Polyploidy

Discovery

Note: What was once called a polyploid is now more accurately referred to as a <u>neopolyploid</u>

https://www.hugodevriesfonds.nl/wp-content/uploads/2018/09/Oenothera_gigas_foto_groot.gif

DeVries 1900

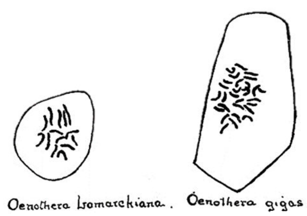
Described a large plant that appeared in *Oenothera* as a gigas (giant) mutant in 1895

- Coined the term mutation

Lutz 1907; Gates 1909

- Polyploidy discovered in *Oenothera gigas* by
    - Lutz in 1907
    - Gates in 1909 → gets the credit

Anne Mae Lutz
1871-1938



Lutz 1907

Winker 1916

Obtained the first artificial polyploid

- A 4x shoot arose from callus on a decapitated nightshade plant
- Credit for term 'polyploid'

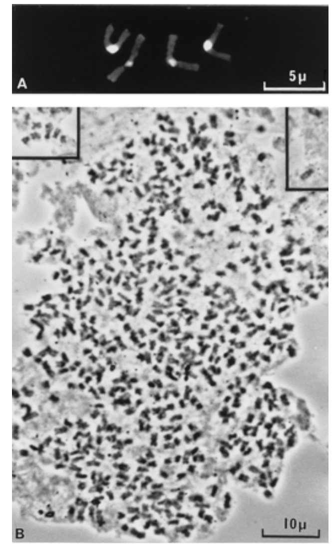
Winkler's drawing of a 4x shoot in a 2x plant.

Chromosome number range

In angiosperms, chromosome number ranges from 2n = 2x = 4 in *Zingeria bierbersteriana* & *Haplopappus gracilis* to the Madagascar palm (*Voanicola gerardii*), 2n = ~ 600

One fern species has 2n = 84x = 1260

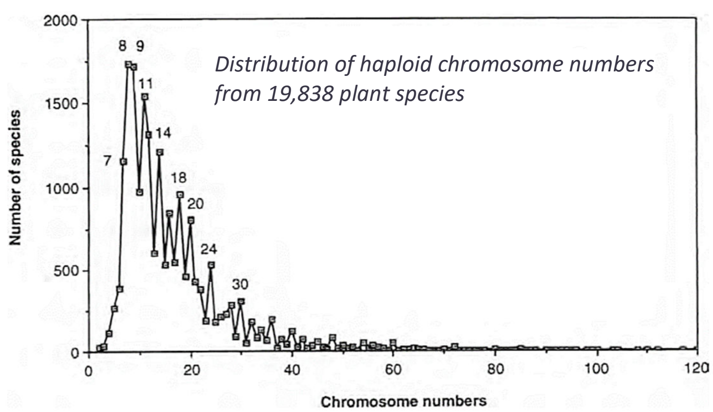
All angiosperms have polyploid origins in the distant past, and thus are known as paleoploids



A) Chromosomes of Zingeria bierbersteriana; B) Voanicola gerardii

Masterson, 1994

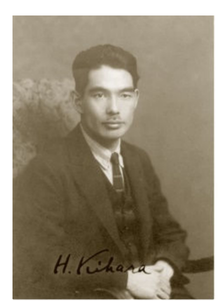
- As many as 70% of all current angiosperms are believed to be neopolyploids, meaning they have become polyploids again
  - Including most agriculturally important plants
  - Defining a polyploid can be challenging



Polyploidy types (Neopolyploidy)

Blakeslee et al, 1923
Recognized two types of polyploidy - ‘tetraploid’ & ‘double diploid’

Kihara and Ono, 1926
Called them auto and allotetraploids based on whether they were genome doubling or genome merger



Hitoshi Kihara
(1893-1986)

Doyle and Egan, 2009

Combining the cytogenetic and taxonomic definitions together:

Taxonomic definition:	Autopolyploidy	Segmental allopolyploidy	Allopolyploidy
Cytogenetic definition:	Polysomic	Mixosomic	Disomic

- Can have a taxonomic allopolyploid behave as a cytogenetic autopolyploid

Incidence

Barker et al, 2016

24% of plants are polyploid classifications

- 13% autoploid
- 11% alloploid

- Taxonomists frequently miss auto4x due to similarity with 2x
    - Auto4x arise 80x more than allo4x, but they do not survive

