

Object — Study of meiosis cell division in temporary squash preparation of grasshopper testis.

Requirements : —

- (i) Grasshopper.
- (ii) Spirit lamp.
- (iii) Slide, Cover glass.
- (iv) Needle, filter paper.
- (v) Sealing material.

Reagents required : —

- (i) 0.67% NaCl solution
- (ii) Carnoy's fluid - I
- (iii) 70% alcohol
- (iv) Aceto carmine stain.
- (v) 45% glacial acetic acid.

Composition of Carnoy's fluid - I : —

- (i) Absolute alcohol → 60 cc.
- (ii) Glacial acetic acid → 20 cc.

Composition of Aceto Carmine Solution : —

- (i) Distilled water → 55 cc.
- (ii) Glacial acetic acid → 45 cc.
- (iii) Carmine powder - 1 gm.

Procedure

- (i) The testis lobes were dissected out from the grasshopper in 0.67% NaCl solution and fixed in Carnoy's fluid - I in a small

tube for 2-12 hours.

(ii) The lobes were fixed in 70% alcohol after fixation.

(iii) For staining, the lobes are taken out from 70% alcohol & placed in aceto-carmine stain for 45-60 min.

(iv) 4-5 lobes are taken out from the staining solution and kept on the centre of the slide for squashing.

(v) 2-3 drops of 45% glacial acetic acid were poured on the lobules on the slide & after about 30 seconds excess acetic acid was removed from the slide with the help of a blotting paper.

(vi) The slide is then kept on the table & lobules are covered with a cover glass. A blotting paper is then placed on the slide & a uniform pressure is applied with the thumb on the cover glass.

(vii) The slide is heated gently to remove the excess glacial acetic acid from the squashed material.

(viii) The squashed material is then sealed with nail polish.

(ix) The slide is thoroughly examined under microscope & different stages of meiosis are observed in properly squashed area.

Observation

Leptotene: Character

- (i) Chromatin threads shows beaded structure
- (ii) Chromosome are extremely long as well as their thread like structure
- (iii) Counting of chromosomes is not possible as they remain entangled with each other.
- (iv) Nuclear membrane is present.
- (v) X-chromosome forms hyperpycnotic irregular deep stained body attached to nuclear membrane.

Hence, it is leptotene stage of Meiosis I

Zygotene: —

- (i) Chromosome number is not countable.
- (ii) Chromosomes maintain their beaded appearance & are not visible distinctly.
- (iii) Lengthwise pairing called synapsis occurs in certain parts of the homologous chromosomes (reported). Each pair is known as bivalent.
- (iv) Nuclear membrane present.
- (v) X-chromosome appear in the form of irregular deep stained hyperpycnotic body ^{at} the periphery.

Pachytene: —

- (i) Chromosomes are apparently visible, each composed of 2 chromatids
- (ii) Each bivalent shows tetrad structure, consist of 4 chromatids
- (iii) Genetical crossing over occurs at certain points called chiasma (reported), but these are not visible because

- Chromosome remain closely apposed to each other.
- (iv) Deep stained X-chromosome with 2-chromatids present at the periphery.
 - (v) Nuclear membrane is still visible.

Diplotene : -

- (i) The bivalent are distinctly visible as they are more or less shortened & thickened.
- (ii) Homologous chromosomes are somewhat pulled away from each other by they remain attached at the certain points called chiasmata.
- (iii) Bivalents are of differt size & shape depending on the number of chasms.
- (iv) 11 bivalents & 1 univalent are present.
- (v) No nuclear nubrane.
- (vi) X-chromosome is hyperpyrenotic & arranged at somewhat peripheral position.

Diakinesis : -

- (i) The chromosomes are too much condensed in structure if being deeply stained.
- (ii) Most of the homologous are ring like or oval in appearance & some are cross-shaped.
- (iii) Terminalisation is completely complete in shorter chromosomes but incomplete in longer ones.

- (iv) X-chromosome & autosome show identical staining.
- (v) Nuclear membrane absent.

