Lab Experiment: Grasshopper testes for meiosis and related features

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Meiosis

- Meiosis is a type of cell division mostly occur in germinal layer that give rise to gametes – gametogenesis.
- Meiosis reduces the number of chromosomes by half.
- Daughter cells are different from parent, and each other.
- It involves two divisions – Meiosis I & Meiosis II.
- It is also preceded by interphase as in mitosis.

What is the difference between mitosis and meiosis?

Meiosis

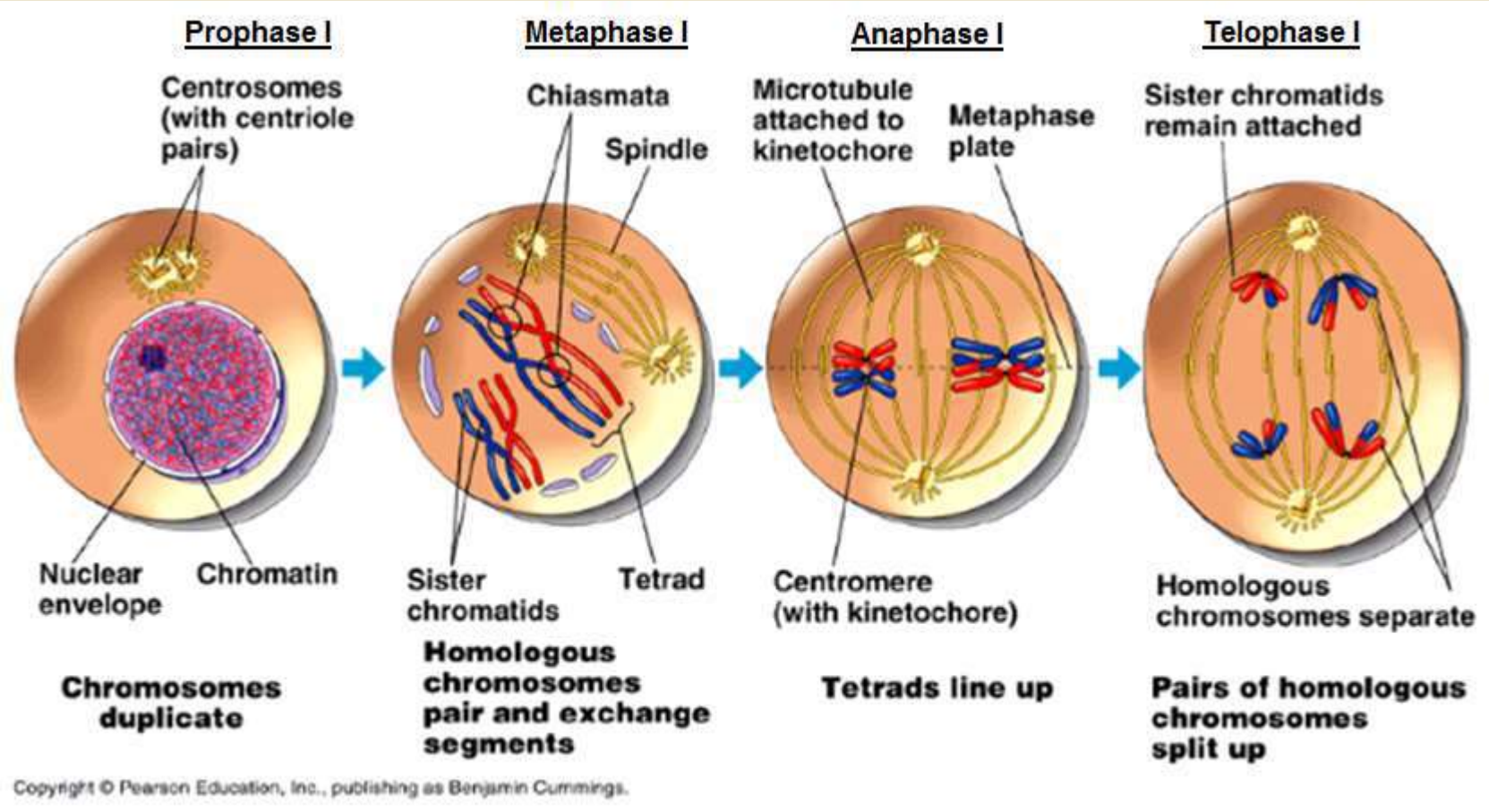
Meiosis I

First division of meiosis; like mitosis it has also for stages; Prophase I, Metaphase I, Anaphase I, and Telophase I; forms 2 diploid cells.

- ***Prophase I:*** Duplicated chromosomes condenses and remains closely associated – called as sister chromatids – forms tetrads. Crossing-over can occur during late phase of this stage.
- ***Metaphase I:*** Homologous chromosomes align at the equatorial plate (tetrad line up), Crossing over completed before this stage.
- ***Anaphase I:*** Homologous pairs separate with sister chromatids remaining together.
- ***Telophase I:*** Two daughter cells are formed with each daughter containing only one chromosome of the homologous pair.

