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Genetic approaches to inflorescence and leaf development in maize

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Summary

The application of genetic methods to the analysis of morphogenesis in maize is described. Several classes of floral mutants are differentiated through developmental studies and tests of epistasis. The results of mosaic and dosage analysis of *Kn1*, a dominant mutation affecting leaf development, are related to molecular studies of the gene.

Key words: maize, morphogenesis, epistasis, mutations, dosage and mosaic analysis.

Introduction

Although it is clear that development is guided to a large extent by information encoded in genes, describing how this is accomplished has been challenging. A detailed description of the process through developmental studies is an important first step, but this approach by itself cannot resolve the activity of individual genes. Molecular studies, by contrast, succeed well at describing gene products, but fail to demonstrate specific functions for them in the larger process.

Genetics affords a solution to this problem by laying a phenomenological framework that is relevant in both developmental and molecular contexts. We describe here several examples that illustrate our use of genetics to understand morphogenesis in maize. We begin with floral development to show how genetics can help define relationships between individual components of a complex process. The focus then shifts to leaf development with discussion of how dosage and mosaic analyses have clarified the nature of the *Knotted* (*Kn1*) gene and provided a framework for molecular studies. Finally we consider strategies for integrating molecular data into genetically grounded models of development.

Developmental analysis of inflorescence mutants

Several aspects of the maize inflorescence make it a convenient system to study morphogenesis. Although the development of the inflorescence is a complex process, it can be readily divided into a sequence of morphologically distinct stages. Because the inflorescence is a reiterated structure, with the oldest, most differentiated florets at its base, departures from the normal sequence of development are easily discerned. Finally, mutants exist that alter specific aspects of inflorescence development (Sheridan, 1989; Coe *et al.* 1988).

Normal development can be divided into two broad phases, as suggested by the classic studies of Bonnett (1940, 1948) and the later more refined SEM analysis of Cheng *et al.* (1983). The first begins with the transformation of a vegetative meristem into a floral axis (Fig. 1A). After the last leaf is initiated, the meristem elongates with spikelet pair primordia forming in an acropetal sequence. These primordia divide to form two individual spikelet initials, each of which initiates a pair of flanking glumes. The spikelet primordia then divide to give upper and lower floret primordia. Each floret axis then produces, in an acropetal sequence, the initials for the glume-like lemma and palea, the stamens and lodicules, and finally, the centrally located pistil. At this point, the immature ear and tassel are bisexual and essentially isomorphic structures that are distinguished primarily by their respective axillary and terminal positions.

During the second phase of development, the tassel (Fig. 1B) and ear (Fig. 1C) become morphologically distinct. The staminate florets of the tassel and the pistillate florets of the ear differentiate by the respective abortion of pistil and stamen primordia. The lower florets of ear spikelets typically disintegrate, whereas they remain in tassel spikelets. In most lines of maize, tassels develop spikelet-bearing lateral branches.

A phenotypic analysis of inflorescence mutants hints at some of the basic logic of floral development. The vegetative growth of most of these mutants is relatively normal, suggesting that the corresponding genes are required only during flowering. Inflorescence-specific mutants can be grouped in a hierarchy. Mutants such as *silkless* (*sk*) (Jones, 1925) or *Vestigial glume* (*Vg*) (Sprague, 1939), each of which alters only one type of

