Meiotic mutations

In terms of mutations affecting the meiotic process, we have talked about mutations that affect:

- pairing
- crossing over
- spindle attachments

Other mutations affect

- Cytokinesis
- Spindle orientation

Lead to the formation of 2n gamete

2n gametes

Review by **Kreinier et al, 2017**

Have known for many years that 4x progeny can be obtained from 2x-4x, 4x-2x, or 2x-2x crosses.

- This is a result of a 2n gamete, i.e., a gamete that has the sporophytic chromosome number.
- This is the only time it is acceptable to use 2n and gamete together.

Rhoades and Dempsey, 1966

Working with the *el* (*elongate*) gene of maize, postulated 5 possible ways to get 2n gametes

- Premeiotic doubling
- Omission or failure of meiosis I, normal meiosis II (First Division Restitution = FDR)
- Replication between meiosis I and II
- Normal meiosis I, omission or failure of meiosis II (Second Division Restitution = SDR)
- Post meiotic fusion

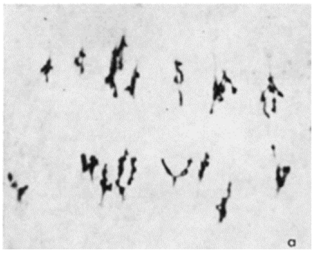


Figure 1. Ana I in an elongate mutant of maize

Mendiburu and Peloquin, 1976

All of the above types fall into 2 categories

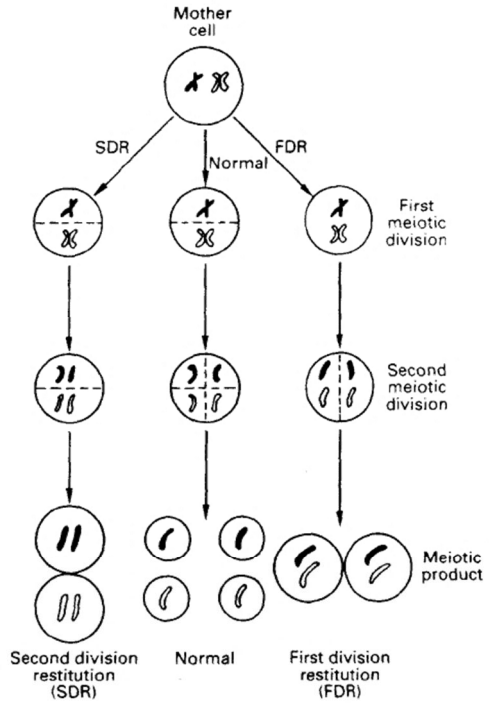
- recover non-sister chromatids in a gamete = FDR
- recover sister chromatids in a gamete = SDR

Mode

Bretagnolle and Thompson, 1995

Mode: Whether sister or non-sister chromatids are recovered

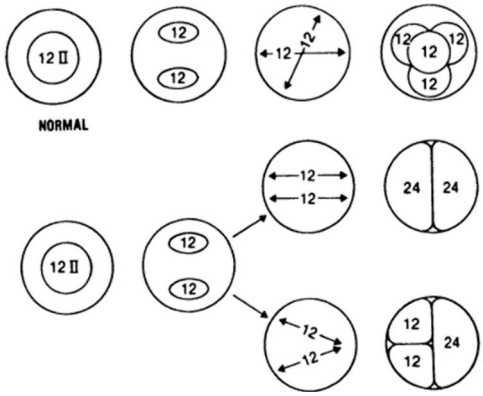
Mechanism: the actual cytological events that lead to a recovery of a 2n gamete



Mechanisms

Peloquin & students

Crossed 4x and 2x potatoes to get 4x potatoes

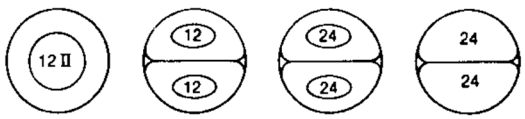


Peloquin, 1983

- Some 4x progeny were very uniform and high-yielding
- Resulted from spindle orientation mutations:
- In this mutation, known as parallel spindles, the metaphase II spindles are on 1 plane, not 2 as is normal, and are either parallel or tripolar, leading to the formation of a dyad or a triad
- This mutation isn't possible in monocots or in eggs, as there is cell wall formation after meiosis I
- Found thus far in alfalfa, sugar beet, carnation, clover, peach, blueberry, pea, trefoil, strawberry, sweet potato, poplar & arabidopsis.
- Each gamete recovers non-sister chromatids, so it is FDR in mode

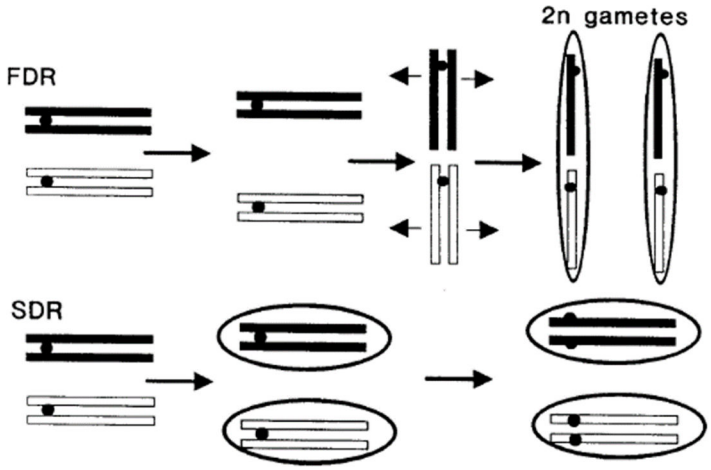
They also found 4x progeny from 4x-2x crosses that were low-yielding:

