Disomic Polyploidy
(Allopolyploidy; Heterogenomic polyploidy)

- A polysomic tetraploid = AAAA
- A disomic tetraploid = AABB

E.g., Wheat evolution

Dvorak et al 1992; Feldman et al., 1997

Wheat has 2n = 6x = 42.

The idea is that the progenitor genomes themselves have a common ancestor

- A genome comes from *Triticum urartu*
- B genome probably comes from the S genome of *Aegilops speltoides*
- D genome comes from *Aegilops squarrosa* = *Ae. tauschii* = *T. tauschii*

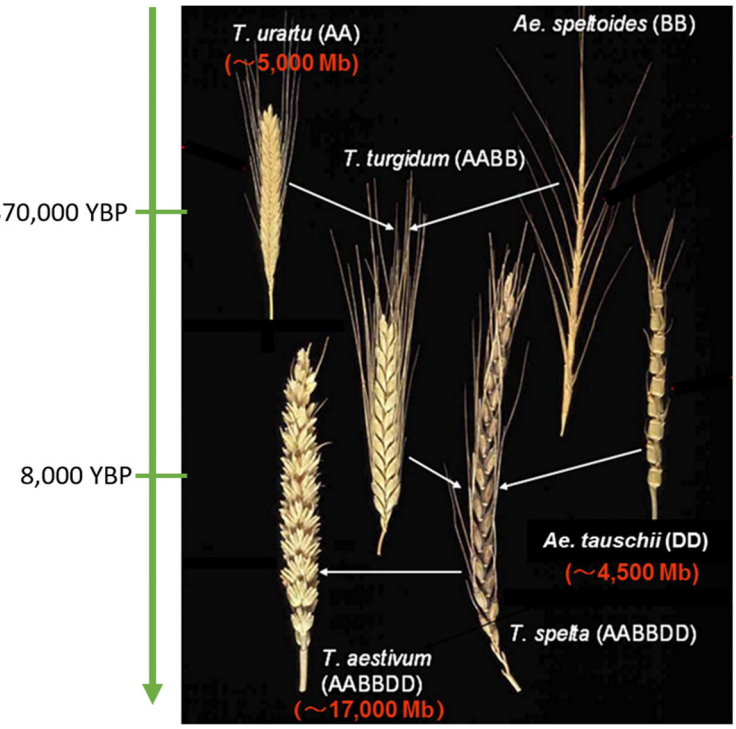


Figure 1. Wheat Genome Consortium

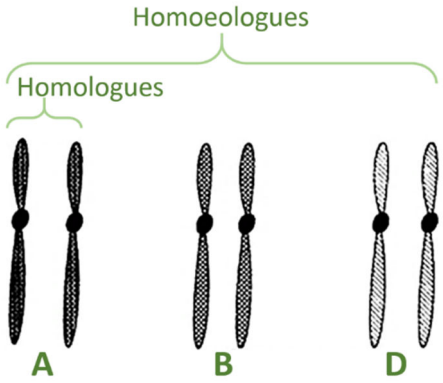
- AABB wheats = *T. turgidum*
  - These are the pasta wheats (durum)
- AABBDD = *T. aestivum*
  - This is bread wheat

Homoeologous chromosomes

Wheat exhibits bivalent pairing

- Chromosomes from the different genomes do not pair with each other
- Yet, chromosomes are so similar they can substitute for each other

- Homos = same
- Homoeo = similar
- Logos = proportion
- Homologous = agreeing
- Homologous chromosomes gradually evolve into homoeologous chromosomes
- Homoeologs have diverged by evolution in different species, but are derived from the same ancestral chromosome
  - Thus the difference between homology and homoeology is simply one of degree
  - And/or the absence or presence of pairing genes



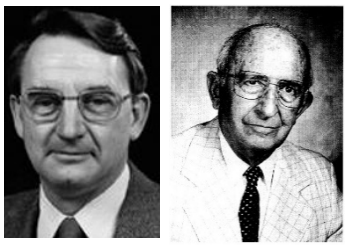
- Theoretically, homoeologous genomes are sufficiently different that pairing between homoeologues (allosyndesis) is restricted
  - Only pairing between homologous chromosomes (autosyndesis) takes place.
  - I.e., AB pairings do not take place, only AA or BB pairings take place.

- Autosyndesis - Pairing of homologous chromosomes (eg, A+A, or B+B, or D+D)
- Allosyndesis – Pairing of homoeologous chromosomes (eg, A+B or A+D or B+D)

Genetic control of autosyndesis

The 5B effect in wheat

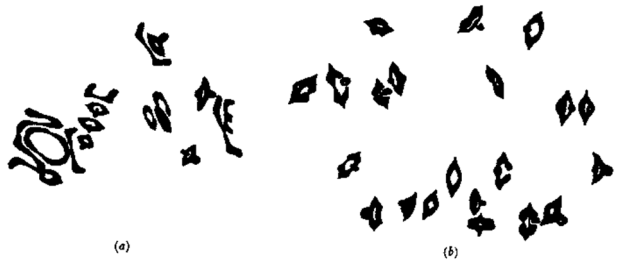
Riley and Chapman, 1958; Sears and Okamoto, 1958



Sir Ralph Riley (1924-1999) Ernest Robert Sears (1910-1991)

Created nullisomics (2n - 2, i.e., missing a pair of homologues) for all 7 chromosome sets

- Found that pairing was affected in some nullisomics
- In 2n - both 5B (i.e., nullisomics for the 5B chromosome pair), pairing occurred between the homoeologues
- In haploids of this (n - 5B), good pairing occurred. Good pairing is not normal in wheat haploids!



Left: PMCs of wheat (a) nullisomic for 5B with 15 II, 1 IV, and 1 VI. (b) normal

- **Riley et al, 1968**: Adding *T. speltoides* genome to wheat has a similar effect.

The *Ph1* locus

Sears, 1977

Used mutagenesis to identify the locus on chromosome 5BL that regulates pairing. It is called *Ph1*, for *pairing homoeologous*.

- Its recessive form permits pairing between homoeologs without having to remove the entire chromosome
- When pairing occurs between homoeologs, the presence of translocations is frequently revealed. E.g., 13 translocations are present in wheat:
  - 9 between homoeologous chromosomes
  - 4 between non-homoeologous chromosomes

Cheng et al., 1994

- PhI "High pairing gene" from *Aegilops speltoides* in one dose suppresses effect of *Ph1* gene
  - Butnot as much as nulli 5B or *ph1b*

Aragón-Alcaide et al., 1997 (Moore lab)
Looked at homologous barley chromosomes in flower buds of a wheat substitution line

- Stage 1 = 3-5 days before meiosis
- Stage 2 = 1-3 days before meiosis
- Stage 3 = meiosis

	Meiocytes			Tapetum cells			Undifferentiated tissue			N
	sep	V	assoc	sep	V	assoc	sep	V	assoc	
Stage 1	--	--	--	--	--	--	72%	23%	5%	134
Stage 2	10%	88%	2%	31%	61%	8%	--	--	--	41 M 128 T
Stage 3	5%	**2%***	93%	6%	9%	85%	--	--	--	61 M 128 T
ph1ph1 Stage 3	**76%***	7%	0%	55%	37%	8%	--	--	--	29 M 67 T

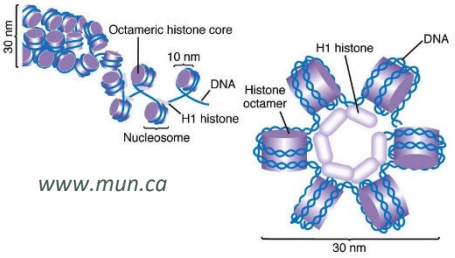
Take-home message– In *Ph1Ph1*, centromeres of homologues pair a few cell divisions before meiosis.

- Similar pairing does not occur in ph1ph1

* Note 76% of chromosomes are unpaired in *ph1ph1* at stage 3 relative to the non-mutant.

Greer et al., 2012

The Ph1 locus is a cluster of defective cyclin-dependent kinases (*Cdk*) genes



- CDK phosphorylates histone H1
- The presence of the defective copies downregulates *CDK* activity → affecting heterochromatin condensation which in turn affects pairing
- It follows that treating with a Ser-Thr phosphatase inhibitor - okadaic acid– should allow pairing of homoeologous pairing
    - That is indeed the case

Zhang et al., 2014

The closest homologue of the Ph1 kinase in arabidopsis also regulates pairing and recombination

Martin et al., 2015

Monitored recombination nodules by looking at MSH1 presence (mismatch repair enzyme)

The *Ph1* mode of action may simply be through a *Cdk* effect on the telomere regions

- Promotes homologue pairing in early meiosis
- Homoeologues pair as well @ pachytene, but do not CO so fall apart
- Ph1 prevents recombination nodules on paired homoeologues from becoming COs later in meiosis
    - Thus suppressing recombination between homoeologues by just preventing the DSBs from becoming COs.

Jenczewski and Alix, 2004

There is good evidence for pairing genes in:

- *Triticum* spp. (wheat)
- *Avena sativa* (oat)
- *Festuca arundinacea* (tall fescue)
- *Brassica napus* (canola)
- *Gossypium hirsutum* (cotton)
- *Gossypium barbadense* (Sea Island cotton)
- *Lolium multiflorum* (annual ryegrass)
- *Lolium rigidum*

The existence of pairing genes can be inferred in the following genera:

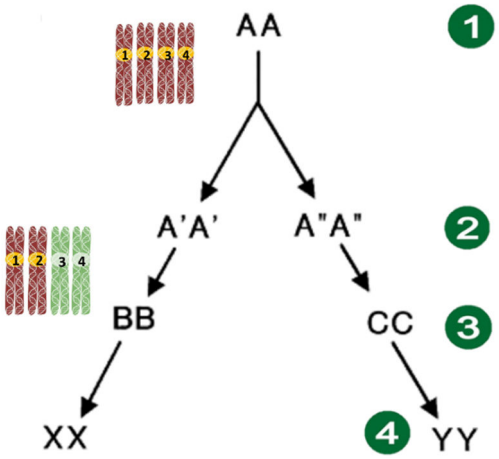
- *Aegilops* (wheat grasses)
- *Hordeum* (barley)
- *Nicotiana* (tobacco)
- *Coffea* (coffee)

Pairing can determine the difference between auto and alloploids, which is why these can be classified according to their pairing behavior

Thus, even if a plant starts as an allo, lack of allosyndetic pairing may convert it to an auto

Divergence & polyploidization

As two populations diverge into separate species, polyploidization can occur at any time during the divergence process. Polyploidization @



1 gives AAAA → autotetraploid or homogenomic tetraploid

3 gives BBCC → allotetraploid, or heterogenomic tetraploid (or, if done by a person, an amphidiploid)

4 is not possible → the species are too divergent to form a viable hybrid

The gray area is at 2. Too much divergence has occurred to be autopolyploid, but not enough to be a genomic allopolyploid--- called segmental allopolyploids by Stebbins (1950) to indicate some chromosomes behave like autopolyploids and some like alloploids

The three categories of polyploids represent a continuum:

This view changed in the 1970's and 80's, based on the writings of Sears and Jackson.

