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CLASS II TASSEL SEED MUTATIONS PROVIDE EVIDENCE FOR MULTIPLE TYPES OF INFLORESCENCE MERISTEMS IN MAIZE (POACEAE)[1]

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The tassel seed mutations *ts4* and *Ts6* of maize cause irregular branching in its inflorescences, tassels, and ears, in addition to feminization of the tassel due to the failure to abort pistils. A comparison of the development of mutant and wild-type tassels and ears using scanning electron microscopy reveals that at least four reproductive meristem types can be identified in maize: the inflorescence meristem, the spikelet pair meristem, the spikelet meristem, and the floret meristem. *ts4* and *Ts6* mutations affect the fate of specific reproductive meristems in both tassels and ears. *ts4* mutants fail to form spikelet meristems from spikelet pair meristems. *Ts6* mutants are delayed in the conversion of certain spikelet meristems into floret meristems. Once floret meristems are established in both of these mutants, they form florets that appear normal but fail to undergo pistil abortion in the tassel. The abnormal branching associated with each mutant is suppressed at the base of ears, permitting the formation of normal, fertile spikelets. The classification of the different types of reproductive meristems will be useful in interpretation of gene expression patterns in maize. It also provides a framework for understanding meristem functions that can be varied to diversify inflorescence architectures in the Gramineae.

Key words: branching; determinacy; flowering; grasses; inflorescence; maize; spikelet; sex determination; *Zea mays.*

Maize plants (*Zea mays* L.) are normally monoecious, with staminate (male) florets in the terminal inflorescence, the tassel, and pistillate (female) florets in the lateral inflorescences, the ears (for reviews, see Dellaporta and Calderon-Urrea, 1993; Veit et al., 1993; Irish, 1996). This condition is derived from the formation of initially perfect (hermaphroditic) flowers and the subsequent selective abortion of pistil primordia in tassel flowers and of stamen primordia in ear flowers. The tassel seed mutations feminize maize plants by the failure to abort pistil primordia (Emerson, 1920). The normal functioning of tassel seed genes is therefore essential to establish monoecy in maize. The function of these genes was acquired during the evolution of modern maize from a perfect-flowered ancestor.

There are five *tassel seed* (*ts*) genes [*ts1, ts2, Ts5* (Emerson, Beadle, and Fraser, 1935), *ts4* (Phipps, 1928), and *Ts6* (Nickerson and Dale, 1955)] that have been mapped and at least that many that have not yet been mapped (unpublished data). All of these *ts* mutations allow pistils to develop in the tassel. There is substantial variation among them, however, in the extent and patterning of feminization and in whether other aspects of inflorescence morphology are affected. So far, only one *ts* gene, *Ts2*, has been cloned (DeLong, Calderon-Urrea, and Dellaporta, 1993). Its sequence predicts that its product is a short-chain alcohol dehydrogenase. It is of interest to learn what the products of other *ts* genes are, how all of these gene interact with each other to effect pistil suppression, and whether they play other roles in the development or physiology of the plant.

Double mutant analysis has revealed that the *ts* mutations can be separated into two groups, Class I and Class II, based on their genetic interactions (Irish, Langdale, and Nelson, 1994). Within a group the mutations interact epistatically but double mutants between the groups exhibit synergistic interactions. The *ts* mutants within each group are also similar in phenotype. Class I *ts* mutants (*ts1, ts2,* and *Ts5*) show simply a conversion of either some or all of the tassel florets from staminate to pistillate. Class II *ts* mutants (the recessive *ts4* and the semidominant *Ts6*) have a more complex phenotype: pistils develop in tassel florets, tassels and ears develop extra short branches along their length, and florets appear disorganized. Fertility of ears is also reduced in Class II *ts* mutants. The Class II phenotypes are exacerbated in double mutant combination with a Class I *ts* (Irish, Langdale, and Nelson, 1994), although as single mutants the Class I mutants show no branching or fertility changes.

The observation that Class I + Class II *ts* double mutants have highly branched, sterile inflorescences provides strong evidence that, in addition to pistil suppression in tassels, all *ts* genes play some role, whether directly or indirectly, in regulating branching of inflorescences and organogenesis of flowers. To examine morphogenesis in Class II *ts* mutants, which even as single mutants exhibit altered inflorescence organization (Nickerson and Dale, 1955), a developmental study using scanning electron microscopy was conducted on inflorescences of isogenic mutant *Ts6/+, ts4/ts4,* and wild-type plants. This study reveals that the primary defect in the Class II *ts* mutations is a delay in or lack of determinacy of specific meristems, leading to a proliferation of meristems and florets, which are, as a consequence, arranged in a complex pattern. Although the florets that are initiated appear normal, pistil suppression fails to occur in these highly branched inflorescences.

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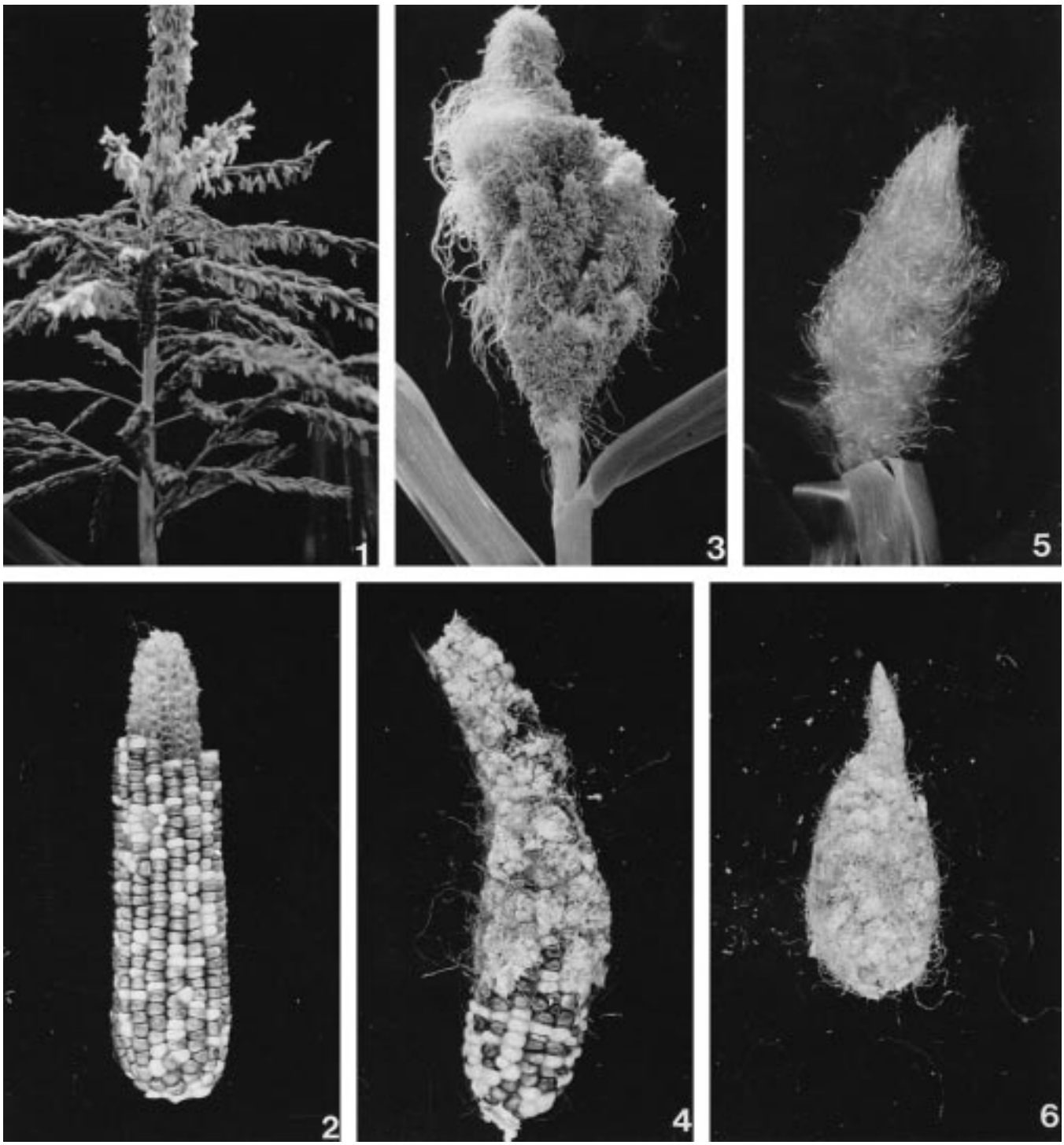


Figs. 1–6. Mature inflorescences and infructescences. **1.** Wild-type tassel. **2.** Wild-type ear. Kernels were removed from tip to show the cob. **3.** *Ts6* tassel. **4.** *Ts6* ear. Note the kernels at the base and short branches adjacent to kernels above the base. **5.** *ts4* tassel. **6.** *ts4* ear. Note the single kernel visible.

