Development 130, 1149-1158

doi:10.1242/dev.00336

Combinatorial control of meristem identity in maize inflorescences

Nicholas J. Kaplinsky and Michael Freeling*

351 Koshland Hall, University of California at Berkeley, Berkeley, CA 94720, USA
*Author for correspondence (e-mail: freeling@nature.berkeley.edu)

Accepted 28 November 2002

SUMMARY

The architecture of maize inflorescences, the male tassel and the female ear, is defined by a series of reiterative branching events. The inflorescence meristem initiates spikelet pair meristems. These in turn initiate spikelet meristems which finally produce the floret meristems. After initiating one meristem, the spikelet pair and spikelet meristem convert into spikelet and floret meristems, respectively. The phenotype of *reversed germ orientation1* (*rgo1*) mutants is the production of an increased number of floret meristems by each spikelet meristem. The visible phenotypes include increased numbers of flowers in tassel and ear spikelets, disrupted rowing in the ear, fused kernels, and kernels with embryos facing the base of the ear, the opposite orientation observed in wild-type ears. *rgo1* behaves as single recessive mutant. *indeterminate spikelet1* (*ids1*) is an unlinked recessive mutant that has a similar phenotype to *rgo1*. Plants heterozygous for both *rgo1* and *ids1* exhibit nonallelic noncomplementation; these mutants fail to complement each other. Plants homozygous for both mutations have more severe phenotypes than either of the single mutants; the progression of meristem identities is retarded and sometimes even reversed. In addition, in *rgo1; ids1* double mutants extra branching is observed in spikelet pair meristems, a meristem that is not affected by mutants of either gene individually. These data suggest a model for control of meristem identity and determinacy in which the progress through meristem identities is mediated by a dosage-sensitive pathway. This pathway is combinatorially controlled by at least two genes that have overlapping functions.

Key words: *rgo*, Maize inflorescence development, Nonallelic noncomplementation, Spikelet, Meristem identity, Gene dosage, *ids1*

INTRODUCTION

Plant growth consists of reiterative developmental events that determine the morphology of the mature organism. Meristems, self organizing pools of undifferentiated cells, give rise to most plant organs including other meristems. The above ground portion of the plant is elaborated from the shoot apical meristem (SAM), which produces leaves, stems and axillary meristems (together called a phytomer) in a repetitive manner (Galinat, 1959; Steeves and Sussex, 1989). If meristem maintenance is disrupted, production of phytomers ceases and plant development arrests (Long et al., 1996; Vollbrecht et al., 2000). Therefore, proper plant development depends on normal meristematic activity. The reproductive structure of plants, the inflorescence, is produced by an inflorescence meristem (IM), which is derived from the SAM and/or axillary meristems. Inflorescences exhibit a wide range of meristematic behaviors in different plants, leading to the varying reproductive morphologies seen in the plant kingdom. Secondary and higher order meristems can be initiated from the primary IM. Each order of meristems represents an order of branching, thus meristem function and inflorescence shape are directly related (Kellogg, 2000). In the grasses, inflorescences range from simple and unbranched spikes to highly branched panicles. This variability can be observed within single species (Chapman and Peat, 1992). During the domestication of corn, *Zea mays*, from teosinte, there has been an increase in inflorescence complexity that reflects human selection for agronomically important traits (Doebley and Stec, 1991; Galinat, 1996; Iltis, 1988).

Maize is a monoecious species that has two types of inflorescences, the male tassel at the apex of the plant, and female ears in the axils of leaves. Tassel and ear development are similar until after flowers are initiated, at which point organ abortion in the tassel and the ear produce separate unisexual inflorescences (Dellaporta and Calderon-Urrea, 1994; Irish, 1996). At the top of the plant the SAM elongates and converts into an IM, which gives rise to the tassel. At approximately the same time, the axillary meristems initiate a prophyll (the first leaf on a shoot) and eight to 14 husk leaves, and then convert into a lateral IM, which produces the ear. The top (youngest) ear primordium grows faster than lower primordia and often develops into a single dominant ear. The IM gives rise to 2°, 3°, and 4° meristems (Fig. 1A). Each IM produces an indeterminate number of spikelet pair meristems (SPM, 2°) in an acropetal (from the base to the apex), polystichous (multiple rows) manner. In the tassel, the first few SPMs become branch meristems and produce long tassel branches that initiate SPMs in a distichous pattern. All other SPMs initiate one spikelet meristem (SM, 3°) and then convert into SMs. Each SM then initiates a pair of bract-like glumes. Subsequently, each SM initiates the lower floret meristem (FM, 4°) and converts into

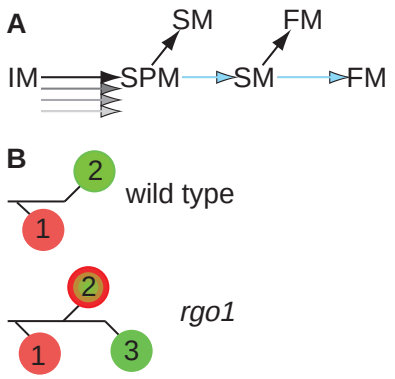

Fig. 1. A model of inflorescence development. (A) The inflorescence meristem (IM, 1°) initiates (black arrows) spikelet pair meristems (SPM, 2°). The SPM then initiates a spikelet meristem (SM, 3°) on its flank, and then converts (blue arrows) into a SM. In a reiterative manner, each SM initiates one floret meristem (FM, 4°) and converts into a FM. Adapted from Irish (Irish, 1997b). (B) In the ear, wild-type spikelets (top) initiate the lower floret (1) which aborts (red) and then the spikelet converts into the upper floret (2) which develops into a functional flower (green). In *rgo1* plants (bottom), the spikelet produces a third flower (3). In many cases the second flower also develops (green/red gradient). Owing to the distichous pattern of floret initiation by the spikelet, any odd numbered flower (1, 3, 5, …) will face the base of the ear, while even numbered flowers (2, 4, 6, …) face the tip of the ear. Any mutation that either increases or decreases floret number may result in a rgo phenotype.

the upper FM. The FMs make the floral organs, the palea/lemma, lodicules, anthers and pistils. The pistils abort in the tassel, while the anthers and lower pistil abort in the ear (Cheng et al., 1983). This results in a single kernel developing from each ear spikelet. A large number of mutants that affect these meristem initiation and identity conversion events have been identified and make the maize inflorescence a powerful system for studying meristem function (McSteen et al., 2000; Veit et al., 1993).

Maize inflorescence mutants affect tassel and ear development in many ways. Interestingly, mutant phenotypes often differentially affect the two inflorescence types (Postlethwait and Nelson, 1964). These mutants interact with each other, often synergistically. The class II tasselseed mutants (*ts4*, *Ts6*) affect both floral organ abortion and branching. Pistil abortion in the tassel is inhibited resulting in a feminized tassel with seeds. In addition, SPMs fail to convert into SMs in *ts4*, and SM to FM transitions are delayed in *Ts6*. Both of these mutations result in extra branching in the ear (Irish, 1997a; Irish, 1997b). The ramosa mutants (*ra1*, *ra2*, *ra3*) have indeterminate SPMs; these make long branches that produce many SMs (Postlethwait and Nelson, 1964; Veit et al., 1993). *indeterminate spikelet1* (*ids1*) mutants delay the SM to FM conversion resulting in the production of extra florets, which is a more branched and less determinate condition (Chuck et al., 1998). *indeterminate floral apex1* (*ifa1*) affects determinacy of all inflorescence meristems, resulting in extra SPMs, SMs and an indeterminate FM. *ids1* and *ifa1* interact synergistically, with SMs converting into branch meristems in the ear and into SPMs in the tassel (Laudencia-Chingcuanco and Hake, 2002).