- This was due to 2n pollen formed by premature cytokinesis after meiosis I
- Recovers sister chromatids in each gamete, so is SDR in mode:



Summary for pollen

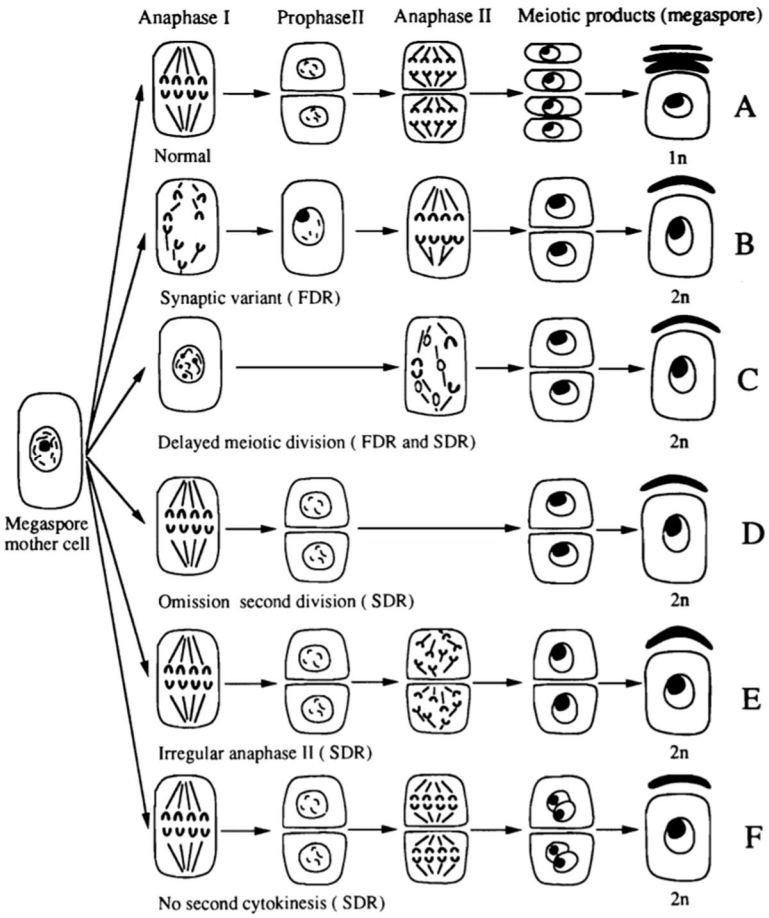
To summarize the movement of chromatids in the parallel spindles and premature cytokinesis mechanisms:



2n egg formation

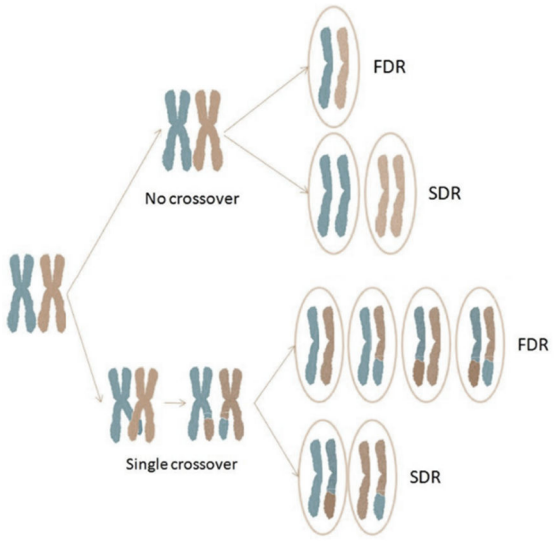
Werner & Peloquin, 1991

There are fewer studies on egg formation due to the difficulty in accessing Egg Mother Cells



Segregation in FDR vs SDR

Cuenca et al. 2015 & Ferris et al, 1992

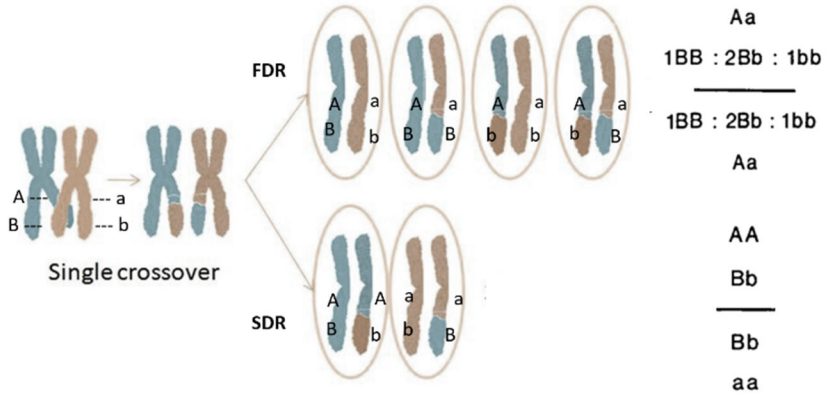


In the absence of crossing over

- FDR maintains the genotype of the parent plant
- SDR loses half of the diversity in the parent plant

