Revisiting the Dioecy-Polyploidy Association: Alternate Pathways and Research Opportunities

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Abstract

The evolutionary transition from hermaphroditism (combined sexes) to dioecy (separate sexes) is associated with whole genome duplication (polyploidy) in several flowering plant genera. Moreover, there is evidence for transitions in the opposite direction, i.e. a loss of dioecy with an increase in ploidy. Here, we review evidence for these associations, synthesize previous ideas on the mechanism underlying the patterns and explore alternative pathways. Specifically, we examine potential ecological and genetic mechanisms, differentiated by whether ploidy or gender (functional sex expression of the plant) changes are the primary cause and whether the effect is direct or indirect. An analysis of 22 genera variable for both ploidy and gender indicates that gender monomorphism (hermaphroditism, monoecy) is more common among diploid than polyploid species, whereas gender dimorphism (dioecy, gynodioecy, subdioecy) is more frequent among polyploid species. The transition from diploid hermaphroditic to polyploid gen-

der-dimorphic taxa may arise directly through changes in gender as a result of genome duplication through genomic rearrangements or homeologous recombination, or changes in gender may result in increased unreduced gamete production leading to polyploid formation. Alternatively, the transition may occur through the indirect effects of genome duplication on mating system and inbreeding depression, which favor selection for unisexuality, or habitat shifts associated with unisexuality may simultaneously cause increased unreduced gamete production. Novel mechanisms for transitions in the opposite direction (from dioecy to hermaphroditism with increase in ploidy) include disruption of genetic sex determination and the benefits of reproductive assurance. We highlight key questions requiring further attention and promising approaches for answering them and better clarifying the genesis of sexual system polyploidy associations. See also the sister article focusing on animals by Wertheim et al. in this themed issue.

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Most angiosperms are hermaphroditic, where each individual possesses both male and female reproductive structures. However, separate sexes (males and females,

Fig. 1. Schematic of a 2-step transition from hermaphroditism (H) to dioecy (D) via gynodioecy (GD). The first step involves the invasion and maintenance of females in hermaphroditic populations, creating gynodioecy. During the second step, males invade either via mutations of small effect (subdioecy – SD) or major effect (trioecy – T). Ultimately, males supplant hermaphrodites and the sexual system becomes dioecious. All of the non-hermaphrodite sexual systems are referred to as gender-dimorphic herein.

known as dioecy) have evolved from hermaphrodite ancestors over 100 times in angiosperms [Charlesworth, 2002]. This transition can occur via a 2-step pathway (fig. 1) [based on Lloyd, 1976; Charlesworth and Charlesworth, 1978; Charlesworth, 1989; Spigler and Ashman, 2012]. In the first step, a male sterility mutation arises creating females, who establish if they have a seed fertility advantage over hermaphrodites. When females are maintained amongst the hermaphrodites (a sexual system known as gynodioecy), their presence leads to selection favoring males (i.e. favoring major or minor female sterility mutations that have negative pleiotropic effects on male fitness). Thus, populations can contain hermaphrodites with low female function and/or males (and present a sexual system known as subdioecy or trioecy). Ultimately, these males supplant the hermaphrodites, and the sexual system is dioecious. Other pathways to/from dioecy are also possible [reviewed in Charlesworth, 1999]. In fact, there are multiple ways plants can express ‘functional gender’ (i.e. the sex expression of a plant) [following Lloyd, 1980; reviewed in Sakai and Weller, 1999; but see Haig, 2000], and thus, populations can be gender-monomorphic (one morph that is both male and female, i.e. hermaphroditism (male and female structures within a flower) or monoecy (male and female flowers on a single plant)) or gender-dimorphic (i.e. dioecy and other systems with more than one sexual morph, gynodioecy, subdioecy, trioecy, and androdioecy). Distinguishing the selective advantages and genetic mechanisms that influence the transitions to gender dimor-

phism is fundamental to our understanding of one of the broadest evolutionary convergences in flowering plants [Sakai and Weller, 1999; Ashman, 2006; Spigler and Ashman, 2012].

Although many ecological and genetic factors have been associated with the transition to separate sexes in plants [Givnish, 1980; Vamوسي et al., 2003; Ashman, 2006; Spigler and Ashman, 2012], one of the most puzzling patterns is the association between dioecy and an increase in polyploidy first noticed by Jennings [1976] and Baker [1984] and highlighted by Miller and Venable [2000]. However, the most often cited hypothesis for this association [Miller and Venable, 2000] has not been explored thoroughly. In addition, there are other possible genetic and ecological interactions between ploidy and sexual system [Pannell et al., 2004], but none of these pathways have been considered fully. Moreover, although the opposite pattern (e.g. dioecious diploids and hermaphroditic polyploids) is also known to occur, the mechanisms underlying this transition (e.g. reproductive assurance) have yet to be considered widely [Pannell et al., 2004]. Because of our incomplete understanding and the fact that polyploidization is arguably one of the most widespread genomic changes and a major mechanism of speciation [Otto and Whitton, 2000; Wood et al., 2009] in land plants, we propose that the associations between polyploidy and gender dimorphism requires further scrutiny.

In this paper, we first review the available evidence for associations between polyploidy and gender dimorphism. To provide the most complete picture of the associations, we consider all sexual systems with gender dimorphism (i.e. dioecy, gynodioecy, subdioecy, trioecy, and androdioecy) relative to those with gender monomorphism (i.e. hermaphroditism and monoecy). We then differentiate several mechanistic pathways from gender-monomorphic diploids to gender-dimorphic polyploids. The majority of mechanisms we propose (especially those involving unreduced gamete production and genomic rearrangements) have previously been underappreciated. We then consider several mechanistic pathways from gender-dimorphic diploids to monomorphic polyploids. For the purposes of this paper, we assume that the most likely direction of the transition is from diploids to polyploids, as it is generally accepted that ploidy is much more likely to increase than decrease in flowering plants [Stebbins, 1950; Levin, 2002; Meyers and Levin, 2006]. We end by highlighting some of the key questions requiring further attention and promising approaches to answer them.