- Under their viewpoint, polyploidization at 3 and 4 is not possible.
- Hybridization at 2 gives an allotetraploid if pairing genes are present.
  - If pairing genes are not present, then it behaves like an autotetraploid.
- I.e., classification is based on cytogenetic behavior of the 4x, not taxonomy of the parents.

In this case, polyploids are classified according to their breeding behavior:

- Disomic: Have diploid genetics, even though they are polyploids. E.g., tobacco, wheat
  - Hence Belling's term "double diploid"

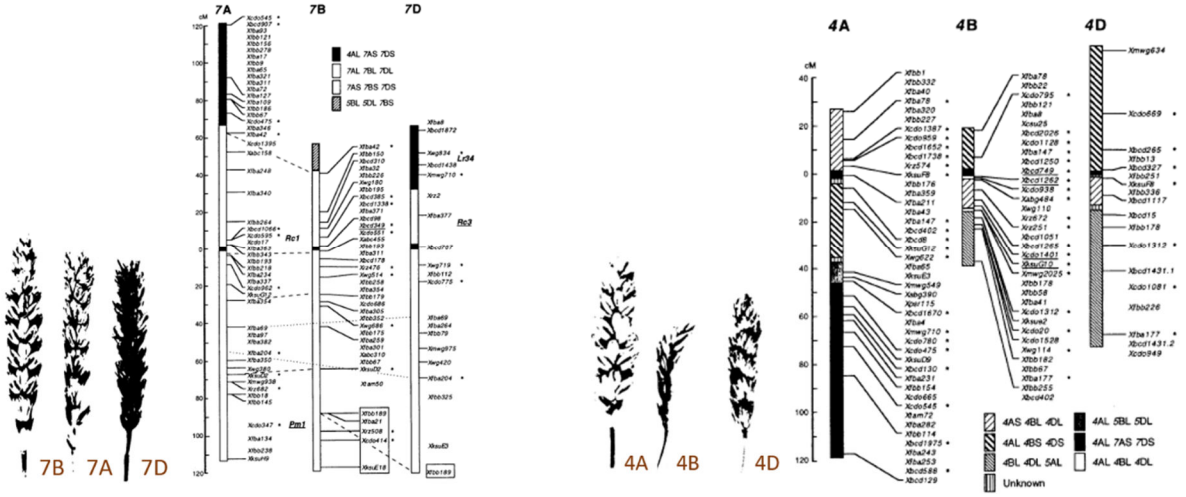
  Polysomic: Have polysomic inheritance. E.g., alfalfa, potato

Recent findings with molecular markers indicate that homoeologs are not as similar as previously thought.

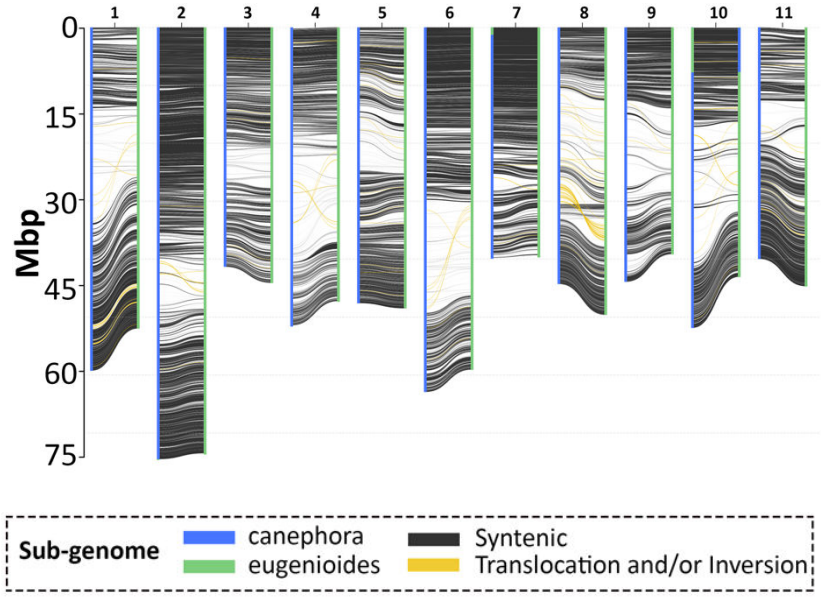
- If these changes occurred before tetraploidization, then the view of Sears and Jackson is not correct
- However, if these changes took place after tetraploidization (good evidence for this), then their view could still be correct

How similar are homoeologous chromosomes

Nelson et al., 1995

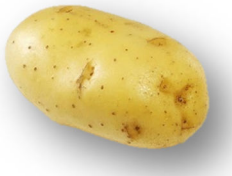


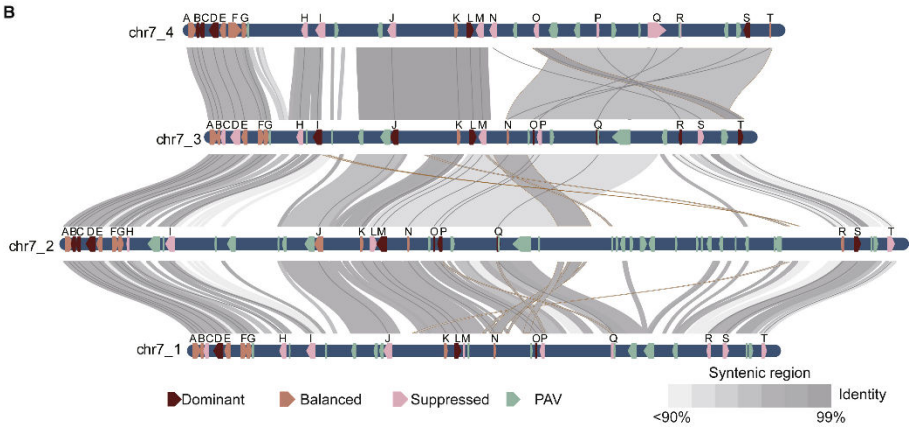
Scalabrin et al. 2024



Compare with homologues from potato

Bao et al. 2022





- Homologs and homoeologs seem to have lots of structural difference
- Supports the idea that the presence or absence of pairing genes determines autoploidy vs alloploidy.

Pairing vs inheritance

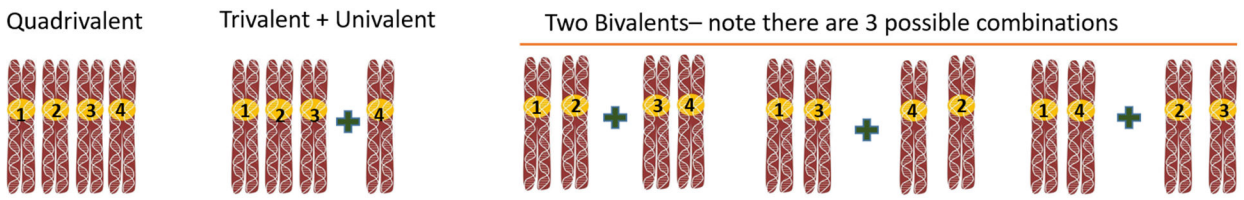
Disomic inheritance

- Found in 2x and alloploids
- Each homolog only has 1 pairing partner

Tetrasomic inheritance

- Each chromosome has 3 possible pairing partners

3 possible pairing configurations

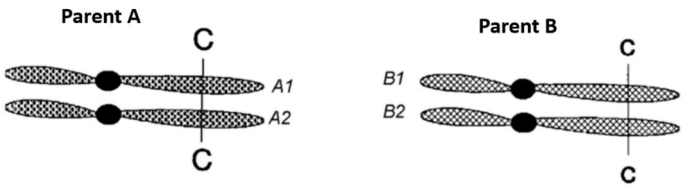


- Pairing is completely random
- Each pair happens 1/3 of time
- So, all homologues pair equally frequently among themselves
- Gives same genetic result as if pairing in quadrivalents

Quantifying allosyndesis vs autosyndesis

For a polyploid from these two parents:

For a species of genomic constitution AABB, with the A genome carrying *CC*, and the B genome having *cc*:



There are 3 pairing possibilities:

1) All pairing in autosyndetic

This is what happens in an allopolyploid.

- Only homologous pairing occurs
- A1 chromosomes only pair with A2 chromosomes
  - and B1 chromosomes only pair with B2 chromosomes.
- Homoeologous pairing (A chromosomes with B chromosomes) does not occur.
  - It is not possible to recover gametes homozygous recessive for c, ie., cc:
- Autosyndesis (A1A2 + B1B2) occurs 100% of the time

		Dominant:recessive ratio	
Type of pairing	Gametes	Backcross	F_2
All autosyndetic	*Cc*	∞:0	∞:0

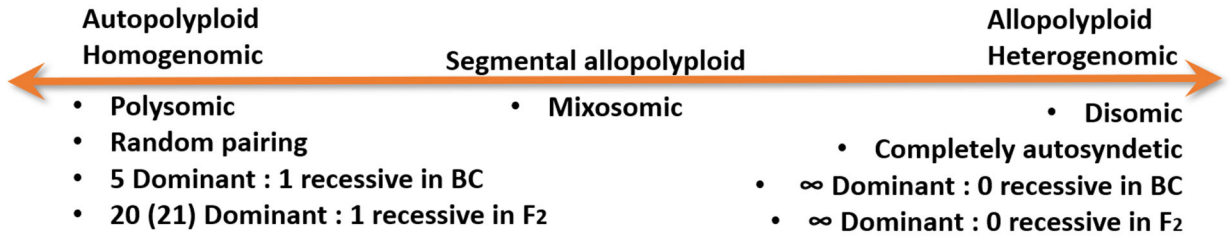
- This is not the same as a digenic ratio in a diploid, which would give a 15:1 ratio

2) All pairing is completely random – what happens in autoploids

- allosyndesis (A1B1 + A2B2) occurs $^1/_3$ of the time
- allosyndesis (A1B2 + A2B1) occurs $^1/_3$ of the time
- autosyndesis (A1A2 + B1B2) occurs $^1/_3$ of the time

		Dominant:recessive ratio	
Type of pairing	Gametes	Backcross	F_2
No preference	1*CC* + 4*Cc* + 1*cc*	5:1	35:1 (20:1)

3) Autosyndesis occurs preferentially, but not to the exclusion of allosyndesis



Pelé et al, 2018

- "Speciation success of polyploid plants closely relates to the regulation of meiotic recombination"

Gerstel, 1963

An illustration of increasing differential affinity with decreasing taxonomic relationship.

