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Review Article

Sex Determination in Plants and its Contribution to Genetic Variability

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Abstract: Sex determination is a process that leads to the physical separation of male and female gamete-producing structures to different individuals of a species. Sexual reproduction is an ancient feature of eukaryote life, yet the sexes as we currently recognize them are relative late comers in the evolution of sex. Sex determination systems in plants have evolved many times from hermaphroditic ancestors (including monoecious plants with separate male and female flowers on the same individual),and sex chromosome systems have arisen several times in flowering plant evolution. Sex chromosome evolution is intimately connected with Y chromosome degeneration. Most current understanding of how the distinctive properties of Y chromosomes evolved comes from theoretical work on the evolution of genomic regions with low recombination. The identification of sex chromosomes in plants is problematic because most of them do not differ morphologically from autosomes or from one another. For example in some species, such as Actinidia deliciosa var. deliciosa, X and Y chromosomes are too small to support observations of their distinguishing characteristics.) In the majority of plants, male and female organs are formed and developed simultaneously, but only up to a point when the growth of either set of sex organs is inhibited. Inhibition of sexual development can vary in character so that in most cases, sexual development is inhibited by the absence of cell division. In many species of bryophytes, heterothallism (unisexuality) has been correlated with the presence of sex chromosomes. 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. Many dioecious species, including those with well-developed sex chromosomes, show 'leaky' or 'inconstant' gender expression, with males and females producing flowers of the other gender. Far from being an aberrant feature of plant reproduction, such phenotypes are typically fully functional for both sexes. The quantitative distribution of gender in a population is under strong selection and can evolve rapidly when circumstances change for example, when males are lost from a population. In angiospermic plant, monoecious species are grouped into 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 plants.
Keywords: Sex, chromosome, plant.

INTRODUCTION

Sexual reproduction is an ancient feature of eukaryote life, yet the sexes as we currently recognize them are relative late comers in the evolution of sex (Beukeboom and Perrin, 2014). The ability to reproduce through sex (fusion of haploid gametes) evolved in the common ancestor of all eukaryotes, but did not involve separate sexes; each individual was able to exchange genetic material with any other of the same species, as is still the case in many modern-day unicellular eukaryotes (Lahr *et al*., 2011). Sex determination is a process that leads to the physical separation of male and female gamete-producing structures to different individuals of a species. Sexual systems in plants are almost overwhelmingly diverse, and understanding their origin and evolution requires data from many different fields, including ecology, developmental biology, and genetics. Sexual system is distribution and function of gamete-producing morphological structures (Sakai AK *et al*., 1999). Genetic control of sex determination is becoming well understood in several animal systems,

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particularly *Drosophila melanogaster, Caenorhabditis elegans* and mammals. In plants, understanding the sex determination system is closely connected with understanding how separate sexes evolved, and current theoretical ideas about this also illuminate the evolution of sex chromosomes. Angiosperms are also of particular interest for empirical studies of sex chromosome evolution, because they probably evolved separate sexes repeatedly and relatively recently. Other plants, particularly Bryophytes (Okada *et al.*, 2001), also have interesting independently evolved sex chromosomes.

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 (Geber *et al.*, 1999).

2. LITRETURE REVIEW
2.1 Evolution of sex chromosomes
The theory outlined the evolution of a rarely recombining chromosome region containing the sex determining genes, an incipient sex chromosome system. The female haplotype carries a recessive male-sterility allele, while the dominant male-determining chromosome would carry female-sterility alleles. Sex chromosome evolution is intimately connected with Y chromosome degeneration. Most current understanding of how the distinctive properties of Y chromosomes evolved comes from theoretical work on the evolution of genomic regions with low recombination. Such regions are subject to several processes, given a sufficiently high rate of deleterious mutations (Charlesworth and Charlesworth, 2000).

Westergaard (Yakubov, *et al.*, 2005) grouped plant sex chromosomes into three types to illustrate different evolutionary stages. The earliest stage of sex chromosome evolution was characterized by having a viable YY genotype where the Y differs from the X only in its sex determination genes. This condition is represented by *Ecballium* and asparagus. The second stage has a YY genotype that is inviable, but the Y chromosome plays a decisive role in sex determination; this condition is represented by papaya and white campion (dephLF, *et al.*, 2010 and Lewis D. 1942). In the third stage the Y chromosome is irrelevant to sex determination and sex is determined by the X: autosome ratio as exemplified by sorrel.

