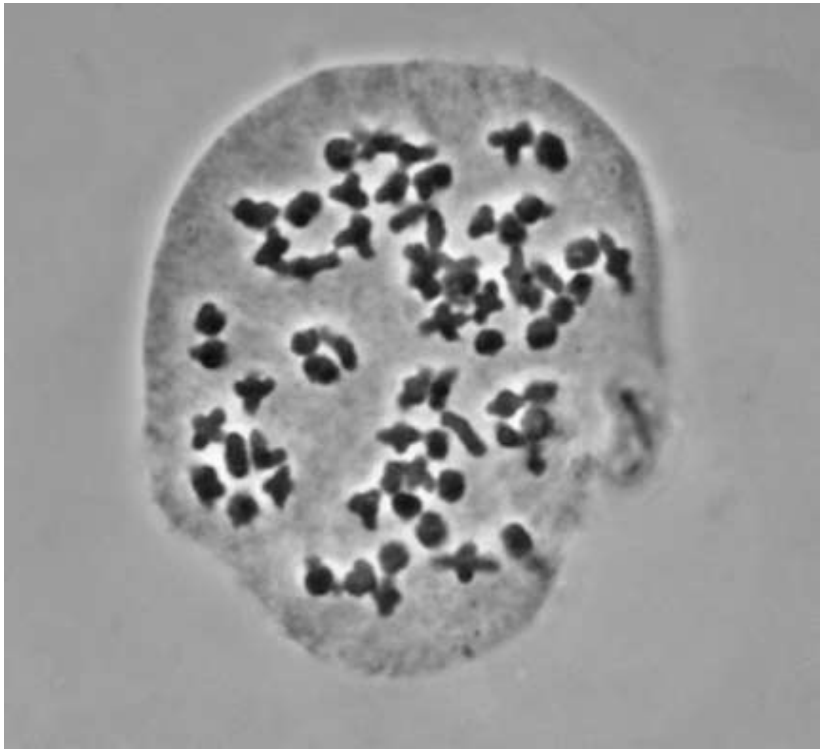
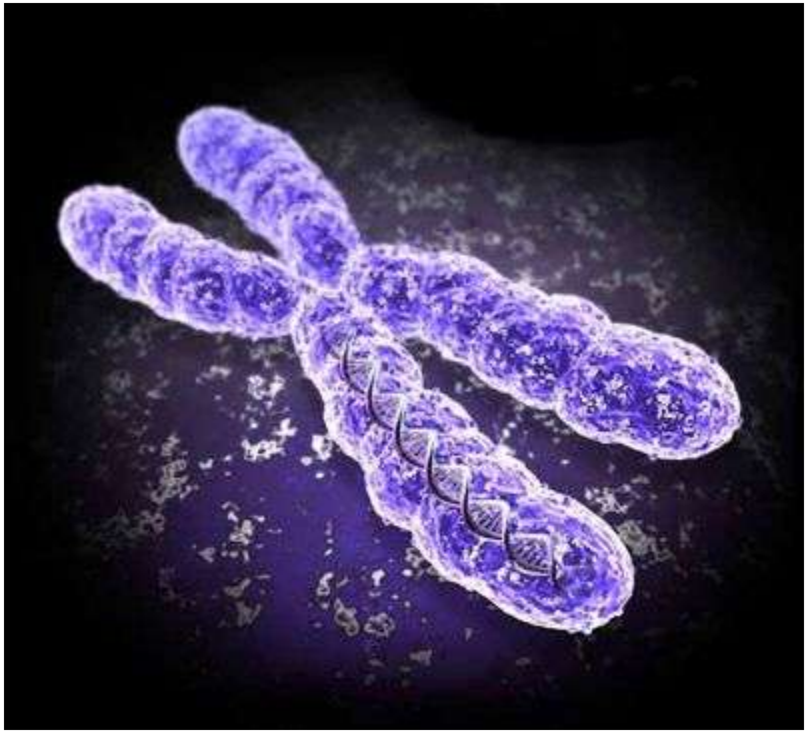
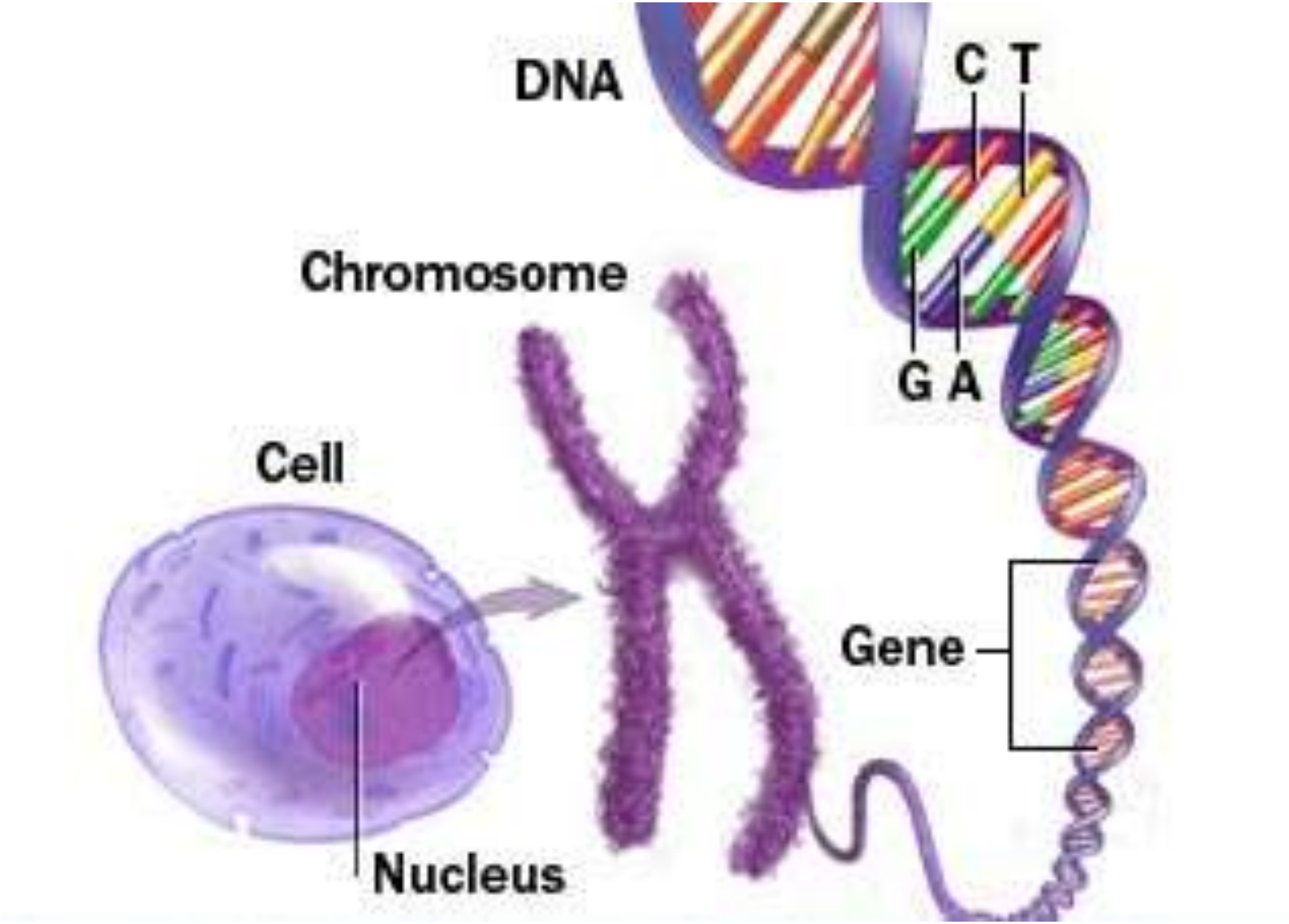
Chromosome Techniques





Chromosomes and Karyotypes



Matthias Jakob Schleiden - 1838 proposes that cells are the basic structural elements of all plants.

Cell Theory

1. All living organisms are composed of one or more cells
2. The cell is the basic unit of structure and organization of organisms
3. All cells come from preexisting cells

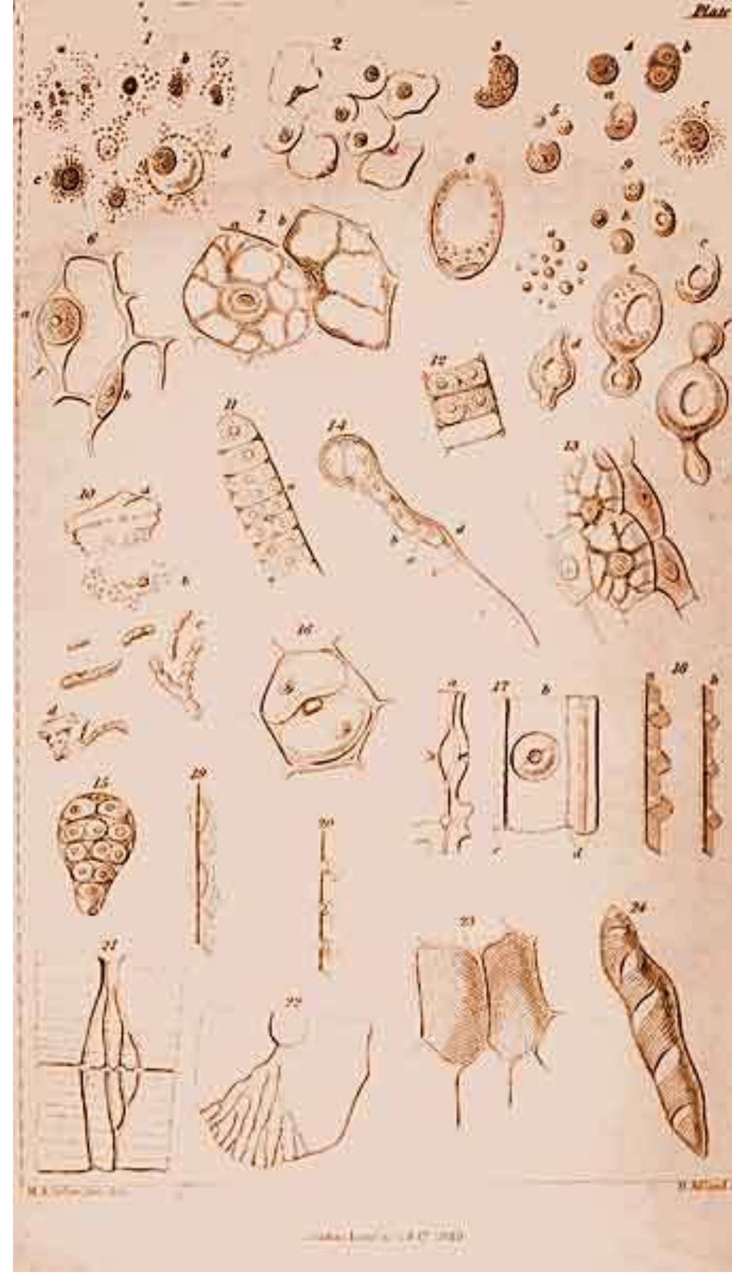
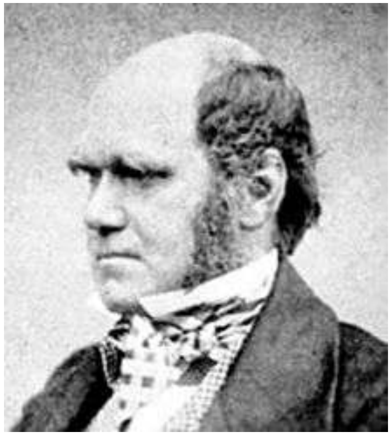
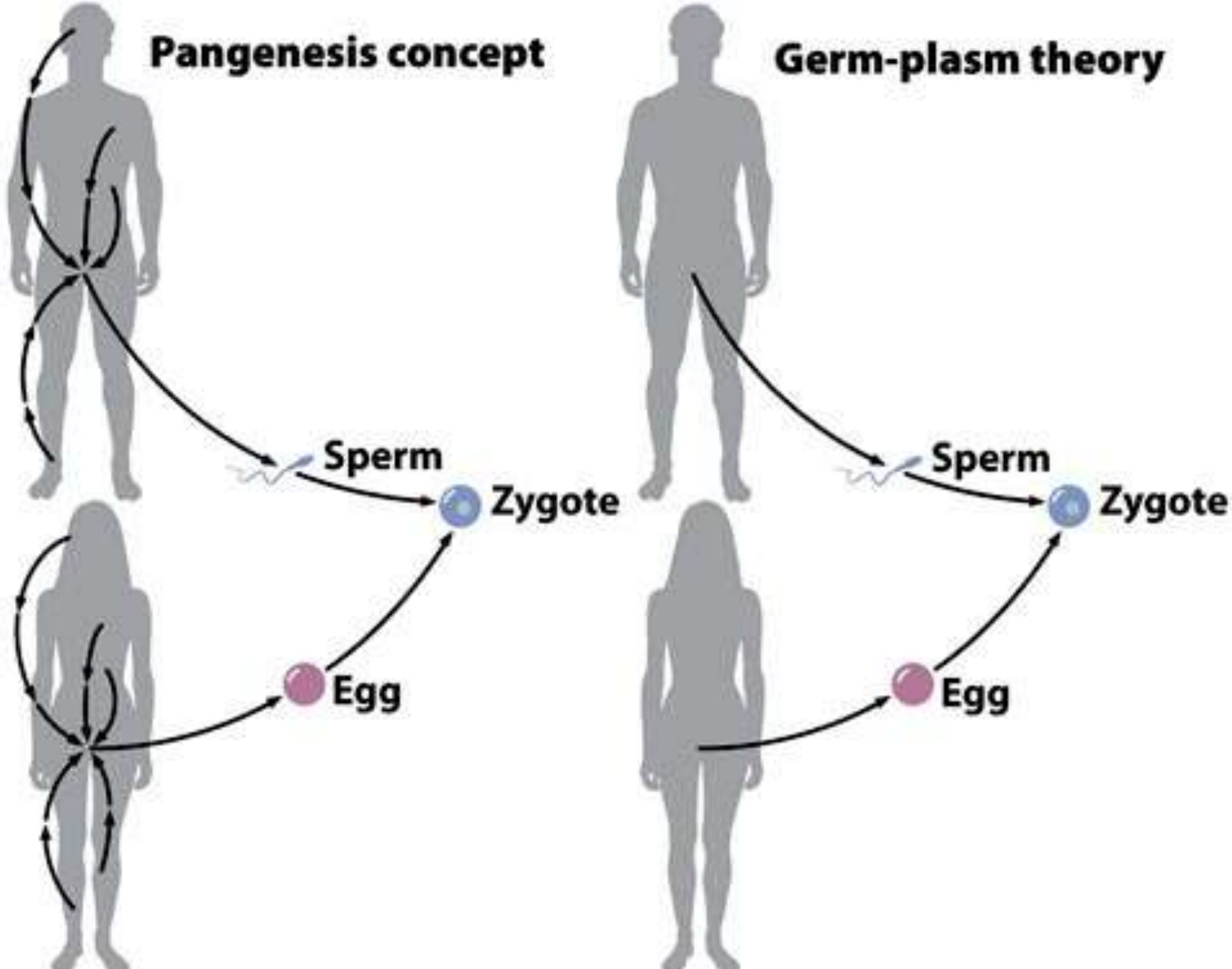
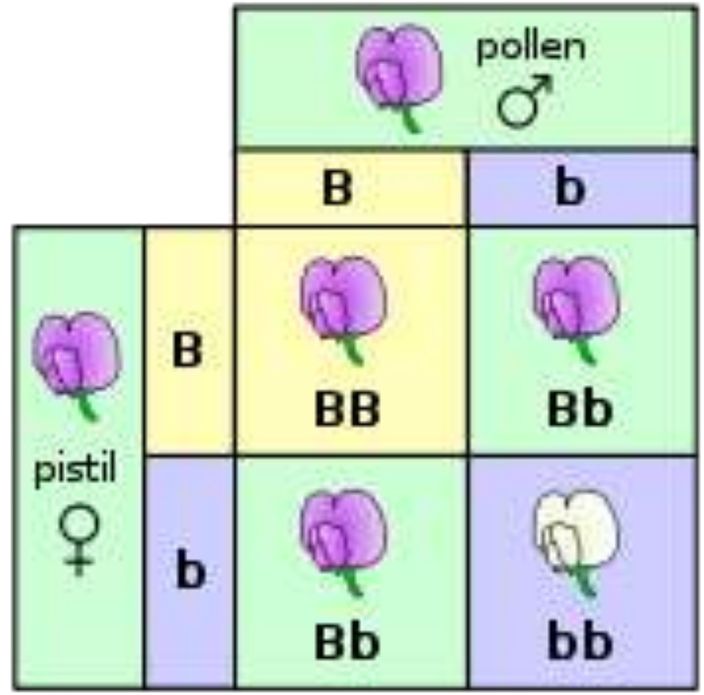


Plate 1 from J. M. Schleiden, Principles of Scientific Botany, 1849, showing various features of cell development





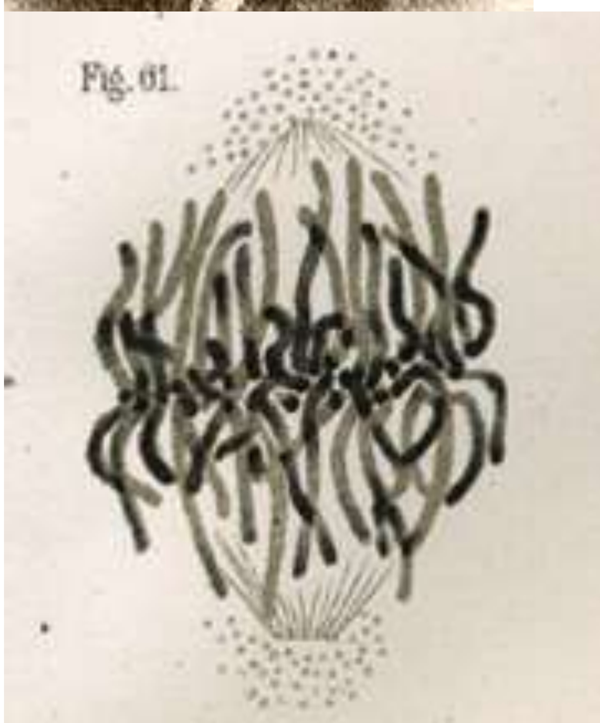
Weismann – Germline
significance of meiosis for reproduction and inheritance - 1890



FIRST LAW:
1. Each trait due to a **pair of hereditary factors** which
2. **segregate during gametogenesis**
SECOND LAW:
3. Multiple sets of hereditary factors **assort independently**

Mendel's work with peas showed that the "blending" explanation was wrong

Walther Flemming - Mitosis



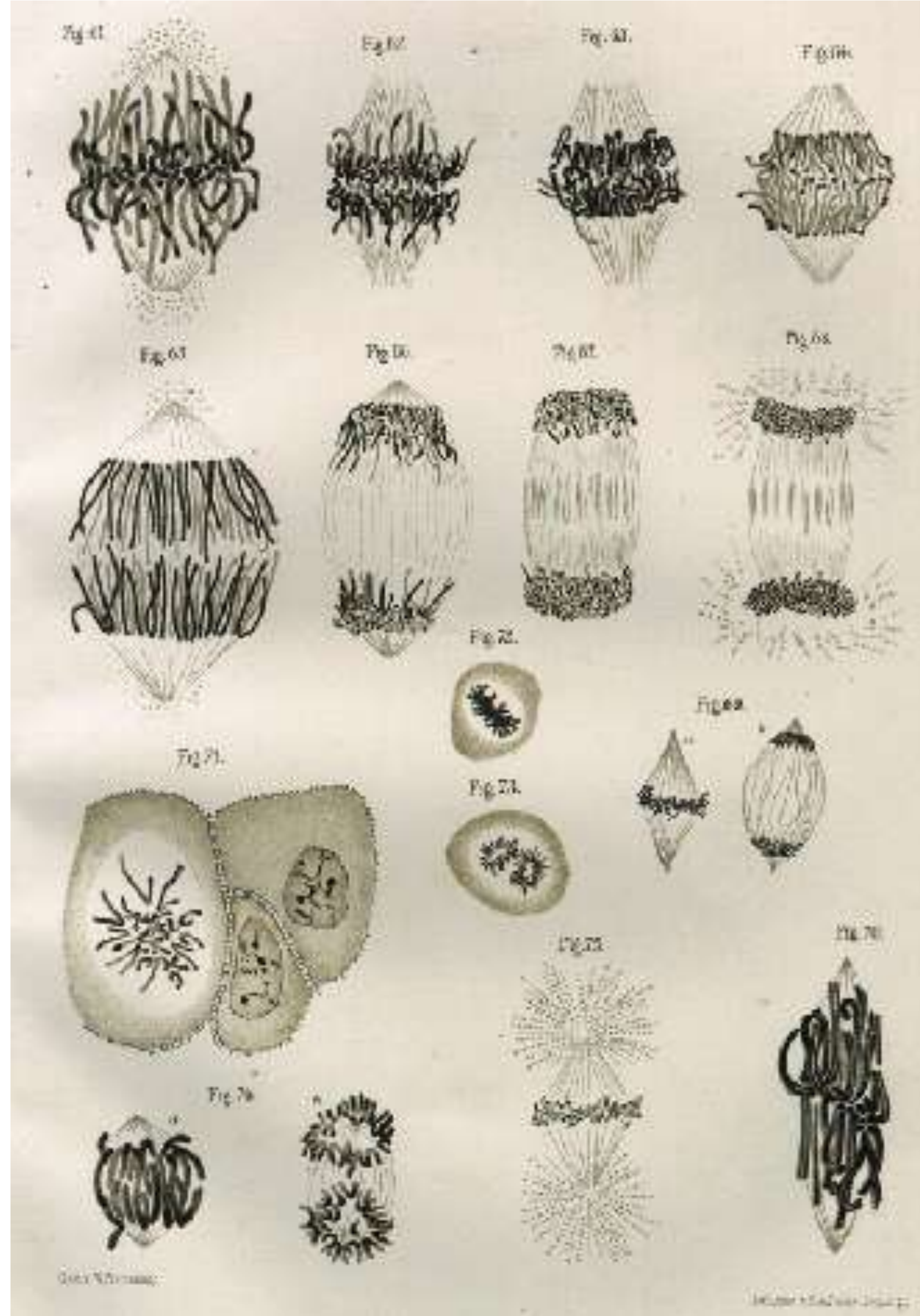
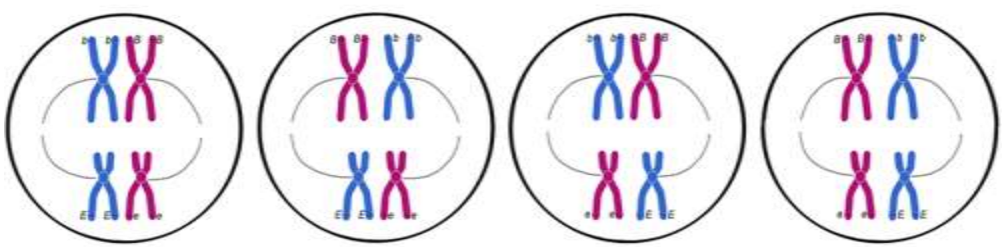
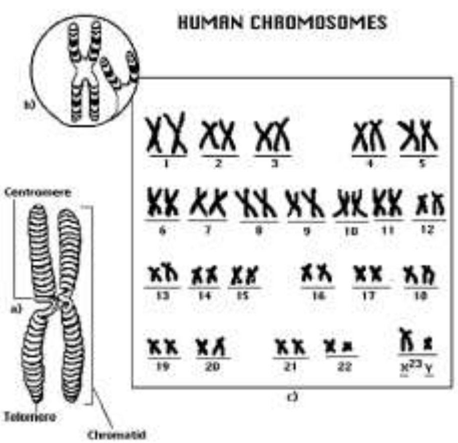



Fig. 61.



