The Sex Determination Process in Maize

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Maize partitions the sexes into different flowers on the plant, a condition called monoecy, which facilitates outcrossing. Sex determination in maize is a complex process involving an interplay between genetic determinants, the environment, and hormones. Unisexuality of flowers is achieved by the process of selective arrest and abortion of the inappropriate organ primordia within a bisexual floral meristem. Floral organ abortion is associated with the degeneration of cells within an immature primordia. Masculinizing genes are required for gynoecial abortion, feminizing genes arrest stamen development, and both types also control secondary sexual traits involving morphological characteristics of floral tissues. Gibberellins, steroid-like plant hormones, appear to play a pivotal role in the stamen abortion process and the feminization of floral tissues.

Most flowering plants bear perfect flowers, which contain both male and female sex organs within each flower. A typical angiosperm flower possesses a male androecium (containing stamens composed of anther and filament) surrounding a central female gynoecium (containing one or more pistils composed of fused or unfused carpels). Specialized cells within these organs undergo gametogenesis and form the haploid pollen or embryo sacs. In more derived forms of flowers, the exact arrangement, symmetry, type, and number of floral organs can vary tremendously. The identity of floral organs within each whorl is determined in a combinatorial fashion by a group of floral organ identity genes, which are expressed in one or more whorls of the developing floral meristem (*1*, *2*).

Various patterns of unisexuality have arisen in the plant kingdom. In general, sexes may be partitioned into unisexual flowers on the same plant (monoecy) or on separate plants (dioecy). Many intermediate forms of monoecy and dioecy also exist in plants (*3*). These various patterns of sexuality are widespread throughout the plant kingdom; it has been estimated that monoecious and dioecious plants represent over 10% of all plant species, distributed among 75% of plant families (*4*).

Plants accomplish unisexuality by various means. Some species, such as spinach (*Spinacia oleracea*), hemp (*Cannabis sativa*), and mercury (*Mercurialis annua*), bypass the formation of the inappropriate sex organs (*5–7*), whereas other species, such as asparagus (*Asparagus officinalis*), maize (*Zea mays*), and white campion (*Silene latifolia*), arrest the development of preformed sex organs at various stages of maturation (*8–10*). Likewise, the genetic basis of unisexuality shows great diversity in plants—active Y, X-to-autosome ratios, and autosomal determinant systems are all represented in the plant kingdom (*11–13*). This developmental and genetic variation suggests that the underlying cellular mechanisms of sex determination in plants may vary considerably.

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The Maize Flower

In maize, the sexes are partitioned into separate pistillate (ear) and staminate (tassel) inflorescences (Fig. 1). Unisexual inflorescences are the principal feature of maize that facilitates efficient hybrid seed production and genetic experimentation. Unisexuality is achieved in an immature bisexual floral meristem by the selective arrest and abortion of the inappropriate sex organs (*14–16*) through the interaction of sex determination genes, hormones, and environmental factors.

Maize shows a determinant growth habit, with shoots terminating in inflorescences bearing staminate or pistillate flowers (also called florets, in grasses). An unbranched pistillate ear forms in one or more of the axillary branches in the upper portion of the main shoot; the main shoot terminates in a branched staminate tassel. In some lines of maize, the lower axillary branches develop into tillers, which reiterate the pattern of one or more axillary pistillate ears and a central staminate tassel.

Despite the gross morphological differences in the tassel and ear at maturity, early events in flower formation are remarkably

