B. Naganowska, P. Kachlicki, P. Krajewski (red.), Genetyka i genomika w doskonaleniu roślin uprawnych, Instytut Genetyki Roślin PAN w Poznaniu, 2009: 11-28

Genome restructuring in hybrids: marriages of inconvenience

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Abstract. New molecular tools have opened up a new dimension in our understanding of how nascent alloploids adjust their genomes to accommodate their 'forced marriages'. There may be differences in genome size, cytogenetic, genetic and epigenetic conflicts, as well as nucleo-cytoplasmic interactions. These conflicts trigger instantaneous and genome-wide adjustments which impact upon phenotypes and also cause new cultivars to vary in ways that breeders neither intended nor are even aware of. In this sense breeding based on morphological, disease or stress resistance traits, for instance, can be seriously flawed. We are working *partially sighted* in many of our crop plants in terms of the dynamic flux of molecular events taking place below the levels of resolution by phenotype or marker. To what extent these issues may apply to chromosome substitutions or introgressed segments is an unknown. These issues are discussed in the context of crop improvement.

Key words: allopolyploids, chromosome changes, sequence changes, epigenetic changes, chromosome elimination, genome balance

Introduction

It is well known that polyploidy has played a major role in plant evolution, and in the domestication and improvement of crop plants. Certain model diploids, maize and Arabidopsis, are also known from RFLP analysis and sequencing to be of ancient polyploid origin (Helentjaris et al. 19998; Arabidopsis Genome Initiative). Nobody knows the full extent of polyploidy, but informed sources suggest between 50-70% of angiosperms have polyploid origins (Stebbins 1971; Wendel 2000), and it generally considered that polyploidy has endowed plants with potential to adapt to a wide range of habitats and diverse ecological conditions (Grime and Mowforth 1982; Levin 1983). These attributes have been long known, but what

is new is the range of molecular tools now available to begin to determine the basis, and the processes of change, that contribute to the benefits of polyploidy, especially where allopolyploids can be newly synthesised and directly compared with their progenitors. Hybrids, and allopolyploids in particular, are the main topic of this contribution.

Allopolyploidisation produces a new species in a single step. In so doing it generates hybridity and duplicate genomes, by placing diverse genomes together in a single nucleus in a common cytoplasm originating from one of the parents (Fig. 1). This allopolyploid *marriage* is not harmonious: it results in an irreversible burst of reorganisation and modification of the genomes involved. There are instant and genome-wide changes at the visible level of chromosomes and phenotypic organisation, as well as cryptic modifications involving gene expression mediated by genetic and epigenetic mechanisms caused by alterations in gene regulatory networks (Matzke et al. 1999; Riddle and Birchler 2003).

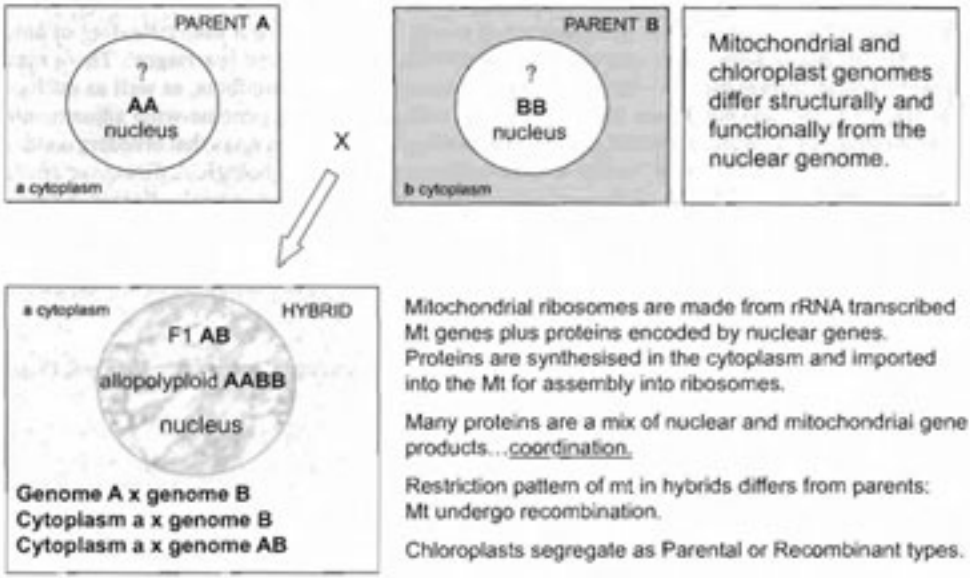

Fig 1. Scheme illustrating nucleo-cytoplasmic interactions in newly formed allopolyploids

