Somatic instability

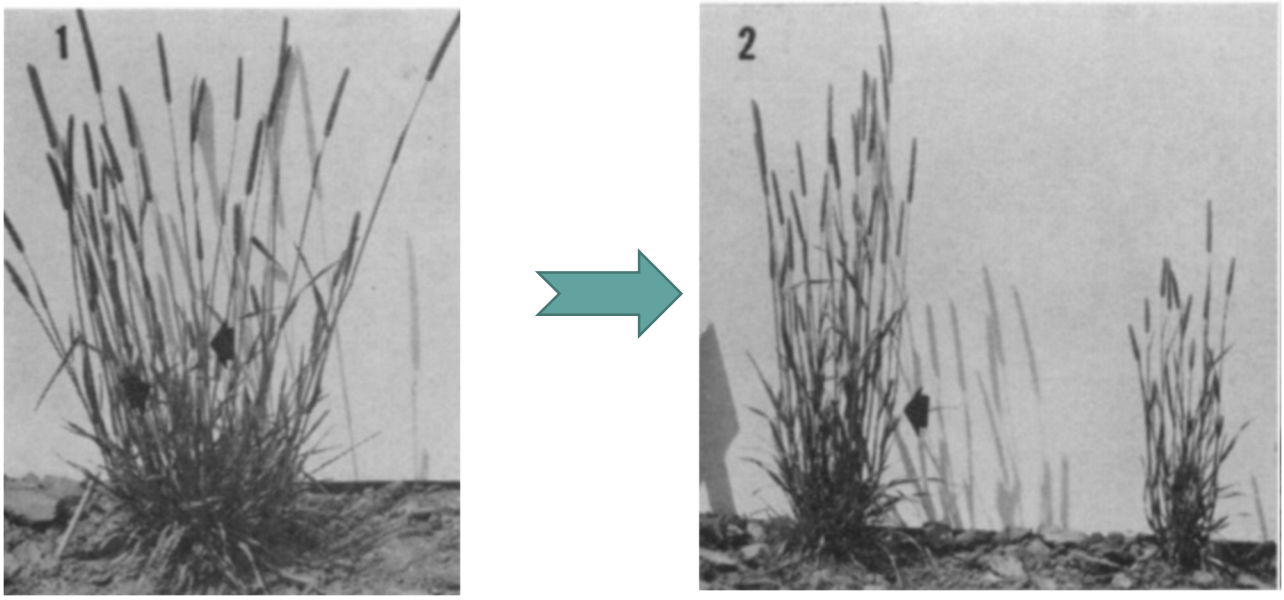
This is a catch-all phrase that includes:

- Aneusomaty
- Chromosome substitution
- Changes in DNA content
- Chromosomal aberrations

Nielsen 1962 - 1968

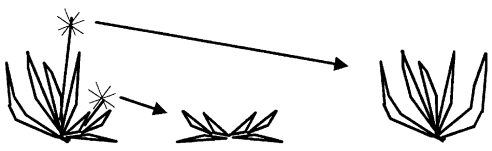
Somatic instability in grasses in relation to breeding and evolution.

- Intra and interspecific hybrids, interploid hybrids, intergeneric hybrids
- Timothy, bromegrass, sudangrass, & sudan-sorghum hybrids
- Found chromosome numbers in root tips of intergeneric hybrids to range in number from 8 to 80.
- Also found intraplant morphological variation

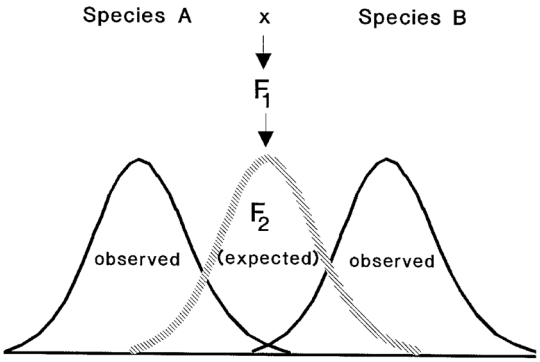


Arrows showing different height of culms in a 5x hybrid of timothy (*Phleum pratense*)

Plants from different corms of the unstable hybrid plant



F2 segregation



In the F_2, more parental types than expected were recovered:

If backcrossed, would get a faster return to parental type than expected

- Due to factors which favor cells with genome from only one species (genome incompatibilities)

