American Journal of Botany 92(6): 979–984. 2005.

THEORY FOR WHY DIOECIOUS PLANTS HAVE EQUAL LENGTH SEX CHROMOSOMES[1]

ROOT GORELICK[2]

School of Life Sciences, Arizona State University, Tempe, Arizona 85287-4501 USA

Dioecy and sex chromosomes almost certainly evolved from ancestral hermaphrodites that only possessed autosomes. There is a growing body of evidence that genes for female or male function were then epigenetically suppressed in some of these hermaphrodites, creating the first males or females and nascent sex chromosomes. The incipient sex-determining epigenetic signals, such as cytosine methylation, then drove Muller's ratchet in many animals, resulting in shorter Y chromosomes. Based on this theory of sex chromosome evolution and limited data on gametophyte gene expression, I argue that plants should be largely immune from Muller's ratchet and therefore retain their ancestral state of equal length sex chromosomes, unless they incur chromosomal rearrangements or large-scale insertions of duplicated genomes. Usually heteromorphic sex chromosomes canalize dioecy, but extensive polyploidy or polysomy can provide an escape from this canalized dioecy. This theory implies that dioecy due to heteromorphic sex chromosomes should be evolutionarily ephemeral in bryophytes and homosporous pteridophytes because of their extraordinarily high incidences of polyploidy. And, if anything, these very high incidences of polyploidy are responsible for translocation or gradual addition of beneficial genes, rather than gradual reduction in the length of a sex chromosome.

Key words: chromatin; cytosine methylation; heteromorphic sex chromosomes; Muller's ratchet.

For better or worse, evolutionary botany is relatively devoid of theory, at least when compared with evolutionary zoology. Yet there is a commonality in much of evolutionary theory amongst all meiotic eukaryotes. Plants can shed light on such evolutionary theory, both in bolstering the commonalities and in illuminating differences, especially when we apply general theory to various distinct lineages. The purpose of this paper is to show how plants reflect on an existing theory of the origins of dioecy and sex chromosomes.

In the first section, I describe the theory of cytosine methylation driving the evolution of dioecy and sex chromosomes, with cytosine methylation being the engine that (1) regulates sex expression and (2) initially establishes sex chromosomes, (3) which then powers Muller's ratchet, (4) eventually causing the evolutionary gradual diminution in length of sex chromosomes. In the second section, I show how plants, as well as some animals, are immune from Muller's ratchet because the haploid stages of their life cycles are subject to extensive selection. I then briefly digress and discuss some other mechanisms for evolution of unequal length (heteromorphic) sex chromosomes. Next, I discuss one of the implications of the general theory—that diminution in the length of sex chromosome canalizes dioecy—and ask whether certain organisms can escape from this canalization. Canalization is an evolutionary sequence leading to a reduction of phenotypic plasticity, possibly via genetic assimilation. I argue that polyploidy provides an escape from dioecy of taxa with heteromorphic sex chromosomes, but that polyploidy is exceedingly rare in animals, somewhat rare in seed plants and heterosporous pteridophytes, and exceedingly common in homosporous pteridophytes and bryophytes. The upshot of this theory is that high incidence of polyploidy in spore-bearing plants renders dioecy as an evolutionarily ephemeral condition in bryophytes and homosporous pteridophytes. On the other hand, seed plants and especially animals retain dioecy in those lineages that have heteromorphic sex chromosomes. I argue that the distinction between dioecious seed plants and dioecious animals is that seed plants should have much lower incidence of heteromorphic sex chromosomes because they are immune from Muller's ratchet. This theory then implies that lack of heteromorphic sex chromosomes should be especially poignant in those seed plants with long-lived gametophytes and those that lack polyploidy, e.g., the cycads.

CYTOSINE METHYLATION DRIVES EVOLUTION OF DIOECY AND MULLER'S RATCHET

It is almost universally believed that all sexual eukaryotes evolved from hermaphroditic ancestors that only had autosomes and no distinct sex chromosomes (Darwin, 1873; Stauffer, 1975; Griffin et al., 2002; Gorelick, 2003). Many ancestrally hermaphroditic/monoecious eukaryotes evolved dioecy, gynodioecy, androdioecy, and other nonhermaphroditic conditions (Lloyd, 1980). Many of these lineages that were not hermaphroditic/monoecious retained equal length sex chromosomes/autosomes. However, once a lineage evolved unequal length (heteromorphic) sex chromosomes, then they could no longer be hermaphroditic/monoecious, but were strictly dioecious (Gorelick, 2003).

Many animals have evolved heteromorphic sex chromosomes (Solari, 1994; Ainsworth, 2000; also see the series of small volumes titled *Animal Cytogenetics* which includes Borganonkar, 1974; Egozcue, 1974; Fregda, 1974; Gustavsson, 1974; Hayman and Martin, 1974; Ohno, 1974; Patton, 1974; Crozier, 1975; White and Webb, 1976; Smith and Virkki, 1978; Hewitt, 1979; Ueshima, 1979; Matuszewski, 1982; Olmo, 1986; Christidis, 1990; King, 1990). Heteromorphic sex chromosomes are a sufficient condition for dioecy and therefore many animals are dioecious (Gorelick, 2003). By contrast, virtually all plants have equal length (homomorphic) sex chromosomes (Ainsworth, 2000; Gorelick, 2003). Despite their lack of heteromorphic sex chromosomes, flowering plants have nonetheless evolved dioecy in roughly two-thirds of their orders (Bawa, 1980) and one-tenth of their species (Yamplos-

[1] Manuscript received 25 September 2004; revision accepted 17 February 2005.

