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# Polyploidy in Animals: Effects of Gene Expression on Sex Determination, Evolution and Ecology

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**Key Words**
Animal polyploidy · Ecology · Evolution · Gene expression · Sex determination

**Abstract**
Polyploidy is rarer in animals than in plants. Why? Since Muller's observation in 1925, many hypotheses have been proposed and tested, but none were able to completely explain this intriguing fact. New genomic technologies enable the study of whole genomes to explain the constraints on or consequences of polyploidization, rather than focusing on specific genes or life history characteristics. Here, we review a selection of old and recent literature on polyploidy in animals, with emphasis on the consequences of polyploidization for gene expression patterns and genomic network interactions. We propose a conceptual model to contrast various scenarios for changes in genomic networks, which may serve as a framework to explain the different evolutionary dynamics of polyploidy in animals and plants. We also present new insights of genetic sex determination in animals and our emerging understanding of how animal sex determination systems may hamper or enable polyploidization, including some recent data on haplodiploids. We discuss the role of polyploidy in evolution and ecology, using a gene regulation perspective, and conclude with a synopsis regarding the effects of whole genome duplications on the balance of genomic networks. See also the sister articles focusing on plants by Ashman et al. and Madlung and Wendel in this themed issue.



Polyploidy is the heritable condition of possessing more than 2 complete sets of chromosomes [Comai, 2005]. This condition can arise as a result of genome duplication(s) within a species (autopolyploidy), or from hybridization of 2 different species (allopolyploidy). A polyploidization event causes a dramatic change in the genome structure and cell organization. It imposes several major challenges on cell cycle processes (e.g. mitoses, meiosis), cell physiology (e.g. metabolism, growth, stoichiometry), regulation of gene expression and genome stability. This 'genome shock' of combining two, either similar or diverged, genomes can lead to a bottleneck of instability [Comai, 2005]. For polyploidy to become stable, genomic reorganizations may occur. Part of the genome reorganization involves a process called 'diploidization'. This is the gradual conversion of polyploidy to diploidy through genetic changes that differentiate duplicated genes and chromosomes, and the loss of many, or most, of the duplicated genes.

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After a polyploidization event, different evolutionary scenarios can unfold. Newly formed polyploid organisms, that cannot overcome the genome instability, or have lowered survival and/or reproduction, may perish and become an 'evolutionary dead-end'. In this case, polyploidy could be considered a catastrophic phenomenon. Alternatively, when the short-term challenges of genome shock can be overcome, polyploidy may result in establishment of a new species (neopolyploids). Subsequent stable persistence and diversification of polyploid lineages depends on the fitness effects and long-term evolutionary consequences of polyploidization. Conversely, polyploidy may be a more transient state that is temporarily endured, followed by the loss of the majority of the duplicated genome. Duplicated genes that are retained can diversify and gain new functions (neo-functionalization) or perform different components of the original common function (sub-functionalization), for example leading to tissue-specific expression and adaptations. Animals and plants can also exhibit a form of localized polyploidy, 'endopolyploidy', in which particular tissues become polyploid after development, for example due to endoreplication (i.e. DNA replication without cell division). In mammals, this process has been reported for the liver, platelets and bone marrow precursor cells [reviewed in Lee et al., 2009]. Moreover, endopolyploidy has been associated with various types of carcinomas and other pathological conditions [Storchova and Pellman, 2004; Erenpreisa et al., 2005; Ganem et al., 2007].

Polyploidy has been recognized as an evolutionary important phenomenon in plants, but less so in animals, where it has long been looked upon as a rare event that was not compatible with the complex development and sexual mode of reproduction [reviewed in Mable, 2004]. Although stable polyploidy indeed seems to be more frequently maintained in plants than in animals, it is not as rare as was originally assumed. In fact, polyploidy exists across all major taxonomic animal groups and occurs even relatively frequently among some groups, particularly in fish and amphibians [Otto and Whitton, 2000; Mable et al., 2011]. Although present existence of polyploid mammals is equivocal [Gallardo et al., 1999, 2006; Svartman et al., 2005; Suarez-Villota et al., 2012], most vertebrate taxa seem to descend from polyploid ancestors [Ohno, 1970, 1999; Sidow, 1996; Spring, 1997; Dehal and Boore, 2005]. Hence, qualifying polyploidy as a marginal process in animal evolution does injustice both to the incidence and the impact of earlier polyploidy events in animal evolution. Nevertheless, what explains the remarkable difference in evolutionary dynamics for polyploidy in animals and plants is still an intriguing question that remains to be solved.

Although addressed multiple times, no single common explanation has been found for polyploidy to be rarer in animals than in plants [Muller, 1925; Orr, 1990; Mable, 2004]. Two major hypotheses have been proposed to explain this fact, based on the notions that animals more often have separated sexes and sex chromosomes. One states that polyploidy disrupts meiosis and the segregation of chromosomes, including sex chromosomes, leading to aneuploidy (i.e. missing copies (monosomy) or extra copies (polysomy) of one or more chromosomes from the full chromosome set). Alternatively, genetic sex determination in animals requires dosage compensation to maintain the genetic balance between sex chromosomes and autosomes [Orr, 1990]. For instance, under XY sex determination, the heterogametic sex has a single X chromosome where the homogametic sex has two. Polyploidization may upset the expression balance of genes in XX females and XY males beyond what can be tolerated. Although this process explains the difficulties of stable polyploidy in some groups of animals (e.g. mammals), it is considered unlikely to apply to 'the animal kingdom as a whole' [Mable, 2004]. Many different mechanisms of sex determination exist among animals (listed as part of table 1), in some groups stable polyploidy does occur, and there has been no universal association between sexual reproduction and polyploidy [Mable, 2004].

Genomic technologies have been hugely informative in revealing both recent and ancient genome evolution processes after polyploidization. For example, comparative genomics of tunicates, fish, mice, and humans identified 2 ancient genome duplication events to lie at the base of the vertebrate lineage. This duplication was followed by the degeneration of the vast majority of the duplicated genes [Dehal and Boore, 2005]. In plants, genome-wide expression studies have shown the changes in the transcriptome and in the epigenetic regulation during the transition from neopolyploid formation to the stabilization of polyploid species [reviewed by Madlung and Wendel, this issue]. Unfortunately, the number of studies on gene expression changes associated with polyploidy in animals has been fairly limited to date.