Figure Abbreviations: w.t., wild-type; IM, inflorescence meristem; B, branch; SP, spikelet pair primordium or spikelet pair meristem; S, spikelet primordium or spikelet meristem; g, glume; ps, pedicellate spikelet; ss, sessile spikelet; u, upper floret meristem; m, meristem; s, stamen; p, pistil; f, floret meristem; si, silk. Note: artifactual bubbles are marked with a horizontal black dash.

MATERIALS AND METHODS

Lines carrying the mutations *Ts6* and *ts4* were originally obtained from R. Scott Poethig, University of Pennsylvania, who had begun introgressing them into the inbred background W23. Introgression into W23 was continued, so that the material examined here had been crossed into W23 nine (*Ts6*) or five (*ts4*) times. As neither mutant sheds much or any pollen, stocks were maintained as heterozygotes and mutants were used as females in crosses. Homozygous recessive *ts4* mutants were generated by self-pollination of heterozygous plants. The semidominant *Ts6* mutation was examined as a heterozygote. Although it is difficult to distinguish *Ts6/+* tassels from *Ts6/Ts6* tassels unambiguously on a macroscopic level, it is very possible that there may be subtle differences due to differences in dosage of the mutant allele. In the absence of markers that could distinguish plants of those two genotypes in families derived from self-pollination of *Ts6/+* plants, I chose to examine only *Ts6/+* plants in families derived from *Ts6/+* × *+/+* crosses. In that way, any variation that might be seen among inflorescences from different plants would have to be due to variation in expression of *Ts6* and not due to dosage of the mutant allele.

Kernels from ears that segregated 1:1 (wild type:*Ts6*) or 3:1 (wild type:*ts4*) were sown in standard greenhouse potting mix in 10-cm pots and grown in the greenhouse. During winter months supplemental lighting provided 16-h days. Once plants had completed vegetative development, as determined from periodic sacrifice and dissection of a few representative plants, sample tassels and ears of mutants and their wild-type sibs were collected over several weeks from a population of sibling plants. The samples used here came from several batches of plants grown in the greenhouse at different times of the year.

Genotypes of the plants were inferred from the morphology of the tassel. Inflorescences were isolated by peeling away all of the enclosing leaves. Once the innermost leaf was removed, molds were made of one side of the inflorescence using dental impression material (Extrude Medium, Kerr, Romulus, MI; Sylvester, Cande, and Freeling, 1990). When the molds had hardened, they were gently lifted off the inflorescences, which were then fixed for other analyses. Molds that exceeded 1 cm (the diameter of the stubs for the microscope) were cut in half with a razor blade and each half was filled separately. Molds were filled with 2-Ton Epoxy cement, which was allowed to set overnight. The casts were mounted on stubs, sputter-coated for 2 min with palladium, and viewed with a Hitachi S-4000 scanning electron microscope at 5 kV accelerating voltage. The advantages of using casts rather than drying and viewing the actual inflorescences were that cell shrinkage could be avoided and the samples could be fixed for histological analyses. The disadvantages of this method are that complete filling of the molds is not always achieved with such complex structures and that leaf exudate deposited on the samples from dissection, if present, was also preserved as an artifactual surface feature.

Observations were made on more than 50 *Ts6* tassels, and ~30 each *Ts6* ears, *ts4* tassels, *ts4* ears, wild-type tassels, and wild-type ears. As in previous studies (Irish and Nelson, 1991, 1993), height of inflorescences was used as a marker for developmental stage. Inflorescences develop in such a way that many developing spikelets are present, and each spikelet is at a stage slightly more advanced than the spikelet above it. This feature greatly aids the interpretation of the morphogenic progression toward the final structures.

