THE PROBLEM OF SEX DETERMINATION MECHANISMS IN FLOWERING PLANTS

The evolution of single-sexed flowers in angiosperms is a complex and confusing topic. The complexity comes from how we define sex determination, how we distinguish genetic vs. environmental sex determination systems, the existence of multiple systems of sexuality in populations from monoecy to dioecy and intermediate systems including hermaphrodites, and the role of selection in establishing different sexual systems and in sexual plasticity. The confusion stems from our desire to generate discontinuous categories and unified processes, when categories are continuous and processes are independent. Basically, the focus of studies in plant sex evolution falls into three independent but overlapping categories and unifed processes, when categories are continuous and processes are independent. Consequently, differential concentrations of plant hormones can regulate whole developmental pathways, providing a mechanism for differential development within isogenic individuals such as seen in monoecious plants. Sex-determining genes in such systems will evolve to generate clusters of coexpressed suites. Coexpression rather than coinheritance of gender-specific genes will define the sexual developmental fate. Therefore, selection for gender type will drive evolution of the regulatory sequences of such genes rather than their synteny. Subsequent mutations to hyper- or hyposensitive alleles within the hormone response pathway can result in segregating dioecious populations. Simultaneously, such developmental systems will remain sensitive to external stimuli that modify hormone responses.

Key words: dioecy; environmental sex determination; evolution; flower development; genetic sex determination; hormone crosstalk; hormone regulation; monoecy; sexual plasticity.

SPECIAL INVITED PAPER—EVOLUTION OF PLANT MATING SYSTEMS

HORMONAL INTERACTIONS AND GENE REGULATION CAN LINK MONOECY AND ENVIRONMENTAL PLASTICITY TO THE EVOLUTION OF DIOECY IN PLANTS

EDWARD M. GOLENBERG AND NICHOLAS W. WEST

Department of Biological Sciences, Wayne State University, Detroit, Michigan 48202 USA

Most models for dioecy in flowering plants assume that dioecy arises directly from hermaphroditism through a series of independent feminizing and masculinizing mutations that become chromosomally linked. However, dioecy appears to evolve most frequently through monoecious grades. The major genetic models do not explain the evolution of unisexual flowers in monoecious and submonoecious populations, nor do they account for environmentally induced sexual plasticity. In this review, we explore the roles of environmental stress and hormones on sex determination, and propose a model that can explain the evolution of dioecy through monoecy, and the mechanisms of environmental sex determination.

Environmental stresses elicit hormones that allow plants to mediate the negative effects of the stresses. Many of these same hormones are involved in the regulation of floral developmental genes. Recent studies have elucidated the mechanisms whereby these hormones interact and can act as switchpoints in regulatory pathways. Consequently, differential concentrations of plant hormones can regulate whole developmental pathways, providing a mechanism for differential development within isogenic individuals such as seen in monoecious plants. Sex-determining genes in such systems will evolve to generate clusters of coexpressed suites. Coexpression rather than coinheritance of gender-specific genes will define the sexual developmental fate. Therefore, selection for gender type will drive evolution of the regulatory sequences of such genes rather than their synteny. Subsequent mutations to hyper- or hyposensitive alleles within the hormone response pathway can result in segregating dioecious populations. Simultaneously, such developmental systems will remain sensitive to external stimuli that modify hormone responses.

Key words: dioecy; environmental sex determination; evolution; flower development; genetic sex determination; hormone crosstalk; hormone regulation; monoecy; sexual plasticity.

GENETIC SEX DETERMINATION VS. ENVIRONMENTAL SEX DETERMINATION: A TRUE DICHOTOMY?

Sex determination systems are often categorized as being genetic sex determination (GSD) or environmental sex determination (ESD). With the greater appreciation of the role of gene regulatory mechanisms in affecting phenotypes compared with older concepts of purely allelic differentiation, this distinction has become rightfully blurred. A more nuanced definition of GSD is the developmental process wherein alternative alleles or linked genomic regions control alternative sexual development by segregation in each generation. ESD is then defined as the process wherein environmental cues trigger the alternative developmental pathways (Charlesworth and Mank, 2010). Of course, it is implicit in the definition of ESD that the alternative developmental pathways are controlled by differential expression of developmental genes and that environmental input regulates that gene expression. We can conceptually rephrase the distinction between GSD and ESD in a different way. In GSD, sexes segregate developmentally by meiotic segregation of...
alternative alleles, whereas in ESD, sexual segregation is due to external regulation of gene expression. In GSD, the expression patterns are mostly invariable while the gene content must vary. In ESD, the gene content is mostly invariable while the expression patterns must vary. This redefinition means that both sex determination systems are genetic, and their distinction should be thought of in terms of the evolution of coding vs. regulatory sequences. Even this distinction need not be exact, as both coding and cis regulatory regions can potentially vary and segregate over generations.

GSD AND CHROMOSOMAL EVOLUTION

Historically, ESD was considered as essentially distinct from having a genetic component. This viewpoint left the problem of the evolution of unisexual systems to be considered tractable only in the restrictive GSD systems. Certainly in the field of plant sexual evolution, the most influential paper is still Charlesworth and Charlesworth (1978). In this paper, the ancestral state of the reproductive system is assumed to be hermaphroditic. In its simplest form, the model postulates three sequential, independent mutational events. In the first mutation, a mutant recessive allele will block the functionality of one sexual output when an individual is homozygous. For theoretical reasons and supported by empirical observations, this mutation is generally feminizing, that is, it blocks male reproductive output. Should the new females be more fit in producing offspring than the hermaphrodites (the conditions of which are reviewed in Charnov (1982)), the population will become gynodioecious, being made up of hermaphrodites and females. A second, independent, dominant mutation that suppresses the second, usually female, sexual output occurs in hermaphrodites. The result is a population with hermaphrodites, males, and females. Again, selection over the hermaphrodites leads to the establishment of a dioecious population. However, to prevent the production of hermaphrodites and neuters in each new generation, a third mutation must occur to prevent recombination between the two independently mutated genes. This suppression of recombination is generally thought to occur by linkage of the two genes and then chromosomal inversion. The chromosomal inversion prevents recombination within the inverted region due to the generation of lethal duplications, deletions, acentic chromosomes, and/or dicentric chromosomes when recombination does occur, thus leading to the incipient establishment of sex chromosomes.

Theoretical population genetic modeling has helped define the conditions under which dioecy can evolve in the Charlesworth and Charlesworth model. Furthermore evidence of intermediate states of gynodioecious and even the less common androdioecious populations supports the evolutionary progression that is predicted by the model. Last, the model’s inherent strength resides in its prediction of evolution of nonrecombining regions or whole chromosomes that are linked to sex determination. Nonrecombining chromosomes will accrue mutations including large insertions or deletions, and these will be detectable in sexually dimorphic chromosomes. Studies of the chromosomal constitution of a few species have been of prime importance in following the evolution of dioecy in plant species as predicted by the model (Clark et al., 1993; Guttmann and Charlesworth, 1998; Atanassov et al., 2001; Lebel-Hardenack et al., 2002; Bergero et al., 2007; Hobza et al., 2007; Ming and Moore, 2007; Ming et al., 2007; Zhluvova et al., 2007; Armstrong and Filatov, 2008; Bergero et al., 2008; Charlesworth, 2008; Blas et al., 2009; Filatov et al., 2009; Yu et al., 2009; Bergero and Charlesworth, 2011; Chibalina and Filatov, 2011). Yet despite these well-documented examples, there are still relatively few unequivocal examples of dimorphic sex chromosomes in plants. Possibly this lack is due to the relatively recent evolution in each of the species such that there has been insufficient time for extensive losses or rearrangements in the nonrecombining chromosomes (Bachtrog, 2011).

