Sex-Determining Mechanisms in Land Plants

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INTRODUCTION

Sex determination is a process that leads to the physical separation of male and female gamete-producing structures to different individuals of a species. Even though sexually reproducing species have only three possible options—to relegate the two sexes to separate individuals, to keep them together on the same individual, or to have a combination of both—plants in particular display a great variety of sexual phenotypes. In angiosperms, a sex-determining process is manifest in species that are monoecious, in which at least some flowers are unisexual but the individual is not, or dioecious, in which unisexual plants produce flowers of one sex type. In plants that produce no flowers and are homosporous, sex determination is manifest in the gametophyte generation with the production of egg- and sperm-forming gametangia on separate individual gametophytes. The determinants of sexual phenotype in plants are diverse, ranging from sex chromosomes in *Marchantia polymorpha* and *Silene latifolia* to hormonal regulation in *Zea mays* and *Cucumis sativa* to pheromonal cross-talk between individuals in *Ceratopteris richardii*. Here, we highlight recent efforts aimed at understanding the genetic and molecular mechanisms responsible for sex determination in several plant species that separate their sexes into two individuals or flowers. Representatives of all major land plant lineages are included to give an evolutionary perspective, which is important in understanding how different sex-determining mechanisms evolved and their consequences in plant development and evolution. Although great progress has been made in genetically identifying the genes that regulate sex expression in these species, few of them have been cloned. Because a “one-size-fits-all” mechanism of sex determination will not account for the variety of sexual systems in plants, future efforts at cloning these genes in several well-chosen model systems will be necessary to understand these processes at the molecular level. There are several excellent recent reviews of sex determination that describe species that have not been included to which the reader is directed (Ainsworth, 1999, 2000; Geber et al., 1999; Matsunaga and Kawano, 2001; Negrutiu et al., 2001; Barrett, 2002; Charlesworth, 2002). We begin with the most basal lineage of the land plants.

THE BRYOPHYES

The bryophytes are a group of plants that includes the modern liverworts, hornworts, and mosses. In this group, the haploid gametophyte is the dominant phase of the life cycle, which is illustrated in Figure 1. Their diploid spore-producing sporophytes are very small, short-lived, and parasitic upon the independent gamete-producing gametophyte generation. All bryophytes are homosporous, producing only one type of spore, yet all three groups of extant bryophytes are represented by species that are homothallic, with one individual gametophyte producing both male and female sex organs (gametangia), and species that are heterothallic, with one individual producing only male or female gametangia (Smith, 1955). Sexual dimorphism in heterothallic species can be extreme, as exemplified by members of the genus *Micromitrium*, in which the dwarf male gametophyte grows on the leaves of the markedly larger female plant (Smith, 1955).

In many species of bryophytes, heterothallism (unisexuality) has been correlated with the presence of sex chromosomes (Smith, 1955). Although the extent of heterothallism and sex chromosomes in the bryophytes has not been assessed systematically, this is the only known group of homosporous plants that uses sex chromosomes in sex determination. To date, studies of bryophyte sex determination have focused on the heterothallic liverwort *Marchantia polymorpha*. In this species, the male and female thalli (vegetative gametophytes) look alike, although males and females can be distinguished easily by differences in the morphology of the sexual structure each produces. A gametophyte bears gametangia on stalked branches called antheridiophores (if male) or archegoniophores (if female) that arise from the upper surface of the thallus (Figure 1). Antheridiophores produce sperm-forming antheridia, and archegoniophores produce egg-forming archegonia. The sex of each haploid gametophyte is determined by cytologically distinct sex chromosomes, with males having one very small Y chromosome and no X chromosome and females having one X chromosome and no Y chromosome (Lorbeer, 1934). In addition to its rapid growth (it is often an invasive weed in greenhouses), its ability to be propagated vegetatively by gemma cups (Figure 1), and its ability to be transformed (Takenaka et al., 2000), *Marchantia* has a relatively small genome size of 280 Mbp distributed among eight autosomes plus one sex chromosome (Okada et al., 2000), making it a worthy model organism amenable to genomics-style investigations.

