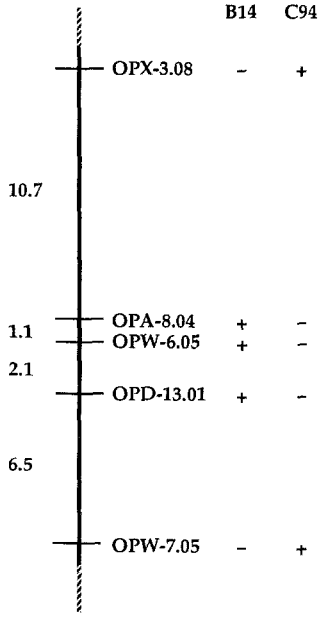

Fig. 3 Linkage relationships and origin of amplification products (marker polarities) for five RAPDs showing a strong association with the expression of trichomes in hybrid H27. Genotypes of the corresponding QTL-A are predicted from a combination of the markers OPX-3.08 and OPA-8.04. Distances in cM.

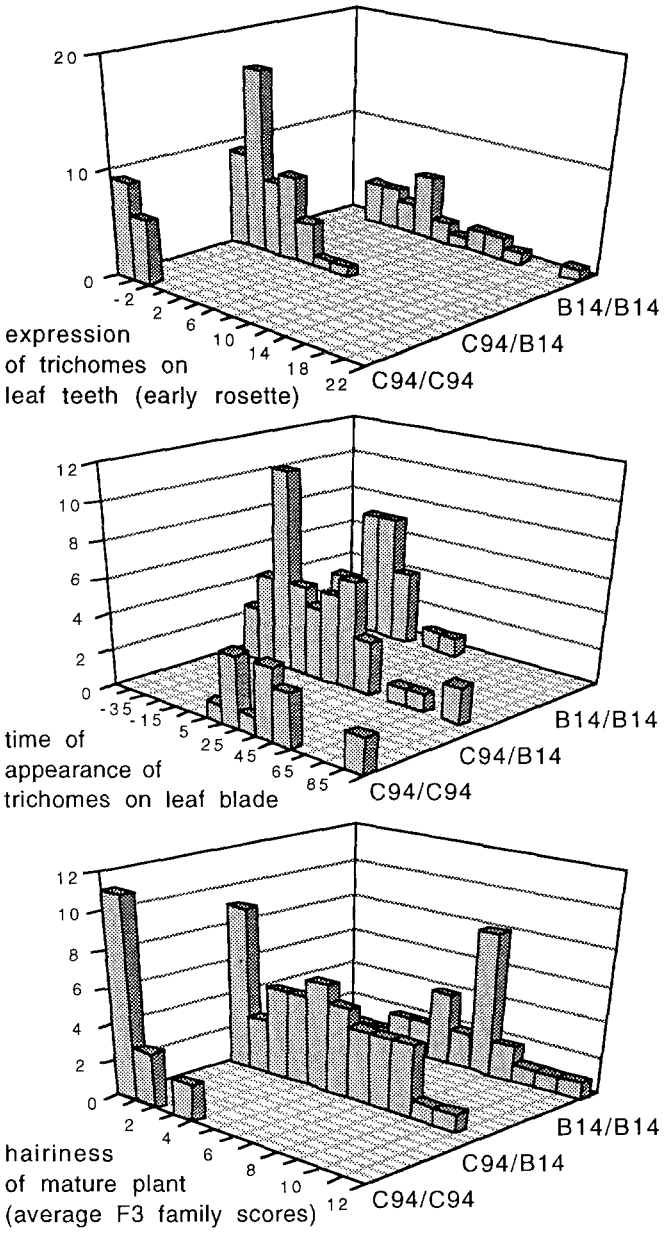

Fig. 4 Pleitropic action of QTL-A. Genotypes are predicted from markers OPX-3.08 and OPA-8.04. Plants with the B14 alleles of QTL-A have more hairs in the angles of the leaf teeth of vegetative rosettes (*upper panel*), more hairs on the leaves of flowering plants (*lower panel*) and produce leaf hairs earlier (*middle panel*)

B14 allele have trichomes on the leaf teeth of early rosettes and, soon thereafter, also on the leaf blades.

