Meiotic Gene Evolution: Can You Teach a New Dog New Tricks?

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Associate editor: Brandon Gaut

Abstract

Meiosis, the basis of sex, evolved through iterative gene duplications. To understand whether subsequent duplications have further enriched the core meiotic "tool-kit," we investigated the fate of meiotic gene duplicates following whole genome duplication (WGD), a common occurrence in eukaryotes. We show that meiotic genes return to a single copy more rapidly than genome-wide average in angiosperms, one of the lineages in which WGD is most vividly exemplified. The rate at which duplicates are lost decreases through time, a tendency that is also observed genome-wide and may thus prove to be a general trend post-WGD. The sharpest decline is observed for the subset of genes mediating meiotic recombination; however, we found no evidence that the presence of these duplicates is counterselected in two recent polyploid crops selected for fertility. We therefore propose that their loss is passive, highlighting how quickly WGDs are resolved in the absence of selective duplicate retention.

Key words: **meiosis, polyploidy, whole genome duplication, evolution, duplication, genome.**

Letter

Whole genome duplications (WGDs) represent an ideal system to study the evolution of meiotic genes; WGD is initially accompanied by irregular meiosis and thereby creates both the necessity to adapt meiotic behavior and the opportunity to do so through diversification of duplicated genes. In this study, we focused on angiosperms, one of the few, if not the only eukaryote lineage(s) that combines two essential attributes to examine the fate of meiotic genes following WGD; flowering plants have one of the highest levels of WGD among eukaryotes (Otto and Whitton 2000) and, at the same time, they are major contributors to meiotic gene discovery (Osman et al. 2011).

Genome-Wide Duplicate Loss Is a Rapid Response to WGD

We first investigated the dynamics of genome-wide duplicate loss through time, an acknowledged gap in our understanding of diploidization following WGD (McGrath and Lynch 2012). This initial analysis examined the pattern of duplicate gene retention/loss following 14 independent WGDs ranging in age from 5–9 to approximately 130 My (supplementary table S1, Supplementary Material online). These data were later used in comparisons with meiotic duplicate retention.

As shown in fig. 1*A*, genome-wide duplicate gene loss follows a remarkably predictable L-shaped pattern when plotted against the rate of synonymous substitutions per synonymous site (K_s). The maximum rate of loss is observed immediately following WGD; fewer than half of the genes are still present as duplicates after the most recent WGDs found in *Brassica rapa* ($K_s \cong 0.25$; 5–9 My) or *Glycine max* ($K_s \cong 0.13$; $<$13 My). The most rapid decay is observed in *Zea mays* (fig. 1*A*), in which only 14% of duplicates were retained after its most recent WGD ($K_s \cong 0.18$; 5–12 My).

Malus domestica ($K_s \cong 0.20$; 30–65 My) and *Populus trichocarpa* ($K_s \cong 0.25$; 60–65 My) display almost the same rate of duplicate loss as that seen from younger WGDs (e.g., *B. rapa*); this slower rate of duplicate gene loss parallels the rate of nucleotide substitution observed in these long-lived perennial tree species (Smith and Donoghue 2008).

These convergent examples of precipitous genome-wide gene loss indicate that fractionation, the process by which duplication is resolved by deleting one gene copy (Freeling 2009; Woodhouse et al. 2010), is probably a rapid response to polyploidy (Sankoff et al. 2010). Although the observed pattern of gene loss was consistent across most species, the

