Indira Gandhi National
Open University
School of Sciences

BBCCL-122
CONCEPTS IN GENETICS
(LABORATORY)

CONCEPTS IN GENETICS

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BBCCL-122: CONCEPTS IN GENETICS

Dear learners, welcome to the practical sessions of Concepts in Genetics. The lab exercises and experiments provided in this manual are based on the syllabus that you have studied in BBCCT-121. The concepts that you have studied in theory course of Concepts in Genetics will be experienced during performing these lab experiments.

This lab course is worth 2 Credits and consists of seven laboratory experiments. The experiments are designed in such a way that you shall be able to experience and connect the theoretical concepts explained so far. Here also the basic concepts on which the experimental procedures are based have been discussed as required.

Expected Learning Outcomes

The broad objective of this lab course is to enable you to:

- Prepare stained squashes of salivary glands of *Drosophila* larva showing well spread banded polytene chromosomes with occasional puffs;
- Induce polyploidy using colchicine and identify polyploid cells in stained squash preparation of onion root tips;
- Independently perform similar experiment in other plant species;
- Calculate the number of Barr body in people with abnormal X- chromosome number;
- Collect and segregate raw data;
- Determine allele and genotype frequency;
- Know the inheritance pattern of PTC tasting in humans;
- Indicate the symptoms, sex and karyotype of representative syndromes;
- Appreciate the importance of maintaining family records and indicate the characteristics of different modes of inheritance; and
- Know the limitations of pedigree analysis.

Study Guide

We advise you to go through respective units of BBCCT-121 before you come to attend the practical sessions. This will enable you to easily understand the purpose of doing experiments and their applications. You should also read the principles of each experiment of this course along with procedure before you start performing the experiment. It is always good to prepare all the reagents freshly and store them under prearranged storage conditions. Adhere to all the safety measures and follow the safety instructions while handling the reagents. One of the good laboratory practices is to maintain your log books up-to-date i.e., enter the observations made while performing the experiments. Carry this laboratory manual and your log book during lab sessions.

Like all other IGNOU laboratory courses, this is an intensive residential exercise requiring one week for completing 2 credits. Everyday there will be two laboratory sessions of 4 hours each. So there will be a total of 14 sessions. The first session will be introductory and the remaining 2nd to 12th sessions will be based on the exercises given in the course. A schedule for laboratory exercises will be given to you in the first session. Sessions 1 to 12 shall have guided exercises under the supervision of the Academic Counsellor. The last two sessions i.e., 13th and 14th will be unguided sessions and that shall be the term end examination. In each session you shall perform exercises for 3 hours and in the remaining 1 hour you are advised to complete your practical note book. The laboratory notebook must be submitted to the counsellor for corrections and grading. 70% marks have been allocated for doing the experiments and for recording it properly. You are aware that there is a time constraint as you will have limited access to laboratory work; therefore, you are required not to miss any of the laboratory sessions.

Assessment of the experiments will be graded and you will have to appear for the *viva-voce* at the end of the practical session. At the end of the laboratory session you should perform the assigned experiment, which will be graded and final assessment will be made based on the continuous performance during the laboratory sessions, maintenance of log books and records followed by *viva-voce*. 30% marks are reserved for the assigned experiments.

For the better understanding of how to use laboratory apparatus few video links shall be provided where ever available. There might be a slight difference in the steps or procedure being explained in the video when compared to the procedure provided in this self-instructional material. However, the principles and reagents remain same. Hence, there is no need to worry about slight modifications adopted in the procedure.

We wish you best in this endeavour!!

IMPORTANT INFORMATION

Attendance is compulsory in the Laboratory Course work held generally at the Study Centre.

The Laboratory Course is worth **2 credits** to be completed over **7 days** duration.

- **6 days** of **Guided** Laboratory work
- **1 day** for the **Unguided** Laboratory work

To successfully complete the laboratory course you will have to pass (at least **35% marks**) in the Guided and Unguided components separately.

EXPERIMENT 1

SQUASH PREPARATION OF SALIVARY GLANDS OF DIPTERAN LARVA TO OBSERVE POLYTENE CHROMOSOMES

Structure

1.1 INTRODUCTION

Polytene chromosomes are giant chromosomes found primarily in well differentiated organs engaged in vigorous metabolic activity. These organs include salivary glands, gut, Malphigian tubules and tracheal walls of dipteran larvae such as *Drosophila* and *Chironomous*. They are also found in several species of protists, plants and even mammals. These tissues grow by an increase in cell size rather than cell number. The giant polytene chromosomes were first observed by **E.G. Balbiani** in 1881. Later in the 1930s, T. S. Painter and colleagues recognized that the size and morphology of polytene chromosomes provide geneticists with unique opportunities to study chromosome structure and gene organization.

Polytene chromosomes (multithreaded) are produced by repeated rounds of DNA replication without the separation of sister chromatids and accompanying cell division **(endomitosis**). The replicated chromosomes are aligned in parallel forming a bundle. The number of times DNA replicates varies with the species and the cell type; in *Drosophila* larvae the DNA replicates nine times (512 strands). These chromosomes are unique in that they are present in the non dividing interphase nuclei. Normally in interphase nuclei the chromatin is diffused and individual chromosomes are not visible. During the development of polyteny, homologous chromosomes are paired (512 x 2 strands) in somatic

cells (a characteristic normally of meiotically dividing cells at prophase -I) and the centromere of all paired chromosomes come together to form a **chromocenter.** The chromosome arms seem to emanate from the chromocenter-five long arms [left and right arms each of chromosomes 2 (2L and 2R) and 3 (3L and 3R) and one of X- chromosome (or chromosome 1) and a very short arm (dot like chromosome 4) in case of *Drosophila melanogaster* (Fig. 1.1).

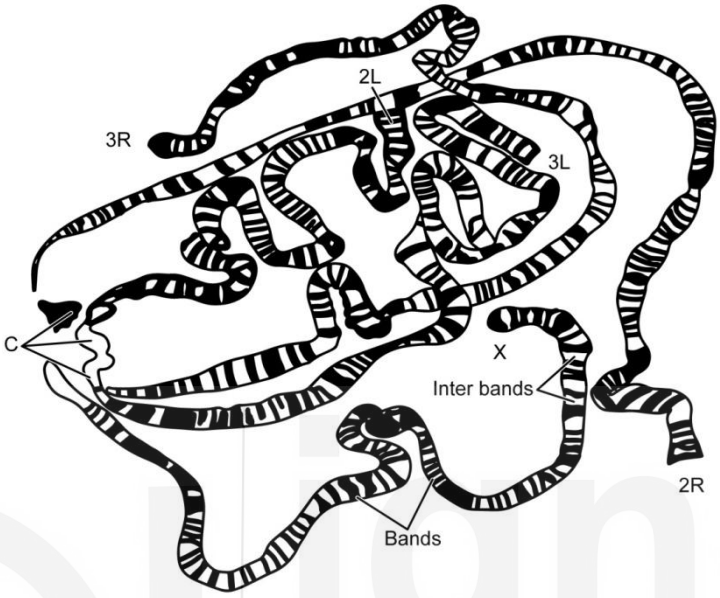


Fig. 1.1 : Polytene chromosomes (Drawn by Dr Neeru Dhamija)**.**

Polytene chromosomes have a reproducible pattern of bands (dark; highly condensed heterochromatin) and inter bands (light; less condensed euchromatin) characteristic of each species. Calvin Bridges published detailed polytene maps that are still used today. They are useful in mapping of genes, detecting chromosomal aberrations and for *in situ* nucleic acid hybridization. Also, transcriptionally active regions are observed as “puffs” (Fig.1.2) or Balbiani rings (large puffs). The exact pattern of puffs differs in different cell types and with changing conditions.

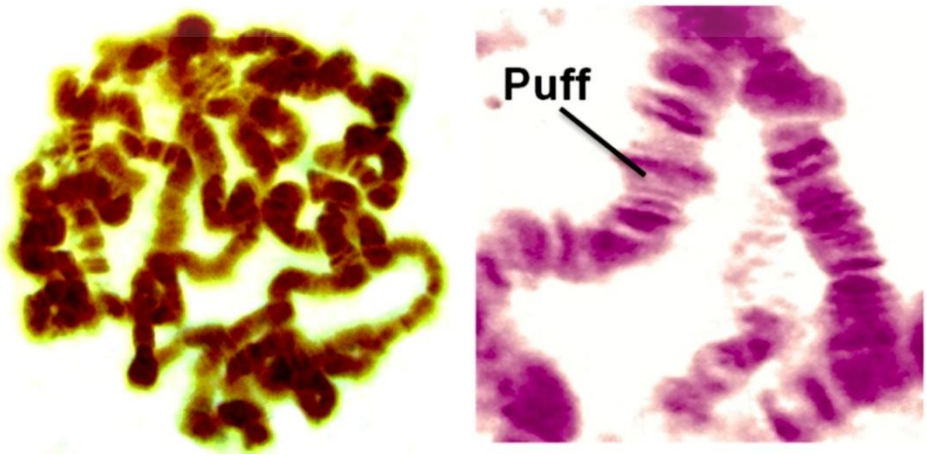


Fig. 1.2: Polytene chromosomes and puffs (transcriptionally active region). (Photo courtesy: Dr. Sunita Joshi).

In this exercise we shall learn to dissect salivary glands from 3rd instar larva; prepare well spread, stained squashes to visualize polytene chromosomes.

Expected Learning Outcomes

After studying and performing this experiment, you should be able to:

- ❖ dissect out the salivary glands from *Drosophila* larva; and
- ❖ prepare stained squashes of salivary glands showing well spread banded polytene chromosomes with occasional puffs.

1.2 PRINCIPLE

The giant polytene chromosomes can be easily observed in stained squashes under light microscope. They are produced by endomitosis and have a consistent pattern of light and dark bands. The latter is more condensed chromatin and so they take up more stain which appears as dark bands. The light bands on the other hand are less condensed. There are also transcriptionally active opened up regions seen as puffs.

1.3 MATERIALS REQUIRED

- Stereo binocular microscope
- Compound microscope
- Bottles (3-4) / tubes (8-10) of healthy 3rd instar larvae
- Teasing needles and fine forceps
- Petri plates
- Blotting paper
- Clean glass slides and cover slips
- Watch glass
- DPX mountant or nail polish.
- Fly food (corn flour, sugar, yeast, agar, preservatives)
- Diethyl ether
- Pasteur pipettes
- Etherizer
- Culture tubes/ bottles.
- Paint brushes
- White tiles (3-4)
- Sponge
- Incubator
- Dropping bottle of acetocarmine or acetoorcein stain (2%)

Dissolve 2g of stain slowly in 45% of boiled acetic acid. Cool the solution and filter it. It is recommended to filter the stain solution before use.

- Dropping bottle of acetic acid (45%)
- Carnoy's fixative: Acetic acid: ethanol (1:3); freshly prepared.
- Insect saline (0.7%)

1.4 PROTOCOL

(A) Pre lab preparation: Culture and growth of fruit fly larvae

i) The fruit fly culture has to be initiated at least ten days in advance so that enough larvae are available for squash preparation of polytene chromosomes by every student. Wild type strains of *Drosophila melanogaster* shall be used in this experiment.

ii) Prepare fly food and pour it into a dozen culture tubes or bottles and allow it to set by leaving them undisturbed, covered with a muslin cloth. Before transferring the flies, sprinkle few grains of active, dry yeast over the surface of the medium.

iii) Anesthetize stock cultures of *Drosophila* adult flies with diethyl ether. **Caution**: Work with ether in a fume hood and do not inhale it. It is extremely inflammable.

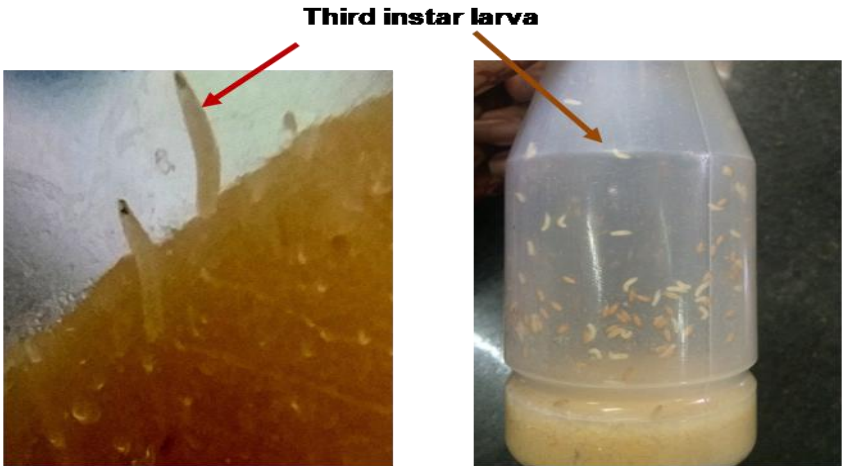
iv) Transfer the anesthetized flies (6-7 females and 4-5males) to dry surface of a fresh culture tube. Keep the tube in a horizontal position until the flies revive from the effect of ether. Repeat the same with all culture tubes or bottles.

v) The cultures are then incubated at 20-23^{0}C in an upright position.

vi) Observe the cultures regularly. When the tiny larvae are first seen feeding in the medium, remove the parents. They may either be transferred to a fresh medium to initiate a fresh culture or killed by dropping into a morgue (70% alcohol).

vii) After a good number of second instar larvae are seen feeding inside the medium, cultures can be transferred to 16-18^{0}C. This step is optional. At lower temperatures, larval growth and development is slower but the third instar larvae usually reach a larger size as compared to 25^{0}C. In addition by carefully controlling larval development, it is possible to obtain enough 3rd instar larvae on the day lab exercise.

viii) The third instar larvae begin to migrate to the sides of the culture vessel (Fig. 1.3). **Remember:** for good larval development, medium must be kept moister than the medium used for performing crosses or maintaining stocks.



