

EVOLUTION OF POLYPLOIDS

Reference: Harlan and deWet (1975)

Origins of Polyploids

Literatures relating to evolution of polyploids have been greatly equivocal.

Winge (1917): Polyploids developed from gametes that were so unlike that chromosomes could not pair and zygote would perish. He speculated that in such zygotes chromosomes might split longitudinally, each making a pair. This allowed the zygotes to develop the hybrid carrying 2x parental number of chromosomes.

Karpechenko (1927): From the cross between *Raphanus* and *Brassica* shown that meiotic failure resulted unreduced gametes.

Buxton and Darlington (1931): From the study on *Digitalis* concluded that “the doubling of the chromosome number is directly determined by the omission of reduction.”

- Most cases the chromosome increase is due to the formation of unreduced gametes. Some cases chromosome doubling in somatic tissues were reported. For example: *Primula kewensis* and *Nicotiana digluta*.

Classification of Polyploids:

Class I: Chromosome increase occurs in the first generation through the union of gametes one or both of which are unreduced. Can occur following self- or cross-pollination.

Class II: Chromosome doubling occurs in offspring of a hybrid with meiotic disturbances leading to unreduced gametes. It's a second generation phenomenon: F₂, BC₁ or F₁ crossed to a third parent.

Class III: Chromosomal doubling occurs in somatic tissues. Mitotic event!

Class I – *first generation event*: From three possibilities:

- 1) $2n + n$ Egg unreduced*
- 2) $n + 2n$ Pollen unreduced
- 3) $2n + 2n$ Both unreduced

* $2n + n$ is most common because $2n$ eggs allow a single plant to produce polyploids via selfing *or* cross-fertilization and usually a $2n$ egg is more viable than $2n$ pollen, which does not compete well with n pollen. Pollens are also more abundant.

Table 1 of Harlan and deWet (1975) shows equal proportion of plants formed between normal gametes and between unreduced female and normal male gametes (612 Vs. 595), whereas very few (only 7) from normal female and unreduced male gametes in *Saccharum* and its allies.

Likewise, very few polyploids were reported to develop from both unreduced male and female gametes (Table 2 of Harlan and deWet 1975)

- Stebbins (1970) believed that nonreduction played a minor role in polyploid formation. But data presented in Table 1 and 2 indicate a major role of unreduced gametes in the development of polyploids.
- In some cases crosses between *Saccharum officinarum* ($2n=80$) x *S. spontaneum* ($2n=64$) produced only $2n=112$ —i.e., $2n + n$ —maybe the only viable interspecific hybrid.
- Frequencies of unreduced gametes lowest among diploids and highest among tetraploid and hexaploid facultative apomictic plants.
- Unreduced gametes were also found among inbreeding population:
 - a. Among maize inbred lines: 0 - 3.5%.
 - b. Triploid rice, robust and vigorous, but less than 2% fertile were identified in farmers field.
 - c. Triploid and tetraploid orchid developed spontaneously.

Class II - *second generation event*

- i. Hybrid has meiotic disturbance and produces unreduced gametes.
- ii. $AA \times BB \rightarrow AB$ which produces an unreduced gamete for further hybridizations.
- iii. Wide crosses sufficiently disturb meiosis that unreduced gametes are the rule.
- iv. Few viable gametes produced; those that are produced are unreduced.
- v. Meiotic failure can occur at either Meiosis I or II—usually MI fails if pairing is very poor and division time is prolonged. In such case second division is equational and, therefore, identical spores developed and no segregation.
- vi. If the first division is completed, then there will be chromosome segregation

Class III - *somatic doubling*

- a. Mitotic rather than meiotic event.
- b. A rare event.
- c. *Primula kewensis*—derived from sterile hybrid of *P. floribunda* x *P. verticillata*—somatic tissue spontaneously doubled to $4x$, which produced a few flowers and set seed.

Success of Polyploids:

Reference: Soltis and Soltis 2000

Polyploidy is very common in angiosperms. Frequency of polyploids can be in the range of ~30- 80% in angiosperms and most estimates are in the neighborhood of ~50%. Most crop plants are polyploids. Not many polyploids are found other major groups of eukaryotes.

Why are the polyploids so common and successful?

- a. Stebbins (1950): vegetative propagation and an out-crossing mating system for the polyploidation to occur.
- b. Polyploids possess better adaptation broader ecological amplitude.
- c. Has better colonization ability.
- d. Higher selfing rate.
- e. Increased heterozygosity.

Genetic role in the success of polyploids:

Allopolyploid: Polyploids from chromosome doubling of F₁s between two species.

Heterozygosity is fixed. Disomic inheritance, bivalent forms in meiosis.

Autopolyploid: Chromosomal doubling of conspecific parents. Multivalents are formed and characterized by polysomic inheritance.

Segmental allopolyploid: Arisen from parents with partially divergent chromosome arrangements such that some chromosomal regions are homologous and others are homoelogenous.

Increased Heterozygosity:

- a. **Allopolyploid:** In allopolyploid heterozygosity is fixed. Depending upon the diversity between parent species involving the wide cross this can be very significant. ~33-43% of the loci are duplicated (polymorphic loci). All allotetraploids are heterozygous and nonsegregating.
- b. **Autotetraploids:** Due to polysomic inheritance autotetraploids maintain a higher level of heterozygosity than diploids. 1:34:1 as opposed 1:2:1 ratio. In polyploids 34/36 are heterozygous.

Higher Outcrossing Rates:

- a. Higher rates of colonization of polyploids is attributed to higher rates of selfing as compared to those of diploids.
- b. Reduced inbreeding depression because of additional genomes. Deleterious alleles are masked by the extra genomes.
- c. Outcrossing rates and levels of inbreeding depression in diploids and polyploids angiosperms are rare. *Epilobium angustifolium*: outcrossing rates are very similar- 0.45 and 0.43 for diploids and polyploids, respectively. However, inbreeding depression was 0.67 for polyploids as opposed 0.95 for diploids.

Recurrent Polyploid Formation:

- a. Application of isozyme analysis and DNA marker technologies indicated repeated polyploid formation.
- b. Molecular marker analysis indicated that nearly all polyploids examined have arisen multiple times from the same diploid species.
- c. Recurrent formation of polyploids makes the polyploid species highly heterogeneous, a significant amount of genetic diversity among polyploids is conserved.

Genome rearrangements:

- a. Rapid genome rearrangements occur following polyploidization. This has been shown by different molecular mapping techniques.
- b. A possible result of polyploidization may be the generation of novel genotypes through genome rearrangements.
- c. Genome rearrangement may occur immediately following the formation of a tetraploid. Intergenomic translocations, may be mediated by transposable elements, could result an important source of novel genetic variation in polyploids.

Polyploids are not evolutionary deadends:

- a. For eudicot, which comprises 75% of the angiosperms, the basal ancestral chromosome number (x) is probably = 8.
- b. Most, if not all, angiosperms have gone through one or more cycle of chromosome doubling.
- c. After polyploidization, diversification continued with subsequent cycles of chromosomal doubling. Therefore, polyploids are a great source of new genetic variation and evolution of novel genotypes/germplasms.

Polyploidy and genetic variation

Soltis and Soltis 1995:

- a. Traditional Views:
 - i. Stebbins (1971) considered that the primary mechanism by which polyploids gain variation was via hybridization with related diploid species.
 - ii. Allopolyploids were considered to be the major force in evolution, whereas autopolyploids were maladaptive and extremely rare in the nature.

b. Current Views:

- i. Recent molecular data revealed the dynamic nature of polyploid genomes.
- ii. Showed that both auto- and allopolyploid form frequently (multiple origin).
- iii. Multiple polyploidation is the rule than exception. It has both genetic and evolutionary significance.
- iv. Autopolyploids are not rare and maladaptive.