Meiosis

Meiosis I



Meiosis

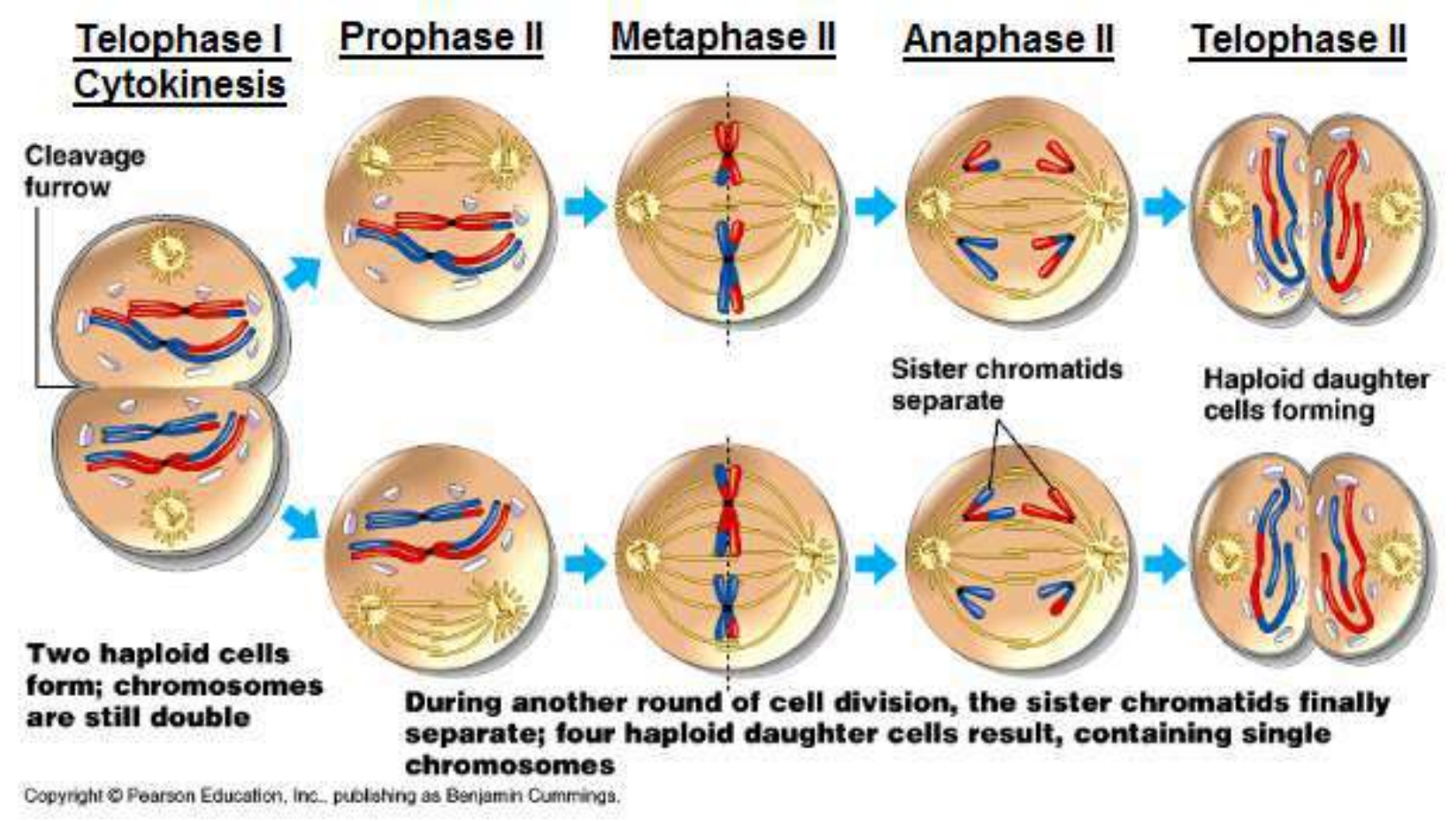
Meiosis II

Second of meiosis; sometimes preceded with brief interphase (lacks 'S-phase')**; it has also for stages; Prophase II, Metaphase II, Anaphase II, and Telophase II; forms 4 haploid gametes.**

- ***Prophase II:*** Chromosomes condenses; nuclear envelope disintegrates; duplicated centrosome (interphase) move away toward opposite poles; new spindle forms.
- ***Metaphase II:*** Nuclear envelope completely disappears; sister chromatids are aligned at the equator of the cell.
- ***Anaphase II:*** Sister chromatids are pulled apart and move toward opposite poles assisted by kinetochrore microtubules.
- ***Telophase II:*** Chromosomes arrive at opposite poles and begin to decondensates; nuclear envelope forms around the chromosomes.