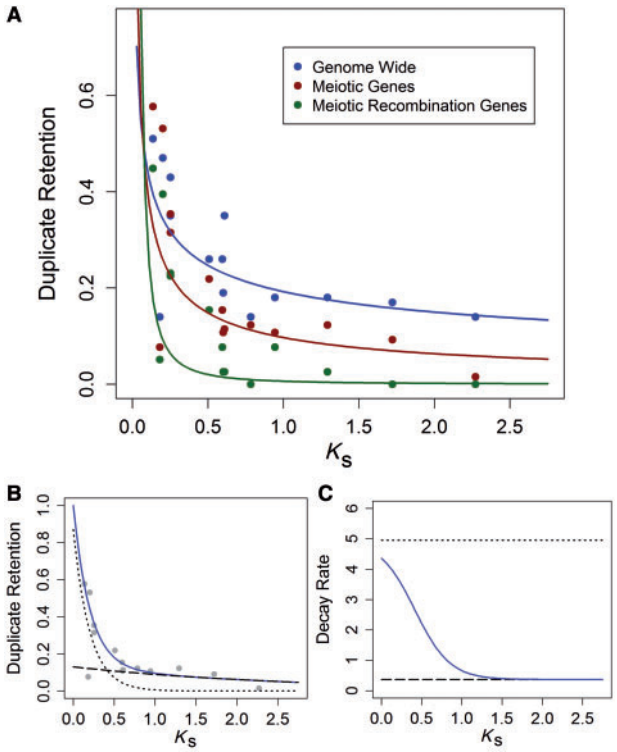

Fig. 1. Duplicate gene loss following angiosperm WGDs. (*A*) Duplicate retention decreases as a WGD's K_s increases; K_s = average synonymous substitutions per synonymous site for all gene pairs arising from a given WGD. Duplicate retention for meiotic genes (red) is lower than observed genome-wide (blue). Meiotic recombination genes (green) are even less retained. Maize (*) is an outlier to the general pattern. Power-law curves were fitted to the data (Maere et al. 2005). (*B*) Maximum likelihood estimates support a two-population model of gene loss (blue line). The best fit to the observed meiotic gene loss (gray circles) was obtained when 87% of duplicates are rapidly lost following WGD (half-life: $K_{s1/2:S} = 0.14$; dotted line) and 13% are retained for longer ($K_{s1/2:L} = 1.87$; dashed line). (*C*) The overall rate of gene loss decreases through time for the two-population model (blue line) line, but is constant within each subpopulation (rapidly lost, dotted line; slowly lost, dashed line).

unexpectedly high rate of fractionation observed in maize serves as a reminder that retention of duplicates is context dependent and will vary with the evolutionary forces acting at the time of the WGD, or after (e.g., mutational and selective landscape, effective population size). Bearing in mind the small sample size, there were no obvious differences in gene loss between species that display genome dominance and those that do not.

Following the initial rapid return of genes to a single copy, duplicate loss progressively slowed through time until eventually reaching a plateau for very old WGDs (fig. 1*A*; supplementary table S1, Supplementary Material online). This indicates that the initial state of rapid gene loss moves toward a state of preferential long-term retention of the remaining duplicates. As discussed in Maere et al. (2005), this is expected if preferentially retained duplicates eventually dominate the remaining population of duplicated genes.

Meiotic Gene-Duplicate Loss Mirrors the Pattern Seen Genome-Wide but Is More Pronounced

We then turned to examine the fate of duplicated meiotic genes. As gene ontologies (GOs) are too equivocal to accurately deal with meiosis or meiotic recombination, we first reviewed and established a list of 65 genes that have been experimentally shown to be involved in plant meiosis (supplementary table S2, Supplementary Material online). This detailed curation was based on the phenotype of mutants and showed genes to encompass a wide range of processes, including meiotic recombination and the control of cell cycle (supplementary table S2, Supplementary Material online). The 65 genes were used as seeds to identify and, when necessary, curate manually homologous sequences in the 18 angiosperm genomes of our survey (supplementary tables S3–S15, Supplementary Material online).

Meiotic gene-duplicate loss reflected the genome-wide pattern, with rapid initial duplicate loss followed by preferential gene retention (fig. 1*A*). The loss, however, was more pronounced, with the 14 WGDs showing on average approximately 30% fewer meiotic gene duplicates than observed genome-wide (supplementary table S1, Supplementary Material online). This trend is already apparent after some of the most recent WGDs of our survey (supplementary table S1, Supplementary Material online).

