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Sex Determination in Flowering Plants

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INTRODUCTION

In most species of flowering plants, cross-pollination (allogamy) is a common breeding mechanism. Outcrossing avoids the deleterious effects of inbreeding depression and promotes heterozygosity, genetic variability, and genetic exchange, the consequences of which are advantageous to the long-term survival and adaptation of a species. Plants have evolved various mechanisms to promote allogamy, including the production of unisexual staminate or pistillate flowers on the same (monoecious) or different (dioecious) plants. A basic description of the various modes of sexuality in plants can be found in Table 1.

Although high rates of outcrossing may confer selective advantage, the incidence of dioecy and monoecy among flowering plants is low. An extensive catalog of sexuality in 120,000 plant species indicates that hermaphrodites are common, representing ~72% of species examined (Yampolsky and Yampolsky, 1922). Approximately one-tenth of all angiosperms are strictly dioecious or monoecious (4 and 7%, respectively). Intermediate forms of sexual dimorphism, including gynodioecy and androdioecy, represent 7% of the species examined, whereas 10% of the species contain both unisexual and bisexual flowers. Various types of sexuality are almost equally distributed among monocot and dicot genera, although dioecy is also more prevalent in tropical than in temperate ecosystems (Ashton, 1969; Tomlinson, 1974).

The extent of outcrossing can vary considerably among species and even within populations. Many factors affect the degree of outcrossing, including the spatial distribution of sexes and their temporal rate of maturation within the flower; population density; pollen characteristics; insect vectors; and environmental factors. The highest outcrossing rates are found among dioecious plants, in which outcrossing is obligatory. Lower rates are found among monoecious plants, in which the spatial separation of sexes within the same individual may favor cross-pollination but does not guarantee it. In dioecious species, sex ratios are usually close to unity but sometimes show significant departures (reviewed by Westergaard, 1958; Lloyd, 1974a). These departures can be biased toward either the staminate or pistillate form (Opler and Bawa, 1978). Sex biases can be due to genetic or environmental factors, such as differential mortality.

Hermaphrodite flowers have evolved many mechanisms to promote allogamy. Many hermaphrodites modify floral development to promote cross-pollination through mechanisms such as dichogamy, heterostyly, incompatibility (see Nasrallah and Nasrallah, 1993, this issue; Newbigin et al., 1993, this issue), and sterility, all of which are described in Table 2. For instance, protogyny, common in the Cruciferae, and protandry, common in the Compositae, result in the asynchronous maturation of female or male sexual organs. Even in species with bisexual flowers that mainly self-pollinate, chasmogamy often results in some degree of outcrossing. Adaptations of flowers to insect (entomophily), wind (anemophily), or water (hydrophily) pollination also promote outcrossing. For insect pollination to be effective, however, plants must produce guides and attractants such as showy petals (see Martin and Gerats, 1993, this issue) and nectaries.

Despite the numerous floral adaptations to promote allogamy, many plant species breed almost exclusively by self-pollination (autogamy). Self-pollination may be advantageous under certain circumstances because the energy cost of separating sexes and its cost in overall reproductive success may be high, especially under conditions that favor rapid reproduction, as is seen in annual weed species. This may explain why some degree of bisexuality is widespread among flowering plants.

BISEXUAL FLOWER

The basic hermaphrodite flower can be subdivided into four whorls, as diagrammed in Figure 1. Whorl 1 contains sepals and whorl 2 contains petals. Collectively, these whorls form the sterile perianth of the flower. In bisexual flowers, sex organs are formed in whorls 3 and 4. These contain the fertile sex organs, stamens (whorl 3), referred to collectively as the androecium, and pistil or carpels (whorl 4), referred to as the gynoecium (see Goldberg et al., 1993, this issue, for a review of stamen development and Gasser and Robinson-Beers, 1993, this issue, for a review of pistil development). Genetic and molecular studies on floral development in Arabidopsis and Antirrhinum have shown that organ position and identity are controlled by the combinatorial action of homeotic genes in three overlapping regions of the floral primordium (referred to as regions A, B, and C; reviewed by Coen and Meyerowitz,

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Table 1. Modes of Sexuality in Flowers, Plants, and Populations

Sexuality	Phenotype	Description
Individual flowers		
Hermaphrodite (bisexual, monoclinous)	☿	Bisexual flower with both stamens and pistil
Diclinous (unisexual)	♀ or ♂	Unisexual flowers
pistillate (carpillate)	♀	Unisexual flower with pistil only (female flowers)
staminate	♂	Unisexual flower with stamens only (male flowers)
Individual plants		
Hermaphrodite	☿	Only hermaphrodite flowers
Monoecious	♀♂	Both pistillate and staminate flowers on the same plant
Dioecious	♀ or ♂	Staminate and pistillate flowers borne on different plants
Gynoecious	♀	Plant bears only pistillate flowers
Androecious	♂	Plant bears only staminate flowers
Gynomonoecious	☿♀	Plant bears both hermaphrodite and pistillate flowers
Andromonoecious	☿♂	Plant bears both hermaphrodite and staminate flowers
Trimonoecious (polygamous)	☿♀♂	Hermaphrodite, pistillate, and staminate flowers on the same plant
Plant populations		
Hermaphrodite	☿	Only hermaphrodites
Monoecious	♀♂	Only monoecious plants
Dioecious	♀ and ♂	Only dioecious plants
Gynodioecious	☿ and ♀	Both hermaphrodite and gynoecious individuals
Androdioecious	☿ and ♂	Both hermaphrodite and androecious individuals
Trioecious (subdioecious)	☿ and ♀ and ♂	Hermaphrodite, pistillate, and staminate individuals

1991; see Coen and Carpenter, 1993, this issue). Sex organogenesis takes place in whorls 3 and 4 by the action of homeotic genes in regions B and C. In whorl 3, the B and C functions are required for stamen determination. C function alone is required in whorl 4 for carpels to form. Hence, the essential difference between stamen and carpel determination resides in the differential action of homeotic genes in regions B and C of the flower.