Cross:	Genomes: n = 13	II in F_1:	IV in amphidiploid	BC Ratio: (exp. 5:1)	Comments:	Most like an:
G. arboreum × *G. herbaceum*	$A_2 \times A_1$	$^{12.9}/_{13}$	$^{\sim 8}/_{13}$	4:1	Closest to a 5:1 ratio*	Autoploid
G. thurberi × *G. raimondii*	$D_1 \times D_5$	$^{12.9}/_{13}$	$^{3.9}/_{13}$	13.4:1	Deviates from 5:1 ratio	????
G. arboreum × *G. raimondii*	$A_2 \times D_5$	$^{6}/_{13}$	$^{0.2}/_{13}$	103.5:1	Approaches an ∞:0 ratio	Alloploid
G. arboreum × *G. thurberi*	$A_2 \times D_1$	$^{8}/_{13}$	$^{0.44}/_{13}$	393.0:1	Approaches an ∞:0 ratio	Alloploid

**Note the high frequency of IV being formed*

Li et al, 2015:

Note: *G arboreum* and *G. raimondii* diverged some 5-10 MYA and hybridized some 1-2 MYA

Modified from **Burnham, 1962**

- Table shows the "theoretical % of recessives in backcross progenies from an F1 duplex for a recessive gene"

- I.e., a cross of AAaa × aaaa in which various levels of autosyndetic pairing are assumed

Pairing	% allosyndesis	% autosyndesis t_1	% recessives in BC	% of BC progeny that are *Aaaa*	Type of pairing
All allosyndetic	100	0.0	25.0	50.00	AB only
	87.5	12.5	21.88	56.25	
	75	25.0	18.75	62.50	
Random *(autoploid)*	66.66	33.33	16.67	66.67	⅔(AB+AB); ⅓(AA+ BB)
	50	50.00	12.50	75.00	
	33.33	66.66	8.33	83.33	
	25	75.00	6.25	87.50	
	12.5	87.50	3.125	93.75	
All autosyndetic (*alloploid*)	0	100.0	0.00	100.00	AA & BB only

Note 3 things from this table:

- The values above the dashed line are mathematically possible, but they are not biologically possible, as they imply that homoeologous chromosomes pair preferentially.
- For each 1% increase in autosyndetic pairing, there is a 0.25% decrease in the recovery of recessives in the backcross progeny.
- For each 1% increase in autosyndesis, there is a 0.5 increase in the recovery of progeny in the backcross generation that received an Aa gamete from the F1 parent.
- These relationships can be expressed by the formula: $t_1 = 1 - {}^{4x}/_{n}$, where
    - t_1 = the proportion of autosyndesis for first backcross data,
    - x = the number of observed recessives, and
    - n = the total number of backcross progeny

Burnham, 1962 using data from **Emerson, 1929**

"Data from the 4x hybrid of recessive maize × perennial teosinte backcrossed to the recessive."

Gene:	Total seeds or plants	Observed recessives		t_1 values (autosyndesis)
		#	%	
g	208	12	5.8	.77
lg	25	1	4.0	.84
su	58	5	8.6	.66
wx	113	11	9.7	.61
TOTAL	404	29	7.2	.71
Expected if pairing is random (*autoploid*):			16.7	.33
Expected if pairing is all autosyndetic (*alloploid*):			0.0	1.00

In the above example, several items become evident:

- All t_1 values are about halfway to the extremes of 0.33 and 1.00
- All % recessives are about halfway to the extremes of 0.0 and 16

To summarize:

	t_1	% autosyndesis MM + TT	% allosyndesis MT + MT
Observed	.71	71	29
Expected for autosyndesis	.33	33	67
Expected for allosyndesis	1.00	100	0

t_1 based on total for all loci tested = 0.71

- This means that 71% (vs. 33% expected) of II were of the autosyndetic type, i.e., MM + TT
- 29% (vs 67% expected) of II were allosyndetic, i.e., MT + MT

Segmental vs genomic allopolyploidy

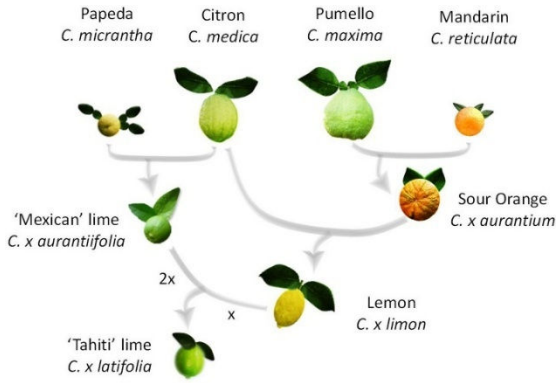
- NOTE– chromosomes which can pair, will not diverge from each other
- Divergence begins once they can no longer pair

Tau statistic

Ahmed et al, 2020

- % heterozygous 2x gametes
  - Calculated as average of all molecular markers on a chromosome
- τ = % of gametes derived from random meiotic pairing = tetrasomic
  - Calculated from markers linked to the centromere
    - 1 = full tetrasomic
    - 0 = full disomic
  - Preferential pairing (PP) = 1-τ
  - β = % double reduction → calculated from telomeric markers

An example from 'Giant Key' lime, which is a 4x version of 'Mexican lime, an F1 interspecific hybrid.



Chromosome	Total number of markers	NDM	Markers in excess of	
			C. micrantha allele	*C. medica* allele
Chr1	19	0	0	19
Chr2	19	0	3	16
Chr3	25	0	1	24
Chr4	19	1	4	14
Chr5	14	0	14	0
Chr6	13	0	0	13
Chr7	14	0	14	0
Chr8	15	7	2	6
Chr9	20	2	0	18
Total	158	10	38	110

NDM, number of markers with no deviation from the expected allelic frequencies in homozygous gametes.

- Gametes transmitted in average 91.17% of the parental interspecific *C. medica/C. micrantha* heterozygosity

Chromosome	PP	τ	DR
Chr1	0.751 ± 0.009	0.249 ± 0.009	0.136 ± 0.065
Chr2	0.82 ± 0.008	0.18 ± 0.008	0.118 ± 0.055
Chr3	0.749 ± 0.008	0.251 ± 0.008	0.167 ± 0
Chr4	0.781 ± 0.034	0.219 ± 0.034	0.084 ± 0.091
Chr5	0.633 ± 0.029	0.368 ± 0.029	0.109 ± 0.076
Chr6	0.669 ± 0.011	0.331 ± 0.011	0.007 ± 0.011
Chr7	0.733 ± 0.055	0.268 ± 0.055	0.132 ± 0.042
Chr8	0.995 ± 0	0.005 ± 0	0.167 ± 0
Chr9	0.945 ± 0.058	0.055 ± 0.058	0.167 ± 0

PP, preferential pairing; τ, tetrasomic rate; DR, double reduction.

- Chromosomes 2, 8, and 9 showed disomic segregation with high preferential pairing values
- Rest have intermediate inheritance with preference towards disomic (ie, preferential pairing) behavior

Characteristics of disomic polyploids

Genetic duplication

Stadler, 1929

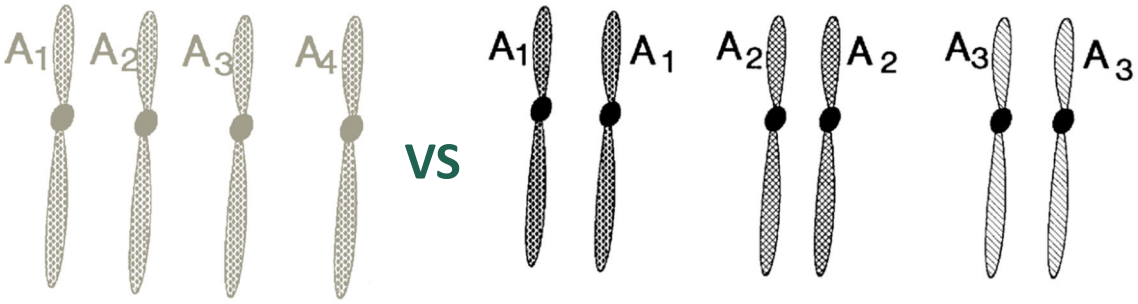
Must get mutations at all homoeologous loci for recessive trait to be expressed

Mutation rate per γ unit per million

2x	4x	6x
10.4	2.0	0.0

Lewis John Stadler
1896-1954

Built-in heterozygosity (Intergenomic heterozygosity)

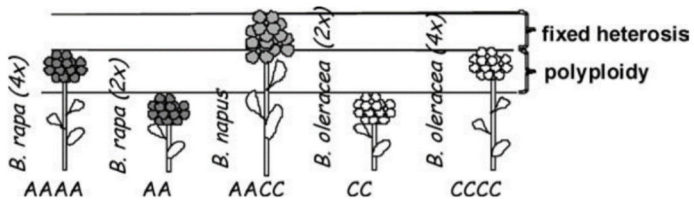


In a polysomic plant, out-breeding maintains heterozygous alleles at a locus (I.e., increased chances of at least 1 dominant allele at each locus)

In a disomic plant, inbreeding ensures homozygosity of alleles homologous chromosomes, but heterozygosity between homoeologous alleles

Eg, **Abel et al., 2005**

The concept of fixed heterozygosity in alloploids. (WP: Not convinced it is greater than the heterozygosity of autoploids)



Thus it is possible to get an inbred disomic polyploid that is equal to an F1 hybrid in performance.

