Sex determination in plants

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Sex determination is an important developmental event in the life cycle of all sexually reproducing plants. Recent studies of sex determination in many plant species, from ferns to maize, have been fruitful in identifying the diversity of genetic and epigenetic factors that are involved in determining the sex of the flower or individual. In those species amenable to genetic analysis, significant progress has been made toward identifying mutations that affect sex expression. By studying the interactions among these genes, pictures of how sex-determining signals are perceived to activate or repress male- or female-specific genes are emerging.

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Abbreviations

A_{CE}	antheridiogen *Ceratopteris*
GA	gibberellin
RDA	representational difference analysis

Introduction

Sex determination is the developmental decision that occurs during the plant life cycle that leads to the differentiation of the two organs or cells that produce the two gametes. In plants, there is great variation in where, when and how this decision occurs. Most angiosperm species produce perfect or hermaphroditic flowers, where male and female reproductive organs form in close proximity to one another within the same flower. At the other end of the spectrum are the dioecious species, where each individual produces only male or female reproductive organs. This variation in sex expression indicates that there are many different sex-determining mechanisms in plants, each having evolved in concert with the ecological niche occupied by each species. Here we review recent studies of sex determination in *Silene latifolia* and *Phoenix dactylifera*, two dioecious angiosperms, *Zea maize*, a monoecious angiosperm, and *Ceratopteris richardii*, a homosporous fern, that together illustrate the diversity of sex-determining mechanisms in plants. Sex determination and the evolution of sexual specialization and dioecy in plants has also been the topic of several recent reviews (see [1,•,2,3••,4,5•,6•]).

Sex determination in *Silene latifolia*

Silene latifolia (=*Melandrium album*) is strictly dioecious. The sex of the individual plant is genetically determined by sex chromosomes (reviewed in [4,5•]). Plants producing only female flowers have *22* autosomes and two X chromosomes; plants producing only male flowers have *22* autosomes, one X and one Y chromosome. As in humans, the sex chromosomes are cytologically distinct. Since plants with one Y chromosome and up to three X chromosomes are predominantly male [7,8], the Y chromosome is thought to contain the dominant determinants of sex type.

During the early stages of floral organ development, all floral organ primordia form in male, female and mutant hermaphroditic flowers. In males, the gynoecium develops as a sterile, undifferentiated rod, while in females, anther development arrests soon after the anther primordia form, then the anthers degenerate [9]. The observation that the MADS-box floral homeotic selector genes of *Silene*, or the SLM (for *S. latifolia* MADS) genes, are expressed appropriately in their signature whorls of the developing flower regardless of their sex indicates that the mechanism underlying sex determination is downstream of floral organ identity decisions made in the flower [10].

Early genetic studies of hermaphroditic and asexual mutants of *S. latifolia* showed that deletions of one arm of the Y chromosome correlate with hermaphroditism, while deletions of the opposite arm correlate with asexuality [11,12]. There are thus at least two sex-determining genes in *S. latifolia* that map to the Y chromosome: one that suppresses female (gynoecium) development, and another that promotes male (androecium) development. Ongoing studies of sex-determining mutants in *S. latifolia* should reveal new genes that are involved in sex determination [4]. Since methods for producing transgenic *Silene* plants are not available at this time, Y-linked sex-determining genes can only be isolated by map-based isolation techniques. Towards this goal, Donnison *et al.* [13••] have recently isolated male-specific DNA markers using representational difference analysis (or RDA). Although they are few in number and repetitive in nature, several of these markers have been mapped to the Y chromosome based on the absence of hybridization of these markers to DNA isolated from various mutant hermaphroditic and asexual plants. While deletions in the Y chromosome and the RDA analysis described above are helpful in explaining the XY male phenotype, it is not clear which genes on the X chromosome (or autosomes) are necessary for the arrest of stamen development and the promotion of gynoecium development in XX females.

Another approach to understanding sex determination and differentiation in *S. latifolia* has been to isolate genes that are differentially expressed in male or female flower buds. This approach has led to the identification

of several genes, one of which has been characterized in detail [14••]. The expression of the *Men-9* (male enhanced) gene is elevated in male flowers compared to female flowers, as well as in pseudohermaphroditic flowers which develop on genetically female (XX) plants that are systemically infected with the smut fungus *Ustilago violacea*. Infected female flowers develop stamens which carry anthers bearing fungal spores rather than pollen. *In situ* hybridizations revealed that *Men-9* gene expression delineates the boundaries of the third whorl of the developing flower primordia. During the later stages of floral development, a second phase of *Men-9* expression is observed in the epidermis and endothecium of stamens of male flowers. Although the sequences of *Men-9* and its inferred protein did not reveal a likely function for this gene, it may be useful as a molecular marker for understanding what regulates expression of male-specific genes during later stages of flower development.