Thanks to Sue Bertram and a pair of anonymous reviewers for their extraordinarily helpful comments.

[2] Author for correspondence (e-mail: cycad@asu.edu)

ky and Yamplosky, 1922). Why have heteromorphic sex chromosomes not evolved in dioecious plant lineages? In an earlier paper (Gorelick, 2003), I argued that the initial stages of evolution of dioecy and sex chromosomes are identical in plants and animals, namely new epigenetic signatures that attach to chromosomes and down-regulate either female or male function. In many animals, these epigenetic changes then drive Muller's ratchet, resulting in ever decreasing length of Y chromosomes over evolutionary time (this theory also applies to Z/W, as well as X/Y, chromosome systems, i.e., it does not matter which sex is heterogametic). Muller's ratchet is a population genetic model in which the minimum number of mutations amongst all individuals increases over evolutionary time. Muller's ratchet is predicated on the mutation rate being higher than the recombination rate. Muller's ratchet is also predicated on selection only acting on the diploid (or polyploid) stages of the lifecycle of sexual organisms (Nei, 1970). Here I argue that plant lineages are effectively immune from the effects of Muller's ratchet because their haploid stages are as subject to selection as their diploid stages and hence maintain their ancestral state of equal length (homomorphic) sex chromosomes.

Cytosine methylation from genomic duplications was probably the initial stage in evolution of dioecy from an ancestrally hermaphroditic condition (Solari, 1994; Griffin et al., 2002; Gorelick, 2003). Genomic duplications—whether they be from transposons, duplication of regulatory genes, or polyploidy—invariably result in high levels of cytosine methylation on the new portions of the genome (Holliday, 1984; Adams et al., 2003). This results in suppressed recombination (Holliday, 1984; Griffin et al., 2002) and down-regulation of genes with methylated promoters (Iguchi-Ariga and Schaffner, 1989; Griffin et al., 2002), which are the hallmarks of incipient Y or W chromosome formation. Thus, if an ancestral hermaphrodite has a gene coding for male function (e.g., production of sperm or male sex hormones) that is suppressed by cytosine methylation, then that individual becomes the first female in the population and the lineage would thereby be considered gynodioecious. Consistent with this mechanism, many lineages have evolved dioecy from an ancestral hermaphroditic condition via gynodioecy or, occasionally, via androdioecy (Charlesworth and Charlesworth, 1978). Liu et al. (2004) described incipient Y chromosome formation in the dioecious plant *Carica papaya* (papaya), noting that the newly non-recombining portion of the Y chromosome contains sex-determining genes, sequence duplications, and retrotransposons. Elsewhere, researchers in this same laboratory showed that the sex-determining locus of papaya is heavily methylated and has severely suppressed recombination (Ma et al., 2004).

There is a growing body of evidence that epigenetic changes, such as nascent cytosine methylation, are responsible for early stages in the evolution of dioecy and sex chromosomes in all eukaryotes (Gorelick and Osborne, 2002; Gorelick, 2003). In date palms (*Phoenix dactylifera*), which like papayas are dioecious with homomorphic sex chromosomes, extra heterochromatin on one of the male chromosomes is believed to determine sex (Siljak-Yakovlev et al., 1996). An almost identical situation occurs in electric eels (*Eigenmannia virescens*) and tilapia (*Oreochromis niloticus*), which are important cases insofar as fish provide crucial windows into the early evolution of sex chromosomes in vertebrates (de Almeida-Toledo et al., 2001; Griffin et al., 2002). Evolution of sex chromosomes is probably far too canalized in mammals to provide any insight into the early stages of sex chromosome evolution because all extant mammals have unequal-length X and Y chromosomes (Graves, 1996). Even in the androdioecious nematode *Caenorhabditis elegans*, which does not possess any cytosine methylation, sex is determined by epigenetic down-regulation of genes coding for female function, although in this instance the epigenetic signal is histone acetylation (Prahald et al., 2003).

In animals, increased cytosine methylation on an incipient Y or W chromosome provides the machinery to drive Muller's ratchet and thereby make the Y or W chromosome shorter than its ancestral homologue (Gorelick, 2003). It does this by first suppressing recombination, which is a prerequisite of Muller's ratchet. Next, cytosine methylation geometrically increases the speed of Muller's ratchet by suppressing recombination in conjunction with increasing mutation rate of cytosine to thymine (Gorelick, 2003). Methylated cytosine undergoes point mutations to thymine at a much higher rate than unmethylated cytosine due to differences in activation energy required for deamination and differences in mismatch repair rates of the intermediate (deamination) products (for details, see fig. 2 and accompanying text in Gorelick, 2003). Speed of Muller's ratchet is proportional to the product of the decrease in recombination rate and increase in rate of per-genome mutation rate, both of which are affected by methylation of cytosine. Finally, cytosine methylation increases the rate of epimutations due to mismatch repair of deaminated 5-methylcytosine. Deamination of unmethylated cytosine yields uracil, which is quickly methylated to form thymine by maintenance methylation enzymes. Deamination of methylated cytosine yields a high-energy tautomer of thymine, which will either be converted to the ordinary, low-energy tautomer of thymine (a mutation) or unmethylated cytosine (an epimutation). Accumulation of either mutations or epimutations provides selective pressure to excise those loci, resulting in a shorter Y chromosome.