Conclusion: Multiple polyploidy events lead to generation of genetic variation in polyploid species –enrich the total gene pool.

Multiple Occurrence of Polyploidy

Allotetraploid species *Tragopogon mirus* and *T. miscellus* formed 9 and 21 times respectively in a small Palouse region of eastern Washington and adjacent Idaho during the last 50 years.

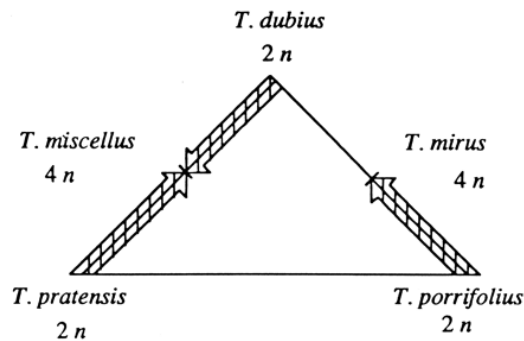


Fig. 1. Parentage and reciprocal origins of tetraploid species of *Tragopogon* in North America. Hatched lines indicate diploid(s) contributing chloroplast to the tetraploids.

- Morphological and cytological data suggested multiple origins of each of these two species, two of *T. miscellus* and three of *T. mirus*, in different locations on the Palouse.
- Recent isozyme and DNA analyses have supported original observations of recurrent origin based on morphological and cytological data (Use of isozyme markers, chloroplast DNA haplotypes, and rDNA markers resulted the number of lineages in *T. mirus* up to 9 and the number in *T. miscellus* up to 21).
- Most crop species, including wheat, maize, sugar cane, coffee, cotton, and tobacco, are polyploid, either through intentional hybridization and selective breeding (e.g. some blueberry cultivars) or as a result of a more ancient polyploidization event.

- Identification of polyploidization events is very challenging. I closely related species carry chromosomes in a polyploid series, then ploidy is evident (e.g. $2n = 16, 32, 64$). Detection of multivalents during meiosis also identify polyploid species.
- The above two criteria work better with recent polyploidization events than ancient ones. Ancient polyploidization events are especially difficult to detect because time erases the signals of duplication; disomic segregation reestablishes, rearrangements scramble chromosomal synteny, and differentiation (or loss) obscures gene copies. Genome wide sequences are helping to identify the ancient polyploids.

Establishment of polyploids

1. Several factors play role in the establishment and survival of polyploids. They are:
 - a. *Gametic screen*: $2n$ gametes must be produced.
 - b. *Sterility screen*: Seed must be produced, though seed set is often low.
 - c. *Vigor screen*: Plants must be able to compete with neighbors, including ancestral species.
2. Thus, vigorous perennials are favored. Likewise vegetative propagation provides an advantage- seed production is not a critical issue.
3. Many new polyploids are not successful: maize remains diploid because diploids outcompete polyploids in the field.
4. Very wide crosses: typically results in weak, even abnormal, plants — unlikely to establish in the wild.
5. Very narrow crosses: polyploid may lack vigor and adaptive advantage over parental species.
6. Intermediate cross: e.g., crosses between ecotypes, races, subspecies, etc. are most successful. Primary tetraploids are most likely cytogenetically closer to autotetraploid than to allotetraploids.
 - a. for example, tetraploid wheat probably developed in two steps: $AA \times A1A1 \rightarrow$ triploid $AAA1$ through A parent producing an unreduced gamete ($2n + n$).
 - b. backcross $AAA1$ to $A1A1$ parent \rightarrow unreduced gamete $AAA1 + A1 \rightarrow AAA1A1$ (again $2n + n$).

- c. The A1 (B) genome does not pair with the A genome not due to divergence, but because of gene *Ph1* that regulates the homologous pairing. Genomes are otherwise not differentiated enough to cause bivalent formation.
7. After polyploids are established, wider crosses are possible due to the buffering capacity of polyploids to withstand alien genomes.
 8. Finally, apomixis is a particularly favorable mechanism to ensure reproduction after polyploidization.

Fate of duplicated genes in polyploids

Force et al. (1999):

Gene duplication can be arisen from tandem gene duplication or from polyploidization. There are several views regarding the fate of duplicated genes.

Classical model:

1. One member of the duplicated pair usually degenerates within a few million years by accumulating deleterious mutations, while the other duplicate retains the original function.
2. One duplicate may acquire a novel function, resulting in the preservation of both members of the pair.
3. Several observations though contradict the classical model.
 - a. A much greater proportion of gene duplicates is preserved than predicted by the classical model. E.g. maize, 72% of the duplicated genes avoided non-functionalization for 11 million years.
 - b. Purifying selection in both copies suggest against degeneration of one copy following duplication (e.g. *Xenopus laevis* - tetraploid ~30 million year ago, Hughes and Hughes 1993).

Note:

Purifying selection: Neutral mutation does not change the amino acid. Non-neutral mutations are deleterious in most of the time. Beneficial mutations from non-neutral mutation occur rarely. Purifying selection acts against deleterious mutations. At the molecular level this can be studied by comparing sequences. Two types mutations are relevant to explain this selection process. Non-synonymous mutations changes the amino acid residue in a specific position, whereas synonymous does not change the amino acid due to redundant nature of codons for most amino acids. The ratio between the non-synonymous mutation and synonymous indicates the type of selection. If it is less than 1, the type selection is purifying selection. Otherwise, it would be the **divergent selection**.

Rare functional mutant from divergent selection would result a novel function for the duplicated gene, and deleterious mutant alleles will perish.

Current View:

Force et al. (1999):

1. Force et al. (1999) proposed the duplication-degeneration-complementation (DDC) model, according to which:
 - i. degenerative mutations in regulatory elements can increase rather than reduce the probability of duplicate gene preservation and,
 - ii. the usual mechanism of duplicate gene preservation is the partitioning of ancestral functions rather than the evolution of new functions. Duplicate genes sub-functionalize through mutations in promoter/regulatory regions that allow, for example, different tissue specificities or different temporal specificities.

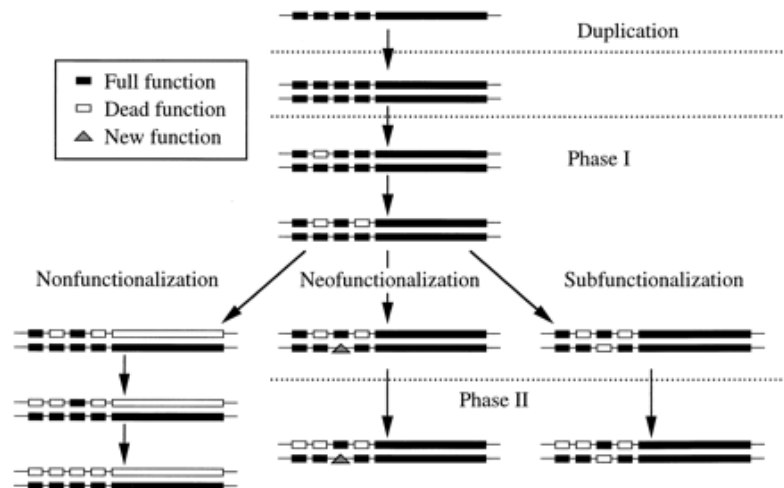


Figure 1. Three potential fates of duplicate gene pairs with multiple regulatory regions. The small boxes denote regulatory elements with unique functions, and the large boxes denote transcribed regions. Solid boxes denote intact regions of a gene, while open boxes denote null mutations, and triangles denote the evolution of a new function. Because the model focuses on mutations fixed in populations, the diagram shows the state of a single gamete. In the first two steps, one of the copies acquires null mutations in each of two regulatory regions. On the left, the next fixed mutation results in the absence of a functional protein product from the upper copy. Because this gene is now a nonfunctional pseudogene, the remaining regulatory regions associated with this copy eventually accumulate degenerative mutations. On the right, the lower copy acquires a null mutation in a regulatory region that is intact in the upper copy. Because both copies are now essential for complete gene expression, this third mutational event permanently preserves

both members of the gene pair from future nonfunctionalization. The fourth regulatory region, however, may still eventually acquire a null mutation in one copy or the other. In the center, a regulatory region acquires a new function that preserves that copy. If the beneficial mutation occurs at the expense of an otherwise essential function, then the duplicate copy is preserved because it retains the original function (From Force et al. 1999).