Overview of the Patterns

Several genera of flowering plants exhibit variability among species for both sexual system and ploidy (table 1; online suppl. table 1, for all online suppl. material, see www.karger.com/doi/10.1159/000353306). Although not exhaustive, table 1 presents the patterns of variation for sexual system, mating system and ploidy in 22 genera across 18 families. Summary data are based on species for which both sexual system and ploidy evidence are available using the primary literature and the Index to Plant Chromosome Numbers database (Missouri Botanical Garden, <http://www.tropicos.org/Project/PCN>) (online suppl. table 1). Many of these genera were identified by Miller and Venable [2000]. Here, we expand the list and also report, whether polyploid species are identified as autopolyploid, allopolyploid or both, as the mechanism of genome duplication may affect gender differently (see below).

Based on table 1, diploid hermaphroditic species are reported from 21 of 22 (95.5%) genera, 11 of which are exclusively hermaphroditic, and 10 contain gynodioecious and dioecious species. In contrast, 21 of 22 (95.5%) genera contain polyploids with sexual dimorphism; 16 genera contain polyploid hermaphroditic species as well. Thus, genera are more likely to contain gender-dimorphic polyploid species than gender-dimorphic diploid species (96% of genera vs. 50%, respectively; $\chi^2 = 10.92$, $p = 0.0011$) and more likely to have hermaphroditic diploid species than hermaphroditic polyploid species (96% vs. 73%; $\chi^2 = 2.72$, $p = 0.09$), although not significantly. Based on the evidence available, allopolyploidy is not more frequently observed (11 of 13 genera) than autopolyploidy (7 of 13 genera; Fisher's exact test, $p = 0.20$), although this may partly reflect the difficulty distinguishing the 2 forms using genetic and cytogenetic criteria [Ramsey and Schemske, 1998].

The 22 genera in table 1 are organized into 3 groups based on the pattern of association between ploidy and gender that can be inferred. Eleven genera (table 1, group A) exhibit unambiguous evidence for a transition from diploid gender monomorphism to polyploid gender dimorphism. Three of these genera are from Cactaceae, 3 from Rosaceae. Ten genera exhibit both sexual systems in both diploid and polyploid species, so the pattern is unclear without additional phylogenetic information (table 1, group B). Two of these genera (*Mercurialis*, *Empetrum*) are often used in the literature as examples of the breakdown of dioecy to hermaphroditism in polyploids [Miller and Venable, 2000]. Finally, one genus (*Isotoma*) provides unambiguous evidence for the loss of dioecy

with increased ploidy (table 1, group C). Information on additional species and phylogenetic relationships for each genus are needed to assess the frequency and incidence of evolutionary transitions along specific lineages within these clades.

Case Studies

Not all genera have been examined in sufficient phylogenetic detail to reveal the evolutionary transitions in ploidy and gender. Here, we describe select genera (table 1) for which informative phylogenetic information is available and can provide more insight.

Transitions from Gender-Monomorphic Diploids to Gender-Dimorphic Polyploids

The genus *Lycium* (Solanaceae) occurs in North America and South Africa and is variable for both ploidy ($2n = 2 \times = 12, 24, 48$) and sexual system (hermaphroditism, gynodioecy and dioecy) [Miller and Venable, 2000]. On both continents, hermaphroditic species are diploid, whereas gender-dimorphic species are consistently tetraploid or octoploid. In North America, polyploidy has evolved once from diploids, giving rise to a clade of 3 species [Miller and Venable, 2000; Miller, 2002]. However, more recently, the placement of *L. californicum*, a polyploid gender-dimorphic species, was revised as having a separate origin of polyploidy [Levin and Miller, 2005]. Furthermore, a detailed population survey has led to the conclusion that this species is also polymorphic for polyploidy and sexual system with populations being either diploid and hermaphroditic or tetraploid and gender-dimorphic [Yeung et al., 2005]. This indicates that the transition to polyploid gender-dimorphic populations is relatively labile even within species.

Fragaria (Rosaceae) consists of 27 wild taxa with centers of biodiversity in America, Europe and Asia. Seventeen of the taxa are hermaphroditic and 10 are gender-dimorphic (gynodioecy, subdioecy, dioecy) [Ashman et al., 2011]. Fifteen of 17 hermaphroditic species are diploid ($2n = 2 \times = 14$), whereas 9 of 10 gender-dimorphic species are polyploid ($4 \times, 6 \times, 8 \times$). Based on cpDNA and nuclear gene phylogenies [Potter et al., 2000; Rousseau-Gueutin et al., 2009], gender dimorphism and polyploidy occur in all major clades, and polyploid dioecious taxa are often sister to diploid hermaphroditic ones [Ashman et al., 2011]. Polyploidy has evolved a minimum of 4 times

Table 1. Summary of angiosperm genera exhibiting variation among species in ploidy and sexual system