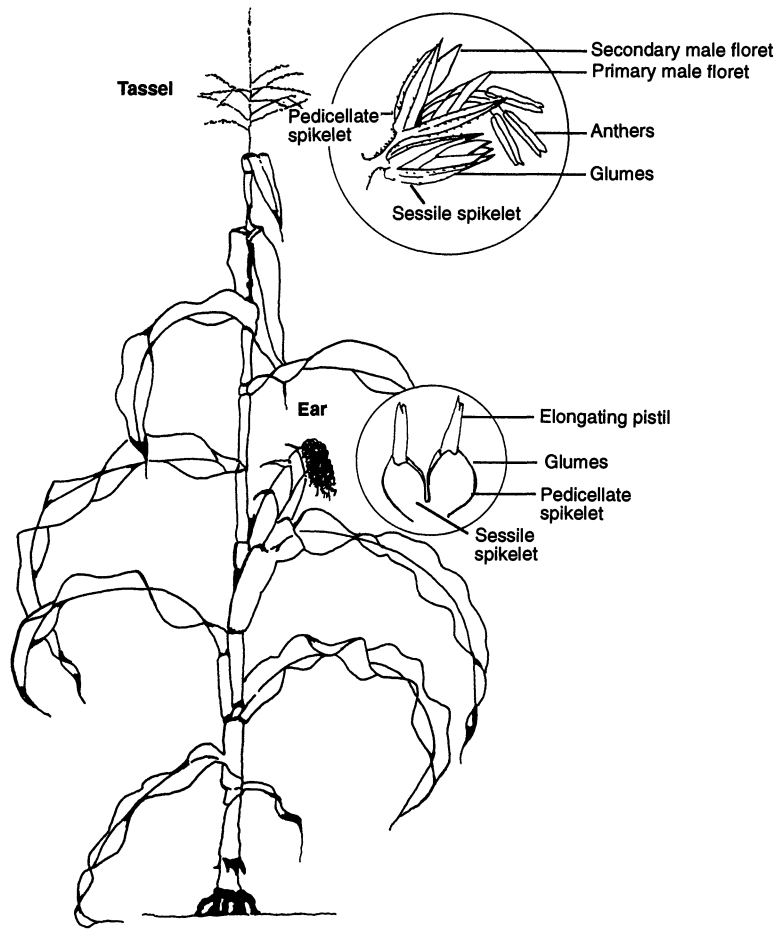

Fig. 1. The maize plant, showing tassel and ear inflorescences. Drawing of a maize plant showing details of pistillate and staminate spikelet pairs. [Redrawn and adapted from (*40*) with permission of the author].

similar. Shoot meristems elongate to form an inflorescence meristem. Branch initials are formed near the base of the tassel inflorescence; other initials in the tassel, and all initials in the ear, bifurcate to form paired pedicellate–sessile spikelet primordia. Both spikelets initiate two subtending glumes (bracts), which will encase two floral meristems. The floral meristem elaborates a series of organs: a lemma, palea, lodicules, stamen initials, and a central gynoecium. At this early point in floral development, both ear and tassel florets are bisexual (perfect) and morphologically indistinguishable (Fig. 2A).

The transition from the immature "bisexual" to the mature "unisexual" stage is marked by profound changes in the morphology of the flower. The primary effect of the sex determination process is to arrest and abort organ primordia of the inappropriate sex—pistil primordia in the tassel and stamen primordia in the ear. In the tassel, cells in the gynoecium become highly vacuolated and eventually degenerate (Fig. 2B); these cellular events appear to be initiated in specific cells in the degenerating organ (*16*). Adjacent stamen initials continue to divide, differentiate, and eventually reach sexual maturity. In primary ear florets, stamen initials are arrested and aborted while gynoecial development continues to sexual maturity. In the secondary ear florets, both androecia and gynoecia abort, leaving a solitary pistillate floret to develop in each ear spikelet. Secondary sexual traits are also controlled by the sex determination pathway in maize. In staminate flowers glume morphology, lodicule formation, trichome distribution, and the degree of pedicel elongation differ markedly from that found in pistillate flowers. Red and purple flavonoid pigments are also differentially deposited in spikelet and floral tissues of pistillate and staminate flowers.

In summary, the determination of unisexuality in maize flowers involves profound changes in the fate of sexual organs as well as marked differences in the morphology of floral tissues. The sex determination program, therefore, must regulate the differential, programmed abortion process of floral sex organs as well as a number of complex pathways controlling tissue morphology and pigmentation patterns.