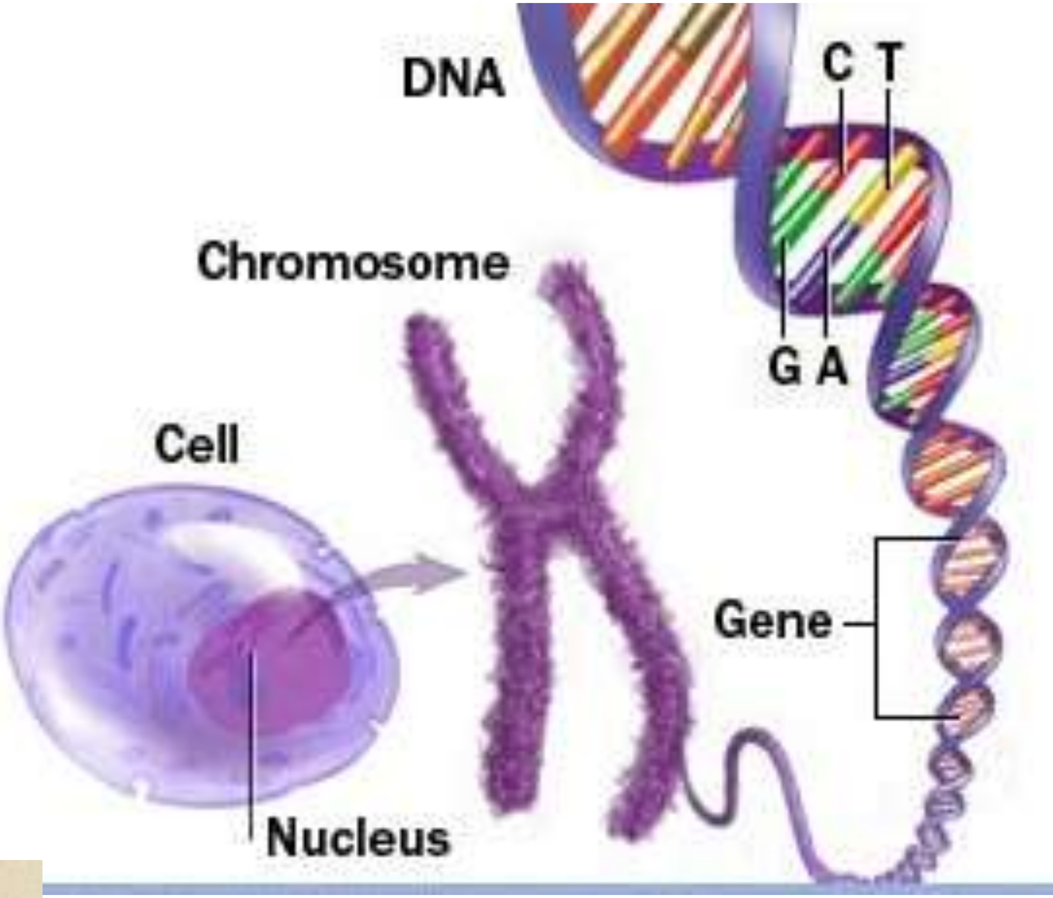
Walter Sutton – Chromosome Theory of Inheritance

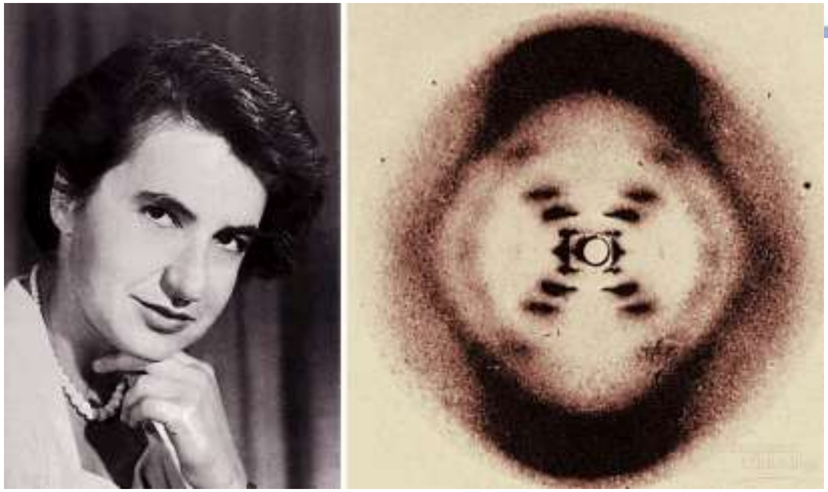
Chromosomes.		Combinations in Gametes.	Combinations in Zygotes.
Somatic Series.	Reduced Series.		
2	1	2	4
4	2	4	16
6	3	8	64
8	4	16	256
10	5	32	1,024
12	6	64	4,096
14	7	128	16,384
16	8	256	65,536
18	9	512	262,144
20	10	1,024	1,048,576
22	11	2,048	4,194,304
24	12	4.096	16,777,216
26	13	8,192	67,108,864
28	14	16,384	268,435,456
30	15	32,768	1,073,741,824
32	16	65,536	4,294,967,296
34	17	131,072	17,179,869,184
36	18	262,144	68,719,476,736



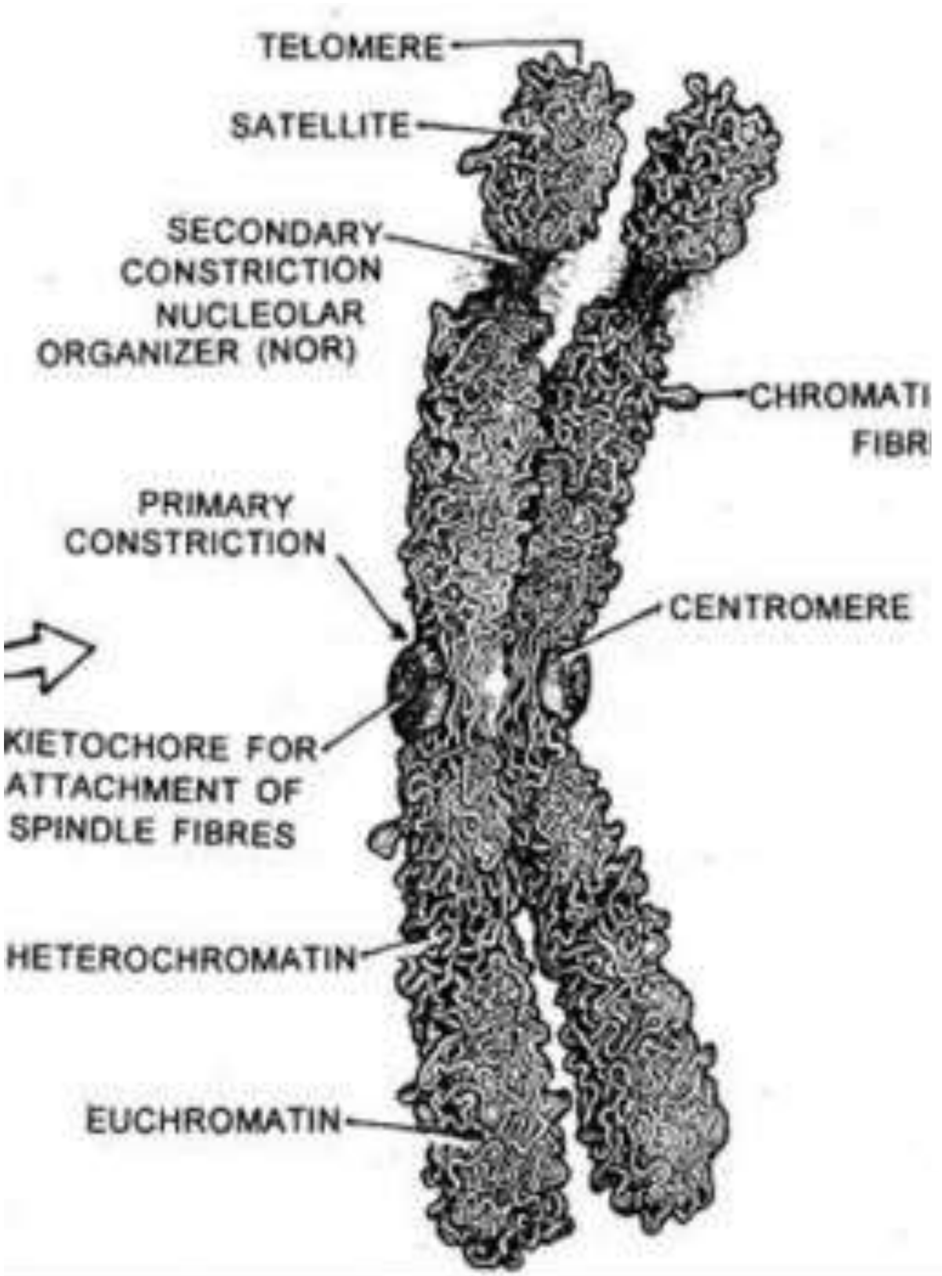
Possible combinations of chromosome pairs at metaphase

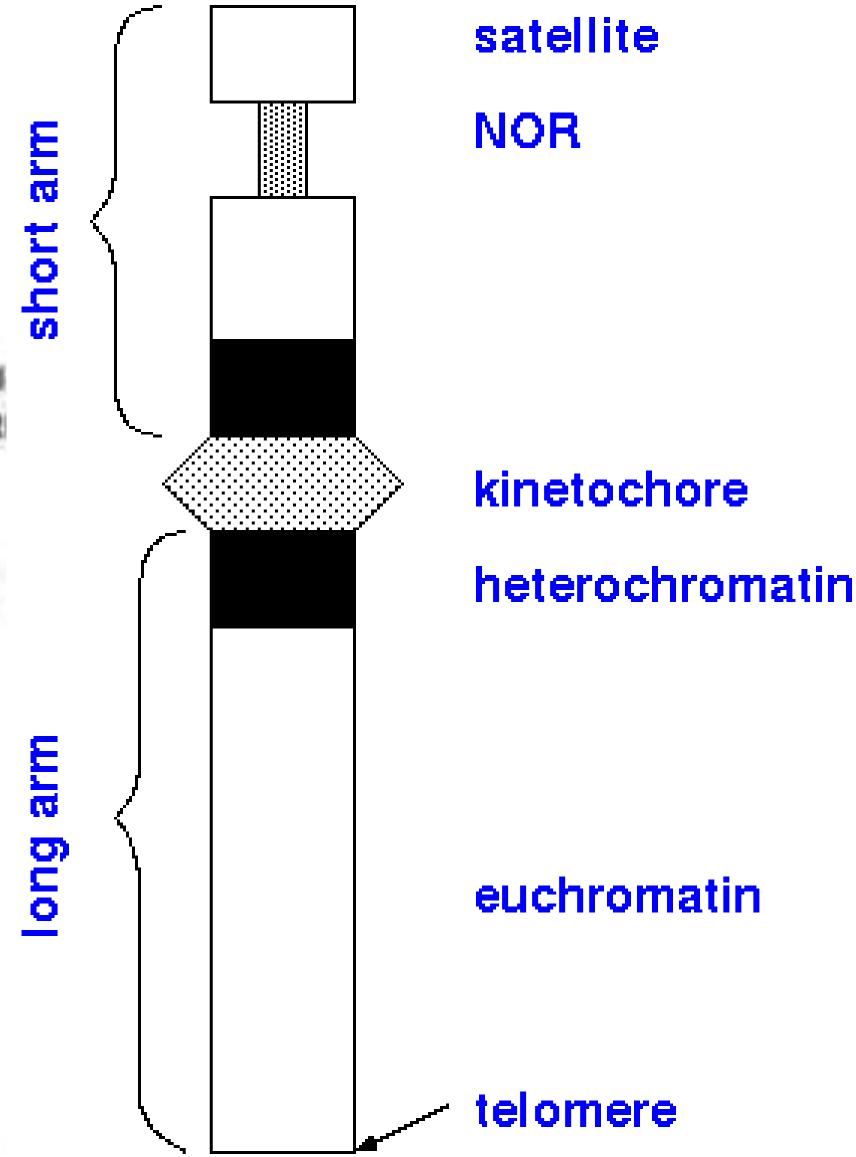
Watson and Crick



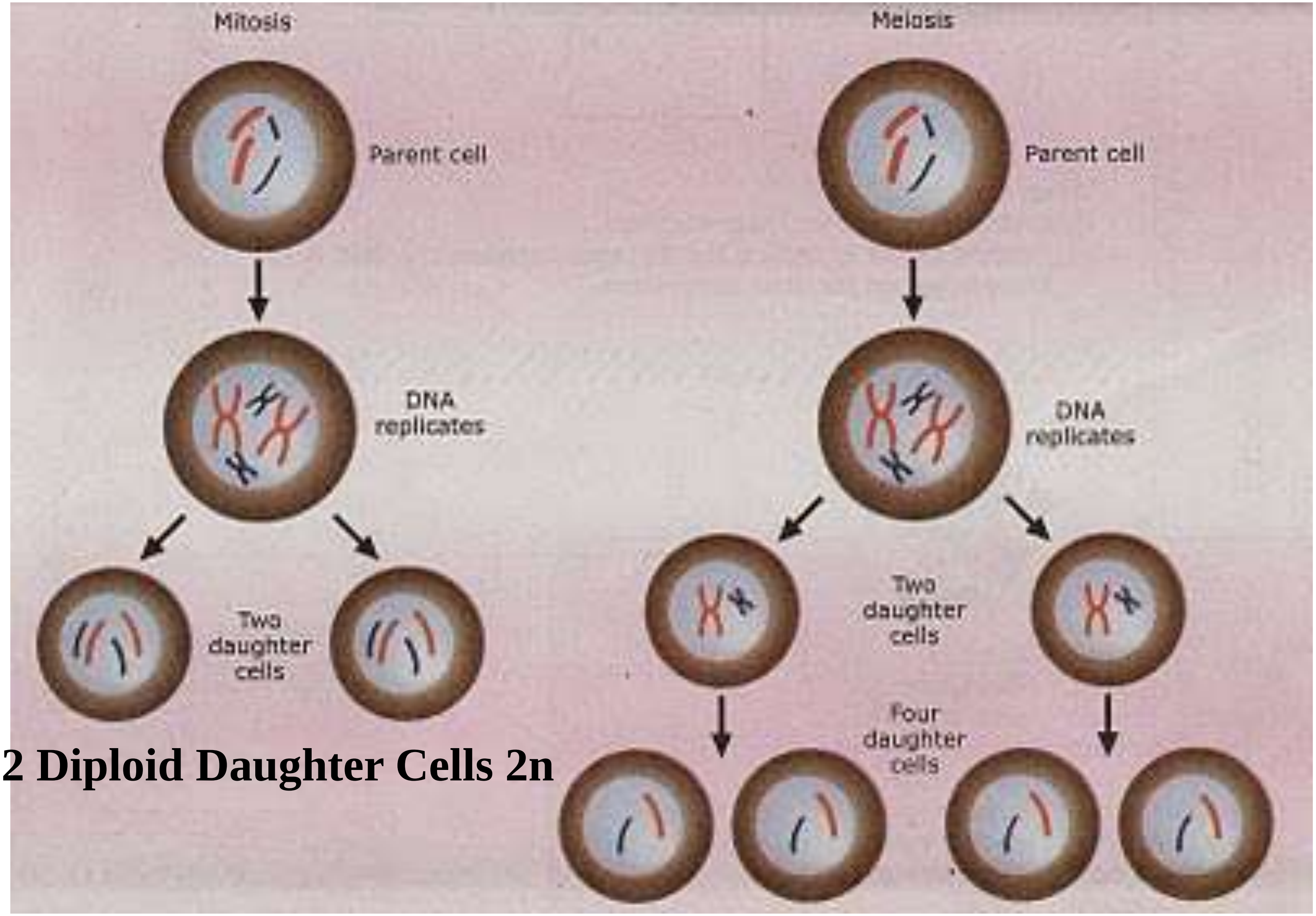


Rosalind Franklin,
X-ray diffraction of DNA


TELOMERE
SATELLITE
SECONDARY CONSTRICTION
NUCLEOLAR ORGANIZER (NOR)
CHROMATI
FIBR
PRIMARY CONSTRICTION
CENTROMERE
KIETOCHORE FOR ATTACHMENT OF SPINDLE FIBRES
HETEROCHROMATIN
EUCHROMATIN


short arm
long arm
satellite
NOR
kinetochore
heterochromatin
euchromatin
telomere

Mitosis **Meiosis**



2 Diploid Daughter Cells 2n

4 Haploid Gametes 1n

Basic Definitions

gene - basic unit of heredity; codes for a specific trait

locus - the specific location of a gene on a chromosome (locus - plural loci)

chromosome - elongate cellular structure composed of DNA and protein - they are the vehicles which carry DNA in cells