Changes at the chromosome level

Studies on established polyploids show that the process of adjustment can involve random translocations as well as species-specific rearrangements in particular chromosomes in all polyploid populations of a species (Leitch and Bennet 1997). The nature of the reorganisation that goes on in newly formed allopolyploids gives

us some insight into the scope and the timing of these events, be they stochastic or otherwise. Chromosome pairing at meiosis can be compromised, more so in F1 hybrids than in allopolyploids; and the way in which DNA is embedded in chromatin, and the activity of histone codes is suddenly altered, and these conflicts must impact on the way in which genomes interact and accommodate to sharing a nucleus (Jones and Pašakinskienė 2003; 2005). In addition, we can anticipate problems due to the spatial separation of genomes and arrangements of nuclear territories. Centromeres may be under separate genetic control, and those from one genome may suffer suppression and silencing by the other, and this could lead to chromosome instabilities, even at a tissue-specific level, with whole genomes, or part of the genome being eliminated as the response. In the resolution of genome conflict the balance of their chromatin may change. Intragenomic recombination may remodel a hybrid, as explained below.

Nucleolar dominance

Navashin (1934) provided an early study of genome conflict with his account of nucleolar dominance (amphiplasty) in *Crepis* hybrids. He cross the F_1 of the species involved back to the parent species and demonstrated that suppression of nucleolus formation was reversible. The information needed to form the nucleolus was suppressed rather than eliminated – what we now call *epigenetic*. Nucleolar dominance is now know in many F_1 hybrids and allopolyploids (Pikard 2000; Viegas et al. 2002). The nucleolus is a highly visible marker for gene expression, but many other non-visible effects probably occur at the same time. In the seventy years since Navashin's discovery we have still not arrived at a definitive explanation for the phenomenon of nucleolar dominance, other than to say that it is an epigenetic process and involves the silencing of genes coding for rRNA in one of the parent species, and that it is normally the species with the largest genome which suffers suppression of its nucleolus.

Chromosome elimination in hybrids

Chromosome elimination of one of the genomes in F_1 hybrids from crosses between different species, or difference genera, is a well known means of producing doubled haploids for plant breeding. The phenomenon was first correctly interpreted in *Hordeum vulgare* × *H. bulbosum* by Kasha and Kao (1970), and since that time the phenomenon has been recorded in many other interspecies and intergeneric crosses.

The key events involved in genome elimination are as follows: (i) species vary in their capacity for genome elimination; and in some crosses stable F_1 plants can be established, while in other genotypes elimination of one of the parental genomes can occur, and when it does the extent to which it happens also varies, so that some plants can remain as mosaics for long periods of time, e.g. *Hordeum vulgare*

× *H. bulbosum* (e.g. Thomas and Pickering 1983; Rier-Lizarazu et al. 1996). (ii) Eliminated chromosomes often fail to congress on to metaphase plate or to reach the anaphase poles during the early divisions of the zygote when the elimination is taking place, as in *Hordeum vulgare* × *H. bulbosum* (Bennett et al. 1976). This failure to congress could be a matter of lack of efficiency in attachment to spindle microtubules; and as later studies in wheat × maize crosses demonstrated, all of the much smaller maize genome chromosomes were lost during the first three cell divisions in most of the embryos. The maize centromeres were either tiny or non-visible and without affinity for spindle attachment, and the maize NORs were also suppressed (Laurie and Bennett 1989). The same is true in wheat × sorghum crosses (Laurie and Bennett 1988). In barley × maize hybrids the maize chromosomes in the zygote had well defined centromeres – but even so they were still eliminated (Laurie and Bennett 1988).

(iii) In stable F_1 hybrids the genomes may show a degree of spatial separation, as in *H. vulgare* cv. Tuleen 346 × *H. bulbosum* (Anamthawat-Jónsson et al. 1983), and the distinctive behaviour of the two parents in the hybrid indicated that their centromere activity must be under separate genetic control. Later studies on the same hybrid (Schwarzacher et al. 1992) confirmed genome separation, as well as the near identical size of the two genomes; and centromere-associated structures of *H. vulgare* were larger than those of the more peripheral *H. bulbosum* chromosomes which has 'weaker', i.e. smaller centromeres. Genome separation has also been found in other for F_1 hybrids (Finch et al. 1981; Leitch et al. 1990; Leitch et al. 1991).

(iv) In the F_1 hybrid *Hordeum marinum* × *H. vulgare* cv. Tuleen 346 elimination of the Tuleen 346 genome occurs in the endosperm, while in the embryo that of *H. marinum* is lost; i.e. there is tissue specific 'alternative elimination' of parental genomes (Finch 1983; Finch and Bennett 1983). The eliminated chromosomes had smaller centromeres and tended to occupy more peripheral positions, and the authors suggest tissue-specific suppression of genes for centromere function. The eliminated chromosomes also showed suppression of their NORs.

