Inversions

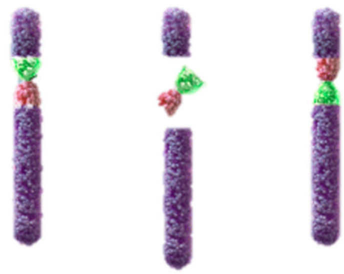
Muller, 1916, working with Drosophila, found C (for crossover reducers) factors reduced or suppressed recombination in certain areas of the chromosome when they were in a heterozygous state

- By 1926, Sturtevant had compared the order of linked genes, and found the order had been inverted
- Inversions can be pericentric (around the centromere) or paracentric (beside the centromere)

A paracentric inversion

From US National Library of Medicine

A pericentric inversion



Paracentric inversions

Russell & Burnham 1950

Crossover suppression

McClintock, 1931; 1933

Provided the cytological explanation as to why crossover was suppressed.

Nomenclature: Eg, the In 2a, S.6-L.6 inversion in maize

In 2a In = inversion
  2 = chromosome 2
  a = the first inversion found on this chromosome

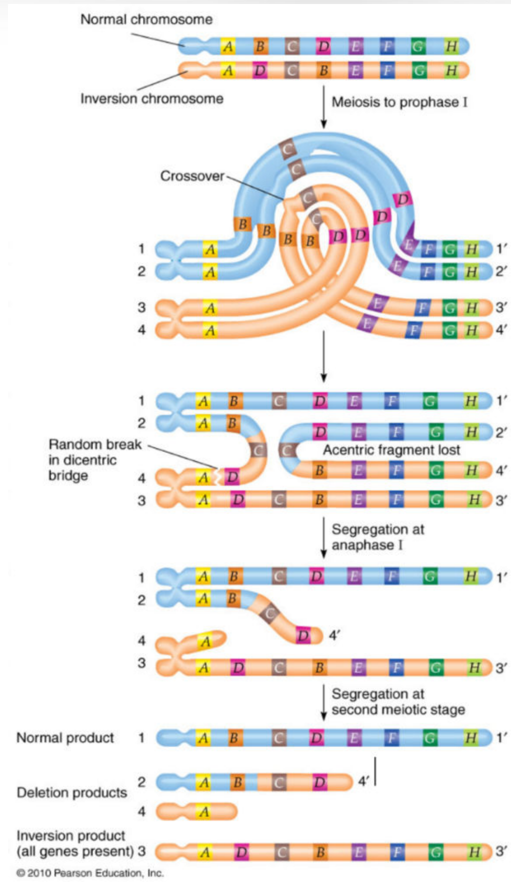
S.6-L.6

- S.6 = breakpoint is at .6 of the distance from the centromere to the end of the short arm
- L.6 = breakpoint is at .6 of the distance from the centromere to the end of the long arm

Working with a paracentric inversion on the short arm of chromosome 8:

Crossover inside loop (right):

- CO outside loop is inconsequential
- Formation of dicentric bridge and fragment at anaphase I The fragment is lost.



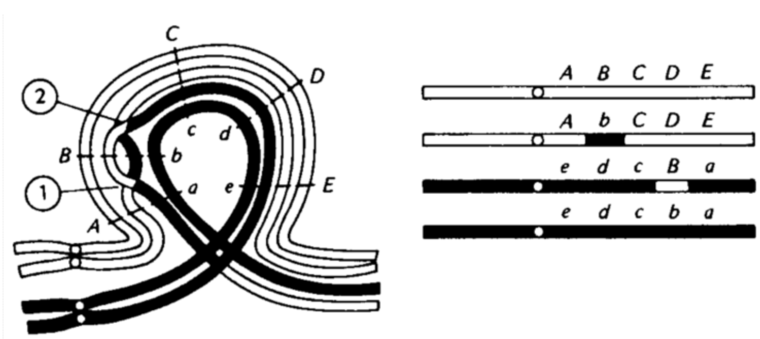
Only functional gametes are those from non-crossover strands. The rest are non-functional.

- This applies to odd numbers of crossovers

Double Crossovers in the Inversion Loops

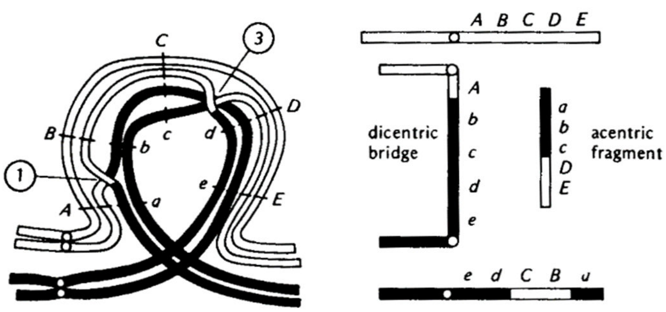
graphics from **Strickberger, 1976**

1) 2-strand double crossover



All gametes are functional. Inversion cannot be detected unless have the appropriate markers.