Upon the evolution of a sex-determining gene, the Y chromosome is expected to begin a process of degeneration because of its reduced population size (between one male and one female, there is only one copy of a Y chromosome and 3 copies of X chromosomes) and reduced recombination that sometimes evolves between the X and Y chromosomes; both factors reduce the effective population size of the Y chromosome and reduces efficacy of selection, which, in turn, can lead to its degeneration. Selection to reduce recombination may be a byproduct of sexual antagonism (SA), where different SA alleles provide higher fitness in males and females; for example, reduced recombination between the sex-determining region and a SA locus can lead to associations between a male benefit (female-detriment) allele and the Y chromosome, and thereby restrict the allele's benefits to males and not expose females to its costs. Cessation of recombination often occurs through inversions that isolate increasingly large stretches of the Y chromosome (Charlesworth *et al.*, 2005). There is evidence that plant sex chromosomes derive from a pair of autosomes (Nicolas *et al.*, 2005; Filatov 2005; Bergero and Charlesworth 2009). Theory predicts that at least two closely linked sex-determining genes are necessary for the birth of sex chromosomes; the so-called "two-gene model" (Charlesworth and Charlesworth 1978) a male sterility mutation (recessive in X/Y systems, dominant in Z/W systems) and a female sterility mutation (dominant in X/Y systems, recessive in Z/W systems.

2.2 Molecular basis of sex determination
Despite a large body of research investigating different species, the molecular mechanisms of sex determination in plants remain relatively unknown. It is generally believed that metabolic processes and the sexual determination pathway have genetic origins. Attempts are made to identify and estimate the number of genes which determine sex in various plant species. An identified gene is often not the primary element in the sex determination mechanism (Ainsworth 2000), but only one of the many genes in the mechanism's cascade. Frankel and Galun (1977) proposed the key gene theory to explain the sex determination mechanisms in plants. When triggered, the key gene activates a cascade of other genes whose expression leads to the development of the respective sex organs. This implies that the genes conditioning the development of male and female sex organs are present simultaneously in every plant, and those sets of loci are activated subject to the allelic architecture of the key gene. A single-gene mechanism controls sex determination in plants such as *Asparagus officinalis* (Gao *et al.*, 2007), *Ecballium elaterium* (Ainsworth 2000), *Pistacia vera* (Hormaza 1994) and *Carica papaya* (Storey 1953).

Understanding of the evolution of plant sex chromosomes and sex determination is advanced by the

use of molecular markers since several groups are searching for. The region containing the sex determining is fully homologous between the two alternative chromosomes. Both X- and Y-linked markers are now being discovered in plants with and without heteromorphic sex chromosomes (Testolin *et al.*, 1995; Harvey *et al.*, 1997; Polley *et al.*, 1997; Zhang *et al.*, 1998; Mandolino *et al.*, 1999). Most markers are, however, anonymous, and cannot tell us which X-linked loci have homologues on the Y chromosomes and which do not.

2.3 Sex chromosomes

Sex chromosomes are a very peculiar part of the genome that have evolved independently in many groups of animals and plants (Bull, 1983). The identification of sex chromosomes in plants is problematic because most of them do not differ morphologically from autosomes or from one another (*Spinacia oleracea*, *Asparagus officinalis*) (Michalik 2009). In some species, such as *Actinidia deliciosa var. deliciosa* (Shirkot *et al.*, 2002), X and Y chromosomes are too small to support observations of their distinguishing characteristics. Heteromorphic sex chromosomes have been discovered in *Rumex acetosa* (Kihara and Ono 1923), *Melandrium album, Melandrium rubrum* (Blackburn 1923), three species of the family *Cannabidaceae,* i.e. *Humulus lupulus*, *Humulus japonicas* and *Cannabis sativa* (Shephard *et al.*, 1999a), as well as in *Rumex nivalis* (Stehlik and Barrett 2005) and three liverwort species: *Sphaerocarpos donnellii, Sphaerocarpos texanus* (Allen 1917) and *Marchantia polymorpha* (Bischler 1986). An occurrence of heteromorphic sex chromosomes is also suspected in mosses of the genus *Macromitrium* (Ramsay 1966).

In diploid species with a chromosomal mechanism of sex differentiation, males are generally heterogametic (XY) and females are homogametic (XX). A rare sex determination mechanism is observed in polyploid, dioecious species of the genus *Fragaria* where female individuals are heterogametic (Dellaporta and Calderon-Urrea 1993). Sex determination is much simpler in haploids where males and females have completely different sex chromosomes: Heteromorphic sex chromosomes are rarely found in angiosperms but have been reported in a number of plant species including Rumex, Cannabis, *Humulus,* and Silene (Parker, 1990). In dioecious Silene, males are the heterogametic sex (XY) and females are homogametic *(XX)* (Westergaard, 1948). The X chromosome appears to be essential in both males and females because only monoploid females can be obtained by in vitro techniques (Ye *et al.*, 1991). Application of hormones, including GA, auxins, and cytokinins, does not result in sex conversion. However, the presence of a single Y chromosome can suppress female development when three X chromosomes are present. Higher X copy number overcomes the Y chromosome masculinization

