PRACTICAL-II
(DBOTL02)
(MSC BOTONY)

ACHARYA NAGARJUNA UNIVERSITY

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M.Sc. Botany (Previous)

Practical-II

CYTOLOGY, GENETICS, PLANT BREEDING AND PLANT PHYSIOLOGY

1. Preparation of common reagents
2. Mitosis from onion root tips
3. Meiosis from onion flower buds
4. Meiosis in polyploids
5. Polytene and B chromosomes
6. Barr bodies from Buccal smears
7. Chromosome banding
8. Assignments in genetics
   - (i) Mendelian inheritance and gene interaction
   - (ii) Multiple alleles
   - (iii) Linkage and chromosome mapping
   - (iv) Probability and chi-square
   - (v) Sex-linked/sex-influenced inheritance
   - (vi) Cytoplasmic inheirtance
   - (vii) Multiple factor inheritance
9. Experiments in plant physiology

1. PREPARATION OF COMMON REAGENTS

(Pretreatment agents, fixatives, chromosome stains)

Prefixatives: Though fresh material can be used, fixed materials give better preparations due to chromosome contraction resulting good staining of chromosomes. Hence, prefixatives are used e.g. 8-Hydroxyquinoline, para-dichlorobenzene and colchicine etc. Some common stains used for chromosome staining are given below:

8-Hydroxyquinoline: Prepare a saturated aqueous solution by dissolving 0.0582 g in 200 ml of distilled water. Keep overnight at 60^{o}C. Filter and store the solution in a refrigerator.

Para-dichlorobenzene: Prepare a saturated aqueous solution by dissolving 0.5 g in 100 ml of freshly prepared distilled water.

α-Bromonaphthalene: Same as para-dichlorobenzene.

Fixatives:

Farmer's fluid: Ethyl alcohol : acetic acid = 3:1. Prepare just before use.

Carnoy's fluid: Ethyl alcohol: Chloroform: Glacial acetic acid = 6:3:1. Prepare just before use.

CHROMOSOME STAINS

Acetoorcein: Dissolve gradually 2g of orcein in 100 ml of boiling 455 acetic acid. Heat the solution for 10 minutes. Allow it to cool and then filter.

Acetocarmine: Dissolve gradually 1.5 or 2.0 g of carmine in 100 ml of boiling 45% acetic acid. Heat the solution for 10 minutes. Allow it to cool and then filter or use reflex condens or during the preparation.

Feulgen: Dissolve 0.5 g of basic fuchsin in 100 ml of boiling distilled water. Allow it to cool and filter. Keep the solution in a dark coloured bottle. Add 3 g of sodium metabisulphite and 30 ml of 1N HCl. Keep the solution overnight in a dark and cool place. Decolourize the solution by adding 7g of activated powdered charcoal. Filter and use.

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2. STUDY OF MITOSIS FROM ONION ROOT TIPS

Plant material: Root tips of *Allium cepa*.

Procedure for acetoorcein/acetocarmine squash preparation:

1. Excise young root tips from the plant.

2. Transfer the root tips to vials containing prefixative (saturated aqueous solution of bromonaphthalene, 8-hydroxyquinoline or para-dichlorobenzene) and keep at 10-14^{o}C for 2-4 hours.

3. Fix the root tips, after washing in water, in glacial acetic acid: ethyl alcohol (1:3) for 24 hours.

A few root tips can be processed further as mentioned below, while others can be stored in 70% ethyl alcohol at 4-10^{o}C for future use.

(a) Acetoorcein squash preparation:

(i) Place the prefixed or fixed root tips (preferably not more than two at a time) in a watch glass containing 9 drops of 2% acetoorcein and 1 drop of 1 N HCl.

(ii) Heat, **without boiling**, over a flame for 10-15 seconds. Allow it to cool.

(iii) Transfer a root tip to a clean slide and add a drop or two of 1% acetoorcein. Put a coverslip, tap gently to scatter the cells and squash by exerting uniform pressure. Tapping can be done with the blunt end of a pencil. Press the slide in the folds of a filter paper with the help of your thumb to spread the cells uniformly and to remove excess stain.

(iv) Observe the preparation. If chromosomes have not separated satisfactorily, repeat warming, tapping and pressing after adding a drop of the stain on the slide.

(v) Sometimes maceration of the tissue is necessary for good spreading. It is done before putting the coverslip.

(b) Acetocarmine squash preparation:

(i) After pretreatment and fixation, hydrolyse the root tips for 10 minutes in 1 N HCl at 60^{o}C.
(ii) Wash them in water to remove traces of HCl.
(iii) Transfer the root tip to a clean slide, add a drop or two of 2% acetocarmine, put a coverslip, warm and squash as described for the aceto-orcein preparation.
(iv) Study the various stages of mitosis.

M I T O S I S

The zygote is starting point of life cycle. In plants, the adult is sporophyte that produces spores by reduction division. The process of cell division is composed of a nuclear and cytoplasmic component. All somatic cells in a multicellular organism are descendants of zygote. The interphase period between successive cell divisions consists of processes associated with growth and preparation for mitosis. Motic division has four major stages. Prophase, metaphase, anaphase and telophase.

Prophase: It is characterized by the shortening of chromosomes. The nuclear envelop disintegrates and nucleolus disappears. As prophase progresses, each chromosome is composed of two identical chromatids connected by a centromere. Mitotic prophase chromosomes appear longitudinally split into the duplicates each is called a chromatid. The condensation of chromatids occur and the chromosomes become free from the nuclear envelope.

Metaphase: It occurs only for a short duration. The condensed chromosomes are arranged on the plane of the spindle. It is called metaphase plate or equatorial plate. The chromosomes lie on the plate with their centromere pointing towards the poles. In plant cells chromosomes are irregularly scattered on the equatorial plate. The chromatids become separated from each other. The chromosomes at metaphase are very distinct. Each chromosome is distinguished by two chromatids and centromere. It is suitable stage for the study of karyotype.

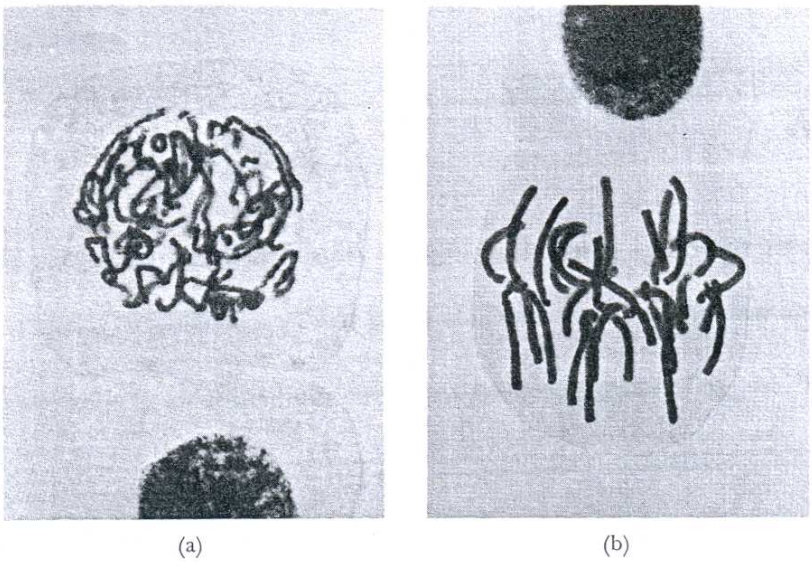


Fig. 1. Prophase (a), metaphase (b), in Lilium regale root tip (x2400).
Reproduced from J. McLeish and B.Snoad, Looking at Chromosomes, Macmillan, London, 1958.

Chromosomes whose centromere is median are described as metacentric, at unequal distance are submetacentric, chromosomes with more terminally placed centromere are acrocentric, centromere at the tip of the chromosome is telocentric. It is shortest of the mitotic phases.

Anaphase: This stage is initiated by the separation of sister chromatids. The sister chromatids are now called as chromosomes. The chromosomes are pulled at centromere by the action of spindle fibers. All the chromatids in the cell separate, with sister chromatids pulled to opposite poles of the cell. The centromere is essential for movement, those chromosomes without a centromere fail to move to the opposite poles. During movement, each chromosome attains a characteristic shape, which is independent on the position of centromere.

The analysis of karyotype includes the study of number, size and morphology of chromosomes. Total length and arm ratios of chromosomes constitute important parameters for karyotypic study of different taxa and are helpful in stystematic and phylogenetic investigations.

Telophase: The chromosomes uncoil increase in length and begin to carry out their physiological functions. A nuclear envelope reforms about each set of chromosomes, nucleoli form and cytokinesis takes place resulting two daughter cells. The nuclear membrane reappears, thus forming a regular nucleus.

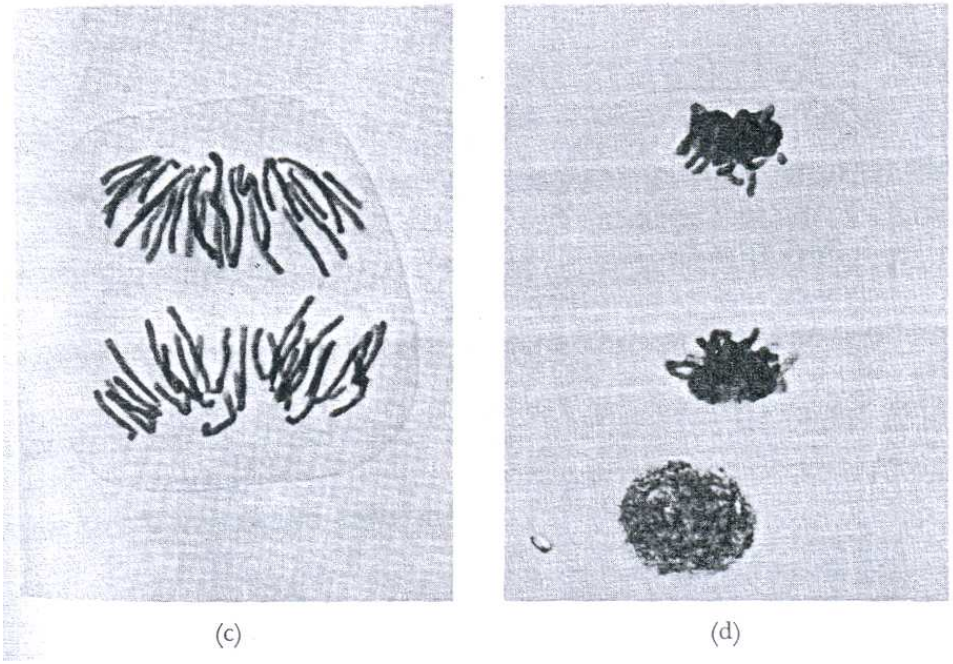


Fig. 2. anaphase (c) and telophase (d), in Lilium regale root tip (x2400).
Reproduced from J. McLeish and B.Snoad, Looking at Chromosomes, Macmillan, London, 1958.

Cytokinesis: Cytoplasm divides in a process called cytokinesis, where cell plate is formed at the centre and spreads laterally to the cell wall in plant cells. In animals, cytokinesis takes place by furrowing. the Furrow begins from either side and join at the centre separating the two daughter cells.

Significance of Mitosis: It is an equational division. Cytokinesis and mitosis result in two daughter cells, each with genetic material identical to that of the one parent cell. There is an exact distribution of the genetic material. Thus the same number of chromosomes are maintained in the resulting two daughter cells.

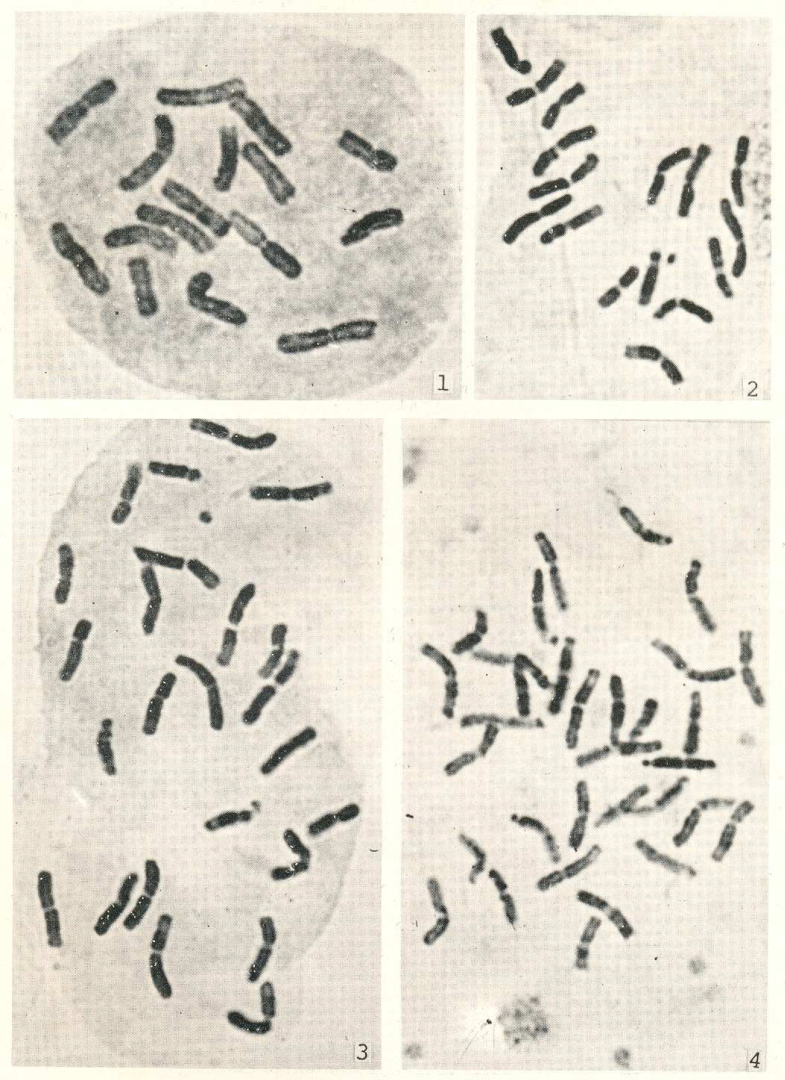
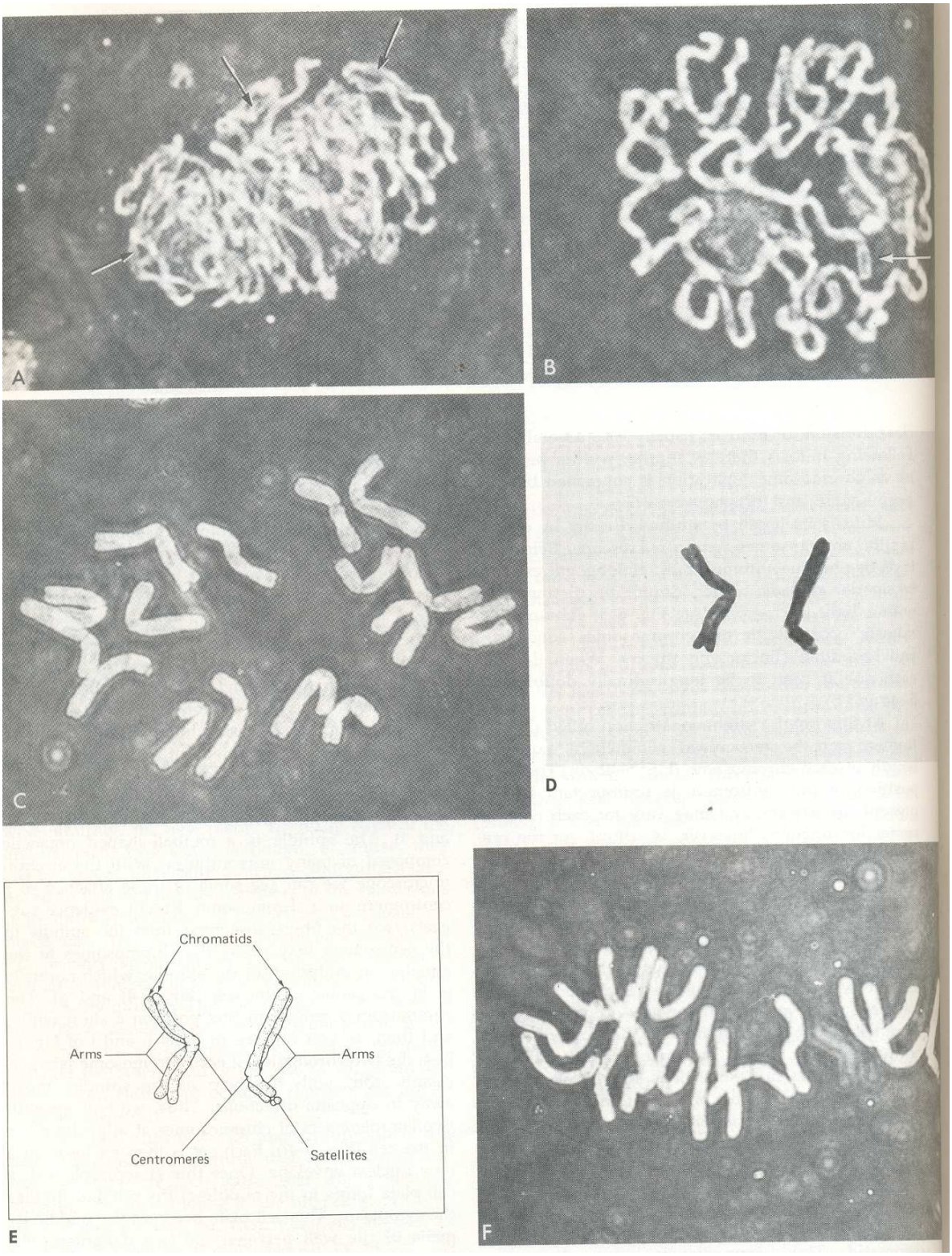
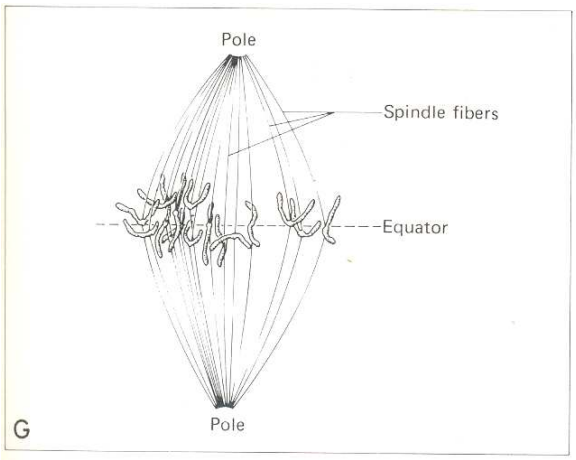
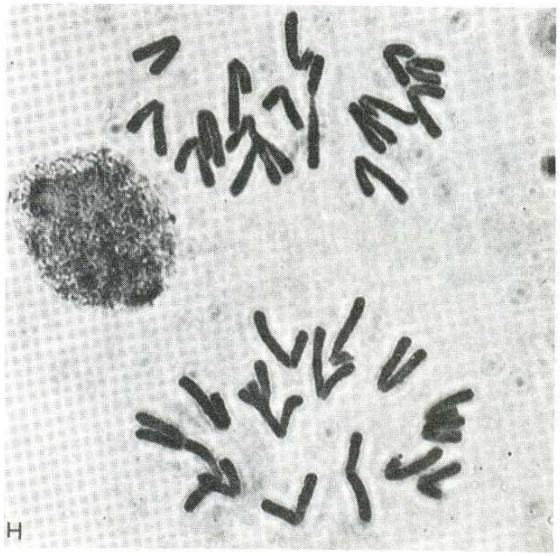
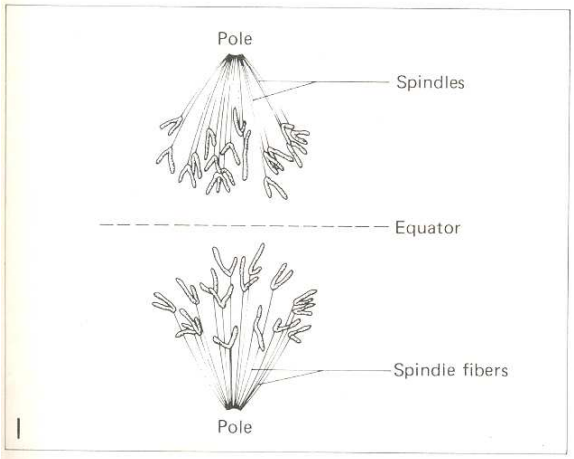


