Sex Determination and Sexual Organ Differentiation in Flowering Plants

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ABSTRACT The research in the genetics of sex determination and the differentiation of reproductive organs in flowering plants has long been a topic in recent years. Understanding the genetic and molecular mechanisms that control sex determination in flowering plants relies on detailed studies of the differentiation of sexual organs. Current theories about sex chromosomes have illuminated the mechanisms of plant sex determination. In addition, recent progress in cloning floral homeotic genes which regulate the identity of the floral organs has generated molecular markers to compare the developmental programs of male, female and hermaphrodite flowers in several species. In this review, the authors focus attention on these recent findings and provide a brief overview of the genetics of plant sex determination and the mechanism of sex determination gene expression and gene programs.

KEY WORDS sex determination, sexual organ, organ differentiation, flowering plant

1 Introduction

In most polymorphic species that have been carefully examined, a common set of floral organs is initiated in all flowers, but further development of stamens or pistils is selective, resulting in unisexual flowers (Durand and Durand 1984). Sex determination is traditionally considered to be this selective abortion of the gynoecium or androecium of initially hermaphroditic floral primordia, but it should also include the differentiation of gametophyte within the pistil or stamen, which occurs in all angiosperm flowers. Most work on sex determination has focused on the differentiation of the pistil and the stamen, with the underlying assumption that their meiotic results are determined as female and male gametes (Durand and Durand 1990, Meng 2000). In plants, understanding the sex determination system is closely connected with the knowledge of how separate sexes evolved. Sex determination is the regulation of sexual differentiation. Sex determinants initiate stamen or carpel development as well as secondary sexual characters. It is thus of importance to analyze the underlying mechanisms that control the development of these specialized organs, i.e., to analyze staminogenesis and carpellogenesis (Lrish and Nelson 1989). Angiosperm species that produce unisexual flowers present the opportunity for floral differentiation and gametogenesis. A large number of literatures describe the genetic and physiological basis of sex determination in these species (Stephen and Calderon 1993, Sabine and Grant 1997, Lou *et al*. 2002). Recently, it has become feasible to pursue the molecular genetic basis of the male and female differentiation programs in certain plant species, as has been profitably undertaken in several animal species.

2 Genetic bases of reproductive organogenesis in flowering plants

2.1 Chromosomal and genic determination of reproductive organs in dioecious plants

2.1.1 Active-Y system of sex determination

One of the sexes, usually the male, is heterogametic, producing two types of gametes and bearing male or female factors. The other, the female, is homogametic and gives rise to only one type of gamete bearing the feminizing factors (Lou *et al*. 2002).

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, Charles *et al*. 1997). 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. The X chromosome appears to be essentia1 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

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sex determination (Westergaard 1946). One end contains a genetic factor (or factors) that suppresses the 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 officinalis is generally a dioecious plant, with sex determined by homomorphic sex chromosomes in which the males (XY) are the heterogametic sex (Bracale *et al.* 1991). Genetic evidence suggests that *asparagus officinalis* 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).

2.1.2 X-to-autosome balance system of sex determination

Approximately 10 dioecious species exist in the genus *Rumex* and subgenus *Acefosa*, in which, in contrast to *Silene*, the X-to-autosome ratio appears to control sex determination (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 (partia1 male/female) or hermaphrodites result in ratios between 0.5 and 1.0. Sex is determined by X-to-autosome ratios even in plants that are trisomic for single autosomes (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 Y1 and Y2 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 *Caenorhabdifis 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, similar to *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 (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).

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 latifolia* and *Asparagus officinalis*, the sex determining mechanism resembles that of mammals in that the Y chromosome plays an active role in female suppression or male activation. In other dioecious genera, 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 of 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.

2.2 Genic determination of reproductive organs in momoecious plants

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 disturb the normal process of sex determination, resulting in a transformation of tassel florets from staminate to pistillate. Such transformation in *ts1* and *ts2* mutants, however, is not a homeotic one. Instead, *ts1* and *ts2* mutations reverse the normal program of organ abortion in the tassel. 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* (Jones 1932), that suppresses function of the lateral pistillate inflorescence.

Secondary sexual characteristics of the inflorescence are also affected by *ts1* and *ts2* mutations. 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 famosa type (Lrish 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. 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.

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), 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.

3 Sex-related differentiation in the expression of homeotic genes

3.1 Bisexual flower

The basic hermaphrodite flower can be subdivided into four whorls, as diagrammed in Fig. 1. 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 (Goldberg *et al*. 1993, Gasser and Robinson-Beers 1993,). 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 regions A, B and C) (Coen and Meyerowitz 1991, Coen and Carpenter 1993, Harles and Gasser 1991). 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.