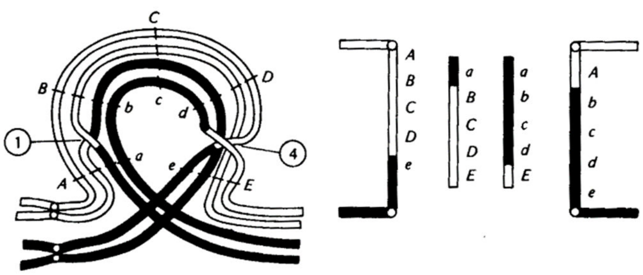
2) 3-strand double crossover



Bridge and fragment seen at Ana I

- 50% sterility
- Of the surviving gametes, 50% will be recombinant

3) 4-strand double crossover



- 2 bridges & 2 fragments at Ana I
- All gametes formed are unviable

If one of the double crossovers occurs outside of the loop

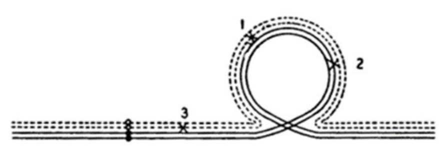
Modified from **McClintock, 1938**

A. Get same configuration for a 1 or 3 strand double crossover
   - Not affected by CO outside of inversion area

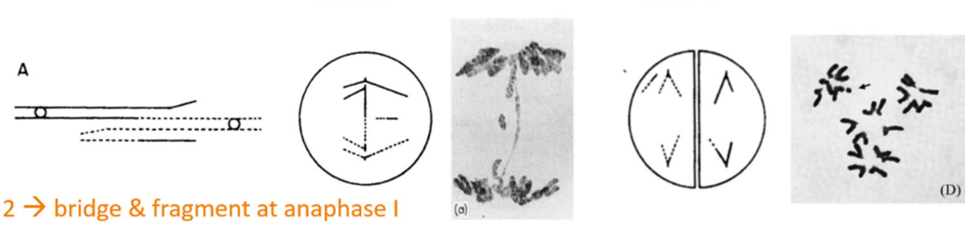
From **Burnham, 1962**, after **McClintock, 1938**

Ana I from Stevens & Bougourd. 1991. Heredity 66: 391-401

Ana II from Wang & Zhang. 2007. Plant Sci 172: 380-392

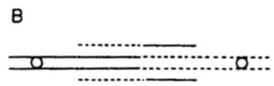


ANA I **ANA II**

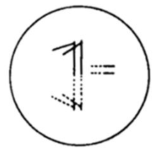


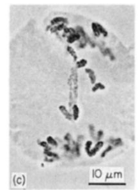
Single Crossover @ 1 or 2 → bridge & fragment at anaphase I

B. Configuration of 4-strand double crossover

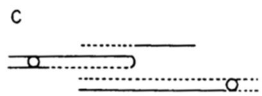


CO @ 1 & 2 → 4 strand double crossover → 2 bridges and two fragments

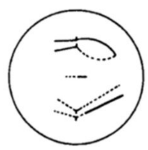


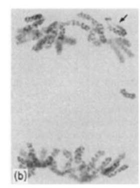


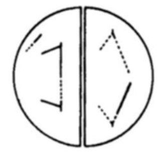
C.



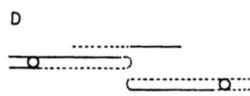
3-strand DCO @ 1 or 2 & 3 → linear & U-shaped fragments @ anaphase I + a bridge @ anaphase II



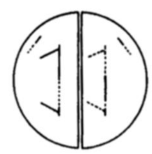




D.



4-strand TCO @ 1 & 2 & 3 → 2 linear fragments + 2 U-shaped fragments at anaphase I and 2 bridges at anaphase II



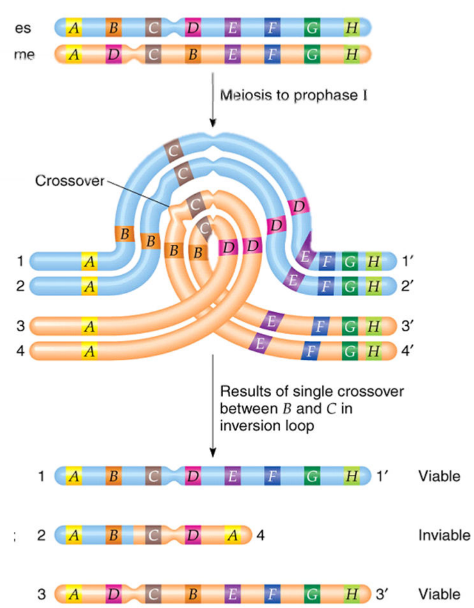
Pericentric inversions

Balanced: involves equal chromosome lengths on either side of the centromere

Unbalanced: involves unequal chromosome lengths on both sides of the centromere

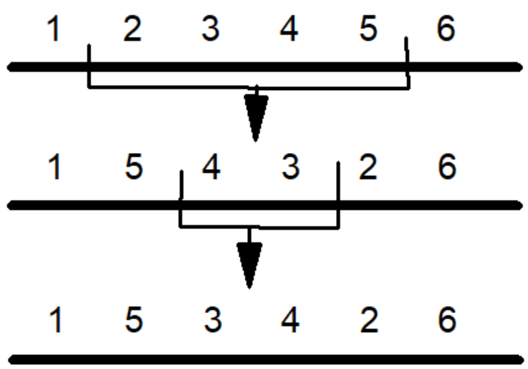
- No bridges or fragments are formed
- Half of the gametes (the recombinant ones) are duplicate-deficient

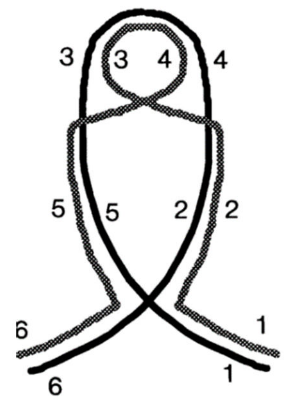
McClintock, 1931



Multiple inversions

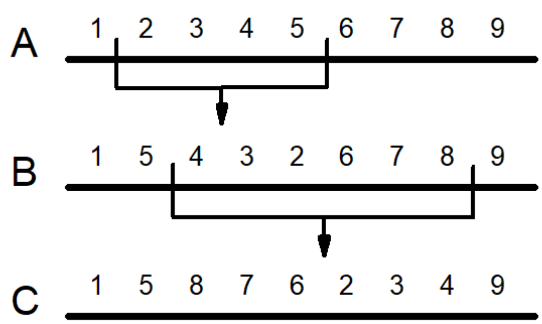
Case 1: Nested inversions

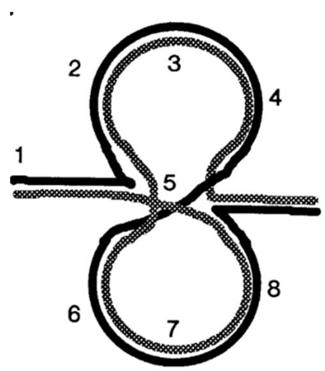




The normal chromosome forms a small loop within the large loop.

