KARPAGAM ACADEMY OF HIGHER EDUCATION

Class: III B.Sc Microbiology

Course Name: Inheritance Biology Practical

Subject code: 17MBU514B

INHERITANCE BIOLOGY - PRACTICAL **(3H – 1C)**

Instruction Hours / week: L: 0 T: 0 P: 3 **Marks:** Internal: **40** External: **60** Total: **100**
End Semester Exam: 6 Hours

SCOPE

This paper imparts knowledge on the different aspects of genetics and pedigree analysis.

OBJECTIVE

- To make students understand the principles of Genetics and inheritance biology.

EXPERIMENTS

1. Mendelian deviations in dihybrid crosses
2. Studying Barr Body with the temporary mount of human cheek cells
3. Studying *Rhoeo* translocation with the help of photographs
4. Karyotyping with the help of photographs
5. Chi-Square Analysis
6. Study of polytene chromosomes using temporary mounts of salivary glands of *Chiromonas / Drosophila* larvae
7. Study of pedigree analysis
8. Analysis of a representative quantitative trait

SUGGESTED READINGS

1. Gardner EJ, Simmons MJ, Snustad DP (2008). Principles of Genetics. 8th Ed. Wiley-India.
2. Snustad DP, Simmons MJ (2011). Principles of Genetics. 6th Ed. John Wiley and Sons Inc.
3. Weaver RF, Hedrick PW (1997). Genetics. 3rd Ed. McGraw-Hill Education.
4. Klug WS, Cummings MR, Spencer CA, Palladino M (2012). Concepts of Genetics. 10th Ed. Benjamin Cummings.
5. Griffith AJF, Wessler SR, Lewontin RC, Carroll SB. (2007). Introduction to Genetic Analysis. 9th Ed. W.H.Freeman and Co., New York
6. Hartl DL, Jones EW (2009). Genetics: Analysis of Genes and Genomes. 7th Ed, Jones and Bartlett Publishers.
7. Russell PJ. (2009). *i* Genetics - A Molecular Approach. 3rd Ed, Benjamin Cummings.

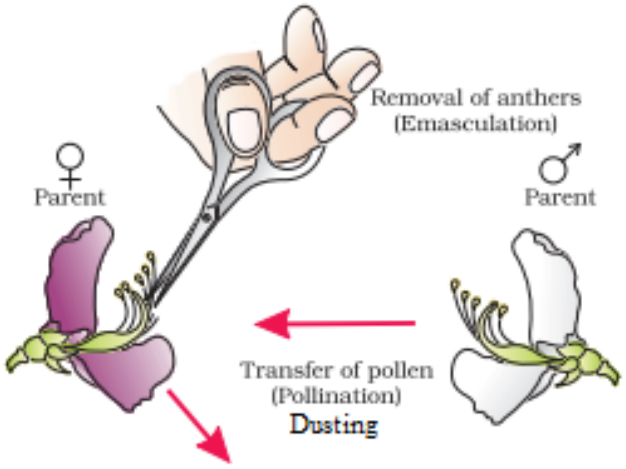
Experiment: 1. Mendelian deviations in dihybrid crosses

Objective

To verify the Mendelian deviation in dihybrid cross assortment.

Principle

In a dihybrid cross, the segregation of one gene pair is independent of the segregation of the other pair. It means that genes of two different traits assort independently to give a probability ratio equal to segregation probability ratio of one allele pair X segregation probability ration of their allele pair which come to, (3:1X3:1)-=9:3:3:1. The rules of meiosis, as they apply to the dihybrid, are codified in Mendel's first law and Mendel's second law, which are also called the Law of Segregation and the Law of Independent Assortment, respectively. The Law of Segregation states that every individual organism contains two alleles for each trait, and that these alleles segregate (separate) during meiosis such that each gamete contains only one of the alleles. An offspring thus receives a pair of alleles for a trait by inheriting homologous chromosomes from the parent organisms: one allele for each trait from each parent. The Law of Independent Assortment states that alleles for separate traits are passed independently of one another. That is, the biological selection of an allele for one trait has nothing to do with the selection of an allele for any other trait. Mendel found support for this law in his dihybrid cross experiments. In his monohybrid crosses, an idealized 3:1 ratio between dominant and recessive phenotypes resulted. In dihybrid crosses, however, he found a 9:3:3:1 ratios. This shows that each of the two alleles is inherited independently from the other, with a 3:1 phenotypic ratio for each. The dihybrid cross is easy to visualize using a Punnett square.