Various other markers show cosegregation with time and strength of trichome expression when the total F_2 segregation is examined or when the residual segregation within QTL-A genotypes is tested separately. The effects of two of these are sufficiently strong and cumulative to indicate additional loci. These are U-13.02 (amplification product from B14; QTL-B) and OPF-13.06 (B14; QTL-C). In addition, there is cosegregation with markers around OPX-3.05 (C94; QTL-D) at the limit of statistical significance. For all three QTLs only markers of one polarity are available.

Table 1 Effects of two modifiers in QTL-A heterozygotes. Date of first leaf hairs (hairdate) and expression of hairs on leaf teeth (hairy teeth) in the F_2 and normalized from F_3/F_2 regression and overall plant hairiness in F_3 are compared between F_2 plants heterozygous for QTL-A and with (B-) or without (CC) the B14 amplification products at the two polymorphic loci

		U-13.02 QTL-B	F-13.06 QTL-C
Hairdate	CC	$51.3 \pm 2.5(12)$	$54.8 \pm 9.1(14)$
(F_2)	B-	$73.6 \pm 22.0(43)$	$73.5 \pm 22.5(41)$
		$t = -3.47$	$t = -3.01$
		$P = 0.00103$	$P = 0.00402$
Hairdate	CC	$-26.0 \pm 9.7(9)$	$-18.0 \pm 6.5(12)$
(normalized)	B-	$6.5 \pm 28.4(37)$	$6.5 \pm 30.1(34)$
		$t = -3.35$	$t = -2.70$
		$P = 0.00103$	$P = 0.00987$
Hairy teeth	CC	$3.3 \pm 2.0(12)$	$3.2 \pm 1.7(14)$
(F_2)	B-	$1.5 \pm 1.7(43)$	$1.3 \pm 1.8(41)$
		$t = 3.10$	$t = 3.39$
		$P = 0.0031$	$P = 0.00133$
Hairy teeth	CC	$2.0 \pm 2.8(9)$	$0.4 \pm 2.9(12)$
(normalized)	B-	$-2.3 \pm 2.1(38)$	$-2.1 \pm 2.5(35)$
		$t = 5.14$	$t = 2.88$
		$P = 5.7 \times 10^{-6}$	$P = 0.00608$
Hairiness	CC	$5.9 \pm 2.9(10)$	$6.0 \pm 2.3(12)$
(F_3 average)	B-	$3.6 \pm 2.8(37)$	$3.5 \pm 2.9(35)$
		$t = 2.35$	$t = 2.75$
		$P = 0.0232$	$P = 0.00845$

Table 1 illustrates the phenotypic segregation of QTL-A heterozygotes independently for QTL-B and QTL-C. The effects of both of these QTLs oppose those of QTL-A: the C94 alleles are associated with an earlier appearance of trichomes and stronger trichome expression. All three QTLs affect all three trichome characters. Attempts to overcome the character correlation and find QTLs associated with trichomes at specific sites or times have failed: residual effects of the three main QTL genotypes override any such effects if they exist.

Since only 1 in 64 F_2 plants is expected to be a triple homozygote, an F_2 population of about 100 plants is too small for a detailed analysis of the interaction of three genes. Table 2 shows the phenotypes for the three QTL-A genotypes, each subdivided into three groups with increasing numbers of C94 alleles for QTL-B and QTL-C together. A comparison between the independent effects of QTL-B and QTL-C on QTL-A heterozygotes (Table 1) and their combined effects (Table 2) shows the roughly additive action of the two modifiers. Table 2 also suggests that the interaction of the three identified QTLs may be sufficient to explain the transgressive segregations found in the F_2.