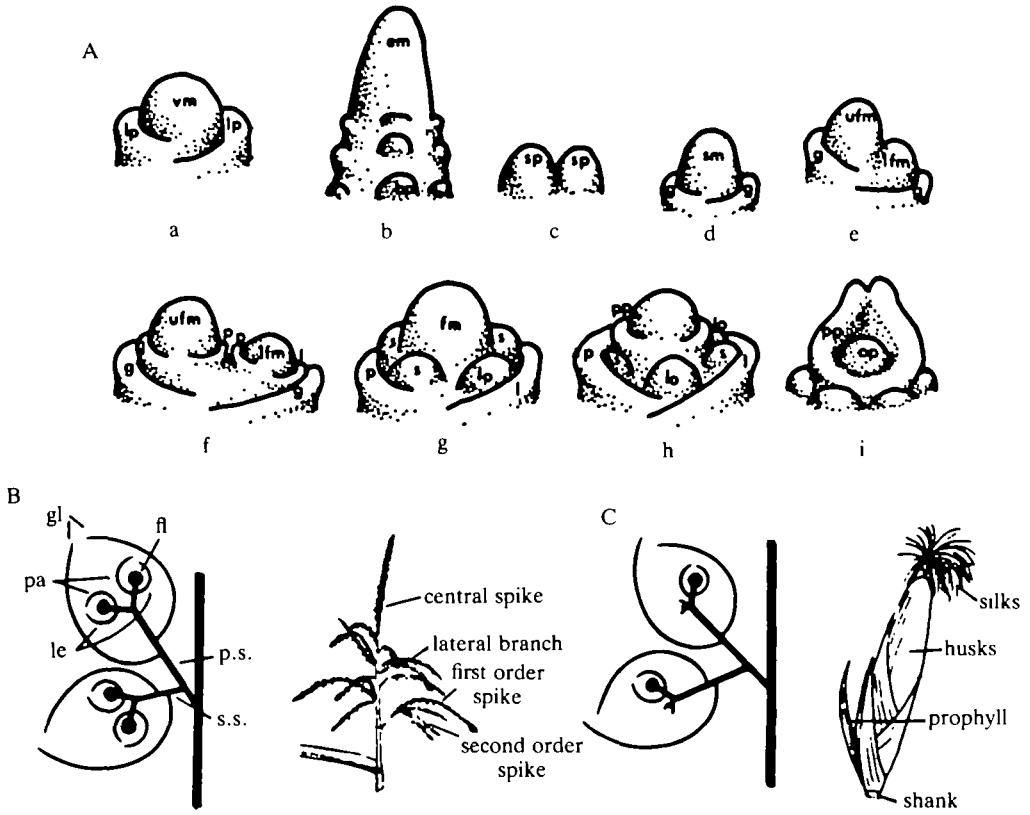

Fig. 1. (A) Diagrammatic representation of the developmental steps of the inflorescence, from Postlethwaite and Nelson (1964). lp, leaf primorida; vm, vegetative meristem; em, elongating meristem; fm, floral meristem; bp, branch primordia (spikelet pair primordia); sp, spikelet primordia; sm, spikelet meristem; g, glumes; ufm, upper floret meristem; lfm, lower floret meristem; l, lemma; p, palea; s, stamens; lo, lodicule; pp, pistil primordia. (B) and (C) Schematic comparison of a pair of tassel spikelets (B), *vs* pair of ear spikelets (C) from Poethig (1982). ps, pedicellate spikelet; ss, sessile spikelet; gl, glumes; le, lemma; pa, palea; fl, floret. Sketches of the corresponding mature inflorescences are shown to the right of each schematic.

inflorescence structure, suggest that gene products pertain exclusively to the development of a single organ. Other mutants show transformations between organ series, suggesting a class of genes that determine organ identity. For example, in the mutant *silky* (*si*), pistil-like structures develop in place of anthers in the ear (Fraser, 1933; B. Veit, unpublished observations). A third class of mutants defines genes that coordinate the development of more than one organ series. For example, mutants such as *ts2* (*tasselseed*) convert the tassel into a kernel-bearing structure by reversing the normal pattern of organ abortion in the tassel. (Emerson, 1920; Nickerson and Dale, 1955; Irish and Nelson, 1989; B. Veit, unpublished observations).

Simple comparisons between mutant and normal patterns of development can suggest the time when a gene is required. Postlethwaite and Nelson (1964) discriminated between several early acting inflorescence mutants, based on the stage at which their phenotypes were first visible. They suggested that such mutants might define critical 'switch points' in development, with normal gene function required to progress to subsequent steps in a developmental pathway. They proposed, for example, that the *ra1* (*ramosal*; Beadle, 1932) mutation interferes with an early decision in which spikelet pair primordia are determined, causing their replacement with less determinate branches.

We have applied this comparative approach to other mutants to make provisional assessments of when the corresponding genes are normally required. This method cannot determine the precise time of gene action since there will always be some undefined lag before differences in gene expression are manifest on a morphological level. Nevertheless, it does define a late limit for the time of gene action. One example (Fig. 2) compares the sequence of development of a normal ear with that conditioned by the mutant *bd* (*branched silkless*; Kempton, 1934). Development proceeds normally up to the point when floret primordia are formed. Although each spikelet gives rise to what appear to be the upper and lower floret primordia, an indeterminate number of abnormal divisions ensue to produce a highly branched structure. Thus, in contrast to *ra1*, which interferes with determination of the spikelet, *bd* appears to block determination of the floret.

Tests of epistasis

Tests of epistasis have proven useful in defining functional relationships between mutationally defined