PLANTS (AND SOME ANIMALS) ARE IMMUNE FROM MULLER'S RATCHET

Even though dioecy probably evolved in most dioecious eukaryotes via genomic duplications triggering cytosine methylation, flowering plants have probably rarely ever evolved heteromorphic sex chromosomes because they are immune from Muller's ratchet. Muller's ratchet in sexual organisms is predicated on the Y or W chromosome being sheltered from the effects of deleterious mutations because a nondeleterious homologue exists on the X or Z chromosome (Nei, 1970). Muller's ratchet in sexual lineages thus has the prerequisite that the haploid stage of the life cycle is barely, if ever, subjected to selection; otherwise the deleterious mutation would no longer be sheltered by the effects of its homologue (Nei, 1970). This prerequisite simply seems to not apply in flowering plants. Although only a limited number of flowering plant taxa have thus far been tested, in each of these, the haploid stages express most of the genes that are expressed in their diploid stages (Willing et al., 1988; Drews and Yadegari, 2002), an empirical result that was presaged by Jim Bull (1978). Much more empirical testing is necessary here. If the gametophytes of all or most flowering plants express most of the genes expressed by their sporophytes, then flowering plants will be immune from Muller's ratchet and will therefore lack heteromorphic sex chromosomes.

Although similar genetic work regarding gene expression in

gametophytes does not appear to have yet been done in nonflowering seed plants, similar results should apply to all other seed plants because they generally have longer-lived and larger haploid stages than do flowering plants. For example, cycad megagametophytes are enormous, occupying much of the volume of what becomes a seed following fertilization (Norstog, 1987). Cycad megagametophytes are long-lived; sometimes there is over a year between pollination and fertilization (Norstog and Nicholls, 1997). Cycad microgametophytes are large and complex compared with other plants or animals (Norstog, 1993). Furthermore, because angiosperms almost certainly descended from an ancestor with large and long-lived gametophytes, the most parsimonious inference is that flowering plants should have haploid stages that express most of their genes, corroborating the arguments made in the previous paragraph.

There are also many examples of animals with homomorphic sex chromosomes (see the many small volumes in the series *Animal Cytogenetics*, cited above, especially for invertebrates; also see Solari, 1994, for examples in fish, amphibians, turtles, and crocodiles). These animals with homomorphic sex chromosomes may still be considered to have X/Y or Z/W chromosome systems, but there is simply no difference in the lengths between the two sex chromosomes. What we see from these lineages with homomorphic sex chromosomes are additional mechanisms by which Muller's ratchet is abrogated for many animals. For example, speed of Muller's ratchet is inversely proportional to effective population size (Nei, 1970; Bell, 1988). Lineages that have always had planktonic larvae, such as many marine invertebrates, should therefore have homomorphic sex chromosomes (Gorelick, 2003). Contrary to the implicit assumption of Nei's model of Muller's ratchet (Nei, 1970), gametes in some animals may be subject to substantial selection. This should especially be true in lineages with planktonic gametes, which therefore should be immune from Muller's ratchet by the same mechanism by which I hypothesize that plants are immune from the ratchet. There are multiple mechanisms that nullify Muller's ratchet, and lineages with any of these nullifications of the ratchet have rarely ever evolved heteromorphic sex chromosomes. And, in the unusual instances when lineages that should be immune from Muller's ratchet have evolved heteromorphic sex chromosomes, it has probably only been via alternate mechanisms, such as chromosomal rearrangements, and not via gradual diminution of the length of the Y or W chromosome over evolutionary time.

EVOLUTION OF SEX CHROMOSOMES WITHOUT MULLER'S RATCHET

The few examples of heteromorphic sex chromosomes in plants (e.g., *Silene latifolia*) have probably evolved via chromosomal rearrangements (Moore et al., 2003). Chromosomal rearrangements are also known in animal lineages with heteromorphic sex chromosomes, e.g., the lizard genus *Sceloporus* (Sites et al., 1992), where some closely related taxa have homomorphic sex chromosomes. In fact, chromosomal rearrangements may be responsible for all later stages of Y or W chromosome evolution in mammals (Graves, 1995).

Two other possible mechanisms for evolution of sex chromosomes are sexually antagonistic genes (Fisher, 1931; Bull, 1983; Rice, 1987b) and genetic hitchhiking (Rice, 1987a). Sexually antagonistic genes are alleles that are favored in only one sex (Fisher, 1931; Rice, 1987b). The model of sex chromosome evolution via sexually antagonistic genes assumes that there are only a few sex-determining loci and that recombination is locally suppressed near these loci (Rice, 1996). Genetic hitchhiking describes a situation in which suppressed recombination can facilitate a mildly deleterious mutation being genetically linked to an advantageous one. With such suppressed recombination, a proto sex chromosome can accumulate deleterious mutations that are sheltered from selection by the linked advantageous mutation. Both of these alternative theories—sexually antagonistic genes and genetic hitchhiking—were developed for the fruit fly family Drosophilidae. *Drosophila* are highly derived and have atypically complicated sex-determining systems (Dübendorfer et al., 2002), hence these models probably do not apply to plants. Sexually antagonistic genes and genetic hitchhiking only accelerate already existing local suppression of recombination, but do not provide an explanation for how one initially suppresses recombination in homomorphic sex chromosomes (Rice, 1996; Gorelick, 2003), other than possibly via nascent cytosine methylation, which will itself drive Muller's ratchet. One of the most poignant arguments in favor of cytosine methylation driving Muller's ratchet is that it explains how recombination was initially suppressed in proto sex chromosomes. By contrast, the sexually antagonistic genes and genetic hitchhiking models require a *deus ex machina* of initial recombination suppression. These two models also have the problem that they do not explain the heterochromatization of the degenerating Y or W chromosome (Steinemann and Steinemann, 1998).