effect (Westergaard, 1958). Autosome ratios have no profound effects on the sex determining factors present on the Y chromosome. This suggests that the Y chromosome is decisive in determining sex in Silene. Three different regions of the Y chromosome have been identified as having separate functions in sex determination (Westergaard, 1946). One end contains a genetic factor (or factors) that suppresses formation of the gynoecium, the opposite end contains a male fertility factor (or factors), and the middle region includes a gene or genes needed for anther initiation. Therefore, the Y chromosome of Silene contains complete linkage between female-suppressor and essential male sex genes. Asparagus is generally a dioecious plant, with sex determined by homomorphic sex chromosomes in which the males (XY) are the heterogametic sex (reviewed by Bracale *et al.*, 1991). Genetic evidence suggests that asparagus is "male dominant" and contains *male-activator-female-suppressor* genetic determinants (Marks, 1973).

Dioecious *Mercurialis annua* has homomorphic sex chromosomes with a small non recombining region despite fairly significant phenotypic differences between the sexes (Durand 1963; Russell & Pannell 2015). Homomorphic sex chromosomes are painstakingly difficult to identify and the hunt for sex chromosomes in poplars has produced controversial results, with some sture porting female heterogamete (ZW females and ZZ males; Paolucci *et al.*, 2010; Yin *et al.*, 2008), and other studies advocating male heterogamete (XY males and XX females; Gaudet *et al.*, 2008; Geraldes *et al.*, 2015; Pakull *et al.*, 2009). The available female poplar genome sequence (Tuskan *et al.*, 2006) did not clarify which system of sex determination is operating, and cytological analyses provided little support for the presence of heteromorphic sex chromosomes (Tuskan *et al.*, 2012). Genetic mapping studies on Populus located the sex-determining region in different positions (Gaudet *et al.*, 2008; Yin *et al.*, 2008; Pakull *et al.*, 2009; Hou *et al.*, 2015).

The primary function of sex chromosomes is to reinforce dioecy, a sexual system that may increase outcrossing in species lacking self-incompatibility (Charlesworth D. 1985. Sex chromosomes are rare in plants because of different factors. 1. Plants are sedentary, and their open architecture (bauplan) with numerous meristems allows them to grow sexual organs in varying numbers, depending on intrinsic and extrinsic factors. 2. Another reason for the rarity of plant sex Chromosomes is frequency of polyploidy in plants. Polyploidy may prevent sex chromosomes from evolving, because the frequency of homozygous recessive alleles in four or eight homologous chromosomes is much lower than in two homologous chromosomes (Wellmer F *et al.*, 2004).

2.4 Mechanisms of Sex Determination in Plants

Most plant species are bisexual by nature. Angiosperms have two types of reproductive structures so that a plant may produce flowers containing both stamens and styles, alternatively, one individual can develop both male flowers (with stamina) and female flowers (with pistils) (Renner and Ricklefs 1995). In the majority of plants, male and female organs are formed and developed simultaneously, but only up to a point when the growth of either set of sex organs is inhibited. Inhibition of sexual development can vary in character so that in most cases, sexual development is inhibited by the absence of cell division, for example in *Rumex acetosa* (Ainsworth *et al.*, 1995) and *Melandrium album* (Farbos *et al.*, 1997), or necrosis of sexual organ cells, which is noted in *Asparagus officinalis* (Caporali *et al.*, 1994) and *Actinidia deliciosa* (Harvey and Fraser 1988).

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. Antheridiophores produce sperm-forming antheridia, and archegoniophores produce egg-forming archegonia. The sex of each haploid gametophyte is determined by cytological 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).

Tandrium and Bombyx, the X/A balance of Drosophila, occur both in the higher animals and among the dioecious seed plants. WESTERGAAD (1948, 1958) does not regard these as different systems but as two stages in the evolution of stable, multigenic mechanisms which ensure dioecism. GOLDSCHMIDT (1955) rejects WESTERGAAD's mutagenic hypothesis and interprets both systems as manifestations of a fundamental F/M balance in development. Both the X/Y and the X/A modes of sex determination occur among the dioecious species of the genus Rumex which thus provides opportunities for investigating the cytogenetic relationships of these differing mechanisms of sex determination within a single genus. An X/Y mechanism controls sex expression in the dioecious Rumex species which comprise the subgenus Acetosella (LOVE 1944). These species exist in a natural

polyploid series: $2n = 14$, 28, 42, and 56, but the male of each species is heterogametic for a single Y chromosome, combined respectively with 1,3,5, and 7 X chromosomes. The expression of maleness results from the strong epistatic effect of the Y chromosome. Females are homogametic with 2, 4, 6, or 8 X chromosomes corresponding to the level of polyploidy in the respective species. An X/A balance controls the expression of sexuality in plants of dioecious *Rumex acetosa* L. *(ONO* 1935; YAMAMOT1O9 38).