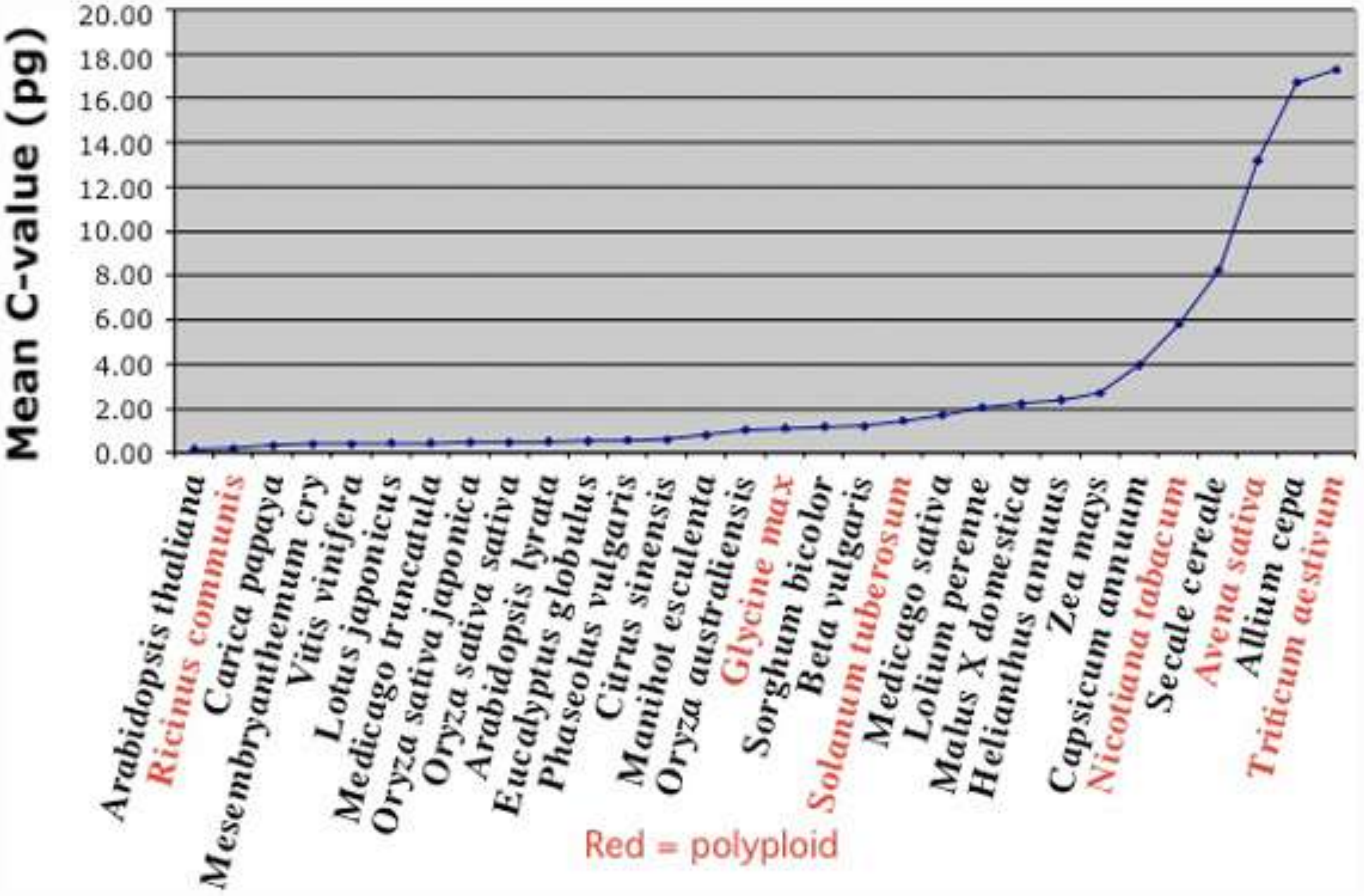
chromatid - one of two duplicated chromosomes connected at the centromere

centromere - region of chromosome where microtubules attach during mitosis and meiosis

diploid (2n) - cellular condition where each chromosome type is represented by two homologous chromosomes

haploid (n) - cellular condition where each chromosome type is represented by only one chromosome

homologous chromosome - chromosome of the same size and shape which carry the same type of genes



Mean C-value (pg)
20.00
18.00
16.00
14.00
12.00
10.00
8.00
6.00
4.00
2.00
0.00
Arabidopsis thaliana
Ricinus communis
Carica papaya
Mesembryanthemum cry
Vitis vinifera
Lotus japonicus
Medicago truncatula
Oryza sativa japonica
Oryza sativa sativa
Arabidopsis lyrata
Eucalyptus globulus
Phaseolus vulgaris
Citrus sinensis
Manihot esculenta
Oryza australiensis
Glycine max
Sorghum bicolor
Beta vulgaris
Solanum tuberosum
Medicago sativa
Lolium perenne
Malus X domestica
Helianthus annuus
Zea mays
Capsicum annuum
Nicotiana tabacum
Secale cereale
Avena sativa
Allium cepa
Triticum aestivum
Red = polyploid

C-Values

The time required for mitosis and meiosis increases with genome size

Species	Picograms per Haploid Genome	Mitosis in Hours	Meiosis in Hours	Plant Habit
Crepis capillaris	1.20	10.8	--	Annual
Haplopappus gracillis	1.85	10.5	36.0	Annual
Pisum sativum	3.9, 4.8	10.8	--	Annual
Ornithogalum virens	6.43	--	96.0	Perennial
Secale cereale	8.8, 9.6	12.8	51.2	Annual
Vicia faba	13.0, 14.8	13.0	72.0	Annual
Allium cepa	14.8, 16.25	17.4	72.0	Perennial
Tradescantia paludosa	18.0	18.0	126.0	Perennial
Endymion nonscriptus	21.8	--	48.0	Perennial
Tulipa kaufmanniana	31.2	23.0	--	Perennial
Lillium longiflorum	35.3	24.0	192.0	Perennial
Trillium erectum	40.0	29.0	274.0	Perennial

Source: Van't Hof, 1965, and Bennett, 1972.

Chromosome Number

Variation exists within genera:
Stylidium (2N = 5-16, 26, 28, 30)
Cardamine (2N = 16, 24, 28, 30, 32)

Variation exists within some species:
Rumex subgenus ***Rumex***: 2N = 2X = 20, 2N = 4X = 40, 2N = 20X = 200.
Chaenactis douglasii: 2N = 12-15, 18, 24-28, 36, 38.

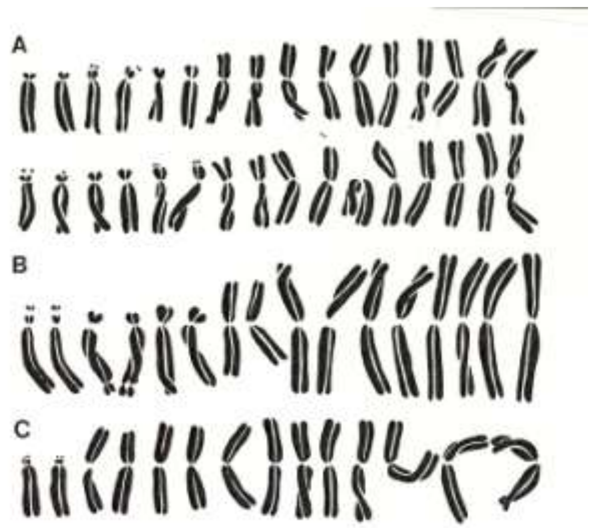


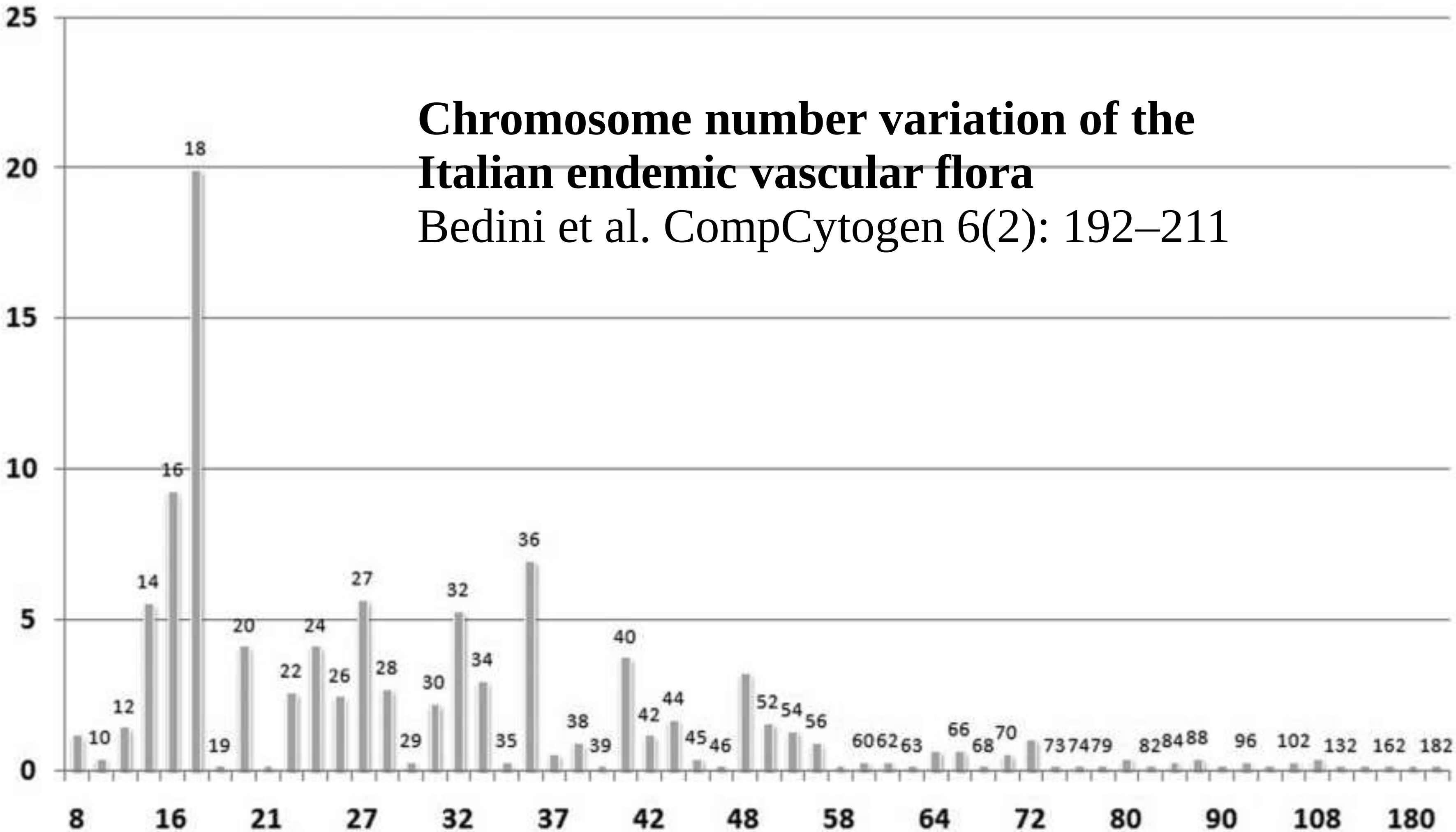
FIGURE 19.4 Drawings of metaphase chromosomes of *Anemone* (Ranunculaceae). A, *A. quinquefolia*, 2n = 32; B, *A. rivularis*, 2n = 16; C, *A. richardsonii*, 2n = 14. x2500. (From Heimburger 1959:592)

Anemone

Chamaecrista

Chromosome number variation of the Italian endemic vascular flora

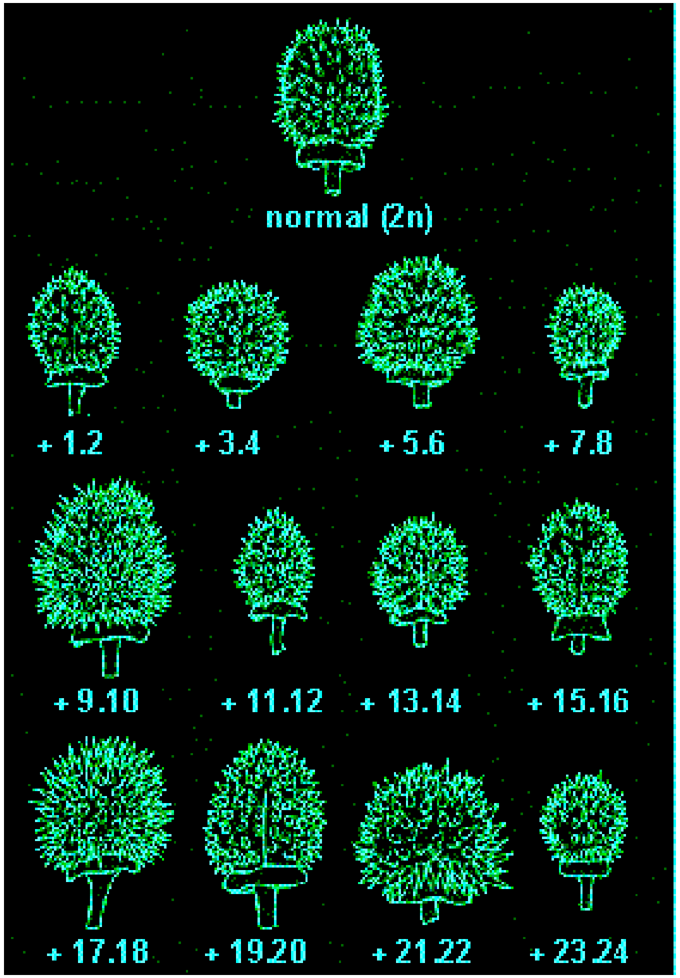
Bedini et al. CompCytogen 6(2): 192–211



Known chromosome numbers in Italian endemics range from 2n = 8 to 2n = 182. **Mean chromosome number for Italian endemics is 2n = 30.68** ± 20.27 (median: 2n = 26, mode: 2*n* = 18).

Aneuploidy

a gain or loss of one or more chromosomes, e.g. 2N -1, 2N + 1, 2N + 2, etc. The most common case are trisomies (sing. trisomy) where a single additional chromosome is present.



Fruits of *Datura*-
Plants On top: Control plant (2n)
Below: Mutants that are characterized by **one additional chromosome** each.

Loss of one or more chromosomes usually has more severe consequences

Aneuploidy

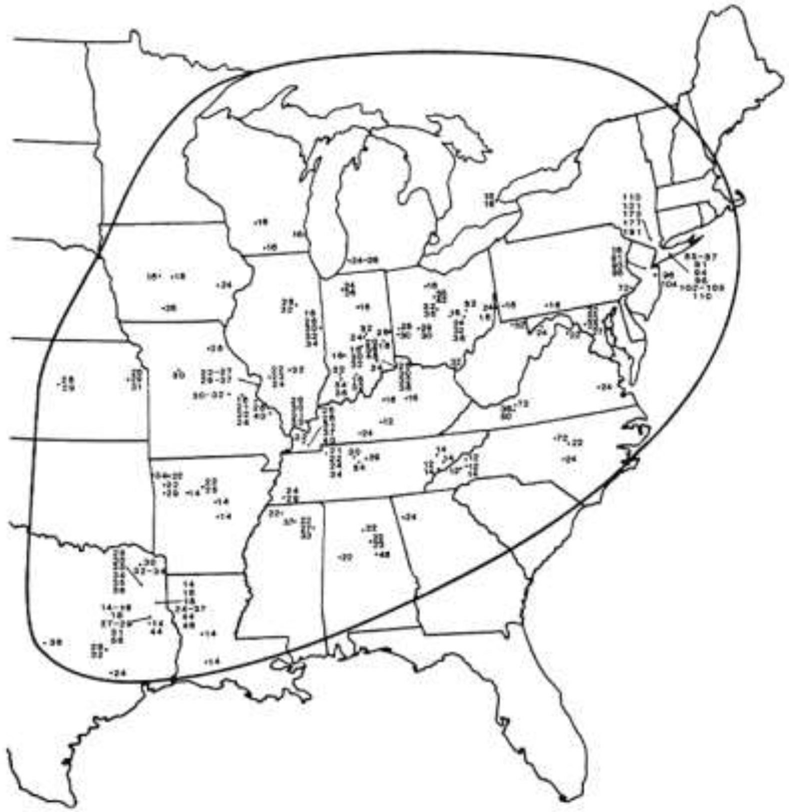


FIGURE 19.2 Variation of diploid chromosome numbers in populations throughout the range (enclosed area) of *Claytonia virginica* (Portulacaceae). (Redrawn from Lewis, Oliver, and Suda 1967:154)

Claytonia virginica Walter Lewis (1970, 1971). Plants have different chromosome numbers in different parts of their ranges and even within same population. and within one individual from year to year.

"I would argue that if an organism does not take its chromosome number seriously, there is no reason why the systematist should" (Walter Lewis).

Aneuploid Series - well known in plants

Carex - long and nearly continuous series from n=6 to n=56

Crepis – series n=6-5-4-3

Nicotiana – series n=12-11-10-9

Crepis – Chromosomes – drawn to scale

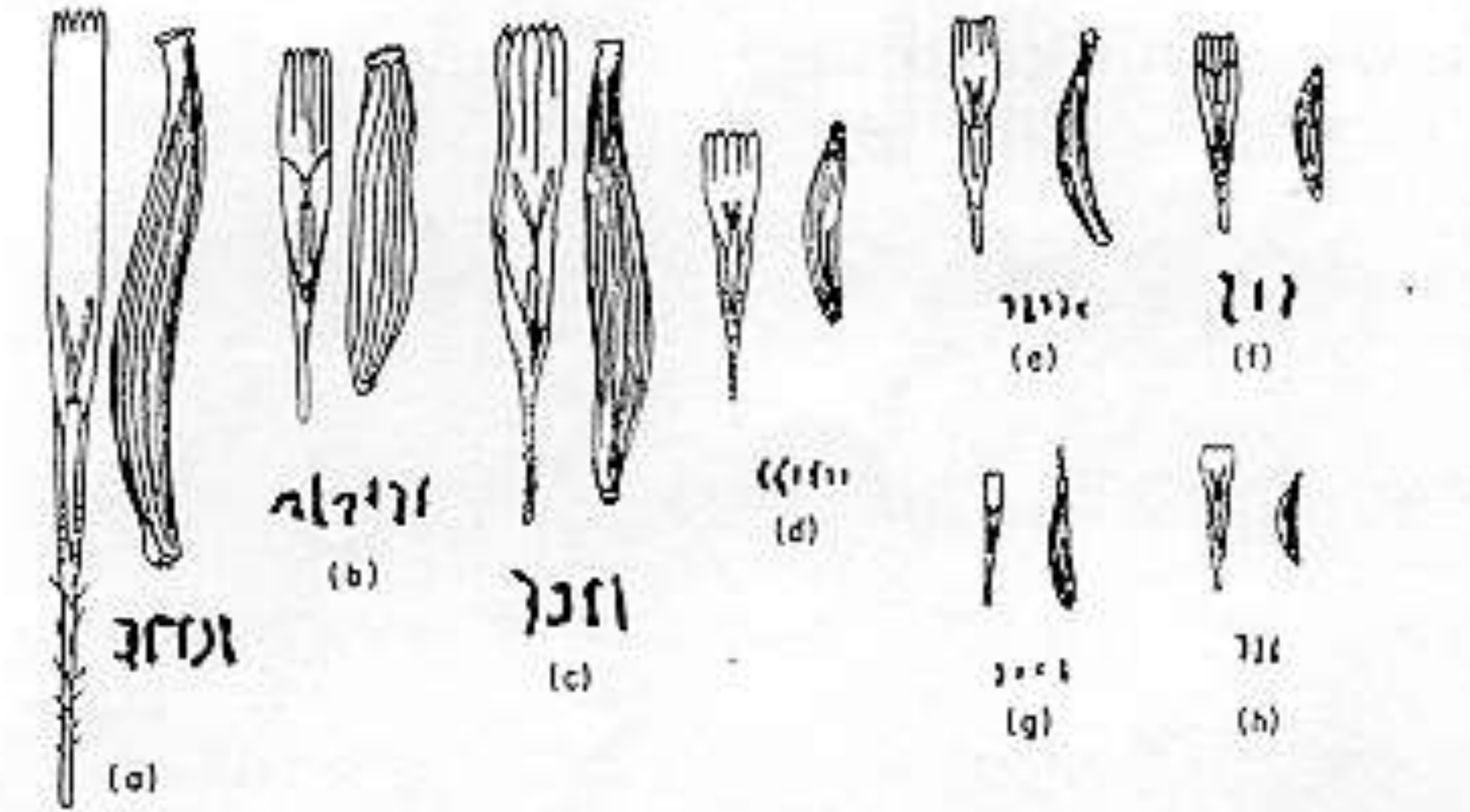
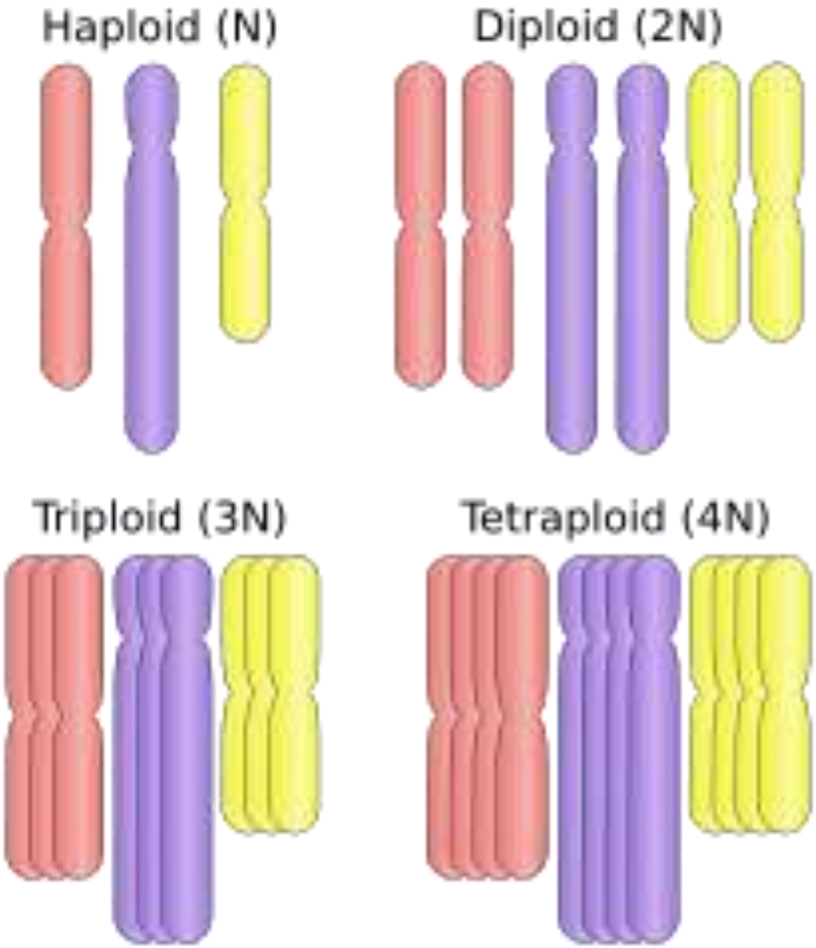


