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Mapped genomic locations for developmental functions and QTLs reflect concerted groups in maize (*Zea mays* L.)

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Abstract For maize, we have analyzed conjointly the map locations reported to-date of genes for growth, development, and stress response. We find that these genes associate into functional clusters, 10–30 cM long, distributed non-randomly along all ten chromosomes. These clusters comprise the loci for environmental and hormonal sensors, the growth machinery genes (e.g., genes for the enzymes of hormone synthesis, mutations disturbing sporophyte and gametophyte development, or genes for programmed cell death) and the master genes presiding over the spatial and temporal transitions in cell growth and differentiation (e.g., genes expressing transcription factors). Taking into consideration mapping accuracy, the putative associations of developmental genes generally coincide with the location of homeotic genes mapped with cDNA probes. The majority of over 800 quantitative trait loci (QTLs) for plant architecture, growth and development in vivo and in vitro, the grain yield as the integer of growth, and ABA accumulation and effects, also map within these clusters. Several physiologically different quantitative traits of plant development and yield are often mapped by one and the same molecular probe. The clusters are redundant, apparently due to several duplication events in the course of maize evolution. We presume that these clusters are the functional units of genes expressed in concert to contribute toward regulating plant development and, apparently, some of the plant responses to abiotic stress. The major QTLs for plant height, earliness and grain yield are visible manifestations of the developmental clusters. The evolutionary and cytogenetic evidence seems to support the adaptive significance of functional gene networks for development. The physiological advantage of the close association of functionally related genes in the clusters may rely on compartmentation and tunneling of signal molecules, which helps to cooperatively recruit the transcription factors into multicomponent regulatory modules of high specificity.

Key words Gene clusters · Homeotic genes · Pleiotropic expression · Quantitative trait loci (QTLs) · *Zea mays* L.

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Introduction

Genetic and molecular analysis has already elucidated many early events determining plant development, including embryogenesis, leaf and shoot growth, and flowering (see Carpenter et al. 1995; Sheridan 1995; Smith et al. 1995; Weigel 1995; Yanofsky 1995 for a review). Less known are those gene interactions governing the life-span development of the whole plant.

Half-a-century ago geneticists working on *Drosophila* and higher fungi had already envisioned the possibility that genes can be distributed in an orderly fashion along chromosomes in relation to their physiological function (see Pontecorvo 1952 for a retrospection of early discussion). The idea of pseudogenes, the heritable clusters of genes arranged to correspond to the successive steps in a particular metabolic or developmental pathway, has finally evolved into the concept of the operon in prokaryotes and later was verified in eukaryotes (Lewis 1978; Spieth et al. 1993). In higher plants, Demarly (1979) also discussed the idea of nonrandom crossing over and put forward the notion of the linkat as a cluster of loci representing co-adapted functions. Whatever may be the attraction of such an idea, however, until now the studies of gene

interactions have indicated regulation *in trans*, e.g., in anthocyanin synthesis in maize and flower development in *Arabidopsis*, rather than *in cis* , e.g., in zein synthesis in maize (see Coe et al. 1988; Dooner et al. 1991; Aukerman and Schmidt 1994; Purugganan et al. 1995; Rounsley et al. 1995 for a review).

Previously we have hypothesized that, in all ten chromosomes of maize, qualitative genes associate into functionally meaningful clusters comprising the loci coding for all essential components of developmental control: genes for the environmental and hormonal sensors, the growth machinery genes, and the master genes supervising all other genes within the particular cluster and presiding over the spatial and temporal transitions in cell growth and differentiation. The initially delineated clusters accounted for most of the naked-eye polymorphisms related to growth and development (Khavkin and Coe 1995 a). To support the cluster hypothesis, we showed that the clusters that manifested the most comprehensive pattern of developmental genes usually included homeotic genes expressing transcription factors. The majority of over 400 major QTLs for plant architecture, growth and development in vivo and in vitro, grain yield as the integer of growth, and abscisic acid (ABA) accumulation and effects, also mapped within the clusters of developmental genes (Khavkin and Coe 1995 b).

In the present study, these clusters were revised in correspondence to the 1995 UMC linkage map (Coe et al. 1995) and verified against the numerous recently mapped MADS-box sequences and QTLs. New data considerably substantiate the cluster phenomenon. In this paper we also addressed the questions of why the developmental clusters are redundant, what is the putative physiological and selective advantage of these gene networks, and how are these gene constellations related to the major loci of agronomically important traits.