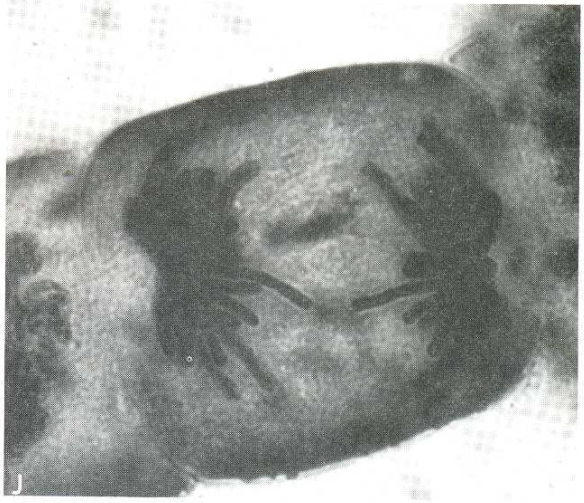
Fig. 1-4. Somatic chromosomes of species of *Theriophonum* (All x 3000).
1. *T. dalzellii* (2n=16), 2. *T. infaustum* (2n=16), 3. *T. minutum* (2n=24), 4. *T. sivaganganum* (2n=32).
(at the courtesy of Dr. Jayalakshmi)


A
B
C
D
Chromatids
Arms
Arms
Centromeres
Satellites
E
F









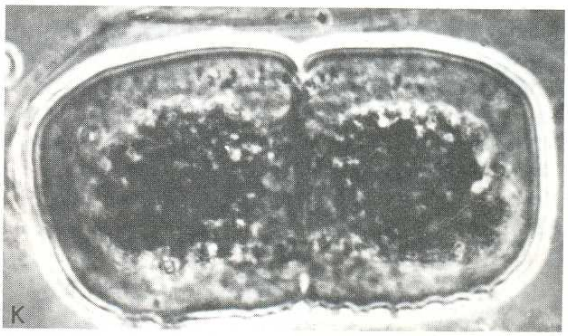


Fig. 5.4. Mitosis in root tips of cultivated onion (*Allium cepa*). Cytologists recognize four stages in mitosis (a,b) prophase chromosomes long and thin, nuclear membrane intact; © nuclear membrane disintegrated, chromosomes compact, spindle fibers attach to centromeres; (d, e) closer view of individual chromosomes at late prophase-metaphase; (f, g) *metaphase*, centromeres align along equator; (h, I) *anaphase*, sister chromatids eparate and become daughter chromosomes, which move toward opposite poles; (j) telophase, anaphase movement completed, new membranes form around the daughter nuclei; and (k) at completion of mitosis, cell plate divides the cell into two daughter cells.

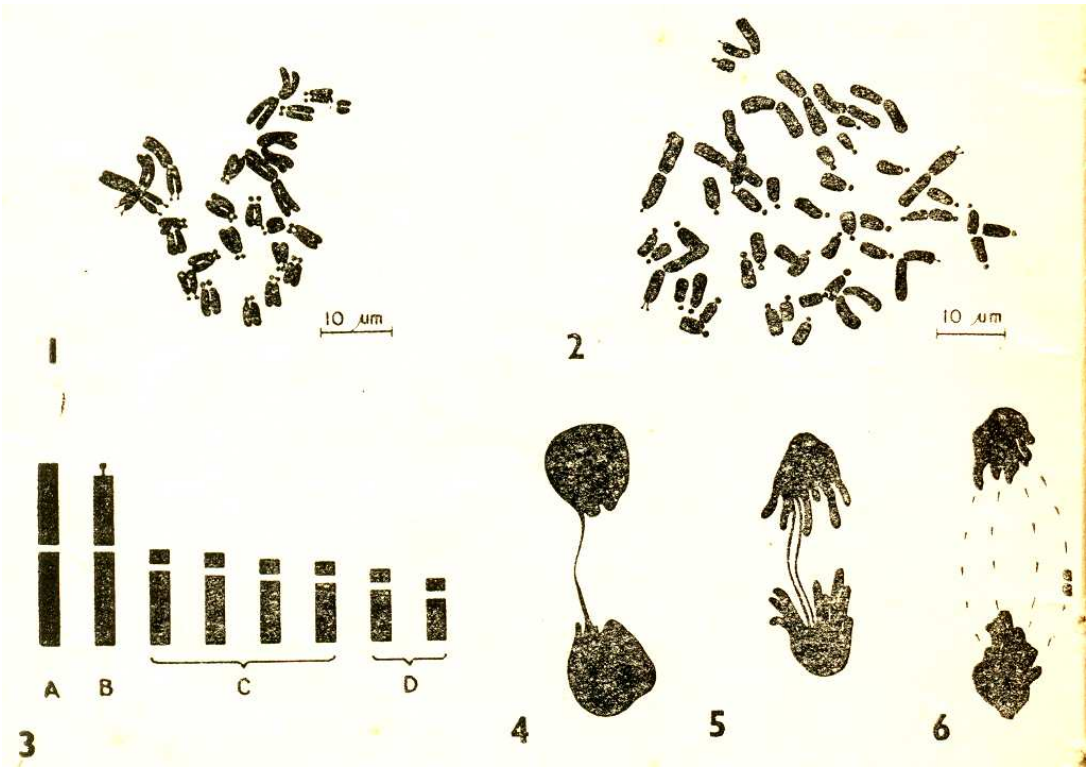


Fig. 1-6. Cytology of endosperm of *Delphinium ajacis* (Courtesy; S.K. Mandal and R.K. Basu).

Fig. 1. Mitotic metaphase with 3n=24 chromosomes. Fig. 2. Mitotic metaphase with 6n=48 chromosomes. Fig. 3. Idiogram showing type ***A*** and type ***B*** chromosomes with nearly median centromeres, the latter with a satellite on the short arm. Type ***C*** chromosomes are with nearly subterminal centromeres, whereas type ***D*** chromosomes are with nearly submedian centromeres. Figs. 4 and 5. Mitotic bridges. Fig. 6. A pair of lagging of fragments.

3. STUDY OF MEIOSIS IN SQUASH PREPARATIONS

Plant material: Young flower buds of *Allium cepa*.

Schedule for acetocarmine squash preparation:

1. Fix flower buds or inflorescences in acetic acid: ethyl alcohol (1:3) for 2-3 days. Store in 70% alcohol.

2. Dissect out anthers from a young flower bud.

3. Transfer one anther to a drop of 2% acetocarmine on a clean slide. Tease it with steel needles (thus also adding a trace of iron). If the anther is quite big, remove the debris after the contents are liberated. Put a coverslip, warm slightly and tap gently to scatter cells. Press and blot excess of stain.

4. Observe and draw different meiotic stages. Locate the positions of chiasmata. Note synchronisation and abnormalities.

N.B.: Stage selection will be easier if buds of different sizes are arranged serially from small size to the suitable size showing the stages. Generally an anther shows mostly one particular stage.

Meiosis: It is a characteristic of organisms, which reproduce sexually and occurs in reproductive cells. It consists of two divisions i.e. Meiosis I and Meiosis II. Meiosis I involves important events as synapsis or pairing of homologous chromosomes, Recombination due to crossing over of chromosome and segregation of homologous chromosomes. The first division reduces the chromosomes. The second division is equational division. There is longitudinal separation of chromatids, as a result four haploid nuclei are produced. The prophase of first division is most extended stage of meiosis. Several such stages have been recognized as Leptotene, Zygotene, Pachytene, Diplotene and Diakinesis.

Leptotene: The chromosomes are long and less coiled present in form of chromatin. Chromatin material is entangled. Chromosomes look long single thread like structures. Nuclear membrane and nucleolus are intact. All the chromatin is lying towards a part of the nucleus.

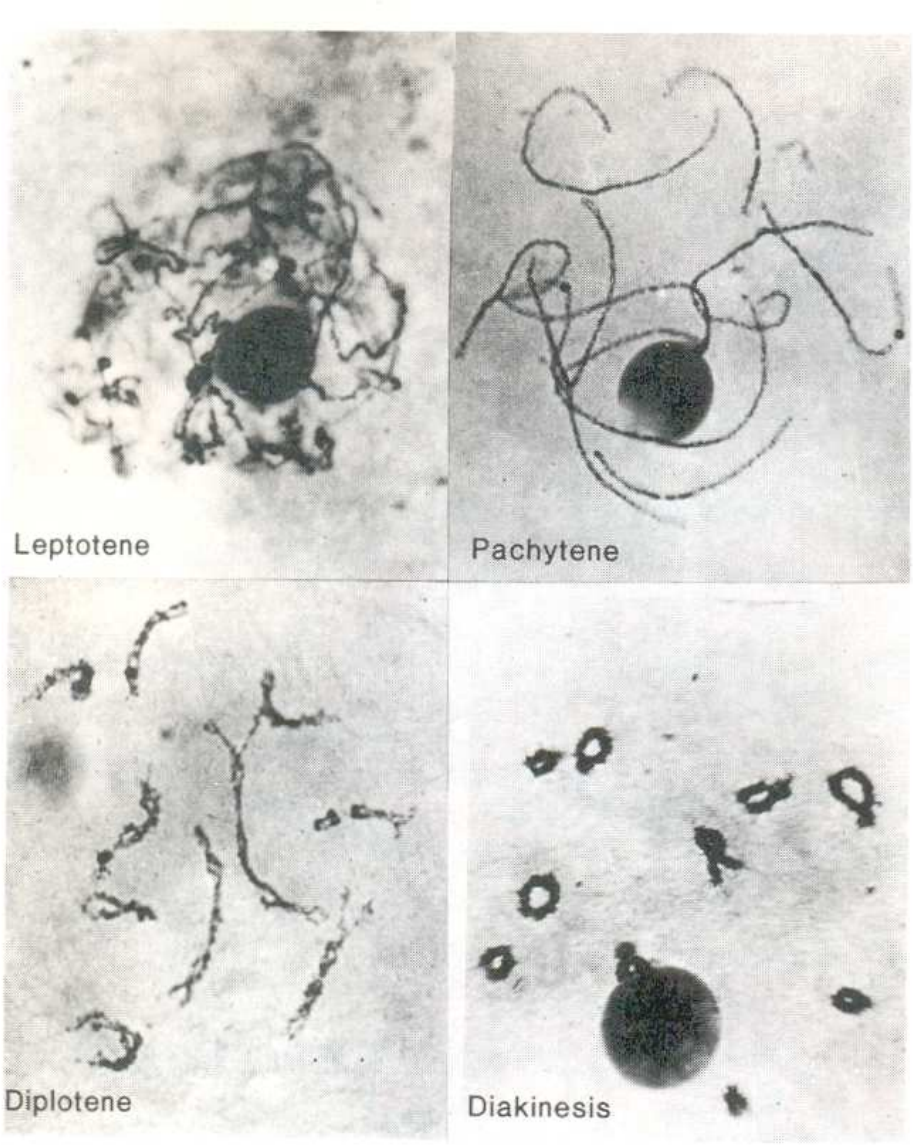


Fig. Meiosis in corn (*Zea mays*)
Courtesy: Dr. M. M. Rhoades, "Meiosis in Maize," Journal of Heredity 41 (1950): 59-67.

Zygotene: The pairing of chromosomes begins. It occurs between homologous chromosomes lengthwise. It may begin at several places, may start at the ends and proceed towards the centromere. By the end of Zygotene pairing completes. Synaptenemal complex is responsible for maintaining pairing. Nuclear membrane and nucleolus are very clear.

Pachytene: Nucleolus and nuclear membrane are distinct. It is a suitable stage to characterize each chromosome by their relative length, arm ratio and constrictions. The nucleolus organizing chromosome is identified by its association with the nucleolus. Pachytene analysis can also be used to study karyotypic studies. The pair of homologous chromosomes is called bivalent. Synaptinemal complex between the paired chromosomes is only visible through electron microscope. It functions to pull chromosomes together helping them to pair more precisely and efficiently. Its association with pachytene chromosomes suggests its functional role in crossing-over or chiasma formation. Pachytene is a stable stage of pairing. The paired threads of each bivalent coil round one another. The chromosomes are thicker than at zygotene and are referred to as bivalents.

Diplotene: The nucleolus is disappearing while nuclear membrane is still intact. Longitudinal separation of paired chromosomes initiates but held together at points called chiasmata. Each bivalent consists of four chromatids, bivalents appear as cross or loop like depending on the number of points of crossing over. The chiasmata may be either interstitial or terminal, the number depends on the length of the chromosome. During this stage, chromosomes start contracting and are thicken. Terminalization occurs due to repulsion in homologous chromosomes. Recombination of chromosome segments results, due to crossing over and chiasma formation, leading to the exchange of genetic material.

Diakinesis: Nuclear membrane and the nucleolus start disappearing. The chromosomes continue shorten by coiling more tightly and thus the number of bivalents are more clear. The bivalents assume more rounded shape. Chiasma move towards the ends of chromosome and terminalization

of chiasmata may be still retained. Depending on the chiasma terminalization, bivalents assume either ring or rod shapes.

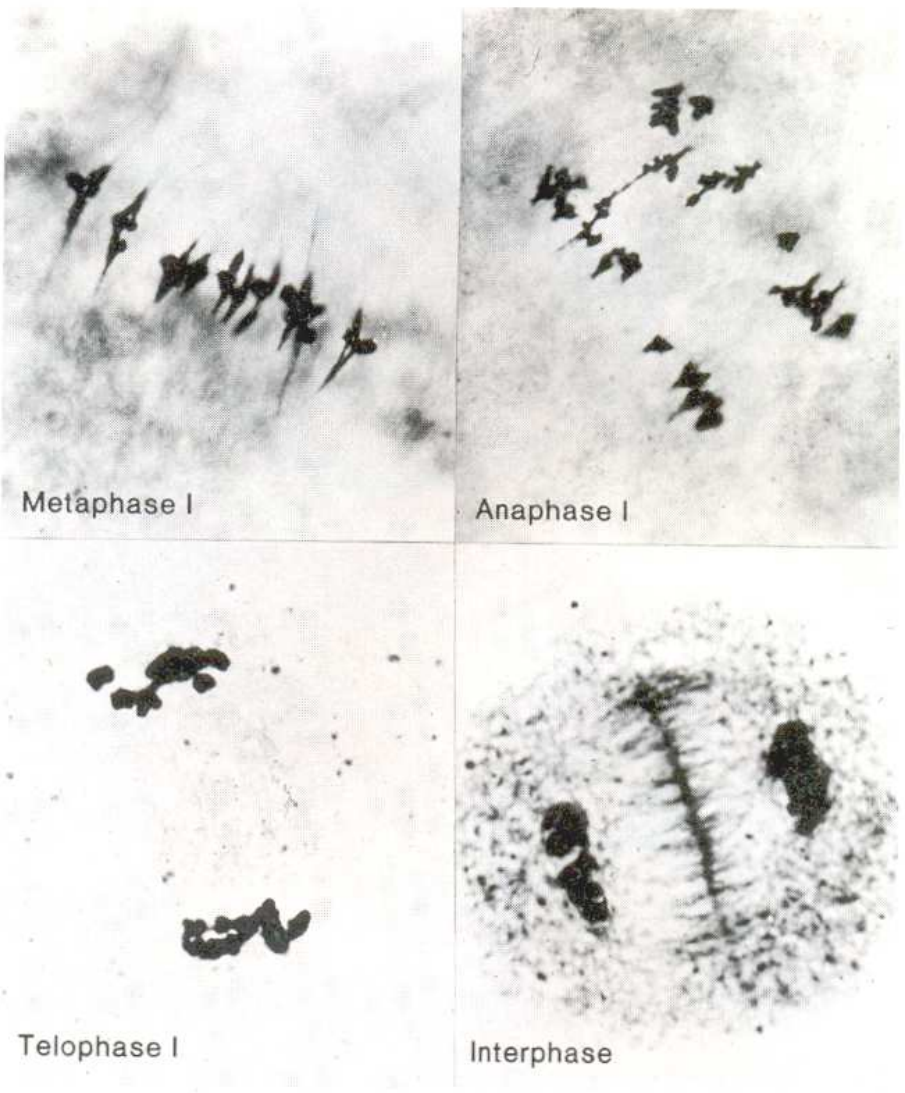


Fig. Meiosis in corn (*Zea mays*)
Courtesy: Dr. M. M. Rhoades, "Meiosis in Maize," Journal of Heredity 41 (1950): 59-67.

Metaphase-I: Nuclear membrane and nucleolus have completely disappeared. Spindle is distinct. Bivalents are oriented on metaphase plate unlike single chromosomes in mitosis. Each bivalent have two undivided centromere. Each chromosome of a bivalent is attached to the spindle fibres by its centromere.

Anaphase-I: Unlike mitosis, in which centromere divides, sister chromatids pass to the opposite poles, centromeres of each bivalent are undivided. The whole chromosome segregate instead of chromatid segregation. There is haploid number of chromosomes at each pole. Reduction in chromosome number is seen. Each group consists of *n* number of chromosomes.

Telophase-I: Nuclear membrane and nucleolus reappear. Two nuclei are seen at the pole. The daughter cells have half the chromosome number to the parent cell. Chromosomes are intermingled and form network. Chromosomes are thin and long, intermingled with one another to form a network.

Meiosis-II : This is like a mitotic division consists of Prophase II, Metaphase II, Anaphase II and Telophase II. However, differences are there between meiosis-II and typical mitosis.

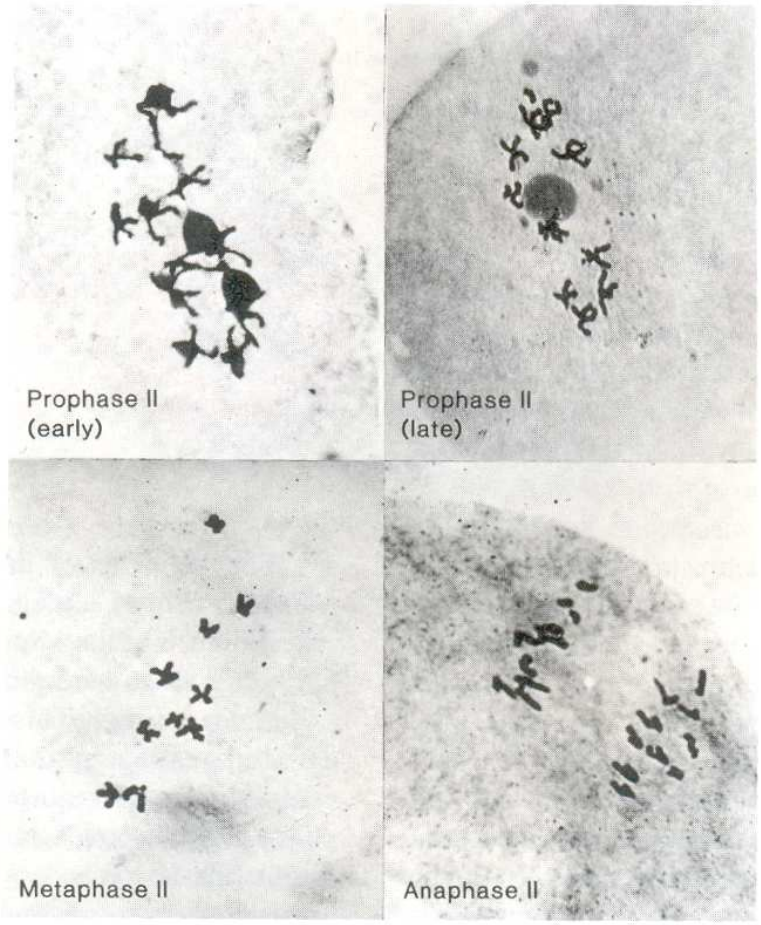


Fig. 3.21 Meiosis in corn (*Zea mays*)

Courtesy: Dr. M. M. Rhoades, "Meiosis in Maize," Journal of Heredity 41 (1950): 59-67.

