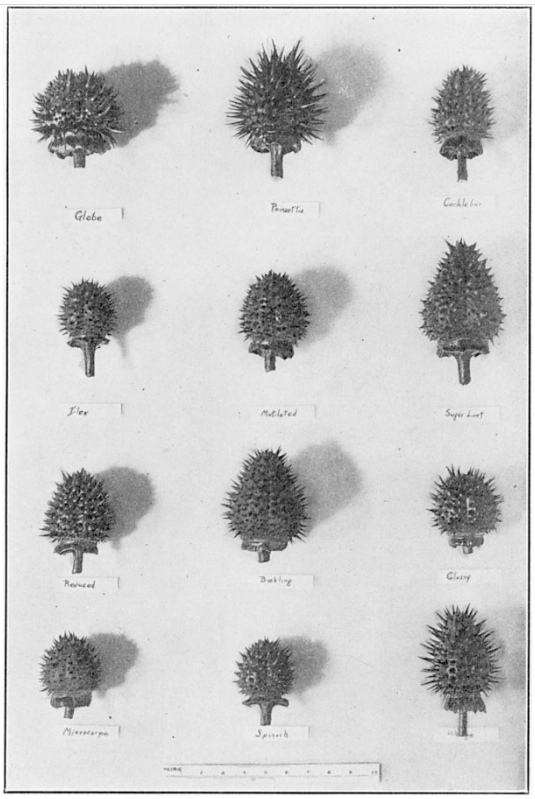
Aneuploidy

Chromosomal mutants of Datura

Albert Francis Blakeslee (1874-1954)



Blakeslee 1921

Jimson weed (*Datura stramonium*), 2n=2x=24, a ⊗ species

1910, Blakeslee found a globe mutant in his class demonstration plot of doubled haploids in Connecticut

- Many different traits were altered in the mutant
- globe × normal → 25% globe
- normal × globe → 0% globe (i.e., not ♂ transmissible)

Blakeslee and Avery, 1919

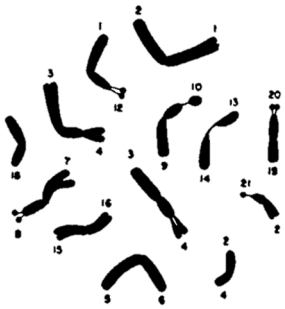
1915: Blakeslee moved to Cold Spring Harbor, and found additional mutants exhibiting similar behavior

Blakeslee 1921 & 1922

Belling found mutant plants had an extra chromosome, and formed trivalents (III)

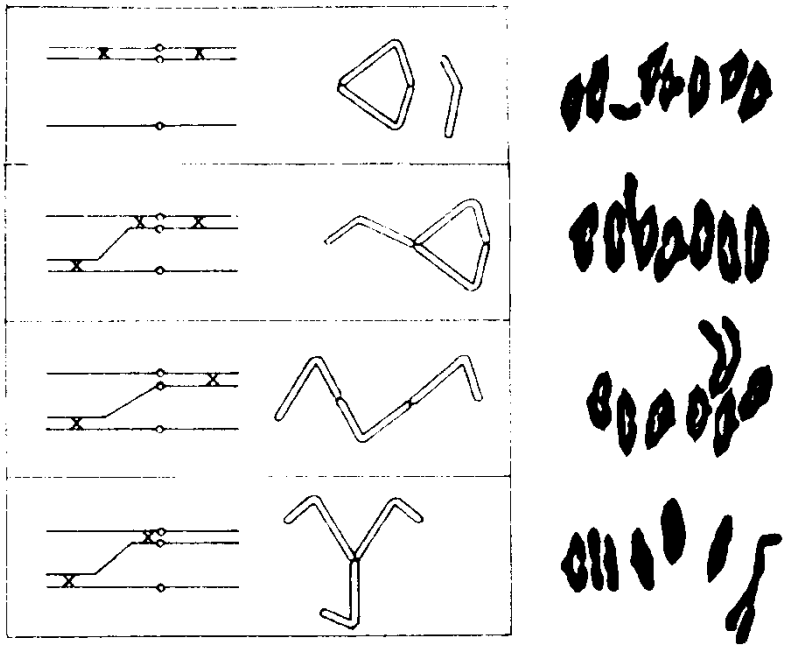
Belling 1920

- Trisomy affects the phenotype of *Datura*, as plants are homozygous, and therefore able to reflect dosage effects.
- Dosage effect is not noticeable in heterozygous backgrounds
- These plants are <u>primary trisomics</u>, i.e., are 2x + 1
- They are obtained from: Primary non-disjunction
    - Progeny of triploids (3x-2x) crosses
    - Progeny of haploids (1x-2x) crosses
    - Progeny of synaptic mutants