One particularly important element that is emerging from gene expression studies in animals and plants, not only in the context of ploidy, is the pervasiveness of complex gene interaction networks. These networks reveal the intricate regulatory control of modules of genes, the high level of interconnectedness among hundreds or even

**Table 1.** The predicted sexes of polyploid individuals under the predominant sex determination mechanisms; sex determination systems that rely on sex chromosome counting (X:A or Z:A balance) are predicted to lead more frequently to intersexes under polyploidy

| Sex determination type | Balanced diploids | Unbalanced diploids | Triploids | Tetraploids | Example [literature] |
|---|---|---|---|---|---|
| **Male heterogamety** | | | | | |
| XY with dominant Y determiner | XX AA = female<br>XY AA = male | X AA = female<br>Y AA = male[a] | XXX AAA = female<br>XXY AAA = male<br>XYY AAA = male | XXXX AAAA = female<br>XXXY AAAA = male<br>XXYY AAAA = male<br>XYYY AAAA = male | |
| XY with X:A ratio | XX AA = female<br>XY AA = male | X AA = male<br>Y AA = male[a] | XXX AAA = female<br>XXY AAA = intersex<br>XYY AAA = male | XXXX AAA = female<br>XXXY AAAA = intersex<br>XXYY AAAA = male<br>XYYY AAAA = male | *Drosophila melanogaster* [Bridges, 1925; Dhobzhansky and Schultz, 1934] |
| XO with X:A ratio | XX AA = female<br>XO AA = male | X AA = male<br>O AA = male[b] | XXX AAA = female<br>XXO AAA = intersex<br>XOO AAA = male | XXXX AAA = female<br>XXXO AAAA = intersex<br>XXOO AAAA = male<br>XOOO AAAA = male | *Caenorhabditis elegans* [Madl and Herman, 1979; Meneely, 1994][c] |
| **Female heterogamety** | | | | | |
| ZW with dominant W determiner | ZW AA = female<br>ZZ AA = male | W AA = female<br>Z AA = male[a] | ZWW AAA = female<br>ZZW AAA = female<br>ZZZ AAA = male | ZWWW AAAA = female<br>ZZWW AAAA = female<br>ZZZW AAAA = female<br>ZZZZ AAAA = male | *Bombyx mori* [reviewed in Traut et al., 2008] |
| ZW with Z:A ratio | ZW AA = female<br>ZZ AA = male | W AA = female[a]<br>Z AA = female | ZWW AAA = female<br>ZZW AAA = intersex<br>ZZZ AAA = male | ZWWW AAAA = female<br>ZZWW AAAA = female<br>ZZZW AAAA = intersex<br>ZZZZ AAAA = male | |
| ZO with Z:A ratio | ZO AA = female<br>ZZ AA = male | O AA = female[b]<br>Z AA = female | ZOO AAA = female<br>ZZO AAA = intersex<br>ZZZ AAA = male | ZOOO AAAA = female<br>ZZOO AAAA = female<br>ZZZO AAAA = intersex<br>ZZZZ AAAA = male | |
| **Haplodiploidy** | | | | | |
| CSD | $A_1A_2$ = female<br>$A_1$ = male<br>$A_1A_1$ = diploid male | | $A_1A_2A_3$, $A_1A_2A_2$, $A_1A_1A_2$ = female<br>$A_1A_1$, $A_2A_2$ = male | $A_1A_2A_3A_4$, $A_1A_2A_3A_3$,<br>$A_1A_1A_2A_2$, $A_1A_1A_1A_2$ = female<br>$A_1A_1A_1A_1$, $A_2A_2A_2A_2$ = male[d] | *Apis melifera* [Mackensen, 1951; Woyke, 1965] |
| MEGISD | AA = female<br>A = male | | AAA = female<br>AA = male | AAAA = female<br>AAA = male | *Nasonia vitripennis* [Whiting, 1960; Beukeboom and Kamping, 2006] |

CSD = Complementary sex determination; MEGISD = maternal effect genomic imprinting sex determination.
[a] Depending on viability of YO and WO individuals. [b] Depending on viability of O individuals. [c] In *C. elegans*, females are hermaphrodites. [d] By extrapolation.

thousands of genes, as well as substantial redundancy in regulators and feedback loops [MacArthur et al., 2009; Davis et al., 2012]. Polyploidization can alter the gene expression patterns in these interaction networks. Although both plants and animals would be sensitive to such changes, the differences in development and growth between animals and plants may lead to different evolutionary outcomes. Investigating the consequences of gene-interaction network disruptions could add valuable information to explain why polyploidy occurs less frequently in animals than in plants.

Here, we review the literature on the association between the regulation of gene expression and the evolution of polyploidy in animals. In particular, we will focus on polyploidy and its associated effects on gene expression in development, sex determination, evolution, and ecology.

## Conceptual Model: Polyploidization Affecting Genome Networks

In this section, we present a conceptual model for the effects of polyploidy on the complex network of gene interactions. Figure 1 illustrates the conceptual model and contrasts various scenarios for changes in expression net-