Perturbations of regular rowing and kernel orientation are sensitive indicators of defects in inflorescence development. Increased or decreased numbers of florets produce an easily recognizable reversed germ orientation (rgo) phenotype (Fig. 2D). Several reversed germ and fused kernel mutants have been previously described in maize. Kiesselbach describes the occurrence of reversed and fused kernels in various inbreds and hybrids, as well as documenting reports of these phenotypes dating back to 1810 (Kiesselbach, 1926). In this and subsequent reports (Jackson, 1996b; Joachim, 1956), the reversed orientation of the kernels was attributed to the development of the lower instead of the upper floret. Fused kernels were attributed to the development of both florets in a spikelet. The development of both florets occurs in Country Gentleman sweet corn and results in reversed kernels (Micu et al., 1983). Diverse patterns of floret development are found in the grasses; basal fertile florets with non-fertile apical florets are found in the pooids, while the opposite pattern occurs in the panicoids (Chapman and Peat, 1992).

The *reversed germ orientation1* (*rgo1*) mutant was isolated by Sachan and Sarkar. They suggested that the phenotype was due to the differential growth of the integuments and the nucellus causing rotation of the kernel (Sachan and Sarkar, 1980). In this paper, we show that the *rgo1* reversed kernel phenotype is actually due to the production of an extra floret by the spikelet meristem. We demonstrate that *rgo1* and *ids1* have overlapping functions in regulating meristem identity. The *rgo1; ids1* double mutant is synergistic and affects more meristem types than either mutant alone. A dosage based model is proposed for the regulation of meristem identity conversion in the maize inflorescence.

MATERIALS AND METHODS

Plant strains and growth

A *rgo1-Sarkar* stock (904G) was obtained from the Maize Genetics Cooperation Stock Center (MGCSC, Urbana, IL) (Jackson, 1996a). This was introgressed seven times into B73 (Pioneer Hi-Bred, Des Moines, IA). The B73 introgression was used for complementation and allelism crosses and double mutant analyses. Plants were grown in fields in Berkeley, CA and San Jose, CA during the summer. Plants were grown in greenhouses in Berkeley, CA and on Molokai, HI during winter seasons. The phenotype of *rgo1* did not vary in these different growth conditions. *ids1-mum1* (Chuck et al., 1998) was a gift from G. Chuck (USDA, ARS, Albany, CA). *ids1-VI* and *ids1-Burr* were gifts from J. Jackson (MGCSC, Urbana, IL). Originally described as *rgo2-VI* and *rgo2-Burr*, complementation and allelism tests with both *ids1-mum1* and *rgo1*, as well as mapping data, showed that they are alleles of *ids1* (Jackson and Kaplinsky, 2000). To observe double mutants, plants used for the *ids1; rgo1* complementation test were selfed. The genotype of representative double mutant individuals was confirmed molecularly using an *ids1* cDNA and umc114 as RFLP probes, and umc1267 primers to detect a *rgo1*-linked SSR. Some double mutants were constructed by crossing *wx1 rgo1* lines generated during mapping (see below) to *ids1* plants. Selfed progeny from this cross segregated *wx1* kernels, which were enriched for *rgo1*. Genetic nomenclature follows the standards for maize genetics (Burr et al., 1995); unlinked genes are separated by a semicolon, linked genes by a space.

rgo1 mapping

Wx1 rgo1 plants were crossed to a *wx1* stock and 33 *wx1* translocation lines representing 18 chromosome arms. The progeny of these crosses were self-pollinated and the resulting waxy (*wx1*) and opaque (*Wx1*)

seed were planted separately. Linkage to an arm is indicated by repulsion from a given translocation marked by *wx1* (Laughnan and Gabay-Laughnan, 1996). After mapping to a chromosome arm, finer molecular mapping was performed using RFLP and SSR markers.

Scanning electron microscopy

1-3 cm developing ears were dissected from 6- to 8-week old plants and fresh tissue was used for imaging. 1-3 cm tassel primordia were fixed in FAA (3.7% formaldehyde, 5% glacial acetic acid, 50% ethanol) overnight at 4°C, dehydrated through an ethanol series, and critical-point dried. Glumes were dissected away to expose the floret primordia, and the samples were then sputter coated with 15 nm of gold/palladium (Polaron SEM coating system). An Electroscan E3 Environmental Scanning Electron Microscope (ESEM) was used to image samples at 20 kV. Images were digitally captured using IAAS software (Electroscan Corp.). Electron microscopy was performed in the Electron Microscope Laboratory at the University of California at Berkeley.

Histology

Tissue was fixed overnight at 4°C in FAA, dehydrated through an ethanol series, and stained with 0.1% Safranin. The samples were then embedded in paraffin (Paraplast, Oxford Labware, St Louis, MO). The samples were mounted on stubs, and 10 μm sections were cut and then mounted on slides. The slides were deparaffinized in Histoclear (National Diagnostics, Atlanta, GA), hydrated through an ethanol series, and stained for 30 seconds to 1 minute with 0.05% Toluidine Blue O. Slides were then rinsed, dehydrated, and mounted with Merckoglas (Mikroskopic, Germany) (Ruzin, 1999). Images were digitally captured using a Zeiss Axiophot light microscope.

Quantitative analysis

Tassel phenotypes were quantified by counting the number of tassel branches, the number of spikelets on the central spike of the tassel, and the number of florets per spikelet. Between 20 and 50 spikelets from the central spike were counted to determine the number of florets per spikelet, and the numbers from siblings were pooled.

RESULTS

The *reversed germ orientation1* phenotype affects both ear and tassel development

To analyze the *rgo1* phenotype, the *rgo1* mutation was introgressed into a B73 background. During vegetative growth, *rgo1* plants are indistinguishable from wild-type siblings. However, *rgo1* ears have irregular rows of kernels compared to wild-type siblings (Fig. 2A,B). Closer examination of *rgo1* ears reveals kernels with their embryos facing both the tip and the base of the ear (Fig. 2B,D). Often, reversed and normal orientation kernels are found in the same spikelet surrounded by a shared pair of glumes. *rgo1* ears also contain fused kernels, each containing an embryo (Fig. 2B). The frequency of fused kernels varies from ear to ear. Open pollination of *rgo1* plants produced fused kernels where one kernel was fertilized by a pollen grain containing a color gene, the other without. Fig. 2E and F show the top and bottom of a fused kernel, each with a visible embryo.

In wild-type female spikelets the upper floret develops into the kernel with the embryo facing the tip of the ear, while the lower floret arrests and aborts. In wild-type male spikelets, two florets develop and produce three anthers each (Fig. 2G). In *rgo1* tassels spikelets have three florets, each with three anthers, for a total of nine anthers per spikelet (Fig. 2H). The penetrance of the tassel phenotype is background dependent, and ranges from 39% in B73 to 91% in Gaspe Bay Flint (GBF). The total number of spikelets and number of tassel branches are not affected in *rgo1* mutants (Table 1).