Pictured: glossy trisomic of *Datura*

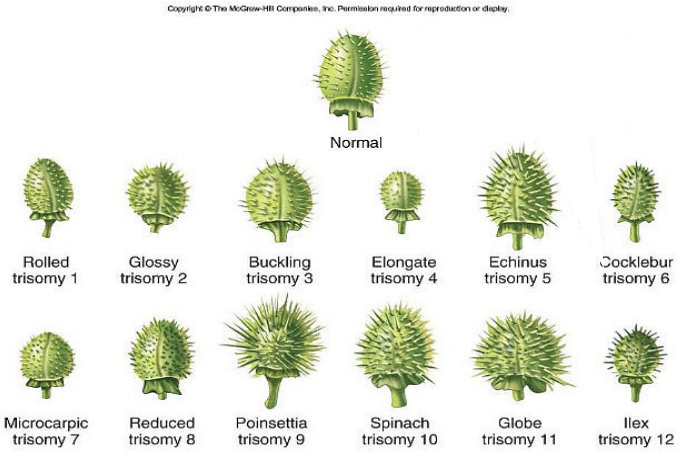
1° Trisomic configurations

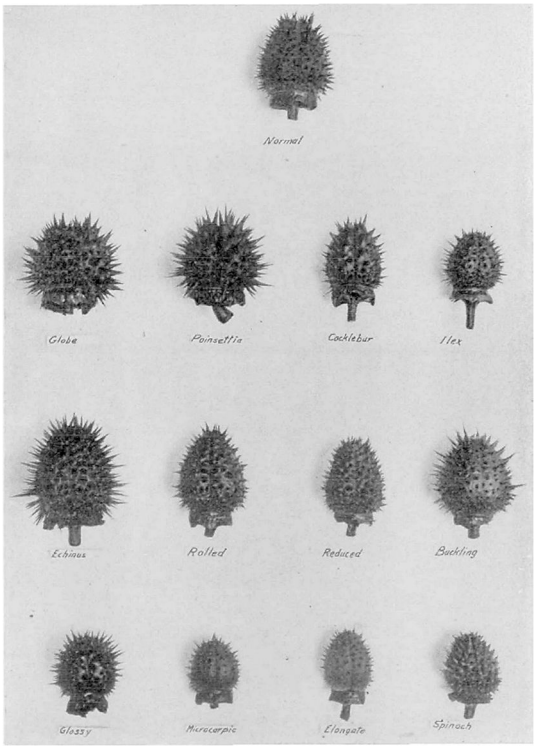


Meiotic configurations of primary trisomics of rye (from Sybenga, 1972)

Primary trisomic series

Blakeslee & Belling, 1924



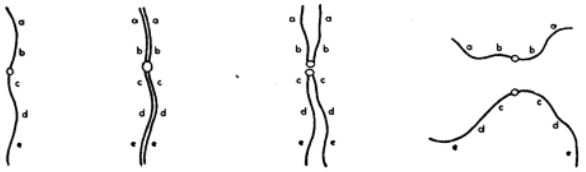


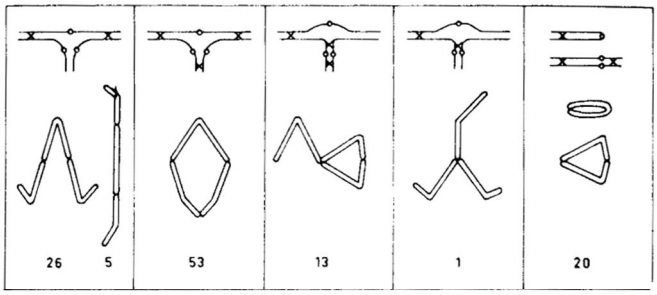
2° Trisomics

Belling and Blakeslee, 1922, 1924

Discovered new phenotypes among the progeny of the 1° trisomics. These had exaggerated features of the 1° trisomics.