Materials and methods

Sources of the data

Naked-eye polymorphisms and other classical genes

This sample included over 170 loci from the 1995 UMC Gene List and Genetic Working Map (Coe et al. 1995) supplemented from the list of newly mapped genes (Coe and Polacco 1996) and with several loci which distinguish maize and teosinte (Szabo and Burr 1996). Though many of these mutations are expressed pleiotropically, with manifold developmental lesions manifested in different tissues of the maize plant and at distant stages of plant development (Coe et al. 1988; Neuffer et al. 1997), we may tentatively classify these genes into four functional groups (Table 1):

(1) naked-eye polymorphisms resulting from mutations manifesting various developmental lesions, i.e., reduced growth (e.g., *brachytic*, *compact plant*, *crinkly leaves*, *dwarf*, *nana plant*), changes in apical dominance and growth habit (e.g., *adherent*, *indeterminate growth*, *lazy plant*, *teopod*), vivipary, malformations and/or displacements in leaf, root, culm and especially inflorescence (e.g., *anther ear*, *barren stalk*, *knotted*, *liguless*, *root-hair defective*, *tassel seed*, *terminal ear*);
(2) mutations mimicking disease *lesions* and *necrotic* mutations;
(3) distortions in gametophyte development (e.g., *gametophyte factor*, *male sterile*);
(4) *blue fluorescent* and *orange pericarp* mutations related to the early steps of auxin production.

Genes mapped with cDNA probes

This category included:

(1) shoot-specific knox (*kn*otted-related homeob*ox*) genes, with their map positions reported by Kerstetter et al. (1994);
(2) *Zea* homologies to *agamous1* and *apetala1* floral MADS-box genes (*zag1*, *zap1* and *zmm1*), transposed MADS-box elements of *Zea* (*tmz1*) and restriction fragment length polymorphisms (RFLPs) containing MADS-box (*mcr*), with their map positions reported by Fischer et al. (1995) and Mena et al. (1995); two more *mcr* loci were reported by Causse et al. (1996);
(3) predominantly meristematic *Zea mays* homeobox genes (*zmhox*) described by Klinge et al. (1996);
(4) several other loci immediately or potentially related to development, mapped with the probes for the *abp*, *cdc*, *obf*, *orp*, *phy*, *pl1*, and *tbp* genes.

QTL database

By the end of 1995, over 800 RFLP-mapped loci were reported for the following growth and developmental manifestations:

(1) growth and differentiation in vitro, i.e., the capacity for callus induction and embryogenesis in immature embryo- and anther-derived cultures;
(2) early and late pollen germinability and pollen-tube growth rate;
(3) plant height, including the length and number of internodes;
(4) earliness, including ear height, days to tassel/pollen and silking, heat units to pollen shed and silking, anthesis – silking interval, and the stay green index;
(5) plant architecture, especially that of the inflorescence;
(6) the yield, including kernel size, kernel weight and total grain yield per acre;
(7) ABA content and ABA-related water-regime parameters.

A more-extended description of qualitative genes, cDNA probes and QTLs is presented in The Maize Genome Database (http://www.agron.missouri.edu). Because of its volume, the complete QTL database was not included in the present paper and can be found elsewhere (Khavkin and Coe, submitted).

Collating map positions

The positions of classical genes were definitely or tentatively assigned to bins (molecular map intervals, 20–30-cM long, defined by the core RFLPs) using the 1995 UMC Gene List and Genetic Working Map (Coe et al. 1995). In this way we could collate the classical genes with the loci mapped by cDNA and RFLP probes. The BNL Molecular Map (Matz et al. 1995) was used as the pivotal interface to conjecture on the bin locations of cDNA and RFLP markers of homeotic genes and QTLs from other molecular maps published by manifold authors.