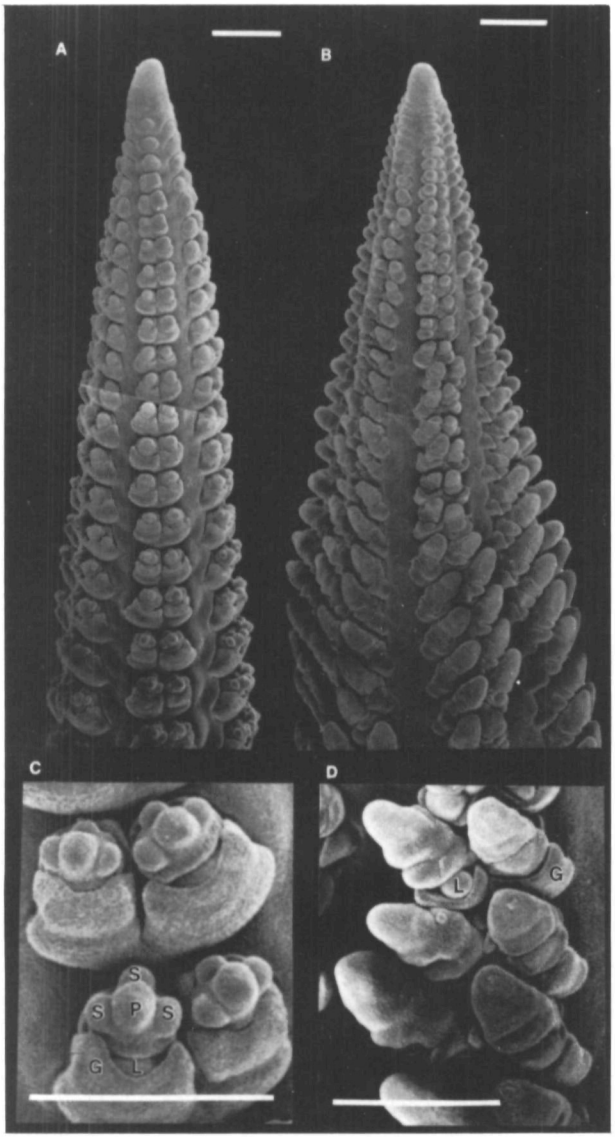

Fig. 2. SEM comparison of immature normal (A) and *bd* (B) ears. Panels illustrate gradient of development with older and more developed spikelets at the base of ears. (C) and (D) closeups of normal *vs bd* immature spikelet pairs. S=stamen, P=pistil, G=glume, L=lower floret. Scale bars for all panels, 0.5 mm.

genes. While the validity of this approach has been most clearly demonstrated in simple microbial systems (reviewed by Botstein and Maurer, 1982), the predictive value of epistasis studies can be seen in examples of *Drosophila* and *Caenorhabditis* development (Nusslein-Vollhard and Wieshaus, 1980; Baker and Belote, 1983; Hodgkin, 1990). The utility of this method in the analysis of plant developmental mutants has been demonstrated in a number of studies (Marx, 1987; Bowman *et al.* 1989). We describe here two inflorescence mutant combinations that illustrate the logic of the approach.

The first simple case examines the combination of *ts2* and *Miniplant* (*Mpl*). *ts2* conditions the development of a completely pistillate tassel. *Mpl* (Harberd and

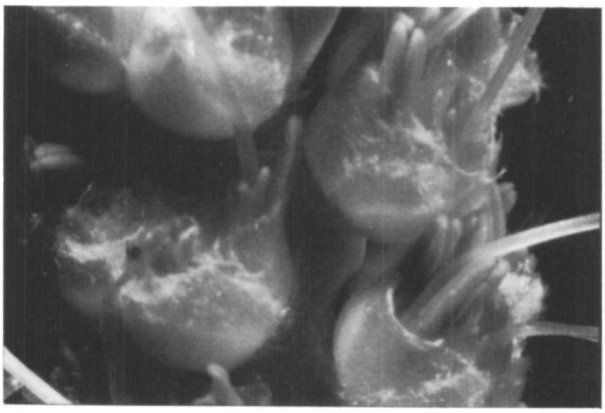

Fig. 3. Closeup view of a lateral tassel branch from a *Mpl ts2* double mutant showing well developed anthers and silks in the same floret.

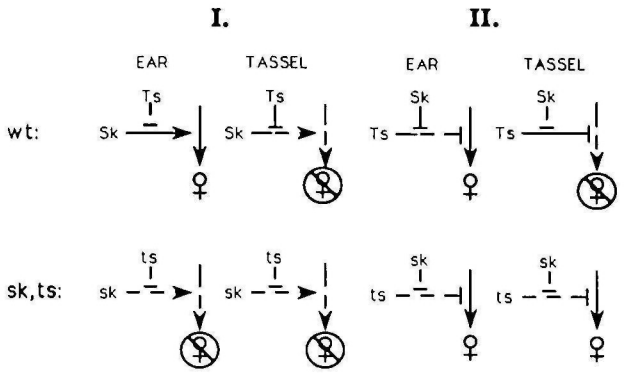

Fig. 4. Comparison of wild type (wt) *vs sk ts2* double mutant development by two distinct models I and II. Sk and Ts represent the wild-type products of the *sk* and *ts* mutations. A line that does not end in an arrow represents a repressor, an arrow represents an activator. A dashed line represents the absence of functional product. The female symbol represents pistillate development.

Freeling, 1987), a dominant dwarf mutant, has relatively little effect on the tassel, but instead blocks abortion of stamen primordia in the developing ear to give perfect florets (anther-ear). The double mutant is an anther-eared dwarf with perfect florets in the tassel (Fig. 3). Neither mutant perturbs the expression of the other (i.e. the double mutant phenotype is a superimposition of those phenotypes associated with the single mutants), suggesting the genes affect pathways that proceed independently of each other.

The second case considers the double mutant combination of *ts2* and *sk* (*silkless*), two genes that appear to interact with each other. If these mutants result from loss of function, as suggested by their recessive character, the wild-type *Silkless* (*Sk*) gene could be thought of promoting formation of silks in the ear, whereas the wild-type *Tasselseed2* gene (*Ts2*) would act to suppress the formation of silks in the tassel.