Meiosis

Meiosis II



Meiosis

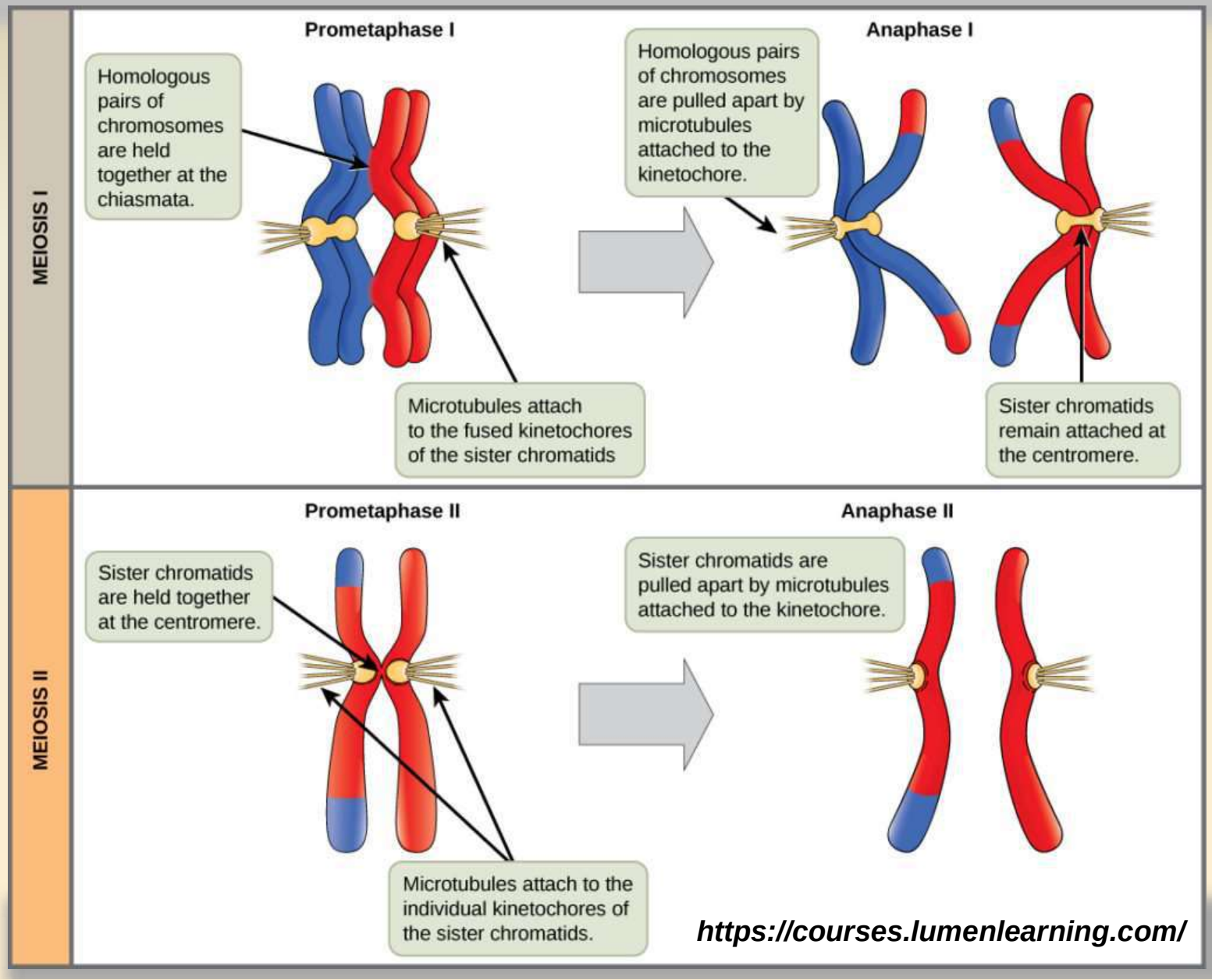
Interphase: Cells grows, replicates its chromosomes, and checks all of its systems to ensure that it is ready to divide. It has 3 sub-phases; G1 (Gap 1), S (Synthesis), and G2 (Gap 2).

- ***Interphase I:*** *It is the stage before prophase I. It is similar to the interphase of mitosis in which cells are preparing for meiotic division.*
- ***Interphase II:*** *It is the stage before prophase II. S sub-phase is absent .*

Prometaphase: It is the intermediate phase between prophase and metaphase in both division, where microtubules attach at the chromosomes' kinetochores and the nuclear envelope breaks down..