The widespread view that all flowering plants arose from a common hermaphrodite ancestor (Cronquist, 1988) suggests that much of the floral developmental program is common to all species. The conservation of this basic program in the taxonomically distinct species Arabidopsis and Antirrhinum tends to support this notion (Coen, 1991). It is also reasonable to speculate that the great diversity in floral form and structure and certain modes of sexuality are modifications superimposed on this basic developmental pathway.

REGULATION OF UNISEXUALITY

Could the production of unisexual flowers be controlled by selectively activating or inactivating homeotic gene function?

Table 2. Floral Modifications in Hermaphrodites[a]

Mechanism	Description
Mechanisms that facilitate self-pollination	
Cleistogamy	Production of closed flowers that self-pollinate
Homogamy	Synchronous maturation of stamens and stigma
Mechanisms that facilitate cross-pollination	
Chasmogamy	Open flowers capable of open pollination
Dichogamy	Differential rate of stamen and stigma maturation
protogyny	stigma receptive prior to anthesis
protandry	anthesis precedes stigma receptivity
Incompatibility	Failure of sexual crosses between genetically similiar individuals
Sterility	Production of nonfunctional sex organs or gametes
Heterostyly	Modification of floral parts

[a] Many of these modifications can be found in diclinous plants as well.

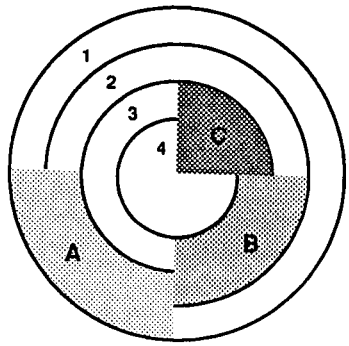

Figure 1. Schematic Diagram of the Four Floral Whorls (1 to 4) and Three Regions (A to C) of Homeotic Gene Action.

Stamen determination occurs in whorl 3 by the action of class *b* and *c* genes; carpels form in whorl 4 by the action of class *c* homeotic genes. Adapted from Coen and Meyerowitz (1991).

The available data, based on mutational analysis of the bisexual flowers of Arabidopsis and Antirrhinum, do not seem to support this idea. Basically, homeotic genes control organ formation in two or more whorls. Phenotypes conditioned by mutant alleles of these genes often result in homeotic transformation of the floral organs of two adjacent whorls into different structures. For instance, mutations in homeotic genes acting in region B cause the transformation of petals into sepals and stamens into carpels (Coen and Meyerowitz, 1991). These patterns are atypical of unisexual flowers found in natural plant populations, in which a single whorl (i.e., stamens or carpels) is usually affected. It is possible that sex determination genes might selectively affect the action of homeotic genes in one whorl, such that stamen development is altered, for example, without secondary effects on carpel formation. Moreover, there are examples of homeotic genes acting in a single whorl: the Arabidopsis homeotic mutation, *flo10*, also known as *superman*, replaces stamens with carpels (Schultz et al., 1991); the *heptandra* mutants of *Digitalis* selectively affect whorl 2, replacing petals with stamens (cited in Coen, 1991); and certain petunia mutants, such as *green petal* and *ph3*, also show defects in just one whorl (van der Krol and Chua, 1993, this issue).

To our knowledge, however, the attainment of unisexuality in flowers by means of homeotic transformation has not been reported as a mechanism of sex determination in natural populations. Unisexuality in plants is usually caused by the reduction or abortion of sex organ primordia; given the available data, a more plausible explanation is that sex determination genes act downstream or independently of homeotic functions. Consistent with this model are detailed morphological studies of several unisexual plants, which have shown that unisexual flowers often pass through a "bisexual stage" in which all floral organs are initiated. Only in *Mercurialis* (mercury) and *Cannabis* (hemp) do the floral primordia lack any vestiges of inappropriate sex organs (see below). The formation of unisexual flowers from this bisexual meristem requires the action of sex determination genes. These genes have been identified in maize by the analysis of mutants that misregulate the normal program of unisexuality.

DEVELOPMENTAL STEPS AFFECTED BY SEX DETERMINATION PATHWAYS

Maize provides an excellent genetic system to study sex determination in a monoecious plant. The unisexual flowers (termed florets) are borne on separate inflorescences. The terminal inflorescence, or tassel, contains only staminate florets, and the axillary inflorescence, the ear, contains only pistillate florets. Several papers are available that describe the development of the maize tassel and ear inflorescences (Bonnett, 1940; Cheng et al., 1983), and Figure 2 illustrates this process (see also Veit et al., 1993, this issue). Briefly, the flowering program begins by induction of the vegetative meristem to form an inflorescence meristem. Initials arise on the inflorescence meristem in an acropetal fashion (Figure 2A). In the tassel, the lowermost initials develop into branch primordia. The remaining initials branch to form two spikelet primordia (Figure 2B). Each spikelet primordium forms two floral bract, or glume, initials, followed by a second bifurcation to form two floral primordia (Figure 2C). Each floral primordium initiates in a sequential fashion a lemma, a palea, two lodicules, three stamens, and a central gynoecium (Figure 2D). Up to this stage, development of the ear and tassel inflorescences is nearly identical, except that in the unbranched ear inflorescence, the lowermost initials form spikelet primordia rather than branch initials.