Example: maize *ZAG1* and *ZMM1* are putative homologues of *AGAMOUS* in *Arabidopsis*. In maize these genes are active at different times in stamens/carpels; *AGAMOUS* is active in both organs during all developmental stages.

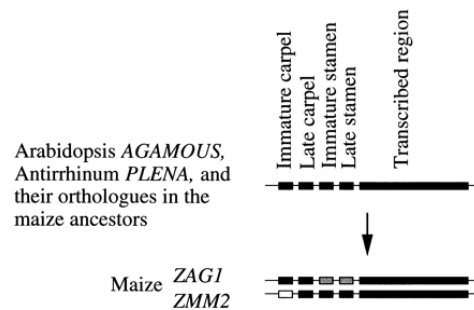


Figure 8. The subfunctionalization hypothesis to explain the expression of duplicated genes in maize. Small solid boxes indicate active regulatory elements, gray boxes indicate regulatory elements with reduced activity, and open boxes indicate null activity alleles for the regulatory element (From Force et al. 1999).

What's after polyploidization or gene duplication?

Dobzhansky (1933) believed that chromosomal doubling results an exact homologue for each chromosome in the hybrid genome, thereby yielding normal pairing between chromosomes and fertility.

However, genomic reunion through sexual hybridization results union of incompatible genes. Those are removed from the population through natural selection against them (Rieseberg 2001, Figure 1a).

In case of chromosomal doubling with a deleterious gene interaction, the genome is fixed and the deleterious gene interaction is preserved – non-Mendelian mechanism (methylation-epigenetic) has to be operative to silence one of the copies and restore fertility (Figure 1b of Riseberg 2001).

Earlier views supported very static nature for polyploids. However, recent molecular data have revealed very *dynamic nature* of polyploids.

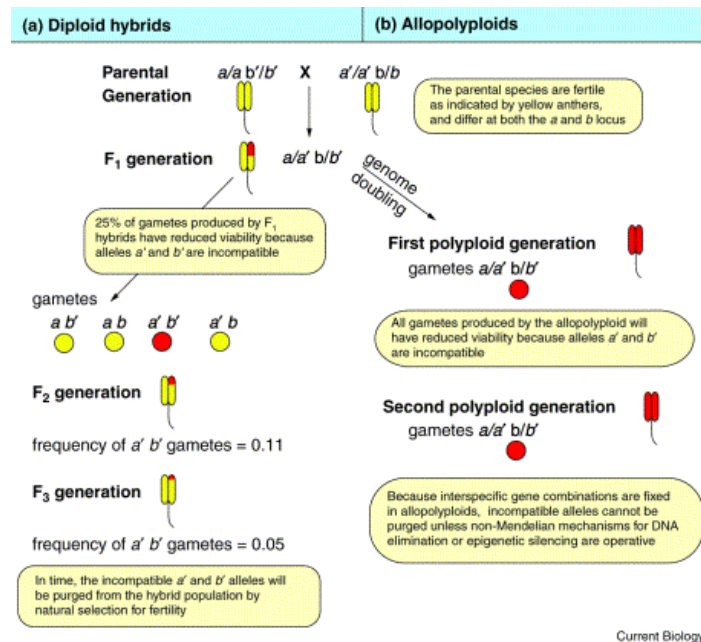


Fig. 1. Fate of incompatible interspecific gene combinations in diploid hybrids (a) and allopolyploids (b). Additive gene action is assumed (From Rieseberg 2001).

Large-scale genome rearrangement triggered by polyploid formation

Recent molecular data surprised our views about polyploids. Following polyploidization newly united genomes go through a very active process of genome reorganization that includes both genetic and epigenetic phenomena.

Example 1:

Synthetic amphidiploid *Brassica*

Song et al. (1995):

- The group made synthetic polyploids representing two natural polyploid species in *Brassica*.
- Three diploid species were hybridized to result natural disomic polyploids. They were: *B. rapa* (A genome), *B. nigra* (B genome) and *B. oleracea* (C genome)
- The natural polyploids they recreated were: *B. juncea* (A x B and B x A) and *B. napus* (A x C and C x A).
- Chromosomes of F₁ were doubled with colchicine treatment and then selfed to form F₂.
- Selfed F₂ individuals to F₅ generation.
- RFLP patterns of parents and all generations were determined for 89 nuclear probes, 6 chloroplast probes, 5 mitochondrial probes.

- g. No changes in the cytoplasmic genomes was observed. Both chloroplast and Mitochondrial probes shown similar patterns among F_5 , F_2 and female parents.
- h. For nuclear genome probes: extensive changes were observed.
 - 1) Gain and loss of parental fragments and generation of novel fragments
 - 2) Changes were detected in each generation from F_2 through F_5 .
- i. Frequency of changes between two polyploids:
 - 1) AB + BA had 2x as many changes as AC + CA
 - 2) A and C are more closely related than A and B
 - 3) Therefore, higher the divergence between parents, the increased the rearrangements following polyploidization.
- j. Was not due to inbreeding alone:
 - 1) Compared an intraspecific cross of two cultivars of *B. rapa*.
 - 2) Found no irregular changes in the F_5 progeny developed by selfing of F_1 hybrids.
- k. Was not due to chromosome losses, all plants except one had normal chromosome numbers.
- l. Chromosomal rearrangements due to *intergenomic (homoelogenous) recombination* probably a major factor.
- m. Observed a high frequency of aberrant meiosis with chromosome bridges, chromosome bridges lagging, and multivalents - leading to new RFLP patterns from segregation of broken chromosomes.
- n. Gene conversion-like processes could also be probably through intergenomic pairing resulting simultaneous loss/gain of parental restriction fragments.
- o. Changes in DNA methylation probably played a minor role.
 - 1) Enzymes used do not recognize methylated bases.
 - 2) Tests with methylation sensitive enzymes did show very minor differences.
- p. Directional genome changes and cytoplasmic effect:
 - 1) Cytoplasm-nuclear interactions: AB amphidiploids A cytoplasm \rightarrow significant changes in B genome than in A genome. Was possibly due a greater loss of B genome than the A genome.
 - 2) B cytoplasm \rightarrow in BA amphidiploid no significant directional effect was observed.
 - 3) AC/CA—similar changes. Variation was not observed, possibly because A & C cytoplasms are more closely related than A and B and there is a better nuclear-cytoplasmic compatibility in the AC and CA polyploids.
- q. Consequences of genome change
 - 1) Rapid divergence of F_5 from F_2 .
 - 2) Also divergence among F_5 individuals within a cross—that is, different individuals gained/lost different bands.

- 3) Marker differences and morphological differences (fertility, leaf shape, branching, etc.)

Implications

- a. Extensive genome changes did occur in early generations.
- b. Many novel genotypes (and phenotypes) are available on which selection can act.
- c. May explain polyploid successes.