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"

- Said another way, classifying strictly on morphology is not reliable

Autopolyploidy

2x & 4x alfalfa

Includes alfalfa, orchardgrass, potato, birdsfoot trefoil

- **All** are cross pollinated - this appears to be an essential requirement
- Also suffer from severe inbreeding depression

Tetrasomic inheritance vs disomic (diploid) inheritance

5 possible allelic compositions at a locus

AAAA	- quadriplex
AAAa	- triplex
AAaa	- duplex
Aaaa	- simplex
aaaa	- nulliplex

Diploid: 3 genotypes possible at a locus with 2 alleles: AA, Aa, & aa

Tetrasomic: 5 genotypes possible at a locus with 2 alleles

>2 alleles are possible at a locus

- monoallelic	$A_1A_1A_1A_1$	(homozygous)
- unbalanced diallelic	$A_1A_1A_1A_2$	(heterozygous)
- balanced diallelic	$A_1A_1A_2A_2$	"
- triallelic	$A_1A_1A_2A_3$	"
- tetraallelic	$A_1A_2A_3A_4$	"

- The result is that autoploids can more easily carry deleterious alleles

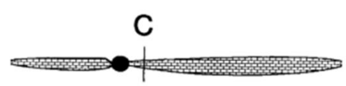
Gametes are diploid and can be heterozygous

- A_1A_1 homoallelic
- A_1A_2 heteroallelic

- Means that the gamete can transmit deleterious alleles that would have been weeded out in a truly haploid gamete

Genetic ratios are complex

Given a gene, C, near the centromere:



Cc ---> *CCcc*

	C	*c*
C		
c		¼ *cc*

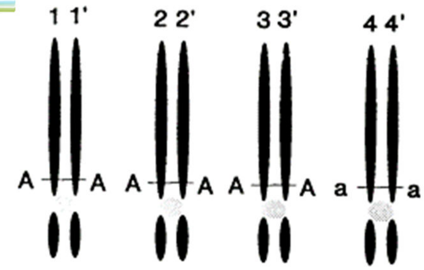
	1 *CC*	4 *Cc*	1 *cc*
1 *CC*			
4 *Cc*			
1 *cc*			$^{1}/_{36}$ *cccc*

1/4 are homozygous recessive

Only 1/36 are homozygous recessive

- The net result is that homozygotes are much more difficult to recover

Random chromosome segregation



Gene is near the centromere.

There are 4 homologous chromosomes, 8 sister chromatids:

- 24 possible combinations of chromatids can be recovered in a gamete:
    - RESTRICTION: cannot recover sister chromatids in a gamete

1-2	1'-2				
1-2'	1'-2'				
1-3	1'-3	2-3	2'-3		
1-3'	1'-3'	2-3'	2'-3'		
1-4	1'-4	2-4	2'-4	3-4	3'-4
1-4'	1'-4'	2-4'	2'-4'	3-4'	3'-4'

Chromatid combinations that can be recovered in a gamete

Gametic products	Genotype	
	AAAa	*AAaa*
AA	12	4
Aa	12	16
aa	0	4
Total:	24	24

Gametic table

Gene-centromere distance affects segregation ratios

However, most genes are not near the centromere.

Random chromosome segregation

CCcc

$\downarrow \otimes$

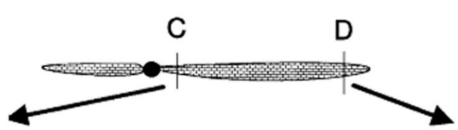
1/36 *cccc*

a) Maximal equational segregation, or
b) Random chromatid segregation

DDdd

$\downarrow \otimes$

ME:1/20.25;
RC: 1/21.8 *dddd*

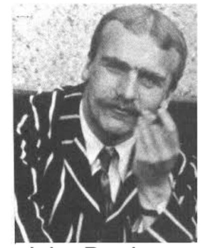


- 1/36 and 1/20.25 are the two limits
- The segregation frequency can be anywhere in between the two limits

Haldane, 1929

- The further a gene gets from the centromere, the probability increases of recovering sister alleles in a gamete, thus altering the segregation ratio

B from chromatid 1 and B from chromatid 1' are sister alleles, as they are from sister chromatids.