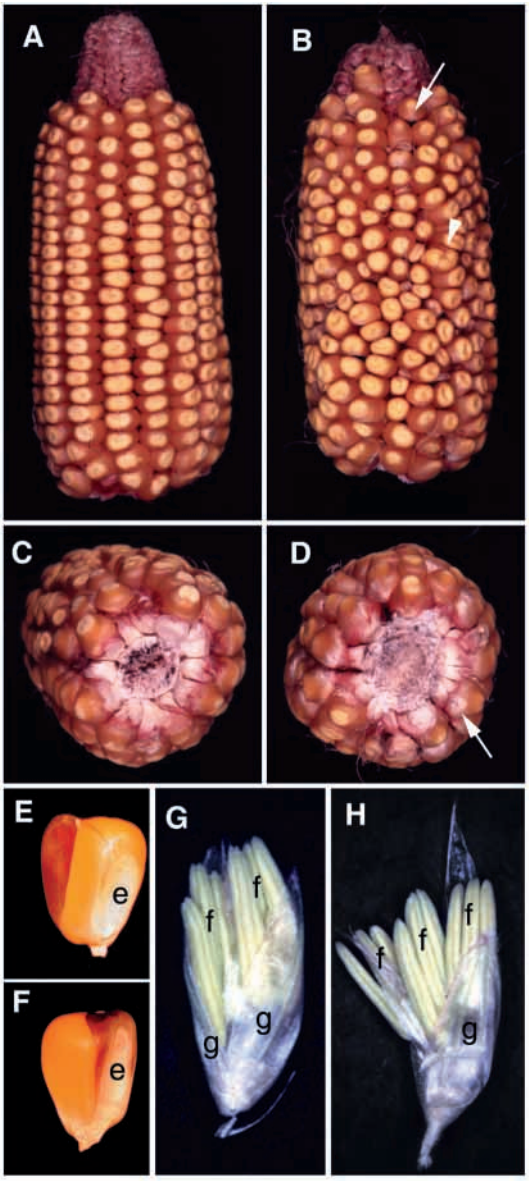

Fig. 2. *rgo1* affects the ear and tassel spikelets. Side views of (A) a wild-type ear with regular rows of kernels and (B) a *rgo1* ear with irregular rows of kernels. The *rgo1* ear has embryos facing the base of the ear (arrow in B and D) and a fused kernel (arrowhead). Bottom views of (C) a wild-type and (D) a *rgo1* ear show embryos facing the base of the ear only in *rgo1* ears. (E,F) Fused kernels have two embryos (e), visible on the two faces (E and F) of this open pollinated example. Wild-type tassel spikelets (G) contain two flowers (f), while *rgo1* spikelets contain three (H). Both are surrounded by leafy glumes (g).

rgo1 is a single recessive mutation and maps to chromosome 9

Self pollinations of heterozygous *rgo1*/+ plants produced 25% (*n*=48) mutant progeny, and crosses of *rgo1*/+ heterozygotes to

Table 1. The effect of genetic background on penetrance of tassel phenotype

Genotype	Florets per spikelet		Spikelets on the central spike		Tassel branch number	
	Mean±s.d.	*n*	Mean±s.d.	*n*	Mean±s.d.	*n*
rgo1^B73x6	2.6±0.5*	154	206.1±25.0	14	6.9±0.9	14
wt sibling	2.0±0.0	450	209.4±21.1	35	6.7±0.9	35
rgo1^GBFx1	2.9±0.3*	138	nd	nd	nd	nd
wt sibling	2.0±0.0	355	nd	nd	nd	nd
(*rgo1*^B73x4 × *ids1-VI*)						
rgo1; ids1 double mutants	3.6±1.0**	120	260.0±19.6	12	6.2±1.7	12
rgo1 and *ids1* single mutants	2.4±0.5*	120	264.5±18.4	23	7.3±2.0	23
wt sibling	2.0±0.0	120	273.4±23.1	23	7.2±2.2	23

The number of florets per spikelet, spikelets on the central spike, and number of tassel branches were counted in mutants and their wild-type siblings from segregating families. *Significantly (P<0.001) different from wild type; **significantly (P<0.001) different from both wild type and single mutants using the Student's two-tailed *t*-test. s.d., standard deviation; *n*, number of spikelets counted (florets per spikelet) or number of plants counted (spikelets per spike, tassel branch number); nd, not determined.

rgo1 homozygotes produced 50.6% (n=81, χ^2=0.012, d.f.=1, P=0.91) mutant progeny. This indicates that *rgo1* behaves as a single recessive mutation when introgressed into B73. *waxy1* (*wx1*) marked reciprocal translocation stocks were used to map *rgo1* to a chromosome arm. *rgo1* was in repulsion to all of the *wx1* marked translocations as well as to a *wx1* stock, indicating that *rgo1* is located on chromosome 9 and linked to *wx1*. Test crosses place *rgo1* and *wx1* approximately 11 cM apart. Further mapping with RFLP and SSR molecular markers locate *rgo1* within 2 cM of the SSR marker umc1267 in bin 3, close to the centromere.

The spikelet meristem initiates an extra flower in *rgo1* mutants

To define the developmental defects in *rgo1* mutants, early ear development was observed using electron microscopy and histology. Inflorescence meristems (IMs) of *rgo1* ears initiate multiple rows of SPMs in the normal polystichous pattern and appear identical to wild-type IMs (Fig. 3A,B). SPMs in *rgo1* plants initiate a SM correctly from the flank of each SPM (Fig. 3C,D), and are thus not responsible for the disrupted rowing observed in *rgo1* ears (Fig. 2B).

Differences between normal and mutant development become evident upon examination of the SM. Normally each SM initiates a set of outer and inner glumes, and then the lower floret meristem (Fig. 3E). The SM itself converts into the upper

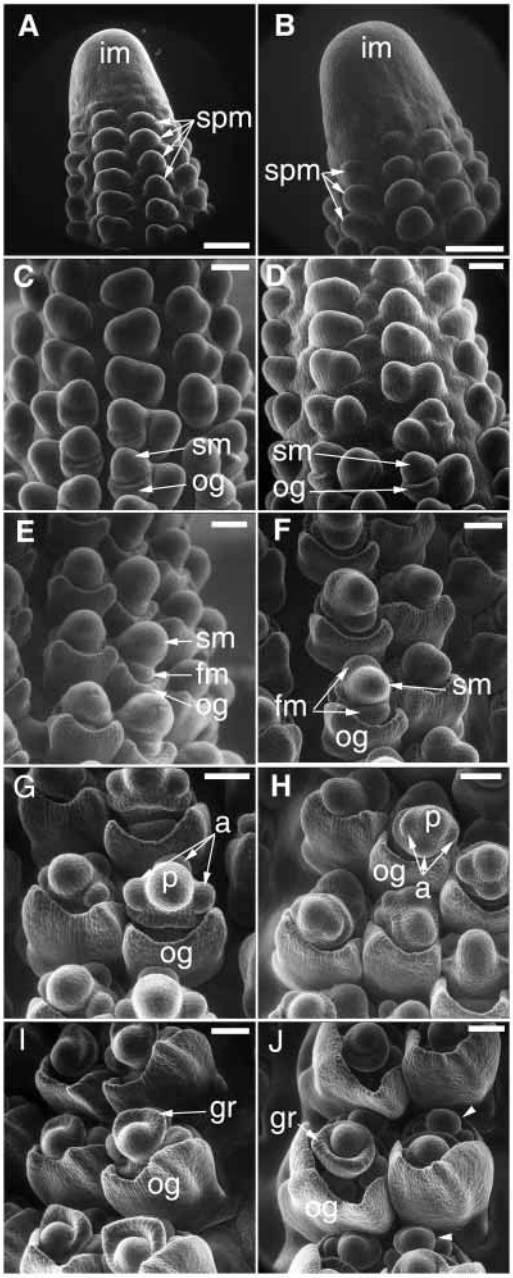

Fig. 3. Wild-type and *rgo1* ear development. These images are a series from the tip to the base of developing ears. (A,C,E,G,I) Wild type and (B,D,F,H,J) *rgo1*. (A,B) Inflorescence meristems (im) producing multiple files of spikelet pair meristems (spm). The spikelet pair meristems produce a spikelet meristem (sm), which initiates the outer glume (og) and then the inner glume (not shown) in wild type (C) and *rgo1* (D). In wild-type development, the spikelet meristem initiates one floret meristem (fm) (E), whereas in *rgo1* mutants each spikelet meristem produces two floret meristems (F). The spikelet meristem then converts into a floret meristem, and initiates floral organs, including three anthers (a) and the pistil primordial (p). In wild-type spikelets the anthers are arranged with the middle anther facing the tip of the ear (G), whereas the anthers face the base of the ear in *rgo1* (H). Finally, each floral meristem produces the gynoecial ridge (gr), which is in the reversed orientationin *rgo1* (J) relative to wild-type (I). The second floret (arrowheads, J) often develops in *rgo1* spikelets. Bars, 200 μm (A,B), 100 μm (C-J).