Fig. 9-19. Species of the genus Crepis showing the size relations of chromosomes, florets (lacking ovaries), and achenes, all drawn to the same magnification so that the sizes are relative to each other. A. *C. sibirica*; B, *C. kashmirica* ; C, *C. conyzaefolia*; D, *C. mungierii;* E, *C. leontodontoides* ; F, *C. capillaris*; G, *C. suffreniana*; H, *C. fuliginosa* (rearranged from Babcock, 1947)

Polyploidy – multiple sets of chromosomes



Diploid – 2 sets
Triploid – 3 sets (watermelon)
Tetraploid – 4 sets (cotton)
Hexaploids – 6 sets (wheat)

Polyploidy

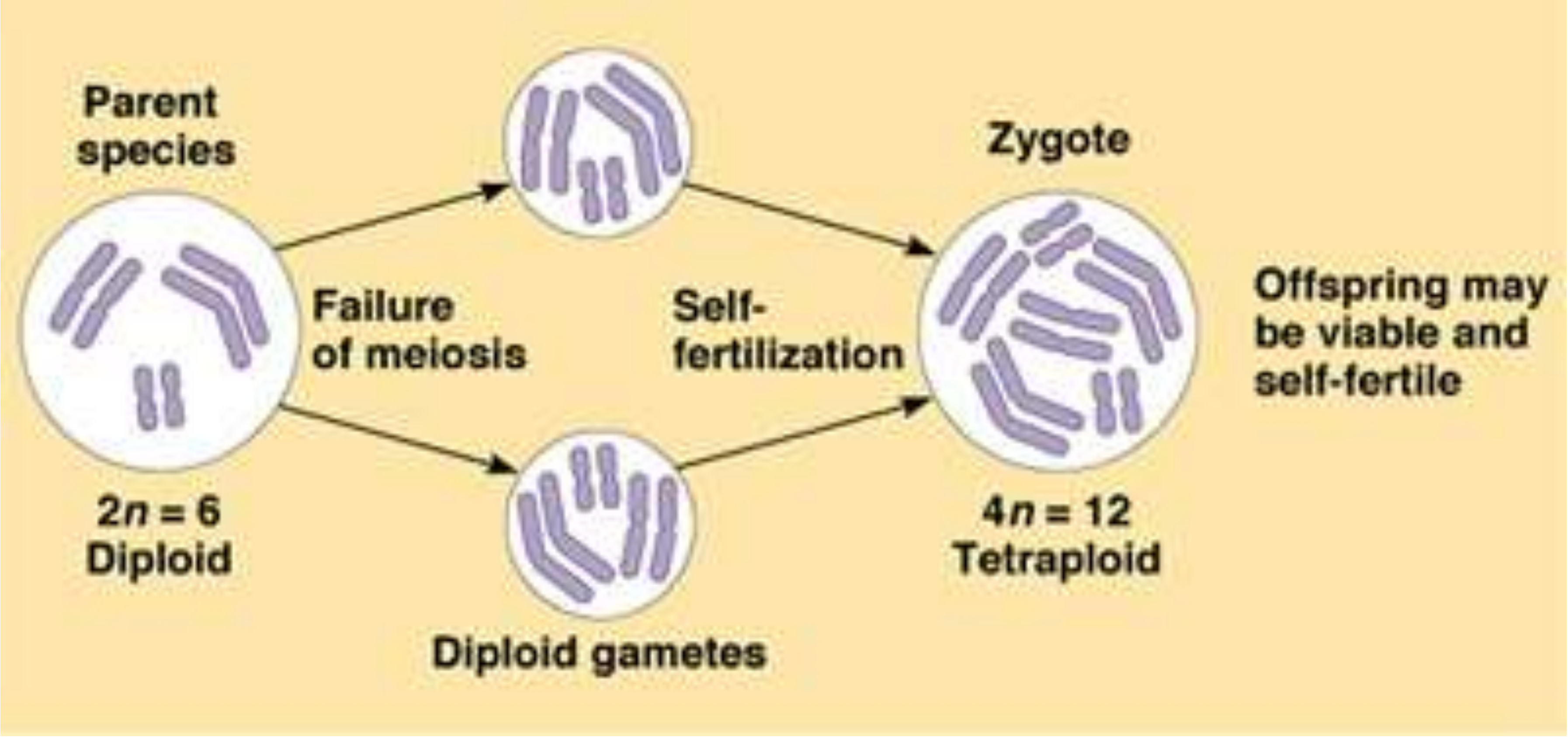
X - The basic chromosome number. The number of different chromosomes.
N -The number of chromosome pairs in mitosis. 2n chromosomes would be seen in mitotic cells.
Diploid -Containing two each of the basic chromosomes. 2n is the diploid chromosome number. In diploids, x = n.
Polyploid - Containing multiple diploid sets of chromosomes.
Tetraploid - Containing four of each chromosome, usually two diploid sets of chromosomes. 4x.
Triploid -Containing three of each chromosome, usual half of a diploid set and half of a tetraploid set. Usually almost sterile. 3x.

Other Common Ploidies

5x = pentaploid
6x = hexaploid
8x = octoploid
10x = decaploid
12x = duodecaploid
16x = hexadacaploid

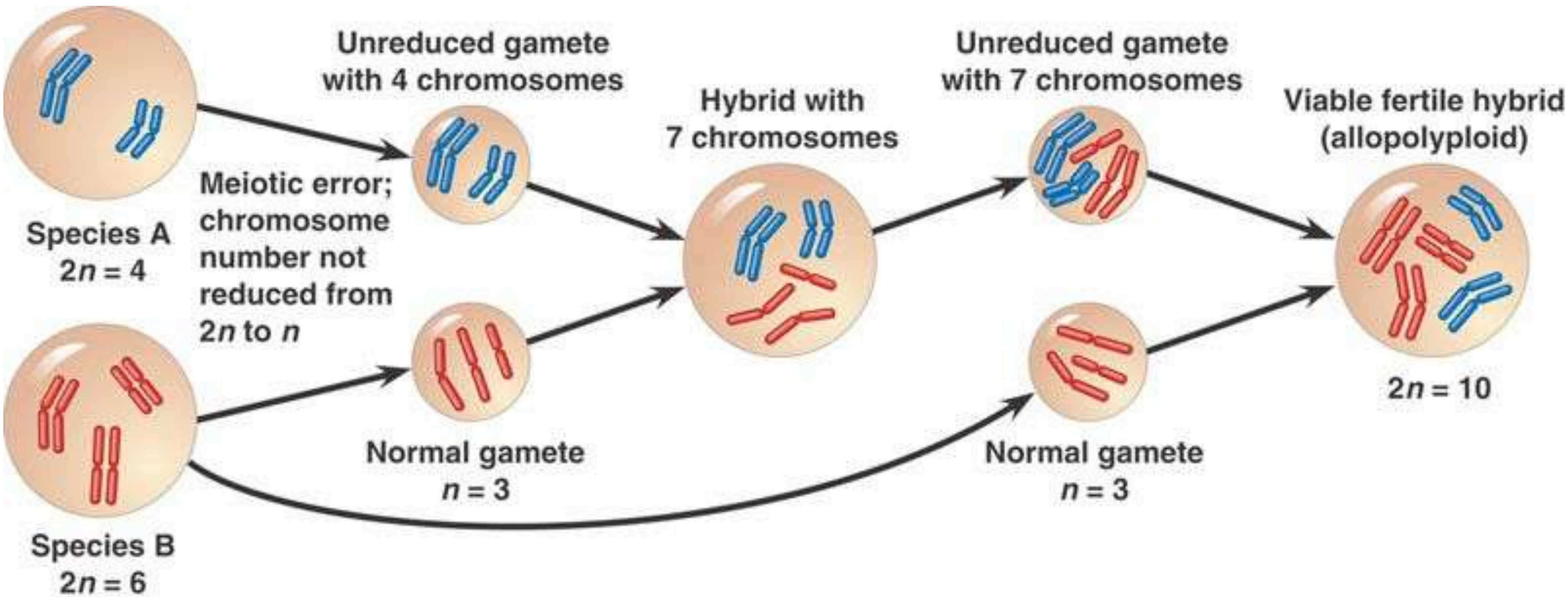
Polyploidy

Autopolyploids: polyploids composed of multiple sets of chromosomes from the same species

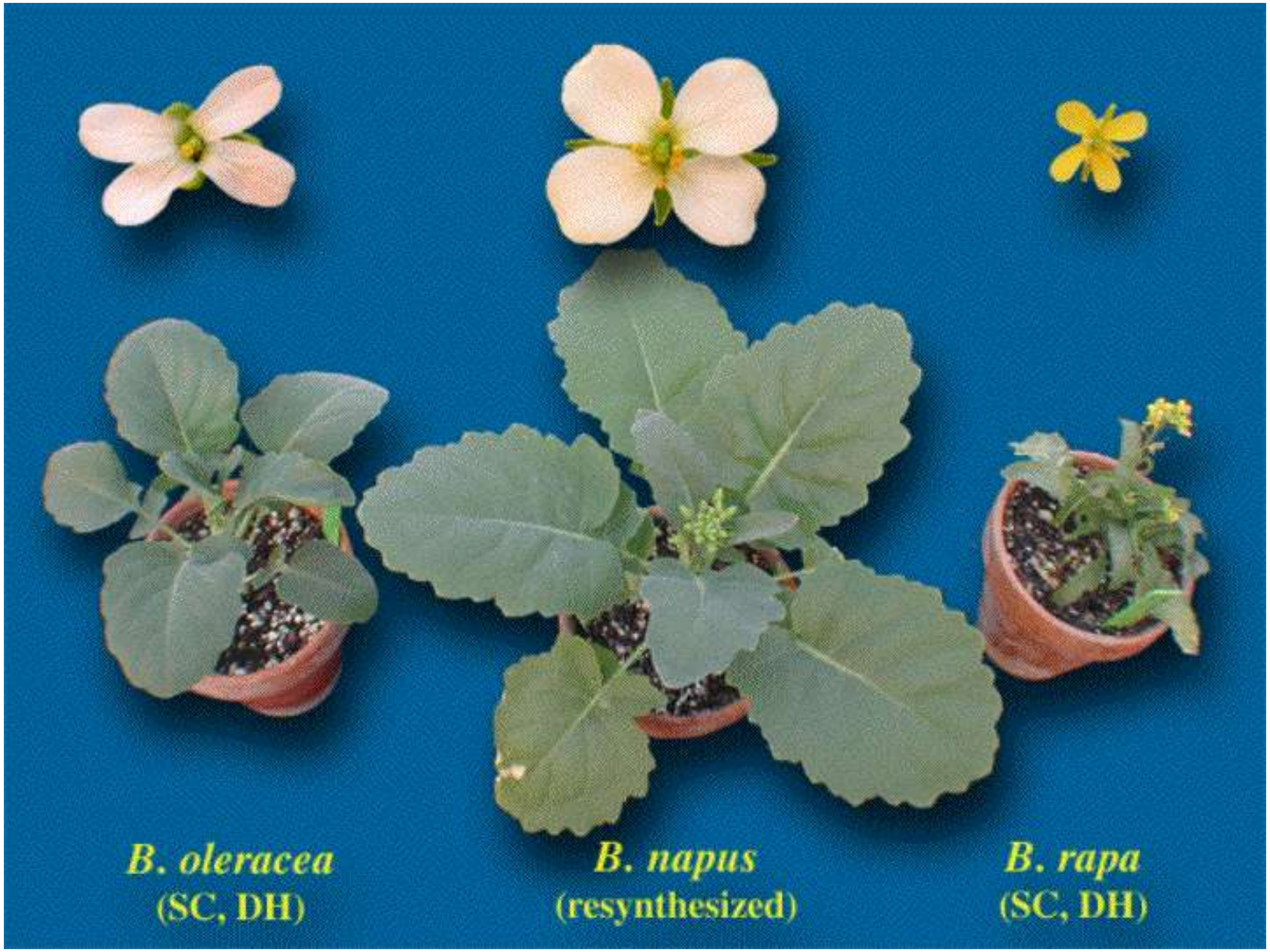


Polyploidy

Allopolyploids: polyploids that are a new species, composed of multiple sets of chromosomes from closely related species

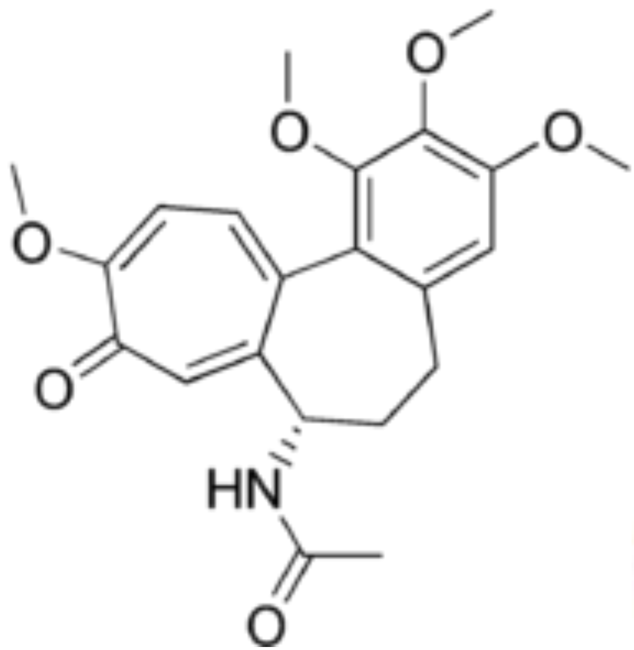


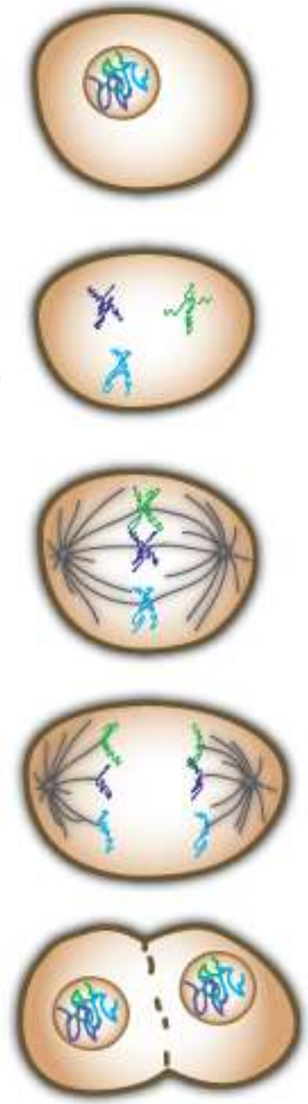
Hybrid Vigor - resynthesized *Brassica napus*



An example of an allopolyploid that shows hybrid vigor over its diploid progenitors is resynthesized *Brassica napus*.

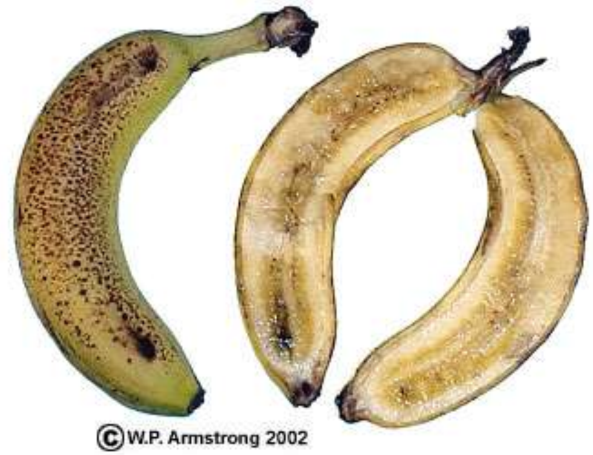
Colchicine – used to double chromosome numbers





Tripoids – 3 sets of chromosomes, generally sterile

Triploid crops: apple, banana, citrus, ginger, watermelon



Musa acuminata (Asian Banana)	X	***Musa balbisiana*** (Asian Banana)	=	***Musa X paradisiaca*** (Hybrid Banana)
AA (fertile)		**BB** (fertile)		**AAB or ABB (etc.)** (sterile)

Origin Of Triploid Banana From Asian Parents

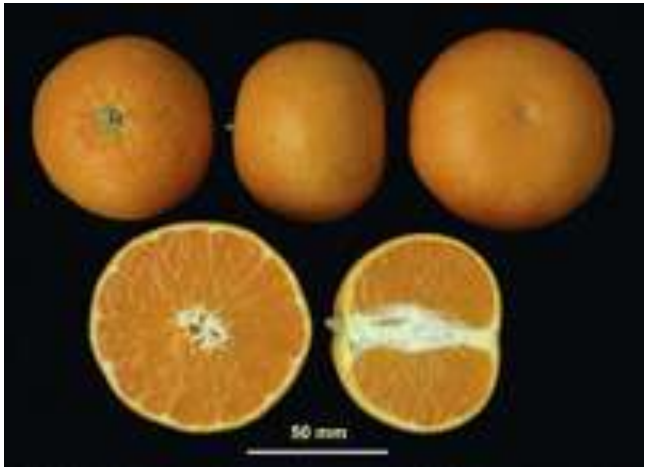
A = one haploid set of chromosomes from *M. acuminata*

B = one haploid set of chromosomes from *M. balbisiana*

Seedless Watermelon

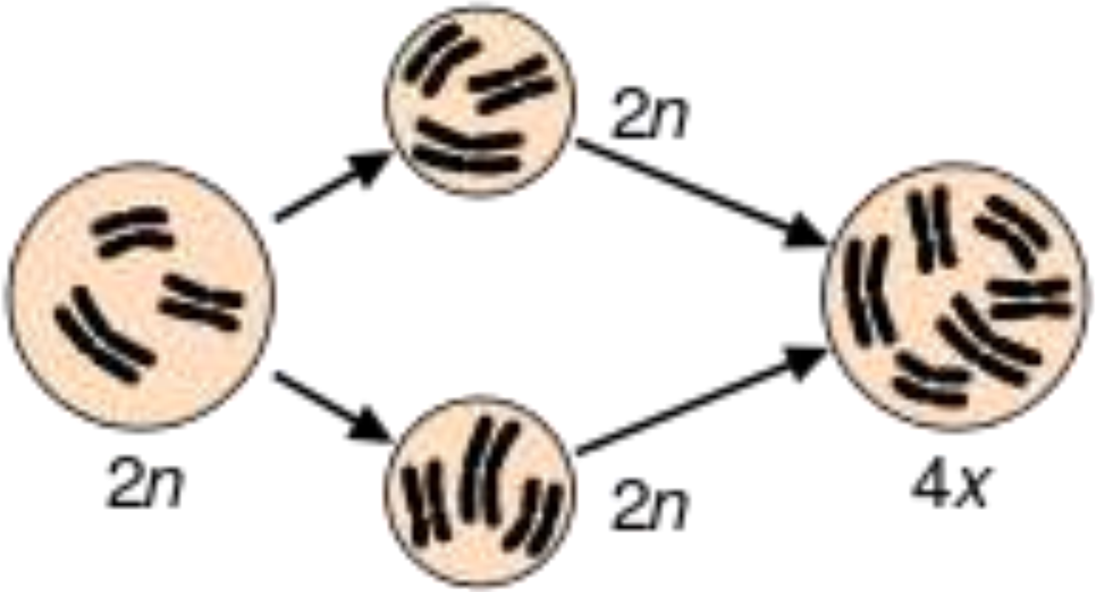
Bramley Apple

Garbi Mandarin



Miscanthus giganteus

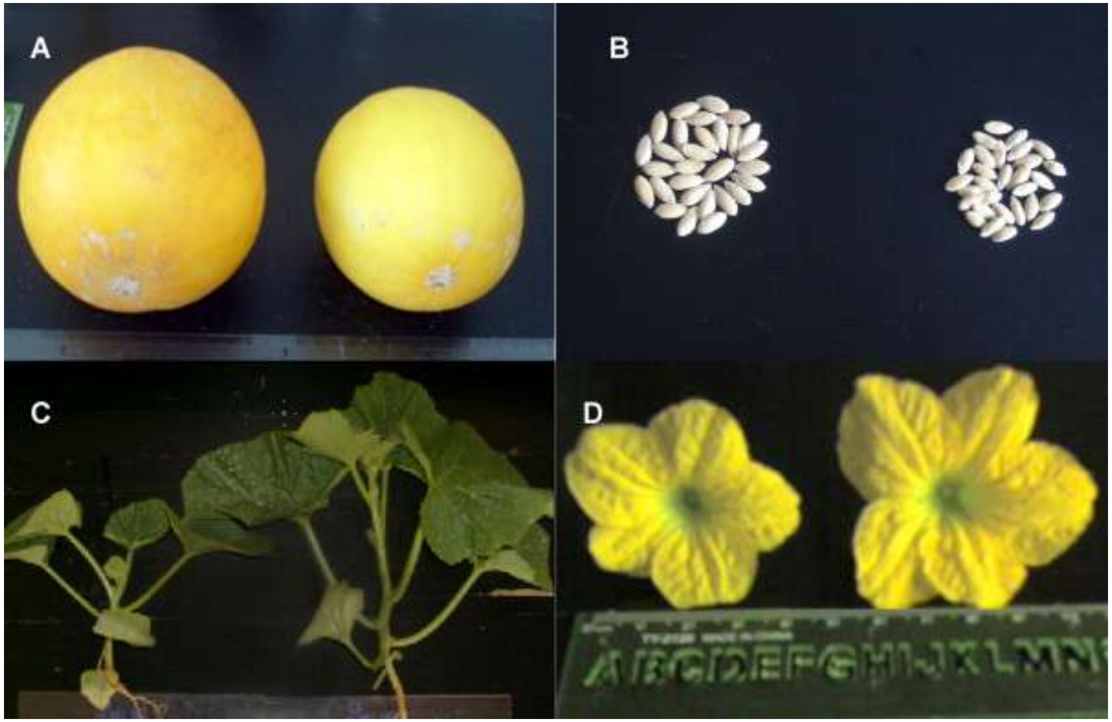
Tetraploids – 4 sets of chromosomes



Failed meiosis, gametes 2N

Tetraploid crops: apple, durum or macaroni wheat, cotton, potato, cabbage, leek, tobacco, peanut, Pelargonium