**Example 2:
Synthetic allopolyploids in wheat**

Ozkan et al. (2001):

1. They studied two classes sequences in a variety of first generation hybrids, synthetic allopolyploids and parents.
2. Five chromosome-specific (CSSs) and three genome-specific sequences (GSSs) were used to analyze 35 interspecific F₁ hybrids, 22 derived allopolyploids and parents.
3. They observed rapid elimination of CSSs and GSSs in newly synthesized allopolyploids. Elimination of GSSs initiated in F₁ plants, whereas elimination of CSSs started in the first allopolyploids generation. In both cases elimination is completed by the second or third allopolyploid generation.
4. Elimination is a nonrandom and reproducible process. The direction of elimination was determined by the genomic combination of the hybrid or the allopolyploid.
5. In most cases elimination was unidirectional, elimination from one parent preserving the sequences from the other.
6. Elimination process was not affected by the genotype of the parental plants, by their cytoplasm, or by the ploidy level.
7. As in case of Brassica this was not the result of intergenomic recombination. The authors concluded that the elimination of certain sequences allow the polyploids to behave like diploid in meiosis, and thereby, results a successful polyploids.

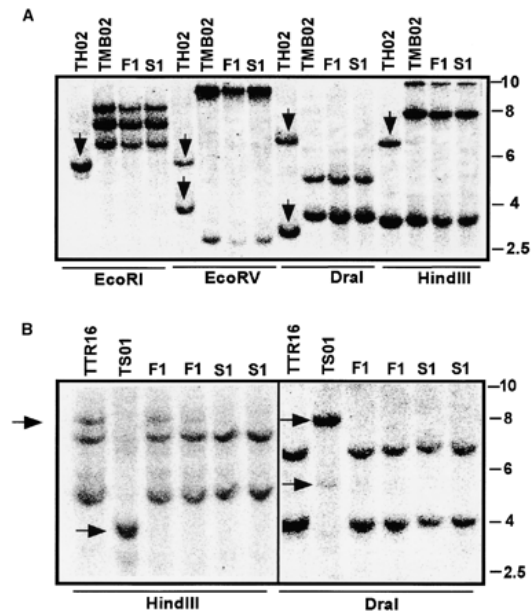


Figure 1. DNA Gel Blot Hybridization of the Genome-Specific Sequence PSR593 to Genomic DNA from F1 Hybrids and Newly Formed Allopolyploids.

(A) Hybridization to genomic DNA from the F1 hybrid between *Ae. sharonensis* (TH02) and *T. monococcum ssp aegilopoides* (TMB02), from the S1 generation of the allotetraploid that derived from this hybrid, and from the two parental plants. DNA was digested using EcoRI, EcoRV, DraI, and HindIII. Arrows indicate the bands from the genome of TH02 that disappeared in F1 and in the S1 generation of the allopolyploid. Fragment size is indicated at right in kilobases.

(B) Hybridization to genomic DNA from the F1 hybrid between *T. turgidum ssp durum* (TTR16) and *Ae. speltoides* (TS01), from the S1 generation of the allohexaploid derived from this hybrid, and from the two parental plants. DNA was digested using HindIII and DraI. Arrows indicate the bands that disappeared in F1 and/or in the S1 generation of the allohexaploid. The upper band of TTR16 is present in F1 of the HindIII digest but is absent in S1. No such difference between F1 and S1 was noted with DraI. This difference between HindIII and DraI probably results from methylation. Fragment size is indicated at right in kilobases (From Ozkan et al. 2001).

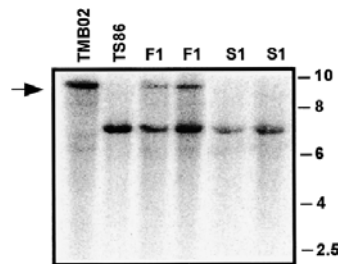


Figure 2. DNA Gel Blot Hybridization of the CSS WPG90 to Genomic DNA from the F1 Hybrid between *T. monococcum ssp aegilopoides* (TMB02) and *Ae. speltoides* (TS86), from the S1 Generation of the Allotetraploid That Derived from This Hybrid, and from the Two Parental Plants.

DNA was digested with EcoRI. The arrow indicates the band from the genome of TMB02 that disappeared in the S1 generation of the allopolyploid. Fragment size is indicated at right in kilobases (From Ozkan et al. 2001).

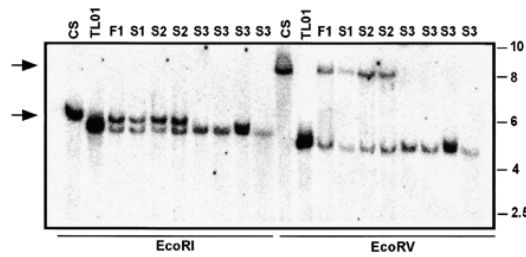


Figure 3. DNA Gel Blot Hybridization of the GSS PSR551 to Genomic DNA from the F1 Hybrid between *T. aestivum* ssp *aestivum* cv Chinese Spring (CS) and *Ae. longissima* (TL01), from the S1, S2, and S3 Generations of the Allooctoploid That Derived from This Hybrid, and from the Two Parental Plants.

DNA was digested using EcoRI and EcoRV. Arrows indicate the band from the genome of TL01 that disappeared only in the S3 generation of the allopolyploid. Fragment size is indicated at right in kilobases (From Ozkan et al. 2001).

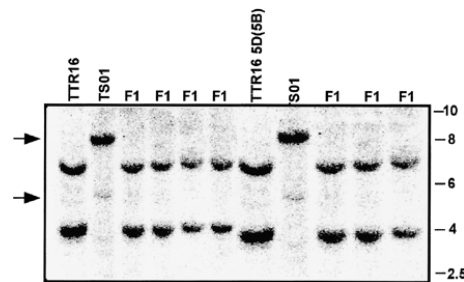


Figure 4. DNA Gel Blot Hybridization of the GSS PSR593 to Genomic DNA from the F1 Hybrid between *T. turgidum* ssp *durum* (TTR16) having *Ph1* and *Ae. speltoides* (TS01), from the F1 Hybrid between *T. turgidum* ssp *durum* (TTR16) Disomic 5D Nullisomic 5B Lacking *Ph1* and *Ae. speltoides* (TS01), and from Their Parental Plants.

DNA was digested with DraI. Arrows indicate the bands from the genome of TS01 that disappeared in the F1 hybrid. Fragment size is indicated at right in kilobases (From Ozkan et al. 2001).

Table 2. Mean Sequence Elimination (%) in F1 Hybrids and S1, S2, and S3 Allopolyploid Generations of *Triticum*, *Aegilops*, and *Secale*^a

Type of Allopolyploid	Generation	CSS						GSS					
		4x		6x		8x		4x		6x		8x	
		n	%	n	%	n	%	n	%	n	%	n	%
Natural	F1	8	3	7	0	–	–	8	31	7	67	–	–
	S1	5	67	6	50	–	–	5	80	6	67	–	–
	S2	3	92	5	75	–	–	4	92	5	100	–	–
Nonnatural	F1	5	0	5	0	10	0	5	10	5	13	10	13
	S1	3	36	2	29	6	10	3	50	2	34	6	28
	S2	–	–	2	58	5	53	–	–	2	50	5	53
	S3	–	–	1	100	3	65	–	–	2	84	3	100

^a *n*, number of combinations; –, data not available; %, mean sequence elimination in every generation was calculated from the percentage of disappearing DNA gel blot bands in every genomic combination (From Ozkan et al. 2001).