Discussion

Tanksley (1993) in his recent review of polygene mapping discusses the statistical aspects of the method and explains how increasing the resolution (mainly by increasing the size of the mapping population) is likely to

Table 2 Cumulative effect of the three main QTLs on trichome characters

QTL-A QTL-B&C	CC B-B-	CC B-CC	CC CCCC	BC B-B-	BC B-CC	BC CCCC	BB B-B-	BB B-CC	BB CCCC	Total F_2 Range maximum	 minimum
Hairdate (F_2)	104.4	89.4	118.0	78.8	54.3	50.8	51.3	58.2	43.0	139.0	34.0
Hairdate (normalized)	47.0	33.2	39.0	13.7	−20.4	−23.7	−27.5	−24.6	−50.5	94.8	−50.5
Hairy teeth (F_2)	0.25	0.60	0.00	1.09	2.67	4.50	5.80	6.36	13.00	14.00	0.00
Hairy teeth (normalized)	−3.98	−3.72	−4.45	−2.74	−0.25	4.41	4.91	5.84	21.16	21.16	−4.45
Hairiness (F_3 average)	0.98	0.68	1.00	2.90	6.21	6.47	7.01	6.20	12.00	12.00	0.00
Number of F_2 plants found	13.0	5.0	1.0	33.0	19.0	4.0	17.0	11.0	1.0		
Number of F_2 plants expected	14.6	9.8	1.6	29.3	19.5	3.3	14.6	9.8	1.6		

reveal ever more genes with ever smaller effects for any quantitative character. Besides identifying individual factors affecting a quantitative phenotype, QTL mapping also orders these according to their relative influence. In our study, for instance, one very strong "main" gene, is followed by two more or less equal modifiers. The fourth QTL is already at the limit of detection of our sample. Between them, the first three QTLs explain most of the parental differences in trichome characters and their F_2 segregation, especially the correlations among strength and timing of trichome appearance and transgressive segregation in the F_2.

The classical examples for genes with alleles regulating time and place of gene expression are the *R* and *B* genes regulating anthocyanin synthesis in maize. Alleles for these loci vary in their tissue specificity, the concentration of anthocyanidins in the tissue, the distribution of the pigment within a tissue and the time of onset of pigmentation, which is related to the final concentration (Styles et al. 1973; Ludwig and Wessler 1990). A similar variation in place, time and strength of the expression of epidermal type D trichomes among natural strains of *Microseris* is primarily determined by alleles of a gene which we have called *hairy inner phyllaries* (*hip*) in *M. pygmaea* (Bachmann et al. 1987; Bachmann and Chambers 1990), and which is most likely identical with the "QTL-A" of this paper.

The maize *R* gene has been cloned by transposon tagging (Dellaporta et al. 1988), and much is known about the molecular biology of anthocyanin biosynthesis in various plant species. *Arabidopsis* plants transformed with a maize *R* gene not only show increased anthocyanin synthesis, they also produce more trichomes (Lloyd et al. 1992). A similar pleiotropic effect is seen with the *Arabidopsis* gene *transparent testa-glabrous*(*ttg*), where mutants lack both anthocyanins and trichomes (Koorneef 1981; Koorneef et al. 1983). It is therefore not impossible that the similarity between trichome genetics in *Microseris* and anthocyanin genetics in various plant species is more than a superficial analogy. However, no correlation between anthocyanin variation and trichomes has yet been found in *Microseris*, and there is no proof that one kind of trichome in one plant is genetically equivalent to another type of trichome in another species.

Trichome mutants have been described in various species. Recently, Hülskamp et al. (1994) have searched specifically for mutants affecting trichomes in *Arabidopisis*. Interesting among those found are the ones in which the developmental regulation changes while the trichomes themselves remain identical. Besides *ttg*, this is the case for *glabrous1* (*gl1*; Koorneef et al. 1983). *GL1* has been isolated (Marks and Feldman 1989; Herman and Marks 1989) and sequenced (Oppenheimer et al. 1991). It codes for a protein of the *myb* transcription factor type. The precise action of *GL1* is still unknown, but the phenotypic effects of *gl1* mutants make it the *Arabidopsis* gene with the greatest similarity to our QTL-A (or *hip*). It is striking that there is ubiquitous natural allelic variation in all of the annual species of *Microseris* for a factor with such a basic role in trichome differentiation.

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