Tetraploid Fruits



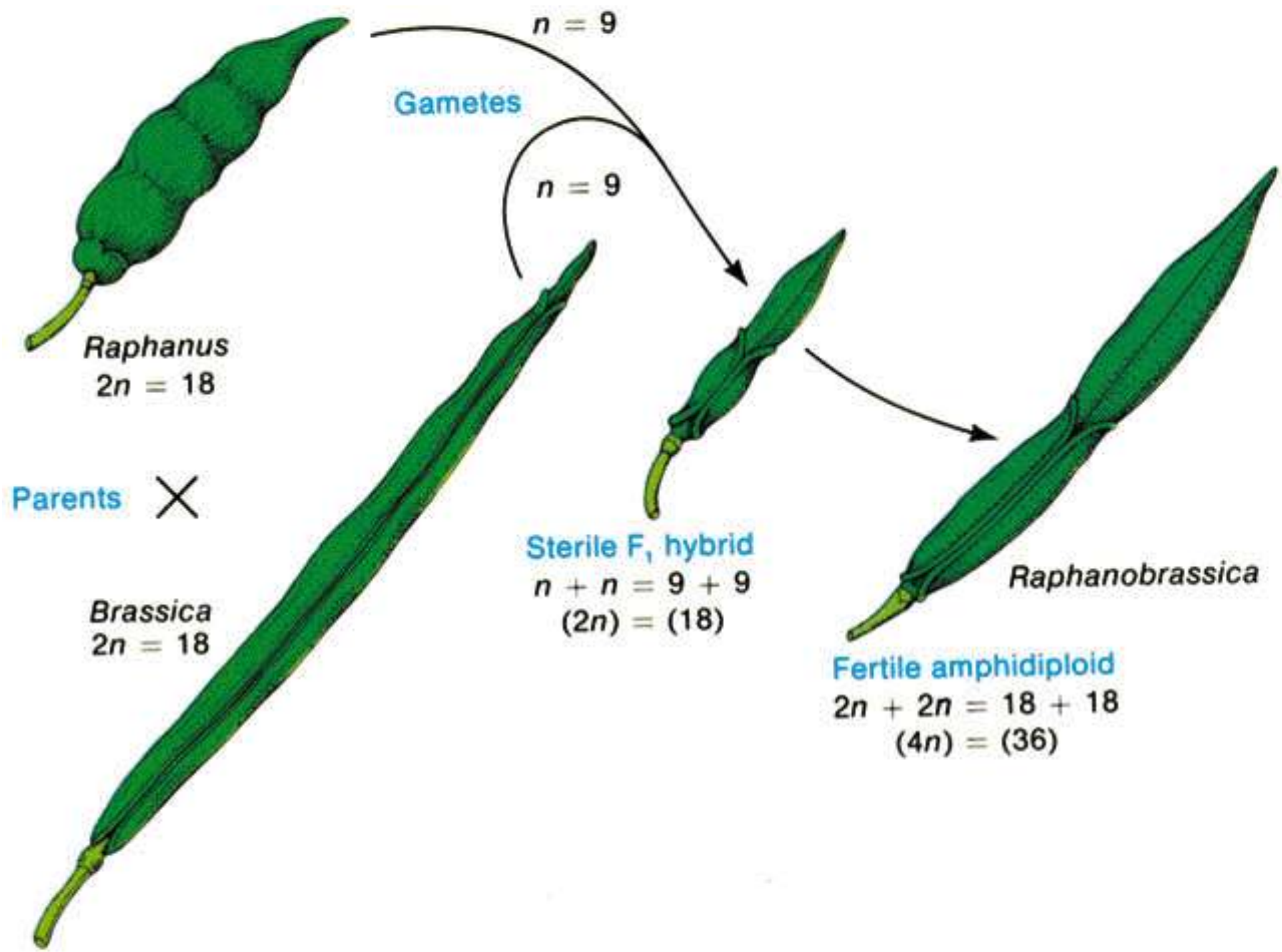
Muskmelon – tetraploid and diploid compared

Passiflora

Raspberries

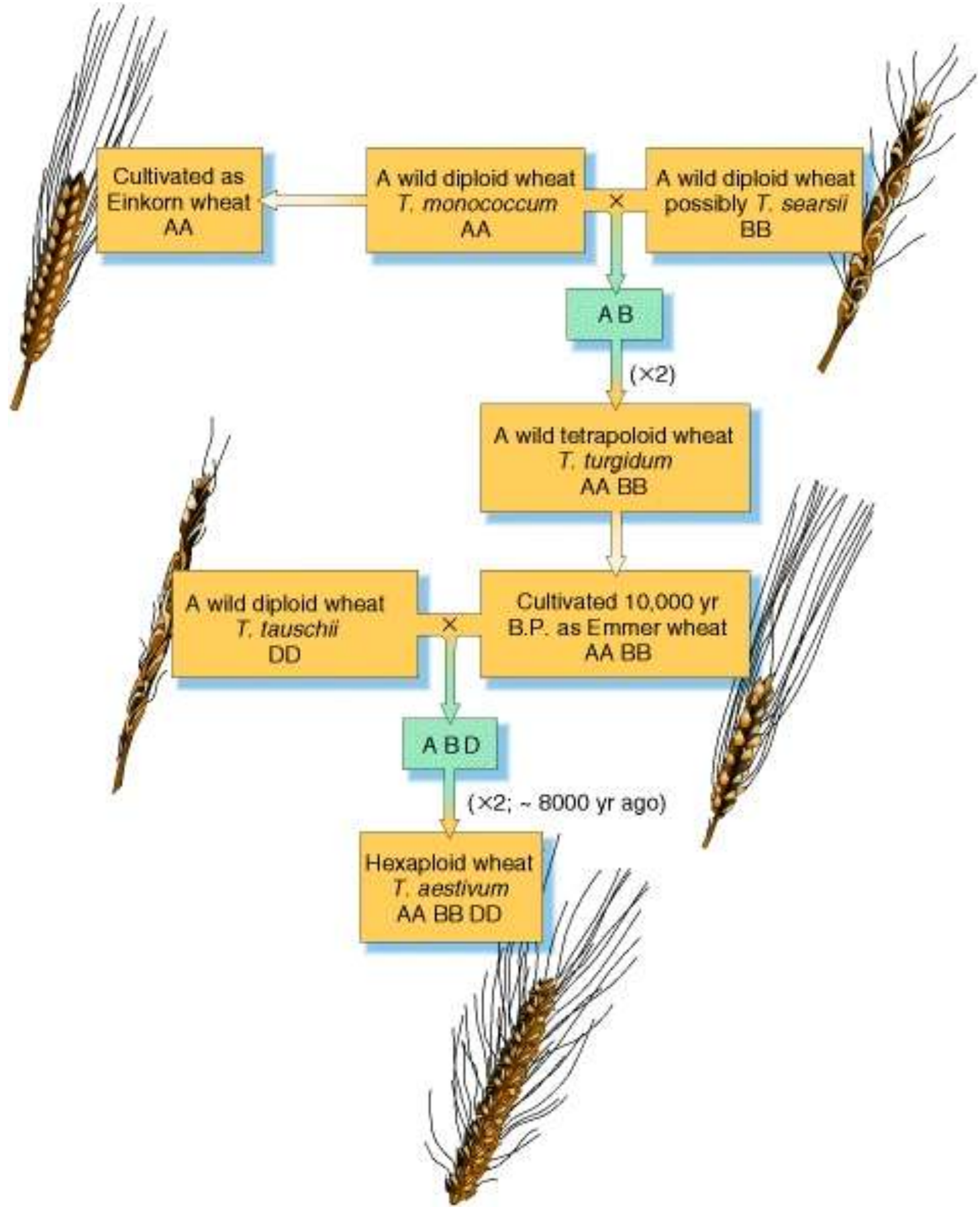
Raphanobrassica - Creation of a new species

1924 - Karpechenko, crossed radish, *Raphanus sativus* (n=9), with cabbage, *Brassica oleracea* (n=9). F1 sterile



Hexaploid – 6 sets of chromosomes

Stages of wheat at or near flowering.

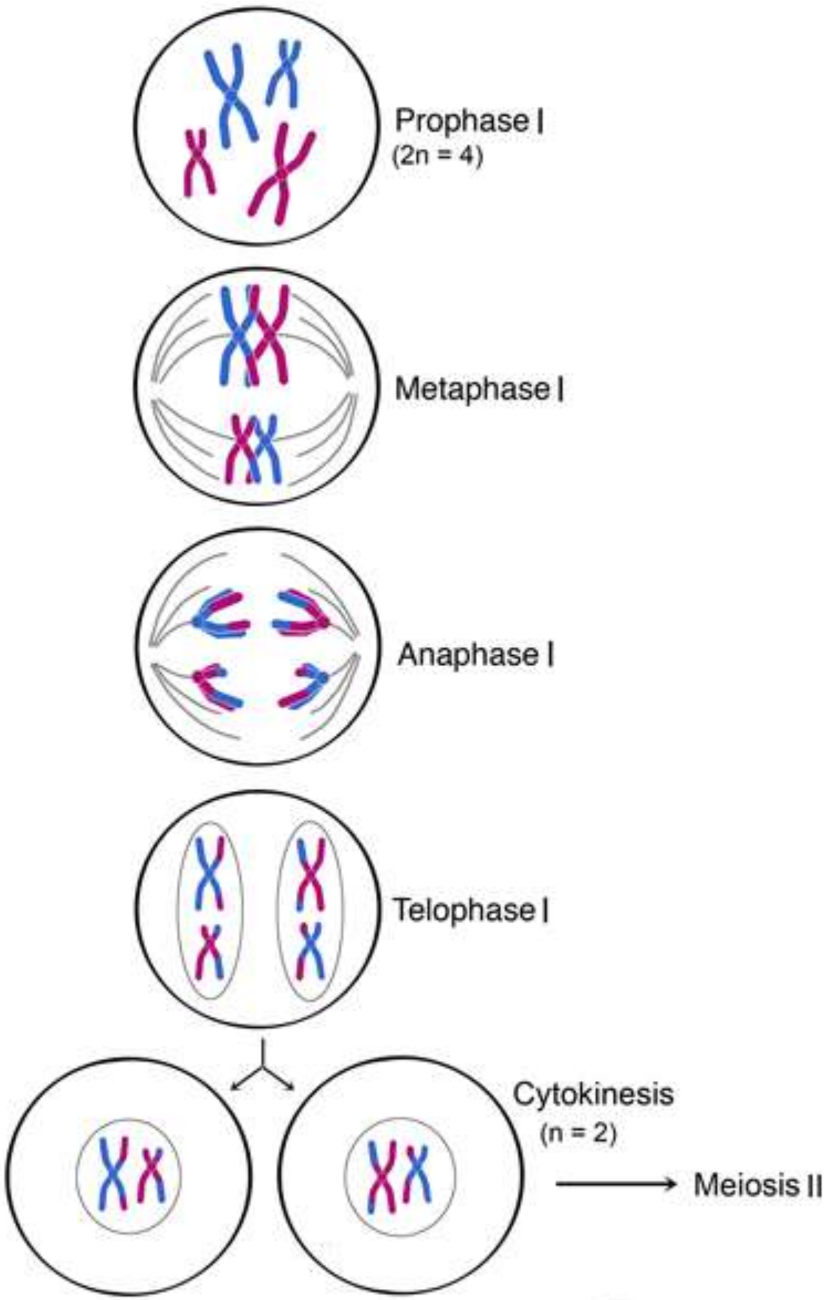


The Problem
Mitosis produces two cells with the same number of chromosomes as the parent cell.
Mitosis of a **diploid** cell (**2n**) produces two diploid daughter cells.
If two diploid cells went on to participate in sexual reproduction, their fusion would produce a tetraploid (**4n**) **zygote**.

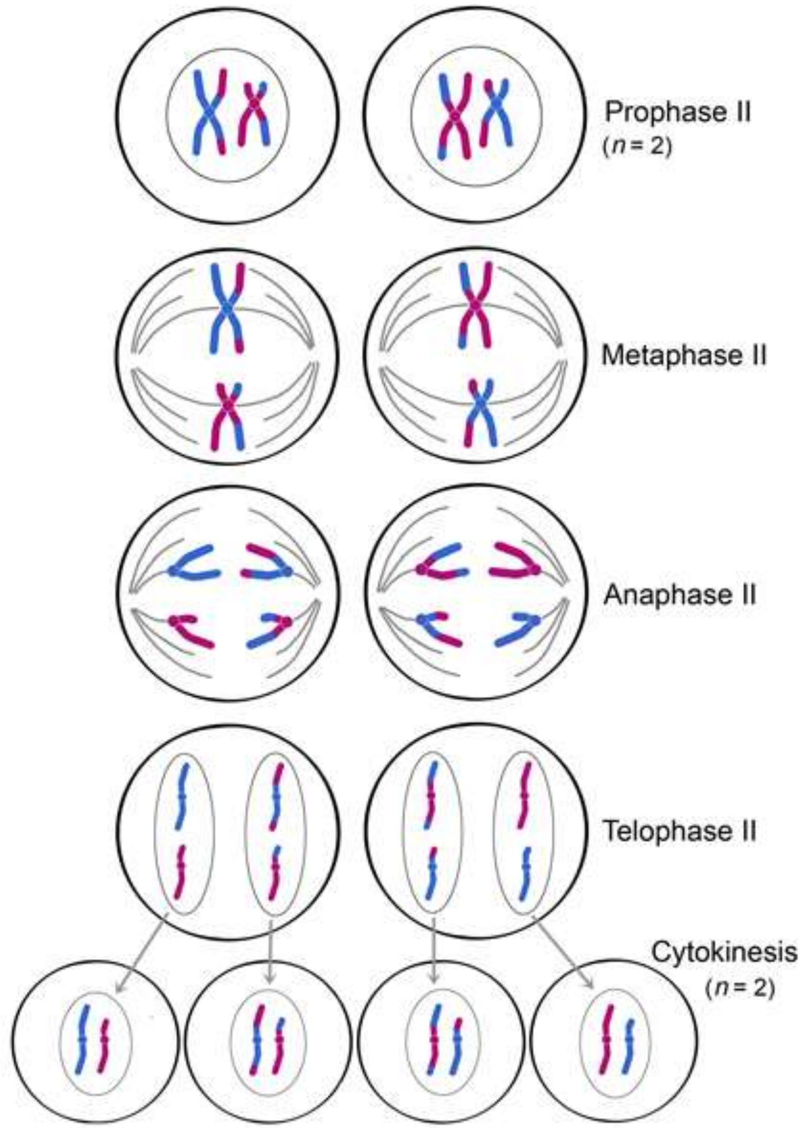
The Solution: Meiosis
Meiosis is a process of cell division in **eukaryotes** characterized by:

- two consecutive divisions: **meiosis I** and **meiosis II**
- no DNA synthesis (no S phase) between the two divisions
- the result: 4 cells with half the number of chromosomes of the starting cell, e.g., **2n** → **n**

Meiosis I – 1st Division



Meiosis II – 2nd Reduction Division



Prophase I –

When the chromosomes first become visible they are already doubled, each homologue having been duplicated during the preceding S phase.

Each dyad consisting of two sister chromatids held together by a protein complex.

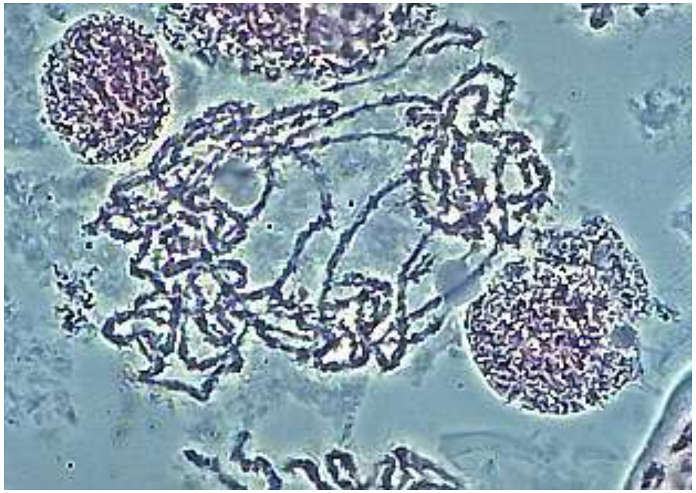
Pairing: Each pair of homologous dyads align lengthwise with each other.

Result: a **tetrad**. These structures are sometimes referred to as **bivalents** because at this stage you cannot distinguish the individual sister chromatids under the microscope.

The two homologous dyads are held together by one or more **chiasmata** (sing. = chiasma) which form between two **nonsister** chromatids at points where they have crossed over.

the **synaptonemal complex** (SC), a complex assembly of proteins (including cohesin)

Meiosis I - Prophase

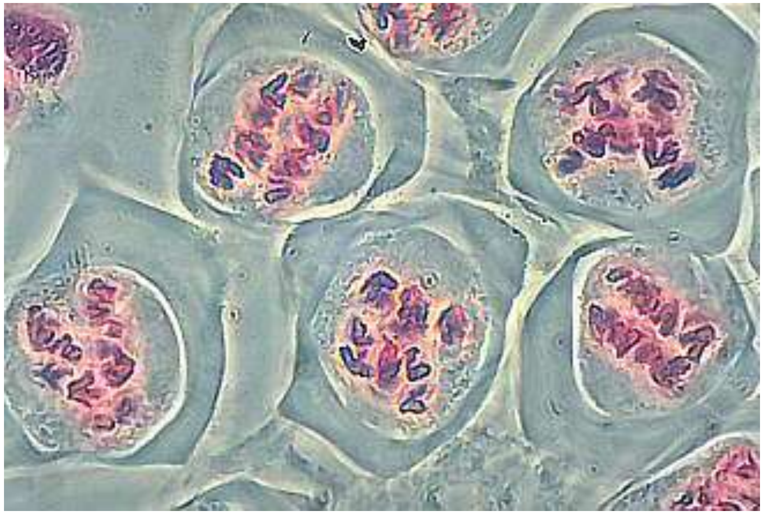


Zygotene / pachyten (the homologous chromosomes can be recognized as thin double strands).

Diplotene (the bivalents can be seen as clear double strands).

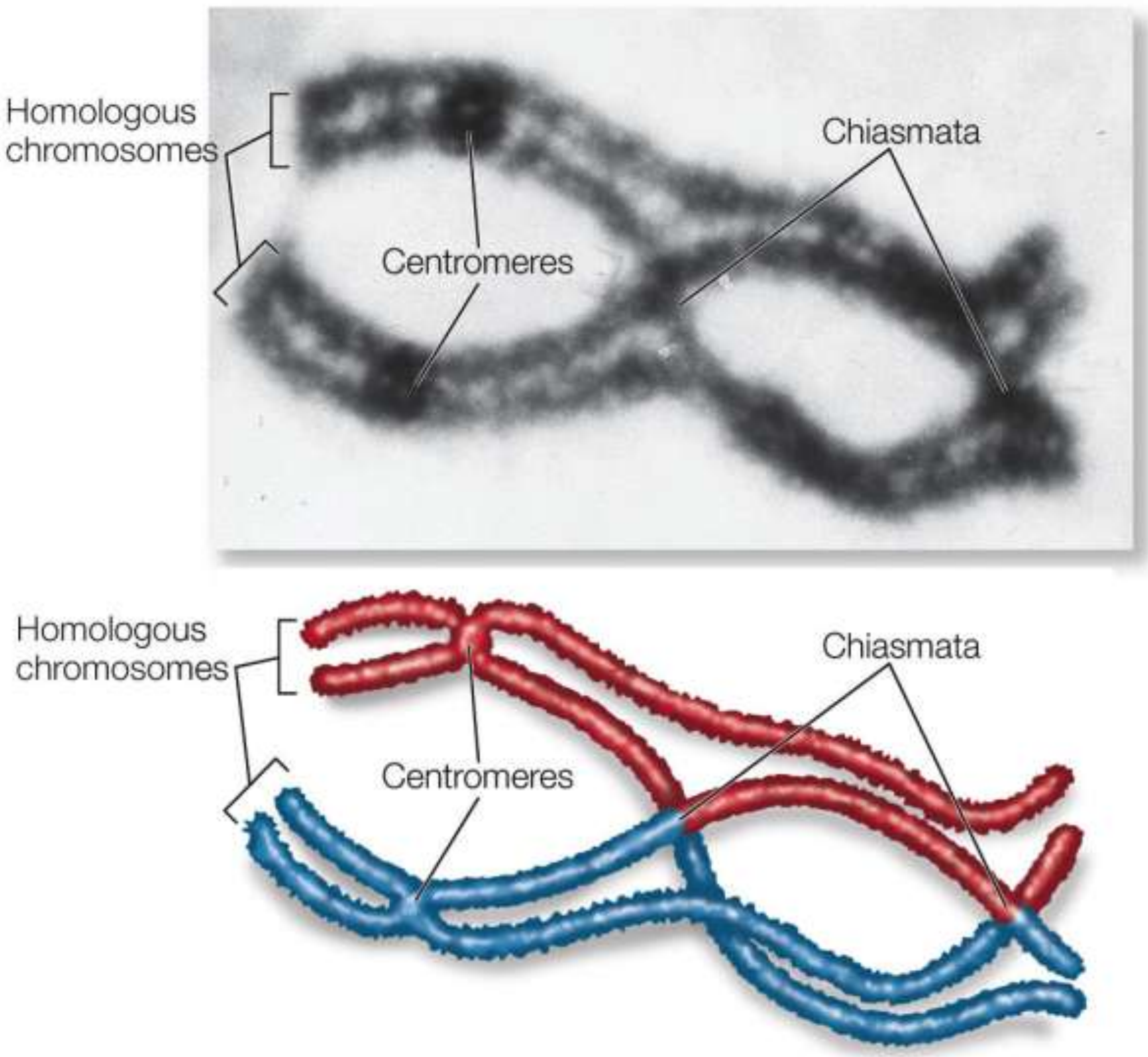
Meiosis I

Metaphase I: side view of all seven bivalents in the equatorial plane. Both series of centromers are already stretched towards the poles.