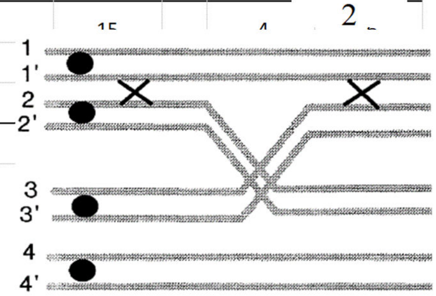
John Burdon Sanderson Haldane (1892-1920)

Can now recover in one gamete

- Sister alleles → probability = α
- Non-sister alleles → probability = 1 - α

1-1'		2-2'		3-3'		4-4'	α
1-2	1'-2						
1-2'	1'-2'						
1-3	1'-3	2-3	2'-3				
1-3'	1'-3'	2-3'	2'-3'				1-α
1-4	1'-4	2-4	2'-4	3-4	3'-4		
1-4'	1'-4'	2-4'	2'-4'	3-4'	3'-4'		

	Genotype			
Gametic products	*BBBb*		*BBbb*	
	α = 0	α = $^1/_7$	α = 0	$\frac{n\times(n-1)}{2}$
BB	12	15	4	
Bb	12			
bb	0			
Total:	24			



There are now 28 possible combinations, and $^4/_{28}$ of these are sister allele combinations: ∴ $\alpha = {^4/_{28}} = {^1/_7}$

In the Random Chromatid model, each chromosome arm can participate in an ∞ number of crossover events

- Notice that chromatid 1' is involved in crossover events with chromatids & 3 in the diagram

Such multiple crossovers are not possible in many plants, especially ones with short chromosomes and low frequencies of chiasmata formation

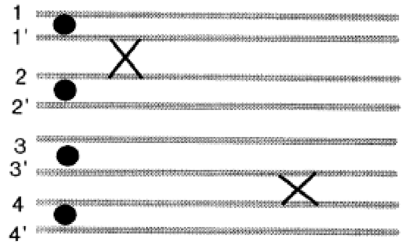
Mather (1935) proposed an alternative model, limiting each chromosome to 1 crossover

Maximum equational segregation

Mather, 1935

The figure shows only one of three possible crossover combinations

- The others are chromosomes 1-3 & 2-4, and 1-4 & 2-3.



In the Maximum Equational model, each chromosome is limited to 1 crossover

Sir Kenneth Mather
(1911-1990)

This restriction prevents the recovery of the 1-2, 3-4, 3'-4', and 1'-2' in a given gamete.

- Thus, we are back to 24 possible combinations of chromatids, and $^4/_{24}$ are sister allele combinations.
- Now $\alpha = {}^4/_{24} = {}^1/_6$.

1-1'		2-2'		3-3'		4-4'	α
~~1-2~~	1'-2						
1-2'	~~1'-2'~~						
1-3	1'-3	2-3	2'-3				
1-3'	1'-3'	2-3'	2'-3'				1-α
1-4	1'-4	2-4	2'-4	~~3-4~~	3'-4		
1-4'	1'-4'	2-4'	2'-4'	3-4'	~~3'-4'~~		

Gametic products	Genotype					
	BBBb			*BBbb*		
	$\alpha = 0$	$\alpha = {}^1/_7$	$\alpha = {}^1/_6$	$\alpha = 0$	$\alpha = {}^1/_7$	$\alpha = {}^1/_6$
BB	12 (1)	15	13	4 (1)	6 (3)	5.33 (2)
Bb	12 (1)	12	10	16 (4)	16 (8)	13.33 (5)
bb	0	1	1	4 (1)	6 (3)	5.33 (2)
Total:	24	28	24	24	28	24

Summary

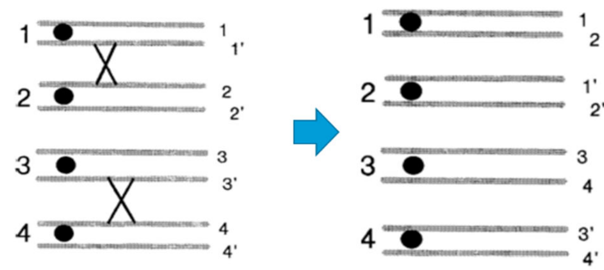
Finally, adding formulae to the table

Gametic products	Genotype							
	BBBb				*BBbb*			
	$\alpha = 0$	$\alpha = {}^1/_7$	$\alpha = {}^1/_6$	Formulae	$\alpha = 0$	$\alpha = {}^1/_7$	$\alpha = {}^1/_6$	Formulae
BB	12 (1)	15	13	${}^1/_2 + {}^1/_4\,\alpha$	4 (1)	6 (3)	5.33 (2)	${}^1/_6 + {}^1/_3\,\alpha$
Bb	12 (1)	12	10	${}^1/_2 - {}^2/_4\,\alpha$	16 (4)	16 (8)	13.33 (5)	${}^4/_6 - {}^2/_3\,\alpha$
bb	0	1	1	$+ {}^1/_4\,\alpha$	4 (1)	6 (3)	5.33 (2)	${}^1/_6 + {}^1/_3\,\alpha$
Total:	24	28	24		24	28	24	