- Were secondary trisomics
- The extra chromosome is an isochromosome, resulting from mis-division of the centromere (form 12II + U)



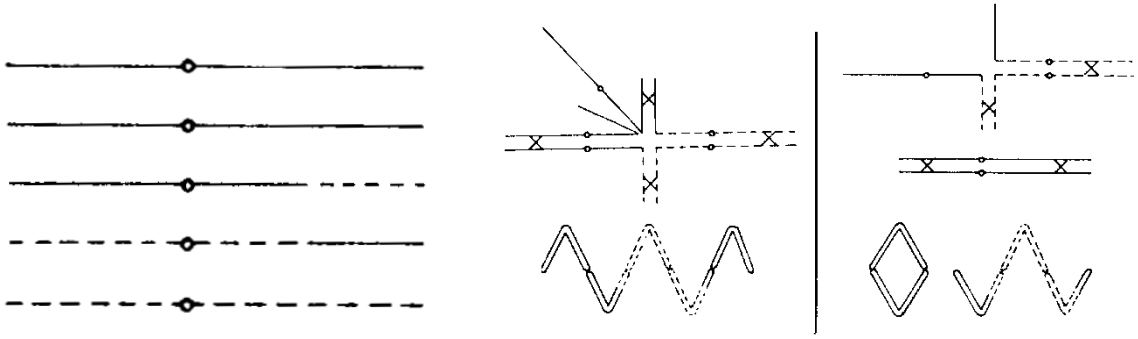


Meiotic configurations of 2° trisomics, Sybenga, 1972, after Belling & Blakeslee, 1924. Numbers are the frequencies of the various configurations (N = 118).

3° Trisomics

In addition, they found trisomics that had traits from two different 1° trisomics.

- These were tertiary trisomics, and the extra chromosome is involved in a translocation
- There are 9 possible pairing configurations for a 3° trisomic. Two are shown



2 of the 9 possible pairing configurations. Sybenga 1972.

Tetrasomics

2x + 2 copies of the same chromosome

- A tetrasomic of Datura ▸
- Pair as 13II or 11 II + 1IV

2x + 1 #11 2x + 2 #11

Double trisomics

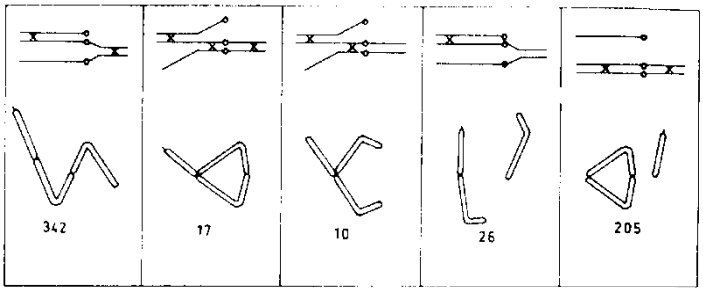
2x + 1 + 1, i.e., has an extra copy of two different chromosomes

- Pair as 12 II + 2I or 10 II + 2 III

Telotrisomics

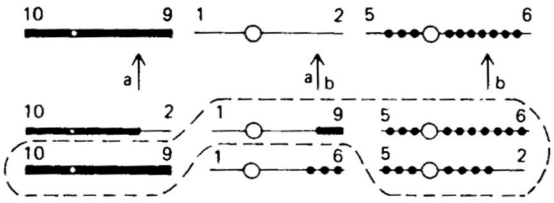
Only have ½ of an extra chromosome

NOTE that more than one crossover per arm is a rare occurrence.



Meiotic configurations of rye telotrisomics. The numbers are the configurations observed in 600 cells. Sybenga, 1972.

Compensating trisomics



Burnham 1962

Are missing an entire chromosome, but this is compensated for by the presence of 2 other chromosomes which together have the equivalent of the missing chromosome.

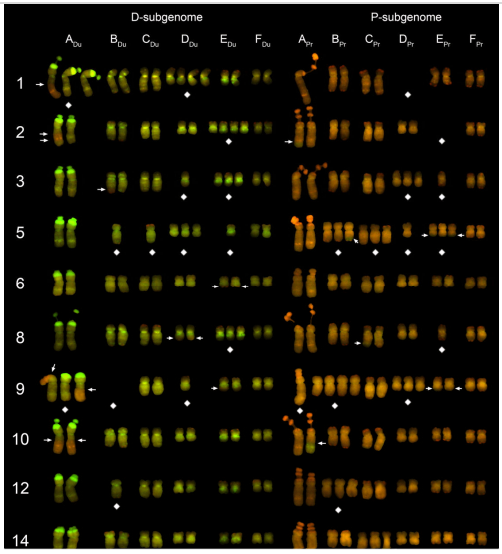
(this e.g., is missing chromosome 1-2)

Arrows indicate break points.