Table 1 The functional components of a typical developmental gene cluster

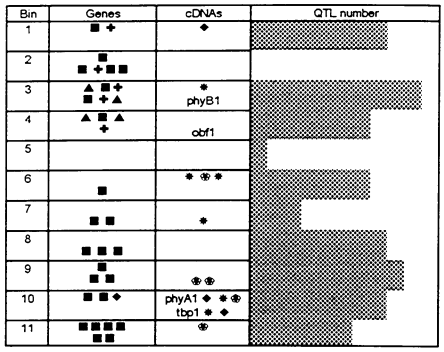
Categories of genes	Physiological function	Examples	References
Qualitative genes located by mutations:			
■ Naked-eye polymorphisms manifesting distortions of plant stature and temporal pattern of development	Genes for hormone sensors and/or response elements;	*d8*, *vp1*	Harberd and Freeling (1989); Vasil et al. (1995)
	Genes for enzymes of hormone synthesis	*an1*, *bf1*, *d3*, *orp1*	Wright et al. (1992); Bensen et al. (1995); Winkler and Helentjaris (1995)
	Putative master genes	*gl15*, *kn1*, *lg2*, *rs1*, *tb1*, *tp1*	Freeling et al. (1992); Dudley and Poethig (1993); Moose and Sisco (1994); Doebley et al. (1995); Smith et al. (1995); Schneeberger et al. (1995)
✚ Mutations mimicking disease lesions and necrotic mutations	Putative genes for the programmed cell death	*les1*, *nec1*	Neuffer et al. (1997)
▲ Distortions in gametophyte development	genes for meiotic and mitotic machinery	*am1*, *as1*, *ga1*, *ig1*, *ms*1, *ms*5	Coe et al. (1995)
Genes mapped with cDNA probes			
Homeotic genes:			
◆-shoot-specific knox (*kn*otted-related homeob*ox*) genes;	Genes for transcription factors	*knox1*	Kerstetter et al. (1994)
✱ -meristematic *Zea mays* homeobox genes		*zmhox1*	Klinge et al. (1996)
❁ ✸-*Zea* homologs to *agamous* and *apetala1* floral MADS-box genes		*zag1*, *zap1*, *zmm1*	Veit et al. (1993); Fischer et al. (1995); Mena et al. (1995)
Other potentially regulatory genes	Genes for the factors of transcription and for cell-division-cycle proteins	*cdc48*, *obf1*, *pl1*, *tbp1*	Dooner et al. (1991); Coe et al. (1995); Jacobs (1995); Zhang et al. (1995)
Genes for environmental and hormonal sensors	Putative receptors of light quality, leaf position and hormones	*abp1*, *phy1*	Schwob et al. (1993); Morgan (1994)

Results

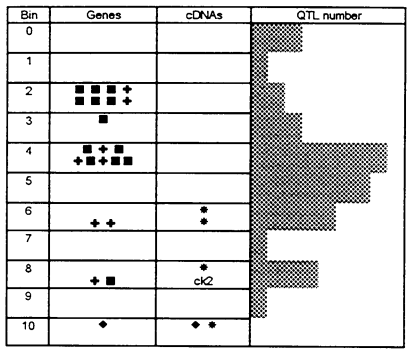
In the maize genetic map we observe numerous sharply focused constellations of closely mapped qualitative genes for plant development alternating with regions where such loci are not found. This pattern becomes somewhat blurred when we distribute genes by bins in the molecular map in order to collate the mutations with the genes mapped by cDNA and/or RFLPs probes and to QTLs. In the molecular map, homeotic loci are scattered non-randomly: six out of ten mapped knox sequences seem to reside side by side (bins 1.10, 5.03/04 and 8.05), and this trend to map by pairs is even more evident among numerous MADS-box sequences, which are closely associated in bins 1.06, 1.10, 4.05, 7.03, 8.03/04, 9.01/02 and 10.03/04. The trend for tandem-duplicated MADS-box regions seems quite substantial, though we must take into account that some of these pairs may be one and the same locus mapped independently in two laboratories (Fischer et al. 1995; Mena et al 1995). QTLs are also unevenly distributed among chromosomes: the better half of their total number maps on chromosomes 1, 3, 5 and 9. The QTL numbers per chromosome apparently differ for the most frequently mapped parameters of plant height, earliness and grain yield; however, such calculations are not very meaningful as the number of RFLP probes used to map each QTL varied considerably for different plant populations in different laboratories (Khavkin and Coe, submitted). Within one and the same mapping population, several physiologically different quantitative traits of plant development and yield are often mapped by one and the same molecular probe.