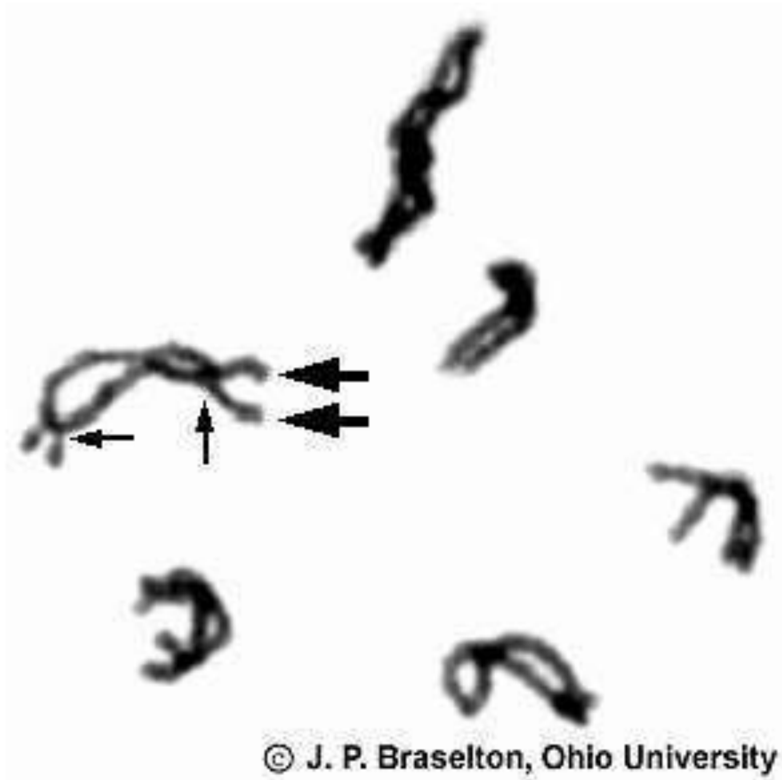


Diakinesis (The homologous chromosomes are drawn to opposite poles. All seven bivalents contain chiasmata.).

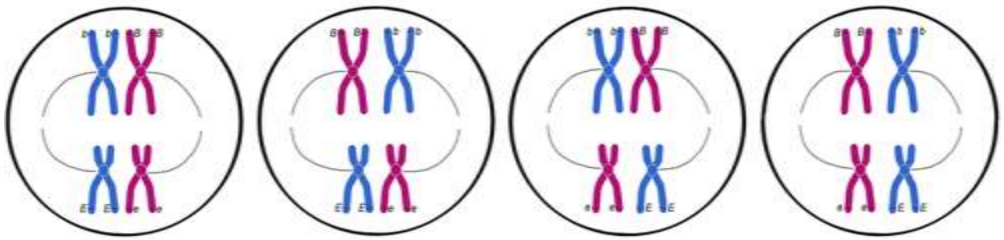
Crossing over introduces genetic variability.

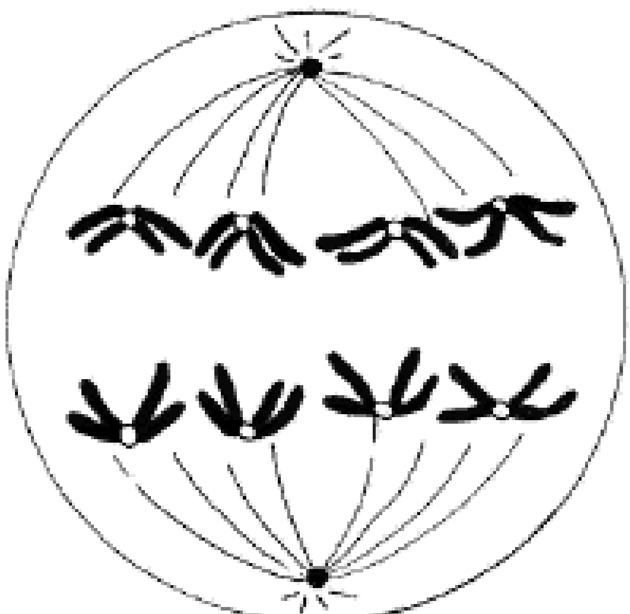


LIFE 8e, Figure 9.17



Diplonema in Mayapple. The six bivalents are clearly seen now that they have shortened and thicken after pachynema. The homologous chromosomes (large arrows) appear to be repelling each other—they remain attached at the chiasmata (singular—chiasma, small arrows). Chiasmata are the cytological evidence that crossing over at the molecular level has occurred.





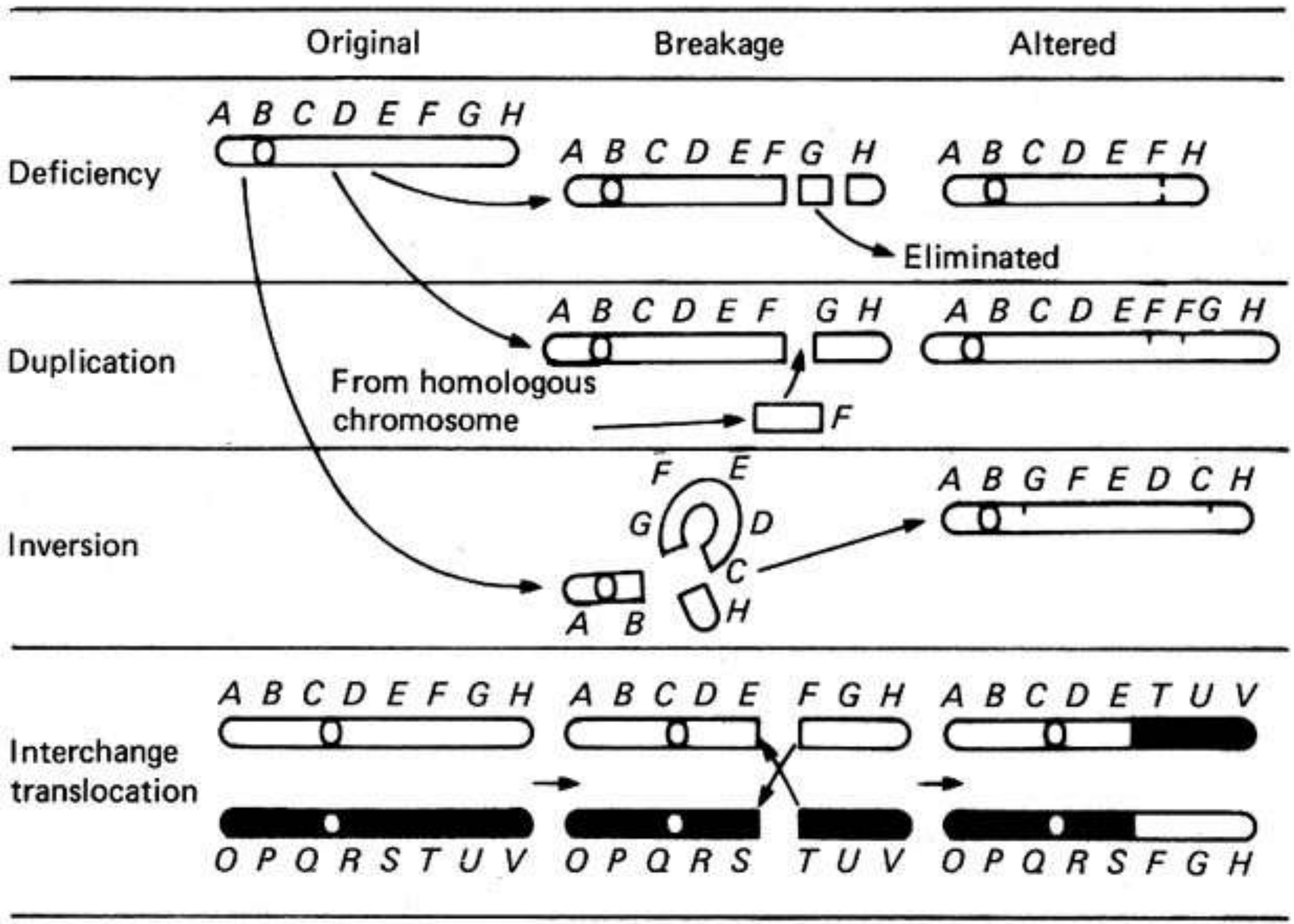
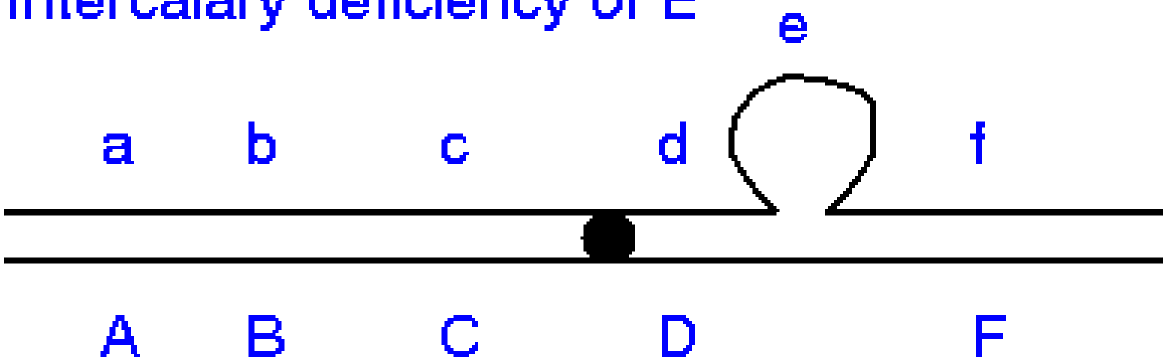


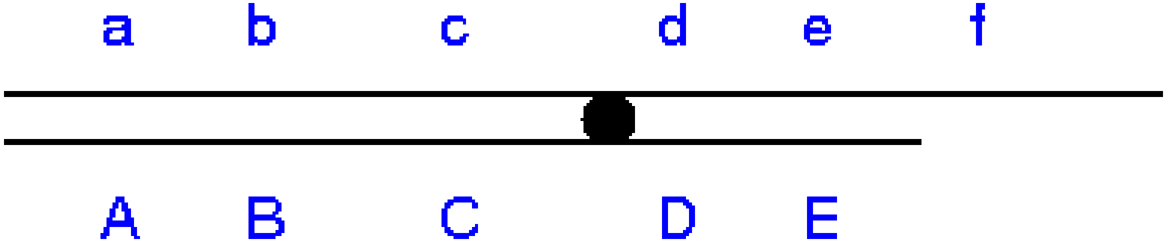
Fig. 6.9. Diagrams to show how chromosome breakage and reunion can give rise to the four principal changes which chromosomes undergo. (After Stebbins, 1966.)