Maximum equational

Note that equational chromosome formation is maximized at 1 CO

- Hence, Mather's <u>Maximum Equational</u> model



As the number of crossovers approaches ∞, the frequency of equational separations drops down from one, and approaches $^6/_7$, as long as each chromosome is free to crossover with its other 3 homologues, as per the Haldane model.

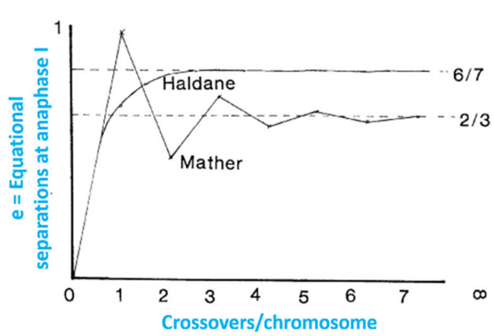
However, if each chromosome is restricted to crossing over with just one other of its homologues (i.e., the Mather model), as the number of crossovers approaches ∞, the number of equational chromosomes formed drops from 1 and approaches $^2/_3$.

Haldane

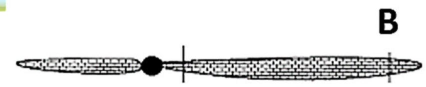
Crossovers per chromosome	Total arrangements	Anaphase I separations		
		Reductional	Equational	Proportion equational
0	4	4	0	0.0
0.5	96	48	48	0.5
1.0	2,304	672	1,632	0.708
1.5	55,296	11,328	43,968	0.795
2.0	1,327,104	223,872	1,103,232	0.8313
2.5	31,850,496	4,892,928	26,957,568	0.84638
3.0	764,411,904	112,630,272	651,781,632	0.85266
3.5	18,345,885,696	2,655,126,528	15,690,759,168	0.85527
∞	∞	$^1/_7$	$^6/_7$	$^6/_7$ = 0.85714
n	4×24^{2n}	$(4 \times 24^{2n}) \times [^1/_7\{1+6(^5/_{12})^{2n}\}]$	$(4 \times 24^{2n}) \times [^6/_7\{1-(^5/_{12})^{2n}\}]$	$^6/_7[1-(^5/_{12})^{2n}]$

Mather

Crossovers per chromosome	Anaphase I separations		
	Reductional	Equational	Proportion equational
0	1	0	0
1	0	1	1
2	$^1/_2$	$^1/_2$	0.5
3	$^1/_4$	$^3/_4$	0.75
4	$^3/_8$	$^5/_8$	0.625
5	$^5/_{16}$	$^{11}/_{16}$	0.688
6	$^{11}/_{32}$	$^{21}/_{32}$	0.656
7	$^{21}/_{64}$	$^{43}/_{64}$	0.672
8	$^{43}/_{128}$	$^{85}/_{128}$	0.6641
∞	$^1/_3$	$^2/_3$	0.6667
n	$1-^2/_3[1-(-^1/_2)^{n/2}]$		$^2/_3[1-(-^1/_2)^{n/2}]$



This is what normally happens in diploid organisms, in which there are only 2 homologues to begin with

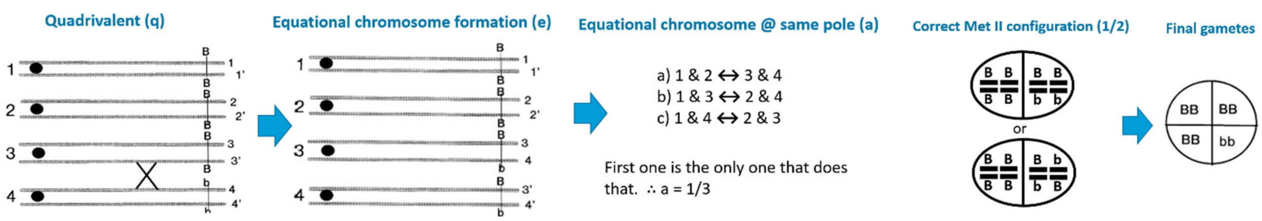


Double reduction

Blakeslee, Belling, & Farnham, 1923

<u>Double reduction</u>: The ability to recover *bbbb* (nulliplex) genotypes from a cross of *BBBb* × *bbbb*