Another approach to studying sex determination in *Silene* has been to investigate the potential role of DNA methylation in this process. The rationale for this approach stems from the observation that one of the two X chromosomes in *Silene* is hypermethylated [15]. This indicates that there may be a dosage compensation mechanism at work that presumably inactivates one of the two X chromosomes by DNA methylation in females, similar to that observed in human females. This dosage compensation mechanism may be responsible for the suppression of gynoecium development.

To test the effects of global demethylation on sex expression, Janousek *et al.* [16••] treated seeds with 5-azacytidine, a drug which demethylates DNA once it has been incorporated into the DNA. This treatment inhibited the suppression of gynoecium development, as demonstrated when 21% of the treated XY male seeds developed as androhermaphrodite plants with mosaic inflorescences consisting of both male and perfect flowers. All androhermaphrodite plants were karyotypically XY and all plants that produced female flowers were karyotypically XX; no gynohermaphroditic plants were observed. By following the heritability of androhermaphroditism for two successive generations, this trait was observed to be semiheritable, but only through the male germline. When 5-azacytidine-treated androhermaphroditic flowers were used as female donors of this trait, none of the progeny seeds developed androhermaphroditic flowers. The observed inhibition of the suppression of gynoecium development suggests that 5-azacytidine either induces inhibition of Y-linked female-suppressing genes, or activates autosomal female-promoting genes. The fact that androhermaphroditism is only semiheritable suggests this effect can be reversed after passage through the female germline [16••]. The same results also indicate DNA methylation is involved in sex determination but not in dosage compensation of the X chromosomes (if it indeed exists) in *Silene*. Since heteromorphic chromosomes usually lead to an imbalance in the dosage of genes located on the sex chromosomes (reviewed in [17]), some form of dosage compensation is likely to occur. Addressing the question of whether and how dosage compensation occurs in this plant is interesting not only in understanding sex determination in *Silene*, but also in comparing mechanisms of dosage compensation in plants and animals.

Sex determination in *Phoenix dactylifera*

How sexual phenotype is regulated is a particularly important problem in dioecious plants that are cultivated for agricultural purposes, as illustrated in a recent study of the date palm, *Phoenix dactylifera*. Date palm is a strictly dioecious species that is cultivated in arid parts of the world. Historically breeding programs to maintain genetic diversity have not been employed because the sex of a date palm cannot be known until it reaches reproductive age (5 to 10 years) [18]. A severe fusariose wilt of date palm caused by *Fusarium oxysporum* has recently destroyed date palms throughout Africa, a problem exacerbated by the lack of natural genetic diversity in date palm populations. While this immediate problem may be overcome by introducing genetic variability into populations (especially for traits which confer disease resistance), the ability to type the sex of seedlings would speed this lengthy process. Sex chromosomes were indistinguishable in this species until Siljak-Yakovlev *et al.* [19•] successfully used chromomycin A3 to stain root chromosomes, thus identifying subtle differences between the heterochromatin of chromosomes isolated from male and female cells. While useful for sex-typing date palm seedlings, this study also illustrates two other important points in understanding sex determination in dioecious species of plants. First, there are often no obvious cytological or genetic differences between male and female plants, and, second, it is often difficult to study the genetic or molecular basis of sex determination in many species of monoecious or dioecious agronomically important plants simply because of their longevity.

Sex determination in maize

Maize is a monoecious plant that develops unisexual female and male flowers within separate inflorescences of the same individual. The male inflorescence, or tassel, forms at the tip of the main shoot. It consists of numerous spikelets, each spikelet having one pair of small staminate florets. The female inflorescence, or ear shoot, develops from an axillary meristem. The spikelets of the ear each contain two florets: one pistillate and the other sterile. Developmental studies of the maize inflorescence have shown that the male and female florets are initially perfect and become unisexual by a process of selective arrest and abortion of pistils in staminate florets of the tassel, and of stamens in pistillate florets of the ear [20,21]. Pistil development is arrested in the lower floret of the ear spikelet, whereas the pistil of the upper floret develops to sexual maturity.