The distribution of developmental genes and cDNA sequences along the maize chromosomes as collated with QTL profiles is schematically presented in Fig. 1. With one bin, as a conservative estimation of the

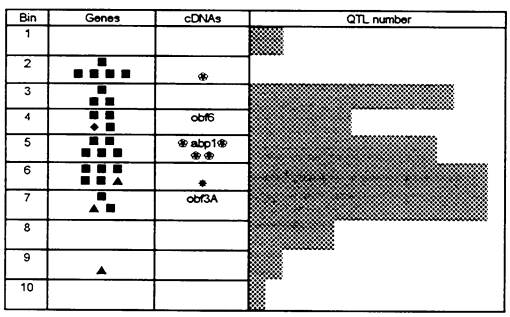
Chromosome 1 (134 QTLs)



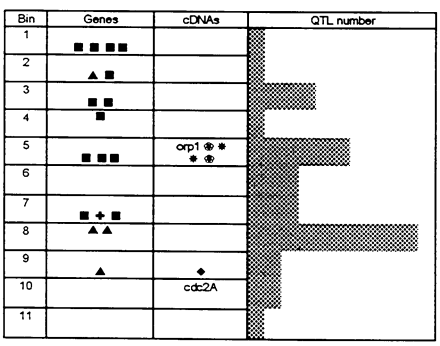
Chromosome 2 (72 QTLs)



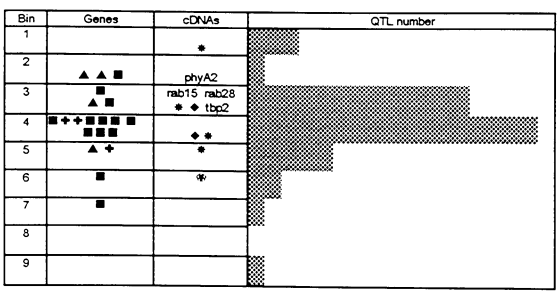
Chromosome 3 (136 QTLs)



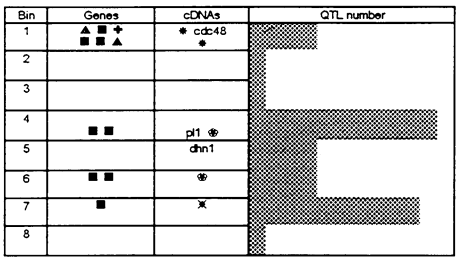
Chromosome 4 (65 QTLs)



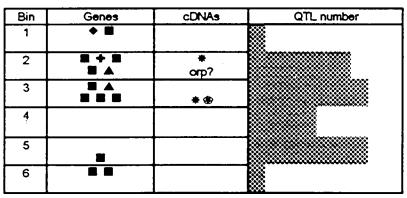
Chromosome 5 (88 QTLs)



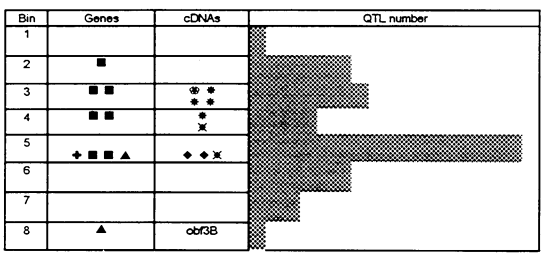
Chromosome 6 (67 QTLs)



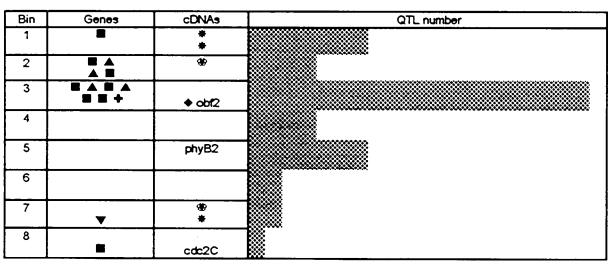
Chromosome 7 (50 QTLs)



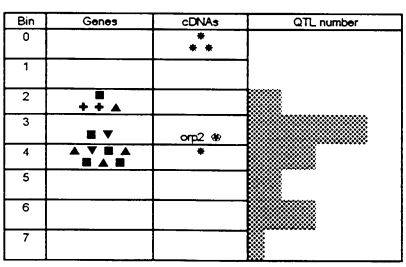
Chromosome 8 (88 QTLs)



Chromosome 9 (94 QTLs)



Chromosome 10 (38 QTLs)



mapping accuracy, we are ready to accept as a proof of matching both the case where qualitative genes coincide with a QTL peak and the situation when the former are immediately adjacent to a QTL maximum. The constellations of qualitative and quantitative loci may be described as developmental gene clusters when they meet three criteria:

(1) a putative cluster includes a "physiologically complete" set of mutations affecting the perception and transduction of external signals, hormone synthesis, the positional pattern of development (morphological and anatomical traits of particular plant tissues or the plant architecture and stature as a whole), the temporal pattern of development, and the death of cells and tissues (Table 1);
(2) this cluster of naked-eye polymorphisms and microscopically discerned qualitative genes is matched by the potential master genes of development (tentatively identified as the genes expressing factors of transcription): the homeotic genes (knox, MADS-box and zmhox sequences) and other genes that express putative factors of transcription in the widest sense of this notion, e.g., the octopine synthase binding factor (*obf*) coding for a bZIP protein that binds to transcriptional enhancer sequences (*ocs*-elements), the purple plant gene (*pl*) encoding the myb-transcriptional activator (though at present described only in flavonoid synthesis and not for developmental genes), and the *tbp* genes encoding the TATA-binding protein component of the transcription initiation factor (Table 1);
(3) the particular cluster of developmental genes coincides with the maximum of QTLs for a variety of parameters of plant growth, development, and grain yield.

The best examples of "complete" clusters can be observed on chromosomes 1, 3 and 5. In bins 3.05/06 we find genes for auxin and ABA sensors (*abp1* and *vp1*), reduced or distorted growth of shoot (*rd3*, *yd2*, and *d2*), leaf (*lg2*, *lxm1*), both the male and female inflorescence (*ba1*, *ig1*, *ig2*, *ts4*), and for reduced plant vigor (*pm1*). Two mutations, *ig1* and *ms23*, affect gametophyte development. Locus *lg2* is a knox gene, and this cluster also contains several MADS-box sequences. Flanking the QTL peak corresponding to this cluster, we observe other mutations distorting leaf (*lg3*, *rg1*) and root (*rt1*) growth and affecting reduced shoot growth (*na1*),vivipary (*rea1*) and male sterility (*ms3*); in addition here we find two loci for a transcription binding factor *obf*. In bins 5.03/04 mutations affecting the development of shoot (*bv1*, *gl17*, *na1*, *td1*), leaf (*nl2*) and tassel (*td1*, *tr1* and *dis1*) are associated with several loci manifesting vivipary (*ps1*, *vp2*), necrotic lesions (*nec3*, *nec6*) and regulating microspore mitosis (*ms5*). In the immediate vicinity, there are genes for environmental (*phyA2*) and gibberellin (*d9*) sensors and two more *male-sterile* genes. These genes are matched by several homeotic sequences and the locus *tbp2* for a transcription factor, as well as by a QTL maximum. In both arms of chromosome 1, there are several gene associations representing more or less "complete" patterns of developmental loci. These clusters are quite compact and well-resolved on the classical map; however, they tend to merge in Fig. 1 when delineated by the bin scale.

More or less similar associations of naked-eye polymorphisms, homeotic genes and QTLs are observed within bins 3.02/03, 6.01, 7.02/03, 9.02/03 and 10.03/04. The chromosome segment 8.04/05 is especially prominent as here we find all three types of homeotic genes, knox, and MADS-box combined together.

The most conspicuous "incomplete" sites are found in bin 2.02, where only a few QTLs correspond to several important developmental genes, while homeotic genes are completely missing; and in bins 6.04, 6.07, 7.05, 9.01 and 10.06, where QTL peaks and several homeotic genes are not adequately matched by naked-eye polymorphisms. However, in several cases when QTL peaks correspond to few qualitative genes, the former are pleiotropic and "compensate" for their insufficient number by exerting manifold developmental manifestations. The best examples are the major ectopic and temporal mutations *ct2* in bin 1.01, *ra2* and *tp3* in bin 3.03, *tga1*/*inc1* in bin 4.05, and *tu1* in bin 4.07. In contrast, in bins 2.10 and 10.00 homeotic genes are not combined with developmental mutations and QTLs.

The approximate combined length of the clusters is about 30% of the total genetic map length. The distribution of associated genes across the maize genome differs from the general pattern of naked-eye polymorphisms: two chromosomes, 1 and 3, seem to carry 40% of all developmental genes, as compared to their 30% proportion of the total number of mutations. The clusters comprise at least three quarters of the qualitative developmental genes already mapped (all *phy* and *obf* loci fall within clusters), the same proportion of knox sequences, and 90% of MADS-box sequences; additionally, the positions of QTLs for plant stature, growth, development and grain-yield components generally match the clusters of qualitative genes (Fig. 1).