Another possible mechanism for evolution of sex chromosomes, retrotransposon traps (Steinemann and Steinemann, 1992, 1998), was also developed for *Drosophila*, but it probably plays a role in plants. The idea here is that Y or W chromosomes accumulate retrotransposons, which then are rendered highly heterochromatic, which in turns shuts down transcription. Plants typically have more—and more obvious—transposons than animals. However, the retrotransposon trap model can be considered a subset or special case of the theory that cytosine methylation drives dioecy and Muller's ratchet. Any newly inserted genomic material is usually highly methylated (Nagl and Ehrendorfer, 1974; Volpe and Eremenko, 1974; Holliday, 1984; Yoder et al., 1997; Matzke and Matzke, 1998; Regev et al., 1998; Colot and Rossignol, 1999; Jones and Takai, 2001; Martienssen and Colot, 2001). Incipient cytosine methylation that drives evolution of dioecy and sex chromosomes could come from any newly inserted portion of the genome, including retrotransposon, duplication of homeotic genes, or even polysomy or polyploidy (Gorelick, 2003). Retrotransposon traps can therefore be subsumed as a special case of the model of cytosine methylation driving Muller's ratchet and consequently should be responsible for the evolution of plant sex chromosomes with different chromatin signatures, but with equal lengths.

DOES SEX CHROMOSOME HETEROMORPHY CANALIZE DIOECY?

A corollary of cytosine methylation driving Muller's ratchet and thereby driving the evolution of sex chromosomes is that individuals with heteromorphic sex chromosomes should be dioecious (Gorelick, 2003). There is, however, one ready escape from this cytosine methylation-induced canalization of dioecy, 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 could simultaneously contain active (up-regulated) genes for both femaleness and maleness.

In seed plants, there are varying levels of polyploidy. In cycads and gingkoes, there exists no evidence of polyploidy. Polyploidy is extremely rare in animals. There are a few examples of polyploidy in animals, such as the pair of ancestral polyploid events in vertebrates (Ohno, 1970) and subsequent polyploid events in fish and amphibians (e.g., Lowcock, 1994). Polyploidy is rare in extant conifers and Gnetales (Lewis, 1980). Tetraploidy exists in a few conifers, e.g., some *Juniperus* spp., and hexaploidy in one species, *Sequoia sempervirens* (Saylor and Simons, 1970; Goldblatt and Johnson, 2003). Tetraploidy exists in a few Gnetales: *Gnetum montanum*, *Welwitschia mirabilis*, and possibly a few interspecific hybrids of *Ephedra* (Khoshoo, 1959; Hizume et al., 1993; Goldblatt and Johnson, 2003). Polyploidy is also rare in heterosporous pteridophytes (Klekowski and Baker, 1966). Because polyploidy is rare in gymnosperms, heterosporous pteridophytes, and animals, there is no escape from the canalization of dioecy that evolved via Muller's ratchet. Thus, this theory predicts that heteromorphy of sex chromosomes should canalize dioecy in virtually all gymnosperms, heterosporous pteridophytes, and animals.

Polyploidy is more common in flowering plants. Depending on which author one cites, from 30 to 80% of extant flowering plants are of polyploid origin (Levin, 2002). So, the above theory predicts some—albeit limited—escapes from canalization of dioecy in those angiosperms with heteromorphic sex chromosomes. But, both the above theory and empirical work show that heteromorphic sex chromosomes (i.e., those of different length) are extremely rare in flowering plants. Therefore there are virtually no instances of canalization of angiosperm dioecy from which to escape.

The rates of polyploidy in seed plants and metazoan animals pale in comparison with bryophytes and homosporous pteridophytes, where well over 95% of taxa are believed to be of polyploid origin (Klekowski and Baker, 1966). With such enormous rates of polyploidy in these spore-bearing plants, the theory herein predicts that dioecy should be a short-lived evolutionary stage, with allopolyploidy restoring monoecy relatively quickly. In fact, polyploidy and polysomy (extra duplication of one chromosome; a form of aneuploidy) of sex chromosomes are believed to restore monoecy from the relatively rare condition of dioecy in mosses (Khanna, 1971).

Some bryophytes possess heteromorphic sex chromosomes. But, unlike with animals and seed plants, existence of heteromorphic sex chromosomes does not correlate with dioecy. The percentage of bryophytes that have heteromorphic sex chromosomes seems roughly comparable to the percentage in animals. Heteromorphic sex chromosomes have been identified in at least 30 species of moss (Bryophyta; Khanna, 1971) and at least two genera of liverwort (Hepatophyta; Allen, 1917; Bischler, 1986). Yet, unlike with most animals, the cause of the length difference in sex chromosomes in spore-bearing plants cannot be attributed to Muller's ratchet because so much selection occurs in their haploid stage. In fact, it is possible (but unstudied) that spore-bearing plants have sporophytes that express a small fraction of those genes expressed in their gametophytes. The theory above then dictates that only options other than Muller's ratchet are available for the evolution of heteromorphic sex chromosomes in spore-bearing plants. This is entirely consistent with Bull's (1978) assertions that sex chromosomes in haploid dioecious organisms (i.e., those in which X gametophytes are female, Y gametophytes are male, and all sporophytes are XY) should not degenerate in length, but instead acquire length dimorphism via addition of beneficial genes. Recent empirical work in dioecious liverworts corroborates this assertion (Okada et al., 2001; Ishizaki et al., 2002). However, Hood et al. (2004), who work with a haploid dioecious fungus (*Microbotryum violaceum*), believe that the genomic additions could be in the form of transposons or noncoding sequences, i.e., deleterious or neutral genomic additions.