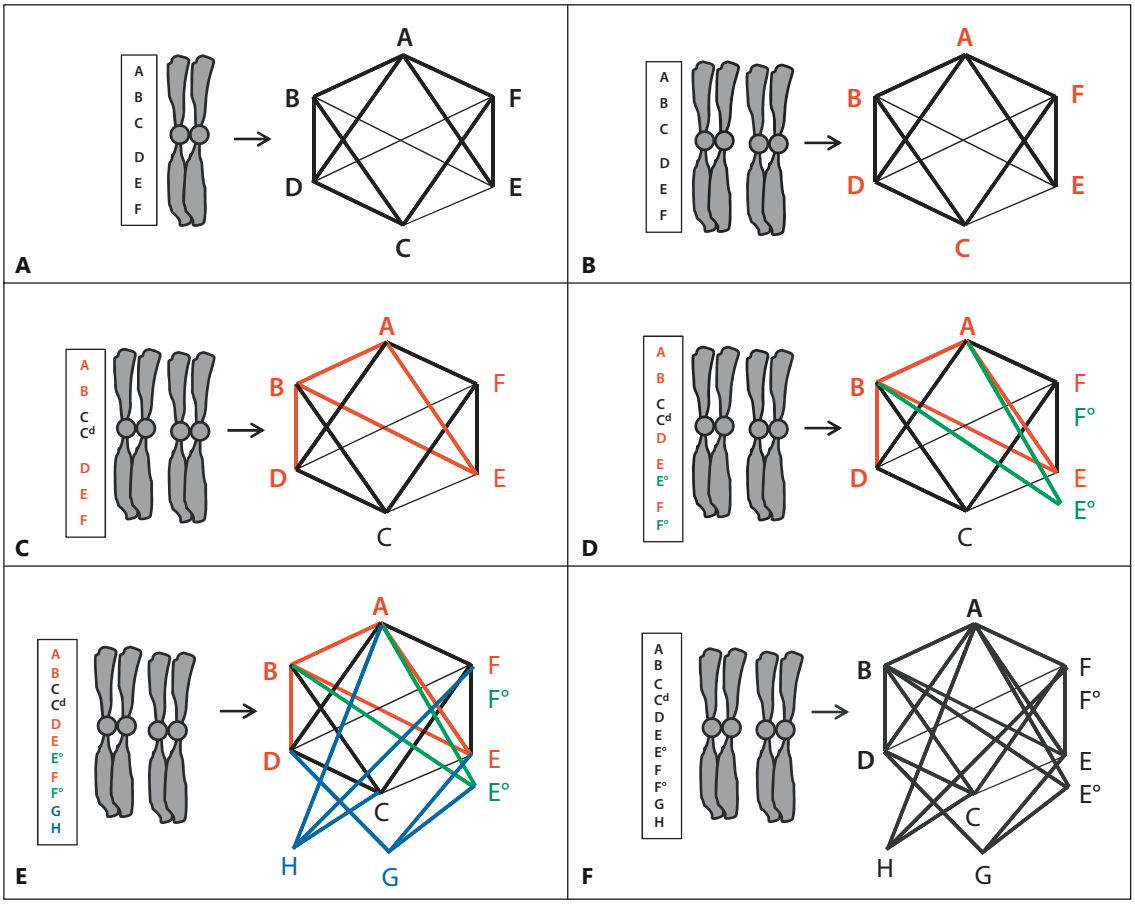


**Fig. 1.** Effects of WGD on gene-expression networks. Depicted is an initial genomic network of 6 genes A to F. **A** Balanced diploid. A diploid genome consists of 'co-adapted' networks of gene expression patterns. Here, a schematic network is presented, where genes are depicted by equal-sized letters and the magnitude of gene-gene interactions is depicted by line thickness. **B** No genomic shock. Upon polyploidization, the relative expression of the genes and/or the co-adapted expression patterns are unaltered, although the expression of all individual genes is doubled (depicted by bold red letters). The relative magnitude of all interactions is unchanged. **C** Transcriptomic shock. Upon polyploidization some gene expression levels increase (bold red letters) or decrease (normal red letters) as a result of dosage compensation. This will cause some interactions to be changed (red lines) while others remain the same (black lines). In addition, some duplicated genes may become silenced pseudogenes and/or decay ($C^d$). **D** Sub- and neofunctionalization. Some duplicated genes continue as paralogs ('ohnologs', green letters). Some new interactions may occur (green lines). **E** Co-option. Some new genes are incorporated into the network (non-ohnologs, blue letters). Completely new interactions become possible (blue lines). **F** Balanced polyploid. The new interaction pathways establish a new, more complex, co-adapted gene expression network. In principle this balanced polyploidy can be considered a new balanced diploid. It is dependent on the newly formed network whether or not a new WGD event is tolerated.

works after genome duplication. This model may provide a comprehensive framework to compare the evolutionary dynamics of polyploidy in animals and plants. Conversely, a better understanding of the mechanisms, constraints and evolution of polyploidy could reveal some fundamental principles of the regulation, coordination and evolution of whole genomes.

Genomic networks consist of interacting genes. Such a network is schematically depicted in figure 1A. Interacting genes are connected by a line, where the thickness of the line represents the magnitude of the interaction. Polyploidization can alter both the dose (indicated by red letters) and the relative expression patterns (indicated by the red lines) of all or some genes. A situation where the increase in gene dose does not change the relative interaction patterns (fig. 1B) is in essence not different from the original situation (fig. 1A). However, when the relative gene interactions change (e.g. through dosage compensa-

tion, silencing of one copy, or through quantitative feedback loops), a 'transcriptomic shock' may occur (fig. 1C). Moreover, duplicated genes may become silenced and decay (fig. 1C), or conversely diversify and gain or subdivide functions (fig. 1D, E, genes $E^o$ and $F^o$, green lines), processes known as neo- and sub-functionalization. Yet other genes may be newly recruited (fig. 1E, genes G and H), a process known as co-option. Eventually, a new balanced genomic network can establish, potentially with increased complexity (fig. 1F).

Currently, there is insufficient direct evidence to critically test the hypothesis of the role of gene interaction networks causing stronger constraints for polyploidy in animals. Yet, there is substantial circumstantial evidence that supports the assumptions and implications of the conceptual framework. The phenomenon of endopolyploidization shows that increased ploidy can lead to upregulated gene expression levels, and that not all genes respond similarly to the polyploidization event [Anatskaya and Vinogradov, 2010]. This means that genome duplication may not simply be a scalar adjustment of expression, but can indeed cause a transcriptomic shock by upsetting the intricate gene interaction networks. Changing the relative expression levels of some genes in co-adapted complexes can arise after polyploidization through, for example, dosage compensation, feedback loops or epigenetic silencing mechanisms. In addition, genome duplication may increase the complexity of gene-interaction networks. Not all co-adapted complexes may be able to sustain these changes or increased complexity, which would impede polyploidization.

Although intricate and spatially variable gene interaction networks occur in both animals and plants, it is conceivable that such networks are more restrictive in animals, for example due to differences in cell fate determination during development and growth. Plants maintain high developmental plasticity through pluripotent meristem cells and exhibit large flexibility in the proportions and frequencies of organs, while animal cell differentiation typically is mostly irreversible. There is significant conservation in the ontogeny of animals, especially in the stages after gastrulation, which has been associated with strong purifying selection on the expression of genes important in early-embryonic development [e.g. Artieri et al., 2009]. Re-establishing expression balance in the expression networks across the various types of terminally differentiating cells might impose severe difficulties. Therefore, overcoming the transcriptomic imbalance after whole genome duplication (WGD) might be more problematic in animals than in plants. Studying the changes in gene expression after polyploidization in the context of these complex regulatory networks may reveal imbalances in polyploid animals and perhaps reveal the constraints and evolutionary processes that shaped polyploidy in animals. In this chapter, we review relevant literature on polyploidy in animals, place it in the context of this conceptual framework and point out promising avenues for further research.

## Polyploidy and Gene Expression Patterns

Many events can change gene expression patterns, including single nucleotide changes, indels, duplications and complete genome duplication. The complexity of the genotype-phenotype relation precludes specific predictions on the impact of any such events, which nevertheless may be profound. Local events, such as the effect of single nucleotide changes, indels and duplications, have been studied intensively over the last decades. With the advent of high-throughput genomic techniques, it has now become possible to functionally address the effect of WGD on gene expression patterns.