Family	Genus	Diploid			Polyploid					Reference
		N _d	mating system	sexual system	N _p	ploidy level	mating system	sexual system	type	
Group A										
Cactaceae	<i>Pachycereus</i>	1	si	h	1	4×	sc	t	u	11
Cactaceae	<i>Mammillaria</i>	3	si	h	6	4×, 6×, 8×, 24×	si	d, g, h	l, u	12, 13, 14, 15
Cactaceae	<i>Echinocereus</i>	2	si	h	5	4×, 6×	sc	d, h	–	16, 17, 18
Geniostomaceae	<i>Labordia</i>	1*	–	h	16	4×	–	d	–	34, 35
Melanthiaceae	<i>Chionographis</i>	2	si	h	1**	4×	sc	g	l	36
Ranunculaceae	<i>Thalictrum</i>	5	–	h	22	4×, 6×, 8×, 10×, 12×, 16×	–	d, h	–	55
Rosaceae	<i>Fragaria</i>	13	sc, si	h, g	9	4×, 6×, 8×, 10×	sc	d, g, h, t	l, u	56, 57, 58, 59
Rosaceae	<i>Potentilla</i>	3	sc, si	h	11	4×, 5×, 6×, 7×, 8×, 10×, 12×, 13×	sc, si	d, h	l, u	57, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72
Rosaceae	<i>Rubus</i>	2	si	h	5	4×, 6×, 8×, 12×	sc	d, h	l	73, 74, 75, 76, 77, 78, 79, 80
Saxifragaceae	<i>Astilbe</i>	23	–	h	1	4×	sc	s	l	81, 82, 83
Solanaceae	<i>Lycium</i>	13	si	h	8	4×, 8×	sc	d, g	u	84, 85, 86, 87
Group B										
Boraginaceae	<i>Phacelia</i>	2	sc	g, h	4	4×, 8×	sc	g, h	–	1, 2, 3, 4, 5, 6, 7, 8, 9, 10
Caryophyllaceae	<i>Silene</i>	3	sc	d, h	4	4×, 6×	sc	d, h	l	19, 20, 21, 22, 23
Fabaceae	<i>Trifolium</i>	8	sc, si	g, h	2	4×	sc, si	g, h	l	29, 30, 31
Onagraceae	<i>Fuchsia</i>	3	sc	g, h	10	4×, 8×	sc	d, g, h	–	37, 38, 39, 40, 41, 42, 43, 44
Poaceae	<i>Bouteloua</i>	8	sc	d, g, h	6	4×, 6×	sc	d, g, h	–	45
Polygonaceae	<i>Rumex</i>	12	–	d, g, h	12	4×, 6×, 8×, 10×	–	d, g, h	–	46, 47, 48, 49, 50, 51, 52, 53, 54
Valerianaceae	<i>Valeriana</i>	1	–	d	6	4×, 8×, 12×	–	g, h	–	88, 89, 90, 91, 92, 93
Euphorbiaceae	<i>Mercurialis</i>	8	–	d, h	4	4×, 6×, 8×, 10×, 12×, 14×	–	a, d, h	l, u	27, 28
Ericaceae	<i>Empetrum</i>	4	si	d, h	4	4×	–	d, h	l	25, 26
Cucurbitaceae	<i>Bryonia</i>	7	sc	d, h	3	4×, 6×	sc	d, h	l, u	24
Group C										
Lobeliaceae	<i>Isotoma</i>	2	sc	d, h	1	4×	–	h	–	32, 33

Group A represents genera with unambiguous evidence for a transition from diploid gender monomorphic to gender dimorphic polyploid species, group B is ambiguous, and group C shows clear evidence for the reverse pattern. The number of diploid (N_d) and polyploid (N_p) species examined, type of polyploid (u = autopolyploid, l = allopolyploid) and ploidy levels were recorded when available. Ploidy level is based on reports from the literature or on the somatic chromosome number, expressed as multiples of the base number for the genus. Sex-

ual system is indicated as h = hermaphrodite (including monoecy), g = gynodioecious, s = subdioecious, t = trioecious, a = androdioecious, and d = dioecious. The mating system (si = self-incompatible, sc = self-compatible) is also reported when available. – = Data not available. Reference numbers correspond to the supplementary reference list (online suppl. table 2).

* Diploid species is currently in the genus *Geniostoma*, as reported in Miller and Venable [2000]; ** one tetraploid species of 3 varieties.

[Rousseau-Gueutin et al., 2009; Njuguna et al., 2013]. The octoploids (*F. chilensis* and *F. virginiana*) are allopolyploids, and the mode of origin for tetraploid and hexaploid species may involve multiple diploid parents, but the complete genealogy is ambiguous based on current phylogenies [Rousseau-Gueutin et al., 2009; Njuguna et al., 2013]. A recent study based on whole chloroplast genome sequencing of 21 species [Njuguna et al., 2013] reinforces the complexities of the evolution of polyploid gender-dimorphic species. Phylogenetic analyses indicate that one diploid subspecies which is gynodioecious

(*F. vesca* ssp. *bracteata*) contributed the maternal genome to the octoploids. Thus, while the loss of male function appears to occur both before and after polyploidization, the loss of female function is observed only after polyploidization [Njuguna et al., 2013].

The genus *Leptinella* (Compositae) comprises 42 taxa that occur in the southern hemisphere [Himmelreich et al., 2012]. Eleven of the 15 taxa that are gender-dimorphic (dioecy and paradioecy) are octoploid or show a higher ploidy level. In this group, gender dimorphism evolved from monoecy, and complete dioecy is only found in 2

tetraploid species, leading the authors to conclude that dioecy evolved at the tetraploid level and was retained during further polyploidization events (16×, 20×, 24×) [Himmelreich et al., 2012].

Transitions Involving Gender-Dimorphic Diploids to Gender-Monomorphic Polyploids

Mercurialis is a genus of western Central Europe and the Mediterranean that is variable for gender in both diploid and polyploid taxa. At the genus level (table 1), the specific transitions toward and away from gender-dimorphic polyploids are unclear, as polyploid and diploid species can be hermaphroditic or gender-dimorphic. Phylogenetic analyses [Krähenbühl et al., 2002] reveal 3 major clades, one of which contains only diploid dioecious species. In a second clade of annual species, considered the *M. annua* species complex, *M. annua* s.s. is diploid and dioecious and is sister to a polyploid (4–12×) complex (*M. ambigua*), which ranges in sexual system from monoecy to androdioecy and subdioecy [Krähenbühl et al., 2002; Pannell et al., 2004]. A third clade (*M. perennis* complex) contains *M. perennis*, which is polyploid and dioecious, and 2 species that are variable for ploidy (diploid and polyploid) and reported as hermaphroditic (*M. leiocarpa*) or dioecious (*M. ovata*), respectively. Therefore, one clade (referred to as *M. annua* s.l. by Pannell et al. [2004]) provides evidence for a shift from gender-dimorphic (dioecy) to monomorphic (monoecy). Interestingly, among the polyploid taxa, autopolyploidy appears to have given rise to monoecious tetraploids, whereas hexaploids, which contain monoecy and androdioecy, are of allopolyploid origin [Obbard et al., 2006].

