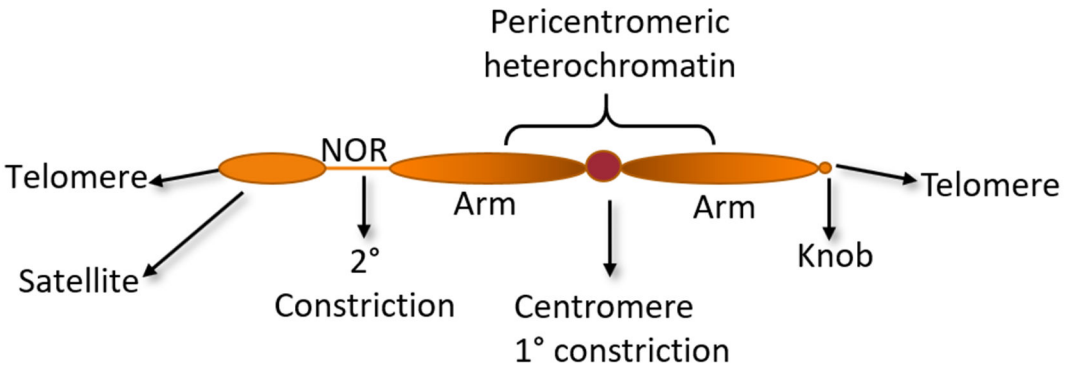
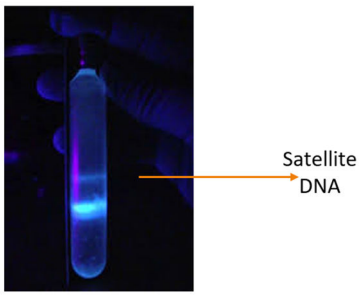
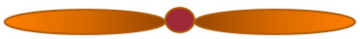
## Landmarks and terminology -See review by **Heslop-Harrison and Schwarzacher, 2011**



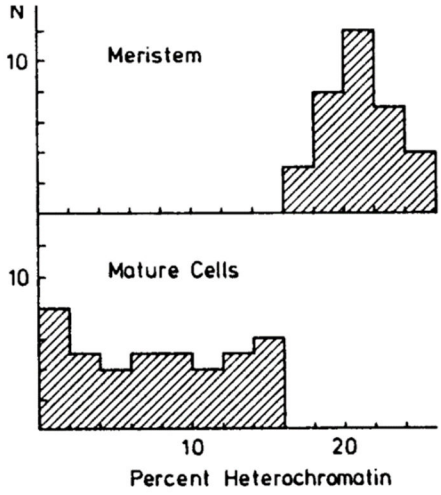




| | | |
|---|---|---|
|  |  |  |
| Metacentric<br>Isobrachial | Submetacentric<br>Acrocentric<br>Heterobrachial | Telocentric |

- **Heitz** (1928, 1933, 1934) discovered euchromatin and heterochromatin

- Euchromatin

- Heterochromatin:

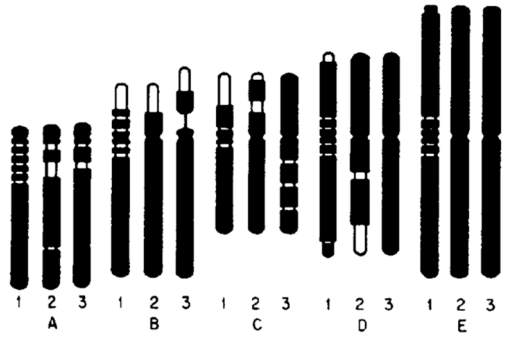



*Changes in heterochromatization of DNA of Dendrobium. Nagl, 1983*

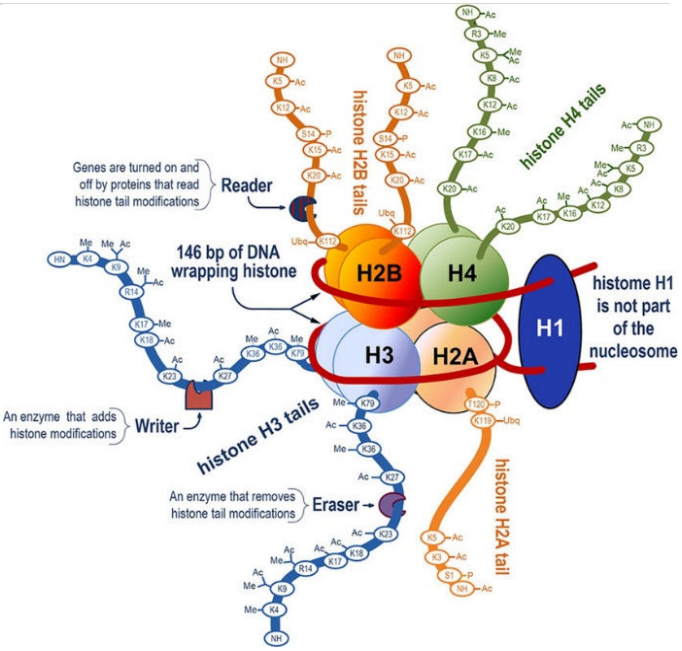
Brown & Nelson-Rees (1961, Genetics 46:983-1007)

- Constitutive:

- Facultative:

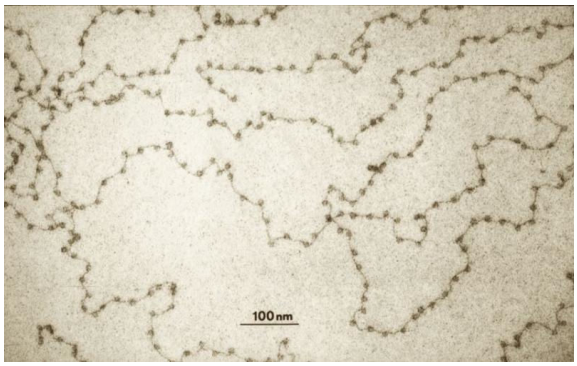



*Location of heterochromatin in 1) Trillium erectum, 2) T. grandiflorum, and 3) T. undalatum*



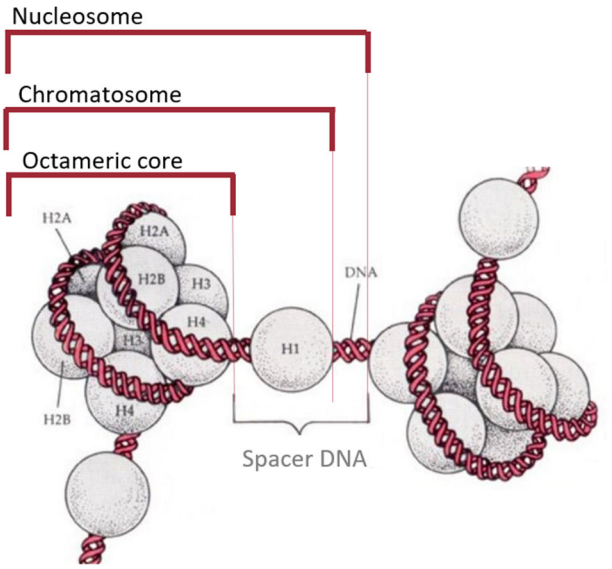

*https://doi.org/10.1021/acs.analchem.7b05007*

## Chromatin organization



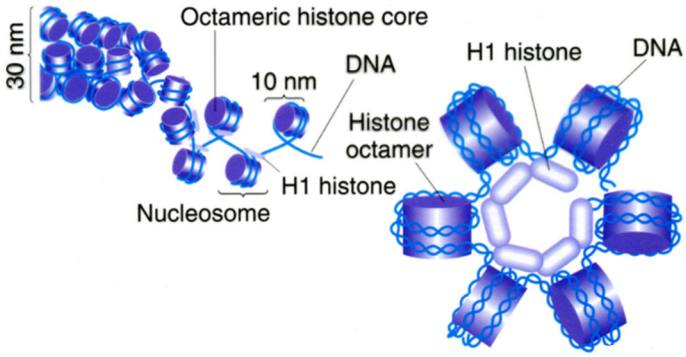

*https://www.ornl.gov/blog/ornl-review/beads-string-discovering-nucleosome*

- **Core particle:** 146 bp DNA + histone octamer
- **Chromatosome:** Core particle + H1 + 22 bp DNA tail
- **Nucleosome:** about 200 bp DNA + chromatosome



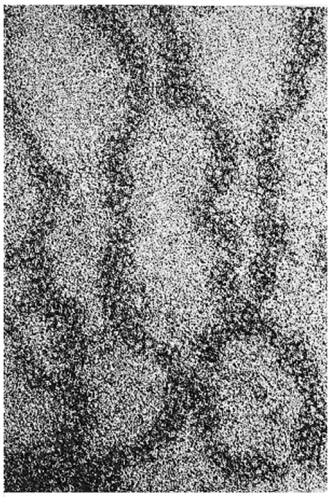

*1Oak.cats.ohiou.edu/~ballardh/pbio475/Heredity/Hierarchical-arrangement.JPG*

## Interphase configuration

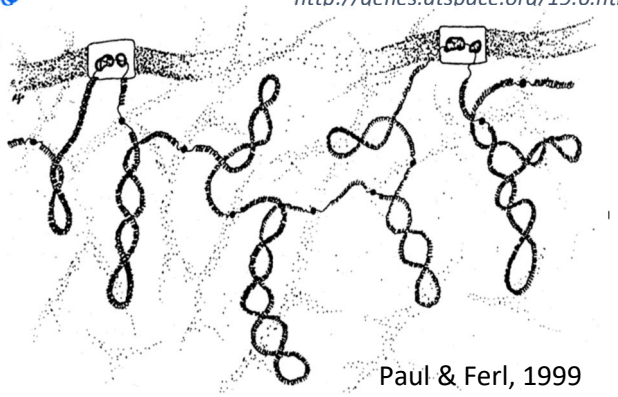



*http://www.discoveryandinnovation.com/BIOL202/notes/lectu*

The 30-nm fiber in .