The concept of genome balance after genome duplication is not new [reviewed in Birchler and Veitia, 2007, 2012]. It has been long recognized that genome balance needs to be re-established after a polyploidization event, both at a structural level (e.g. extensive intra- and inter-chromosomal rearrangements ensuring normal chromosome pairing in meiosis) and at a regulatory level (e.g. epigenetic remodeling to silence parts of the duplicated genome) [Soltis and Soltis, 1999]. Orr [1990] proposed that a sudden change in genotype disrupts development in animals more than in plants, because of the requirement of dosage compensation in sex determination. He coined the importance of maintaining a balanced genome in sex determination to explain the rarity of polyploidy in animals. Later perspectives on the rarity of polyploidy in animals proposed that genome balance may have wider implications than just in the context of sex determination. In fact, the lethality of polyploids in mammals and birds may be due to a general disruption of development, not to problems restricted to sex determination [Otto, 2007]. This assertion was based on the observation that in these taxa polyploidy typically is fatal early in development before sex determination is relevant and leads to more severe defects than a trisomy of the sex chromosomes [Otto and Whitton, 2000; Otto, 2007].

Comai [2005] observed that in polyploids the regulatory networks that were established and optimized before

duplication or polyploidization are changed (fig. 1A–C). To re-establish the transcriptional balance, both increase and decrease of gene expression is needed across the genome. Comai treated the changes in regulatory dynamics of gene expression as part of the disadvantages of polyploidy, but also realized that there may be advantages. A lack of data concerning the effect of ploidy on gene expression patterns in animals precludes any strong inferences at this stage. Recently, Birchler and Veitia [2007, 2012] formulated the 'gene balance hypothesis' to emphasize that stoichiometry of gene expression patterns is required to achieve and maintain genomic balance in the context of (partial) polyploidization.

A good illustration of the transcriptional balance concept is the vertebrate fish complex of *Squalius alburnoides*, originating through a hybridization event between the sympatric species *Squalius pyrenaicus* (maternal ancestor, contributing the P genome) and a presently extinct species (paternal ancestor contributing the A genome) related to *Anaecypris hispanica* [Alves et al., 2001]. Pala et al. [2008] investigated gene expression in allotriploid (PAA) *S. alburnoides* and found that transcription levels were 'adjusted' to conform to the diploid state. Surprisingly, it was not a whole haplome that was inactivated (one A or the P), but the allelic expression patterns indicated a gene-specific silencing pattern, independent of A or P origin. Although the A genome was expressed preferentially, and some genes *(vasa, β-actin)* and tissues (liver, gonads) were expressed even exclusively from the A genome, the gene-silencing patterns differed over various tissues, indicating a specific regulation mechanism. In a recent paper, Inácio et al. [2012] demonstrated the involvement of small RNAs in this phenomenon and showed increased microRNA expression in triploid PAA *S. alburnoides*. This illustrates the complexity of restoring genome balance in animals across intricate and spatially variable gene interaction networks.

The importance of the genome balance hypothesis in animals has mostly been discussed at the single chromosome level. In 1967, Ohno proposed that X-linked genes have increased expression to compensate for the difference with autosomal gene expression as a result of degenerate Y chromosomes, allowing for mono-X in males and an inactivation of one X chromosome in females [Ohno, 1967]. This postulate is easily expanded to whole X chromosome gene expression and could explain why dosage compensation results in stoichiometric imbalance of gene expression upon polyploidization (see also section on sex determination). However, Ohno's hypothesis is based on single gene expression events and high-throughput studies of genome-wide expression indicate that Ohno's hypothesis does not apply in general. Xiong et al. [2010] reanalyzed microarray gene expression data for human and mouse that were reported to sustain Ohno's hypothesis [Gupta et al., 2006; Nguyen and Disteche, 2006]. They concluded that the higher resolving power of RNA-seq analysis leads to the rejection of higher expression levels for genes located on X chromosomes compared to those on autosomes [Xiong et al., 2010]. This conclusion was then challenged by Deng et al. [2011] who attributed the pattern to the skewed gene content on X chromosomes for genes involved in sexual reproduction and proposed that dosage compensation may only be applicable to those genes that are not silent or repressed. This paper culminated in a debate in *Nature Genetics* by Kharchenko et al. [2011], Lin et al. [2011] and He et al. [2011]. The debate was reviewed recently by Birchler [2012] and Forsdyke [2012] and put into perspective of differential compensation of specific dosage-sensitive genes. Recently, Lin et al. [2012] refueled the debate by reporting that only ~5% of mammalian X-linked genes were upregulated. This debate may serve to illustrate how sex-dependent dosage compensation can be (partially) involved in the process of restoring genome balance upon polyploidization, but also reveals the multilevel complexity associated with this phenomenon.

## Polyploidy and Sex Determination

The question that we like to pose in this section is whether polyploidy can disturb the genetic regulation of sex determination. The issue is relevant to the question 'why polyploidy is rarer in animals than in plants' as was first addressed by Muller [1925] and revisited by Orr [1990] and Mable [2004]. Here we only consider the sex determination argument with regard to animals. Separate sexes (dioecy or gonochorism) are rare in plants but common in animals in which sex is often genetically determined [Bull, 1983]. Muller [1925] argued that this restricts the evolution of polyploidy in animals but not in plants, because sex determination is disrupted in gonochoristic animals.

Muller based his observations on crosses with *Drosophila melanogaster* in which sex is determined by the X chromosome to autosome (X:A) ratio, i.e. the decision to develop as female or male is not determined by the presence of the Y per se, but rather by the balance between sex chromosomes and autosomes. Diploid individuals with 2 X chromosomes have an X:A ratio of 1.0 and de-

velop into females. Individuals with 1 X and 1 Y chromosome have an X:A ratio of 0.5 and develop into males. Studies with mutant individuals that deviated in the normal number of X and Y chromosomes revealed that ratios of 0.5 and lower lead to maleness and ratios of 1.0 and higher to femaleness. For example, triploids with 1X:3A are males, but with 3X:2A and 3X:3A are females (table 1). Individuals with 2X and 3A (X:A = 0.67) are sexual mosaics with mixtures of cells developing as female or male. Muller further observed that tetraploidy does not lead to fertile individuals as the cross between a tetraploid and a normal diploid individual produces sterile intersexes. Similar X:A ratio effects on sex determination have been found in experimental manipulation of ploidy levels in *Caenorhabditis elegans* [Madl and Herman, 1979; Meneely, 1994].