- Due to the recovery of sister alleles in a gamete (in other words, α).
- So, for double reduction to occur:
    - Must get formation of a quadrivalent (q) (i.e., must have 4 copies of a given chromosome)
    - Formation of equational chromosomes (e).
        - This requires a crossover between gene pair and centromere, leading to the formation of an equational chromosome pair
    - There are 3 possible Ana I disjunctions for the quadrivalent
        - Only 1 results in the equational chromosomes at the same pole
        - Thus a = $^1/_3$
    - Then there are 2 possible Met II orientations, so this value is always $^1/_2$



Burnham, 1962

Detection of double reduction depends on

- Sample size of progeny
- Frequency of IV formation [really, 2 II's at random]
- Type and frequency of centromere segregation
- Gene-centromere distance
    - α= 0 at the centromere, =$^1/_6$ or $^1/_7$ at telomere, with a continuum in between

In summary

α	=	q	×	e	×	a	×	½		
Mather	=	1		1		$^1/_3$		½	=	$^1/_6$
Haldane	=	1		$^6/_7$		$^1/_3$		½	=	$^1/_7$

Where $^1/_6$ = 0.1666 and $^1/_7$ = 0.142857

Haynes & Douches, 1993

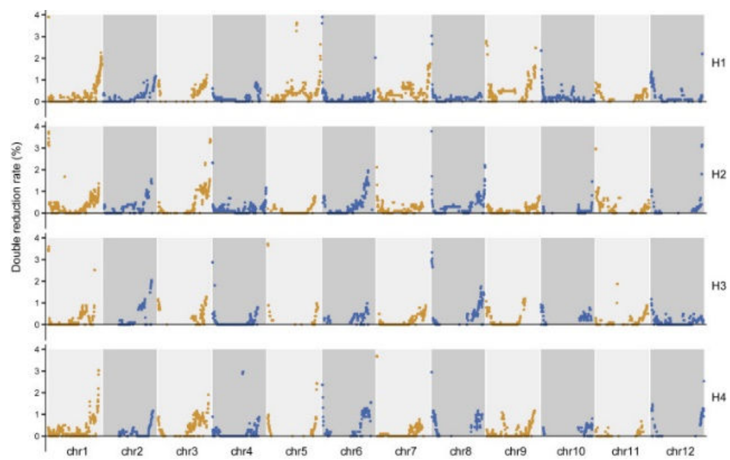
Measuring α as determined from 4x-2x crosses in potato

- Note that α decreases as gene gets closer to the centromere

Locus	Total progeny	Double reduction products	α	±SE	Gene-centromere distance (cM)
Mdh-1	283	7	0.099*	0.037	33.5
6-Pgdh-3	214	7	0.131*	0.049	30.1
Pgi-1	122	3	0.098ns	0.057	26.0
Idh-1	314	2	0.025ns	0.018	18.4
Mdh-2	144	1	0.063ns	0.044	n/a

Bao et al, 2022

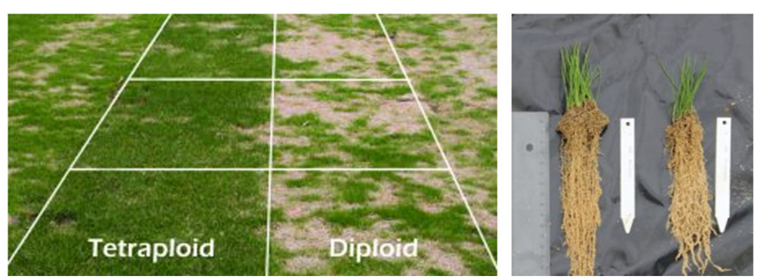
Used genome sequencing to measure the number of double reduction events represented in one genotype of potato



Why autotetraploids?