Many explanations have been put forward over the years:

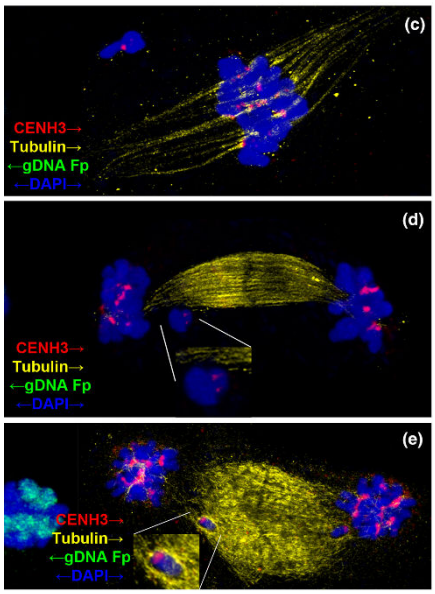
- Somatic cell selection
- Premeiotic selection
- Prophase I lethality
- Gamete selection
- Affinity of chromosomes of one species to stick together
- ¿Related to nuclear architecture?
- **¿Species specificity of centromeres**? → actually, of kinetochores

- Happens when two species that have been separated a long time (and consequently evolved apart) are brought together
- Somatic instability most frequently associated with hybrid backgrounds.
- Chromosome substitution can also happen in polyploids
- Important in evolution, as is a source of variation, especially for asexual or apomictic species
- Attributed to "genome incompatibilities" or "nuclear-cytoplasmic incompatibilities"

Majka et al, 2023
Working with *Lolium* × *Festuca* (ryegrass × fescue) hybrids

- Loss of fescue chromosomes
- Kinetochore proteins NDC80 and NNF1
  - Exclusively expressed from the *Lolium* allele
  - *Festuca* alleles get silenced

Summary – fescue chromosomes do not attach well to spindles when *Lolium* kinetochore proteins are used.

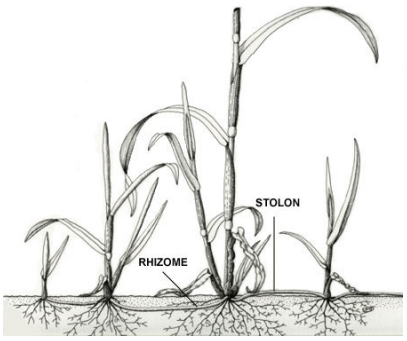


(c) Met I and (d) Ana I with fescue univalent not attached to the spindle. (e) Ana I with Lolium univalent attached to the spindle.

Bermudagrass

Caetano-Anollés, 1998

Heterogeneity from seed mixtures gives the same appearance as from somaclonal variation



Turfgrass grows horizontally from rhizomes and stolons and can propagate asexually through mitosis alone (thelawninstitute.org)

Burton developed a series of hybrids of native Bermudagrass crossed to a wild relative from Africa. These are used as forage (Coastal Bermudagrass released in 1943) and turf grasses:

Tifgreen - a turfgrass released in 1956

- 2n = 3x = 27, derived from *Cynodon dactylon* × *C. transvaalensis*
- gets patches of contrasting morphology and performance
- leads to legal problems

Tifdwarf - 1965 mutation from Tifgreen

Tifway - also a 3x hybrid
- mutation frequency is 18x less than that of Tifgreen and Tifdwarf
- off-types result from contamination

Cultivar Breakdown/Decline

Jensen, 1965

A cultivar does not need to be an interspecific hybrid to break down. All cultivars are said to "breakdown", manifested as loss of yield & other desirable traits over time

- Due to mechanical mixtures, accumulation of diseases, outcrossing, residual heterozygosity
- Some of this is the result of somatic instability
- Not an issue when a cultivar replaced every 2-3 years

Suarez & Favret, 1986

	Aneuploidy		
	Present	Absent	Percentage
Tall plants	19	6	76%
Tall plants with short dent glumes	2	1	67%
Early plants	4	13	23%
Short plants	2	-	100%
Spikes from tall plants	1	-	100%
Short spikes	1	1	50%
Spikes with high sterility	2	1	67%
Spikes with supernumerary spikelets	1	1	50%

Fernandes et al., 1991

	Cultivar		
PMCs with:	IAS 55	Londrina	Sonora 64
Unpaired chromosomes	11%	4%	28%
Broken chromosomes	4%	4%	4%
Lagging chromosomes	2%	5%	13%
Aneuploidy	7%	0%	4%
Normal tetrads	95%	98%	89%