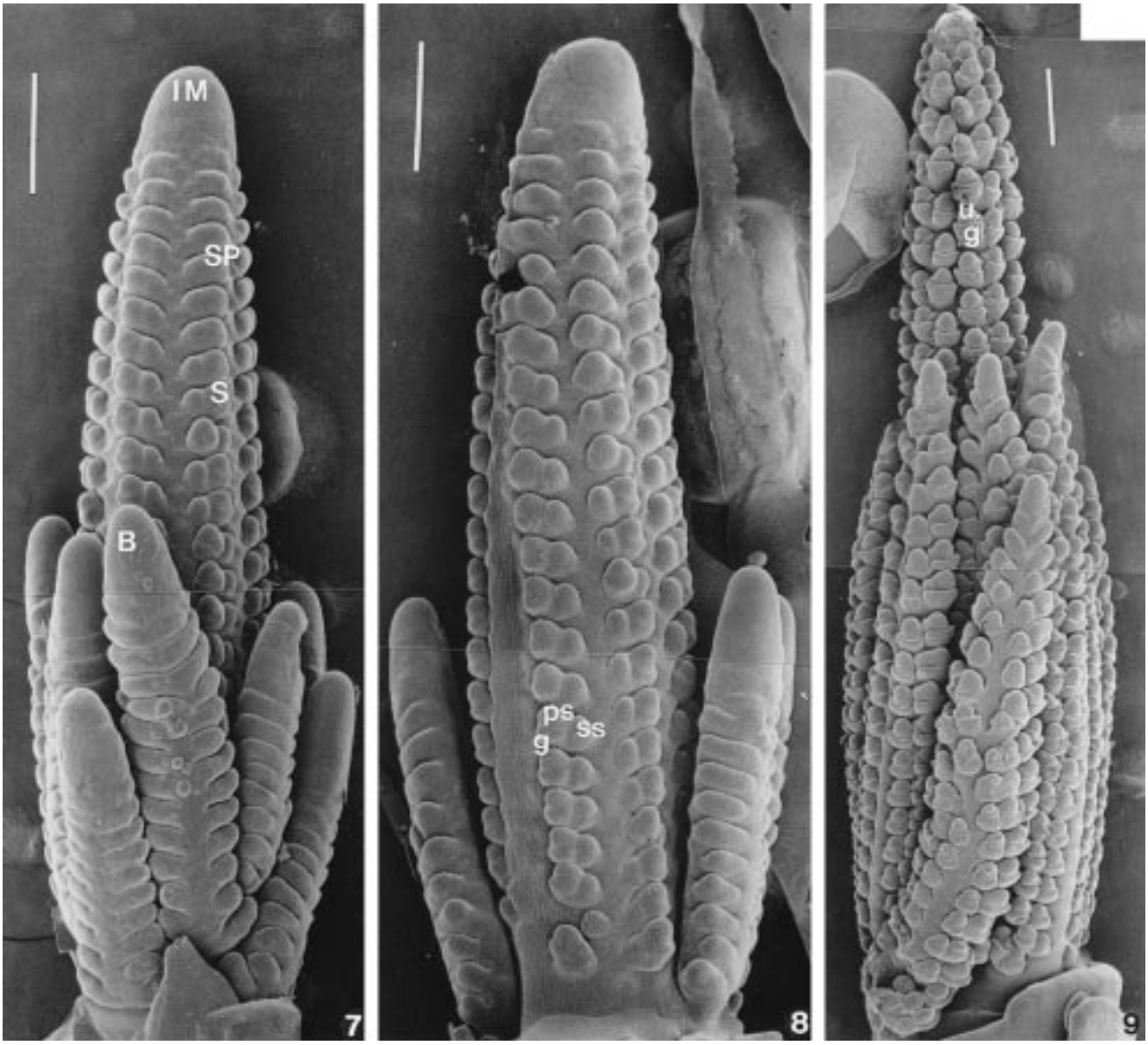
RESULTS

Morphology of mature inflorescences—The morphology of maize inflorescences has been described numerous times (Bonnett, 1948; Nickerson and Dale, 1955; Veit et al., 1993), but will be reviewed here. The tassel of wild-type maize at anthesis consists of a thin main rachis with several long, thin branches which are restricted to the base (Fig. 1). Flowers are borne in two-flowered spikelets, the basic unit of grass inflorescences. Each spikelet is subtended by a pair of glumes (bracts) and is arranged in pairs along the length of the main rachis and the branches. Each pair of spikelets consists of one pedicellate spikelet and one sessile spikelet. Each spikelet consists of two staminate florets, the upper floret being developmentally in advance of the lower. Florets consist of two different bracts at the base, the palea and the lemma, and a flower. The mature ear of wild-type maize (Fig. 2) differs from the tassel in several aspects: there are no basal branches, the main rachis is thick, the distinction between sessile and pedicellate spikelets is modest, each spikelet has only a single functional floret, and the flowers are pistillate, developing a kernel after fertilization.

The most obvious trait that distinguishes tassels of *Ts6* (Fig. 3) and *ts4* (Fig. 5) mutants from wild-type tassels is the presence of well-developed, functional pistils, as *ts* mutants fail to suppress the pistils that are initiated and subsequently aborted in tassel florets of normal plants. The tassels of both mutants are branched along their length instead of only at the base, and the branches are much shorter than those that do form in wild-type tassels. Stamens are also present in tassels of the two mutants, but shed little pollen.

The ears of *ts4* (Fig. 6) are highly branched, similar to tassels of *ts4*. Although many silks are present, they have very low fertility: an average of 45.6 kernels ($N = 7$, SD = 47) develop on a controlled-pollination ear (note the single kernel visible in Fig. 6), compared to wild-type sibs that develop over 600 kernels per ear. Kernels tend to be hidden under the sterile branches on *ts4* ears. Stamens are absent from flowers of these ears, as in the wild type. *Ts6* ears (Fig. 4) are similar to *ts4* ears but have a less severe phenotype. The distal two-thirds of *Ts6* ears exhibit short, sterile branches dispersed among normal kernels. At their bases, *Ts6* ears appear identical to the wild type except that kernels are crowded such that paired ranks are less obvious. Stamens are also absent from *Ts6* ears.

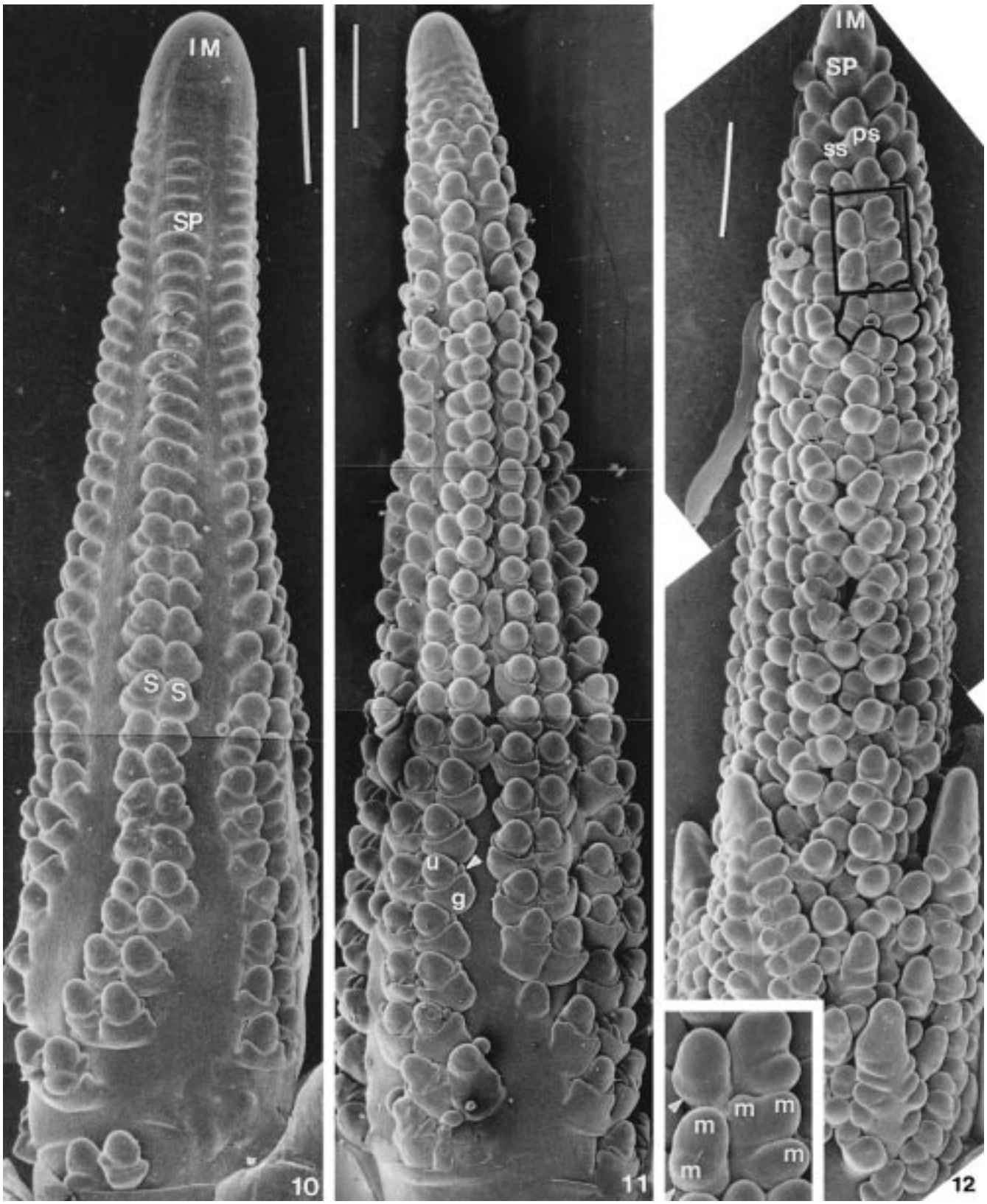
Development of inflorescences: Wild-type—Development of wild-type tassels and ears has been well described previously (Cheng, Greyson, and Walden, 1982; Stevens et al., 1986) and will be reviewed briefly here to emphasize the normal steps in their morphogenesis. The tassel develops from the shoot apical meristem after it has initiated a complete set of leaves or nodes (Irish and Nelson, 1991). Ears develop from meristems in the axils of leaves at nodes in the midportion of the stem (Bonnett, 1948), after those meristems have first initiated a number of husk leaves. In both tassel and ear development, the meristem elongates and initiates outgrowths, called spikelet pair primordia. Spikelet pair primordia are initiated in multiple ranks and in an acropetal sequence (Figs. 7, 10). The spikelet pair primordia at the base of tassels develop into long branches, on which are initiated additional spikelet pair primordia, usually arranged in two ranks. All other spikelet pair primordia (on ears, on tassel branches, and on the distal portion of the main axis of the tassel) enlarge asymmetrically and divide unequally (Figs. 7, 8) to produce two spikelet primordia. The orientation of the asymmetry (right vs. left side larger) appears random. The two spikelet primordia are initially



Figs. 7–9. Wild-type tassels. Bar = 0.5 mm. **7.** A 4-mm tassel. **8.** A 4-mm tassel with basal branches removed to show most mature spikelets. **9.** A 6.7-mm tassel, showing paired spikelets with glumes which cover the site of the formation of the lower floret meristem.

