Fundamental concepts

Frequency of chiasmata = frequency of CO

Brown and Zohary, 1955

Used a genotype of *Lilium formosanum* that had a chromosome with a terminal deficiency (obtained by exposing the pollen to X-rays), and therefore formed a heteromorphic pair at meiosis.

- Looked at metaphase I and anaphase I:

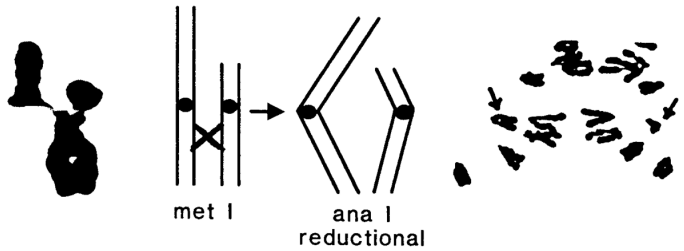


Figure 1. Photographs and interpretive drawing of events leading to a reductional anaphase I.

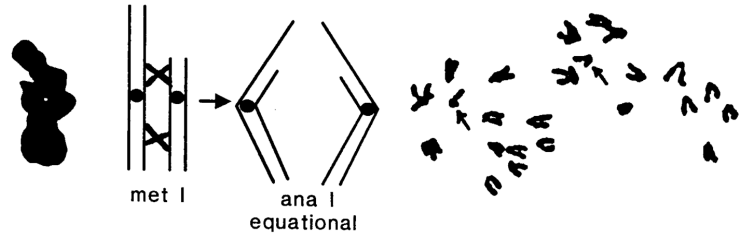


Figure 2. Photographs and interpretive diagram of events leading to equational anaphase I.

	Metaphase I		Anaphase I		P
	X-mata in heteromorphic arm	no X-mata	Equational	Reductional	
Year: 1951	71% (452)	29% (183)	71% (172)	29% (69)	>0.50
Year: 1953	51% (120)	49% (114)	55% (081)	45% (67)	>0.50

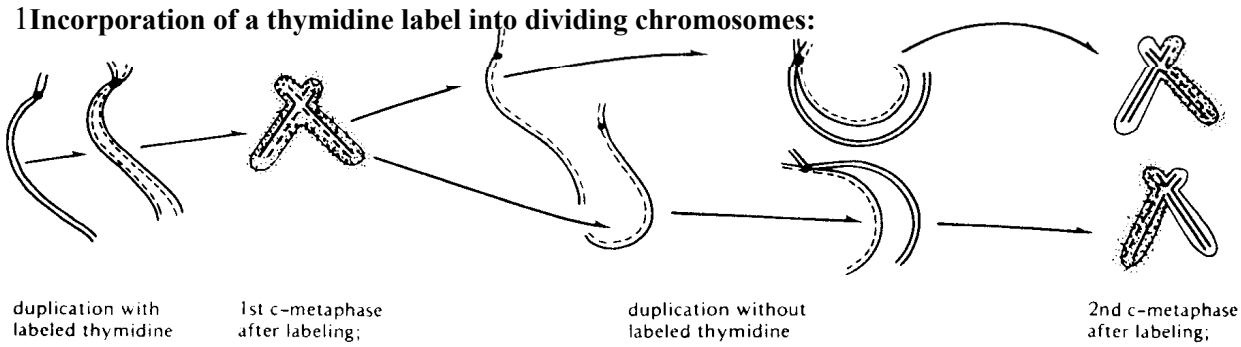
This work also shows that:

- A given crossover only involves 2 chromatids
- Exchange is between non-sister chromatids

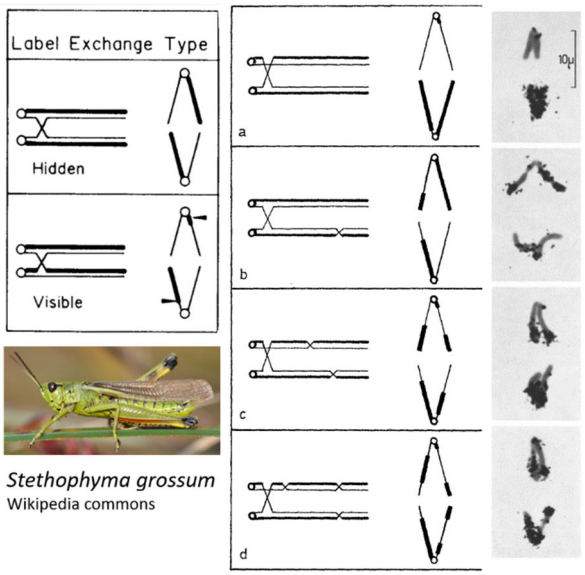
Jones, 1971

Studied crossing over in the ♂ grasshopper (*Stethophyma grossum*), which has very localized chiasma, i.e., they always occur in the same spot on the chromosome

- Treated them with tritiated thymidine at the next-to-last S phase prior to meiosis, effectively radiolabeling one sister chromatid of each chromosome.
- Found that half of all chiasmata produced a labeled exchange



- Some argued that his observations were an artifact of the radiation, but results have since been verified using bromodeoxyuridine labelling, which color-labels one DNA strand.
- The observation that 1 crossover = 1 chiasma has been called the <u>chiasmatype hypothesis</u>
- Although postulated in 1909, it was not considered proven until 1971.



Stethophyma grossum
Wikipedia commons

Recombination takes place between homologs

Stern, 1931 (Drosophila)
Creighton & McClintock, 1931 (Maize)

Need a chromosome pair with heteromorphic at both ends and with genetic markers on both arms.

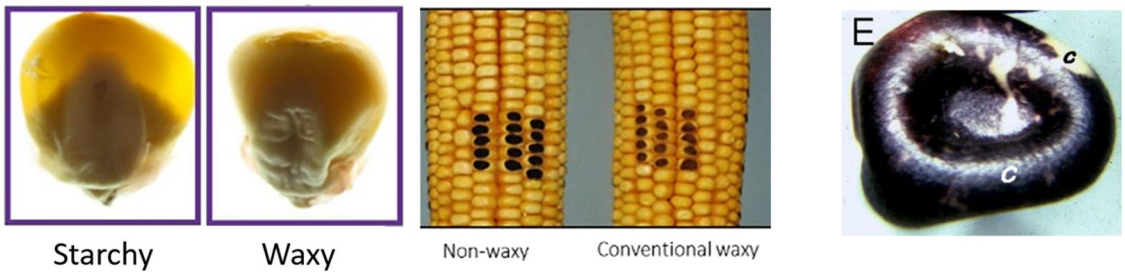
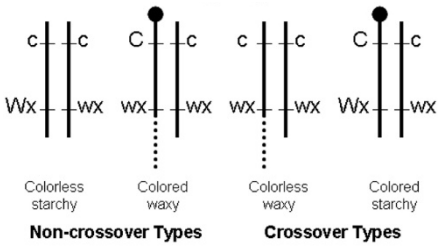