Dihybrids cross experiment

Materials and Methods

Plastic beakers, Plastic beads each of yellow, green

Procedure

(1) Selection of parent s and obtaining pure lines: For dihybrid cross, mental selected pea plant having yellow and round seeds (YYRR) as the female parent and pea plant having green and wrinkled (yyrr) seeds as the male parent. He obtained pure line by selfing these plant for three generations. He confirmed that pea plant having yellow and round seeds are producing yellow and round seeds and pea plant having green and wrinkled seeds are producing green and wrinkled seeds.

(2) Emasculation, dusitn and raising F1 generation: a. Emasculation is a process of removal of stamens before the formation of pollen grains (Anthesis). This is done in the bud condition. The bud is carefully open and all stamens (9+1) are removed carefully.

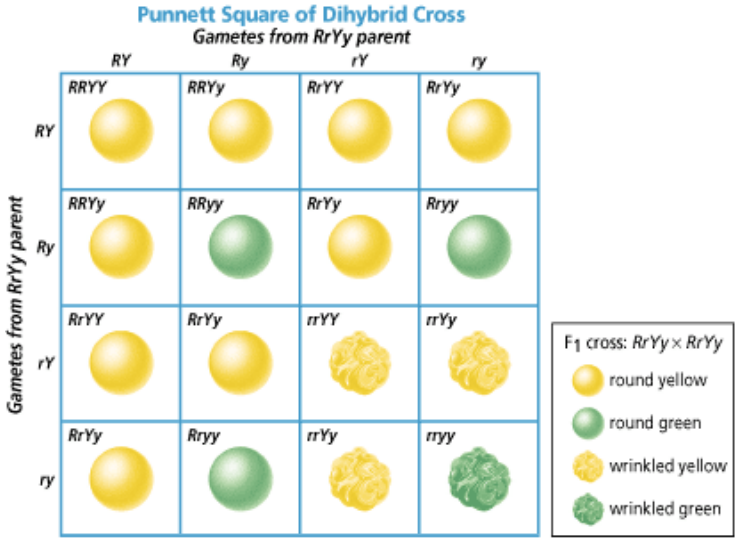
b.Dusting and rasing F1 generation: the pollens from selected male flower are dusted on the stigma of the emasculated female flower. This is artificial cross. Mendel crossed many flowers, collected seeds and raised F1 generation. The female plant produced gametes with genes YR while male plant produced gametes with gens yr.

c. Yellow and round are dominant alleles, hence all F1 generation was with yellow and round seeds. All the plants produced in F1 generation with yellow and round seeds (YyRr), which are heterozygous for both the alleles and are called dihybrid.

d. Mendel allowed natural pollination in each F1 hybrid, collected seeds separately and F2 generation is obtained.

Results

He found seeds of four types, yellow round, yellow wrinkled, green round and green wrinkled in the ratio 9:3:3:1. Our of these four types, two were parental combinations, are may yellow round and green wrinkled and two were new combinations like yellow wrinkled and green round. All the mendelian dihybrid crosses the ratio in which four different phenotypes occurred was 9:3:3:1. This ration is called dihybrid ratio.



Ratio:

- The 9 represents the proportion of individuals displaying both dominant traits: RRYY + 2 x RRYy + 2 x RrYY + 4 x RrYy
- The first 3 represents the individuals displaying the first dominant trait and the second recessive trait: RRyy + 2 x Rryy
- The second 3 represents those displaying the first recessive trait and second dominant trait:
  rrYY + 2 x rrYy
- The 1 represents the homozygous, displaying both recessive traits: rryy

Conclusion

The four phenotypic classes in the F2 generation are in ratio of 9:3:3:1 as expected from the Law of Independent Assortment. The genotypic ratio would be (1:2:4:2): (2:1):(2:1):1.