Shaked et al. (2001):

1. They provided evidence for epigenetic changes following polyploid formation.
2. They applied a AFLP based sensitive PCR technique MSAP (methylation-sensitive amplification polymorphism) to identify loci that affected by cytosine methylation (criterion for epigenetic gene regulation) following polyploidization.
3. Alterations in cytosine methylation occurred in 13% of the loci, either in the F1 hybrid or in the allopolyploid.
4. Again they observed elimination of sequences is one of the major and immediate responses of the wheat genome to wide hybridization or allopolyploidy,
5. In one cross between *Ae. sharonensis* x *Ae. umbellulata*, 14% of the loci from *Ae. sharonensis* were eliminated compared with only 0.5% from *Ae. umbellulata*, with most changes occurring in the F1 hybrid.
6. In contrast, crosses between *Ae. longissima* x *T. urartu* showed that sequence elimination was more frequent after chromosome doubling.
7. For eight of nine bands that were isolated, the sequences that underwent elimination corresponded to low-copy DNA, whereas alterations in methylation patterns affected both repetitive DNA sequences, such as retrotransposons, and low-copy DNA in approximately equal proportions.

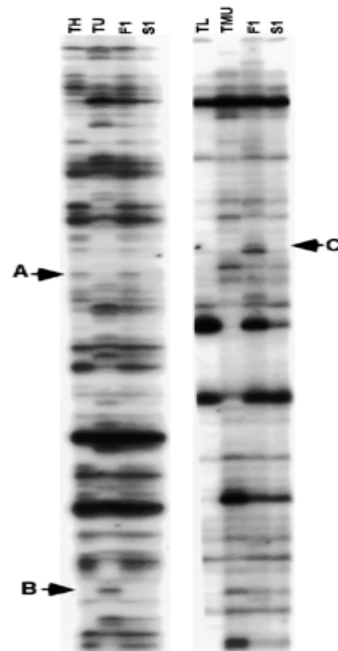


Figure 1. AFLP Fingerprints of Genomic DNAs in Diploid and Allopolyploid Wheat.

The panel at left (EcoRI+AAC/MseI+CTA) included *Ae. sharonensis* (TH), *Ae. umbellulata* (TU), the F1 interspecific hybrid between TH and TU (F1), and the descendant allotetraploid (S1). The panel at right

(EcoRI+ACC/MseI+CAG) included *Ae. longissima* (TL), *T. urartu* (TMU), the F1 hybrid, and the descendant allotetraploid (S1). Arrows indicate three different situations: disappearance of a band in only the allotetraploid plant (A); disappearance of a band in the F1 plant that was maintained in the allotetraploid plant (B); and a rare event in which a new band appeared in the F1 plant (C) (From Shaked et al. 2001).

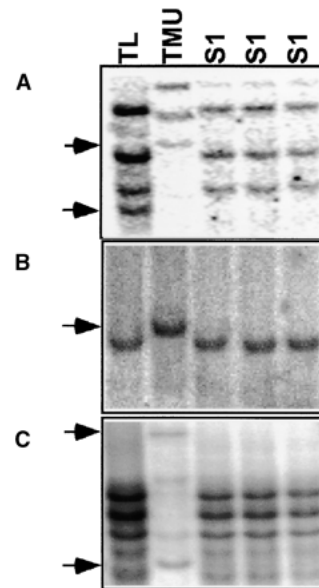


Figure 2. DNA Gel Blot Hybridization with Three Different AIF Probes That Showed Band Disappearance with the AFLP Method.

Genomic DNA of *Ae. longissima* (TL; genome S¹S¹), *T. urartu* (TMU; genome AA), and three independently made newly synthesized allotetraploids (S1 generation; genome S¹S¹AA) was digested and blotted on a nylon membrane.

(A) AIF1 probe (Table 2) was isolated from *T. urartu* and hybridized to EcoRV-digested genomic DNA. Sequence elimination was found in both *Ae. longissima* (bottom arrow) and *T. urartu* (top arrow).

(B) AIF2 probe (Table 2) was isolated from *T. urartu* and hybridized to BamHI-digested genomic DNA. This was the only probe that showed a single-copy fragment in both parents. The *T. urartu* band, shown by the arrow, disappeared in the allotetraploid.

(C) AIF6 (Table 2) was isolated from *T. urartu* and hybridized to HindIII-digested genomic DNA. Two bands from *T. urartu*, shown by arrows, disappeared in the allotetraploid. This pattern of elimination also was shown with enzymes that are not sensitive to methylation, such as DraI, with all of the probes (data not shown) (From Shaked et al. 2001).

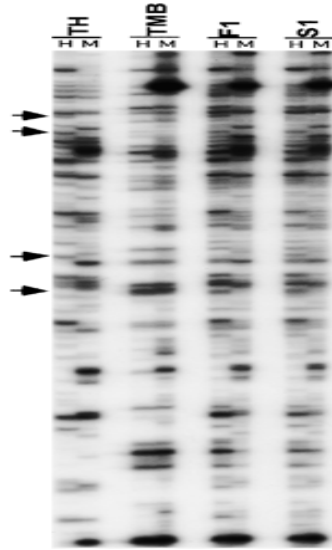


Figure 3. MSAP Patterns Detected in the Two Diploid Parents, the F1 Hybrid, and the Allotetraploid.

The primer combination used was HM+TCAA/E+ACG. Lines 1 to 4 are *Ae. sharonensis* (TH), *T. monococcum* ssp *aegilopoides* (TMB), the F1 hybrid (F1), and the allotetraploid (S1), respectively. H and M refer to digestion with EcoRI+HpaII and EcoRI+MspI, respectively. Bands that showed alteration in methylation pattern in the F1 hybrid and/or the allotetraploid are shown by arrows.

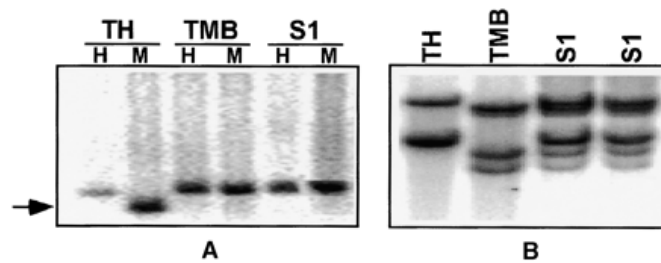


Figure 4. DNA Gel Blot Analysis Using MIF8 Probe That Showed an Alteration in MSAP Pattern in the Allotetraploid (S1) of the Cross between *Ae. sharonensis* (TH) and *T. monococcum* ssp *aegilopoides* (TMB).

(A) Each DNA sample was digested with EcoRI+HpaII (H) and EcoRI+MspI (M), and the two digests were loaded on the gel side by side (HpaII digest on the left and MspI digest on the right). The arrow points to the fragment whose methylation is altered in the allotetraploid.

(B) DNA samples were digested with DraI (not sensitive to cytosine methylation) using the same hybridization probe. MIF8 was obtained using primer combination HM+TCAA/E+ACT (Table 5).

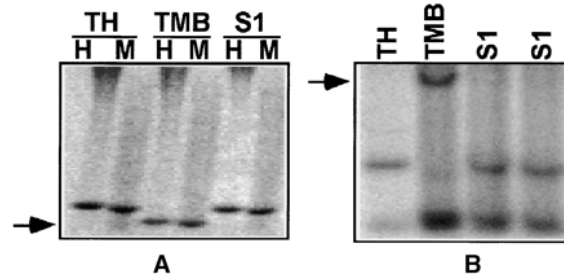


Figure 5. DNA Gel Blot Analysis of a Band Isolated from MSAP That Did Not Show Alteration in Methylation but Rather Showed Band Disappearance.

The bands obtained by both isoschizomers in parent *T. monococcum ssp. aegilopoides* (TMB) were eliminated in the first generation of the allotetraploid (S1). TH, *Ae. sharonensis*.

(A) Each DNA sample was digested with EcoRI+HpaII (H) and EcoRI+MspI (M), and the two digests were loaded on the gel side by side (HpaII digest on the left and MspI digest on the right).

(B) DNA samples were digested with EcoRV using the same hybridization probe. The probe was obtained using primer combination HM+TCAA/E+AAG. Arrows indicate the band of TMB that disappeared in the allotetraploid.