(a) (b)

Fig. 1.3: Drosophila larva: (a) The 3rd instar larva is seen moving out of food; (b) The larvae (and few pupae) have settled on the dry surface of the bottle (Photo courtesy: Dr. Sunita Joshi).

(B) Dissection of salivary glands from third instar larva

i) Take a clean glass slide and place a drop of insect saline.

ii) Remove a large, **active larva** sticking to the sides of fly bottle with the help of a brush. The 3rd instar larvae have crawled out of the food.

iii) Transfer it initially to a wet filter paper placed on a Petri plate. This will help in freeing the motile larva of any food particles sticking to it.

iv) Next transfer the larva to a drop of saline placed on the slide.

v) Using the stereo microscope, dissect the larva by placing one teasing needle on the posterior end (blunt) and another needle at the anterior end (pointed) close to the mouth parts (Fig. 1.4). The position of the needle may even be in the middle instead of the posterior end.

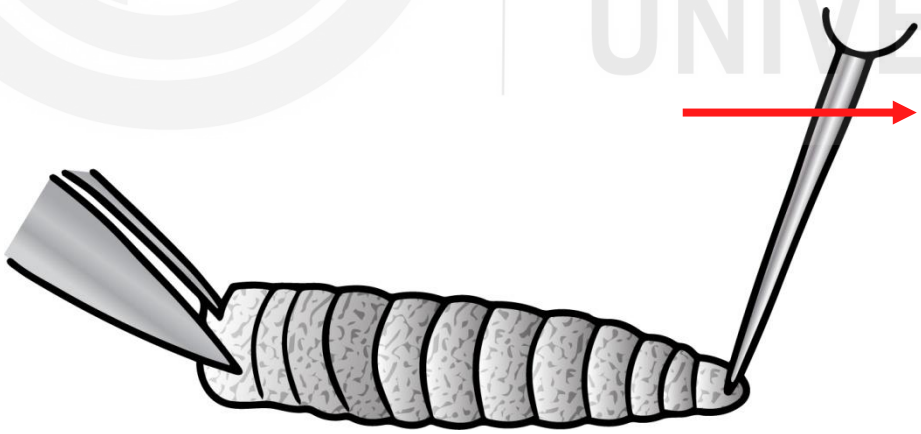


Fig. 1.4: Technique for dissecting salivary glands from *Drosophila* larva.

i) While firmly grasping the posterior end /middle of the larva, pierce through the head and pull forward the mouth parts away from the body of the larva (Fig.1.4; note the direction of the arrow). If this step is performed neatly, two salivary glands will be pulled out along with the head, leaving the rest of the digestive system behind**.** The salivary glands are a pair of white translucent structures connected to a salivary duct. Each salivary gland has a distinct, darkly pigmented fat body at one end (Fig. 1.5a).

Note: The greatest problem is in accurate positioning of the needle while the larva is still active. The most common error is to mistake the digestive system for salivary glands. The dissection procedure requires practice and patience.

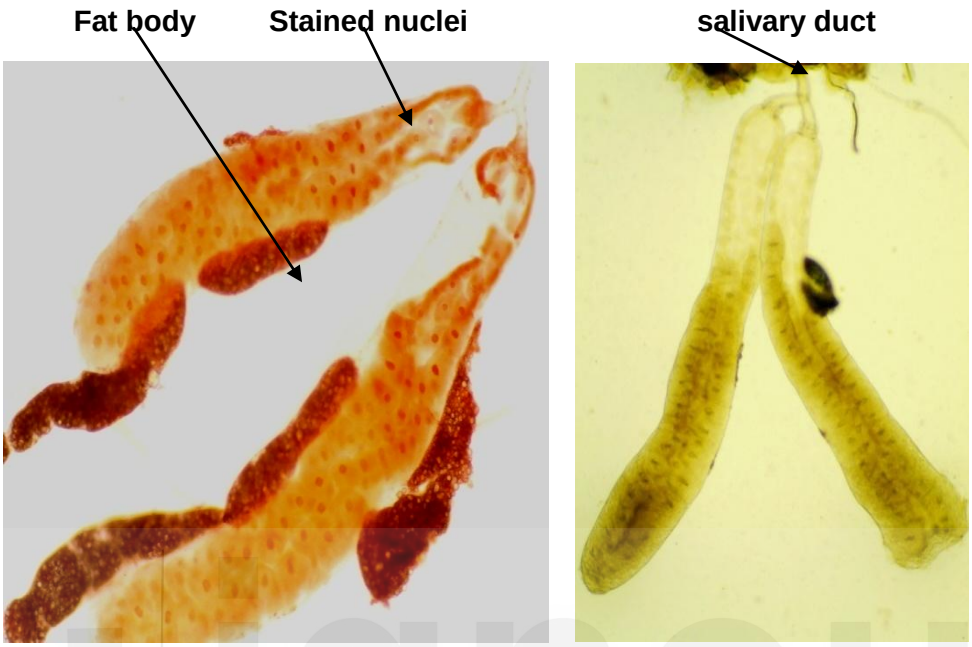


Fig. 1.5: (a) Salivary glands showing stained nuclei (b) fat body removed. (Photo courtesy: Dr. Sunita Joshi and Ms. Kajal Gupta).

With the help of the needle clear the debris and separate the fat body (darkly pigmented) as much as possible from each salivary gland (Fig 1.5b).

i) Clean the salivary glands with a few drops of fresh saline.

(C) Staining and squash preparation

i) Drain out excess saline and pass freshly prepared fixative by holding the slide in a slanted position. Remove excess fixative by soaking carefully with a tissue/ filter paper. **This step should not exceed 1 minute.**

ii) Add few drops of acetocarmine stain and leave the slide covered with a watch glass or in a humid chamber for 4-5 minutes. The time can vary depending upon the temperature.

iii) Remove excess stain by blotting with a filter paper and destain with 45% acetic acid, if required.

iv) Place carefully a cover slip on the stained salivary glands and transfer the slide between the folds of filter paper. Apply moderate pressure in a vertical direction with your thumb.

v) Seal the cover slip with DPX or nail polish to prevent drying.

vi) Observe the salivary gland squash at 40X and 100X (oil immersion) under compound microscope.

vii) You can use mobile phone camera to click images of polytene chromosomes & / draw it.

1.5 OBSERVATIONS

Observe the stained squash under the microscope and report the following:

(a) Draw or click pictures of polytene chromosomes.

(b) Count the number of chromosome arms seen in your preparation.

(c) Try to identify the chromosome(s) by comparing their ends from Fig 1.1.

(d) Label dark and light bands.

(e) Try to locate at least one puff.

1.6 RESULTS

Report your results and comment on the quality of squash preparation. Your instructor will guide you.

1.7 PRECAUTIONS

i) It is recommended to select active larvae that are still moving because salivary glands and other larval structures are histolyzed as the 3rd instar larva begins to pupate and are no longer suitable for polytene squashes.

ii) Avoid excessive tapping as this may lead to breakage of individual chromosomes. With practice one can optimize tapping in order to obtain a well spread preparation.

iii) The salivary gland squash increases in size from its original size. If that does not happen it means the tissue was not kept in sufficient saline/ stain and has dried to some extent. It is better to try again.

iv) Any traces of grease on slides or cover slips hamper spreading of chromosomes. The slides and the cover slips must be cleaned with alcohol and then dried before use.

v) Do not allow the cover slip to move laterally as this would ruin the squash

vi) At no time should the squash run dry.

EXPERIMENT 2

TO OBSERVE POLYPLOIDY IN COLCHICINE TREATED SQUASH OF ONION ROOT TIPS

Structure

2.1 INTRODUCTION

Polyploidy is a numerical change in whole set of chromosomes in a cell or organism such as triploid, tetraploid or higher multiples. Polyploidy is found in some organisms; it is especially common in plants where it has played an important role in their evolution. It also occurs in some somatic tissues of diploid organisms such as liver. Polyploidy often leads to an increase in cell size and the resultant organisms are larger, more robust and high yielding than their diploid counterparts.

There are two broad classes of polyploids viz, autopolyploid and allopolyploids. The former group has multiple sets of chromosomes from the same species while the latter has chromosome complements from different species, generally closely related. Many polyploids are sterile and reproduce asexually such as bananas (triploid) are cultivated from cuttings; tulips from bulbs and Baldwin apples by grafting. Some tetrapolyploids are fertile and reproduce sexually. Allopolyploids are of great importance to plant breeders as advantages possessed by different species can be combined. This has also occurred many times during plant evolution. One of the best examples is of modern bread wheat (a hexaploid of three related diploid species) and mules among animals.

Polyploidy can occur due to failure of chromosomes to separate either during mitosis or meiosis. They may arise spontaneously or can be induced using chemical treatment. Colchicine and a related substance colcemid are commonly used to arrest cell division and induce an increase in ploidy. In this lab exercise we shall induce polyploidy in onion root tips and observe cells in stained squashes under light microscope.

Expected Learning Outcomes

After studying and performing this experiment, you should be able to:

- ❖ induce polyploidy using colchicine;
- ❖ identify polyploid cells in stained squash preparation of root tips; and
- ❖ independently perform similar experiment in other plant species.

2.2 PRINCIPLE

Polyploidy is induced in onion root tips (*Allium cepa*) by exposing them to an anti mitotic agent, colchicine. The root tips of plants have meristems which keeps generating new cells by cell division. The diploid chromosome number of onions is 2n=16. Polyploidy can be observed in the stained cells by an increase in size of the cells and chromosome number [3n (24), 4n (32) and so on]. A variety of nuclear stains are available for staining such as acetocarmine.

Colchicine is a plant alkaloid isolated from *Colchicum s*pecies. It binds irreversibly to free tubulin. Colchicine (Fig. 2.1) bearing tubulin dimer can add to growing microtubules (MT) which then prevent subsequent addition or loss of tubulin subunits. Due to the disruption of MT dynamics the mitotic spindle is not formed and the cells are arrested in metaphase.

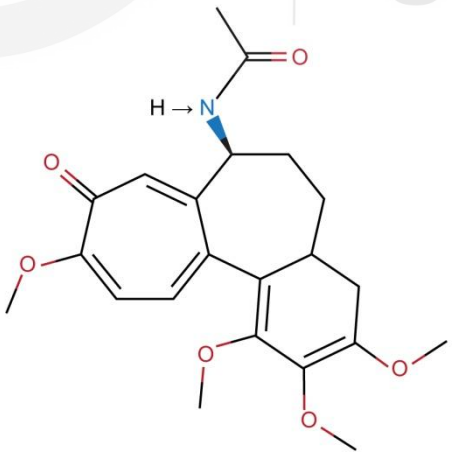


Fig. 2.1: Colchicine- an anti mitotic drug.

The figure below (Fig. 2.2) shows onion cells arrested in mitosis, with variations in ploidy.

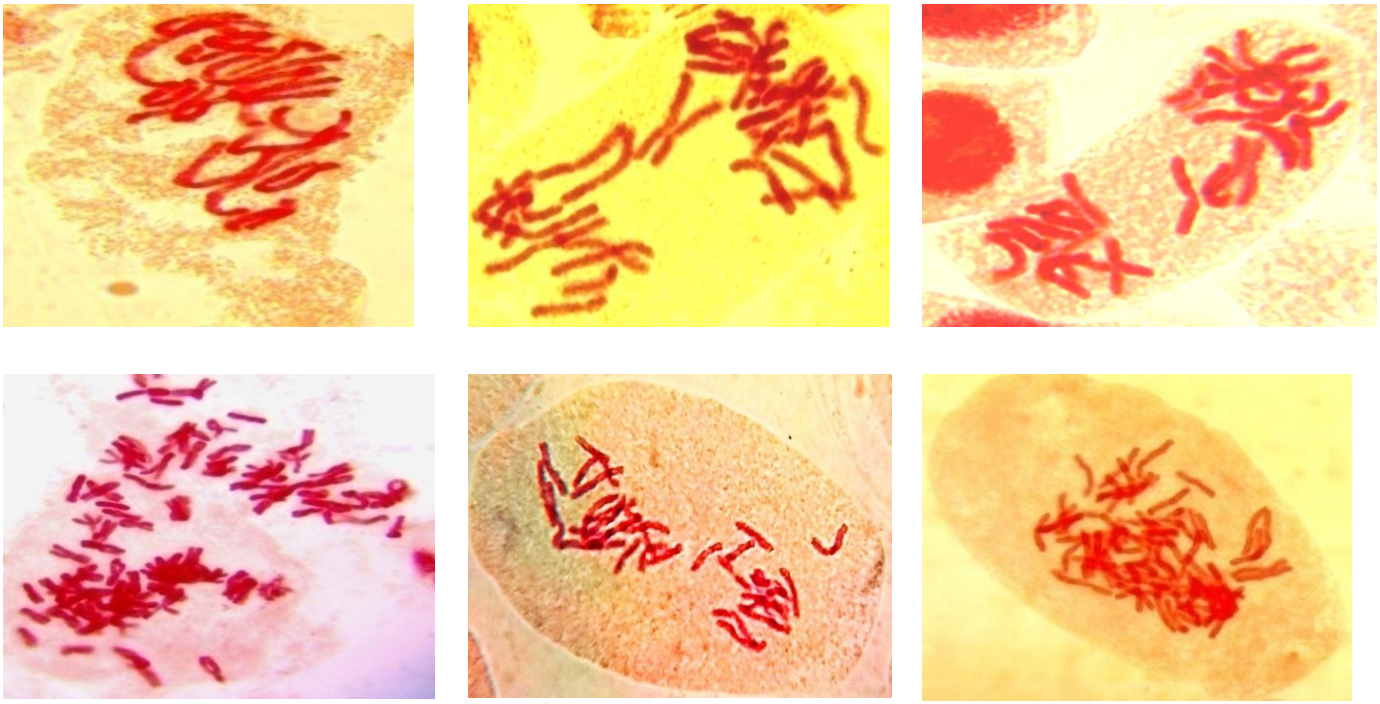


Fig. 2.2: Polyploid cells from colchicine treated onion root tips (Dr. Sunita Joshi).