The transition from gender-dimorphic diploid to gender-monomorphic polyploid has also been reported from genera such as *Empetrum* [Miller and Venable, 2000] and *Bryonia* [Volz and Renner, 2008]. Both genera contain diploid and polyploid dioecious and hermaphroditic species. However, current phylogenetic hypotheses for *Empetrum* [Anderberg, 1994] are not fully resolved, making it difficult to isolate specific ancestor-derivative relations. Phylogenetic hypotheses for *Bryonia* [Volz and Renner, 2008] suggest that dioecious polyploid taxa (*B. cretica*, *B. marmorata*) are derived from dioecious diploids, likely through both autopolyploidy and allopolyploidy. One other species (*B. aspera*) contains diploid and tetraploid individuals, but the sexual system is uniformly hermaphroditic. Thus, there is currently little support for an association between ploidy and sexual system in these groups.

Causal Pathways

In the following section, we describe the multiple pathways that can lead to associations between polyploidy and gender dimorphism. We include ideas put forth to date [Miller and Venable, 2000; Pannell et al., 2004] and present other novel alternatives. We classify all of these according to the timing of character transitions (simultaneous and sequential) (table 2). Simultaneous (direct) pathways refer to cases in which ploidy and gender changes occur in concert due to genetic linkages, whereas sequential (indirect) pathways refer to cases, where change in one trait (gender or ploidy) triggers changes in selection in a direction that favors an evolutionary change in the other trait. First, we discuss transitions from gender-monomorphic diploids to gender-dimorphic polyploids and second, transitions from gender-dimorphic diploids to gender-monomorphic polyploids (table 2).

Gender-Monomorphic Diploids to Gender-Dimorphic Polyploids

Simultaneous Pathways

Simultaneous (direct) pathways include those in which whole genome duplication (WGD) or sexual system change is the direct cause, and gender change or increase in ploidy, respectively, is the effect (table 2).

WGD Causes Gender Change

In this pathway, WGD either in conjunction with hybridization or on its own creates allo- or autopolyploids, respectively, and this instantaneously leads to male and/or female sterility. These sterility mutations then create the raw material for sexual system evolution, i.e. unisexual individuals. If male sterility mutations are created by WGD, this can initiate the transition from hermaphroditism to gynodioecy, whereas if female sterility mutations occur in a lineage that already has females, i.e. is gynodioecious (e.g. *Fragaria*, Njuguna et al. [2013]), then, WGD may facilitate the transition to dioecy [Ashman et al., 2011; Spigler and Ashman, 2011].

In theory, loss of function phenotypes can result from gene expression changes triggered by genetic and epigenetic changes well known to follow WGD [Comai et al., 2000; Lai et al., 2005; Gaeta et al., 2007; Johnson and Tricker, 2010]. Structural genomic changes can include insertions (duplication) or deletions and homoeologous rearrangements (transposition and translocations) [Lim et al., 2008; Buggs et al., 2012], and these can contribute

Table 2. Predicted pathways for associations between sexual system and polyploidy

Timing	Pathway		Mechanisms
	cause	effect	
Monomorphic diploid to gender dimorphic polyploid*			
Simultaneous	WGD	instantaneously affects gender expression	<ul style="list-style-type: none">WGD (allopolyploidy) causes male sterilityWGD affects gender expression, male and female sterility, leading to gender dimorphismsex determination genes increase UG production, leading to WGD
	gender shift	instantaneously increases ploidy	
Sequential	WGD	creates conditions for the transition to gender dimorphism	<ul style="list-style-type: none">WGD causes to loss of self-incompatibility, increased selfing, increased inbreeding depression selects for gender dimorphismWGD leads to colonization of novel, harsh environments, favors gender dimorphismWGD increases sex allocation plasticity, leads to gender dimorphismgender dimorphism leads to UG production, promoting WGDinvasion of stressful environments by gender dimorphic species leads to an increased UG production leading to WGD
	gender shift	creates conditions for the transition from diploid to polyploid	
Gender dimorphic diploid to monomorphic polyploid**			
Simultaneous	WGD	instantaneously affects gender expression	<ul style="list-style-type: none">WGD disrupts sex determination leading to gender monomorphismsex determination genes increase UG production, leading to WGD
	gender shift	instantaneously increases ploidy	
Sequential	WGD	creates conditions for the transition to gender monomorphism	<ul style="list-style-type: none">WGD causes reduced inbreeding depression in polyploids, allows selection for monomorphismpolyploids expand to marginal habitats, selection for reproductive assurance favors monomorphismgender monomorphism increases selfing, enhances likelihood of union of UG, thereby facilitating establishment of polyploidsshifts to gender monomorphism in peripheral populations with harsh conditions, stimulates UG production and polyploid formation
	gender shift	creates conditions for the transition to higher ploidy	
<p>* Transitions from diploid gender monomorphism (hermaphroditism or monoecy) to polyploid gender dimorphism (gynodioecy, subdioecy, dioecy, or androdioecy).</p> <p>** Transitions from diploid gender dimorphism to polyploid gender monomorphism. These pathways involve whole genome duplication (WGD) and unreduced gamete (UG) production.</p>			

directly or indirectly to novel gene expression patterns and phenotypes. Translocations (and chromosome loss, aneuploidy), in particular, can reduce fertility because of the production of duplication and deficiency gametes. These are generally expected to be at a selective disadvantage [Gaeta and Pires, 2010], although under specific ecological conditions (see below), they could be favored. Transposition of genes can also lead to novel expression patterns and phenotypes [Muñoz and Sankoff, 2012], including loss of function phenotypes (male and female sterile plants). Translocations and inversions could not only lead to male sterility (or female sterility), but translocations can bring male and female sterility loci together [Wiens and Barlow, 1979; Goldberg et al., 2010], and rearrangements can reduce recombination. As linkage be-

tween male and female sterility loci and recombination suppression between them are key features of nascent sex chromosomes [see Ming et al., 2011 for review], genomic rearrangements can facilitate the creation of a sex-determining region.

Whether rearrangements underlie the reductions in male and female function and sterility that have been observed in natural or synthetic polyploids from hermaphroditic diploids, remains to be seen for most species, but some intriguing observations have been made. For instance, in a comparative mapping study, Spigler et al. [2008] noted evidence of a translocation near the sex-determining region in subdioecious octoploid *F. virginiana* relative to the hermaphroditic diploid map. In addition, a duplicated segment of chromosome 12 in rice is respon-

sible for pollen sterility (i.e. male sterility) as well as hybrid male sterility [Zhang et al., 2011]. The extent of rearrangement may be associated with the pathway by which the polyploid was formed (i.e. via somatic doubling alone or in conjunction with hybridization), the genetic distance between the progenitors or the specific parental genotypes involved [Adams et al., 2003; Szadkowski et al., 2011].

