ON THE NATURE OF THINGS: ESSAYS
New Ideas and Directions in Botany

Cytonuclear responses to genome doubling[1]

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Plant cells contain genomes in three distinct compartments: the nucleus, mitochondrion, and plastid. This tripartite distribution of DNA has profound consequences for plant biology. Key metabolic processes, including aerobic respiration and photosynthesis, require the intimate coordination of cytoplasmic and nuclear genomes (Rand et al., 2004). Because of the extensive and ongoing history of gene transfer from organelles to the nucleus (Kleine et al., 2009), many nuclear-encoded proteins destined for the mitochondria or plastids must directly interact with the gene products of cytoplasmic genomes in multi-subunit complexes such as Rubisco, the enzymes that comprise the mitochondrial electron transport chain, and the organellar ribosomes. Additionally, the replication and expression of cytoplasmic genomes are highly dependent on nuclear-encoded proteins, as are many of the biosynthetic and signaling functions of mitochondria and plastids. It is perhaps unsurprising, then, that changes in one genomic compartment can influence evolution in other genomes within the plant cell (Sloan, 2015).

Whole-genome duplications (WGDs), in which the number of nuclear genome copies is elevated as a result of autopolyploidy or allopolyploidy, underlie many of the major episodes of diversification in land plants (Wendel, 2015; Soltis and Soltis, 2016; Van de Peer et al., 2017) and remain a prominent process in plant speciation (Barker et al., 2016). From an economic perspective, polyploidy is tremendously important because many crops (Renny-Byfield and Wendel, 2014) and invasive weeds (te Beest et al., 2012) are neopolyploids, and *all* flowering plants have undergone one or more rounds of WGD at some point during their evolutionary history (Soltis and Soltis, 2016). Hence, understanding WGD is central to our understanding of basic plant biology. Enormous progress has been made in investigating the myriad genomic and transcriptomic consequences of polyploidy, which include biased patterns of gene loss, recombination, and/or gene conversion between duplicated gene copies (homoeologs), genome-wide alteration of gene expression, and epigenetic reprogramming, among other phenomena (Yoo et al., 2014; Soltis et al., 2015; Song and Chen, 2015).

Notwithstanding these many important advances, the genetic and evolutionary forces that WGD imposes upon *cytoplasmic* genomes are less well understood, despite the central role that cytonuclear interactions play in plant function and fitness. There are several reasons to believe that WGDs perturb cytonuclear interactions, including the imposition of sudden changes in gene copy number and stoichiometry (Wendel, 2000; Birchler and Veitia, 2012), but we still know little about the developmental and evolutionary responses to these disruptions.

Polyploid lineages face the critically important task of maintaining coordinated gene expression of interacting gene products in the face of a suddenly doubled nuclear gene copy number, which may alter assembly dynamics for multi-subunit complexes (Birchler and Veitia, 2012). This problem of stoichiometric imbalance may be especially relevant to cytonuclear interactions. Cytoplasmic genomes are commonly described as "effectively haploid" because they are often inherited from a single parent, but each of these genomes actually exists in dozens to thousands of copies per cell. Thus, even in diploid species there are complex stoichiometric relationships between nuclear and cytoplasmic genomes. While the number of mitochondrial and plastid genomes within a cell varies tremendously across tissue types and development, these numbers are likely to be perturbed by WGD, as cytoplasmic genomes exist in a cellular environment in which all nuclear genes have been instantly doubled. We therefore expect selection to favor compensatory mechanisms that maintain coordinated expression between cytoplasmic and nuclear genes immediately following polyploidization (e.g., downregulated

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expression of mitochondrial- and plastid-targeted genes in the nucleus, upregulated cytoplasmic genome copy number, elevated levels of organelle biogenesis) as well as over longer timescales (e.g., subfunctionalization, pseudogenization) (Fig. 1).