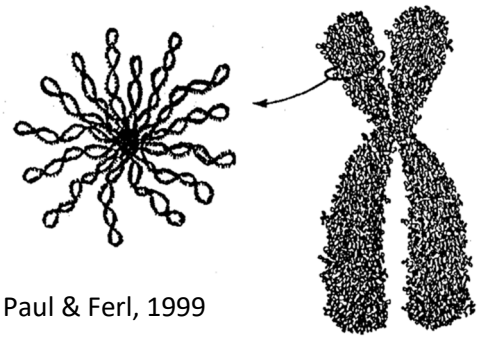


*http://genes.atspace.org/19.6.html*

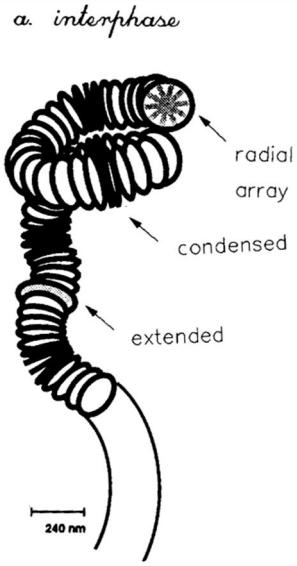


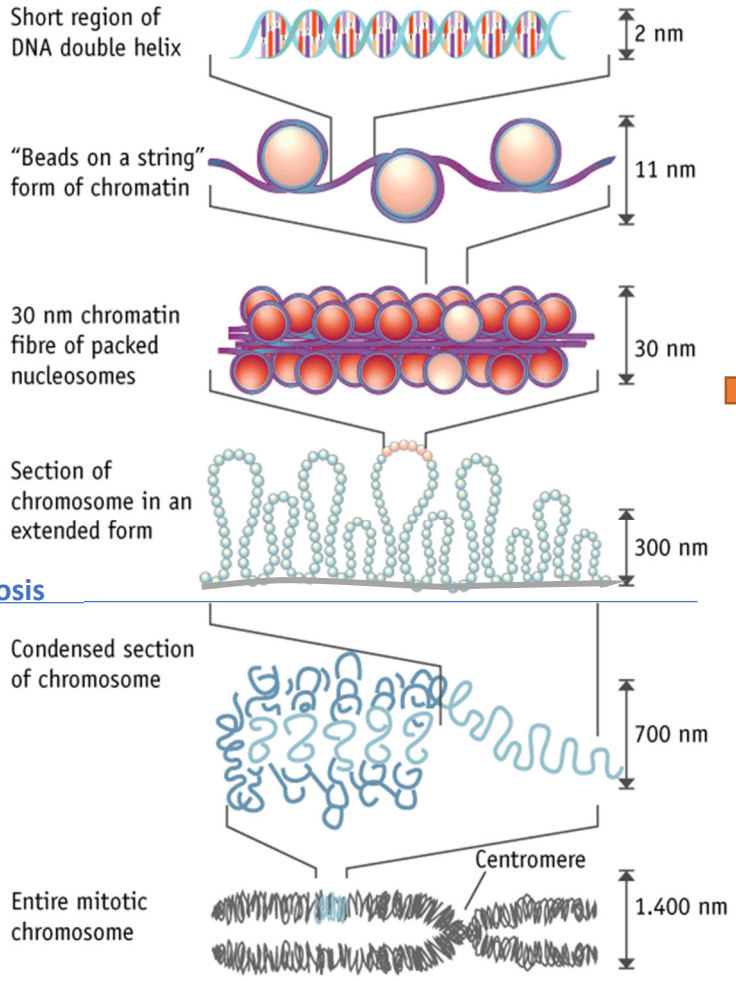
Paul & Ferl, 1999

## Mitotic configuration



Paul & Ferl, 1999

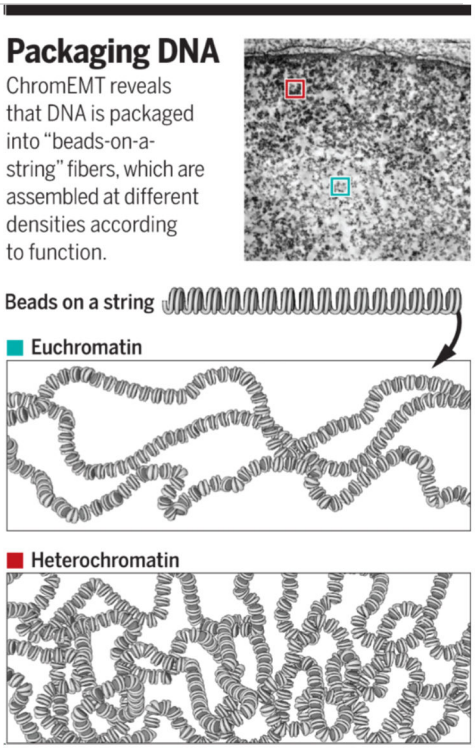


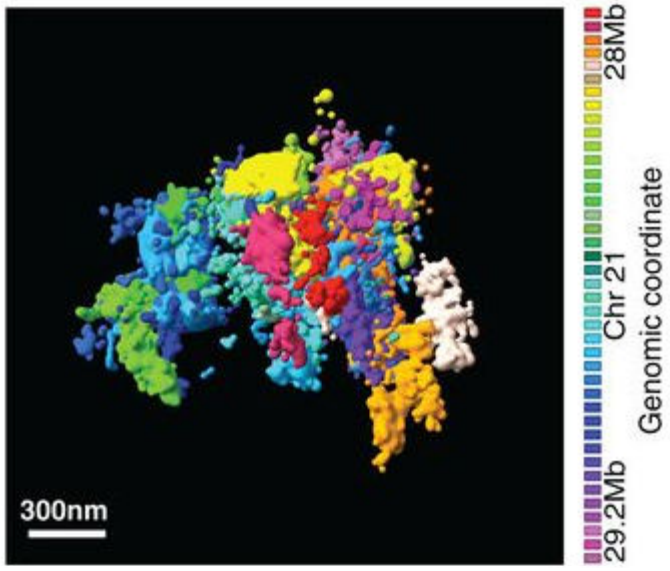

May only exist in isolated DNA

Mitosis

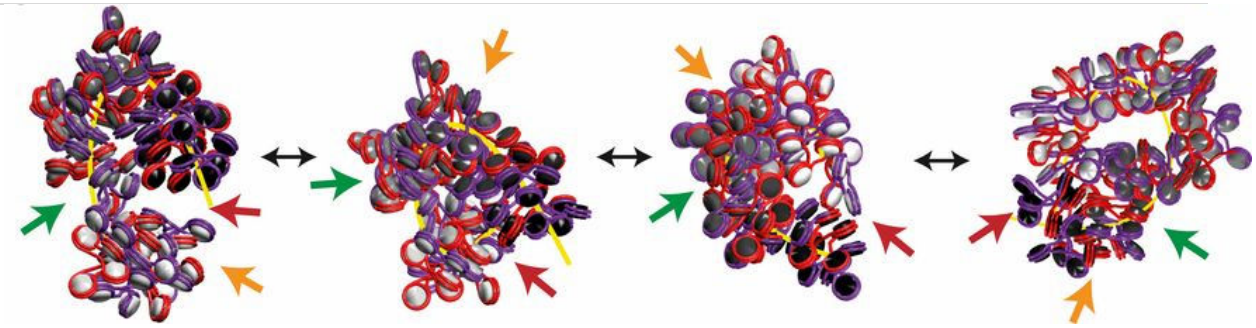
# Chromatin organization




Science 28 Jul 2017. 357:354-356




*Bintu et al., 2018; DOI: 10.1126/science.aau1783*

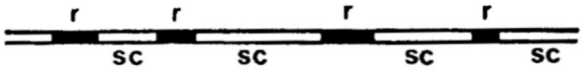


Sridhar et al, 2020. https://doi.org/10.1073/pnas.1910044117

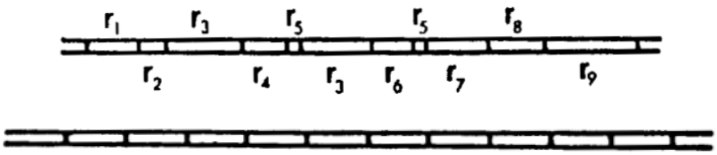
# Landmarks & DNA arrangements

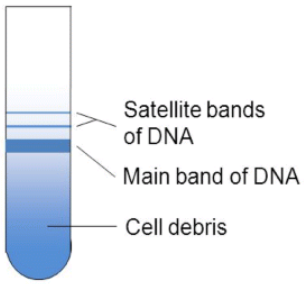
## CHROMOSOME LANDMARKS

a. **Interspersed**:
(Flavell 1980)



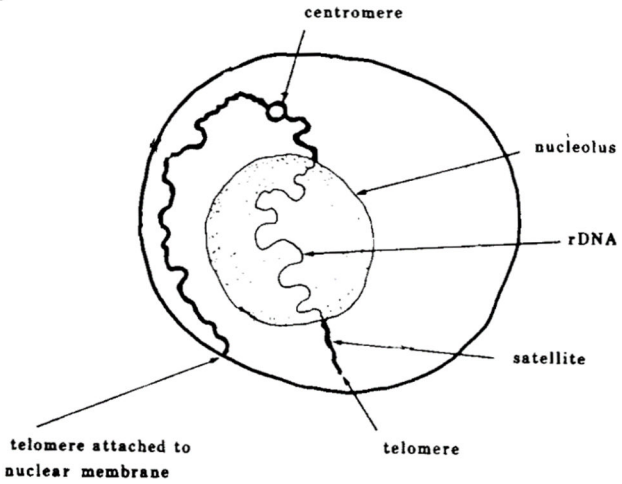

b. **Tandem**
(Flavell 1980)



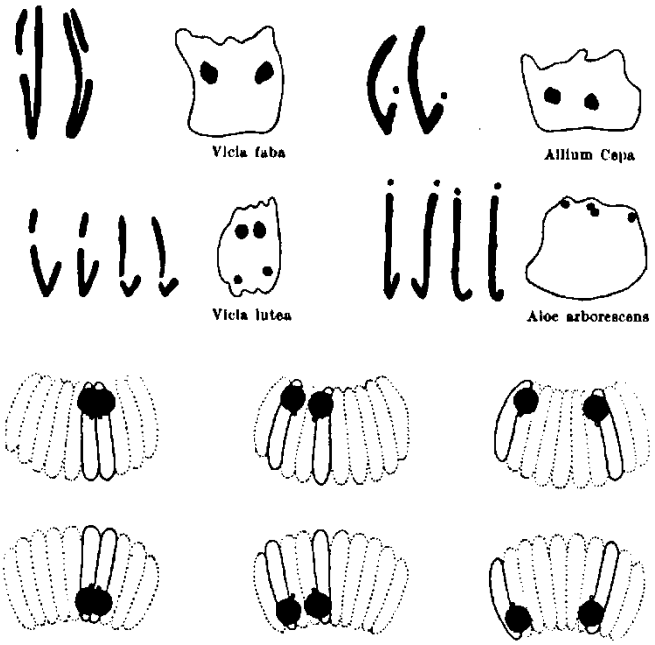




*http://duwyjaza17.dva4.ru.net/go/ebevaruwu-satellite-dna-ppt.html.*

## THE NUCLEAR ORGANIZING REGION



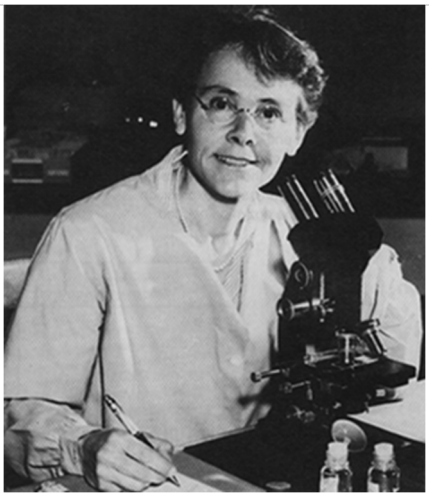

**1931: Heitz**

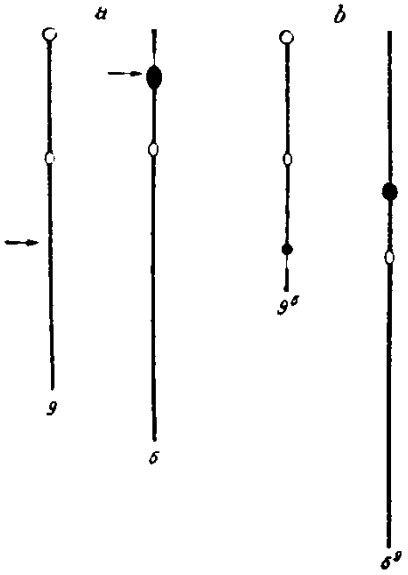


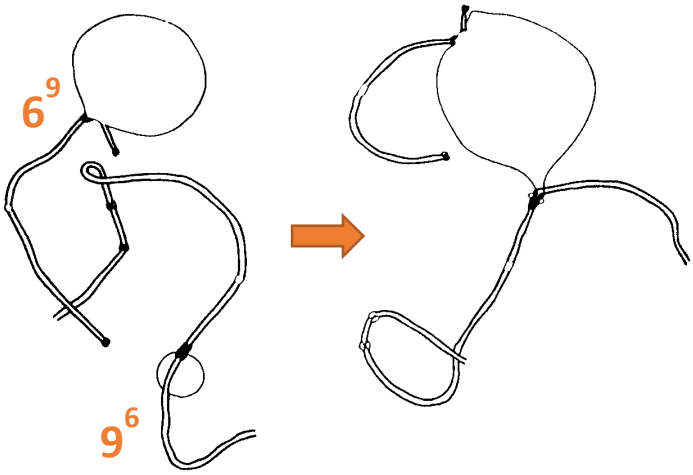

Emil Heitz, From *Passarge 1979*

**1934: McClintock**

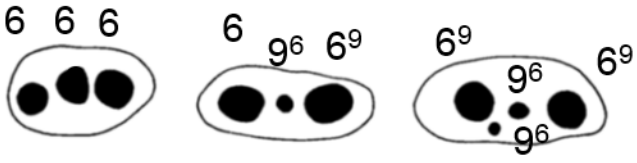
*Question - what does the 2° constriction do?*