Metaphase I -

- (i) Chromosomes are highly condensed & deeply stained.
- (ii) Bivalent chromosomes are arranged in either a circular pattern (polar view) or a linear array (side view) at the equatorial region.
- (iii) The centromeres (2 in number) of each bivalent lie equidistant on opposite side of the equatorial line, while chiasma is on the equatorial line.
- (iv) Nuclear membrane completely disappears.
- (v) X-chromosome is slightly light-stained & displaced from the rest.

Date _____

Anaphase I



- (i) Homologous chromosomes are separated from each other forming 2 sets of univalent chromosome, each set is facing opposite to each other.
- (ii) All the chromosomes are V-shaped in structure, the centromere of each chromosome is directed toward the pole, while telomere toward the equator.
- (iii) One side contain an extra chromosome (X-chromosome) than the other side.
- (iv) X-chromosome is identified as light stained rod like structure.
- (v) Nuclear membrane is absent.

Telophase-I

- (i) Each set of chromosomes are at the opposite pole where they remain entangled with each other.
- (ii) A few V-shaped chromosomes can be distinguished in each clump.
- (iii) Chromosome number is not countable.

Prophase-II

- (i) Chromosomes are light stained and haploid in number.
- (ii) Each chromosome consist of 2 chromatids attached to the centromere without chiasma.
- (iii) No nuclear membrane.

Date ___ / ___ / ___

Metaphase II: —

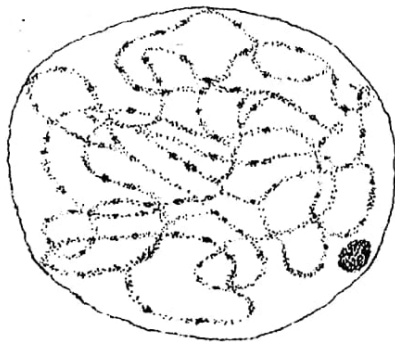
- (i) The dark stained chromosomes are arranged in a circular conformation (polar view) or in a linear array (side view) on the equator.
- (ii) Each chromosome consists of 2 chromatids, therefore they appear in the form of paired rod.
- (iii) Centromeres remain on the equator, while the arms are directed towards the periphery.
- (iv) The chromosome number is haploid.
- (v) X-chromosome is not clearly visible.

Anaphase II: —

- (i) Chromosomes are separated from each other forming 2 sets of chromatids, each containing equal number of chromatids (daughter chromosome).
- (ii) Chromosomes are rod shaped and move towards the poles.
- (iii) In a polar view, monads appear like a circle of thick rod.
- (iv)