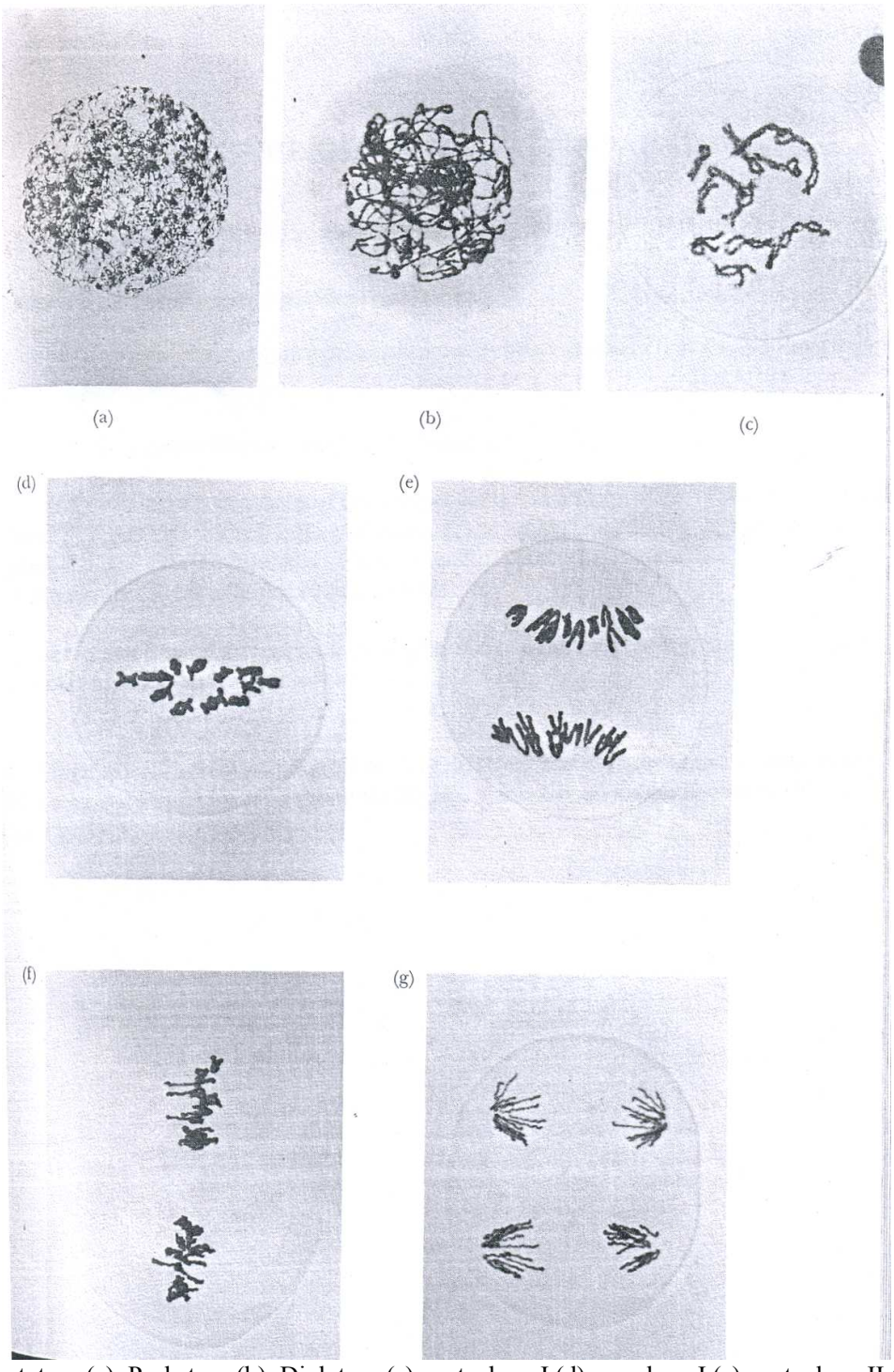


Fig. Leptotene (a), Pachytene (b), Diplotene (c), metaphase I (d), anaphase I (e), metaphase II (f), and anaphase II (g) in a pollen mother cell of *Lilium regale* (x1700).

Reported from J. McLeish and B. Snoad, Looking at Chromosomes, Macmillan, London, 1958.

1) The chromosomes in each nucleus are haploid.

2) No replication of DNA takes place before this division.

3) Chromatids of each chromosome are widely separated.

4) Chromatids are genetically different, while in mitosis, chromatids are qualitatively and quantitatively similar.

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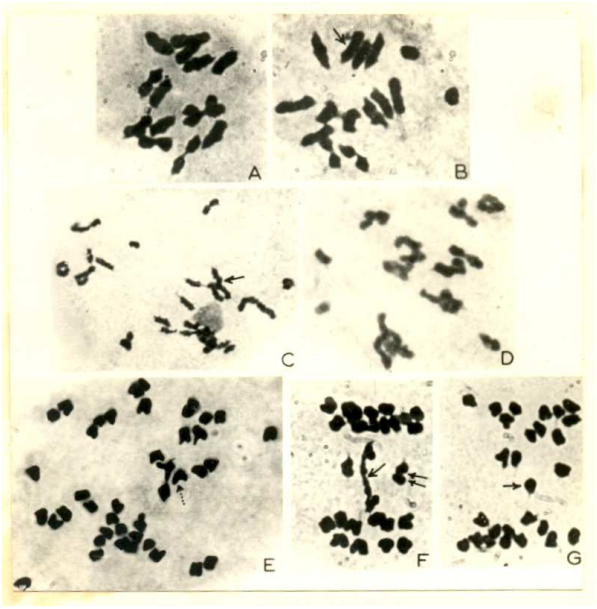
4. MEIOSIS IN POLYPLOIDS

Since there are more than two sets of homologous chromosomes in autopolyploids, they pair as multivalents. The association of chromosomes into multivalents rather than bivalent is one of the important criteria used for distinguishing auto and allopolyploids. In some cases, multivalent formation may be suppressed and only bivalents may be observed due to the presence of a diploidizing system, which forces an autopolyploid to behave like a diploid.

Among autopolyploids more particularly with an odd number of genomes, (triploids, pentaploids) exhibit higher degree of meiotic abnormalities. This results from the unequal distribution of chromosomes, which leads to the presence of lagging chromosomes. In allopolyploids, formation of bivalents is a common feature.

The frequency of quadrivalents vary in cells of a material. They may associate as a trivalent and a univalent instead of a quadrivalent. The presence of multivalents often leads to unequal distribution of chromosomes, production of laggards at anaphase I and II, production of micronuclei at diad and tetrad stages. These features result in pollen sterility and reduced seed set. These abnormalities described above are shown from different materials in Figures.

Autotetraploids (A-D) in *Papauer syriacum* (4x=28) and *Papuer sommiferum* (opium pogpy) 6x=42



(courtesy from Prof. T.N.Mary)

A – Early metaphase-I *P. syriacum* (4x), $I_{IV} + 13_{II}$

B – Metaphase-I showing $I_{IV} + 11_{II} + 2_{I}$
Note preponderance of rod bivalents

C – Diakinesis in *P. somniferum*. Note the presence of quadrivalent near the nucleous

D – Early Metaphase-I in *P. sommiferum* (4x)
Autohexaploid 6x (E-G)

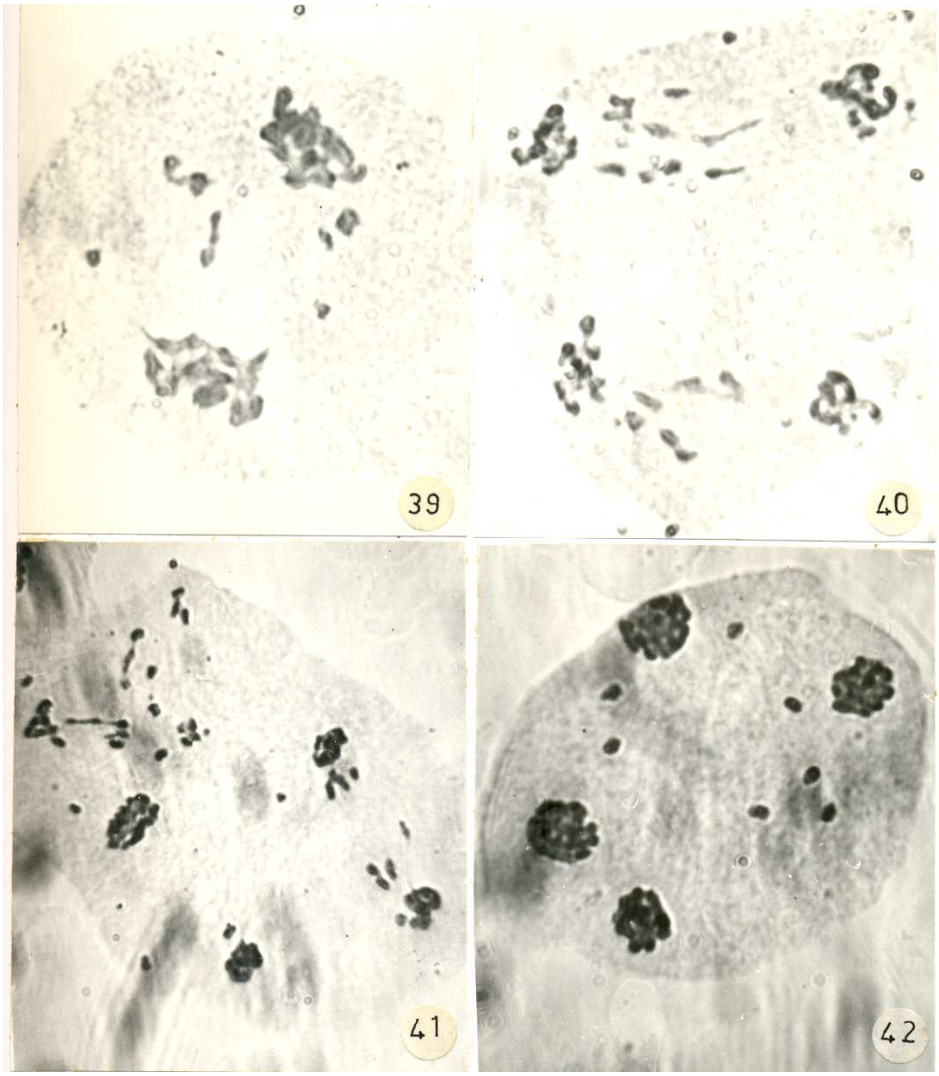
E – Anaphase-I, distribution ($\frac{20}{21}$ + 1) in autohexaploid *P. rhoeas* (6x=42)

F – Anaphase-I with $\frac{15}{11}$ + 2, distribution (4x=28)

G – Anaphase-I with $\frac{11}{11}$ + 4, distribution 4x27 (one chromosome missing)

12

(a) (b)



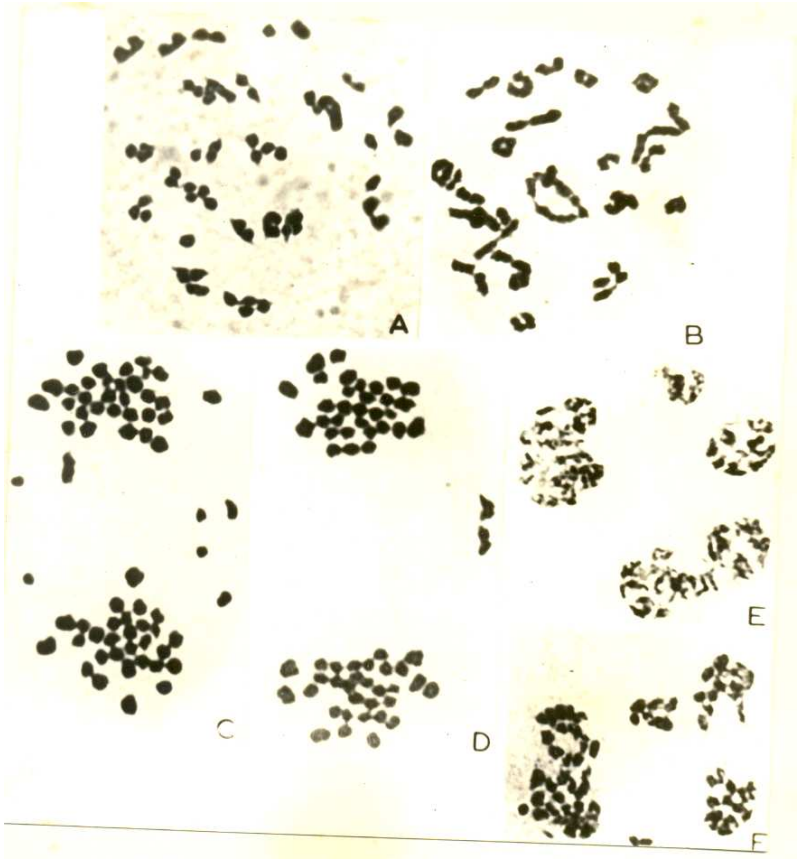
(c) (d)

Figs. Post meiotic abnormalities in tetraploids of *Capsicum annuum*.

Fig. (a). Anaphase I with laggards x 2250
Fig. (b). Anaphase II with 4 groups of chromosomes and laggards x 2250
Fig. (c). Anaphase II with 5 groups of chromosomes and laggards x2250
Fig. (d). Telophase II with 4 nuclear groups and 7 micronuclei x2250.

At the courtesy of Prof. N. Lakshmi

Meotic stages in an amphiploid of *P. somniferum* x *P. setigerum*



A - Metaphase-I, $1_{IV} + 1_{III} + 28_{II} + 3_{I}$ $6x = 62$

B - Diakinesis. Observe the presence of a ring quadrivalent near the nucleolus.

C - Anaphase-I, $^{32}/_{30}$ distribution with precociously dividing chromosomes in the centre.

D - Anaphase-I, $^{31}/_{33} + 2$ distribution.
Mark the presence of early dividing chromosomes.

E-F - Telophase-II, Note the presence of non-synchronous division and unequal sized micronuclei.

(courtesy of Prof. T.N. Mary)

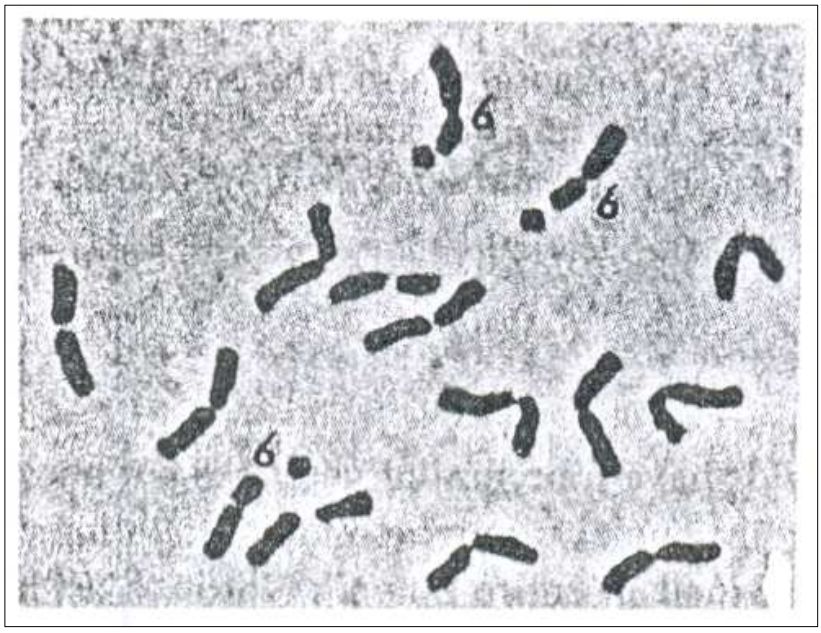
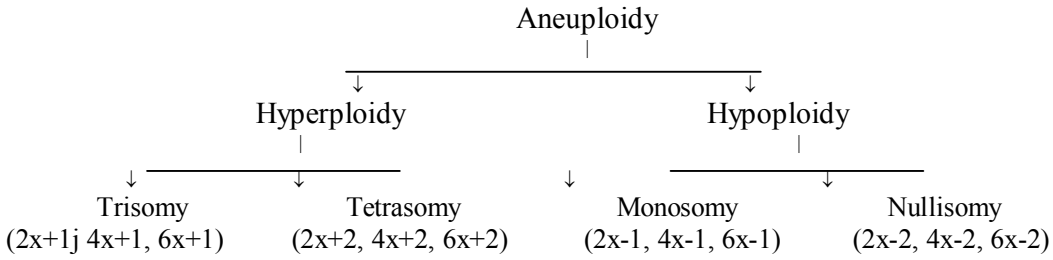


Fig. Trisomic barley, showing 3 chromosomes of chromosome no.6 of metaphase primary trisomic. (Courtesy: T. Tsuchiya)

Aneupoloidy: The presence of a chromosome number, different from an exact multiple of a basic chromosome number is called Aneuploidy. It can be either due to gain of one or more chromosomes or due to loss of one or more chromosomes.

In plants aneuploids have been studied such as tobacco, wheat, oat and trisomics in diploid species e.g. Datura, maize, bajra, tomato, rye, pre and spinach etc. A trisomic is a primary trisomic, if the extra chromosome is the same as one of the haploid genome. In a secondary trisomic, the additional chromosome is an isochromosome (two arms of the chromosome are identical). A tertiary trisomic has a translocated chromosome as an extrachromosome.