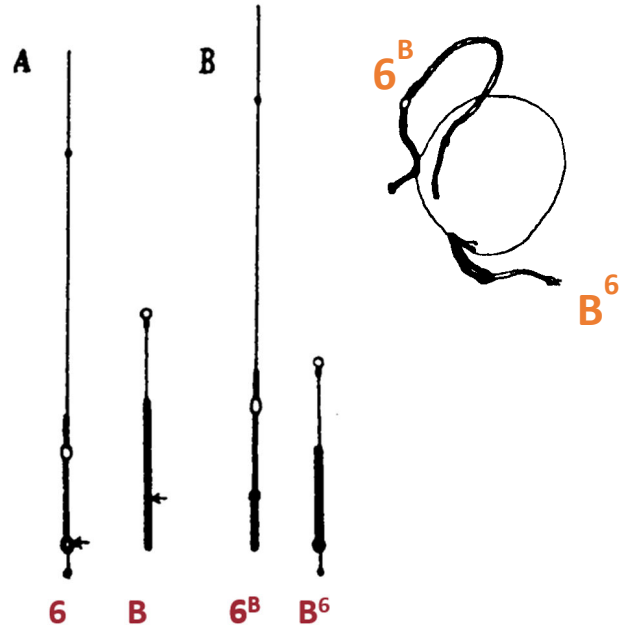



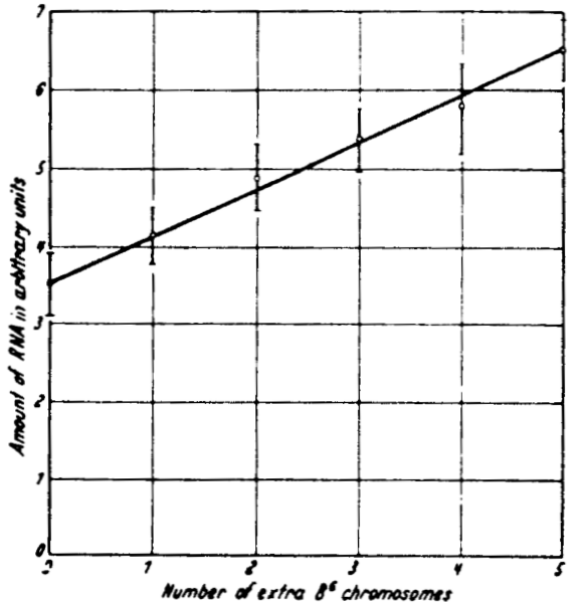

**Root-tip cells of trisomics:**

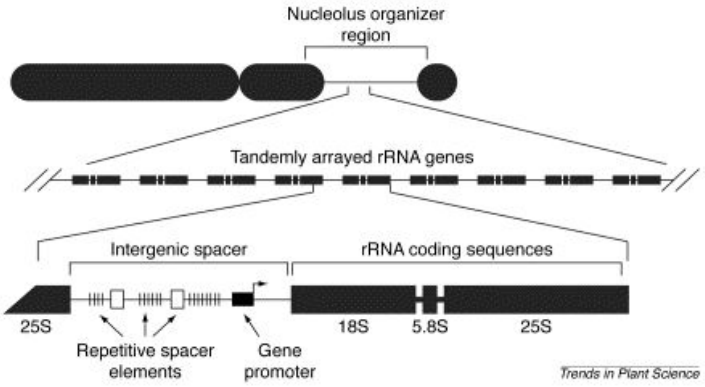


**1955: Lin**

*Question - what does the NOR do?*

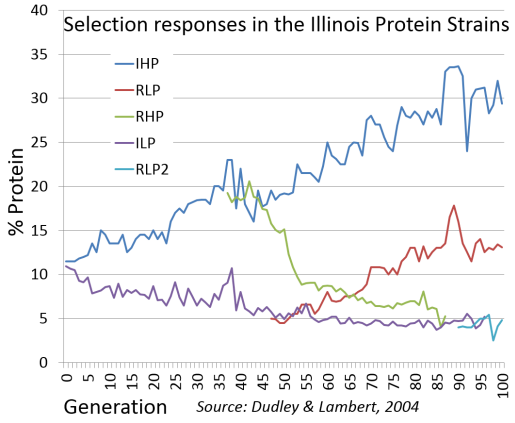


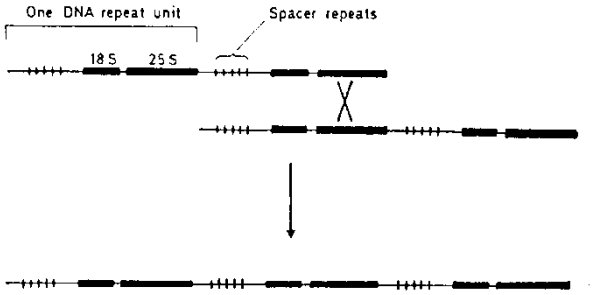



**1971, 1978: Phillips**

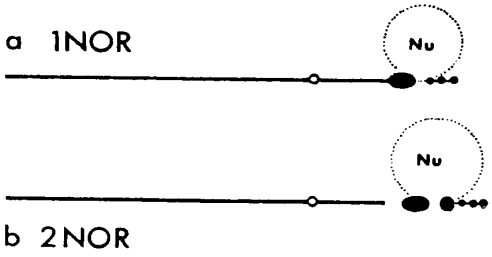
- Curcurbits have 27,000 rRNA genes
- Jerusalem artichoke has 1400 rRNA genes
- Corn:
    - "Sticky mutant" 3,300 copies
    - W23 5,000 copies
    - W127 17,000 copies
    - "Reverse high protein" 23,100 copies



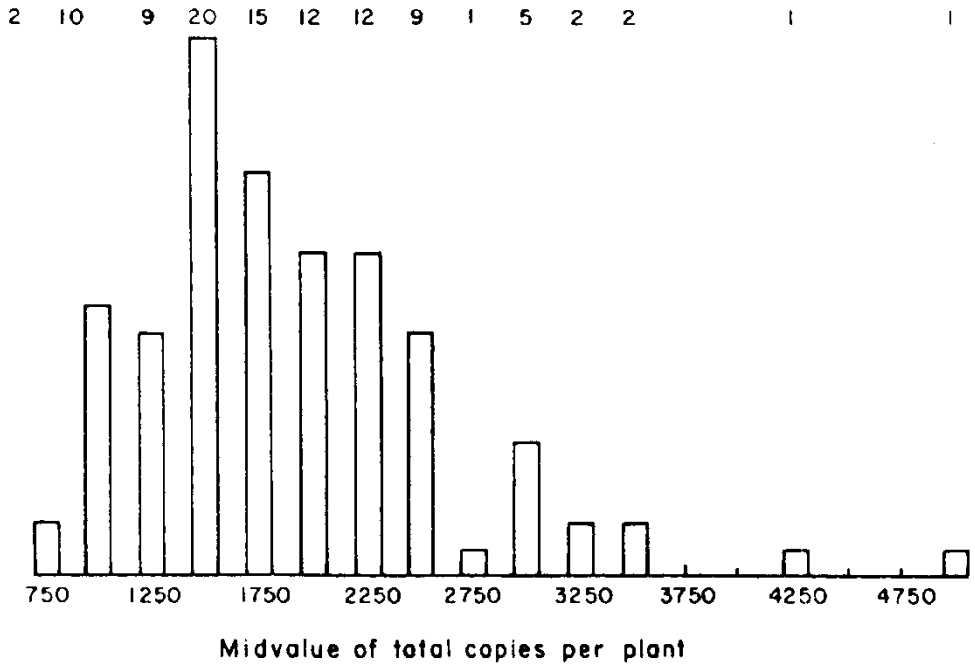

(The NOR is immediately to the right of a heterochromatic region, here depicted as a large knob. The duplication includes part of the heterochromatic region in addition to the NOR).




*Flavell, 1985*




**1990: Zhang et al (Allard):**




**Fultz et al, 2023:**

## TELOMERES

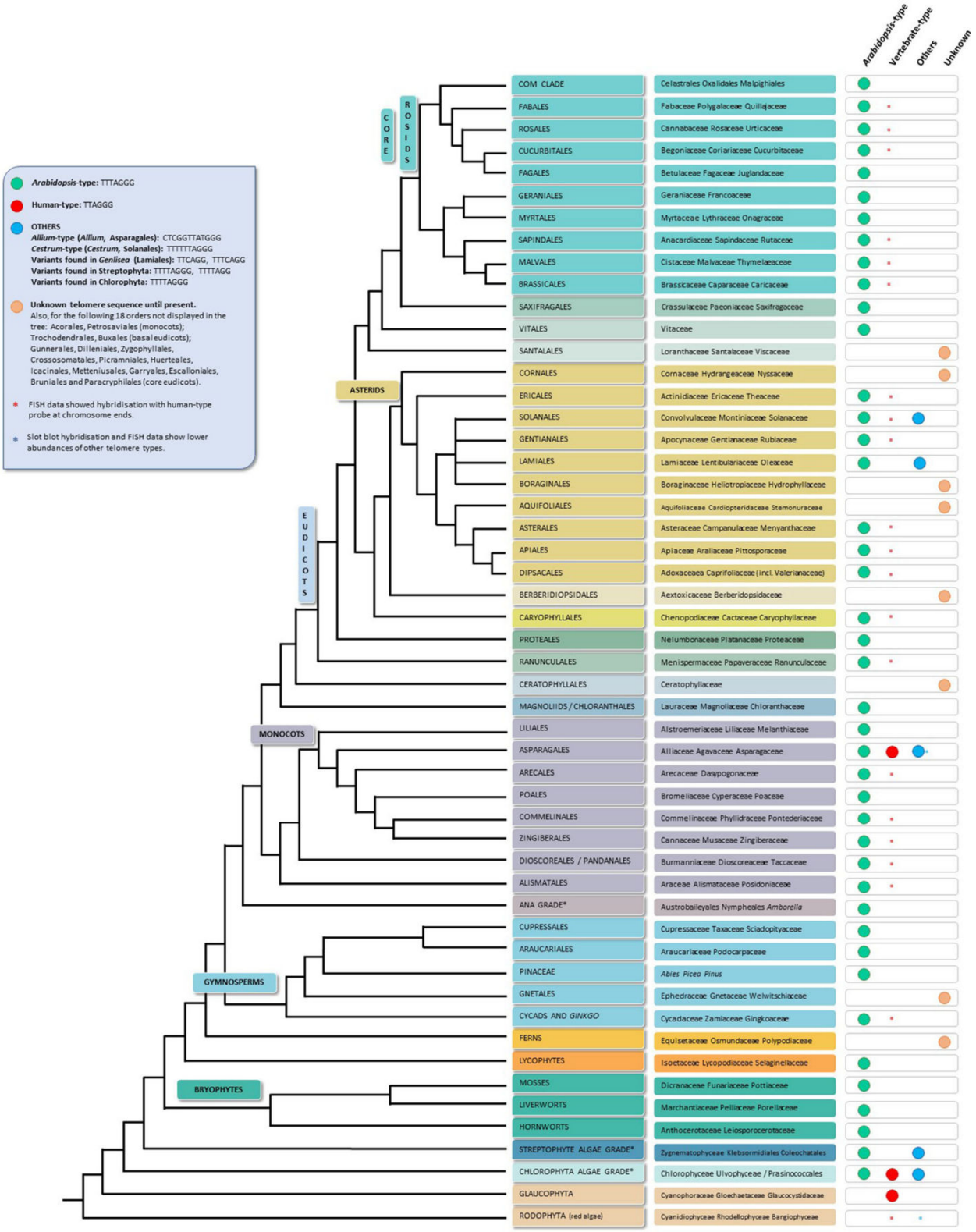
Reviews by **Peska & Garcia, 2020 & Shakirov et al., 2022**