Sex determination in maize takes place subsequent to this common "bisexual" stage (Figures 2D to 2F). In most maize lines, the stamen initials and the secondary floral primordium of each ear spikelet abort; the gynoecium continues to develop to sexual maturity (Figure 2F). In the tassel, both florets of the spikelet remain functional. The preformed gynoecial initials abort, while the stamens continue to develop to sexual maturity (Figure 2E). Gynoecial cells enlarge and become vacuolated prior to their disintegration (Cheng et al., 1983). Secondary sexual characteristics also become apparent during this period. The ear glumes remain short, thin, and translucent, and the paired spikelets remain sessile. The tassel spikelets develop long glumes, and one pedicel of each spikelet pair remains sessile while the other elongates. In summary, the process of sex determination in maize involves the programmed cell death of preformed sex organs and modifications of secondary sexual characters in the inflorescence.

Several other plant species follow a sex determination pathway that also involves the arrest of preformed sexual organs in bisexual primordia, as shown in Figure 3. In wild species of cucumber (*Cucumis sativum*), clusters of staminate flowers and solitary female flowers form on the same plant. All

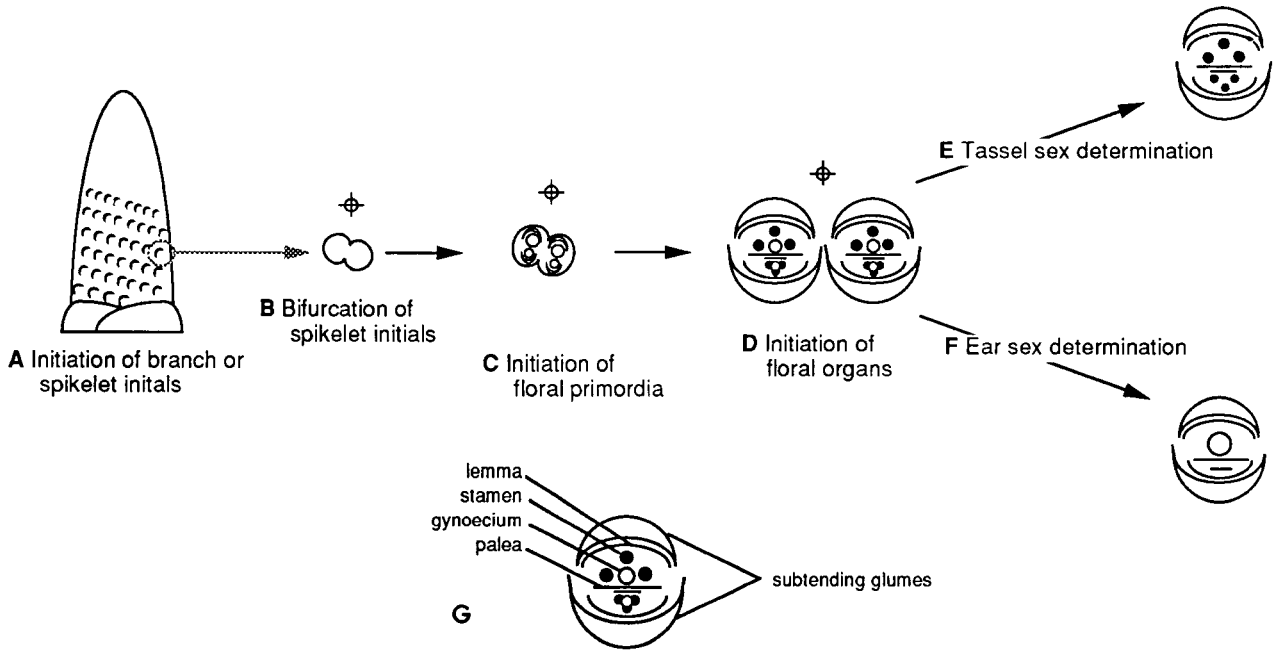

Figure 2. Summary of the Sexual Development of Male and Female Florets in the Inflorescences of Maize.

(A) Initiation of branch meristems or spikelet intials on the inflorescence meristem.
(B) Spikelet initials bifurcate to form two spikelets. The main axis of the inflorescence is indicated by crosshairs.
(C) Two subtending glumes are formed on each spikelet. The central region bifurcates a second time to form two floral primordia.
(D) Each floral primordium initiates an outer lemma and an inner palea, three stamen initials (closed circles), and a central gynoecium (open circle) composed of three fused carpels (lodicules not shown). Up to this point, floral development in both ear and tassel inflorescences is nearly identical. The action of sex determination genes causes selective abortion of preformed floral organs.
(E) In the tassel, staminate florets form by aborting gynoecial cells and allowing stamen initials to develop to sexual maturity. Gynoecial abortion and stamen development require the action of the *Ts1* and *Ts2* genes.
(F) In the ear, pistillate florets are formed by the selective abortion of stamen initials in the primary floret and abortion of the entire secondary floret. Each spikelet contains a single functional ovule. Genetic blocks in GA metabolism cause failure of stamen initials to abort.
(G) Schematic drawing of floret components. From DeLong et al. (1993).

immature floral buds contain stamen and pistil primordia, and sex differences are established by the arrested development of the inappropriate sex organs (Atsmon and Galun, 1960; reviewed by Malepszy and Niemirowicz-Szczytt, 1991). In dioecious *Silene* (campion; also referred to as *Melandrium*) species, both stamen and carpel primordia are present in both sexes, with the developmental arrest of the inappropriate sex occurring at early stages of floral development (reviewed by Ye et al., 1991). The stage of arrest is later than that of maize, when organ primordia are well defined but prior to their full maturation and meiosis.