EXCEPTIONS TO GSD AND CHROMOSOMAL EVOLUTION: MONOECY AND ENVIRONMENTAL PLASTICITY

An alternative explanation for the scarcity of dimorphic chromosomes in dioecious species is that the Charlesworth and Charlesworth model may not apply in all cases. The model predicts the development of unisexual flowers and dioecy through intermediate steps of gyno- or androdioecy. The model is not at all applicable to the development of unisexual flowers in monoecious (or gyno- or andromonoecious) species or when dioecy evolves through an intermediate step of monoeocy. This progression is actually very common (Renner and Ricklefs, 1995; Renner and Won, 2001). Early, Yampolsky (1920) reported that 47 families with dioecious species also had monoecious species and developed through a monoecious or polygamous grade compared to 14 families that had dioecious species with no monoecious or polygamous species. 42 families had only monoecious or monoecious and hermaphroditic species. Indeed, the Charlesworth and Charlesworth model is useful only when unisexual flower development is controlled by GSD sensu stricto, that is, by allelic segregation. In monoecious species, the development of unisexual flowers cannot be due to allelic segregation within the individual plants among individual floral meristems. Key sex-determining genes must exist in these species, but the alternative expression states of such genes cannot be regulated by segregation of alleles. Rather independent cues, either external environmental or internal physiological, must regulate their expression. Internal heterogeneity within a plant can be sufficient to determine sexual, developmental differentiation (P. K. Diggle, University of Colorado at Boulder, personal communication).

Examples of independent cues for differential sexual development are common and usually are presented as either environmental cues (ESD s.s.) or hormonal cues. In either case, the developmental processes must be put within an evolutionary context precisely because individual reproductive success and hence individual fitness is directly affected. Because of this, sexual development in response to environment, especially sexual plasticity by which individuals can change sex in dioecious populations or the proportion of one sex to another in monoecious individuals, is often studied in terms of adaptive responses.

The distribution of genders or sex ratio is a common example of frequency-dependent selection (Fisher, 1930; Charnov, 1982). In all diploid organisms undergoing sexual reproduction, the male parent will contribute one half of the genome, and the female parent will contribute one half. In other words, the contributions to the autosomal nuclear genome are equal. When sex ratios deviate from 50:50, individuals from the rarer sex will on average have a higher contribution to the next generation, that is, they will have a higher fitness. The strength of the fitness
differential decreases as the population approaches a 50:50 ratio. Thus, other things such as cost of development and differential survivorship being equal, selection will drive and maintain population sex ratios at 50:50.

The question becomes different when individuals can change sex or can differentially allocate their own proportion of maleness vs. femaleness. Under these scenarios, an individual could differentially increase its fitness by either changing sex or changing the proportion of the sexes in response to varying conditions. These environmental conditions include not only sex ratios themselves, but also resource and energy availability. Energetic costs for gamete production and zygotic development differ between males and females. Limited resources therefore can affect the number of viable gametes or zygotes produced, and severe energy sinks into reproduction can reduce viability. So while the total male and female contribution to the offspring generation must remain 50:50 and costs for gamete and offspring development may remain constant, deviations in sex ratios and environmental constraints can make sexual plasticity advantageous to an individual in a temporally variable environment. Further deviations from the conditions of a simple 50:50 sex ratio occur when individuals contribute differently to the subsequent generation such as under conditions of gynodioecy or in haploid-diploid sexual systems (Charnov, 1982).

Most commonly, changes in sex ratios are studied in relation to environmental stresses that are assumed to put energy constraints on individual plants. It is usually assumed that ovule and seed development are energy-demanding processes, but they have a higher probability of contributing to offspring than does pollen production. Therefore environments that lead to robust growth should make being female advantageous. Alternatively, low resource availability and reduced productivity would reduce ovule production, decrease seed and fruit development, and, perhaps, lower survivorship. Plants that can switch sex could increase their inclusive fitness by increasing their maleness, thereby potentially contributing to the next generation through energy-cheap pollen (Lloyd, 1979; Charnov, 1982). Therefore, the ability to switch sex in response to the environment in such a way that the individual's number of offspring increases over its lifetime would be considered adaptive in an ecological sense. Of course, to be adaptive in an evolutionary sense, this sexual plasticity, including the mechanisms to perceive and developmentally respond to environmental stress, must have had a polymorphic, genetic basis.

ENVIRONMENTAL STRESS, PLANT HORMONES, AND SEX DETERMINATION

What evidence is there for environmental cues and sexual plasticity in plant populations? Korpelainen (1998) wrote an extensive review of studies of environmentally induced sex change in plants. Multiple studies since that time have added to the database of sexual plasticity (Wolfe and Shmida, 1997; Decker and Pinson, 2000; Dafni and Shmida, 2003; Dorken and Barrett, 2004; Dorken and Pannell, 2008; Stehlik et al., 2008; Adam et al., 2011). We will pay specific attention to examples among angiosperms. We summarize a sampling of the data in Table 1A and list species and their response by specific environmental stresses.

Some broad patterns appear to occur. Environmental conditions that stress the plants or reduce their growth or productivity tend to bias plants away from developing seed-producing, either female or hermaphroditic flowers. Drought tends to be a common masculinizing stress. Thirteen of 14 species respond to drought by increasing masculine output either by increasing the proportion of staminate flowers on monoecious individual, by increasing staminate plants in dioecious or androdioecious populations, or by increasing the proportion of hermaphrodites in gynomonoecious or gynodioecious populations. Pickett (1915) and Atkinson (1898) reported increased ratios of male to female flowers in *Artsaema trilophyllum* in response to drought. Similar responses were reported in *Ochradenus baccatus*, *Colchicum stevenii*, *Solanum carolinense*, *Elaeis guineensis*, and *Atriplex canescens* (McArthur, 1977; Solomon, 1985; Dafni and Shmida, 2003; Adam et al., 2011). Yet exceptions to this pattern exist. *Acer rufinerve* produced more female flowers and seed in response to drought (Nanami et al., 2004). Indeed, Nanami et al. (2004) reported greater feminization in response to stress as seen in dying trees or in trees with reduced growth. Additional environmental conditions that would be expected to increase growth and productivity such as high light intensity vs. shade or highly fertilized soil vs. nutrient poor soil also tend to increase the ratio of females to males (Lovett-Doust and Cavers, 1982). Here too, exceptions occur. Solomon (1985) reported that the male ratio increases in *Solanum carolinense* in response to shade and drought as expected, but also increases in fertilized soil. Increased light intensity actually increases the male to female ratio in *Cannabis sativa* (Bothwick and Scully, 1954). Last, plant size, a summary measure of prior growth success and a surrogate measure of available energy, is correlated with feminization. Policansky (1981) plotted the reproductive gain with size in terms of male vs. female productivity. He found that the rate of gain by mass is steeper for females than males. The y intercepts also differed as the costs for producing female flowers and seeds are relatively high. He predicted the size at which the sex ratio should shift to mostly female by the intercept of the reproductive success lines. This prediction was closely supported by field data. Dafni and Shmida (2003) reported greater feminization with increased corn size, and Condon and Gilbert (1988) reported sex changes to female plants with size in *Gurania* and *Psiguria*. Yet, here, too, exceptions exist (Solomon, 1985).