Genetic and Hormonal Determinants of Sex

The genetic dissection of sex determination in maize presently suffers from several limitations. Several of the genes affecting sex determination are defined only by single alleles of unknown nature. Certain alleles of these genes show differing penetrance and expressivity in various inbred backgrounds and hybrids. Moreover, because the maize genome is extensively duplicated (*17*), many genes, including those involved in sex determination, may be refractory to standard mutagenesis because of functional redundancy. Nevertheless, from the many mutations that affect the flowering process in maize, a subset has been identified that is somewhat specific to the sex determination process, that is, mutations that interfere with selective abortion of floral organs (Fig. 3) rather than those that affect floral organ identity or number. These mutations are described here as feminizing or masculinizing genes depending on the inferred function of the wild-type gene product from mutant phenotype analysis.

Feminizing genes. Several mutations have been described that perturb the process of stamen abortion or gynoecial development in pistillate flowers. One general class of mutations affecting stamen abortion is the *dwarf (d)* mutants, *d1*, *d2*, *d3*, *d5*, *anther ear1 (an1)*, and *D8*. In addition to their short stature, all six mutants are andromonoecious (perfect flowers in the primary ear florets and staminate flowers in the tassel and secondary ear florets) (Fig. 4). Stamen production in the ear is due to the failure of the normal stamen abortion process in the primary and secondary florets. Pistil development in the primary ear florets and gynoecial abortion in the secondary florets are often unaffected, although in some severe *dwarf* alleles, pistils may fail to develop and anthesis may be blocked (*18*).

The *d1*, *d2*, *d3*, and *d5* mutations block specific steps in the gibberellin biosynthetic pathway. Gibberellins (GAs) are steroid-like plant hormones implicated in many aspects of plant growth and development (*19*). These dwarf mutations have low concentrations of endogenous GAs, specifically GA_1, the gibberellin that controls shoot elongation in maize (*20*, *21*). In wild-type lines, sustained GA_3 treatment (*22*) can cause partial to complete staminate-to-pistillate

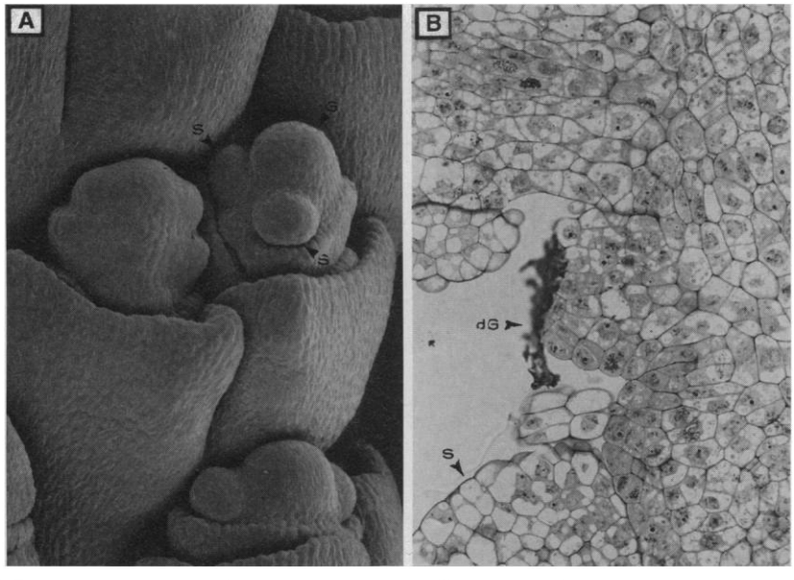

Fig. 2. Transition from bisexual to unisexual flowers. (**A**) Scanning electron micrograph of a spikelet pair at the bisexual stage showing stamen initials (S) and the central gynoecium (G). [Figure 3B from (*35*) by copyright permission from Cell Press.] (**B**) Transmission electron micrograph of a staminate floral meristem at a later stage of development showing the degenerated gynoecium (dG), whereas stamen initials (S) continue to develop. [Figure from (*41*), reproduced with permission of the author.] Original magnification, ~×174 for (A) and ~×300 for (B).