(v) The most compelling evidence we have to date that the centromere underlies chromosome instabilities in hybrids comes from the application of DNA fibre-fluorescence *in situ* to addition lines of individual maize chromosomes added to oat(Jin et al. 2004). In this material the CEN-DNA is intermingled with centromere specific retrotransposons (CRM), and collectively these components make up a range of sizes varying between ~300 to 2,800 kb for individual maize centromeres. The point of most interest here is that in addition lines with two different genes coding for the Cen-H3 histone, one from maize and one from oat, the oat gene is dominant and the oat Cen-H3 becomes incorporated into the maize centromeres. The maize Cen-H3 gene is silenced in the oat background, and the oat Cen-H3 functions to organise the kinetochore on the maize chromosomes. The fibre-FISH

patterns therefore remain identical whether a maize centromere is within its natural maize environment or in the oat background. Furthermore in oat × maize hybrids it is the maize chromosomes which are eliminated, and the maize centromeres which are impaired in the hybrid condition, and we still have to make sense of these apparent contradictions. In the oat × maize crosses the elimination of the maize chromosomes is more gradual than it is in crosses such as wheat × maize and barley × maize (Riera-Lizarazu et al. 1996), but nonetheless it does occur, and least we can now say is that the centromeres find themselves compromised in hybrids, and are subject to suppression and silencing together with the NOR rRNA genes.

Synaptic adjustment

A large difference in genome size is not necessarily a factor in creating genome conflict in a hybrid. The diploid hybrid ryegrass, *Lolium temulentum* × *L. perenne* (2n=2x=14), for example, has two sets of chromosomes which are structurally and genetically dissimilar, differing in DNA amounts by about 50%, and yet they form a stable F_1 hybrid with regular bivalents at meiosis and have chiasma frequencies which are similar to those of the parents. The hybrid displays a remarkable capacity to resolve differences, in terms of its synaptonemal complex irregularities and genome size differential, and to produce homoeologous bivalents with functional and morphological integrity (Jenkins and White 1990).

Instabilities in allopolyploids

Changes at the whole genome/chromosome level can be visualised using the GISH (Pašakinskienė and Jones 2005). This differential 'painting' of species chromosomes provides us with a rich cytological resource for studying genome conflict in hybrids.

It is known that *F. arundinacea* is a natural allohexaploid which originated as a hybrid between *F. pratensis* and *F. glaucescens*, and that it has the genome composition *FpFpFgFgFgFg* (Humphreys et al. 1995). In the last few years two cases of 'dramatic' genome rearrangements taking place in somatic tissues were recorded in *Lolium multiflorum* × *Festuca arundinacea* hybrids. Firstly, in colchicine doubled F_1C_0 octoploids of *L. multiflorum* × *F. arundinacea* (2n=8x=56) some genotypes restructured themselves as 'novel diploids' by processes of diploidisation (2n=14) and somatic recombination (Pašakinskienė et al. 1997). GISH showed them to be new genomic variants derived from *F. pratensis*, *L. multiflorum* and *F. glaucescens*, with genomes of the three species being represented in different proportions, and as variable patterns, but in any case with *F. pratensis* chromatin making the genomic basis of the 'novel diploids'. Secondly, similar events were recently found in a selected F_2C_1 hybrid from the same population of plants. The plant concerned was a hexaploid genotype F_2 3-18 (2n=6x=42), and was characte-

rised as a 'super-recombinant'. GISH revealed its genome to have a high number of recombinant chromosomes, with some of them being constructs composed of chromatin from the three species, *L. multiflorum - F. pratensis - F. glaucescens.* Such a recovery of a functional tri-specific chromosome set is unique in the history of investigations of *L. multiflorum* × *F. arundinacea* hybrids. This genotype was observed for a number of years, and has high vigour complemented by a good level of fertility. Its instability showed in phenotypic segregation, as it was multiplied vegetatively. The initial hexaploid gave rise to a few somatic segregants which again preferred the diploid chromosome number of 2n=2x=14, for both *Festuca* and *Lolium* phenotypes (Pašakinskienė and Jones 2005).