2.3 MATERIALS REQUIRED

- Onions
- Teasing needle and forceps
- Razor blades
- Coplin jars
- Watch glass
- Pasteur pipettes
- Tissue paper / blotting paper
- Colchicine solution (0.04%)
- Ethanol 70%)
- HCl (2N)
- Carnoy's fixative (Acetic acid-ethanol; 1:3)
- Acetic acid (45%)
- Compound microscope
- Glass slides and cover slips
- DPX mountant or nail polish.

2.4 PROTOCOL

(A) Pre lab preparation (Induction of polyploidy in onion root tips)

The pre lab preparation is schematically depicted in Fig 2.3.

i) Clean 3-4 onion bulbs, clear dried roots and make few cuts to induce rooting.

ii) Place them with their root side down in contact with water in a Coplin jar or beaker.

iii) After 2-3 days, depending on the season, roots (2-3 cm) can be observed.

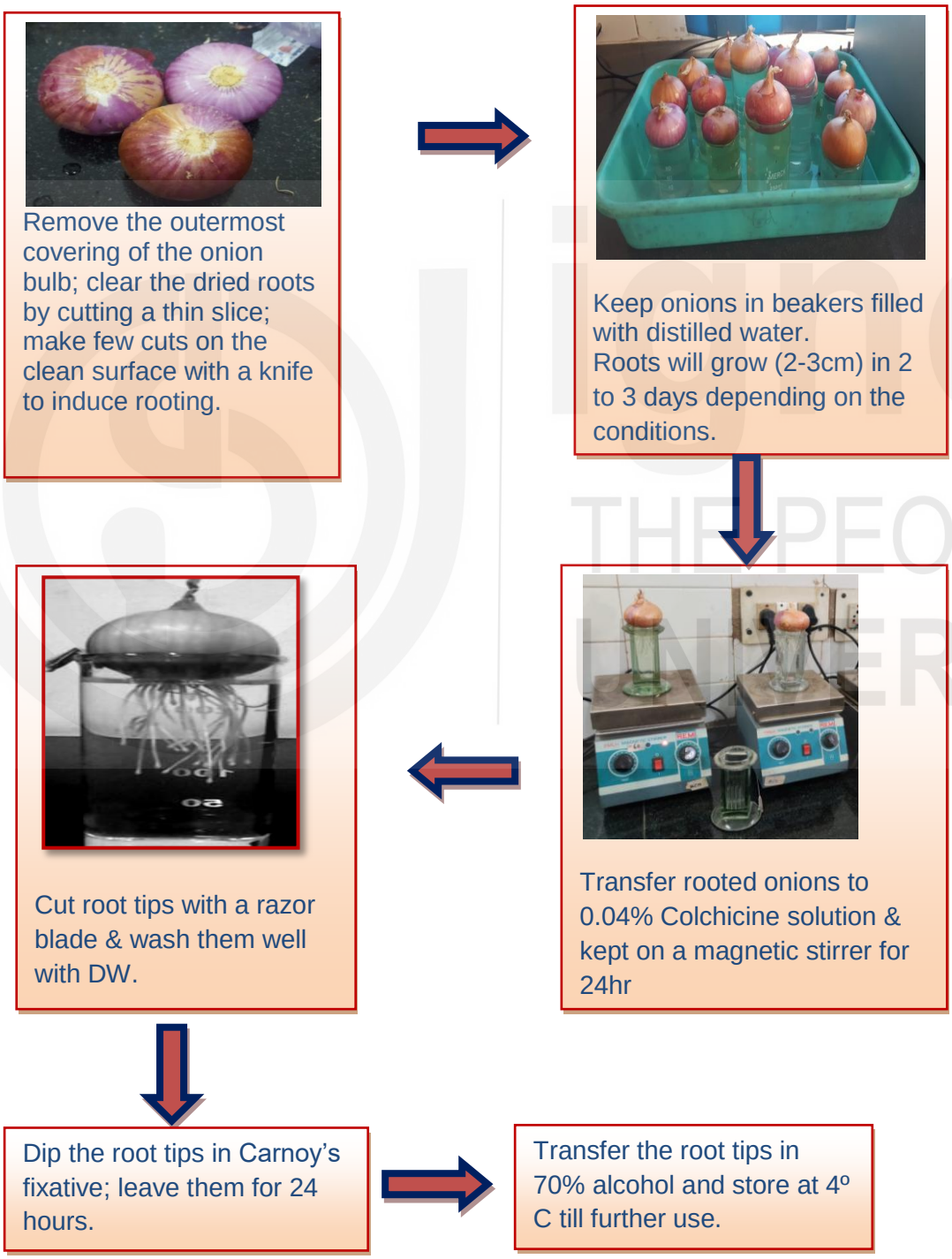


Fig. 2.3: Steps to induce polyploidy in onion roots (Dr Neeru Dhamija).

iv) Transfer the rooted onions to another Coplin jar containing 0.04% colchicine solution for 24-30 hours.

v) Aerate the solution continuously during the incubation period using either a magnetic stirrer or an aquarium pump.

vi) Wash the treated roots with distilled water and excise them with a razor blade.

vii) Wash the root tips again with distilled water and transfer them to Carnoy's fixative for 24 hrs.

viii) Transfer the root tips to 70% alcohol (storage solution) and store them at 4º C till further use.

(B) Squash preparation and staining

i) Transfer the root tips from storage solution to distilled water in a watch glass.

ii) Wash the root tips thoroughly with distilled water 2-3 times and leave them in distilled water or hypotonic solution for 30 -45 min.

iii) Remove excess water with the help of a Pasteur pipette and transfer the tips to 2N HCl for 20 minutes.

iv) After hydrolysis drain off HCl and wash the tips again with distilled water.

v) Remove water and add few drops of acetocarmine dye.

vi) Cover the watch glass and leave it for 10-15 minutes.

vii) Transfer carefully the stained tips (2-3) to a clean slide. Very gently tease the extreme tip of the root to dislodge some of the root cap cells.

viii) Cut the darkly stained edge of the root tip (rich in dividing cells) with the help of a blade.

ix) Add a few drops of 45% acetic acid for 1-2 minutes to remove excess stain.

x) Carefully place a coverslip over the stained tips.

xi) Place the slide between the folds of filter paper and apply moderate pressure in a vertical direction with your thumb or flat end of a pencil.

xii) Seal the coverslip with nail polish.

xiii) Examine the slides under the microscope at 40x and 100x.

xiv) Record your observations.

2.5 OBSERVATIONS

Record the following observations:

(a) Draw and click pictures of few cells arrested in metaphase.

(b) Count and segregate at least in one field cells that are polyploid vs normal (diploid) and comment on their cell size.

2.6 RESULTS

Cells arrested in metaphase would be seen as shown in figure 2.2.

Try to count the number of chromosomes in few cells and tabulate their ploidy.

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2.7 PRECAUTIONS

1. The growing end of root tips has dividing cells which should be used for the experiment.
2. The tips may be kept in hypotonic solution for some time so that they can swell and the chromosome will spread out better.
3. Try to remove the root cap.
4. Avoid air bubbles while placing the coverslip. You may take the help of a needle.
5. Coverslips should not slip laterally during squash preparation.
6. Do not let the squash run dry.

EXPERIMENT 3

DEMONSTRATION OF SEX CHROMATIN IN BUCCAL EPITHELIAL CELLS BY SMEAR TECHNIQUE

Structure

3.1 INTRODUCTION

In many organisms sex is determined at the time of gametic fusion by virtue of differences in sex chromosomes (number or genes) of potential males and females. It took a long time to establish that humans have 46 chromosomes (J.H Tijo and Albert Levan, 1956) of which 22 pairs are autosomes and one pair is sex chromosomes XX or XY. Subsequently other indicators of cellular sex were discovered which are relatively simpler and less time consuming to determine sex than analysis of chromosomes.

The second indicator of nuclear sex was a chance discovery in 1949 by Murray L. Barr and E.G. Bertram. They observed that the nerve cells of a cat have a deeply staining body in the nucleus, close to the nucleolus. Further investigations revealed that it is present only in females and the differentiating structure came to be known as sex chromatin or Barr body (Fig. 3.1a) after its discoverer. It is late replicating and is generally present towards the periphery of the nucleus.

A few years later another useful indicator of nuclear sexing was discovered in polymorphonuclear leukocytes (PMN; neutrophils) by William M. Davidson and David R. Smith. They observed a round body attached to one of the lobes of the nucleus by a thin stalk (Fig. 3.1b) and called it ‘drumstick’. It is present in a small percentage of PMNs of females.

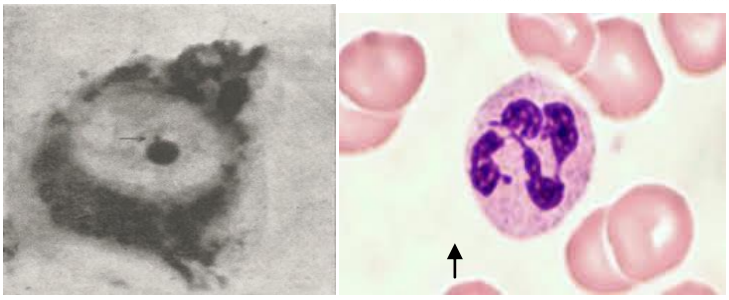


Fig. 3.1: (a) Sex chromatin (b) Neutrophil drumstick.

Barr body (sex chromatin) and drumstick occurs in non dividing cells whereas chromosomes are visible in cells that are dividing. The first indications that Barr body and drumstick are related to X-chromosomes emerged from studies of abnormities in sexual development such as Klinefelter's syndrome. Their nuclear sex is female as they have Barr body in buccal epithelial cells and drumsticks in PMNs. It was Susumu Ohno who identified Barr body as densely packaged X-chromosome.

Mary Lyon, a British geneticist suggested that Barr body results from inactivation of one of the X-chromosomes. She proposed that one of the X-chromosome is inactivated randomly in each somatic cell of mammalian females. This process is initiated during gastrulation. Once a given X-chromosome is selected for inactivation then all its descendents will have the same X-inactivated. The sequence of events is called **Lyonization**. The inactive X chromosome is highly condensed (heterochromatin) and transcriptionally silent. The number of Barr bodies or drumstick in a cell is one less than the number of X chromosomes (n-1), where n= no. of X chromosomes (Fig.3.2). The inactivation of X-chromosome is reversible, as in oogenesis.

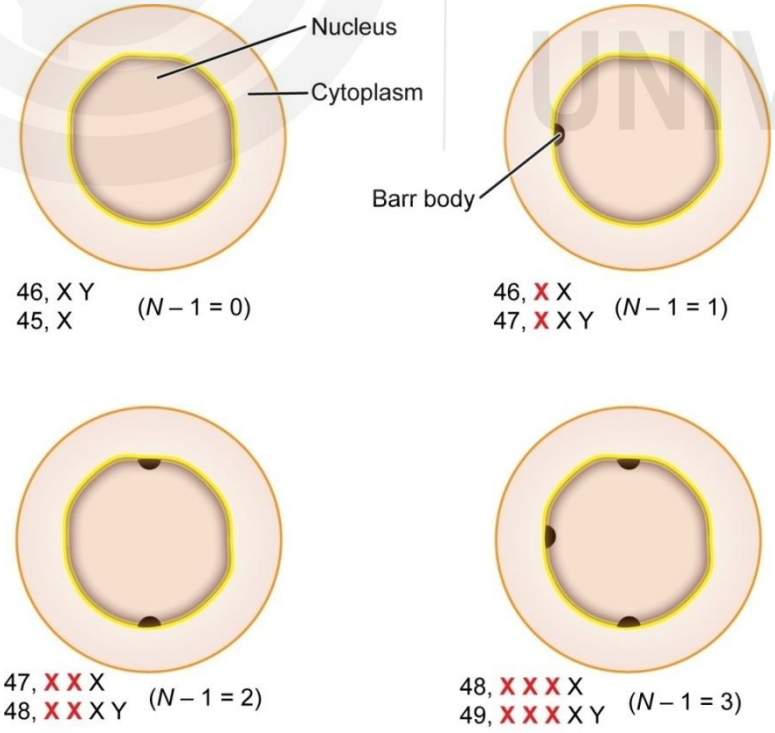


Fig. 3.2: The number of Barr body in humans with extra copies of X chromosome (Adapted from Snustad).

Fig. 3.3: Calico cat.