The critical stage for sex determination in dioecious *Asparagus officinalis* (garden asparagus) occurs much later in floral development. Flower buds from females and males are phenotypically indistinguishable until the onset of meiosis (Lazarte and Palser, 1979; Bracale et al., 1991). At this time, pollen formation is arrested in female flowers and embryo sac formation is arrested in male flowers, so that the mature flowers are functionally unisexual. It appears that the defect in stamen maturation in pistillate flowers is the precocious degeneration of the tapetal cells and the collapse of the microspore mother cells; in staminate flowers, degeneration begins in nucellar and integumentary cells and progresses to the megaspore mother cell (Lazarte and Palser, 1979). There is some variation in the timing of megaspore degeneration that may be genotype dependent.

In some species, unisexual flowers show no evidence of the missing sex, and male and female flowers may differ radically in general morphology and size. In *Cannabis sativa*, female flowers result from the direct "pass-over" from perianth initials to carpel initials; these flowers never form any vestiges of stamen initials (Mohan Ram and Nath, 1964). The genus *Mercurialis* contains both dioecious and monoecious species, with unisexual flowers devoid of rudiments of organs of the opposite sex (Durand and Durand, 1991). Yet under certain conditions, sexuality can be reversed by hormone treatment, and in some cases, both stamens and carpels can form in the same flower (Heslop-Harrison, 1957). The occurrence of hermaphroditism and sex reversal indicates that mercury floral primordia are sexually bipotent.

GENETIC REGULATION OF MONOECY

In monoecious plants, the process of sex determination is developmentally regulated by sex determination genes. The recessive *tasselseed* (*ts*) mutations of maize provide a working model to explain the action of sex determination genes. Mutations in the *ts1* and *ts2* genes of maize perturb the normal process of sex determination, resulting in a transformation of tassel florets from staminate to pistillate. The transformation from a staminate to pistillate inflorescence in *ts1* and *ts2* mutants is not a homeotic transformation, however. Instead, *ts1* and *ts2* mutations reverse the normal program of organ abortion in the tassel. In wild-type tassels, the preformed gynoecium aborts early during floral development, whereas stamen initials develop to sexual maturity (Figure 2E). In *ts2* mutant tassels, stamen initials abort, whereas the gynoecium develops to sexual maturity. The same mutant phenotype is found in *ts1* plants. This reversal in the normal sex determination program results in the formation of a terminal pistillate inflorescence. These mutations have little effect on the vegetative development of the plant but rather affect the sexual characters of the plant specifically. Interestingly, the *ts1* and *ts2* mutations cause mutant plants to become gynoecious. In a population, segregation of *Ts* and *ts* alleles will result in a gynodioecious population. Unisexual maize plants (dioecious maize) can be derived from this gynodioecious condition by the addition of mutations, such as *silkless* (*sk*; Jones, 1932), that suppress function of the lateral pistillate inflorescence.

Secondary sexual characteristics of the inflorescence are also affected by *ts1* and *ts2* mutations. In wild-type plants, the glumes or floral bracts completely enclose the staminate florets and eventually become photosynthetic and covered with trichomes. In the ear, spikelet pedicules are sessile and glumes remain short, thin, and translucent, without encasing the pistillate floret. In *ts2* mutant tassels, pedicules are sessile and glumes are short, thin, and translucent—characteristics of the pistillate inflorescence of the ear. Thus, *ts2* mutations tend to feminize the tassel inflorescence, although other sexual features of the terminal inflorescence are unaffected. The *ts2* mutant tassel retains the branching characteristics of the wild-type tassel; the inflorescence remains thin, with the development of both florets. Branching characteristics appear to be regulated by a different genetic pathway, which is defined by mutations of the *ramosa* type (reviewed by Irish and Nelson, 1989). The *ts2* mutation also has an effect on the development of the ear inflorescence. In most inbred lines of maize, the secondary florets of each spikelet abort, leaving a single fertile floret in each spikelet for fertilization (Figure 2F). In *ts2* mutant ears, this secondary floret often develops to maturity, resulting in double kernels in each spikelet after fertilization. These additional kernels cause crowding and irregular rowing on the mature ear.

Ts5, a dominant mutation, also affects the sex determination process (Irish and Nelson, 1989). *Ts5* mutant tassels show a base-to-tip developmental gradient of pistillate to staminate florets. Because this locus is defined by a single dominant allele, it is difficult to interpret the significance of this phenotype. Yet, because *Ts5* specifically affects the selective abortion of the gynoecium, it may define an important step in the pathway