floral meristem. *rgo1* SMs also initiate two glumes and a FM, but then continue to make a second FM at 180° from the first FM (Fig. 3F). Subsequently both wild-type and *rgo1* SMs become FMs and initiate floral primordia (Fig. 3G,H). However, the orientation of the floral organs on *rgo1* spikelets is at 180° from normal, with the middle anther primordium on the same side as the outer glume (Fig. 3H), as opposed to the inner glume as in wild-type spikelets (Fig. 3G). Later in development (further down the developing ear), the FM makes the gynoecial ridge, two fused carpels that elongate to become the silk (Nickerson, 1954). Like the anther primordia, the orientation of the developing silk on the top flower is reversed in *rgo1* spikelets (Fig. 3I,J). In *rgo1* spikelets the lowest FM arrests and aborts similar to wild-type development, but in many spikelets the second flower continues to develop (arrowheads in Fig. 3J). The developmental defects in tassel spikelets mirror those observed in ear spikelets, with no defects observed until FMs are initiated. Wild-type SMs initiate only one FM (Fig. 4A), while *rgo1* SMs initiate two (Fig. 4B). The orientation of the third flower in *rgo1* tassel spikelets is reversed compared to wild-type apical florets (Fig. 4C,D).

rgo1 affects floral abortion of subapical florets producing fused kernels and disorganized rowing

During normal development the lower flower aborts while the upper flower makes a silk (Fig. 5A). The flower is then fertilized, and develops into a kernel. The lower flower undergoes programmed cell death which is detectable using the TUNEL assay for DNA fragmentation (Jones et al., 2001) and the accumulation of autoflorescent compounds in the cell wall (data not shown). After cell death, collapsed cell walls are all that remains of the aborted flower (af in Fig. 5E). In *rgo1* plants, developing flowers have several fates, reflecting the variable expressivity of the mutation. Mirroring wild-type development, the apical flower can develop while the two lower flowers abort (Fig. 5B,F). However, in many *rgo1* spikelets the second flower does not abort and the two upper flowers develop while the lowest flower aborts (Fig. 5C,G). Two kernels are produced in spikelets where the two apical flowers develop. The top (third) flower produces a reversed kernel, while the middle (second) flower's kernel has a normal orientation (Fig. 1). These two flowers can fuse during development, producing fused kernels (Fig. 5D,H). In spikelets with two developing flowers, the flowers are crowded and displace each other resulting in the disrupted rows of kernels observed in mature *rgo1* ears (Fig. 2B).

rgo1 and *ids1* exhibit nonallelic noncomplementation

indeterminate spikelet1 (*ids1*) is a maize gene with homology to the AP2 class of transcription factors. Mutations of the *ids1*

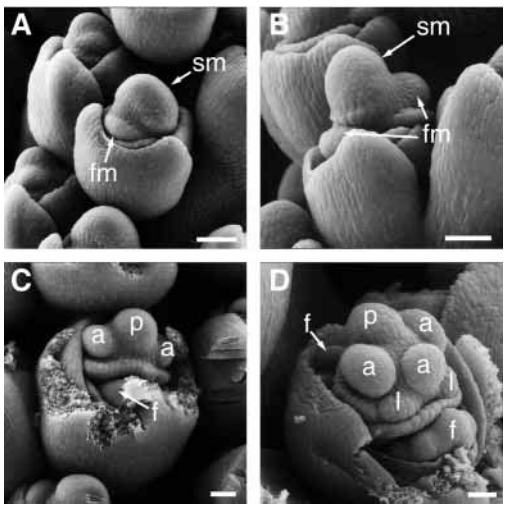

Fig. 4. *rgo1* tassel defects are similar to ear defects. (A) Wild-type tassel spikelet meristems (sm) initiate one floret meristem (fm) while (B) *rgo1* spikelet meristems initiate two. Earlier events (not shown) and later events including floral organ initiation (C, wild type; D, *rgo1*) are unaffected. a, anther primordia; p, pistil primordia; l, lodicule. Bars, 100 μm (A), 200 μm (B), 50 μm (C,D).

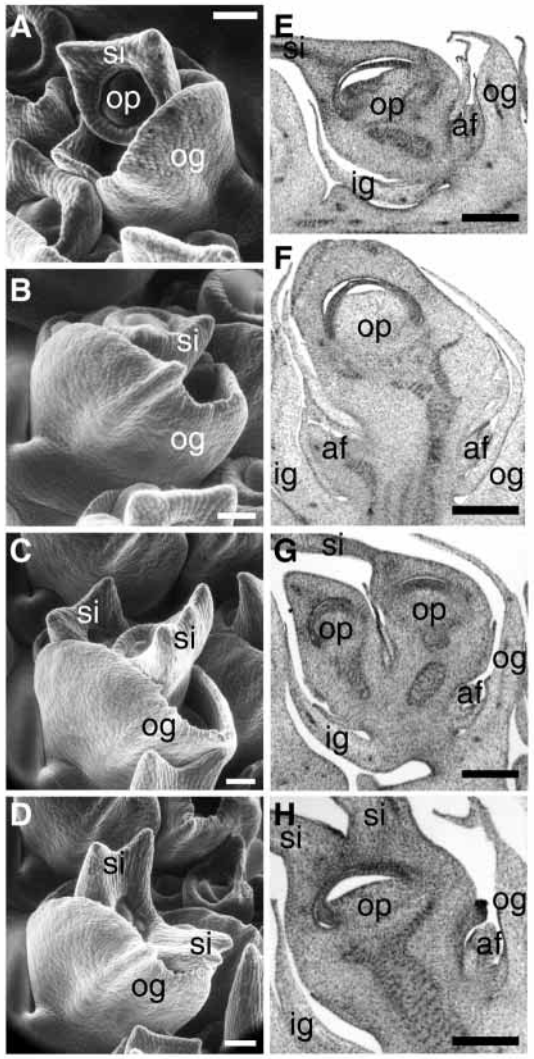

Fig. 5. *rgo1* spikelet development has several possible outcomes. (A,E) Wild type spikelets have one silk (si), a single ovule primordium (op) and an aborting lower floret (af) surrounded by the outer glume (og) and inner glume (ig). (B-D,F-H) *rgo1* mutant spikelets have three florets. The two lower florets abort in some spikelets (B,F). In other spikelets, only the first floret aborts, resulting in two functional flowers (C,G) and a pair of opposed kernels. Rarely, the two developing florets are fused (D,H), and produce a fused kernel. Bars, 100 μm (A-D), 250 μm (E-H).