Telephase II: —

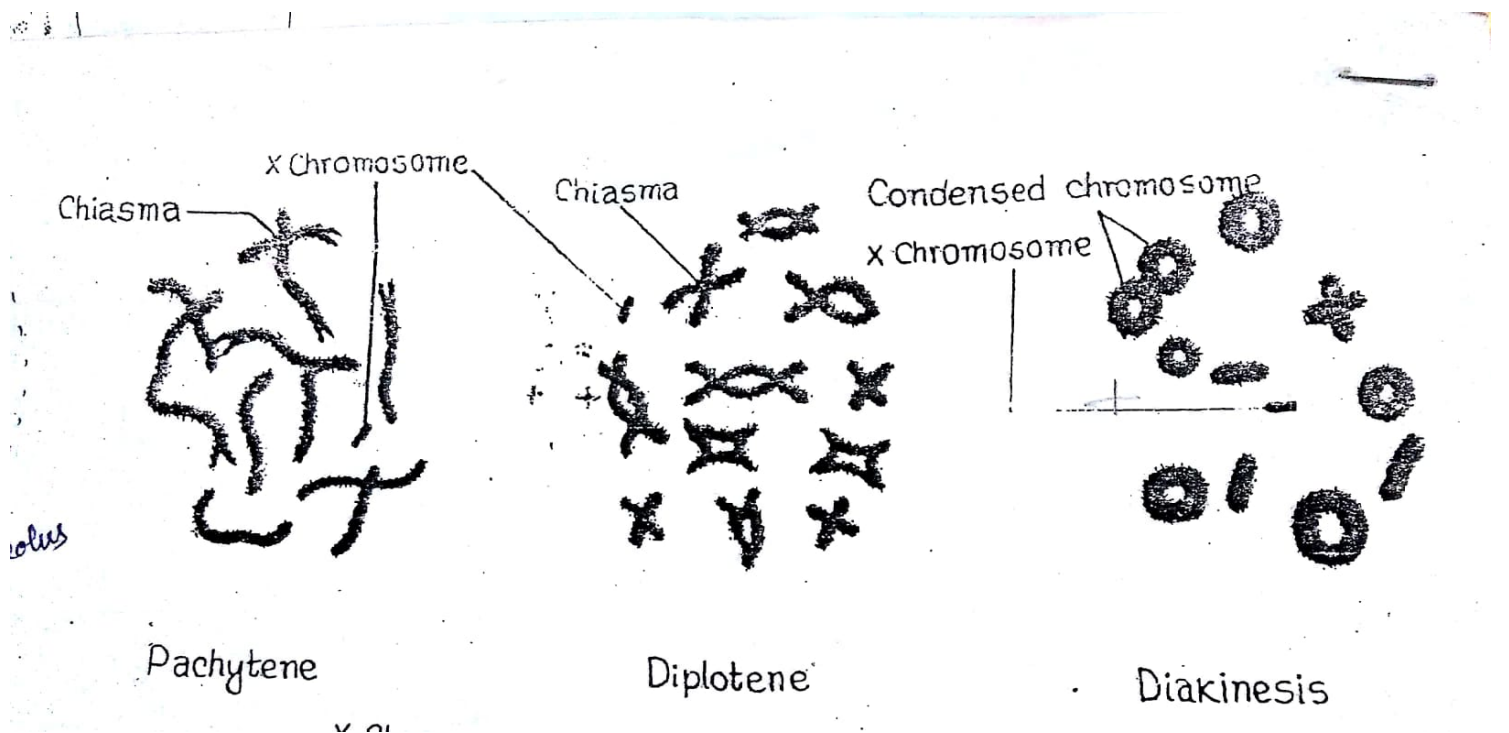
- (i) Chromosomes are at the opposite poles of the cell.
- (ii) Chromosomes are thin and entangled with each other at the pole.
- (iii) Nuclear membrane present.