Problem - 1: Crosses that Involve 2 Traits -

Consider: RrYy x rryy

The square is set up need to shown

Experiment: 2. Studying Bar Body with the temporary mount of human Cheek Cells under the Microscope

Objective

To prepare a temporary mount of human cheek epithelial cells and to study its characteristics.

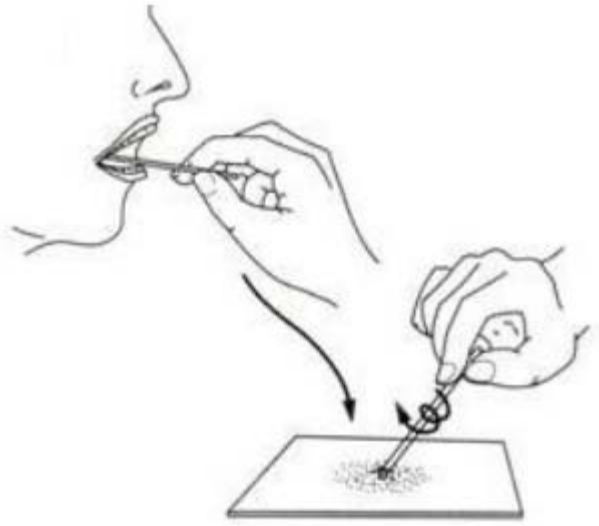
Principle

In human being the sex can be identified by observing the nucleus of their resting cells (the interphase nucleus) in the cells of female. A darkly stained chromatin matrix is observed on the slide in the interphase nucleus. This is known as sex chromatin or Barr body after the name of its discoverer Murray Barr in 1940.

The body of all animals including humans is composed of cells. Unlike plant cells, animal cells do not have cell wall. The outermost covering of an animal cell is a cell membrane. The cytoplasm, nucleus and other cell organelles are enclosed in it. Epithelial tissue is the outermost covering of most organs and cavities of an animal body.

Buccal epithelial cells especially have Barr body structure, which are considered to play a major role for sex determination. This small round Barr body is located either in the border of nuclear membrane or sometimes inside of nucleus. This Barr body may be single or more in number in some cases. These structures are present only in the female sex.

Condition	Sex Chromosomes	Number of Barr Bodies
Normal Female	XX	1
Normal Male	XY	None
Trisomy X	XXX	2
Turner's Syndrome	X	None
Kleinfelter's Syndrome	XXY	1



Materials required

Glass microscope slides
cover slips
Paper towels or tissue
Methylene Blue solution (0.5% to 1% (mix approximately 1 part stock solution with 4 parts of water))
Plastic pipette or dropper
Sterile, individually packed cotton swabs

Procedure

1. To rinsed our mouth with water.
2. Take a clean cotton swab and gently scrape the inside of your mouth.
3. The scrapped material is transferred into a drop of water and taken on a clean side.
4. Smear the cotton swab on the centre of the microscope slide and dry it for few mins.
5. Add a drop of methylene blue solution and place a coverslip on top.
6. Remove any excess solution by allowing a paper towel to touch one side of the coverslip.
7. Place the slide on the microscope, with 4 x or 10 x objective in position and find a cell. Then view at higher magnification.

Results

Epithelial cells are small, polygonal in shape and compactly arranged to form a continuous layer. The cells are without cells wall. Cell membrane encloses a distinct nucleus and

a vacuole. Epithelial tissue forms the outermost covering of almost all the organs and various cavities of animals and human.

Note: Methylene blue stains negatively charged molecules in the cell, including DNA and RNA. The cells seen are epithelial cells from the outer epithelial layer of the mouth. The small blue dots are bacteria from our teeth and mouth.

Result

The epithelial cell dark stained heterochromatin (Barr body) is observed usually periphery of the nucleus, clearly under high power microscope.

S. No.	Feature	Observation
1.	Shape of cells	Ploygonal
2.	Arrangement of cells	Compact
3.	Inter-cellular spaces	Present
4.	Cell membrane	Present
5.	Cell wall	Absent
6.	Cell contents	Nucleus, Mitochondria
7.	Vacuoles	Absent

- Large irregularly shaped cells with distinct cell walls.
- A distinct nucleus at the central part of each individual cell (dark blue in color).
- A lightly stained cytoplasm in each cell.