- The bottom rows are the F1 hybrid, (middle = a translocation, bottom = b translocation).

If the chromosomes in the dotted line get included in a gamete and crossed to a normal plant, a compensating trisomic is the result (new plant will have only 1 intact 1-2 chromosome.

- The other chromosome is made up of translocated parts of 1-9 and 2-5. Plant will be trisomic for -9 and -5)



Compensated aneuploids in Trapogon miscellus, an allo4x from T. dubius (green) & T. pratensis (orange). Chester et al., 2012

Monosomic: 2n – 1

- Usually not possible in a diploid, as a nullisomic gamete is required, which is lethal.
- In *Datura*, would pair as 11 II + 1I (if *Datura* were able to have monosomics!- example only)

Nullisomic: 2n – 2

- A homologous pair is missing
- This condition is lethal in a true diploid
- Pair as 11 II

Double monosomic: 2n - 1 – 1

- I.e., 2 different chromosomes are missing
- Pair as 10 II + 2I
- The haploid gametophyte serves as a sieve, and prevents monosomics from reaching the sporophytic generation
- In allopolyploids, the gametophyte is still 2x or 3x, so loss of a chromosome is not lethal

Monosomics in a diploid

The *r-x1* deficiency in maize, induced with X-rays by **Satyanara & Kermicle**, and described by **Plewa & Weber, 1973**

Leads to the production of monosomics.

This deficiency includes the R locus on chromosome 10.

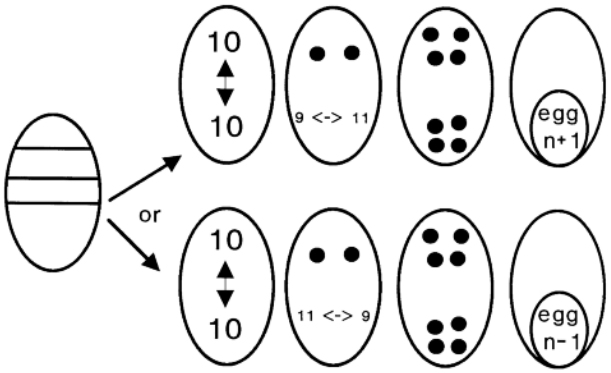
- Permits the recovery of 11% trisomics and 11% monosomics
- This is the only known example of monosomics in a diploid

Lin & Coe, 1986

Due to nondisjunction during the second mitotic division of megagametogenesis:

If nondisjunction was occurring during the first division (as originally was thought), then a monosomic embryo would always come with a double trisomic endosperm, and vice versa.

- This is not the case



The deficiency is not lethal in the egg, as other cells in the egg sac still have the missing chromosome.

Transmission

Blakeslee & Avery, 1938

In general, transmission of trisomics is poor.

However, enough transmission does take place to alter genetic ratio.

Data are from Blakeslee & Avery, 1938 (who looked at 28,566 progeny from ⊗ plants of the 12 trisomics)

Trisomic	Total	2n	2n + 1	% 2n + 1	2°	Unrel-ated 2°	Unrel-ated 1°	4x	1x
1.2	2049	1780	213	10.40	6	0	27	23	0
3.4	2089	1634	452	21.64	0	0	1	1	1
5.6	2367	1591	725	30.63	2	2	24	18	0
7.8	2080	1865	208	10.00	0	0	6	1	0
9.10	2160	1454	686	31.76	1	0	13	3	0
11.12	2228	1716	491	22.04	2	1	14	1	1
13.14	2033	1451	538	26.46	0	1	29	7	1
15.16	2278	1788	458	20.11	1	0	21	2	0
17.18	2140	1565	558	26.07	1	0	11	2	2
19.20	4758	4498	141	2.96	7	4	100	33	1
21.22	2340	1626	686	29.32	0	0	4	11	0
23.24	2044	1371	665	32.53	0	0	1	6	0
Average all 12 trisomics:				22.08					