Numerous studies have also recorded the effects of hormones on alternative or plastic sexual development. As with the environmental effects, many hormones have similar masculinizing or feminizing effects in independent species; however, there are no purely masculinizing or feminizing hormones (Table 1B). Gibberellic acid has a masculinizing effect in *Solanum carolinense*, *Asparagus officinalis*, *Coriandrum sativum*, *Cannabis sativus*, and *Spinacia oleracea* (Atal, 1959; Amruthavalli, 1978; Chailakhyan and Khryanin, 1978; Lazarte and Garrison, 1980; Solomon, 1985) and has a feminizing effect in *Luffa acutangula*, *Hyoscyamus niger*, and *Zea mays* (Resende and Viana, 1959; Bose and Nitsch, 1970; Hansen et al., 1976). Auxin has a feminizing effect in *Opuntia stenopetala*, *Cannabis sativus*, *Silene pendula*, *Spinacia oleracea*, and *Cucumis* sp., (Heslop-Harrison and Heslop-Harrison, 1958; Chailakhyan and Khryanin, 1978; Malepszy and Niemirowicz-Szczytt, 1991; Orozco-Arroyo et al., 2012), but is masculinizing in *Mercurialis annua* and *Cloe men spinosa* (de Jong and Bruinsma, 1974; Hamdi et al., 1987; Durand and Durand, 1991). Similarly, cytokinins have generally been found to be feminizing in *Mercurialis annua*, *Vitis vinifera*, *Spinacia oleracea*, *Asparagus officinalis*, and *Solanum carolinense* (Negi and Olmo, 1966; Chailakhyan and Khryanin, 1978;
Chung et al., 1979; Dauphin-Guerin et al., 1980; Lazarte and Garrison, 1980; Solomon, 1985). Ethylene is among the most tracked hormone; its feminizing role in cucurbit flower development has been very well studied (Atsmon and Tabbak, 1979; Yin and Quinn, 1992, 1995; Trebitsh et al., 1997; Kahana et al., 1999; Krupnick et al., 1999; Yamasaki et al., 2001; Mibus and Tatlioglu, 2004; Boualem et al., 2008; Martin et al., 2009). Yet, even here, exceptions occur as ethylene is masculinizing in watermelon (Rudich cited in Grumet and Taft, 2011). Last, a number of additional plant hormones such as abscisic acid (feminizing in Solanum carolinense), and jasmonate and brassinosteroids (both masculinizing in Zea mays) have been reported to contribute to sexual differentiation (Solomon, 1985; Browse, 2009; Hartwig et al., 2011).

PLANT HORMONES IN RESPONSE TO ENVIRONMENTAL STRESS

The relationship of hormonal response to environmental stresses and sex determination is rarely defined, although the hormonal response to environmental stress alone is well studied in many cases. Recent reviews have outlined the role of ethylene and other phytohormones in a plant’s response to abiotic and biotic stress (Anderson et al., 2005; Glazebrook, 2005; Broekaert et al., 2006; Bari and Jones, 2009; Kazan and Manners, 2009). Ethylene is known to be produced in all plant cells during development with the highest rates of production seen in meristematic, stressed and ripening tissues (Abeles et al., 1992). Ethylene pathway or response genes have been activated to confer tolerance to salt stress in tobacco (Park et al., 2001), to low and high temperatures, mechanical wounding, salt exposure and flooding in potatoes (Nie et al., 2002; Lee et al., 2007), and flooding in rice (Xu et al., 2006). Abiotically induced osmotic stress such as caused by drought or salt accumulation triggers the increased production of abscisic acid (Nambara and Marion-Poll, 2005). Among the biotically induced hormones, both auxin and jasmonic acid are involved in response to necrotrophic fungal pathogens, whereas salicylic acid is activated in some bacterial pathogenic responses (Kazan and Manners, 2009). Herbivory, an additional biotically induced stress, triggers jasmonic acid accumulation that then activates a defense response (McConn et al., 1997; Devoto et al.,

### Table 1

Sample list of species that are reported to manifest changes in sexual composition of individuals in response to external stimuli.