Meiotic Recombination Genes Show the Fastest Return to a Single Copy

The overall trend of preferential meiotic duplicate loss is opposite to that reported for photosynthetic (Coate et al. 2011) or circadian clock gene families (Takata et al. 2010; Lou et al. 2012), which have both expanded following the WGD events studied. These opposing trajectories are evident when considering meiotic genes versus photosynthetic or clock genes as a whole, but they are not necessarily true when considering specific gene families.

Genes involved in meiotic cell-cycle progression or coordinating entry into meiosis were overrepresented among the most commonly retained genes (supplementary tables S16 and S17, Supplementary Material online) echoing results in *Drosophila* (Reis et al. 2011). As in *Drosophila*, in which preservation of single-gene duplicates is not attributable to dosage sensitivity (i.e., selection to maintain members of a genetic network in the same ratio: See Freeling 2009), there are indications that some of these WGD duplicates have acquired "something new and useful to do." For example, omission of second division (OSD1) and tardy asynchronous meiosis (TDM), which are part of the same regulatory network (Cromer et al. 2012), have *Arabidopsis* α duplicates with nonredundant function (Glover et al. 1998; Hase et al. 2006); this suggests that the α WGD may have created a new network of subfunctionalized genes that more specifically regulate cell-cycle progression during meiosis. Likewise, genes related to *CDKA;1* (among the most retained genes in our survey) that is a regulator of the meiotic cell cycle have been

implicated in the cytological diploidization of allopolyploid wheats (Griffiths et al. 2006; Greer et al. 2012), drawing a tempting link between retention of such regulatory genes and polyploid meiotic adaptation.

In contrast, gene loss observed in the subset of meiotic genes involved in recombination was even more striking than for meiotic genes as a whole, with no "plateau" and essentially all genes returning to a single copy by K_s 0.75 (fig. 1*A*). Accordingly, the meiotic recombination genes were among the least retained gene duplicates (supplementary tables S16 and S18, Supplementary Material online). Although very strong, this trend for meiotic recombination genes to rapidly return to a single copy is not absolute. A counterexample is the meiotic DNA repair gene *XRI1* that is the most retained gene following recent WGDs ($K_s < 0.6$) (supplementary table S19, Supplementary Material online), demonstrating that the fates of individual gene families are unique and may run counter to those of the wider functional classes to which they belong.

Together, these results confirm and extend previous observations based on protein domains (Paterson et al. 2006) or GO categories (Maere et al. 2005; Wang et al. 2011). However, given the breadth of many GO terms and inaccuracies in their assignment (especially regarding meiosis), our use of evidence-based biological definitions enabled a more detailed understanding of gene retention/loss in their specific biological context: that is, within well-defined biochemical pathways (see above) and well-established protein complexes (supplementary table S20, Supplementary Material online).

The Rate of Gene-Duplicate Loss Decreases through Time

Given the apparent disparity in the rate of loss of meiotic recombination genes compared with other meiotic genes, we questioned whether meiotic duplicate loss could be modeled by considering two populations of duplicates, one that rapidly returns to a single copy and a second that is retained for longer. Maximum likelihood estimates show that the observed data better fit the two-population model than a single population (uniform decay) model ($P = 0.0018$, fig. 1*B*). A consequence of the two-population model is that the total rate of duplicate loss decreases over time until it approximates that of the more retained duplicates (fig. 1*C*).

This model also predicts that duplicates remaining from older WGDs would primarily belong to the limited number of gene families comprising the more-retained population. In line with these predictions, we observed that duplicates from the *Mei2-like*, *AtK1*, and *ASK1* gene families were frequently retained following old WGDs ($K_s > 0.6$), whereas even older duplicates, predating the monocot–dicot divergence more than approximately 165 Ma, were found in the *Mei2-like* and RPA gene families (supplementary figs. S1 and S2 and table S4, Supplementary Material online). These gene families show the highest levels of expansion through WGD.