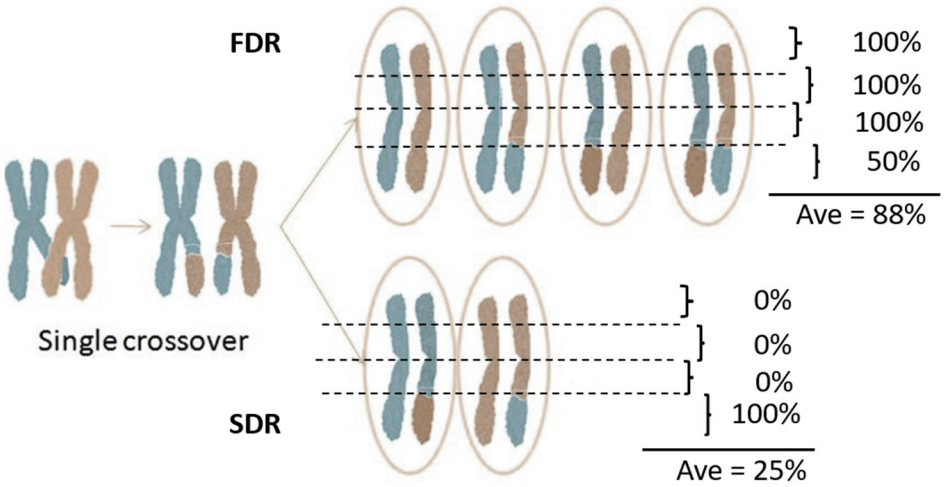
If there is a crossover:

- FDR will lose heterozygosity from the CO to the telomere
- SDR will transmit heterozygosity from the CO to the telomere



Heterozygosity in FDR vs SDR

Cuenca et al. 2015



Heterozygosity past the 2nd crossover

(NOTE: A 2-plane model of crossing over has been used to help emphasize the differences between FDR and SDR. This does not imply that the 2-plane model is correct.)

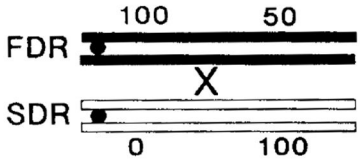
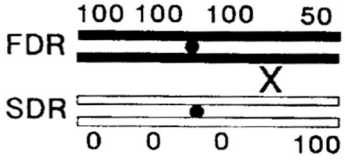
For FDR, 2nd interval:
1 + 3 = 0
1 + 4 = 100
2 + 3 = 100
2 + 4 = 0
=========
= 200/4 = 50%

For SDR, 2nd interval:
1 + 2 = 100
3 + 4 = 100
=========
= 200/2 = 100%

- FDR recovers non-sister chromatids. Consequently, all heterozygous loci are preserved from the centromere to the first crossover. 50% of heterozygous loci are preserved between the 1st and 2nd crossovers, and 100% after the 2nd crossover, so 75% is preserved past the 1st CO when there is more than 1 CO per arm. This averages about 80% per chromosome.
- SDR recovers sister chromatids. Consequently, all heterozygosity between the centromere and the first crossover is lost. 100% heterozygosity is retained between the 1st crossover and the 2nd crossover, none after the 2nd crossover, so on average and 50% of the heterozygosity gets transmitted when there is more than 1 crossover per arm. This means that about 40% of the heterozygosity is preserved per chromosome.

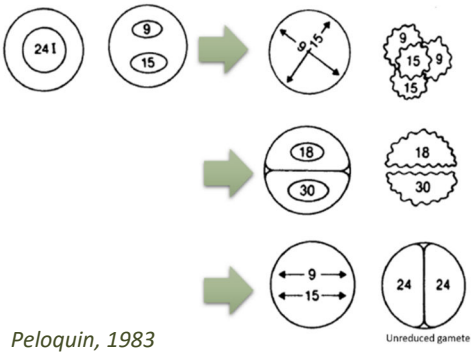
Effect of centromere position

The amount of heterozygosity transmitted depends on the morphology of individual chromosomes. Limits are defined as follows:

	FDR	SDR
	75% (100 + 50)/2	50% (0 + 100)/2
	88% (100 + 100 + 100 + 50)/4	25% (0 + 0 + 0 + 100)/4
Average for all chromosomes:	80%	40%

Synaptic mutants, odd ploids, & 2n gametes

Hesse, 1971; Peloquin, 1983; Aboobucker et al, 2023



Peloquin, 1983

Note that parallel spindles will restore fertility to plants with pairing problems, i.e., synaptic mutants and plants with odd ploidy (1x, 3x, 5x, etc.)

- Premature cytokinesis will not
- Discovered in monoploid peaches, and found in synaptic mutants of red clover and potato

As no pairing occurs in asynaptic mutants with parallel spindles, no crossing over occurs.

- Therefore each gamete has the exact genotype of the parent plant. These are called unreduced gametes, because neither chromosomal or genetic reduction has taken place.

Reduction

- Chromosomal: The halving of chromosome number that occurs during meiosis
- Genetic: The loss of genetic heterozygosity. This is normally associated with chromosomal reduction, but can also result from crossing over during 2n gamete formation:
  - SDR: Reduction is from centromere to 1st crossover
  - FDR: Reduction is after the first crossover

The term "unreduced gamete" is frequently abused by using it incorrectly to refer to 2n gametes in general.