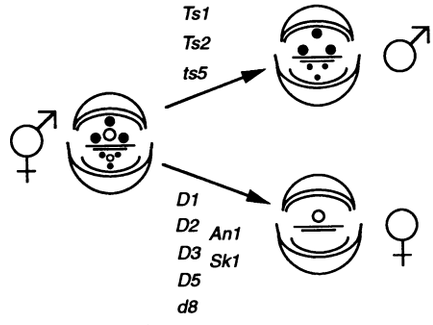

Fig. 3. Genes required for the transition from bisexual to unisexual flowers. Maize floral diagrams of a spikelet containing two bisexual floral meristems (left) converted either to a tassel spikelet containing two staminate florets (top, right) or to an ear spikelet containing a solitary pistillate floret (bottom, right) by the action of the genes indicated. The following genes promote gynoecial abortion: *Tasselseed* (*Ts*) *1*, *2*, and *ts5*, a gene defined by the dominant *Ts5* mutation. The following genes promote stamen abortion and feminization: *Dwarf (D) 1, 2, 3, 5, Anther ear1 (An1)*, *Silkless1 (Sk1)*, and *d8*, a gene defined by the dominant dwarf *D8*. The floral diagrams shows gynoecia (○), stamen initials (●), palea (⁼), lemma (⊃), and glumes (⊜) when present; lodicules are not shown.

conversion of the tassel when plants are treated before the determination of sexuality (*23–25*). Environmental treatments that feminize tassels, such as short day length and low light, have also been correlated with increases in endogenous GA-like substances in the tassel; application of GA biosynthesis inhibitors can completely reverse this feminization process (*25, 26*). In addition, GA_3 promotes pistillate flower formation in cultured inflorescences (*27, 28*). Collectively, these genetic and physiological studies support a pivotal role for GAs in the feminization of maize flowers and specifically in the stamen abortion process.

The *silkless1 (sk1)* mutation of maize (*29*) prevents the formation of pistils in the ear, which results in barren florets (Fig. 4) even though secondary sexual characteristics (glume morphology, anthocyanin production, and trichome patterns) remain female. Staminate florets in the tassel are unaffected. This phenotype suggests that the silkless1 gene product is required only for pistil development. As discussed below, however, double-mutant analysis suggests that *silkless1* may lie in the sex determination pathway, possibly as a target of sex determination functions such as *tasselseed2* (*30, 31*).

Masculinizing genes. Mutations with specific effects on the production of staminate flowers in the tassel are the *tasselseed* (*ts1, ts2*) mutations. Mutant plants produce functional pistillate flowers in the tassel that, after pollination, form viable seeds in the tassel inflorescence. The staminate-to-pistillate conversion in tassels is due to the failure of the normal pistil abortion process and the concomitant induction of ectopic stamen abortion. Secondary sexual characters, such as glume morphology, anthocyanin deposition, and pedicel elongation, are completely feminized in the mutant tassel florets; only early decisions such as tassel branching patterns appear to be unaffected (*31, 32*). Complete loss-of-function *ts2* mutations cause a complete feminization of all tassel florets. The *ts1* and *ts2* mutations affect the pistillate florets of the ear and secondary sexual characters as well. The normal pattern of secondary floret abortion is disrupted; secondary gynoecia fail to abort, yielding spikelets containing two functional pistillate flowers (*31, 32*). This suggest that *Ts1* and *Ts2* genes function to abort gynoecia and to direct the sexual fate of immature spikelet and floral tissues.