Working on the assumption that sex-determining factors exist on the *Marchantia* sex chromosomes, Okada and colleagues...
(2000) set out to identify these factors by constructing separate male and female P1-derived artificial chromosome (PAC) libraries and identifying clones specific to either the male or the female genome. Their screen resulted in 70 male-specific PAC clones that hybridized only the Y chromosome by fluorescence in situ hybridization. No female-specific clones were found, indicating that the X chromosome does not harbor long stretches of unique sequences, as does the Y chromosome. To date, two male-specific PAC clones with insert sizes totaling 126 kb have been sequenced (Okada et al., 2001; Ishizaki et al., 2002). This and other analyses have revealed that approximately one-fourth to one-third of the 10-Mb Y chromosome of Marchantia consists of an estimated 600 to 15,000 copies of an element of variable length (0.7 to 5.2 kb) that contains other smaller repetitive elements (Okada et al., 2001; Ishizaki et al., 2002). Of the six putative protein-encoding genes found embedded within the repeats, all are present in multiple copies on the Y chromosome based on DNA gel blot hybridization. Two of these genes, named ORF162 (Okada et al., 2001) and M2D3.5 (Ishizaki et al., 2002), are unique to the Y chromosome; the remaining four genes are present in low copy number on the X chromosome or the autosomes. ORF162 encodes a putative protein with a RING finger domain; M2D3.5 is a member of the same gene family. ORF162 transcripts are detectable only in the male sexual organs, indicating that the gene family represented by ORF162 and M2D3.5 may be important in the development of the antheridiophore. Of the four genes also present on the X chromosome or the autosomes, only one (M2D3.4) is restricted in its expression to the male gametophyte, indicating that the M2D3.4 X or autosomal homolog might be a pseudogene.  

M2D3.4 encodes a putative protein similar to a Lilium longiflorum gene that is expressed exclusively in the male gametic cells. The remaining three genes are not sex specific in their expression. The functions of the Y chromosome–encoded genes are as yet unknown.

Although only a small portion of the Marchantia Y chromosome has been sequenced, it is sufficient to make meaningful comparisons with the euchromatic male-specific region (MSY) of the human Y chromosome, the sequence of which was published recently (Skaletsky et al., 2003; see also Hawley, 2003). The sequence of the human MSY region and limited comparative sequencing of the MSY regions in great apes (Rozen et al., 2003) have added new insights to our understanding of how the mammalian testis gene families on the Y chromosome have been maintained over the course of evolution. As will be shown, the similarities between the human and liverwort Y chromosomes are striking and may reflect a common mechanism underlying the evolution of the Y chromosome in these two disparate organisms. The MSY region of the human Y chromosome is made up of three classes of sequences: X transposed, X degenerate, and ampliconic, the latter representing ~30% of the MSY euchromatin. Because the Marchantia X chromosome has not been sequenced, it is only possible to make comparisons between the human ampliconic sequences, which are Y specific, and the Marchantia Y chromosome sequences. Like the Marchantia Y chromosome sequences, the ampliconic regions of the MSY consist of highly repetitive sequences unique to the Y chromosome, although the sizes, sequences, and stoichiometries of the repetitive elements vary considerably between the two species. Protein-encoding genes.
or gene families (six genes in Marchantia and nine gene families in human) occur within repetitive elements, and all are present in multiple copies on the Y chromosome. In both organisms, homologs also may be present on the X or autosomal chromosomes in low copy number. Although all protein-encoding genes found within the human ampliconic sequences are expressed only in the testis, at least some of the protein-encoding genes identified in Marchantia are male organ specific in their expression.

According to the prevailing theory of mammalian sex chromosome evolution (Graves and Schmidt, 1992; Jegalian and Page, 1998; Lahn and Page, 1999), the X and Y chromosomes are derived from an ancient autosomal pair of chromosomes. The Y chromosome acquired genes, especially those that enhance male fertility, by a series of autosomal transpositions that were then amplified on the Y chromosome, whereas the X chromosome maintained its ancestral genes. Other genes present on the Y chromosome (i.e., X homologs) were lost, probably aided by a lack of X-Y recombination, leading to a mostly degenerate Y chromosome. The recent sequencing results suggest that the a lack of X-Y recombination, leading to a mostly degenerate Y chromosome (i.e., X homologs) were lost, probably aided by a lack of X-Y recombination, leading to a mostly degenerate Y chromosome. The recent sequencing results suggest that the uniformity of the ampliconic repetitive sequences of the human Y chromosome is maintained from generation to generation by intrachromosomal Y-Y gene conversion. This occurs at relatively high rates: ~600 nucleotides per newborn human male are estimated to have undergone Y-Y gene conversion (Rozen et al., 2003). Although it is not known if there are higher order palindromic repeated sequences in Marchantia as there are in humans (these palindromic sequences may be necessary for gene conversion), the homogeneity of repetitive sequences in the Marchantia Y chromosome and the relatively high frequency of male-specific genes within these repetitive elements suggest gene conversion playing a role in maintaining the repetitive elements of the Marchantia Y chromosome.