Case 2: Overlapping inversions





Can be used to trace the history of inversions and reconstruct evolutionary trees. In this example, the possibilities are:

A to B to C or A ← B → C or C → B → A

Paracentric inversions can be common in some organisms in natural populations

- By preventing recombination, isolate large blocks of genes of adaptive value
- Pericentric inversions - source of heteromorphic arms in orthopteran insects

Role of inversions

Dobzhanski, with Drosophila:

Certain inversions or inversion combinations are adapted to particular ecological combinations. These differ with altitude and with seasons

Conclusions

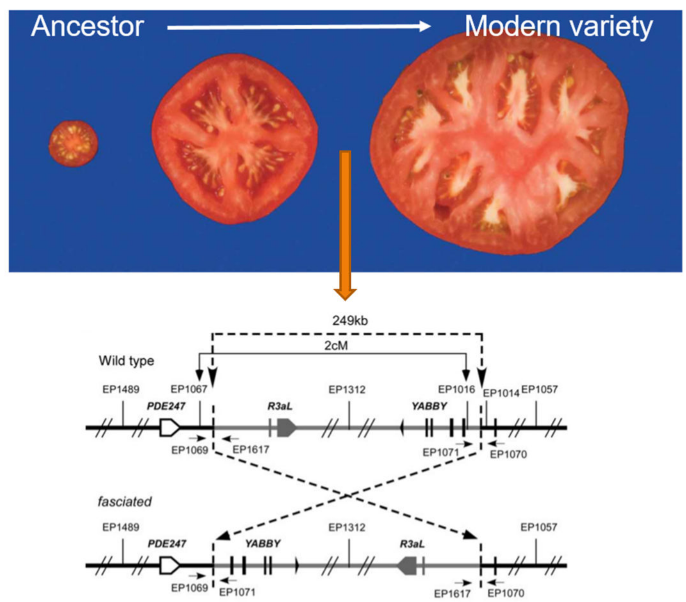
1. Inversion complex has selective value
2. Population is in a state of flux
3. Heterozygotes have a greater selection value than either homozygote
4. Optimal size to inversions

Roles of inversions

1) Genes within inversions may diverge
2) ¿Could serve as initial barriers for speciation?

Structural variants - inversions

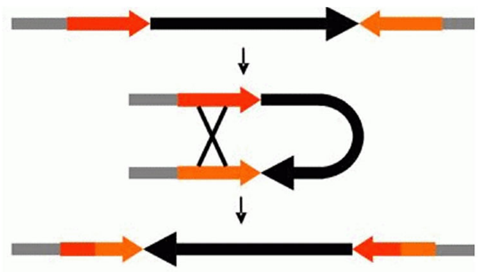
Huang & van der Knaap, 2011



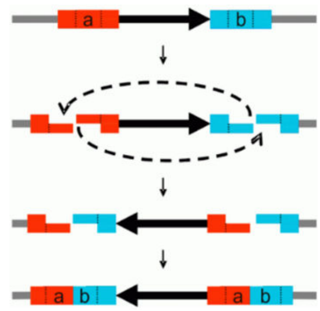
Origin of inversions

Casals & Navarro, 2007

Model 1: Duplications lead to inversions



Model 2: Staggered breaks



Left: Ectopic recombination model

- Most accepted
- Duplications lead to inversions

Right: Staggered breaks model – eg., as might happen with transposon excision or radiation

- Inversion leads to duplication

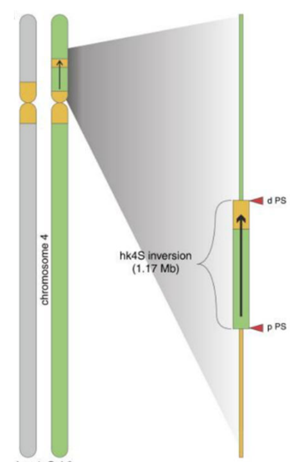
Models are not mutually exclusive!

Reversing inversions

Schmidt et al, 2020

Because inversions act as recombination suppressors, genes in inverted areas are not amenable to breeding.

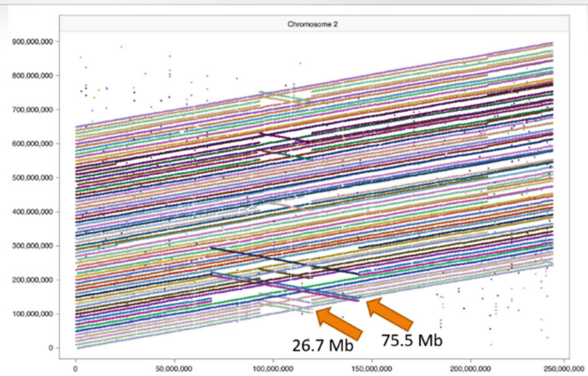
- In arabidopsis, chromosome 4 in Columbia has a paracentric inversion relative to Landsberg Erecta
- Using CRISPR to make simultaneous cuts, they were able to reverse the inversion with a 0.5% frequency.



Schwartz et al, 2020

Some corn inbreds have a 26.7 Mb or 75.5 Mb inversion relative to B73.