Chromosomal rearrangements provide the most likely origin of bryophyte and homosporous pteridophyte heteromorphic sex chromosomes because of their extraordinarily high rates of polyploidy and polysomy (Såstad, in press). Duplication of entire chromosomes results in an immediate addition of epigenetic signals, at least in flowering plants (which is the one taxon from which data currently exists on this phenomenon). In cotton (*Gossypium*; Malvaceae), one-quarter of the genes tested appeared to be epigenetically silenced immediately following polyploid formation (Adams et al., 2003). Adams et al. (2003) tentatively attributed this epigenetic silencing to changes in cytosine methylation and chromatin levels. Similarly, Wang et al. (2004) report that 3–11% of homeologous genes are epigenetically reciprocally silenced following polyploid formation in *Arabidopsis* (Brassicaceae), and this silencing appears to be caused by changes in cytosine methylation (or the machinery that maintains it, such as dihydroxypropyladenine inhibiting methyltransferase activity). At this juncture, no other studies have been published regarding polyploidy or polysomy epigenetically silencing genes on reciprocal homeologous genes, but this may simply reflect that only a few taxa have yet been examined. However, similar empirical evidence and theoretical arguments for reciprocal silencing of homeologous genes in polyploids was put forwards a dozen years earlier for homosporous pteridophytes (Gastony, 1991; Werth and Windham, 1991), albeit none of these earlier authors asserted that the silencing was of epigenetic origin. High levels of cytosine methylation following polyploid formation may result in chromosomal rearrangements such as translocations (Lim et al., 2004), which may have provided the genesis of sex chromosomes in highly polyploid plant lineages. High levels of cytosine methylation also result in high levels of heterochromatin (Scarbrough et al., 1984), especially constitutive heterochromatin (Buzek et al., 1998). Nascent additions of constitutive heterochromatin probably made the sex chromosomes of the liverworts *Sphaerocarpos donnellii* and *S. texanus* readily visible to Charles Allen almost a century ago (Allen, 1917, 1919).

DISCUSSION

There is growing evidence that nascent epigenetic signals, especially cytosine methylation, are essential for the evolution of dioecy from hermaphroditic ancestors for those lineages that have had autosomes but no well-defined sex chromosomes. This cytosine methylation does more than just down-regulate genes that control female or male function. It also provides the initial suppression of recombination, thereby starting Muller's ratchet of Y or W chromosome evolution, as well as accelerating the rate of Muller's ratchet by increasing

the rates of mutations and epimutations. If Muller's ratchet is truly the mechanism by which Y and W chromosomes degenerate over evolutionary time, then we may have an elegant explanation for why dioecious seed plants lack heteromorphic sex chromosomes, so long as tentative empirical work on high levels of gene expression in gametophytes generalizes to all flowering plants. Muller's ratchet cannot work in plants because, unlike with animals, plant haploid stages (gametophytes) appear to not be largely immune from selection. For better or worse, the theory in this paper crucially depends upon the premise that plant gametophytes are subject to approximately as much selection (if not more) as their sporophytes, whereas animal gametes (except those with planktonic larvae) are largely immune from selection when compared with their diploid stages. Heteromorphic sex chromosomes can thereby only arise in plants by other means, such as chromosomal rearrangements or gradual acquisition of beneficial genes. Extensive chromosomal rearrangements may themselves be a result of extensive polyploidy, as appears to have occurred in bryophytes and homosporous pteridophytes.

In animals, heteromorphic sex chromosomes canalize dioecy in previously hermaphroditic lineages. However, extensive polyploidy (or polysomy of the sex chromosomes) can provide an escape from this canalized dioecy. Extensive polyploidy is lacking in animals and most seed plants, but is present in bryophytes and homosporous pteridophytes. Therefore, the above theory implies that dioecy due to heteromorphic sex chromosomes can exist, but should be evolutionarily ephemeral in bryophytes and homosporous pteridophytes due to polyploidy. Additionally, very high incidences of polyploidy in bryophytes and homosporous pteridophytes are probably responsible for differential growth in the length of X and Z chromosomes, due to translocations or possibly additional duplication of beneficial genes.

LITERATURE CITED

ADAMS, K. L., R. CRONN, R. PERCIFIELD, AND J. F. WENDEL. 2003. Genes duplicated by polyploidy show unequal contributions to the transcriptome and organ-specific reciprocal silencing. *Proceedings of the National Academy of Sciences, USA* 100: 4649–4654.

AINSWORTH, C. 2000. Boys and girls come out to play: the molecular biology of dioecious plants. *Annals of Botany* 86: 211–221.

ALLEN, C. E. 1917. A chromosome difference correlated with sex differences in *Sphaerocarpos*. *Science* 46: 466.

ALLEN, C. E. 1919. The basis of sex inheritance in *Sphaerocarpos*. *Proceedings of the American Philosophical Society* 58: 289–316.

BAWA, K. S. 1980. Evolution of dioecy in flowering plants. *Annual Review of Ecology and Systematics* 11: 15–39.