A broad classification of aneuploidy is presented here:



Meiotic stages in a trisomic (2n=15) *P. rhoeas*

A – Metaphase-I of *P. rhoeas* control showing 7_{II} 2n =14

B-E – Metaphase-I stages in *P. rhoeas* trisomic plant (2n+1) 2n = $7_{II} + 1_{I}$ (15)

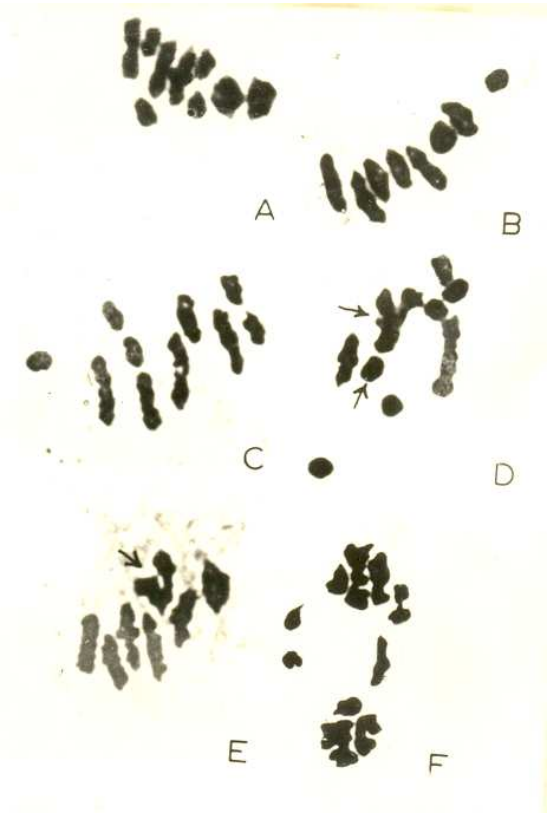
B - $7_{II} + 1_{I}$

C - $6_{II} + 3_{I}$

D - $1_{III} + 3_{II} + 6_{I}$

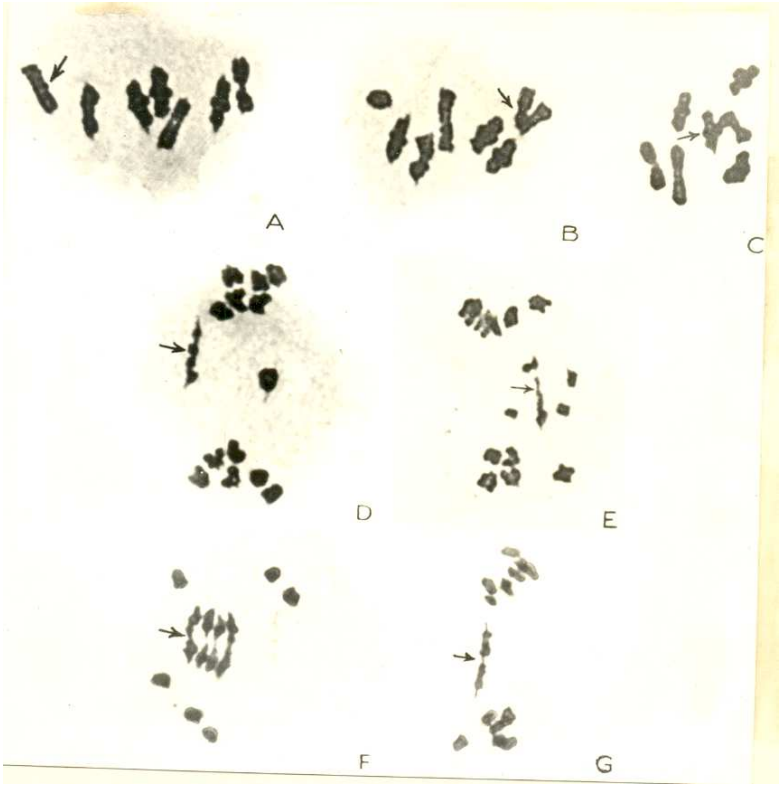
E – $1_{III} + 6_{II}$

F – Late Anaphase-I, showing lagging chromosomes



(At the courtesy of Prof. T.N. Mary)

Meiotic stages of F_2 and complex crosses in some *Papaver* species



At the courtesy of Prof. T.N.Mary

A-C - Metaphase-I, from F_2 of *P. syriacum* x *P. rhoeas* 2n = 14

A - 7_{II}, observe presence of only rod bivalents
B - Some of them are heteromorphic, showing $1_{III} + 5_{II} + 1_{I}$
C - Pollen Mother Cells (PMC) with $1_{IV} + 5_{II}$

D - Cell with Anaphase-I distribution of $^{6}/_{6} + 1$
Note the presence of a bridge and a fragment (→)

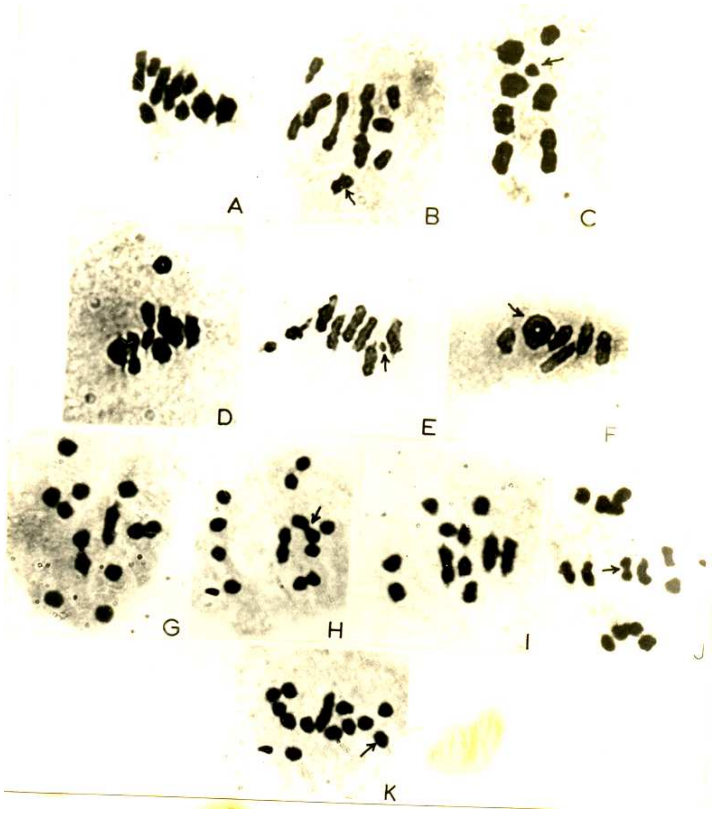
E - Anaphase-I, stages in (*P. rhoeas* x *P. glaucum*) x *P. syriacum* with $^{6}/_{5}$+3 distribution. Bridge and a fragment configuration along with misdividing chromosome in the centre are also observed.

F - Anaphase-I, showing $^{3}/_{3}$ distribution, four lagging bivalents on the equator are

also seen.

G - Anaphase-I, $^{6}/_{6}$ distribution, one bivalent showing late disjunction.

Some of the meiotic stages in *P. rhoeas* treated with Maleic hydrazide (MH) and gamma-rays (A-F = following MH; G-K = Gamma-rays)



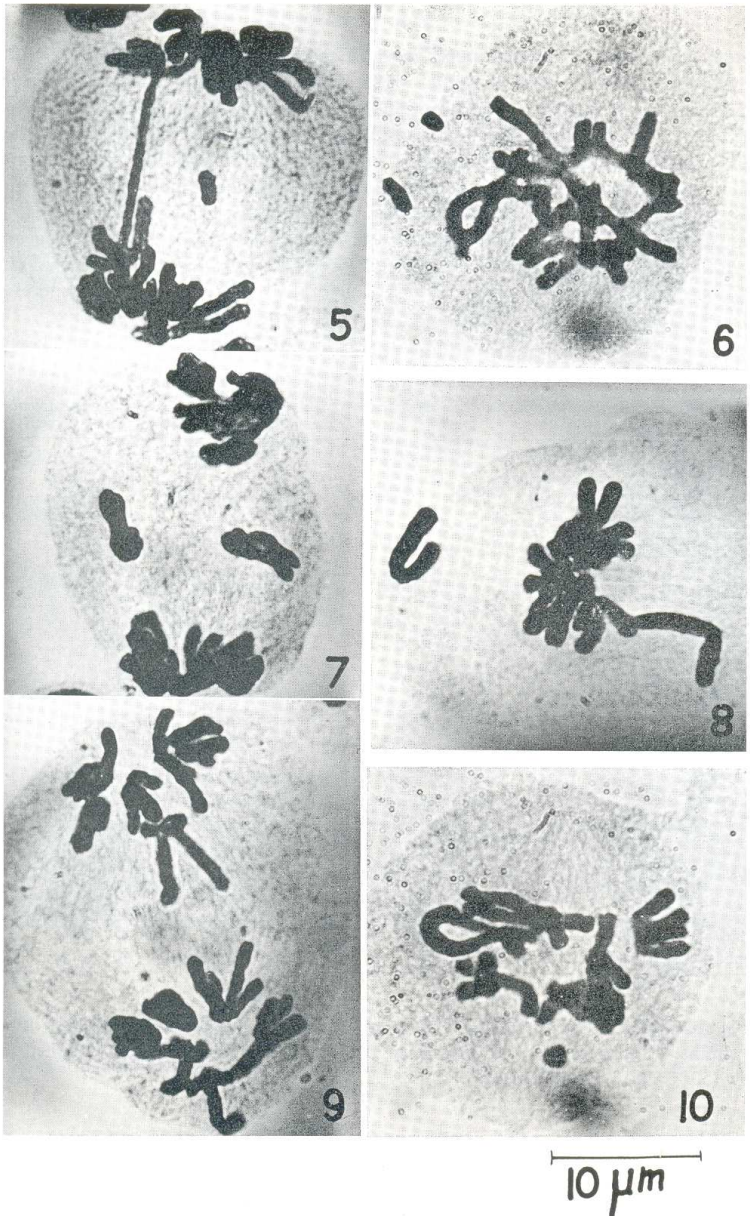
Courtesy of Prof. T.N. Mary

A - Metaphase-I, 7_{II} (control)
B - Metaphase-I, $5_{II} + 4_{I}$. Note early disjunction of rod bivalent.
C - Early Metaphase-I 7_{II}. Observe fragment lying close to bivalents.
D - Metaphase-I 7_{II}. Note the presence of the teromorphic bivalents.
E - Metaphase-I, 7^{II}. Mark the presence of heteromorphic bivalents and fragment attached.
F - Metaphase-I showing $1_{IV} + 5_{II}$
G - Late Metaphase-I showing $2_{II} + 10_{I}$
H - Late Metaphase-I, $1_{II} + 12_{I}$.
Observe the two attached univalents giving the appearance of a bivalent.
I - Metaphase-I, $4_{II} + 6_{I}$

Note early disjunction bivalent in the centre.

J - Anaphase-I $^{4}/_{4}$ + 6 distribution. Mark precociously diving chromosomes (→)

K - Metaphase-I, $I_{II} + 12_{I}$



Figs. 5-10. Meiotic stages of *Tulbaghia violacea* 2n=12

5, anaphase I with a dicentric bridge and acentric fragment.
6, metaphase I showing proximal localization of chiasmata and two fragments.
7, anaphase I with two laggards.
8, metaphase I with two univalents.
9, anaphase I showing 6:6 disjunction.

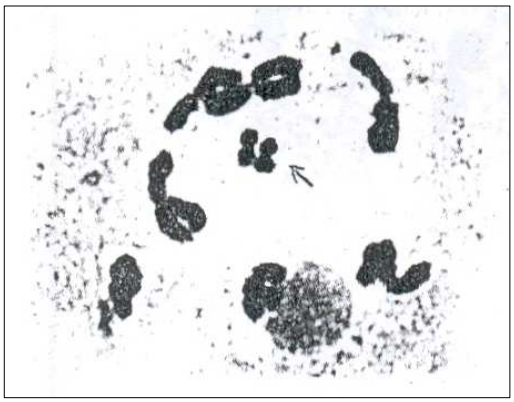
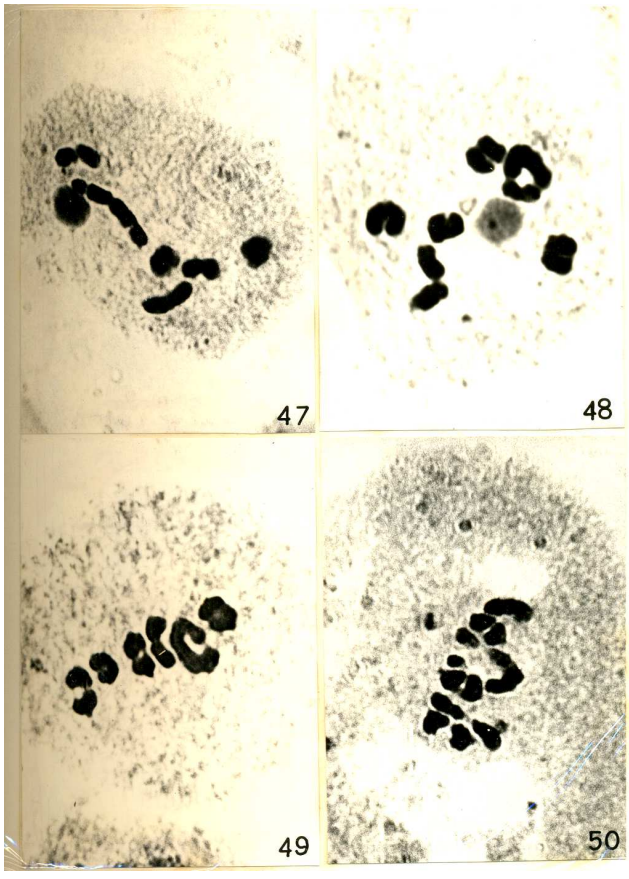


Fig. Meiosis in Triploid *Pennisetum americanum* showing Trivalents and 4B chromosomes. (courtesy Dr. J.V. Pantulu)

10, metaphase I with 1 fragment. At the courtesy of Prof. N. Lakshmi



Figs. a-d. Meiotic stages of a Translocation heterozygote

Fig. a. Diakinesis showing type 1 chain with two short chromosomes at the ends, x1500

Fig. b. Diakinesis with the translocation ring composed of two large and two small chromosomes, x1600.

Fig. c. Adjacent-1 co-orientaton of interchange complex at metaphase I, x1600.

Fig. d. Metaphase I showing adjacent-2 co-orientation of interchange complex, x1600.

(Courtesy of Prof. Z.Vishnuvardhan)

5. POLYTENE CHROMOSOMES

In insects belonging to the order *Diptera*, the cells show extra replication of each chromosome in a nucleus. They were first reported by Balbiani (1881) while observing the larval cells of *Chironomous. Drosophila* larvae show giant size polytene chromosomes in salivary gland cells (2n=8), unusually these large chromosomes arise as a result of endomitosis. In this process, the chromosomes replicate but the cells do not divide. Hence, results very thick chromosomes that magnify any differences in density along their length. Such density differences produce a precise banding pattern that serves to identing any particular chromosome. Each band is chromatin multiplied many times, thus consists of 85% of DNA, interband represents only 15% of DNA.

The two homologous chromosomes of each diploid pair are lined up side by side, so only four very thick and long chromosomes appear. Fourth pair is very small chromosome. *Drosophila* chromosomes are in a continual prophase condition. The four polytene chromosomes are linked together by regions near the centromeres that aggregate to create a large chromocenter. The precise banding pattern serves to identify a particular chromosome. The number of bands vary between species but are constant for the members of any particular species.

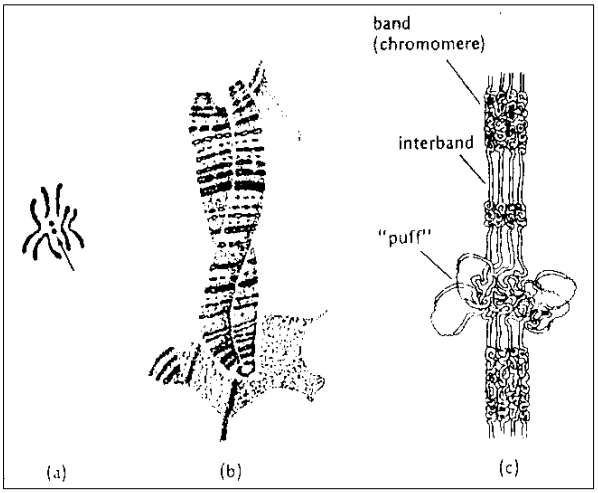


Fig. Comparison of mitotic (a) and salivary chromosomes (b, c). (a) Chromosome constitution (greatly enlarged) of a *Drosophila melanogaster* female. Arrow points to chromosome 4. (b) The same chromosome in the larval salivary glands, (c) Diagram visualizing the salivary bands as light coils of chromatid material (chromomeres) and the interband spaces as uncoiled chromatid lengths. Puffing is shown to be caused by expansion of the same chromomere in many paired chromatids. (a and b after Bridges; c after DuPraw).

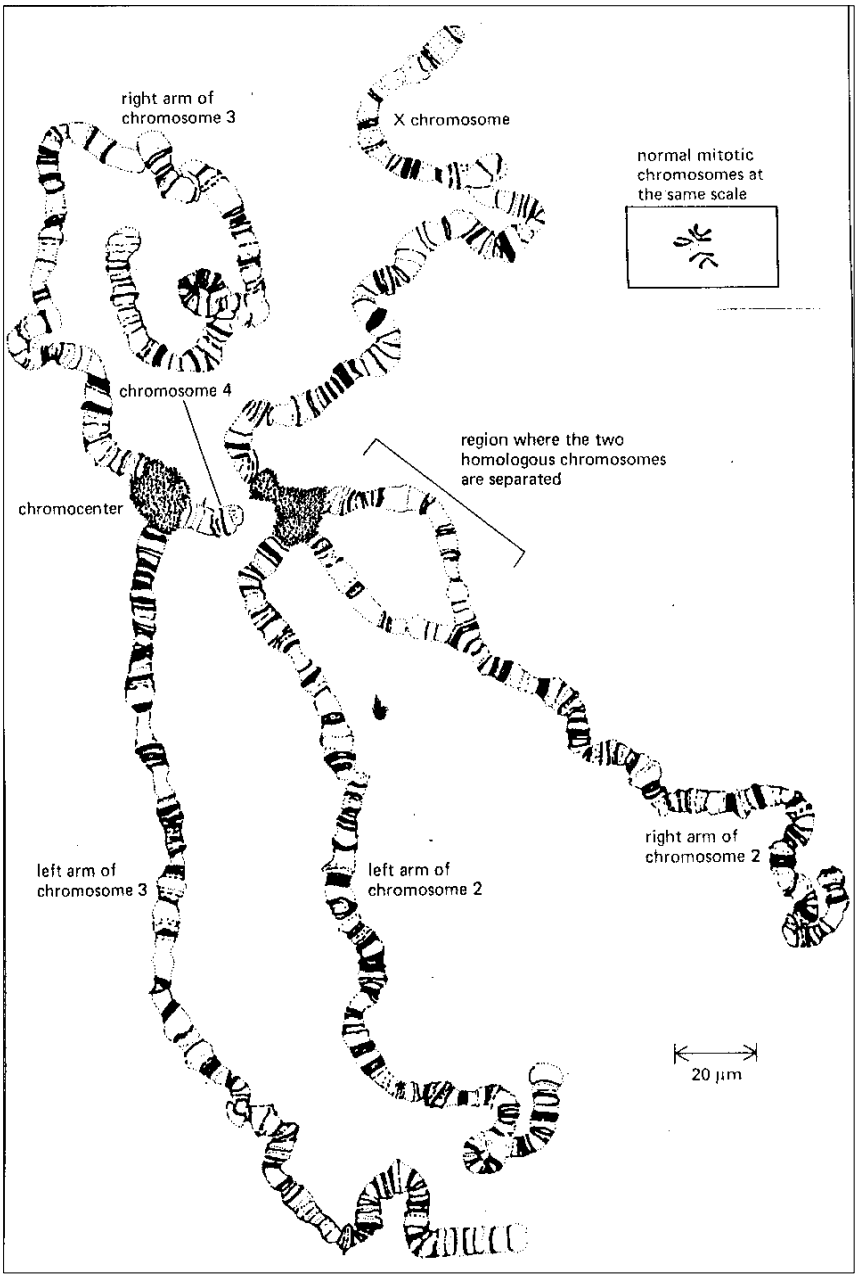


Fig. A detailed sketch of the entire set of polytene chromosomes in one *Drosophila* salivary cell. These chromosomes have been spread out for viewing by squashing them against a microscope slide. *Drosophila* has four chromosomes, and there are four different chromosome pairs present. But each chromosome is tightly paired with its homologue (so that each pair appears as a single structure), which is not the case in most nuclei (except in meiosis). The four polytene chromosomes are normally linked together by regions near their centromeres that aggregate to create a single large "chromocenter"; in this preparation, however, the

chromocenter has been split into two halves by the squashing procedure used. (Modified from T.S. Painter, J. Hered. 25: 465-476, 1934).