The instabilities described above in the *L. multiflorum* × *F. arundinacea* hybrids are genotype-specific, and the segregated plants which become diploid (2n=2x=14) are constructed *de novo* as a resolution of the tri-specific genomic conflict involving *L. multiflorum – F. pratensis – F. glaucescens.* In most cases the chromosomes of the novel diploid are very similar to the chromosomes of the pure *F. pratensis* diploid, but in other cases the *F. pratensis* genome has gained various-sized blocks of *L. mulitflorum* chromatin, and the presence of *F. glaucescens* is not obvious in all of the segregants. In any case the reconstructed chromosomes are different from those existing in the *F. pratensis* as a constituent genome of *F. arundinacea*, according their GISH-banding pattern (Pašakinskienė et al. 1998). We assume that the 'novel diploids' could have resulted from concerted transposition, where at the some stage the entire newly made allopolyploid genome was a ferment of rearrangement of its constituent species-specific parts. It is clear the centromeres have most likely also played an important role in fixation of the 'novel diploids'. We speculate that the centromeres must be 'novel' as well, built on the basis of the centromeric components of the parental species involved in the chromosome set of the hybrid genome.

Genome balance in allopolyploids

GISH has enabled the discovery of changes to 'genome balance', in what are otherwise stable *Lolium-Festuca* allopolyploids. In the F_8 population of a tetraploid hybrid 'Prior' between *Lolium perenne* and *Festuca pratensis* meiosis in the early generations, as shown by conventional staining with acetocarmine, was characterized as stable with a high level of bivalent formation. A GISH study, however, revealed that extensive recombination had taken place between homoeologues of the two genomes, and that the balance of chromatin was not equal (Canter et al. 1999). The substitution of *Festuca*-origin chromosomes by those of *Lolium*-origin resulted in a mean of 17.9 *Lolium* and 9.7 *Festuca* chromosomes per genotype. In terms of chromatin amounts this equates to a mean length of 62.1% *Lolium* and 37.9 % *Festuca*. Clearly the genome conflict had been dealt with by a change in the genome balance, over the eight cycles of sexual repro-

duction, in favour of the dominant *Lolium* genome. In an earlier study with the hybrids *L. multiflorum* × *F. pratensis* (Zwierzykowski et al. 1998) it was shown that the proportion of the genomes occupied by the *L. multiflorum* chromatin ranged from 49.2-66.7%, and this likewise confirms the balance in favour of the *Lolium* over *Festuca*. A new example of *Lolium*-dominant behaviour, has recently been found in the Lithuanian variety 'Punia', made from a cross at the tetraploid level of *F. pratensis* × *L. multiflorum* (Pašakinskienė and Jones 2003). The reasons for the dominance of *Lolium* over *Festuca* in this way are not understood. Various theories have been proposed, such gametic competition, pollination effects or selection for vigour in the early stages of seedling growth, but no definitive answers have yet emerged. In the light of recent knowledge of the centromere organization and function we could conjecture that the *Lolium* centromeres are more competitive than those of *Festuca*, and this may account for the predominance of *Lolium* chromatin in these hybrids. Does centromere drive operate here, at female meiosis, in a way we have hitherto not even suspected, and could this account for the dominance of the *Lolium* chromatin over that of *Festuca*? In this particular genomic conflict where *L. perenne* or *L. multiflorum* competes against *F. pratensis* the *Lolium* chromosomes are the ones that behave as selfish and tend to colonize the genomic space in these *Lolium-Festuca* allotetraploids. The phenotypic outcome of this dominant *Lolium* chromatin is also evident in the hybrid variety 'Punia', which clearly expresses characteristics of root cell growth and response to low temperatures closer to that of *L. multiflorum* than to that of *F. pratensis* (Šimkūnas and Pašakinskienė 2003). The interesting question remains as to which of the *Lolium* chromosomes out of the whole set have dominant centromeres and become retained as multiples, and which ones of *Festuca* loose-out in the competition. A similar story to that of *Festulolium* was earlier described by Anamthawat-Jónsson (1999). Using GISH she recovered a unique set of chromosomes in *Triticum* (2n = 4x = 28, AABB) × *Leymus* (2n = 4x = 28, NNXX) hybrids. The allopolyploids stabilised over a number of years as a set of 6 pairs of *L. mollis* and 15 pairs of the wheat parent (Anamthawat-Jónsson 1999). The unique composition probably resulted from the stable replacement of one pair of *Leymus* chromosomes by the addition of one pair from wheat, together with the selective elimination of 8 pairs from *Leymus*. It is clear the conflicts and chromosome instabilities in the allopolyploids have a higher degree of complexity and a larger variety of outcomes. There are more conflicts to resolve and more ways in which resolutions can occur.