Figure 2. Figure 6. Corteva; Fedoroff NV. 2012. PNAS 109: 20200-20203; https://doi.org/10.1073/pnas.1215482109

- Creighton and McClintock worked with maize, with chromosome 9, which sometimes has a knob at the end of it.
- In addition, it had an arm of chromosome 8 translocated (i.e., interchanged) onto it, making the arm visibly longer.
- The genes C, for colored aleurone, and Wx, for waxy endosperm, are on chromosome 9, and linked to the knob.
- A cross was set up as follows, leading to 4 types of progeny

Colored, starchy × colorless, starchy

$$\frac{knobless \cdot c \cdot Wx \cdot normal}{knob \cdot C \cdot wx \cdot interchange} \times \frac{knobless \cdot c \cdot Wx \cdot normal}{knobless \cdot c \cdot wx \cdot normal}$$



Progeny types	Cytological Features	Attributed cytological mechanism
Colored, waxy (*C_*, *wxwx*)	Knob, Interchanged	Non-CO
Colorless, waxy (*cc*, *wxwx*)	Knobless, Interchanged	Crossovers
Colored, starchy (*C_*, *Wx_*)	Knob, normal	Crossovers
Colorless, starchy (*cc*, *Wx_*)	Knobless, Interchanged	Crossovers
	Knobless, normal	Non-CO

Figure 3. https://www.nature.com/scitable/content/mcclintock-and-creighton-s-work-in-maize-41556/

"Conclusions. - Pairing chromosomes, heteromorphic in two regions, have been shown to exchange parts at the same time they exchange genes assigned to these regions."

Crossing over occurs at the 4-strand stage

The linear sporads of *Neurospora*

In *Neurospora*, the spores are recovered in a linear order, permitting tetrad analysis:

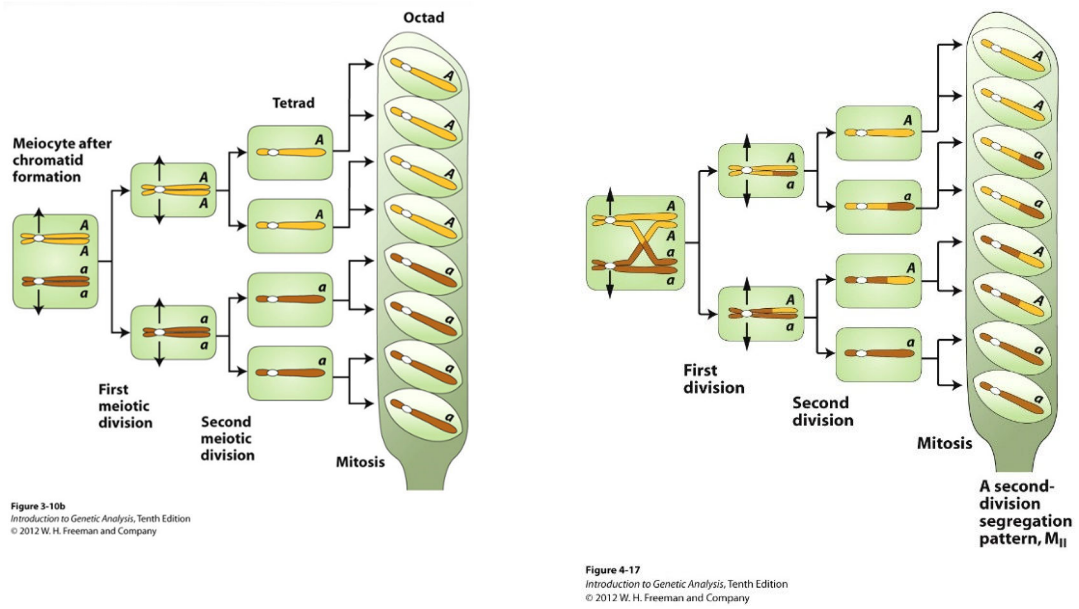


Figure 3-10b
Introduction to Genetic Analysis, Tenth Edition
© 2012 W. H. Freeman and Company

Figure 4-17
Introduction to Genetic Analysis, Tenth Edition
© 2012 W. H. Freeman and Company

- Half of the recovered spores are recombinant types, and half are parental types
- If crossing over occurred at the 2-strand stage, it would be impossible to recover any parental types
    - All would be crossover types:

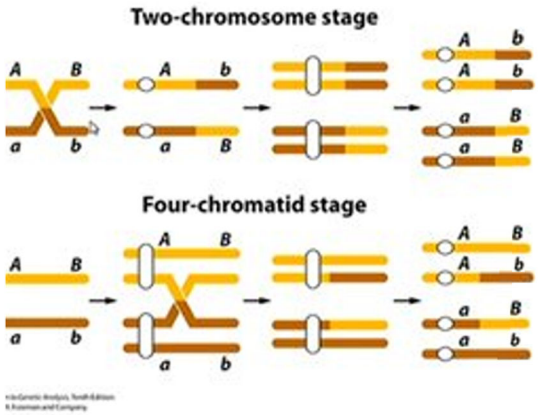


Figure 4. https://quizlet.com/

Models of chiasma

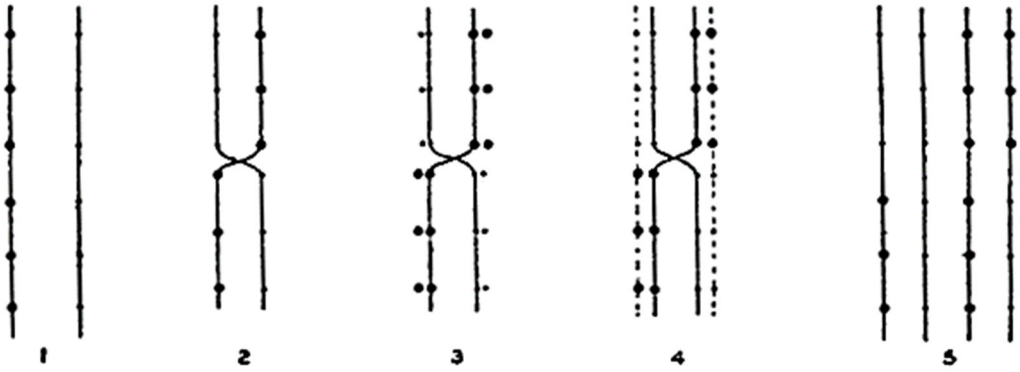
Sax, 1932

Gets the observations right, but arrives at wrong conclusion. Any model of CO must account for these facts:

- Crossing over occurs at the 4-strand stage
- No (little) DNA synthesis occurs during crossing over
- It is precise - no DNA is lost, gained, or changed (not true for repetitive DNA)
- It involved 2 out of the 4 strands
- Chromosome interference occurs
- There is no (little) chromatid interference
- The cytological events related to the genetic event
- No more segregation occurs after meiosis
- DNA replication is semi-conservative
- Genetic maps are linear
- Presence of a synaptonemal complex

The copy choice model

Belling 1933; Frease 1957

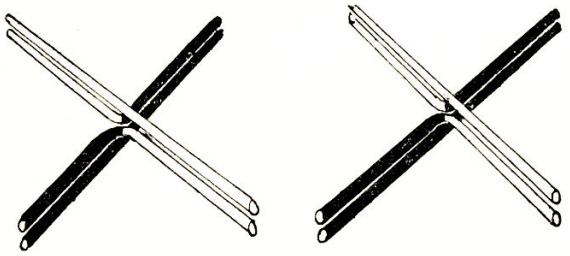


Ruled out because:

- Cannot explain 3 or 4 strand crossovers, and would require substantial DNA synthesis when none is known to occur.
- Also requires conservative DNA replication.