Genetics of 2n gamete formation

The vast majority of mutations that affect meiosis are:

1) single, recessive genes in the homozygous condition

- 2n gametes are no exceptions:

- jason	*parallel spindles*	arabidopsis	pollen	De Storme & Geelan, 2011
- AtPS1	*parallel spindles 1*	arabidopsis	pollen	d'Erfurth et al., 2008
- cc	*calyx carpellaris*	pea	pollen	Myers et al., 1984
- d_2	*dyad formation*	*Datura*	pollen	Satina & Blakeslee, 1935
- el	*elongate*	maize	egg	Rhoades & Dempsey, 1966
- fc	*failure of cytokinesis*	potato	egg	Werner & Peloquin, 1990
- min	*minute*	barley	egg	Prasad et al, 1983
- ms_1	*male sterile 1*	soybean	pollen	Kennell & Horner, 1985
- os	*omission of second div.*	potato	egg	Werner & Peloquin, 1990
- pc	*premature c-mere div.*	tomato	pollen	Clayberg, 1959
- pc_1	*premature cytokinesis*	potato	pollen	Mok & Peloquin, 1975
- pc_2	*premature cytokinesis*	potato	pollen	Mok & Peloquin, 1975
- ps	*parallel spindles*	potato	pollen	Mok & Peloquin, 1975
- rp	*restitution pollen*	alfalfa	pollen	McCoy, 1982
- tri	*triploid inducer*	barley	egg	Finch & Bennett, 1979

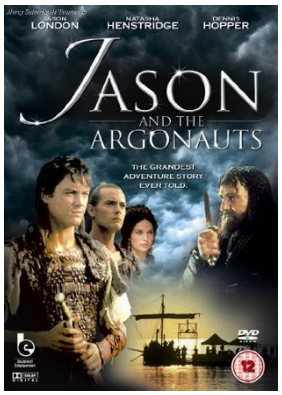
Neither are 4n gametes:

jp	jumbo pollen	alfalfa	pollen	McCoy & Smith, 1983

2) subject to large environmental effects (i.e., have incomplete penetrance & expressivity
   - Variable expressivity in potato: 0-80% of microsporocytes can express *ps*, depending on:
     - Genetic background
     - Environment (varies from 0 to 50%)
     - Location within the anther locule

Jason and Medea

de Storme & Geelan, 2001



EMS mutants of arabidopsis led to the discovery of 2 mutants, *atps1* and *Jason*

- Atps1 - mode of action thought to with microtubule organization & mRNA stability
- *Jason* regulates levels of *Atps1* mRNA
- *Jason* & *Atps1* have a synergistic effect

	AtPS1	Jason	Both
% 2n pollen	**~60%**	**~53%**	**~90%**

Called *Jason* as found in a MEDEA background– maternal effect embryo lethal in arabidopsis

- Medea murdered her 2 children by Jason when he left her for the daughter of the king of Corinth
- The *Medea* gene is a ploidy sensor that kills wrong-ploids through endosperm abortion

Gene-centromere mapping

Half-tetrad analysis for gene-centromere mapping

Rhoades and Dempsey, 1966

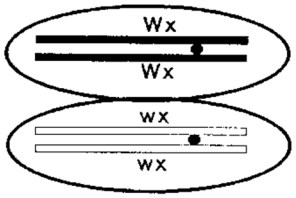
Used the el gene in maize, which gives 2n SDR eggs

Cross	Progeny	Map Distance
Wx wx × *wx wx wx wx*	39.5% *wx wx wx wx*	10.5
Sh sh × *sh sh sh sh*	19.2% *sh sh sh sh*	30.8

To calculate the map distance between the gene and the centromere:

- **50% - % nulliplex (i.e., homozygous recessive) progeny**
- Where the % nulliplex = the % of non-crossover gametes

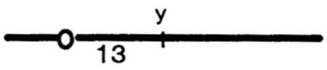
- The reason this works is that in the absence of all crossing over, and as sister chromatids are recovered in SDR, 50% of all gametes will be *wx wx*:
- Any crossing over between the gene and the centromere decreases the percent of homozygous gametes by the percent of crossover.



Mendiburu et al., 1979

Used the *ps* and *pc* genes in potato to map the *Y* gene, which codes for yellow tuber flesh

Cross	Progeny	Map Distance
y y y y × *Y y* (SDR)	38% *y y y y*	50 - 38 = 12
y y y y × *Y y* (FDR)	7% *y y y y*	7 × 2 = 14



For FDR, % recombination = % recessive x 2

- Without recombination, all gametes are heterozygous, and there would be no recessive progeny
- With recombination, get Aa + Aa (non-recombinants) and AA + aa (recombinants)
- Only ½ of the recombinant gametes give recessive progeny, so the amount of recessive progeny is ½ the amount of recombination that takes place.
- Therefore, % recombination = % recessive x 2

Co-Dominant markers

With codominant markers for FDR, % recombination = % homozygous in 1 category

- In the case of codominants in SDR, only get heterozygotes if there is a CO.
  - Each CO leads to 2 heterozygotes, so therefore must divide % heterozygotes by 2.
  - Amount of heterozygotes/2 = gene-centromere distance

Codominant markers in SDR

YY	*Yy*	*yy*
38%	24% ÷ 2 =12%	38% = 50 - % homozygotes

- Alternatively, gene-centromere distance = 50 - % of any one homozygous class (barring segregation distortion)
- Average if the 2 homozygote classes are not equal

Mode vs segregation

- As long as a gene is near the centromere, can also use gene-centromere mapping to deduce if a 2n gamete is SDR or FDR.
- Once the gene gets too far from the centromere, FDR and SDR formulae give the same distance