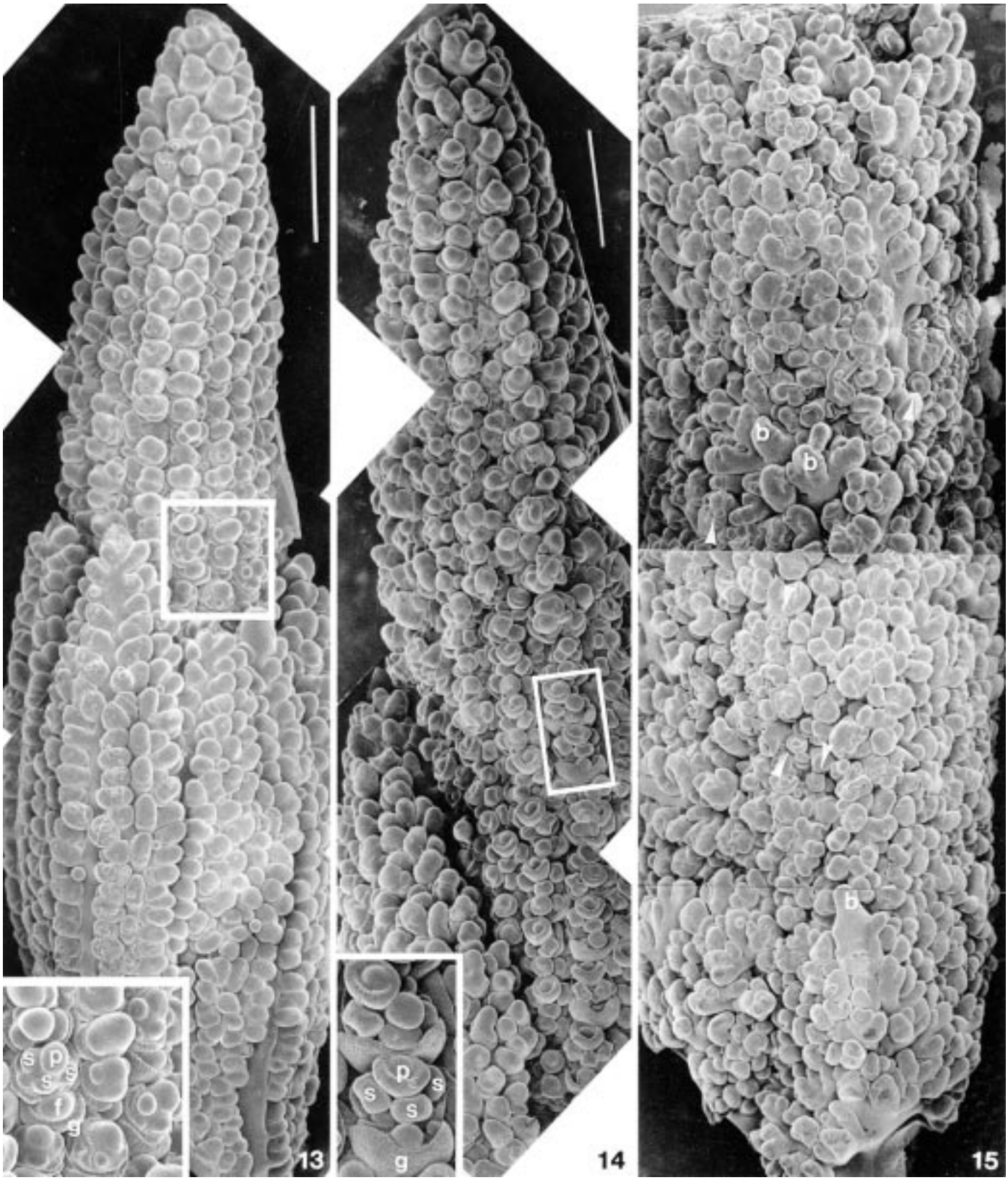
unequal in size (Figs. 8, 10), but become equal (Figs. 9, 11). The spikelet primordium that was larger initially develops into the pedicellate spikelet and the smaller into the sessile spikelet. Each spikelet primordium initiates a pair of glumes (Figs. 9, 11), then divides unequally to form the upper and lower floret primordia. At early stages the lower floret primordium is obscured by the outer glume but is visible in sectioned material (Cheng, Greyson, and Walden, 1982). A floret consists of a lemma, a palea, and a flower (Bonnett, 1940). Thus, the primary difference between developing tassels and ears from wild-type plants is that the spikelet pair primordia at the base of tassels develop into lateral branches, while basal ear spikelet pair primordia develop into a pair of two-flowered spikelets, as do those on tassel and ear tips and on lateral tassel branches. Later in the development of the ear, the lower floret of each spikelet becomes arrested, in contrast to tassels, on which all florets develop fully (not shown).

Ts6 tassels—The first events in the formation of *Ts6* tassels were identical to those of normal tassels, such that wild-type and *Ts6* plants from a segregating family were indistinguishable when their tassels were <4 mm long (not shown). In both genotypes, the shoot apical meristem elongated and initiated spikelet pair primordia in an acropetal sequence. Before *Ts6* (and wild-type) tassels reached 4 mm in length, the only primordia that had developed past initiation were those at the base, which formed branches. Also like the wild type, spikelet pair primordia above and on the lateral branches increased in size asymmetrically and divided to form two spikelet pri-