Conclusion

In this experiment is the basic structure of a cell and its major parts. For easy identification of the parts, will help them identify different parts with ease.

Experiment: 3. Studying Rhoeo translocation with the help of photographs

Objective

To demonstrate the process of plasmolysis (using Rhoeo discolor leaf/ onion bulb)

Materials required:

Onion bulb, watch glass, petri dish, slides, cover slips, forceps, brush, needles, microscope, water and 50% concentrated sucrose solution.

Procedure:

- Take an onion bulb/ rhoeo leaf, with the help of forceps pull a thin transparent peel.
- Keep this peel in water filled watch glass
- Transfer the peel gently on a clean slide in a drop of water with the help of a brush and needle
- Examine it under high power (40X) of a light microscope
- Observe the individual cells note down the observation in the table 1.(Observation 1)
- With the help of dropper put the sucrose solution on the side of the cover slip so that it reaches the peel under the cover slip
- Examine the peel after 10 minutes and note down the observation in the table 1. (Observation 2)
- Drain out the concentrated sugar solution from the peel and add few drops of water into the peel
- Observe the cells again after 10 minutes and note down the observation in table 1.(Observation 3)
- Make a sketch of the cell showing the cell wall and cell membrane during all the three observations.

Observation

After half an hour we can observe that cells in sodium chloride 0.1% solution appear turgid, while cells in the sodium chloride 5 % solution show plasmolysis.

Conclusion

When plant cells are immersed in sodium chloride 5 % solution or concentrated salt solution, water moves through the cell membrane into the surrounding medium because the water concentration inside the cell is greater than that which is outside the cell. Ultimately the protoplasm causes shrinkage and assumes spherical shape. This is called plasmolysis. When a plant cell is immersed in sodium chloride 0.1% solution or dilutes salt solution, the water moves into the cell because of the higher concentration of water outside the cell than inside the cell. The cell then swells and becomes turgid.

Prepared by Dr. P. Thirunavukkarasu, Assistant Professor, Dept, of Microbiology, KAHE

Experiment: 4. Karyotyping with the help of photographs

Objective

The karyotype analysis to separate the chromosomes and observe the chromosomes in mammal cells with a microscope.

Material requirements

- 10 cm cell culture dishes (Trueline Valve Corpotation, catalog number: TR4002)
- Centrifuge and tube
- CO_2 incubator
- Centrifuge at room temperature (using "relative centrifugal force, rcf")
- Water bath
- Coplin staining jars with lids (Lipshaw 107)
- Forceps
- Glass slides
- Glass tube (2 mL, conical)
- Green filter for microscope
- Kimwipes or any coarse, grease-free tissues
- Microscope with 100X oil immersion objective and 40X phase-contrast objective
- Pasteur pipettes (~0.2-mL capacity, drawn to deliver 10-µL drops)
- Water bath preset to 60°C-65°C
- Whatman 3MM filter paper

Procedure

Day 1

1. Seed 5 x 10^5 cells on 10 cm cell culture dishes for attachment.
2. Cells were incubated at 37 °C, 5% CO_2 for 48 h.

Dary 3

3. Add 0.1 ml colcemid, which can collapse mitotic spindles and prevent the completion of mitosis, to each dish and mix gently. Incubated at 37 °C, 5 % CO_2 for 2 h.
4. Transfer medium to centrifuge tubes from the cell culture dishes. Use PBS to wash the dishes, and remove PBS. Then, add 1 ml 0.1% Trypsine into the dishes at 37 °C, 5% CO_2 for 2 min. Also, transfer the mixture (Trypsine and cells) into the centrifuge tubes and mix with the medium which is transferred to centrifuge tubes before we use PBS to wash the dishes. Further, centrifuge at 100 rcf for 10 min at room temperature.
5. Discard the supernatant and leave 0.5 ml medium to mix the pellet gently.
6. Resuspend the pellet in 5-7 ml 37 °C hypotonic solution and mix thoroughly. Incubate in water bath at 37 °C for 10 min.
7. Centrifuge at 100 rcf for 10 min at room temperature.
8. Discard the supernatant and leave 0.5 ml solution to mix the pellet gently.