Two alternative models would relate the activities of *Ts2* and *Sk* (Fig. 4). The first supposes that *Sk* has the potential to promote silk development in both the ear

and tassel, but is suppressed in the latter by the activity of the *Ts2* gene. The model supposes that *Ts2* is normally not expressed in the ear, leaving the silk-promoting *Sk* gene unsuppressed. An alternative model supposes that *Ts2* has the potential to suppress silk development in both the ear and tassel, but is suppressed in the ear by *Sk*. While both models account equally well for the single mutant phenotypes, only the second correctly predicts the observed tasselseed phenotype of the double mutant (Jones, 1932, 1934).

The long term aim of this approach is to develop internally consistent models for gene interactions by examining many double mutant combinations. Studies that are in progress have so far revealed relatively few cases of epistasis. With a few exceptions, such as *ts2* and *sk*, the majority of double mutants show additivity of mutant phenotypes, especially in those cases where mutants affect the development of different organs. Nonetheless, these data should provide a useful foundation for molecular studies of inflorescence development.

Dosage and mosaic analysis of the *Kn1* locus

In contrast to the preceding analysis, which aims to describe temporal and functional relationships between genes, we have also approached development by focusing on a single gene, *Knotted* (*Kn1*), that affects leaf development. The normal pattern of leaf development has been described in a number of studies (Sharman, 1942; Dale, 1988; Langdale *et al.* 1989; Nelson and Langdale, 1989). In 1922, *Kn1-O*, a mutant with hollow, finger-like outpocketings of tissue on its leaves, was discovered in a row of Walden Dent corn. The *Kn1* locus is now defined by several additional dominant mutations which are considered alleles to the extent that they share similar phenotypes and a common map position, near *Adh1* on the long arm of chromosome 1 (Freeling and Hake, 1985).

Although knots conditioned by *Kn1* can lend a grotesque appearance to affected plants (Fig. 5), it would be wrong to dismiss them as 'teratologies'. On the contrary, several aspects of the *Kn1* phenotype display a positional specificity that suggest the *Kn1* gene participates in the process of normal leaf development. For example, knots form on the blade portion of the leaf, and are found much less frequently on the more proximal sheath (Fig. 6). Within the blade, the knots are centered over the lateral veins. These veins, even when lacking knots, show a relatively consistent thickening in which parenchymatous cells replace the bundle sheath and associated upper sclerenchyma. In contrast, the smaller intermediate veins appear normal. Further specificity is seen in the characteristic differences that characterize different *Kn1* alleles (Freeling and Hake, 1985). For example, knots conditioned by *Kn1-O* (O=original) occur most frequently on leaves initiated late in development, while those conditioned by *Kn1-2F11* tend to occur on embryonic leaves. The two mutants also differ with respect to displacement of

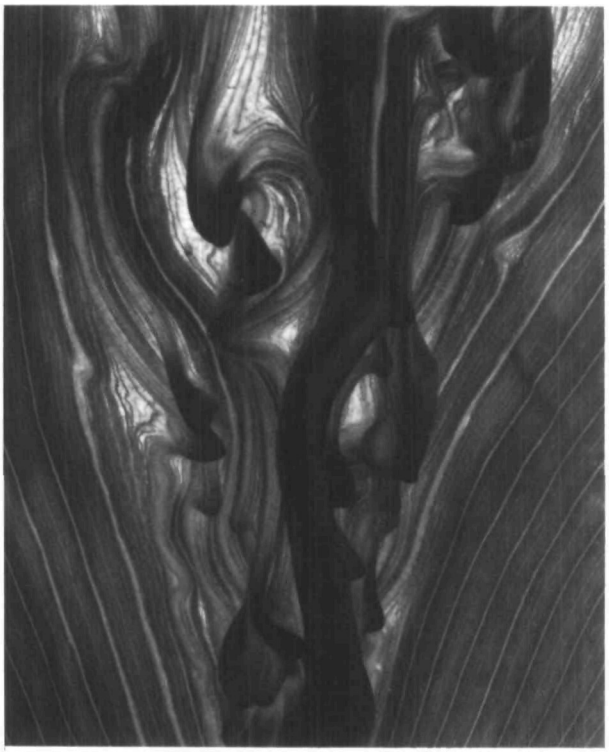

Fig. 5. Backlit photograph of the abaxial (lower) surface of a *Kn1-O* leaf blade showing severe knots centered over lateral veins.

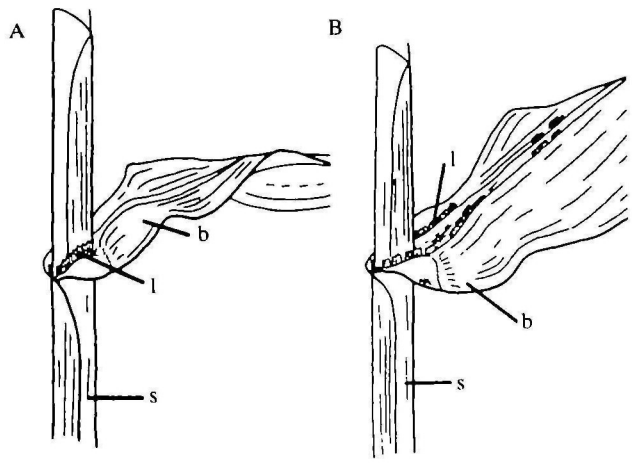

Fig. 6. Line drawings illustrating normal maize leaf parts (A) and ligule displacement associated with the *Kn1-O* mutant (B). b, blade; l, ligule; s, sheath.

the ligule, a flap of tissue that normally forms at the junction of the blade and sheath (Fig. 6). While displacement is relatively common in *Kn1-O*, with ectopic ligule running parallel to lateral veins, it rarely is seen in *Kn1-2F11*.