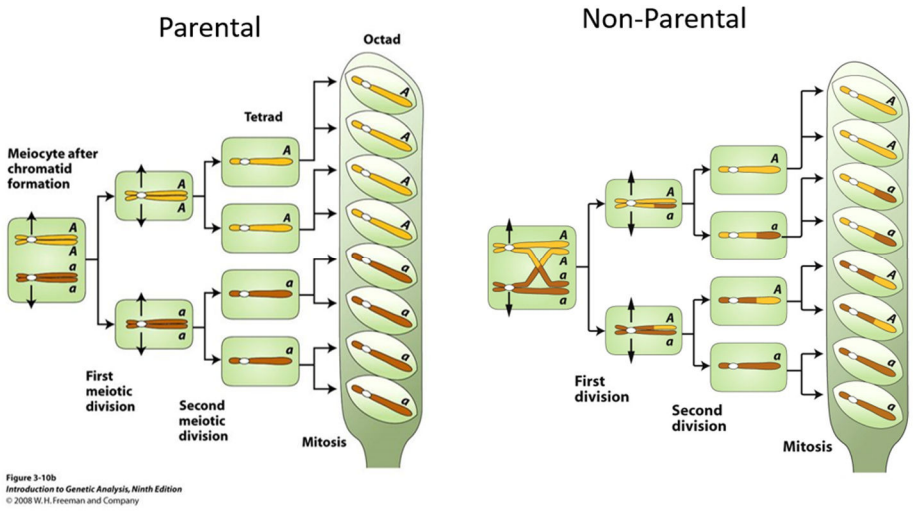
Cross	Progeny	Map Distance	
Wx wx × wx wx wx wx	39.5% *wx wx wx wx*	10.5	39.5 x 2 > 50, so rule out FDR
Sh sh × sh sh sh sh	19.2% *sh sh sh sh*	30.8	19.2 x 2 = 38.4, so cannot tell if FDR or SDR

Can calculate a 95% confidence interval for the gene-centromere distance (p), where p hat = the frequency of recessive progeny, q hat = frequency of dominant progeny, and N = the number of plants in the population.

$$\hat{p}-2\sqrt{\frac{\hat{p}\hat{q}}{N}} < p < \hat{p}+2\sqrt{\frac{\hat{p}\hat{q}}{N}}$$

Mapping with ordered tetrads

In fungi, where ordered tetrads are recovered, it is possible to use tetrad analysis to map gene-centromere distances. = ordered tetrad analysis



Gene-centromere distance =

$$\frac{1}{2}x\frac{Non\ parental}{Parental + Non\ parental}$$

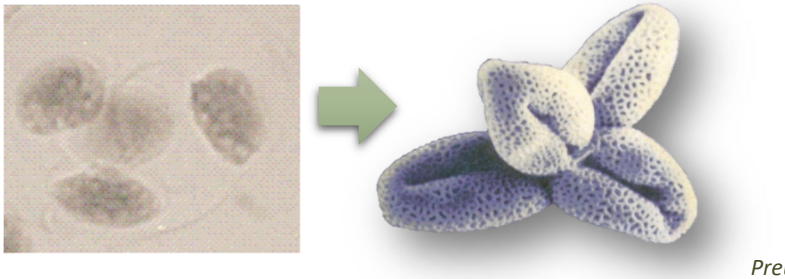
= (CO/total) x 1/2, as 1 CO only involves 2/4 of the chromatids

Unordered tetrad analysis

Copenhaver et al., 1998

In plants, the discovery of mutants in which the individual pollen grains remain attached led to the speculation that tetrad analysis would be possible in plants as well.

- Naturally occurring species that retain their tetrads also exist.
- However, such tetrads have not been overly useful for gene-centromere mapping, as they are symmetrical, which makes it impossible to determine which pollen grains contain sister chromatids - transgene tags overcome this limitation.



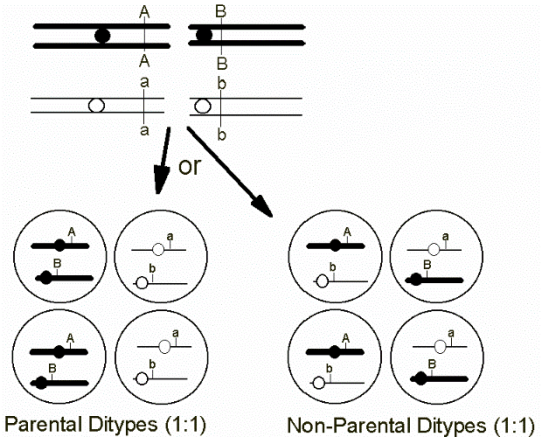
Preuss et al., 1994. Quartet mutant of arabidopsis.

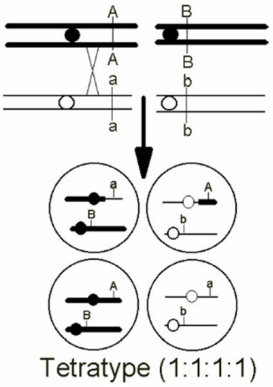
Thus, mapping with symmetrical tetrads requires 2 chromosomes where

- 1 has gene being mapped
- 1 has a centromeric marker, ie, a genetic marker tightly linked to the centromere

Then, in the absence of crossover,

- Parental genotypes are recovered 50% of the time. –> called parental ditypes
- Due to independent assortment, NonParental Ditypes are recovered the other 50% of the time





$$\frac{\mathrm{TT}[1/2]}{\text{all tetrads}}$$

- However, if get a crossover between gene and centromere, the result is a tetratype
- Frequency of tetratypes ÷ 2 = gene-centromere distance
- I.e., tetratypes = crossover frequency (earlier said that recombination = ½ CO frequency)
- Only ½ of spores in a tetratype are recombinant