Supernumerary or B chromosomes

In many plants and animals, cells contain in addition to the normal complement, one or more extra chromosomes. They are referred to as accessory, supernumerary or B chromosomes. They are usually smaller than normal chromosomes. They are unstable and vary in number from organism to organism or even in different meiocytes within an organism. They are genetically inert and hence produce little or no phenotypic expression. They are mostly heterochromatic and genetically inert and are non-essential for normal growth and development. Exceptions are there, they are euchromatin in maize. They show abnormal meiotic behaviour and yet persist in populations. Some workers have suggested that Bs may confer adaptive advantage under certain conditions of severe selection pressure. They probably arose form normal chromosomes through structural repatterning followed by their heterochromatinization. They are both euchromatic and heterochromatic. They split up but undergo non-disjunction. Accumulation of a number of supernumeraries in an organism may result loss of vigour.

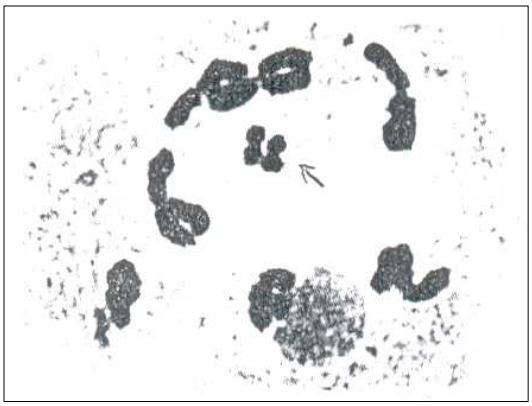
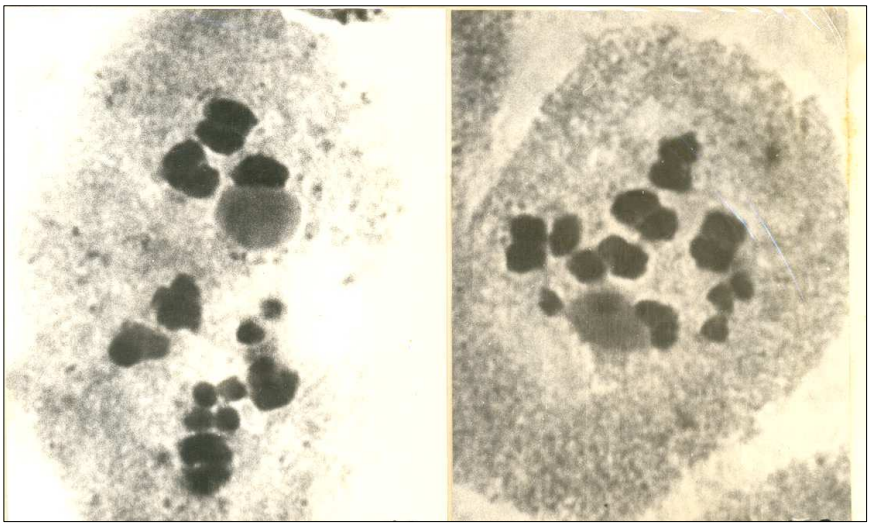


Fig. Meiosis in Triploid *Pennisetum americanum* showing Trivalents and 4B chromosomes (Courtesy Dr. J.V. Pantulu)



(a) **(b)**

Fig. a. Diakinesis showing 7 II and 6 B-chromosomes, x 1825
Fig. b. Diakinesis with 7 II and 4 B-chromosomes (1 III + 1 I), x1800

(courtesy of Prof. Z.Vishnuvardhan)

7. Barr body analysis from Buccal smears

- Rinse out the mouth thoroughly with tap water.
- With metal or wooden spatula inside of the cheek is scraped.
- First scrapping in adults is usually discarded and subsequent scrappings are spread over the glass slide.
- For temporary slide preparation acetocarmine squash technique can also be followed.
- For permanent slides – The slides are immersed in 95% ethyl alcohol : ether (1:1). The slides are immersed in the fixative for 15 to 30 min.
- The slides are now hydrated through 75 or 50 per cent ethyl alcohol to water and stained with either Feulgen Aceto-orcein or aceto-lacmoid or carbolfuchsin.

XY chromosome system of sex determination exist in humans. Females have XX and males have only one X chromosome. The question arises as to how the organism compensates for this dosage difference of XY chromosomes. A mammalian female with two X chromosomes, will have one of these chromosomes somatically inactive so that the dosage relationship between sex. Chromosomes and autosomes is same as in the somatic tissues of the male. The necessary dosage compensation is met by inactivation of one of the X chromosomes in females. Mary Lyon suggested the Barr body in Females is as a result of inactive X chromosome, which is tightly coiled into heterochromatin. It is seen near the outline of the membrane tightly coiled, as a visible mass of heterochromatin. It may be sometimes paternally derived X chromosome or maternally derived X chromosome since females receive X chromosomes from both Father and Mother. The expression of the character depends on the inheritance of loci arranged on these X chromosomes. It is one of the examples studied under regulation of gene expression in eukaryotes.

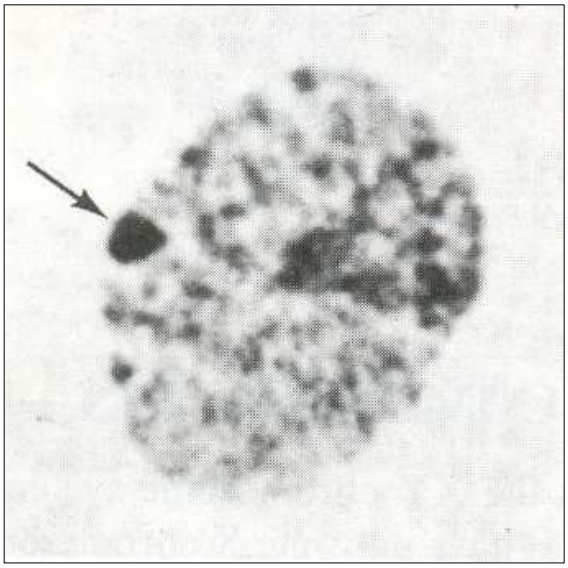


Fig. 5.20 Barr body (**arrow**) in the nucleus of a check mucosal cell of a normal female. This visible mass of heterochromatin has been identified as an

inactivated X chromosome.

7. CHROMOSOME BANDING

One of the ultimate goals of any cytogenetic study is to understand the chromosomal organization and to use this information to explain various genetic phenomena of evolutionary significance. Chromosome banding techniques play a significant role by providing means for the linear differentiation of chromosomes, understanding homology between the chromosomes.

The specific binding of heterochromatin with fluorochromes has been widely investigated and more recently, experiments on DNA hybridization have stimulated many publications on the banding patterns of chromosomes. The original 'banding' experiments were done by Jones and Corneo (1971) and Pardue and Gall (1970), and both involved alkaline denaturation of the chromosomal DNA followed by renaturation in a suitable buffer at the correct temperature and pH.

The principles involved in the banding techniques are:

1. Denaturation of part or all of the DNA/histone content, by
   (a) Alkaline Treatment
   (b) Enzyme Treatment
   (c) Heat Treatment

2. Renaturation of part of the DNA if denaturation was complete by incubation in a suitable buffer.

3. Staining – Giemsa of Leishman is the usual stain. The staining technique can be varied by altering:
   (a) pH of the stain
   (b) Temperature at which slide was stained
   (c) Time of staining
   (d) Concentration of the stain.

The heat-denaturation method seemed to be the most promising and experiments are being continued involving this process.

The chromosome banding are categorized as Fluorescence and non-fluorescence types. Fluorescence is the property where light energy of a particular wavelength is absorbed and emitted at a different wavelength. The chemicals that show fluorescing property are called flurochromes. These dyes selectively stain some regions of metaphase chromosome more intensely than other regions producing banding pattern specific for individual chromosomes. The banding techniques are based on the identification of chromosome segments that consists of either GC or AT rich regions of constitutive heterochromatin. A variety of different kinds of bands Q, C, G or R etc., have been studied in animal material.

The chromosome banding nomenclature adopted at Paris conference 1971, recognises the following types of banding (See rederic and Disteche, 1973).

Q-band : by quinacrine staining and fluorescence
G-band : by Giemsa, after simple saline, alkali or trypsin treatment.
R-band : banding reverse to Q-bands
C-band : for constitutive heterochromatin
N-band : for nucleolar organizer region
CT_band : for centromere-telomere region

Although the molecular basis for regularity of chromosomal bands remains unknown they serve as useful landmarks along the length of each chromosome to distinguish the chromosomes of similar size and shape. The differences between the chromosomes are explained through the number, distribution, intensity and thickness of the bands. Thus chromosome banding solve many problems in Taxonomy, Biosystematics and phylogeny.

C-banding: It is non-fluorescent banding. The bands are observed at the centromeric regions of chromosome. They represent constitutive heterochromatin surrounding the centromeres. The appearance of C-bands is due to differential condensation of euchromatin and heterochromatin material. The DNA stained is satellite DNA found around centromeres with numerous repetitions of short sequences. Thus C-bands can be noted at centromeric, telomeric and secondary constriction regions of the chromosomes.

One of the most commonly used C banding technique is described.

The utility of Giemsa C-banding in chromosome identification involved the following steps.

1. Prepare the slide for chromosomes through conventional squash technique.
2. Treat the slide with 0.2 N HCl for 10-30 min.
3. Wash the slide in deionized water.
4. Treat the slide with NaOH for 2 min.
5. Rinse in ethanol several times.
6. Incubate in 6x SSC (Sodium Saline Citrate) at 60^{o}C.
7. Stain in Giemsa solution
8. Wash and mount in DPX.

Quinacrin mustard is a fluorescent compound or a fluorochrome. The bands appear only when chromosomes are exposed to UV light. Ultraviolet irradiation causes some of the quinacrine molecules that have inserted into the chromosome to emit energy, those parts of chromosome shine brightly other parts remain dark. Using these bands, cytogeneticists can identify particular chromosome in a cell.

Thus chromosome banding solve many problems in Taxonomy, biosystematics, phylogeny and evolution.

Q-banding: It is more reliable, safe and specific for detection of heterochromatin but less successful with plant chromosomes. The bright fluorescence of Q bands is due to more aggregation of A-T rich repetitive sequences.

The differences can be noted in the intensity of fluorescene and size of Q bands. Such variations are referred as band heteromorphism. Q bands are heterochromatic in nature having highly or mildly repetitive sequences of DNA. Q band heteromorphism is a common feature in populations of animals and man; whereas C-band heteromorphism has been reported in plants. Heteromorphism of bands enables organism for a rapid adaptation to the changed environmental conditions.

Band polymorphism can be used as a finer method of analysis to distinguish varieties, cultivars and strains among populations growing in different climatic conditions.

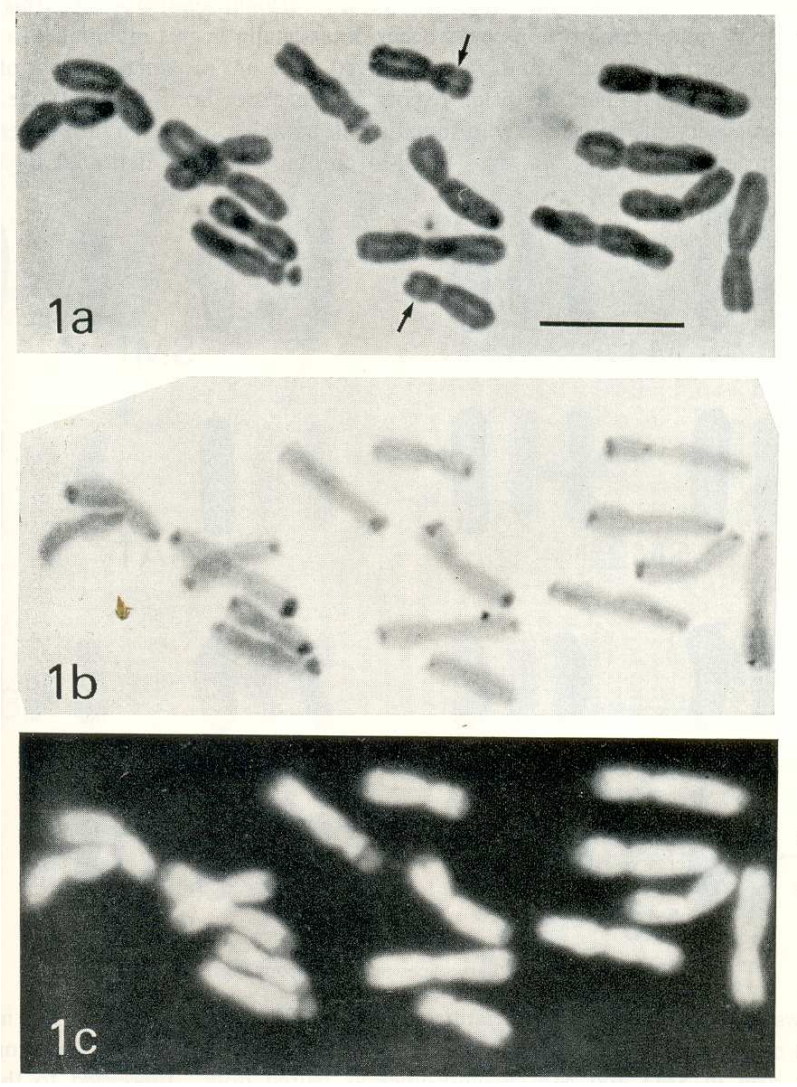


Fig. 1a-c. Root-tip chromosomes stained sequentially in one and the same cell; a, aceto-orcein, b, C-banded, and c, Q-banded preparation. Arrow indicates a gap of SM4 chromosome pair. Bar represents 10 micra.

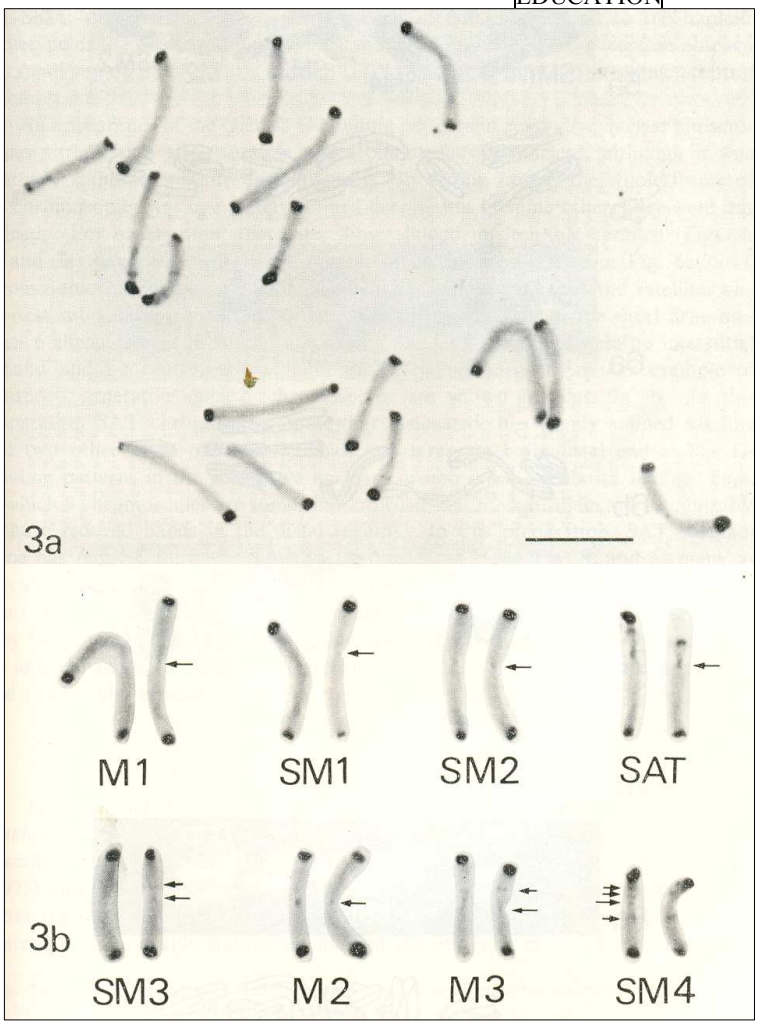


Fig. 3a-b. C-banded root-tip chromosomes with centromeric (long arrows) and interstitial (short arrows) bands
(courtesy of Dr. Satyesh Chandra Ray)

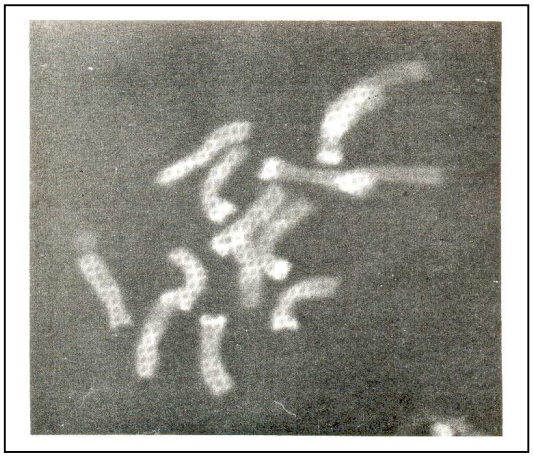
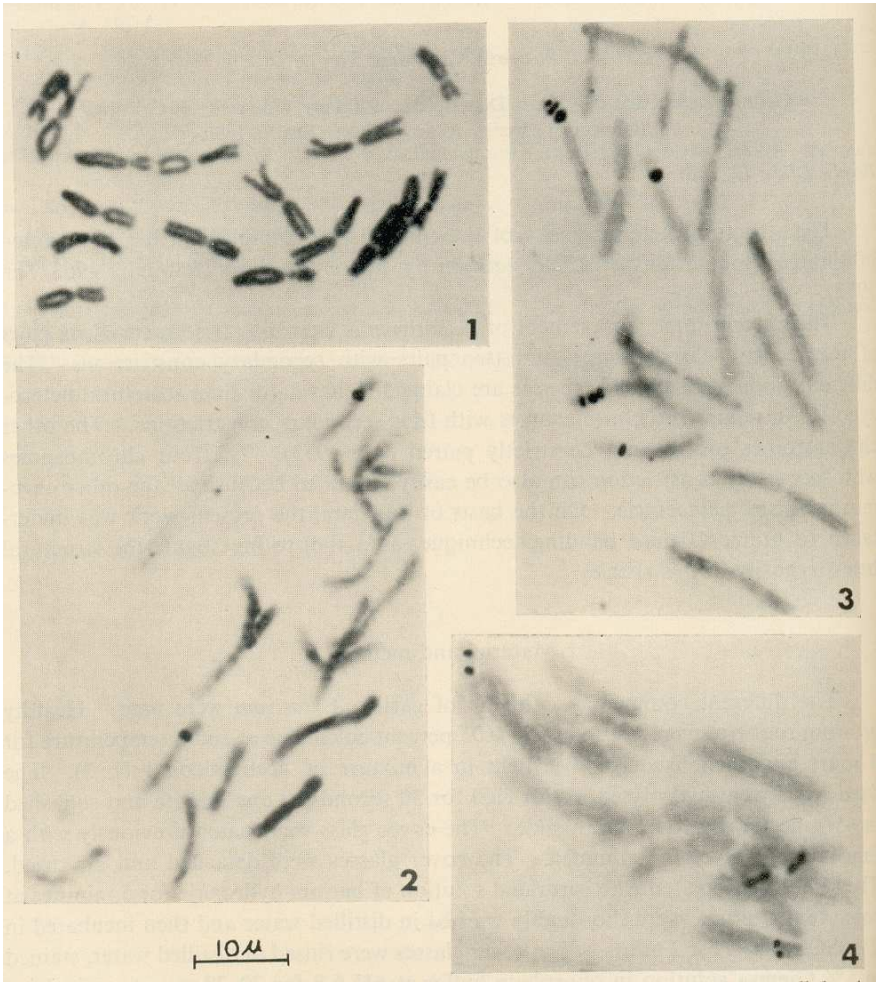


Fig. 1. Kanpur cultivar of *Lathyrus sativus* L. (2n=14)
Note the bright telomeric Q-bands in eight chromosomes (x2500)
(courtesy of Dr. Saran, Kumar and Jain)

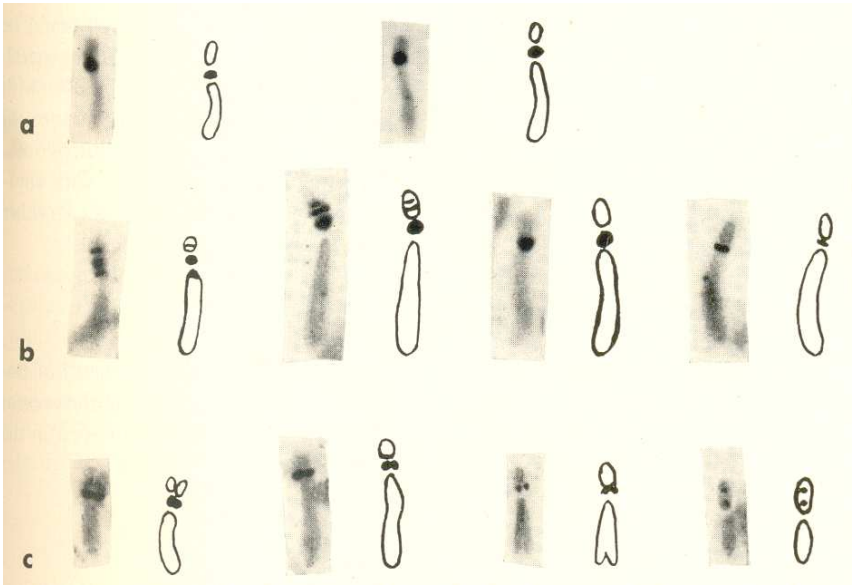
Polymorphism in Giemsa banding patterns by *Allium sativum*

Based on the intensity of the colour, constancy of its occurrence, the banding pattern is recognized, three groups of chromosomes are detected, structural heterozygosity is evident in this species.