One indication that polyploidy has substantial stoichiometric consequences for cytonuclear interactions is that organelle-targeted genes appear to be one of the first and most overrepresented classes of nuclear genes to return to single copy following a WGD (De Smet et al., 2013), although there may be considerable variation in sensitivity to gene dosage imbalance across different functional pathways within the organelles (Coate et al., 2011). Additionally, the well-known positive relationship between nuclear genome size and cell size (Beaulieu et al., 2008) indicates that plants may be capable of harboring more organelles per cell as nuclear genome copy number and cell size increases (Fig. 1). Indeed, cotton and alfalfa polyploids exhibit elevated chloroplast number per cell relative to diploids (Bingham, 1968; Krishnaswami and Andal, 1978), and chloroplast number per cell, but not chloroplast size, scales with ploidy in maize (Rhoades and Dempsey, 1966). Many of the specific mechanisms controlling organelle biogenesis have yet to be elucidated. Interestingly, Kawade et al. (2013) concluded that cell size may be a more direct determinant of chloroplast proliferation than nuclear ploidy. The anterograde (nucleus-to-organelle) and retrograde (organelle-to-nucleus) signaling pathways that are known to participate in organelle biogenesis and DNA replication would be prime candidates for coordinating cytonuclear stoichiometry following WGD. However, we still lack systematic studies on how the number and size of organelles, the copy number of cytoplasmic genomes, the expression level of cytoplasmic genes, and anterograde/retrograde signaling pathways change in response to WGD.

The close association between polyploidy and hybridization in plants means that allopolyploids must face the additional challenge of maintaining successful interactions between nuclear and cytoplasmic genes that were inherited from different species. The typical uniparental inheritance of cytoplasmic genomes means that they should be more closely "matched" to maternal copies of organelle-targeted genes than to paternal homoeologs, leading to the expectation that selection will favor the maintenance of maternal homoeologs.

