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References

Galagan JE, Henn MR, Ma LJ, Cuomo CA, Birren B. 2005. Genomics of the fungal kingdom: insights into eukaryotic biology. *Genome Research* **15**: 1620–1631.

Kamoun S. 2006. A catalogue of the effector secretome of plant pathogenic oomycetes. *Annual Review of Phytopathology* **44**: 41–60.

Kamoun S, Hogenhout SA. 2001. Agricultural microbes genome 2: first glimpses into the genomes of plant-associated microbes. *Plant Cell* **13**: 451–458.

Kroken S, Glass NL, Taylor JW, Yoder OC, Turgeon BG. 2003. Phylogenomic analysis of type I polyketide synthase genes in pathogenic and saprobic ascomycetes. *Proceedings of the National Academy of Sciences, USA* **100**: 15670–15675.

Leach JE, Gold SE, Tolin S, Eversole K. 2003. A plant-associated microbe genome initiative. *Phytopathology* **93**: 524–527.

Mehrabi R, Zwiers LH, de Waard MA, Kema GH. 2006. MgHog1 regulates dimorphism and pathogenicity in the fungal wheat pathogen *Mycosphaerella graminicola*. *Molecular Plant-Microbe Interactions* **19**: 1262–1269.

Payne GA, Nierman WC, Wortman JR, Pritchard BL, Brown D, Dean RA, Bhatnagar D, Cleveland TE, Machida M, J. 2006. Whole genome comparison of *Aspergillus flavus* and *A. oryzae*. *Medical Mycology* **44**: 9–11.

Richards TA, Dacks JB, Jenkinson JM, Thornton CR, Talbot NJ. 2006. Evolution of filamentous plant pathogens: gene exchange across eukaryotic kingdoms. *Current Biology* **16**: 1857–1864.

Sagaram US, Butchko RAE, Shim W-B. 2006. The putative monomeric G-protein GBP1 is negatively associated with fumonisin B1 production in *Fusarium verticillioides*. *Molecular Plant Pathology* **7**: 381–389.

Tyler BM, Tripathy S, Zhang X, Dehal P, Jiang RH, Aerts A, Arredondo FD, Baxter L, Bensasson D, Beynon JL, Chapman J, Damasceno CM, Dorrance AE, Dou D, Dickerman AW, Dubchak IL, Garbelotto M, Gijzen M, Gordon SG, Govers F *et al.* 2006. Phytophthora genome sequences uncover evolutionary origins and mechanisms of pathogenesis. *Science* **313**: 1261–1266.

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Polyploidy: genome obesity and its consequences

Polyploidy workshop: Plant and Animal Genome XV Conference, San Diego, CA, USA, January 2007

Polyploidy is a major evolutionary feature of many plants and some animals (Grant, 1981; Otto & Whitton, 2000). Allopolyploids (e.g. wheat, cotton, and canola) were formed by combination of two or more distinct genomes, whereas autopolyploids (e.g. potato, sugarcane, and banana) resulted from duplication of a single genome. Both allopolyploids and autopolyploids are prevalent in nature (Tate *et al.*, 2004). Recent research has shown that polyploid genomes may undergo rapid changes in genome structure and function via genetic and epigenetic changes (Fig. 1) (Levy & Feldman, 2002; Osborn *et al.*, 2003; Chen, 2007). The former include chromosomal rearrangements (e.g. translocation, deletion, and transposition) and DNA sequence elimination and mutations, whereas epigenetic modifications (chromatin and RNA-mediated pathways) give rise to gene expression changes that are not associated with changes in DNA sequence. Over time, polyploids may become 'diploidized' so that they behave like diploids cytogenetically and genetically. Comparative and genome sequence analyses indicate that many plant species, including maize, rice, poplar, and *Arabidopsis*, are recent or ancient diploidized (paleo-) polyploids.