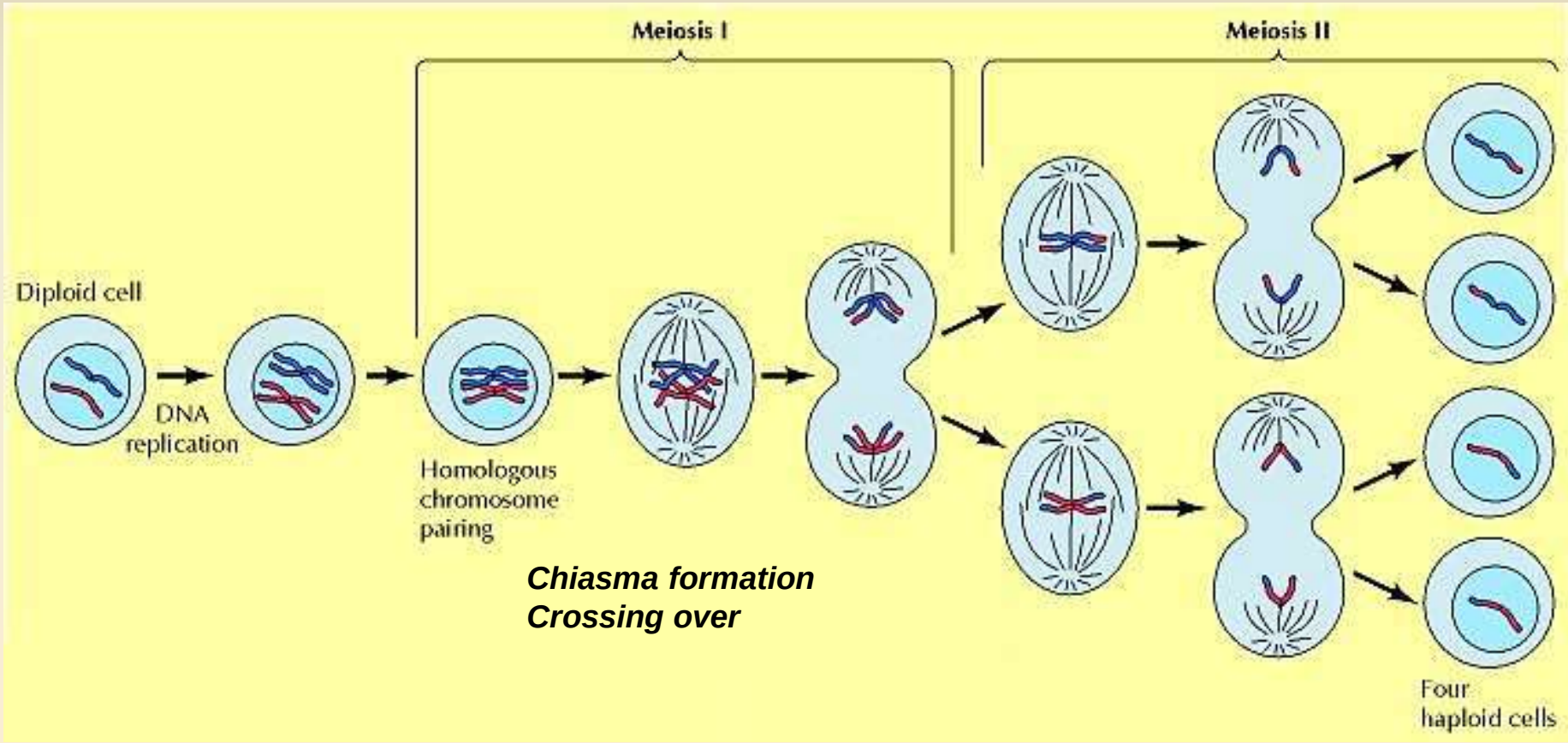
Gap 2 is the last sub-phase & S is longest sub-phase.

Meiosis



https://courses.lumenlearning.com/

Meiosis



It is also called reductional division.