Wheat varieties differ in their stability, with Norin 10 germplasm being most unstable

- Norin 10 was used to develop the short wheats of the Green Revolution
- Drastic climate changes, agrichemicals, soil acidity, viral and fungal diseases can all increase instability (***McClintock, 1984***)

Norin 10 germplasm

"*Veteran in a New Field*" (1865) Winslow Homer

"You know in the song 'America the Beautiful,' we talk about amber waves of grain? That was not written about modern wheat. Rather than sway in the wind, the shorter modern wheat stalks only nod." --Thom Leonard, Flagpole Magazine 27 Jul 2016

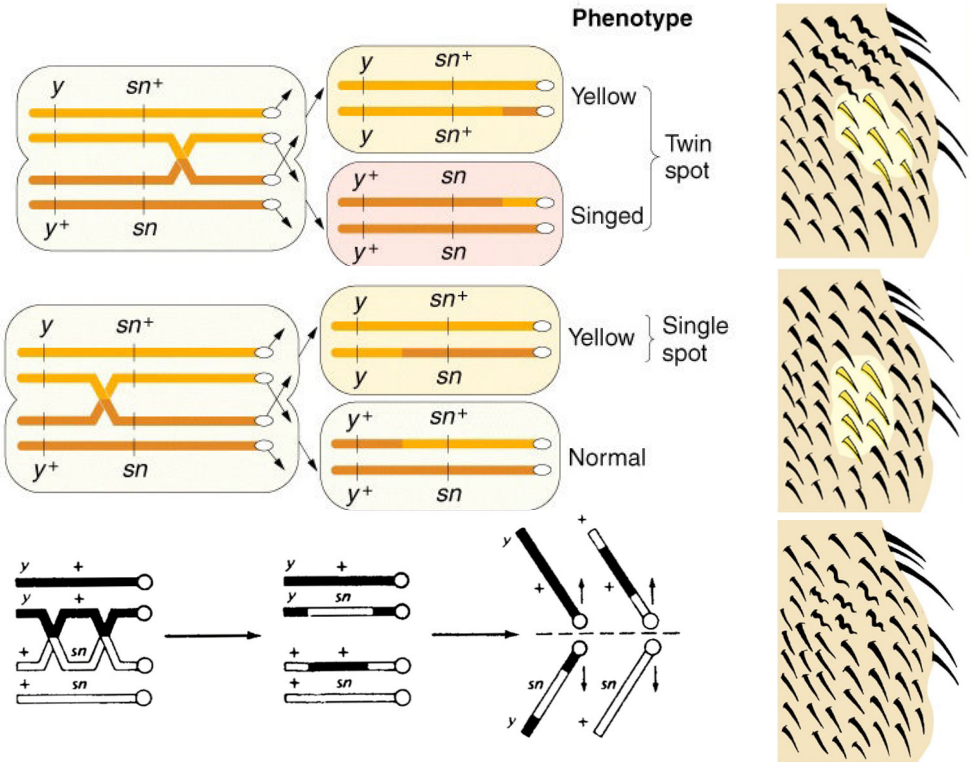
Cultivar breakdown is reason why certified seed got imposed – US Federal Seed Act of 1940.

- During the seed increase process, off-types are removed (rogued) before seed set
- Prevents cultivar breakdown

Somatic crossing over

Stern, 1936. Somatic crossovers in the Drosophila X chromosome which was heterozygous for the yellow (y) and singed (sn) loci. Figure credit: Alamy

- The phenomenon responsible for individuals with eyes of different color
- Much less common than meiotic crossing over
- Phenotype is a single or twin spots of recessive phenotype in a dominant background
- The earlier in development that the cross over occurs, the larger the spot
- Does not involve chiasma formation
- Overall, somatic crossing over is rare in nature, but is important in fungi which lack a sexual stage, depending on a parasexual cycle for recombination.
- Discovered by Stern, 1936, in the Drosophila X chromosome which was heterozygous for the *yellow* (*y*) and *singed* (*sn*) loci (B&W graphics from Strickberger):



http://www.mun.ca/biology/desmid/brian/BIOL2250/Week_Five/mitcroso.jpg
Peter J. Russell, iGenetics