One important distinction between Marchantia and humans is that X-X recombination cannot occur in Marchantia because all diploid sporophytes are X-Y. This suggests that forces other than homologous interchromosomal recombination are responsible for preventing the degeneration of the X chromosome, although size alone may not be an indicator of chromosome degeneracy. Future efforts to sequence the entire Y and X chromosomes in Marchantia will be very important in understanding how sex chromosomes specify sexual phenotype in this species and how both the X and Y chromosomes evolved in this ancient lineage of plants.

THE LYCOPHYTES

Another group of land plants deserving attention from an evolutionary perspective is the lycophyte lineage, which includes the modern Lycopodiales genera Selaginella and Isoetes. This lineage is most closely related to the earliest vascular plants that first appeared on land ~250 to 400 million years ago (Kenrick and Crane, 1997). Although the earliest lycopods and extant members of the Lycopodiales are homosporous and produce only one type of spore, Selaginella and Isoetes are heterosporous, with their sporophytes producing free-living megaspores and microspores that give rise to the female and male gametophytes, respectively. Of the lycophocytes, Selaginella has great potential as a useful comparative system for the study of sex determination in plants, in part because many species have very small genome sizes (J.A. Banks, unpublished observations). Selaginella produces microsporangia and megasporangia that are born on the same strobilus or in different strobili of the same plant, depending on the species; strictly unisexual species have not been reported. In Selaginella moellendorffii, for example, each strobilus bears both kinds of sporangia, with microsporangia at the bottom and macrosporangia toward the top of each strobilus. In such plants, sex determination would be viewed as the process that regulates the sexual identity of the sporangia in the strobilus, a mechanism that has clear parallels with floral organ identity in angiosperms that produce perfect flowers. Although we know virtually nothing about this group of plants beyond descriptive biology, this lineage holds important clues for understanding the evolution of heterospory from homospory, a switch that occurred many times during land plant evolution (Stewart and Rothwell, 1993) and that has had a major impact on the timing of sex determination from the gametophyte to sporophyte generation in plants (Sussex, 1966). The question of how heterospory evolved from homospory is difficult to study in the heterosporous angiosperm lineage because their homosporous progenitors are probably extinct.

THE HOMOSPOROUS FERNS

Recent phylogenetic analyses of vascular seed–free plants group the leptosporangiate and eusporangiate ferns and members of Equisetum and Psilotum into a monophyletic clade that is sister to the seed plants (Pryer et al., 2001). With few exceptions, they are homosporous plants. The one plant for which a sex-determining pathway has been genetically well defined is the leptosporangiate fern Ceratopteris richardii. Like Marchantia, Ceratopteris is homosporous and produces only one type of haploid spore. Although the sex of the Marchantia gametophyte is determined genetically by sex chromosomes, the sex of the Ceratopteris gametophyte (male or hermaphroditic) is determined epigenetically by the pheromone antheridiogen. Since their discovery by Dopp (1950) in the fern Pteridium aquilinum, antheridogens have been identified and characterized from many species of leptosporangiate ferns (reviewed by Naf, 1979; Yamane, 1998), suggesting that it is a common mode of regulating sexual phenotypes in this group of plants. Although the structure of the Ceratopteris antheridiogen is unknown, all other fern antheridiogens characterized to date are mostly novel gibberellins (Yamane, 1998).

The Ceratopteris male and hermaphroditic gametophytes are easy to distinguish not only by the type of gamete produced but also by the presence or absence of a multicellular meristem. As illustrated in Figure 2, the hermaphrodite forms a single meristem and meristem notch that gives the hermaphrodite its heart-shaped appearance. Cells of this meristem differentiate as egg-forming archegonia, sperm-forming antheridia, or simply enlarge, adding to the growing sheet of photosynthetic parenchyma cells that make up most of the hermaphrodite prothallus. A Ceratopteris spore grown in isolation always develops as a hermaphrodite. A male gametophyte develops from a spore only if the spore is placed in medium that had previously...
supported the growth of a hermaphrodite. Lacking a multicellular meristem, almost all cells of the male gametophyte terminally differentiate as antheridia. The male-inducing pheromone that is secreted by the Ceratopteris hermaphrodite is called ACE for antheridiogen Ceratopteris. Based on physiological studies in Ceratopteris (Banks et al., 1993), ACE is not secreted by the hermaphrodite until after it loses the competence to respond to its male-inducing effects, which corresponds to the initiation of the meristem. A gametophyte will develop as a male only if it is exposed continuously to ACE from a very young age, between 2 to 4 days after spore inoculation. Thus, in a population of spores, those that germinate first become ACE-secreting meristic hermaphrodites, whereas those that germinate later become amestic males under the influence of ACE secreted by its neighboring hermaphrodites.