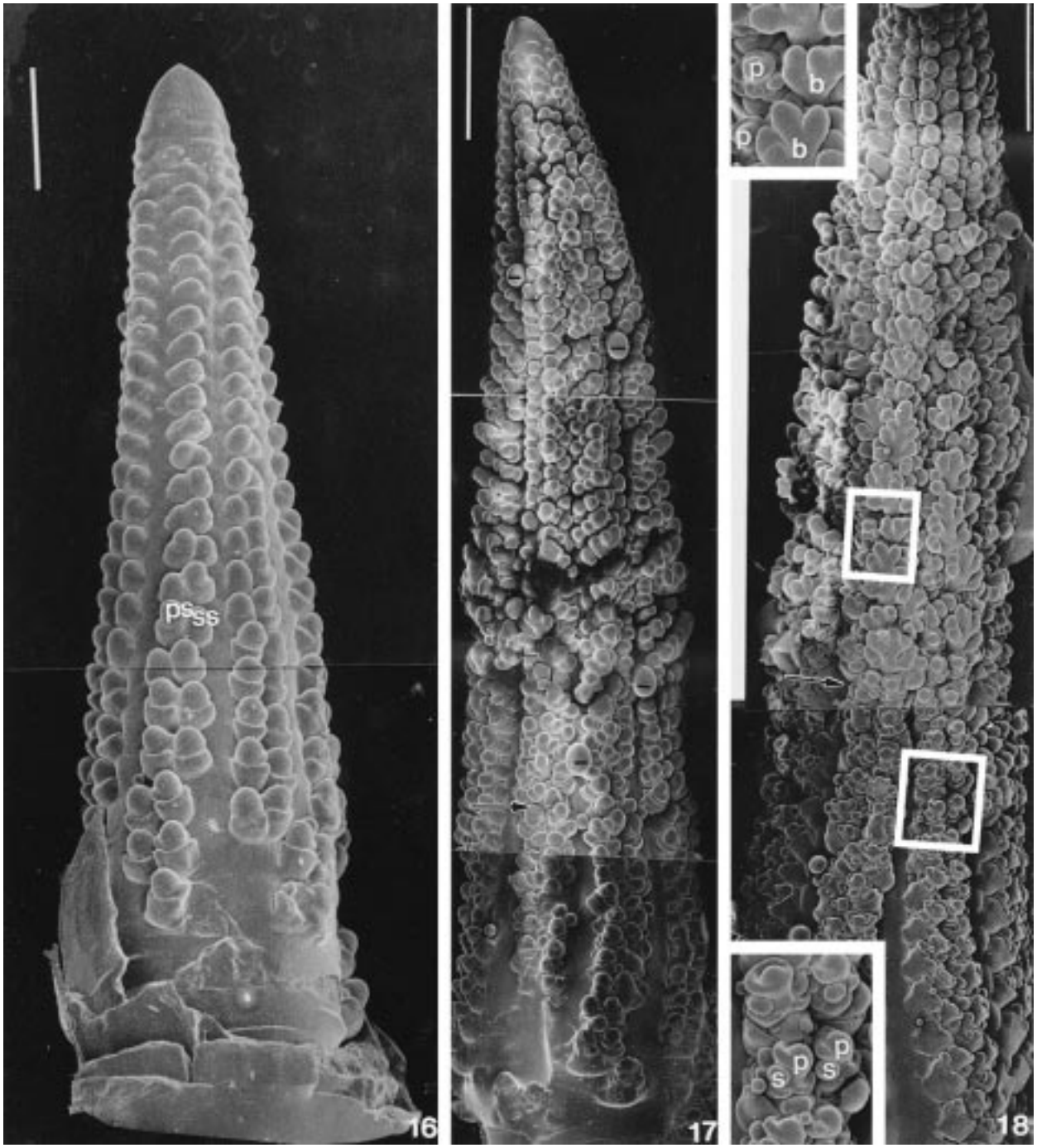


Figs. 10, 11. Wild-type ears. Bar = 0.5 mm. **10.** A 4.3-mm ear. Spikelet pair meristems give rise to two spikelets, which initially are unequal in size but become equal and initiate glumes. **11.** A 5.6-mm ear. Equal-sized spikelet meristems give rise to an upper and a lower floret, the latter being covered by the outer glume. The arrowhead points to the inner glume of a spikelet.

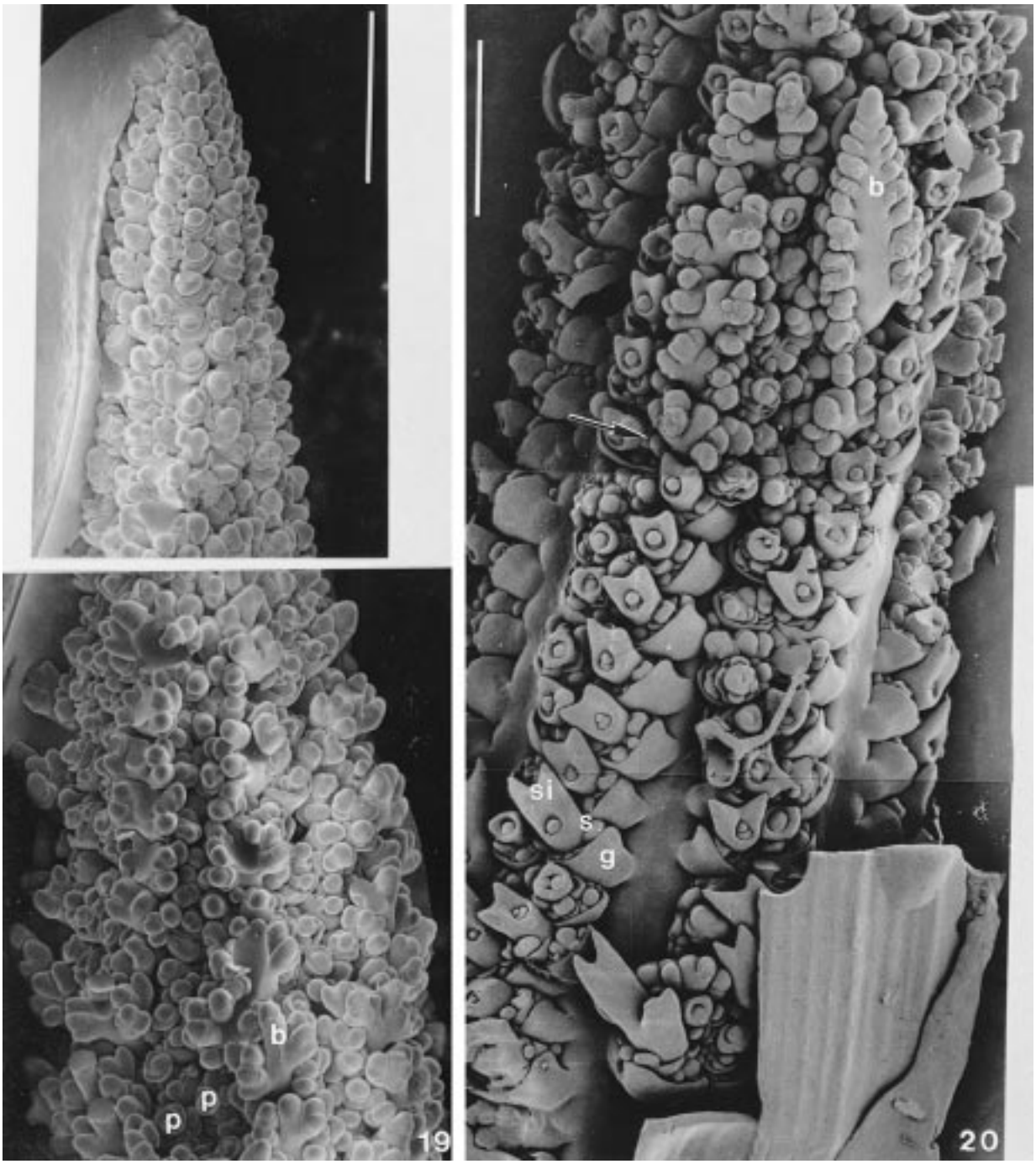
Figs. 12–15. *Ts6* tassels. Bar = 0.5 mm (Fig. 12); bar = 1.0 mm (Figs. 13–15). **12.** A 5.1-mm tassel. Spikelet pair primordia divide unequally, as in the wild type. The sessile spikelet meristem functions normally. The pedicellate spikelet meristem initiates extra floret meristems. The



arrowheads point to glumes at the base of a pedicellate spikelet. The derivatives of one spikelet pair primordium is outlined. The boxed region shows a higher magnification view of two successive clusters of meristems. Each cluster is derived from a single spikelet pair primordium. **13.** A 6.5-mm tassel. Floret meristems initiate normal flowers with stamen and pistil primordia. Boxed area shows a higher magnification view of several florets at various stages of floral organogenesis. **14.** A 9-mm tassel. Boxed area shows a higher magnification view of two successive two-flowered spikelets. The lower floret in each is partially hidden by a glume. **15.** Base of a 15-mm tassel. Florets (arrowheads) are interspersed among meristems on branches.



Figs. 16–20. *Ts6* ears. Bar = 0.5 mm (Fig. 16); bar = 1.0 mm (Figs. 17–20). **16.** A 4-mm ear. The mutant ear is identical to wild-type ears at this stage. **17.** A 9-mm ear. Normal spikelets, each with two florets, developed at the base. Higher up on the ear (above the arrow), branches are growing out adjacent to normal spikelets. **18.** A 10-mm ear. Note continued growth of pedicellate spikelet meristem into a branch. Arrow indicates the transition from normal pairs of two-flowered spikelets to branches. Lower boxed area shows a higher magnification view of pairs of two-flowered spikelets. Upper boxed area shows a higher magnification view of a normal spikelets adjacent to an indeterminate branch. Each pair of a normal spikelet and an indeterminate branch was derived from a single spikelet pair meristem. Figs. 19, 20. Tip and base of a 15-mm ear. **19.** Two-flowered spikelets form from sessile spikelet meristems, while indeterminate branches form from pedicellate spikelet meristems. **20.** The upper florets of each spikelet show well-developed pistils with growing silks but the stamens have been arrested, indicating that sex determination mechanisms (stamen suppression) can operate in ears of *Ts6* mutants. The lower florets are not as advanced as the upper florets but nonetheless have pistil primordia larger than would be present if lower floret suppression occurred as in the wild type (Irish and Nelson, 1993). Arrow indicates the point of transition from normal spikelets to spikelets with extra florets and branches.