Despite Their Rapid Rate of Loss, Meiotic Gene Duplicates Are Probably not Counterselected

We next extended our analysis to *Triticum aestivum* (bread wheat) and *B. napus* (oilseed rape), two species that have undergone very recent WGDs (<10,000 years ago), to determine whether meiotic recombination duplicates return to a single copy after only a few thousand generations. An important component of this extended analysis was to question whether meiotic recombination duplicates might be detrimental, in which case iterative restoration to a single copy could result from selective pressure to eliminate "deleterious" duplicates (De Smet et al. 2013). Given that intertwined changes in (epi)genome and transcriptome in newly formed polyploids can generate sufficient phenotypic variation for selection to act within a few generations (Pires et al. 2004), we reasoned that a few thousand generations would be amply sufficient to allow selective elimination of detrimental duplicates. This is particularly true given that these genes are essential for fertility, a phenotype that has been under intense selection in these crops bred for high yield.

Counter to the above prediction, we obtained no evidence of physical gene loss in either wheat or oilseed rape (supplementary table S21, Supplementary Material online), despite analyzing a subset of 19 meiotic recombination genes that were found to have almost always returned to single copy following older WGDs in other species. Even copies that are partially lost in *B. rapa* (one of the parents of *B. napus*; supplementary fig. S3, Supplementary Material online) remain unchanged in oilseed rape. In addition, we observed no mutations in these genes that would suggest a loss of function. In wheat, some additional copies were found that presumably result from tandem or segmental duplication following the divergence of diploid wheats.

We then investigated whether the homologous copies were still expressed in wheat and oilseed rape. All observed genes were expressed from multiple copies (supplementary figs. S4 and S5, Supplementary Material online). It is therefore unlikely that meiotic recombination gene duplicates are detrimental and, thus, counterselected. In line with this hypothesis, all retained meiotic recombination duplicates in all species show evidence for purifying selection and no evidence for divergent rates of evolution, irrespective of the age of the WGD (supplementary fig. S6 and table S22, Supplementary Material online).

Conclusions

Although early gene duplications were instrumental in establishing the eukaryotic core meiotic toolkit (Malik et al. 2008), we show that iterative WGDs in angiosperms have only occasionally been conducive to further diversification. This is particularly true for genes involved in meiotic recombination, which passively return to a single copy within a few million years. If "you can't teach an old dog new tricks," it may be because most diploid species already have the tools required to correctly segregate chromosomes in a polyploid state.

Meiotic adaptation observed in established polyploids may therefore require "fine-tuning" the progression or the effectiveness of meiosis/meiotic recombination. This assertion is consistent with recent findings from autotetraploid *A. arenosa*, in which improved chromosome segregation seems to be achieved through the selection of specific alleles at known meiotic recombination genes, which may ultimately result in decreased crossover frequencies (Yant et al. 2013). As some of the WGDs of our survey could be ancient autotetraploidies (Garsmeur et al. 2013), selection of genetic variants at pre-existing loci, rather than diversification of new duplicates, may have contributed to ensure regular meiosis in ancient polyploids.

The foregoing hypothesis would explain why meiotic recombination genes are not maintained in duplicate but not why they are lost more rapidly than genome average. As genome-wide data also best fit a two-population model of duplicate loss (supplementary fig. S6, Supplementary Material online; $P = 1.3 \times 10^{-6}$), we propose that genome-wide retention is elevated due to the inclusion of genes selectively maintained in duplicate. The precipitous decline of meiotic recombination genes therefore highlights how WGDs are resolved when there is no (or little) selective force opposing duplicate loss. Our results, encompassing 18 species with differing rates of evolution, confirm and extend gene-loss data in yeast (Scannell et al. 2006), suggesting that this is a general pattern among all eukaryotes.

Supplementary Material

Supplementary tables S1–S22 and figures S1–S6 are available at *Molecular Biology and Evolution* online (http://www.mbe.oxfordjournals.org/).

Acknowledgments

The authors thank Olivier Garsmeur for his contribution to the analysis of the banana genome data. They also thank Mathilde Grelon, Raphael Mercier, Fabien Nogue, Catherine Darmerval, Jeremy Timmis, Marta Cifuentes, and Wayne Crismani for critical reading and discussion of the manuscript. This work has been funded through the ANR project ANR-2010-BLANC-628 SVSE7-DUPLIC. The authors also acknowledge Céréales Vallée competitiveness cluster for financial support of the project.

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