Kashkush et al. (2002):

1. Analyzed 3072 transcripts in the first generation of a synthetic allotetraploid (genome S^lS^lA^mA^m), which resembles tetraploid wheat (genome BBAA), and in its two diploid progenitors *Aegilops sharonensis* (S^lS^l) and *Triticum monococcum ssp. aegilopoides* (A^mA^m). Applied cDNA-AFLP.
2. The expression of 60 out of 3072 transcripts was reproducibly altered in the allotetraploid: 48 transcripts disappeared and 12 were activated.
3. Transcript disappearance was caused by gene silencing or by gene loss. Gene silencing affected one or both homeologous loci and was associated in part with cytosine methylation.
4. Gene loss or methylation had occurred already in the F₁ intergeneric hybrid or in the allotetraploid, depending on the locus.
5. The silenced/lost genes included rRNA genes and genes involved in metabolism, disease resistance, and cell cycle regulation.
6. The activated genes with a known function were all retroelements. These findings show that wide hybridization and chromosome doubling affect gene expression via genetic and epigenetic alterations immediately upon allopolyploid formation.
7. These events contribute to the genetic diploidization of newly formed allopolyploids.

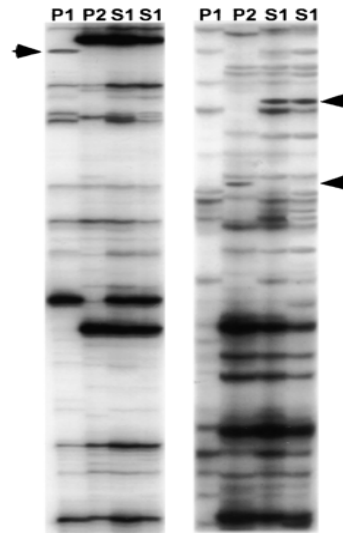


Figure 1. cDNA-AFLP patterns detected in the two diploid parents, *Ae. sharonensis* (P₁), and *T. monococcum* ssp. *aegilopoides* (P₂), and the first generation of the derived synthetic allotetraploid (S₁). (Left) The arrows show examples of differential fragments between the parents and the allotetraploid in the high-resolution part of the gel.

Table 1. cDNA-AFLP analysis in an allotetraploid and its diploid progenitors

Tissue	Total no. of transcripts ^a	Expression alteration in allopolyploid ^b		
		Transcript absent in allopolyploid (%)	New transcript in allopolyploid (%)	Total alterations (%)
Leaves	1872	29 (1.6)	10 (0.5)	39 (2.1)
Root tips	1200	19 (1.6)	2 (0.2)	21 (1.8)
Total	3072	48 (1.6)	12 (0.4)	60 (2.0)

^a Each cDNA-AFLP band was considered as a different transcript. The 1872 leaf transcripts are derived from 37 pairs of selective primers and the 1200 root-tip transcripts are from 25 pairs of selective primers. Monomorphic transcripts (similar in both parents) were scored only once.

^b Only qualitative alterations were scored, namely, disappearance of parental transcripts in the allotetraploid or appearance of a new transcript in the allotetraploid. The percentage of altered bands is out of the total number of transcripts.

Table 2. Molecular characterization of cDNA clones obtained by cDNA-AFLP

Clone identification	cDNA AFLP pattern ^a			Sequence similarity ^b
	S ^l S ^l	A ^m A ^m	S ^l S ^l A ^m A ^m	
RAIF1-3	+	-	-	None
RAIF4-6	+	-	-	ORF (putative proteins)
RAIF7	+	-	-	Rice EST
RAIF8	+	-	-	<i>Ae. speltooides</i> genome-specific DNA
RAIF9	+	-	-	Wheat Wis 2-1A retrotransposon
RAIF10-11	-	+	-	None
RAIF12-16	-	+	-	ORF (putative proteins)
RAIF17-18	-	+	-	Wheat DNA for ORF flanked by ARS
RAIF19-23	-	+	-	Wheat EST
RAIF24-26	-	+	-	Rye 26S rRNA
RAIF27	-	+	-	Gene cluster 21-kb wheat chloroplast DNA
RAIF28	-	+	-	Wheat rubisco-large subunit
RAIF29	-	+	-	Wheat mitochondrion rrn26 gene for rRNA
RAIF30	-	+	-	Rice alpha 2 subunit of 20S proteasome
RAIF31	-	+	-	<i>Agropyron cristatum</i> -RAPD marker
RAIF32	-	+	-	Wheat acetyl-coenzyme A carboxylase
RAIF33	-	+	-	Wheat retrotransposon Tar1
RAIF34	-	+	-	Barley NBS/LRR resistance gene RPM1
RAIF35	-	+	-	Human cell cycle regulatory protein p95
RAIF36	+	+	-	ORF (putative proteins)
RAIF37	+	+	-	Wheat EST
RAIF38	+	+	-	<i>Arabidopsis thaliana</i> putative retroelement
RAIF39	+	+	-	Succinyl-diaminopimelate desuccinylase
RAIF40	+	+	-	<i>A. thaliana</i> Ring-h2 finger protein
RAIF41-42	+	+	-	<i>A. thaliana</i> acylaminoacyl-peptidase
RAIF43-44	-	-	+	None
RAIF45	-	-	+	Maize retrotransposon (gag)
RAIF46-48	-	-	+	Wheat Wis2-1A retrotransposon

^a +, transcript present; -, transcript absent.

^b The search was done using the BLAST package. Similarity was considered as significant for E-values < e⁻¹⁰.

RAPD, randomly amplified polymorphic DNA.

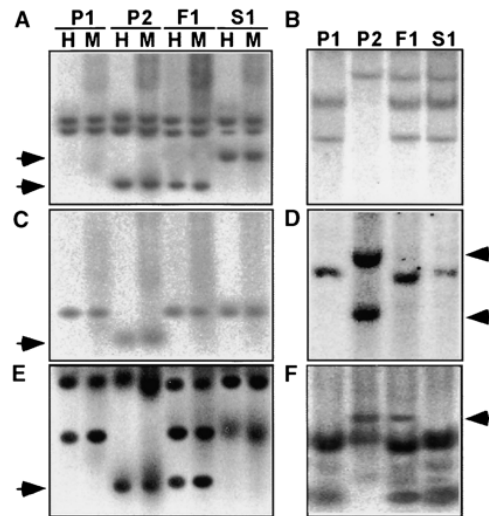


Figure 3. Southern blot analysis of three genes that showed an alteration in gene expression in the F₁ hybrid and the first generation allotetraploid (S₁) of the cross between *Ae. sharonensis* (P₁) and *T. monococcum* ssp. *aegilopoides* (P₂). (A, C, and E) DNA samples of P₁, P₂, F₁, and S₁ digested with the two isoschizomers *Hpa*II (H) and *Msp*I (M) loaded on the gel side by side and hybridized with ³²P-labeled probes RAIF34, RAIF16, and RAIF32, respectively. (B, D, and F) DNA samples probed with RAIF34, RAIF16, and RAIF32, respectively, after digestion with *Dra*I (B) and *Eco*RI (D and F). See Table 2 for probe description.

Example 3:**Synthetic allopolyploids in cotton**

Cronn et al. (1999); Liu et al. (2001):

1. Earlier Wendel group had shown from sequence analysis of 16 loci that in AADD genomes of cultivated cotton and its AA and DD diploid progenitors A genomes are similar at both ploidy levels, and likewise, the D genomes are also similar at both ploidy levels.
2. They concluded that duplicated genes in allopolyploid cotton evolved independently of each other and at a similar rate as those in their diploid ancestors.
3. Recently they studied nine sets of newly synthesized allotetraploid and allohexaploid plants and their parents. Using both methylation-sensitive and methylation-insensitive enzymes AFLP was performed and about 22,000 loci were compared.
4. They did not see any significant deviation in band patterns as observed in synthetic polyploids of the wheat group or brassica.
5. Likewise from the use of methylation-sensitive enzymes they did not see any evidence of epigenetic changes following the synthesis of polyploids.