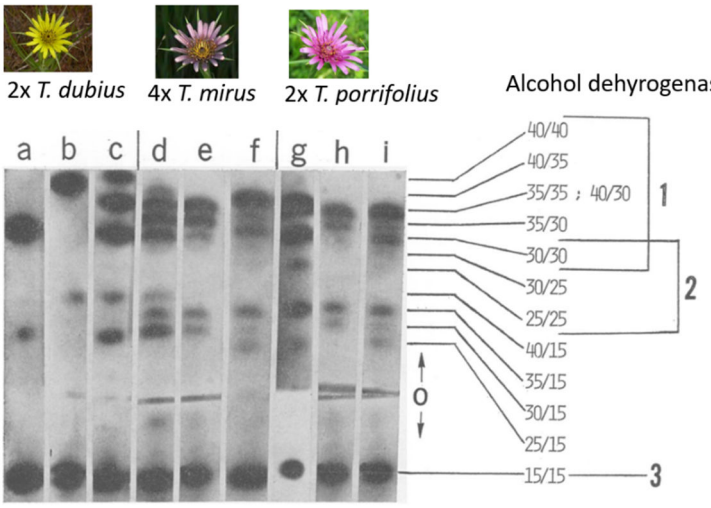
This is not possible with diploids. This is the reason that crops like hybrid wheat have never become popular—With the technology available till now, seed is expensive to produce, and breeders can derive inbreds of equal or better performance.

On the other hand, farmers cannot save seed for the next crop if plant hybrid wheat, so it is to the seed companies' benefit.

Enzymatic diversity

Eg, **Roose & Gottlieb, 1976**

Trapogon mirus and *T. miscellus*



- Show enzyme multiplicity and novel heteromeric enzymes as a consequence of tetraploidy

- This is a wonderful compromise between fitness (selfing) and flexibility (heterozygosity), that helps explain the evolutionary success of allopolyploids

Metabolic richness

Levy & Levin, 1971

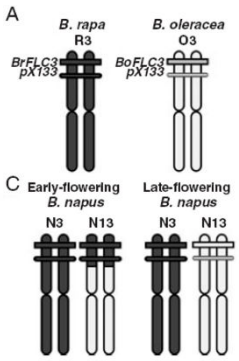
Working with *Phlox* spp

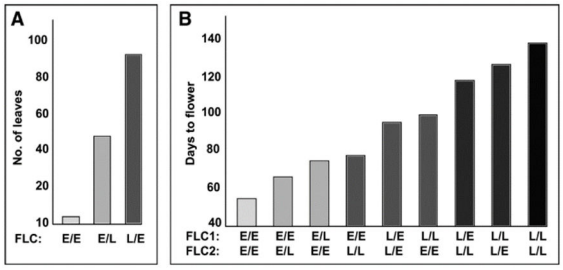
- The 4X spp make 5 flavonoids not found in the 2x spp

It follows that with more enzymes, can produce more metabolites

Phenotypic plasticity

Osborn, 2004





Note for panel: the third bar should be labeled L/L

Note that polyploidy allows multiple genotypes at a locus, and hence enhanced phenotypic diversity and thus adaptable to more ecological niches

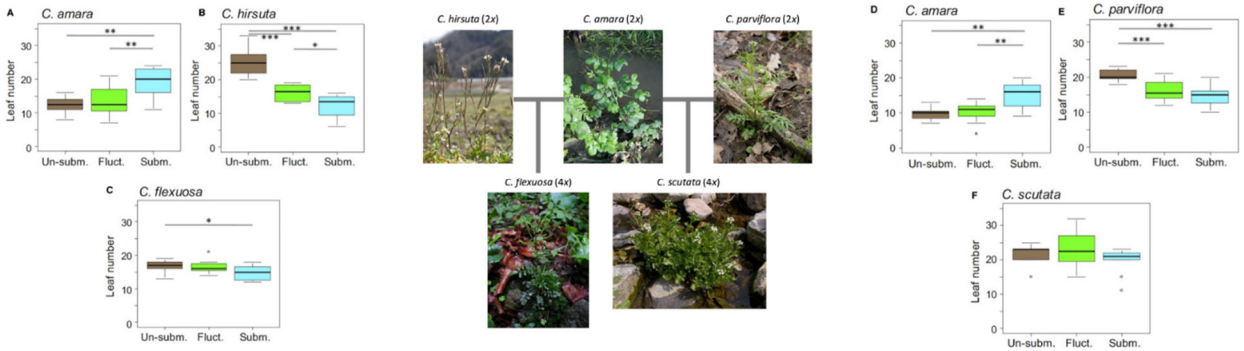
Multiple copies of FLC in polyploid *Brassica* lead to variation in flowering time from early (E) to late (L), as more allelic combinations are possible; such variation could eventually lead to reproductive isolation and speciation.

General purpose genotypes

Shimizu-Inatsugi et al, 2017

Working with *Cardamine* spp, the diploids occupy particular dry or wet niches, while the 4x derived from them can tolerate either environment

- Hence, Stebbins described allotetraploids as 'general purpose genotypes'



Distinguishing allo from autotetraploids

May have to use more than one criterion

Segregation ratios

If tetrasomic, the "2" alleles can pair with the "1" alleles

	1*AA*	4*Aa*	1*aa*
1*AA*			
4*Aa*			
1*aa*			$^1/_{20\text{-}36}$ *aaaa*

If an allo, the "2" & "1" alleles do not pair, they behave as 2 independent loci ➔ **Digenic** inheritance, ie, a 2-gene ratio

	A_1A_2	A_1a_2	a_1A_2	a_1a_2
A_1A_2				
A_1a_2				
a_1A_2				
a_1a_2				$^1/_{16}$ $a_1a_1a_2a_2$

Sample sizes necessary to test ratios

Problems

- Large number of plants required
- Tough to distinguish between a $^1/_{16}$ and a $^1/_{20}$ ratio

AAaa ⊗→ 35:1 if tetrasomic, 15:1 if digenic } Need 270 individuals to distinguish between these at p = 0.05

Backcross → 5:1 if tetrasomic, 3:1 if digenic } Need 350 individuals to distinguish between these at p = 0.05

To determine if gene is at the centromere (35:1) or at the end of a maximum equational chromosome (20:1): → 1000 plants required

To determine if the gene is in the middle of the chromosome arm (i.e., differs significantly from both ends) → need 18,000 plants

Expected ratios after selfing an autotetraploid

Barone et al., 2002

Genotype	Disomic inheritance	Tetrasomic inheritance		Distinguish auto from allo?
		Random chromosome	Random chromatid	
AAAa	1A	1A	∝, eg, 738A : 1a	No (Very tough)
AAaa	1A or 1A:1a	35A : 1a	20.8A : 1a	Yes
Aaaa	3A:1a	3A : 1a	2.5A : 1a	Very tough

Note: in $A_1A_1A_2a_2$, the homozygous recessive would never be recovered in a disomic polyploid

- Other digenic ratios: 3:1, 15:1, 63:1, 9:7, 27:37

Chromosome pairing

Jackson, 1982

Chromosomes in haploids of disomics usually do not pair at meiosis, whereas those of tetrasomics do.
E.g.,

- Haploid of wheat ~ 1 II/cell
- Haploid of potato ~ 12 II/cell

WARNING: This is not a fool proof method.

- The first haploid of potato examined had 24 I/cell, so assumed potatoes were allotetraploids. It turned out they were looking at a synaptic mutant!
- Also, knocking out the *Ph1* gene in wheat leads to pairing in the haploid

Clausen et al., 1945

Contrary to popular belief, pairing in the polyploid is not a reliable indicator either

In theory, autoploids have multivalents, and alloploids have bivalents.

- However, mutants allow multivalent formation in alloploids (eg, wheat)
- In autoploids, multivalents need not form. Eg, alfalfa, which has II formation
  - In fact, formation of II plays a role in the fertility of autotetraploids

Chromosome number

Autoploids are 2n = 4x = multiples of 4

- E.g., alfalfa =32; potato = 48

Alloploids don't have to be multiples of 4

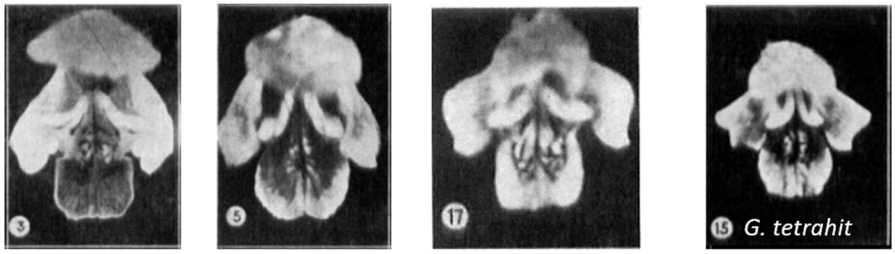
E.g., *Brassica* = 34

Resynthesizing hybrid from ancestral species

First done by **Müntzing, 1932**

Considered most valuable evidence, *if the parents still exist*

Galeopsis. pubescens × *G. speciosa* → artificial tetrahit that was like, and crossable with, *G. tetrahit*



Karyotype

- Chromosomes in set of 4's → autotetraploid
- @ least some chromosomes in sets of 2 → allotetraploid
- Asymmetry → allotetraploid

Karyotype of Milium montianum. Bennett & Bennett, 1992. Allopolyploid derived from M. vernale x unknown spp.