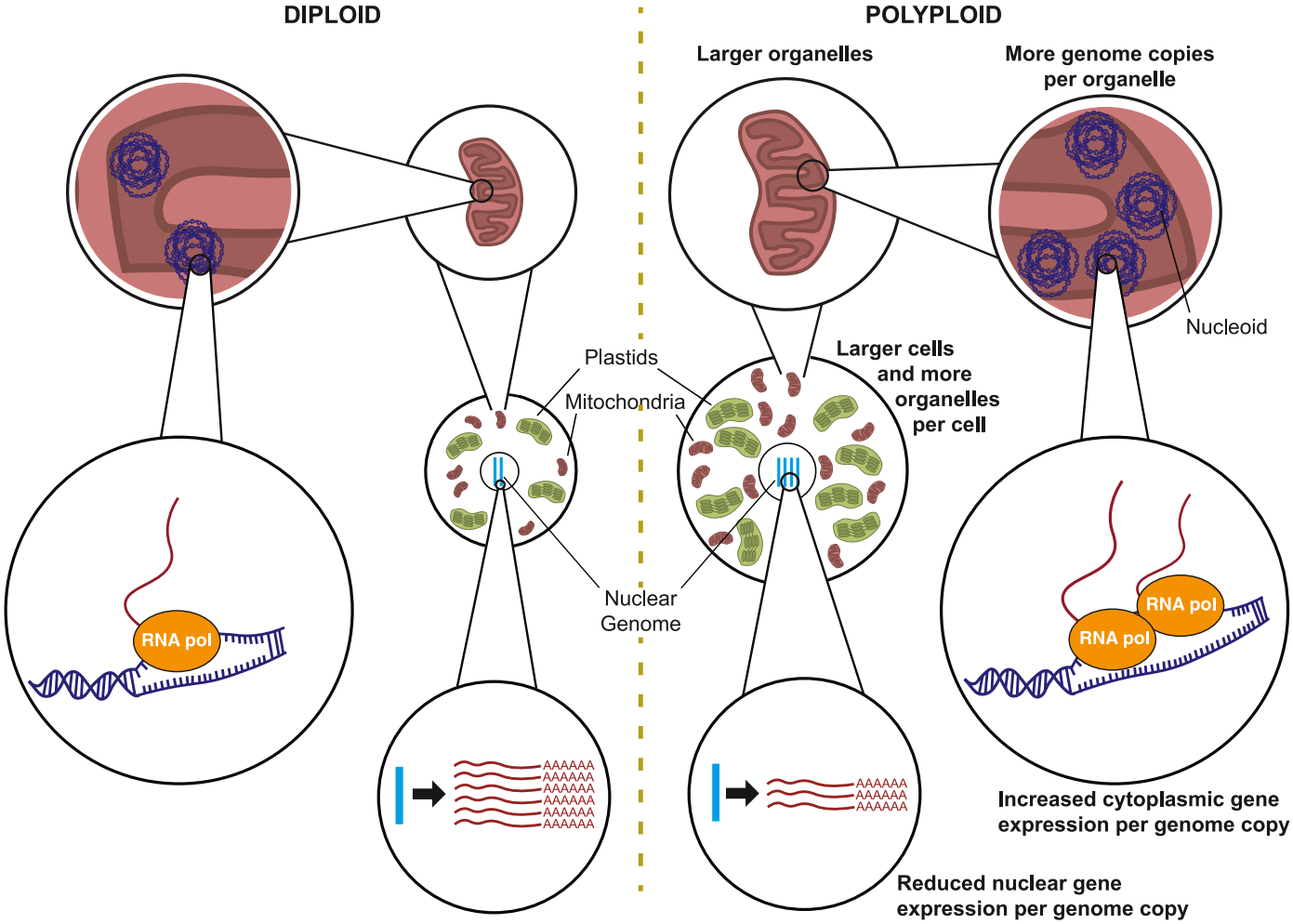

FIGURE 1 Potential mechanisms to maintain cytonuclear stoichiometry in polyploid plants. Gene dosage imbalance of cytonuclear genetics in the wake of a WGD event likely has important consequences for organismal function both in the immediate aftermath and as an evolved response to polyploidy. The elevated nuclear gene copy number resulting from polyploidization may require compensatory mechanisms to maintain coordinated gene expression between organelle-encoded and organelle-targeted genes. Some mechanisms that aid in maintaining cytonuclear stoichiometry may scale allometrically with ploidy level (e.g., cell size, organelle number, and organelle size), while others likely require regulatory control by the nuclear genome (e.g., cytoplasmic genome copy number and nuclear/cytoplasmic gene expression regulation).