To understand how ACE represses the development of female traits (i.e., archegonia and meristem) and promotes the development of male traits (i.e., antheridia) in Ceratopteris, a genetics approach has been used to identify the genes involved in this response (Banks, 1998; Strain et al., 2001). To date, five phenotypic classes of mutants have been identified; representatives of each class are illustrated in Figure 2. In addition to those that are always hermaphroditic (the hermaphroditic mutants), always male (the transformer [tra] mutants), or always female (the feminization [fem] mutants) regardless of the absence or presence of ACE, there are mutants that produce excessive antheridia (the many antheridia mutants) as well as the feminizing mutants that often lack a meristem notch (the notchless mutants). By comparing the phenotypes of double mutant gametophytes to each single mutant gametophyte parent, the epistatic interactions among these genes have been assessed. One particularly informative phenotype is the fem1 tra1 double mutant, also illustrated in Figure 2. Unlike other double mutant combinations, this one has a novel phenotype unlike that of either parent. This finding suggests that these two genes (FEM1 and TRA1) define two separate pathways, one specifying male development and the other female development. The sex-determining mutants have been ordered into a genetic sex-determining pathway, illustrated in Figure 3, that is most consistent with the genetic data. In this pathway, the sex of the gametophyte ultimately depends on the activities of two genes, one specifying the development of male traits (FEM1) and the other specifying the development of female traits (TRA). These genes also repress each other, so that when TRA is active, FEM1 is not and visa versa. What determines which of these two genes is expressed in the gametophyte (and thus its sex) is ACE, which ultimately represses the TRA genes, as described in the legend to Figure 3.

In comparing mechanisms of gametophytic sex determination in homosporous bryophytes and ferns, one obvious question that arises is what drove Marchantia to an X-Y chromosomal mechanism of sex determination and Ceratopteris to an epigenetically regulated mechanism dependent on pheromonal cross-talk between individuals? The answer to this question probably lies in the different ratios of males and females or hermaphrodites that occur in the populations of each species. In Marchantia, the segregation of X and Y sex chromosomes during meiosis in the sporophyte ensures that each gametophyte progeny has an equal probability of being either male or female, barring selection. In Ceratopteris, the ACE response allows the ratio of males to hermaphrodites to vary depending on the density of the population, such that as the population density increases, the proportion of males also increases. Although the underlying sex-determining mechanism is inflexible in...
Marchantia, it is flexible enough in Ceratopteris to allow each individual to determine its sex according to the size of the population in which it resides and the speed at which it germinates relative to its neighbors. The flexibility of the Ceratopteris sex-determining mechanism is reflected in its sex-determining pathway, and this becomes especially apparent compared with the sex-determining pathways known from other organisms, including *Drosophila melanogaster* and *Caenorhabditis elegans*, which are illustrated in Figure 3. In both of these animals, an individual's sex (male or female in *D. melanogaster* and male or hermaphrodite in *C. elegans*) is determined genetically by the ratio of X to autosomal chromosomes. This ratio is read and either activates or represses the activities of downstream genes in each pathway. In both animals, the sex ultimately depends on the state of the terminal gene in each linear pathway, *TRA1* in the case of *C. elegans*. The Ceratopteris sex-determining pathway is distinctly different from those of *D. melanogaster* and *C. elegans* in that it is not linear and there are two sex-determining genes, one for male and one for female development. Their ability to repress each other endows each Ceratopteris spore with the flexibility to determine its sex upon germination based on environmental cues.

So why would a flexible mechanism of sex determination that allows sex ratios to vary be adaptive in ferns but not in bryophytes? The answer to this question may lie in the ephemeral nature of the fern gametophyte. Although the gametophytes of bryophytes are persistent, the gametophytes of ferns are not. In Ceratopteris, for example, gametophytes reach sexual maturity only 14 days after spore inoculation and die once they are fertilized. The limited time that a fern gametophyte is able to be crossed by another might favor a sex-determining system that would promote outcrossing by increasing the proportion of males when population densities are high and ensuring a high proportion of hermaphrodites capable of self-fertilization when population densities are low. Because there are a variety of sex-determining mutants available in Ceratopteris, the hypothesis related to the consequences of variable versus fixed sex ratios can be tested easily, at least under defined laboratory conditions.

Future studies to clone the sex-determining genes in Ceratopteris will be necessary to understand their biochemical functions and to test their interactions predicted by the genetic model. Although the size of the Ceratopteris genome is probably very large (n = 37), the ability to inactivate genes in the Ceratopteris gametophyte by RNA interference (Stout et al., 2003; G. Rutherford, M. Tanurdzic, and J.A. Banks, unpublished observations) provides an important means to study the effects of inactivating potential sex-determining genes in the Ceratopteris gametophyte.