Finding 2n gametes

Interploid crosses

4x progeny from 2x-4x or 4x-2x crosses

Cytologically

Observe meiosis in a sporocyte

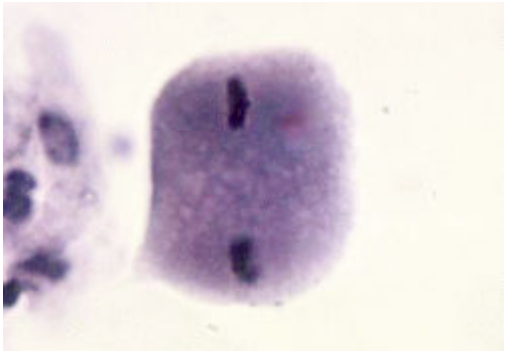


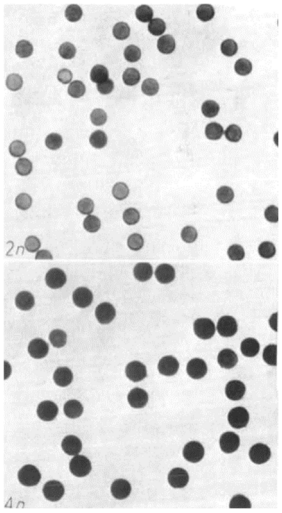
Figure 2. Parallel spindles at Met II in red clover

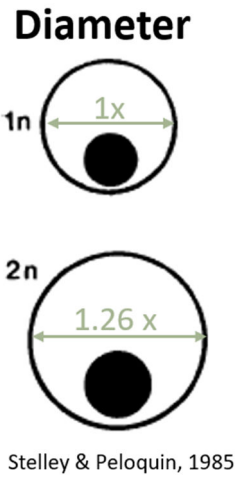
Cell diameter

Blakeslee & Avery, 1937

$$V = \frac{4}{3}\pi r^3$$

Nuclear diameter of 2n is 1.26x that of the n volume, reflecting the doubled nuclear volume





Stelley & Peloquin, 1985

Germination pores

Dijkstra and Speckman, 1965

The number of pollen germ pores increases in pollen with more than 1 ploidy

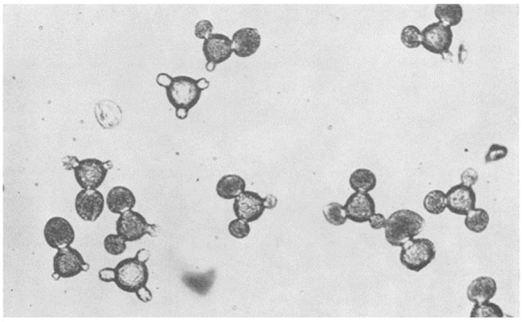


Figure 4, Haploid pollen of red clover showing 3 pores

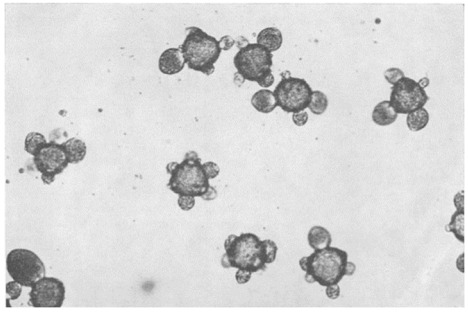


Figure 3. Diploid pollen of red clover showing multiple pores

Shape

Hutton & Peak, 1954

In some plants, shape of pollen is ploidy dependent

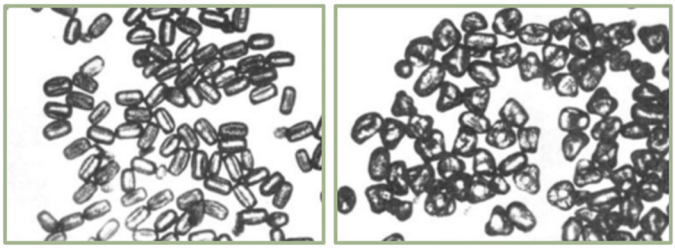


Figure 5. n (left) and 2n (right) pollen of red clover. from ***Taylor et al 1976***

DNA content

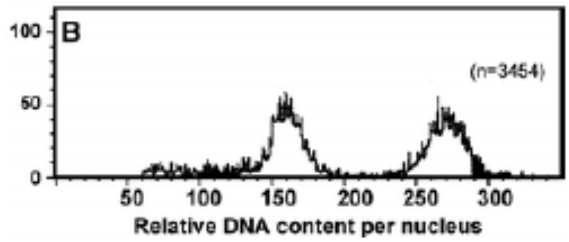


Figure 6. Lily pollen showing a mixture of n and 2n grains.
Akutsu et al. 2007

Breed for it

Tavoletti et al, 1991; Smith, 1986

	% 2n pollen				% 2n eggs	
Cycle of selection	red clover	alfalfa	% 4x from 4x-2x crosses (alfalfa)		alfalfa	% 4x from 2x-4x crosses (alfalfa)
0	0.04	9.09	0.38		50.00	4.62
1	3.09	54.79	4.97		82.01	11.46
2	17.86	77.82	13.11		79.20	26.40
3	47.38					