Allopolyploids involve hybridization of at least 2 species, and hybridization itself may lead to changes in sexual system [Obbard et al., 2006; Yakimowski, 2013]. This could result from its effects on gene regulation and reactivation of epigenetically silenced transposons [Marfil et al., 2006; Michalak, 2009]. Distorted regulatory interactions (gene silencing, subfunctionalization or novel activation) are frequently observed after WGD [Wang et al., 2006; Chen, 2007; Hegarty and Hiscock, 2008], and these changes can lead to novel phenotypes and be heritable [Richards, 2006; Verhoeven et al., 2010]. Moreover, because transposable elements are subject to epigenetic modification, their derepression can affect the expression of linked genes [Michalak, 2009]. For instance, in diploid melon (*Cucumis melo*), sex of a flower is determined by 2 genes, and the transition of male to female flowers is a function of transposon-induced epigenetic changes in the promoter of one of them [Martin et al., 2009].

In addition, allopolyploids may be subject to gene expression changes that result from hybridization such as cytonuclear incompatibilities. For instance, Barr and Fishman [2010] showed that hybrids between 2 hermaphroditic species, *Mimulus guttatus* and *M. nasutus*, segregated for male sterility as a result of nonmatching cytoplasmic male sterility elements in the mitochondrial genome and nuclear genes that restore male fertility in the parental species. In the context of the gender dimorphism-polyploidy association, it is of interest to note that cytoplasmic male sterility is determined by chimeric mitochondrial genes [reviewed in Touzet, 2012] and thought to arise from recombination events, such as those that could occur in heteroplasmic cells [Woloszynska, 2010]. Heteroplasmy may result from paternal leakage that may be more common following hybridization [Woloszynska, 2010] and thus in allopolyploids or during fusion of 1n and 2n gametes (see below). For instance, Guo et al. [2006] concluded that observed recombination of mtDNA in a triploid carp was the result of paternal leakage of the 2n sperm. Because the products of nuclear genes and mitochondrial genes will experience dosage imbalance [Freeling, 2009] directly following WGD, polyploidization may directly alter gender expression, when cytonuclear interactions are important in control-

ling fertility expression (i.e. nuclear restorers of cytoplasmic male sterility) [Kubo et al., 2011]. Moreover, if WGD occurs only once in a species with cytoplasmic male sterility, then, it will instantly create a bottleneck and fixation of cytoplasmic male sterility, leaving segregating nuclear genes alone (i.e. restorers of fertility) to influence sex expression.

Taking all of the genomic changes associated with polyploidization into account, it may be predicted that the simultaneous pathway (table 2) will be more common in allopolyploids than in autopolyploids and that gender dimorphism will more often be associated with allopolyploidy than autopolyploidy. Our limited survey of types of polyploidy for genera in table 1 only weakly supports this prediction, but a more complete test is needed.

Changes in Sexual System Cause WGD

In this pathway, changes in gender (male or female sterility) stimulate WGD and the formation of polyploids. This process may occur if genes controlling gender (sterility) are linked or pleiotropic to genes affecting the formation of polyploids. The dominant mechanism of polyploid formation is likely through the union of unreduced (2n) gametes, which contain the somatic chromosome number [de Wet, 1980; Bretagnolle and Thompson, 1995; Ramsey and Schemske, 2002]. Unreduced gametes can result from a range of meiotic abnormalities (chromosomal nondisjunction, failed cytokinesis) but commonly occur due to the failure of spindle formation in metaphase I (first restitution nuclei) and metaphase II (second restitution nuclei) (fig. 2). In both cases, the meiotic products have the somatic chromosome number, however, they differ with respect to the degree of heterozygosity present in the parent. Unreduced gametes produced through the disruption of the first meiotic division are expected to retain the heterozygosity of the parents, whereas second division restitution gametes will be more homozygous [Bretagnolle and Thompson, 1995].

Polyploid formation depends not only on the rates of unreduced gamete production in eggs and sperm but also on the viability/fertility of 1n + 2n zygotes [Husband, 2004] and the patterns of mating (selfing vs. outcrossing) [Ramsey and Schemske, 1998]. Autopolyploids can be formed in a single step through the union of 2 2n gametes ($2n + 2n = 4x$) or through 2 steps, which involve (a) the union of a reduced and an unreduced ($1n + 2n = 3x$) gamete (resulting in a triploid, for diploid taxa) and then, (b) backcrossing of the triploid to a diploid parent or selfing of the triploid. Triploids that are fertile often produce high

Fig. 2. Schematic of the major stages in gametogenesis, which give rise to typical (TYP), first division restitution (FDR) and second division restitution (SDR) gametes. The fate of 2 homologous chromosomes is shown through all stages. Note that first division restitution nuclei maintain heterozygosity, whereas second division restitution nuclei do not.

frequencies of unreduced ($2\times$ and/or $3\times$) gametes [Mok et al., 1975], which give rise to polyploids when crossed to $1n$ gametes from diploids. The 2-step process – involving a triploid bridge – can be an important mechanism for recurrently generating new polyploid progeny [Husband, 2004]. In addition to this, the likelihood of polyploid formation will also depend on the degree of self-fertilization versus cross fertilization in a population [Ramsey and Schemske, 1998]. Ramsey and Schemske [1998] estimate that allopolyploid formation is 34 times more likely, and autopolyploid formation 1.7 times more likely with selfing than outcrossing (i.e. backcrossing). This is largely the result of an elevated role of triploid bridge.