- One distinct band was found in each of two chromosomes.
- Banding is found in four chromosomes with secondary constrictions.
- Heteromorphy in banding pattern, having 2 bands in one metacentric chromosome and one band in one chromosome with secondary constriction.



Figs. 1-4. *Allium sativum.* 1, somatic plate showing 2n=16 chromosomes following orcein squash. 2, cell showing two distinct bands in two chromosomes after Giemsa staining. 3, showing bands in four chromosomes with heteromorphic nature in one pair only. 4, two chromosomes showing heteromorphicity in having two bands in one metacentric chromosome and one in a chromosome with secondary constriction.



Courtesy of Satyesh Chandra Roy

Fig. 2. a, two chromosomes with secondary constrictions showing banding pattern in the first population. b-c, showing heteromorphicity in banding pattern in the second population. *A. rativum.*

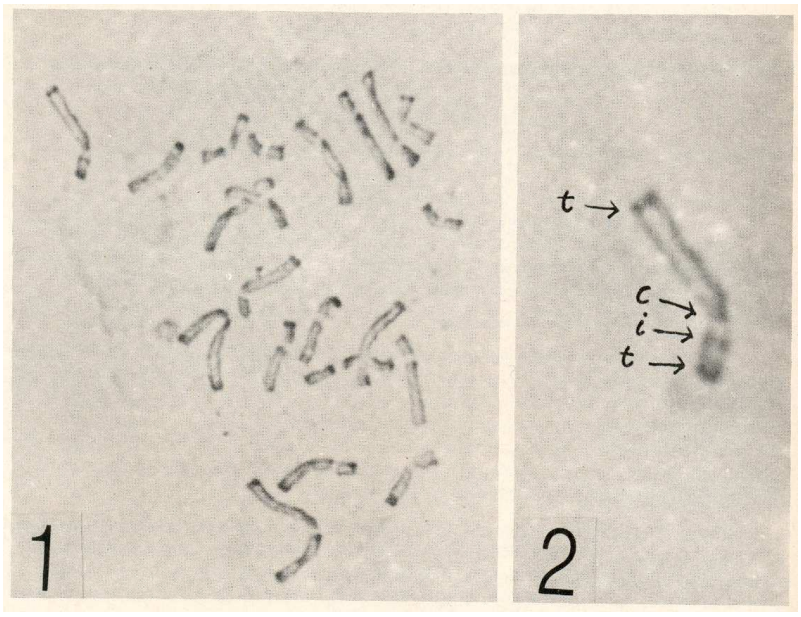


Fig. 1-2 *Crinum latifolium*. 1. Phase contrast photomicrograph of somatic metaphase.

Note darker bands (x 1500). 2. A single magnified chromosome with telomeric (t), centromeric (C) and intercalary bands (i) (x 3000).
(courtesy of Dr. Saran *et al*.)

8. ASSIGNMENTS IN GENETICS

8.(i) MENDELIAN INHERITANCE AND GENE INTERACTION

1. Corn (*Zea mays*) has a diploid number of 20. How many chromosomes would be expected in (a) a meiotic product (microspore or megaspore), (b) the cell resulting from the first nuclear division (karyokinesis) of a megaspore, (c) a polar nucleus, (d) a sperm nucleus, (e) a microspore mother cell, (f) a leaf cell, (g) a mature embryo sac (after degeneration of non-functional nuclei), (h) an egg nucleus, (i) an endosperm cell, (j) a cell of the embryo, (k) a cell of the pericarp, (l) an aleurone cell?

Ans: (a) 10, (b) 20, (c) 10, (d) 10, (e) 20, (f) 20, (g) 30, (h) 10, (I) 30, (j) 20, (i) 20, (l) 30)

2. If a plant homozygous for tall is crossed with one homozygous for dwarf, what will be the appearance of f_i, of f_2, of the offspring of a cross of f_1 with its tall parent; with its dwarf parent?

A) Tall dominant = TT, homozygous dwarf recessive = tt

TT x tt
| |
Gametes T t

F1 – Tt all tall heterozygous

If a cross is made between F1 plants

Gametes Tt x Tt
| |
T t
t t

	T	t
T	TT	Tt
t	Tt	tt

Tall - 3
Dwarf - 1

If F1 plants crossed with Tall parents

Gametes Tt x TT
| |
T T
t

	T
T	TT
t	Tt

All tall

If F1 plants crossed with dwarf parents

Gametes Tt x tt
| |

	t
T	Tt

½ tall
½ dwarf

T t Tall : Dwarf = 1:1

t t

	tt

2) A tall plant crossed with a dwarf one produces offspring of which about one-half are tall and one half dwarf what are the genotypes of the parents?

A. To produce one half tall or dwarf one parent should produce only one kind of gametes and the other should produce 2 kinds of gametes. This is possible when F1 plants are backcrossed with its recessive parent.

One parent genotype may be - Tt

Other parent's genotype - Tt

t

Gametes Tt X tt T ½ tall

|	|	½ dwarf
T	t	
t		

Tt	
t	

4. In the following crosses in which genotypes of the parents are given, what are the gametes produced by such parent and will be the flowers colour of the offspring from each cross Rr x RR; rr x Rr; in four-o clock plant flowers red flower colour R is in completely dominant over white r, the heterozygousplants being pink flowered.

R

A) I Gametes Rr x RR Red flowered – 0.5

↓ ↓ Pink flowered – 0.5

R R

r

R

RR	
Rr	

r

ii) rr x Rr R r Pink flower plant = 0.5

Gametes ↓ ↓

r R

r

Rr	rr

5. Yellow coat color in guinea pigs is produced by the homozygous genotype C^YC^Y, cream color by the heterozygous genotype C^YC^W, and white by the homozygous genotype C^WC^W. What genotypic and phenotypic ratios are matings between cream colored individuals likely to produce?

$C^YC^W \times C^YC^W$

Ans: ¼C^YC^Y = yellow : ½ C^YC^W = cream : $^1/_4C^WC^W$ = white

6. In *Drosophila*, sepia colored eyes are due to a recessive allele s and wild type (red eye color) to its dominant allele s^+. If sepia eyed females are crossed to pure wild type males, what phenotypic and genotypic ratios are expected if the F_2 males are backcrossed to the sepia eyed parental females?

Sepia Wild
P Ss x S^+s^+
F_1 S^+s Wild

Back cross → Sepia x SS x S^+s Wild
S^+S; SS

Ans: ½ wild type s^+s : ½ sepia ss

7. Suppose the Mendel had produced an F_1 heterozygous for all seven allelic pairs. What would be the minimum number of the F_2s he would need to display the expected F_2 phenotypic ratio?

Ans: When one pair of contrasting character is present, in F_2 there will be 4 plants to represent all possible phenotypes. If there are 2 pairs of characters, the minimum number of F_2s needed to express all the phenotypic combinations is 16. If there are 3 pairs of characters, the needed F_2 plants are 256. Thus we can correlate a mathematical table between the number of genes involved in the cross; number of visibly different F_2 classes of individuals (if the dominance is complete); Number of different kinds of gametes produced by F_1 hybrids; number of genotypes and number of possible combinations in F_1 gametes.

The relation between the number of genes involved in a cross and the number of phenotypic and genotypic classes in F_2 are:

Number of genes involved	No. of visibly different F_1 individuals	No. of different kinds of gametes formation F_1	No. of genotypically different combinations	No. of possible combinations in F_1 gametes
1	2	2	3	4
2	4	4	9	16
3	8	8	27	64
n	2^n	2^n	3^n	4^n

Suppose that Mendel had produced an F_1 heterozygous for all seven allelic pairs. Then for the minimum number of F_2 plants that he would need to display, the expected F_2 phenotypic ratio, is as follows:

Number of genes involved in the cross	No. of visibly different F_1 hybrids	No. of gametes produced by F_1 hybrids	No. of genotypes types in F^2	No. of possible combinations in F_1
7	2^7	2^7	3^7	4^7

Then the number of gametes produced = 128
The number of genotypes produced = 2187
The number of possible combinations = 16,384

If Mendel produces an F_2 of the above cross, he would need a minimum of 16,384 plants, to display 128 phenotypes and 2187 genotypes.

Dihybrid/Trihybrid cross

1. In the garden pea, Mendel found that yellow seed color was dominant to green ($Y > y$) and round seed shape was dominant to shrunken ($S > s$). (a) What phenotypic ratio would be expected in the F_2 from a cross of a pure yellow, round x green, shrunken? (b) What is the F_2 ratio of yellow : green and of round : shrunken?

Solution:

(a) P: *YY SS* x *yy ss*
yellow, round green, shrunken

F_1: Yy Ss
yellow, round

F_2:
$\frac{9}{16}$ *Y-S-* yellow, round
$\frac{3}{16}$ *Y-ss* yellow, shrunken
$\frac{3}{16}$ *yyS-* green, round
$\frac{1}{16}$ *yy ss* green, shrunken

(b) The ratio of yellow : green = ($^{9}/_{16}$ yellow, round + $^{3}/_{16}$ yellow, shrunken) : ($^{3}/_{16}$ green, round + $^{1}/^{16}$ green, shrunken) = 12 : 4 = 3 : 1. The ratio of round : shrunken = ($^{9}/_{16}$ yellow, round + $^{3}/_{16}$ green, round) : ($^{3}/_{16}$ yellow, shrunken + $^{1}/_{16}$ green, shrunken) = 12 : 4 = 3 : 1. Thus at each of the individual loci a 3 : 1 F_2 phenotypic ratio is observed, just as would be expected for a monohybrid cross.

2. Tall tomato plants are produced by the action of a dominant allele, *D*, and dwarf plants by its recessive allele *d*. Hairy stems are produced by a dominant gene *H*, and hairless stems by its recessive allele *h*. A dihybrid tall, hairy plant is testcrossed. The F_1 progeny were observed to be 118 tall, hairy : 121 dwarf, hairless : 112 tall, hairless : 109 dwarf, hairy. (a) Diagram this cross. (b) What is the ratio of tall : dwarf; of hairy : hairless< (c) Are these two loci assorting independently of one another?

Solution:

(a) Parents: Dd Hh (tall, hairy) x dd hh (dwarf, hairless)

Gametes: DH Dh dH dh dh

F_1:

Genotypes	Number	Phenotypes
Dd Hh	118	tall, hairy
Dd hh	112	tall, hairless
dd Hh	109	dwarf, hairy
dd hh	121	dwarf, hairless

Note that the observed numbers approximate a 1:1:1:1 phenotypic ratio.

(b) The ratio of tall : dwarf = (118 + 112) : (109 + 121) = 230 : 230 or 1:1 ratio. The ratio of hairy : hairless = (118 + 109) : (112 + 121) = 227 : 233 or approximately 1:1 ratio. Thus the testcross results for each locus individually approximate a 1:1 phenotypic ratio.

(c) Whenever the results of a testcross approximate a 1:1:1:1 ratio, it indicates that the two gene loci are assorting independently of each other in the formation of gametes. That is to say, all four types of gametes have an equal opportunity of being produced through the random orientation which non-homologous chromosomes assume on the first meiotic metaphase plate.

3) When three genes tall, yellow, round in pea plants vs dwarf, green, wrinkled are involved in a cross, show by forking method or dichotomous method, what is trihybrid ratio (phenotypic ratio)

P1 - Tall plants X Dwarf plant
Yellow seeds, Round seeds Green, wrinkled
TT YY RR ↓ tt yy rr

F1 Tall Yellow Round
Tt Yy Rr
↓ Selfing

F2

3 YY
- 3RR – 27 tT YY RR – Tall yellow round
- 1rr – 9 TT YY rr – Tall yellow wrinkled

3 TT

1 YY
- 3RR – 9 TT YY RR – Tall Green round
- 1 rr – 3 TT yy rr - Tall green wrinkled

3 YY
- 3RR – 9 tt YY RR – Tall yellow round
- 1rr – 3 tt YY rr – Dwarf yellow wrinkled

1 tt

1 yy
- 3RR – 3 tt yy RR – Dwarf Green round
- 1 rr – 1 tt yy rr - Dwarf green wrinkled

Phenotypic Ratio

27 Tall yellow Round	9 Tall yellow round
9 Tall yellow wrinkled	3 – Dwarf yellow wrinkled
9 Tall green round	3 – Dwarf green round
3 Tall green wrinkled	1 – Dwarf green wrinkled

Epistatise gene interactionss

1. Two white flowered strains of the sweet pea (*Lathyrus odoratus*) were crossed, producing an F_1 with only purple flowers. Random crossing among the F_1 produced 96 progeny plants, 53 exhibiting purple flowers and 43 with white flowers. (a) What phenotypic ratio is approximated by the F_2? (b) What type of interaction is involved? (c) What were the probable genotypes of the parental strains?

Solution:

(a) To determine the phenotypic ratio in terms of familiar sixteenths, the following proportion for white flowers may be made: 43/96 = x/16, from which x = 7.2. That is, 7.2 white : 8.8 purple, or approximately 7:9 ratio. We might just as well have arrived at the same conclusion by establishing the proportion for purple flowers: 53/96 = x/16, from which x = 8.8 purple.

(b) A 7 : 9 ratio is characteristic of **duplicate recessive** genes where the recessive genotype at either or both of the loci produces the same phenotype.

(c) If *aa* or *bb* or both could produce white flowers, then only the genotype *A-B-* could produce purple. For two white parental strains (pure lines) to be able to produce an all purple F_1, they must be homozygous for different dominant-recessive combinations. Thus

P: aaBB x AAbb
white white

F_1: AaBb
purple

F_2: 9/16 A-B- = 9/16 purple
3/16 A-bb
3/16 aaB- } = 7/16 white
1/16 aabb }

2. On chromosome three of corn there is a dominant gene (A_1) which, together with the dominant gene (A_2) on chromosome nine, produces colored aleurone. All other genetic combinations produce colorless aleurone. Two pure colorless strains are crossed to produce an all colored F_1. (a) What were the genotypes of the parental strains and the F_1? (b) What phenotypic proportions are expected among the F_2? (c) What genotypic ratio exists among the white F_2?

Ans: (a) P:$A_1A_1a_2a_2$ x $a_1a_1A_2A_2$; F_1:$A_1a_1A_2a_2$
(b) 9/16 colored : 7/16 colorless
(c) $^1/_7A_1A_1a_2a_2$: $^2/_7A_1a_1a_2a_2$: $^1/_7a_1a_1A_2A_2$: $^2/_7a_1a_1A_2a_2$: $^1/_7a_1a_1a_2a_2$

3. A plant of the genus *Capsella*, commonly called "shepherd's purse", produces a seed capsule the shape of which is controlled by two independently assorting genes. When dihybrid plants were interpollinated, 6% of the progeny were found to possess ovoid-shaped seed capsules. The other 94% of the progeny had triangular-shaped seed capsules. (a) What two factor epistatic ratio is approximated by the progeny? (b) What type of interaction is operative?

Ans: (a) 15 triangular : 1 ovoid (b) duplicate dominant gene interaction

Dihybrid → AaBb

	AB	Ab	aB	ab
AB	AABB 1	AABb 2	AaBB 3	AaBb 4
Ab	AABb 5	AAbb 6	AaBb 7	Aabb 8
aB	AaBB 9	AaBb 10	aaBB 11	aaBb 12
ab	AaBb 13	Aabb 14	aaBb 15	aabb 16

*****

8.(ii) Multiple alleles

1. In rabbis, full colour (C), Himalayan albinism (c^h) and albinism (c^a) form a series of multiple alleles with dominance in the order given. What will be the appearance of the offspring of the following crosses:

a. Coloured x Himalayan (both homozygous)
b. F_2 from *a*
c. Himalayan x albino (both homozygous)
d. F_2 from *c*
e. F_1 from *a* x F_1 from *c*.

A. (a) Cross between homozygous coloured x Himalayan

cc x C^hC^h

|

cCh All coloured

(b) F_2 from *a*

Cch x C^hc

|

CC Cc^h Cc^h c^hc^h

Coloured : Himalayan

3 : 1

(c) Homozygous Himalayan x albino

C^hc^h x c^ac^a

|

C^hC^a All Himalayan

(d) F_2 from *C*

C^hC^a x C^hC^a

C^hC^h, C^hC^a, C^hC^a, C^aC^a

Himalayan Albino

3 : 1

(e) F_1 from *a* x F_1 from *C*

cC^h x C^hC^a

|

cC^h, cC^a, C^hc^h, C^hc^a

Wild coloured : Himalayan

1 1

2. What will be the phenotype, as to blood groups, of offsprings of parents of the following genotypes for blood groups: *lA; x lB I; lA lB x lBi; lB I x lBi:*

A. (a) $l^A I$ x $l^B i$

$l^A l^B, l^A i, l^B i, ii$

AB	:	A	:	B	:	O
1	:	1	:	1	:	1

(b) $l^A I^B$ x $l^B i$

$l^A l^B, l^A i, l^B l^B, I^B i$

AB	*:*	*A*	*:*	*B*
1	:	1	:	2

(c) $I^B i$ x $I^B i$

$I^B I^B, I^B i, I^B i, ii$

B	:	0
3	:	1

3. If a person of blood group AB maries one belonging to group O, what will be the blood groups of their children? In the three following problems on blood groups, determine the genotypes of the parents.

A. The marriage between AB person x 0 person

$I^B I^B$ x *ii*

$I^A i, I^B i$

A	:	B
1	:	1

50% of the children will have A group of blood and 50% of the children will have B group of blood.

*****

8. (iii) LINKAGE AND CHROMOSOME MAPPING

1. Linkage

When two or more genes reside in the same chromosome, they are said to be **linked**. They may be linked together on one of the autosomes or connected together on the sex chromosome. Genes on different chromosomes are distributed into gametes independently of one another (Mendel's Law of Independent Assortment). Genes on the same chromosome, however, tend to stay together during the formation of gametes. Thus the results of testcrossing dihybrid individuals will yield different results, depending upon whether the genes are linked or on different chromosomes.