BELL, G. 1988. Sex and death in protozoa: the history of an obsession. Cambridge University Press, Cambridge, UK.

BISCHLER, H. 1986. *Marchantia polymorpha* L. s. lat. karyotype analysis. *Journal of the Hattori Botanical Laboratory* 60: 105–107.

BORGANONKAR, D. 1974. Animal cytogenetics: Chordata 4: Mammalia II: Placentalia: 4. Insectivora and Chiroptera. Gebrüder Borntraeger, Stuttgart, Germany.

BULL, J. J. 1978. Sex chromosomes in haploid dioecy: a unique contrast to Muller's theory for diploid dioecy. *American Naturalist* 112: 245–250.

BULL, J. J. 1983. Evolution of sex determining mechanisms. Benjamin/Cummings, Menlo Park, California, USA.

BUZEK, J., I. EBERT, M. RUFFINI-CASTIGLIONE, J. SIROKY, B. VYSKOT, AND J. GREILHUBER. 1998. Structure and DNA methylation pattern of partially heterochromatinised endosperm nuclei in *Gagea lutea* (Liliaceae). *Planta* 204: 506–514.

CHARLESWORTH, B., AND D. CHARLESWORTH. 1978. Model for evolution of dioecy and gynodioecy. *American Naturalist* 112: 975–997.

CHRISTIDIS, L. 1990. Animal cytogenetics: Chordata 3b: Aves. Gebrüder Borntraeger, Stuttgart, Germany.

COLOT, V., AND J.-L. ROSSIGNOL. 1999. Eukaryotic DNA methylation as an evolutionary device. *BioEssays* 21: 402–411.

CROZIER, R. H. 1975. Animal cytogenetics: Insecta 7: Hymenoptera. Gebrüder Borntraeger, Berlin, Germany.

DARWIN, C. R. 1873. On the males and complemental males of certain cirripedes, and rudimentary structures. *Nature* 8: 431–432.

DE ALMEIDA-TOLEDO, L. F., F. FORESTI, E. VIEGAS-PÉQUIGNOT, AND M. F. Z. DANIEL-SILVA. 2001. XX:XY sex chromosome system with X heterochromatinization: an early stage of sex chromosome differentiation in the neotropic electric eel *Eigenmannia virescens*. *Cytogenetics and Cell Genetics* 95: 73–78.

DREWS, G. N., AND R. YADEGARI. 2002. Development and function of the angiosperm female gametophyte. *Annual Review of Genetics* 36: 99–124.

DÜBENDORFER, A., M. HEDIGER, G. BURGHARDT, AND D. BOPP. 2002. *Musca domestica*, a window on the evolution of sex-determining mechanisms in insects. *International Journal of Developmental Biology* 46: 75–79.

EGOZCUE, J. 1974. Animal cytogenetics: Chordata 4: Mammalia II: Placentalia: 5. Primates. Gebrüder Borntraeger, Stuttgart, Germany.

FISHER, R. A. 1931. The evolution of dominance. *Biological Reviews and Biological Proceedings of the Cambridge Philosophical Society* 6: 345–368.

FREGDA, K. 1974. Animal cytogenetics: Chordata 4: Mammalia II: Placentalia: 2. Carnivora. Gebrüder Borntraeger, Stuttgart, Germany.

GASTONY, G. J. 1991. Gene silencing in a polyploid homosporous fern: paleopolyploidy revisited. *Proceedings of the National Academy of Sciences, USA* 88: 1602–1605.

GOLDBLATT, P., AND D. E. JOHNSON. 2003. Index to plant chromosome numbers. Missouri Botanical Garden, St. Louis, Missouri, USA (also available at website http://mobot.mobot.org/W3T/Search/ipcn.html).

GORELICK, R. 2003. Evolution of dioecy and sex chromosomes via methylation driving Muller's ratchet. *Biological Journal of the Linnean Society* 80: 353–368.

GORELICK, R., AND R. OSBORNE. 2002. Inducing sex change and organogenesis from tissue culture in the endangered African cycad *Encephalartos woodii* (Cycadales, Zamiaceae). *South African Journal of Science* 98: 114–117.

GRAVES, J. A. M. 1995. The origin and function of the mammalian Y chromosome and Y-borne genes: an evolving understanding. *BioEssays* 17: 311–320.

GRAVES, J. A. M. 1996. Mammals that break the rules: genetics of marsupials and monotremes. *Annual Review of Genetics* 30: 233–260.

GRIFFIN, D. K., S. C. HARVEY, R. CAMPOS-RAMOS, L.-J. AYLING, N. R. BROMAGE, J. S. MASABANDA, AND D. J. PENMAN. 2002. Early origins of the X and Y chromosomes: lessons from tilapia. *Cytogenetic and Genome Research* 99: 157–163.

GUSTAVSSON, I. 1974. Animal cytogenetics: Chordata 4: Mammalia II: Placentalia: 3. Ungulata. Gebrüder Borntraeger, Stuttgart, Germany.

HAYMAN, D. L., AND P. G. MARTIN. 1974. Animal cytogenetics: Chordata 4: Mammalia I: Monotremata and Marsupialia. Gebrüder Borntraeger, Stuttgart, Germany.

HEWITT, G. M. 1979. Animal cytogenetics: Insecta 1: Orthoptera: grasshoppers and crickets. Gebrüder Borntraeger, Berlin, Germany.

HIZUME, M., Z. GU, Z. YUE, AND K. KONDO. 1993. Karyomorphological study on *Gnetum montanum* collected in Yunnan, China. *Chromosome Information Service* 54: 23–25.