The genetic links between gender and unreduced gamete production are not well understood. There are many genes coding for various cytological stages of meiosis that may influence gametic nonreduction. Some of them are sex-specific [Kaul and Murthy, 1985], while others are not [Jongedijk and Ramanna, 1989; Jongedijk et al., 1991a, b] creating the possibility that at least, a shift in gender from egg and pollen production to just one gamete type can directly affect rates of unreduced gamete production. Few studies have tested for unreduced gametes in both eggs and pollen in the same individuals. In one case involving *Dactylis glomerata* [De Haan et al., 1992], the mean rates for eggs and pollen were similar. However, in this and other studies, variation in unreduced pollen production among individuals was not correlated with variation in unreduced egg production [Ramanna, 1983; Megalos and Ballington, 1988; Veronesi et al., 1990; van

Santen et al., 1991; De Haan et al., 1992] suggesting that meiotic irregularities are not always expressed equally in male and female gametes. The weak association between $2n$ eggs and pollen may reflect different genetic mechanisms of gametic nonreduction in the male and female gametophytes. It may also be related to different environmental influences experienced during pollen and egg formation [McHale, 1983].

For shifts in gender toward dioecy to stimulate polyploid production, unisexual individuals would have to produce more unreduced gametes than hermaphrodites. It is conceivable that unreduced gamete production is elevated in unisexuals if plant hormones controlling the expression of hermaphroditism suppress unreduced gamete production. For example, gender expression in *M. annua* is highly labile and is controlled in part by hormonal balance. Exogenous applications of auxins have a masculinizing effect, whereas cytokinins are feminizing [Hamdi et al., 1987; Durand and Durand, 1991]. These alterations in hormones may simultaneously influence meiotic irregularities and unreduced gamete production. This seems plausible given that hormonal changes often accompany parthenogenesis, and many environmental factors that influence parthenogenesis also influence chromosome doubling [Asker, 1980], but direct study is needed.

Sequential Pathways

Sequential (indirect) pathways (table 2) include those in which WGD, either allo- or autopolyploidy, creates

conditions, specifically (i) change in mating system, (ii) ecological effects or (iii) sex allocation plasticity that promote an evolutionary transition in sexual system, or conversely, gender shifts lead to the evolution of polyploidy via unreduced gametes.

WGD Promotes Evolution of Gender Dimorphism via Mating System Shifts

In this particular pathway, WGD causes increased rates of self-fertilization in hermaphrodites, which in turn, favor selection for unisexual genotypes. This idea was first articulated by Baker [1984], when he speculated ‘...another historical process that could lead to the loss of self-incompatibility and the subsequent need for a relatively easily evolved substitute outcrossing mechanism like dioecy, is polyploidy’. Miller and Venable [2000] elaborated on this idea, hypothesizing that WGD in a self-incompatible hermaphroditic diploid causes the loss of self-incompatibility in the polyploid species. This, in turn, leads to increased selfing and expression of sufficient inbreeding depression to favor the invasion by females and, later, selection for males (i.e. fig. 1) [Charlesworth and Charlesworth, 1978].

This hypothesis has been in the literature for much longer than most others and in some ways has been subject to more direct scrutiny. Evidence to date is supportive in particular cases but is more complex in others. For example, diploids with self-incompatibility were reported in 11 of 16 genera in table 1, while polyploid species were self-incompatible in only 3 of 15 genera ($\chi^2 = 7.46$, $p = 0.0063$). Moreover, there is convincing evidence that polyploids are more likely to be self-fertilizing than diploids [Barringer, 2007; Husband et al., 2008], although the pattern appears to be weaker in autopolyploids than allopolyploids [Husband et al., 2008]. The mechanism underlying this shift in mating system, however, is ambiguous; increased selfing may reflect changes in pollination [Segraves and Thompson, 1999], floral morphology [Vamosi et al., 2007] or inbreeding depression [Husband and Schemske, 1997] as well as loss of self-incompatibility [Borges et al., 2012]. Polyploidy can indeed affect the strength of the self-incompatibility reaction, although the effect is not consistent [Mable, 2004], occurs mostly in groups with gametophytic self-incompatibility and can occur without change in sexual system [e.g. Borges et al., 2012]. This review and specific case studies confirm that loss of self-incompatibility is not always involved in the transition to self-fertilizing polyploids. For example, in *Acacia mangium* [Griffin et al., 2012], experimental diploids were strongly outcrossing, and synthetic tetraploids

were almost exclusively selfing, but this appears to be a result of masking of inbreeding depression as a result of genome duplication, not a loss of self-incompatibility. Similarly, in the genus *Fragaria*, diploid progenitors (*F. vesca*, *F. iinumae*) of octoploid gender-dimorphic species are self-compatible, rather than self-incompatible [Njugu et al., 2013].

The assumption that inbreeding depression in self-fertilizing polyploids promotes the spread of male sterile individuals also requires closer examination. Theoretical studies show that inbreeding depression caused by partial dominance is expected to decline, not increase, upon WGD [Lande and Schemske, 1985, although see Ronfort 1999]. Indeed, empirical studies generally find that inbreeding depression is reduced in polyploids compared to diploid relatives [Alexander, 1960; Dewey, 1966, 1969; Johnston and Schoen, 1996; Husband and Schemske, 1997; Rosquist, 2001; Barringer and Geber, 2008; Husband et al., 2008]. What is more important is whether the magnitude of inbreeding depression is sufficient to favor the evolution of inbreeding avoidance. For example, in the widespread plant fireweed (*Chamerion angustifolium*), extant polyploids have almost half the inbreeding depression of diploids, but the magnitude is still sufficiently strong ($\delta = 0.61$) to favor the evolution of outcrossing mechanisms. The most important question is, how much inbreeding depression is present in newly formed polyploids, but this is less understood for natural populations. In fireweed, synthetic neopolyploids have no detectable inbreeding depression [Husband et al., 2008], suggesting that selfing, not outcrossing, would be favored, unless the magnitude of inbreeding depression increases over successive generations of selfing [Miller and Venable, 2000]. The limited evidence available is in support for this possibility [Ozimec and Husband, 2011], but more study is needed.