Uses

Assigning genes to chromosomes

McClintock and Hill, 1931

Primary trisomics to assign genes to chromosomes

- Possible due to poor ♂ transmission

	colored	colorless	ratio	Comments
Rr ⊗	608	204	3:1	
Rr × *rr*	1161	1196	1:1	
rr × *Rr*	132	135	1:1	
RRr ⊗	396	41	10:1	Approaches 8:1 (autotriploid ratio)
RRr × *rr*	819	213	4:1	
rr × *RRr*	941	486	2:1	Poor ♂ transmission

Maize trisomic for chromosome 10

Mapping with telotrisomics

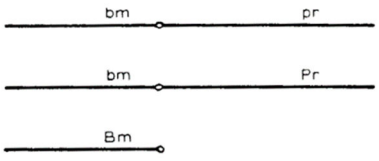
Rhoades, 1936

Locate genes on to chromosome arms

- Need dominant allele on telotrisome
- Smaller size makes them less likely to have deleterious effects, and consequently more likely to be transmitted through the gamete
    - Advantage over trisomics

Disadvantages

- Xma interference will lower CO relative to a straight deficiency
- Smaller size also makes them less likely to form a chiasma, and thus have a greater chance of being lost



Segregation	2n (normal)	2n + telo short, broad leaf	Total	Ratio
Pr : *pr*	63 : 64	31 : 35	94 : 99	1 : 1
Bm : *bm*	1: 171	85 : 0	86 : 171	1 : 2

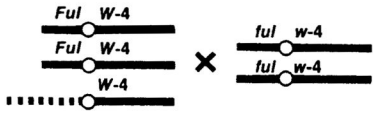
The one *Bm* plant that was not a telotrisome had to come from a crossover between the *Bm* locus and the centromere, which happened in one out of 172 plants.

- This frequency ($^{1}/_{172}$ = 0.6% × 2 = 1.2 cM) would also represent the distance between *Bm* and the centromere. [multiply × 2 because only ½ the gametes have the trisomic]
- This has been the method used to map centromeres in cereals
- Note that the calculated distance may be lower than it actually is, due alterations in pairing caused by the trisomic– i.e., an arm involved in 1 CO may not CO effectively with the 3rd arm.
- Half-tetrad analysis considered to be more accurate

Assign genes to chromosomes with tertiary trisomics

Khush & Rick, 1967

Localization of the tomato *w-4* (*wiry-4*, which makes for narrow leaves) gene on the long arm of chromosome 4, using F2 data:



	2n progeny			2n + 1 progeny		
	Dominant	Recessive	Ratio	Dominant	Recessive	Ratio
fulgens[1]	107	33	3:1	73	26	3:1
wiry-4	107	33	3:1	99	0	∞:0

[1] fulgens = yellow leaves

Place traits in the absence of genetic markers

Carlson, 1972

In this case, trisomics were used to map dehydrogenase (dh) enzymes and hexokinase for which alternate forms were not known.

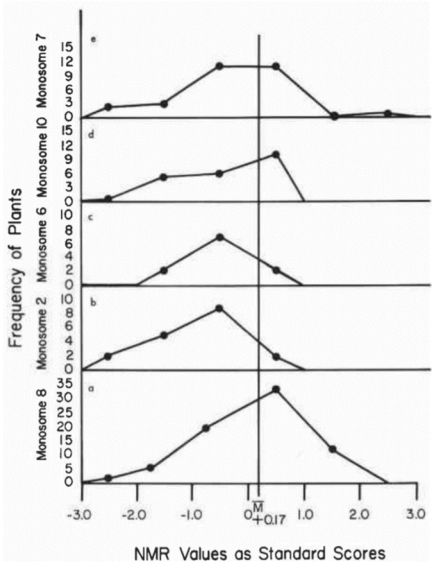
- Based on the assignment of relative activity of 100 to each enzyme in which two copies of the gene are present.
  - A 1° trisomic would have 150 activity units of an enzyme, and a 2° would have 200.