We now know the molecular basis of this disruption of sex determination in *Drosophila* under polyploidy. A set of X-linked genes known as X chromosome signal elements (XSEs), such as *scute (sc), sisterless A (sisA), runt (run)*, and *outstretched (os)*, act in a dose-sensitive fashion to activate the early promoter of a single target gene, *Sex lethal (Sxl)* [recent reviews in Cline et al., 2010; Salz and Erickson, 2010]. Consequently, embryos with 2 X chromosomes have a high level of XSE proteins that induce splicing of *Sxl* into the female form *($Sxl^F$)* which promotes its own production through a positive feedback loop. Presence of a single X chromosome results in the male splice form *($Sxl^M$)*, because the level of XSE protein complex is insufficient to initiate the production of $SXL^F$ protein and its subsequent autoregulation. $SXL^F$ regulates the female-specific splicing of the downstream gene *transformer (tra)*, which, in turn, regulates female-specific splicing of the switch gene *doublesex (dsx)*. Newly arisen polyploids will produce offspring with unbalanced ratios of sex chromosomes and autosomes resulting in aberrant XSE protein levels. According to Erickson and Quintero [2007], this is due to a shift in the temporal window at which these transiently expressed elements function during blastoderm formation. In haploid embryos, the number of embryonic cells in which the sex is assessed is increased, and in triploids it is decreased. The authors argue that sex determination in *Drosophila* should therefore be viewed as determined by the number of X chromosomes rather than the X:A ratio.

Muller believed that an X:A ratio type of sex determination would be the most prevalent among animals, but it is now known that male heterogamety often relies on dominant Y-linked factors [Orr, 1990]. Is a dominant Y system (or dominant W system in female heterogamety) less restrictive for sustaining polyploidy? This question was addressed by Otto and Whitton [2000] who realized that in a newly arisen population of tetraploids the sex ratio will be strongly biased towards the heterogametic sex, as XXXY, XXYY, XYYY individuals will all be male and only XXXX individuals will be female (table 1). However, sex ratio selection in favor of the X chromosome may quickly restore the balance. In contrast, in female heterogametic systems three-quarters of individuals would be female assuming random segregation of W and Z chromosomes. Since sex ratio selection may be less strong in female-biased populations, polyploidy could persist longer or more easily in ZW than in XY systems under dominant drivers.

Another obstacle for the evolution of polyploidy in gonochorists is the disruption of dosage compensation [Orr, 1990]. Orr argued that polyploidization disrupts the balance between sex chromosome and autosome gene dose which is normally maintained by dosage compensation. As a result, polyploidy should only be rare in animal groups that have degenerate Y (or W) chromosomes and have developed mechanisms to balance gene dosage of the single sex chromosome (X or Z) in the heterogametic sex. These considerations led Otto and Whitton [2000] to predict that polyploidy should be more common in animal taxa (1) with asexual and hermaphroditic reproduction, (2) with sex determination based on a Y-linked sex determiner rather than an X:A ratio, and (3) with non-degenerate sex chromosomes and absence of dosage compensation. Some evidence is available in support of all 3 predictions. It is beyond the scope of this chapter to systematically test these predictions, and we restrict ourselves to some general observations. In support of the first prediction, polyploidy and parthenogenesis are very often associated in animals [Suomulainen et al., 1987; Stenberg and Saura, 2009 and this issue]. Purely on the basis of sex chromosome segregation alone, polyploidy and parthenogenesis may arise more easily in XX than in ZW females, because the latter would produce 50% of ZZ males [Engelstaedter, 2008]. To our knowledge, the second prediction of an association between a Y-linked sex determiner versus an X counting system and polyploidy, in the absence of parthenogenesis, has not been formally tested. However, a simple analysis of the predicted sexes of polyploids under Y or W dominance versus X or Z chromosome counting systems reveals that the latter may be more prone to produce intersexes (table 1). The reason is that a dominant male (on the Y) or female (on the W) determiner may be rather insensitive to an additional copy of the homologous

chromosome, whereas under a dosage mechanism, every added sex chromosome will directly affect the sex chromosome to autosome balance. Unfortunately, there are very few systems in which information is available on the sexes of various polyploids to test these predictions (e.g. *Drosophila, Bombyx, C. elegans*). Amphibians form an example in support of the third prediction related to the degeneracy of the sex chromosomes: heteromorphic sex chromosomes are rare [for possible reasons, see Perrin, 2009] and polyploidy is common [Schmid et al., 1991; Eggert, 2004; Schartl, 2004].

In haplodiploid organisms, (heteromorphic) sex chromosomes do not exist; males are haploid and develop from unfertilized eggs, whereas females are diploid and arise from fertilized eggs. Dosage compensation is believed to be absent, which would predict that polyploidy can evolve with fewer constraints. However, the particular details of the genetic regulation of sex determination may pose a problem. Many species of Hymenoptera have complementary sex determination (CSD) in which the sex of an individual is determined by the allelic state of a single locus; heterozygotes develop into females, whereas hemi- and homozygotes become males [Whiting, 1960]. This system bears some similarity to the evolution of polyploidy and self-incompatibility in plants [Miller and Venable, 2000]. Homozygous diploid males are frequently sterile or inviable [van Wilgenburg et al., 2006]. Homozygosity at the sex locus may therefore prevent evolution of polyploidy in those groups that rely on this mode of sex determination (table 1). A related observation is that diploid males, if viable, typically produce diploid sperm, showing that (mitotic) spermatogenesis depends on maleness and not on ploidy level. Interestingly, in a few CSD species, diploid males appear to be fertile and reproductive [El Agoze et al., 1994; Cowan and Stahlhut, 2004; Elias et al., 2009]. Why a ploidy increase is lethal in some haplodiploids but not others, and how gene regulation and spermatogenesis is altered in polyploid males, remains unknown.

In *Nasonia* wasps, sex is not determined by CSD but by maternal imprinting. In haploid eggs, the *transformer* gene is silenced, and a paternal genome in diploid fertilized eggs is required to initiate zygotic *transformer* transcription for female development [Verhulst et al., 2010]. Triploid and tetraploid strains exist in which gene expression is apparently not so strongly affected to disrupt sex determination (table 1). This would predict that polyploidy can evolve more easily in non-CSD species. However, triploid and tetraploid females are unbalanced polyploids and have low fecundity due to production of high proportions of aneuploid offspring as a result of meiotic oogenesis [Beukeboom and Kamping, 2006]. Diploid *Nasonia* males are viable and fertile and produce diploid sperm. Interestingly, the imprinting sex determination appears to be sometimes disrupted in polyploids, resulting in the production of haploid females and gynandromorphs [Beukeboom et al., 2007]. Study of gene regulation and sex determination under different ploidy levels in haplodiploids may be informative about how development and gene regulation constraint the evolution of polyploidy in the absence of sex chromosomes.