WGD Promotes Gender Dimorphism through Ecological Effects

Polyploidy may promote the evolution of gender dimorphism through its consequences on geographic ranges and colonization of novel environments. Specifically, physiological or morphological changes associated with polyploidy, such as greater vegetative size, slower development or drought tolerance [Maherali et al., 2009; Hodgson et al., 2010; Herben et al., 2012], could lead to invasion of new or marginal habitats [Stebbins, 1950; Hijmans et al., 2007; Ainouche et al., 2009]. Polyploid species have long been associated with geographic range shifts (e.g. to habitats more extreme in salinity or cold) or

novel environments compared to their diploid progenitors [Stebbins, 1950]. Evidence for a change in geographic range associated with WGD is extensive, ranging from individual case studies [Hijmans et al., 2007] to floristic analyses [Clausen et al., 1945] and meta-analyses [Stebbins and Dawe, 1987; Petit and Thompson, 1999; Martin and Husband, 2009] of geographic/ecological ranges. These shifts necessarily expose populations to new and more extreme environments, some of which are conducive to the evolution of separate sexes, e.g. dry or nutrient-poor habitats [Darwin, 1877; reviewed in Ashman, 2006]. Stressful habitats are expected to favor separate sexes because resource limitation can (1) hinder seed production of hermaphrodites more than females [e.g. Spigler and Ashman, 2011], (2) intensify inbreeding depression [e.g. Armbruster and Reed, 2005] and (3) cause changes in plant morphology or pollination that increase selfing [e.g. Case and Ashman, 2007]. Extreme cold or northern latitudes (or high elevation) may themselves be associated with changes in pollination [e.g. Alonso, 2005]. If polyploidy is a facilitator of colonization, then polyploids may more often be found in conditions that strongly favor the evolution of dioecy. The benefits of genomic changes that facilitate niche exploitation and expansion into new habitats (e.g. greater ecological tolerance or ecological amplitude) [reviewed in te Beest et al., 2012] may be strong enough to overcome fertility costs that might also be associated with genomic rearrangements (see above). Such a scenario could leave a pattern of association of ploidy and dimorphic sexual system in novel habitats (high altitude or harsh environments) that is seen in at least some taxa (e.g. *F. chiloensis*), but not in others (e.g. *M. annua*) [Pannell et al., 2004, Ashman et al., 2011].

Plant-antagonist interactions are another ecological interaction worth considering because they can be altered by WGD [Thompson et al., 2004], and they can also affect the outcome of sexual system evolution [Ashman, 2002]. For instance, *Greya politella* prefers ovipositing on capsules of tetraploid *Heuchera grossulariifolia* over those of diploids [Thompson et al., 2004]. And in gender-dimorphic plants, males and hermaphrodites are damaged to a greater extent than females [e.g. Ashman, 2002; Ashman and Penet, 2007]. Considering these studies together, one might predict that the direction of selection could be altered by polyploidy if WGD generally leads to enhanced chemical defenses or chemical attractants [Hull-Sanders et al., 2009]. However, to date, we have only a few empirical studies of antagonist interactions in mixed ploidy systems [Hull-Sanders et al., 2009], and we have none in those that also have gender polymorphism, thus we can-

not yet evaluate whether polyploidy will enhance or retard the evolution of dioecy.

WGD Promotes Evolution of Gender Dimorphism via Increased Sex Allocation Plasticity

Ecological context can mediate sexual system evolution, and phenotypic plasticity in sex expression has been implicated as influential in this process, especially via its effect on sex ratio [Delph, 1990; Delph and Wolf, 2005; Spigler and Ashman, 2011]. If polyploids are more phenotypically plastic than diploids because they have higher levels of heterozygosity [Mitton and Grant, 1984] or because epigenetic effects (described above) can be modulated by the environment [Leitch and Leitch, 2008; Chinnusamy and Zhu, 2009], then polyploids may be more sexually plastic than diploids. If the underlying mechanism of sex expression plasticity following WGD is a function of epigenetic modification, then transition into a new environment may provide additional novel heritable variation for selection to act on [Richards et al., 2010].

Gender Shifts Create Conditions for Increased Unreduced Gamete Production and Polyploid Evolution

Shifts from hermaphroditic to unisexual plants may have no immediate genetic impact on the expression of polyploidy, but it may influence the conditions governing the formation and establishment of polyploids. All else being equal, the formation of polyploids may actually be diminished in unisexuals because of the absence of selfing and the reliance on cross-fertilization. Selfing contributes significantly to the evolution of polyploids by either increasing the likelihood that 2 unreduced gametes unite [Ramsey and Schemske, 1998] or enhancing the establishment of polyploids through assortative mating, which minimizes the demographic costs of producing less fit hybrids [Levin, 1975; Husband and Sabara, 2004]. Selfing in hermaphrodites will also produce more homozygous progeny than outcrossing, and the expression of deleterious recessive mutations (inbreeding depression) may increase the rates of unreduced gametes, though this has never been quantified.

One condition that may favor polyploid evolution is, when unisexuals are able to colonize novel or more extreme environments than their hermaphroditic progenitors (see above). The expression of meiosis genes is influenced by environment [Bretagnolle and Thompson, 1995]. Unreduced gamete production is highly sensitive to environmental fluctuations such as high and low tem-

perature [reviewed in Hermesen, 1984; Felber, 1991]. For example, *Solanum tuberosum* grown in field conditions produced 76% and 80% unreduced pollen but 35% and 27% in the greenhouse [McHale, 1983]. This suggests that a shift in habitat by unisexuals may be sufficient to cause at least a short term change in 2n gamete production. If the new habitat is more extreme, one might expect 2n gamete frequency to increase. Currently, research is sparse on the relative contributions of genotype and environmental change to variation in 2n gamete production, especially in relation to gender dimorphism.

Gender-Dimorphic Diploids to Gender-Monomorphic Polyploids

Simultaneous Pathways

WGD Causes Gender Change

Losses of dioecy with WGD may be the consequence of breakdown of nuclear sex determination [Westergaard, 1958], sex chromosome duplications (and thus genes for both male and female fertility) associated with allopolyploidy [Gorelick, 2003], whole chromosome loss in neopolyploids [see Xiong et al., 2011], or other immediate effects of genome duplication on sex determination (*Mecurialis*) [Pannell et al., 2004]. Perhaps, the most convincing example of the effects of genome duplication on gender is observed in mosses [Jesson et al., 2011], where male gametophytes are haploid for the X chromosome and females haploid for Y, and only in polyploids, in which the gametophytes are diploid, it is possible to be hermaphrodite (XY). None of these mechanisms, however, has been directly tested in flowering plants. However, a breakdown in dioecy has been observed in synthetic polyploids [Pannell et al., 2004]. For example, progeny of synthetic polyploids in *Silene* and *Rumex* and F2 progeny of neotetraploid *Mercurialis* contained hermaphroditic morphs [Westergaard, 1958; Durand, 1963].