The purpose of X-inactivation in placental mammals is to compensate the difference in dosage of X-linked genes between sexes (XX Vs XY). It is also accompanied by an increase in the expression of dosage sensitive genes on the X-chromosome of both males and females to balance with autosomal expression. The present estimates indicate that only 75% of genes are inactivated; rest escape completely such as genes on pseudoautosomal region while others vary between females. Since the process is random and once a cell inactivates an X-chromosome it is maintained through successive divisions, a female is a genetic mosaic for genes she is heterozygous. She expresses both alleles but only one in a given cell. A clearly visible example of this is of calico cats (Fig. 3.3) which has sectors of orange and black hair color, each sector represents descendants of a different X-linked allele being functional.

The inactivation of X-chromosome is an epigenetic silencing process which exhibits mitotic heritability throughout its lifespan. The process of inactivation requires a counting mechanism, choice of X chromosome, initiation, progression and maintenance. Some aspects of X-inactivation are partially understood in molecular terms. The X-inactivation centre (XIC) is necessary and sufficient for inactivation. The first detectable event is the transcription of a long non coding RNA (lncRNA; 17Kb) from XIC which finally determines the X-chromosome that will become inactive. The X-inactivation specific transcript (XIST) coats the X-chromosome in cis and recruits chromatin modifier complexes. These complexes bring about DNA methylation, alteration in histone marks and recruitment of histone variants which remodel chromatin to a condensed state.

In this lab exercise you shall learn to prepare smear of buccal epithelial cells, stain cells and observe them under the microscope for possible presence of Barr body.

Expected Learning Outcomes

After studying and performing this experiment, you should be able to:

- prepare thin smears of cells / piece of skin in fluid;
- explain what a Barr body is;
- calculate the number of Barr body in people with abnormal X-chromosome number;
- indicate the purpose of having a Barr body in mammalian females; and
- repeat the experiment in buccal epithelial cells of males; neutrophils and if possible, samples of patients suspected with abnormal X-chromosome constitution.

3.2 PRINCIPLE

Barr bodies can be observed easily in buccal smears of females. They can also be seen in vaginal smears and hair follicles. The uniformly spread cells are fixed, freed of debris by HCl treatment and stained with nuclear stains like Giemsa, Feulgen, Leishman stain and fluorescent dyes. The condensed sex chromatin is stained more intensely than other chromosomes in interphase nuclei. Excess stain is washed to reduce background and observed under the microscope. Simple techniques for determination of sex from buccal smear were developed by Moore and Barr.

3.3 MATERIALS REQUIRED

- Tooth picks / spatula
- Glass slides and cover slips
- Coplin jars
- Needles
- Dropper
- Savlon antiseptic solution
- 90% alcohol
- 6N HCl (Measure 52.6 ml of conc. HCl and make up the volume to 100ml).
- Giemsa stain / Leishman stain
- Phosphate buffer (pH 7)
- Distilled water
- Xylene
- DPX mountant
- Immersion oil
- Diamond marker
- Compound microscope
- Tissue paper

3.4 PROTOCOL

i) Rinse your mouth thoroughly with water.

ii) Gently scrape the inner side of your cheek using the broad side of a toothpick or a clean spatula (dipped in a solution of savlon). Discard the first scraping.

iii) Repeat step (ii) and gently spread the scrapping on a clean dry slide. Leave it for air drying.

iv) Fix the cells by dipping the slide in a Coplin jar containing 90% alcohol for 1 minute and air dry the slide again.

v) Next transfer the slide to a Coplin jar filled with 6N HCl for 10 minutes at room temperature.

vi) Wash the cells with distilled water and add Leishman stain / phosphate buffered 4% Giemsa stain for 1 / 10 minute(s), respectively.

vii) Then add buffer and keep for 5 minutes.

viii) Rinse the slide with water for few minutes and air dry.

ix) Clear the slide with xylene and mount with DPX.

x) Observe the cells at 40X and 100X (oil immersion) for the presence of Barr body.

3.5 OBSERVATIONS

Once you are ready with a stained smear of buccal epithelial cells observe them under the microscope and record the following information:

i) Locate a cell with a Barr body at 40X and then apply a drop of immersion oil before switching to oil immersion objective for a better view.

ii) Observe the shape and position of the sex chromatin.

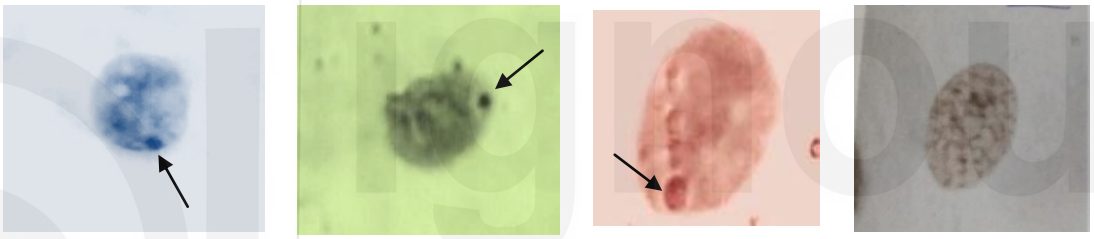
iii) Count the number of Barr bodies / cell.

iv) Calculate the % of cells with Barr body.

Can you think why only a small percentage of buccal epithelial cells have a Barr body?

3.6 RESULTS

(i) Expected results



(a) (b) (c) (d)

Fig. 3.3: Barr body- marked in (a), (b) & (c) cells; absent in (d) (Pic courtsey Dr. Sunita Joshi).

(ii) Record your results and infer the sex of the person.

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3.7 PRECAUTIONS

1. Slides should be clean and dry.
2. First scrapping should ideally be discarded to minimise food chunks, if any.
3. Smear should be uniform, thin (single layer) and without too many cells.
4. Labeling the slide using diamond marker as 90% alcohol erases labels written even with a permanent glass marker.
5. After staining leave the slide dipped in water for sufficient time to remove background stain. It is always advisable to observe the slide after a few minutes before deciding the actual duration for leaving in water.

EXPERIMENT 4

TESTING PTC TASTING ABILITY IN A RANDOM POPULATION AND CALCULATION OF ALLELE & GENOTYPE FREQUENCY USING HARDY WEINBERG LAW

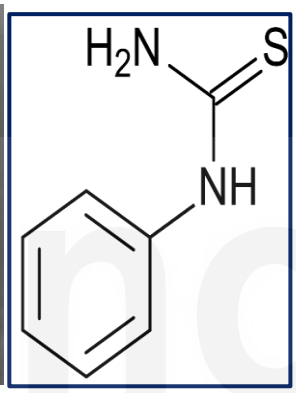
Structure

4.1 INTRODUCTION

The differential ability of humans to taste phenylthiocarbamide (PTC), also known as phenylthiourea (PTU was found accidently by **Arthur Fox** in 1931. He was pouring powdered PTC into a bottle when some of it accidentally blew into the air and a colleague standing nearby complained that the dust tasted bitter. Fox surprisingly tasted nothing at all. Fox then had his friends and family try the chemical and asked them how it tasted. Some people tasted nothing; some found it intensely bitter and still others thought it tasted only slightly bitter. It is now known to be a genetically determined characteristic, inherited in a simple Mendelian fashion.

The PTC gene, **TAS2R38**, was discovered in 2003. It is a 1000 base pair gene located on chromosome 7. There are two common alleles of the PTC gene and at least five rare forms. One of the common forms is a tasting allele while

the other is a non-tasting allele. Each allele codes for a bitter taste receptor protein with a slightly different shape. The shape of the receptor protein determines how strongly it binds PTC. All diploid organisms including humans have two copies of every gene, combinations of the bitter taste gene variants determine whether someone finds PTC intensely bitter (fast tasters), somewhat bitter (slow tasters), or no taste (non tasters) at all. The ability to taste (T/-) is dominant over non tasting (tt). The ratio of tasters to non-tasters varies among populations but every group has both tasters and non-tasters. On an average, 75% of people can taste PTC while 25% cannot. It was even used in paternity testing before the advent of DNA profiling techniques. In general the ability to discern bitter tastes probably evolved as a mechanism to avoid early humans/ grazing animals from eating poisonous plants. PTC bears structural resemblance to some plant toxins.



Arthur Fox **Phenylthiocarbamide (PTC)**

In this exercise we shall determine allele and genotype frequency of taster and non taster alleles in a random population. The term population refers to a group of organisms of the same species that live in a given geographic area and interbreed randomly (without regard to genotype). They share the same gene pool and combinations of alleles in the population result in individuals with different genotypes. Changes in birth rate, death rate, migration, or contact with other populations can lead to variations in the gene pool. Allele frequencies are calculated from genotype frequencies (refer to calculation in section 4.6) and they both add up to 1. An allele with a frequency of 1is said to be fixed (monomorphic) whereas an allele whose frequency is zero is lost from that population.

Hardy-Weinberg law describes the relationship between allele and genotype frequencies in an ideal population. Ideal refers to an infinitely large population, randomly mating, not subject to evolutionary forces such as mutation (no new alleles originate from the existing ones), migration or selection (individual of all genotypes have equal rates of survival and equal reproductive success). For an ideal population, allele frequency in a gene pool does not change over time. If two alleles (A and a) at a locus, are considered, then after one generation of random mating, the frequency of genotype AA: Aa: aa in a population can be calculated from equation (i).

$$p^2 + 2pq + q^2 = 1 \text{ ---------- (i)}$$

Where, p= frequency of allele A

q= frequency of allele a

A population that meets these criteria is said to be in Hardy–Weinberg equilibrium. It is rare for a real population to conform totally to the Hardy–Weinberg equilibrium and for all allele and genotype frequencies to remain unchanged generation after generation.

Expected Learning Outcomes

After studying and performing this experiment, you should be able to:

- collect and segregate raw data;
- determine allele and genotype frequency;
- know the inheritance pattern of PTC tasting in humans;
- indicate how PTC tasting ability varies among individuals;
- prepare PTC coated discs; and
- independently perform this /similar experiment(s).

4.2 PRINCIPLE

PTC testing is based on the principle of differential tasting ability by individuals in a random population. It is due to presence of allelic variants of the gene, TAS2R38 in the population. The homozygous dominant and heterozygotes are **tasters** while homozygous recessive individuals are non tasters. The frequency of tasters and **non tasters** varies in different populations.

PTC tasting is often used as an example of a simple Mendelian trait with dominant inheritance. However, tasters vary greatly in their tasting response. The wild type PTC gene has about 85% of the total influence on being a taster but there are many other factors that affect the sensitivity of PTC tasting such as dry mouth and whether one ate or drank before testing. The data generated shall be used to calculate allele and genotype frequency.

4.3 MATERIALS REQUIRED

- 3mm Whatman paper
- Auto pipettes and tips
- Punching machine,
- Large Petri plates (90mm)
- Small Petri plates (60mm)
- Forceps
- Phenylthiocarbamide (2mg/ ml): Dissolve 20mg of PTC in a few drops of water; add 200µL of ethanol and mix well. Make up the volume to 10ml.

4.4 PROTOCOL

(A) Pre Lab Preparation (Preparation of PTC Discs)

i) Make small discs of 3mm Whatman paper using a punching machine.

ii) Dilute PTC stock solution for preparation of 2.5 µg and 5µg coated discs; make 1:1v/v dilution of stock solution to get 1mg/ml (1µg/µ) solution and again dilute 1mg/ml solution 1:1 to obtain 0.5µg/µL solution.

iii) Use 5µL of 2mg / ml for 10µg discs; 5µL of 1mg / ml for 5µg discs and 5µL of 0.5 mg / ml for 2.5 µg discs.

iv) Add carefully 5µL of the prepared solutions with the help of autopipettes onto the Whatman paper discs. Leave them to air dry. The PTC discs are now ready to use. The number of discs must be made in excess of the expected requirement.

v) Keep the discs of different amount in three separate Petri plates and label the plates.

(B) Testing PTC Tasting Ability

i) The raw data is collected on two days for each subject and tabulated as given in table 1.

ii) On day 1, ask the subjects from a random population to taste a 5µg PTC disc.

iii) On the basis of their tasting ability, record their response as fast tasters (FT), slow tasters (ST) or non tasters (NT). You can also ask how much bitter it tastes and grade them in three levels (+++ / ++ / +).

iv) On day 2, ask the slow tasters and non tasters to taste 10µg PTC disc and record the change, if any.

v) Similarly make the fast tasters to taste 2.5µg PTC disc and ask the difference.

vi) Be sure that all non tasters do not taste even10µg PTC.

Table 1: Collection of raw data

	Day 1	**Day 2**	**Day 2**
Name of subject	**5µg PTC**	**10µg PTC**	**2.5µg PTC**
A	ST	ST / FT	-
B	FT	-	FT / ST
C	NT	NT	-
D	NT	NT / ST	-

ST- Slow tasters; **FT-** fast tasters and **NT**- Non tasters

It is important to carefully analyse raw data and remove entries that are incomplete or incorrect, for instance a faster taster at low concentration is not likely to be a non taster at higher concentration. If possible the subject may be asked to taste again.

4.6 CALCULATIONS

The raw data is segregated into homozygous dominant (TT), heterozygous dominant (Tt) and homozygous recessive (tt). A hypothetical data is given in table 2.

(a) Table 2: Observation table (hypothetical data)

PTC tasting ability	Genotype	Number of individuals tested
Slow tasters	Tt	96
Fast tasters	TT	150
Non tasters	tt	225
Grand total		**471**

Each one of you shall collect data of 5-10 people and then enter the consolidated information in the table below. The raw data can be consolidated in your lab notebook.

