

REPRODUCTIVE BIOLOGY  
CHAPTER 20

*Evolution of Dioecy and Sex Chromosomes in  
Cycads*

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## Abstract

Sex determination in cycads—and most other plants and animals—is a function of cytosine methylation and/or chromatin formation downregulating genes that control female or male function. Sex change probably occasionally occurs in cycads because there is no genetic assimilation of these epigenetic signals. In plants, allopolyploidy is the only escape from such canalized dioecy, but allopolyploidy never occurs in cycads. Cycads never developed a pair of unequal length sex chromosomes because their haploid stages are large, complex, and long-lived.

## Resumen

La determinación del sexo en las cícadas—y la mayoría de otras plantas y animales—es una función de la metilación de la citosina y/o la formación de cromatina que inhibe a los genes que controlan la función masculina o femenina. El cambio de sexo en cícadas probablemente ocurre ocasionalmente por ausencia de la asimilación genética de estas señales epigenéticas. En las plantas, la alopoliploidía es el único escape de tal dioicismo canalizado, pero la alopoliploidía no ocurre en las cícadas. Las cícadas nunca han desarrollado un par de cromosomas de sexo in iguales de largo dado que sus etapas haploides son muy largas, complejas y longevas.

## Introduction

Living cycads are considered strictly dioecious (i.e., have separate female and male reproductive parts on separate individuals) but contain no discernible sex chromosomes (Norstog & Nicholls, 1997). In this paper, we propose an evolutionary theory that describes how we believe this situation arose in cycads.

Charles Darwin hypothesized that ancestral lineages were hermaphroditic (each individual produces both functional female and male gametes) and that dioecy evolved as a derived condition (Darwin, 1873; Stauffer, 1975). In plants and animals, evolution from hermaphroditism to strict dioecy almost certainly occurred via an intermediate stage that involved individuals who were both hermaphroditic and either functional females or males (Darwin, 1873; Charlesworth & Charlesworth, 1978; Charlesworth & Guttman, 1999; Gorelick, 2003a). These intermediate stages are referred to as gynodioecy (functional females) or androdioecy (functional males).

Cycads are an ancient lineage. They have existed in roughly modern form for 275 to 300 million years (Zhifeng & Thomas, 1989). Cycads were never diverse (Harris, 1961, 1964). Dioecy appears to be quite ancient among cycads; no bisexual cycad cones or individuals have ever been found in the fossil record. The only instances of anything other than strict dioecy that we have ever seen in cycads are those examples of sex change discussed in the accompanying paper (Osborne & Gorelick, 2007). Thus, we must explain how cycads evolved strict dioecy, maintained it for hundreds of millions of years, yet

managed to maintain enough phenotypic plasticity to permit sex change under extreme environmental conditions.

## Gene Regulation and Sexual Function

In all living organisms, genes are regulated by epigenetic signals that bind to regulatory portions of the gene (Riggs et al., 1996). The most ancestral such epigenetic signal is cytosine methylation (Poole et al., 2001). If sufficient methylation occurs on the regulatory portion of a gene, then that gene is turned off (Finnegan et al., 1993; Tate & Bird, 1993). This is an essential process, because most genes are not expressed in any given cell. Thus, even though all cells in an individual plant contain identical DNA, only certain genes are turned on in leaf cells, whereas other genes may be turned on in root cells, and so on. Furthermore, which genes are turned on and off depends on the age of the tissue.

In almost all living organisms (there are a few highly derived exceptions among animals and fungi, but not plants), cytosine methylation controls gene regulation. Cytosine methylation and other chemicals (e.g., heterochromatic proteins and histones)—that attach to DNA nucleotides are types of epigenetic signals. Epigenetic simply means “on top of” genetic. These epigenetic signals act largely by blocking those sites on the chromosomes to which the enzymes that mediate transcription of messenger RNA would ordinarily bind (see Gorelick 2003a, 2003b, for review of these mechanisms).