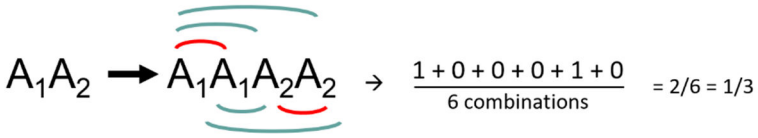
For the red clover example, given eggs = 3.36% 2n eggs & pollen = 45.5% 2n pollen, then the expected frequency of 4x progeny = 0.0336 x 0.4545 = 0.0153 = 1.5%. Observed frequency = 1%

Capitalizing on ploidy in breeding

http://biologicalexceptions.blogspot.com/2013/01/an-evolutionary-ploy-employing.html

Knew that many naturally occurring 4x plants are superior to their 2x forms (e.g., alfalfa and potato)

- With the discovery of colchicine, found that artificially derived 4x plants were usually worse than the original 2x forms
  - For decades, failed to recognize that somatic doubling of chromosomes gives F = 0.33.
  - This is equivalent to the inbreeding obtained from 2.2 - 3.8 generations of selfing



- So the question becomes how do polyploids arise in nature to avoid inbreeding
- At the early part of the 20th century, there were 2 opposing hypothesis:
  - Darlington (1920's): polyploidization via 2n gametes
  - Winge (1917): "interspecific hybridization followed by chromosome doubling"
    - Became the predominant view until very recently

1975 - a comprehensive review of reports of spontaneous tetraploidization in the literature review, "*On Ö. Winge and a Prayer*" by Harlan & DeWitt found almost no support for Winge, but almost universal occurrence of 2n gametes.

Now recognize that almost all polyploidization takes place via 2n gametes

Unilateral sexual polyploidization:

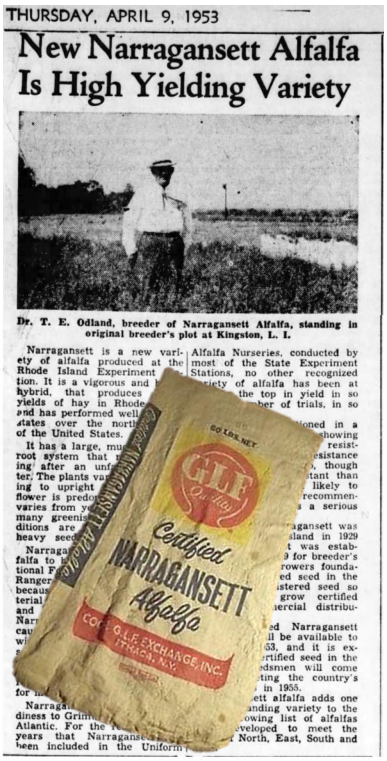
2x × 2x → 3x

3x × 2x → 4x

Bilateral sexual polyploidization

-2x × 2x → 4x

History in breeding



THURSDAY, APRIL 9, 1953

New Narragansett Alfalfa Is High Yielding Variety

Dr. T. E. Odland, breeder of Narragansett Alfalfa, standing in original breeder's plot at Kingston, L. I.

Narragansett is a new variety of alfalfa produced at the Rhode Island Experiment Station. It is a vigorous and hybrid, that produces yields of hay in Rhode and has performed well states over the north of the United States.

It has a large, mu root system that ing after an unfa ter. The plants va ing to upright flower is predo varies from ye many greenis ditions are heavy see

Narraga alfa to tional F Rangers becaus terial and Nar cau wi for

Narraga diness to Grimm Atlantic. For the years that Narragans been included in the Uniform

Alfalfa Nurseries, conducted by most of the State Experiment Stations, no other recognized riety of alfalfa has been at the top in yield in so ber of trials, in so

tioned in a showing resist- sistance , though tant than likely to recommen- a serious

ganasett was sland in 1929 t was estab- for breeder's rowers founda- ed seed in the istered seed so grow certified mercial distribu-

d Narragansett ll be available to 53, and it is ex- rtified seed in the dsmen will come ting the country's in 1955. ett alfalfa adds one anding variety to the owing list of alfalfas veloped to meet the North, East, South and

'Narragansett' alfalfa (4x) was derived from a 1929 cross between 'Grimm' and 'Don'

- In 1951, realized that 'Don' was 2x, raising the question of how a 4x plant was obtained from a 4x-2x cross
- Realized in retrospect that this was first indication of the value of 2n gametes in a breeding program

Alternative to move germplasm between ploidy levels

Plant on left and in center are 4x.
That on the right is 2x plant on the left was derived via nitrous oxide somatic doubling.

The center plant was derived via an FDR pollen grain from the 2x male, indicating the importance of heterozygosity in autotetraploid plants.

Figure 7. Crop Sci. 24:499-472

Reminder, it is not the heterozygosity per se that is important; it is maximizing the odds of having at least 1 dominant allele at each locus.