<table>
<thead>
<tr>
<th>Species</th>
<th>Stress</th>
<th>Effect</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Response to environmental stresses</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acer grandidentatum</td>
<td>Drought</td>
<td>Masculinizing</td>
<td>Barker (1985)*</td>
</tr>
<tr>
<td>Acer rufinerve</td>
<td>Drought</td>
<td>Feminizing</td>
<td>Nanami et al. (2004)</td>
</tr>
<tr>
<td>Elaeis guineensis</td>
<td>Drought</td>
<td>Masculinizing</td>
<td>Adam et al. (2011)</td>
</tr>
<tr>
<td>Colchicum stevenii</td>
<td>Drought</td>
<td>Masculinizing</td>
<td>Dafni and Shmida (2003)</td>
</tr>
<tr>
<td>Solanum carolinense</td>
<td>Drought</td>
<td>Masculinizing</td>
<td>Solomon (1985)*</td>
</tr>
<tr>
<td>Zea mays</td>
<td>Resource abundance</td>
<td>Feminizing</td>
<td>Minina (1938)*</td>
</tr>
<tr>
<td>Solanum carolinense</td>
<td>Resource abundance</td>
<td>Masculinizing</td>
<td>Solomon (1985)*</td>
</tr>
<tr>
<td>Cucumis melo</td>
<td>Resource abundance</td>
<td>Feminizing</td>
<td>Minina (1938)*</td>
</tr>
<tr>
<td>Mercurialis annua</td>
<td>High density</td>
<td>Masculinizing</td>
<td>Dorken and Pannell (2008)</td>
</tr>
<tr>
<td>Rumex nivalis</td>
<td>High density</td>
<td>Feminizing</td>
<td>Orozco-Arroyo et al. (2012)</td>
</tr>
<tr>
<td>Cannabis sativa</td>
<td>High light intensity</td>
<td>Masculinizing</td>
<td>Borthwick and Scully (1954)*</td>
</tr>
<tr>
<td>Arisaema triphyllum</td>
<td>High light intensity</td>
<td>Feminizing</td>
<td>Lovett-Doust and Cavers (1982)*</td>
</tr>
<tr>
<td>Elaeis guineensis</td>
<td>High light intensity</td>
<td>Feminizing</td>
<td>Berti (1982)*</td>
</tr>
<tr>
<td>Solanum carolinense</td>
<td>Shade</td>
<td>Masculinizing</td>
<td>Solomon (1985)*</td>
</tr>
<tr>
<td>Catasetum ventricosum</td>
<td>Shade</td>
<td>Masculinizing</td>
<td>Gregg (1973)*</td>
</tr>
<tr>
<td>Zea mays</td>
<td>Shade</td>
<td>Feminizing</td>
<td>Rood (1980)</td>
</tr>
<tr>
<td>Cynoches densiflorum</td>
<td>Shade</td>
<td>Feminizing</td>
<td>Gregg (1973, 1975)*</td>
</tr>
<tr>
<td>Xanthium pennsylvanicum</td>
<td>N abundance</td>
<td>Masculinizing</td>
<td>Neidle (1939)</td>
</tr>
<tr>
<td>Cleome spinosa</td>
<td>N abundance</td>
<td>Feminizing</td>
<td>De Jong and Bruinsma (1974)*</td>
</tr>
<tr>
<td>Cucumis melo</td>
<td>N abundance</td>
<td>Feminizing</td>
<td>Brantley (1960)*</td>
</tr>
<tr>
<td>Spinacia oleracea</td>
<td>N abundance</td>
<td>Feminizing</td>
<td>Thompson (1955)*</td>
</tr>
<tr>
<td>B. Response to exogenous hormone applications</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cannabis sativa</td>
<td>Auxin</td>
<td>Feminizing</td>
<td>Harilbach (1956)*</td>
</tr>
<tr>
<td>Cucumis sativus</td>
<td>Auxin</td>
<td>Feminizing</td>
<td>Laibach (1950a,b); Wittwer (1954)*</td>
</tr>
<tr>
<td>Humulus lupulus</td>
<td>Auxin</td>
<td>Masculinizing</td>
<td>Harilbach (1963)*</td>
</tr>
<tr>
<td>Mercurialis annua</td>
<td>Auxin</td>
<td>Masculinizing</td>
<td>Hamdi et al. (1987)</td>
</tr>
<tr>
<td>Silene pendula</td>
<td>Auxin</td>
<td>Feminizing</td>
<td>Harilbach and Harilbach (1958)*</td>
</tr>
<tr>
<td>Opuntia sternopetala</td>
<td>Auxin</td>
<td>Feminizing</td>
<td>Orozco-Arroyo et al. (2012)</td>
</tr>
<tr>
<td>Cannabis sativa</td>
<td>Gibberellin</td>
<td>Masculinizing</td>
<td>Atal (1959); Chailaklyan and Khryanin (1978)</td>
</tr>
<tr>
<td>Cucumis sativus</td>
<td>Gibberellin</td>
<td>Masculinizing</td>
<td>Friedlander (1977)*</td>
</tr>
<tr>
<td>Coreopodium latumatum</td>
<td>Gibberellin</td>
<td>Feminizing</td>
<td>Arthryathali (1978)*</td>
</tr>
<tr>
<td>Hyoscyamus niger</td>
<td>Gibberellin</td>
<td>Feminizing</td>
<td>Resende and Viana (1959)*</td>
</tr>
<tr>
<td>Spinacia oleracea</td>
<td>Gibberellin</td>
<td>Masculinizing</td>
<td>Chailaklyan and Khryanin (1978)</td>
</tr>
<tr>
<td>Cucumis sativus</td>
<td>Ethylene</td>
<td>Feminizing</td>
<td>McMurrray and Miller (1968)*</td>
</tr>
<tr>
<td>Cucumis melo</td>
<td>Ethylene</td>
<td>Feminizing</td>
<td>Byers et al. (1972)</td>
</tr>
<tr>
<td>Citrullus lanatus</td>
<td>Ethylene</td>
<td>Masculinizing</td>
<td>Rudich (1990)</td>
</tr>
</tbody>
</table>

PLANT HORMONES AND FLORAL DEVELOPMENT

How can hormone fluctuations affect sexual development or floral development in general? The normal development of a plant flower involves the orchestration of numerous environmental and developmental conditions that would allow successful seed production for the particular species. This assessment is then translated into the commitment to reproductive growth from vegetative growth. There are generally considered to be three pathways that can trigger the conversion to reproductive growth or the initiation of the floral meristem, the autonomous pathway, the vernalization pathway, and the photoperiod pathway (Albani and Coupland, 2010; Amasino, 2010). These independent pathways eventually converge through suppression of stamen development, jasmonate has recently been observed to be involved in phyllotactic leaf initiation in which coordinated polar auxin transport causes areas of high auxin concentration that fore-shadow the location of a new leaf. Based on the development of local auxin maxima during vegetative growth, a recent simulation accurately predicted the organization of floral patterning by modeling the probable location of auxin maxima in a developing floral meristem (van Mourik et al., 2012). This highlights auxin’s crucial function in floral morphogenesis.

Cytokinin is best known for its involvement in regulating the size of a plant’s apical meristem. Less is known about cytokinin’s involvement in floral development. A recent study of cytokinin overproduction in floral tissues suggested that the hormone influences the number and development of flowers through the regulation of floral meristem size (Li et al., 2010b). The transgenic plants overexpressing cytokinin produced an excess of flower primordia, and the flowers developed extra organs per-whorl. The mechanism by which cytokinin affects flower size and organ number is through its regulation of the meristem maintenance gene CLAVATA 1 (CLV1) and its regulatory effect on WUSCHEL (WUS) (Lindsay et al., 2006). In addition to cytokinin’s early influence over flower development, the hormone has also been observed to be involved in the development of gynoecia. Recent studies have observed that cytokinin plays a role in gynoecia and fruit morphogenesis and patterning (Marsch-Martinez et al., 2012) as well as having an effect on seed number (Barrtrina et al., 2011).

Jasmonic acid and its metabolites are also involved in flower development. It has been known for some time that jasmonate signaling is required for stamen and pollen maturation (Stintzi and Browse, 2000; Ishiguro et al., 2001; Park et al., 2002). Mutants that are defective in the synthesis or signaling of jasmonates are male-sterile. A recent study has shown that the floral organ identity gene AG controls late stage stamen maturation in Arabidopsis though regulation of a jasmonate biosynthesis gene (Ito et al., 2007). In addition to its involvement in stamen development, jasmonate has recently been observed to