The chiasmatype or 1-plane model

Janssens, 1909



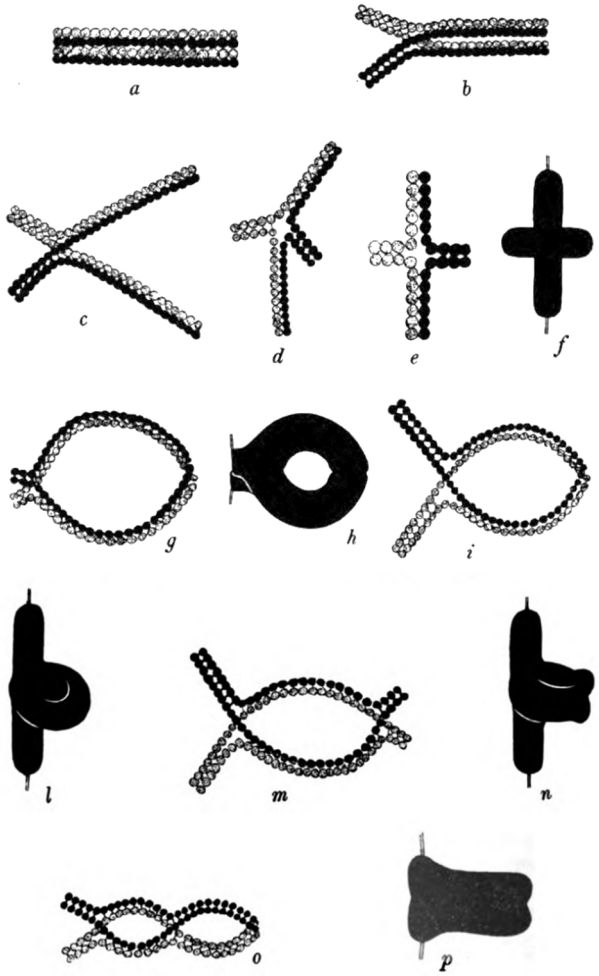
Frans Alfons Janssens
1865-1924

- In this model, chromatids do not exchange pairing partners
- Highly controversial and not accepted for 6 decades

The 2-plane model

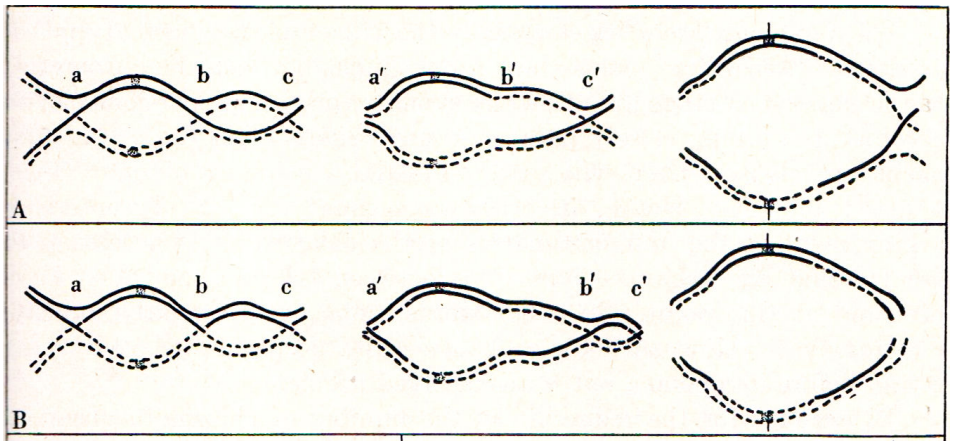
Granata, 1910

- Key feature is the switch in pairing partners



1-plane and 2-plane models

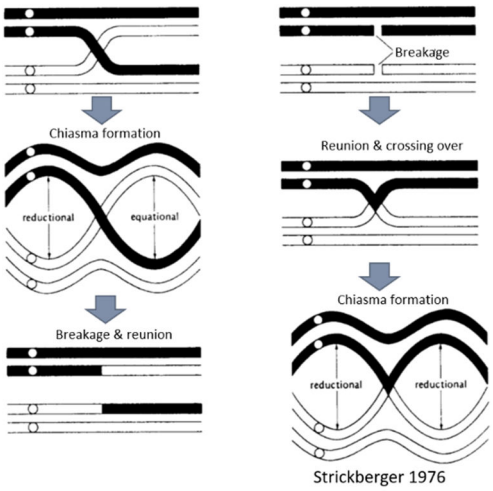
First summarized by **Sharpe, 1934**, who did not pick one over the other



Classical or 2-plane model

Blamed on **Sharp, 1934**, based on McClung and Sax, but it precedes him – he just explained what others thought

- Chiasmata cause crossing over
- CO would occur at MetI to AnaI



Strickberger 1976

Chiasmatype or 1-plane model

Janssens, 1909, with follow-up by **Darlington, 1930**

- Crossing over leads to chiasma

Evidence for the chiasmatype hypothesis

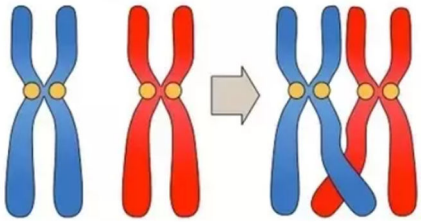
The synaptonemal complex

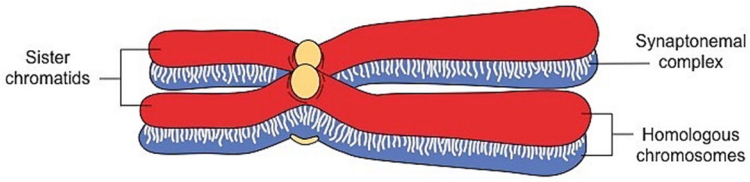
Chiasmatype model requires sister chromatids to change pairing partners.

- Means the synaptonemal complex would have to dissolve and reform, not something known to happen.