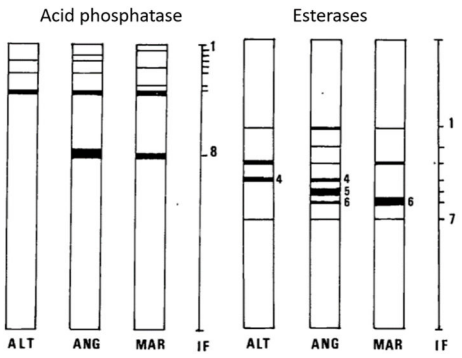
Morphology

- Alloploids resemble 2 species
- Autoploids resemble 1 species
- But
  - Differences may be obscure
  - One parent may exhibit dominance
  - **Clausen et al, 1945**
    - "Fairly safe examples of true autoploids can be recognized only in essentially monotypic genera and sections, and in those groups that have been thoroughly investigated cytogenetically"

Biochemical traits

Eg, **Guénégou et al., 1988**

E.g., allozymes: used to show that *Spartina anglicana* (2n = 4x = 122) was derived from *S. alterniflora* (2n = 62) × *S. maritima* (2n = 60)



Breeding behavior

- Self-pollinated → allo
- Cross pollinated → auto or allo

Barrington, 2007

- 36% of 2x spp. are self-pollinated
- 53% of polyploids are self-pollinated
- Caveat- Did not distinguish between allo and autoploidy

Many if not most alloploids are self-pollinated species

- Some, while considered selfers, have high degrees of outcrossing (e.g., cotton)
- A very few are outcrossers
  - e.g., white clover, tall fescue, switchgrass

Perennial polyploids that are selfers are allopolyploid

- E.g., tobacco, coffee, cotton

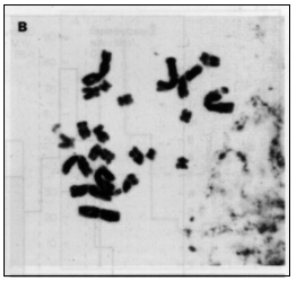
This is in contrast to autopolyploids, which are always cross pollinated, barring small populations

- Selfed progeny have high inbreeding and tend to not survive
- Outcrossing is believed to be necessary to maintain maximum heterozygosity in tetrasomic plants and help limit inbreeding
- In allopolyploids, self-pollination may help maintain control of autosyndesis

Nuclear architecture & 2° associations

SECONDARY ASSOCIATIONS

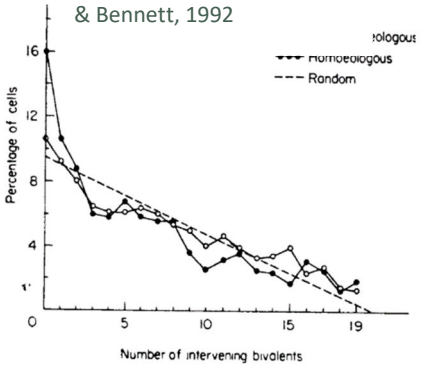
- Refers to the fact that homoeologs are closer together in the nucleus than expected out of chance alone
- May be due to the presence of nuclear domains



Secondary associations visible in Milium montianum. Bennet & Bennett, 1992

Kempanna and Riley, 1964

Used heteromorphic pairs of homoeologs (chromosomes 1A and 1B) and of non homoeologs (chromosomes 1A & 2B; 1B & 2B) and measured the distance between them by counting the number of the remaining 19 II between the marked pairs at M I.

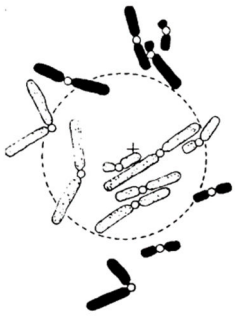


- The distance between the non-homoeologs fit the expected random curve very well
- The homoeologous pair, however, was closer than predicted through random chance alone

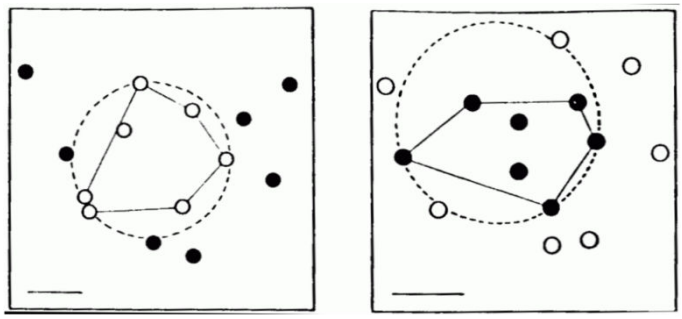
Bennett, 1984; 1987

Possible explanation for 2° associations based on nuclear architecture

- Used 3-D computer reconstructions of EM sections through a nucleus
- In allopolyploids, each parental genome can occupy its own domain
  - usually arranged as concentric spheres or adjacent spheres (concentric or lateral domains)



Root tip chromosomes of an F1 between barley (stippled) and H. bulbosum (solid). Bennett, 1984



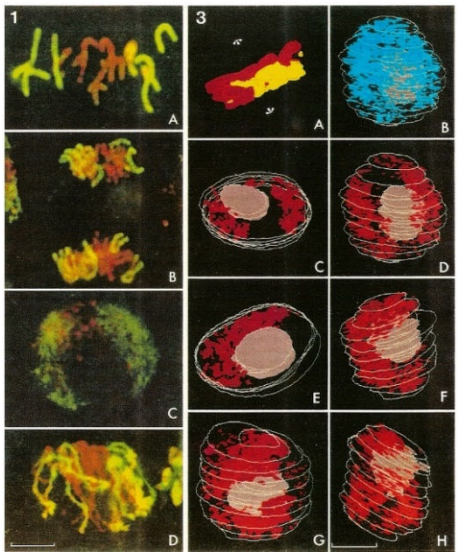
Hordeum (white circle) & Secale (black circle) centromeres R: H. vulgare x S. africanum L: H. chilense x S. africanum (Bennett, 1987)

Swarzachter, Leitch, Bennett, & Heslop-Harrison, 1989

Looked at a hybrid between *Secale africanum* and *Hordeum chilense*

Found a probe from *S. africanum* that would hybridize exclusively to the *Secale* chromosomes.

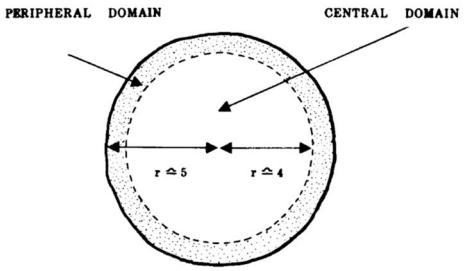
- Biotinylated the probe and hybridized it to the chromosomes
- It gave all *Secale* chromosomes a yellow color.
- Counterstained with fluorescein, which gave all *Hordeum* chromosomes a red color
  - Thus it was possible to distinguish the chromosomes from both species



Found that the chromosomes from both species did not mix

- Each genome occupied a separate domain
- Also verified the Rabl configuration

Bennett, 1987



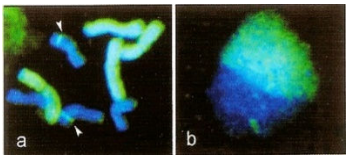
CENTRAL DOMAIN

- More condensation of chromosomes at mitotic metaphase
- Less effect of genes here on plant phenotype
- NOR genes tend to be expressed

PERIPHERAL DOMAIN

- Chromosomes tend to condense less at mitotic metaphase
- Genes here tend to dominate plant phenotype
- NOR genes here tend to be repressed
  - Alternatively, NOR genes may be eliminated altogether from one genome

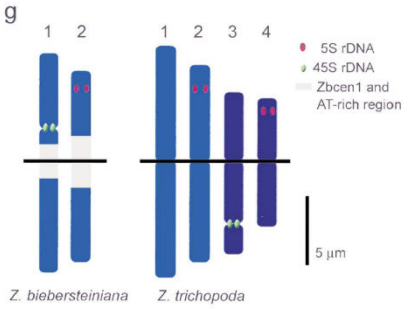
Kotseruba et al., 2003



- *Zingeria bierbersteiniana* → 2n = 2x = 4
- *Z. trichopoda* → 2n = 4x = 8, an alloploid between *Z. bierbersteiniana* and an unknown sp.

First, note presence of domains in *Z. trichopoda,* seen in a metaphase spread and in an interphase nucleus, after staining with a *Z. bierbersteiniana*-specific probe

- C & D = *Z. bierbersteiniana*.
  - In D, the 5S rDNA loci are stained in red, the 45S rDNA is stained in yellow
- E & F = *Z. trichopoda*, with the *Z. bierbersteiniana* chromosomes in dark blue
- Only 1 pair of homologues has 5S rDNA loci on them
- In F, the 5S rDNA loci are stained in red, the 45S rDNA is stained in yellow



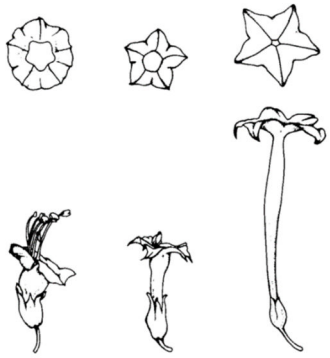
The 45S loci from Z. bierbersteiniana have been lost in the allotetraploid.

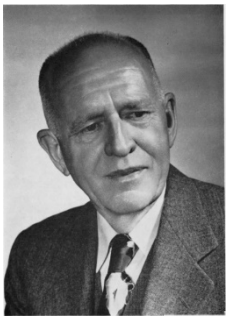
Take home message: The 45S loci from *Z. bierbersteiniana* have been lost in the allotetraploid.

Identification of progenitor species

Chromosome pairing

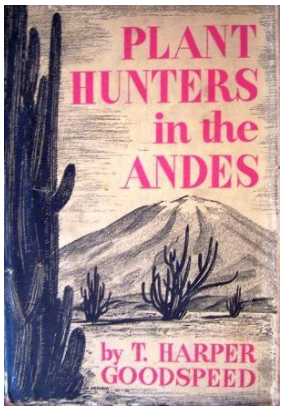
Goodspeed & Clausen, 1927; 1928
Clausen 1932





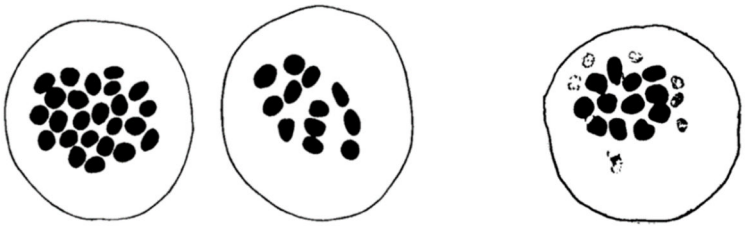
Roy Elwood Clausen
(1891-1956)

Thomas Harper Goodspeed
(1887-1967)



Tobacco and its putative ancestors. *Nicotiana tomentosa* (2x = 24); *N. tabacum* (4x = 48); *N. sylvestris* (2x = 24). Goodspeed, 1954.