The consequences of polyploidy have been of long-standing interest in genetics, evolution, and systematics (Wendel, 2000; Soltis *et al.*, 2003). Research interest in polyploids has been renewed in the past decade following the discovery of multiple origins and patterns of polyploid formation (Soltis *et al.*, 2003) and rapid genetic changes in resynthesized allotetraploids in *Brassica* (Song *et al.*, 1995) and wheat (Feldman *et al.*, 1997). Rapid technological advances have also facilitated genomic-scale investigation of polyploids and hybrids (Wang *et al.*, 2006). Many ongoing studies are focused on investigation of: (i) the evolutionary consequence of gene and genome duplications in polyploids; (ii) genomic and gene expression changes in resynthesized allotetraploids; (iii) genetic and gene expression variation in natural populations of polyploids; and (iv) comparison of genetic and gene expression changes in resynthesized and natural polyploids (Wendel, 2000; Osborn *et al.*, 2003; Soltis *et al.*, 2003; Comai, 2005; Chen, 2007). The presentations given at the Polyploidy workshop, Plant and Animal Genome XV Conference (http://www.intl-pag.org/), reflected these current research themes, reporting on ancient polyploidy events in *Glycine*, expression evolution of duplicate genes in *Arabidopsis*, gene expression changes in resynthesized *Brassica* and wheat allopolyploids, hybridization barriers in *Arabidopsis*, and tissue-specific and stress-induced expression patterns of duplicate genes in cotton and hybrid *Populus*.

'... expression of duplicate genes in response to developmental programs is more strongly correlated than that of duplicate genes in response to environmental stresses, suggesting rapid evolution of duplicate genes in response to external factors'

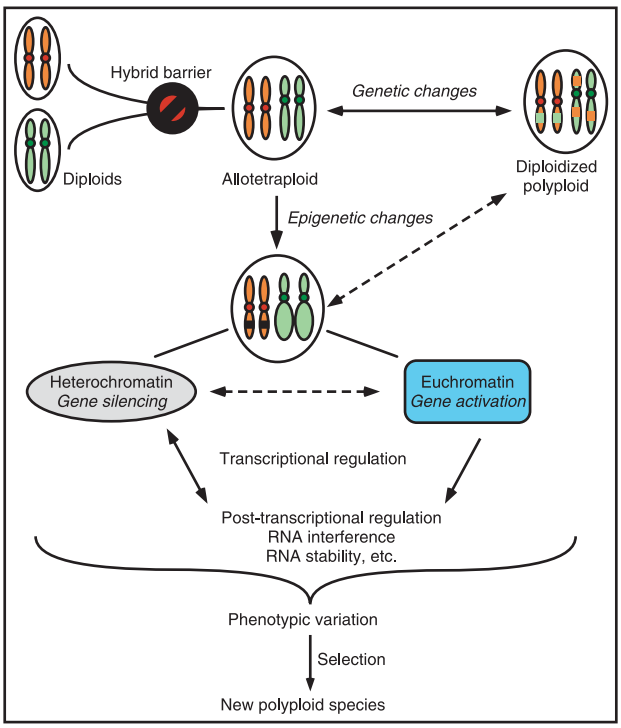

Fig. 1 Diagram of allopolyploid formation and evolution. A hybrid (not shown) derived from two diploid species can be induced to form a stable allotetraploid via spontaneous chromosome doubling or by colchicine treatment. Alternatively, an allotetraploid can be formed by fusing two unreduced gametes from two diploids or by hybridization of two autotetraploids (not shown) (Chen, 2007). Allotetraploid formation is usually impaired by the hybridization barrier between the two species (red stop sign). Once an allotetraploid is formed, it may undergo rapid genetic changes (e.g. chromosomal rearrangements, loss, and transposition) and epigenetic changes (e.g. chromatin modifications and post-transcriptional regulation). Chromosomes from the two different species are colored orange and green, respectively. The chromosomes (orange or green) in different species are orthologous, and they become homoeologous (orange and green) in the allotetraploid. Over time, allopolyploids may evolve to become diploidized polyploids because of rapid changes in chromosomal structure and sequence composition. In many instances, epigenetic changes predominate in allopolyploids. Interspecific hybridization or allopolyploidy may induce formation of heterochromatin and euchromatin, resulting in gene silencing or activation via transcriptional and post-transcriptional mechanisms. RNA interference induces and maintains heterochromatin formation. These changes in allopolyploids will lead to alteration of gene expression and phenotypic variation. Both genetic and epigenetic changes can be selected by natural or artificial forces that facilitate adaptive evolution of new polyploid species. Solid and dashed arrows indicate observed and predicted changes, respectively.