9. Resuspend the pellet in 5 ml cold fixative (drop by drop and flick the tube between drops to prevent cell clumping).
10. Put the centrifuge tube on ice at least 20 min.
11. Centrifuge at 100 rcf for 10 min at room temperature.
12. Discard the supernatant and add 3-5 ml cold fixative.
13. Repeat steps 9-10 three times or until the supernatant is clear and the pellet become white.
14. After the final centrifugation, suspend the cells in a few drops of cold fixative to give a slightly opaque suspension.
15. Drop 1-2 drops onto wet and clean slides (Before that, the slides have to be rinsed by 1-2 drops of cold fixative.)
16. Dry the slides at room temperature.
17. Observe the chromosomes with the microscope (Olympus DP71 with 200x magnification).
18. Count the number of chromosomes about 50 cells (A normal human cell have 23 pairs of chromosomes.).

Observation

This method is most useful for examining chromosomal translocations, especially ones involving the Y chromosome. Taken together, these banding techniques offer clinical cytogeneticists an arsenal of staining methods for diagnosing chromosomal abnormalities in patients.

Experiment: 5. Chi-Square Analysis

Objective

To determine whether the association between two qualitative variables is statistically significant

Principle

The Chi-square goodness of fit test is used to test whether the distribution of a set of data follows a particular pattern. For example, the goodness-of-fit Chi-square may be used to test whether a set of values follow the normal distribution or whether the proportions of Democrats, Republicans, and other parties are equal to a certain set of values, say 0.4, 0.4, and 0.2.

The Chi-square test for independence in a contingency table is the most common Chi-square test. Here individuals (people, animals, or things) are classified by two (nominal or ordinal) classification variables into a two-way, contingency table. This table contains the counts of the number of individuals in each combination of the row categories and column categories. The Chi-square test determines if there is dependence (association) between the two classification variables. Hence, many surveys are analyzed with Chi-square tests.

Chi-Square Formula

$$\chi^2 = \sum \frac{(\text{Observed Value} - \text{Expected Value})^2}{(\text{Expected Value})}$$

Procedure

To determine whether the association between two qualitative variables is statistically significant, researchers must conduct a test of significance called the Chi-Square Test. There are five steps to conduct this test.

Step 1: Formulate the hypotheses ϖ Null Hypothesis: H0: There is no significant association between students' educational level and their preference for online or face-to-face instruction.

Step 2: Specify the expected values for each cell of the table (when the null hypothesis is true)

Step 3: To see if the data give convincing evidence against the null hypothesis, compare the observed counts from the sample with the expected counts, assuming H0 is true.

Step 4: Compute the test statistic I.The chi-square statistic compares the observed values to the expected values. II. This test statistic is used to determine whether the difference between the observed and expected values is statistically significant.

Step 5: Decide if chi-square is statistically significant I. The final step of the chi-square test of significance is to determine if the value of the chi-square test statistic is large enough to reject the null hypothesis. II. Statistical software makes this determination much easier.

Result

Uses of the Chi-Square Test Use the chi-square test to test the null hypothesis H 0 : there is no relationship between two categorical variables when there is a two-way table from one of these situations: I. Independent random samples from two or more populations, with each individual classified according to one categorical variable. II. A single random sample, with each individual classified according to both of two categorical variables.

Conclusion

One of the most useful properties of the chi-square test is that it tests the null hypothesis “the row and column variables are not related to each other” whenever this hypothesis makes sense for a two-way variable.

Experiment: 6. Study of polytene chromosome using temporary mounts of salivary glands of Chiromonas/ Drosophila larvae

Objective

Study of polytene chromosome isolation and to separate the chromosome allow to temporary mount of salivary glands of Chiromonas/Drosophila larvae.