- *The SC was unknown at the time.*

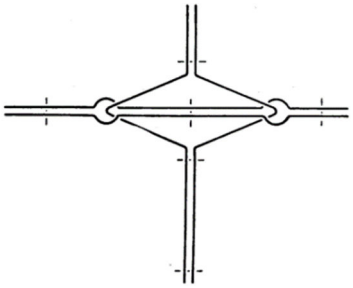
Pairing and chiasma as commonly depicted



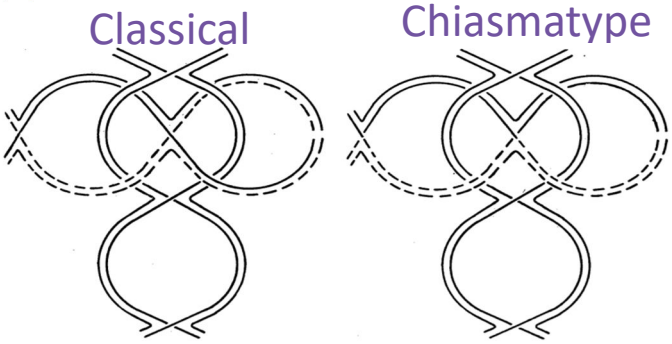


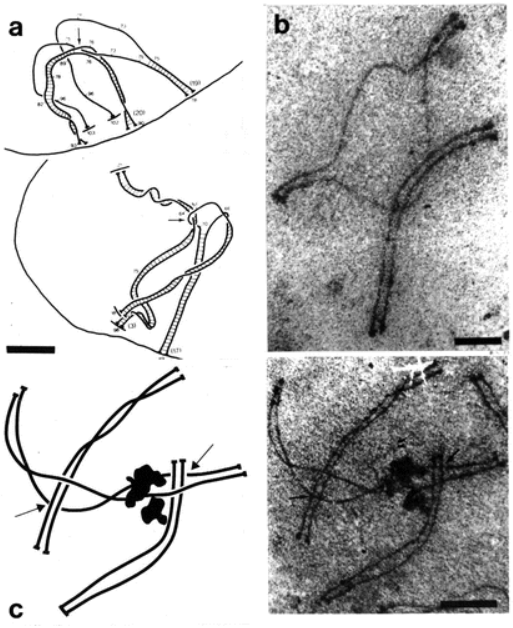
Interlocking bivalents

Mather, 1934 noted that chromatids would need to switch pairing partners to explain the interlock, and decided that was unlikely, hence supporting the chiasmatype model



Interlocking bivalent in Lilium regale. https://www.jstor.org/stable/pdf/2471717.pdf

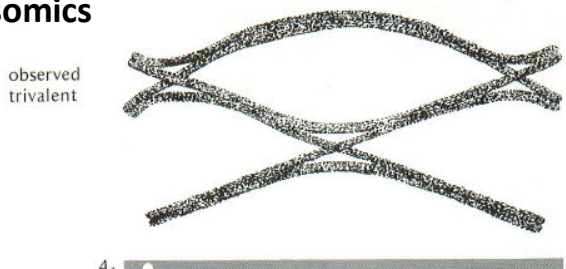


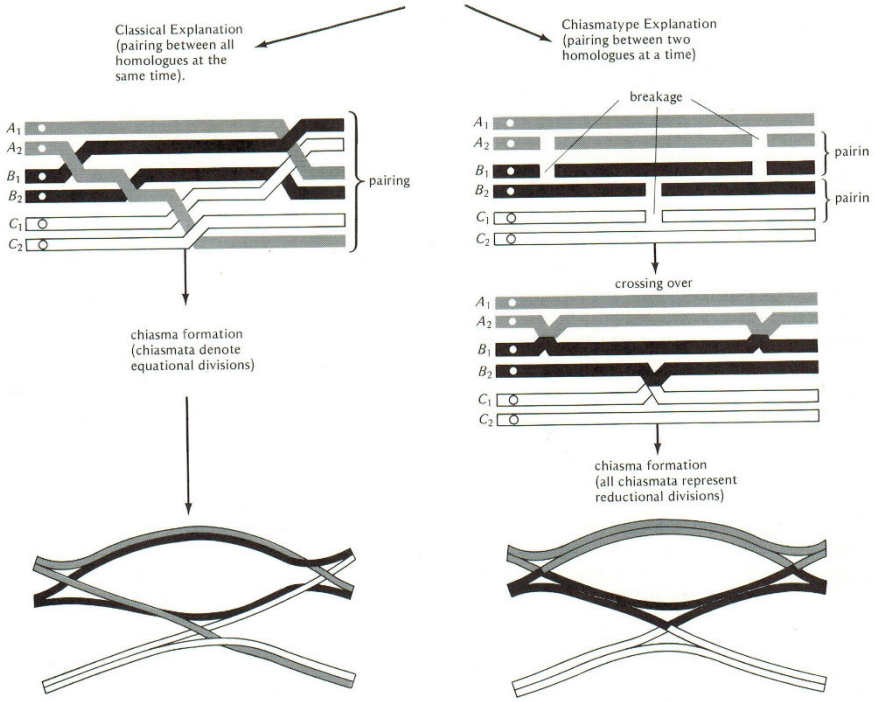


Examples of interlockings seen in Bombyx mori. Zickler & Kleckner, 1999

Ji et al., 1999. After homologues pair, double stranded breaks take place, only a few of which will lead to crossovers. This can lead to the interlocking chromosomes.

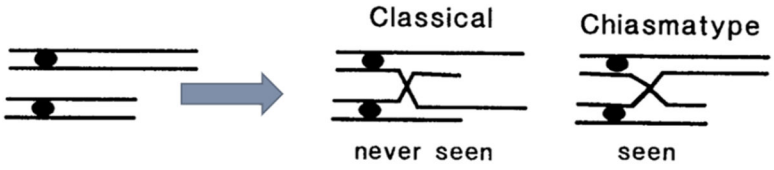
Chiasmata in trisomics





Heteromorphic pairs @ pachytene

No evidence from heteromorphic pairs @ pachytene for the classical model-



Pairing and crossing over

Synapsis

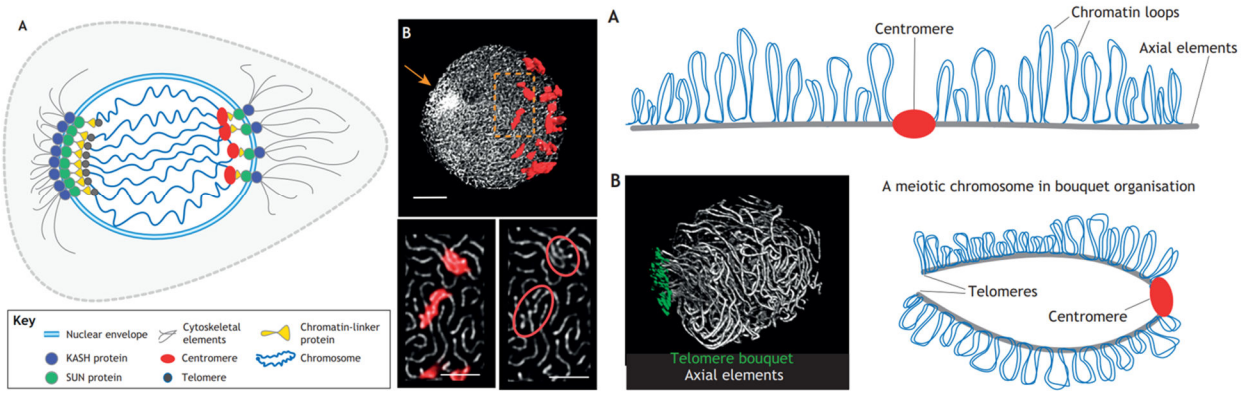
The pairing that occurs between homologues (chromosomes with the same sequence of genes) during meiosis only (zygotene and pachytene)