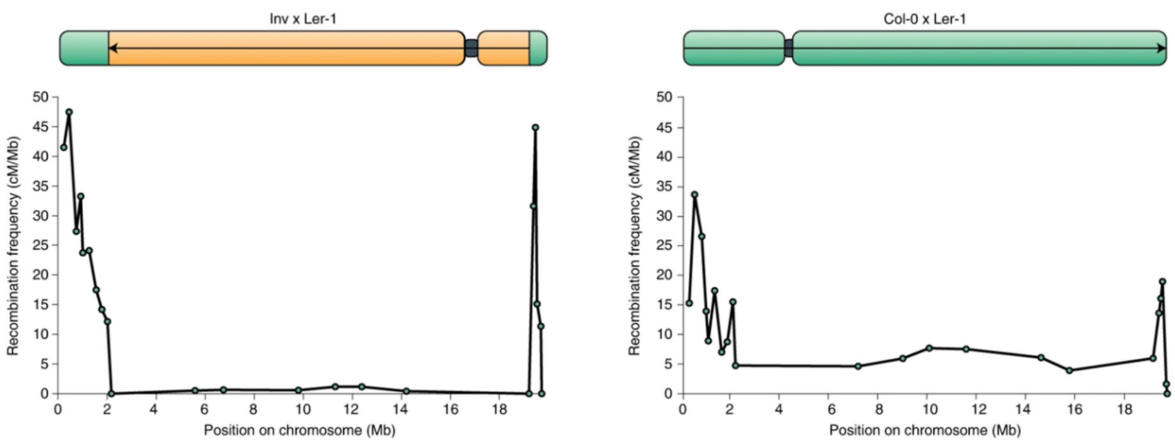
The large inversion has now been reversed in one inbred



Creating inversions to preserve linkage blocks

Rönspies et al, 2022

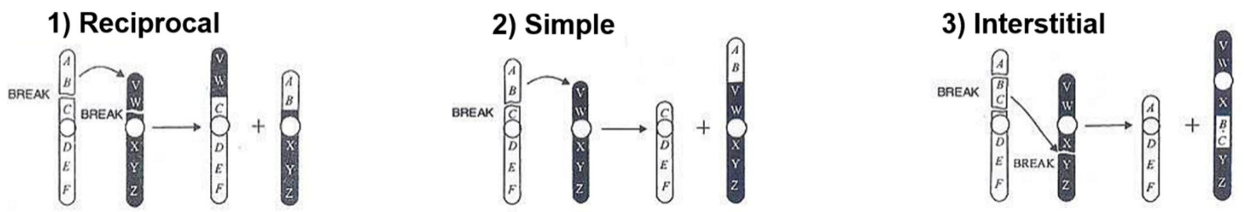
If inversions can be reversed, they can also be created, thus forming permanent linkage blocks without recombination, but for recombination from DCOs in the loop (visible in the 10-14 Mb segment on the figure on the left below.



TRANSLOCATIONS or INTERCHANGES

Exchanges between non-homologous chromosomes

- Three types



- Simple translocations are rare
  - Normally need broken ends to bind with a fragment

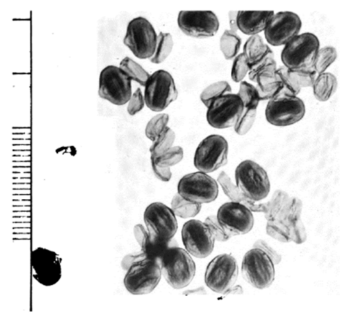
Semisterility in Florida velvet bean

Belling 1914/1915

Working with the Florida velvet bean, *Stizolobium deeringianum* = *Mucuna pruriens*

- Found plants with 50% aborted pollen and 50% seed set -- called this semisterility
- Half of offspring were normal, half were semisterile.

https://www.etsy.com/listing/635026498/mucuna-pruriens-velvet-bean-cowitch-raw



K/K l/l x k/k L/L

↓

KL K/l k/L k/l

viable viable

Postulated 2 genes, K and L, such that

- Presence of K or L gave fertility

In 1920, moved to Cold Spring Harbor, and started working on Jimson weed plants ('B' white) that were not breeding in a Mendelian manner

Belling & Blakeslee 1924; 1925

1924: Belling & Blakeslee concluded that non-homologous chromosomes could exchange segments

1925: Associated translocations with cause of semisterility

Blakeslee 1928

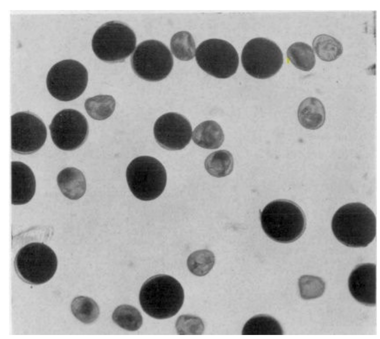
Found a ring of 4 (⊙ 4) in crosses of 'B' white x other plants of *Datura* (Jimson weed)

1926-1930: Translocations in *Drosophila* by Stern, Painter, and Muller

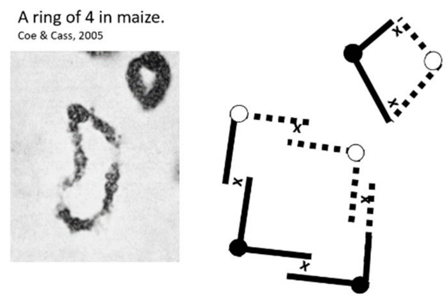
Semisterility in corn

Brink, 1927; Brink & Burnham, 1929

Find semisterility in corn
(the same year McClintock karyotyped maize)