Meiosis

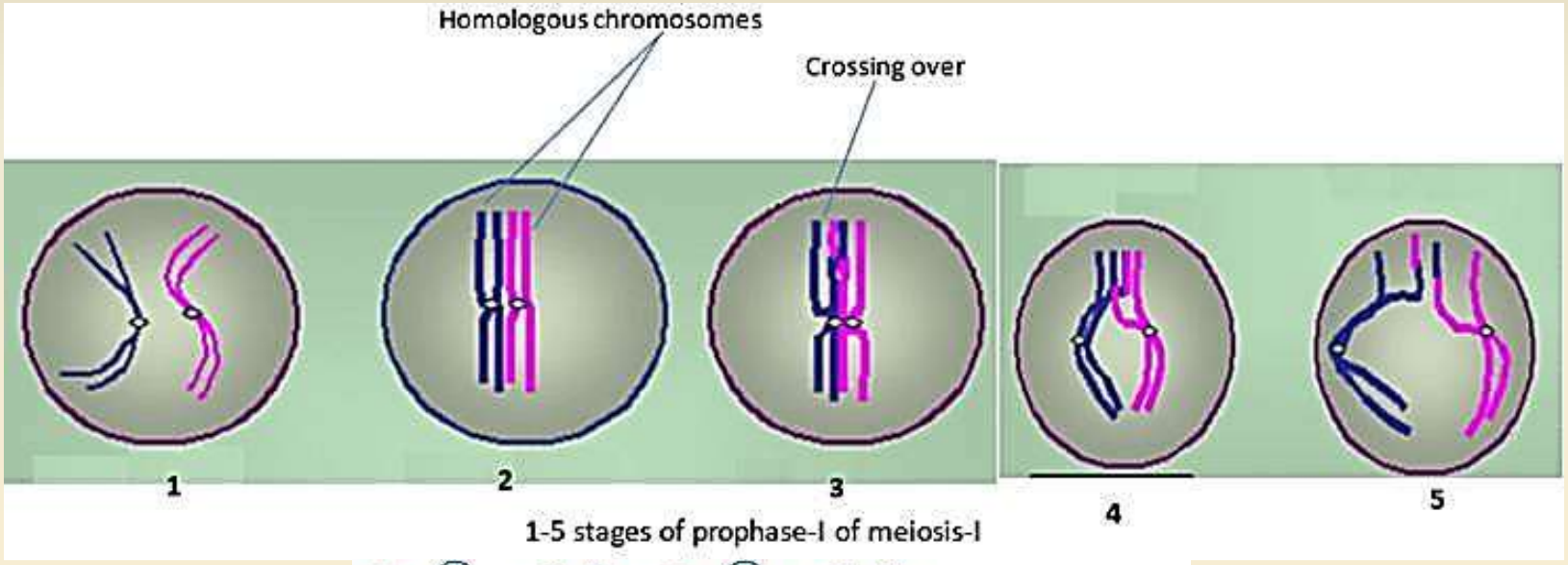
Different sub-stages of prophase I

Prophase I can be divisible into 5 sub-stages.

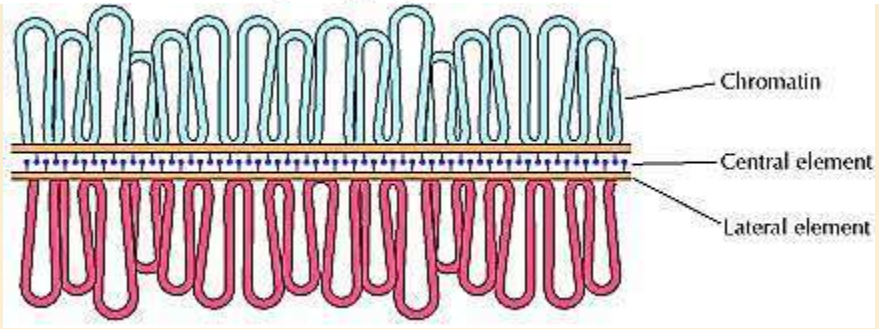
- ***Leptotene:*** Chromosomes begin to condense and attain a compact structure during leptotene.
- ***Zygotene:*** Chromosomes start pairing (chromosomal synapsis), and synaptonemal complex forms. Chromosomes become bivalent or tetrad.
- ***Pachytene:*** Crossing over of non-sister chromatids of homologous chromosomes occurs at recombination nodules. Chromosomes remain linked at the sites of crossing over.
- ***Diplotene:*** Synaptonemal complex dissolves; homologous chromosomes separate, however they remain attached at the sites of crossing over. . Chiasmata form during separation (chiasma formation).
- ***Diakinesis:*** Chiasmata terminates and spindle assembly begins.