Dosage analysis has been used to address the dominant expression pattern that characterizes all *Kn1* mutants (Freeling and Hake, 1985). Knots do not appear to result from reduced expression of a normal gene product since plants which are hypoploid for the

long arm of chromosome 1 (which would contain the hypothetical wild type *Kn1* gene) are normal. Conversely, increasing the dose of a smaller 18 map unit segment of chromosome 1 from the normal 2 copies to 4, fails to produce knots, suggesting that *Kn1* conditions the phenotype through expression of a novel gene product or a substantial overexpression of a normal product.

Further insight into the nature of the *Kn1* gene product has been gained through mosaic analysis. Clonal sectors of tissue which lacked *Kn1* were generated by X-irradiating young plants which were heterozygous for *Kn1*. Sectors lacking *Kn1* are easily recognized by the absence of chlorophyll that results when the closely linked gene *lw* (*lemon white*) on the opposite homologue is uncovered. Pure white sectors were unknotted, suggesting that Kn1 activity does not readily diffuse in a lateral direction (Hake and Freeling, 1986). More refined analyses were performed to ask in which cell layers *Kn1* must be expressed for knots to form. Examination of numerous mericlinal and sectorial chimeras in which one or more layers lacked the *Kn1* gene indicated that *Kn1* need only be present in the internal layers corresponding to the bundle sheath to perturb the development of all cell layers (Sinha and Hake, 1990). Thus, knotting provides an example of induction in plants, in which the developmental fate of one cell layer is determined by a *Kn1*-dependent signal that originates in an adjacent layer.

Molecular analysis of *Kn1*

The cloning of *Kn1-2F11* has provided opportunities to test and refine models based on genetic analysis. One such approach seeks to define a structural basis for phenotypic differences that characterize different *Kn1* alleles. *Kn1-2F11* was cloned by virtue of its association with the previously cloned transposable element, *Ds2* (Hake *et al.* 1989). Moreover, analysis of revertants suggests *Ds2* is responsible for the mutant phenotype, since knotting is absent in lines where the *Ds2* has transposed away.

Comparison of *Kn1-2F11* with several *Kn1* alleles (designated *Kn1-mum*) that arose in lines carrying Robertson's Mutator (Robertson, 1978) reveals some similarities. Like *Kn1-2F11*, the *Kn1-mum* alleles are associated with transposon insertions (Walko and Hake, manuscript in preparation). The inserts, members of the one Mutator class of transposons, lie near where the *Ds2* element is inserted in the *Kn1-2F11* (Fig. 7A). However, in contrast to the mild knots conditioned by *Kn1-2F11*, *Kn1-mum* knots are more severe and are found on leaves initiated later in development. *Kn1-O*, an allele which like the *Kn1-mums* conditions knots late in development, lacks any sizeable insertion element. Instead, the allele is associated with a tandem duplication of a 17 kb region (Fig. 7B). Knotting is absent in derivatives in which the duplication has been lost or disrupted, while gain of a third repeat copy exacerbates knotting (Veit *et al.* 1990).

The sequence alterations associated with various *Kn1* alleles, involving a relatively small region (<2 kb), prompted an analysis of transcription (Vollbrecht *et al.* 1991). Probes derived from sequences lying between the novel duplication junction of *Kn1-O* and the *Ds2* insertion site of *Kn1-2F11* detect a single 1.7 kb polyA+ transcript in both *Kn1-O* and normal plants. Near full length cDNAs corresponding to the transcript were cloned from normal or *Kn1-O* plants and found