Enzyme:	Trisomic:																	
	1.2	3.4	3.3	5.6	5.5	6.6	7.8	9.10	9.9	10.10	11.12	13.14	15.16	17.18	17.17	19.20	21.22	23.24
Alcohol dh																157		
Lactate dh				138	102	177												
Malate dh								139	87	171								
Isocitrate dh		147	190															
6-Phospho-gluconate dh				144	81	185												
Glucose-6-phosphate dh															141	94		
Glyceral-dehyde 3-phosphate dh															135	169		
Glutamate dh																	162	
Hexokinase		140	88															

Plewa & Weber, 1973

Oil content of monoploids, showing reduced oil for monosomics for chromosomes 2, 6, & 10

- Hence, these chromosomes have genes that affect oil quantity

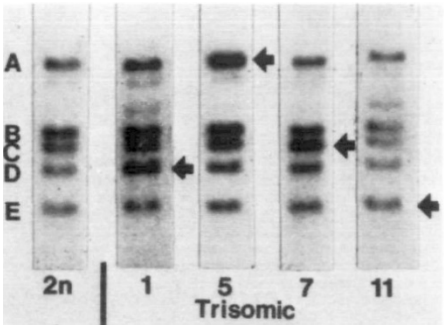


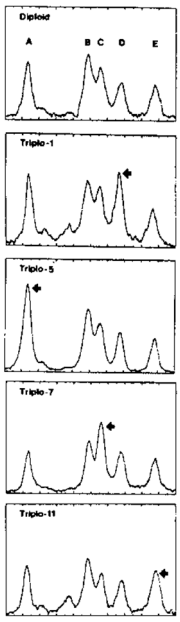
Place markers on linkage groups

Young, Miller & Tanksley, 1987

The same principle holds.

- An RFLP probe belonging to the trisomic chromosome will make a band that is 1.5x more intense than a band made by a probe from a disomic chromosome





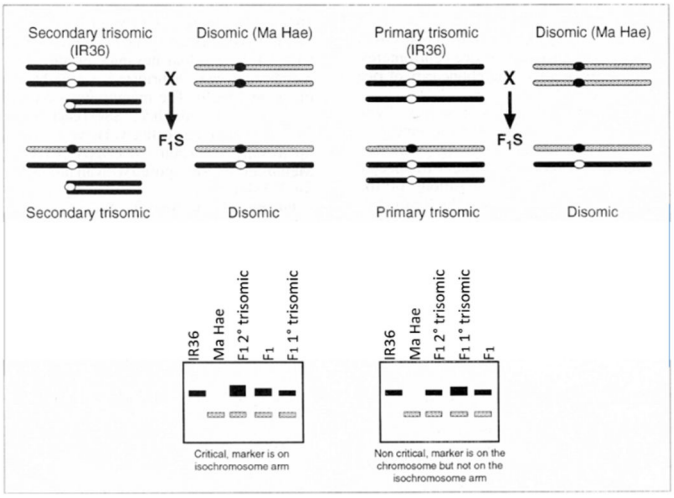
In addition, the X-ray film can be run through a densitometer, an instrument that measures the darkness of each band, thus detecting the band that comes from the trisome

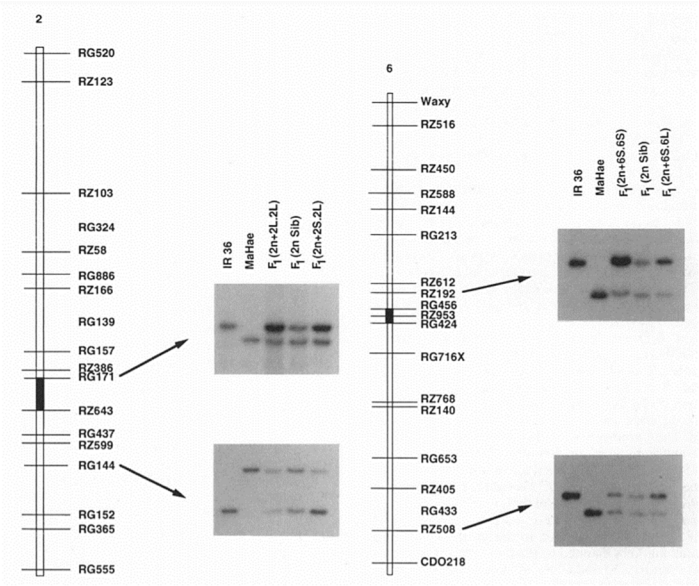
Localize centromeres

Singh et al., 1996

As one goes down the list of markers in a map, they will show up as being in one arm or the other.