- The gigas effect – auto4x found in nature are much larger and more vigorous than their diploid counter parts
- Upon the discovery of colchicine in the 1920's and 30's, there was a lot of speculation that the creation of auto4x crops would lead to great yield advances
- But, raw auto4x plants are slow growing, low yielding, and low in fertility
- Consequently, autotetraploid breeding fell out of favor

Auto4x and 2x perennial ryegrass (*Lolium perenne*)



http://www.greenkeepingeu.com/greenkeeping-feature-tetraploid-perennial-ryegrass-technology-explained/

Which diploids make good autotetraploids?

Levan, 1942; Åkerberg et al., 1961

Raw autotetraploids suffer from inbreeding and from low fertility

- The fact that chromosome doubling leads to inbreeding was not recognized until much later

2x and auto4x daylilies. https://plantlet.org/autopolyploidy-multiplying-same-genome/

Identified criteria from to predict if an artificial autotetraploid is likely to be successful

- A starting diploid that
  - has a low chromosome number
  - is allogamous
  - grown for foliage or vegetative tissues
- The inbreeding depression data explain why the crop should be allogamous rather than autogamous
- Plants with low chromosome number have fewer fertility issues, but still have low seed set.
  - The lower seed set are why crops grown for foliage or vegetative tissues work best

Inbreeding at the tetrasomic level

All known autotetraploids are cross pollinated. Though selfing can occur, seedlings from selfing usually do not survive, and all auto 4x plants suffer greatly from inbreeding.

F = the coefficient of inbreeding, and is defined as the probability that two alleles are identical by descent. For example, for $A_1A_1A_2A_2$, obtained by doubling A_1A_2, both copies of A_1 are descended from the same allele, making them identical by descent.

- Auto4x can have a heavy genetic load
- Deleterious mutations can become homozygous upon selfing
- Coefficient of inbreeding

- When there is random mating in a 2x population: F = $\frac{3\alpha}{2+\alpha}$
- Selfing a 2x: F = $\frac{1}{2}(1+F')$
- Selfing an auto4x: F = $\frac{1}{6}[1+2\propto(5-2\propto)F']$
  - Where FT(L) = current level of inbreeding and
  - F' = the previous level of inbreeding

Thus 1 generation of selfing gives 50% in breeding in a 2x plant (F = ½), but only 17% inbreeding (F=1/6) for an autotetraploid (if α = 0)

Somatic chromosome doubling

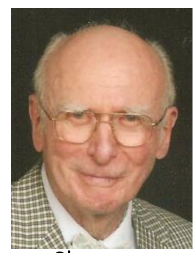
Note that somatic chromosome doubling leads to an inbreeding of F = $^1/_3$:

$$A_1A_2 \rightarrow A_1A_1A_2A_2 \rightarrow \frac{1+0+0+0+1+0}{6 \text{ combinations}} = 2/6 = 1/3$$

In the above examples, there are 6 possible pairs of alleles. Out of these 6 possible pairs, two (indicated in red) are pairs of alleles that consist of alleles that are identical by descent. The resulting F = $^1/_3$ is what one would obtain with a little more than 2 generations of selfing.

The fact that somatic chromosome doubling leads to inbreeding was not recognized until much later

Analytic breeding



Sherrett Spaulding Chase 1918 - 2021

Chase 1962

Designed a breeding scheme to maximize heterozygosity, called it **analytic breeding**

Chase 1963

Designed analytic breeding for potato, extracting 2x potatoes from 4x, selecting at 2x level, and converting to 4x via analytic breeding

							A_1A_2	$F = 0$
							↓	
1×2		3×4		5×6		7×8	$A_1A_1A_2A_2$	$F = {}^1/_3$
↓		↓		↓		↓	↓	
A	×	B		C	×	D	Single Cross Hybrids	$F = {}^1/_9$
	↓				↓		↓	
	E		×		F		Double Cross Hybrids	$F = {}^1/_{81}$
			↓				↓	
			G				Double-Double Cross Hybrids	$F = {}^1/_{243}$

- Maximizes heterozygosity. It is not the heterozygosity that is important. Instead, it maximizes the odds of having at least 1 dominant allele at each locus → capitalizes on additive genetic variance

Fertility in autotetraploids

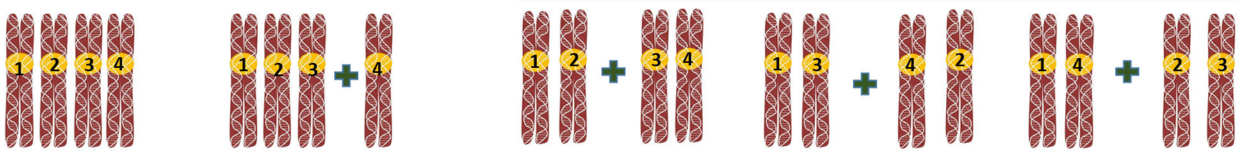
Even if vigor issues can be resolved, there are still fertility/seed set issues to overcome