| Organism | Type | Telomere sequence |
|---|---|---|
| *Euplotes* | protozoan | TTTTGGG |
| *Tetrahymena thermophila* | protozoan | TTGGGG |
| Vertebrates, slime molds, and trypanosomes | | TTGGGA |
| *Arabidopsis thaliana* | angiosperm | TTTAGGG |
| *Dictyostelium* | | $AG_{1-8}$ |
| *Plasmodium* | | (C/T)TTGGGA |
| Baker's yeast | | $TG_{1-3}$ |

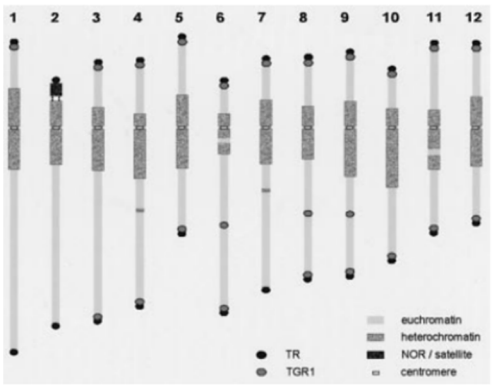
- The number of copies differs for between species and between chromosomes:

| | | |
|---|---|---|
| Maize | | = 150-380 copies/chromosome |
| Rye & barley | 1-6 kb/chromosome | = 140 - 850 copies/chromosome |
| Humans | 3-20 kb/chromosome | = 500 - 3000+ copies/chromosome |

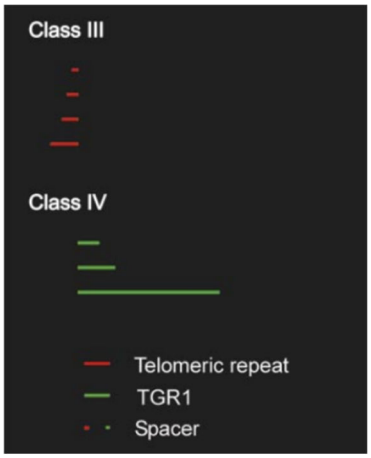
**Peska & Garcia, 2020**



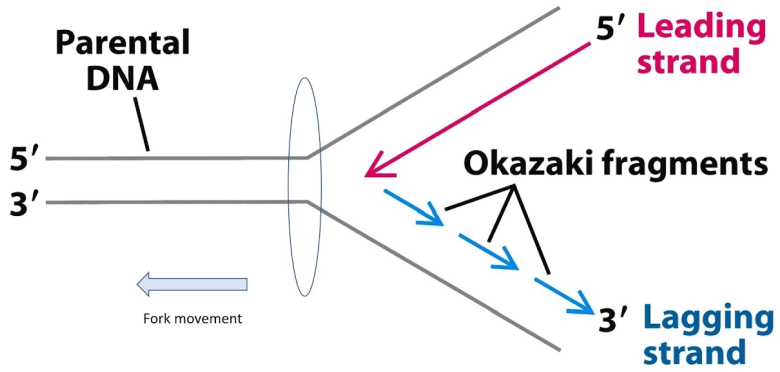
# Subtelomere repeats – Zhong et al, 1998



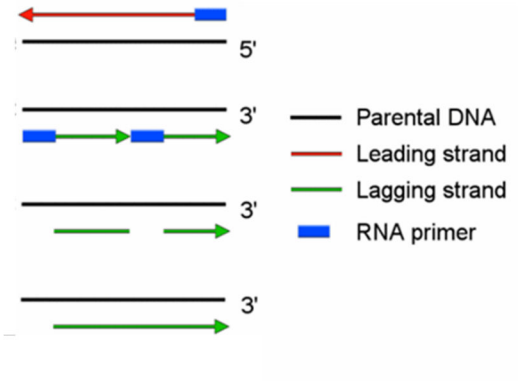


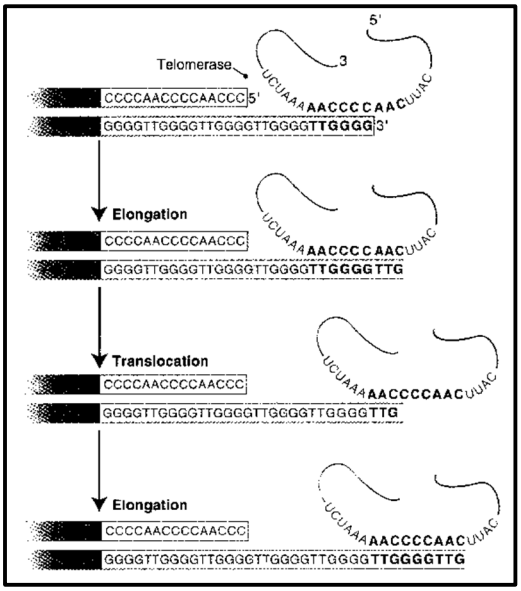


**Function:**

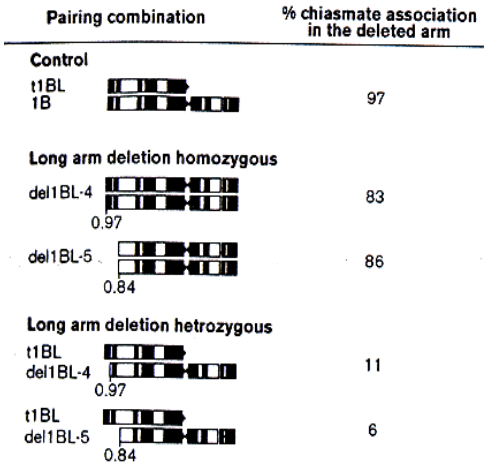



*Youtube.com/watch?v=pz3vZ7HDnvQ*



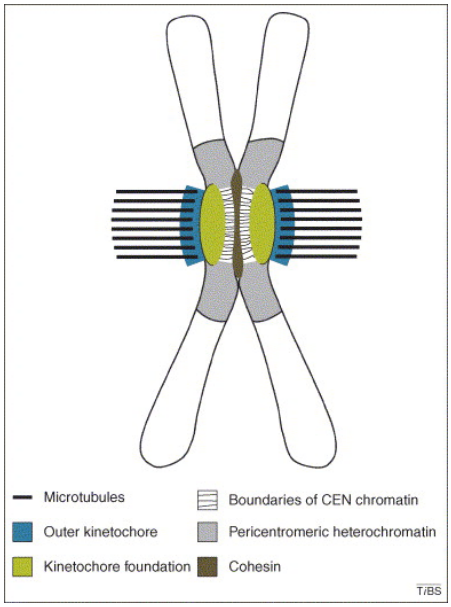
**Function:**
4. Meiosis pairing
**Lukaszewski, 1997**

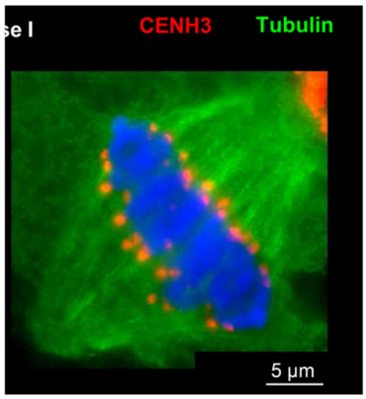



Gill & Friebe

## CENTROMERES

Based on **Zhang et al., 2005; Topp et al., 2004** (Dawe Lab), and **Birchler et al., 2011; Presting, 2018**




*CenH3 localization in mitosis. V Schubert, A Ruban & A Houben. 2016. Chromatin ring formation at plant centromeres. Frontiers in Plant Science. 7: 28*

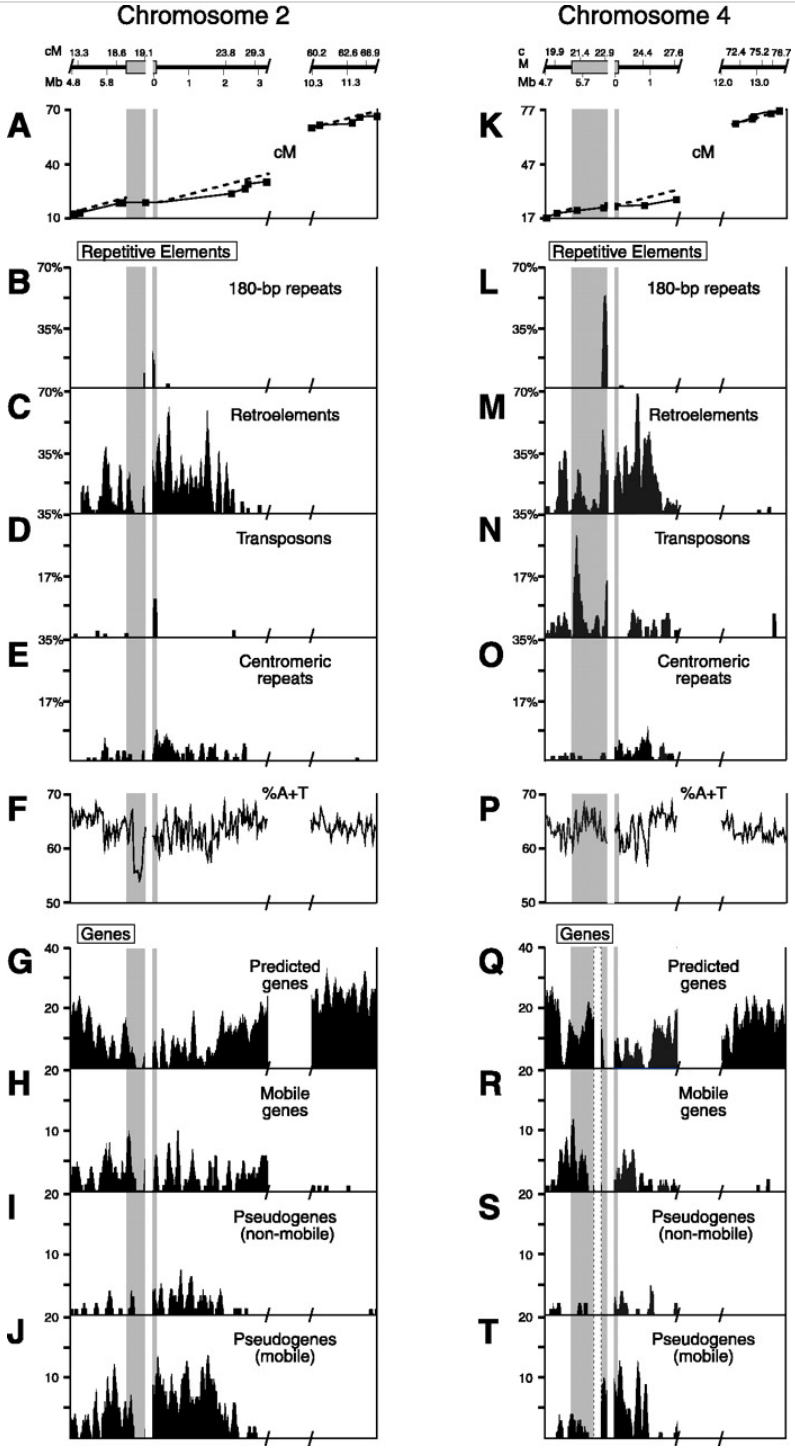
Based on ***Richards & Dawe, 1998; Jin et al., 2004***