Evans & Paddock, 1976

Frequency of somatic crossing over in plants ranges from:

- Soybean 5.74×10^{-5}
- Tobacco 7.70×10^{-6}

with cotton and tomato having intermediate values

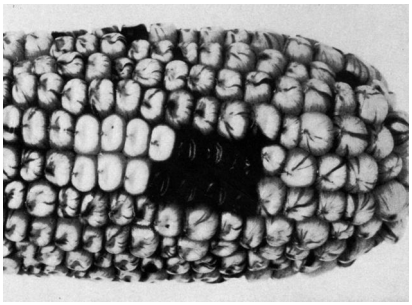
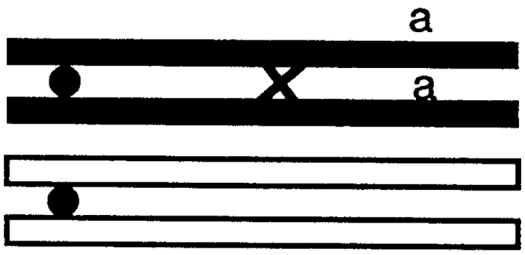


Figure 1 Twin spots in maize. Brink & Nolan, 1952

Sister Strand Crossovers

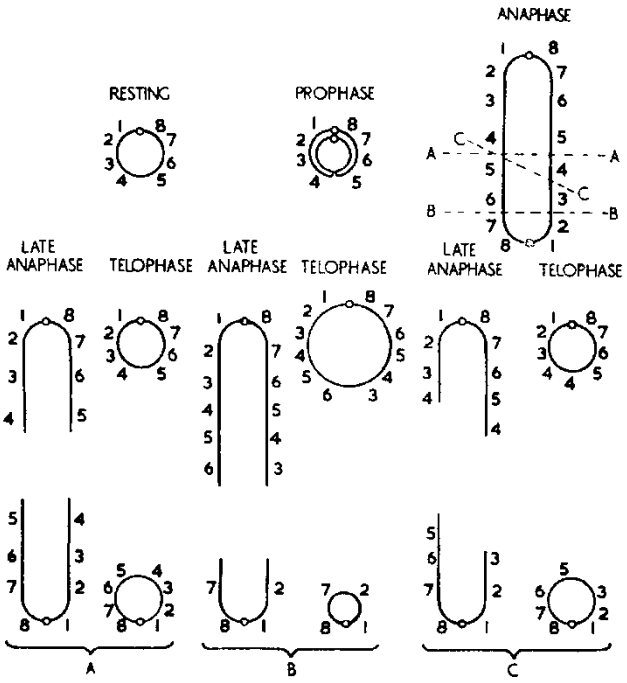
Cannot be observed genetically as sister chromatids have the exact same alleles.



McClintock, 1938; 1941

Provided he first evidence for sister strand crossing over

- Two breaks in chromosome 5 of maize, followed by joining of the broken ends resulted in the formation of a ring-shaped chromosome
- The ring chromosome changed size during mitosis. McClintock explained it as:
- In this scenario, sister strand crossover during prophase leads to the formation of a larger ring during anaphase.
- This ring breaks at random (3 possible locations are labelled here as A, B, and C). Reannealing of the broken ends leads to the formation of new rings of varying sizes.



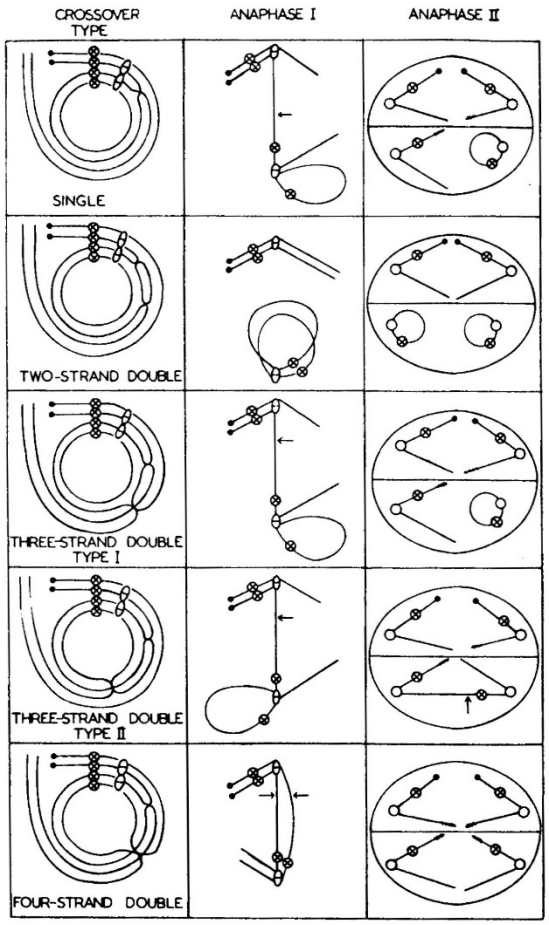
Schwartz, 1953 (not covered)