A ring of 4 in maize.
Coe & Cass, 2005

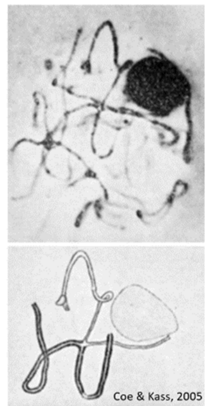


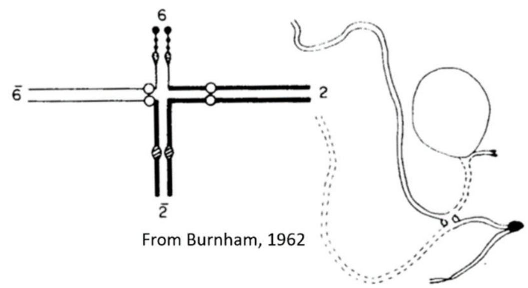
Burnham, 1930

Finds a ⊙ 4

McClintock, 1930

Identified cross-shaped configuration at pachytene

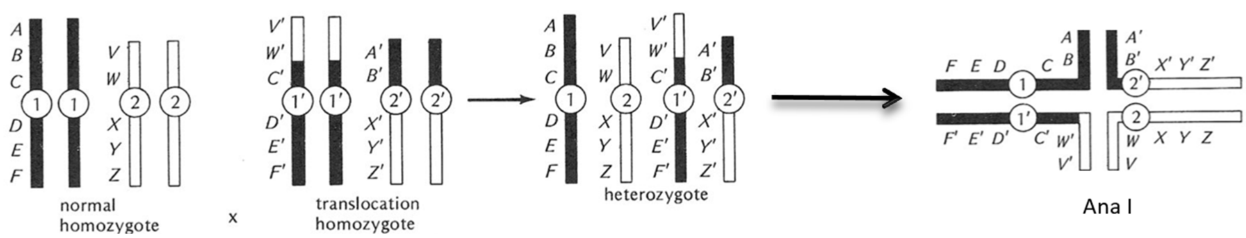




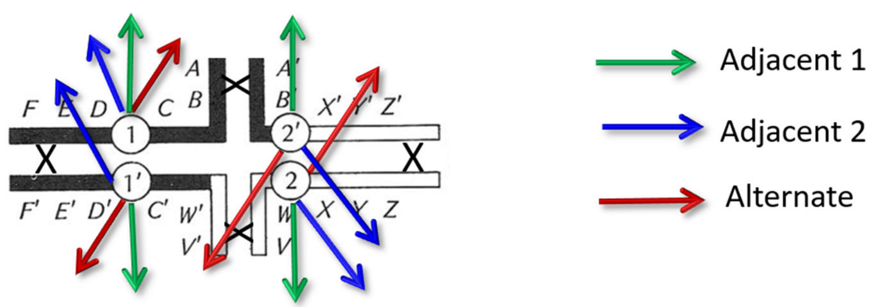
From Burnham, 1962

Meiosis in translocation heterozygotes
graphics from **Strickberger, 1976**

Normal × translocation homozygote to give a translocation heterozygote. Forms cross @ pachytene.



3 possible disjunctions

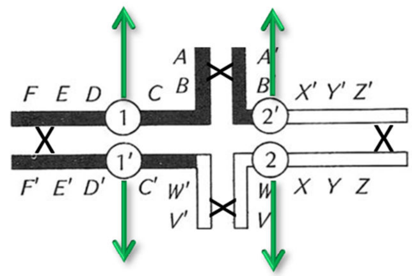


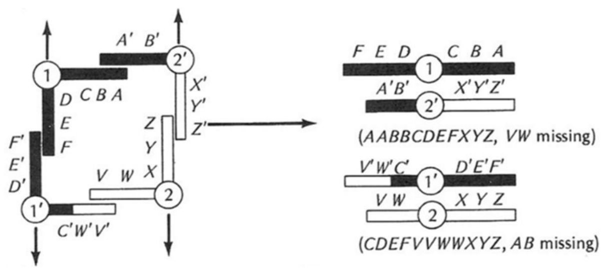
Case 1 – Crossovers in the Arms

Adjacent 1 separation

Non-sister centromeres go to the same pole & end up in separate daughter cells)

- Leads to formation of non-functional (duplicate-deficient) gametes



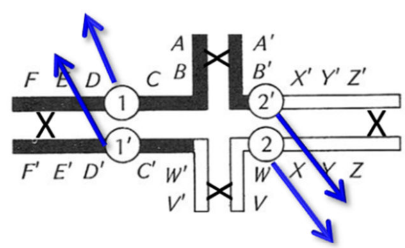


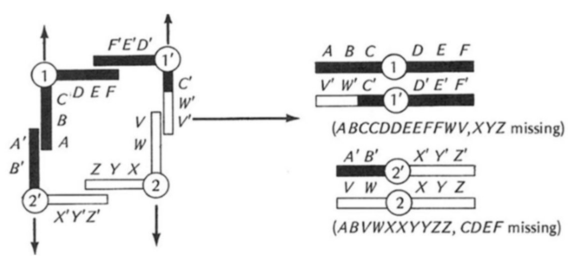
All gametes abort

Adjacent 2 separation

Sister centromeres go to the same pole & end up in the same daughter cell

- Gametes formed are also duplicate-deficient



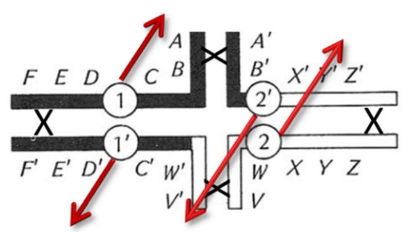


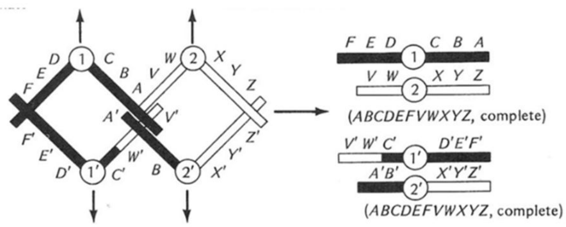
All gametes abort

Alternate separation

Sister centromeres end up in the same daughter cell

- Gives a figure 8 configuration
- All gametes are functional
- Always recover #1 and #2 together
  - There is no independent assortment