PTC tasting ability	Genotype	Number of individuals tested
Slow tasters	Tt	
Fast tasters	TT	
Non tasters	tt	
Grand total		

(b) Calculations

(i) **Ratio of tasters to non tasters: 052: 0.48** (phenotypic frequency)

(ii) Allele frequencies

$$\boldsymbol{allele\ frequency} = \frac{\boldsymbol{no.of\ copies\ of\ an\ allele\ in\ a\ population}}{\boldsymbol{total\ no.of\ all\ alleles\ for\ that\ gene\ in\ a\ population}}$$

(a) For allele T:

$$\boldsymbol{allele\ frequency} = \frac{\mathbf{150(2) + 96(1) + 225(0)}}{\mathbf{471(2)}} = \frac{\mathbf{396}}{\mathbf{942}} = \mathbf{0.42}$$

Allele frequency of T (p) = **0.42**

(b) For allele t:

$$\boldsymbol{allele\ frequency} = \frac{\mathbf{150(0) + 96(1) + 225(2)}}{\mathbf{471(2)}} = \frac{\mathbf{546}}{\mathbf{942}} = \mathbf{0.58}$$

Allele frequency for t (q) =**0.58**

p + q= 0.42+0.58 = 1

(iii) Genotype Frequencies

$$\boldsymbol{genotype\ frequency} = \frac{\boldsymbol{no.of\ individuals\ with\ that\ genotype\ \ in\ a\ population}}{\boldsymbol{total\ no.of\ individuals\ in\ the\ population}}$$

Fast tasters (TT)

$$\boldsymbol{genotype\ frequency\ (TT)} = \frac{\mathbf{150}}{\mathbf{471}} = \mathbf{0.32}$$

Non-tasters (tt):

$$\boldsymbol{genotype\ frequency(tt)} = \frac{\mathbf{225}}{\mathbf{471}} = \mathbf{0.48}$$

Slow tasters (Tt):

$$\boldsymbol{genotype\ frequency(Tt)} = \frac{\mathbf{91}}{\mathbf{471}} = \mathbf{0.20}$$

Genotype frequencies = 0.32+0.48+0.2= 1

(iv) Assuming random mating the expected genotype frequencies can be calculated from allele frequency using Hardy- Weinberg equation (Eqn i).

TT (p^2) = $(0.42)^2$ = **0.1764**

tt (q^2) = $(0.58)^2$ = **0.336**

Tt (2pq) = 2 x 0.42 x 0.58 =**0.487**

These values do not tally with our results because most populations are far from ideal.

Calculate allele and genotype frequency of your data.

(i) Allele frequencies

(a) Allele T:

(b) Allele t:

(ii) Genotype frequencies

(a) Fast tasters (TT):

(b) Slow tasters (Tt):

(c) Non tasters (tt):

4.7 RESULTS

(i) Report your results in the table below.

Allele	Allele frequency
T	
t	

Genotype	Genotype frequency
TT	
Tt	
tt	

(ii) DISCUSSION

Analyse your result and report your findings with a resoning

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4.8 PRECAUTIONS

1. Discs should be loaded accurately and carefully to avoid discrepancies such as loading incorrect amounts or not loading at all.
2. Forceps and Petri dishes should be properly cleaned and dried before using.
3. Make sure that the population chosen should be random.
4. Before collecting data explain clearly to the subjects what they have to assess.
5. Use separate tips for loading different concentrations of PTC.
6. Take care to ask the subjects to rinse their mouth with water and preferably have food after the test.

EXPERIMENT 5

KARYOTYPING (DRY LAB EXERCISE)

Structure

5.1 INTRODUCTION

Humans are diploid organisms with 22 pairs of autosomes and a pair of sex chromosomes. The females are homogametic with a pair of X chromosomes while the males are heterogametic (XY). The Y chromosome in humans is the key determinant of male sexual phenotype. Generally the pairs of chromosomes of an organism are cut out from a photograph and arranged according to their sizes. This type of organised photographic profile is called **karyotype**.

The human karyotype is arranged into seven groups (A to G), from largest to smallest. The autosomes are arranged in pairs (numbered 1 through 22) and the 23rd pair is of sex chromosomes (Fig 5.1). The centromere divides the chromosome into two arms, the short arm is denoted by the letter p (for petite) and long arm by q (the letter following p in English alphabets). Based on the position of centromere chromosomes are classified as metacentric, sub metacentric, acrocentric and telocentric.

Chromosomes are stained using various non-fluorescent and fluorescent stains. The reproducible banding patterns produced by each of them are useful in identifying particular chromosomes in a cell, especially those of similar size and shape; analyzing fine details of chromosome structure and detecting structural and number abnormalities.

In this dry lab exercise you shall be introduced to five abnormal human karyotypes and their symptoms, sex and prevalence. Then you will search from literature two more karyotypes and indicate the sex of the affected individual and the symptoms associated with them.

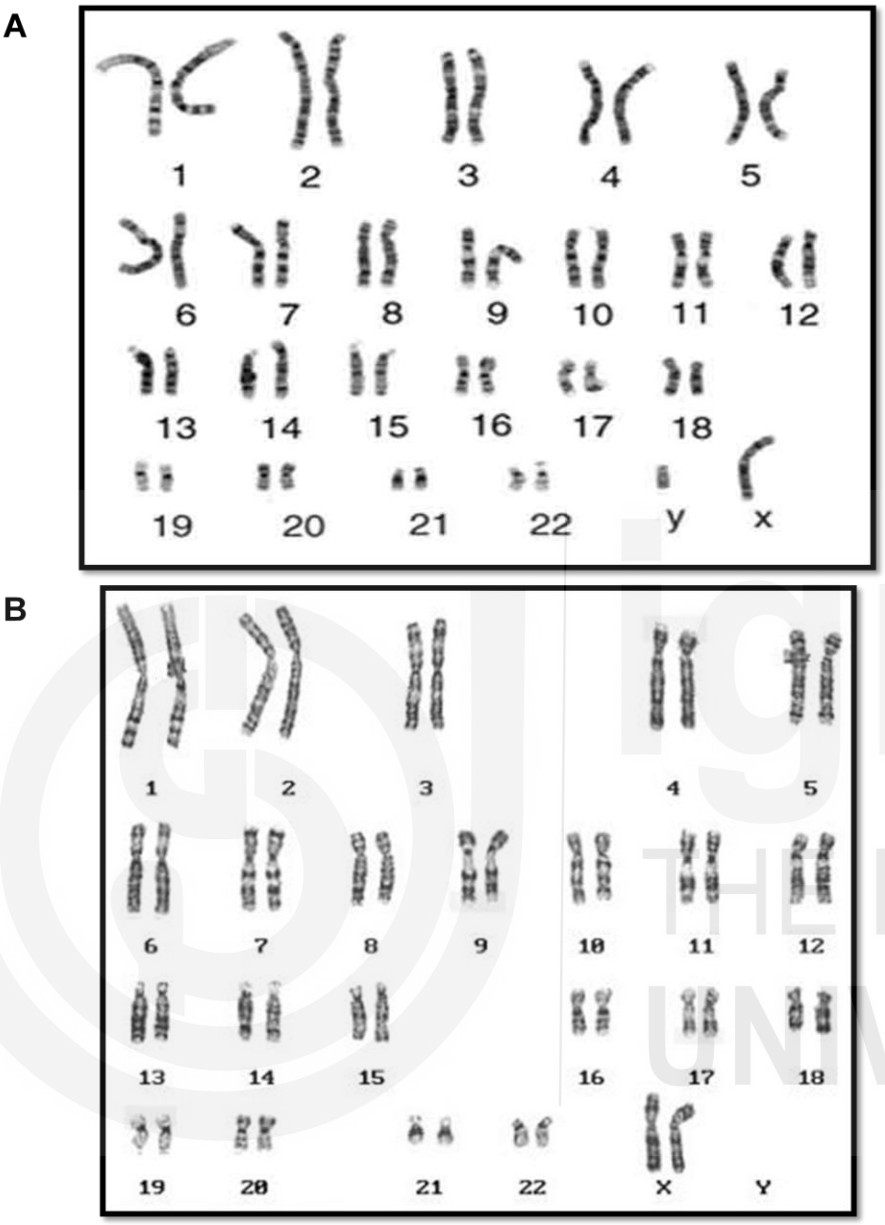


Fig. 5.1: The karyotype of a normal human (A) male & (B) female.

Expected Learning Outcomes

After studying this experiment, you should be able to:

- ❖ understand the impact of aberrations in chromosome structure and number;
- ❖ indicate the symptoms, sex and karyotype of representative syndromes; and
- ❖ pick up at least two examples and describe the nature of aberrations and symptoms associated with them.

5.2 PRINCIPLE

All chromosomal aberrations fall into two broad classes. They are either due to **structural abnormalities** (deletions, duplications, translocations and inversions) or **variations in chromosome number** (aneuploids). These aberrations are responsible for certain inherited human disorders.

The imbalances in dosage in many cases are lethal and about 50% of spontaneous abortions result from abnormality in chromosome number. In general trisomy (a diploid organism with an extra copy of an individual chromosome) of chromosome 13, 18 or 21 (small chromosomes) and variations in the number of X- chromosomes survive better than trisomy of other large autosomes or monosomies (a diploid organism with a missing copy of an individual chromosome) of autosomes. The small chromosomes are gene poor and therefore imbalances are better tolerated. In cases where a child survives for variable period we have information about the consequences of specific chromosome imbalances. We shall pick up few examples of well characterised syndromes.

5.3 MATERIALS REQUIRED

- Abnormal karyotypes
- Images of children / adults highlighting symptoms

5.4 GUIDED STUDY OF REPRESENTATIVE HUMAN CHROMOSOMAL ABERRATIONS

5.4.1 Primary Down Syndrome (47, +21)

Down syndrome is one of the most common autosomal aneuploidy which occurs in 1 in 750 live births even though most fetuses of trisomy 21 are aborted. It was first described by a British physician, John Langdon Down (1866) who noticed a striking resemblance among a number of his mentally retarded patients. Their karyotype (Fig. 5.2 A) revealed the presence of an additional chromosome 21 (trisomy; 47, XY, 21+).

Primary Down syndrome usually arises from spontaneous non disjunction primarily during oogenesis. The incidence of Down syndrome increases with mother's age. Most children with Down syndrome are born to normal parents. The failure of disjunction is not a familial trait. These children are mentally retarded (IQ ranges from 20-50) but are responsive to the love and encouragement of their family. They easily get happy. Other symptoms of familial Down syndrome are highlighted in Fig. 5.2 B.

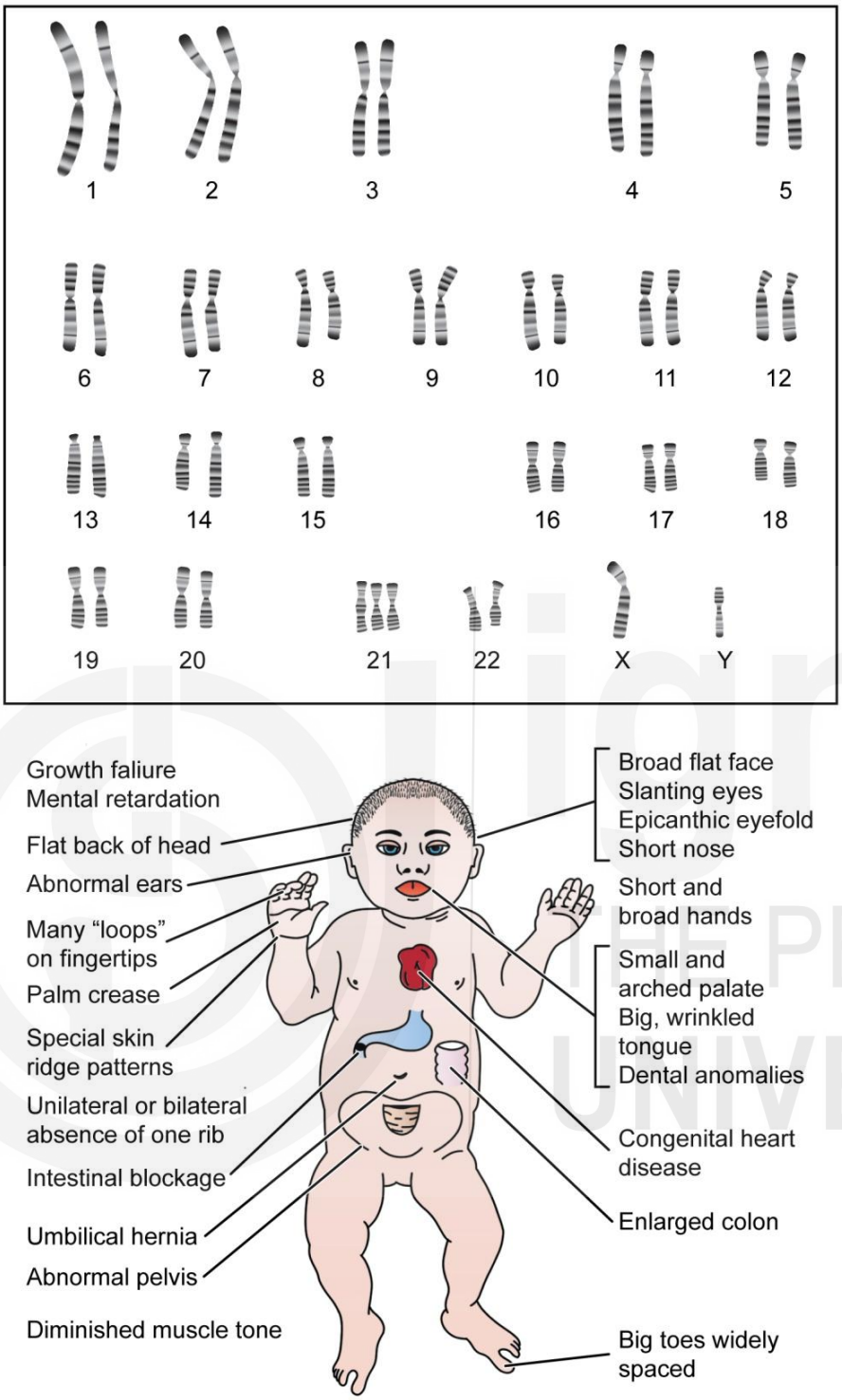


Fig. 5.2: Down Syndrome (primary) child (Adapted from Griffith**).**

In addition children born with Down syndrome have low birth weight; delayed speech and motor development; their ears may be small and low set and they have a single crease across the palm (simian crease).They have a bigger than normal space between the first and second toe (the 'sandal gap'). Females may be fertile but not males. Their mean life expectancy is about 17 years.