**THE FLOWERING PLANTS**

Although unisexuality is very common in animals, hermaphroditism is the rule in angiosperms. Approximately 90% of all angiosperm species have perfect flowers with specialized organs producing microspores or megaspores from which the male or female gametophytes develop. Of the remaining species, approximately half are monoeocious, producing unisexual flowers of both sexes on the same individual, and the other half are dioecious, with unilateral male and female flowers arising on separate individuals (Yampolsky and Yampolsky, 1922). The distribution of dioecy and monoeocy within the angiosperm phylogenetic tree strongly favors the evolutionary scenario in which unisexual flowers evolved from perfect flowers multiple times in the angiosperm lineage (Lebel-Hardenack and Grant, 1997; Charlesworth, 2002). Not surprisingly, there are a variety of sex determination mechanisms in the angiosperms. For organizational purposes only, sex determination in monoeocious and dioecious species is treated separately in this review.
The Monoecious Angiosperms

There are numerous terms to describe the variety of sexual phenotypes observed in monoecious plant species (defined and compiled by Sakai and Weller, 1999). Although this rich nomenclature is appropriate, it tends to confound the problem of sex determination in this group of plants. For simplicity, monoecious species here are grouped into two categories: those that produce only unisexual male and female flowers on the same plant, and those that produce both unisexual and perfect flowers on the same plant.

*Zea mays* (maize) is an example of a monoecious species that produces only unisexual flowers in separate male and female inflorescences, referred to as the tassel and ear, respectively. Unisexuality in maize occurs through the selective elimination of stamens in ear florets (flowers) and by the elimination of pistils in tassel florets (reviewed by Irish, 1999). Two general classes of sex-determining mutants have been identified in maize, including those that masculinize ears and those that feminize tassels. The *anther ear* (*an1*) and dwarf (*d1, d2, d3, and d5*) mutants of maize are recessive and masculinize ears by preventing stamen abortion in the female florets (Wu and Cheung, 2000). The dominant dwarf mutation, *d8*, has a similar phenotype. The *D1, D3*, and *AN1* genes encode enzymes involved in the biosynthesis of the plant hormone gibberellin (GA) (Bensen et al., 1995; Winkler and Helentjaris, 1995; Spray et al., 1996). The *D8* gene encodes the maize homolog of *GIBBERELLIN INSENSITIVE REPRESSOR OF gal-3* (Peng et al., 1999), a family of transcription factors that negatively regulate GA responses in plants (Richards et al., 2001; reviewed by Olszewski et al., 2002). GA signaling is thought to be derepressed in the *d8* mutant, resulting in a dominant phenotype. The molecular identity of these genes provides direct evidence that endogenous GAs have a feminizing role in sex determination in maize.

The *tasselseed1* (*ts1*) and *ts2* mutants of maize feminize male florets in the tassel, as illustrated in Figure 4. In addition to the tassel phenotype, the second floret of the ear spikelet, which normally fails to develop, develops normally in the *ts* mutants, leading to a double-kerneled spikelet in the ear (Dellaporta and Calderon-Urrea, 1994; Irish, 1999). The *TS2* gene has been cloned and shown to encode a putative short-chain alcohol dehydrogenase with signature motifs of steroid dehydrogenases (DeLong et al., 1993). *TS2* mRNA is expressed in the subepidermal layer of the gynoecium before its abortion, which correlates well with the timing and location of cell death responsible for the pistil abortion in wild-type male florets (Calderon-Urrea and Dellaporta, 1999). The expression of *ts2* requires the wild-type *TS1* gene. Although the pistils of ear florets also express *TS1* and *TS2*, they are protected from a deathly fate by the action of another gene, *SILKLESS1*, that interacts directly or indirectly with *TS2* (Calderon-Urrea and Dellaporta, 1999). Although the cloning of the sex-determining genes in maize demonstrates that GAs and possibly other steroid-like hormones play a pivotal role in stamen abortion and feminization of flowers, the spatial distribution of these molecules could have an effect on the sex determination process, as exemplified by a steep gradient in GA abundance along the maize shoot (Rood et al., 1980), which correlates well with the male-suppressing and female-promoting phenotypic effects of GA. How the synthesis or transport of these molecules is regulated, and the identity of the downstream targets of these hormones, remain to be discovered.

Another monoecious plant, *Cucumis sativus* (cucumber), has served as a model system for sex determination studies since the 1950s and early 1960s, driven by breeding programs for hybrid seed production. Cucumber plants are mostly monoecious but can be dioecious or hermaphroditic, depending on the genotype. Regardless of their sex, all floral buds are initially hermaphroditic, and it is the arrest of stamen or pistil development that leads to unisexual flowers. In monoecious cucumbers, male flowers form at the bottom and female flowers form at the top of each shoot. There are three major genes that affect the arrangement of unisexual flowers or their sex; they are designated the F, A, and M genes (following the nomenclature of Pierce and Werner, 1990). The F gene is semidominant and affects the expression of female-promoting phenotypic effects of GA. How the synthesis or transport of these molecules is regulated, and the identity of the downstream targets of these hormones, remain to be discovered.