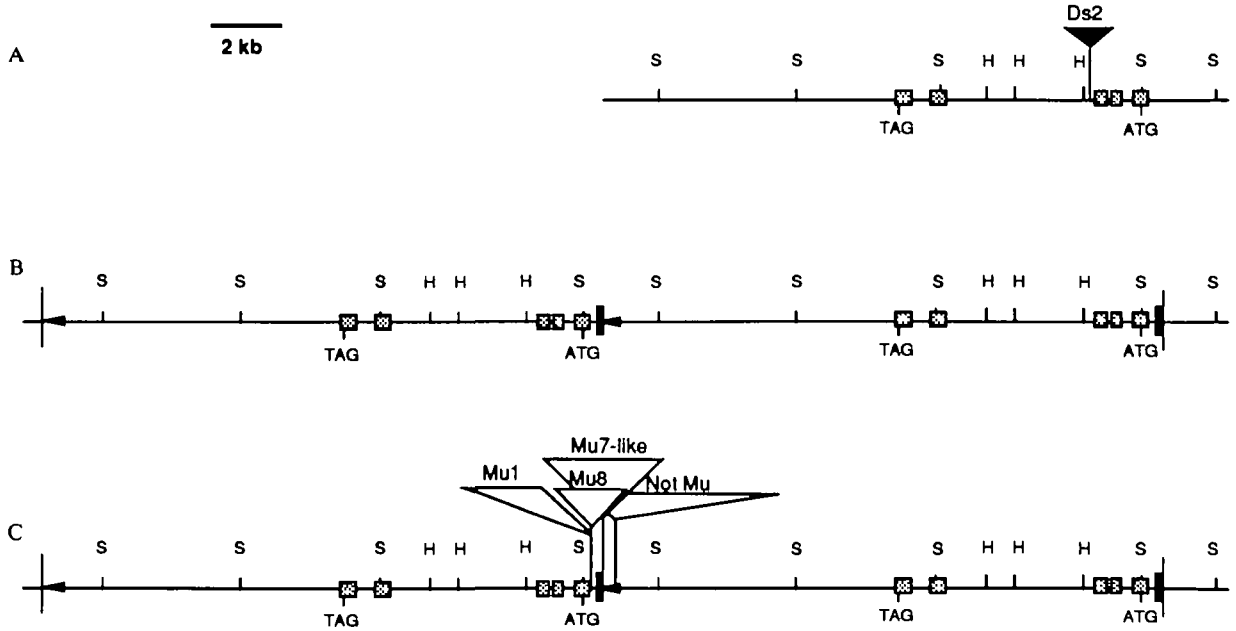

Fig. 7. Comparison of transposon induced *Kn1* alleles with *Kn1-O*. (A) Relative positions of transposon insertions (triangles) in *Kn1-2F11*. (B) Duplicated structure of *Kn1-O*. (C) Positions of transposon insertions that knockout *Kn1-O*. Exons of *Kn1* gene are shown as small boxes superimposed on map. S, *Sac*I; H, *Hin*dIII.

to be essentially identical (Fig. 7B). The gene spans 8 kb and includes 4 introns. Primer extension and S1 analysis locate the transcribed portion of the gene entirely within the *Kn1-O* repeat unit. The unusually large third intron contains the insertion sites of transposons associated with the *Kn1-2F11* and *Kn1-mum* alleles (Fig. 7A). A homeodomain sequence motif is found in the fifth exon, suggesting that the *Kn1* gene product is a transcription factor.

Thus far, we have detected no qualitative differences between transcripts expressed in normal *vs. Kn1-O* or *Kn1-2F11* plants. Quantatitive differences are observed only for *Kn1-O* in which expression is proportional to gene copy number. While this difference might suggest that the knotted phenotype results from a simple increase in levels of a normal transcript, two lines of evidence argue against this model. First, as shown by previously discussed dosage analysis, doubling the normal dose of *Kn1+* with segmental translocations does not produce knots; however, we have not yet discounted the possibility that dosage compensation is effected at the level of transcription by these larger duplications. A transposon mutagenesis study (Veit *et al.* 1990) casts further doubt on the simple overexpression model. Nine normal plants were found in a screen of 10000 *Kn1-O* heterozygotes. Of the normals, five had lost one of the tandem duplication copies, while the remaining four had sustained insertions within the tandem duplication. The distribution of insertions appears non-random (Fig. 7C). Although the *Kn1* gene is represented twice in the duplication, all four insertions fall within a 2 kb segment that is immediately 5′ to one of the gene copies. Thus it appears that the two gene copies are not equivalent.

The clustered distribution of insertions that knockout *Kn1-O* suggests that the novel junction it contains, lying immediately 5′ to one of the gene copies, might condition knotting through aberrant expression of a normal transcript. This model is supported by *in situ* localization studies that show a novel distribution of transcripts in *Kn1* plants. Signal that is normally concentrated in the schlerenchyma around lateral veins is more widely distributed in *Kn1* plants, with lower levels of hybridization seen in sclerenchymatous tissue and in the bundle sheath of intermediate veins (Sinha, 1990). By analogy with the enhancer element located within the intron of the immunoglobulin heavy chain gene (Banerji *et al.* 1983), it is conceivable that similar expression patterns are conditioned by the transposons of *Kn1-2F11* and *Kn1-mum* alleles. Alternatively, these transposons might condition knotting by serving as initiation sites for truncated, misexpressed transcripts.

Identifying other genes that interact with *Kn1*

Although the cloning of *Kn1* represents a significant step towards understanding leaf development, a mechanistic explanation for *Kn1* action awaits characterization of other components. Genetics provide some clues to their nature. Many components are likely to correspond to mutants that, like *Kn1*, interfere with normal leaf development. Their relationship to *Kn1* is being addressed by approaches similar to those that have been applied to inflorescence development. Evidence for other components can be seen in the form of background effects that mute or enhance the expression of *Kn1*. Unfortunately, attempts to resolve individual modifiers of *Kn1* have generally been unsuccessful, suggesting a polygenic basis for most of the background differences. One exception, *sok* (*suppressor of Knotted*), was resolved genetically as a recessive suppressor of *Kn1-O* (B. Greene, unpublished observations). *sok* by itself does not appear to condition abnormal leaf development. It is not yet known whether *sok* acts in an allele-specific manner.