5.4.2 Familial Down Syndrome

Familial Down syndrome or translocation Down syndrome is a rare condition which occurs in 4% of people with Down syndrome. They have 46 chromosomes but an extra copy of part of chromosome 21 is attached to another chromosome**.** This condition is termed **familial Down syndrome** because it has a tendency to run in families

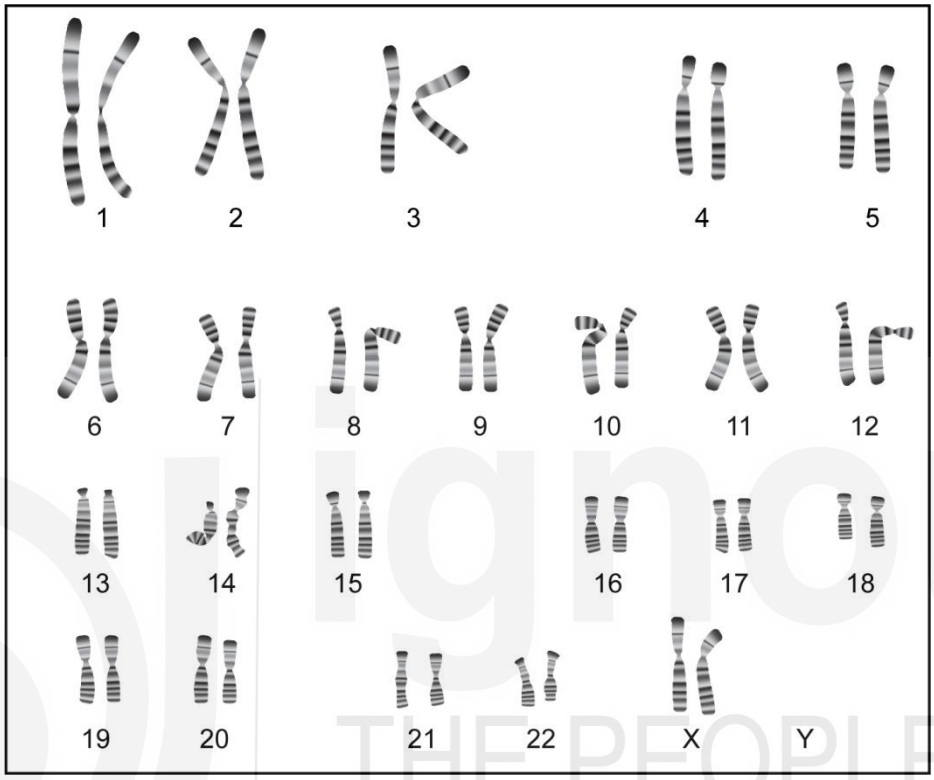


Fig. 5.3: Karyotype of a familial Down syndrome child.

It arises in offspring whose parent(s) are phenotypically normal with 45 chromosomes. Generally one of them is a carrier of chromosomes that have undergone **Robertsonian translocation**, most commonly between long arm of chromosome 21 and chromosome 14 (Karyotype: **46, XY,-14, +t (14q21q)**. It is a kind of non reciprocal translocation between two non homologous chromosomes in which their centromeric ends become fused to form a single centromere. The exchange produces a chromosome that includes the long arms of chromosomes 14 and 21, and a very small chromosome that consists of the short arms of both chromosomes. The small chromosome is generally lost after several cell divisions Fig. 5.3.

When a translocation carrier produces gametes, the translocated chromosome may segregate in three different ways to produce six types of gametes. Half of these gametes when fused to normal gamete will produce children that are translocation carriers or have familial Down syndrome or they are normal offspring's. The rest 50% of gametes will result in aborted embryos (aneuploids). The phenotypic characteristics of familial Down syndrome are the same as those of primary Down syndrome but the risk of having this condition is not linked to maternal age.

5.4.3 Turner syndrome (45, X; Sex Chromosome Aneuploidy)

Turner syndrome is due to monosomy of X-chromosome **(**Karyotype 45; XO). It was first described by Henry H. Turner in 1938. They are phenotypically females due to the absence of Y chromosome. Many Turner women have some cells that are XX and other cells are XO (somatic mosaics). The salient features and karyotype of the syndrome are highlighted in Fig.5.4 (A & B).

Most women are sterile. They generally have swelling of hands and feet; broad chest (shield chest); obesity; high waist-to-hip ratio; webbed neck (congenital, usually bilateral skin fold that runs along the sides of the neck down to the shoulders); low-set ears; hearing loss and visual impairments. Many suffer from nonverbal learning disabilities (NLD) such as problems with interpreting body language and spatial relationships. Some suffer from attention Deficit / Hyperactivity Disorder or ADHD in which they have problems with concentration, memory, attention with hyperactivity, seen mostly in childhood and adolescence.

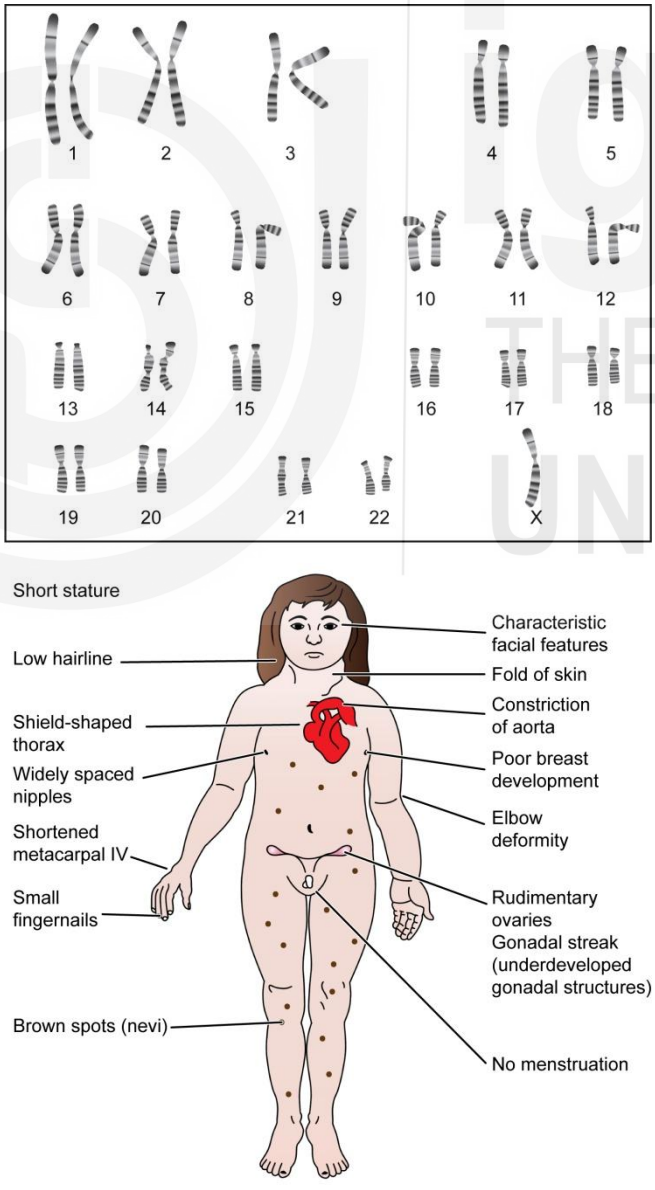


Fig. 5.4: Karyotype and symptoms of a Turner female (Adapted from Griffith).

5.4.4 Klinefelter's Syndrome (47, XXY)

Klinefelter's syndrome is a genetic disorder resulting from additional X chromosome in males (Karyotype: 47, XXY). It is generally due to non-disjunction of sex chromosomes in males during meiosis I. The fertilisation of an XY bearing sperm with a normal egg (X) produces an XXY offspring. It can also result from non-disjunction in females during meiosis-II, if sister chromatids of an X-chromosome fail to separate (disjoin). An XX egg fertilising a normal sperm (Y), yields XXY offspring.

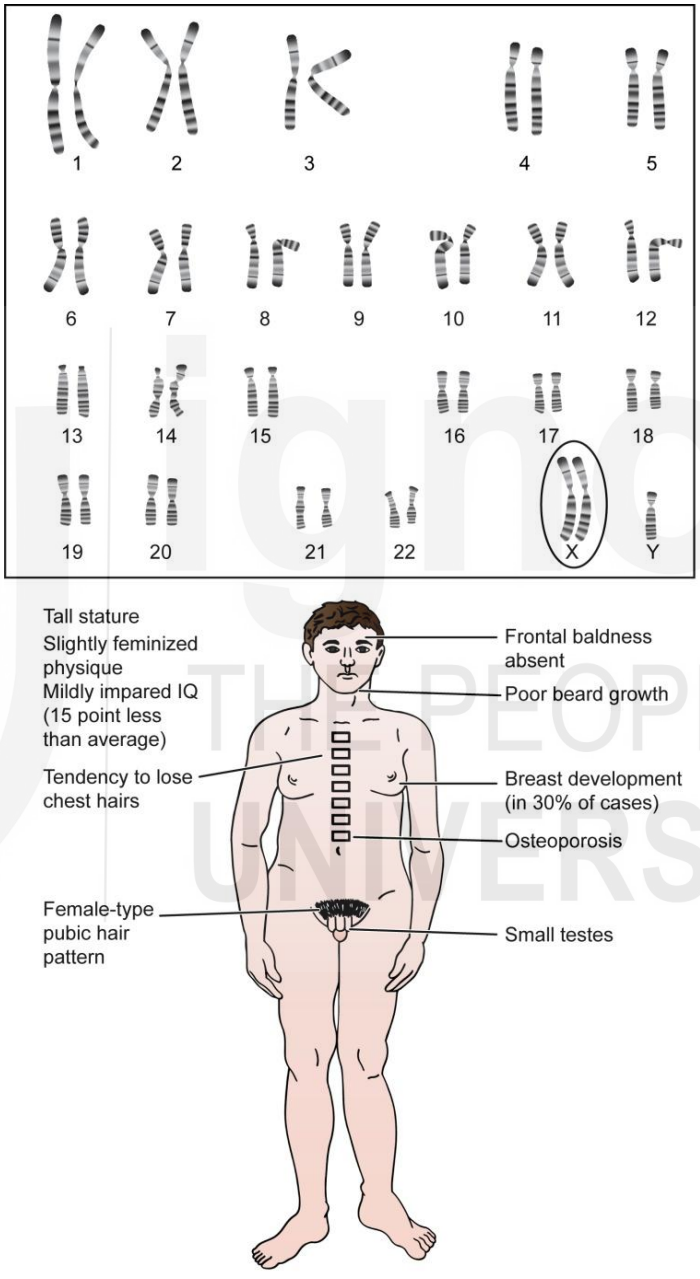


Fig. 5.5: Karyotype and symptoms of Klinefelter's syndrome *(*Adapted from Griffith).

The karyotype and salient features of Klinefelter's syndrome are given in Fig. 5.5. These men are sterile and have low serum testosterone but high FSH and LH levels. Their motor development is delayed. They are prone to develop certain auto immune disorders, breast cancer and osteoporosis.

5.4.5 Cri du Chat Syndrome

The syndrome gets its name from the distinctive cat like mewing cries made by affected infants. This is due to abnormal development of the glottis and larynx. It is a structural aberration resulting from deletion in chromosome 5 (Karyotype: 46, 5p-; meaning that the individual has all 46 chromosomes but some or the entire p arm (the petite arm) of one member of chromosome 5 pair is missing) Fig. 5.6. Other manifestations of the syndrome are microencephaly (abnormally small head) and a moonlike face; mental retardation; infants may exhibit anatomic malformation including gastrointestinal and cardiac complications; fatality rates are low and many with this condition reach adulthood.