N. tabacum	×	*N. sylvestris*	→	F1 = 12 II + 12 I
n = 24		n = 12		n = 18
TTSS		SS		TSS



N. tabacum (24 II) and N. sylvestris (12 II). Right: N. sylvestris × N. tabacum (12 II + 12 I)

N. tabacum	×	*N. tomentosa*	→	F1 = 12 II + 12 I
n = 24		n = 12		n = 18
TTSS		T'T'		TT'S

Left: N. tabacum (24 II); Right: N. tabacum x N. tomentosa (12 II + 12 I).

N. sylvestris	×	*N. tomentosa*	→ F1 = 24 I
n = 12		n = 12	n = 24
SS		T'T'	T'S
			↓ doubling
			T'T'SS → 24II (amphidiploid)

N. tabacum	×	amphidiploid	→ F1 = 24 II
n = 24		n = 24	n = 24
TTSS		T'T'SS	

These results suggest that *N. sylvestris* and *N. tomentosa* are the ancestors of tobacco

- However, the F_2 segregates, suggesting that all genes in the amphidiploid are not identical to those in tobacco

Gerstel, 1963

- Made hybrids between tobacco and *N. tomentosa*, *N. otophora*, and *N. tomentosiformis*, all of which are T'T'
- This gave him TT'S hybrids → doubled to get TTT'T'SS → ⊗

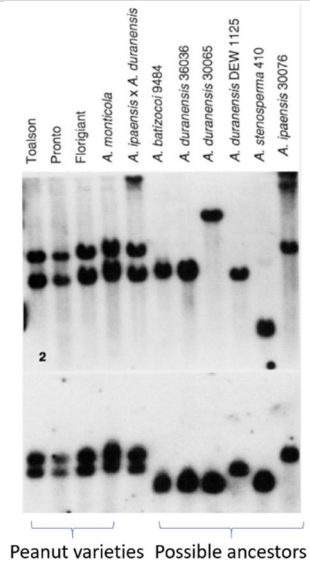
Then:

- If autopolyploid, should segregate 35:1 (want this) from genes on T genome
- If allopolyploid, should get ∞:0.

N. tomentosiformis gave the best results in terms of phenotypic resemblance, so considered to be the most likely ancestral species.

- In this case, *sylvestris* is the pivotal genome, i.e., the genome that changed the least

Molecular markers



Kochert et al., 1996

Idea was to find RFLP markers in possible diploid progenitors, which match those in the tetraploid

- Technique would be most effective for recently derived tetraploids, before markers have a chance to diverge.

In this example, the non-pivotal genome of peanut had been difficult to identify. It now appears that peanut was derived from a cross between *Arachis duranensis* × *A. ipanensis*

Genomic In Situ Hybridization (GISH)

Makes use of repetitive DNA that has colonized the genome of a specific species

- Again, only works with recently derived tetraploids.
- Eventually, the repetitive DNA will colonize all genomes of the polyploid, and this technique will no longer work

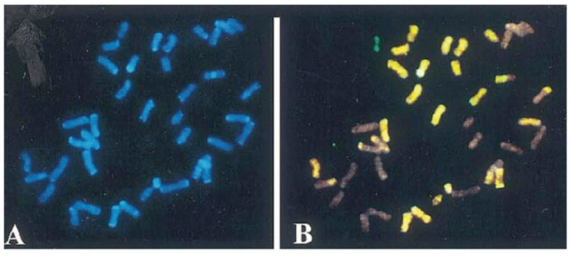
Brysting et al., 2000

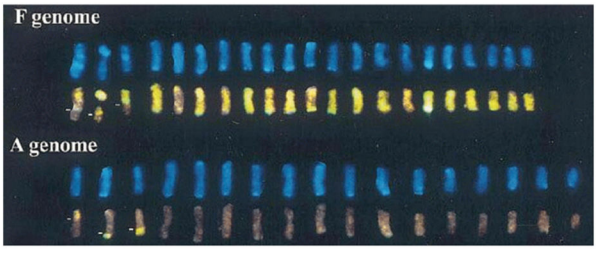
Figure below is the original karyotype for a grass from Scandinavia, *Poa jemtlandica*, 2n = 38, thought to be allopolyploid between *P. alpina* and *P. flexuosa*, based on its intermediate morphology and isozyme variation. It only has vegetative propagation.

Karyotype of Poa jemtlandica, 2n = 38, ¿allopolyploid between P. alpina & P. flexuosa?

- Figure A below left shows the chromosomes from *Poa jemtlandica*
- B shows the same spread after hybridization with DNA from *P. flexuosa*, the chromosomes of which are glowing yellow.

Poa jemtlandica *Poa jemtlandica + P. flexuosa DNA*



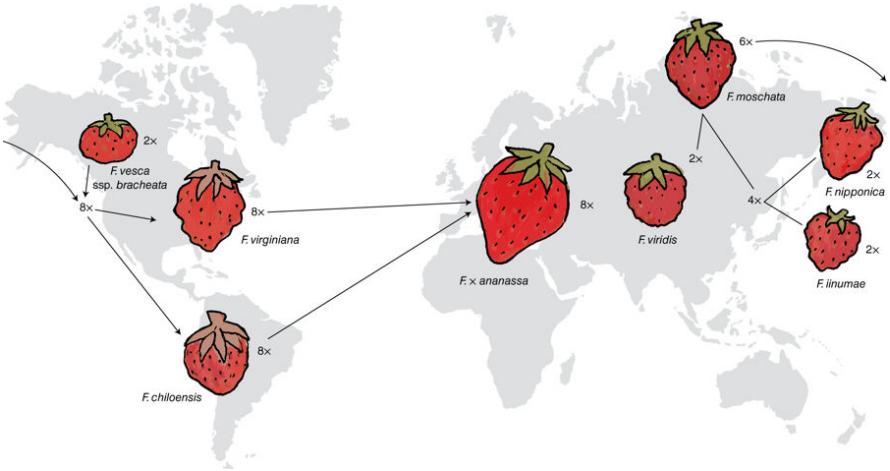


Above right is the revised karyotype, showing the two genomes

- Note the presence of 3 reciprocal translocations between genomes

Genome sequencing

Eg., Edger et al, 2019



https://www.nature.com/articles/s41588-019-0365-3/figures/1

2x *F. nipponica* × 2x *F. iinumae* → 4x sp. × 2x *F. viridis* → 6x *F. moschata* × 2x *F. vesca* → *8x F. virginiana* & 8x *F. chiloense* which crosses to get *F. ×ananassa* in the 1740's

Determining the maternal parent

Usually determined based on chloroplast sequences

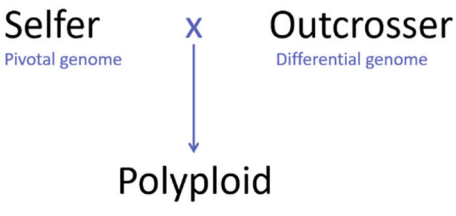
- Most of the time, angiosperms have maternal inheritance of the chloroplast
-

What happens during allopolyploidization?

Concept of the pivotal genome

Mac Key, 1970

Mirzaghaderi & Mason, 2017



The genome from the outcrosser has the heterozygosity that permits it to change and adapt to the genome of the selfer

- It can change so much that the progenitor species becomes difficult to identify
- The genome from the self-pollinated species remains constant, much like a pivot
  - One can still identify the progenitor species. Hence, it is called the pivotal genome
- Reason that the origin of the B genome in wheat has been so difficult to identify

Rapid effects of allopolyploidization

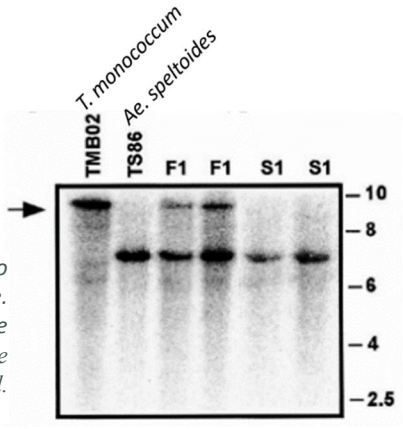
There are both rapid and long-term effects of polyploidization, with rapid effects occurring immediately after polyploidization and long-term effects occurring during the life of the polyploid

- Includes rapid changes in gene expression, with both genetic and epigenetic effects

Loss/ gain of AFLP or RFLP fragments in polyploid relative to parents

Ozkan et al., 2001

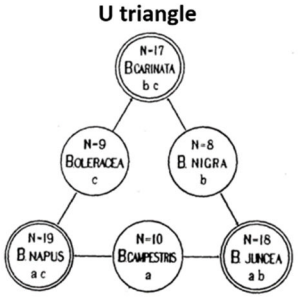
Loss of *T. monococcum* DNA in the S1 generation



Ozcan et al., 2001. DNA gel blot of the chromosome-specific sequence WPG90 to genomic DNA from the F1 between T. monococcum ssp aegilopoides (TMB02) and Ae. speltoides (TS86) from the S1 generation of the allotetraploid. The arrow indicates the band from the genome of TMB02 that disappeared in the S_1 generation of the allopolyploid.