Figs. 16–20. Continued.

mordia (Fig. 12). Both spikelet primordia initiated glumes, as in wild type, but were much less prominent than in wild type, relative to the size of the spikelet primordia (compare Figs. 8, 12). The sessile spikelet primordium, which was derived from the smaller portion of the spikelet pair primordium, developed into two floret primordia, an upper and a lower. The upper floret meristem of the sessile spikelet was developmentally in advance of the lower, as in the wild type, but both developed into florets with well-formed (i.e., not aborted) stamens and pistils (Figs. 13, 14).

The major difference between the wild-type and *Ts6* mutant tassels was seen in the fate of the pedicellate spikelet primordium (Fig. 12). After initiating glumes, instead of dividing unequally to form exactly two floret primordia, it formed several floret primordia (Figs. 12, 13). The floret primordia were initiated in a pattern reminiscent of the formation of spikelet primordia on wild-type tassel branches (Fig. 9): that is, in an acropetal sequence and a distichous arrangement. The number of florets formed by the pedicellate spikelet primordium was small, approximately three to five, and typically the

spikelet primordium eventually developed into a terminal floret primordium (Fig. 14). The increased number of florets derived from a single spikelet pair primordium (Figs. 13, 14) is consistent with the mature tassel phenotype (Fig. 3). The morphology of each floret after all floral organs had been initiated was identical to wild-type, although they were oriented in various directions, as judged using the asymmetry of the pistil (Figs. 14, 15). Nonetheless, neither pistil nor stamens were aborted (not shown), leading to a tassel seed phenotype.

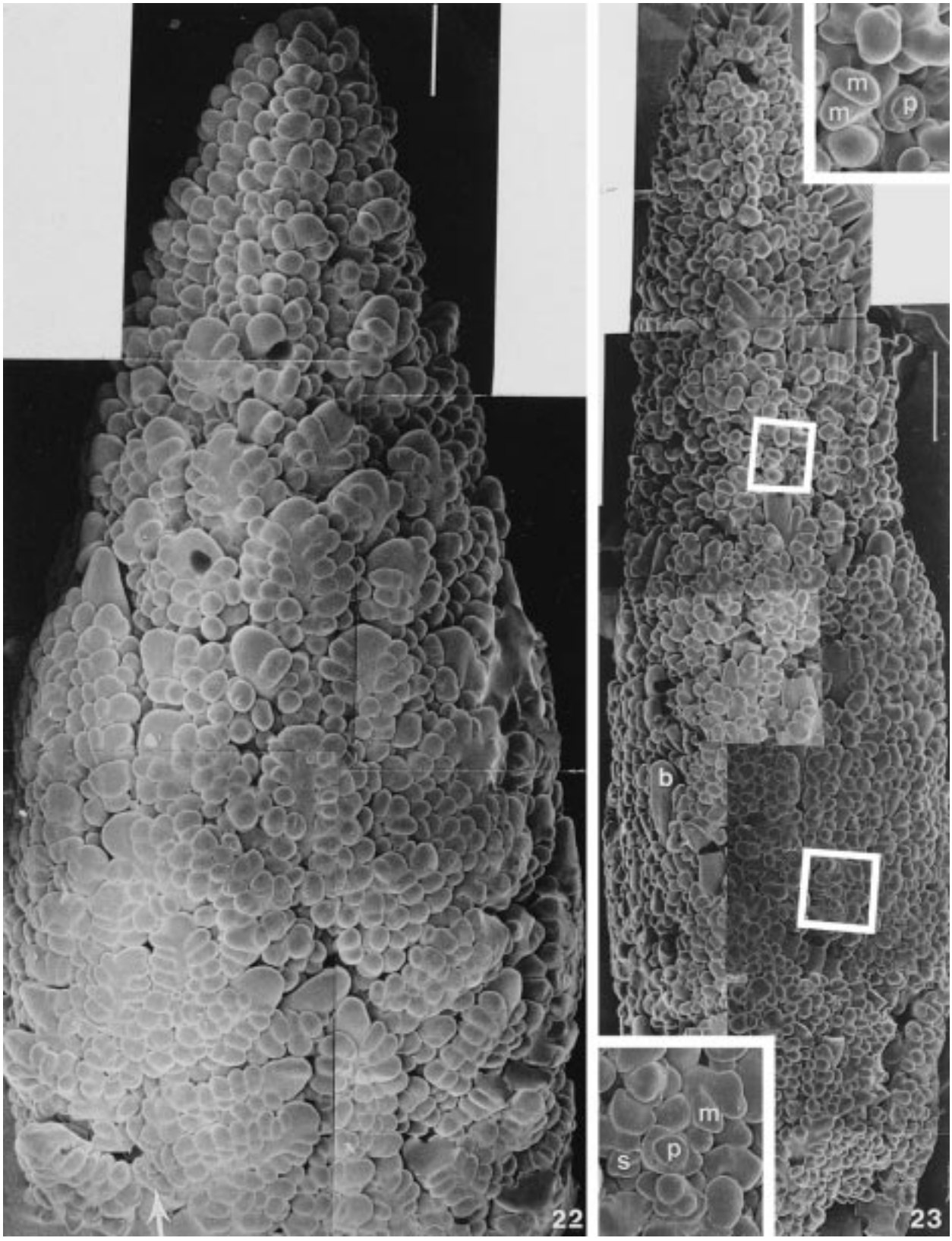
The continued activity of pedicellate spikelet primordia that failed to differentiate into a floret primordium resulted in branch formation. Even tassels as long as 15 mm exhibited branches derived from pedicellate spikelet primordia, in which the spikelet primordium had not yet differentiated a terminal floret primordium (Fig. 15). In normal inflorescences of plants of the inbred background W23, enlarged stamens or pistils, marking the occurrence of sex determination, are apparent when tassels and ears have reached 1 cm in length (Irish and Nelson, 1993). Growth of stamens and pistils of the florets in *Ts6* tassels was delayed relative to floret development of wild-type inflorescences as only a small proportion of florets at the base of tassels 16–20 mm long had matured past the stage at which sex differentiation-associated organ abortion occurs. In those florets there was no sign of organ abortion in those florets (not shown), consistent with the mature tassel phenotype.

Ts6 ears—The ears of *Ts6* mutants, like the tassel, exhibited early stages of morphogenesis identical to the wild type, and, as a result, ears less than 4 mm of wild type and mutants were indistinguishable. In ears longer than 4 mm, two distinct zones could be observed on *Ts6* ears. At the base of *Ts6* ears, both sessile and pedicellate spikelets gave rise to only two florets, so that except for lack of suppression of the lower floret in the mutant, this zone on *Ts6* ears (Fig. 16) was like wild-type ears. Above the basal zone of near-normal development, the sessile spikelet was also normal, producing exactly two floret primordia, but the pedicellate spikelet primordium gave rive to supernumerary floret primordia, forming a short branch (Figs. 17–20), as in *Ts6* tassels. All florets that were initiated continued to develop, but sex differentiation occurred in mutant ears as in the wild type, where stamens were initiated but subsequently aborted (Fig. 20). Development of the lower floret in the sessile spikelet (Fig. 20) resulted in kernel crowding on pollinated ears (Fig. 4).

ts4 tassels—Despite superficial similarities between tassels of *ts4* and *Ts6*, these two mutants show major differences during morphogenesis of their inflorescences. All spikelet pair primordia on *ts4* tassels developed into branches (Fig. 21) bearing additional spikelet pair primordia (Fig. 22; note lack of glumes, which are at the base of spikelets but not spikelet pairs). *ts4* tassels could be distinguished from wild-type tassels as early as at 2 mm in length, as a result of the continuous branching of the spikelet pair primordia from the time that they were initiated. This branching was reiterated on the branches, so that the inflorescence became a mass of meristems (Fig. 22). Rarely was a normal floret formed. Each had typical floret organization but its pistil was not aborted (Fig. 23).

ts4 ears—The ears of *ts4* were more like the wild type than were the tassels of the mutant. Like *Ts6* ears, the basal zone developed normal pairs of two-flowered spikelets (Figs. 24, 25). Distal to the normal zone, branches formed from the pedicellate spikelet primordia. In the central zone of *ts4* ears, sessile spikelet primordia formed, as indicated by the presence of glumes surrounding meristems that differentiated into floret primordia.