In addition to the differences in the sex of the florets (the primary sex characteristics), there are a number of secondary sex characteristics associated with each inflorescence, spikelet or floret, including differences in inflorescence architecture, glume morphology, trichome distribution, and pigment deposition. The presence of secondary sex characteristics that can manifest themselves early in plant development makes maize among the most interesting yet challenging systems for the study of sex determination in plants. Our understanding of sex determination in maize (reviewed in [2,6•]) comes mainly from the analysis of mutations that affect either primary sex characteristics alone or both primary and secondary sex characteristics.

Early studies demonstrated that the application of gibberellin (GA), or changes of growth conditions that result in an increase of GA levels, tend to feminize the tassel [22–24]. Mutants deficient in GA biosynthesis or perception, such as the *dwarf* (*d*) and *anther-ear* (*an*) mutants, have perfect upper ear florets and staminate lower ear florets [25,26]. Tassel development in GA-deficient mutants is unaffected. These observations indicate that GAs promote pistil development and suppress stamen development in the ear florets, although the two processes may differ in their sensitivity to GA levels in the plant.

Two other types of sex-determining mutations, *silkless* (*sk1*) and tasselseed (*ts*), have been identified. In *sk1* plants, the pistils and stamens of both ear florets are aborted although secondary sexual traits remain female, indicating that *Sk1* is necessary for the formation of a functional pistil in the upper ear floret [27]. In *ts* plants, functional pistillate florets form in the tassel [28]. Several nonallelic *ts* mutations have been described: *ts1* and *ts2* affect the primary sex characteristics only, whereas *ts4* and *ts6* affect secondary sex characteristics as well. The *ts6* mutation, for example, feminizes the tassel and disrupts the pattern of branching in inflorescences [29•]. This indicates that the control of primary and secondary sex characteristics is interactive yet the two groups of characteristics are still genetically separable. Based on their mutant phenotypes, the *Ts* genes are thought to control sex expression in the flower by promoting stamen development and either promoting pistil abortion or suppressing pistil development in florets of the tassel. The *Ts2* gene has been cloned by transposon tagging and its product found to be related to hydroxysteroid dehydrogenases [30]. The *Ts2* gene is expressed in the subepidermal layer of the developing pistil in tassel florets, indicating that it may be involved in pistil abortion in the tassel. The expression of *Ts2* in developing ear florets has not been reported and its role in abortion of the pistil of the lower floret of the ear spikelet remains unclear.

Many of the epistatic interactions between the sex-determining mutations of maize have been investigated. The *ts2 d1* double mutants have an additive phenotype, indicating that the development of the unisexual male and females flowers is controlled by independent pathways, one regulated by GA and the other regulated by Ts2 [31]. The *sk1 ts2* double mutant forms ear spikelets with a *ts2* phenotype (both upper and lower florets are pistillate), and various tassel florets depending on its position in the tassel, with staminate florets developing distally (*sk1* phenotype) and pistillate florets developing proximally (*ts2* phenotype) [31]. This phenotype indicates that *ts2* is epistatic to *sk1* in the ear, whereas *sk1* partially suppresses *ts2* in the tassel. Dellaporta and Urrea-Calderon [2] propose that *Sk1* and *Ts2* mutually repress each other's expression such that in the tassel floret *Ts2* is expressed, *Sk1* is repressed, and pistils abort while stamens develop to maturity. In the ear florets, the expression or repression of *Ts2* and *Sk1* would be reversed. What regulates the abortion of the pistil of one ear floret is unclear. The cloning of the remaining sex-determining genes in maize will be important and necessary to understand the molecular basis of the interactions between the *Ts* and *Skl* genes and their control of sexual phenotype in maize.

Sex determination in *Ceratopteris richardii*

In contrast to the heterosporous seed plants, many non-seed plants, such as ferns, lycopods and horsetails, are homosporous. Although one type of haploid spore is produced in homosporous plants, the haploid gametophytes that develop from these spores are often sexually dimorphic, either male or female (dioecious) or male or hermaphroditic (androdioecious). Sex determination in these plants is a question of how the sex of the gametophyte is determined independently of its genotype. Among the homosporous plants, sex determination in the homosporous fern *Ceratopteris richardii* has been studied in the most detail.