<table>
<thead>
<tr>
<th>Hormone</th>
<th>Effect</th>
<th>Proposed mode of action</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gibberellic acid</td>
<td>Initiates flowering</td>
<td>Activation of SCL, LFY expression</td>
<td>Blázquez et al. (1998); Blázquez and Weigel (2000)</td>
</tr>
<tr>
<td>Gibberellic acid</td>
<td>Promotes petal, stamen, and anther development</td>
<td>Controls cell elongation in petals and filaments, required for microsporangial development into pollen</td>
<td>Cheng et al. (2004); Hou et al. (2008)</td>
</tr>
<tr>
<td>Auxin</td>
<td>Initiation of flowering, gynoecium structure, floral organ abscission, silique ripening</td>
<td>Activation of ARF2 YUCCA1 activated by STY1</td>
<td>Ellis et al. (2005); Sohlgberg et al. (2006)</td>
</tr>
<tr>
<td>Cytokinin</td>
<td>Cell division, suppression of stamen development, enhancement of carpeloid development</td>
<td>Activation of WUS Activated by SUP</td>
<td>Gordon et al. (2009); Ishiguro et al. (2001); Nibau et al. (2010)</td>
</tr>
<tr>
<td>Jasmonate</td>
<td>Stamen maturation, anther morphogenesis, dehiscence</td>
<td>Activated by AG</td>
<td>Ito et al. (2007)</td>
</tr>
<tr>
<td>Brassinosteroid</td>
<td>Anther and pollen development, initiation of flowering</td>
<td>Activate SPLINZZ, TDF1, AMS, MS1, MS2 Suppression of FLC</td>
<td>Yu et al. (2008); Li et al. (2010)</td>
</tr>
<tr>
<td>Ethylene</td>
<td>Delays flowering</td>
<td>Promotes accumulation of DELLA proteins. Suppresses SCL1, LFY</td>
<td>Achard et al. (2007)</td>
</tr>
</tbody>
</table>

Notes: Gene identifications not noted in the text: AUXIN RESPONSE FACTOR2 (ARF2); STYLISH1 (STY1); SUPERMAN (SUP); SPOROCYTELESS/NOZZLE (SPLINZZ); TAPETAL DEVELOPMENT AND FUNCTION1 (TDF1); ABORTED MICROSPORES (AMS); MALE STERILITY1 (MS1); MALE STERILITY2 (MS2); FLOWERING LOCUS C (FLC).
play a role in the late stages of petal development (Brioudes et al., 2009).

Brassinosteroids have been shown to be involved with cell expansion and growth in addition to being required for male fertility. Mutant studies exhibiting a deficiency of brassinosteroid signaling caused dwarf plants (Choe et al., 2000) and reduced male fertility (Ye et al., 2010). In maize, mutants in the brassinosteroid synthesis pathway results in dwarf and tasselseed phenotypes (Hartwig et al., 2011). Earlier in the flowering pathway, brassinosteroids have been demonstrated to induce flowering through suppression of FLOWERING LOCUS C (FLC) (Yu et al., 2008). Much research has been focused on signal transduction (Clouse, 2011) and identification of genes involved in brassinosteroid signaling (Shang et al., 2011), leaving the hormone’s involvement in flower development a relatively untapped field.

Ethylene plays a role in floral development, fruit ripening, and senescence. Its role in fruit ripening has long been known, and much study has occurred in industrially relevant species including tomato, avocado, apple, and banana. In tomato, ethylene was observed in the pistils (Llop-Tous et al., 2000), while in tobacco, ethylene was observed in the stigma, style, and ovary but not in its pollen and anthers (De Martinis and Mariani, 1999). Expression of ethylene receptors have been observed in the inflorescence, floral meristems, and developing petals and ovules of Arabidopsis (Sakai et al., 1998). Additionally ethylene has been implicated in the expression of floral organ identity genes in tomato. The expression of TAG-1, an AG orthologue, was suppressed by the ethylene inhibitor 1-MCP (Bartley and Ishida, 2007). Furthermore, stress-induced ethylene can delay flowering by repressing GA levels (Achard et al., 2007). As a result, repressor DELLA proteins accumulate and suppress SOC1 and LFY expression.

MECHANISMS OF HORMONE ACTION

From these discussions, we can recognize that environmental stress, as well as direct applications of plant hormones, can effect sexual plasticity in some species, environmental stress can cause up and down activation of plant hormones, and plant hormones are intrinsically involved in floral developmental regulation even under nonstress growth conditions. We can conclude that environmental regulation of plant sex development or ESD must be mediated by hormone response pathways. Of course, this conclusion is not completely novel. Heslop-Harrison and Heslop-Harrison (1957) postulated a relationship between light and auxin on sex determination. Also, Yin and Quinn (1992) proposed a general model wherein a single hormone could control sexual development. The functional relationship of environment and hormones is, however, often ignored. One reason for this is that the modes of actions and their effects on sexual development are largely unknown, and those that are becoming clearer to us are not universal. As discussed earlier, no specific hormone can be identified as being a masculinizing or feminizing hormone. However, in several species, groups of hormones do appear to act antagonistically. Abscisic acid and auxin generally have the opposite sexual developmental effect than does gibberellic acid. Cytokinins also appear to have the opposite effect to gibberellic acid, although it may also conflict with auxin in its effects. It is tempting to interpret this duality in light of the Charlesworth and Charlesworth model introduced earlier, where male-suppressing (feminizing) and female-suppressing (masculinizing) pathways independently evolve. Thus, in a given species, cytokinin may trigger the female flower development and gibberellic acid may control the male flower development. Each of these pathways would be independent. However, the evidence is that the activities of these hormones are not at all independent. For example, some of the actions of the hormones are not considered to be direct. de Jong and Bruinsma (1974) argued that the masculinization through pistil abortion in Cleome by auxin was rather a result of the loss of cytokinin due to the auxin concentration. Similarly salicylic acid can block the synthesis and effects of auxin. Increased salicylic acid or secondary metabolites due to bacterial pathogens can interfere with auxin by reducing auxin availability, auxin receptors (TIR1), or auxin transport (Peer and Murphy, 2007; Wang et al., 2007; Kazan and Manners, 2009).

Recent advances in our molecular understanding of hormone receptors and their modes of actions bring the conflicting phenotypic effects and anecdotal negative correlations into a clearer mechanistic framework. The overarching picture is that hormone effects are indeed not independent, and alternative hypotheses due to hormonal input are not simply the result of alternative activation of separate response pathways. Rather, individual hormones affect the response pathways of others through complex interactions based on regulation of biosynthesis/degradation, transport, or repressor proteins, all commonly lumped together under the concept of hormone crosstalk.

To begin to understand the nuances of hormone crosstalk and the relation of this crosstalk to environmental and hormonal sex determination in plants, it is useful to review the mechanisms of hormone regulation on gene expression. The first important general observation is that the majority of plant hormones cause activation of their downstream response elements through derepression rather than through activation. The second general observation is that the regulatory networks often use protein digestion through the 26S proteosome. Many response pathways use both elements.