Darlington 1932

Autotetraploids can suffer from low fertility due to 3 ↔ 1 disjunction of IVs

- Each chromosome has 3 possible pairing partners

Quadrivalent Trivalent + Univalent Two Bivalents– note there are 3 possible combinations



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

Randolph, 1941

First to realize that fertility in an auto4x could be selected for


Lowell Fitz Randolph
1894 - 1980

Gilles & Randolph, 1951

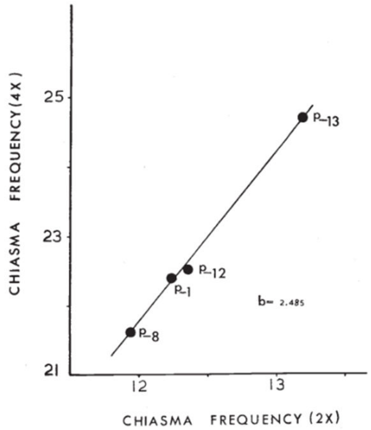
Over ten years, there was a shift to fewer IV and more II

- From 8.47 to 7.46 IV/cell

Plant no.	Frequency of cells with ten to five quadrivalents 10	9	8	7	6	5	Total no. of cells examined
48-326 — 15	8	22	7	2	2	–	41
— 21	7	24	9	1	–	–	41
— 22	3	15	16	3	3	–	40
— 1	3	18	13	2	5	–	41
— 27	3	18	17	2	1	–	41
— 13	6	18	11	4	2	–	41
— 24	6	18	11	4	2	–	41
— 26	4	16	13	5	3	–	41
— 52	3	19	12	4	3	–	41
— 53	5	16	14	4	2	–	41
— 75	4	15	15	4	3	–	41
Total	52	199	138	35	26	–	450
per cent	11.5	44.2	30.7	7.8	5.8		

Plant no.	Frequency of cells with ten to five quadrivalents 10	9	8	7	6	5	Total no. of cells examined
48-327 — 5	–	6	16	11	6	2	41
— 14	–	5	18	12	5	1	41
— 22	–	5	14	15	6	1	41
— 37	–	6	12	13	10	2	43
— 39	–	5	13	16	6	2	42
— 50	–	4	18	13	6	1	42
— 17	–	4	21	11	5	–	41
— 24	–	5	19	13	3	1	41
— 26	–	3	15	15	7	1	41
— 44	–	6	18	13	4	–	41
— 72	–	7	17	12	5	–	41
per cent	–	12.0	40.0	31.6	13.8	2.4	
Total	–	56	181	144	63	11	455

Hazarika and Rees, 1967



Pointed out a clear relationship between Xma frequency at 2x and derived 4x levels.

Summary by **Stebbins, 1971**

Problem: artificial autotetraploids suffer from low fertility

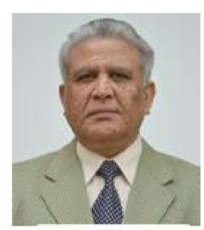
1906 - 2000

- Due to 3 ← → 1 disjunction of IVs
- Best is to avoid formation of IV and III altogether
  - Requires acrocentric chromosomes or chromosome interference across the centromere
  - Also, shorter chromosomes that can only support 1 CO
    - e.g., alfalfa, accomplished by short chromosomes that can only handle 1 CO per chromosome, with distal chiasmata localization
- Mentioned earlier that chiasma formation is under genetic control.

Should be able to select 2x plants with a low level of chiasma formation

- Look for 2x with rod II's (i.e., those that only have 1 CO per chromosome) as opposed to ⊙ II's.