Translocation separations

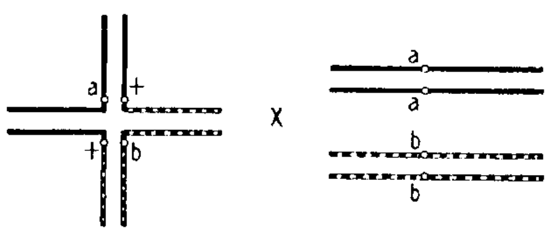
If all three configurations occurred with equal frequency, $^2/_3$ of gametes would be nonviable. However, this is not observed.

Type:	Expected	Drosophila	Maize	Barley
Adjacent 1	$^1/_3$	30	30	30
Adjacent 2	$^1/_3$	20	20	-
Alternate	$^1/_3$	50	50	70

Therefore, obviously, alternate separation takes place preferentially

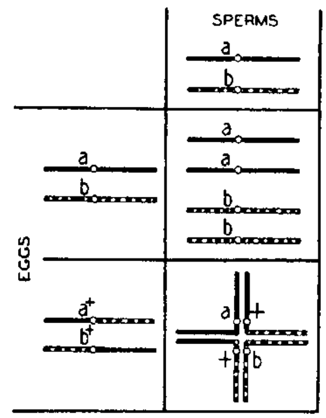
Pseudolinkage

From **Sturtevant & Beadle, 1939**



Apparent linkage of genes on nonhomologous chromosomes

- Result of negation of independent assortment



All genes on the 2 chromosomes involved in a translocation behave as a single linkage group

The only phenotypes recovered are the double recessive (*aabb*) or the double dominants (*AB*).

Dominant/recessive combinations (*Abb* or *aaB*) are never recovered. I.e., the effects of independent assortment are negated

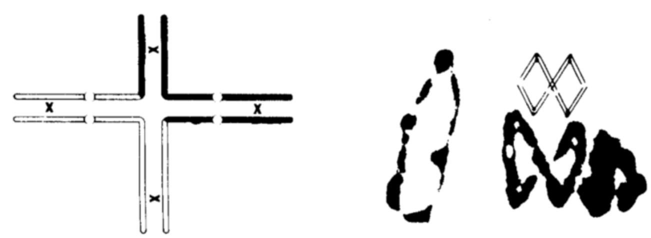
Effect on linkage maps

Homozygote: segments of the maps will be interchanged, reflecting change in the physical order of the genes on the chromosomes

Heterozygotes: gives a 4-armed map corresponding to the pachytene configuration

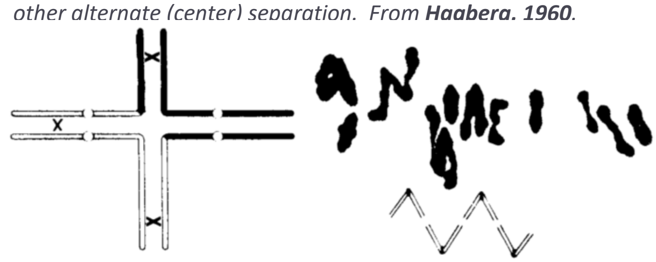
Effect of crossover number

A crossover event in each of the 4 arms of a cross gives a ⊙4



*Two rings of 4 in barley, one undergoing adjacent (left) and the other alternate (center) separation. From **Hagberg, 1960**.*

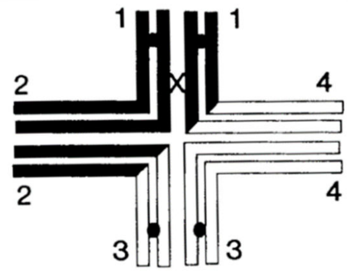
A crossover in 3 of the 4 arms gives a chain of 4



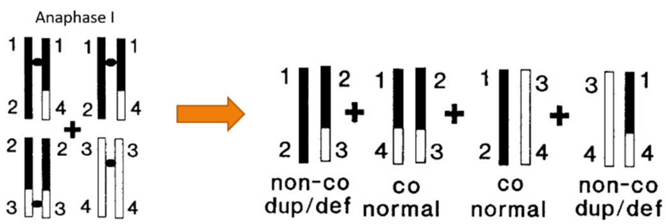
*Two chains of 4 in Agrostis. After **Jones, 1956**.*

Case 2 – Crossovers in the interstitial region

I.e., the region between the centromere and the exchange point



Adjacent 1 separation



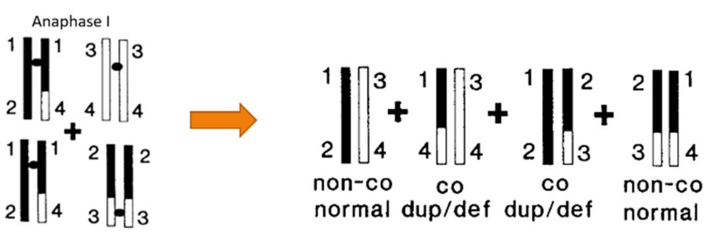
Net result is that 50% of gametes are functional.

- These result from the crossover event.

Adjacent 2 separation

Will not occur, or is rare, its occurrence depends on length of the interstitial region

Alternate



Net result is 50% of gametes are non-functional.