In addition to the sex-determining genes, plant hormones have long been implicated in the sex-determining process in cucumber. GA and ethylene application and the use of GA and ethylene inhibitors can subvert the genotypic constitution of the plant, with GA acting mainly as a masculinizing agent and ethylene acting as a feminizing agent (Perl-Treves, 1999). By treating monoecious and andromonoecious cucumber plants with various...
combinations of GA and ethrel or GA and ethylene inhibitors, Yin and Quinn (1995) demonstrated that ethylene is the main regulator of sex determination, with GA functioning upstream of ethylene, possibly as a negative regulator of endogenous ethylene production. These findings led them to propose a model for how sex determination might occur (Yin and Quinn, 1995), with ethylene serving both as a promoter of the female sex and an inhibitor of the male sex. The basic tenets of the model are that the F gene should encode a molecule that would determine the range and gradient of ethylene production along the shoot, thereby acting to promote female sex, whereas the M gene should encode a molecule that perceives the ethylene signal and inhibits stamen development above threshold ethylene levels. This model is consistent with how unisexual flowers might arise very early and very late during shoot development; however, the model also predicts an entire range of intermediate types rarely or never seen in cucumber. As suggested by Perl-Treves (1999), variations in the model of Yin and Quinn and the incorporation of additional factors in the sex-determining process in cucumber could account for the observed lack of intermediate types.

Recent results from several laboratories have provided molecular evidence in favor of the ethylene theory of sex determination in cucumber. Two 1-aminocyclopropane-1-carboxylic acid synthase genes, CS-ACS1 and CS-ACS2, have been identified in cucumber, and one of them (CS-ACS1) maps to the F locus (Trebitsh et al., 1997). The monocious cucumber genome has only one copy (Cs-ACS1), whereas the gynoecious genome has both copies. The expression of both genes correlates with sexual phenotype, with gynoecious plants accumulating more transcript than monocious or andromonoecious plants (Kamachi et al., 1997; Yamasaki et al., 2001). Although these studies are consistent with the female-promoting effects of ethylene, they do not address the question of how ethylene inhibits stamen aborption in gynoecious and not andromonoecious plants. Yamasaki et al. (2001) provided evidence suggesting that the product of the M locus mediates the inhibition of stamen development by ethylene (i.e., M affects sensitivity to ethylene). This finding indicates that ethylene concentration, which is likely to be dependent on the F locus, and the differential sensitivity of males and females to ethylene, which is likely to be dependent on the M locus, are both important in regulating sexual phenotype in cucumber. Definitive cloning of the M and F genes will allow these hypotheses to be tested directly.

Because sex determination in cucumber and most other angiosperm species occurs via selective abortion of flower organs, Kater et al. (2001) set out to establish whether this abortion is based on organ identity or positional information within the flower. The availability of cucumber homologs of the MADS box ABC homeotic genes and the ability to express them ectopically in cucumber allowed these authors to show that the sex determination machinery in cucumber selectively aborts sex organs based on their position rather than their identity (i.e., in male flowers, carpels are aborted only in the fourth whorl, and in female flowers, stamens are aborted only in the third whorl). In addition, because nonreproductive organs that develop in the inner whorls of a C-class homeotic mutant are not aborted, Kater et al. (2001) speculated that C-class gene products might be targets of the sex-determining process. Even though this is a tempting speculation, it leaves the question of abortion timing unresolved.

Several studies have addressed the role of the MADS box floral homeotic genes in the sex determination process in many monocious and dioecious plants, including Asparagus officinalis (Park et al., 2003), Betula pendula (Elo et al., 2001), Gerbera hybrida (Yu et al., 1999), Populus deltoides (Sheppard et al., 2000), Rumex acetosa (Ainsworth et al., 1995), Silene latifolia (Hardenack et al., 1994), and maize (Heuer et al., 2001). In all cases, the expression of B- or C-function floral homeotic genes in carpels or stamens was shown to decline in organs targeted for abortion in the unisexual flower. However, it is not clear if the changes in expression of these genes are a cause or a consequence of organ abortion. Furthermore, evidence that sex-determining mutants cosegregate with MADS box genes is lacking. That plants may not use MADS box genes for sex determination would not be surprising given that the unisexual flowers of most monocious and dioecious plants are derived from bisexual flowers with all sex organs present.