Pairing in disomic polyploids

1. The correct pairing and segregation of chromosomes during meiosis is essential for genetic stability and subsequent fertility.
2. This is more difficult to achieve in polyploid species, such as wheat, because they possess more than one diploid set of similar chromosomes.
3. Feldman et al. 1997 suggested that homologous pairing in allopolyploids is facilitated by two complementary systems: (1) elimination of DNA sequences in the initial allopolyploids and (2) subsequent evolution of gene systems that determine exclusive bivalent pairing of homologues by suppressing pairing between homoeologues.
4. Genes systems were identified in several allopolyploid plant species, *e.g.*, *Triticum aestivum*, *Avena sativa*, *Festuca arundinacea*, *Lolium multiflorum*, and *Verbena oubleita*.
5. The best-characterized at the cytological level is the *Ph1* gene of common (hexaploid) wheat, *T. aestivum*. This gene, located on the long arm of chromosome 5B, suppresses the pairing of homoeologous chromosomes and allows bivalent formation.
6. In the absence of *Ph1*, multivalent formation between homoeologous chromosomes is resulted.

Two hypotheses for the mode action (Feldman et al. 1998):

1. The **presynaptic** hypothesis assumes that pairing occurs in at least two stages.
 - a. In the first stage, homologous chromosomes approach each other during premeiotic interphase, leading to their premeiotic alignment.
 - b. In the second stage, they pair at zygotene. Chromosomes that are premeiotically aligned will pair at meiotic prophase; failure of this alignment results in univalents at first metaphase.
 - c. The presynaptic hypothesis suggests that *Ph1* determines the pattern of premeiotic alignment of homologous and homoeologous chromosomes.
 - d. In the absence of the gene, both homologues and homoeologues lie near each other in the premeiotic nucleus, resulting in some multivalent pairing at first meiotic prophase and metaphase.
2. The **synaptic** hypothesis assumes that *Ph1* affects the stringency of both synapsis and crossing over at meiotic prophase.
 - a. In the presence of *Ph1*, synapsis occurs with a high stringency, and crossing over is confined to homologous chromosomes.
 - b. The absence of the gene leads to decreased stringency, permitting crossing over and recombination between partially homologous chromosomes.

Ph1 in specific somatic and meiotic centromere association

Martinez-Perez et al. (2001):

Moore group, John Innes Centre, England, earlier showed that centromeres associate in pairs before meiosis in flowers of polyploid cereals, but not until the beginning of meiosis in their diploid progenitors.

1. Recently they have reported similar observation in xylem vessel of roots of bread wheat (Martinez-Perez et al. 2001).
2. The level of centromere association in xylem vessel cells of wheat is unaffected by the presence of *Ph1*, as shown for floral development earlier.
3. Although centromeres associate in the xylem vessel cells in the presence and absence of *Ph1*, they are not associated in other root tissues.
4. They studied homologue association in these vessel cells by labeling specific pairs of rye chromosomes in wheat–rye addition lines.
5. These rye homologues associate at a high level through their centromeres during vessel development in the presence of *Ph1* (25/25 cells OK) but not in its absence (22/25 cells did not show association).

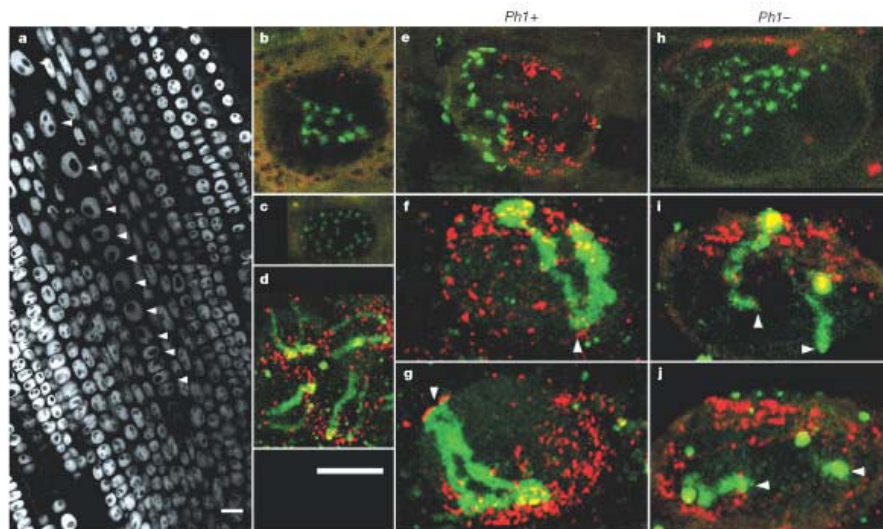


Figure 1 Centromere and homologue behaviour during root development. **a**, Root section labelled with DAPI (4',6-diamidino-2-phenylindole dihydrochloride). Arrowheads indicate the column of developing xylem vessel cells. **b**, Polytene vessel nucleus in *T. monococcum*, $2n = 14$, showing 13 centromeric sites (green). **c**, Non-polytene root nucleus in hexaploid wheat, $2n = 42$, showing 35 centromeric sites (green). **d**, Non-polytene root nuclei in hexaploid wheat, showing the homologues unassociated (green). **e**, **h**, Polytene vessel nuclei in hexaploid wheat, showing about 21 centromere sites (green) in both the presence (**e**) and absence (**h**) of *Ph1*. **f**, **g**, Polytene vessel nuclei in hexaploid wheat, showing homologues (green) associated through their centromeres (arrowheads). **i**, **j**, Polytene vessel nuclei (*Ph1* mutant), showing homologues (green) unassociated; centromeres are indicated by arrowheads. Telomeres are red in **b–j**, which are all at same magnification. Scale bars, 10 μm [From Martinez-Perez et al. (2001)].

6. In both the presence and absence of *Ph1*, the number of centromere sites seen indicates centromere pairing. This suggests that in the presence of *Ph1* most centromeres are homologously associated (more means less pairing, less means more pairing).
7. In other root cells, neither the centromeres nor the homologues are associated.
8. In xylem cells in which the homologues are associated at their centromeres, associations also occur at interstitial sites in 67% of the cells. Centromere association may precede interstitial association.
9. To study if *Ph1* promotes the specificity of the association to true homologues, and this specificity could be achieved by reducing non-homologous centromere associations they determined the centromere behaviour during meiosis of developing flowers and developing xylem vessels in hybrid lines possessing only non-homologous chromosomes, in both the presence and absence of the *Ph1* locus. These lines are wheat-rye hybrids, and contain a haploid set of 21 wheat chromosomes and a haploid set of 7 rye chromosomes, making 28 chromosomes in total.
 - a. In most root tissues, centromeres are not associated (Table 1).
 - b. During xylem vessel development in the absence of the *Ph1* locus, however, the centromeres associate, reducing to a mean of 16 centromere sites, whereas in the presence of the *Ph1* locus, a mean of 20 sites is seen. Thus, in these somatic cells, *Ph1* can reduce but not eliminate non-homologous association.

