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

Andrew Lloyd, Marion Ranoux, Sonia S. Vautrin, Natasha Marie Glover, Joëlle Fourment, Delphine Charif, Frédéric Choulet, Gilles Lassalle, William Marande, Joseph Tran Ngoc Danh, et al.

MBE Advance Access published April 1, 2014

Type:
Letter - Discoveries

Title:
Meiotic gene evolution: can you teach a new dog new tricks?

Authors:

Lloyd AH[1,2], Ranoux M[3], Vautrin S[4], Glover N[3,4], Fourment J[5], Charif D[1,2], Choulet F[3,4], Lassalle G[6], Marande W[5], Tran J[1,2], Granier F[1,2], Pingault L[3,4], Remay A[3,4], Marquis C[1,2], Belcram H[7], Chalhoub B[7], Feuillet C[3,4], Bergès H[5], Sourdille P[3,4], Jenczewski E[1,2].

Author affiliations:

[1] INRA, UMR1318, Institut Jean-Pierre Bourgin, RD10, F-78000 Versailles, France
[2] AgroParisTech, Institut Jean-Pierre Bourgin, RD10, F-78000 Versailles, France
[3]INRA UMR1095 Genetics Diversity and Ecophysiology of Cereals, F-63039 Clermont-Ferrand, France.
[4]University Blaise Pascal, UMR1095 Genetics Diversity and Ecophysiology of Cereals, F-63039 Clermont-Ferrand, France.
[5] INRA UPR 1258, Centre National des Ressources Génomiques Végétales, Castanet-Tolosan, France
[6] INRA, UMR1349-IGEPP, Le Rheu Cedex, France.
[7] INRA-CNRS-UEVE, Unité de Recherche en Génomique Végétale, Evry, France.

Corresponding author:

Dr Eric Jenczewski.
e: eric.jenczewski@versailles.inra.fr

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 counter-selected 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.

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 behaviour 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 combine 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 diploidisation 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 ~130 MYA (Table S1). These data were later used in comparisons with meiotic duplicate retention.

As shown in Fig. 1A, genome-wide duplicate-gene loss follows a remarkably predictable L-shaped pattern when plotted against the rate of synonymous substitutions per synonymous site (Ks). 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* (Ks≅0.25; 5-9 million years, MY) or *Glycine max* (Ks≅0.13; <13 MY). The most rapid decay is observed in Maize (Fig. 1A), in which only 14% of duplicates were retained after its most recent WGD (Ks≅0.18; 5-12 MY).

Malus domestica (Ks≅0.20; 30-65 MY) and Populus trichocarpa (Ks≅0.25; 60-65 MY) display almost the same rate of duplicate loss as that seen from younger WGDs (e.g. Brassica rapa); this slower rate of duplicate gene loss parallels the slower 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). While the observed pattern of gene loss was consistent across most species, the 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. 1A; Table S1). 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 (Table S2). 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 (Table S2). The 65 genes were used as seeds to identify and, when necessary, curate manually homologous sequences in the 18 angiosperm genomes of our survey (Tables S3-S15).

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

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 vs 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 co-ordinating entry into meiosis were overrepresented among the most commonly retained genes (Tables S16-S17) 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, OSD1 and TDM, which are part of the same regulatory network (Cromer et al. 2012), have Arabidopsis α duplicates with non-redundant 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) which is a regulator of the meiotic

cell-cycle, have been implicated in the cytological diploidisation 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 Ks 0.75 (Fig. 1A). Accordingly, the meiotic recombination genes were among the least retained gene duplicates (Tables S16 & S18). Although very strong, this trend for meiotic recombination genes to rapidly return to a single copy is not absolute. A counter example is the meiotic DNA repair gene *XRI1* which is the most retained gene following recent WGDs (Ks < 0.6) (Table S19), 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: i.e. within well-defined biochemical pathways (see above) and well-established protein complexes (Table S20).

The *rate* of gene-duplicate loss decreases through time

Given the apparent disparity in the rate of loss of meiotic recombination genes compared to other meiotic genes, we questioned whether meiotic duplicate loss could be modelled 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 ($p = 0.0018$, Figure 1B). 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 (Figure 1C).

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 (Ks > 0.6), while even older duplicates, pre-dating the monocot-dicot divergence more than ~165 MYA, were found in the *Mei2-like* and RPA gene families (Figures S1-S2; Table S4). These gene families show the highest levels of expansion through WGD.

Despite their rapid rate of loss, meiotic gene-duplicates are probably not counter-selected

We next extended our analysis to *Triticum aestivum* (bread wheat) and *Brassica napus* (oilseed rape), two species that have undergone very recent WGDs (< 10,000 YA), 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 (Table S21), despite analysing 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 *Brassica rapa* (one of the parents of *B napus;* Fig. S3) 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 homeologous copies were still expressed in wheat and oilseed rape. All observed genes were expressed from multiple copies (Figures S4-S5). It is therefore unlikely that meiotic recombination gene duplicates are detrimental and, thus, counter selected. 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 (Figure S6 and Table S22).

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 (Figure S6, $p = 1.3 \times 10^{-6}$), 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, confirms and extends gene-loss data in yeast (Scannell et al. 2006), suggesting that this is a general pattern among all eukaryotes.

Acknowledgements : We would like to thank Olivier Garsmeur for his contribution to the analysis of the banana genome data. We would also like to 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. We also acknowledge Céréales Vallée competitiveness cluster for financial support of the project.