From the above discussion it is clear that the mechanism of sex determination does affect the evolution of polyploidy in animals, but several additional factors need to be invoked to explain its rarity [Mable, 2004]. For example birds, which have a ZZ/ZW mechanism of sex determination, do not have global dosage compensation but also lack polyploid lineages. As discussed above, the reason for this may be a general disruption of development in polyploids rather than problems due to having chromosomal sex determination. Similarly, in mammals, including humans, absence of polyploidy has been attributed to general developmental disruption due to imprinting, i.e. the necessity to have 1 copy of the genome of either parent [see Otto and Whitton, 2000, for further discussion of these arguments].

Plants often have polygenic sex determination and mixtures of genetic and epigenetic (plastic) sex determination, which may be less vulnerable to changes in chromosome copy number. Many animals also have epigenetic sex determination and are hermaphroditic. We use the term 'epigenetic' here in its broadest sense [Holliday, 1990], including any non-genetic cues that lead to sexual differentiation of cells or individuals. Such cues can be intrinsic, based on the position of a cell or tissue in the body, or extrinsic, like many environmental factors [Beukeboom and Perrin, in preparation]. Orr [1990] predicted that animal taxa with environmental sex determination would be less constrained in evolving polyploidy than taxa with genotypic sex determination. Indeed many taxa with hermaphroditic reproduction and epigenetic sex determination appear to have evolved polyploidy [Mable, 2004]. However, whether and how alteration of gene dosage as a result of polyploidization is tolerated in such groups is hard to predict as the molecular genetic regulation of epigenetic sex determination is currently poorly understood. A particular amenable case for further study may be the African clawed frog *Xenopus laevis* which is tetraploid. It has female heterogametic (ZZ/ZW) sex determination, but sex determination is

also partly epigenetic [Kobel and Du Pasquier, 1986], although the molecular regulatory details are not yet clear [Bewick et al., 2010].

### Polyploidy and Evolution

After the original arising of polyploidy, duplicate copies of the genes may start to diverge, either in protein sequence or in their regulatory control (fig. 1D–F). The duplication event may release (at least) 1 copy of the gene from constraints associated with its original function [Lynch, 2004]. For example, different members of gene families can start to show divergence in the tissues or developmental stages in which they are expressed [e.g. Adams et al., 2003]. This latter process could constitute a neo-functionalization process when the gene family members acquire different functions or produce a novel result or gene product in a particular tissue or cell type (fig. 1E). A prime example of the latter are the Hox gene clusters, which control cell fate and patterning during embryogenesis. After the 2 WGDs at the base of the vertebrate lineage, the 4 duplicated Hox gene clusters diverged towards distinct expression patterns due to the recruitment of novel *cis*-regulatory elements that exert concerted control over whole Hox gene clusters [Deschamps, 2007; Tschopp and Duboule, 2011]. Similarly, we hypothesize that divergent evolution after gene or genome duplication for modulators of signal transduction pathways (such as the rich and rapidly evolving family of serine-type endopeptidases) could have led to the very different results of a single signaling cascade in different tissues. For example, enhanced *Toll* expression in the liver/fat body leads to the production of antimicrobial peptides, while it leads to blood cell proliferation in the hematopoietic organs [Jang et al., 2006; Mulinari et al., 2006].

By providing duplicated genes, polyploidization leads to extensive genetic variation that can form the raw material for various evolutionary processes [Lynch, 2004]. The multiple copies of the same gene in neopolyploids increase the allelic or genetic complexity, which can be advantageous in terms of providing more allelic or gene variants that selection might favor under particular (new) conditions, masking recessive deleterious mutations and ameliorating inbreeding depression [reviewed in Otto, 2007]. Although the latter 2 advantages may be highly relevant for the establishment of the neopolyploid species, which often experience a severe bottleneck after the initial genome shock and during initial establishment, these short-term benefits would lessen over time because polyploidy also masks both beneficial mutations and the accumulation of deleterious mutations [Otto, 2007]. The increase in variants available for selection, however, primarily provides long-term benefits, because it requires time for evolution to act upon and fine-tune that available variation. Finally, the merging of 2 genomes may result in immediate fitness effects that selection could act on or against, such as increased or reduced hybrid vigor, reproduction and/or survival, and tolerance to extreme conditions [Otto, 2007]. A main source of allelic or genetic variation that evolution could act on is the variation in the regulation of gene expression in the duplicated genes [Adams and Wendel, 2005].

At the arising of polyploidy from a sexual reproductive event, e.g. through hybridization, polyspermy (multiple sperm fertilizing a single egg) or fertilization of/by unreduced gametes, more than 2 alleles may be present from the onset of the genome duplication. This is in contrast to endopolyploidization, genome duplication by selfing or parthenogenesis, or intragenomic gene duplications where only the 2 original alleles are being duplicated. Therefore, the origin of the polyploidization events determines the original amount of genetic variation, which would typically be more extensive with larger genetic distance between the progenitors of the neopolyploid. For plants, it has been suggested that it is especially the disruption in the regulation of gene expression in allopolyploidy (e.g. through changes in methylation, disruption of heterochromatin, activation of transposable elements, alterations in imprinting) that may have immediate effects on the phenotype, even outweighing the effect of ploidy itself [Otto, 2007].

The genomic diversity that results from polyploidization may become a driver of diversification and speciation [Evans, 2008; Mable et al., 2011]. For some taxa, a WGD appears at the base of the lineage, such as for flowering plants [Bowers et al., 2003] and vertebrates [Dehal and Boore, 2005], and additional genome duplications occurred in various fish and amphibian families [reviewed in Mable et al., 2011]. Although it is tantalizing to speculate that the WGD may be the reason for the burst of morphological complexity and diversification that followed, it proves very hard to verify these adaptionist hypotheses [Donoghue and Purnell, 2005; Otto, 2007]. Modeling studies have also shown that genome or gene duplications may passively lead to increased biodiversity through silencing of alternate copies of the duplicated genes, effectively creating post-mating reproductive barriers. Following genome duplication, stochastic nonfunctionalization processes (i.e. the loss of function of a

duplicated gene by accumulation of mutations) would lead to accumulated silenced copies, that may rapidly result in incompatibility between species members [Lynch and Conery, 2000]. Notwithstanding these considerations and without any universal claims on the role of genome duplication in the evolution of complexity and biodiversity, polyploidy has the potential to provide a rich source of genomic variation that may be exploited in evolutionary processes.