Deficiencies

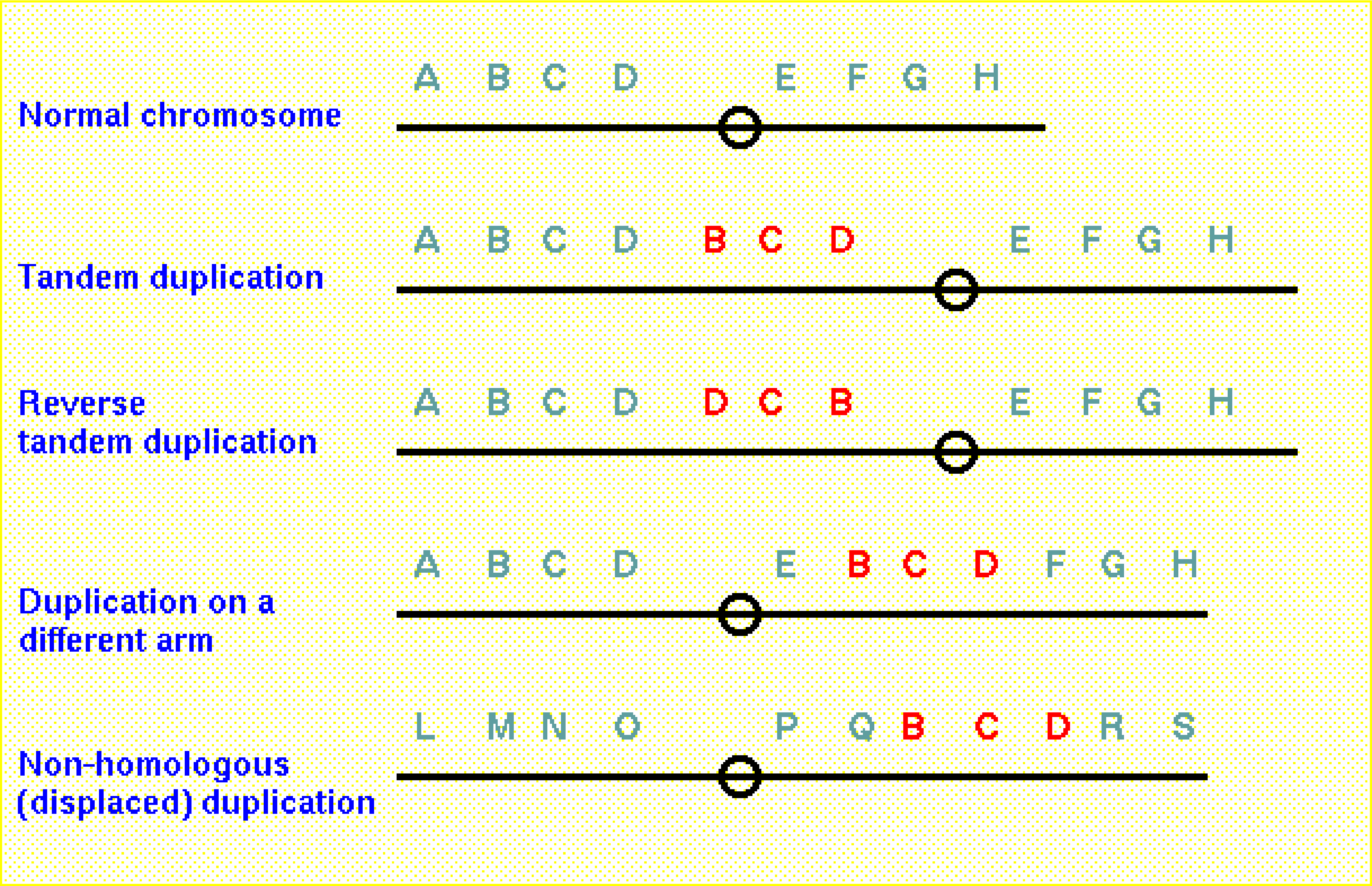
Intercalary deficiency of E

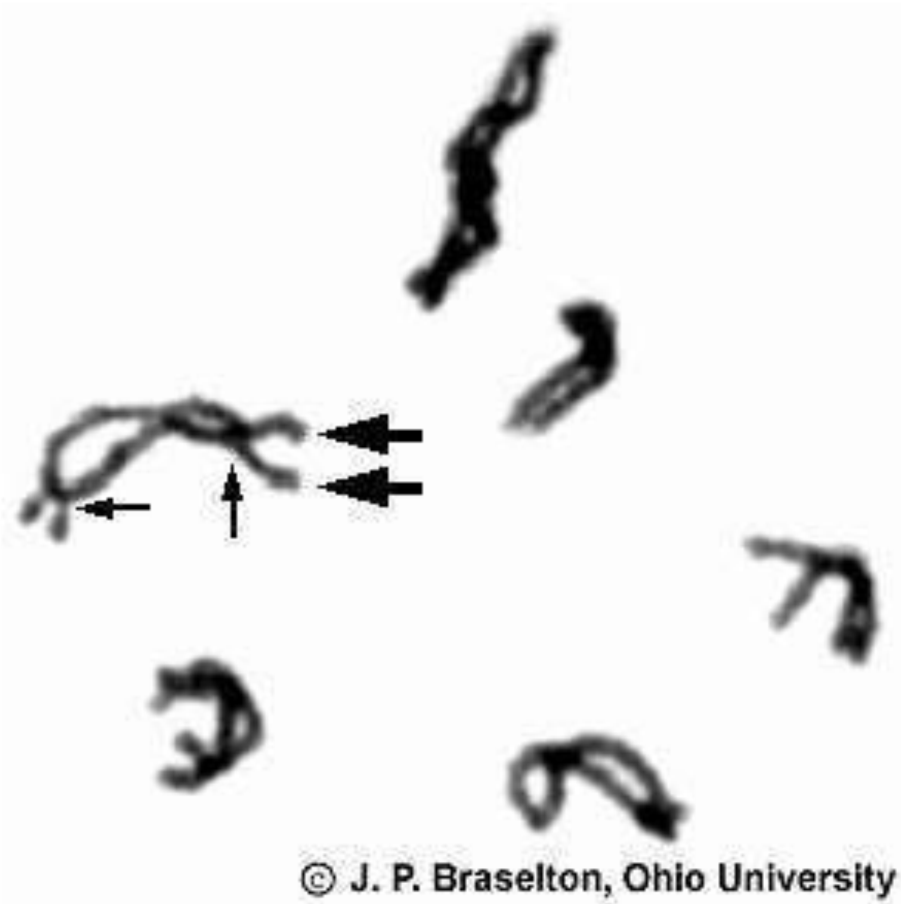


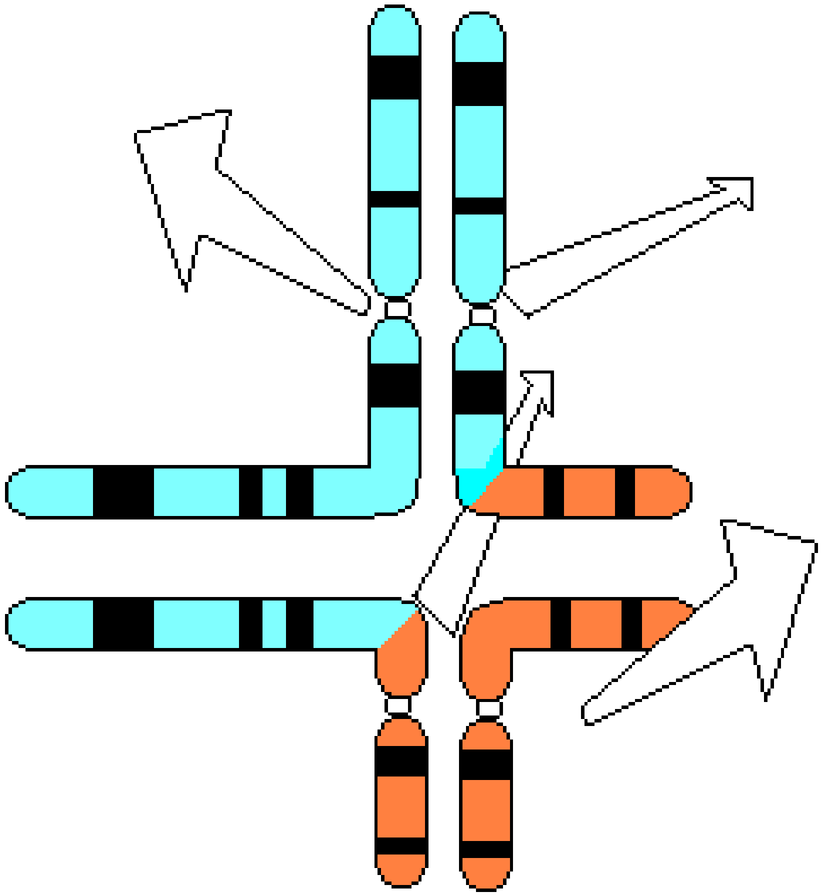
Terminal deficiency of F

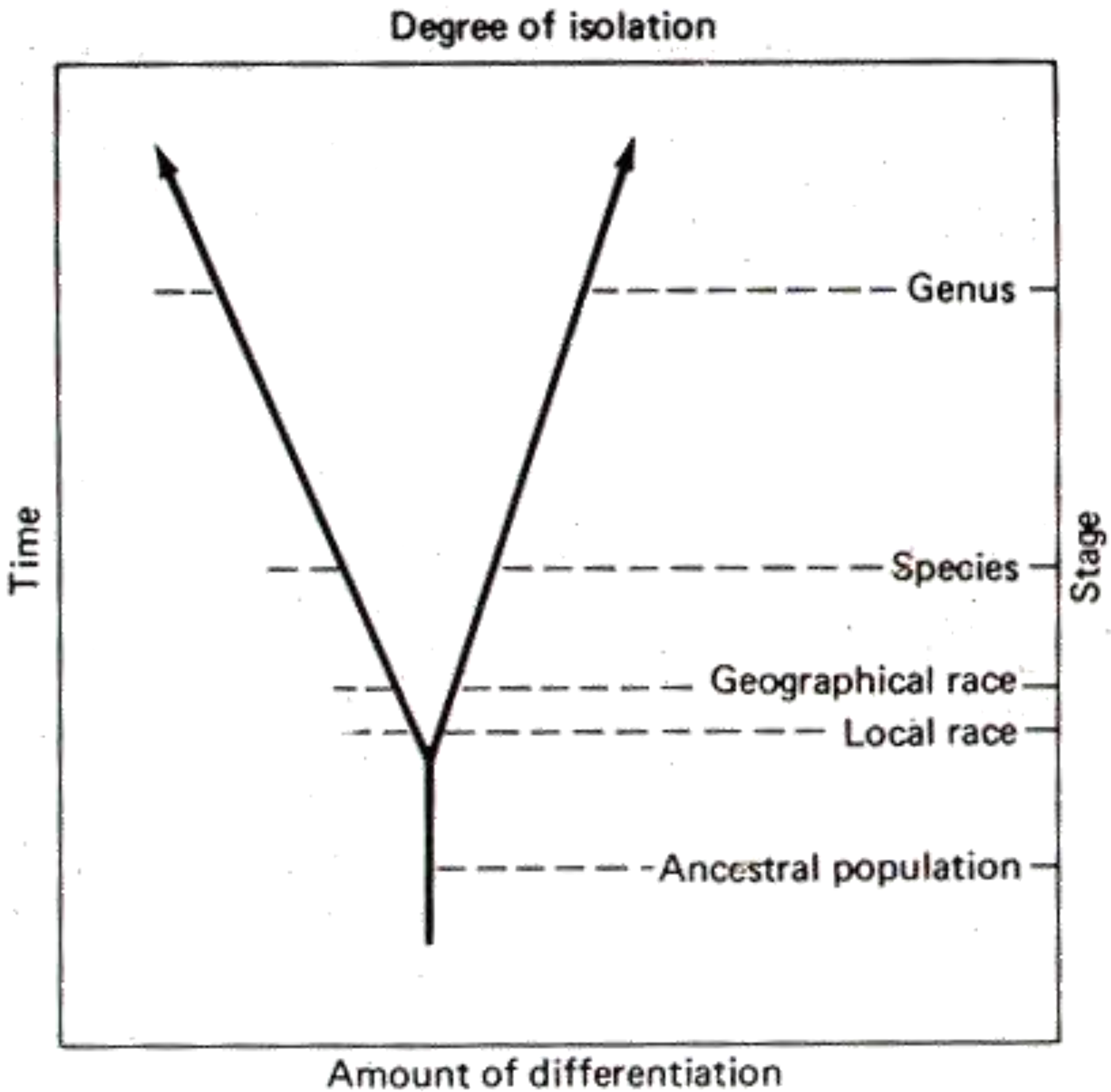


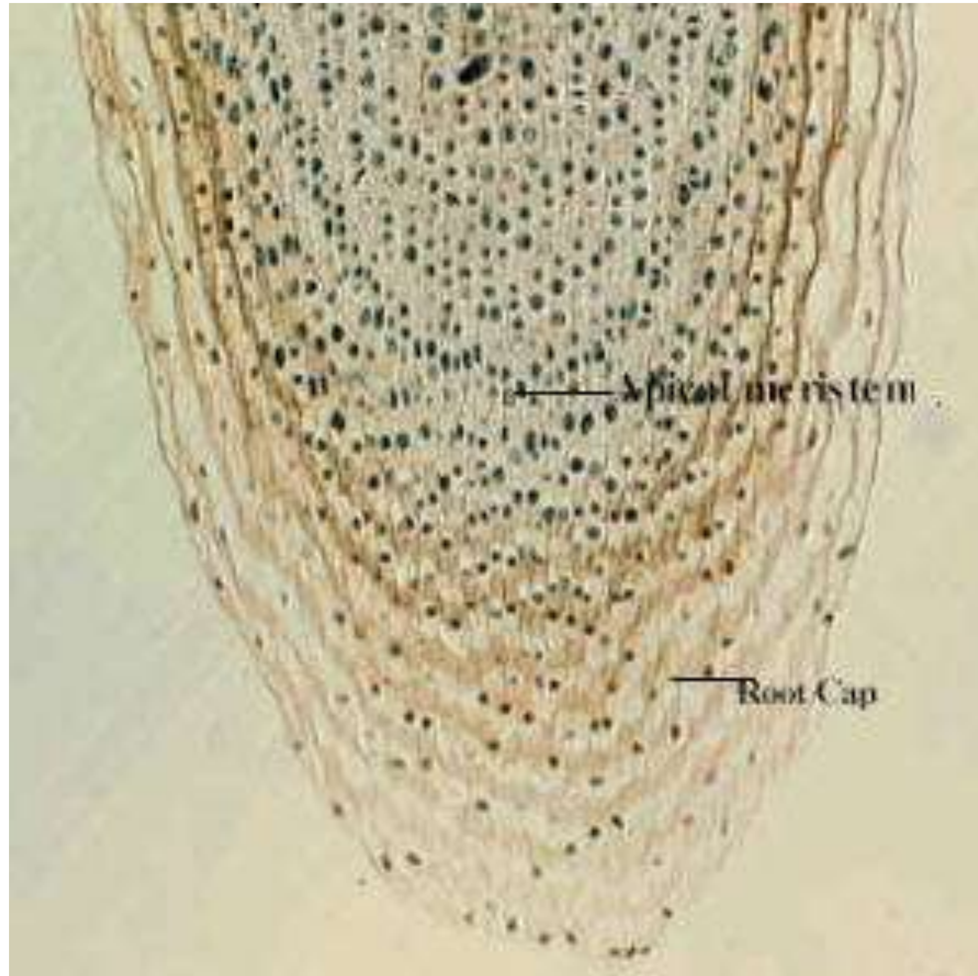
Duplications




© J. P. Braselton, Ohio University




Degree of isolation
Genus
Species
Geographical race
Local race
Ancestral population
Time
Stage
Amount of differentiation


Apical meristem
Root Cap

Pre-treatment of Roots

8-hydroxyquinoline -cause contraction and improve spreading of chromosomes

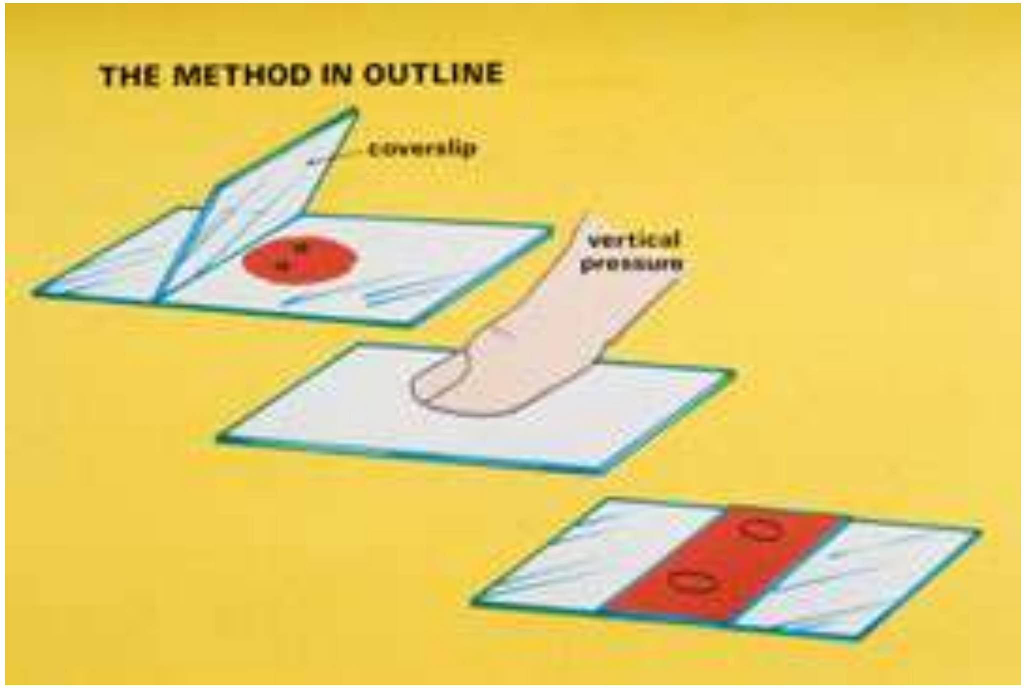
Fixation

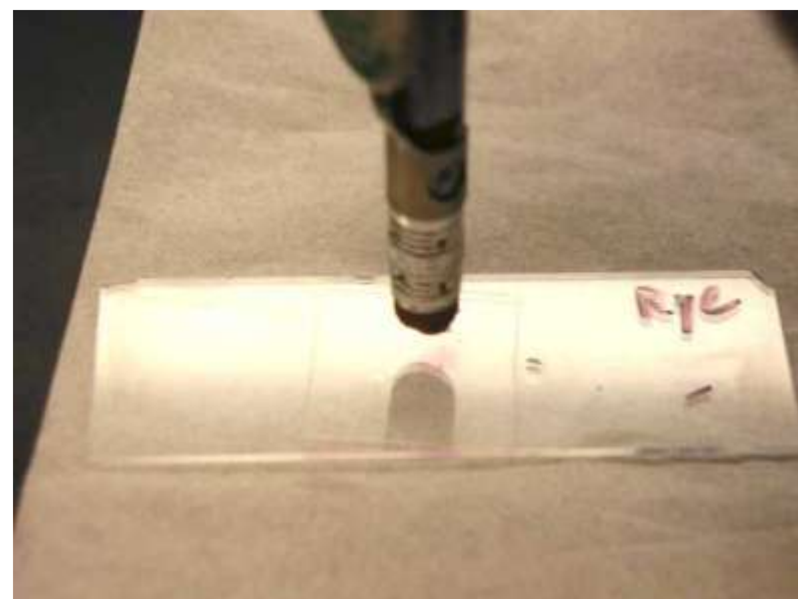
Prevent autolysis of chromosome, denaturation of proteins.
Prevent bacterial decomposition of cells.
Prevents swelling and shrinking of cells and tissues.
Rapid penetration to reach the interior of the cell
Mixtures of chemicals are used, freshly prepared

Carnoy’s: ethanol-acetic acid (3:1) or

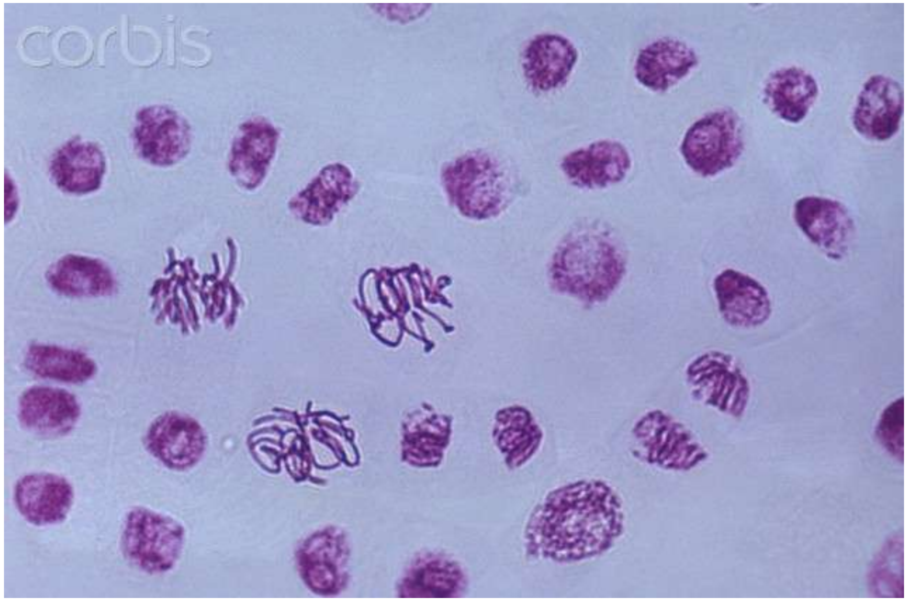
Farmer’s: ethanol-chloroform-acetic acid (6:3:1) (2:1:1)

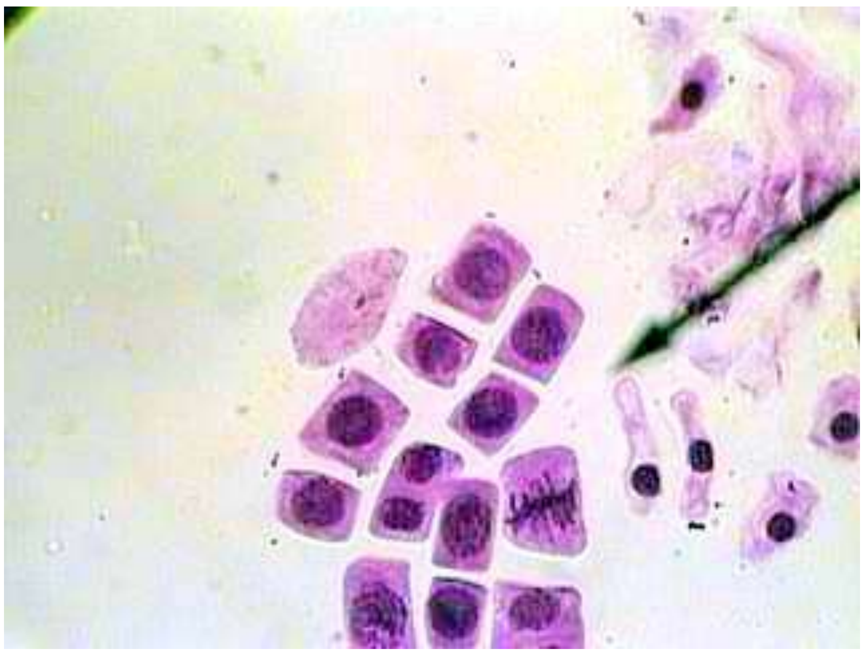
Fixation time 30 minutes- 24 hours – 36 hours (maximum).
After fixation the material is preserved in 70% alcohol


THE METHOD IN OUTLINE
coverslip
vertical pressure

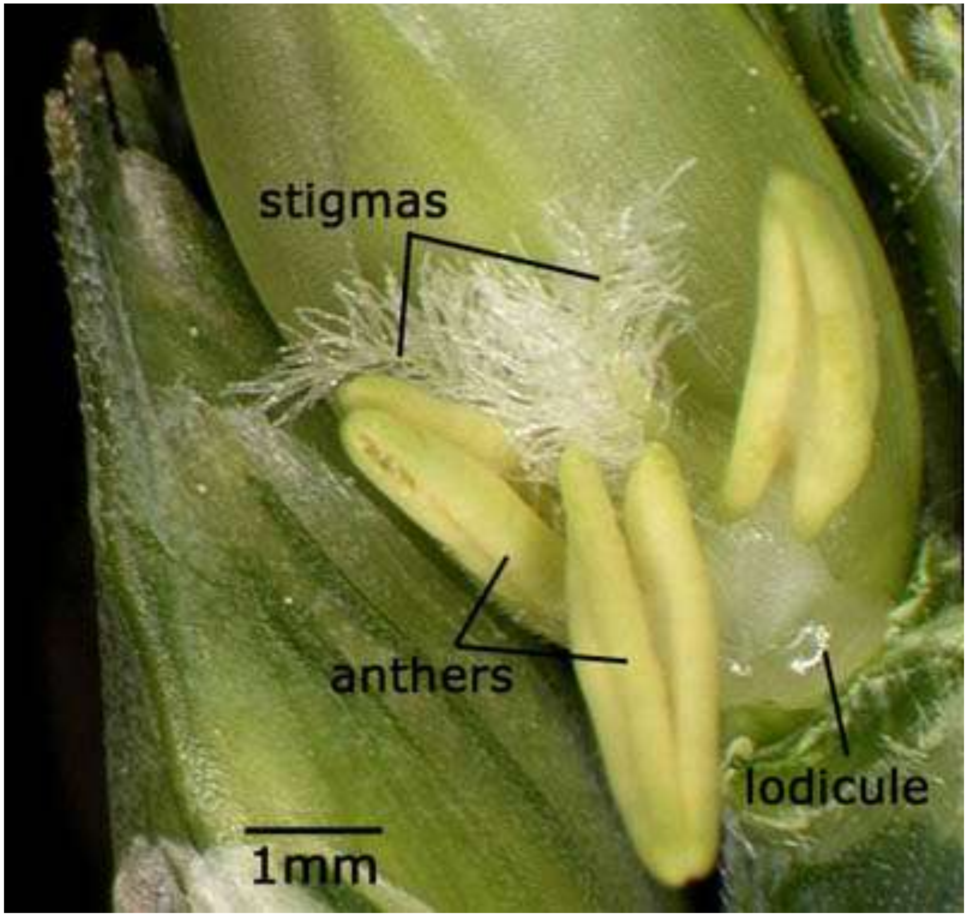

Rye

Onion Root Tip Squash





Anther – Pollen Mother Cells



Stages of wheat at or near flowering.

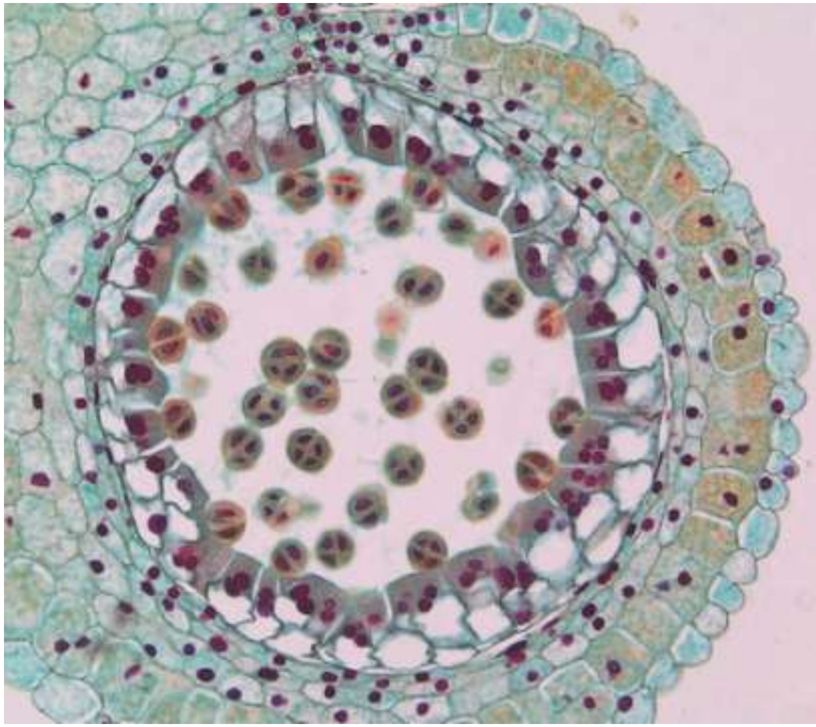
Older florets are at the bottom, and newer florets are at the top