A dominant mutation, *Tasselseed5* (*Ts5*) (*33*), also affects staminate flowers (Fig. 4), but the degree of staminate-to-pistillate conversion is less severe and positional. Pedicellate spikelets contain staminate florets, whereas the sessile spikelet is pistillate but often nonfunctional in the proximal region of the tassel, perfect in the medial region, and staminate in the distal regions. Other tasselseed phenotypes (*ts4* and *ts6*, for instance) are more complex in nature and often result in the proliferation of floral tissues in addition to pistil formation in tassels. These genes may act early in establishing organ identity and number rather than represent specific steps in the sex determination pathway (*31, 34*).

Genetic mosaic analysis has addressed issues of timing and autonomy of tasselseed action. Sectors of male spikelets can be generated in an otherwise completely feminized tassel by use of an unstable, transposon-induced *ts2* mutation (Fig. 5) (*35*). Staminate sectors can occur as small as single spikelets or can encompass large regions of the tassel inflorescence. The presence of mixed pistillate-staminate paired spikelets on these plants indicates that sex can be determined as late as the bifurcation of initials to form paired spikelet primordia. The transposon excision events that restore *tasselseed2* activity take place in subepidermal lineages (*35*). It is these subepidermal lineages that form the bulk of the floral tissue and undergo meiosis to give rise to the gametophyte (*36*). Both subepidermal and overlying epidermal cells in these sectors assume a male fate. Male sexual characteristics expressed in the epidermis include the deposition of anthocyanin in certain cells and the production of abundant surface hairs or trichomes. This raises the intriguing possibility that the cell nonautonomous action of the tasselseed2 product is to direct the sexual fate of floral tissues through a diffusible morphogen.

Little is currently known about the molecular biology of the sex determination process in maize or any other plant. The maize *tasselseed2* gene, which has been cloned, encodes an alcohol dehydrogenase–like protein with similarity to steroid dehydrogenases (*35*). The implicated role of GA hormones in feminizing the ear florets and double-mutant studies, discussed below,

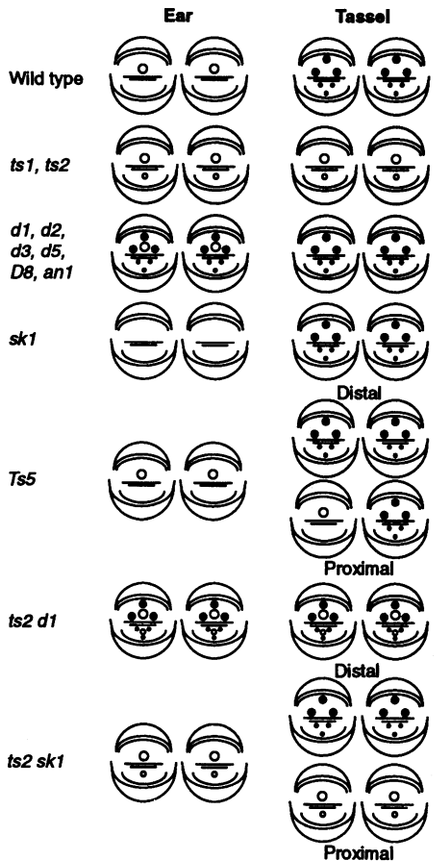

Fig. 4. Floral diagrams of spikelet pairs in wild-type and mutant lines of maize. Shown are floral diagrams of paired spikelets in ears and tassels of wild-type, single-, and double-mutant lines of maize. (See legend to Fig. 3 for allele designations and an explanation of the floral diagrams. The pedicellate spikelet is shown to the right of the spikelet pair and individual genotypes are separated by commas.)