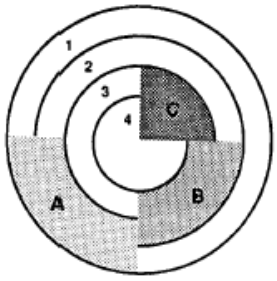

FIGURE 1 Schematic diagram of the four floral whorls (1 to 4) and three region (A to C) of homeotic gene action
Stamen determination occurs in whorl 3 by the action of class b and c genes. Adapted from Cone and Meyerowitz (1991)

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, Xu 1998) 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.

3.2 Regulation of unisexuality

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

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 inappreciate sex organ primordial (Shou 2000); 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 severa1 unisexual plants, which have shown that unisexual flowers often pass through a "bisexual stage" in which all floral organs are initiated. Only in *Mercurialis* and *Cannebis* do the floral primordia lack any vestiges of inappropriate sex organs. Floral primordial seems to avoid the hermaphrodite condition, suggesting that sex determinants act earlier than the appearance of floral primordial. 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.

3.3 Homeotic genes and sexual organ differentiation

A number of genes affected by the homeotic mutations have been cloned and characterized, including the snapdragon *deficiensA* gene and the *Arabidopsis agamous, apetala1,* and *apetala3* genes. They encode related proteins belonging to a class of transcription factors containing a conserved region known as the MADS domain. The MADS box is the region of these genes encoding the DNA binding MADS domain of these protein transcription factors (Anusak 2003). The MADS-box homeotic genes tend to be highly conserved among the angiosperms (Davies and Schwarz-Sommer 1994, Weigel and Megerowitz 1994). This makes it possible to identify genes in dioecious and monoecious species that potentially have a common evolutionary origin, and related function to the homeotic genes of *Arabidopsis*, simply by their sequence homology and tissue specificity.

Comparisons of mRNA expression patterns of MADS-box genes have been carried out in two dioecious dicotyledons (Hardenack *et al.* 1994, Ainsworth *et al.* 1995) and in the monoecious monocotyledon maize (Mena *et al.*1996, Schmidt *et al.* 1993).

One of these studies focused on white campion, in which both male and female floral meristems undergo a hermaphrodite phase early in their development (Hardenack *et al.* 1994). The development of the carpel primordium in male flowers is arrested immediately after their initiation, whereas the stamen primordia in female flowers are not aborted until tapetum initiation in the anthers (Grant *et al.* 1994). The MADS-box genes corresponding to the A-, B- and C-function genes were found to be expressed in developing male and female flowers, as they are in hermaphrodite flowers. However, the spatial expression pattern of the petal- and stamen-specific genes, *SLM2* (*Silene latifolia* 'MADS')(a *PI* homolog) and *SLM3* (an *AP3* homolog), differed between male and female flowers. In both sexes, the expression of these genes was confined to the regions that give rise to petal and stamen primordial. However, *SLM2* and *SLM3* transcripts were expressed in a more central region of the male flower meristem than in the equivalent female meristems. Their expression patterns indicated that the fourth whorl is smaller in male flowers than female flowers, and that the third whorl is the same size in both sexes. This was confirmed in morphological comparisons of early stages of male and female flower development (Grant *et al.* 1994). The gynoecium primordium that forms in a male flower is much smaller

than the corresponding primordium in a female flower. A hermaphrodite mutant flower resembles a female flower in the size of all the floral whorls. These results suggest that genes suppressing gynoecium development are responsible for limiting the growth and division of cells in the carpel whorl, such as *Cla1* (*Clavata1*) (Clark *et al*. 1993) and *Sup* (*Superman*) (Sakai *et al.* 1995), have been isolated from *Arabidopsis*. It remains to be determined whether homologous genes that lead to gender dimorphism are differentially regulated in male and female white campion.

A similar study was also carried out in sorrel (Ainsworth *et al.* 1995). The flowers of this species consist of two outer whorls of sepaloid perianth organs with either an inner whorl of three stamens in male flowers or a gynoecium in female flowers. There is no proliferation of cells in the center of male flowers (internal to the stamen priomordia) and stamen primordia are barely visible in developing female flowers. In each sex, one whorl appears tobe repressed extremely early in development; here, expression of a stamen- and carpel-specific MADS-box gene, *RAP1* (*Rumex acetosa* '*Plena*-like'), revealed differences between the sexes. The sequence of *RAP1* is homologous to *AG*, which specifies the identity of the stamen and carpel organs in *Arabidopsis.* In the male and female floral meristems of very young sorrel, *RAP1* expression was observed briefly in both stamen and carpel whorls, indicating that even the male flowers, which lack morphologically detectables carpel primordial, show the initial signs of a hermaphrodite stage. However, the expression of *RAP1* became undetectable in the inappropriate primordial as soon as these primordia were arrested in their development. The disappearance of *RAP1* expression in the repressed primordia contrasts with the continued expression of the *AGAMOUS*-homolog *SLM1* in repressed sex organs of white campion. The sorrel sex determination mechanism leads to the early repression of a gene potentially important for organ identity, but the white campion mechanism functions without much influence on the expression of the related gene.