Example 1. Genes on different chromosomes assort independently, giving a 1:1:1:1 testcross ratio.

Parents	AaBb		x		aabb			
Gametes	AB	Ab	aB	ab	ab			
F_1	¼ AaBb : ¼ Aabb : ¼ aaBb : ¼ aabb							

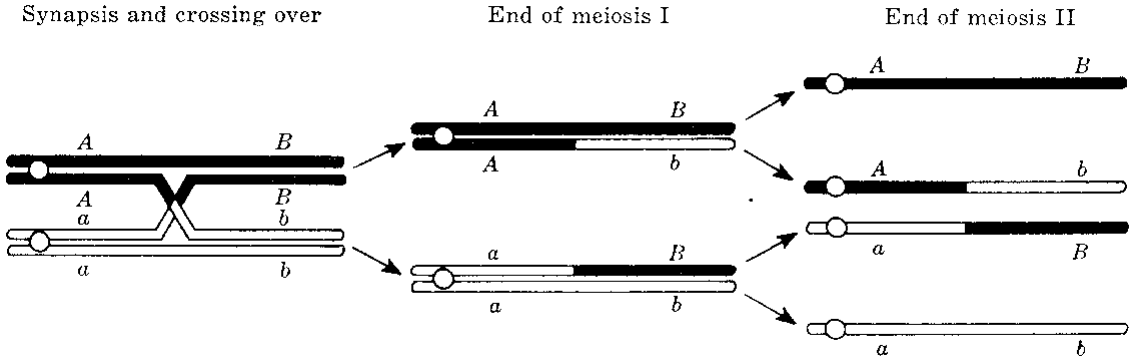
Example 2. Linked genes do not assort independently, but tend to stay together in the same combinations as they were in the parents. Genes to the left of the slash line (/) are on one chromosome and those to the right are on the homologous chromosome.

Parents:	AB/ab		x		ab/ab
Gametes:	AB	ab		ab	
F_1 :	½ AB/ab : ½ ab/ab				

Large deviations from a 1: 1 : 1 : 1 ratio in the testcross progeny of a dihybrid could be used as evidence for linkage. Linked genes do not always stay together, however, because homologous non-sister chromatids may exchange segments of varying length with one another during meiotic prophase. Recall from meiosis that homologous chromosomes pair with one another in a process called "synapsis" and that the points of genetic exchange, called "chiasmata", produce recombinant gametes through crossing over.

2. Crossing over

During meiosis each chromosome replicates, forming two identical sister chromatids; homologous chromosomes pair (synaps) and crossing over occurs between non-sister chromatids. This latter process involves the breakage and reunion of only two of the four strands at any given point on the chromosomes. In the diagram below, a crossover occurs in the region between the A and B loci.



Notice that two of the meiotic products (*AB* and *ab*) have the genes linked in the same way as they were in the parental chromosomes. These products are produced from chromatids that were not involved in crossing over and are referred to as *non-crossover* or *parental* types. The other two meiotic products (*Ab* and *aB*) produced by crossing over have recombined the original linkage relationships of the parent into two new forms called *recombinant* or *crossover* types.

The alleles of double heterozygotes (dihybrids) at two linked loci may appear in either of two positions relative to one another. If the two dominant (or wild type) alleles are on one chromosome and the two recessives (or mutants) on the other (*AB/ab*), the linkage relationship is called *coupling phase*. When the dominant allele of one locus and the recessive allele of the other occupy the same chromosome (*Ab/aB*), the relationship is termed *repulsion phase*. Parental and recombinant gametes will be of different types, depending upon how these genes are linked in the parent.

Example 1. Coupling Parent: AB/ab

Gametes:
- Parental: AB, ab
- Recombinant: Ab, aB

Example 2. Repulsion Parent: Ab/aB

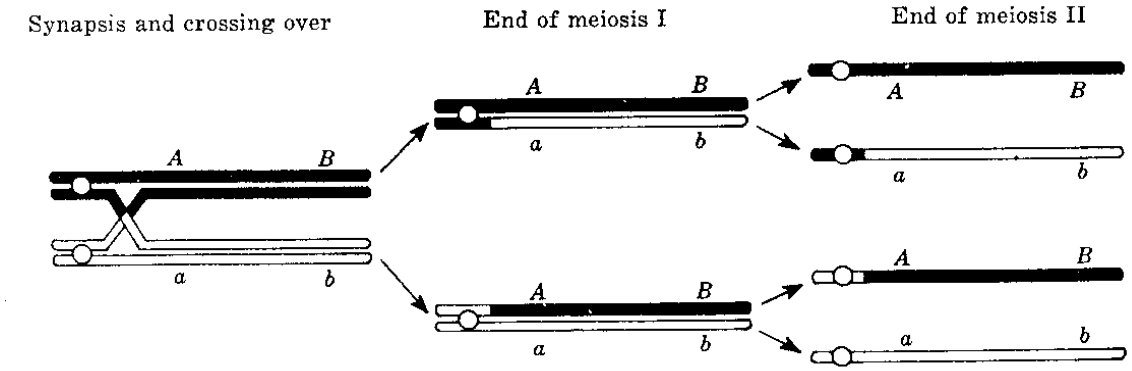
Gametes:
- Non-crossover: Ab, aB
- Crossover: AB, ab

3. Chiasma Frequency

A pair of synapsed chromosomes (bivalent) consists of four chromatids called a *tetrad.* Every tetrad usually experiences at least one chiasma somewhere along its length. Generally speaking, the longer the chromosome the greater the number of chiasmata. Each type of chromosome within a species has a characteristic (or average) number of chiasmata. The frequency with which a chiasma occurs between any two genetic loci also has a characteristic or average probability. The further apart two genes are located on a chromosome, the greater the opportunity for a chiasma to occur

between them. The closer two genes are linked, the smaller the chance for a chiasma occurring between them. These chiasmata probabilities are useful in predicting the proportions of parental and recombinant gametes expected to be formed from a given genotype. The percentage of crossover (recombinant) gametes formed by a given genotype is a direct reflection of the frequency with which a chiasma forms between the genes in question. Only when a crossover forms *between* the gene loci under consideration will recombination be detected.

Example 1: Crossing over outside the A-B region fails to recombine these markers.



When a chiasma forms between two gene loci, only half of the meiotic product will be of crossover type. Therefore chiasma frequency is twice the frequency of crossover products.

Chiasma % = 2 (crossover %) or Crossover % = ½ (chiasma %)

Example 2: If a chiasma forms between the loci of genes A and B in 30% of the tetrads of an individual of genotype *AB/ab*, then 15% of the gametes will be recombinant (*Ab* or *aB*) and 85% will be parental (*AB* or *ab*).

Example 3: Suppose progeny from the testcross *Ab/ab* X *ab/ab* were found in the proportions 40% *Ab/ab*, 10% *AB/ab* and 10% *ab/ab*. The genotypes *Ab/ab* and *ab/ab* were produced from crossover gametes. Thus 20% of all gametes formed by the dihybrid parent were crossover types. This means that a chiasma occurs between these two loci in 40% of all tetrads.

1. Two dominant mutants in the first linkage group of the guinea pig govern the traits pollex (Px), which is the ativistic return of thumb and little toe, and rough fur (R). When dihybrid pollex, rough pigs 9with identical linkage relationships) were crossed to normal pigs, their progeny fell into four phenotypes: 79 rough, 103 normal, 95 rough, pollex and 75 pollex, (a) determine the genotypes of the parents. (b) Calculate the amount of recombination between Px and R.

Solution:

(a) The parental gametes always appear with greatest frequency. In this case 103 normal and 95 rough, pollex. This means that the two normal genes were on one chromosome of the dihyrbid parent and the two dominant mutations on the other, (i.e., coupling linkage).

P : Px R / px r x px r / px r
pollex, rough normal

(b) The 79 rough and 75 pollex types are recombinants, constituting 154 out of 352 individuals = 0.4375 or approximately 43.8% recombination.

2. An individual homozygous for genes cd is crossed with wild type and F_1 back crossed with double recessive. The appearance of the offspring is as follows:

903 + +
897 *c d*
98 + d
120 *c* +

Explain the result, giving the strength of the linkage between *c* and *d*. If assortment between *c* and *d* were independent, what should be the result of this cross?

A. An individual homologous for genes *c* and *d* is crossed with wild type and the F_1 crossed back with the double recessive.

$$\frac{c\ d}{c\ d} \times \frac{+\ +}{+\ +}$$

F_1 genotypes: $\frac{+\ +}{c\ d}$

When this is crossed to its double recessive parent.

$$\frac{+\ +}{c\ d} \times \frac{c\ d}{c\ d}$$

The genotypes of progeny $\frac{+\ +}{c\ d}$ = 903

$\frac{+\ d}{c\ d}$ = 98

$$\frac{c +}{c\ d} = 102$$

$$\frac{c\ d}{c\ d} = \frac{897}{2000}$$

In this case $+ d$ and $c +$ are recombinants and the parental combinations $\frac{+ +}{c\ d}$ and $\frac{c\ d}{c\ d}$ are high in frequency. So were the chromosome (Loci) are in coupling phase. The recombinants are 200 out of 2000. So the percentage of Recombination = $\frac{200 \times 100}{2000} = 10\%$

So the strength of linkage is = 10%

If the assortment of *c* and *d* is independent the progeny will be in 1 : 1 : 1 : 1 ratio.

3. Three recessive genes in linkage group V of the tomato are *a* producing absence of anthocyanin pigment, *hl* producing hairless plants, and *j* producing jointless fruit stems (pedicels). Among 3000 progeny from a trihybrid testcross, the following phenotypes were observed:

259 hairless	268 anthocyaninless, jointless, hairless
40 jointless, hairless	941 anthocyaninless, hairless
931 jointless	32 anthocyaninless
260 normal	269 anthocyaninless, jointless

(a) How were the genes originally linked in the trihybrid parent?
(b) Estimate the distance between the genes.

Solution:

(a) The most frequent phenotypes observed among the offspring are the jointless (931) and anthocyaninless, hairless (941). Hene *j* was on one chromosome of the trihybrid parent, *a* and *hl* on the other. The double crossover (DCO) types are the least frequent phenotypes; jointless, hairless (40) and anthocyaninless (32).

Case 1. If jointless is in the middle, we could not obtain the double crossover types as given:

P: $\frac{A\ j\ Hl}{a\ J\ hl}$

F_1: DCO → *A J Hl* = normal

DCO → *a j hl* = triple mutant

Case 2. If *h* was in the middle, the double crossover types could be formed. Therefore the parental genotype is as shown below:

P: $\frac{J\ hl\ a}{j\ Hl\ A}$

F_1: DCO
- *J Hl a* = anthocyaninless
- *j hl A* = jointless, hairless

(b) Now that the genotype of the trihybrid parent is known, we can predict the single cross-over type.

P: $\frac{J\ hl\ a}{j\ Hl\ A}$

F_1: Single crossovers (SCO) between j and hl (region I) yield:

SCO (I)
- *J Hl A* = normal (260)
- *j hl a* = jointless, hairless, anthocyaninless (268)

Therefore, the percentage of all crossovers (singles and doubles) that occurred between j and hl is 260 + 268 + 32 + 40 = 600/3000 = 0.2 = 20% or 20 map units.

Similarly, the single crossovers between *hl* and *a* (region II) may be obtained.

P: $\frac{J\ hl\ a}{j\ Hl\ A}$

F_1: SCO (II)
- *J hl A* = hairless (259)
- *j Hl a* = jointless, anthocyaninless (269)

259 + 269 + 32 + 40 = 600 / 3000 = 0.2 = 20% or 20 map units

Note the similarity of numbers between the SCO (II) jointless, anthocyaninless (269) and the SCO (I) triple mutant (268). Attempts to obtain map distances by matching pairs with similar numbers could, as this case proves, lead to erroneous estimates. The single crossover types in each region must first be determined in order to avoid such errors.

J 20 hl 20 a

J Hl A

4. Chromosome Mapping in Maize (Chromosome-7 (Emerson, Beadle Fraser, 1935)

v = Virescent seedling
gl = Glossy seedling
va Variable sterile

$$\text{Test cross } \frac{+\ +\ +}{v\ gl\ va} \times v\ gl\ va$$

Test cross Progeny			Percentage of Recombinations
Wild type Virescent, Glossy, Variable Sterile	+ + + v gl va	235 Parental 270 Combinations	
Virescent Glossy, Variable Sterile	v + + + gl va	60 Single Cross Overs 65 Reg-I	18.3
Variable Sterile Virescent, Glossy	+ + va v gl +	40 Single Cross Overs 48 Reg-II	13.6
Glossy Virescent, variable sterile	+ gl + v + va	7 Double Cross 4 Overs	

Total Chromosome Map

v gl va

18.3 Units 13.6 Units

31.9 Units

Interference and Coincidence

Independent crossing over assumes that when two events are independent, the probability of their simultaneous occurrence is the product of their separate probabilities.

In the example given above in *Drosophila*

ec-cv … 10.2 per cent crossing over
cv-ct … 8.4 per cent crossing over

Hence, the frequency of expected double crossovers should be:

$$10.2 \times 8.4 = 0.86$$

But, in the actual experiment (observed) the frequency of double crossovers is 0.15 per cent. It seems, then, that the occurrence of crossing over at one point in the chromosome decreases the probability of its occurrence elsewhere in the same chromosome and the phenomenon is termed *Interference*. An inverse measure of interference is called *Coincidence*. It is defined as the ratio of observed double crossovers to the expected D.C.Os.

$$\text{Coefficient of coincidence} = \frac{\text{observed D.C.O.}}{\text{expected D.C.O.}}$$

$$\text{C.O.C.} = \frac{0.15}{0.86} = 0.17$$

17 per cent of the expected doubles are actually found. Interference is greatest in short distance and no double crossing over can be concluded when the coincidence = 0 and no interference when coincidence = 1. Complete interference gives a coincidence value of 0; coincidence of 1.0 indicates no interference whatsoever.

5. In corn, the following allelic pairs have been identified in chromosome 3:

+/b = Plant color booster vs. non booster
+/lg = liguled vs. liguleless
+/v = green plant vs. virescent

A test gross involving triple recessives and F_1 plants heterozygous for the three gene pairs gave in the progeny the following phenotypes:

+ v lg	305
b + lg	128
b v lg	18
+ + lg	74
b v +	66

+ + +	22
+ *v* +	122
b + +	275

Give the gene sequence, the map distances between genes, and the coefficient of coincidence.

Ans: According to the data
Parental combinations are

+ *v lg*	--	305
b + +	--	275

Double cross over types

b v lg	--	18
+ + +	--	22

If we note from three point test cross data, where allelic pair is transposed in order to make double cross over from parental type chromosomes, that allelic pair must be situated between the other two.

∴ Actual gene order may be

Parental types	*Double cross over types*
v + *lg*	*v b lg*
+ b +	+ + +

Single cross over at R-I

v + *lg*	+ + *lg* --	74
X		
+ *b* +	*v b* + --	66

Single cross over at R-II

v + *lg*	*v* + + --	112
X		
+ *b* +	+ *b lg* --	128

Double cross over at R-I and R-II

v	+	*lg*	*v* *b* *lg* --	18
X		X		
+	*b*	+	+ + + --	22

			Total no. of individuals =	1,000

The distance between v and b

Single cross over at R-I	+	+ *lg* --		74
	v	*b* + --		66
Double cross overs	*v*	*b* *lg* --		18
at R-I and R-II.	+	+ + --		22

				180

$\therefore$ The map distance between *v* and + = $\frac{180 \times 100}{1000}$ = 18 Units

The distance between b and lg

Cross over at R-II

	v	+ + --		112
	+	b lg --		128
Double cross over	v	b lg --		18
at R-I and R-II	+	+ + --		22

				280

$\therefore$ The map distance between *v* + and *lg* = $\frac{280 \times 100}{1000}$ = 28 Units

The distance between *v* and *lg*.

Single cross over at R-I

	+	+ lg --		74
	v	b + --		66
Double cross over at R-II	v	b lg --		112
	+	b lg --		128

$$\therefore \text{ The map distance between v and lg} = \frac{380 + 80}{1000} \times 100 = 46 \text{ units}$$

On construction of the genetic map

v 18 b 28 lg

|----------|----------|

Coefficient of Coincidence

$$\text{It is calculated by} = \frac{\text{observed number of double cross over}}{\text{expected number of double crossovers}}$$

When two events are independent, the probability of their simultaneous occurrence is the product of their separate probabilities.

If the crossing over in two regions (R-I and R-II) is independent we can expect = 0.18 x 0.28 = 0.0504

$\therefore$ Probability for double cross over in one individual = 0.0504

For 1000 individuals = 1000 x 0.0504 = 50.4

i.e. expected number of double cross overs = 50.4 observed double cross overs = 18 + 22 = 40

$$\therefore \text{ Coefficient of coincidence} = \frac{40}{50.4} = 0.7938$$

*****

8. (iv) PROBABILITY AND CHI-SQUARE TEST

1. (a) A coin is tossed 10 times and lands heads up 6 times and tails up 4 times. Are these results consistent with the expected 50-50 ratio? (b) If the coin is tossed 100 times with the same relative magnitude of deviation from the expected ratio, is the hypothesis still acceptable? (c) What conclusion can be drawn from the results of parts (a) and (b)?

Solution:

(a)

Phenotypic Classes	Observed (o)	Expected (e)	Deviations (o-e)	$(o-e)^2$	$(o-e)^2/e$
Heads	6	½ (10) = 5	1	1	$^1/_5 = 0.2$

Tails	$^4/_{10}$	$\frac{1}{2}(10) = \frac{5}{10}$	$\frac{-1}{0}$	1	$^1/_5 = \underline{0.2}$ $x^2 = 0.4$

Two mathematical check points are always present in the chi-square calculations. (1) The total of the expected column must equal the total observations. (2) The sum of the deviations should equal zero. The squaring of negative deviations converts all values to a positive scale. The number of degrees of freedom is the number of phenotypes minus one (2-1 = 1). We enter Table 7.2 on the first line (df = 1) and find the computed value of 0.4 lying in the body of the table between the values 0.15 and 0.46 corresponding to the probabilities 0.7 and 0.5 shown at the top of the respective columns. This implies that the magnitude of the deviation in our experimental results could be anticipated by chance alone in more than 50% but less than 70% of an infinite number of experiments of comparable size. This range of values is far above the critical probability value of 0.05 or 5%. Therefore, we accept the null hypothesis and conclude that our coin is conforming to the expected probabilities of heads = ½ and tails = ½ .

(b) In (a), heads appeared in 60% and tails in 40% of the tosses. The same relative magnitude of deviations will now be considered in a sample of size 100. In problems such as this, where expected values are equivalent in all the phenotypic classes, chi-square may be calculated more rapidly by adding the squared deviations and making a single division by the expected number.

Phenotypes	o	e	(o – e)	$(o - e)^2$
Heads	60	½ (100) = 50	10	100
Tails	40/100	$\frac{1}{2}(100) = \frac{50}{100}$	$\frac{-10}{0}$	100 ----- $x^2 = 200/50 = 4.0$

with df = 1, this x^2 value lies between 6.64 and 3.84 corresponding to the probabilities 0.01 and 0.05 respectively. This means that a deviation as large as or larger than the one observed in this experiment is to be anticipated by chance alone in less than 5% of an infinite number of trails of similar size. This is in the "critical region" and we are therefore obliged to reject the null hypothesis and conclude that our coin is not conforming to the expected 50-50 ratio. Either of two explanations may be involved: (1) this is not a normal well-balanced coin or (2) our experiment is among the one in twenty (5%) expected to have a large deviation produced by chance alone.

(c) The results of parts (a) and (b) demonstrate the fact that large samples provide a more critical test of a hypothesis than small samples. Proportionately larger deviations have a greater probability of occurring by chance in small samples than in large samples.