HOLLIDAY, R. 1984. The biological significance of meiosis. *In* C. E. Evans and H. G. Dickinson [eds.], Controlling events in meiosis, 381–394. Cambridge University Press, Cambridge, UK.

HOOD, M. E., J. ANTONOVICS, AND B. KOSKELLA. 2004. Shared forces of sex chromosome evolution in haploid-mating and diploid-mating organisms: *Microbotryum violaceum* and other model organisms. *Genetics* 168: 141–146.

IGUCHI-ARIGA, S. M. M., AND W. SCHAFFNER. 1989. CpG methylation of the cAMP-responsive enhancer/promoter sequence TGACGTCA abolishes specific factor binding as well as transcriptional activation. *Genes and Development* 3: 612–619.

ISHIZAKI, K., Y. SHIMIZU-UEDA, S. OKADA, M. YAMAMOTO, M. FUJISAWA, K. T. YAMATO, H. FUKUZAWA, AND K. OHYAMA. 2002. Multicopy genes uniquely amplified in the Y chromosome-specific repeats of the liverwort *Marchantia polymorpha*. *Nucleic Acids Research* 30: 4675–4681.

JONES, P. A., AND D. TAKAI. 2001. The role of DNA methylation in mammalian epigenetics. *Science* 293: 1068–1070.

KING, M. 1990. Animal cytogenetics: Chordata 2: Amphibia. Gebrüder Borntraeger, Stuttgart, Germany.

KHANNA, K. R. 1971. Sex chromosomes in bryophytes. *Nucleus* 14: 14–23.

KHOSHOO, T. N. 1959. Polyploidy in gymnosperms. *Evolution* 13: 24–39.

KLEKOWSKI, E. J., AND H. G. BAKER. 1966. Evolutionary significance of polyploidy in Pteridophyta. *Science* 153: 305–307.

LEVIN, D. A. 2002. The role of chromosomal change in plant evolution. Oxford University Press, Oxford, UK.

LEWIS, W. H. 1980. Polyploidy: biological significance. Plenum Press, New York, New York, USA.

LIM, K. Y., R. MATYASEK, A. KOVARIK, AND A. R. LEITCH. 2004. Genome evolution in allotetraploid *Nicotiana*. *Biological Journal of the Linnean Society* 82: 599–606.

LIU, Z. Y., ET AL. 2004. A primitive Y chromosome in papaya marks incipient sex chromosome evolution. *Nature* 427: 348–352.

LLOYD, D. G. 1980. Sexual strategies in plants. III. A quantitative method for describing the gender of plants. *New Zealand Journal of Botany* 18: 103–108.

LOWCOCK, L. A. 1994. Biotype, genomotype, and genotype: variable effects of polyploidy and hybridity on ecological partitioning in a bisexual–unisexual community of salamanders. *Canadian Journal of Zoology-Revue Canadienne De Zoologie* 72: 104–117.

MA, H., P. H. MOORE, Z. LIU, M. S. KIM, Q. YU, M. M. M. FITCH, T. SEKIOKA, A. H. PATERSON, AND R. MING. 2004. High-density linkage mapping revealed suppression of recombination at the sex determination locus in papaya. *Genetics* 166: 419–436.

MARTIENSSEN, R. A., AND V. COLOT. 2001. DNA methylation and epigenetic inheritance in plants and filamentous fungi. *Science* 293: 1070–1074.

MATUSZEWSKI, B. 1982. Animal cytogenetics: Insecta 3: Diptera I: Cecidomyiidae. Gebrüder Borntraeger, Berlin, Germany.

MATZKE, M. A., AND A. J. M. MATZKE. 1998. Polyploidy and transposons. *Trends in Ecology and Evolution* 13: 241.

MOORE, R. C., O. KOZYREVA, S. LEBEL-HARDENACK, J. SIROKY, R. HOBZA, B. VYSKOT, AND S. R. GRANT. 2003. Genetic and functional analysis of *DD44*, a sex-linked gene from the dioecious plant *Silene latifolia*, provides clues to early events in sex chromosome evolution. *Genetics* 163: 321–334.

NAGL, W., AND F. EHRENDORFER. 1974. DNA content, heterochromatin, mitotic index, and growth in perennial and annual *Anthemidea* (Asteraceae). *Plant Systematics and Evolution* 123: 35–54.

NEI, M. 1970. Accumulation of nonfunctional genes on sheltered chromosomes. *American Naturalist* 104: 311–322.

NORSTOG, K. J. 1987. Cycads and the origin of insect pollination. *American Scientist* 75: 270–279.

NORSTOG, K. J. 1993. Spermatogenesis in *Microcycas*: evolutionary significance of male gametes of seed plants. *In* D. W. Stevenson and K. J. Norstog [eds.], The biology, structure, and systematics of the Cycadales, 270–278. Palm & Cycad Societies of Australia, Milton, Queensland, Australia.

NORSTOG, K. J., AND T. J. NICHOLLS. 1997. The biology of cycads. Cornell University Press, Ithaca, New York, USA.

OHNO, S. 1970. Evolution by gene duplication. Springer-Verlag, Berlin, Germany.

OHNO, S. 1974. Animal cytogenetics: Chordata I: Protochordata, Cyclostomata and Pisces. Gebrüder Borntraeger, Stuttgart, Germany.