Kn1 is also suppressed in plants which are monosomic for 1L (Freeling and Hake, 1985). Interestingly, suppression is not observed in plants in which *Kn1* is heterozygous with a small deficiency (<2 map units), implying that *Kn1* expression may be modified by specific dosage-sensitive factors on 1L. Alternatively, the altered growth characteristics generally associated with hypoploidy might in some way suppress knotting. Tests are in progress to distinguish these models.

Although we are attempting to clone some of these other genes through transposon tagging, the cloning of *Kn1* enables several alternative strategies. One strategy is suggested by the observation that many genes involved in *Drosophila* larval development share a conserved homeodomain motif (Hoey and Levine, 1988). These and other studies (reviewed by Biggin and Tjian, 1989) suggest a prominent role for structurally related transcription factors in regulating animal development. That plant development may be regulated in a similar manner has been suggested by the presence of a DNA-binding motif in *Kn1*, as well as in two genes that affect floral development, *agamous* from *Arabidopsis* (Yanofsky *et al.* 1990) and *deficiens* from *Antirrhinum* (Sommer *et al.* 1990). Hybridization experiments suggest the existence of a number of such homeodomain genes in maize and other species which we are now characterizing in more detail (B. Lowe, E. Vollbrecht and R. Walko, unpublished observations).

Given that *Kn1* appears to be a transcription factor, we may ask the obvious question of what it binds to. Recent technical innovations have made cloning of such target sequences possible (Sompayrac and Danna, 1990). Similarly, *Kn1* itself is likely to be a target of transcriptional regulation. We are using transient expresssion systems to define *cis* elements involved in such regulation with the hope they will lead us to *trans* acting factors (Vinson *et al.* 1988).

These approaches will almost certainly yield clones, but they say little about a gene beyond it having certain molecular properties that led to its isolation. By contrast, the functions of genes isolated by transposon tagging are often suggested by the altered phenotypes that result from insertions of the tagging elements. In some cases, it may be possible to show that genes isolated by molecular methods correspond to mutationally defined genes whose mutant phenotypes

would then be relevant. In maize, a preliminary assessment can be obtained very quickly by mapping with recombinant inbred lines (Burr *et al.* 1988).

While other types of correlative evidence may also be instructive, the most definitive tests of gene function will require transgenic methods, whereby the consequences and causes of modified expression patterns can be studied directly. Clones that correspond to loss of function mutations can be approached directly through complementation studies. For other clones, dominant phenotypes resulting from artificially induced expression might suggest function. In the case of *Kn1*, itself a dominant, our goal is to resolve those elements that are required to induce the knots by which we came to know the gene.

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References

Baker, B. and Belote, J. (1983). Sex determination and dosage compensation in *Drosophila melanogaster*. *A. Rev. Genet* **17**, 345–393.

Benerji, J., Olson, L. and Schaffner, W. (1983). A lymphocyte-specific cellular enhancer is located downstream of the joining region in immunoglobulin heavy chain genes. *Cell* **33**, 729–740.

Beadle, G. W. (1932). Genes in maize for pollen sterility. *Genetics* **17**, 413–431.

Biggin, M. D. and Tjian, R. (1989). Transcription factors and the control of *Drosophila* development. *Trends Genet.* **5**, 377–383.

Bonnett, O. T. (1940). Development of the staminate and pistillate inflorescences of sweet corn. *J. agric. Res.* **60**, 25–37.

Bonnett, O. T. (1948). Ear and tassel development in maize. *Ann. Mo. bot. Gard.* **35**, 269–287.

Botstein, D. and Maurer, R. (1982). Genetic approaches to the analysis of microbial development. *A. Rev. Genet.* **16**, 61–83.

Bowman, J. L., Smyth, D. R. and Meyerowitz, E. M. (1989). Genes directing flower development in *Arabidopsis*. *The Plant Cell* **1**, 37–52.

Burr, B., Burr, F. A., Thompson, K. H., Albertson, M. C. and Stuber, C. W. (1988). Gene mapping with recombinant inbreds in maize. *Genetics* **118**, 519–526.

Cheng, P. C., Greyson, R. I. and Walden, D. B. (1983). Organ initiation and development of unisexual flowers in the tassel and ear of *Zea mays*. *Am. J. Bot.* **70**, 450–462.

Coe, E. H., Jr, Neuffer, M. G. and Hoisington, D. A. (1988). The genetics of corn. In *Corn and Corn Improvement*, (ed. G. F. Sprague). Madison: Am. Soc. Agronomy.

Dale, J. E. (1988). The control of leaf expansion. *Ann. Rev. Plant. Physiol. Plant Mol. Biol.* **39**, 267–295.

Emerson, R. A. (1920). Heritable characters of maize. II – Pistillate flowered maize plants. *J. Hered.* **11**, 65–76.

Fraser, A. C. (1933). Heritable characters of maize XLIV – silky ears. *J. Hered* **24**, 41–46.

Freeling, M. and Hake, S. (1985). Developmental genetics of mutants that specify knotted leaves in maize. *Genetics* **111**, 617–634.

Hake, S., Vollbrecht, E. and Freeling, M. (1989). Cloning *Knotted*, the dominant morphological mutant in maize using *Ds2* as a transposon tag. *EMBO J.* **8**, 15–22.