Principle

In any immunohistochemical experiment there will be advantages and disadvantages to different methods of fixation and sample preparation, the relative merits of which must be balanced. Thus, for any new antigen of interest, it is necessary to optimize the chosen fixative and fixation conditions. In the case of studies directed towards RNA polymerase II elongation control, the challenge is to identify conditions that suitably preserve the chromatin structure, allowing accessibility to antibodies without stripping away key proteins or blocking critical epitopes.

Materials requirments

- Drummond dissection forceps (2)
- Petri dishes (60 x 15 mm)
- Frosted microscope slides (Fisher No. 12-544-3) (poly-lysine-coated)
- 22 x 22 mm No. 15 cover slips (Fisher No. 12-520B) (coated with Sigmacote; Sigma #SL2)
- 22 x 40 mm No. 15 cover slips (Fisher No. 12-530B)
- Kim-wipes
- Phase contrast microscope with 20X objective
- Small Dewar (e.g., vacuum flask or thermos bottle)
- Long forceps
- Razor blades
- Coplin Jar
- Rubber-Maid or Tupperware tray (or equivalent sealable tray)
- Parafilm (cut into 22 mm squares)
- 175 g weights

Procedure

Use your forceps to remove 8-10 actively crawling larvae from the sides of the cultivation bottle. Place them on a dry microscope slide (slightly to one side).

2. Use a Pasteur pipette to place a drop of saline solution in the middle of the same microscope slide. Use your forceps to transfer one of the larvae to the saline drop, with its tracheal tubes facing up (See Figure 3).

3. Use one pair of forceps to hold the anterior end of the larva in place at the spot just above the neural ganglion in Figures 3 and 4, while using a second pair of forceps to grab the top

layer of cuticle from a position on the larva at about one third body length from the anterior end (Figure 4).

4. The salivary glands and ventral ganglion (brain) will usually remain attached to the head region, separate from the rest of the body, after a clean dissection. A diagram of this is shown in Figure 5, and a photograph is shown in Figure 6. Figure 7 shows a photograph of salivary glands after clean dissection from the larval body and head regions. You may have to use your forceps to search for the salivary glands among the dissected material. If this fails, simply start over with a new larva. These dissections are difficult to master, but reasonable results can be obtained with a little practice.

5. As you dissect the salivary glands, you can either keep them set aside in a separate saline drop on the slide used for your dissections or transfer them to a separate saline drop on a different slide. In handling the salivary glands, either grab them from their less fragile smallest diameter base (Figure 7, arrow) or use your forceps to scoop them from underneath.

6. After you have accumulated 5-6 salivary glands, prepare a slide for a squash of 1-2 glands. Use a Kimwipe to wipe its surface clean, then blow any dust from its surface before placing a 10µl drop of Lacto/Aceto Orcein stain on the slide. One partner of the lab group can handle this task while the other (probably the partner who did the dissection) handles step # 7 below.

7. Then place a drop of 45% Acetic Acid (HOAc) fixative on the slide next to the drop of saline holding your salivary glands and use your forceps to transfer the salivary glands from the saline to it and hold in place for ~30 seconds. Then immediately transfer the glands to the drop of Lacto/Aceto Orcein stain on the slide from step # 6.

8. Place a clean coverslip (dust blown from its surface) onto the surface of the glands and use your forceps to gently tap the surface of the coverslip in a circular pattern. The coverslip should rotate somewhat freely on the surface of the microscope slide; this is needed to burst the membranes of the polytene nuclei and allow the chromosome arms to spread.

9. Place the slide on your microscope. Using the lowest power objective and standard light source, scan around the slide until you find the squashed material. It is often useful to first locate the squashed material on the slide by eye. Then place the slide on the microscope stage with this material centered in the light beam. This will make it easier to find your chromosomes under the microscope. First locate the focal plane of your chromosomes by adjusting the focus knob until the fluid background material becomes visible. You can then begin to scan around the slide to find the chromosomes.

10. If the chromosomes look sufficiently well spread (See Example in Figure 1), you can now invert the slide and coverslip onto a sheet of Kimwipe. Then use your thumb to apply pressure to the coverslip while using the middle and index fingers of your other hand to secure the position of the slide and coverslip (Diagram in Figure 8). This squashing action will bring about further spreading of the chromosomes and remove excess liquid between the slide and coverslip to allow maximal attachment of the chromosomes to the slide.