Plant gender is fundamentally a quantitative trait (Lloyd, D.G., and Bawa, K.S. (1984). Whereas individuals in most gonochoristic animals are either fully male or female, gender in plants is often better viewed on a quantitative scale, measured in terms of the relative allocation to each sexual function, or the proportion of genes transmitted through sperm versus eggs (Lloyd, D.G. (1980). Many dioecious species, including those with well-developed sex chromosomes, show 'leaky' or 'inconstant' gender expression, with males and females producing flowers of the other gender. Far from being an aberrant feature of plant reproduction, such phenotypes are typically fully functional for both sexes. The quantitative distribution of gender in a population is under strong selection and can evolve rapidly when circumstances change for example, when males are lost from a population (Dorken, M.E., and Pannell, J.R. (2009). Such shifts in sex allocation can occur at any level in the modular hierarchy of plant development, from the number of pollen grains produced in anthers, to the number of floral or inflorescence primordia that develop with male versus female functions (Lloyd, D.G., and Bawa, K.S. (1984). Such variation allows for multiple pathways to sex differentiation and potentially sex determination. While the distribution of quantitative gender in a population may have a strong genetic component, individuals can also modify their sex expression in response to context-dependent opportunities or costs (Lloyd, D.G., and Bawa, K.S. (1984). Plastic sex expression applies not only to the quantitative nature of sex allocation in functional hermaphrodites (Delph, L.F., and Wolf, D.E. (2005), but, more rarely, to complete sex change (Schlessman, M.A. (1988). In jack-in-the-pulpit (Arisaema species), young plants are fully male, whereas, in later seasons, they become fully female or hermaphroditic Bierzychudek, P. (1984). Such 'sexual diphase' is expected to evolve both if the marginal benefits of reproducing as male versus female change with plant size (Zhang, D.Y., and Jiang, X.H. (2002), and if there are additional benefits to sexual specialization at a particular point in time (e.g., if fitness is compromised by interference between simultaneous male and female functions) (Charnov, E.L., and Bull, J. (1977). Plasticity in sex allocation, and sexual diphasy specifically, indicate that sex may be determined not by a genetic dimorphism, but by the differential expression of genes shared by all

individuals in a population in response to physiological and environmental cues.

The distribution of dioecy and monoecy 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). There are a variety of sex determination mechanisms in the angiosperms. In angiospermic plant monoecious species here are grouped into 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). 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.

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).

Isolation of male-specific cDNAs from developing flower buds or reproductive organs has not yet led to discovery of sex determining genes (Matsunaga *et al*, 1996; Barbacar *et al*, 1997), probably because sex-determi (Hardenack *et al*, 1994; Ainsworth *et al*, 1995). This is not surprising, as these mutations change floral organ identities, whereas in unisexual flowers apparently normal reproductive organs merely stop developing, as predicted by the genetic model above. Both X- and Y-linked expressed loci have now been identified in *S. latifolia*. One approach is to directly search for sex-linked genes (Guttman and Charlesworth, 1998). This has identified the X-linked *MROS-X* (male reproductive organ specific) gene and its Y-linked homologue, *MROS3-Y*, which appears to have degenerated. *MROS3-Y* contains only a short region of homology to the *MROS3-X* sequence. This region has been evolving in a neutral manner, with a ratio of silent to replacement substitutions, Ka/Ks, of 0.974, close to unity, as expected for a sequence evolving without selective constraints (Nei, 1987).

3. CONCLUSION AND RECOMMENDATION

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 the economic value in manipulating the sexual phenotypes of crop plants. 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.

Production of unisexual flowers has evolved independently in many plant species, different and nove1 mechanisms may be operational. There is probably not one unifying mechanism that explains sex determination in plants. Currently, an important signpost on the road to the exploration of these mechanisms seem to be molecular sex-linked markers. Although these markers alone do not explain the molecular basis of sex determination in dioecious plants, but their number, structure of sequence or sequence homology between the typical, male and female sequences represent a foothold in the study of the mechanisms of sex determination.

Diversity of sex-determination mechanisms in plants reflects the important fact that separate sexes tend to have evolved independently in different lineages and much more recently than in most animal lineages. Plasticity and genetic polymorphism remains puzzling as why a chromosomal polymorphism that determines sex in the gametophytes of bryophytes has never replaced environmental sex determination in ferns or

lycophytes. Mutations in many flower development genes have the potential to cause male or female sterility, thereby leading to monoecy, gynodioecy, androdioecy, and dioecy. DNA methylation on sex chromosomes is known to affect sex expression in plants. Sex reversal under different environmental conditions is likely under epigenetic control and needs to be explored. The often young sex chromosomes in angiosperms provide a unique system compare the nascent sex chromosomes with their homologous autosomes in sister species.

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