Recent studies involving allotetraploids of *F. pratensis* × *L. perenne* have tracked changes in the balance of chromatin in somatic cells over each of six successive generations of open pollination (Zwierzykowski et al. 2006). There is extensive recombination between chromosomes of the two parental genomes, as well as substitution of whole *Festuca* chromosomes by whole *Lolium* chromosomes. The total

number of *Lolium* chromosomes increased progressively from a mean of 14.36 in the F_2 to 16.26 in the F_6, and the total number of *Festuca* chromosomes decreased correspondingly from a mean of 13.57 down to 11.56. The proportion of total genome length contributed by *L. perenne* chromatin increased from about 50% in the F_2 to about 60% in the F_6, raising speculation about far this chromatin replacement could go over further cycles of open pollination. It is conceivable, and it remains to be demonstrated, that *Lolium* could take over the chromatin of the whole genome of the original allopolyploid, at least in terms of the GISH component; thus negating the original objective of the breeding programme. The mechanism therefore appears to be progressive by small incremental shifts at each generation. One possible explanation for this change could be the low level intergenomic recombination which compromises the stability of the *Festulolium* hybrid, together with preferences for homologous versus homoeologous centromeres in their spindle attachments and movements to the poles at anaphase I (Zwierzykowski et al. 2008).

Changes at the sequence level

In addition to the visible changes at the level of chromosomes and NORs, newly synthesised allopolyploids undergo rapid genetic modifications involving dosage-related and novel patterns of transcription (Pikaard 2001) and gene expression (Ramsey and Schemske 1998), interactions between regulatory network elements such as promoters and protein complexes (Riddle and Birchler 2003;) and epigenetic mechanisms involving DNA methylation and posttranslational histone modifications. New traits may also be apparent. The extent of these molecular adjustments and interactions will depend on the degree of divergence of the progenitors, and implicit in the study of these effects is the direct comparison between the new allopolyploids and their parental progenitors. The molecular tools used to study these rapid changes at sequence level involve analysis of DNA fragments, as RFLPs and/or modified AFLPs and AFLP-cDNA, in resynthesised allopolyploids of *Brassica* species and in wheat (Ozkan et al. 2001; Shaked et al. 2001; Kashkush et al. 2002; Han et al. 2003). DNA methylation changes have been observed in newly formed allopolyploids of Arabidopsis (Madlung et al. 2002) and wheat (Shaked et al. 2001), and are responsible for the maintenance of silenced RNA genes and protein-coding genes in Arabidopsis allopolyploids (Chen and Pikaard 1997; Lee and Chen 2001). Blocking of DNA methylation using either chemical inhibitors, or RNAi, depresses the silenced genes in allopolyploids (Lee and Chen 2001; Lawrence et al. 2004; Wang et al. 2004); and this effect indicates a causal relationship between DNA methylation and the gene silencing. Knowledge on changes at the DNA sequence level is especially informative from studies on wheat and Arabidopsis, as detailed below.

Wheat

An early study on short-term changes induced by allopolyploidisation in the wheat *Aegilops-Triticum* group by Feldman et al. (1997), using restriction analysis and Southern blotting, found that a number of genome-specific low copy sequences were rapidly eliminated in the F1, and that the loss of chromosome-specific sequences were initiated quickly in the allopolyploids and completed as soon as the second or third generations. Ozkan et al. (2001) later studied the rate and timing of allopolyploidy-induced genome changes in the *Aegilops-Triticum* wheat group, using DNA gel blots. They looked at eight DNA sequences over 35 interspecific and intergeneric F_1 hybrids, and 22 derived allopolyploids compared with the parent plants. Rapid sequence elimination occurred in both chromosome-specific (CSS) and genome-specific sequences (GSS). The loss of GSSs was initiated in F_1 plants and was completed by the second or third generation allopolyploid generation, whereas the CSS elimination began in the first allopolyploid generation and ended in the second or third generation. Sequence loss depended on the genomic combination of the hybrid or allopolyploid concerned, and was unaffected by the genotype of the parental plants, ploidy level or cytoplasm. Controlled experiments using naturally occurring allopolyploids (unreduced gametes) also established that polyploidisation itself, and not colchine treatment used to double F_1 hybrids was responsible for the changes. Shaked et al. (2001) extended the analysis further, with F_1 hybrids between diploid wheats from the same *Aegilops-Triticum* wheat group, and their derived allotetraploids by screening a large number of loci, using AFLPs and DNA gel blots, and confirmed extensive and rapid sequence elimination, possibly occurring as early as the zygote, and in a reproducible manner (same loci involved in the same patterns of elimination). They also found differences in cytosine methylation patterns in 13% of loci in both F_1s and allopolyploids. Sequences undergoing elimination corresponded to low-copy DNA, whereas changes in methylation affected repetitive sequences, including retrotransposons. The mechanism of elimination was unknown (gene conversion, excision?). Kashkush et al. (2002) investigated the transcriptome response by analysing 3,072 transcripts in a first generation synthetic bivalent-forming allopolyploid and its two diploid progenitors *Aegilops sharonensis* and *Triticum monococcum*. cDNA-AFLP band patterns revealed that 60 out of the 3072 transcripts were altered in a reproducible way. Forty-eight transcripts disappeared and 12 were activated. The disappearance of transcripts was due to gene silencing, or in some cases by gene loss, as confirmed by sequencing. Silencing included genes for RNA, metabolism, disease resistance and cell cycle regulation; and these changes occurred via genetic and epigenetic alterations immediately after the polyploids were formed. Gene loss is an irreversible process, whereas silencing by methylation is epigenetic. The authors conclude that wide hybridisation and / or chromosome doubling triggers a 'genome shock' as proposed by McClintock (1984).