- Studied pairing between chromosome 6 of maize and its ring form
- Predicted the number of single and double bridges that would form if crossovers a) were random, and b) were limited to non-sister strands

	Anaphase I			Ana II (daughter cell pairs)		
Bridges:	Single	Double	None	Single	Double	None
N	368	81	171	166	47	262
Observed (%)	59	13	28	35	10	55
Expected (%)	60	20	20	20	0	80

Found significant deviations from the expected:

- Increase in single bridge frequency at anaphase II attributed to a single non-sister crossover + a sister strand crossover in the ring
- Double bridges attributed to a sister strand crossover in the ring, associated with no or double crossover between non-sister strands



Expected meiotic configurations in the absence of sister strand crossing over in a homologous pair consisting of a rod chromosome and a ring chromosome (after Schwartz, 1953).

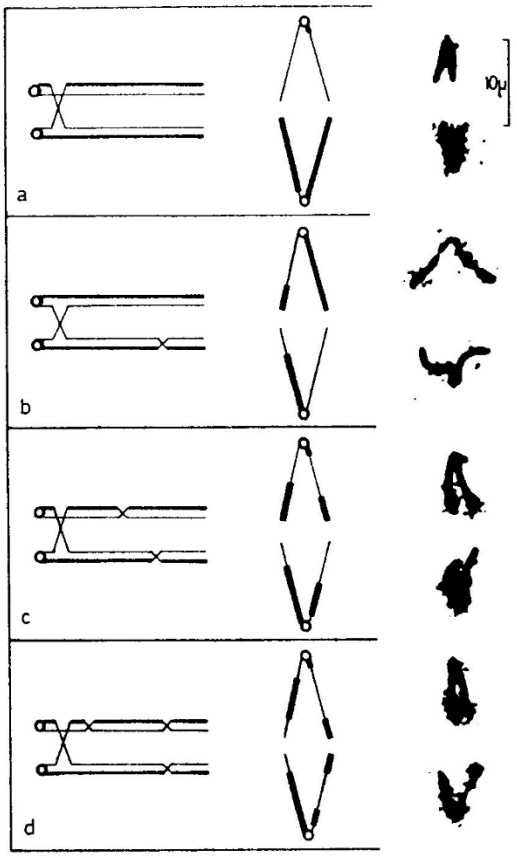


Diagram from Jones, 1971: Effect of sister strand crossovers chromosomes of Stethophyma grossum labeled with BrdU, and engaged in a single, proximal chiasma.

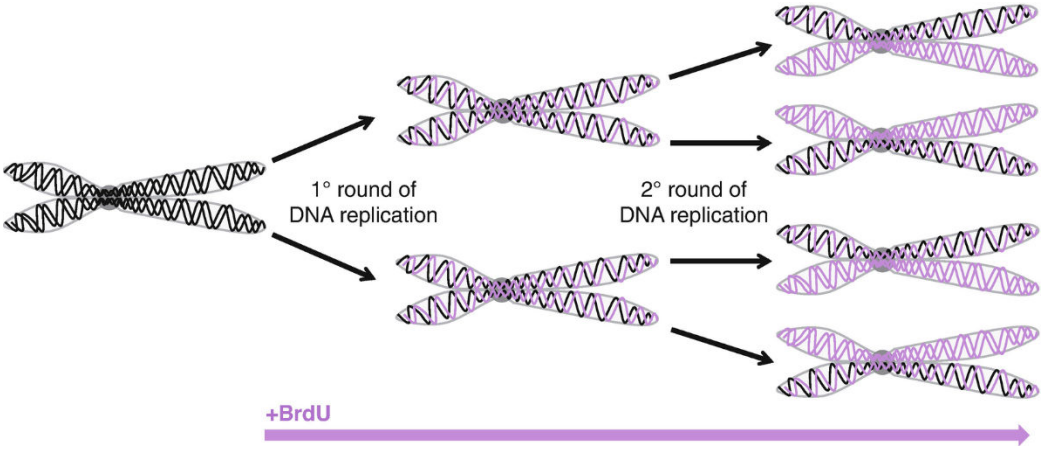
Jones, 1971 (not covered)