U, 1935

Relationship between the 2x and allo4x species of *Brassica*



Song et al., 1995 (Osborn lab)

Had RFLPs of the diploid *Brassica* species

- Resynthesized the amphidiploid species
- Selfed the new plants to the F5 generation

				Types of Fragment Changes						
					Loss/Gain				Loss	Gain
Line	# Plants	# Probes	# Probes detecting changes	# Fragments changed	A	B	C	Shared Fragments	F_2 Fragments	Novel Fragments
AB F_5	9	82	43	96	9/13	25/12		9/1	9	19
BA F_5	9	82	59	95	8/12	14/0		4/1	5	51
AC F_5	9	89	23	38	7/1		19/4	3/0	4	1
CA F_5	9	89	31	51	15/1		16/5	7/0	3	4

Where: Loss = fragments present in parents and F_2, but not F_5
Gain = diploid parental fragments absent in F_2 but present in F_5
F_2 fragments = fragments found in the F_2 but not in either diploid parent
Novel fragments = fragments found only in F_5 plants

Liu et al, 1998

And to show this also happens in grasses:

Amphiploid	Generation	# seqs. studied # polymorphic	# seqs. changed	Loss	Loss + gain of new band
SSAA	S_6	9/6	6	5	1
SSDD	S_3	9/7	7	4	3
AABBDD	S_5	9/7	7	6	1
AABBDD	S_5	9/8	8	7	1
AABBDDSS	S_3	9/8	8	8	0

Loss/gain of DNA after allopolyploidization is

Rapid

Levy & Feldman, 2004

Starts in F1 hybrid and usually completed by second or third alloploid generation

Extensive

14% of chromosome-specific and genome-specific sequences lost in allotetraploid wheat

Nonrandom & repeatable

Blanc & Wolfe, 2004

- Serves to increase differences between homoeologues
- Facilitates disomic pairing, hence ensuring fertility
- Changes are non-random and repeatable

- Duplicate genes involved in transcription, signal transduction, metabolism, and regulatory functions preferentially retained
- All members of a network or pathway tend to be preferentially retained or lost as a group = "concerted divergence"
- Duplicate genes involved in DNA repair, defense, and encode transmembrane receptors or organellar proteins preferentially lost

Retrotransposon activation

Gu et al., 2004

Genetic changes can also involve disruption of genes through retrotransposon activation and gene conversion

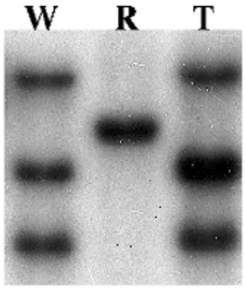
- 3 genes in the glutenin locus in hexaploid wheat disrupted by insertion of retrotransposon

Preferential loss of DNA from one parental genome

Jones & Flavell, 1982
Ma et al., 2004

In triticale (rye × wheat)

- 70% of repetitive DNA from rye is lost
- including subtelomeric heterochromatic repeats
- ~70% AFLP fragments from rye subgenome lost
- compared to only ~26% of fragments from wheat subgenome



The figure shows probe CDO595 present in all 3 wheat (Chinese Spring) genomes and in rye (Imperial) but not in the amphiploid. Ma et al., 2004

Silencing

Comai et al., 2000, 2003

Obtained allo4x plants from auto4x *Arabidopsis thaliana* × 4x *Cardaminopsis arenosa*, thus recreating *A. suecica*

- Looked at expression of 700 genes, and found 20 (0.4%) to be silenced

Wang et al., 2004

In allotetraploid *Arabidopsis suecica* (*A. thaliana* × *A. arenosa*) about 1% of the transcriptome silenced and 1.3% silenced in more than one independent line

He et al., 2003

Expression differences between synthetic hexaploid wheat and the parental *Aegilops tauschii* and *Triticum turgidum*

- Data are derived from cDNA and AFLP display, and given as percentage of reduced or induced polymorphic bands

	Origin		
	DD	AABB	Total
Bands Scored	1650	1750	2800
Bands polymorphic between two parents	1050	1150	2200
Bands reduced or missing in hexaploid	122 (11.6%)	38 (3.3%)	160 (7.3%)
Bands induced in hexaploid		8 (0.4%)	

Involves nucleolar dominance

Genes of both parental species are not necessarily equally expressed

- Particularly true of the nucleolus

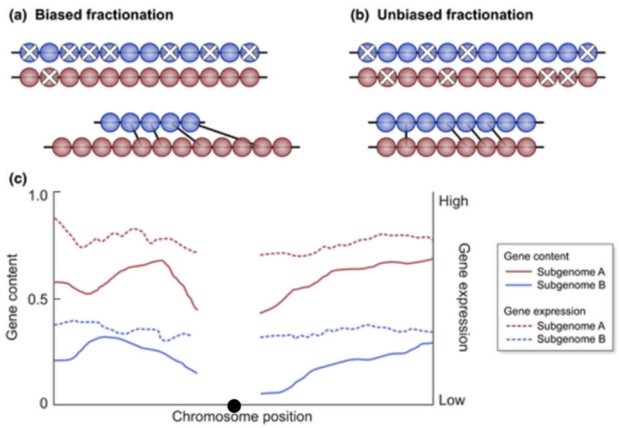
Plant	Reference	Effect
allotetraploid *Arabidopsis suecica* Genome is AACC: • AA from *A. thaliana* • CC from *A. arenosa*	**Chen et al., 1998**	In the AACC tetraploid • rDNA from *A. thaliana* silenced When backcrossed to both parents to get ACCC or AAAC • The rDNA from *A. arenosa* is silenced
Solanum allopolyploids from tomato × potato	**Komarova et al., 2004**	• Tomato (*S. lycopersicon*) provides 95-100% rRNA relative to potato (*S. tuberosum*)
allotetraploid cotton	**Adams et al., 2004**	• Twice as many genes from paternal subgenome silenced relative to maternal subgenome

Fractionation vs genome dominance

Bird et al (Edger lab), **2018**

In this model, biased fractionation leads to genome dominance

Note- No one has shown if this is related to nuclear architecture

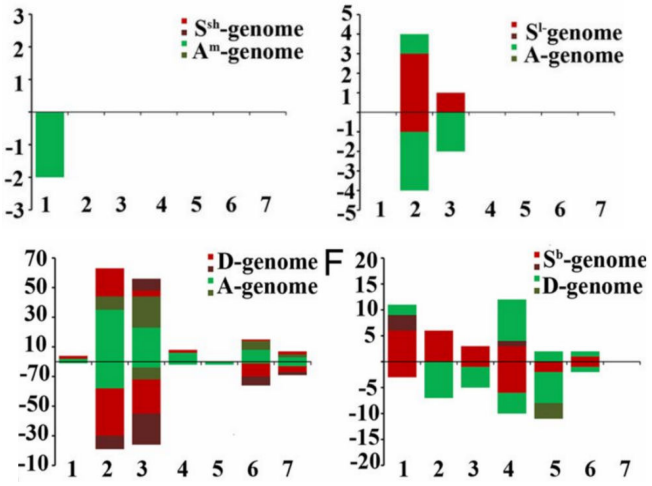


Some genomes are > compatible than others

Zhang et al, 2013

Looked at A genome × S (=B) or D genome wheat crosses

- AS crosses much more stable than AD crosses
- AS crosses show lots of intrachromosomal changes
- AD crosses show gain/loss of chromosomes and other rearrangements that lead to loss of fertility



Chromosomal gain/loss in different crosses

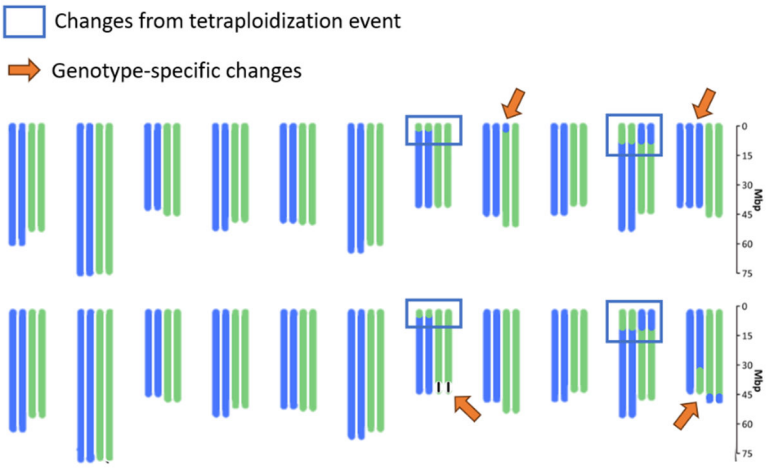
Chromosomal gain/loss and configurations comes from homoeologous pairing

- A genome has pairing genes to prevent such pairing, and thus gives rise to more stable plants
- "Therefore, it is likely that the survived and eventually established nascent polyploids are those that are able to fine-tune the balance of mutability and karyotype stability."

Instability remains in alloploids

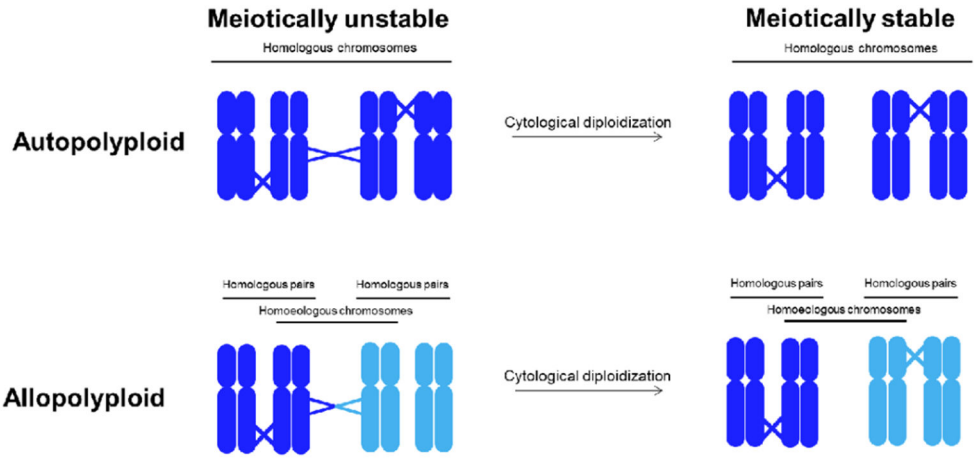
Scalabrin et al. 2024

- Alloploids can have homoeologous translocations and other genotype-specific aberrations
    - Aneuploidies, deletions, duplications and translocations



Alloploid cytological diploidization

Gonzalo, 2022



Fate of Duplicated genes

Birchler & Yang, 2022

Involves more lasting genomic changes such as duplicate gene functionalization, transposition, & introgression

Blanc and Wolfe, 2004

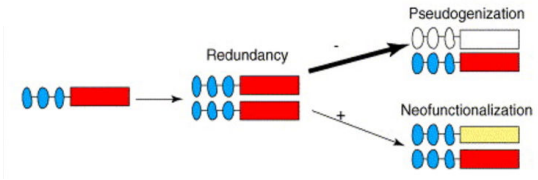
Greater than half of the gene pairs formed by most recent polyploidy event in *Arabidopsis* have significantly different gene expression patterns and 62% have undergone functional diversification