Ethylene, abscisic acid, and brassinosteroid response pathways use kinases and phosphatases to regulate their downstream effects. Ethylene binds to a transmembrane receptor ETR1. When bound with ethylene, ETR1 represses the function of the CTR1 serine/threonine kinase. In the absence of the ethylene-triggered repression, CTR1 represses the activity of the positive transcription factors EIN2, EIN3, and EIL3, and these proteins are degraded by digestion in the proteosome (Alonso and Stepanova, 2004). The abscisic acid response pathway also uses a phosphatase/kinase signaling system (Umezawa et al., 2012). PP2C phosphatases act as negative regulators by dephosphorylating the activator SnRK2 kinase in the absence of abscisic acid (Umezawa et al., 2009; Vlad et al., 2009). Abscisic acid is bound to the PYR/PYL/RCAR receptor through the PYR1 subunit (Nishimura et al., 2009; Santiago et al., 2009). The abscisic acid-bound receptor complex then binds to PP2C phosphatases, inhibiting the PP2C activity and thereby derepresses the response system. Similar to the ethylene pathway, the brassinosteroid response pathway utilizes a membrane bound kinase, BR1, which becomes phosphorylated in the presence of brassinosteroid. The phosphorylated BR1 dissociates from the kinase inhibitor BKI1 and associates with an additional transmembrane kinase, BAK1. This phosphorylated complex inhibits the activity of the repressor kinase BIN2. In a remarkably analogous system to the CTR1 function in the ethylene pathway, BIN2 phosphorylates and inactivates the positive transcription factors BES1 and BZR1. Thus, brassinosteroids ultimately act to cause
dephosphorylation of BES1 and BZR1, and hence, once again, triggers activation of downstream response genes through derepression (Clouse, 2008).

Auxin, gibberellic acid, and jasmonate have strikingly analogous response pathways based on transcription repressor proteins. All three have cytoplasmic receptors. The first auxin receptor identified was TIR1, an F box protein that, in the presence of auxin, binds to a family of transcription repressor proteins AUX/IAA (Dharmasiri et al., 2005; Kepinski and Leyser, 2005). GID1, an F box protein first identified in rice, serves as the gibberellic acid receptor and similarly binds to the DELLA transcription repressor proteins in a GA-dependent manner (Ueguchi-Tanaka et al., 2005; Nakajima et al., 2006; Eckardt, 2007; Ueguchi-Tanaka et al., 2007; Shimada et al., 2008). Last, COI1, another F box protein, binds jasmonic acid and binds the repressor JAZ proteins in a JA-dependent manner (Devoto et al., 2002; Xu et al., 2002; Feng et al., 2003; Gfeller et al., 2006; Chini et al., 2007; Thines et al., 2007; Fonseca et al., 2009). All three receptor–hormone–repressor complexes interact with the SCF ubiquitination complexes to effect E3 ligase ubiquitination of the respective transcription repressor proteins. These proteins are then targeted for degradation by the 26S proteosome (Sparz and Gray, 2008). Remarkably, a recently described receptor for the hormone strigolactone also is an F box protein and forms SCF complexes in the presence of the hormone (Stirnberg et al., 2007; Hamiaux et al., 2012). An anticipated repressor protein has not been identified yet for this system.

HORMONE CROSSTALK

The similar modes of action of multiple hormones, particularly through the ubiquitination and degradation of repressors proteins, suggests a possible interaction due to simultaneous use of E3 SCF ubiquitin ligase complexes and the 26S proteosome. However, there is no evidence of competition for the ubiquitin ligases or for limiting rates of proteolysis through the proteosome. The major components of the SCF complexes in Arabidopsis are SKP-1 (ASK-1 in Arabidopsis), CULLIN-1, and RBX-1 (Patton et al., 1998; Gray et al., 1999; Zhao et al., 2010). The F box proteins provide the substrate specificity and, as previously discussed, often serve as hormone receptors thereby activating their interaction with target substrate. There are about 700 F box proteins proposed in Arabidopsis (Gagne et al., 2002).

There is growing evidence that either hormone-specific repressor proteins or the targets of those repressor proteins directly interact. MYC transcription factors activate defense pathways in response to various biotic and abiotic stresses. JAZ repressor proteins directly interact with MYC2 proteins and block their ability to activate downstream genes. In response to external stress, jasmonic acid (JA) is expressed and is perceived by the COI1 F box receptor. The COI1-JA complex then targets JAZ proteins for degradation. The degradation of the JAZ proteins releases the repression on MYC2 and thereby activates JA-mediated stress response (Chini et al., 2007). At the same time, JAZ proteins also interact with DELLA repressor proteins of the gibberellic acid (GA) pathway (Yang et al., 2012). These interactions sequester a pool of each of the repressor proteins from interacting with their target hormone response transcription factors, establishing an interactive balance (Fig. 1). An increase in one hormone in response to an environmental cue triggers the degradation of a repressor protein, thereby releasing the second repressor protein to further interact and repress its target transcription factor (Kazan and Manners, 2012). For example, PIF3 (Phytochrome Interacting Factor 3) activates cell elongation and hence plant growth. DELLA proteins bind to PIF3 and block its effect on growth (de Lucas et al., 2008; Feng et al., 2008). Thus, when JA levels increase due to stress, JAZ proteins are degraded. The MYC2 transcription factor activates stress response. Simultaneously, the degradation of the JAZ proteins releases DELLA proteins that interact with PIF3. Growth is thereby constrained while the plant responds to the external assaults. Alternatively, when GA levels increase, DELLA proteins are degraded, releasing JAZ proteins to interact with MYC proteins and thus suppressing defense responses (Yang et al., 2012). The result is the alternative regulation of two pathways through a single switchpoint. This interaction between repressor proteins is not limited to the gibberellic acid and jasmonate pathways. TOPLESS, a corepressor protein in the auxin response pathway, interacts with repressor proteins in the jasmonate, salicylic acid, and ethylene pathways (Arabidopsis Interactome Mapping Consortium, 2011).

There are several examples of hormone crosstalk at the biosynthesis, degradation, or transport levels. For example, brassinosteroid, another pathogen-response hormone, blocks GA 20-oxidase, which is the last step of the bioactive GA biosynthetic pathway and induces GA 2-oxidase, which deactivates bioactive GA (De Vleesschauwer et al., 2012). Analogously, ethylene can regulate the biosynthesis of auxin (Fig. 2). Ethylene activates expression of WEIZ/ASAI and WEIZ/ASBI (Stepanova et al., 2005). These are subunits of an enzyme involved in the rate-limiting step of Trp biosynthesis that then leads to increased auxin production in Arabidopsis root tips (Stepanova et al., 2005). In contrast, salicylic acid blocks the expression of the TIR1 gene. This gene encodes for the F-box auxin receptor protein that is required for the proteosomal degradation of the repressor AUX/IAA proteins. As a result, downstream auxin inductive genes are not transcribed. Additionally salicylic acid blocks transcription of auxin pump proteins AUX1 and PIN7 and thus interferes with the ability to transport and concentrate auxin (Wang et al., 2007). The plant hormone, strigolactone (Tschiya and McCourt, 2009) also is known to suppress auxin production in the stem and transport in the stem and roots through suppressing of PIN1 (Crawford et al., 2010; Ruyter-Spira et al., 2011; Ruyter-Spira et al., 2012). In contrast, gibberellic acid can facilitate auxin concentration. It is required for auxin pump PIN protein stability in roots, inflorescences, and meristems as the proteins are degraded in the vacuoles in the absence GA (Willige et al., 2011).