11. If the nuclei were found to be still intact, you can attempt to rupture them if there is evidence of liquid between the slide and coverslip as evidenced by movement of background material. To rupture the nuclei, use your forceps to tap the surface of the coverslip as described

in Steps 8-10. It may be necessary to start over with some newly dissected salivary glands. Preparing polytene chromosome squashes is part science/part art. It takes a lot of practice and a little luck to get the quality of squash.

Results

There should be numerous well spread polytene chromosomes. The double labeled with a marker for interband regions in green and with a dye that stains the banded regions in blue. If insufficient spreading is obtained, the chromosomes will look like little balls. On the other hand, if too much spreading has occurred, the chomosomes will be too thin and extended or in some cases fragmented into small pieces.

Conclusion

This concludes the procedure for viewing the polytene chromosome in larvae of the fruit fly Drosophila melanogaster

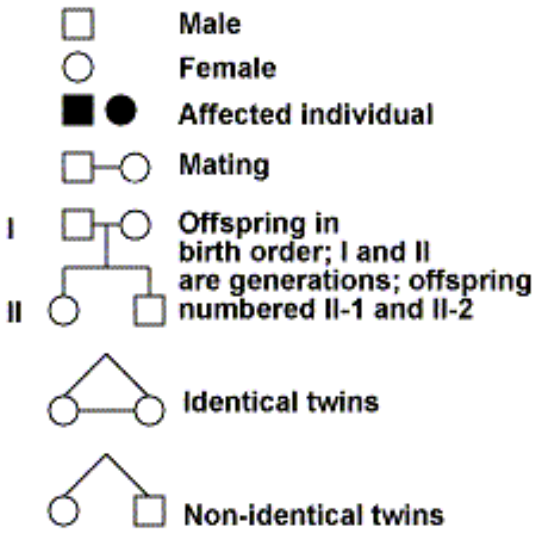
Experiment: 7. Study of Pedigree analysis

Objective

Preparation and analysis of Pedigree charts

Principle

The Mendelian concept of dominance and segregation can also be studied in humans by preparing and then analyzing the pedigree charts. The internationally approved symbols for indicating males and females , various generation (I, II, ,III) etc. This is always the case when studying human genetics. Scientists have devised another approach, called pedigree analysis, to study the inheritance of genes in humans. Pedigree analysis is also useful when studying any population when progeny data from several generations is limited. Pedigree analysis is also useful when studying species with a long generation time.



Materials requirements

Information about characters

Traits in a family for more than one generation

Procedure

Select a family in which any one of the monogenic traits such as tongue rolling, widow's peak, blood groups', red-green colour blindness, dimple in the cheek, hypertrichosis of ear, hitch-hiker's thumb, etc., is found. Ask the person exhibiting the trait to tell in which of his/her parents, grandparents (both maternal and paternal), their children and grand children the trait in question is present. Among surviving individuals the trait may also be examined. The information made available is the basis for the preparation of pedigree chart using the appropriate symbols. A careful examination of the pedigree chart would suggest whether the

gene for the character is autosomelinked dominant or recessive, X - chromosome linked dominant or recessive, Y- chromosome linked or not.

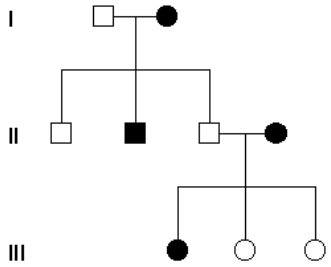
Results

For those traits exhibiting dominant gene action:

- affected individuals have at least one affected parent
- the phenotype generally appears every generation
- two unaffected parents only have unaffected offspring

The following is the pedigree of a trait controlled by dominant gene action.

Dominant Pedigree

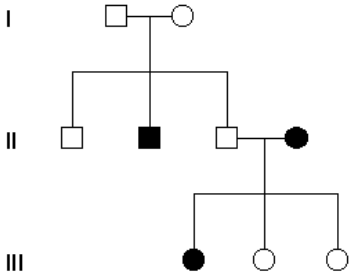


And for those traits exhibiting recessive gene action:

- unaffected parents can have affected offspring
- affected progeny are both male and female

The following is the pedigree of a trait controlled by recessive gene action.