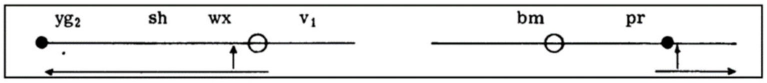
- These result from the crossover

Relative frequency of disjunctions worked out by McClintock (unpublished) and Burnham, 1950, using the NOR (chromosome 6) as a marker.

	Alternate	Adjacent 1	Adjacent 2
Short interstitial	50-57%	19-31%	19-26%
Long interstitial	55-56%	41-45%	0-3%

Effect of Translocations on recombination

Given the T5-9a translocation (i.e., the first translocation found between chromosomes 5 and 9) of maize:



Chromosome 5 (R) and part of chromosome 9 (L) showing break points. Horizontal arrows indicate regions showing variable pairing.

Chromosome	Standard	Heterozygous T5-9a as male	Homozygous T5-9a
Chromosome 9			
yg_2-sh	23	11	--
sh-wx	20	5	18.6
wx-v_1	12	11	independent
Chromosome 5			
bm-pr	27	32	--
pr-wx	independent	28	23.8

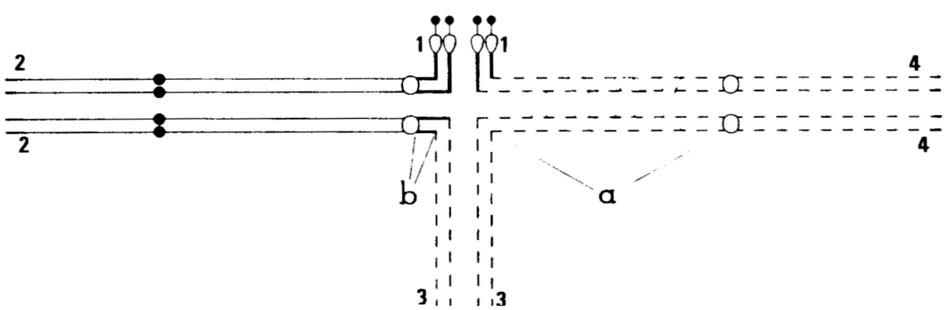
The tendency is for a reduction in crossover frequency

In addition, whenever crossing over takes place in the interstitial region, alternate disjunction results in unviable gametes.

This leads to an overall decrease in the number of crossovers recovered, compressing the map distance

Frequency of crossing over in the interstitial region

- Worked out by McClintock (unpublished) and Burnham, 1950, using the NOR (chromosome 6) at the tetrad stage and pollen abortion as markers in translocations between chromosomes 6 & 5



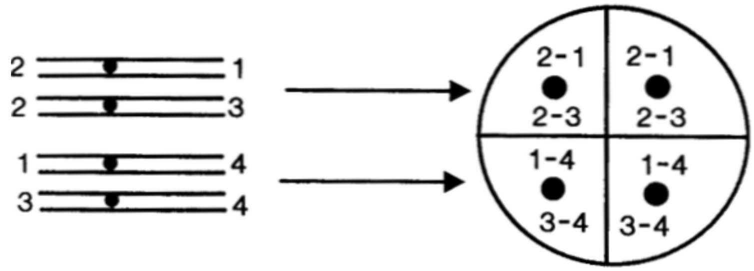
Pachytene configuration of a 5-6 translocation. The interstitial regions are denoted as a and b. (Burnham, 1960).

- Number of NOR = number of nucleoli
- If no NOR is present, nucleolar material remains scattered in small droplets

IF NO CROSSING OVER OCCURS:

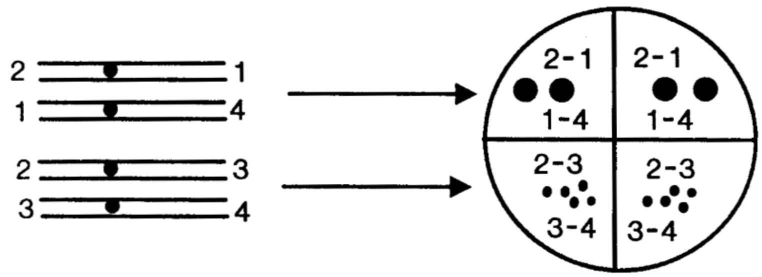
1) Adjacent 1:

- 2 have 2 nucleoli
- 2 have diffuse nucleolus
- All abort



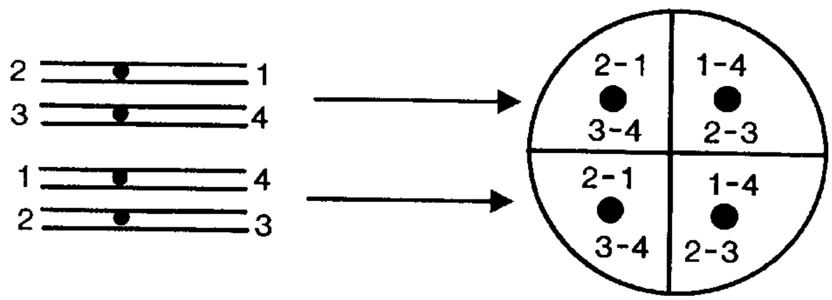
2) Adjacent 2:

- All microspores have 1 nucleolus
- All abort



3) Alternate:

- All have 1 nucleolus
- All are viable
- Can't tell from adjacent 2 by looking at tetrad



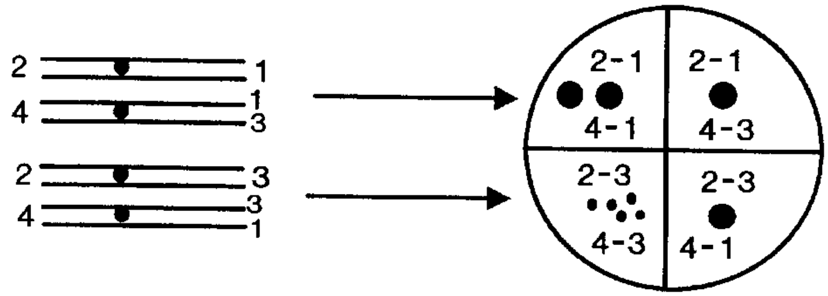
IF A SINGLE CROSSOVER TAKES PLACE AT a:

Adjacent 2 - will not occur

Adjacent 1:

- 2 are viable

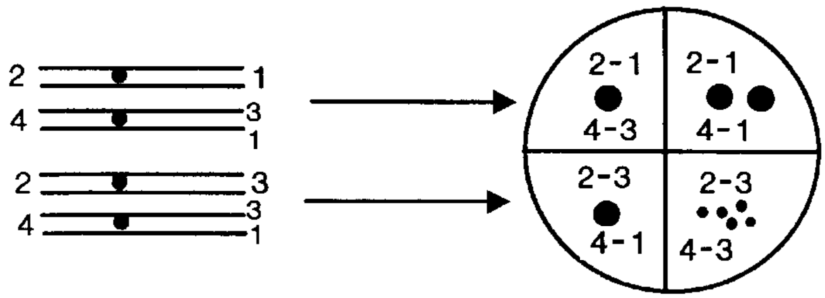
- 2 abort (1 diffuse)



Alternate:

- 2 viable

- 2 abort (1 diffuse)



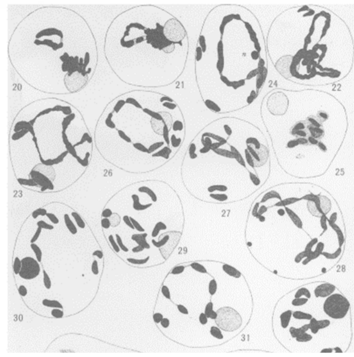
Problem: Cannot tell adjacent 1 apart from alternate, as both tetrads are the same.

-The # of tetrads with 1 diffuse nucleolus gives the # of crossover tetrads
- The # of NCO separations is made of 3 types:
  - Adjacent 1 = frequency of tetrads with 2 diffuse nucleoli
  - Alternate = amount of viable pollen [alternate] - viable pollen from crossover tetrads [adj1 with CO + alternate dis] = % tetrads with 1 diffuse × 2
  - Adjacent 2 = amount of aborted pollen - adjacent 1 - aborted pollen from crossover

Permanent translocation heterozygotes

Gates 1908; Golczyk, Massouh & Greiner, 2014

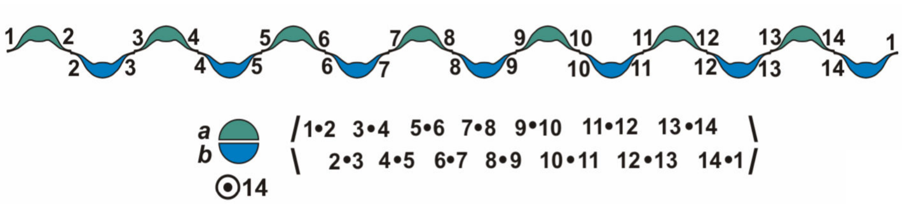
(see section 5d on *Oenothera* cytogenetics)

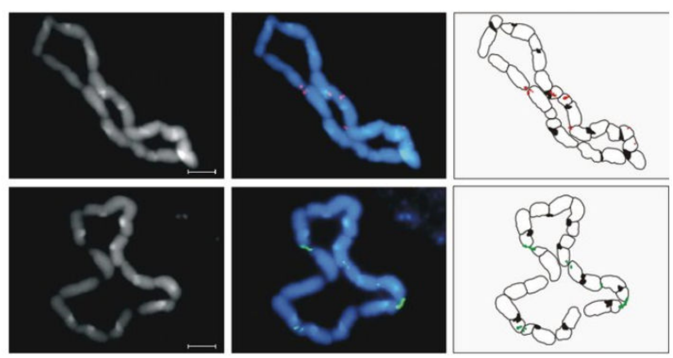


Oenothera

It is possible to get every chromosome involved in a translocation

Originally found in *Oenothera* by Gates, 1908

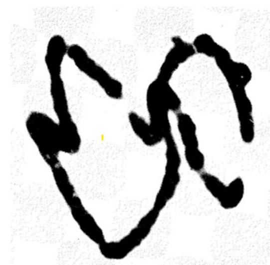




Use of translocations in mapping & breeding

Burnham 1946, Sisodia & Shebeski, 1965; review by **Farré et al., 2014**

- Used for chromosome mapping, assigning linkage groups to chromosomes
- Used for development of physical maps
- Burnham (1946) tried to get *Oenothera*-type translocation complexes in maize and barley, but failed
    - Benefits: could get true-breeding hybrids from seed!
    - As translocations increased, fertility decreased
    - Sisodia & Shebeski. 1965. Got ring chromosomes, but plants were sterile



⊙ 14 in barley, Sisodia & Shebeski, 1965

- Works in *Oenothera*, but only because it is in conjunction with lethals, etc.
    - **Golczyk et al, 2014:** The end-segments are deprived of canonical telomeres but capped with constitutive heterochromatin
- Works better with metacentric chromosomes

- Also found in *Rhoeo discolor*, *Paeonia californica*, *Viscum fischeri*, and *V. engleri* (East African mistletoes).
    - In these mistletoes, 2n = 23♂, 22♀.
    - The ♂ has 4 X and 5 Y chromosomes, all involved in translocations with the autosomes, so its gametes have 12 chromosomes.
    - The ♀ has 8 X chromosomes, none of which are involved in translocations.
        - It forms 11 II, and its gametes have 11 chromosomes.