As mentioned already in the context of its interaction with gibberellic acid, jasmonic acid controls response to biotic and abiotic stresses and restricts growth. However, jasmonic acid is not the only stress response hormone, and plants have an ability to regulate responses so that only one pathway is dominant. For example, jasmonic acid and salicylic acid are both activated in response to both biotic pathogens and herbivory, but they differ in their specific response spectra. Part of this differential response is due to direct antagonism between SA and JA. SA activates the NPR1 (NONEXPRESSOR OF PATHOGENESIS-RELATED GENES1) gene that blocks JA effects downstream of JA synthesis (Fig. 3). Ethylene further modifies this antagonistic pathway by making the SA repression of JA response independent of NPR1 (Leon-Reyes et al., 2009; Thaler et al., 2012). Likewise, abscisic acid induced by drought can downregulate JA-regulated
ethylen are both known to be involved in initiation of flowering in response to environmental cues. Halliday et al. (2003) reported that phytochrome induction of flowering was temperature sensitive and that this sensitivity was regulated through FLOWERING LOCUS T (FT) transcription, a central regulator of flowering induction. Kumar et al. (2012) recently mechanistically tied PIF4 to induction of FT transcription in response to higher temperatures during short days. PIF4 expression increases at higher temperatures. However, PIF4 availability alone is insufficient to activate FT. The heat stress activation is dependent on the destabilization of the histone protein H2A.Z at higher temperatures that then allows PIF4 to bind. Yet, as with stem growth, PIF4 activity in flower initiation as a transcription factor is compromised by DELLA protein binding. Intriguingly, Achard et al. (2008) reported that the C-repeat/drought-responsive element binding factor, which is induced in cold, activates GA 2-oxidase expression. GA 2-oxidase inactivates GA. Without the GA-induced ubiquitination and degradation, DELLA proteins accumulate and suppress growth and, likely, flowering. These results suggest a further mode of cooperative interactions between GA and ethylene in flowering response to temperature.

**COMBINING ESD, SEXUAL PLASTICITY, AND MONOECY WITH A HORMONE-BASED MODEL**

With this rapidly expanding understanding of hormone response mechanisms and highly reticulate interactive networks, what new insights can we make concerning sexual development in plants? We can begin with explicitly recognizing that...
Environmental stresses do not act on plant development and physiology through unknown processes. Environmental stresses elicit hormone responses that trigger specific growth and physiological alterations. Historically, the functional relationship between environmental stress and hormone response has been overlooked or at least not expressed in studies of sexual plasticity and environmental sex determination. Perhaps this oversight was due to the emphasis in such studies on environmental plasticity itself rather than on the mechanisms for that plasticity. Whatever the reason, today, when we look at patterns of sexual response to stresses such as drought or heat, it is important to translate these into the actual plant response hormone and define the sexual developmental response in terms of the hormone rather than the environmental stress. Such a translation is not merely a semantic point, as by defining the sexual response in terms of hormonal response, it is possible to determine the genetic regulatory network involved in sex determination. Again, while tying the environment to regulatory networks may seem obvious when so stated, no such attention to underlying genetic systems is generally given in environmental studies.

A second concept that is often overlooked in the role of environment in sex determination is that hormone responses are highly pleiotropic in plants. Again, this concept is not new, but it is important to recognize when assessing the evolutionary role, or more specifically, the fitness adaptation of sexual plasticity or sex determination in response to environmental stresses. Individual plant responses to localized external insults such as drought (osmotic) stress or temperature extremes typically address viability concerns rather than fecundity. Many of these responses are indeed adaptive in the evolutionary sense as genetic variation in a population is directly selected. However, given that plant hormones are involved in multiple aspects of development, including flower development, and even floral organ development, it is not unexpected that stress-induced hormone levels may alter flower development. It is also not unexpected that floral developmental responses may be similar when facing similar stresses in independent species precisely because the underlying developmental mechanisms and stress responses are well conserved among angiosperms. Yet, because of this developmental conservation, observations of similar patterns of shifts to pistillate or staminate flowers are not sufficient evidence to support the role of natural selection in sexual plasticity. Natural selection may play a role in the morphological or physiological plasticity that enhances viability, but the sexual response may simply be a hitchhiker effect. Some hormones have well defined regulatory effects in floral and inflorescence gene regulation. Therefore, it is not unreasonable that some convergence in response to environmental stress is seen in floral development. Furthermore, as pointed out already, sex ratios are under high frequency selection pressures. If whole populations become predominantly male or female in response to broad spatial stresses such as drought or temperature, that response will have a negative effect on individual fitness. Such unidirectional sexual responses will not be deleterious only if there remains environmental heterogeneity within the population to keep effective population sex ratios close to 50:50.

As discussed earlier, there do not appear to be plant sex-specific hormones per se across all species as is the case in animals. Given that monoecy and dioecy have evolved multiple times independently, the absence of universal sex-specific hormones...
Hormone levels commonly vary by location within a single plant. By being the trigger to alternative developmental pathways, hormone levels can control differential male vs. female flower development where each reflects regulation of specific genes without requiring allelic segregation within an individual plant. From this recognition of the importance of hormone cross-talk and the potential role that differential hormone levels can have on sexual development, we can understand the role of hormones in flower development in monoecious species such as Cucumis melo or Zea mays. Just as importantly, it is possible to conceive how dioecy can evolve from monoecy, even in species that are considered to be under GSD. Moneocious and sub-monoecious (gynomonoecious, andromonoecious) species have evolved mechanisms to trigger or switch to unisexual development. The triggers or switches must be activated by hormone concentrations because the receptor or response elements must be genetically identical within the individual plant. Traditionally, these receptors or response elements that lead to unisexual development of the individual floral meristem have not been considered sex determining genes because they do not segregate. However, once these alternative developmental pathways are fixed in a population, a subsequent mutation that either modulates the effector hormone concentration within the inflorescence or floral meristem or mediates the response to the hormone concentration by becoming hyper- or hyposensitive to the hormone can arise and segregate allelicly. At this stage, the population then will be dioecious with only male and female plants or subdioecious with unisexual plants, male and/or female, is to be expected. The underlying genetic mechanisms of sex determination are expected to vary, and hence, to the extent that hormones are involved in the gene regulation, we expect that the specific hormones associated with the developmental process will also vary. Yet, groups of hormones do appear to have opposite effects on sex determination across species, consistent with the theory that masculinization and feminization evolve and are governed by two independent pathways. However, the third important concept that has emerged is that hormones do not act independently within a plant. As reviewed already, some act antagonistically and some synergistically. Because of these interactions, we cannot conclude that opposing sex determining effects of hormones necessarily reflect two independently evolved developmental pathways, each controlled by a single hormone. An alternative model is that one hormone controls the major differentiation process, while the second hormone modifies the availability of the first hormone or its response pathway, without directly regulating its own pathway. An additional model is that each hormone may indeed control a sex-specific developmental cascade, but the regulation of the switch is controlled only by one of the hormones. The reason this distinction is important is 2-fold. First, this model acknowledges that hormone levels, even single hormone levels can regulate two alternative growth paths. If this scenario is true, then the sex determining mutations that respond to these hormone levels can occur at a single gene, rather than necessarily requiring two genes to generate male vs. female development. Second, this model suggests a mechanism whereby species can become monoecious. Hormone levels commonly vary by location within a single plant. By being the trigger to alternative developmental pathways, hormone levels can control differential male vs. female flower development where each reflects regulation of specific genes without requiring allelic segregation within an individual plant. From this recognition of the importance of hormone crosstalk and the potential role that differential hormone levels can have on sexual development, we can understand the role of hormones in flower development in monoecious species such as Cucumis melo or Zea mays. Just as importantly, it is possible to conceive how dioecy can evolve from monoecy, even in species that are considered to be under GSD. Moneocious and sub-monoecious (gynomonoecious, andromonoecious) species have evolved mechanisms to trigger or switch to unisexual development. The triggers or switches must be activated by hormone concentrations because the receptor or response elements must be genetically identical within the individual plant. Traditionally, these receptors or response elements that lead to unisexual development of the individual floral meristem have not been considered sex determining genes because they do not segregate. However, once these alternative developmental pathways are fixed in a population, a subsequent mutation that either modulates the effector hormone concentration within the inflorescence or floral meristem or mediates the response to the hormone concentration by becoming hyper- or hyposensitive to the hormone can arise and segregate allelicly. At this stage, the population then will be dioecious with only male and female plants or subdioecious with unisexual plants, male and/or female,
and monoecious plants. The newly mutated gene would fit the strict definition of being a sex-determining gene because the genotype of the individual at that gene would determine the proclivity toward one sexual development or the other. Note that the genotype defines the proclivity or tendency toward a sexual development rather than canalizing the sexual development. As this system evolves from a monoecious system, it is the hormone level or the perception of the hormone level that triggers the floral developmental fate. Therefore, environmental stress can still influence the sexual development of the individual plant or floral meristem and will result in sexual plasticity or even environmental sex determination.