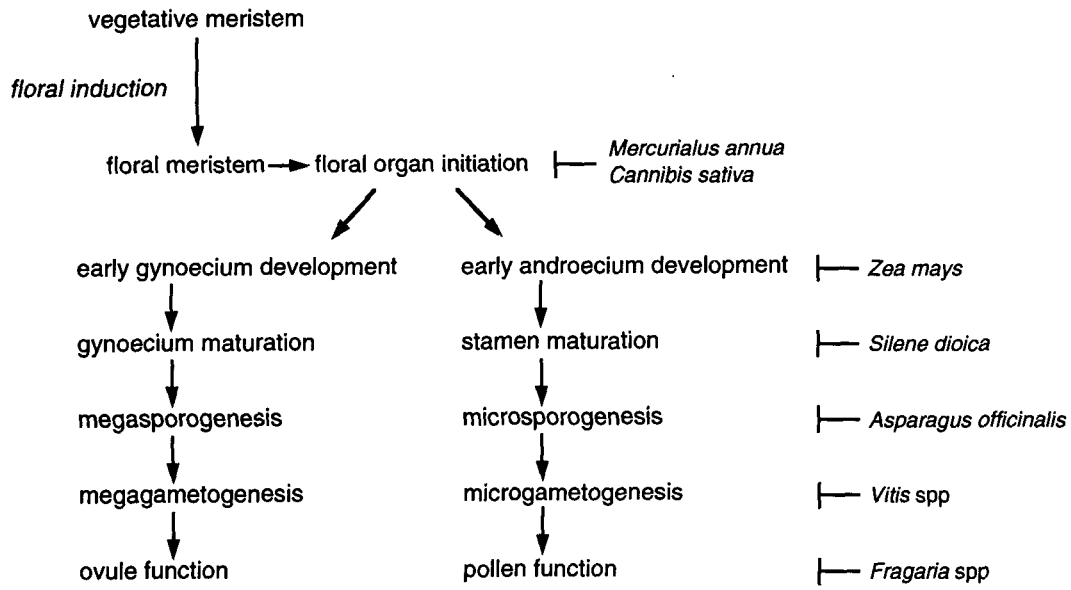

Figure 3. Developmental Steps in Floral Development Blocked during the Sex Determination Process for Representative Monoecious and Dioecious Plants.

of sex determination. The dominant nature of the mutation suggests a gain of function in a process such as signal reception or transduction.

No phenotype opposite to that of *ts1* and *ts2* (i.e., completely staminate ear) has been described. Mutations affecting the gibberellic acid (GA) biosynthesis pathway (*dwarf* mutations) result in shortened internodes in the plant, and many of these mutations also perturb the normal process of stamen abortion in the ear without affecting the gynoecia. Therefore, stamens develop in the ear, resulting in hermaphrodite flowers (Emerson and Emerson, 1922). The tassels are normal and staminate. This suggests that GA may be involved in the process of stamen abortion, although the effects may be indirect because these mutations have pleiotropic effects on vegetative growth. Double mutant studies with *ts2* and *dwarf* mutations show that these mutations have additive effects, producing hermaphrodite florets in the ear and pistillate florets in the tassel (T. Nelson and E. Irish, personal communication). This would suggest that *Ts2* and GA act independently of one another.

Many additional maize mutations affect floral development, but do not specifically alter the sexual fate of the floral primordia in the way that *ts1* and *ts2* mutations do (Irish and Nelson, 1989; see Veit et al., 1993, this issue). Other *ts* mutations, *ts4* and *Ts6*, cause the irregular proliferation of floral tissue, aberrant floral organ initiation, and the development of inappropriate sex organs in both tassel and ear. Because these mutations affect floral processes other than abortion of the inappropriate sex organs, their roles in sex determination may be indirect. In experiments in which double mutants between *ts1* or *ts2* and *ts4* or *Ts6* have been examined, additive effects are mainly observed: tassels are completely feminized (*ts1* or *ts2* effects), whereas floral and vegetative structures show the aberrant phenotypes associated with the *ts4* or *Ts6* mutations (T. Nelson and E. Irish, personal communication).

Cases of clear epistasis between *ts1* or *ts2* and other floral mutations are rare. Mutations in the *sk* gene suppress pistil development in the ear, but tassels are normal. Double mutant plants (*ts2 sk*) have double florets with pistils in the ear (Jones, 1932; T. Nelson and E. Irish, unpublished data), suggesting that *ts2* is epistatic to *sk*. This interaction suggests that the wild-type *Sk* product may act to suppress the action of *Ts2* in the ear. The tassel inflorescence of the double mutant often contains both staminate and pistillate florets, which suggests that *sk* mutation can partially correct the *ts2* phenotype in the tassel.

It is not yet possible to distinguish whether *Ts1* and *Ts2* act in an independent pathway of sex determination or whether they act downstream of other genes involved in floral determination. What is clear is that the sex determination roles of *Ts1* and *Ts2* are late-acting functions, consistent with their role in the selective abortion of the gynoecium after floral organ determination steps are complete. The sex-reversal phenotype of the mutants suggests that the wild-type functions of the *Ts1* and *Ts2* genes are required for both gynoecial abortion and stamen development. The evidence suggests that stamen abortion in maize directly or indirectly requires the action of GA. The *Ts*- and GA-controlled pathways may act independently or coordinately in sex determination.

The molecular cloning of the *Ts2* gene has provided clues to its function in the sex determination process (DeLong et al., 1993). In the inflorescence meristem, *Ts2* mRNA levels are highest in subepidermal cells of the gynoecia just prior to their degeneration; much lower levels of expression are seen in other floral whorls. Sequence analysis indicates that *Ts2* encodes a short-chain alcohol dehydrogenase, which shows greatest similarity to steroid dehydrogenases. These results suggest the intriguing possibility that the *Ts2* product is directly responsible for modifying a feminizing substance, such as a plant hormone, that then allows for male sexual development.