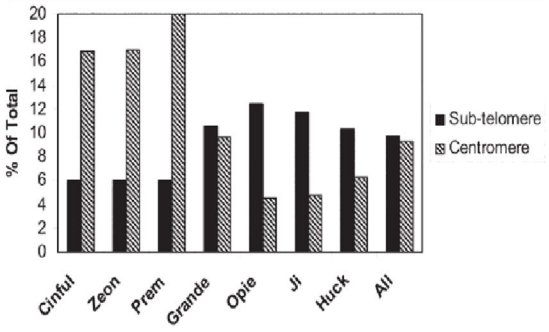
***Nagaki et al.*, 2004; *Wu et al.*, 2004**

***Copenhaver et al.*, 1999**

**The arabidopsis centromeres**

•

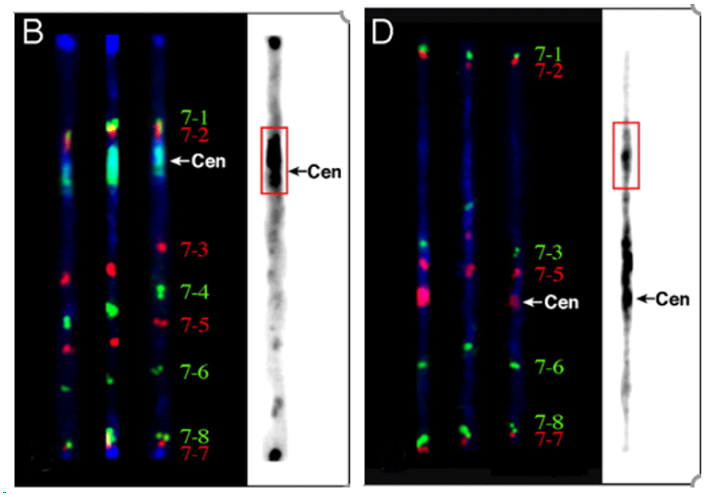





***Fengler et al., 2007***

***Nakano et al., 2003*, *Black et al.*, 2004; *Birchler* et al., 2011**

**Han et al., 2009**

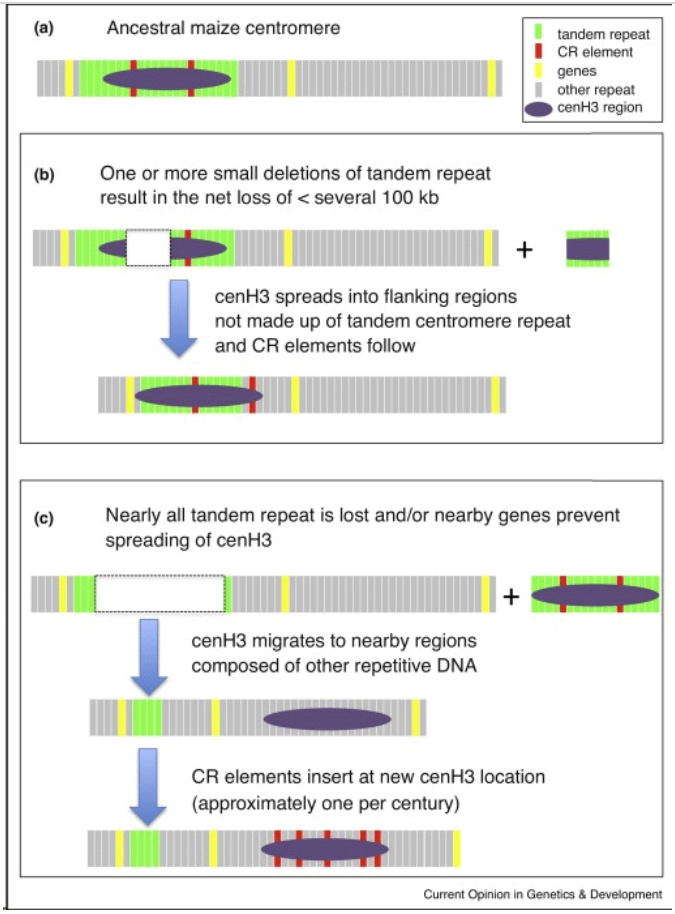



*Han et al., 2009. B= cucumber chromosome 7. C, its melon corresponding chromosome, II. The red box in D is where the melon centromere used to be. Note that the flanking markers have not moved.*

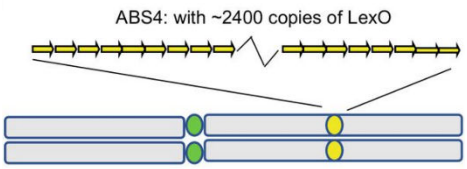
**Additional evidence for epigenetic control**

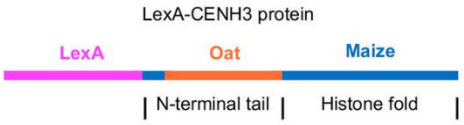
# Centromere dynamics

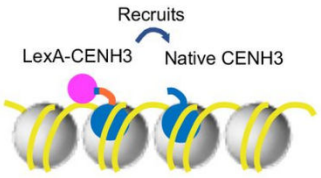
**Presting, 2018**

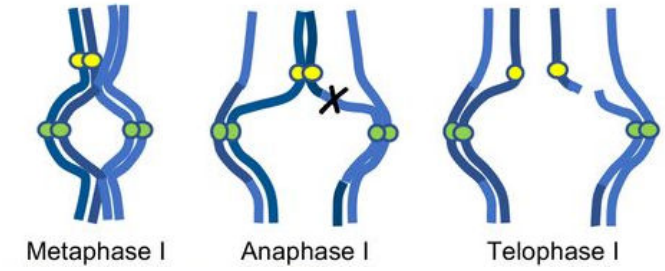


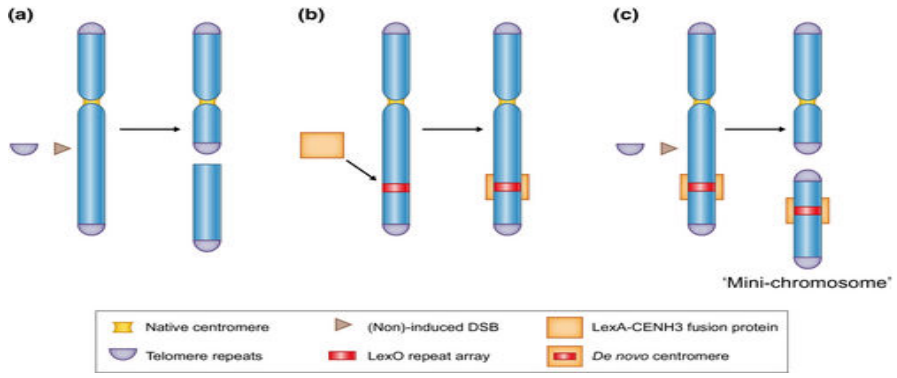

**Synthetic centromeres - Dawe et al., 2022**



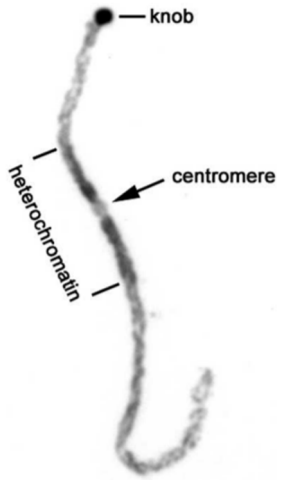




*Puchta & Houben, 2023*

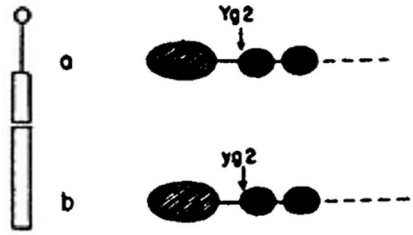
## Pericentromeric heterochromatin




*Anderson et al, 2007. Gen Res 16:115-122.*

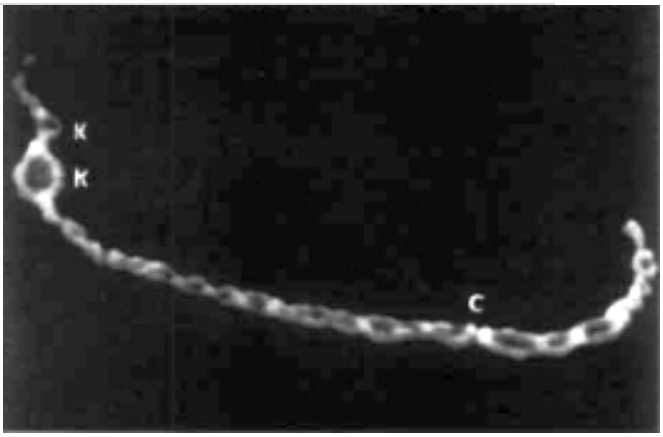
## KNOBS

After **Ananieval et al, 1998**




*McClintock, 1929- 1st drawing of a knob. 1944- A terminal knob and two chromomeres.*

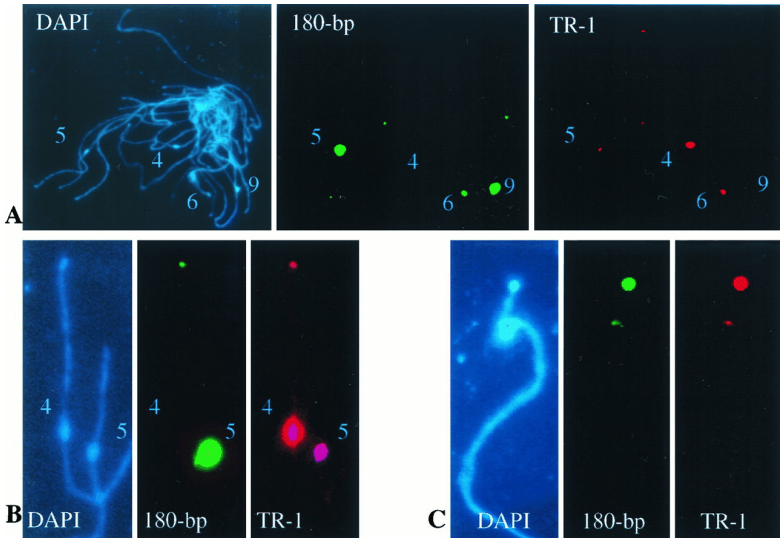
**Chromomeres vs Knobs**




*Caixeta and de Carvalho. 2000. Digested pachytene chromosomes with trypsin. Chromomeres (bright spots) and knobs (K) on chromosome 8 of maize*