Duplication of resistance genes

Species of *Glycine* (soybean and relatives) are complex paleopolyploids that underwent at least two rounds of polyploidzation events, estimated to be *c.* 15 and *c.* 50–60 million years ago (Mya), respectively. To elucidate the complexity of the *Glycine* genome, Jeff Doyle (Cornell University, Ithaca, NY, USA), a member of the plant genome project led by Roger Innes (Indiana University, Bloomington, USA), reported progress in sequencing two homoeologues of a 1 Mb region that contains several disease resistance gene clusters (R-genes) in two soybean varieties and relatives of soybean. The homoeologous regions were derived from genome duplication which occurred 15 Mya. The gene densities of the two homoeologues in soybean are very different, mainly because of differences in the number of transposable element insertions. The two homoeologues also differ in their R-gene composition, with the gene-poor homoeologue also being degenerate for R-genes. Patterns of R-gene evolution are complex, with apparent recombination among copies and a considerable amount of copy-number variation among lineages. Little of this has been the result of polyploidy, however; only one of over 20 duplication events inferred from phylogenies appears to be related to the 15 Mya duplication, and most expansion has been tandem and much more recent. Variation in R-gene content also occurs among *Glycine* species, and even between soybean cultivars, suggesting recent and rapid changes. In other regions that do not contain resistance genes, gene densities and repeats tend to be very similar between homoeologues (Schlueter *et al.*, 2006), raising the question of whether the marked differences between homoeologues reported here are the result of evolutionary properties of R-gene clusters. Although much of the change in these two homoeologous regions has occurred recently, it is possible that the divergent evolution of the two homoeologues was set in motion by the polyploid event and has been ongoing subsequently.

Expression evolution of duplicate genes

The evolutionary fate of duplicate genes is poorly understood. Theory predicts that duplicate genes will eventually be lost or mutated. However, many gene duplicates are retained in the genome, probably via neofunctionalization or subfunctionalization (Lynch & Force, 2000). To test these hypotheses, Misook Ha (University of Texas at Austin, TX, USA), analyzed expression divergence of *c.* 2000 pairs of gene duplicates that resulted from a single duplication event that occurred 20–40 Mya (Blanc *et al.*, 2003). The gene expression microarrays measured at various conditions were used to test whether the expression patterns of gene duplicates diverge rapidly compared with the randomly paired genes in response to environmental and developmental changes. The data presented indicate that duplicate genes have a higher similarity of expression patterns than randomly paired genes. Moreover, expression of duplicate genes in response to developmental programs is more strongly correlated than that of duplicate genes in response to environmental stresses, suggesting rapid evolution of

duplicate genes in response to external factors. To explain these patterns of expression divergence between duplicate genes after whole genome duplication, Ha proposed a model whereby expression of duplicate genes diverges rapidly in response to changes in abiotic and biotic stresses, whereas the expression of duplicate genes diverges relatively slowly in response to developmental changes that are associated with complex biological networks.

Developmental regulation and subfunctionalization of duplicate genes

Functional divergence of homoeologous genes is manifested by tissue- or organ-specific expression patterns of duplicate genes, which were first observed in the allopolyploids *Brassica* and *Gossypium* (cotton). The silenced rRNAs genes in leaves subjected to nucleolar dominance in *Brassica* allotetraploids were reactivated in floral organs, suggesting developmentally regulated gene expression (Chen & Pikaard, 1997). Adams *et al.* (2003) found that developmental regulation of gene expression occurs in 10 out of 40 genes examined in cotton allopolyploids, suggesting tissue-specific regulation of homoeologous genes or subfunctionalization of duplicate genes in allopolyploids. Current work in the Adams laboratory (University of British Columbia, Vancouver, Canada) has focused on using a fluorescence-based semi-quantitative assay (snapshot) to distinguish expression differences between homoeologous loci in different tissues and organs and in cold and water submersion stresses. Adams reported that the expression ratios of homoeologous genes change not only in different tissues, but also under different stress (cold and water submersion) conditions. The data from *Arabidopsis* and cotton suggest that paralogous and homoeologous genes may have similar fates in response to changes in environmental cues and developmental programs.