Ozkan et al. (2003) addressed the question of what happens to the C value in newly made allopolyploids, relative to the midparent value of their progenitors, using allotetraploids and allohexaploids comprised of various wheat types. They found a significant loss of DNA in the first generation of the allopolyploids, and this loss became fixed in the second generation: it was independent of ploidy level. A critical element in these kinds of measurements, indeed of all of this genome reorganisation work, is to compare newly made allopolyploids directly with their progenitor plants from which they made, and the lack of such a control may account for some of the inconsistencies in C value data from earlier studies.

In Triticale there are similar results, but with some additional observations (Ma et al. 2008). There is extensive nonadditive sequence change, but the degree of modification seems to be higher than in other nascent allopolyploids. Some of the changes were non-random, and related to genome relationships, ploidy levels and sequence types. Additionally the frequency of changed parental bands, based on AFLP and RFLP analysis, was much higher for the rye than for the wheat parental genome, and the frequency of lost bands was much higher those gained. In terms of timing, 68% of changes occurred immediately following wide hybridisation, and the changes subsequent chromosome doubling happened much more slowly over five or six generations. The analysis also showed, contrary to some of the wheat hybrids (Ozkan et al. 2001), that cytoplasm also played a role in determining the direction, timing and rate of change.

Arabidopsis

Early studies by Song et al. (1995), working with various newly synthesised polyploids made from a number of diploid Brassica species (*B. rapa, B. nigra, B. oleracea*), using RFLPs and Southern hybridisation, showed that extensive genome changes involving loss and/or gain of parental restriction fragments, together with the appearance of some novel fragments, over several generations. Although the there was no sequence data one of their main conclusions was that the extent of the change was partly determined by the degree of divergence between genomes involved. Widely divergent species tended to show more extensive changes than closely related ones. They also made the interesting suggestion that the extent of change depended on parent of origin, and that the parent contributing both nucleus and cytoplasm suffered much less change than the one contributing only its nuclear genome.

Lee and Chen (2001) used AFLP-cDNA for genome-wide screen of gene silencing in the allopolyploid *A. suecica* (*A. thaliana* × *arenosa*). Ten silenced genes were identified from one parent or the other, in various chromosomes and affecting a number of RNA and protein genes, including four transcription factors. Silencing was due to hypermethylation and could be reactivated by blocking methylation. Wang et al. (2004) later analysed gene expression in both newly formed and na-

tural allotetraploid lines of Arabidopsis, in relation to epigenetic changes. They designed experiments to study *when* differential patterns are established in selfing lines following polyploidisation; whether or not the *same set of genes* is silenced within and between lineages and what is the mechanism for maintaining silenced genes. Synthetic *A. suecica*-like allotetraploid lines (2n=4x=26) were produced by cross pollinating the autotetraploid *A. thaliana* (2n=4x=20) with autotetraploid *A. arenosa* (2n=4x=32), and one of the four lines produced was then selfed for several generations, and across several independent lines derived from the same parents. Silencing of selected genes occurred independently in different lines, indicating that certain loci may be intrinsically be more susceptible to epigenetic change than others. The epigenetic nature of silencing was indicated by reactivation of these genes with RNAi inhibition of either *MET1* or *DDM1*. AFLP-cDNA display was performed with RNA from pooled plants in four selfing generations, using leaves and flower buds. Line 605A was crossed with *A. suecica* in the third generation. S1 to S3 showed stable chromosome inheritance with the expected set of 13 chromosomes. Phenotypic variation and changes in gene expression were observed. Some genes expressed in parents remained silenced in progeny for 3 or more selfing generations, showing rapid establishment of an altered regulatory state, while other genes were expressed in only a few generations – expression is stochastic. Novel patterns were also found, presumably due to reactivation of genes not expressed in the parents. In survey of ~2430 cDNA fragments ~11% displayed changes in S2-S4 generations, relative to parents: ~4% of changes were relative to *A. thaliana*, 5% to *A. arenosa*, 1% to both parents, 1% to neither parents, i.e. novel expression. 43 candidate genes were cloned and sequenced, encoding proteins for transposons, cell division, cell metabolism, protein transport, signal transduction pathways, and unknown function. No clustering of silenced genes. AFLP-cDNA was confirmed with RT-PCR. The pattern of silencing varied in different lines, although there were common elements.