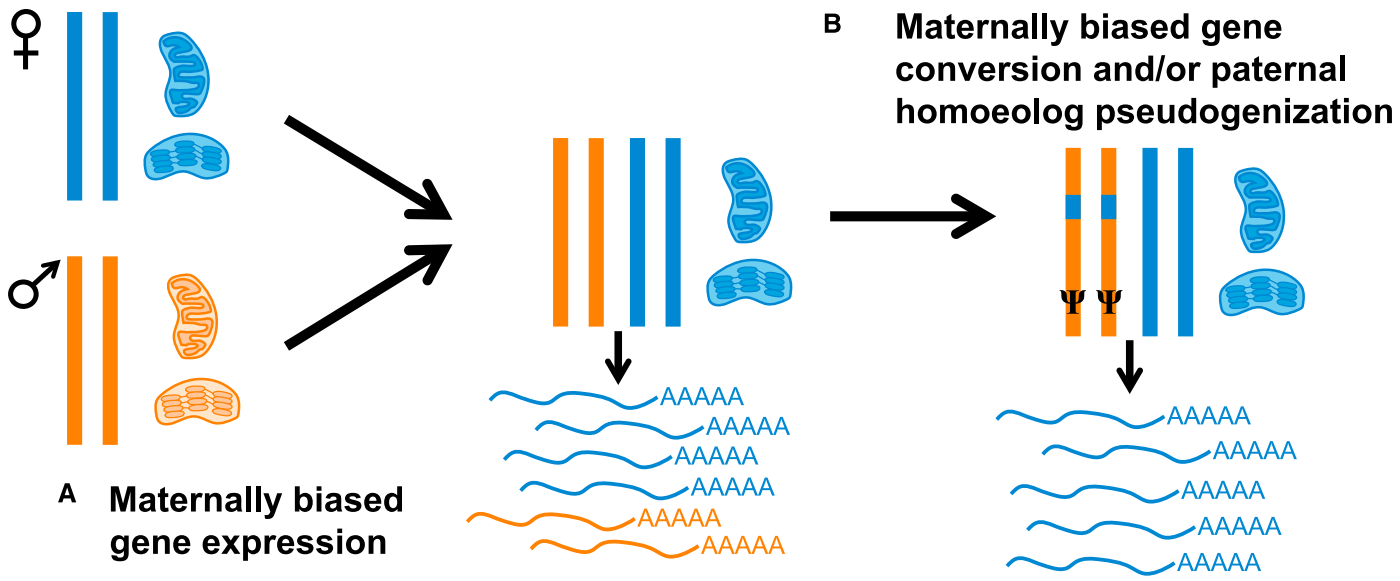

FIGURE 2 Cytonuclear incompatibility and molecular evolution of organelle-targeted genes in allopolyploid plants. Maternal inheritance of cytoplasmic genomes results in a more recent shared evolutionary history with the maternal nuclear subgenome than with the paternal nuclear subgenome in allopolyploids. As such, maternally encoded genes targeted to organelles are expected to interact with cytoplasmic genomes in hybrids more successfully than paternally encoded genes. Preferential expression of maternal transcripts is predicted to prevent cytonuclear mismatch in the short term (A), while pseudogenization (denoted by Ψ symbol) of paternally inherited organelle-targeted genes and/or maternally biased gene conversion may evolve over longer timescales (B).

Because establishing successful cytonuclear interactions plays a central role in the success of hybrid lineages (Burton et al., 2013), two predominant evolutionary outcomes of allopolyploidization might be expected: (1) gene silencing and relaxed selection, leading to eventual loss of function of the paternal homoeolog for organelle-targeted genes, and (2) homoeologous recombination resulting in preferential gene conversion to the maternal homoeolog (Fig. 2). To date, tests of these predictions, although limited to only a single protein complex (Rubisco), have provided support for the hypothesis that differential gene loss/expression and asymmetric gene conversion can play a role in coordinating cytonuclear interactions in allopolyploids (Gong et al., 2012, 2014; Sehrish et al., 2015).

A more complete understanding of the mechanisms that maintain cytonuclear interactions in polyploids will require expanding research efforts beyond Rubisco to include the numerous enzyme complexes and pathways involved in mitochondrial and plastid function. Such investigations can take advantage of taxa with relatively ancient allopolyploidization events (e.g., *Gossypium*, ~1–2 Ma) in which we expect higher levels of pseudogenization and gene conversion vs. recent and ongoing allopolyploidization events (e.g., *Tragopogon*, ~80 yr ago), which may exhibit few differences in gene content but still show patterns of gene expression bias. Repeated bouts of polyploidization and hybridization should accelerate rates of diploidization and biased genome fractionation, making systems that have experienced multiple rounds of allopolyploidization (e.g., *Triticum*, *Brassica*) useful for understanding rates of asymmetric gene loss and pseudogenization. Finally, allopolyploids in which cytoplasmic genomes are paternally inherited (e.g., *Actinidia*, *Medicago*) offer the opportunity to flip our predictions on their head and test whether organelle-targeted genes are preferentially expressed and maintained by the paternal subgenome.

The proliferation of genomic data and the development of bioinformatic tools to dissect the inherently complex history of polyploid genomes make this an exciting time to uncover biological mechanisms that contribute to and ameliorate stoichiometric imbalances and epistatic incompatibilities between cytoplasmic and nuclear genomes. More broadly, polyploidy and cytonuclear interactions represent major contributors to angiosperm innovation, diversification, and reproductive isolation, so understanding the processes that maintain cytonuclear coordination in the face of WGD will ultimately shed light on plant evolution and diversification.

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LITERATURE CITED

Barker, M. S., B. C. Husband, and J. C. Pires. 2016. Spreading Winge and flying high: The evolutionary importance of polyploidy after a century of study. *American Journal of Botany* 103: 1139–1145.

Beaulieu, J. M., I. J. Leitch, S. Patel, A. Pendharkar, and C. A. Knight. 2008. Genome size is a strong predictor of cell size and stomatal density in angiosperms. *New Phytologist* 179: 975–986.

Bingham, E. T. 1968. Stomatal chloroplasts in alfalfa at four ploidy levels. *Crop Science* 8: 509–510.

Birchler, J. A., and R. A. Veitia. 2012. Gene balance hypothesis: Connecting issues of dosage sensitivity across biological disciplines. *Proceedings of the National Academy of Sciences, USA* 109: 14746–14753.

Burton, R. S., R. J. Pereira, and F. S. Barreto. 2013. Cytonuclear genomic interactions and hybrid breakdown. *Annual Review of Ecology, Evolution, and Systematics* 44: 281–302.