Used bromodeoxyuridine to label grasshopper chromosomes. Got harlequin chromosomes indicating sister strand crossovers had occurred

- Some still said that sister strand crossing over could be an artifact of the ring condition, or that it may be induced by the BrdU label itself, and that it would not happen under normal circumstances

Schubert, 1994

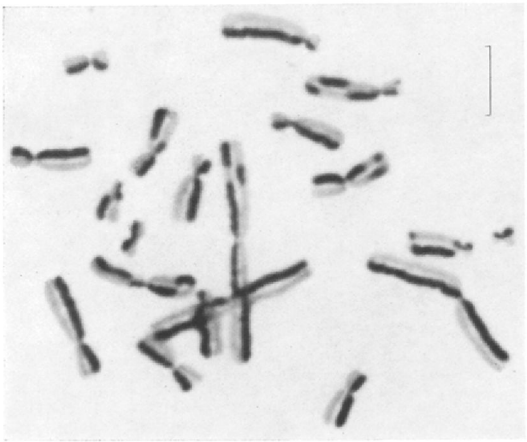
- Sister chromatids can also undergo crossovers during mitosis
- Since genetically identical, cannot be detected with genetic markers
- Used thymidine labels to identify somatic crossovers:



Tumini E., Aguilera A. (2021) The Sister-Chromatid Exchange Assay in Human Cells. In: Aguilera A., Carreira A. (eds) Homologous Recombination. Methods in Molecular Biology, vol 2153. Humana, New York, NY.

https://doi.org/10.1007/978-1-0716-0644-5_26

Skeptics attributed SSCOs to an artifact of radiation {Wolf, 1964. Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis. 1(4): 337-343}

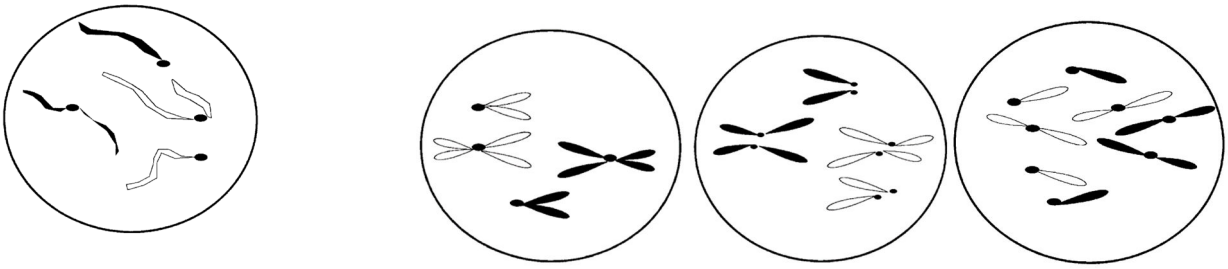


Harlequin chromosomes. Wolff S & P Perry. 2004. Differential giemsa staining of sister chromatids and the study of sister chromatid exchanges without autoradiography. DOI:10.1007/BF00290991

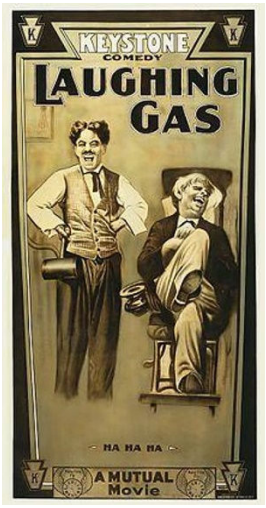
Species		SCE frequency/ chromosome
Allium cepa	2n=16	44.8
Allium sativa	2n=16	104.0
Hordeum vulgare	2n=14	20.6
Secale cereal	2n=14	11.06
Tradescantia paludosa	2n=12	43.5
Triticum aestivum	2n=6x=42	15.2
Zea mays	2n=20	3.7
Crepis capillaris	2n=6	4.96
Solanum tuberosum	X=12	0 to 8
Vicia faba	2n=12	20.6
Nicotiana plumbaginifolia	2n=20,40	7.7, 5.4
Picea abies	2n=24	36.9
Pinus sylvestris	2n=24	36.2

Frequency of sister chromatid exchanges in plants varies greatly

C-mitosis = colchicine mitosis = endomitosis



As with regular mitosis, starts with chromosome condensation in prophase. There is no pro-metaphase. Then you have C-metaphase, C-anaphase, and C-telophase. No nuclear or cell division.