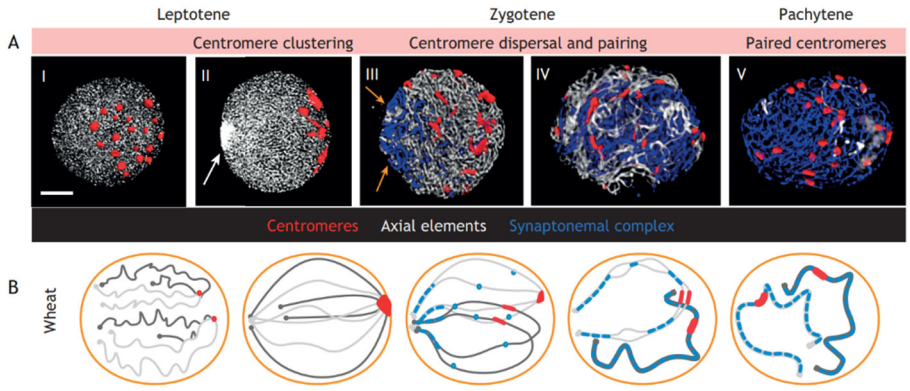
Lukaszewski, 1997; Abirached-Darmeny et al., 1983

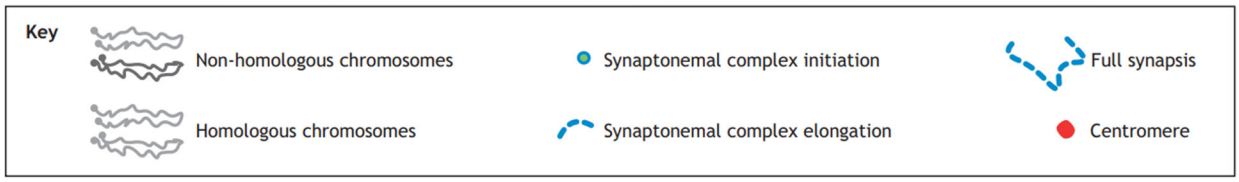
Homozygous no deletion | Hemizygous terminal deletion | Homozygous terminal deletion

- Conclude that - Pairing starts at chromosomal ends
  - Homology of chromosomal ends is necessary for pairing
- Hence importance of clustering telomeres and centromeres before meiosis

Sepsi & Schwarzacher, 2020







The synaptonemal complex

Paired chromosomes form a synaptonemal complex

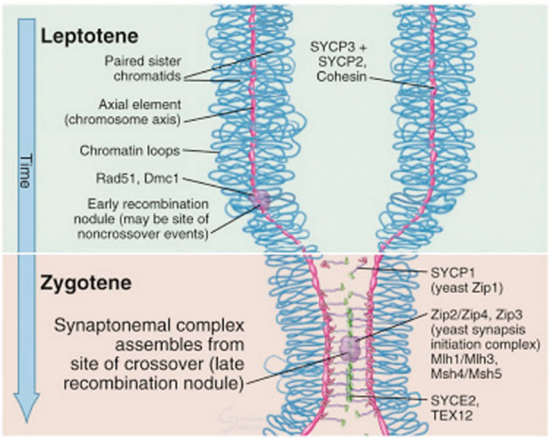


Figure 5. Cell Biology, third edition (2017)

- Discovered by Moses and Westergard. The whole thing is about 1800 nm wide.
- The central element is about 300 nm wide and consists of a protein called adhesin.
- The lateral elements are of RNA and protein, and are about 450 nm wide.
- Synaptonemal complexes do not form in ♂ Drosophila, ♀ silk moth, and haploid tomatoes

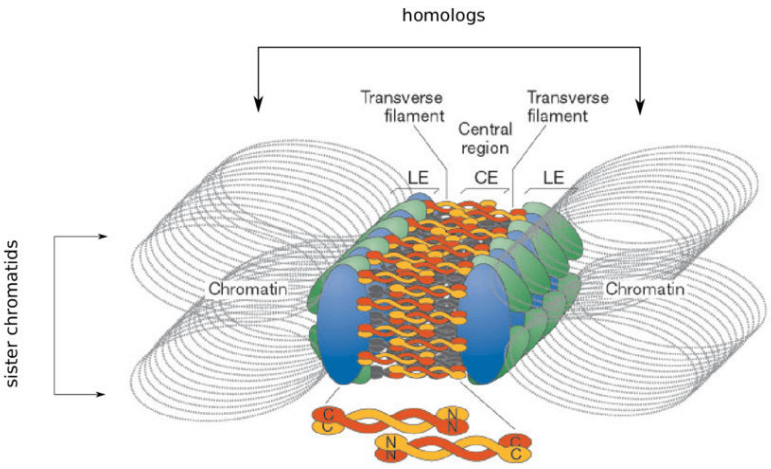
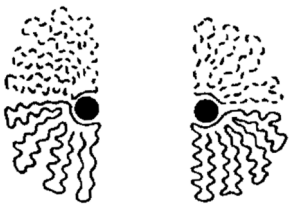


Figure 6. Alexandra Popa. The evolution of recombination and genomic structures: a modeling approach.. Bioinformatics [q-bio.QM]. Université Claude Bernard - Lyon I, 2011. English. <tel-00750370>



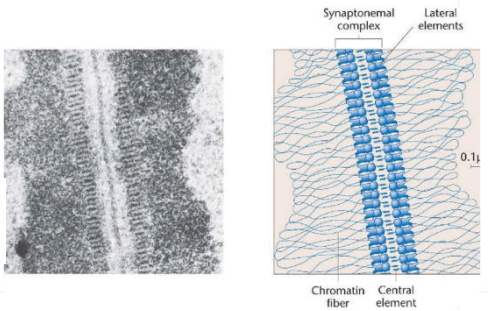


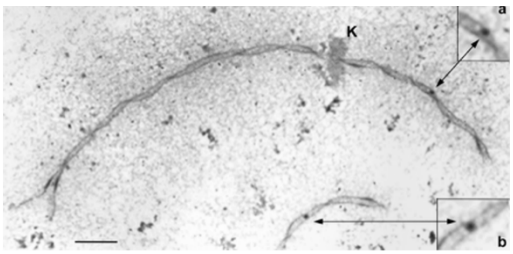
Figure 7. http://bio3400.nicerweb.net/Locked/media/ch02/02_14-synaptonemal_complex.jpg