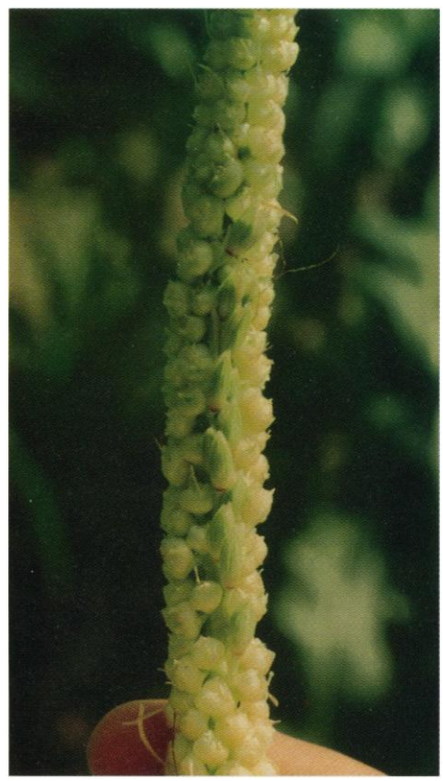

Fig. 5. Sector of staminate spikelets in a mutant pistillate tassel. Plants homozygous for the *Ac*-induced *Ts2* mutation, *ts2-m1*, show instability of the staminate-to-pistillate conversion phenotype due to excision of *Ac* in subepidermal lineages which restores Ts2 function (*35*). Pistillate-to-staminate reversion is associated with a complete restoration of male traits including gynoecial abortion, stamen development, and secondary male traits such as glume morphology and anthocyanin deposition patterns in floral tissues. [Figure 2E from (*35*) by copyright permission from Cell Press.]

suggest that tasselseed genes act antagonistically to the feminizing effects of GA. This antagonism may be direct, by inactivating GAs or redirecting biosynthesis, for instance, or indirect, by inducing pistil abortion and in turn blocking GA production. In situ localization indicates that the tasselseed2 RNA is located in gynoecial cells before cell death, consistent with its role in gynoecial abortion (*35*).

Model for Sex Determination in Maize

A genetic hierarchy is beginning to emerge from the analysis of single and double mutants in sex determination genes. Double-mutant studies between *ts2* and *sk1* indicate that these genes may interact to control floral sexuality. Homozygous *sk1 ts2* tassels show partial suppression of *ts2*-induced tassel feminization (Fig. 4) (*31*, *37*). Partial epistasis of *silkless1* in the tassel might be expected if the *silkless1* mutation were not a complete loss-of-function allele (*31*). The epistasis of *ts2* to *sk1* in ear (Fig. 4) suggests that *Sk1* may also function to suppress *Ts2* action in the primary ear florets. Hence, tasselseed products may be required for the process of pistil abortion in a *Sk1*-dependent manner; the stamen abortion phenotype associated with *ts1* and *ts2* mutations seen in tassel florets may be the consequence of the presence of functional pistils, perhaps associated with GA production.

Double-mutant studies have been conducted between *tasselseed2* and *dwarf* mutations. In the *tasselseed2* and *dwarf1* double mutant, florets in the ears and tassels are perfect (Fig. 4) (*31*). This additive phenotype suggests that any interaction between *ts2* and *d* genes may be an indirect one. Nonetheless, the phenotype of the secondary florets in these single and double mutants is informative; these secondary florets are bisexual only when both *Ts2* and *D1* action are impaired. The wild-type pattern of secondary floret abortion in ears, therefore, may reflect a combined role of *Ts2* (gynoecia abortion) and GA (stamen abortion).

These genetic and physiological observations can be reconciled in a scenario in which the interaction between *tasselseed2*, *silkless1*, and GAs regulate the sexual fate of floral meristems (Fig. 6). According to this model, *Sk1* would promote pistil development in the primary ear florets by blocking *Ts2* action, whereas *Ts2* acts to abort pistils in tassel florets and in secondary florets of the ear by suppressing *Sk1*. Consistent with this model is the localization of Ts2 expression to subepidermal cells in developing gynoecia in tassel florets (*35*) and the observed ear and tassel phenotypes in the *ts2 sk1* double mutant (*31*, *37*). The model also predicts that the *sk1* mutation would permit ectopic Ts2 expression in primary ear florets.