C-mitotic agents

Other doubling agents (c-mitotic agents) include:

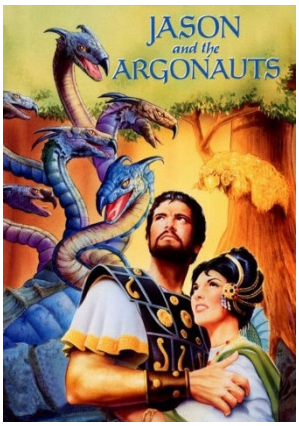
- Temperature shocks (high or low)
  - Randolf 1932 – high temperature in maize
- Pressure
- Nitrous oxide, oryzalin

https://www.imdb.com/

Colchicine

Eigsti, 1955; Weismann, 2009

Originally comes from the autumn crocus: 0.1 in flowers to 0.8% colchicine in corms

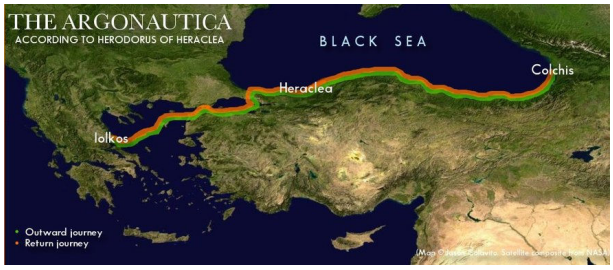


Autumn crocus is from Colchis, a kingdom on the Black Sea in what is now the Republic of Georgia

3000 years ago, ruled by King Aeëtes, whose daughter, Medea, specialized in herbal remedies

- The term 'medicine' is derived from 'Medea'
- Colchine was used to treat gout among the royalty
- Both a pharmaceutical and a poison

- Jason from Lolcos got sent to Colchis to get colchicine, a.k.a, Golden Fleece



Colchicum autumnale from Dictionnaire de botanique by Henri Ernest Baillon. Route map from http://www.argonauts-book.com/maps.html. Jason & the argonautes, 1963 version. Not the 2000 minie series version.

- Jason in Jason & the Argonauts – "We have a flower in Colchis"
  - Assume King Pelias was suffering from gout!
- Medea agreed to help Jason get the Golden Fleece if he would marry her. He did and they returned to Lolcos.

Pernice, 1889, Eigsti, 1938

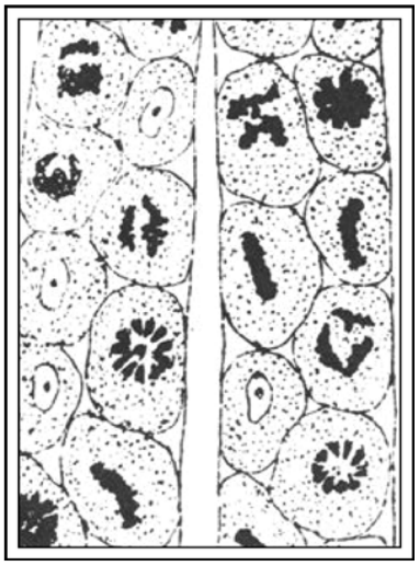
C-mitotic effect discovered in dog intestines by Biaggio Pernice in 1889, who was trying to induce gastroenteritis

1936/7 – Use in plants reported by Eigsti, 1938

- Animal researchers had started using it to study animal chromosomes. He heard about it and decided to try it.

Very effective in doubling chromosome number by preventing the assembly of tubulin subunits to make the mitotic spindle.

- Cytokinesis (cleavage furrow or cell plate formation) is also dependent on microtubules, so is therefore prevented



. Pernice 1889 drawing of c-mitosis in dog intestinal cells

- Is soluble in water and in ethanol
- Effective over a wide range of concentrations
  Can be applied in many ways
- Effective in most organisms
- Appears to only affect the spindle
- Usually causes no permanent damage
  - Can be toxic at some concentrations
  - Has been known to induce mutation