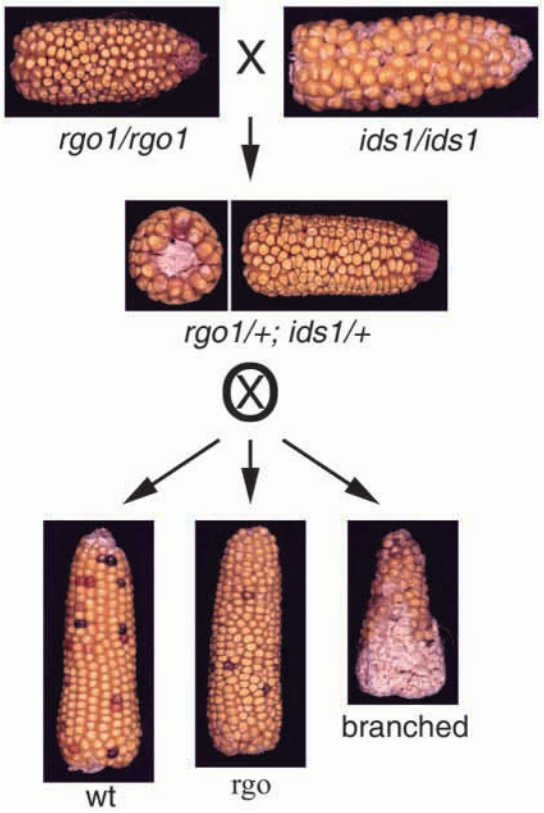

Fig. 6. *rgo1* and *ids1* exhibit nonallelic noncomplementation and have a synergistic double mutant phenotype. *rgo1* and *ids1* homozygous plants were crossed (top). The double heterozygote progeny had a reversed kernel and disrupted row phenotypes (middle). When these plants were self-pollinated, wild type (wt), *rgo1*-like (rgo), and double mutants with long branches (branched) were produced (bottom).

gene produce more than the usual two florets per spikelet. This phenotype has been interpreted as having less determinate SMs. In some backgrounds the spikelet becomes totally indeterminate. *ids1* is expressed in both SPMs and SMs, although the *ids1* phenotype only affects spikelet meristems (Chuck et al., 1998). Because of the production of extra florets, *ids1* mutant ears also have a reversed germ and disturbed rowing phenotype (Fig. 6). The lack of a SPM phenotype despite *ids1* mRNA expression suggests redundancy with another gene or genes, and given the similarity of the *rgo1* and *ids1* phenotypes, we investigated whether the double mutant would display a SPM phenotype.

Homozygous *rgo1* and *ids1* mutants were crossed to each other. The resulting *ids1/+; rgo1/+* plants were grown and self-pollinated to produce the double mutant. Since *ids1* maps to chromosome 1 and *rgo1* maps to chromosome 9, it was a surprise that the ears of the doubly heterozygous plants had disturbed rows and reversed kernels, reminiscent of the *ids1* and *rgo1* single mutants (Fig. 6). When two recessive mutations at different loci fail to complement each other, the genetic behavior is known as nonallelic noncomplementation (Yook et al., 2001). Penetrance of this phenotype was 26% in *ids1-Burr*, 84% in *ids1-mum1* and 100% with *ids1-VI*. Penetrant *ids1-VI* and *ids1-mum1* ears were used for further studies. Nonallelic noncomplementation suggests that genetically, *rgo1* and *ids1* have overlapping functions. The occurrence of a mutant phenotype with the loss of two functional copies (either a *rgo1* or *ids1* homozygote or the *ids1/+; rgo1/+* double heterozygote) implies that at least three wild-type copies of these two genes are needed for correct regulation of spikelet meristem activity.

rgo1; ids1 double mutants affect multiple meristems in the ear

In order to observe double mutants, families of the selfed progeny of double heterozygote plants were grown. Three classes of F_2 ears were observed in these families: normal ears, ears with reversed kernels and disrupted rowing like *rgo1* or *ids1* single mutants, and ears with proliferated spikelets and long branches (Fig. 6). The expectation based on the observation of nonallelic noncomplementation is that 11/16 of the plants (all plants missing two or more copies of *ids1* or *rgo1*) would exhibit a mutant phenotype, and 1/16 would be double mutants. Table 2 lists the observed numbers of ears in each of these phenotypic classes. All families segregated the three phenotypic classes as expected, although the severity of the double mutant phenotype varied from family to family.

While single mutants only affect the SM, the *ids1; rgo1* phenotype affects the SM as well as the SPM. To analyze the double mutants, we define meristems that produce glumes or florets as SMs, and meristems that produce spikelets as SPMs. A range of effects on the SM is observed, from retardation of identity transitions to identity reversions. As expected from the single mutant phenotypes, extra florets are produced by each SM, resulting in reversed and fused kernels (Fig. 7A,E). In addition, the double mutant SMs make supernumerary glumes (Fig. 7B,G). The SM can initiate one or more florets, elongate into a branch, and then initiate one or more spikelets (Fig. 7C,E). Both the primary and reiterated spikelets are identified by the presence of subtending glumes (Fig. 7C,D).

Table 2. Numbers of ears in each phenotypic class

Family	Pedigree	Wild-type ears	Ears with reversed kernels	Ears with long branches	χ^2 *P* value, n.n.	χ^2 *P* value, no n.n.
NK612	(*rgo1*^*W23/M14x2* × *ids1-VI*)*	37	92	5	0.25	8.5×10^{-13}
NK1153	(*rgo1*^B73x4 × *ids1-VI*)*	63	115	13	0.80	1.9×10^{-10}
NK1580/1	(*rgo1*^B73x4 × *ids1-VI*)*	100	207	18	0.83	7.7×10^{-22}
NK1154/7	(*ids1-mum1* × *wx1 rgo1*)*, *wx1* kernels	4	35	10	0.69	n/a

Families of *rgo1/+; ids1/+* heterozygotes were selfed (*). F_2 ears were scored as either wild type, *rgo1*- or *ids1*-like (reversed kernels), or double mutants with long branches (long branches). χ^2 *P* values are indicated for each family, and do not rule out the possibility of nonallelic noncomplementation (n.n.) with a double mutant phenotype. The χ^2 *P* values for the null hypothesis, no nonallelic noncomplementation, were all $<1\times10^{-9}$. For families 612, 1153, 1580 and 1581 the expected ratio of wt:rgo:long was 5:10:1 (n.n.) and 9:6:1 (no n.n.). Families 1154 and 1157 consisted of only *wx1* kernels from an ear segregating *wx1* linked to *rgo1*. The expected ratios (0.9:12:3.1) were based on a 11% recombination rate between *rgo1* and *wx1*.