Although hybridization events are generally associated with reduced fitness of the newly arisen individual, polyploidization may facilitate the origin of new species by hybridization. Combining the partially diverged genomes of 2 species often leads to F2 hybrid breakdown as a result of negative epistatic gene actions [Coyne and Orr, 2004]. These negative interactions are typically partially recessive and rescued by a dominant interaction of 2 gene copies from one of the original parental species [Turelli and Orr, 1995]. Polyploidy could be beneficial in rescuing the negative effects of hybridization in 2 ways: (1) by supplying 2 properly interacting gene copies from one of the parental species, and (2) by providing 2 recessive and negatively interacting gene copies from one of the parental species. The latter may sound counterintuitive, but when the negative interaction is due to an insufficient amount or action of specific gene products, a double dose of this suboptimal product could rescue the particular cellular process. Some evidence for such a positive effect of a ploidy increase on hybrid viability was recently found by Koevoets [2012].

The constraints that appear to exist for animal polyploidy (as described before) might be traced in the vertebrate genomes after the 2 rounds of WGD. All genes are duplicated upon WGD, and have been termed 'ohnologs' by Wolfe [2000]. Nakatani et al. [2007] reconstructed the vertebrate ancestral genome by comparing human and medaka fish genome sequences to retrace the 2 WGD events. Ohnologs were defined by comparison to invertebrate genomes. This ohnolog set was validated using the chicken genome, revealing a contrast of slow karyotype evolution after the second WGD in gnathostome, osteichthyan and amniote ancestors and rapid, lineage-specific genome reorganizations in teleosts, amphibians, reptiles, and marsupials. Makino and McLysaght [2010] surveyed the ohnologs in the human genome and noted that genes that were preserved after WGD are dosage-balanced, have low copy number variation and are not subject to small-scale duplications. This balanced dose dependency is not very flexible and may prevent the human genome to sustain another round of WGD. The human situation may be extrapolated to other animal genomes to explain the rarity of polyploidy.

This brings the focus back to the genomic balance hypothesis. Storchová and Pellman [2004] and Storchová et al. [2006] described the genomic constraints on polyploidy in yeast [reviewed in Thorpe et al., 2007]. They identified mutations that affected genomic stability in tetraploids through altering important structural features such as mitotic spindle formation, homologous recombination and chromosome cohesion. Selective pressure on genes involved in these processes is probably higher in polyploids, and may prove essential if a polyploidization event were to lead to survival of the resulting individual. More evidence for the existence of essential genes or gene complexes under polyploidization comes from the work of Chain and Evans [2006] and Chain et al. [2011]. They describe how retained expression of 290 expressed paralogs (duplicated genes within the genome) in the tetraploid frog *X. laevis* is realized by mechanisms that preserve stoichiometry and spatiotemporal maintenance of expression levels. *X. laevis* is closely related to the diploid frog *Silurana tropicalis.* WGD has occurred in the genus *Xenopus* but not in the genus *Silurana*. Chain et al. [2011] investigated 2 sets of genes: (1) a 'single' gene of *S. tropicalis* and the through WGD duplicated genes (ohnologs) in *X. laevis* and (2) a 'single' gene of *S. tropicalis* and the *X. laevis* ortholog of which 1 copy has been lost after WGD. By logistic regression, the authors demonstrated that the genetic and expression characteristics of genes in the diploid species are indicative for duplicate gene persistence in the tetraploid species. The total expression pattern and the evenness of expression across tissues and through development were the main determinants. In addition, slow evolutionary rate of the encoded protein and high gene information density (few exons, short introns) were also positively correlated with persistence of paralogs after polyploidization. This shows that a particular subset of genes in the genomic network is key to establishing a new balance after a polyploidization event (fig. 1).

## Polyploidy and Evolutionary Ecology

The evolutionary potential of polyploidization is only realized when polyploidization has a fitness effect that is sustainable, or at least not severely deleterious, and when some form of assortative mating is established. Without these prerequisites, the chances of a polyploid species to establish are exceedingly small [Mable, 2004]. Interestingly, some physiological and ecological circumstances

seem to promote the arising or establishment of polyploidy. In this section, we explore whether a better understanding of the consequences of polyploidy on gene expression could potentially be associated with the conditions that appear to drive polyploidy development or persistence.

Abiotic stress has been associated with an increased production of polyploidy. In plants, latitudinal and altitudinal clines have shown that cold environments harbor proportionally more polyploids than tropical regions [reviewed in Mable, 2004]. In fish and amphibians, polyploidy is primarily found in taxa that reproduce in temperate freshwater environments, where the zygotes are potentially exposed to temperature stress [Mable et al., 2011]. What is unclear, however, is whether this correlation with particular environments is due to a higher probability of the production of unreduced gametes (e.g. as a side effect of abiotic stress), or whether there is any fitness advantage to polyploidy under extreme conditions [Soltis and Soltis, 1999; Mable, 2004; Mable et al., 2011].

The earlier discussed fish hybrid species complex of *S. alburnoides* is sympatric with 2 species with which hybridization has occurred: *S. pyrenaicus* in the south (PA, PAA, PPA, and PPAA genomes) and *S. carolitertii* (CA, CAA, CCA, and CCAA genomes) in the north of the Iberian Peninsula. Pala et al. [2008, 2010] demonstrated that for southern triploid PAA hybrid fish predominantly the A allele was expressed. In contrast, northern triploid hybrid fish containing a C allele showed bi-allelic expression of C and A alleles. This demonstrates environmental effects on hybrid gene expression, but may also reflect differential interaction of hybridizing genomes [Pala et al., 2010].

So far, there is no universal pattern for polyploid species inhabiting a wider range of environments than their diploid ancestors [Mable, 2004; Mable et al., 2011]. That does not refute the possibility that polyploid species are better in coping with an extreme environment than their progenitors. Gene expression studies have frequently been used to compare environmental stress responses among genotypes and species in common-garden experiments [e.g. Gasch, 2007; Grishkevich et al., 2012] or among different tissues [e.g. Cossins et al., 2006]. Although expression studies are insufficient to provide an integrative understanding of how a particular genome determines the ability to cope with environmental stress [Feder, 2007], a similar common-garden transcriptomics approach between diploid and polyploid organisms may reveal the changes in the regulatory networks in response to environmental stress. Ideally, such studies would compare genome-wide expression under benign and stressful conditions between diploid and polyploid individuals with similar fitness (under benign conditions), not to triploids with reduced overall fitness.