2n gametes in ornamental plants

Xie et al, 2022

- Naturally found in 40 genera/60 spp of ornamentals
- Artificially induced in 10 genera

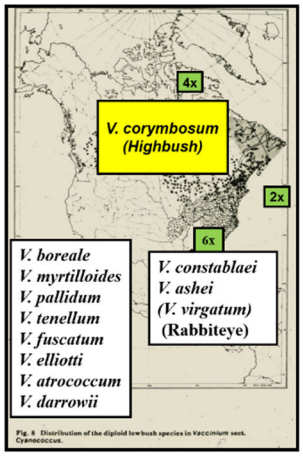
Agave 'Cimarrón' | Dianthus 'Promesa' | Lantana 'Radiation' | Phalaenopsis 'Timothy Christopher'

https://www.pinterest.com/pin/588282770059706631/ https://www.pinterest.com/pin/142496775682792735/
https://westernstarnurseries.com/plants/lantana-radiation/ https://www.orchidsbyhausermann.com/phal-timothy-christopher.html

A little background on blueberry

(Information and graphics courtesy of James Hancock)

- There are two major types of blueberry "southern" and "northern"
- Wild spp contain several desirable traits



- Domestication of the highbush blueberry is only a century old
- SHB were developed about 50 years ago through interspecific hybridization using southern native species
  - Using 2n gametes to make the interploidy crosses

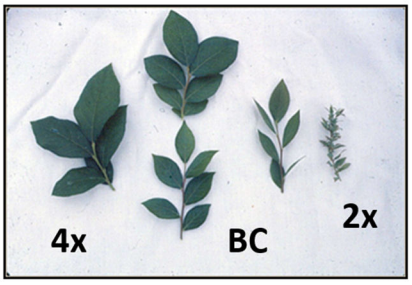


Table of species background of SHB cultivars developed by Florida breeding program:

- The point is that SHB are mostly northern types with ~10-20 of their genome from wild spp.

	Star	Millennia	Misty	Emerald	Snowchaser	Flicker	Meadowlark
Northern							
COR 4x	78%	81%	86%	82%	65%	66%	75%
ANG 4x	8%	5%	1%	2%	5%	< 1%	
CON 6x							
Southern							
DAR 2x	7%	1%	6%	14%	8%	12%	13%
ELL 2x					19%	19%	
FUS 2x		13%					
TEN 2x	1%		1%	> 1%	1%	1%	
ASH 6x	6%	2%	6%	2%	2%	1%	
ARB 2x							12%

Use in potato

Use of 2n gametes is most advanced in potato

- Cultivated forms are 4x, but most wild germplasm is 2x
- Surveys revealed a high frequency of 4x progeny following 4x-2x crosses

Yield

Mendiburu & Peloquin, 1977

Table shows potatoes in pounds of tubers per hill (ave. 2 locations)

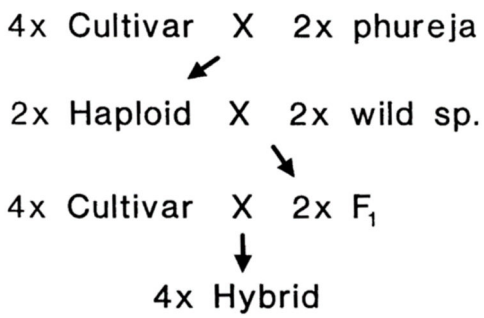
- Note, cultivated potatoes are usually 4x, while the wild ones tend to be 2x

F = coefficient of inbreeding = probability that 2 alleles are identical by descent

Cross:	Yield	F
4x - 2x (FDR)	6.0	0
4x - 2x (SDR)	4.1	0.167
4x - 4x	3.9	0.111 - 0.144
Parental means:		
4x	4.4	
2x (FDR)	1.8	
2x (SDR)	1.9	

Note the FDR 4x progeny are the highest yielding

Yerk and Peloquin, 1988



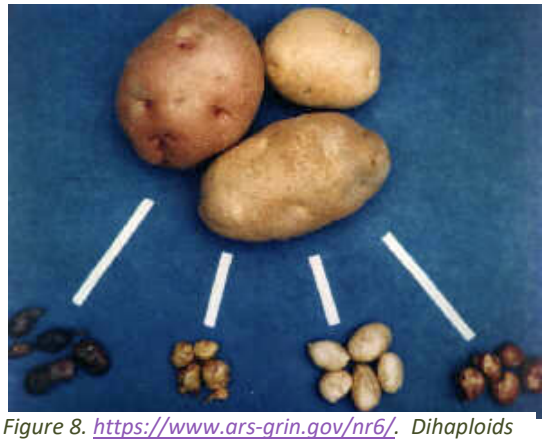


Figure 8. https://www.ars-grin.gov/nr6/. Dihaploids can also be intercrossed with each other at the 2x level.

1) Crossing a 4x cultivar with 2x subspecies *phureja* results in the formation of haploids

2) The resulting haploid (still 2x!) is crossed with a diploid wild species
- The diploid species has been previously selected for production of 2n pollen

3) The resulting 2x F1 plant is crossed with another 4x cultivar

4) The resulting 4x hybrid is 3/4 adapted germplasm, and only 1/4 unadapted germplasm
    - While crossing the 4x cultivar directly with the 2n pollen-producing 2x wild species would result in a hybrid that is ½ adapted and ½ unadapted germplasm.

True potato seed (TPS)

Potatoes are cross pollinated, and are therefore highly heterozygous.
- Since they are normally vegetatively propagated, a field of potatoes is genetically uniform.
- Same genetic uniformity as a field of hybrid corn or a grafted apple orchard

Seeds segregate. If a field were planted from seed, the plants would be very uneven and heterogenous. This has limited potato production from seed.

In LDCs, vegetative production is a problem. Tubers accumulate viruses, which decrease yields. Furthermore, few have adequate facilities to store the tubers without rotting.

Potato Flowers > Potato Berries > True Potato Seeds

Figure 9. https://www.cultivariable.com/the-absolute-beginners-guide-to-true-potato-seed-tps/

Alternative: True seed produced by FDR 2n gametes (or, even better, unreduced gametes) will give seed of nearly identical genotypes, providing enough uniformity to make cultivation feasible.

- This is becoming a breeding practice in some parts of the world.