Cytosine methylation regulates production of female and male sex hormones, and these sex hormones are believed to be the primary determiner of sex in plants (Jost & Saluz, 1993; Grant, 1999). Furthermore, sex hormones probably regulate the production of male and female gametes in plants (Charlesworth & Charlesworth, 1978; Gorelick & Osborne, 2002; Gorelick, 2003a). Female reproductive structures are those on which the genes for female function have been upregulated (unmethylated), whereas the genes for male function have been downregulated (methylated) (Janousek et al., 1996; Iannello et al., 2000). This is true in both hermaphroditic and dioecious lineages.

## Evolution of Dioecy

Consider a hermaphroditic lineage with no distinct sex chromosomes. It is then possible to obtain the first female or male in that lineage by simply downregulating its gene for

maleness or femaleness, respectively. This downregulation was almost certainly triggered by extra methylation near the sex-determining gene.

Often only one gene has to be downregulated to impede either female or male function. That is, sex of an individual is controlled by a single locus for each sex. This certainly appears to be true in most mammals, with their *SRY* gene for maleness (Graves, 2002). Likewise, turtles all have their sex determined by egg temperature, which turns their *Dmrt1* gene on and off (Murdock & Wibbels, 2003). It is, however, not known whether sex is determined by one or multiple genes in cycads.

The above hypothesis begs the question of how or why extra methylation originally arose on a gene for female or male function. We propose that this was somewhat serendipitous. Excess cytosine methylation is invariably incorporated onto and nearby those portions of the genome that are new (Yoder et al., 1997; Gorelick, 2003b). This is a mechanism by which cells protect themselves against genomic parasites, such as insertions of viral or bacterial DNA. If, say, a transposon (a piece of parasitic DNA) is inserted near or within the gene for maleness in a hermaphroditic individual, then that individual would likely become the first female of the lineage (Gorelick, 2003a).

Methylation patterns, including those that determine the sex of an individual, are highly heritable. Thus, once dioecy, androdioecy, or gynodioecy is established in a lineage, that sexual system is retained until other evolutionary forces intercede.

At this juncture, the lineage has evolved to a condition of gynodioecy or androdioecy. Other selective pressures then take over in driving the lineage to strict dioecy, especially in those lineages in which sex is determined by two or more genes (Charlesworth & Charlesworth, 1978; Dorken & Barrett, 2004). Strict dioecy could arise via either methylation of genes for femaleness in male individuals or methylation of genes for maleness in female individuals, and with, in either case, suppression of recombination (crossing over) between the genes for femaleness and maleness. Suppressed recombination arises as a by-product of increased cytosine methylation (Griffin et al., 2002; Gorelick, 2003b; Liu et al., 2004), making this process much more likely.

Once plants evolve strict dioecy via the epigenetic mechanism of cytosine methylation suppressing male function in females and suppressing female function in males, this condition could become more permanent over evolutionary time through genetic assimilation. Genetic assimilation means that epigenetic control of phenotypes is transferred to genetic control; that is, there is a switch from coding by cytosine methylation to coding

by the nucleotides themselves (Waddington, 1957; Pál & Miklós, 1999). This may have occurred in some cycads but probably not in those lineages for which environmental shocks can cause sex change (Osborne & Gorelick, 2007). Cytosine methylation provides a convincing mechanism for generating and maintaining phenotypic plasticity (Gorelick, 2004), which has apparently not been fully lost in cycad sex determination. Because sex change has been documented in each of the extant families of cycads and in six of the ten extant genera, genetic assimilation probably did not occur in cycads (unless, in the very unlikely situation, the genetic assimilation occurred along with substantial nascent genotype-by-environment interactions).

In general, there is one ready escape from dioecy that evolved via cytosine methylation, namely allopolyploidy. Allopolyploids are formed via the hybridization of individuals from two distinct lineages (e.g., two different species). The allopolyploid offspring contain all of the chromosomes from both of their parents; hence, the offspring could simultaneously contain active (upregulated) genes for both femaleness and maleness. However, polyploidy has never been documented or even suspected in any cycads, extant or fossil. This may help explain why cycads have retained their strictly dioecious condition for so many millions of years, even without genetic assimilation arising in cycads.