**Looking at knobs & chromomeres at the molecular level**

Ananiev, EV, RL Phillips, and HW Rines, 1998



# The identification of individual chromosomes

Landmarks are often not enough to distinguish chromosomes from each other


*Yang F, L Lingjiao, E Rong, Y-b He, X Zhao & Y Wu. 2015. Karyotype analysis of obtained tetraploid in medicinal plant (Platycodon grandiflorus). Journal of Medicinal Plants Research. 9: 294-300*

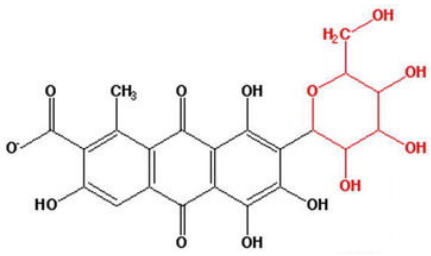
## The acetocarmine stain

**Dapson, 2007**

*Mirzaghaderi G. 2010. A simple metaphase chromosome preparation from meristematic root tip cells of wheat for karyotyping or in situ hybridization. African J Biotechnol 93: 314-318*

*Carmine is made by cochineal insects feeding on Opuntia cactus. Originally used to dye clothes. Still used as a natural food coloring.*

Cochineal harvest- Eadweard Muybridge photo, 1875

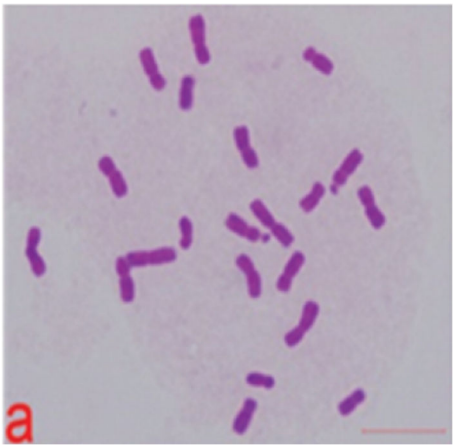



## The carbol fuchsin stain

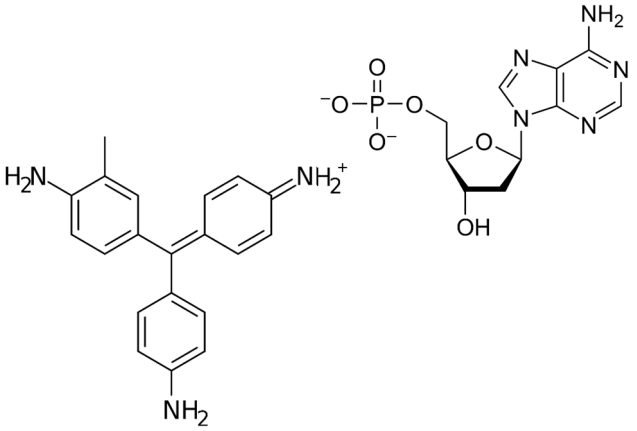
August Wilhelm von Hofmann

1818 - 1892

*Wikipedia commons*

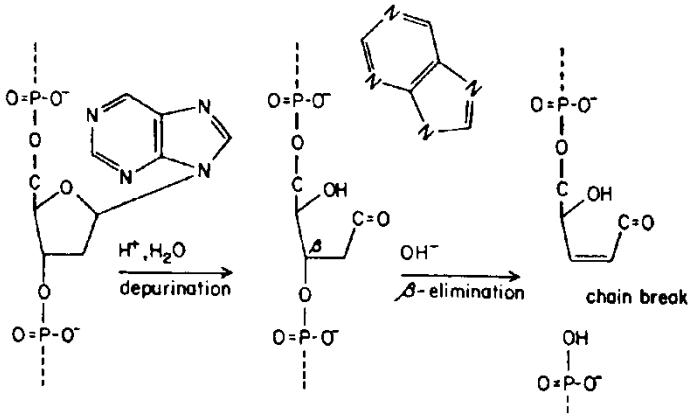



*Iqbal MZ, Cheng M, Zhao Y, Wen X, Zhang P, Zhang L, Ali A, Rong T, Tang QL (2018) Mysterious meiotic behavior of autopolyploid and allopolyploid maize. Comparative Cytogenetics 12(2): 247-265.*

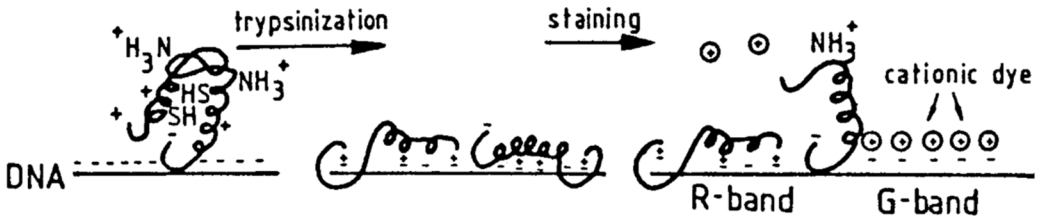



## DNA pretreatments

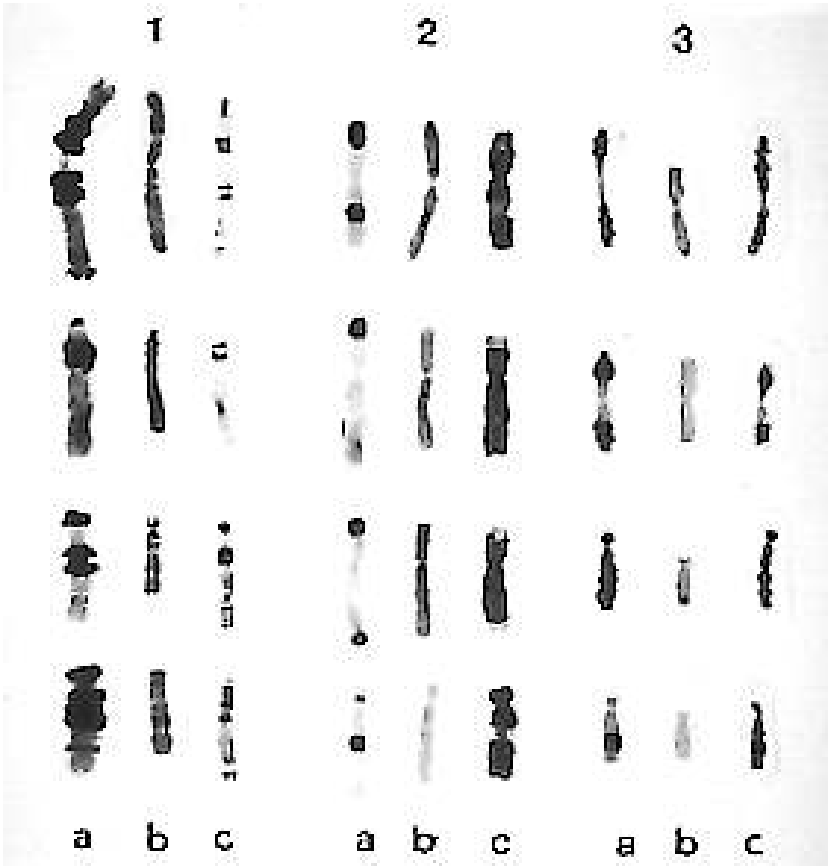
### Acid hydrolysis & β elimination




### Trypsinization




## Stain comparison



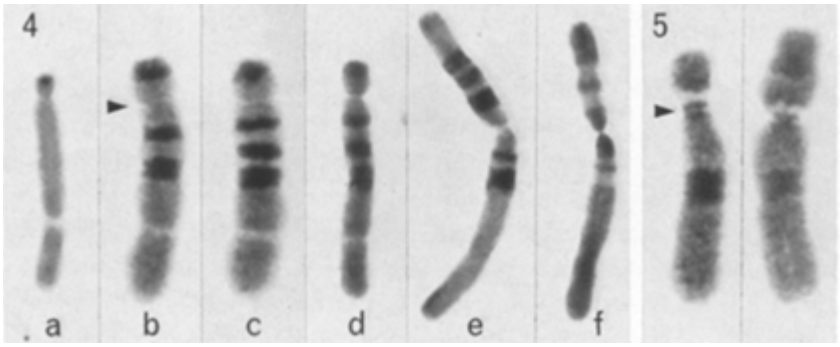

Chromosomes of 1. *Anemone blanda* 2. *Scilla sibirica* 3. *Fritillaria lanceolata*. Chromosomes in column a) show G-bands. Column b & c) Feulgen with long and short hydrolysis. Marks, 1983.

## G bands

**Schweizer, 1973**

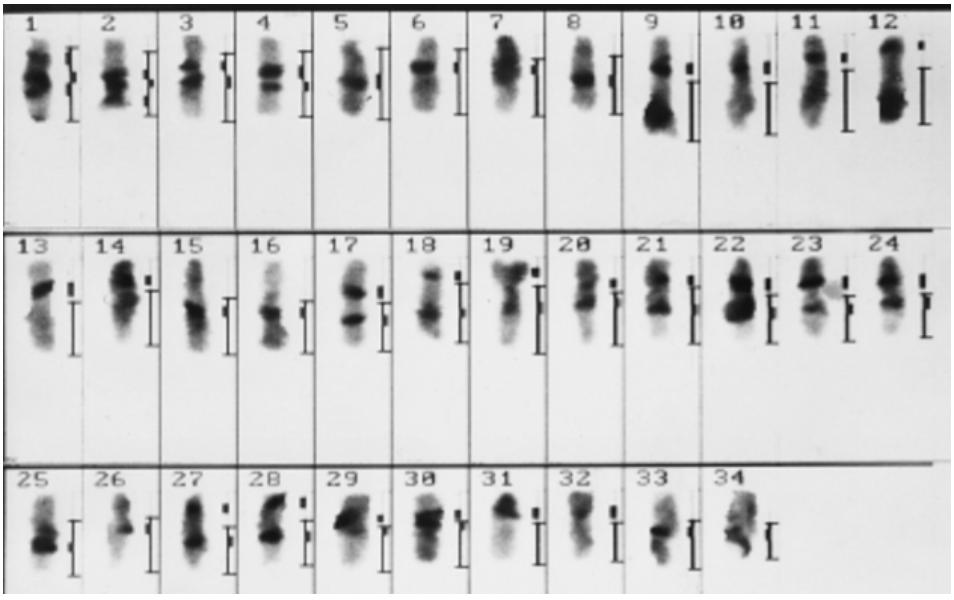
- Giemsa + alkali: = G bands
- Giemsa at high temperature: DNA rich in CG pairs = R bands (for reverse Giemsa)

- Works well in mammals
- Some plants with large chromosomes
- Stains AT-rich regions




*G-banded chromosomes of Fritillaria recurva*

- Resolution in plants was never good, as compared to animals. EG, G-banding in sunflower