Interestingly, endopolyploidy can occur in a subset of the cells in a tissue, enabling the direct comparison of gene expression and cell physiology in diploid and polyploid cell types under the same conditions. The generation of polyploid cells, from cell fusions, endoreplications and abortive cell cycles, has been correlated to various cellular stressors, such as oxidative damage and hypertension [Storchova and Pellman, 2004]. While it is not clear whether these correlations represent adaptive or pathological responses, increased ploidy can have tangible effects on cell size, cellular physiology and metabolic load on the organ [Storchova and Pellman, 2004]. Gene expression studies of polyploid and diploid cells showed that endopolyploidy mostly leads to up-regulated gene expression, frequently involving genes in tissue-specific functions, metabolism and in stress response and protection [Anatskaya and Vinogradov, 2010]. Moreover, endopolyploidy can protect cell vitality and favor energy conservation [Anatskaya and Vinogradov, 2010]. Hence, it was suggested that endopolyploidy may provide a fast mechanism to respond to unfavorable conditions and to optimize energy efficiency in particular tissues. Correspondingly, the endopolyploidy of flight muscles in many Hymenoptera has been suggested as a mechanism to yield more energy [Aron et al., 2005]. However, the frequent occurrence of altered ploidy in cancer cells and the association between malignancy and inactivation of *p53*, a checkpoint gene for tetraploidy, emphasize that excessive genome duplications in cells can also constitute a liability [Storchova and Pellman, 2004].

A particular type of biotic interactions that has been associated with polyploidy is parasitism and parasite resistance [Nuismer and Otto, 2004; Osnas and Lively, 2006; M'Gonigle and Otto, 2011; King et al., 2012]. A role for polyploidy in immunity has been inferred in the context of (1) allelic and genetic diversity and (2) endopolyploidy (next paragraph). Firstly, both innate and acquired immune systems comprise highly duplicated gene families coding for pattern recognition receptors that distinguish between self and non-self [Palm and Medzhitov, 2009; Kawai and Akira, 2010; Birnbaum et al., 2012]. Having multiple alleles may be beneficial when it allows for recognition of a wider range of pathogens or facilitates co-evolutionary adaptation to local parasite communities [M'Gonigle and Otto, 2011]. Pattern recognition genes are among the fastest evolving defensive genes, presum-

ably as a result of the arms races and co-evolutionary dynamics in host-parasite interactions [Obbard et al., 2009]. Theoretical approaches have been used to compare the consequences of haploidy and diploidy in both the parasite and the host [Nuismer and Otto, 2004, 2005; M'Gonigle and Otto, 2011]. Assuming single loci governing both recognition and virulence, these studies concluded that haploidy is usually favored in parasites, as it reduces the expression of antigens that may be recognized by the host, while diploidy/polyploidy is favored in hosts as it increases the ability to detect multiple varieties of the antigens [Nuismer and Otto, 2004; M'Gonigle and Otto, 2011]. Adding additional loci to these models, to reflect the co-adapted gene complexes that underlie most host-parasite interactions, may provide a better insight in the evolution of co-expression of various alleles, modulators and polymorphisms [Nuismer and Otto, 2004, 2005].

Endopolyploidy has been reported for several cell types specifically involved in host immunity (e.g. platelets, megakaryocytes and liver cells) [Lee et al., 2009]. Additionally, several parasitoid wasps (i.e. insects that lay their eggs in other insects, where the developing parasitoid larvae kill the host during their development) produce teratocytes and inject these in the host during parasitization. These polyploid cells are considered virulence factors that control host growth and development, as well as repress the host's immune responses [e.g. Strand and Wong, 1991; Dahlman et al., 2003]. Both the arguments of enriched allelic diversity and genetic complexity, and the observation of so many immune-competent tissues showing endopolyploidy, suggest that polyploidy may be favored in the evolution of resistance and immunity.

## Conclusions and Outlook

Wagner [2000, 2011] emphasized the importance of taking 'genotype networks' into account when studying the complexity of genotype-phenotype maps. Similarly, Schadt [2009] stressed the importance of 'molecular networks', consisting of RNA, proteins and metabolites as intermediate phenotypes in the genotype-phenotype complexity. This way of considering genomic networks in relation to phenotypes has exposed different layers of complexity, such as genome-genome interactions, genome-environment interactions, gene redundancy, phenotypic plasticity, and homeostasis. A combination of the new genomic technologies and systems biology may find ways to tackle these stacked levels of complexity, of which polyploidy surely is a part.

Although stable polyploidy in animals is not as rare as originally thought, polyploidy does seem to be more constrained in animals than in plants. The lower incidence of polyploidy in animals does not appear to be due to limitations at the initial polyploidization step. Yet, the subsequent genome reorganization to maintain a balanced genome after polyploidization might be more problematic in animals. This could be related to the complex regulatory control of gene expression leading to intricate gene regulatory networks and tissue-specific expression patterns. Although tissue-specific and intricate regulatory control of signaling pathways is also prevalent in plants [e.g. Adams et al., 2003; Nakashima et al., 2009], differences between animals and plants in development, genomic architecture, as well as sex determination may provide a combined explanation for the rareness of polyploidy in animals. In particular, it appears more difficult to re-establish genome balance after a polyploidization event in animals, because (1) intricate gene interaction networks vary among terminally differentiated cells in the various tissues and organs, (2) animal body plans are inflexible (e.g. the proportions and frequency of organs is fixed), and (3) separation of sexes is the norm, which has resulted in regulatory mechanisms to compensate for expression balance of genes on the sex chromosomes and autosomes. All these key features of animals could hamper the re-establishment of genome balance after genome shock.

The remark of Orr [1990] that 'we are left without an adequate explanation for the remarkable difference between animal and plant speciation' is still valid, but it has now become a tractable challenge. Although gene expression studies in plant polyploidy have bloomed in recent years, similar studies in animals are much more limited. This is unfortunate, as contrasting gene expression patterns in polyploids could help to explain the differences in evolutionary dynamics of polyploidy effects in plants and animals. Some aspects of the consequences of WGD for these gene expression networks are presented in figure 1. The difficulty has been in finding model organisms where expression could be compared between diploidy and polyploidy under otherwise equal conditions. Perhaps endopolyploidy provides such a situation that may be exploited for elucidating the consequences of ploidy on gene interaction networks. Additionally, a possible way to filter out sex determination effects, or at least sex chromosomal effects, may be to study hermaphroditic and haplodiploid species. We hope that the conceptual model of the effects of polyploidization on genomic networks as emerged from the literature may serve as a framework to unify the various explanations for the rareness of polyploidy in animals.

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