Meiosis

Different sub-stages of prophase I



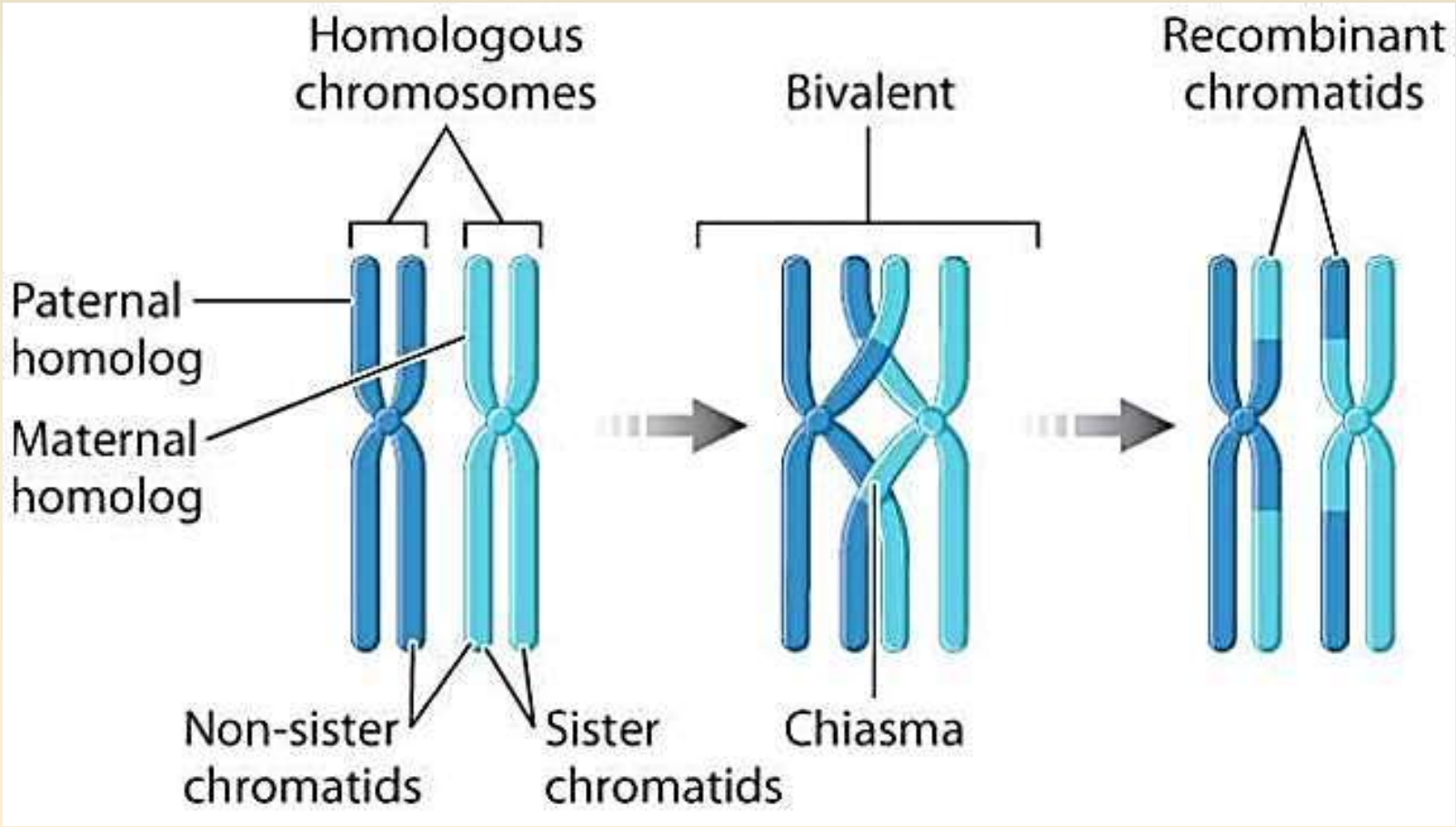
1-5 stages of prophase-I of meiosis-I



Synaptonemal complex

Meiosis

Different sub-stages of prophase I



Meiosis

Different sub-stages of prophase II

The five stages of Meiosis I are not represented in prophase II.

No crossing over occurs in prophase II.

Lab Experiment

Visualization of meiosis in the testes of grasshopper

Introduction

- Squash technique for the observation of meiosis in the testes of grasshopper is simple technique and is widely used.
- It consists of applying a gentle pressure on a small piece of previously stained tissue to flatten the cells and spread the chromosomes.
- It helps to study a single layer of large cells in their entirety.

Objective

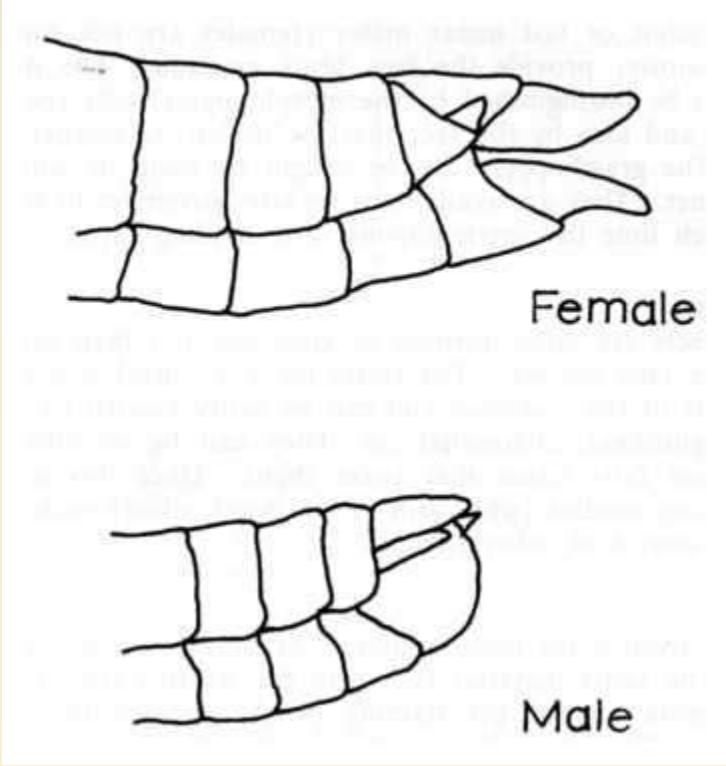
This lab exercise enables the students:

To make squash preparations of sample of animal origin, such as the testes of grasshopper, and to observe meiosis.

Why Grasshopper

Poekilocerus pictus (Fabricius, 1775)

- Grasshoppers are easily available insects having relatively few number of diploid chromosomes (2n = 23; XO) system of sex determination).
- Further all chromosomes of grasshopper are large and metacentric.
- Chiasmata are very clearly seen in diplotene and diakinesis allowing its study with ease.



Young adult or last instar males are taken.

Requirement

Sample

- Grasshopper Testes

Equipment

- Compound microscope
- Dissecting tray & Dissection box
- Spirit lamp
- Petri dishes
- Slides & Cover slips
- Blotting paper

Reagents

- Chloroform
- Glacial acetic acid
- Ethanol (70%)
- Acetocarmine/acetoorecin stain
- HCL (N/10)
- Molten wax/nail polish

Solution

- ***Fixative:*** *Aceto-alcohol solution (1:3:: glacial acetic acid : ethanol)*
- ***Stain:*** *Acetocarmine (*1% solution of carmine in 45% acetic acid**;** Dissolve 10 g carmine (Fisher C579-25) in 1 L of 45% glacial acetic acid, add boileezers, and reflux for 24 h. Filter into dark bottles and store at 4°C) or *aceto-orecein (*1% solution in 45% acetic acid; prepared by pouring 55 ml boiling glacial acetic acid over 1 g orcein powder, cooled, 45 mL of distilled water added, and filtered).