Figs. 21–23. *ts4* tassels. Bar = 0.5 mm (Figs. 21, 22), bar = 1.0 (Fig. 23). **21.** A 5-mm tassel. All spikelet pair meristems develop into branches that bear additional spikelet pair meristems. **22.** An 8-mm tassel. Arrow indicates a primary branch, which bears three additional orders of branches. **23.** A 16-mm tassel. Even at late stages of development, the tassel of *ts4* is primarily a mass of branches and meristems with a few normal florets. The boxed areas show higher magnification views of florets (indicated by pistils and stamens) adjacent to meristems.



Figs. 21–23. Continued.

Paired with the sessile spikelets were branches lacking glumes but bearing numerous meristems. Thus, in *ts4* ears, spikelet pair primordia at the base developed into four florets, and in the center they developed into a sessile spikelet with two florets and an indeterminate branch. At the tip of *ts4* ears there was no evidence of glume formation, so that this portion of the ears resembled *ts4* tassels in the reiteration of the formation of spikelet pair primordia by the spikelet pair primordia.

DISCUSSION

This comparison of the development of inflorescences of the wild type and Class II *ts* mutants suggests that, in maize, there are at least four different types of reproductive meristems that can be identified, based on the order in which they arise, the type of derivative organs they produce, and which mutations affect them. The first reproductive meristem to arise is the inflorescence meristem, which is formed directly by the conversion of a vegetative shoot meristem. The derivatives of the inflorescence meristem are a second type of reproductive meristem, the spikelet pair meristem. Spikelet pair meristems initiate a third type of meristem, the spikelet meristem. Spikelet meristems initiate first a pair of lateral organs, glumes, and then initiate floret meristems. Floret meristems initiate only lateral organs: a palea, a lemma, and the floral organs, lodicules, stamens, and carpels. I propose that the relatively simple organization of inflorescences of wild-type maize is the consequence of early determinacy of certain of these meristems, so that each outgrowth from the inflorescence meristem gives rise to exactly four florets (Fig. 26). The Class II *ts* mutations perturb the fate of specific reproductive meristems, leading to extra branching in the inflorescence.

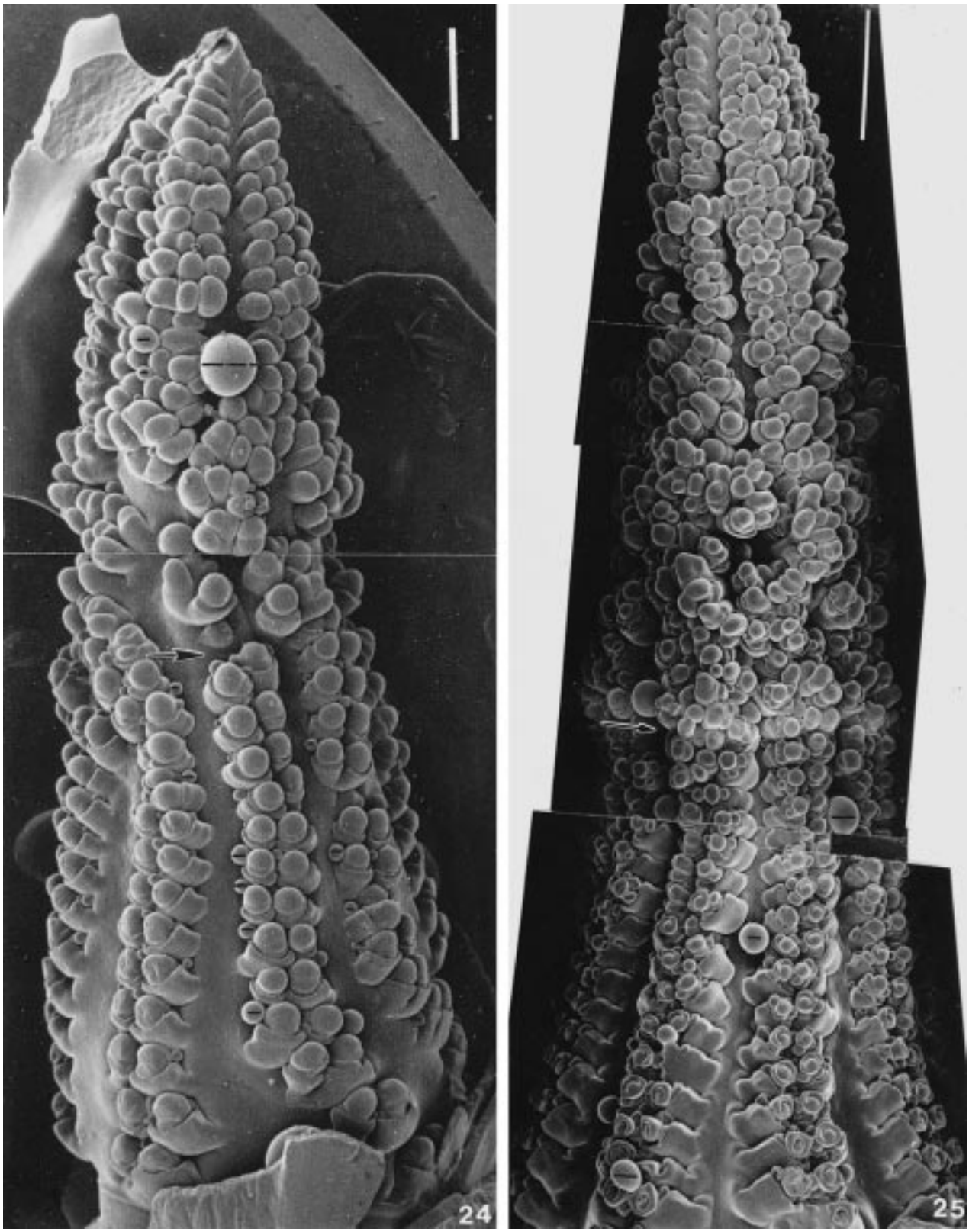
According to this scheme (Fig. 26), in wild-type tassels, the first-formed spikelet pair meristems are indeterminate. Each produces a basal branch that reiterates the structure of the main axis of the inflorescence. Later formed spikelet pair meristems on the central rachis and lateral branches of the tassel and all spikelet pair meristems on ears are determinate. They produce a single derivative, a spikelet meristem. Their determinacy is marked by their subsequent conversion to the derivative type of meristem, the spikelet meristem, so that exactly two spikelet meristems are derived from the spikelet pair meristem. Spikelet meristems produce one pair of glumes, and in normal maize are also determinate, each producing only a single derivative, a lower floret meristem, before itself becoming converted to a floret meristem. Thus, in normal maize, a spikelet pair meristem can have two very different fates: to form a long branch bearing many florets, or to form a short, once-branched branch bearing four florets.

Conversion of a meristem into a derivative type as a way of conferring determinacy is seen also in the vegetative shoot, as vegetative development is terminated when the shoot apical meristem converts into an inflorescence meristem (Irish and Nelson, 1991). Even though the derivative meristems have to be initiated before the conversion of the meristem that formed them, in both cases the converted meristems (pedicellate spikelet, upper floret) develop faster than the derivatives (sessile spikelet, lower floret). As a result, the spikelets of wild-type maize are cymose even though the inflorescence overall is racemose. This pattern of terminal flowers of determinate inflorescences being developmentally advanced is also seen in the *Antirrhinum* mutant *centroradialis* (Bradley et al., 1996). Like spikelets of *ts4* and *Ts6* maize mutants, the inflorescence of wild-type snapdragon is indeterminate. Indeterminacy is bestowed upon wild-type snapdragon inflorescences by the action of *centroradialis*, which prevents the expression of *floricaula*, a gene required for the formation of floral meristems, in the apical meristem. Whether a maize homologue of *floricaula* is expressed in pedicellate spikelet and upper floret meristems in wild-type maize but not in *ts4* and *Ts6* mutants has yet to be determined.