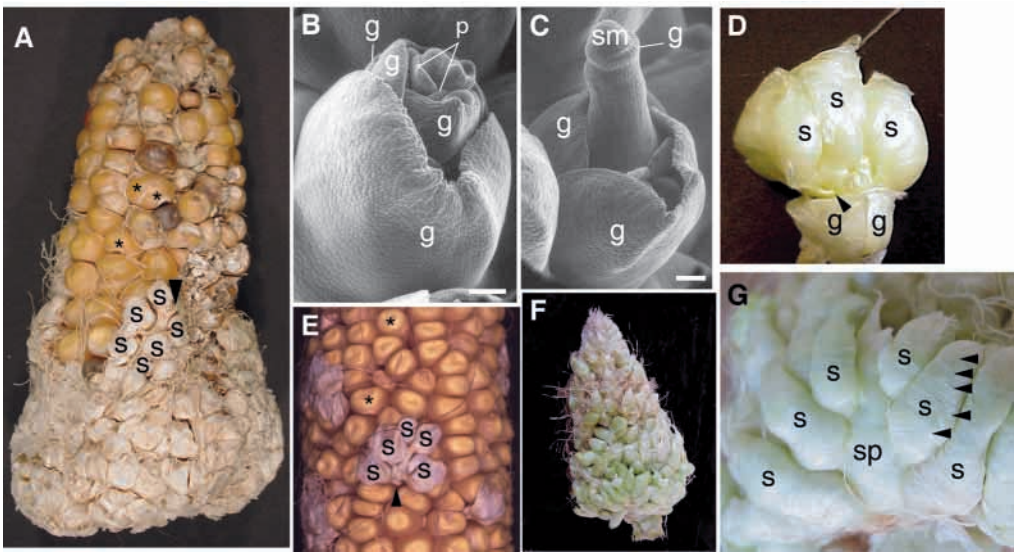

Fig. 7. *rgo1; ids1* double mutant phenotypes. (A) Double mutant plants have reversed and fused kernels (*) and SPMs that elaborate long branches (arrowhead) with spikelets (s) in a distichous pattern. (B) Individual spikelets develop multiple glumes (g) as well as multiple florets in the axils of paleas (p). (C) A SM (sm) that has elongated and reverted into a SPM, initiating another glume. These SMs can be identified by their subtending glumes (g in C,D). The spikelets that they produce, sometimes in pairs (arrowhead, D) are also surrounded by glumes. (E) A SM that has elongated and produced several spikelets. (F) Severe double mutants exhibit increased SPM and SM branching, producing a highly branched ear with no kernels and very few silks. (G) A close examination of a spikelet pair axis (sp) at the base of this ear shows at least six spikelets (s) in a distichous pattern. Each of these spikelets has initiated multiple glumes (arrowheads). Bars, in B and C 100 μm.

In cases where the SM is producing spikelets, it reverts to SPM identity.

SPMs produce too many spikelets in the double mutant. This phenotype can be restricted to the base of the ear (Fig. 6, Fig. 7A) where the SPM produces multiple SMs. In the most severe cases, all of the SPMs on the ear become indeterminate (Fig. 7F), and produce multiple spikelets (Fig. 7G). These spikelets make many glumes (arrowheads, 7G) and only rarely produce florets as evidenced by the absence of silks. Since glumes are produced by the SM, in these spikelets the transition from producing glume primordia to floret primordia is delayed.

Unlike the synergistic interaction observed in the ear, *rgo1; ids1* tassels have an additive phenotype. More florets are produced by each spikelet, but there is no evidence that the SMs in the tassel have changed identities or that SPMs are affected (Table 1).

DISCUSSION

rgo1 is required for conversion from spikelet to floret meristem identity

The progression from vegetative to floral meristem identities during plant growth is critical for normal plant reproduction. We have shown that progression of meristem identity from the spikelet to the floret stage is retarded (i.e. the spikelet meristem does not convert into a floret meristem, and thus continues to initiate florets) in the *rgo1* mutant (Fig. 8A,B). As a result, extra flowers are produced, and because of the distichous pattern of floret primordia initiation on the spikelet axis, kernels with a reversed germ orientation are observed (Fig. 1B). Many *rgo1* ear spikelets form two fertile flowers. Some of these bi-floral spikelets have fused flowers (Fig. 5D). This may indicate that conversion of the SM to FM identity occurred before two distinct flowers formed. The abnormal survival of sub-apical florets may be a result of a lack of suppression by the apical flower (Novoplansky, 1996).

If *ids1* is placed into the framework of meristem identity changes it would perform a similar role as *rgo1* (Fig. 7C), promoting the SM to FM change. The overlapping functions of *rgo1* and *ids1* revealed by their mutants and genetic interactions support the idea that these genes perform similar

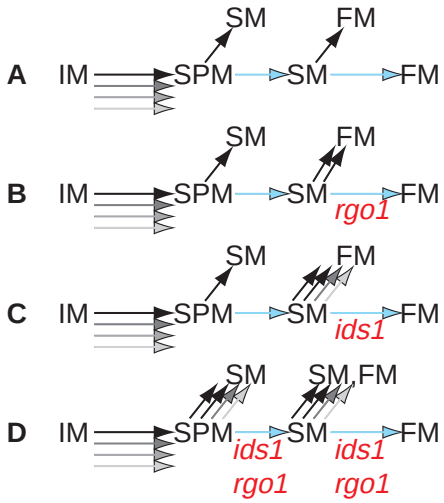

Fig. 8. A model of *rgo1* and *ids1* combinatorial control of spikelet and spikelet pair meristem identity. (A) During wild-type development the inflorescence meristem (IM) produces many spikelet pair meristems (SPM). Each SPM initiates one spikelet meristem (SM) and converts into a SM. Each SM initiates one floret meristem (FM) and converts into a FM. (B) *rgo1* mutants are similar to wild type except that each SM initiates two FMs before converting into an FM. (C) *ids1* mutants are similar to *rgo1* except that each SM can initiate multiple FMs before converting into an FM. (D) *rgo1; ids1* double mutants exhibit a synergistic phenotype. SPMs initiate multiple SMs. SMs initiate multiple SMs in a reiterative fashion and can eventually produce FMs. All FMs initiate floral organs. Black and grey arrows represent meristem initiation. Blue arrows are meristem identity conversions.

biological functions. In some double mutant spikelets, the SM reiterates more SMs (Fig. 7D,E) or makes supernumerary glumes (Fig. 7G) before initiating any FMs. The phenotype indicates that SM identity changes are inhibited or even reversed. This is consistent with a meristem identity role for both *rgo1* and *ids1*. In addition, in the double mutant the SPM also produces long branches with multiple SMs (Fig. 7A,F). Like the SM, SPM conversion is defective in *ids1* and *rgo1* mutant plants (Fig. 8D). The *ids1; rgo1* double mutant phenotype resembles the *ids1; ifa1* phenotype in several ways. In the latter, the ear SM is converted into an indeterminate branch meristem which makes SPMs, while the tassel SM is converted into a SPM (Laudencia-Chingcuanco and Hake, 2002). Both double mutants have more severe effects in the ear than in the tassel. In both cases, meristem identity has changed and results in a decrease in determinacy. *rgo1* and *ifa1* do not exhibit a synergistic interaction like *ids1* and *ifa1* do (data not shown), although *ids1* interacts synergistically with both *rgo1* and *ifa1*. This illustrates that although *rgo1* and *ids1* functions overlap, these genes play unique roles in inflorescence development.

The conversion model of maize inflorescence development proposes that SPMs initiate a SM and then convert into SMs, and SMs intiate a FM and then convert into FMs (Fig. 8A). This model is based on the class II tasselseed phenotypes, in which meristem identity conversions are delayed (Irish, 1997a). Determinacy is simply a function of correct identity transitions in the conversion model. A SM that converts into a FM is determinate, while a SM that never changes its identity is indeterminate. An alternative model of inflorescence development, the lateral branching model, does not involve identity conversion (Chuck et al., 1998). Instead, Chuck et al., suggest that all florets, including the terminal floret, are initiated laterally by the SM. A band of *ids1* expression between the developing upper and lower floret was interpreted to be the remnant SM, which is not suppressed in *ids1* mutants. This theory was partially reconciled with the conversion model by Irish who suggested that the remnant of the SM is morphologically indistinguishable but still present on the flank of the FM (Irish, 1998). In the lateral branching model, *ids1* functions to suppress indeterminate growth of the SM. One of the predictions of this model is that *ids1* mutant SMs should make more FMs, but as SM identity does not change, only FM primordia should be produced by the SM.