GENETICS OF SEXUALITY IN DIOECIOUS PLANTS

Active-Y System of Sex Determination

Heteromorphic sex chromosomes are rarely found in angiosperms but have been reported in a number of plant species including *Rumex*, *Cannabis*, *Humulus*, and *Silene* (Parker, 1990). In dioecious *Silene*, males are the heterogametic sex (XY) and females are homogametic (XX) (Westergaard, 1948). As is the case in mammals, *Silene* has an active-Y system of sex determination, with dominant male factors and female suppressing factors mapping to the Y chromosome (van Nigtevecht, 1966). The X chromosome appears to be essential in both males and females because only monoploid females can be obtained by in vitro techniques (Ye et al., 1991). Application of hormones, including GA, auxins, and cytokinins, does not result in sex conversion. However, the presence of a single Y chromosome can suppress female development when three X chromosomes are present. Higher X copy number overcomes the Y chromosome masculinization effect (Westergaard, 1958). Autosome ratios have no profound effects on the sex-determining factors present on the Y chromosome. This suggests that the Y chromosome is decisive in determining sex in *Silene*. Three different regions of the Y chromosome have been identified as having separate functions in sex determination (Westergaard, 1946). One end contains a genetic factor (or factors) that suppresses formation of the gynoecium, the opposite end contains a male fertility factor (or factors), and the middle region includes a gene or genes needed for anther initiation. Therefore, the Y chromosome of *Silene* contains complete linkage between female-suppressor and essential male sex genes.

Asparagus is generally a dioecious plant, with sex determined by homomorphic sex chromosomes in which the males (XY) are the heterogametic sex (reviewed by Bracale et al., 1991). Genetic evidence suggests that asparagus is "male dominant" and contains *male-activator–female-suppressor* genetic determinants (Marks, 1973) similar to those postulated for *Silene* (Westergaard, 1958). In addition to these major sex determination genes, genetic modifiers can influence the stage

of stylar degeneracy (Peirce and Currence, 1962; Franken, 1970; Bracale et al., 1991). In the dioecious populations, male plants with a few perfect flowers are occasionally found (Rick and Hanna, 1943; Franken, 1970; Lazarte and Palser, 1979). These flowers can self-pollinate and produce homogametic males (YY). Because males are the desired sex in commercial applications, due to their increased vigor, selected YY male and XX female plants are used as parents for producing all-male F_1 hybrid seed. In summary, the absence of heteromorphic chromosomes and the viability of the YY genotype suggest that dioecy in asparagus may have been derived relatively recently.

X-to-Autosome Balance System of Sex Determination

Approximately 10 dioecious species exist in the genus *Rumex*, subgenus *Acetosa*, in which, in contrast to *Silene*, the X-to-autosome ratio appears to control sex determination (Ono, 1935, as cited in Parker, 1990; Parker and Clark, 1991). Females are XX and males XY_1Y_2 (2n = 14 and 2n = 15, respectively); however, diploid plants with XXY and XXY_1Y_2 genotypes are fertile females. The Y chromosomes are late replicating and heterochromatic. In polyploids, an X-to-autosome ratio of 1.0 or higher is female; X-to-autosome ratios of 0.5 or lower are males. Intersexes (partial male/female) or hermaphrodites result from ratios of between 0.5 and 1.0. Sex is determined by X-to-autosome ratios even in plants that are trisomic for single autosomes (Yamamoto, 1938, as cited in Parker and Clark, 1991).

The Y chromosomes in *Rumex* are required for pollen fertility but do not seem to be required for stamen development. Both Y_1 and Y_2 appear to be required for normal progression of microspore mother cells through meiosis. In contrast to *Silene*, Y chromosomes of *Rumex* do not inhibit female gynoecium development. Thus, the situation in *Rumex* is remarkably similar to that in Drosophila and *Caenorhabditis elegans*, in which the primary determinant of sex is the X-to-autosome ratio (Hodgkin, 1990).

Two species of the genus *Humulus* (hops) are dioecious, with a sex determination system similar to that of *Rumex* (Winge, 1929; Jacobsen, 1957). The sex chromosomes of two species (*H. lupulus* and *H. japonicus*) are heteromorphic, and, as with *Rumex*, females (2n = 14 + XX) and males (XY_1Y_2) are determined by X-to-autosome ratios rather than by the presence or absence of the Y chromosome (reviewed by Parker and Clark, 1991). In cultivated hops, an XX female–XY male system is found, and multiple X systems ($X_1X_1X_2X_2$ females, $X_1Y_1X_2Y_2$ males) are found in Japanese varieties (*H. jupulus* cv *cordifolius*). However, the existence of an XX–XO sex determination system has not been demonstrated convincingly in plants (Westergaard, 1958).

An unusual case of sex determination is found in the genus *Fragaria*. This is one of the plant species in which sex chromosomes are heteromorphic and the heterogametic sex is female (Valleau, 1923; Staudt, 1952, 1955, as cited by Westergaard, 1958). *Fragaria* species form a polyploid series with 2n = 14, 28, 42, and 56. All diploid species are hermaphrodites, and wild polyploid species are dioecious (Westergaard, 1958). Sex is determined late in floral development, after microspore or megaspore mother cell formation but prior to meiosis.