CONCLUSION AND FUTURE DIRECTIONS

Sex determination systems in plants have been categorized as either GSD (genetic sex determination) or ESD (environmental sex determination) for too long. This distinction is too strict and not helpful in suggesting analytical approaches to dissect the developmental mechanisms that generate unisexual flowers or the evolution of those mechanisms from hermaphroditic ancestral states. Plant developmental geneticists have made enormous strides in discovering genes that are directly involved in the growth and differentiation of specific tissues and organs. Extraordinarily elegant models of gene regulation have exposed the mechanistic pathways through which plant hormones activate, repress, or derepress these genes. Plant physiologists have traditionally identified how plants, being sessile organisms, have generated morphological and physiological responses to inescapable environmental stresses through hormone production and response. Furthermore, in the last 10 years, we have witnessed an explosion in our knowledge of how these hormones work, move, and interact.

With these novel insights, we are poised to reevaluate the evolution of plant sexual systems and their dynamic or plastic responses within a new theoretical continuum. There are dioecious species that have evolved directly from hermaphrodites and that have evolved incipient or true sex chromosomes. It is likely that multiple, independent developmental pathways are present in such species that are controlled by genes that are linked chromosomally to facilitate coinheritance and to suppress recombination. However, there are dioecious species that have evolved either from hermaphrodites or monoecious ancestral species. These species do not have discernable sex chromosomes because there is no selective pressure to suppress recombination or to facilitate coinheritance. Instead, coexpression rather than coinheritance of gender-specific genes defines the sexual developmental fate. As such systems are being defined experimentally, the recurrent theme is that pre-existing hormonal pathways determine this coordination of coexpression. Therefore, evolution of the regulatory sequences of such genes rather than their synteny will be molded by selection for gender type. Simultaneously, such developmental systems will remain sensitive to external stimuli that modify hormone responses. Environmental plasticity thus can be used as a key to unlocking sex-determining genes rather than being the ecologically interesting but genetically obscuring phenomenon that it has been.

Whole genome sequencing and de novo chromosomal sequence construction are becoming powerful tools for the understanding of chromosomal evolution in those species that show evidence of sex chromosomes. Three key issues need to be addressed in such systems: fine-scale identification of nonrecombining regions on chromosomes that segregate with sex determination, detection of the dynamic nature of such regions over evolutionary time both in terms of the span of the regions and the genomic content, and the demonstration of function of genes within the nonrecombining regions that mechanistically regulate alternative sexual development. High-density single-nucleotide polymorphism (SNP) maps within a population will address the issue of identification of nonrecombining chromosomal regions. However, comparative studies of syntenic regions in related hermaphroditic species are required to uncover evidence of evolutionary change once sex-determining chromosomal regions have been elucidated. In particular, studies should focus on detecting evidence for expansion of nonrecombining regions, including capturing of sexually antagonistic genes, gene degradation and generation of pseudogenes, large deletion mutations, and accumulation of transposable elements, all of which are predicted in single-copy, nonrecombining chromosomes. Such studies should not be limited to comparisons of rates of nucleotide substitutions or evidence of directional or purifying selection, as a rapidly reduced effective population size for these regions plus linkage of all genes may bias patterns. Perhaps of the utmost importance, studies must uncover true sex-determining genes within these regions and dissect their true function in single-sex development. The lack of identification of sex-determining genes and their actions in well-studied species with proposed sex chromosomes remains the most tantalizing and disturbing gap in our knowledge for these systems.

For dioecious species that evolve through monoecy or demonstrate classically defined ESD, comparative transcriptome analyses will be the initial keys to discovering the evolution of sex specific gene expression complexes. Sexual evolution will not be identified by nonrecombining genes, but by the evolution of new expression modules or eigen-genes. New classes of sex determining genes and their ancillary sexually antagonistic genes will be identified through their expression patterns and eventually through the evolution of their coordinated cis response elements in comparison with closely related hermaphroditic species. As in chromosomally determined sexual development, the key gap in our knowledge remains the identity and modes of action of sex-determining genes. However, differing from the chromosomal systems, the means of segregating these alternative sex determination pathways is as integral as, and indeed will be integrated with, the actual modes of action of sex determination. We must anticipate the possibility that, analogously to the chromosomal systems, once established, sexual systems will continue to evolve. However, instead of the predicted gene capture and genic and chromosomal degradation predicted for sex-specific nonrecombining regions, the genome may evolve through the accumulation of new genes in coexpression modules driven by cis element evolution that will link their expression to the same cues as are used by the sex determination genes themselves. Of course, secondary sexual genes can become coexpressed by being regulated downstream in a regulatory cascade in both the chromosomally based models and the hormonally based models. The distinction of what drives the coexpression will need to be resolved by studying the evolution of the cis control elements among the genes. Following these patterns of genomic evolution under both models will be an exciting extension to the study of sex determination itself.

Finally, sex determination pathways will vary among lineages just as the developmental processes themselves are already
recognized as varying. However, once key regulatory systems are determined for independent lineages, phylogenetic studies will be powerful in retracing the mutational events and intermediate phenotypes required for unisexual flower development to evolve, as opposed to the evolutionary changes that occur after dioecy is achieved. Having hypotheses of intermediate mutational stages opens the exciting possibility testing ancestral phenotypes through transgenic transformation of modern plants.

LITERATURE CITED