The antagonistic role of pistils on stamen development in *ts2* mutant tassels could be accounted for if pistils were emitters of a feminizing factor, such as GAs, that suppressed stamens. This proposed antagonism predicts that the *tasselseed2*, *dwarf* double mutants should contain bisexual florets due to lack of *Ts2* (gynoecial abortion) and *D* action (GA production) in mutant pistils, the observed phenotype of the *ts2 d1* double mutant (Fig. 4) (*31*). Lastly, the failure of stamen development in mutant *sk1* ears, which lack pistils, might be due to suppression of stamens from high concentrations of endogenous GAs found in this region of the plant, as opposed to upper regions in the tassel. In vivo measurements of GA-like substances show relatively high concentrations in the ear but only trace amounts in maturing tassels (*26*). Additional tests of this model are the predicted phenotypes associated with *sk1 d* double and *ts2 sk1 d* triple mutations, which await analysis.

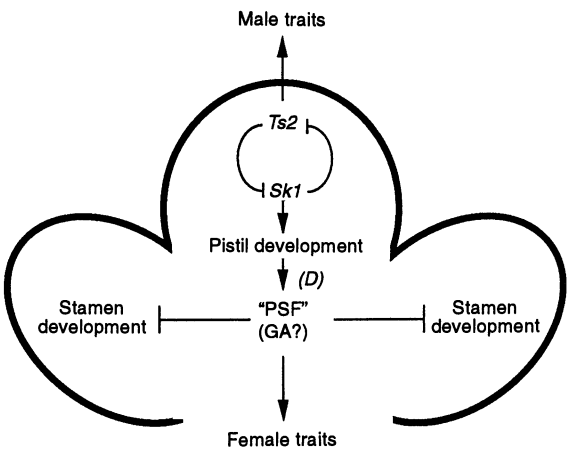

Fig. 6. Model for the sex determination process in maize. Diagram of an immature floral meristem showing a central gynoecium and two of the three stamen primordia. Proposed action of sex determination genes in promoting floral organ arrest or development is outlined. In brief, *Sk1* promotes development of pistils, which produce a "pistil-specific factor" (PSF), possibly a GA-like substance, that inhibits stamens and feminizes floral tissues (pistillate florets). *Sk1* may also prevent *Ts2* action (gynoecial abortion) in primary ear florets. Conversely, when *Ts2* is functional, *Sk1* is suppressed, blocking pistil development and preventing PSF production and stamen arrest (staminate florets). *Ts2* is shown to also promote stamen development and masculinization of floral tissues, perhaps mediated through the action of a diffusible morphogen.

Conclusions

Plants offer excellent opportunities to study the evolution and mechanism of sex determination. Unisexuality has evolved independently in many different species of plants, providing an opportunity to study a variety of genetic, molecular, and cellular processes. Remarkable similarities exist between the sex determination processes in both plants and animals. The sex determination process in maize shows that plants, like animals, are basically bisexual with the potential to develop toward either sex. As in animals, sexual fate in plants is determined at a critical developmental stage and controlled by genetic factors, programmed cell death, and steroid-like hormones. Nevertheless, the actual details in the regulation and mechanism of unisexuality are likely to be quite unique to each organism.

Furthermore, understanding sex determination in plants is valuable for agricultural purposes. Unisexuality in maize is an important agricultural trait that facilitates the large-scale production of hybrid offspring. Hybrid maize seed now represents the vast majority of acreage planted in maize worldwide, and hybrid vigor (heterosis) is responsible for tremendous increases in grain yield. Now that synthetic sex determination systems have been developed that allow unisexual flowers to be engineered in hermaphrodites (*38*, *39*), unisexual flowers will continue to have profound effects on crop yields and agricultural practices.

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Signaling the Arrest of Pollen Tube Development in Self-Incompatible Plants

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Self-incompatibility (SI), the cellular recognition system that limits inbreeding, has served as a paradigm for the study of cell-to-cell communication in plants since the phenomenon was first described by Darwin. Recent studies indicate that SI is achieved by diverse molecular mechanisms in different plant species. In the mustard family, the mechanism of SI shows parallels to the signaling systems found in animals that are mediated by cell-surface receptors with signal-transducing protein kinase activity.