OKADA, S., ET AL. 2001. The Y chromosome in the liverwort *Marchantia polymorpha* has accumulated unique repeat sequences harboring a male-specific gene. *Proceedings of the National Academy of Sciences, USA* 98: 9454–9459.

OLMO, E. 1986. Animal cytogenetics: Chordata 3a: Reptilia. Gebrüder Borntraeger, Stuttgart, Germany.

PATTON, J. L. 1974. Animal cytogenetics: Chordata 4: Mammalia II: Placentalia: 1. Rodentia. Gebrüder Borntraeger, Stuttgart, Germany.

PRAHALD, V., D. PILGRIM, AND E. B. GOODWIN. 2003. Roles of mating and environment in *C. elegans* sex determination. *Science* 302: 1046–1049.

REGEV, A., M. J. LAMB, AND E. JABLONKA. 1998. The role of DNA methylation in invertebrates: developmental regulation or genome defense? *Molecular Biology and Evolution* 15: 880–891.

RICE, W. R. 1987a. Genetic hitchhiking and the evolution of reduced genetic activity of the Y chromosome. *Genetics* 116: 161–167.

RICE, W. R. 1987b. The accumulation of sexually antagonistic genes as a selective agent promoting the evolution of reduced recombination between primitive sex-chromosomes. *Evolution* 41: 911–914.

RICE, W. R. 1996. Evolution of the Y sex chromosome in animals. *Bioscience* 46: 331–343.

SÅSTAD, S. M. In press. Patterns and mechanisms of polyploid speciation in bryophytes. *In* F. T. Bakker, L. W. Chatrou, B. Gravendeel, and P. Pelser [eds.], Plant species-level systematics: new perspectives on pattern and process, 317–334. Ganter Verlag, Ruggell, Liechtenstein.

SAYLOR, L. C., AND H. A. SIMONS. 1970. Karyology of *Sequoia sempervirens*: karyotype and accessory chromosomes. *Cytologia* 35: 294–303.

SCARBROUGH, K., S. HATTMAN, AND U. NUR. 1984. Relationship of DNA methylation level to the presence of heterochromatin in mealybugs. *Molecular and Cellular Biology* 4: 599–603.

SILJAK-YAKOVLEV, S., S. BENMALEK, M. CERBAH, T. COBA DE LA PEÑA, N. BOUNAGA, S. C. BROWN, AND A. SARR. 1996. Chromosomal sex determination and heterochromatin structure in date palm. *Sexual Plant Reproduction* 9: 127–132.

SITES, J. W., J. W. ARCHIE, C. J. COLE, AND O. FLORES-VILLELA. 1992. A review of the phylogenetic hypotheses for lizards of the genus *Sceloporus* (Phrynosomatidae): implications for ecological and evolutionary studies. *Bulletin of the American Museum of Natural History* 213: 1–110.

SMITH, S. G., AND N. VIRKKI. 1978. Animal cytogenetics: Insecta 5: Coleoptera. Gebrüder Borntraeger, Berlin, Germany.

SOLARI, A. J. 1994. Sex chromosomes and sex determination in vertebrates. CRC Press, Boca Raton, Florida, USA.

STAUFFER, R. C. 1975. Charles Darwin's *Natural Selection*: being the second part of his big book written from 1856 to 1858. Cambridge University Press, Cambridge, UK.

STEINEMANN, M., AND S. STEINEMANN. 1992. Degenerating Y chromosome of *Drosophila miranda*: a trap for retrotransposons. *Proceedings of the National Academy of Sciences, USA* 89: 7591–7595.

STEINEMANN, M., AND S. STEINEMANN. 1998. Enigma of Y chromosome degeneration: neo-Y and neo-X chromosomes of *Drosophila miranda* a model for sex chromosome evolution. *Genetica* 102/103: 409–420.

UESHIMA, N. 1979. Animal cytogenetics: Insecta 6: Hemipetera II: Heteroptera. Gebrüder Borntraeger, Berlin, Germany.

VOLPE, P., AND T. EREMENKO. 1974. Preferential methylation of regulatory genes in HeLa cells. *FEBS Letters* 44: 121–126.

WANG, J. L., L. TIAN, A. MADLUNG, H.-S. LEE, M. CHEN, J. J. LEE, B. WATSON, T. KAGOCHI, L. COMAI, AND Z. J. CHEN. 2004. Stochastic and epigenetic changes of gene expression in *Arabidopsis* polyploids. *Genetics* 167: 1961–1973.

WERTH, C. R., AND M. D. WINDHAM. 1991. A model for divergent, allopatric speciation of polyploid pteridophytes resulting from silencing of duplicate gene expression. *American Naturalist* 137: 515–526.

WHITE, M. J. D., AND G. C. WEBB. 1976. Animal cytogenetics: Insecta 2: Blattodea, Mantodea, Isoptera, Grylloblattodea, Phasmatodea, Dermaptera and Embioptera. Gebrüder Borntraeger, Berlin, Germany.

WILLING, R. P., D. BASHE, AND J. P. MASCARENHAS. 1988. An analysis of the quantity and diversity of messenger RNAs from pollen and shoots of *Zea mays*. *Theoretical and Applied Genetics* 75: 751–753.

YAMPLOSKY, E., AND H. YAMPLOSKY. 1922. Determination of sex forms in the phanerogamic flora. *Bibliographia Genetica* 3: 1–62.

YODER, J. A., C. P. WALSH, AND T. H. BESTOR. 1997. Cytosine methylation and the ecology of intragenomic parasites. *Trends in Genetics* 13: 335–340.