To determine how silent genes are maintained RNAi was used to silence *AtDDM1* or *AtMET1*, methyl transferase genes, and this resulted in a significant reduction in the target genes and in the levels of DNA methylation. Estimated that ~3-11% of progenitor genes are susceptible to change in expression in new allopolyploids. The genome of an allopolyploid is faced with gene redundancy, enabling reprogramming of gene regulation, leading to silencing or the activation of genes which were silent in progenitor plants, in a largely stochastic way, although the precise mechanism of the rapid change is not known, which is why we talk about genome interaction and what McClintock (1984) called genome shock.

Wang et al. (2006) later carried out genomewide analysis of synthetic allopolyploids using microarrays, and detected >15% divergence between the progenitors, and 3923 (~ 15%) genes were highly expressed in *A. thaliana* (2105) and *A. arenosa* (1818) respectively, representing ~43% of the genome. >65% of nonadditively

expressed genes (different from the midparent value) are repressed, and >94% of these repressed genes are match the genes that are expressed *A. thaliana* higher levels in *A. thaliana* than in *A. arenosa*, consistent with the silencing of *A. thaliana* rRNA genes subjected to nucleolar dominance and with the overall suppression of the *A. thaliana* phenotype in synthetic allotetraploids and natural *A. suecica*. The majority of nonadditively expressed genes showed expression divergence between the two parents involving various biological pathways, thus providing a molecular basis for *de novo* variation for the selection and adaptation of new allopolyploid species. In terms of phenotype *A. arenosa* appears to be dominant over *A. thaliana* in the allotetraploids. *A. thaliana* and *A. arenosa* diverged ~5.8 MYA, i.e. ~ 20 million generations, (Koch et al. 2000).

RNAi of *met1* reduces DNA methylation and induces genome-specific changes in gene expression in Arabidopsis allopolyploids (Chen et al. 2008). RNAi of *met1* altered expression of ~ 200 genes, many of which code for transposons, predicted proteins, and centromeric and heterochromatic RNAs. Reduced DNA methylation occurred frequently in promoter regions of the upregulated genes. A high level of *A. thaliana* centromeric small RNA accumulation was correlated with hypermethylation of *A. thaliana* centromeres. The greater effects of reduced DNA methylation on transposons and centromeric repeats in *A. thaliana* than in *A. arenosa* are consistent with the repression of many genes that are expressed at higher levels in *A. thaliana* than in *A. arenosa* in the resynthesised allopolyploids. It seems that many *A. thaliana* genes are transcriptionally repressed in resynthesised allopolyploids, and a subset of *A. thaliana* loci, including transposons and centromeric repeats, are heavily methylated and subjected to homoeologous genome-specific RNA-mediated DNA methylation in natural allopolyploids.

Many expression changes in the *met1*-RNAi lines are derived from transposable elements and repetitive DNA, whereas the genes that are nonadditively expressed in the resynthesised allopolyploids encode proteins that are involved in various biological pathways important to plant growth and development (Wang 2006). The reactivated transposons and repetitive DNA in the *met1*-RNAi lines are preliminarily derived from the *A. thaliana* parent, suggesting repression of these elements in natural allopolyploids, which is reminiscent of genome wide repression of *A. thaliana* genes in resynthesised allotetraploids (Wang 2006). Therefore, in both interspecific hybridisation (or allopolyploidisation) and DNA methylation affect the *A. thaliana* genes that are generally repressed in the allopolyploids (Chen 2007), but the interspecific hybridisation affects primarily the protein-coding genes, whereas RNAi of *met1* affects transposons and centromeric repeats. Compared with *A. arenosa*, the *A. thaliana* homoeologous genome (as maternal parent) is hypersensitive to interspecific hybridisation and DNA methylation perturbation in allopolyploids.