In summary, sex determination in plants can be controlled genetically by mechanisms also found in the animal kingdom. In some dioecious species, such as *Silene* and *Asparagus*, the sex determining mechanism resembles that of mammals in that the Y chromosome plays an active role in female suppression/male activation. In other dioecious species, such as *Rumex* and *Humulus*, the X-to-autosome ratio determines the sexual fate of floral primordia, similar to the situation found in Drosophila and *C. elegans*. It should be noted, however, that even though both Drosophila and *C. elegans* share overall genetic similarity in having an X-to-autosome determination of sexuality, the underlying molecular mechanisms that regulate sexual dimorphism are quite different (Hodgkin, 1990). Therefore, we can assume the mechanistic basis of sex determination in plants will also be species specific. The variations in underlying mechanism are reflected in the physiological control of sex determination in plants.

HORMONAL REGULATION OF SEXUALITY

Mercurialis annua is a dioecious species with homomorphic chromosomes and male heterogamety. Sex is determined by three independently segregating genes, *A1*, *B1*, and *B2* (Louis, 1989; Durand and Durand, 1991). Either an *A1* dominant gene together with recessive alleles of *b* genes or an *a1* recessive allele together with dominant *B* alleles induce femaleness. Male determination requires complementary gene action—the presence of a dominant *A1* allele together with one additional dominant *B* allele. The degree of "maleness" is determined by the *B1-B2* genotype. The dominant *B* genes influence the degree of "maleness" or sensitivity to feminizing cytokinin hormones. Together, *B1* and *B2* induce resistance to feminization by cytokinins, whereas *B1* or *B2* alone induces sensitivity to feminization (Louis, 1989; Durand and Durand, 1991). Exogenous auxin treatment has also been shown to transform females into males (Hamdi et al., 1987).

High levels of endogenous cytokinin, *trans*-zeatin, in mercury appear to be correlated with induction of floral primordia to carpels (Dauphin-Guerin et al., 1980). In males, the zeatin nucleotide, rather than the free base, accumulates (Dauphin-Guerin et al., 1980; Louis et al., 1990). The qualitative and quantitative variation of zeatin in males and females has been shown to be under genetic regulation by the sex determination genes. Feminization of pistillate flowers by cytokinin treatment on genetically male individuals has also been noted in several other dioecious plants, including species of *Vitis*, *Spinacia*, and *Cannabis* (Negri and Olmo, 1966; Chailakhyan and Khryanin, 1978; Galoch, 1978). The ability to reverse the sex determination mechanism by hormonal treatment suggests that the floral

primordia, even when lacking vestiges of the inappropriate sex, are still sexually bipotent and that sex determination genes regulate alternative programs of sexuality, possibly through a signal transduction mechanism that modifies endogenous levels of auxins and cytokinins.

In summary, sex reversal by hormone application indicates that in some plants, genes required for the development of the androecium or gynoecium are functional but suppressed. The action of particular hormones in feminizing or masculinizing flowers appears to be species dependent. The same hormone can have completely opposite effects in different plants. For example, GA feminizes maize but has the opposite effect on cucumber. Cytokinins cause male-to-female conversion in mercury but the opposite reaction in other species. In some dioecious species, such as *Silene*, hormone applications have little or no effect on the sexuality of flowers. This variation seen in plants may reflect different underlying mechanisms of sex determination.

EVOLUTION OF SEXUAL DIMORPHISMS IN PLANTS

Plants offer a unique opportunity to study the evolution of sex determination because plants with unisexual flowers have arisen recently and multiple times from bisexual ancestors. The process of evolving unisexual flowers has been postulated to require at least two independent events, as outlined in Figure 4 (Charlesworth, 1991). The first change is a mutation causing male sterility (gynoecy), resulting in a gynodioecious population. Alternatively, androdioecy could result from a female-sterile mutation, but this is a rare condition in plants (Lloyd, 1975). The first change is more likely to involve male sterility than female sterility because in partially selfing species, the lack of access of pollen to a proportion of ovules means that male function is genetically less valuable than female function; hence, a male-sterile mutant has less loss of fitness than a female-sterile mutant (Charlesworth and Charlesworth, 1978). Once females are established, increased male fertility and decreased female fertility in hermaphrodites might have a selective advantage by promoting further outcrossing. Decrease in fitness due to selfing and a redistribution of resource allocations may drive such changes (Charlesworth and Charlesworth, 1987). This second event may involve the loss of female fertility by a single mutation (female sterility) or by a gradual reduction in female fertility through a progression from gynodioecy, subdioecy (see Table 1) to full functional dioecy.

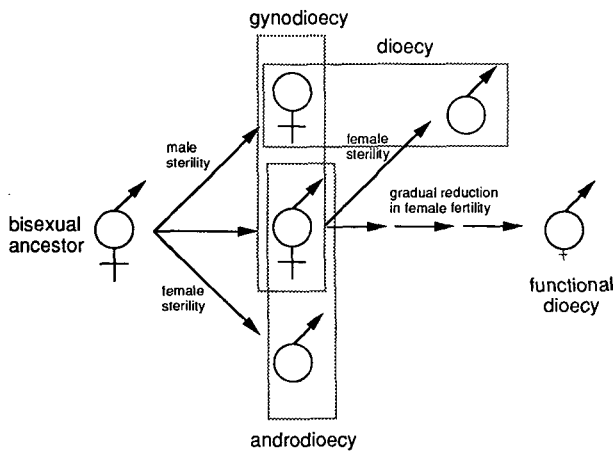

Figure 4. Major Pathways in the Evolution of Dioecy.

The first step is the establishment of females in the population by a male sterility mutation, forming a gynodioecious population. The pathway to androdioecy is rare in plants. A second process involving the loss of female fertility of the hermaphrodite leads to dioecy. Adapted, with permission, from Charlesworth (1991).