References

Coate JE, Schlueter J a, Whaley AM, Doyle JJ. 2011. Comparative evolution of photosynthetic genes in response to polyploid and nonpolyploid duplication. Plant Physiol. 155:2081–2095.

Cromer L, Heyman J, Touati S, et al. 2012. OSD1 promotes meiotic progression via APC/C inhibition and forms a regulatory network with TDM and CYCA1;2/TAM. PLoS Genet. 8:e1002865.

Freeling M. 2009. Bias in plant gene content following different sorts of duplication: tandem, whole-genome, segmental, or by transposition. Annu. Rev. Plant Biol. 60:433–453.

Glover J, Grelon M, Craig S, Chaudhury A, Dennis E. 1998. Cloning and characterization of MS5 from Arabidopsis: a gene critical in male meiosis. Plant J. 15:345–356.

Greer E, Martín AC, Pendle A, Colas I, Jones AME, Moore G, Shaw P. 2012. The Ph1 locus suppresses Cdk2-type activity during premeiosis and meiosis in wheat. Plant Cell 24:152–162.

Griffiths S, Sharp R, Foote TN, Bertin I, Wanous M, Reader S, Colas I, Moore G. 2006. Molecular characterization of Ph1 as a major chromosome pairing locus in polyploid wheat. Nature 439:749–752.

Hase Y, Trung KH, Matsunaga T, Tanaka A. 2006. A mutation in the uvi4 gene promotes progression of endo-reduplication and confers increased tolerance towards ultraviolet B light. Plant J. 46:317–326.

Lou P, Wu J, Cheng F, Cressman LG, Wang X, McClung CR. 2012. Preferential retention of circadian clock genes during diploidization following whole genome triplication in Brassica rapa. Plant Cell 24:2415–2426.

Maere S, De Bodt S, Raes J, Casneuf T, Van Montagu M, Kuiper M, Van de Peer Y. 2005. Modeling gene and genome duplications in eukaryotes. Proc. Natl. Acad. Sci. U. S. A. 102:5454–5459.

Malik S-B, Pightling AW, Stefaniak LM, Schurko AM, Logsdon JM. 2008. An expanded inventory of conserved meiotic genes provides evidence for sex in Trichomonas vaginalis. PLoS One 3:e2879.

McGrath CL, Lynch M. 2012. Evolutionary Significance of Whole-Genome Duplication. In: Soltis PS, Soltis DE, editors. Polyploidy and Genome Evolution. Berlin: Springer-Verlag. p. 1–20.

Osman K, Higgins JD, Sanchez-Moran E, Armstrong SJ, Franklin FCH. 2011. Pathways to meiotic recombination in Arabidopsis thaliana. New Phytol. 190:523–544.

Otto SP, Whitton J. 2000. Polyploid Incidence and Evolution. Annu. Rev. Genet. 34:401–437.

Pires JC, Zhao J, Schranz ME, Leon EJ, Quijada A, Lukens LN, Osborn TC. 2004. Biological relevance of polyploidy : ecology to genomics Flowering time divergence and genomic rearrangements in resynthesized Brassica polyploids (Brassicaceae). :675–688.

Reis M, Sousa-Guimarães S, Vieira CP, Sunkel CE, Vieira J. 2011. Drosophila genes that affect meiosis duration are among the meiosis related genes that are more often found duplicated. PLoS One 6:e17512.

Sankoff D, Zheng C, Zhu Q. 2010. The collapse of gene complement following whole genome duplication. BMC Genomics 11:313.

Scannell DR, Byrne KP, Gordon JL, Wong S, Wolfe KH. 2006. Multiple rounds of speciation associated with reciprocal gene loss in polyploid yeasts. Nature 440:341–345.

De Smet R, Adams KL, Vandepoele K, Montagu MCE Van, Maere S. 2013. Convergent gene loss following gene and genome duplications creates single-copy families in flowering plants. Proc. Natl. Acad. Sci. U. S. A. 110:2898–2903.

Smith S a, Donoghue MJ. 2008. Rates of molecular evolution are linked to life history in flowering plants. Science 322:86–89.

Takata N, Saito S, Saito CT, Uemura M. 2010. Phylogenetic footprint of the plant clock system in angiosperms : evolutionary processes of Pseudo-Response Regulators. BMC Evol. Biol. 10.

Wang XX, Wang HHH, Wang JJJ, et al. 2011. The genome of the mesopolyploid crop species Brassica rapa. Nat. Genet. 43:1035–1039.

Woodhouse MR, Schnable JC, Pedersen BS, Lyons E, Lisch D, Subramaniam S, Freeling M. 2010. Following tetraploidy in maize, a short deletion mechanism removed genes preferentially from one of the two homologs. PLoS Biol. 8:e1000409.

Yant L, Hollister JD, Wright KM, Arnold BJ, Higgins JD, Franklin FCH, Bomblies K. 2013. Meiotic Adaptation to Genome Duplication in Arabidopsis arenosa. Curr. Biol. 23:2151–2156.

Figure 1 Duplicate gene loss following angiosperm WGDs. **A)** Duplicate retention decreases as a WGD's Ks increases; Ks = 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 (grey circles) was obtained when 87% of duplicates are rapidly lost following WGD (half-life: $Ks_{1/2:S}$ = 0.14; dotted line) and 13% are retained for longer ($Ks_{1/2:L}$ = 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 sub-population (rapidly lost, dotted line; slowly lost, dashed line).