Changes in Gender Cause WGD

Similar to the pathway above, here, loss of separate sexes (gain of male or female fertility) may alter production of unreduced (2n) gametes. Although there are no empirical tests of this, unreduced gametes produced in a hermaphrodite would have an increased opportunity to unite and thus form new polyploids. Genetic mechanisms are the same as those proposed above.

Sequential Pathways

Whole Genome Duplication Promotes Evolution of Gender Monomorphism via Mating System Shifts

WGD could create conditions that promote the transition to hermaphroditism by reducing inbreeding depression (cites above) or by promoting invasion of habitats that select for reproductive assurance [Pannell et al., 2004]. In the latter case, new polyploids expanding into marginal habitats would encounter selection for reproductive success under low population densities [Pannell et al., 2004]. Such selection would favor single propagules that are self-fertile (i.e. Baker's law), and there is some evidence for this. Specifically, decaploid hermaphrodite *F. iturupensis* evolved from octoploid gender-dimorphic species and is associated with long-distance dispersal to an island [Njuguna et al., 2013]. In *M. annua*, gender-monomorphic (monoecious) populations are small and isolated, whereas gender-polymorphic (androdioecious) ones are large and widespread [Pannell et al., 2004].

Gender Shift Creates Conditions for Increased Unreduced Gamete Production and Polyploid Evolution

A shift to hermaphroditism from gender dimorphism will allow selfing which can facilitate the establishment of polyploids by either increasing the likelihood that 2 unreduced gametes unite [Ramsey and Schemske, 1998] or by avoiding the costs of minority cytotype exclusion [Husband and Sabara, 2004; Pannell et al., 2004]. If hermaphrodites are also found in peripheral populations that are especially harsh (e.g. *M. annua*) [Pannell et al., 2004], they could have increased levels of unreduced gamete production as discussed above for dioecious species under the same ecological stresses.

Key Questions and Promising Approaches

Our analysis reveals several ecological and genetic scenarios that may give rise to associations between polyploidy and gender dimorphism, the relative significance and underlying mechanisms of which can only be resolved through future research. Here, we outline some directions and approaches that may be profitable to pursue.

Currently, our understanding of the incidence of the joint evolution of ploidy and gender rests on a limited number of genera (table 1) [Miller and Venable, 2000]. Several unlisted genera (e.g. *Passiflora*, *Salvia*, *Geranium*, *Gentiana*, and others) exhibit variation among species for both ploidy and gender and should be explored more ex-

haustively to understand the actual frequency of occurrence of gender-dimorphic polyploids and the conditions favoring their evolution. In some cases, this will require additional research to determine the distribution of chromosome number and gender within and among populations. A large scale survey of genera and species will also assist in determining, whether certain plant taxa are predisposed to give rise to dioecious polyploids. For example, the evolution of gender and polyploidy may depend on the genetic basis of gender or the specific modes of self-fertilization represented within particular taxa. Further, more complete phylogenetic analyses of a breadth of taxonomic groups will provide a historical framework needed to test the direction and order of evolutionary transitions of polyploidy and gender [Brunet and Liston, 2001].

Experimental studies are essential for testing the underlying causal relationships between ploidy and gender. The pathways outlined in table 2 suggest a number of predictions and relationships that could be tested empirically. For instance, it will be important to begin to look deeper into the ecological and genetic causes of the association, and the groups already known to exhibit the ploidy-gender dimorphism pattern (table 1) are a good place to start. We can assess the degree of sex allocation plasticity in diploid and polyploid species or compare the incidences of unreduced gamete production across taxa. It will likewise be profitable to examine the genetic and genomic basis of gender determination in diploid and polyploid congeners or the ecological distribution and mating patterns in association with the lineages exhibiting diploid hermaphrodite and polyploid dioecious species. These types of investigations will provide the foundation for assessing the likelihood of proposed mechanistic underpinnings in table 2.

An even more powerful approach to evaluate mechanisms will be to create synthetic polyploids (neopolyploids), by inducing chromosome doubling in diploid plants or crosses to unite naturally occurring unreduced gametes (fig. 1). Such neopolyploids offer an important tool for examining the effects of genome duplication [Ramsey and Schemske, 2002], as they share the same genic constitution as their diploid progenitors but differ in genome copy number. Thus, they represent the immediate effects of genome duplication with little or no effects of selection. Synthetic polyploids can be compared with diploid progenitors in controlled environment or as transplants in more natural settings to evaluate their effects on gender, mating system, plasticity, and habitat differentiation. They can also be useful for testing, whether

after hybridization, WGD is more likely to influence gender than autopolyploids. Such polyploids have mostly been used to understand the genetic and genomic effects of WGDs [Schranz and Osborn, 2000; Madlung et al., 2005; Eilam et al., 2009] and mostly in agricultural species or model systems [Ramsey and Schemske, 2002]. However, this approach is increasingly being used to understand basic morphological, ecological [Vamosi et al., 2007; Oswald and Nuismer, 2011] and evolutionary [Griffin et al., 2012; Martin and Husband, 2012] effects of genome duplication.

Ploidy has long been associated with changes in sexuality, including increased incidence of parthenogenesis and self-fertilization in both plants and animals [Vandel, 1938; Stebbins, 1950; Otto and Whitton, 2000], but in all cases, it has rarely been clear, whether polyploidy is an evolutionary cause, a consequence or both. In the hopes of stimulating more research on polyploidy and sexual systems, here, we have reviewed multiple pathways in which polyploidy is either a direct genetic cause or consequence of separate sexes. Regardless of the hypothesis, the availability of genetic resources and growing interest in the phylogenetic history, causes and molecular effects of genome duplication converge to create an unprecedented opportunity to make progress on our understanding of evolutionary transitions in sexual system and polyploidy.

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