Hake, S. and Freeling, M. (1986). Analysis of genetic mosaics shows that the extra epidermal cell divisions in *Knotted* mutant maize plants are induced by adjacent mesophyll cells. *Nature* **320**, 621–623.

Harberd, N., Hake, S. and Freeling, M. (1987). Programmed periclinal divisions of epidermal cells during glume development. *Maize Genet. Coop. News Lett.* **61**, 23–24.

Hodgkin, J. (1990). Sex determination compared in *Drosophila* and *Caenorhabditis*. *Nature* **344**, 721–728.

Hoey, T. and Levine, M. (1988). Divergent homeobox proteins recognize similar DNA sequences in *Drosophila*. *Nature* **332**, 858–861.

Irish, E. E. and Nelson, T. (1989). Sex determination in monoecious and dioecious plants. *Plant Cell* **1**, 737–744.

Jones, D. F. (1925). Heritable characters of maize. XXIII – *Silkless*. *J. Hered.* **5**, 339–341.

Jones, D. F. (1932). The interaction of specific genes determining sex in dioecious maize. *Proceedings of the Sixth International Congress of Genetics* **2**, 104–107.

Jones, D. F. (1934). Unisexual maize plants and their bearing on sex differentiation in other plants and in animals. *Genetics* **19**, 552–567.

Kempton, J. H. (1934). Heritable characters in maize. XLVII – *Branched silkless*. *J. Hered.* **25**, 29–32.

Langdale, J. A., Lane, B., Freeling, M. and Nelson, T. (1989). Cell lineage analysis of maize bundle sheath and mesophyll cells. *Devl Biol.* **133**, 128–139.

Marx, G. A. (1987). A suite of mutants that modify pattern formation in pea leaves. *Plant Molec Biol. Rep.* **5**, 311–335.

Nelson, T. and Langdale, J. A. (1989). Patterns of leaf development in C4 plants. *The Plant Cell* **1**, 3–13.

Nickerson, N. H. and Dale, E. E. (1955) Tassel modifications in *Zea mays*. *Ann. Mo. bot. Gard.* **42**, 195–212.

Nüsslein-Volhard, C. and Wieschaus, E. (1980). Mutations affecting segment number and polarity in *Drosophila*. *Nature* **287**, 795–801.

Poethig, R. S. (1982). Maize – the plant and its parts. In *Maize for Biological Research* (ed. W. F Sheridan), pp 9–18 Charlottesville: Plant Molec. Biol. Assoc.

Postlethwaite, S. N. and Nelson, O. E. (1964). Characterization of development in maize through the use of mutants. I. The *Polytypic (Pt)* and *Ramosa-1 (ra1)* mutants. *Am. J. Bot.* **51**, 238–243.

Robertson, D. S. (1978). Characterization of a mutator system in maize. *Mutat. Res.* **51**, 21–28.

Sharman, B. C. (1942). Developmental anatomy of the shoot of *Zea mays* L. *Ann. Bot.* **6**, 245–284.

Sheridan, W. F. (1988). Maize developmental genetics: Genes of morphogenesis. *A. Rev. Genet.* **22**, 353–385.

Sinha, N. and Hake, S. (1990). Mutant characters of *Knotted* maize leaves are determined in the innermost tissue layers. *Devl Biol.* **141**, 203–210.

Sinha, N. (1990). *Developmental Analysis of the Knotted Mutant in Maize*. Ph.D. dissertation, University of California, Berkeley.

Sommer, H., Beltran, J.-P., Huijser, P., Pape, H., Lönnig, W.-E., Saedler, H. and Schwarz-Sommer, Z. (1990). *Deficiens*, a homeotic gene involved in the control of flower morphogenesis in *Antirrhinum majus*: the protein shows homology to transcription factors. *EMBO J.* **9**, 605–613.

Sompayrac, L. and Danna, K. J. (1990). Method to identify genomic targets of DNA binding proteins. *Proc. natn. Acad. Sci. U.S.A.* **87**, 3274–3278.

Sprague, G. F. (1939). Heritable characters in maize – *Vestigial glume*. *J. Hered.* **30**, 143–145.Veit, B., Vollbrecht, E., Mathern, J. and Hake, S. (1990). A tandem duplication causes the *Kn1-O* allele of *Knotted*, a dominant morphological mutant of maize. *Genetics* **125**, 623–631.

Vinson, C. R., Lamarco, K. L., Johnson, P. F., Landschulz, W. H. and McKnight, S. L. (1988). *In situ* detection of sequence-specific DNA binding activity specified by a recombinant bacteriophage. *Genes Dev.* **2**, 801–806.

Vollbrecht, E., Veit, B., Sinha, N. and Hake, S. (1991). The developmental gene *Knotted-1* is a member of a maize homeobox gene family. *Nature* **350**, 241–243.

Yanofsky, M. F., Ma, H., Bowman, J. L., Drews, G. N., Feldmann, K. A. and Meyerowitz, E. M. (1990). The protein encoded by the *Arabidopsis* homeotic gene *agamous* resembles transcription factors. *Nature* **346**, 35–39