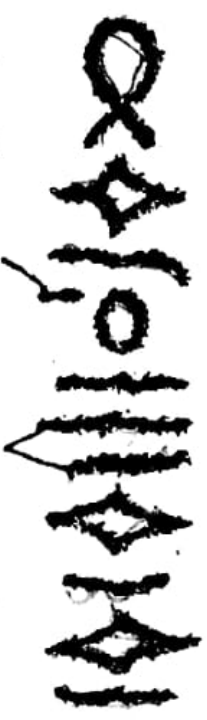
Leptotene : prophase-I



Zygotene : prophase-I



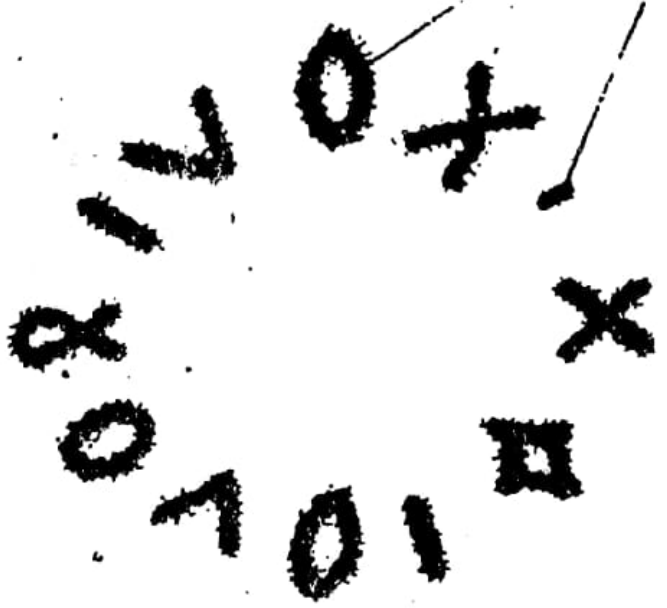
X Chromosome
Chromosomes



X Chromosome

Chromosomes at
equatorial plane

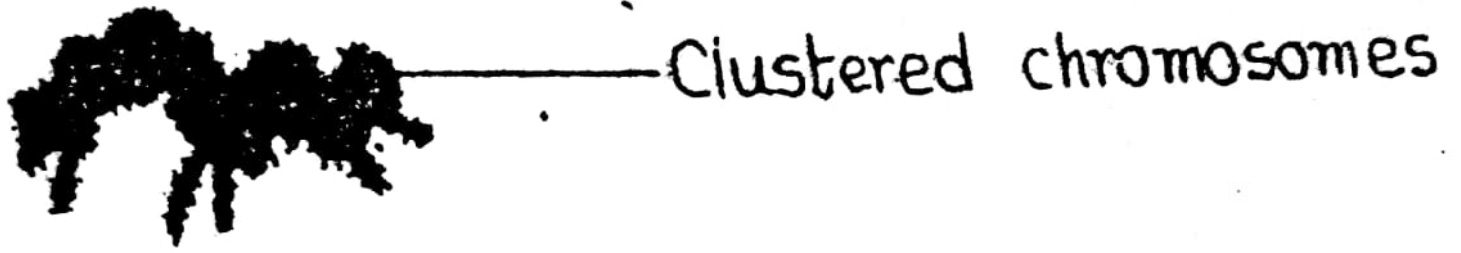
Metaphase I (Side view)



Metaphase I (Polar view)



Anaphase - I.



Equator

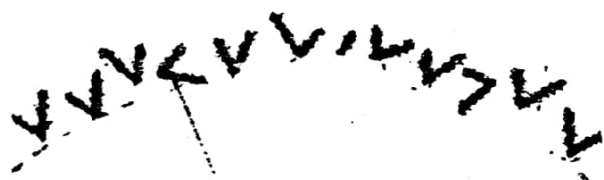


Telophase I



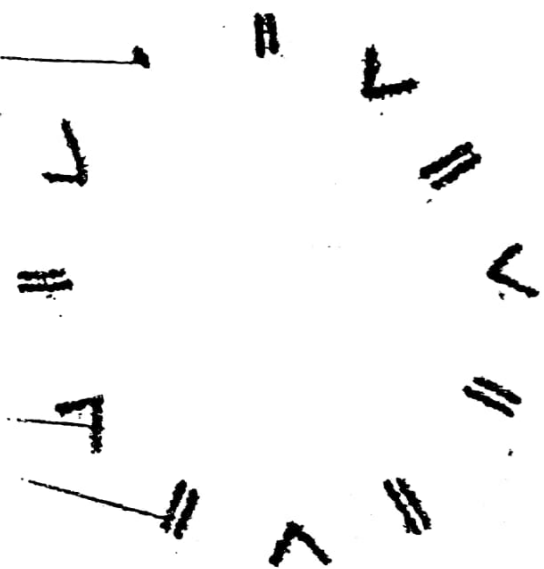
Prophase- II

X-Chromosome



Chromosomes

Metaphase II (Side view)



Metaphase II (Polar view)



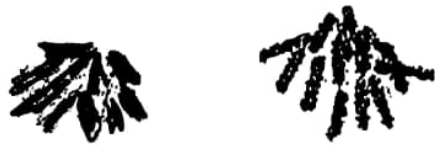
Anaphase - II

• 012

— Bar
Shaped
Chromosomes



) Anaphase II (Polar view)



Telophase - II