2. Determine the probability of obtaining 6 heads and 3 tails in 9 tosses of a coin by applying formula Binomial method.

Solution:

In tossing a coin, we may consider heads to be a success (a) and talls a failure (f). The probability of obtaining 6 successes and 3 failures in 9 trials is

$$\frac{n!}{s!\,f!}\,p^s q^f = \frac{9!}{6!\,3!}\,p^6 q^3 = \frac{9 \cdot 8 \cdot 7 \cdot 6!}{1 \cdot 2 \cdot 3 \cdot 6!}\,p^6 q^3 = 84\,p^6 q^3$$

If the probability of obtaining a head (p) is equal to the probability of obtaining a tail (q) = ½, then $84p^6q^3 = 84(½)^9 =$

$$84 \times \frac{1}{2} \times \frac{1}{2} \times \frac{1}{2} \times \frac{1}{2} \times \frac{1}{2} \times \frac{1}{2} \times \frac{1}{2} \times \frac{1}{2} \times \frac{1}{2} \times \frac{1}{2} = \frac{84}{512} = \frac{21}{128}$$

3. Assuming a 1:1 sex ratio, what is the probability that a family of 6 children will consist of

(a) 3 boys and 3 girls?
(b) a boy, a girl, a boy, a girl, a boy, and a girl in this order?
(c) all girls?
(d) all the same sex?
(e) at least 4 girls?

Ans: If the family has 6 children assuming the sex ratio as 1:1 the probability of

(a) 3 boys and 3 girls will be $\frac{6!}{3!3!}\left[\frac{1}{2}\right]^3\left[\frac{1}{2}\right]^3$

$$P = \frac{5}{16} = 0.3125$$

The probability of getting 3 boys and 3 girls in a family of 6 children is = 0.3125

(b) A boy, a girl, a boy, a girl, a boy and a girl will be = ½ x ½ x ½ x ½ x ½ x ½ = $^1/_{64}$ = 0.01563

Since the sex ratio is 1:1, there is 0.5 probability to get a boy or girl. The product of their probabilities will give the probability to get the children in the above order.

(c) To get 4 girls out of 6 children, we have to consider the other 2 children.

Out of 6, we have 4 girls. So the rest 2 may be both boys, girls or a boy and a girl. To get the probability of at least 4 girls, we have to *sum* the probabilities of those other 2 children also.

So assuming that other 2 are boys

$$P = \frac{6!}{4!\,2!} (½)^4 (½)^2 = 15/64 \quad (1)$$

Assuming that there are 5 girls and 1 boy, then the probability of 5 girls

$$P = \frac{6!}{5!\,1!} (½)^5 (½)^1 = 6/64 \quad (2)$$

Assuming that all 6 children are girls, their probability is $= \frac{6!}{6!\,0!} (½)^6 (½)^0 = {}^1/_{64}$ (3)

Rule: (The probability that one of several mutually exclusive events will occur is equal to the *sum* of their probabilities).
So the total sum of each individual probability will give the probability of getting at least 4 girls out of 6 children.

$\therefore$ $P = {}^{15}/_{64} + {}^{6}/_{64} + {}^{1}/_{64} = {}^{22}/_{64} = 0.344$

4. In the garden pea, yellow cotyledon color is dominant to green, and inflated pod shape is dominant to the constricted form. When both of these traits were considered jointly in self-fertilized dihybrids, the progeny appeared in the following numbers: 193 green, inflated: 184 yellow, constricted: 556 yellow, inflated: 61 green, constricted. Test the data for independent assortment.

Solution:

P: *Gg Cc* x *Gg Cc*
yellow, inflated yellow inflated

F_1: (expectations)
${}^9/_{16}$ *G-C-* yellow, inflated
${}^3/_{16}$ *G-cc* yellow, constricted
${}^3/_{16}$ *gg C-* green, inflated
${}^1/_{16}$ *gg cc* green, constricted

Phenotypes	Observed	Expected	Deviation d	d^2	d^2/e
yellow, inflated	556	9/16(994)=559.1	-3.1	9.61	0.017
yellow, constricted	184	3/16(994) = 186.4	-2.4	5.76	0.031
green, inflated	193	3/16(994) = 186.4	6.6	43.56	0.234
green, constricted	61	1/16(994) = 62.1	-1.1	1.21	0.019
	994	994.0	0		x^2=0.301

$$df = 4 - 1 = 3,\ p > 0.95$$

This is not a significant chi-square value, and thus we accept the null hypothesis, i.e. the magnitude of the deviation (o – e) is to be expected by chance alone in greater than 95% of an infinite number of experiments of comparable size. This is far above the critical value of 5% necessary for acceptance of the hypothesis. We may therefore accept the data as being in conformity with a 9:3:3:1 ratio, indicating that the gene for cotyledon color assorts independently of the gene for pod form.

5. The flowers of four o'clock plants may be red, pink, or white. Reds crossed to whites produced only pink offspring. When pink flowered plants were crossed they produced 113 red, 129 white and 242 pink. It is hypothesized that these colors are produced by a single gene locus with codominant alleles. Is this hypothesis acceptable on the basis of a chi-square test?

$$\sum \frac{(O-E)^2}{E}$$

Phenotypes	Observed	Expected	Deviation	d^2	d^2/E
Red	113	$\frac{1}{4} \times 484 = 121$	-82	64	$\frac{64}{121}$
Pink	242	$\frac{1}{2} \times 484 = 242$	- 0	0	$\frac{0}{484}$
White	$\frac{129}{484}$	$\frac{1}{4} \times 484 = 121$	8	64	$\frac{64}{121}$ -----

$$= \frac{64}{121} + \frac{0}{484} + \frac{64}{121} = \frac{512}{484} = 1.06$$

Ans: Yes. $X^2 = 1.06$; p = 0.5-0.7; acceptable

Table 1 Chi-Square Distribution

Degrees of Freedom	Probability										
	0.95	0.90	0.80	0.70	0.50	0.30	0.20	0.10	0.05	0.01	0.001
1	0.004	0.02	0.06	0.15	0.46	1.07	1.64	2.71	3.84	6.64	10.83
2	0.10	0.21	0.45	0.71	1.39	2.41	3.22	4.60	5.99	9.21	13.82
3	0.35	0.58	1.01	1.42	2.37	3.66	4.64	6.25	7.82	11.34	16.27
4	0.71	1.06	1.65	2.20	3.36	4.88	5.99	7.78	9.49	13.28	18.47
5	1.14	1.61	2.34	3.00	4.35	6.06	7.29	9.24	11.07	15.09	20.52
6	1.63	2.20	3.07	3.83	5.35	7.23	8.56	10.64	12.59	16.81	22.46
7	2.17	2.83	3.82	4.67	6.35	8.38	9.80	12.02	14.07	18.48	24.32
8	2.73	3.49	4.59	5.53	7.34	9.52	11.03	13.36	15.51	20.09	26.12
9	3.32	4.17	5.38	6.39	8.34	10.66	12.24	14.68	16.92	21.67	27.88
10	3.94	4.86	6.18	7.27	9.34	11.78	13.44	15.99	18.31	23.21	29.59
	Non-significant								**Significant**		

Source: R. A. Fisher and F. Yates,- Statistical Tables for Biological, Agricultural and Medical Research (6th edition), Table IV, Oliver & Boyd, Ltd., Edinburgh, by permission of the authors and publishers.

8(v). SEX-LINKED / SEX INFLUENCED (INHERITANCE)

1. A sex-linked recessive gene c produces red-green color blindness in humans. A normal woman whose father was color blind marries a color blind man. (a) What genotypes are possible for the mother of the color blind man? (b) What are the chances that the first child from this marriage will be a color blind boy? (c) Of all the girls produced by these parents, what percentage is expected to be color blind? (d) Of all the children (sex unspecified) from these parents, what proportion is expected to be normal?

Normal woman Color blind man

a) X^CX x X^CY

X X

b) X^CY, XY, X^CX^C, XX^C

Colour blind boy	Normal boy	Color blind girl	Normal but carrier girl

Ans: (a) Cc or cc (b) ¼ (c) 50% (d) ½

2. A narrow reduced eye called "bar" is a dominant sex-linked condition (B) in *Drosophila*, and the full wild type eye is produced by its recessive allele B^+. A homozygous wild type female is mated to a bar-eyed male. Determine the F_1 and F_2 genotypic and phenotypic expectations.

B^+B^+ x BB

F_1 B^+B bar eyed females

B^+Y wild type males

F_2 Females

F_2 males

3. A certain type of white forelock in humans appears to follow the sex-influenced mode of inheritance, being dominant in men and recessive in women. Using the allelic symbols w and w′, indicate all possible genotypes and the phenotypes thereby produced in men and women.

Ans:

Genotypes	Men	Women	Genotypes
w dominant in men:			w′ dominant in men:
ww	forelock	forelock	w′w′
ww′	forelock	normal	(or) w′w
w′w′	normal	normal	ww

8(vi). CYTOPLASMIC INHERITANCE

1. The hermaphroditic snail *Limnaea peregra* can reproduce either by crossing or by self-fertilization. A homozygous dextral snail is fertilized with sperm from a homozygous sinistral snail. The heterozygous F_1 undergoes two generations of self-fertilization. (a) What are the phenotypes of the parental individuals? (b) Diagram the parents, F_1, and two selfing generations, showing phenotypes and genotypes and their expected ratios.

Solution:

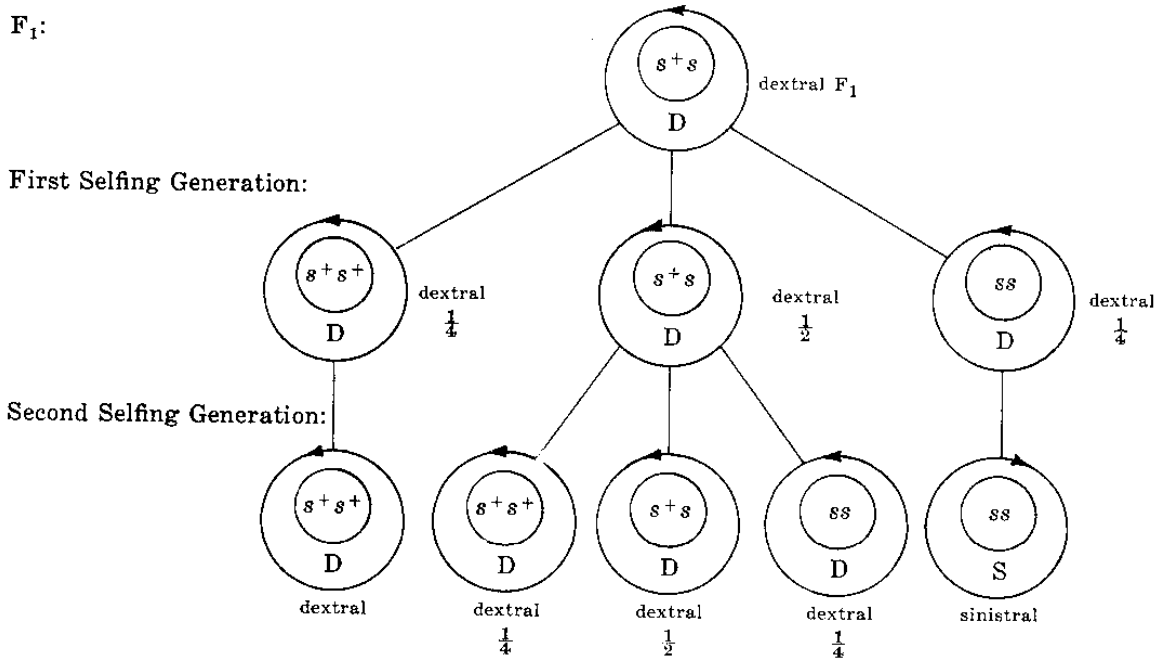
(a) Although we know the genotypes of the parents, we have no information concerning the genotype of the immediate maternal ancestor which was responsible for the organization of the egg cytoplasm from which our parental individuals developed. Therefore, we are unable to determine what phenotypes these individuals exhibit. Let us assume for the purpose of diagramming part (b) that the material parent is dextral and the paternal parent is sinistral.

(*b*) Let D = dextrally organized cytoplasm, S = sinistrally organized cytoplasm.

Parents:

dextral egg parent

sinistral sperm parent



Notice that the F_1 is coiled dextrally, not because its own genotype is s^+/s, but because the maternal parent possessed the dominant dextral gene s^+. Likewise in the first selfing generation, all are phenotypically dextral regardless of their own genotype because the F_1 was s^+/s. In the second selfing generation, we expect the following:

First Selfing Generation		Second Selfing Generation		
$\frac{1}{4}\ s^+s^+$	×	all s^+s^+	=	$\frac{1}{4}\ s^+s^+$
$\frac{1}{2}\ s^+s$	×	$\frac{1}{4}\ s^+s^+$	=	$\frac{1}{8}\ s^+s^+$
		$\frac{1}{2}\ s^+s$	=	$\frac{1}{4}\ s^+s$
		$\frac{1}{4}\ ss$	=	$\frac{1}{8}\ ss$
$\frac{1}{4}\ ss$	×	all ss	=	$\frac{1}{4}\ ss$

Summary

Genotypes				Phenotypes
s^+s^+	= $\frac{1}{4}$	+ $\frac{1}{8}$	= $\frac{3}{8}$	$\frac{5}{8}$ dextral
s^+s	= $\frac{1}{4}$		= $\frac{2}{8}$	
ss	= $\frac{1}{8}$	+ $\frac{1}{4}$	= $\frac{3}{8}$	sinistral

2. The killer trait in *Paremecium* is due to symbiotic particles called kappa in their cytoplasm. A killer paramecium of genotype Kk conjugates with a sensitive paramecium of the same genotype without cytoplasmic transfer. One of the exconjugants is killer, the other is sensitive. Each of these conjugants undergoes autogamy. Predict the genotypes and phenotypes of the autogamous products.

Solution:

Reciprocal fertilization in paramecia produces identical genotypes in the exconjugants. In the micronuclear heterozygotes Kk, which of the four haploid nuclei produced by meiosis (K, K, k, k) will survive depends on chance. Thus whether cytoplasmic mixing occurs or not, both exconjugants are expected to be KK 25% of the time, Kk 50% of the time, and kk 25% of the time. Autogamy of the exconjugants cannot produce any genotypic change in the homozygotes, but does produce hymozygosity in heterozygotes. Half of the autogamous products of the monohybrid Kk are expected to be KK and half kk.

Exconjugants with Kappa (Killers)		Autogamous Products	
¼ KK	=	¼ KK	½ KK (killer)
½ Kk	=	½ KK = ¼ KK	
	=	½ kk = ¼ kk	½ kk (unstable)
¼ kk	=	¼ kk	

The same genotypic ratios would be expected in the autogamous products of exconjugants without kappa, but phenotypically they would all be sensitive.

3. Male sterile plants in corn may be produced either by a chromosomal gene or by a cytoplasmic factor. (a) At least 20 different male sterile genes are known in maize, all of which are recessive. Why? Predict the F_1 and F_2 results of pollinating (b) a genetic male sterile by a normal, (c) a cytoplasmic male sterile by a normal.

Solution:

(a) A plant in which a dominant genic male sterile gene arose by mutation of a normal gene would be unable to fertilize itself and would be lost unless cross-pollinated by a fertile plant. the gene would be rapidly eliminated from heterozygotes within a few generations by continuous back crossing to normal pollen parents. (b) F_1: +/*ms*, fertile; F_2: ¼ +/+, ½ +/*ms*, ¼ *ms*/*ms*; ¾ fertile, ¼ male sterile. (c) male sterile cytoplasm is transmitted to all F_1 progeny; a selfed F_2 cannot be produced because none of the F_1 plants can make fertile pollen.

4. When a killer strain of paramecia conjugates without cytoplasmic transfer with a sensitive strain, half of the exconjugants are sensitives and half are killers. Autogamy of the killer exconjugants produces only killers; autogamy of the sensitive exconjugants produces only sensitives. What are the genotypes of (a) the two original strains, (b) the exconjugants, (c) the autogamous products?

Ans: (a) (b) (c) All killers are KK with kappa; all sensitives are KK without kappa.

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8(vii). MULTIPLE FACTOR INHERITANCE

1. Assume that in squashes the difference in fruit weight between a 3-pound type and a 6-pound type is due to three factor pairs, *AA, BB* and *CC*, each factor contributing ½ pound to fruit weight. Cross a 3-pound plant (*aa bb cc*) with a 6-pound one. What will be the phenotypes of the F_1; of the F_2?

Ans: In squashes the difference in fruit weight is due to three gene pairs *AA, BB, CC*, each dominant gene contributing ½ pound to the fruit weight.

The weight of the triple recessive plant is 3 pounds and of the triple dominant is 6 pounds.

when these plants (*AA BB CC* x *aa bb cc*) are crossed

	AA BB CC	x	*aa bb cc*	
	ABC		*abc*	
F_1	---	*Aa Bb Cc*	---	all 4.5 pound plants

Since the F1 plants have 3 dominant genes, these will add 3 halves, i.e. 1½ pounds to the triple recessive fruit weight, making the total weight of F_1 plants = 4.5 lbs. When these 4.5 lbs F_1 plants are selfed to raise F_2 progeny.

Aa Bb Cc x *Aa Bb Cc*

F_2 Phenotypes

	Plants	Weight
	1	6.0 lbs
	8	5.5 lbs
	15	5.0 lbs
	19	4.5 lbs
	15	4.0 lbs
	7	3.5 lbs
	1	3.0 lbs
Total =	64	

Quasi-Quantitative Traits

2. Two homozygous varieties of *Nicotiana longiflora* have mean corolla lengths of 40.5 mm and 93.3 mm. The average of the F_1 hybrids from these two varieties was of intermediate length.

Among 444 F_2 plants, none was found to have flowers either as long as or as short as the average of the parental varieties. Estimate the minimal number of pairs of alleles segregating from the F_1.

Solution:

If four pairs of alleles were segregating from the F_1, we expect $(¼)^4 = {}^1/_{256}$ of the F_2 to be as extreme as one or the other parental average. Likewise, if five pairs of alleles were segregating, we expect $(¼)^5 = {}^1/_{1024}$ of the F_2 to be as extreme as one parent or the other. Since none of the 444 F_2 plants had flowers this extreme, more than four loci (minimum of five loci) are probably segregating from the F_1.

2. The mean internode length of the Abed Binder variety of barley was found to be 3.20 mm. The mean length in the Asplund variety was 2.10 mm. Crossing these two varieties produced an F_1 and F_2 with average internode lengths of 2.65 mm. About 6% of the F_2 had an internode length of 3.2 mm and another 6% had a length of 2.1 mm. Determine the most probable number of gene pairs involved in internode length and the approximate contribution each gene makes to the phenotype.

Solution:

With one pair of genes were expect about ¼ or 25% of the F_2 to be as extreme as one of the parents. With two pairs of genes we expect approximately ${}^1/_{16}$ or 6.25% as extreme as one parent. Thus we may postulate two pairs of genes. Let *A* and *B* represent growth factors and *a* and *b* represent null genes.

P: *AA BB* *x* *aa bb*
Abed Binder Asplund
4 growth genes = 3.2 mm No growth genes = 2.1 mm

F_1: Aa Bb
2 growth genes = 2.65 mm

The difference 2.65 - 2.10 = 0.55 mm is the result of two growth genes. Therefore each growth gene contributes 0.275 mm to the phenotype.

F_2:

No. of Growth Genes	Mean Internode Length (mm)	Frequency	Genotypes
4	3.200	${}^1/_{16}$	AABB
3	2.925	¼	AaBB, AABb
2	2.650	${}^3/_8$	Aabb, AaBb, aaBB
1	2.375	¼	aaBb, Aabb
	2.100	${}^1/_{16}$	aabb (physiological minimum due to residual genotype)