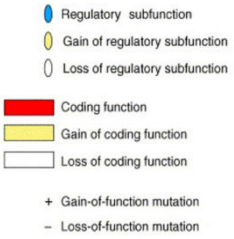
Moore & Purugganan, 2005

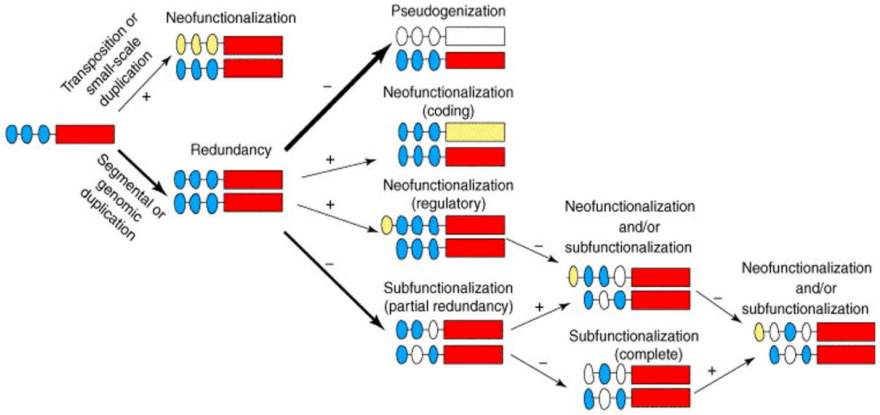
The original view was that duplicated genes would either accumulate mutations till they were non-functional, or they would evolve and acquire new functions

- In reality, there are many more possibilities
    - Changes can be in regulatory or coding sequences
    - Duplicate genes can acquire tissue-specific gene expression

Neofunctionalization– new functions due to promoter differences as opposed to new functions acquired by changes in coding sequence





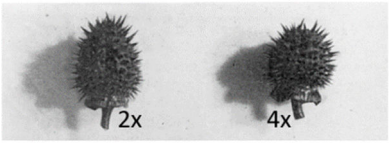


Summary

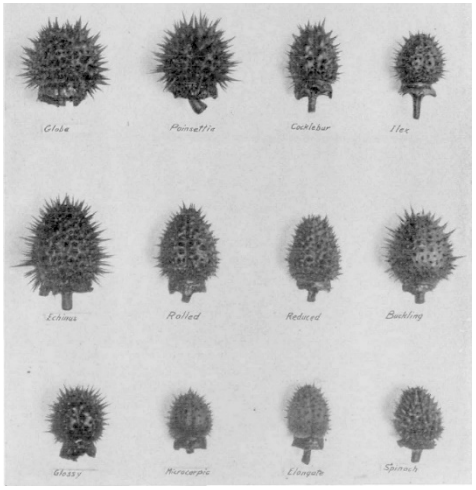
Modified from **Cheng et al, 2018**

	Needed mutations	
Phase 1: Pre-allo and continuing	• Genome differences pre-exist between progenitors	• Transposons being silenced
Phase 2: The new polyploid	• New epigenetic subgenome differences	• Permanent heterozygosity • Gene dominance • Homoeologue interference

Gene dosage & balance in plants



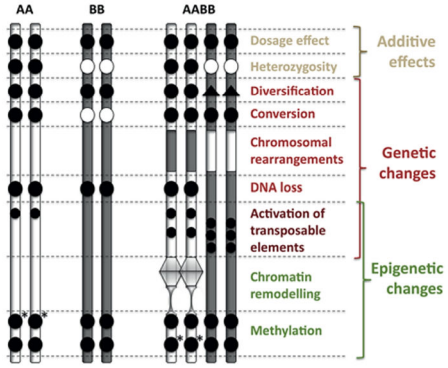
Blakeslee, 1922



Blakeslee & Belling, 1922

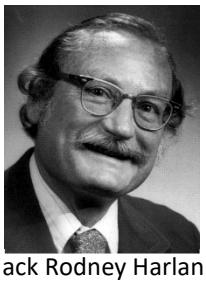
Phase 3: Moderate time post-allo and continuing	• Expression fine-tuning • Chromosomal breaks and reunions	• Dosage rebalancing by fractionation • Continued RdDM-dependent transposon and gene silencing
	• Fractionation of genes/cis-sites • Canonical mutations (SNPs and INDELs) begin	• Mutation under non-functionality • Subfunctionalization, (duplication, degeneration, complementation) • Continued homoeologue interference • Innovation, amplification and divergence
	• Neofunctionalization (rare and multiple mutations)	• Gene product neofunctionalization • Expression/regulatory neofunctionalization
Phase 4: Establishment of polyploids	• Evolution of new gene networks. • Very rare, multiple mutation combinations	• Unstable environment • Ecological niches • Adaptive radiation • Novel physiological diversifying selection

Tayalé & Parisod, 2013



Origin of neopolyploidy

Harlan and deWet, 1975


Jack Rodney Harlan
(1917-1998)

Artificial

Use of colchicine, oryzalin, N2O, wounding, cold shock = amphiploid

Somatic doubling

Winge, 1917

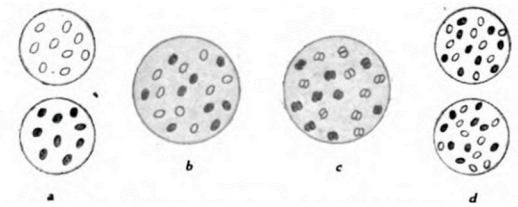
Noticed a high mortality of interspecific *Chrysanthemum* zygotes

- Reasoned that chromosomes must pair in the zygote for zygote to survive
- If chromosomes were so different that they could not pair
  - Then the only way that a zygote could survive was if chromosomes doubled in the zygote, thus having a pairing partner
- "Interspecific hybridization followed by chromosome doubling"


Öjving Winge
(1886-1964)

Little evidence to support hypothesis. It has two major problems:

- Shortage of examples: *Primula kewensis* and little else
- Inbreeding depression caused by somatic doubling
- Limited germplasm pool if only 2 gametes contributed towards formation of new population



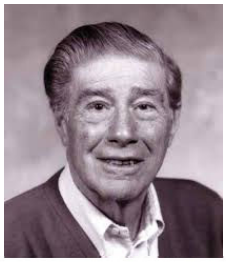
Occurrence of doubled chromosome numbers through hybridization

2n gametes

Mendiburo & Peloquin, 1976; Soltis et al, 2010

Proposed by Darlington in the 1920's, but Winge's hypothesis was too popular, and people did not take notice.

Two possible ways to get polyploids



Stanley J. Peloquin
(1921-2008)

Bilateral sexual tetraploidization

One-step

2x × 2x → 4x

Triploid bridge

Two-step

2x × 2x → 3x; 3x × 2x → 4x (probably the most common)

- The intermediary triploid is also called 'triploid bridge' after Harlan and DeWet, 1975

Unilateral sexual tetraploidization

Once the tetraploid is established, it can continue to cross with members of the population

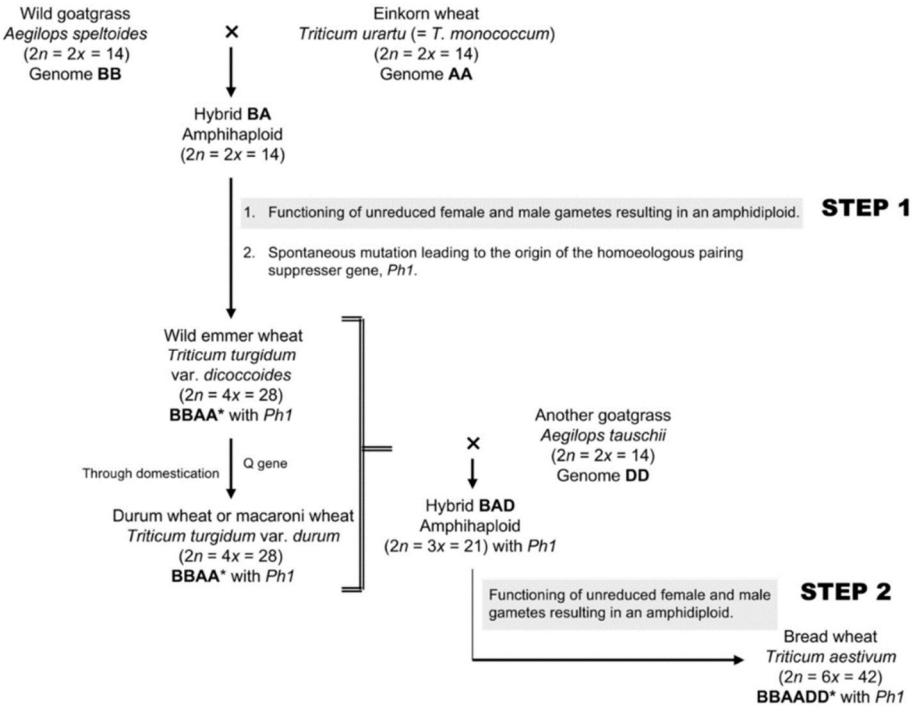
2x × 4x → 4x

- This permits germplasm to be continuously introgressed from the 2x level to the 4x level

Steps in the evolution of bread wheat

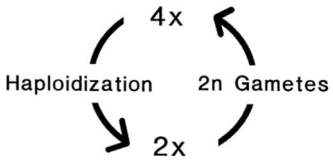
Jauhar,2007

Superimposing 2n gametes formed by interspecific hybrids, we get the following scenario for the evolution of wheat (): Q gene = free-threshing



Repeated neopolyploidization

Raven & Thompson, 1965



Proposed that polyploidy is not a 1-way street

- Sometimes 4x × 2x crosses yield 2x plants (e.g., alfalfa and potato).
- It is theoretically possible to get germplasm moving in a cycle between ploidy levels.
- So far, only shown to exist in populations of *Dicanthium*
- This works for autoploids.
    - No evidence this is the case for alloploids

Soltis, 2005

Polyploids were once thought of as a one-way street, and thus an evolutionary dead end (eg., Stebbins)

- It is now apparent that allopolyploids can form repeatedly
    - Therefore, have a lot of genetic diversity in them
- Crosses between alloploids of different origins allow for a diverse, variable, and adaptable population
- May be key for reason for their success

Hybridization without polyploidization

Pesakinskiené et al., 1996

Interspecific hybridization does not necessarily lead to allopolyploid formation

- Diploid hybrids are recovered
- However, a polyploid step may be involved

2x *Lolium multiflorum* × 6x *Festuca arundinacea*
⇓
4x hybrids
⇓
8x × 8x
⇓
3-5 % 2x plants

The 2x plants are mostly *L. multiflorum*, with some fescue segments introgressed.

- Probably a result of chromosome elimination, as with barley, or somatic reduction