Another plant within the monocious group of angiosperms that has been studied is Carica papaya (papaya). Papaya is a polygamous species with three sexes—females, males, and hermaphrodites. In this species, sexual phenotype is controlled by a single gene with three alleles: the dominant M (for male), the dominant F (for hermaphrodite), and the recessive m (for female) alleles (Storey, 1938). The progeny of self-fertilized hermaphrodites (genotypically M*m) segregate segregates hermaphrodites and females in a 2:1 ratio. The lack of male progeny indicate that the M allele is lethal, perhaps because of a lethal gene closely linked to the M locus. This and other crosses led early workers to the conclusion that all males are genotypically M*M. Thus, females are the homogametic and males the heterogametic sex. Papaya flowers of different sexes also display secondary sexual characteristics that cosegregate with the M allele. The apparent lack of recombination between the loci responsible for primary and secondary sex traits indicates that both loci are tightly linked and inherited en bloc, much like a sex chromosome (Storey, 1953), although heteromorphic chromosomes do not exist (Kumar et al., 1945).

The economical importance of papaya has driven sex determination research in this species, because the pyriform fruits from hermaphroditic trees are preferred by consumers more than the spherical fruits produced by the female trees. Because it is only upon flowering that the sex of the individual papaya tree can be determined, molecular markers that cosegregate with the M alleles have been sought intensively. To date, two groups have reported randomly amplified polymorphic DNA markers that are highly specific for males and hermaphrodites but absent in females (Deputy et al., 2002; Urasaki et al., 2002). One randomly amplified polymorphic DNA marker was also shown by DNA gel blot hybridization to be completely absent from the female genome (Urasaki et al., 2002), providing the first molecular evidence for genomic differentiation between the sexes. Although papaya is a tropical tree and not considered a model plant system, it has a small genome of 371 Mbp (Arumuganathan and Earle, 1991) and may be transformable (Fitch et al., 1992). These facts, coupled with its economic importance in tropical and subtropical regions of the world, make it a species worthy of further study.
The Dioecious Angiosperms

Silene latifolia is a dioecious species with individual plants producing either all female or all male flowers. As it is by far the best characterized dioecious species to date, our review of sex-determining mechanisms in dioecious plants will focus on this species. In male and female S. latifolia flowers, the gynoecium and androecium initiate but arrest development prematurely, leading to functionally unisexual flowers (Grant et al., 1994). The sexual phenotype of individuals is determined by sex chromosomes; males are heterogametic (XY) and females are homogametic (XX). Early cytogenetic studies of sex-determining mutants in S. latifolia led Westergaard (1946, 1958) to conclude that the Y chromosome is divided into three regions relevant to sex expression: one required for the suppression of female development and two required for the promotion of male development. None of these regions would be necessary for the development of female reproductive organs, because these functions would reside on the X or autosomal chromosomes. Additional sex-determining mutants have been generated recently by x-ray mutagenesis of pollen and selecting both hermaphrodites and asexual F1 progeny (Farbos et al., 1999; Lardon et al., 1999; Lebel-Hardenack et al., 2002). These mutants verify the earlier work of Westergaard (1946, 1958; his lines were apparently lost) and have resulted in the identification of two additional classes of mutants, those that are not Y linked and hermaphroditic and those that are Y linked and asexual. The hermaphroditic deletion mutants are likely to contain a gene(s) necessary for the female-suppressing function, whereas the asexual deletion mutants likely contain the male-promoting gene(s). Genetic screens to identify mutant XX hermaphrodites or asexuals have not been reported. Although these sex-determining genes have not been cloned, the construction of an amplified fragment length polymorphism map of the Y chromosome using lines deleted for overlapping regions of the Y chromosome will be useful for genetic and physical mapping of the sex-determining mutants (Lebel-Hardenack et al., 2002) and may ultimately lead to their cloning.

Another approach to identify sex-determining genes in S. latifolia has been to clone genes that are expressed specifically in the male flowers and determine their linkage to the Y chromosome (reviewed by Charlesworth, 2002). Of the >50 isolated genes that have been correlated with sex expression in S. latifolia to date, only the four listed in Table 1 have been shown to be linked to the Y chromosome. Genes linked exclusively to the Y chromosome have not been found, because all of the Y-linked genes have X or autosomal homologs. These data indicate that the S. latifolia Y chromosome most closely resembles the X-degenerate class of sequences of the human Y chromosome. Genes within this class have an X homolog, the X and Y homologs encode similar but nonidentical isoforms, many of them are ubiquitously expressed, and all are present in low copy number in the genome (Skaletsky et al., 2003). As shown in Table 1, many of these features are shared with the Y-linked genes of S. latifolia.