Recombination nodules

Sherman and Stack, 1995

The synaptonemal complex contains recombination nodules (RNs)

- Are spherical to ellipsoid, about 100 nm Ø
- Lie in central region of the synaptonemal complex during mid to late pachytene
- Are sites of CO and future xmata
- Every synaptonemal complex has at least one recombination nodule



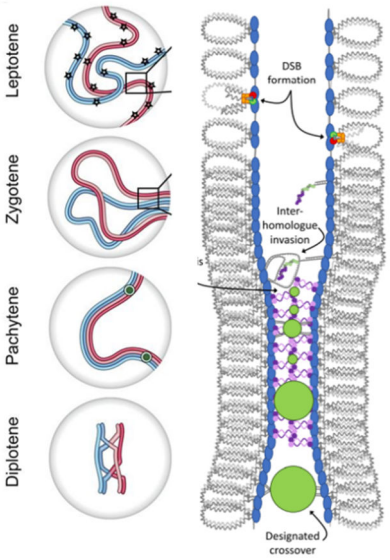
Anderson et al., 2003. *Two recombination nodules in early diplotene chromosomes – note the large chromosome has started to desynapse. K=kinetochore.*

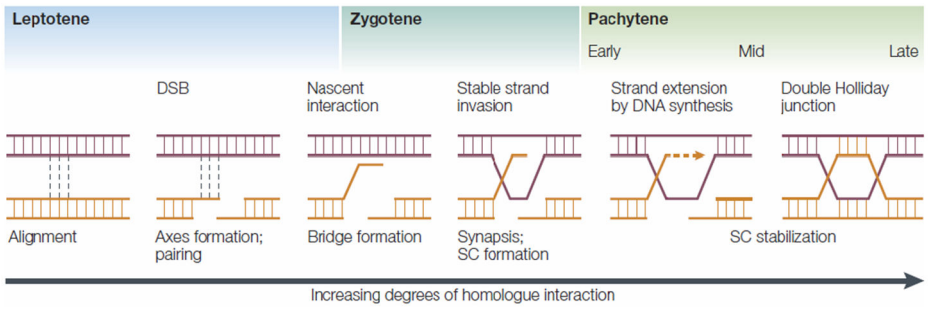
Crossing over

Gerton and Hawley, 2005; Lambing et al, 2018; Sepsi & Schwarzacher, 2020; Lloyd, 2022

Pairing at the molecular level:

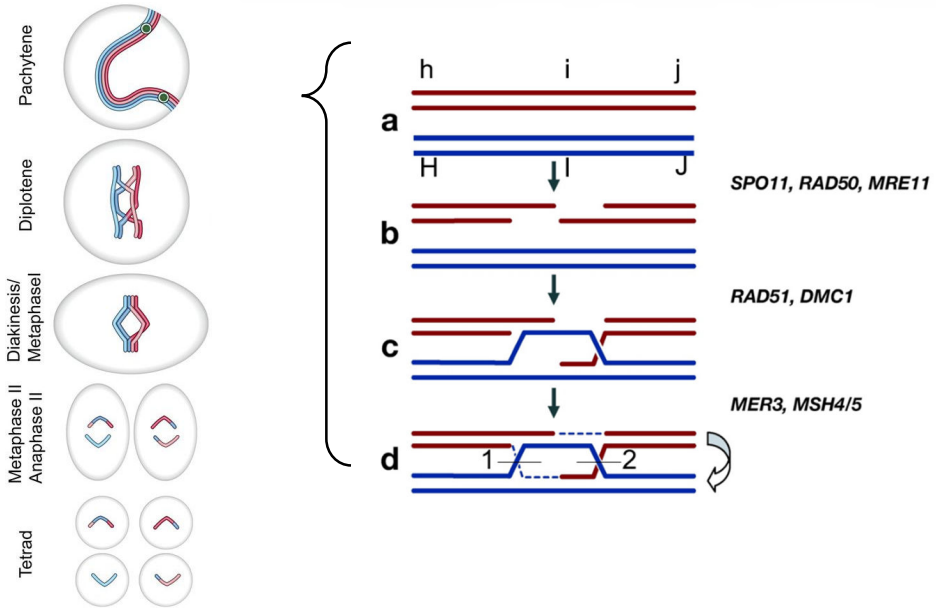
- Double strand breaks leave ssDNA ends. These find their homologue, lead to pairing:





Notice that DNA in loops will not recombine in any given meiosis

Modified from **Ma, 2005; Lambing et al, 2018**



Looking at the chiasmatype hypothesis in more detail:

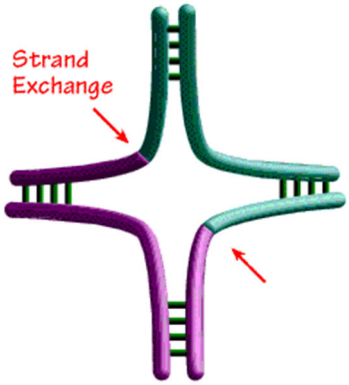
a) Original homologues at 4-strand stage

b) A double-strand break is made in the chromosomes, followed by 5' to 3' exonuclease digest of the exposed 5' ends

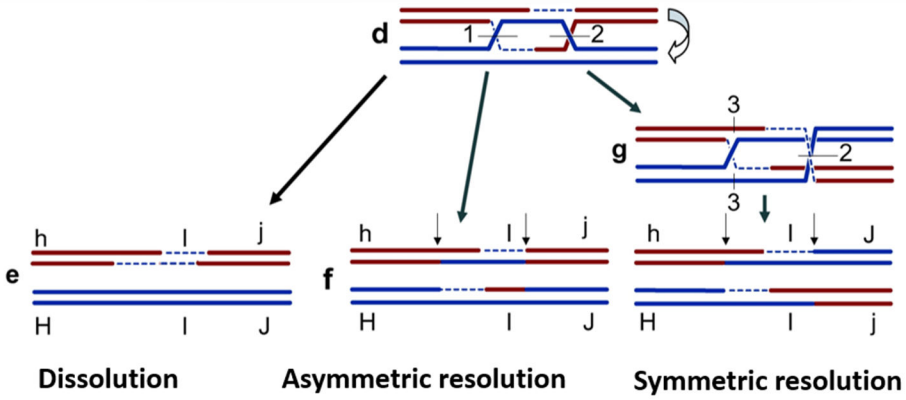
c) Strand invasion. 3' ends serve as primers for DNA synthesis. The uncut homologue serves as template.

d) 4-strand intermediate with 2 Holliday junctions

Holliday junction



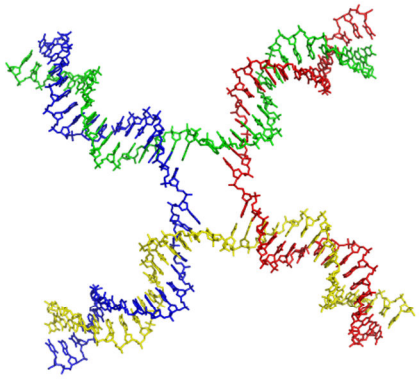
Illustrates how two DNA strands will cross over in the chiasmatype model
http://emergentcomputation.com/Images/Holliday3D.gif