Observations from our research favor the conversion model, which can explain mutant behavior without invoking remnant meristems. In the double mutant, SMs produce supernumerary FMs, but also initiate additional glumes and SMs, indicating that SM identity has changed (Fig. 8D). The lateral branching model does not implicate *ids1* in meristem identity changes because in this model the SM never becomes a FM. The conversion model explicitly implicates identity change as a mechanism, and thus can explain the double mutant phenotype. The lateral branching model was partially based on the observation that *ids1-mum1* spikelets do not terminate in florets, because in this model the florets are lateral branches of the spikelet. Upon introgression into B73, *ids1-mum1* plants can make functional terminal florets, as do the *ids1-Burr* and *ids1-VI* alleles. *ids1-VI* ears make kernels and do not exhibit indeterminate spikelets on the ear (Fig. 6). Thus, in certain genetic backgrounds, *ids1* mutant spikelets can terminate in fertile florets, suggesting that *ids1* is involved in the conversion of SMs into FMs.

Meristem identity is regulated in a dosage-sensitive manner by multiple genes

Nonallelic noncomplementation can be explained by a genetic pathway that is sensitive to the total dosage of two genes (Stearns and Botstein, 1988; Yook et al., 2001). Based on the nonallelic noncomplementation and synergistic double mutant phenotypes of *rgo1* and *ids1*, we propose a dosage based model for meristem conversion. In this model, SPM→SM and SM→FM conversions are regulated by both *ids1* and *rgo1*, although the threshold for normal behavior is lower in SPMs than in SMs (Fig. 9). Signals from the vegetative portion of the maize plant are known to affect meristem identity transitions. In the maize *indeterminate 1* (*id1*) mutant the transition from vegetative to reproductive development is extremely delayed or never happens. *id1* is expressed in immature leaves, but acts in a non cell-autonomous manner and affects the apical meristem (Colasanti et al., 1998). Meristem culture experiments also support the idea that the SAM integrates signals from the whole plant and does not keep track of its identity internally (Irish and Karlen, 1998; Irish and Nelson, 1991). If *rgo1* and *ids1* are in the genetic pathway that responds to these signals coming from the rest of the plant, these mutants could make the meristem less sensitive to the signal needed for meristem conversion (this is equivalent to raising the signaling threshold).

Control of meristem identity transitions in the maize inflorescence is under the combinatorial control of multiple genes. The penetrance of the *ids1/+; rgo1/+* nonallelic noncomplementation phenotype and the severity of the *ids1; rgo1* double mutant phenotype are variable (Fig. 7), suggesting that there are genetic modifiers in the maize genome (and probably environmental effects as well) that affect this phenotype. Three additional lines of experimental evidence provide support for the presence of modifiers. To test the dosage model of nonallelic noncomplementation, we produced plants missing one copy of the long arm of chromosome 1 (which carries *ids1*) using B centromere translocation lines (Birchler, 1996). Chromosome 1L hypoploids exhibited a reversed kernel phenotype. This shows that there are other genes on 1L (possibly *tb1*, *kn1*, and/or *ts6*, which all have effects on meristematic behavior) that in combination with a missing dose of *ids1* have an effect on spikelet meristem identity. Similarly, a directed *Mutator* transposon mutagenesis experiment aimed at cloning *rgo1* produced hundreds of plants with *rgo1*-like phenotypes. Only one or two disruptions of the *rgo1* gene would be expected, suggesting that *rgo1* heterozygotes uncover many branching modifiers. Finally, a large screen of over 50,000 F_1 *Mutator* active ears for dominant or semi-dominant reversed germ

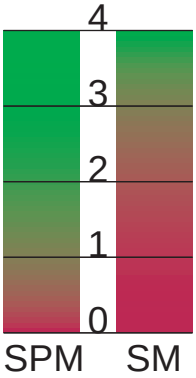

Fig. 9. Gene dosage model for SM and SPM identity. SPMs are wild type (green) with even one dose of *ids1* or *rgo1*, and mutant (red) in the absence of any *ids1* or *rgo1*. SMs are more sensitive to *ids1* and *rgo1* dosage, with a threshold somewhere between two and three doses.

phenotypes identified several ears with strong reversed germ phenotypes. These ears transmit fascicled (ears with more than one main spike due to multiple IMs), ramosa (ears with long branches at their base due to indeterminate SPMs), and reversed germ (SM) phenotypes in the F_2 families in a recessive manner. In these families, heterozygotes uncover a reversed germ (i.e. SM) phenotype. However, homozygotes affect both the SM and SPM and IMs. These data suggest there are many genes in the maize genome that, when disrupted, can contribute to the dosage effect that creates reversed kernels.

Several other examples of dosage-sensitive developmental pathways have been described in plants. *LEAFY (LFY)* is an *Arabidopsis* gene that regulates the transition to flowering by promoting and maintaining floral meristem identity (Weigel et al., 1992). Heterozygous *lfy/+* plants are normal under long day conditions. However, in short day conditions floral reversion results in inflorescences being elaborated from the floral meristem (Okamuro et al., 1996). A second example can be found in the regulation of meristem maintenance. The *CLAVATA1* (*CLV1)* and *CLV3* genes in Arabidopsis are involved in maintaining meristem size by balancing the rates of cell division and primordial initiation. *CLV1* encodes a receptor kinase, and *CLV3* is its ligand (Trotochaud et al., 2000). *CLV1* and *CLV3* exhibit a form of dosage sensitivity. The weak *clv1/+* phenotype is strongly enhanced in a *clv3/+* background (Clark et al., 1995). In addition, *stm/+* heterozygotes suppress *clv1* semidominance (Clark et al., 1996). Finally, hemizygosity of either *liguleless1* or *liguleless2*, genes necessary for ligule development in maize, affect the null phenotype of the other mutant (Harper and Freeling, 1996). These examples may reflect a general sensitivity to dosage in many aspects of meristem function.

Conclusion

Kellogg (Kellogg, 2000) has proposed a generalized model for inflorescence development. The model states that meristems can produce either more meristems or a set of determinate floral organs on their flanks. In a reiterative manner, any subsequent meristems must make the same developmental decision. This simple model can explain the wide variety of inflorescence forms found in plants. If our results can be generalized, then progress through the various orders of meristem identity will depend on the dosage of *ids1* and *rgo1*-like genes and the concentration of their products. Meristem identity is established by varying a threshold, with different thresholds for different identities. Duplication or loss of these genes would explain variations in the number of iterations of this basic process, and thus could be responsible for the great diversity in inflorescence forms.

We thank Christopher Turbeville for his help with cytology and the staff of the Electron Microscopy Laboratory for their expert assistance. We are grateful to the staff of the Oxford Tract facility, for managing the greenhouse and fields. J. Pfluger, R. Langham, D. Lisch, D. Braun, P. McSteen, G. Chuck and S. Hake provided comments and suggestions. Funding was provided by NIH grant 2R01-GM42610. NJK was supported by a NSF pre-doctoral fellowship.

REFERENCES

Birchler, J. A. (1996). Dosage analysis using B-A translocations. In *The Maize Handbook* (ed. M. Freeling and V. Walbot), pp. 328-330. New York: Springer Verlag.

Burr, B., Chandler, V., Coe, E., Dooner, H., Fauron, C., Langdale, J., Polacco, M., Sachs, M., Scanlon, M. and Stinard, P. (1995). A standard for maize genetics nomenclature. *Maize Genetics Cooperation Newsletter* **69**, 182-184.