Two sorrel genes with homolog to the *AP3* gene of *Arabidopsis* were also found, *RAD1* and *RAD2* (*Rumex acetosa* '*DEFICIENS*-like'). They were both expressed in the stamen whorls of male and female flowers. The *RAD* gene expression persisted in the stamen primordial of both sexes after *RAP1* transcripts disappeared in expressed sex organ primordial.

The effects of sex determination on MADS-box gene expression reveal that the mechanisms of sex determination are different in the two species examined. An intriguing question arises: could homeotic MADS-box genes play an active role in sex determination? It is conceivable that a female flower could form by a homeotic conversion of stamens to carpels. For example, a mutation in the *Arabidopsis* gene *PI* (necessary for petal and stamen development), leads to a female flower, because petals are converted into sepals and stamens are converted into carpels. Hometic genes could be directly involved in sex determination in some unisexual species, particularly those in which floral meristems seem to bypass the hermaphrodite stage, such as hemp, *Mercurialis* or spinach. However, in the species that have been examined, sexual differentiation involves repression of primordium development, instead of a change in the organ identity of some primordia.

4 Developmental steps affected by sex determination pathways

Maize provides an excellent genetic system to study sex determination in a monoecious plant (Bonnett 1940, Cheng *et al*. 1983, Veit *et al.* 1993). Sex determination in maize takes place subsequent to this common "bisexual" stage. 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. In the tassel, both florets of the spikelet remain functional. The preformed gynoecial initials abort, while the stamens continue to develop to sexual maturity. 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 tasse1 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**.** In wild species of cucumber**,** clusters of staminate flowers and solitary female flowers form on the same plant. All 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, Malepszy and Niemirowicz-Szczytt

1991). In dioecious *Silene* (campion) 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 (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, Yang 1998). 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.

5 Sex organ differentiation in *Cymnocladus dioicus* (Kentucky coffeetree)

Kentucky coffeetree is a medium to large deciduous tree in the Pea Family. It grows to heights of 23 to 34 m and bole diameters of 60 to 90 cm. Kentucky coffeetree is used chiefly as an ornamental and also to some extent for posts and crossties (Harrar and Harrar 1946). It has been reported that early settlers of Kentucky and Tennessee used the seeds as a substitute for coffee and the pulp of the green fruit in medicines (Harrar and Harrar 1946). There has been some research into the insecticidal properties of certain unusual amino acids isolated from the seeds (Evans and Bell 1978, Rehr *et al* 1973). Kentucky coffeetree offers no significant disease or pest problems, and should be more widely planted in open spaces that can afford its large size and beauty at maturity. Since it does not fruit at an early age, determination of gender may take a number of years, since the seedless males offer less of a cleanup problem due to the absence of fallen fruit pods and seeds.

Kentucky coffeetree is a dioecious plant and produces unisexual flowers. In our opinion, strictly dioecious plants are particularly well adapted for the study of stamenogenesis or carpel organogenesis and greatly facilitates the research of sexual determination genes: male and female individuals naturally contain different regulator genes (genes or heterochromosomes of sex determination), controlling the appearance of each kind of reproductive organ.

The authors used a combination of cytological and histological and scanning electron microscopy analysis to refine the comparative study and description of the staging of male compared to female flower development in Kentucky coffeetree. The results show that the sex differentiation process first undergo a hermaphroditic stage when the staminate and pistillate primordiums of male and female flowers were indistinguishable, but thereafter they undergo different morphogenetic pathways. In the male flower, the pistillate priomordium degenerated and the stamen fully developed, while in the female flowers, the staminate priomordium stopped growing and the pistil continued to grow. Male developmental arrest occurs in female flowers at the meiotic division, a particular crucial step in which sporophytic and gametophytic differentiation actively takes place in adjacent cellular domain. A female "suppression" function acts at least as early as the beginning of macrosporocyte.

Based on these studies, Kentucky coffeetree appears to be a system of choice in the study of early mechanisms controlling differentiation processes in reproductive organs, a black box, so far, in flower organogenesis.

6 Future prospects

In many ways, flowering plants offer unique systems through which to study sex determination. This view mainly deal with the mechanisms of sex determination and differentiation from a molecular level since sexual organogenesis is one of the most impor-

tant topics in plant development and growth which can serve as a model for the study of plant organogenesis. 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 flowering 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. In addition, the relationships or interactions between the determining regulars and the genes expressed in sexual differentiation remain however an unsolved problem in plant developmental biology. 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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