The ability to distinguish between self and nonself and to allow only legitimate cellular unions during fertilization is an essential component of sexual reproduction in plants as in other organisms. However, in flowering plants, fertilization is preceded by a series of cell-cell interactive events dictated by the anatomy of the reproductive structures (Fig. 1A). Pollen grains are released from the anther in a desiccated state. They will hydrate and germinate to produce a pollen tube when delivered to the appropriate cell surface, which is generally that of the specialized epidermal cells of the stigma, a structure located at the distal end of the pistil. In species with solid pistils, the pollen tube, which extends by tip growth, must then invade the pistil and grow in the intercellular matrix of the central transmitting tissue of the stigma, style, and ovary before gaining access to the ovule (Fig. 1B) and delivering its cargo of sperm cells that will effect the double fertilization events unique to plants (*1*).

The pistil acts as an efficient screen of pollen grains, promoting the germination and growth of "appropriate" pollen tubes but not of "inappropriate" pollen tubes. Thus, gametes are preserved by interspecific pollination barriers that prevent the activation or growth of foreign pollen that is indiscriminately disseminated by wind, insect, or animal pollinators. Because the proximity of pistil and anther in plants with perfect flowers renders self-pollination more likely than cross-pollination, intraspecific barriers to pollination have also evolved, presumably as a means to avoid the deleterious effects of inbreeding and to promote outbreeding. One class of such barriers, namely genetic self-incompatibility (SI), is a prezygotic barrier that is widely distributed in both dicots and monocots. In self-incompatible plants, highly polymorphic loci control the ability of the pistil to inhibit the germination or subsequent development of self-related, but not genetically unrelated, pollen. SI systems can vary greatly between plant families with respect to site of pollen inhibition (in stigma, style, or ovary) and mode of genetic control (by one or more loci; sporophytic or gametophytic control of pollen phenotype) (*2*).

The specificity of self-incompatible pollen-pistil interactions has provided plant biologists with the opportunity to investigate the basis of plant cell-cell communication. In this article, we present an overview of our current understanding of the molecular basis and diversity of genetic SI systems, with a particular emphasis on the SI system of the crucifer (mustard) family. A consideration of the underlying molecular mechanisms is not only critical to the understanding of plant reproduction, but is also likely to shed light on the ways in which a plant cell interacts with neighboring plant cells during differentiation and morphogenesis, as well as with pathogens and symbionts.

Pollination Responses and Self-Incompatibility in Crucifers

The sequence of postpollination events that lead to successful fertilization is conserved in all cruciferous plants (*3*, *4*), whether they are members of self-compatible genera in which no SI has been reported, such as *Arabidopsis*, or of largely self-incompatible genera, such as *Brassica*. In *Arabidopsis*, the four phases of pollen tube growth proceed at an astoundingly rapid rate, and the pollen tube reaches the ovary 2 hours after the pollen grain is delivered to the stigma surface (Fig. 1B). Particularly rapid are the very early events that take place at the pollen–papillar cell interface, including the establishment of a zone of adhesion between the pollen grain and the "dry" surface of a papillar cell (Fig. 2A), hydration of the pollen grain, establishment of polarity within the pollen grain in preparation for germination (Fig. 2B), tip growth and elaboration of the pollen tube, and ingress of the pollen tube into the papillar cell wall, presumably through the action of hydrolytic enzymes located at the pollen tube tip.

It is during this one-on-one interaction between a pollen grain and papillar cell that the SI reaction is manifested: A pollen tube is either not formed or is unable to invade the papillar cell wall. The SI reaction is highly localized to the site of contact, because in mixed pollinations of a papillar cell with incompatible and compatible pollen grains, the latter are not arrested (*5*).

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