Procedure

- **Dissection of grasshopper:**
  - The insects are chloroformed or eutherised and then dissected on the dissecting tray using saline water.
  - The testes lie in a dorsal position in the anterior half of the abdomen and can be easily located by making a dorsal, longitudinal, abdominal cut.
  - They can be identified by the orange-yellow fatty tissue that cover them.
  - The testes are then removed and put on the petri dish in saline.
  - Each testis can be seen to consists of many follicles.

Grasshopper during dissection

Grasshopper testes on the petriplate

Grasshopper testes under microscope

https://www.youtube.com/watch?v=e-yKVs_5AsA

Procedure

- **Fixation:**
  - Put testis material in the fixative for at least 5 min (ideal fixative is 1:3 acetic alcohol).
  - Store testis material for future, if necessary either in fixative solution or in 70% ethanol.
- **Staining:**
  - Place a small drop of the stain (either acetic-carmine or aceto-orcein) in the middle of a clean slide.
  - Take 3 or 4 testis follicles from the fixative, drain or soak excess moisture with blotting paper, and leave in the stain for 3 to 5 min. Break the follicles by firmly tapping with glass rod.
  - Remove any large piece of tissue material, and place a clean cover slip over the specimen.
  - Heat the slide gently over the flame (stain must not boil).
  - Place the slid in between two pieces of blotting paper and squash firmly with vertical thumb pressure

Procedure

- **Observation of slide:**
  - Observe the slide under compound microscope.

- **Preparation of permanent slide (Conger & Fairchild, 1953):**
  - Freeze the preparation by inverting the slide on a flat piece of dry ice for 30 sec to 1 min.
  - Prise the cover slip off with a scalpel or razor blade (material should remain attached to the slide).
  - Immerse the slid e in absolute ethanol, while material is still frozen, for 10 min.
  - Place the slide in fresh absolute ethanol for at least another 10 min.
  - Put a small drop of euparal or Canada balsam in the middle of the squashed material and apply a clean cover slip.
  - Heat the slide gently to remove any large bubble and then leave it in a dust-free place to harden.