Although molecular approaches have not yet succeeded in identifying the major regulatory sex-determining genes in S. latifolia, this work has and will continue to test theories of how Y chromosomes evolved from an ancestral pair of autosomes in plants (Charlesworth, 1996, 2002; Negrutiu et al., 2001). These theories state that once mutations that result in genetically determined males (where female genes are repressed) and females (where male genes are repressed) occur, recombination between the sex-determining genes must be suppressed to avoid recombinant asexual or hermaphroditic offspring. Another consequence of nonrecombination is the certainty that unisexual male and female offspring will be produced in equal ratios. Recombination between homologous chromosomes often is suppressed by the accumulation of chromosomal inversions on one homolog (in this case, the Y). The lack of X-Y recombination would eventually lead to degeneracy and loss of gene function on the Y chromosome, with the exception of genes required for male fertility and those necessary to suppress female fertility. The S. latifolia Y chromosome appears not to fit this paradigm for several reasons. First, the Y chromosome is 1.4 times larger than the X chromosome and is largely euchromatic, indicating that it may not be degenerate (Ciupercescu et al., 1990; Grabowska-Joachimiak and Joachimiak, 2002). Second, measurements of DNA polymorphism in sex-linked gene pairs have revealed that although there is no Y chromosome by S. latifolia (Farleigh et al., 1999), it is likely that the S. latifolia Y chromosome is at a relatively early stage of evolution (Charlesworth, 2002). Comparative sequencing of the S. latifolia sex chromosomes will be important in understanding the evolution of the Y chromosome in plants, especially compared with the Y chromosome of Marchantia and the sex-determining chromosome region of papaya.

Table 1. Sex Chromosome–Linked Genes in S. latifolia

<table>
<thead>
<tr>
<th>Y-Linked Gene</th>
<th>X-Linked Gene</th>
<th>Male-Specific Expression</th>
<th>Function</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>SIY-1</td>
<td>SIX-1</td>
<td>SIY-1 yes, SIX-1 no</td>
<td>WD-repeat protein</td>
<td>Delichere et al., 1999</td>
</tr>
<tr>
<td>SIY-4</td>
<td>SIX-4</td>
<td>No</td>
<td>Fructose-2,6-bisphosphatase</td>
<td>Atanassov et al., 2001</td>
</tr>
<tr>
<td>MRO3-Y&lt;sup&gt;a&lt;/sup&gt;</td>
<td>MRO3-X&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Yes</td>
<td>Unknown</td>
<td>Matsunaga et al., 1996; Gutman and Charlesworth 1998</td>
</tr>
<tr>
<td>DD44Y</td>
<td>DD44X</td>
<td>No</td>
<td>Oligomycin sensitivity-conferring protein</td>
<td>Moore et al., 2003</td>
</tr>
</tbody>
</table>

<sup>a</sup>The Y homolog is inactive and the X homolog is active.
FUTURE DIRECTIONS

Sex determination in plants is a fundamental developmental process that is particularly important for economic reasons, because the sexual phenotypes of commercially important crops dictate how they are bred and cultivated. Although most crop plants are not considered model systems—and sex determination is not a problem that can be addressed in the model angiosperm Arabidopsis—the economic value in manipulating the sexual phenotypes of crop plants should continue to drive interest in this area of research. Recent studies of sex-determining mechanisms have demonstrated clearly that angiosperms, including crop plants, have evolved a variety of sex-determining mechanisms that involve a number of different genetic and epigenetic factors, from sex chromosomes to plant hormones. Although the determinants of sexual phenotype are diverse, determining whether the downstream master sex-regulatory genes that specify male or female development are held in common or not will require cloning the sex-determining genes from a variety of plant species.

Choosing to study sex determination in plants representing other major land plant lineages will allow several broader developmental and evolutionary questions to be addressed. One unresolved question is how heterospory evolved from homosporous sexual phenotypes. By identifying the sex-determining genes in homosporous plants such as Ceratopteris and examining the expression of possible homologous genes in closely related heterosporous species, one can test the hypothesis that the switch from homosporous to heterosporous involved a heterochronic shift in the timing of expression of these genes from the gametophyte to the sporophyte generation. Other questions to be resolved are how sex chromosomes evolved in plants and whether similar processes led to distinct sex chromosomes in plants and animals. Comparing the Y chromosome sequences of Marchantia and *S. latifolia*, for example, will be invaluable in understanding how male-promoting genes and female-suppressing genes became localized to a Y chromosome and how recombination between the Y and its homolog was and continues to be suppressed.

ACKNOWLEDGMENTS

Support was provided by the National Science Foundation (MCB-9723154). This is journal paper 17271 of the Purdue University Agricultural Experiment Station.

Received August 25, 2003; accepted January 19, 2004.

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