Coate, J. E., J. A. Schlueter, A. M. Whaley, and J. J. Doyle. 2011. Comparative evolution of photosynthetic genes in response to polyploid and nonpolyploid duplication. *Plant Physiology* 155: 2081–2095.

De Smet, R., K. L. Adams, K. Vandepoele, M. C. Van Montagu, S. Maere, and Y. Van de Peer. 2013. Convergent gene loss following gene and genome duplications creates single-copy families in flowering plants. *Proceedings of the National Academy of Sciences, USA* 110: 2898–2903.

Gong, L., M. Olson, and J. F. Wendel. 2014. Cytonuclear evolution of rubisco in four allopolyploid lineages. *Molecular Biology and Evolution* 31: 2624–2636.

Gong, L., A. Salmon, M. J. Yoo, K. K. Grupp, Z. Wang, A. H. Paterson, and J. F. Wendel. 2012. The cytonuclear dimension of allopolyploid evolution: An example from cotton using rubisco. *Molecular Biology and Evolution* 29: 3023–3036.

Kawade, K., G. Horiguchi, N. Ishikawa, M. Y. Hirai, and H. Tsukaya. 2013. Promotion of chloroplast proliferation upon enhanced post-mitotic cell expansion in leaves. *BMC Plant Biology* 13: 143.

Kleine, T., U. G. Maier, and D. Leister. 2009. DNA transfer from organelles to the nucleus: The idiosyncratic genetics of endosymbiosis. *Annual Review of Plant Biology* 60: 115–138.

Krishnaswami, R., and R. Andal. 1978. Stomatal chloroplast number in diploids and polyploids of *Gossypium*. *Proceedings of the Indian Academy of Sciences B* 87: 109–112.

Rand, D. M., R. A. Haney, and A. J. Fry. 2004. Cytonuclear coevolution: The genomics of cooperation. *Trends in Ecology & Evolution* 19: 645–653.

Renny-Byfield, S., and J. F. Wendel. 2014. Doubling down on genomes: Polyploidy and crop plants. *American Journal of Botany* 101: 1711–1725.

Rhoades, M. M., and E. Dempsey. 1966. Induction of chromosome doubling at meiosis by the elongate gene in maize. *Genetics* 54: 505–522.

Sehrish, T., V. V. Symonds, D. E. Soltis, P. S. Soltis, and J. A. Tate. 2015. Cytonuclear coordination is not immediate upon allopolyploid formation in *Tragopogon miscellus* (Asteraceae) allopolyploids. *PLoS One* 10: e0144339.

Sloan, D. B. 2015. Using plants to elucidate the mechanisms of cytonuclear coevolution. *New Phytologist* 205: 1040–1046.

Soltis, P. S., D. B. Marchant, Y. Van de Peer, and D. E. Soltis. 2015. Polyploidy and genome evolution in plants. *Current Opinion in Genetics & Development* 35: 119–125.

Soltis, P. S., and D. E. Soltis. 2016. Ancient WGD events as drivers of key innovations in angiosperms. *Current Opinion in Plant Biology* 30: 159–165.

Song, Q., and Z. J. Chen. 2015. Epigenetic and developmental regulation in plant polyploids. *Current Opinion in Plant Biology* 24: 101–109.

te Beest, M., J. J. Le Roux, D. M. Richardson, A. K. Brysting, J. Suda, M. Kubesova, and P. Pysek. 2012. The more the better? The role of polyploidy in facilitating plant invasions. *Annals of Botany* 109: 19–45.

Van de Peer, Y., E. Mizrachi, and K. Marchal. 2017. The evolutionary significance of polyploidy. *Nature Reviews. Genetics* 18: 411–424.

Wendel, J. F. 2000. Genome evolution in polyploids. *Plant Molecular Biology* 42: 225–249.

Wendel, J. F. 2015. The wondrous cycles of polyploidy in plants. *American Journal of Botany* 102: 1753–1756.

Yoo, M. J., X. Liu, J. C. Pires, P. S. Soltis, and D. E. Soltis. 2014. Nonadditive gene expression in polyploids. *Annual Review of Genetics* 48: 485–517.