Fig. 1 Genes and QTLs for growth and development: (■) naked-eye polymorphisms for reduced growth, changes in apical dominance and growth habit, vivipary, and leaf, root, culm and inflorescence malformations and/or displacements; (✚) *lesions* and *necrotic* mutations; (▲) mutations in gametophyte development; (▼) *blue fluorescent* and *orange pericarp* mutations; (◆) *knox* genes; (❁) *MADS-box* genes; (✸) MADS-box containing RFLPs (*mcr*); (✲) *zmhox* genes; for other genes mapped with cDNA, their standard abbreviations were used. See Table 1 for further details and references. For QTLs, *bar length* corresponds to the number of loci mapped to a particular bin

Discussion

The cluster phenomenon

The three independent sources of mapping evidence – (1) mutations, (2) cDNA and RFLPs for qualitative genes, and (3) QTLs mapped with RFLP probes – present concurring support to the cluster phenomenon: the hypothesis that the genes that regulate maize development are combined into several functional associations. The complete clusters embrace the loci for environmental and hormonal sensors, the growth machinery genes (e.g., genes for the enzymes of hormone synthesis, mutations disturbing sporophyte and gametophyte development, and genes for programmed cell death) and the master genes governing the spatial and temporal transitions in cell growth and differentiation. Taking into consideration mapping accuracy, the associations of mutations generally coincide with the location of homeobox and MADS-box genes, and various other genes expressing factors of transcription. The majority of QTLs for plant architecture, growth and development in vivo and in vitro, the grain yield as the integer of growth, and ABA accumulation and effects, also map within these clusters. We want to emphasize that the concept of developmental cluster does not necessarily presume tight linkage of the component loci: the developmental gene associations are apparently much looser than the clusters of tandem-duplicated genes for zeins (bins 4.01/02 and 7.01/02), ribosomal RNAs (bin 6.01) and rust resistance (bin 10.01).

When accepted as a working model, the phenomenon of clusters as the functional units of developmental genes poses numerous questions which at present can be answered only tentatively, mostly by addressing the available evidence from other sources.

Qualitative and quantitative loci for development: the meaning of major QTLs

The search for major QTLs as the principal target of marker-assisted selection for maize disclosed several loci for plant height and grain yield each explaining 10–20% of the phenotypic variation (Beavis 1994; Stuber 1995). All these major QTLs coincide with the clusters of developmental genes. When QTLs for these and other development-related parameters are collated on the molecular map with qualitative loci, two questions evidently arise.

First, why are several physiologically different quantitative traits of plant development mapped by one and the same molecular probe? We may envision QTLs as projections onto the phenotype of the key structural loci providing for the various essential elements of growth and development. The most evident examples are mutations with already known molecular manifestations, like *an1*, *d3*, *phy1*, *ts2*, *vp5 and vp9*, or such master switches of development as knox and MADS-box genes expressing transcription factors. Such loci must be pleiotropic by definition. In addition, numerous QTLs collate with extremely pleiotropic qualitative genes of as-yet unknown molecular function, like *ad1*, *ig1*, *tan1*, *tb1*, *td1*, *te1*, *tga1* and *tp1* (see Neuffer et al. 1997 for a detailed description of the manifold morphological manifestations of these mutations.)

The physiological interpretation of QTLs has been attempted in several laboratories. Persuasive evidence from a direct approach to this problem is exemplified by the study of an important QTL site for plant height in bin 9.03: the candidate gene for this QTL was defined as the pleiotropic locus *d3* by its map position and physiological criteria and then, with the help of transposon tagging and sequence analysis, was identified as a gene encoding a cytochrome P450 enzyme of the early gibberellin biosynthetic pathway (Touzet et al. 1995; Winkler and Helenjaris 1995).

More often, the indirect approach is used to relate QTLs for a particular trait within one and the same plant population. In this way one may attempt to dissect the major QTLs into their components and, for example, to represent plant height as a function of node number and internode length (Phillips et al. 1992). Our results of such an analysis based on the data by Edwards et al. (1992), Phillips et al. (1992), and Beavis (1994) were rather ambiguous. QTL "complementation" was not very consistent; the major QTLs matched the putative master genes of development (*tlr1*, *ts2*, *rs2*, *tu1*, *hsf1*, *pt2*, *lg4*, *des17*, *gl15*, and homeobox and MADS-box genes) rather than the particular naked-eye polymorphisms for stunted growth (see Khavkin and Coe, submitted, for further discussion).