Recessive Pedigree



Conclusion

The pattern of phenotypic expression can be quite useful in determining the mode of transmission for a given characteristic. In fact, geneticists often study the expression of particular traits in family lineages, or pedigrees, in order to gain insight into the mode of expression for a given character trait. Not only can pedigree analyses provide insight into the mode of transmission, but importantly, they can be used to predict the genotype of particular individuals.

Experiment: 8. Analysis of a representative quantitative trait

Objective

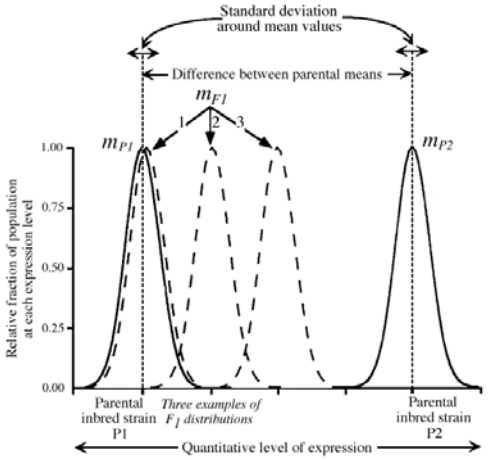
Study of representative quantitative trait either a large population of mice/human that all differ in their genotypes or compare several strains, each of which has a fixed genotype.

Principle

Polygenic inheritance refers to inheritance of a phenotypic characteristic (trait) that is attributable to two or more genes and can be measured quantitatively. Multifactorial inheritance refers to polygenic inheritance that also includes interactions with the environment. Unlike monogenic traits, polygenic traits do not follow patterns of Mendelian inheritance (discrete categories). Instead, their phenotypes typically vary along a continuous gradient depicted by a bell curve. Many traits are polygenic, that is, determined by the cumulative contributions of a large number of genes. Furthermore, many of these traits are capable of being measured on a scale at least as strong as the interval scale; such traits are quantitative traits. If a polygenic trait Y is quantitative and the contributions from its governing genes are assumed to combine additively, we have the additive genetic model. This type of approach is used in order to better characterize the phenotypic variation in and evolution of human traits.

Procedure

- Geneticists are, obviously, most interested in the genetic contribution to a quantitative trait.
- A genetic contribution cannot be demonstrated by looking at individuals from a single inbred strain alone.
- The comparison of expression levels must be made on sets of animals from two different inbred strains.
- The statistical approach can be used to determine formally whether two strains differ significantly in the expression of the quantitative trait.
- If a significant strain-specific difference is demonstrated, and all other variables have been controlled.
- it becomes possible to attribute the observed difference in quantitative expression to allelic differences that distinguish the two strains.
- Quantitative trait is most amenable to genetic analysis in mice and other experimental organisms with a pair of inbred strains that show non-overlapping distributions in measured levels of expression among at least 20 members of each group.



Idealized distributions for the expression of a quantitative trait in two inbred strains and the F_1 hybrid between them. In this example, the mean levels of expression for two inbred parental populations (m_{P1} and m_{P2}) are separable from each other by 16 standard deviations

Note: To apply this test, one needs to use a pair of only three values derived from an analysis of the expression of the trait in sets of animals from each inbred strain. First is the number of animals examined in each inbred set (N_1 and N_2). Second is the mean level of expression for each set (m_1 and m_2) calculated as:

Conclusion

QTL mapping in animals provides a valuable, though preliminary, contribution to the search for genes contributing to human alcoholism. The identification of QTLs even though these regions typically contain about 15 million DNA base pairs and an estimated 500 to 1,000 genes provides a significant advance in the daunting task of elucidating the genetic bases of alcohol effects. The subsequent identification of novel candidate genes and the clarification of their mechanisms of influence contribute to the eventual understanding of functional variability in humans that confers differential vulnerability to alcoholism.