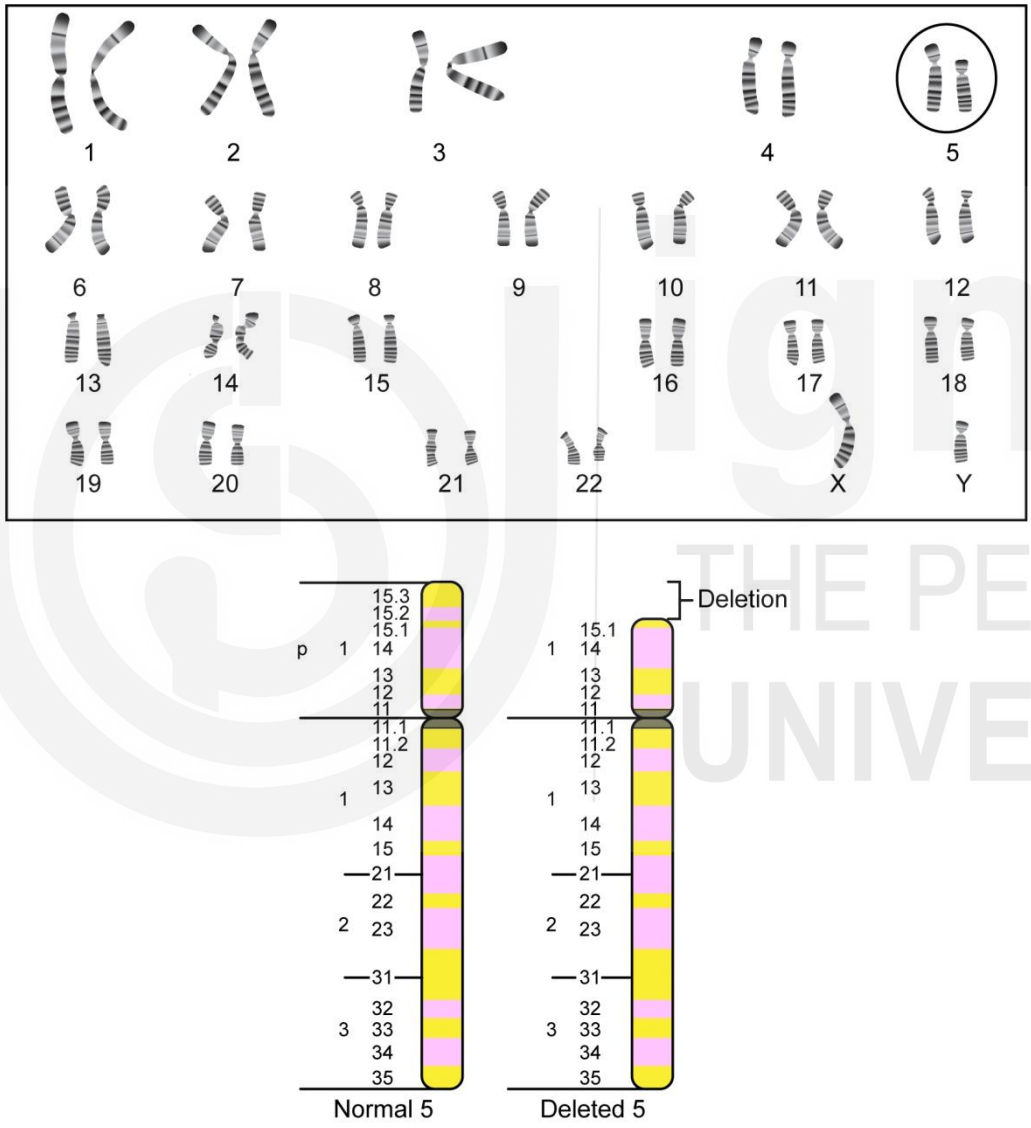


Fig. 5.6: Karyotype of cri du chat syndrome.

EXPERIMENT 6

STUDY OF HUMAN PEDIGREES (DRY LAB)

Structure

6.1 INTRODUCTION

The application of Mendelian principles to human genetics began soon after the rediscovery of Mendel's work in 1900. Although many human diseases have a genetic component but the study of human genetic characteristics is not always simple. In humans, controlled mating is not possible. They have a long generation time unlike bacteria with generation time of 20 minutes or *Drosophila* of 10-12 days ; reach reproductive age around 12 -14 years and do not produce many progeny, making it difficult to discern Mendelian ratios. In addition incomplete penetrance, mistaken paternity and incomplete data affects the predictive ability of an investigator. Yet the drive to understand human genetics is much stronger than the problems encountered. Pedigree analysis offers an alternative tool to study the inheritance of human traits and is the subject matter of this dry lab exercise.

Expected Learning Outcomes

After studying this unit, you should be able to:

- Appreciate the importance of maintaining family records;
- Indicate the characteristics of different modes of inheritance;

- ❖ Predict wherever possible the likelihood of appearance of a trait in the offspring;
- ❖ Independently workout the most likely mode of inheritance in the given pedigrees and defend your choice; and
- ❖ Know the limitations of pedigree analysis.

6.2 PRINCIPLE

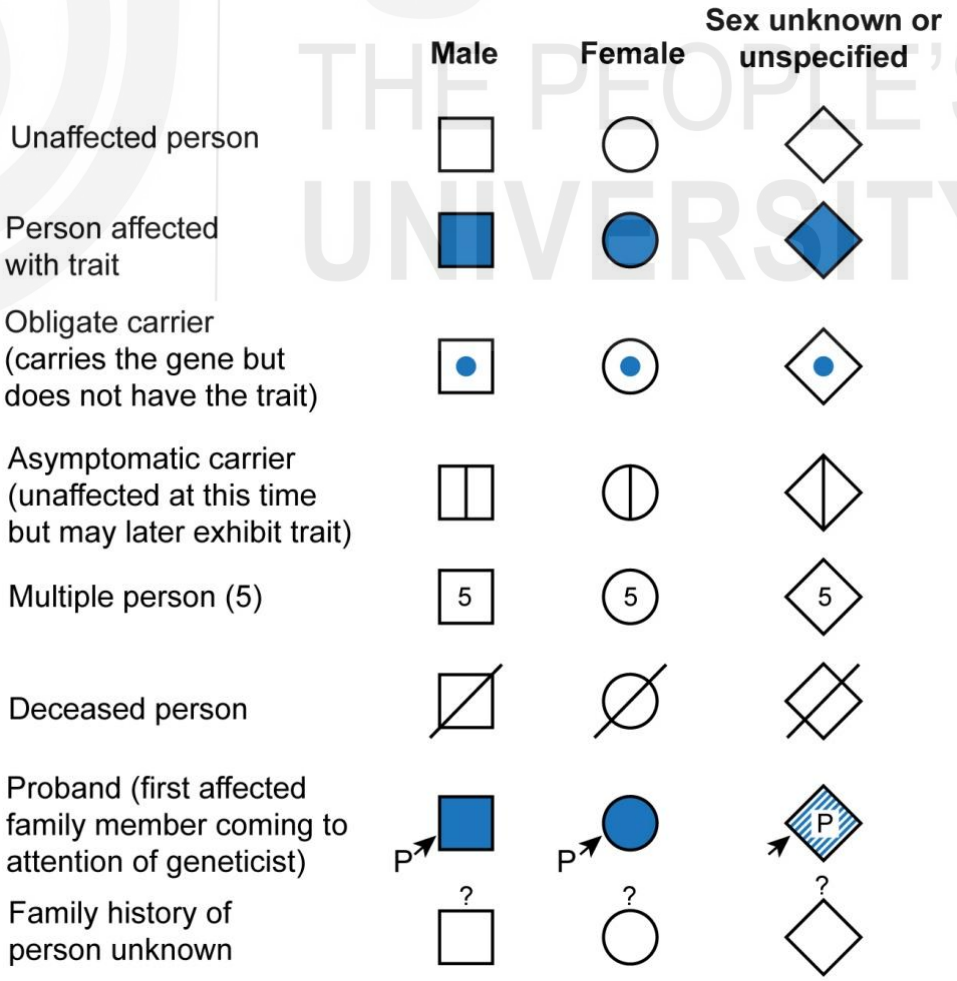
The genetic analysis of human heredity depends on family records, which are often incomplete. **Pedigrees** are diagrams that show relationships between members of a family. We shall concentrate on five major pattern of inheritance. They are autosomal dominant (PTC tasting ability); autosomal recessive (Tay Sachs disease); X linked dominant (familial vitamin D resistant rickets); X linked recessive (red-green colour blindness) and Y linked (hair on pinna and other holandric characteristics). The characteristics of each of the above pattern of inheritance are given in Table 6.1.

Table 6.1: Characteristics of Pattern of Inheritance.

Autosomal recessive trait
1. Usually appears in both sexes with equal frequency. 2. Tends to skip generations. 3. Affected offspring are usually born to unaffected parents. 4. When both parents are heterozygous, approximately one-fourth of the offspring will be affected. 5. Appears more frequently among the children of consanguine marriages.
Autosomal dominant trait
1. Usually appears in both sexes with equal frequency. 2. Both sexes transmit the trait to their offspring. 3. Does not skip generations. 4. Affected offspring must have an affected parent unless they possess a new mutation. 5. When one parent is affected (heterozygous) and the other parent is unaffected, approximately half of the offspring will be affected. 6. Unaffected parents do not transmit the trait.
X-linked recessive trait
1. Usually more males than females are affected. 2. Affected sons are usually born to unaffected mothers; thus, the trait skips generations. 3. Approximately half of a carrier (heterozygous) mother's sons are affected. 4. Never passed from father to son. 5. All daughters of affected fathers are carriers.

X-linked dominant trait
1. Both males and females are usually affected; often more females than males are affected. 2. Does not skip generations. Affected sons must have an affected mother; affected daughters must have either an affected mother or an affected father. 3. Affected fathers will pass the trait on to all their daughters. 4. Affected mothers (if heterozygous) will pass the trait on to half of their sons and half of their daughters.
Y-linked trait
1. Only males are affected. 2. Passed from father to all sons. 3. Does not skip generations.

They are extremely useful helpful in pedigree analysis. The knowledge gained from such an analysis facilitates a genetic counsellor to educate the patient and family about the disease and more importantly the probability of transmitting the condition to future generations. They also guide about the options available to them for genetic testing and help them reach a decision if woman is bearing a child with a deleterious mutation which is untreatable. The standard symbols used in depicting pedigrees are summarised in Fig. 6.1.



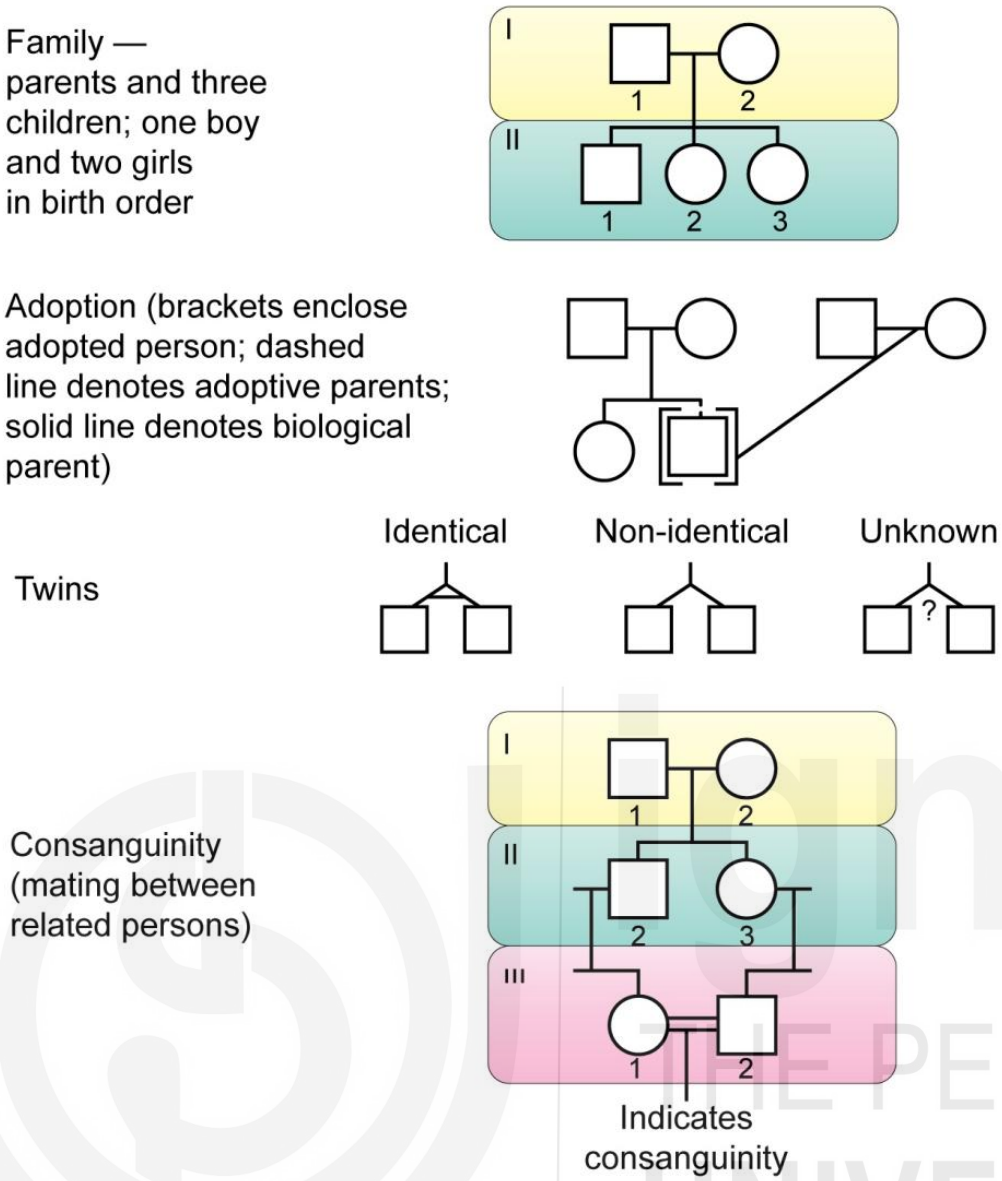


Fig. 6.1: Standard symbols and characteristics of patterns of inheritance (from Genetics by Benjamin Pierce).