Genetic and epigenetic changes in resynthesized *Brassica* allotetraploids

Gene expression changes may also be associated with either genetic or epigenetic mechanisms (Osborn *et al.*, 2003; Chen, 2007) (Fig. 1). Robert Gaeta (University of Wisconsin, Madison, WI, USA) reported chromosomal rearrangements and changes in DNA methylation among 50 resynthesized lines of *Brassica napus*-like plants. There is a correlation between changes in gene expression and chromosomal rearrangements and transposition (insertion of a fragment from one homoeologous chromosome to another). For example, Flowering Locus C expression is dependent on dosage caused by chromosomal rearrangements in 50 allopolyploid lineages. Similar changes were also reported in previous independent studies using resynthesized *B. napus*-like plants (Pires *et al.*, 2004). Interestingly, the frequency of changes in the restriction length fragment polymorphism (RFLP) among 50 lines is relatively low in the first generation following allopolyploid formation but high in the progeny after six generations of selfing. Furthermore, the frequency of DNA methylation changes is fairly constant in selfing progeny. Importantly for those interested in resynthesized polyploids, there is no obvious difference of genomic and gene expression changes in the progeny derived from allotetraploids that are derived from spontaneous chromosome doubling or colchicine-treatment. Chromosomal rearrangements and epigenetic modifications may explain a wide range of morphological changes observed in 50 different lineages of *Brassica* allotetraploids. As in *Arabidopsis* allopolyploids (Wang *et al.*, 2006), changes in gene expression are also frequently observed in resynthesized wheat allohexaploids. Bikram Gill (Kansas State University, Manhattan, KS, USA) reported high amounts of gene expression changes using microarray in comparison with wheat diploids, tetraploids, and hexaploids.

From hybridization barriers to the success of allopolyploids

Hybridization between the species that are separated for millions of years encounters barriers between alien cytoplasm and nuclear genomes and between two divergent genomes (Comai, 2005; Chen, 2007) (Fig. 1). These barriers are partly reflected by the changes in dosages of maternal and paternal genomes and imprinting patterns of gene expression (Bushell *et al.*, 2003). Comai (University of California at Davis, CA, USA) and colleagues have shown that the expression of PHERES1 and MEDEA is altered in resynthesized *Arabidopsis* allotetraploids (Josefsson *et al.*, 2006). Although reciprocal crosses of *Arabidopsis* allotetraploids cannot be made, the data suggest maternal and paternal effects of gene expression on seed fertility in the allopolyploids. Brian Dilkes (University of California at Davis, CA, USA), reported mapping a locus, named after Dr Strangelove (DSL1), in the triploid progeny of *Arabidopsis*. DSL1 is predicted to be a homologue of TRANSPARENT TESTA GLABRA (TTG2), a WRKY transcription factor. *Arabidopsis* TTG2 is strongly expressed in trichomes and in the endothelium of developing seeds and subsequently in other layers of the seed coats, and in developing roots. DSL1 does not show imprinting patterns, suggesting that post-zygotic barriers and seed fertility may also be affected by proper development of maternal tissues (ovules).

Perspectives

Polyploidy is a fascinating biological phenomenon that is a source of the raw genetic materials for adaptive evolution and crop domestication. Polyploid cells are often associated with carcinogenesis in animals, and polyspermy (fertilization

of more than one sperm into one ovum) usually causes abortive human triploids (McFadden *et al.*, 1993), suggesting why polyploidy is rarer in animals than in plants. The molecular changes observed in various polyploid plant systems will improve our understanding of why polyploid plants are so successful during evolution and why and how plants can tolerate genome obesity (increase in genome dosage) better than animals, especially mammals.