Spores of the homosporous fern *Ceratopteris* have the potential to develop as either male or hermaphroditic gametophytes. Hermaphrodites produce antheridia, archegonia and a lateral meristem that forms a two-dimensional sheet of cells. Males produce antheridia and no archegonia or meristem (they are ameristic). The sex of the gametophyte is determined by a pheromone, referred to as A_{CE} for antheridiogen Ceratopteris, that is secreted by the hermaphrodite and induces the development of male gametophytes if they are exposed to A_{CE} very early in their development [32]. Since the exposure to A_{CE} results in the development of an ameristic male, it seems that A_{CE} promotes maleness and suppresses the development of the meristem and archegonia in the gametophyte. In contrast to many organisms in which the sex of the haploid individual is fixed early in development, male sex expression is reversible in *Ceratopteris*: male gametophytes that are removed from medium containing A_{CE} will eventually form a hermaphroditic prothallus. The antheridiogens of homosporous ferns are gibberellins or gibberellin-like compounds (see [33,34], for example).

Although the sex of the *Ceratopteris* gametophyte is epigenetically determined by the presence or absence of A_{CE}, many of the genes that are regulated by A_{CE} have been identified by isolating mutations that alter the sex of the gametophyte. The epistatic interactions among these genes have been examined and a genetic regulatory pathway controlling sex determination in *Ceratopteris* defined [1,35,36••]. In this pathway, there are two master regulatory genes, or sets of genes, that regulate the sexual phenotype of the gametophyte. One includes the *TRANSFORMER* or *TRA* genes which, when active, simultaneously promote femaleness (the development of archegonia and meristem) and repress maleness (the development of antheridia). The other includes the *FEMINIZATION1* or *FEM1* gene. When active, the *FEM1* gene simultaneously promotes maleness and represses femaleness. The *TRA* genes are thought to repress maleness by indirectly repressing the activity of the *FEM1* gene, while the *FEM1* gene represses femaleness by repressing the activity of the *TRA* genes. The repression of *FEM1* by *TRA* requires another gene called *MAN1* (for many antheridia); the *TRA* genes positively regulate *MAN1*, which then negatively regulates *FEM1* [37]. The sex of the gametophyte, male or female, ultimately depends on which of the two master regulatory genes is expressed first. This decision is determined by A_{CE}, which activates the A_{CE} signal transduction pathway defined by the *HERMAPHRODITIC* or *HER* genes. When activated by A_{CE}, the *HER* genes, together with *FEM1*, repress *TRA* and the gametophyte develops as a male. When A_{CE} is absent, the *TRA* genes are not repressed, *FEM1* is repressed, and the gametophyte develops female traits.

While the sex determining pathway is useful for understanding how the sex determining genes interact to determine the sex of the *Ceratopteris* gametophyte, this pathway reveals a few fundamental points about sex determination that might also be expected to occur or that have been observed in other plant species. The first is that sex determination in *Ceratopteris* involves both genetic factors (defined by mutation) and pheromones/hormones, or epigenetic factors. The same is true for maize, as previously described. The second point is that the mechanism involved in sex determination in *Ceratopteris* is inherently plastic in that the sex of a gametophyte can be reversed in response to changes in the environment. A good example of another species showing labile sex expression is *Thymelaea hirsuta*, where individuals may change their sex from year to year in response to changes in the environment [38,39•]. The final point is that the sex of an individual involves two master regulatory genes, each promoting one sex and repressing the other sex. Thus, for an individual to be female, the female-promoting regulatory gene(s) must be expressed and the male-promoting regulatory genes(s) must be repressed; to be male, the opposite must occur. In *Ceratopteris* and maize, the two plants where these master regulatory genes have been identified, the evidence thus far indicates that the simultaneous promotion of one sex and repression of the opposite is accomplished by the two master regulatory genes which, directly or indirectly, exclude each other's expression. Whether and how these predicted interactions occur can be tested once these genes have been cloned.

Conclusions

There has been significant progress in our understanding of sex-determining mechanisms in plants, particularly in species that are amenable to a genetic analysis of mutations that affect sex expression. Continued studies of sex determination in diverse species that have been identified as model systems, such as *S. latifolia*, *Z. maize* and *C. richardii*, are important for two reasons. First, the diversity of sex-determining mechanisms in plants can only be understood by studying several species displaying different patterns of sex expression. Second, the problem of sex determination gets to the heart of the fundamental but poorly understood question of how plant cells choose between two developmental fates (male or female). Studying the genetic and molecular basis of sex determination in several species of plants should reveal how such decisions are made, and how each decision is or can be influenced by external (environmental) factors and internal (genetic) factors.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

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