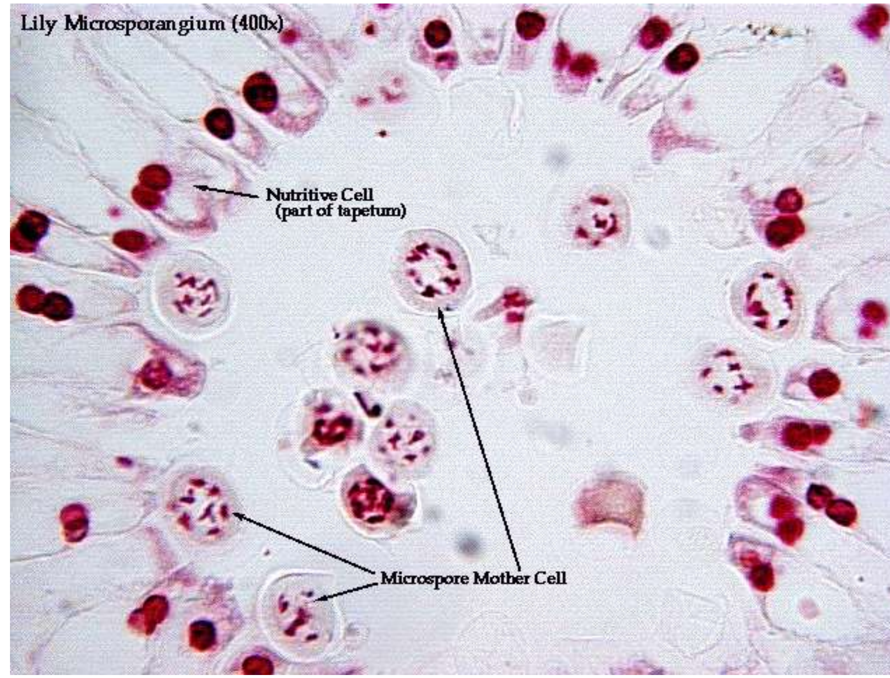
Add stain

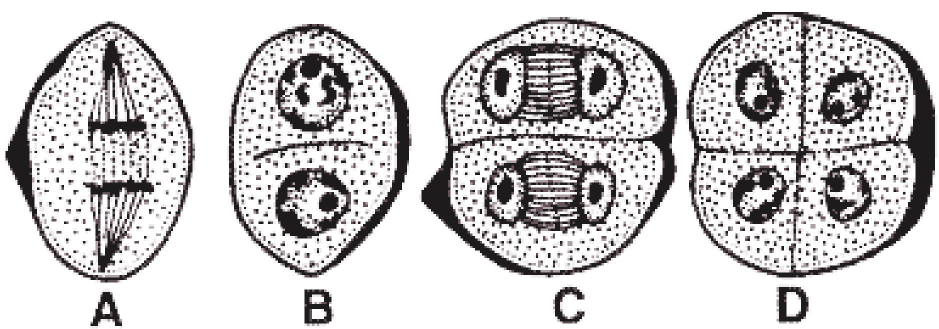
Warm slightly

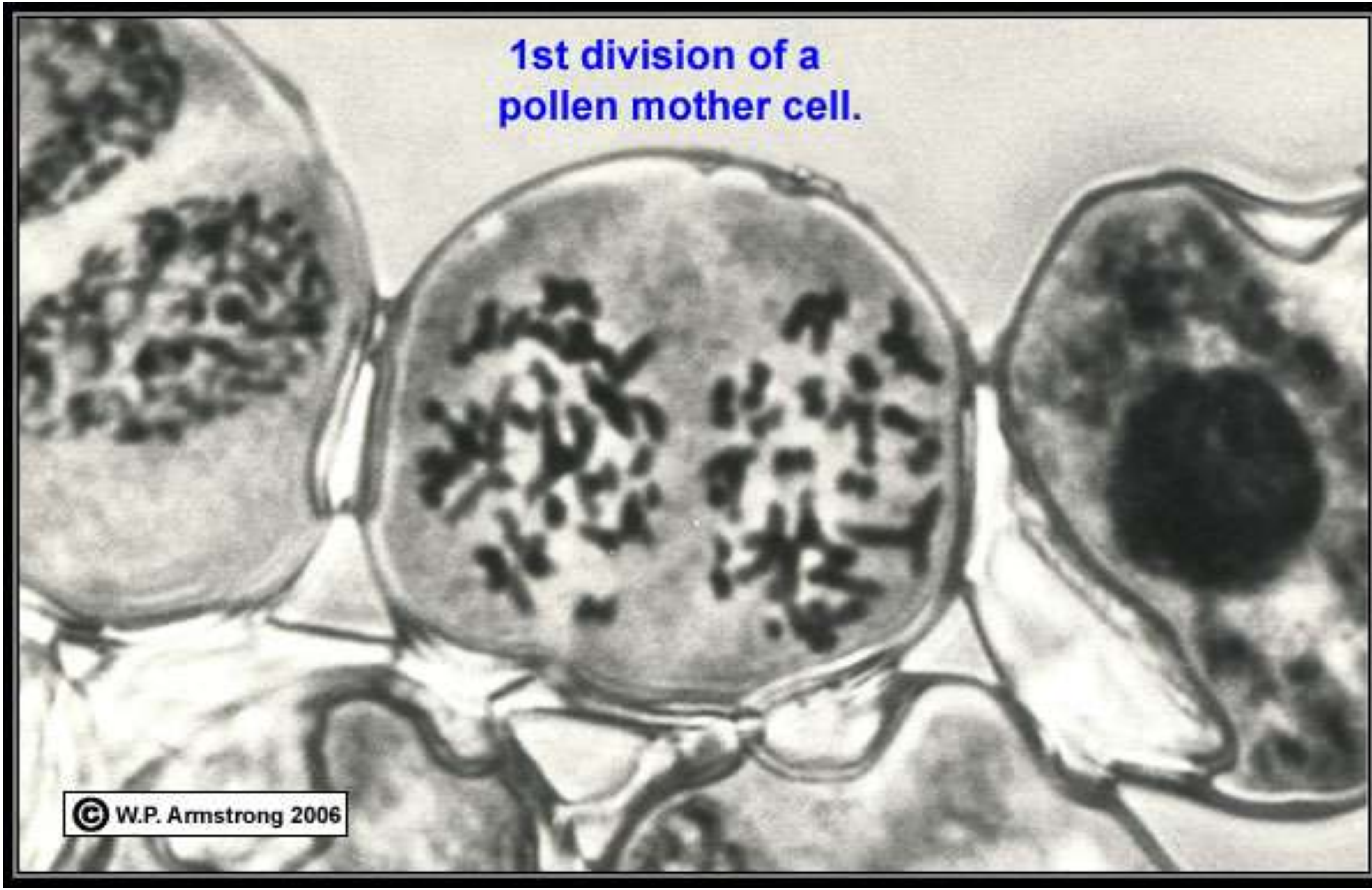
Squash gently

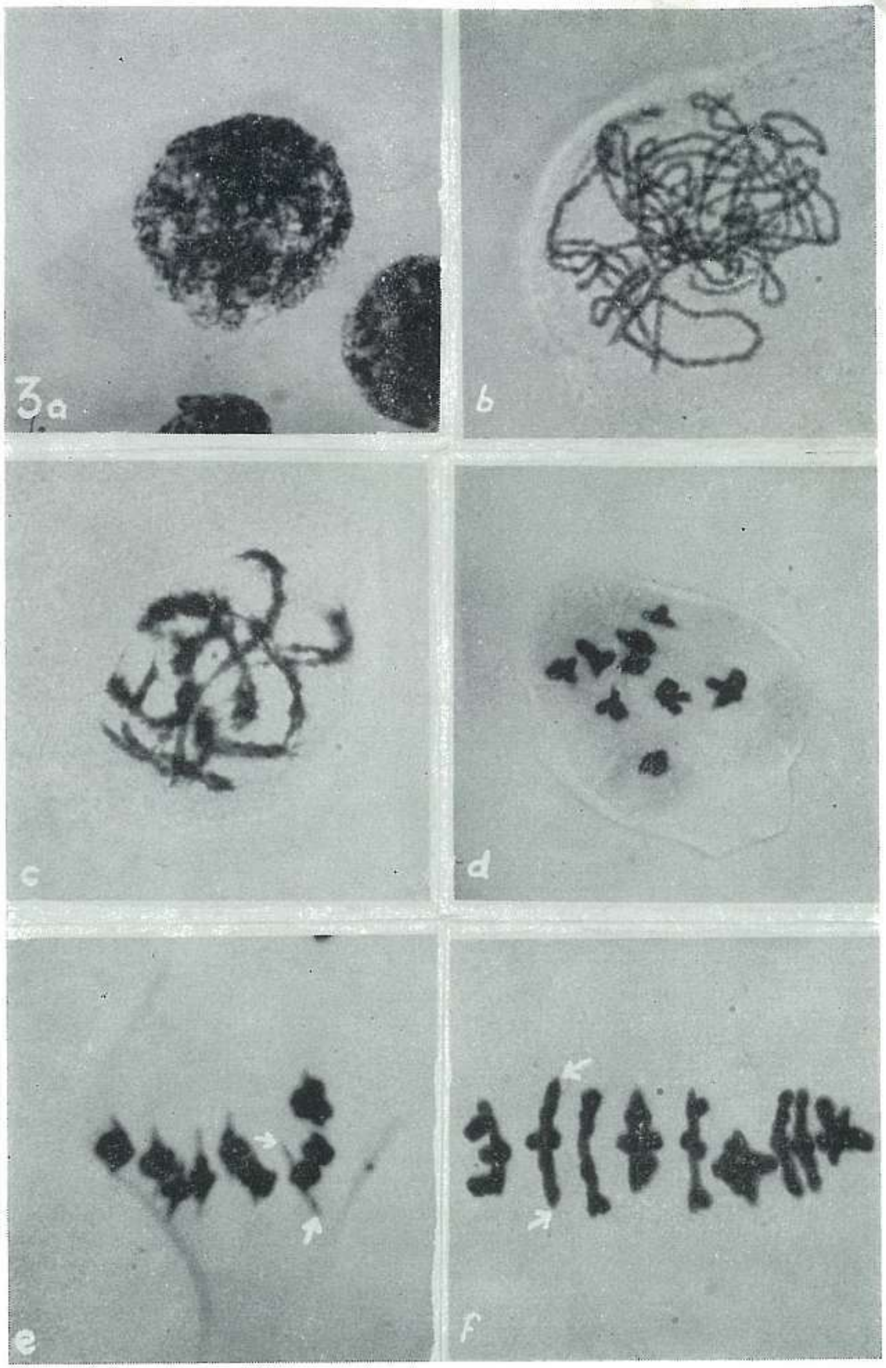
Pollen Mother Cells







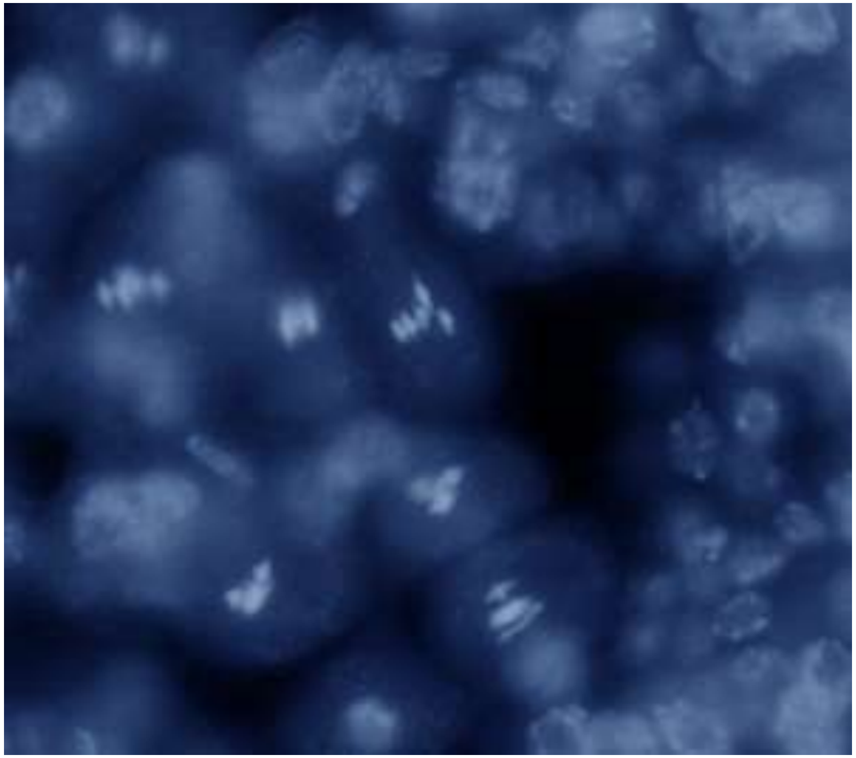

1st division of a
pollen mother cell.
© W.P. Armstrong 2006



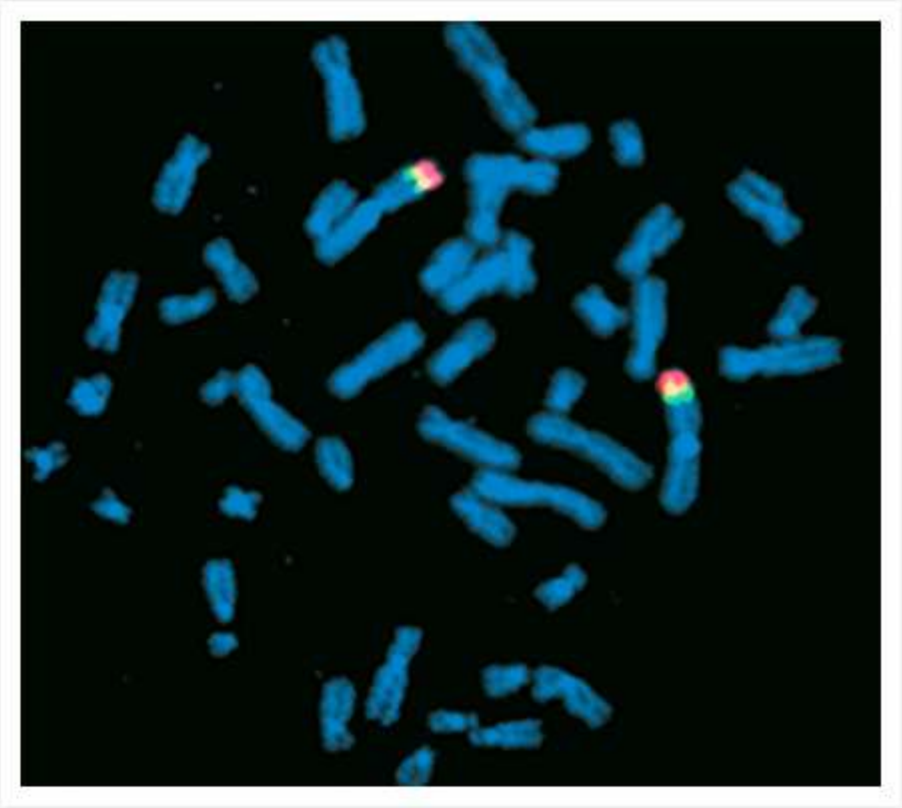
Stages of meiosis from squash preparations of pollen mother cells of Allium triquetrum, stained in Feulgens.

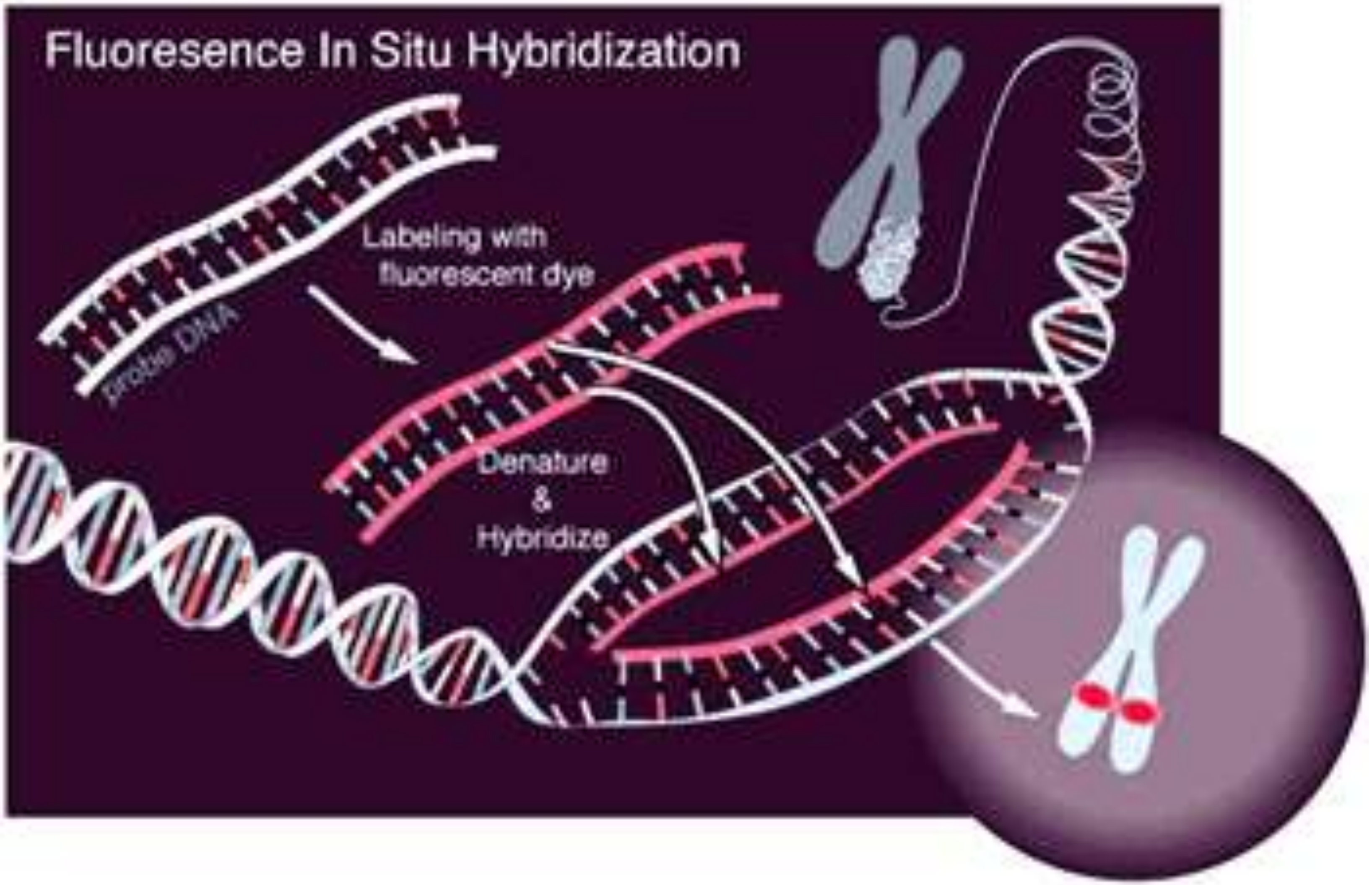
a, leptotone;
b, pachytene;
c, diplotene;
d, diakinesis of prophase

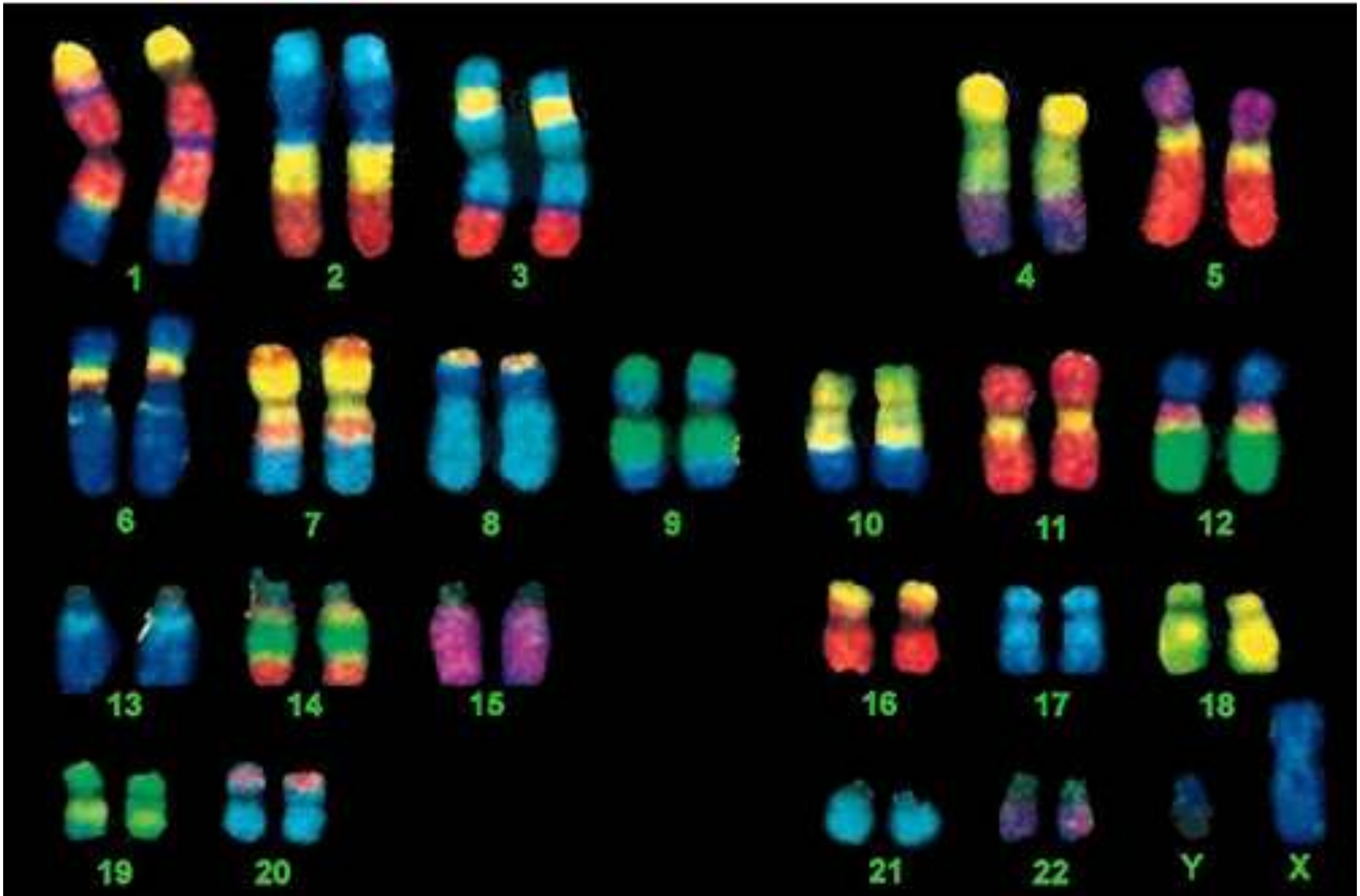
DAPI Fluorescent Stain



FISH Labeled




Fluoresence In Situ Hybridization
Labeling with
fluorescent dye
probe DNA
Denature
&
Hybridize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Y
X

End