Second, why is one and the same developmental trait mapped to several widely distant loci? The first answer is that the loci defined as different genes can manifest one and the same physiological trait (e.g., reduced growth). Drawing an analogy from metabolic regulation, we suggest that the position of a bottleneck locus in one and the same developmental pathway may change in different genotype-by-environment interactions, and thus different key genes are manifested in the various segregating populations employed for QTL mapping. The second answer is the extensive duplication of the namesake loci characteristic of the maize genome (see the next section).

Why so many clusters?

One partial answer to the evident redundancy of developmental clusters is the hypothesis of paleopolyploid corn origin; in addition, earlier events of gene duplication and diversification could contribute to the redundancy (Helentjaris 1995). It is remarkable that most

clusters border the centromeres (bins 2.04, 3.05, 4.05, 5.04, 6.01, 7.02, 7.02, 9.03 and 10.03) where Helentjaris (1995) observed most duplicated regions. The most evident examples of duplicated clusters are found when we compare chromosome segments corresponding to bins 1.03 vs 9.03, 1.10 vs 5.02, 3.07 vs 8.03 and 4.05 vs 7.02 and 10.04. Chromosome 1 presents the most complicated case of redundancy with several segments at the centromere and both telomeres similar to the clusters in other chromosomes.

MADS-box genes are found practically in all the regions recognized by Helentjaris (1995) as containing duplicate loci; in addition MADS-box sequences are sometimes duplicated in one and the same locus (Fischer et al. 1995; Mena et al. 1995) resembling Hox genes in animals (Lewis 1978; Kenyon 1994) and the clusters of zein and rRNA genes in maize. Fischer et al. (1995) distinguish between tightly linked and strongly dispersed MADS-box genes in maize resulting from two favorable forms of genome evolution (Wagner 1994). In fact, the two alternatives merge together if we assume that the whole cluster is duplicated as a network of genes encoding development, and due to duplication of clusters, MADS-box genes and other twin components of each network disperse; meanwhile, within a cluster, MADS-box genes duplicate in a tandem series. The number of additional copies varies between maize inbreds (Fischer et al. 1995; Mena et al. 1995), indicating recent duplication events in the maize genome.

In what way may homologous and non-homologous genes for physiologically similar functions (e.g., plant height) and the whole clusters as functional networks interact when functionally redundant and located on different chromosomes? The recently described phenomenon of homology-dependent gene silencing (Matzke and Matzke 1995; Meyer and Saedler 1996) may hopefully provide a partial answer to this question in the near future. Redundancy between non-homologous genes, and especially in genetic networks with cross-regulatory connections, will require a more complex interpretation (Pickett and Meeks-Wagner 1995).

Physiologically incomplete clusters

The physiologically "incomplete" clusters present two different and very intriguing potential tasks. First, by considering these sites as white spots on the genetic map, one can foresee mapping new genes to perform the already known functions or, by speculating on the missing physiological components of the cluster, to predict new functional identities for the already known qualitative loci. Due to their major role as master genes, the highly conserved homeobox and MADS-box sequences are obviously the first choice for such a search. A second group of candidate genes to supplement the existing clusters can be located within the established duplicated chromosome segments by probing the "incomplete" clusters with the cDNAs for their putative twin qualitative genes.

The second task is to investigate whether these *semi*-clusters can complement *in trans*. Regretfully, we do not know much about the modes of chromosome interaction in the interphase nucleus. An encouraging support for this highly speculative assumption of *trans*-complementation comes from the observations of the ordered dispositions of chromosomes of barley and rye; Bennett (1982) presumed that the spatial association of genes on the superdomains of associated arms belonging to heterologous chromosomes provided for a higher efficiency of gene action and might have further selective advantage.

What is outside of the developmental clusters

One might argue that the developmental clusters have no specific meaning and reflect the generally uneven distribution of structural genes. The well-known suppression of recombination in the chromosome region surrounding the centromere (Causse et al. 1996; Gill et al. 1996) would explain the preferential localization of these clusters in the pericentromeric region of chromosomes. We are prepared to respond to this argument: in maize, the chromosomal sections outside of the developmental clusters are by no means empty. This is substantiated by the gene and QTL clusters that are not immediately related to plant development.