## Lack of Unequal Length Sex Chromosomes

In many animals (but only in a handful of plants), sex chromosomes of different lengths can be identified. For example, in humans, the Y chromosome is much shorter than the X chromosome. Evolution of sex chromosomes is usually explained by a population genetic model known as Muller's ratchet (Nei, 1970; Griffin et al., 2002; Gorelick, 2003a). Although the mathematics is complicated, the principle underlying Muller's ratchet is simple. Consider a population in which there are some individuals with no mutations. Follow that population over evolutionary time. If the mutation rate is large compared with the recombination rate, then eventually the population will reach a point at which every individual has at least one mutation. In fact, the minimal number of mutations among all individuals ratchets up over time. As the Y chromosome accumulates more mutations, there is strong selective pressure to expunge those portions of the Y chromosome, which thereby becomes shorter. This result depends crucially on there being very little recombination, such as occurs on Y chromosomes, especially if the Y chromosome is highly methylated. Instead,

if recombination rates are high, then number of mutations in the population can decrease over time, because some gametes contain fewer mutations than expected due to fortuitous crossing over (some gametes also contain more mutations).

One of the prerequisites for Muller's ratchet for sexual organisms is that the haploid stage of the life cycle be largely immune from selection. This is the case in most animals, which generally have small and short-lived gametes. Plants, on the other hand, have large multicelled and long-lived haploid stages (gametophytes), with cycads being extreme in this regard. Cycad gametophytes are enormous, occupying much of the volume of what becomes a seed following fertilization (Norstog, 1987). Female cycad gametophytes are also long-lived; sometimes more than a year passes between pollination and fertilization (Norstog & Nicholls, 1997). Male gametophytes are also large and complex compared with other plants or animals (Norstog, 1993). Although there are no data concerning whether cycad haploid stages express most of the genes expressed by their diploid stages (as there are for some flowering plants; Willing & Mascarenhas, 1984; Willing et al., 1988; Drews & Yadegari, 2002), the structural complexity, size, and age of cycad gametophytes indicate that they should be largely immune from the effects of Muller's ratchet. Therefore, the chance of cycads (and most other plants) developing unequal length sex chromosomes is remote (Gorelick, 2003a, 2005).

Possession of different length sex chromosomes means that it should not be possible to change the sex of an individual, except possibly via large chromosomal rearrangements (Gorelick, 2003a). Because we know that sex change does occur in cycads, this implies that cycads must have sex chromosomes of the same length.

We expect cycad sex chromosomes to be only distinguishable by differential methylation and associated chromatin formation at sex-determining loci. Heterochromatic proteins typically bind to cytosine methylation, and this can be seen under a light microscope as a small chromosomal band that is different on female versus male plants (Gorelick, 2003a).

## Summary

Occasional sex change in cycads provides us with a window into the evolution of their sex-determining mechanism and sex chromosomes. Cycads appear to have retained an ancestral form of dioecy, with the sex of an individual being determined by cytosine methylation downregulating genes responsible for production of gametes or sex hormones. Cycads have

thereby retained the phenotypic plasticity to change sex via removing methylation in the face of large environmental perturbations. It is not obvious how many other plants have retained this plasticity in sex determination. It does not appear that any genetic assimilation of the epigenetic mechanism of sex determination has occurred in cycads. However, such canalization of dioecy may have been unnecessary because cycads cannot revert to a hermaphroditic condition via allopolyploidy. Finally, it appears that cycads (and most other plants) are immune from Muller's ratchet because they have haploid stages that express most of the genes expressed in their diploid stages. Microarray studies could be used to test this assertion at the molecular level. But until then, we base this inference of immunity from Muller's ratchet on the large size, complexity, and longevity of cycad gametophytes.

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