Conclusions

Newly synthesised hybrids and allopolyploids are unstable through conflicts in their regulatory mechanisms. These conflicts trigger a plethora of genome changes at all levels of organisation from whole chromosomes down to DNA sequences; involving protein coding genes as well as other elements such as transposons. The overview of the genetic and epigenetic mechanisms underlying these changes summarised in Fig. 2. Thus far most of our knowledge of these marriages of

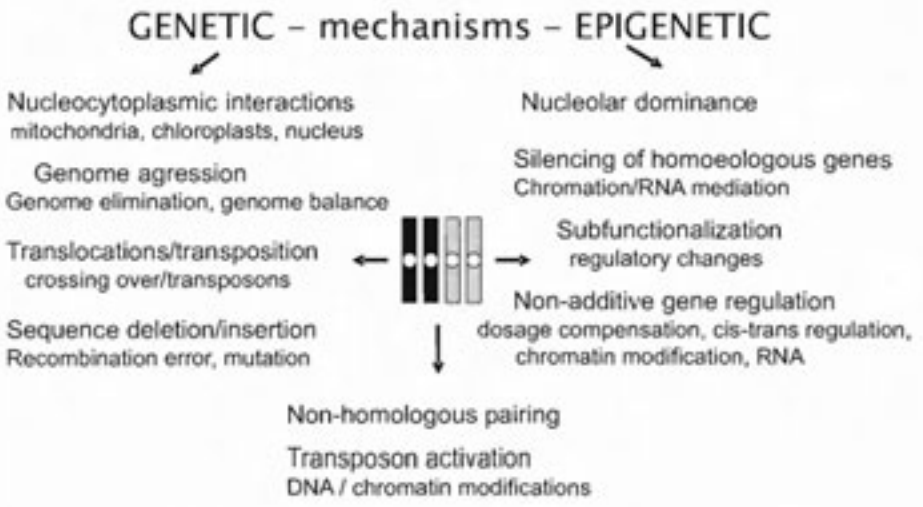

Fig 2. Mechanisms of genetic and epigenetic changes in new allopolyploids

inconvenience comes from relatively few species and hybrids, namely wheats and brassicas, with Arabidopsis providing the strongest knowledge source. At the same time we have few doubts that knowledge transfer can extend the story to other less well known plants; although cotton (*Gossypium*) appears to be one exception which differs from other species in its response to allopolyploidisation: there are changes in the copy number of repeated sequences and in the expression profile of transposons, but low copy sequences appear to escape both genetic and epigenetic changes (Liu et al. 2001; Riddle and Birchler 2003).

One of the key questions we can now ask about all these genome instabilities is how can we integrate then into a coherent scheme, if at all, dealing with F_1 hybrids and allopolyploids?

The first clue comes from the observation of the linkage between whole genome elimination in wide F_1 crosses with nucleolar dominance, where it is clear in some cases that the genome which is eliminated is also the one which suffers nucleolar suppression, and is invariably the larger of the two genomes involved: so genome

size is also connected. Centromeres also fit into the picture, since their function appears to be compromised by their reduced size and 'strength' in the eliminated genome, possibly due to epigenetic effects. We also know from addition lines of single maize chromosomes into an oat background that centromere dominance operates; and that it is the maize centromeres which incorporate the oat CenH3 histone, and not their own distinctive form.

Another significant question to be addressed is how far the knowledge gained from model species can be translated into crop plants which have yet to be studied in the same way? There is a hint that in *Festuloliums* the centromeres may play a role in genome balance/dominance, in respect of *Lolium* versus *Festuca*; and in the diploidisation associated with *L. multiflorum* × *F. arundinacea*. In terms of substitution and introgression lines, of *F. pratensis* into *L. perenne*, it is assumed that the substituted *F. pratensis* chromosome behaves in the same way in the *Lolium* background as it does in its natural background (King et al. 2002, Armstead et al. 2001; 2006). It can be argued that the closeness of the two species makes this assumption valid, but this still does not account for the difference in the GISH profiles or the known story about genome replacement of *Festuca* by *Lolium*, as already described. It is also of interest to note that when monosomic or disomic additions of one, or two, chromosomes of *F. pratensis* are introgressed in autotetraploids of *L. multiflorum*, that the introgressed *F. pratensis* chromosomes show very low preferences in their pairing at MI of meiosis between partners of *L. multiflorum* or *F. pratensis:* for the monosomics there is no discrimination (Kopecký et al. 2008). How then do the introgressed traits maintain their expression? Does genome drift in *Lolium* hybrids, and in introgression lines, also involve gene silencing, or gene reactivation, and if it does so how are the two processes related? Clearly there is need to extend the molecular analysis of genome conflict to the *Festuloliums*, and to other crop plants, and to make a stronger link between model species and crop plants. There is also a need to understand how the expression divergence between the two progenitor parents in nascent allopolyploids can provide a molecular basis for *de novo* variation for the selection and adaptation of new allopolyploid crop plants.

A marriage of inconvenience is one thing: divorce is another. We can only ask the question about what would happen in established allopolyploids were we to make a divorce and extract out the original diploid progenitors, and then compare them with their natural diploid forms. Would the changes associated with being 'married' result in new life, and possibly new forms of genetic variation, after the 'divorce'?

Acknowledgement

Howard Thomas is supported by a Leverhulme Emeritus Fellowship.

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