Observation

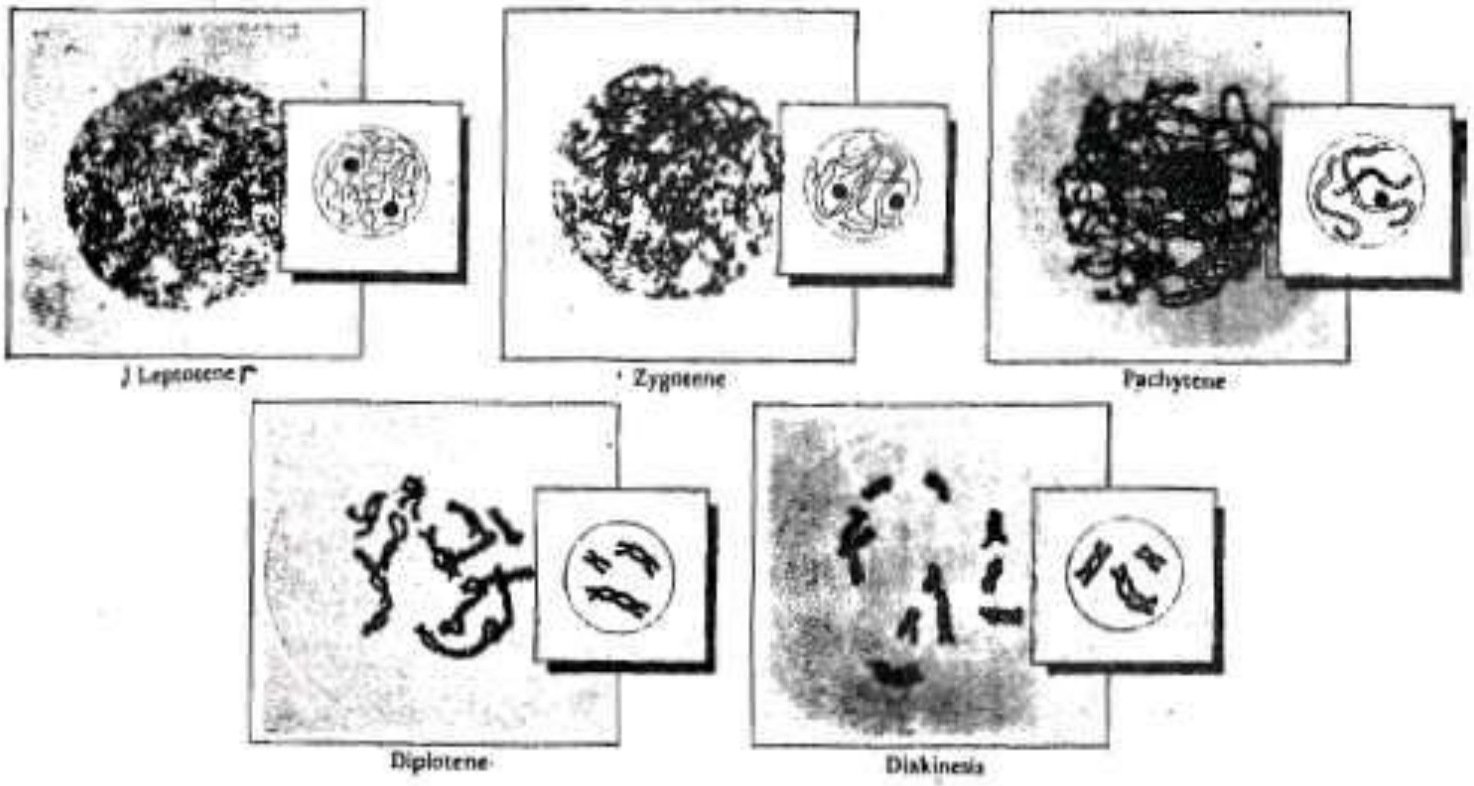


Fig. 7.2 : Stages in meiosis (Prophase I)

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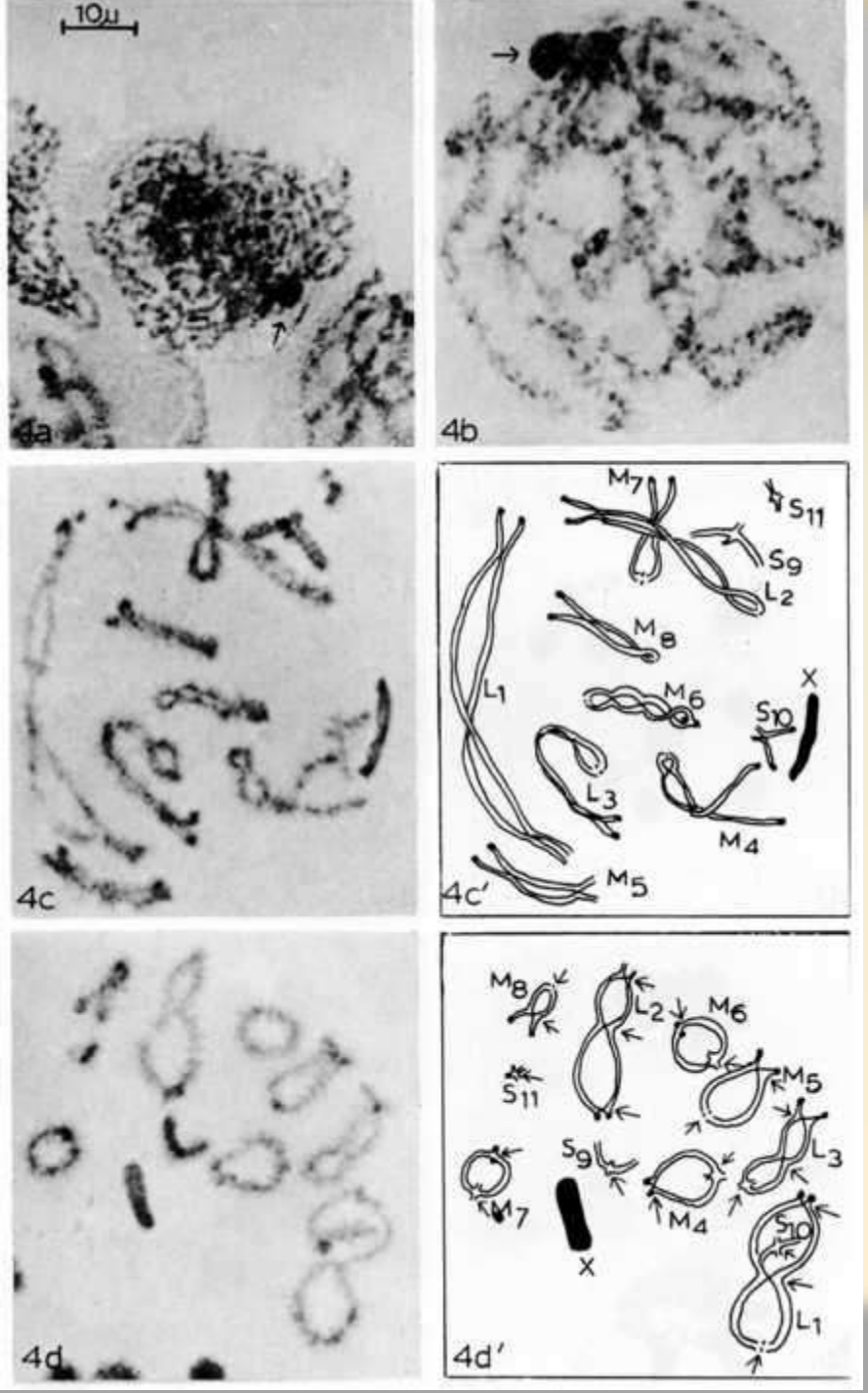

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Thank you!