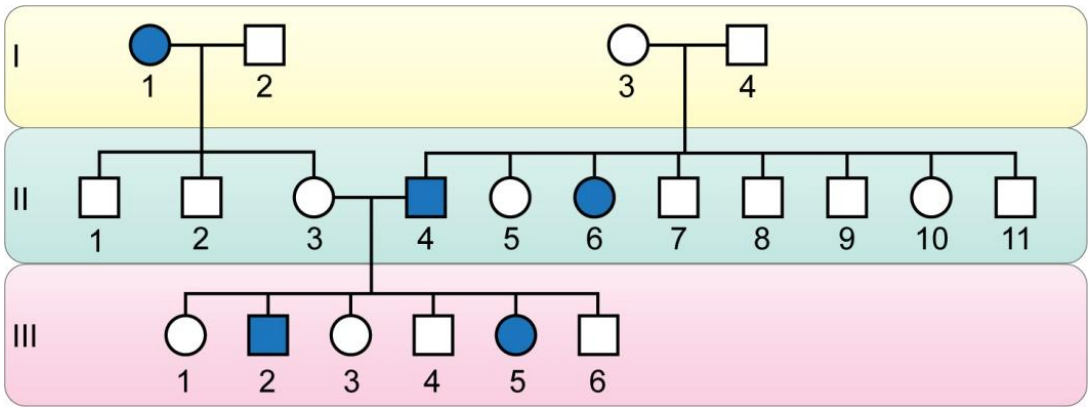
6.3 REQUIREMENTS

Different pedigrees. You can refer suggested readings and internet for pedigrees. Some examples are given below for understanding and analysis.

6.4 GUIDED STUDY OF REPRESENTATIVE HUMAN PEDIGREES

We shall analyse three pedigrees and predict the likely mode of inheritance. The approach is to defend our choice by excluding other possibilities.

6.4.1 Pedigree 1



What is the most likely mode of inheritance of pedigree 1? Defend your choice.

Solution

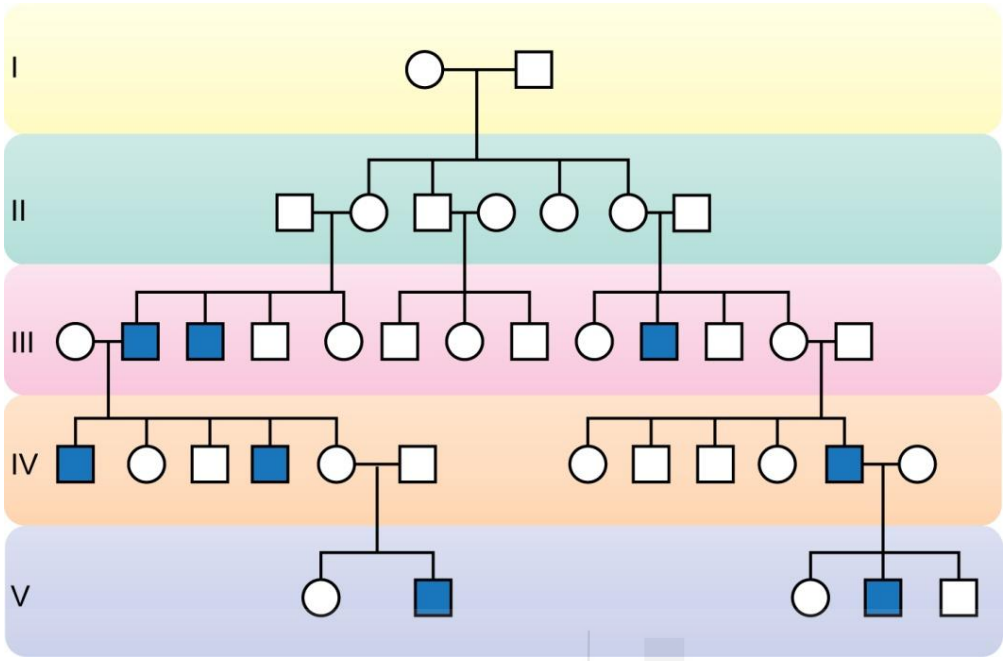
The most likely mode of inheritance of pedigree 1 is **autosomal recessive.** An important feature of autosomal recessive trait is that affected progenies are generally born to unaffected parents (heterozygous carriers).

1. In this pedigree also two unaffected individuals (I-3 and I-4) have affected progeny (II-4, II-6). In addition, 25% (2/8) of the progeny is affected (II-4 and II-6 of II4-11) which is also expected in mating between two carriers.
2. The trait is transmitted from either parent to both sons and daughters. Females and males are equally likely to be affected.
3. Tends to skips generations as evident from unaffected II-1, II-2, II-3.

Why other possibilities are excluded?

- Y-linked is ruled out because females are affected.
- X-linked dominant pattern is not possible because:
    - Unaffected mother (I-3) has an affected son (II-4);
    - Affected mother (I-1) has unaffected progenies.
    - Affected father (II-4) has unaffected daughters (III-1 and III-3)
    - It is skipping generation (II-1 II-2 and II-3 are unaffected)
- X-linked recessive is not feasible because affected mother (I-1) has unaffected sons (II-1 and II-2).
- Autosomal dominant is ruled out because all affected progenies do not have an affected parent.

6.4.2 Pedigree 2



The pedigree above is for a rare disease called spastic paraplegia, a nervous disorder. What mode of inheritance is suggested by the pedigree? (Adopted from Griffith)

Solution

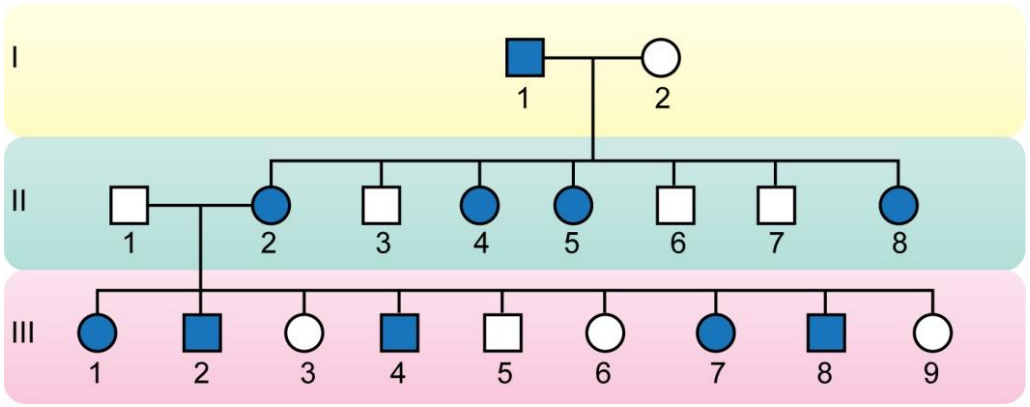
In this pedigree **only males (8) are affected** and they are born to unaffected mothers. This is expected for rare **X-linked recessive** disorders.

- The mothers of all affected sons are heterozygous carriers. Daughters of all affected men must be heterozygous. In addition, I-1 must be heterozygous.
- The trait skips generations.
- A criss cross of inheritance pattern is observed [father (III-2) to daughter (IV-5) to grandson (V-2)].

Why other modes of inheritance are excluded?

- Y-linked is excluded because unaffected fathers (II-1, II-7) have affected sons (III-1; III-3; III-10). Also, the trait is seen to skip generation, which is not the case with Y-linked pattern of inheritance.
- It is inconsistent with X-linked dominant traits because an affected father (III-2) has unaffected daughters (IV-2; IV-5).
- Autosomal dominant is excluded as all affected individuals do not have an affected parent.
- Autosomal recessive is also ruled out since daughters are unaffected. Autosomal traits are transmitted from both parents to sons and daughters alike.

6.4.3 Pedigree 3



1. What is the mode of inheritance in pedigree 3? Defend your choice.
2. Can we exclude the possibility of an autosomal dominant or X linked recessive pattern of inheritance? If yes, why?

Solution

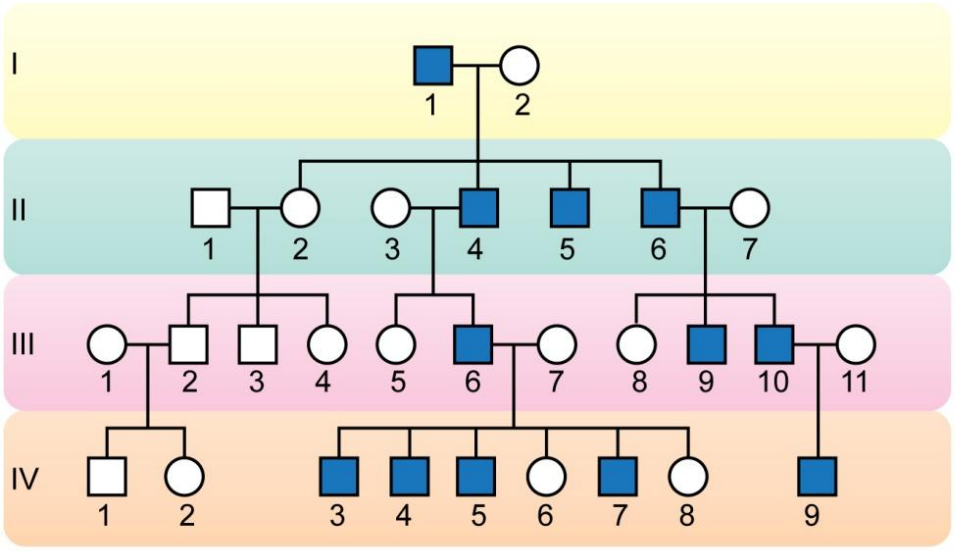
1. It is an **X-linked dominant** pattern of inheritance because :
   - Affected father (I-1) and unaffected mother (I-2) had only affected daughters (II-2, II-4, II-5, and II-8)
   - Both males and females are affected; more females than males are affected.
   - Affected sons and daughters are born to affected mothers.
   - It shows a criss-cross pattern of inheritance [Father (I-1) to daughter (II-2) to grandson (III-2)].
   - The trait does not skip generations; a characteristic of dominant traits.
2. **(a)** An **autosomal dominant** mode of inheritance is excluded because the affected father (I-1) has passed the disease only to the daughters (II-2, II-4, II-5, II-8) and sons are not affected.

   (b) An **X-linked recessive** pattern is unlikely because:
   - Unaffected father (II-1) had affected daughters (III-1; III-7).
   - X-linked recessive traits are more common in males than in females; however, reverse is seen in the pedigree.
   - All affected mothers are expected to have affected sons which is not the case with III-5, who is an unaffected son of an affected mother (II-2).

6.5 ACTIVITY

Indicate the mode of inheritance in the given pedigrees. Analyse carefully the two pedigrees given below and predict the most likely mode of inheritance. Defend your choice.

6.5.1 Pedigree A



What is the most likely mode of inheritance? Defend your choice. (Adapted from Pierce)

Solution (*Hint:* Only males are affected).

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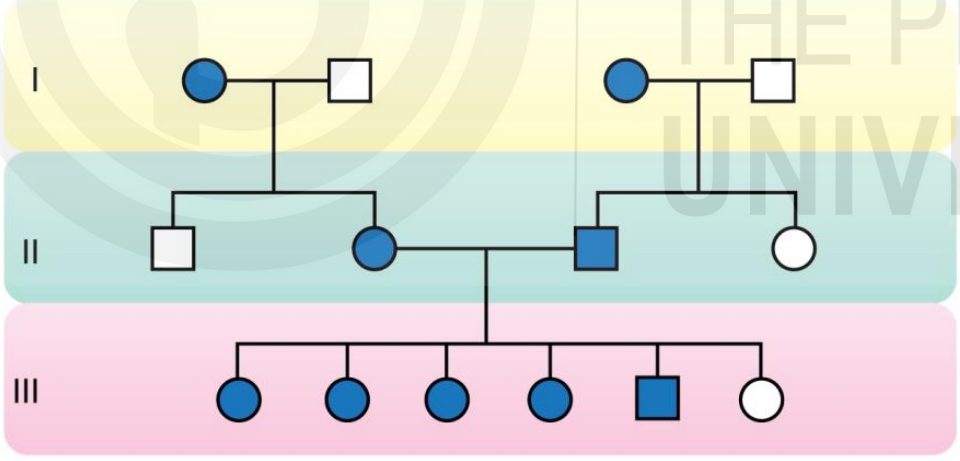
6.5.1 Pedigree B



Assume that this pedigree is straight forward, with no complication, such as illegitimacy.
Phenotype W, found in the individuals represented by shaded symbols, is rare in the general population, (Adapted from Griffith).

Solution

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SUGGESTED READINGS

1. Gasque, C.E. (1989) A manual of laboratory experiments in Cell Biology, published by Universal book stall in India.

2. Mittwoch, U (1963), Sex differences in cells, Scientific American , July 1963.