Lavania, 1991



Umesh Chandra Lavania

Correlation between rod II formation in 2x and III + IV formation in the 4x is r = -0.776*

- Ie, as % of rods in the 2x increases, the % of II in the 4x also increases

- Correlation between chiasma formation at 2x and 4x levels is r = 0.851*
- Correlation between chiasma formation at 4x level and
  - ⊙ II formation: r = 0.764*
  - Seed set: r = -0.755*

	2x		4x C_2			
	% rod II	% ⊙ II	II	III	IV	I
Lolium perenne	82↑	18	46↑	--	53↓	--
Amaranthus hypochonriacus (C_1)	71	29	43	1	55	--
A. caudatus	66	34	33	1	64	1
Hyoscyamus muticus	65	30	28	4	67	1
A. edulis	60	40	30	--	68	2
H. niger	58	41	10	2	76	2
H. algus	59	38	7	6	84	3

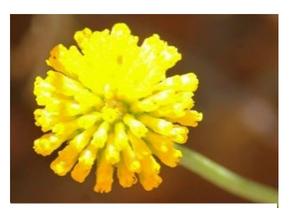
In other words, can select for fertility by selecting for rod II formation and against IV, III, and ⊙II formation

Inversely, selecting the 4x for seed production also selects for fewer IV formation

Species	Generation	% IV	% Seed Set
Hyoscyamus muticus	C_0	45↓	65
	C_1	--	--
	C_2	30	78↑
H. niger	C_0	27↓	75
	C_1	24	80
	C_2	22	92↑
H. albus	C_0	24↓	43
	C_1	17	54
	C_2	12	83↑

Rivero-Guerra, 2008

Auto4x individuals of *Santolina pectinata*: "Chromosome number doubling produces statistically significant decreases in the lengths of the short arm, long arm, and whole chromosome"



Santolina pectinata

Key issue, because they only have 1 CO per chromosome pair, they cannot form trivalents or quadrivalents

- Most fertile auto4x plants have II + II pairing, not IV pairing

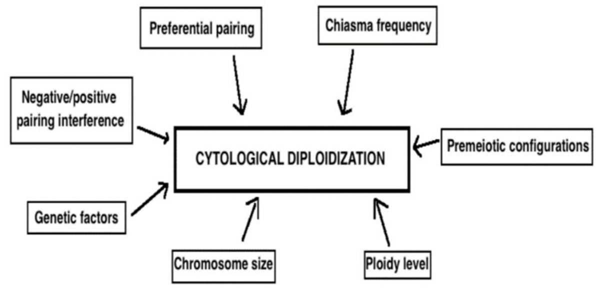
As long as pairing is random, any given pair only pairs $^1/_3$ of the time, leading to tetrasomic segregation

- Thus lack of IV cannot be used as to infer whether it is an auto or alloploid

The process has been called cytological diploidization (although it still has polysomic genetics)

Cytological diploidization

Dorone, 2013

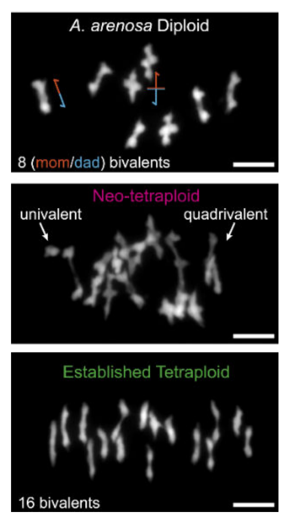


Arabidopsis arenosa as a model

- Auto 4x
- Outcrossing
- Short lived perennial
- Has 2x versions
- Dates to at least last ice age

https://extremeplants.org/species/arabidopsis-arenosa/

Hollister et al., 2012; Yant et al., 2013 (Bomblies lab)
Certain alleles of meiosis-specific genes are over-represented in the auto4x versions of *Arabidopsis arenosa*

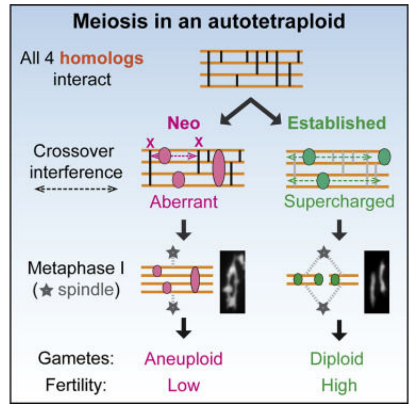


Evolution of CO interference

Morgan et al, 2021

- 2x plants have rod and ⊙ IIs
- Raw tetraploids have multivalent formation and low fertility
- The established 4x have rod II's

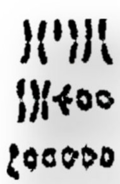
Conclude evolution of rapid and strong interference



Prevalence

Rice et al., 2019

In general, neopolyploidy is associated with the harsher environments– poles, dry seasons, lots of competition.



Fewer COs at lower temperatures → less issues with fertility?