For example, a tandem cluster for zein genes in bin 7.01/02 maps within the limits of a developmental cluster, whereas another zein cluster in bin 4.01/02 does not coincide with a considerable association of developmental genes. Goldman et al. (1993) and Berke and Rocheford (1995) mapped numerous QTLs for protein, starch and oil concentration in maize kernels mostly to positions outside of developmental clusters, though some of these QTLs co-mapped with QTLs for kernel weight. Several QTLs for protein, starch and oil concentration formed the clusters of functionally related loci: some of these clusters overlapped or neighbored the associations of developmental genes (bins 2.05/06, 3.04, 5.03, 9.03 and 9.06/07); in another case, six QTLs for protein and starch concentration associated around *sh2* in bins 3.08/09, outside of the span of developmental gene clusters. A similar distribution, both within and beyond the developmental gene clusters, is characteristic of the loci for kernel pigmentation and enzymes of general metabolism (Coe et al. 1995). McMullen and Simcox (1995) described the clusters of genes and QTLs for disease and insect resistance in all maize linkage groups, except chromosomes 7 and 9. Some of these clusters were tightly linked and some were as loose as most developmental clusters. Several resistance clusters coincided with the position of developmental clusters (bins 1.06/07, 3.04/05, 6.01), whereas other significant clusters were found in the chromosome sections devoid

of developmental loci (bins 4.02/03, 8.06, 10.01). Multiple genes of resistance to rust are clustered in bin 10.01 (Hu and Hulbert, 1996) which is devoid of genes for development. In some of these cases, gene clusters which are not immediately related to plant development are found at the distal end of chromosome arms 6S, 7S and 10S.

Adaptive advantage of developmental gene associations

Evolutionary and cytogenetic evidence lends indirect support to the adaptive significance of developmental clusters. The prospect that selective pressure maintains such polygenic complexes against recombination may indicate the advantage of gene association. Indeed, the clusters comprising relatively few pleiotropic genes and QTLs for plant height, kernel size, seed disarticulation and day-length-insensitive flowering have been maintained as integral units throughout the evolution of the Poaceae (Lin et al. 1995; Paterson et al. 1995). Bingham et al. (1994) used their evidence of greater heterosis in autotetraploid alfalfa as compared to the diploid form to argue that the superiority of the linkage blocks (gene clusters) may reside in the complementary gene interaction within a conserved chromosome segment.

Physiological significance of clusters

We presume that the clusters are the functional units of genes expressed in concert to contribute to plant growth, development and, apparently, some of the plant responses to stress. The general idea of a physiological advantage of gene clusters is borrowed from the field of metabolic regulation. The close association of functionally related genes in the clusters would contribute to the compartmentation and tunneling of signal molecules, and thus would protect, stabilize and amplify the environmental and hormonal signals. The spatial and temporal control over gene expression must also benefit from a clustering of transcription factors, though the model linking developmental events that occur wide apart in time may present considerable difficulties.

In this context, the physiological advantage of functional gene units is best justified by recent evidence concerning Hox genes in animals and MADS-box genes in a diverse range of eukaryotes, from yeasts to mammals. The focal argument is the ability of these genes to act in concert due to heterodimerization of their products, i.e., transcription factors with subtly different DNA-binding specificities (Kenyon 1994; Shore and Sharrocks 1995). Several dozens of MADS-box genes are already known in *Arabidopsis*, *Antirrhinum*, maize and other plant species that considerably differ in their spatial and temporal specificity (Carpenter et al. 1995; Mena et al. 1995; Purugganan et al. 1995; Rounsley et al. 1995; Weigel 1995; Yanofsky 1995). The interaction of homeobox and MADS-box genes within a developmental gene cluster would cooperatively recruit the various transcription factors into multicomponent regulatory modules of higher specificity and thus would facilitate the fine tuning of plant growth and development. Similar interactions of the proteins expressed by different *obf* genes are probably important for promoter regulation (Zhang et al. 1995).

Agronomic implications of the concept of developmental gene clusters

On the collective evidence presented above we suggest that clusters of developmental genes are in fact the major QTLs. The fact that plant response to drought stress was also found to relate to the associations of developmental genes presumes that many plant reactions to abiotic stresses when mediated by the growth machinery rely on these gene clusters. The same is apparently true in the case of microbial infections (*Agrobacterium*, *Plasmodiophora*, *Rhizobium*) and such intruders as gall-forming insects and nematodes that modify normal cell differentiation to provide for their own habitat in plant tissues. *Lesions* and *necrotic* components of the developmental gene clusters, as well as octopine synthase binding factors (*obf*), may participate in manifold defense responses to pathogens. The possibility that the associations of developmental genes could somehow participate in plant resistance apparently deserves further consideration.

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