The inflorescences of *ts4* and *Ts6* mutants exhibit extra branching. This is due to a delay in or lack of determinacy of specific meristems. *ts4* tassels show the most extreme phenotype, which can be interpreted as a lack of determinacy in all spikelet pair meristems and their production of more spikelet pair meristems instead of spikelet meristems. *ts4* is a recessive mutation, suggesting that the phenotype is the result of the lack of some product that is required for the transition to spikelet meristem activity. *ts4* mutants are not completely sterile as some functional florets are formed. This implies that either *ts4* is not a null mutation or there is some redundancy in the function performed by the wild-type allele of *ts4*. Two additional mutant alleles of *ts4* have phenotypes identical to the reference allele used in this study (not shown), and a fourth, radiation-induced allele had greater fertility than the reference allele (Nickerson and Dale, 1955). That none of these alleles confers complete sterility suggests that determinacy can eventually be conferred by the product of another gene in *ts4* mutants. Nevertheless, *Ts4+* is required for the normal early determinacy of spikelet pair meristems and for the differentiation of their derivatives as spikelet meristems.

Pedicellate spikelet meristems of *Ts6* mutants initiate extra floret meristems before converting to floret meristems. Thus, wild-type *ts6+* function is required for determinacy of the pedicellate spikelet meristem. The pedicellate spikelet meristem is a continuation of the leading growing point initiated as the spikelet pair primordium. Its derivatives show normal determinacy but it retains its indeterminate, meristematic activity longer in *Ts6* mutants than in the wild type. This interpretation is supported by supernumerary foci of expression of *Knotted*, a shoot meristem-specific gene in developing *Ts6* tassels (Irish, 1997). As *Ts6* is a semidominant mutation, one possible explanation for its phenotype is that it overproduces a factor that maintains indeterminacy in the leading meristems. Unlike *ts4* mutants, there is only a delay in determinacy. Once floret meristems are formed in either mutant, they form normal florets.

The *Ts6* and *ts4* mutations affect spikelet pair meristem determinacy only in the mid to upper portion of the ear. This suggests that basal branching on ears is suppressed by the activity of an independently acting gene whose effect is not overcome by the Class II *tassel seed* mutations. Where branching is suppressed (or determinacy is achieved) *ts4* and *Ts6* mutants are capable of producing normal florets. Nonetheless, normal functioning of *ts4+*



Figs. 24–25. *ts4* ears. **24.** A 5.3-mm ear. The basal zone has normal paired spikelets consisting of two floret meristems, the lower of each spikelet hidden behind the outer glume. Above this zone (arrow) branches without basal glumes are adjacent to sessile, normal spikelets. At the tip, no normal florets are apparent, and branches form from all meristems. **25.** A 10-mm ear. In the basal zone, normal florets are present, in which the pistil is advancing past the stages associated with sex determination, while the stamens appear arrested in development. Above the basal zone, a proliferation of meristems on short branches is apparent.

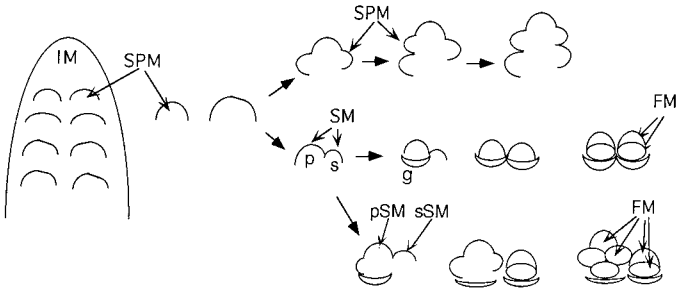


Fig. 26. Model for maize inflorescence morphogenesis. Spikelet pair meristems at the base of the wild-type tassel are indeterminate, producing numerous additional spikelet pair meristems to form the lateral branches (upper sequence). Those spikelet pair meristems, like those above the branches on tassels and all those on ears, are determinate, initiating a single spikelet meristem before converting to become a spikelet meristem. Spikelet meristems initiate glumes, then a single floret meristem, and then convert to floret meristem activity also (central sequence). *Ts6* inflorescences gain extra branching through the delayed determinacy of the pedicellate spikelet meristems (lower sequence). *ts4* inflorescences are more branched (and less fertile) than wild-type because most spikelet pair meristems on tassels and the distal zone of ears are indeterminate and their derivatives are also all indeterminate spikelet pair meristems (reiterating upper path). The basal zone of *ts4* and *Ts6* ears are near-wild-type, as a result of determinacy of spikelet pair and spikelet meristems (central sequence). FM, floret meristem; g, glume; IM, inflorescence meristem; p, pedicellate; s, sessile; SPM, spikelet pair meristem; SM, spikelet meristem.

and *Ts6+* is required at the base of ears to suppress the development of the lower floret, like other *ts* genes.

Although each meristem type (spikelet pair primordium, spikelet primordium, floret meristem) is morphologically similar, each can be distinguished by what types of organ it produces and which mutations affect it. The mutation *Suppressor of sessile spikelets* (*Sos*) prevents the initiation of the sessile spikelet meristem by the spikelet pair meristem, but does not prevent the conversion of the spikelet pair meristem to a spikelet meristem or its subsequent initiation of floret meristems (Doebley, Stec, and Kent, 1995). As a result, florets are found in unpaired spikelets in *Sos* mutants. According to the model presented above, the mutation *ts4* prevents the transition to the spikelet meristem state, and *Ts6* delays the conversion of a spikelet meristem to a floret meristem. This interpretation of *ts4* and *Ts6* is consistent with the phenotype of the *ts4 + Ts6* double mutant, which is no more severe than that of *ts4* (Irish, Langdale, and Nelson, 1994).

The obvious question that stems from this study is: how are branching and pistil suppression related? Branching in inflorescences is a process that amplifies the possible number of flowers that can be formed from a shoot apical meristem and is found in a multitude of angiosperm species (Weberling, 1989). The absence of floral determination in a meristem formed by an inflorescence meristem will result in the development of an inflorescence branch, as is seen in the homoeotic mutations *leafy* (Huala and Sussex, 1992) and *apetala 1* (Irish and Sussex, 1990) of Arabidopsis and *floricaula* (Carpenter and Coen, 1990) of snapdragon. However, in order for flowers to be formed in fertile plants, the balance must shift from multiplying meristems to floral determination and determinacy of those meristems. The wild-type alleles of *Ts4+* and *ts6+* are clearly involved in regulating this balance in maize. One explanation for pistil development in tassels of *ts4* and *Ts6* mutants is that the extra branching causes florets, when they do develop, to be in a developmental context in which the processes that result in organ abortion cannot function correctly. An observation that argues against this explanantion is that stamen suppression does occur normally in the ears of both of these mutants. An alternate explanation is that branching and pistil development both directly require the normal functioning of *Ts4+* and *ts6+*.

Monoecy in maize is a derived condition. As a rule, grasses develop perfect flowers, but all of the Maydeae have the ability to suppress stamen or pistil development in florets in specific locations on the shoot (Kellogg and Birchler, 1993). A characteristic of the Gramineae, however, is the variation and complexity of architecture of the inflorescences (Clifford and Watson, 1977; Dahlgren, Clifford, and Yeo, 1985; Clifford, 1988). Genes involved in controlling meristem function may have become subverted to act in pistil suppression during the evolution of maize. In addition to *ts4* and *Ts6*, the mutation *ramosa 3* results in development of perfect tassel flowers and basal branching of the ear (Coe, Neuffer, and Hoisington, 1988). However, mutations that alter branching in inflorescences do not always affect sex determination. Staminate tassels and pistillate ears are formed by *ramosa 1* mutants, in which the spikelet pair meristems are indeterminate throughout the tassel and ear, and by *unbranched* mutants, in which all spikelet pair meristems are determinate, resulting in unbranched tassels. *Branched silkless* mutants, in which the spikelet meristems are indeterminate in the tassel, also are able to suppress pistils in tassel florets. Therefore, only a subset of genes involved in inflorescence architecture also regulates sex determination in maize.

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