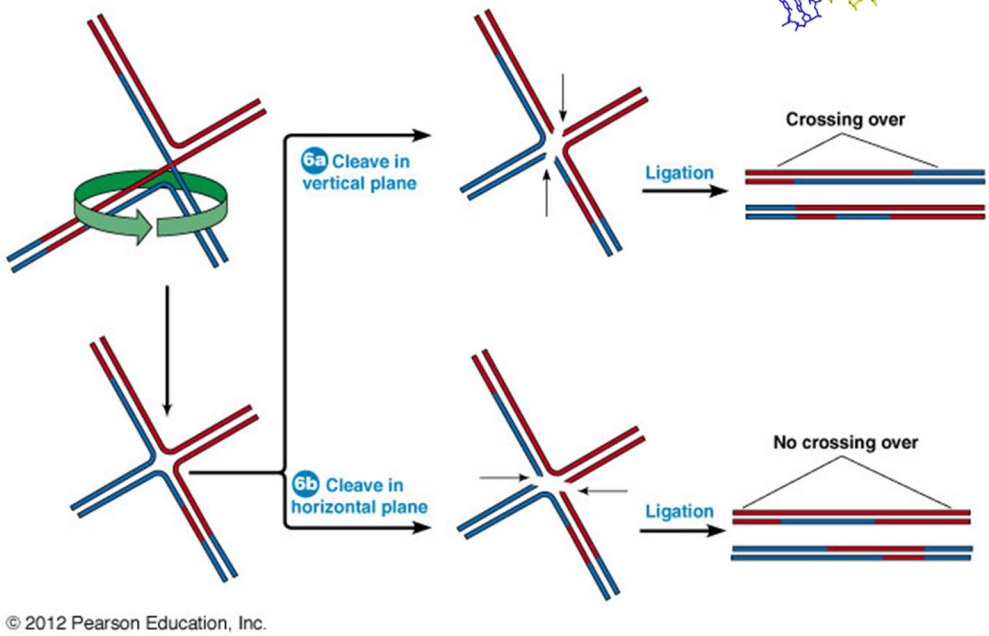


e) Dissolution, with conversion but no recombination. Arrows indicate area of gene conversion.

f) Asymmetric resolution, with conversion but no recombination. Due to cutting and re-ligation at 1 and 2. Arrows indicate area of gene conversion.

g) The second possible outcome- symmetric resolution, with recombination, following cuts and re-ligation at positions 2 and 3. Gene conversion still took place at the original DNA repair site. Notice that the site of recombination (left arrow) is not where the conversion track ends (right arrow).





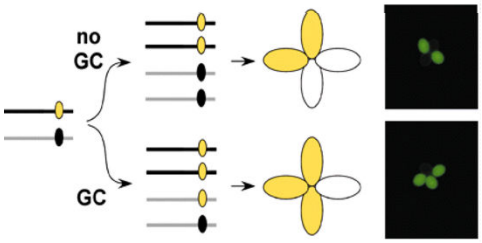
Gene conversion

Pollen tetrad of arabidopsis. Preuss et al., 1994

Francis et al., 2007; Sun et al., 2012

The frequency of gene conversion was measured in arabidopsis, based on the repair of a defective yellow fluorescent protein transgene.

- There is a mutation in arabidopsis where the 4 microspores do not fall apart, allowing conversion to be easily detected.
- Frequency = 1 per 366 meioses
- = 3.5×10^{-4} conversions per locus per meiosis



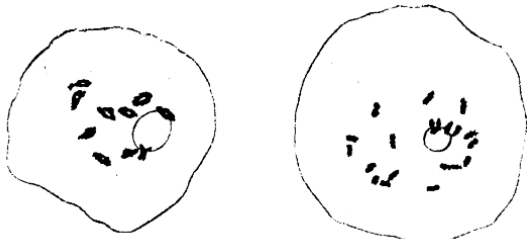
The limitation is that conversion events are not detectable if they there are no markers. Overcome with Whole Genome Sequencing

Yang et al., 2012

- WGS of 40 arabidopsis F2 plants
- Conversions 10 - 20 kb in size are >90x more frequent that COs
- Causes 600x more protein sequence alterations than mutations do!

Pairing mutants

Soost, 1951



Diakinesis in normal and asynaptic maize (Beadle, 1930)

Asynaptic –

Pairing never occurs. ∴ no crossing over

Desynaptic –

Pairing occurs, but bivalents fall apart prior to diplotene.

- Tends to reduce the amount of crossing over that can occur.

Synaptic mutants –

As it is difficult to distinguish between asynapsis and desynapsis (pachytene studies are required), Soost, 1951, termed both of these collectively as synaptic mutants

- These mutations are conditioned by single, recessive genes.

Metanaphase

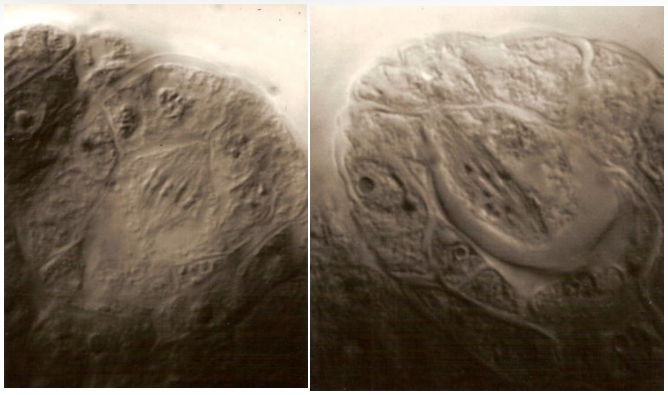


Figure 8. (left) Metaphase I in normal and (right) Metanaphase I in asynaptic megaspores of potato.

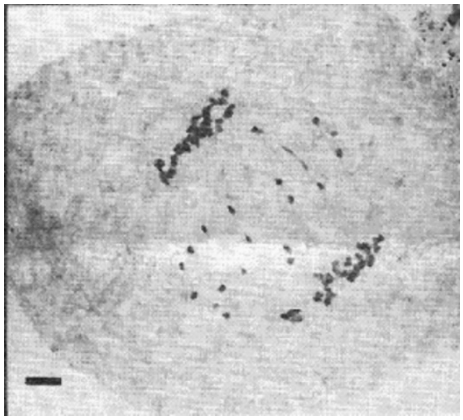
Pairing is necessary to provide the correct orientation of chromosomes during metaphase I and regulate the separation of homologues during anaphase I.

Without pairing of homologues, meiosis is disturbed.

Synaptic mutants have a 'metanaphase' stage instead of diakinesis, metaphase I, and early anaphase I stages. These stages are indistinct due to the lack of metaphase plate formation [terminology = **Beadle, 1930**]. Separation at Anaphase I is entirely at random.