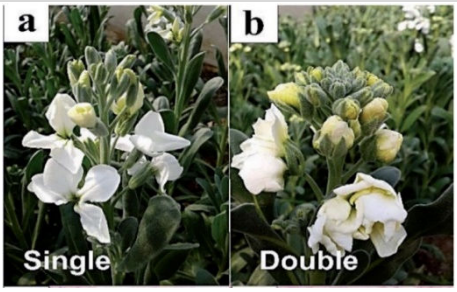
- There will come a point where a marker is in one arm and the next marker is in the other arm.
- The centromere must between those two markers





Plant breeding/male sterility

Frost & Lesley, 1954



Single & double flowers of Hoary stock, Matthiola incana. Irani & Arab, 2017

Matthiola is an ornamental flower.

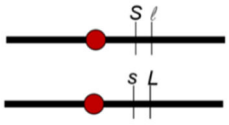
- In some genotypes, the stamens and carpels are converted into petals.
- Such double-flowered types are valued by the industry, but they are sterile.
- *Thus, seed for doubled flowered plants must be obtained from single-flowered types.*

The doubled flowered phenotype comes from a recessive mutation in the agamous gene, known as the *S* gene in matthiola.

- Thus, the homozygous recessive (*ss*) gives double-flowers.

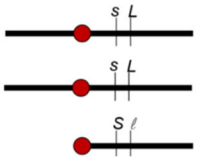
The *S* locus is very tightly linked to the L locus, which gives pollen lethality when recessive.

- Thus, in heterozygous plants, *Sℓ* pollen aborts, so ½ the progeny will be single flowered and the other half will be double flowered.
- The limitation to the system is that the grower must wait for the plants to flower before knowing if they will be single or double-flowered.



Male → Female ↓	Sℓ (pollen lethal)	sL	
Sℓ		SsLℓ	Normal single
sL		ssLL	Normal double

There is a phenotype that appears, called slender, that is due to a telotrisomic with the *SL* loci on it.



F/M → ↓	SsLℓ (poor pollen transmission)	sL	
SsLℓ		SssLLℓ	Slender single
sL		ssLL	Normal double

A particularly useful method of seed production can be obtained when the Sℓ alleles are carried on the telosome, as shown at left.

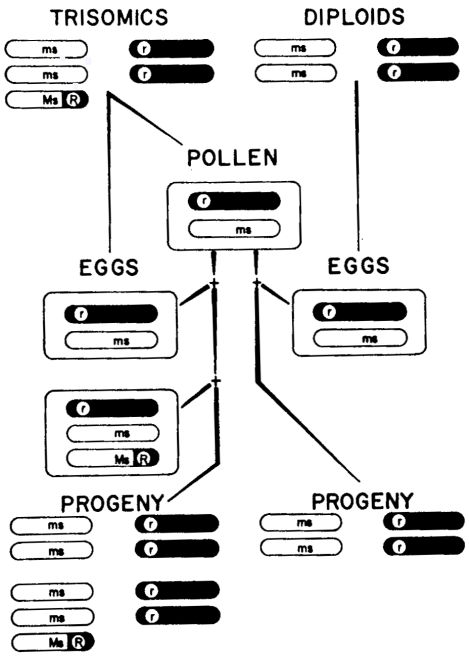
The telosome has very poor male transmission, and bad female transmission.

- Regardless, its transmission will result in a single-flowered type, but with the slender phenotype.
- Lack of transmission of the telosome results in doubled flower plants with normal phenotype.

- Because the telosome transmission is low, doubled-types will predominate in the seed. Furthermore, seedlings with the slender phenotype can be eliminated, leaving only doubles behind.

Ramage, 1965

"Balanced tertiary trisomics for use in hybrid seed production"



- Proposed a way to get male sterile plants to use as ♀ parents for hybrid seed production
- Balanced tertiary trisomics - 3° trisomics that have:
  - Dominant allele of a marker gene that is near the break point
  - The recessive allele on the two normal chromosomes

Notice:

- The extra chromosome of the trisomic is not transmitted through the pollen, so only one type of pollen is produced
- The extra chromosome is transmitted through the eggs, so 2 types of eggs are produced by the trisomic plants
- All gametes formed must have one complete copy of each chromosome.
  - If they only have one of the normal chromosomes plus the 3° chromosome, they will be deficient and will abort
- The diploid parent is male sterile, so it only produces one type of egg
- All trisomics will be red and male fertile.
  - These can be ⊗ for propagation
- All disomics will be green and male sterile

In practice, in barley, 70% of all progeny is diploid, 29 % is 3° trisomic, and 1% is 1° trisomic

Bottleneck: Getting the right translocation with the correct markers