Comparative studies support the view that dioecy has evolved from an ancestral gynodioecious condition (Darwin, 1877; Lewis, 1942; Westergaard, 1958), although there are a few instances in which monoecy appears to be ancestral to dioecy (Cronquist, 1988). The gradual conversion of hermaphrodites into functional males is commonly seen in unstable gynodioecious populations (Darwin, 1877; Carlquist, 1966; Arroyo and Raven, 1975). Reduction in female fertility is often characteristic of gynodioecious species in which male sterility is caused by nuclear factors (Lewis, 1941; Ross, 1970; Lloyd, 1974b). This reduction in female fertility can approach or even reach dioecy when the male-fertile partner becomes essential female sterile, forming the basis of a functionally dioecious population. Nevertheless, stable forms of gynodioecy do exist, suggesting the existence of some mechanism that maintains female fertility in hermaphrodites. Stable gynodioecy often shows cytoplasmic inheritance of male sterility; this may prevent evolution toward dioecy because linkage cannot develop between nuclear female sterility and cytoplasmic male sterility (Ross, 1978).

Other factors may have contributed to the origins of plant sex determination pathways, such as the association of dioecy with homomorphic or heteromorphic sex chromosomes, the suppression of recombination over part or most of the X and Y chromosomes, male heterogamety, or Y-active or X-to-autosome determinants of sexuality (Charlesworth, 1991). A dioecious population would contain heterogametic males and homogametic females, the most common situation found in dioecious inheritance, when recessive male sterility and dominant female infertility factors are involved. With male heterogamety, the "Y" chromosome will have the dominant female-suppressor and the "X" will carry a recessive male-suppressor. This type of genetic control of sex determination is essentially what is found in *Silene* and several other species (Lewis, 1942; Westergaard, 1958). If the two mutations are loosely linked, the equilibrium populations would contain males, females, and hermaphrodites (Charlesworth and Charlesworth, 1978), a condition that is found in a number of plant species (Fryxell, 1957; Westergaard, 1958).

Relatively recently evolved systems of dioecy may represent intermediates in the process of Y chromosome evolution.

"Proto-sex" chromosomes may have evolved from homologous chromosomes carrying linked recessive genes for both male sterility and female fertility (proto-X) and linked dominant factors for male fertility and female suppression (proto-Y) (Charlesworth, 1991). Recombination between male and female determinants would need to be suppressed to stabilize the dioecious condition in the population. Such a situation would result in homomorphic sex chromosomes with heterogametic males (XY) and homogametic females (XX), as is the case for the sex determination system of *Asparagus* (Rick and Hanna, 1943). The recent evolutionary origin and overall similarity between the homomorphic sex chromosomes of *Asparagus* may explain the viability and male fertility of the YY genotype (Franken, 1970).

Heteromorphic sex chromosomes in plants may represent more advanced forms of dioecy through a degeneration process of the Y chromosome. Restriction of recombination between male and female fertility factors may have been the first step in establishing heteromorphic sex chromosomes (Muller, 1914, 1918). More extensive restrictions on recombination between sex chromosomes may have evolved by selective advantage if sex-linked alleles have opposite effects on fitness in the two sexes (Bull, 1983). This reduction of recombination between sex chromosomes would tend to cause deleterious mutations linked to sex determination factors to accumulate on the Y chromosome in the heterogametic sex because there would be no opportunity for such mutations to be eliminated by recombination. These mutations would build up in a finite population through the stochastic action of "Muller's ratchet" or genetic "hitchhiking," leading eventually to degeneracy of the Y chromosome (Charlesworth and Charlesworth, 1978). Recombination in the homogametic (XX) sex would tend to eliminate deleterious mutations from the X chromosome. *Silene*, with its well-defined heteromorphic sex chromosomes, may represent such a situation in plants. The existence of XX dihaploid lines of *Silene* but not YY dihaploids suggests that essential information has been lost from the Y chromosome in its evolution (Ye et al., 1991).

Despite their variety, all sex determination mechanisms in plants may have a common origin—selective pressures for increased genetic fitness. As with animal species, however, the underlying mechanistic basis may be quite different from plant to plant. Morphological and physiological studies suggest that developmental arrest of inappropriate sex organs can occur at any stage of sex organ development. This variability may reflect different molecular mechanisms operational to arrest further development of the inappropriate sexual organs. Therefore, an understanding of the evolution of mechanisms of unisexuality in plants will be complicated by its multiple histories and different regulatory circuits.

SUMMARY

In many ways, plants offer unique systems through which to study sex determination. Because the production of unisexual flowers has evolved independently in many plant species, different and novel mechanisms may be operational. Hence, there is probably not one unifying mechanism that explains sex determination in plants. Advances in our understanding of sex determination will come from the analysis of the genetics, molecular biology, and biochemistry of genes controlling sexual determination in plants. Several excellent model systems for bisexual floral development (Arabidopsis and Antirrhinum), monoecy (maize), and dioecy (*Silene*, asparagus, and mercury) are available for such analyses. The important questions that remain concern the mechanism of action of sex determination genes and their interrelationship, if any, with homeotic genes that determine the sexual identity of floral organ primordia. At the physiological level, the connection between hormone signaling and sexuality is not well understood, although significant correlations have been discovered. Finally, once the genes that regulate these processes are identified, cloned, and studied, new strategies for the manipulation of sexuality in plants should be forthcoming.

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