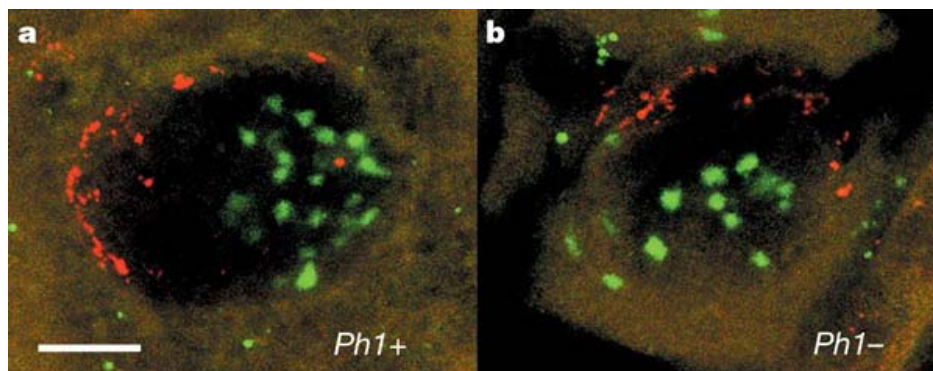


Figure 2 Centromeres (green) and telomeres (red) in root xylem vessel cells of wheat-rye hybrids. **a**, Presence of *Ph1*, showing 20 centromere sites. **b**, Absence of *Ph1*, showing 14 centromere sites. Scale bar, 10 μm [From Martinez-Perez et al. (2001)].

10. At the telomere-bouquet stage, in the absence of the *Ph1* locus the centromeres reduce to a mean of 14 sites, showing complete but non-homologous pairing. In the presence of *Ph1* the mean number of sites increases to 24 (Table1).
11. Non-homologous centromere associations are mostly eliminated at the telomere-bouquet stage of meiosis in wheat-rye hybrids in the presence of *Ph1*, but not in its absence.

Table 1 Statistics of the number of centromeres

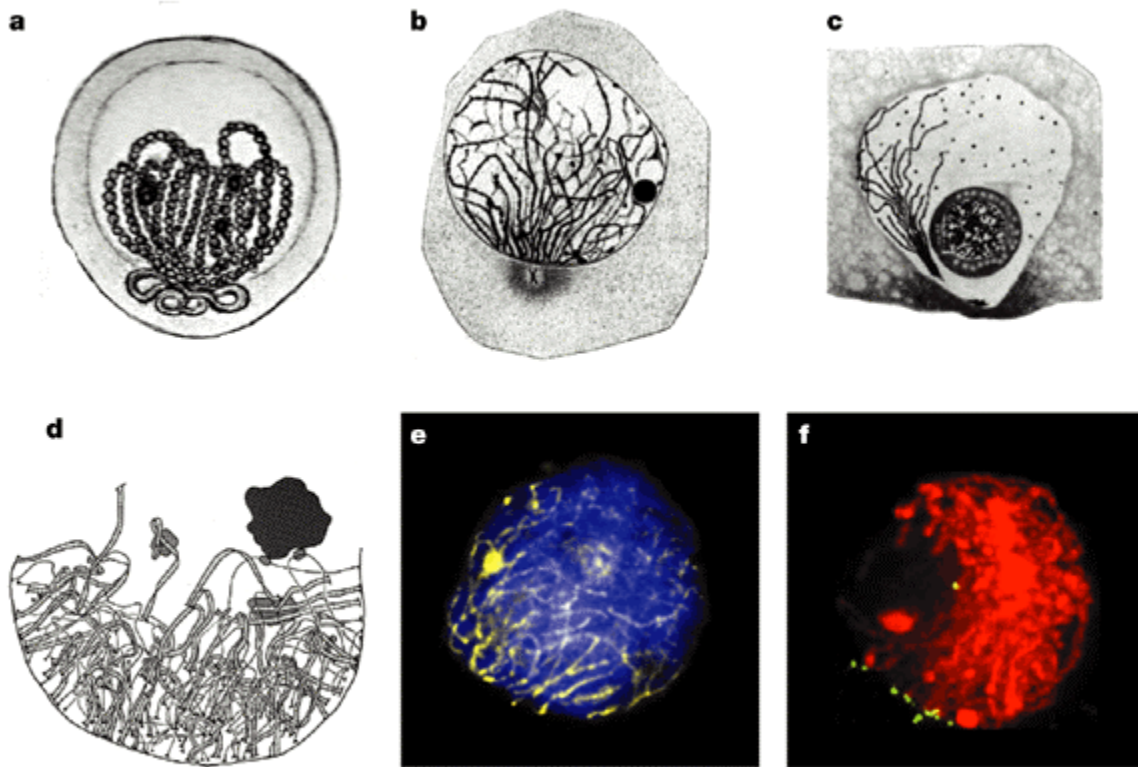
		<i>Ph1+</i>	<i>Ph1-</i>	t-test
Wheat-rye	Premeiosis	19.5 (2.7)	16.3 (2.9)	$P < 0.001$
	Telomere bouquet	23.5 (1.5)	13.7 (2.1)	$P < 0.001$
	Xylem vessel	20 (1.7)	16.3 (1.8)	$P < 0.001$
	Non-polytene root	24.9 (1.2)	25.2 (1.3)	$P = 0.6$
Wheat	Xylem vessel	21.7 (2.1)	21.5 (1.8)	$P = 0.798$
<i>T. monococcum</i>	Xylem vessel	12.56 (0.8)		

The s.d. is given in parentheses. Student's t-test was used to test the null hypothesis that the two means in the presence and absence of *Ph1* are the same. The null hypothesis can be discounted in all comparisons except the wheat xylem and the wheat-rye non-polytene root. All centromere sites were counted on the original three-dimensional confocal stacks.

12. This premeiotic association of centromeres provides an initial sorting of the chromosomes. Earlier the group showed that the sites remain paired through floral development to the telomere-bouquet stage of meiosis. At the telomere-bouquet stage, any non-homologous centromere associations separate in the presence of *Ph1*, enabling a further sorting of these chromosomes.
13. In the absence of *Ph1*, non-homologous associations made before meiosis are maintained. Thus, telomere-led synapsis results in a high level of multivalents (involving a total of up to 17 chromosomes).
14. Deletion of the *Ph1* locus has two major phenotypic effects: first, chromosome pairing is disrupted, which leads to the:
 - a. synapsis of non-homologous chromosomes;
 - b. non-homologous recombination is induced.

Conclusion: Data in this paper suggest that the effect of *Ph1* on premeiotic alignment and correction during synapsis are most likely the results of a mechanism involving centromere association. *Ph1* functions both in presynaptic and synaptic stages.

Note: Next page-



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Figure 1 | Chromosomal bouquets of different species. **a** | Early drawing (1885) of a bouquet nucleus of *Helix pomatia* (reproduced from Ref. 63). **b** | Zygotene bouquet of *Myxine glutinosa*. Note the centrosome at the bouquet base (reproduced from Ref. 18). **c** | Bouquet arrangement during Coccid (intracellular parasite) prophase I (polarization involves only one telomere). The large round sphere represents the nucleolus (reproduced from Ref. 21). **d** | Crowded early zygotene bouquet base in achiasmate meiosis of tetraploid *Bombyx mori* female (reproduced from Ref. 64, with permission from S. Rasmussen and P. Holm, The Carlsberg Laboratory). **e** | Late leptotene bouquet of a human spermatocyte. DNA is stained with DAPI (blue), axial cores by immunofluorescence with antibodies against SCP3 (yellow, false colour). The resemblance of the SCP3 staining and the chromosome structures depicted by the early cytologists (compare with **a**) suggests that these structures resemble axial elements or complete synaptonemal complexes. **f** | Projection of optical sections covering 5 μm near the centre of a DAPI-stained (red, false coloured) three-dimensionally preserved maize bouquet nucleus after telomere fluorescence *in situ* hybridization (green signals). Telomeres are grouped at the lower left of this nucleus (reproduced with permission from Ref. 65 © (1997) The Rockefeller University Press).

From Nature Reviews Molecular Cell Biology 2; 621-627 (2001).