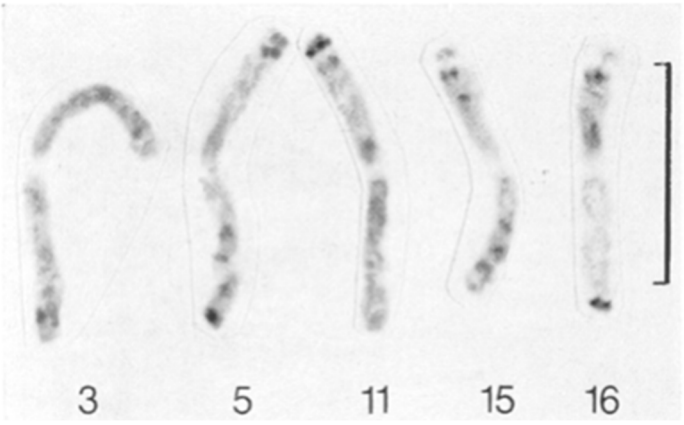



*Schrader O, R Ahne, J Fuchs & Schubert. 1997. Karyotype analysis of Helianthus annuus using Giemsa banding and fluorescence in situ hybridization. Chromosome Research 5:451-456*

## C bands (Hy bands/Feulgen bands)

**Developed by Greilhuber, 1973**

- Acid hydrolysis for depurination
- ß-elimination of depurinated DNA by hot salt
- Renaturation in SSC or $Ba(OH)_2$ buffer
- Staining
  - Aceto carmine
  - Feulgen

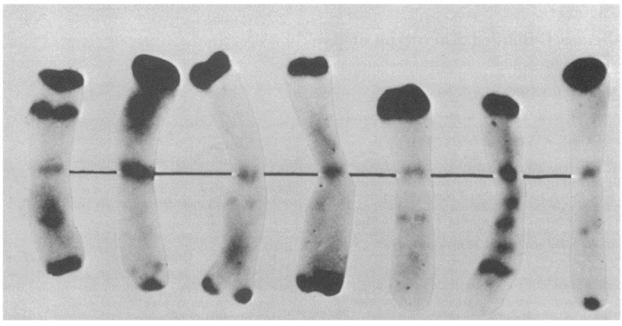



*C-banded chromosomes of Allium carinata*

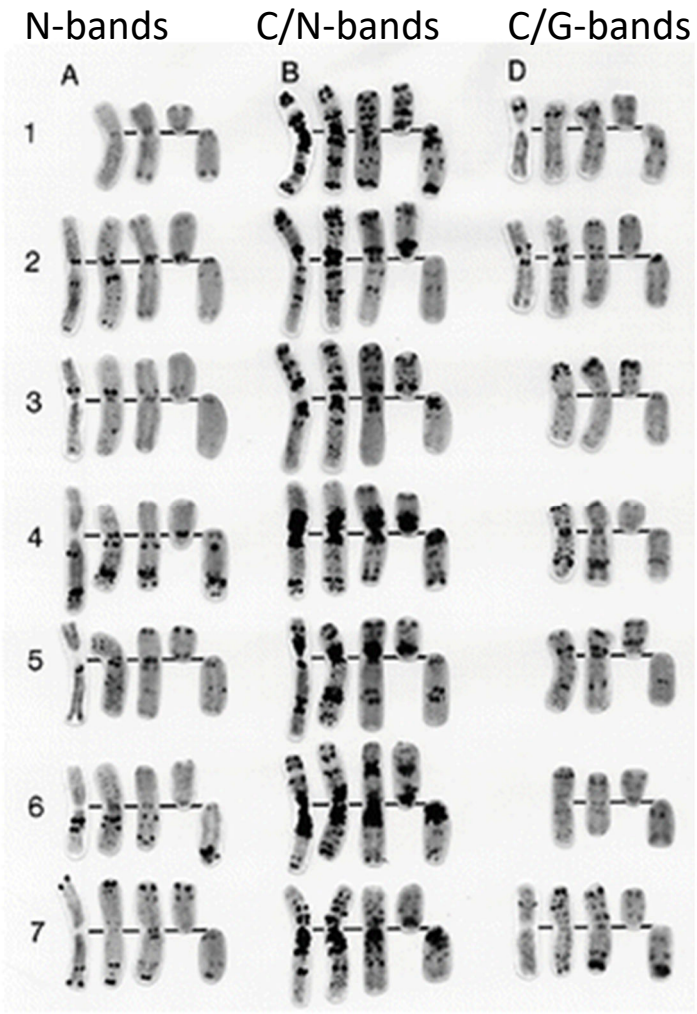
## C+G bands

**Gill + Kimber, 1973**

*C+G banded chromosomes of rye*



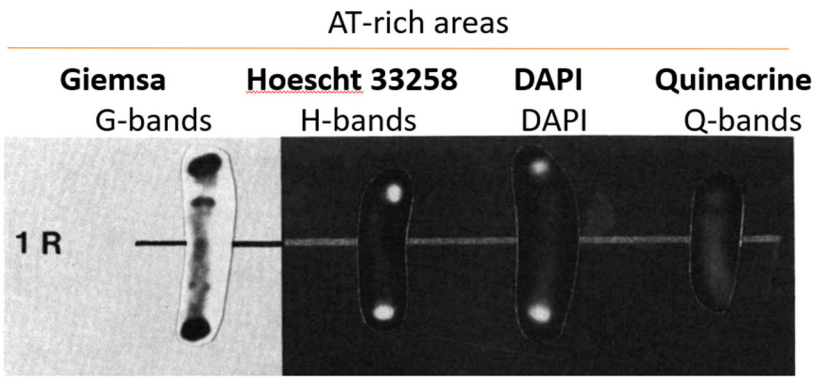
## Other stains




*Gill BS, Friebe B, Endo TR (1991) Standard karyotype and nomenclature system for description of chromosome bands and structural aberrations in wheat (Triticum aestivum). Genome 34:830–839*

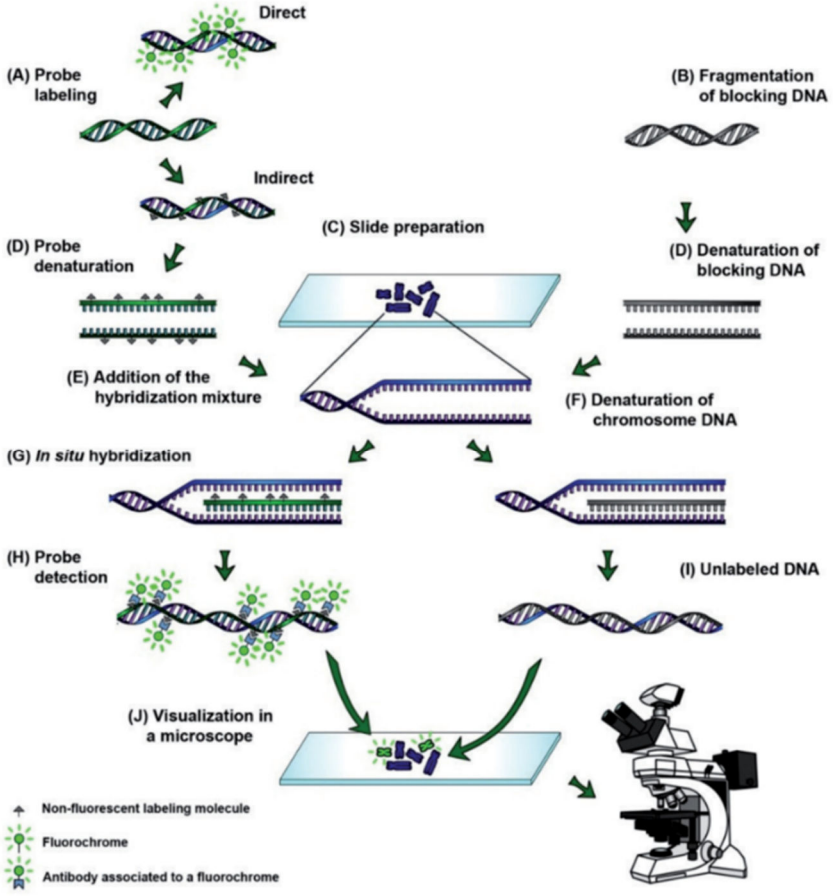
- N = acetocarmine → hot NaH2PO4 → Giemsa stain
- C/N = acetocarmine → hot NaH2PO4 → Barium hydroxide → Giemsa stain
- C/G = acetic acid → dehydration → acid hydrolysis → Giemsa stain

- Giemsa and other stains in general target AT-rich areas



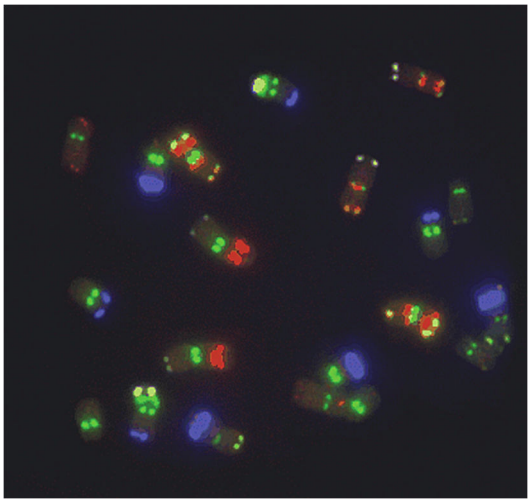

*Martin J & CU Heseman. 1988. Cytogenetic investigations in wheat, rye and triticale. I. Evaluation of improved Giemsa C- and fluorochrome banding techniques in rye chromosomes. Heredity. 61: 459-467.*

## FISH/GISH




*Brammer et al., 2013*

**Individual chromosomes in a genome can now be identified by:**



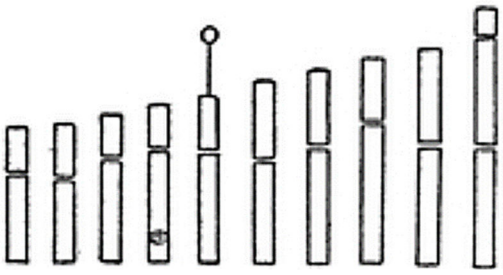
*Kato et al, 2004*

| | |
|---|---|
| 1 | |
| 2 | |
| 3 | |
| 4 | |
| 5 | |

## **KARYOTYPES** (whole genomes)

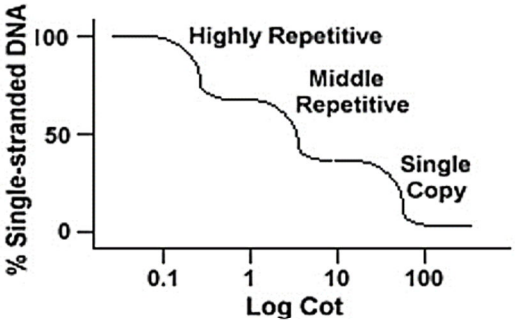
Genome (**Winkler**, ):

**McClintock, 1929 -**



*McClintock, B. 1929. Chromosome morphology in Zea mays. Science, 69, 629*

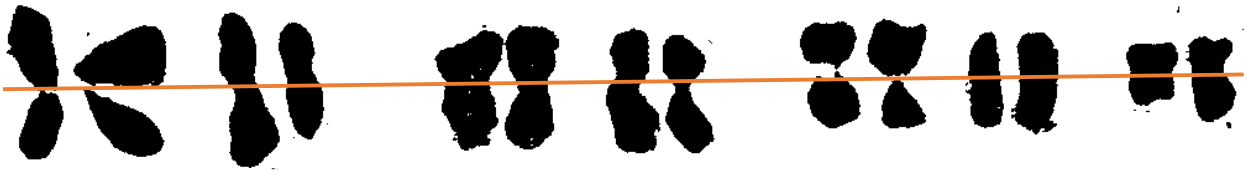
## Whole genomes can be identified by:



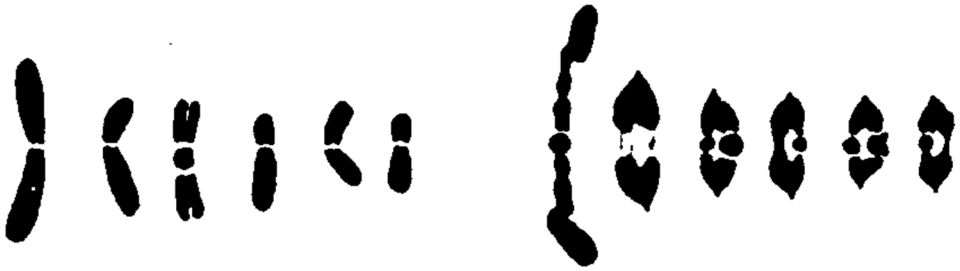

*www.slideshare.net/StevensonThabah/repetitive-sequences-in-the-eukaryotic-genome*

| | |
|---|---|
| 1 | |
| 2 | |
| 3 | |
| 4 | |
| 5 | |
| 6 | |
| 7 | |
| 8 | |
| 9 | |
| 10 | |
| 11 | |
| 12 | |

Karyotype:

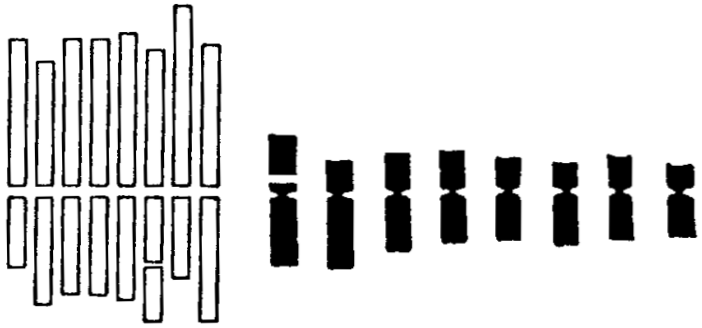


*Karyotype of Trifolium pratense (red clover)*



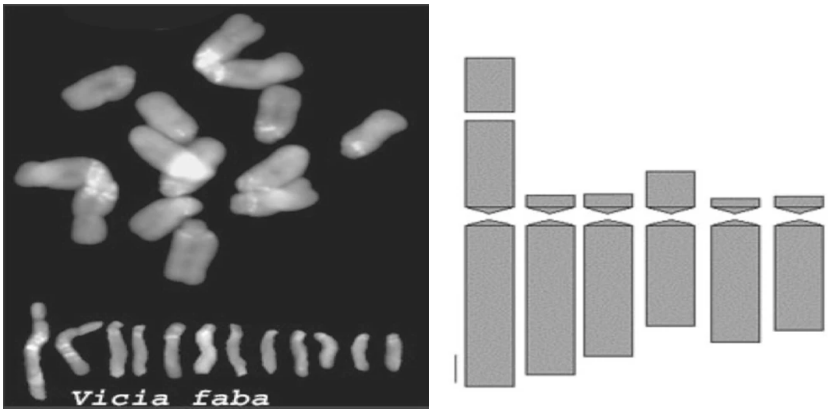
*Idiograms of root tip (left) and meiotic (right) chromosomes of Trifolium subterraneum (sub clover).*

Idiotypes: Schematic representations of chromosome sets. NOR depicted as a gap.



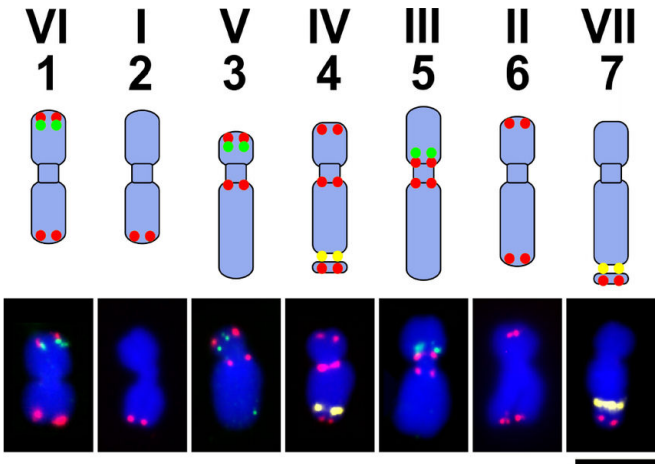
*Idiotypes of Trifolium subterraneum (left) and T. masaiense (right)*

## Hybrid presentations




*Faba bean karyotype & idiotype. Osman, S.A., Ali, H.B., El-Ashry, Z.M. et al. 2020. Karyotype variation and biochemical analysis of five Vicia species. Bull Natl Res Cent 44, 91*

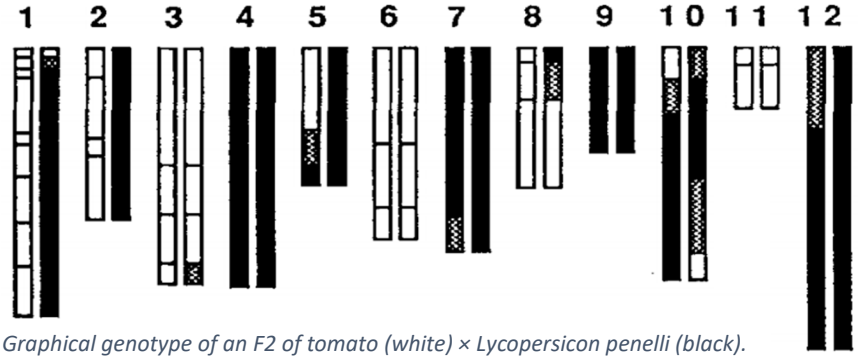
*Pea idiotype and karytoype.*
*PisTR-B (red), 5S rDNA (green), and 45S rDNA (yellow)*



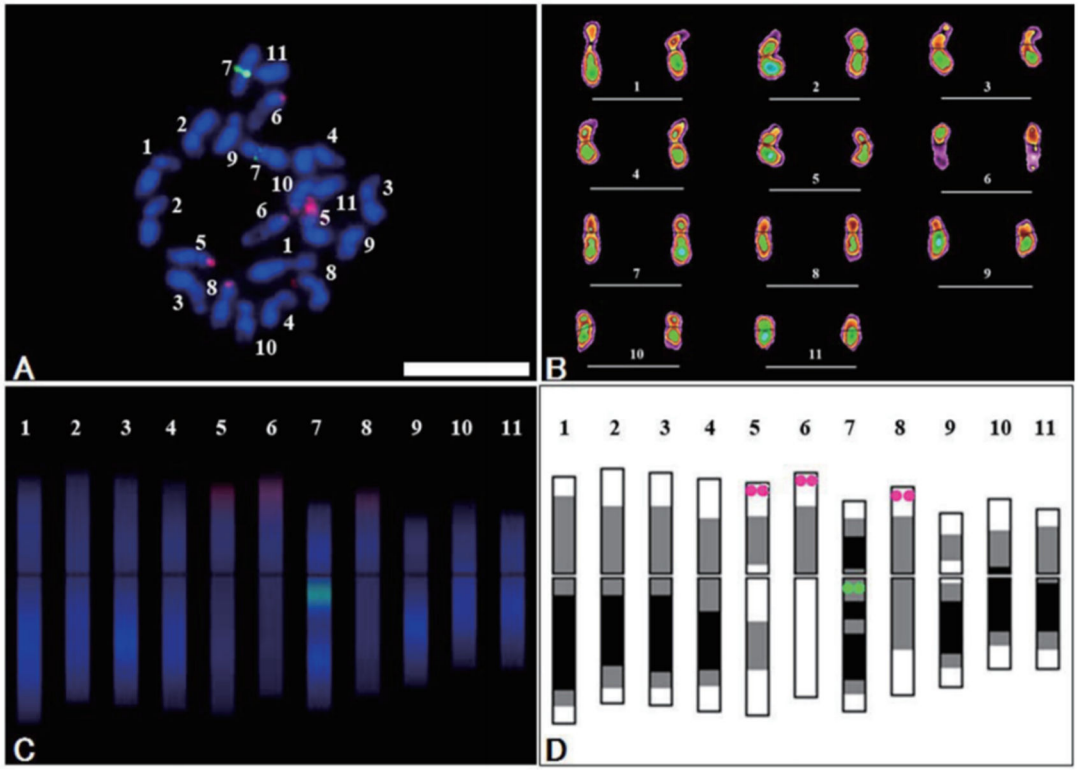

*Smýkal P; Aubert G; Burstin J; Coyne CJ; Ellis NTH; Flavell AJ; Ford R; Hýbl M; Macas J; Neumann P; McPhee KE; Redden RJ; Rubiales D; Weller JL; Warkentin TD. 2012. Pea (Pisum sativum L.) in the Genomic Era. Agronomy 2: 74-115.*

## Graphical genotypes

Young and Tanksley, 1989




*Graphical genotype of an F2 of tomato (white) × Lycopersicon penelli (black).*

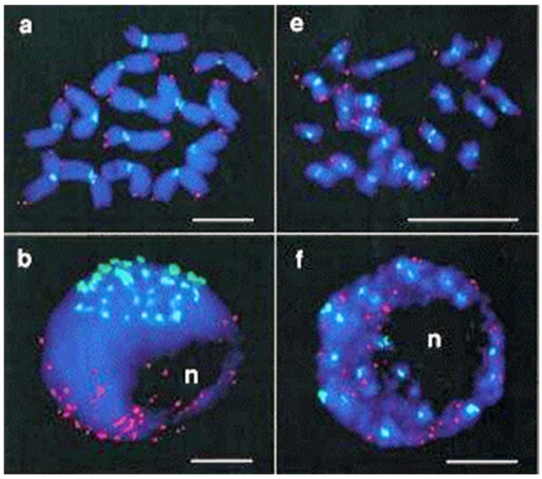



*Ohimodo et al., 2013. Chromosome Science 16: 17-21, 2013*

# Nuclear architecture

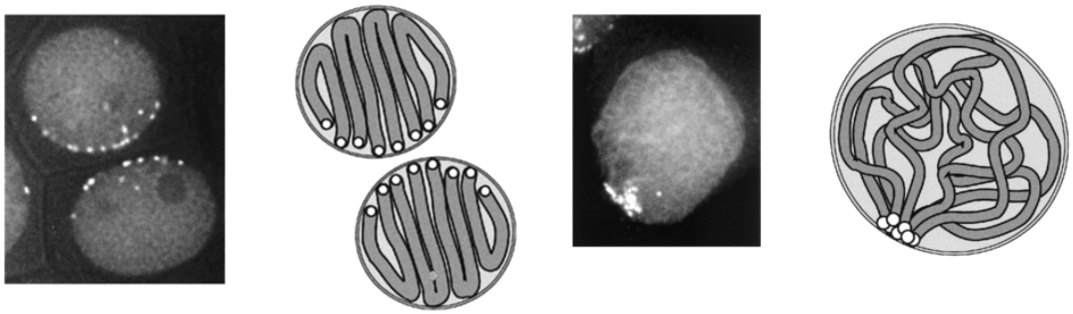
## Rabl 1985

- **Bouquet stage**
- **<u>Polfeld</u>**
- **Rabl configuration**




*Dong & Jiang, 1998. A&b = rye; e & f = sorghum. A& e = metaphase; b& f = interphase*

***Cowan et al., 2000***



# Chromosomal arrangements

Long-time question: - Are chromosomes arranged in any particular order relative to each other, ie, does each chromosome occupy a specified "chromosome territory"?

**Fransz et al., 2002**

**Berr & Schubert, 2007**