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References

Adams KL, Cronn R, Percifield R, Wendel JF. 2003. Genes duplicated by polyploidy show unequal contributions to the transcriptome and organ-specific reciprocal silencing. *Proceedings of the National Academy of Sciences, USA* **100**: 4649–4654.

Blanc G, Hokamp K, Wolfe KH. 2003. A recent polyploidy superimposed on older large-scale duplications in the *arabidopsis* genome. *Genome Research* **13**: 137–144.

Bushell C, Spielman M, Scott RJ. 2003. The basis of natural and artificial postzygotic hybridization barriers in arabidopsis species. *Plant Cell* **15**: 1430–1442.

Chen ZJ. 2007. Genetic and epigenetic mechanisms for gene expression and phenotypic variation in plant polyploids. *Annual Review of Plant Biology* **58**: 377–406.

Chen ZJ, Pikaard CS. 1997. Transcriptional analysis of nucleolar dominance in polyploid plants: biased expression/silencing of progenitor rrna genes is developmentally regulated in *Brassica*. *Proceedings of the National Academy of Sciences, USA* **94**: 3442–3447.

Comai L. 2005. The advantages and disadvantages of being polyploid. *Nature Reviews Genetics* **6**: 836–846.

Feldman M, Liu B, Segal G, Abbo S, Levy AA, Vega JM. 1997. Rapid elimination of low-copy DNA sequences in polyploid wheat: a possible mechanism for differentiation of homoeologous chromosomes. *Genetics* **147**: 1381–1387.

Grant V. 1981. *Plant speciation*. New York, NY, USA: Columbia University Press.

Josefsson C, Dilkes B, Comai L. 2006. Parent-dependent loss of gene silencing during interspecies hybridization. *Current Biology* **16**: 1322–1328.

Levy AA, Feldman M. 2002. The impact of polyploidy on grass genome evolution. *Plant Physiology* **130**: 1587–1593.

Lynch M, Force A. 2000. The probability of duplicate gene preservation by subfunctionalization. *Genetics* **154**: 459–473.

McFadden DE, Kwong LC, Yam IY, Langlois S. 1993. Parental origin of triploidy in human fetuses: evidence for genomic imprinting. *Human Genetics* **92**: 465–469.

Osborn TC, Pires JC, Birchler JA, Auger DL, Chen ZJ, Lee HS, Comai L, Madlung A, Doerge RW, Colot V, Martienssen RA. 2003. Understanding mechanisms of novel gene expression in polyploids. *Trends in Genetics* **19**: 141–147.

Otto SP, Whitton J. 2000. Polyploid incidence and evolution. *Annual Review of Genetics* **34**: 401–437.

Pires JC, Zhao JW, Schranz ME, Leon EJ, Quijada PA, Lukens LN, Osborn TC. 2004. Flowering time divergence and genomic rearrangements in resynthesized *brassica* polyploids (brassicaceae). *Biological Journal of the Linnean Society* **82**: 675–688.

Schlueter JA, Scheffler BE, Schlueter SD, Shoemaker RC. 2006. Sequence conservation of homeologous bacterial artificial chromosomes and transcription of homeologous genes in soybean (glycine max 1. Merr.). *Genetics* **174**: 1017–1028.

Soltis DE, Soltis PS, Tate JA. 2003. Advances in the study of polyploidy since plant speciation. *New Phytologist* **161**: 173–191.

Song K, Lu P, Tang K, Osborn TC. 1995. Rapid genome change in synthetic polyploids of *brassica* and its implications for polyploid evolution. *Proceedings of the National Academy of Sciences, USA* **92**: 7719–7723.

Tate JA, Soltis PS, Soltis DE. 2004. Polyploidy in plants. *The evolution of the genome*. New York, NY, USA: Academic Press.

Wang J, Tian L, Lee HS, Wei NE, Jiang H, Watson B, Madlung A, Osborn TC, Doerge RW, Comai L, Chen ZJ. 2006. Genomewide nonadditive gene regulation in arabidopsis allotetraploids. *Genetics* **172**: 507–517.

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

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