Chapman, G. P. and Peat, W. E. (1992). An introduction to the grasses: (including bamboos and cereals). Wallingford, Oxon, UK; Tucson, AZ, USA: CAB International.

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

Chuck, G., Meeley, R. B. and Hake, S. (1998). The control of maize spikelet meristem fate by the APETALA2-like gene indeterminate spikelet1. *Genes Dev.* **12**, 1145-1154.

Clark, S. E., Jacobsen, S. E., Levin, J. Z. and Meyerowitz, E. M. (1996). The CLAVATA and SHOOT MERISTEMLESS loci competitively regulate meristem activity in *Arabidopsis*. *Development* **122**, 1567-1575.

Clark, S. E., Running, M. P. and Meyerowitz, E. M. (1995). CLAVATA3 is a specific regulator of shoot and floral meristem development affecting the same processes as CLAVATA1. *Development* **121**, 2057-2067.

Colasanti, J., Yuan, Z. and Sundaresan, V. (1998). The indeterminate gene encodes a zinc finger protein and regulates a leaf-generated signal required for the transition to flowering in maize. *Cell* **93**, 593-603.

Dellaporta, S. L. and Calderon-Urrea, A. (1994). The sex determination process in maize. *Science* **266**, 1501-1505.

Doebley, J. and Stec, A. (1991). Genetic analysis of the morphological differences between maize and teosinte. *Genetics* **129**, 285-296.

Galinat, W. C. (1959). The phytomer in relation to floral homologies in the American *Maydeae*. *Harvard University Botanical Museum Leaflets* **19**, 1-32.

Galinat, W. C. (1996). The patterns of plant structures in Maize. In *The Maize Handbook* (ed. M. Freeling and V. Walbot), pp. 61-65. New York: Springer Verlag.

Harper, L. and Freeling, M. (1996). Interactions of liguleless1 and liguleless2 function during ligule induction in maize. *Genetics* **144**, 1871-1882.

Iltis, H. H. (1988). *Maize Evolution and Agricultural Origins*. Washington, DC, USA: Smithsonian Institution Press.

Irish, E. E. (1996). Regulation of sex determination in maize. *BioEssays* **18**, 363-369.

Irish, E. E. (1997a). Class II tassel seed mutations provide evidence for multiple types of inflorescence meristems in maize (Poaceae). *Amer. J. Bot.* **84**, 1502-1515.

Irish, E. E. (1997b). Experimental analysis of tassel development in the maize mutant Tassel seed 6. *Plant Physiol.* **114**, 817-825.

Irish, E. E. (1998). Grass spikelets: A thorny problem. *BioEssays* **20**, 789-793.

Irish, E. E. and Karlen, S. (1998). Restoration of juvenility in maize shoots by meristem culture. *Int. J. Plant Sci.* **159**, 695-701.

Irish, E. E. and Nelson, T. M. (1991). Identification of multiple stages in the conversion of maize meristems from vegetative to floral development. *Development* **112**, 891-898.

Jackson, J. D. (1996a). Recovery of *rgo1*. *Maize Genetics Cooperation Newsletter* **70**, 66.

Jackson, J. D. (1996b). Reverse germ orientation mutants. *Maize Genetics Cooperation Newsletter* **70**, 66.

Jackson, J. D. and Kaplinsky, N. J. (2000). The reverse germ orientation2-VI mutation maps to chromosome 1L near Ts6 and shows allelism to ids1. *Maize Genetics Cooperation Newsletter* **74**.

Joachim, G. S. (1956). Observations on the morphology of 'reversed germ' in two lines of dent corn. *Proc. Minnesota Acad. Sci.* **24**, 37-43.

Jones, A. M., Coimbra, S., Fath, A., Sottomayor, M. and Thomas, H. (2001). Programmed cell death assays for plants. In *Methods in Cell Biology. Apoptosis* (ed. L. M. A. J. D. E. Schwartz), pp. 437-451. London, San Diego: Academic Press Inc.

Kellogg, E. A. (2000). A model of inflorescence development. In *Monocots: Systematics and Evolution* (ed. K. L. Wilson and D. A. Morrison), pp. 84-88. Melbourne: CSIRO.

Kiesselbach, T. A. (1926). Fasciated kernels, reversed kernels, and related abnormalities in maize. *Amer. J. Bot.* **13**, 35-39.

Laudencia-Chingcuanco, D. and Hake, S. (2002). The *indeterminate floral apex1* gene regulates meristem determinacy and identity in the maize inflorescence. *Development* **129**, 2629-2638.

Laughnan, J. R. and Gabay-Laughnan, S. (1996). The placement of genes using *waxy*-marked reciprocal translocations. In *The Maize Handbook* (ed. M. Freeling and V. Walbot), pp. 255-258. New York: Springer Verlag.

Long, J. A., Moan, E. I., Medford, J. I. and Barton, M. K. (1996). A member of the KNOTTED class of homeodomain proteins encoded by the STM gene of Arabidopsis. *Nature* **379**, 66-69.

McSteen, P., Laudencia-Chingcuanco, D. and Colasanti, J. (2000). A floret by any other name: Control of meristem identity in maize. *Trends Plant Sci.* **5**, 61-66.

Micu, V. E., Palii, A. F. and Rotar, A. I. (1983). Genetic study of maize mutants with development of both florets in the female spikelet. *Genetica* **19**, 1020-1023.

Nickerson, N. H. (1954). Morphological analysis of the maize ear. *Amer. J. Bot.* **41**, 87-92.

Novoplansky, A. (1996). Hierarchy establishment among potentially similar buds. *Plant Cell Environ.* **19**, 781-786.

Okamuro, J. K., Den, B. B. G. W., Lotys-Prass, C., Szeto, W. and Jofuku, K. D. (1996). Flowers into shoots: Photo and hormonal control of a meristem identity switch in Arabidopsis. *Proc. Natl. Acad. Sci. USA* **93**, 13831-13836.

Postlethwait, 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.

Ruzin, S. E. (1999). *Plant Microtechnique and Microscopy.* New York: Oxford University Press.

Sachan, J. K. S. and Sarkar, K. R. (1980). Reversed germ orientation – a developmental mutant in maize. *Indian J. Genet. Plant Breeding* **40**, 281-284.

Stearns, T. and Botstein, D. (1988). Unlinked noncomplementation: Isolation of new conditional-lethal mutations in each of the tubulin genes of Saccharomyces cerevisiae. *Genetics* **119**, 249-260.

Steeves, T. A. and Sussex, I. M. (1989). *Patterns in Plant Development*. Cambridge, UK; New York: Cambridge University Press.

Trotochaud, A. E., Jeong, S. and Clark, S. E. (2000). CLAVATA3, a multimeric ligand for the CLAVATA1 receptor-kinase. *Science* **289**, 613-617.

Veit, B., Schmidt, R. J., Hake, S. and Yanofsky, M. F. (1993). Maize floral development: New genes and old mutants. *Plant Cell* **5**, 1205-1215.

Vollbrecht, E., Reiser, L. and Hake, S. (2000). Shoot meristem size is dependent on inbred background and presence of the maize homeobox gene, *knotted1*. *Development* **127**, 3161-3172.

Weigel, D., Alvarez, J., Smyth, D. R., Yanofsky, M. F. and Meyerowitz, E. M. (1992). LEAFY controls floral meristem identity in Arabidopsis. *Cell* **69**, 843-859.

Yook, K. J., Proulx, S. R. and Jorgensen, E. M. (2001). Rules of nonallelic noncomplementation at the synapse in Caenorhabditis elegans. *Genetics* **158**, 209-220.