Figure 9. Lagging chromosomes in a pollen mother cell of buffelgrass. Bar = 10 µm

Behavior of univalents



- Can separate at 1st division, segregate at random in the 2nd division
- Can remain off the plate at first division, divide during the second
- Mis-divide, forming either isochromosomes or telosomes
- Premature separation of sister chromatids can also occur
- Can be lost altogether

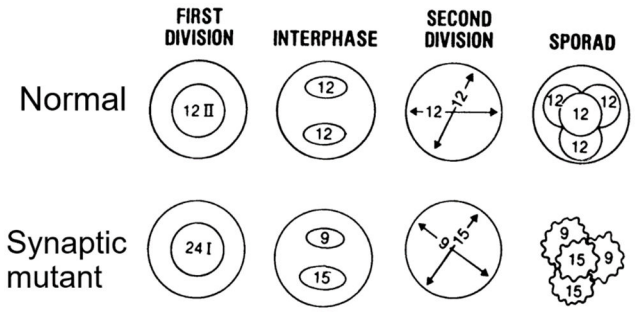
Photo: Visser NC, JJ Spies, and HJT Venter. The presence of synaptic and chromosome disjunction mutants in Cenchrus ciliaris (Poaceae: Paniceae). Bothalia 29:327-334.

Meiosis in synaptic mutants

Peloquin 1983

When separation at Anaphase I is entirely random, it results in microspores with the wrong chromosome complement.

- These usually abort, resulting in male sterility.



Top: normal meiosis and microsporogenesis. Bottom: microsporogenesis in a synaptic mutant. Peloquin 1983

- A microspore with an extra chromosome (or a megaspore with 1 or 2 extra chromosomes) can be viable.
    - This is a great source of trisomics.

- The presence of lagging chromosomes is indicative of synaptic mutants.
- These lag behind and get excluded from the telophase nuclei.
    - Each one can form a micronucleus.
- A sporad containing nuclei and micronuclei is called a polyad.

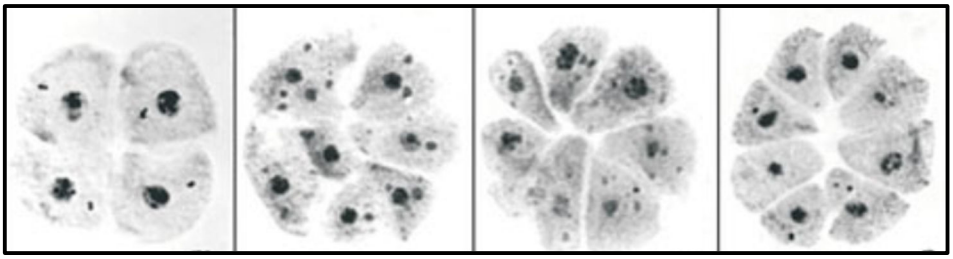


Figure 10. Polyads from 4x Brachyaria brizantha. Mendes-Bonato et al., 2009.

Disjunction in synaptic mutants

Belling & Blakeslee, 1927

Any spore that deviates too much from the n chromosome number will abort.

- Synaptic mutants are highly sterile.

In a synaptic mutant, each chromosome has a p = ½ chance of going to the correct pole.

- $(½)2^x$ of the pollen mother cells will result in viable spores.
    - $(½)^x$ is the probability all the chromosomes from 1 haploid genome will go to one pole.
    - However, their homologues must also go to the other pole, and that probability is $(½)^x$.

E.g., for red clover, which is 2n = 2x = 14:

- $(½)^7 = {}^1/_{128}$. Thus, $(½)^{14} = {}^1/_{128} \times {}^1/_{128} = {}^1/_{16384}$ is the number of PMCs that will give fertile pollen with the correct x number, as long as separation at ANAI is random, that is, follows a binomial distribution.
- However, separation at AnaI is not always completely random.

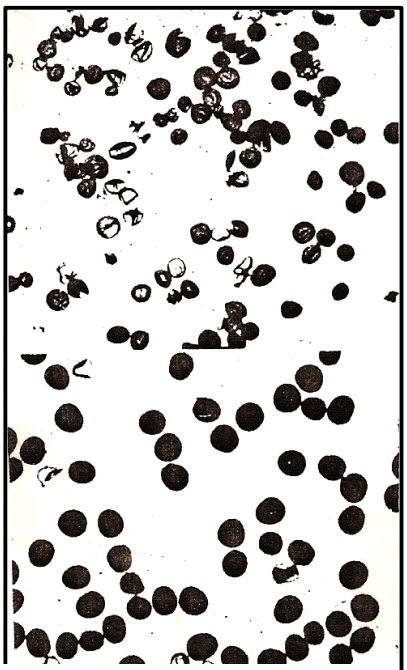
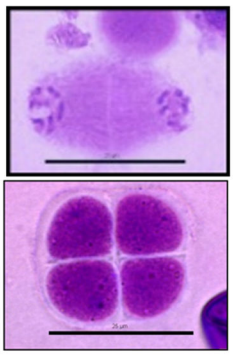


Figure 11. Pollen from asynaptic (top) and normal maize. Notice even normal maize has some aborted pollen grains [Beadle, 1930]

Correction for small *n*

Jackson and Jordan, 1975

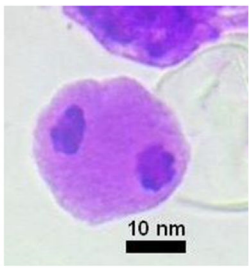
$(½)^n$ does not hold in cases where n is small, and there is a large probability that one of the products after telophase I would have no chromosomes in it.



Class alum Doug Heckart
Seashore paspalum

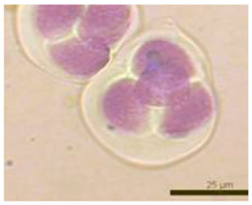
In this case, for monocots, use:

$$\frac{4}{4(2^n)-2}$$



Class alumnus Aaron Hoskins
Jalapeño pepper

- For eudicots, use: $\frac{4}{4(2^n)-4}$



Class alumna Rebecca Tashiro
White clover

Where:

- numerator 4 = total number of spores expected
- denominator 4 = maximum number of potential spores
- n = chromosome number
- 2 = number of potential spores lost if cytokinesis occurs after meiosis I
- 4= number of potential spores if cytokinesis is not until Meiosis II

Chromosome #	$(0.5)^n$	$4/[4(2^n)-2]$	$4/[4(2^n)-4]$
1	50.00	66.67	100.00
2	25.00	28.57	33.33
3	12.50	13.33	14.29
4	6.25	6.45	6.67
5	3.13	3.17	3.23
6	1.56	1.57	1.59
7	0.78	0.78	0.79
8	0.39	0.39	0.39
9	0.20	0.20	0.20
10	0.10	0.10	0.10
11	0.05	0.05	0.05
12	0.02	0.02	0.02
13	0.01	0.01	0.01