Practical Manual

On

Principles of Genetics and Cytogenetics

HFS 102 3(2+1)

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Syllabus:

Study of fixatives and stains. Squash and smear techniques. Demonstrations of permanent slides and cell division, illustration in plant cells, pollen fertility and viability, determination of gametes, Solving problems of monohybrid, dihybrid, and test cross ratios using chi-square test, gene interactions, estimation of linkages using three point test cross from F_2 data and construction of linkage maps. Genetic variation in pea.

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Practical No. 1

Objective: To study different parts of microscope

Material Required: ……

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Procedure: ……

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Properties:……

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Practical No. 2

Objective: To prepare fixatives and write its properties

Material Required: ……

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Procedure: ……

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Properties: ……

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Practical No. 3

Objective: To prepare squash and learn smear techniques

Material Required: ……

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Procedure: ……

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Properties:……

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Practical No. 4

Objective: To study structure of a plant cell

Material Required: ……

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Procedure: ……

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Properties:……

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Draw well-labelled structure of a plant cell

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Practical No. 5

Objective: Study of Mitosis and demonstration of permanent slides

Material Required: ……...

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Procedure: ……

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Observed under the microscope and draw different stages of mitosis

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Practical No. 6

Objective: Study of meiosis and demonstration of permanent slides

Material Required: ……...

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Procedure: ……

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Observed under the microscope and draw different stages of meiosis

Practical No. 7

Objective: To study pollen fertility and viability in laboratory

Material Required: ……

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Procedure: ……

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Properties:……

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Questions:

1. Count and write viable and non-viable pollens in different microscopic fields?

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2. Calculate the sterility percentage from above data?

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3. Draw picture as observed in microscopic field.

Practical No. 8

Objective: To determine the gametes and solve the problems

Problem 1: What will be the appearance of (a) F_1 and (b) F_2 progenies when a pure (homozygous) tall pea plant is crossed with a pure (homozygous) dwarf pea plant?

Solution: ……

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Problem 2: Work out the probabilities of the off-springs in a case when heterozygous tall hybrids of F_1 generation are self-fertilized.

Solution: ……

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Problem 3: Work out for the genotypes of the parents of the cross between a tall and a dwarf pea plant which result into about one half of the tall and one half of dwarf off-springs.

Solution: ……

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Problem 4: What will be the gametes formed from the parents and the genotypes of the offspring obtained from the following matings?

1) AaBB × aabb 2) Aabb × AABB 3) AaBb × AaBb

Solution: ……

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Problem 5: In the garden pea, Mendel found that yellow seed colour was dominant to green and round seed shape was dominant to shrunken.

a) What phenotypic ratio would be expected in the F_2 from a cross of a pure yellow round x green shrunken?
b) What is the F_2 ratio of yellow: green and of round: shrunken?
c) Give the test cross ratio.

Solution: ……

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Problem 6: What will be the gametes formed from the parents and the genotypes of the offspring obtained from the following matings and give the ratio of F_2 generation?

1. PPQQRR ×ppqqrr 2. PpQqRr ×PpQqRr

Solution: ……..

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Practical No. 9

Objective: Chi square test and to determine test cross ratios

Chi-square test :……

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It can be determined by the following formula Chi square value (χ^2) =

Test cross: ……..

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Uses of test cross:

1. ……..

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Q 1: Some experiment results are given below in garden pea. Test each for goodness of fit to the given hypothesis.

S. No.	Cross	Progeny	Hypothesis
a	Green x yellow pods	(F_2) 428: 152	3:1
b	Violet red x white flowers	(F_1)47 : 40	1:1
c	Round yellow x wrinkled green seeds	(F_1) 31:26:27:26	1:1:1:1

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(a)

	O	E	(O-E)	$(O-E)^2$	$(O-E)^2 / E$
	428	435 (3/4 x180)			
	152	145 (1/4 x 580)			
Total	580	580			χ^2=0.451, df=1

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(b)

	O	E	(O-E)	$(O-E)^2$	$(O-E)^2 / E$
	47	43.5 (1/2 x 87)			
	40	43.5 (1/2 x 87)			
Total	87	87			χ^2=0.5632, df=1

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(c)

	O	E	(O-E)	$(O-E)^2$	$(O-E)^2 / E$
	31	27.5			
	26	27.5			
	27	27.5			
	26	27.5			
Total	110	110			χ^2=0.5632, df=3

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Q 2: In rice, there are varieties with short and long outer glumes. When 2 pure breeding lines with short and long glumes were crossed, F_1 was intermediate. On selfing, the progenies segregated as short glume (436), long glume (412) and intermediate (808). Interpret the segregation and prove by chi square test.

Solution: ……

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Practical No. 10

Objective: Study of Gene Interactions

Gene interactions: ……...

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It can be categorised in two categories (i) between allele of the same locus (intralocus) intra allele, and (ii) interaction between alleles at different loci (interlocus).

There are three types of **Intralocus Interactions**-

a. Dominance-

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b. No dominance or additive dominance or in complete dominance-

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c. Co- dominance –

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Interlocus Interactions –

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Epistatic gene action:

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Example: In summer squash white colour of fruit is controlled by a dominant gene 'W' and yellow colour by a dominant gene 'G'. The gene 'W' is epistatic to the gene 'G'. Find out the phenotype of F_1 and segregation ration in F_2 generation (White colour of fruit F_2 controlled by 'W' and Yellow colour of fruit is controlled by G; Gene W is epistatic to gene G)

WW gg × wwGG

White × yellow

Gamete ……. ……

Phenotype of F_1 WwGg Selfing

Segregation ratio F_2

Gametes: WG, Wg, wG, wg

Gametes	WG	Wg	wG	wg
WG				
Wg				
wG				
Wg				

WW= Produce white Gg = Produce yellow

WwGg = W mask the expression of G Wwgg = Green colour

Phenotypic Ratio = 12:3:1 (masking gene action) (White : Yellow : Green)

Supplementary gene action:

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Example: In maize, interaction of two dominant alleles P & R Produces purple colour of grain, R alone produce Red colour, P alone produce white colour. Determine phenotype of F_1 generation & Phenotypic segregation ratio in F_2 from a cross between PPRR x pprr

Solution: Interaction of P & R produce purple colour of grain, while R alone produce Red and P Produce white phenotype of F_1 generation

Parent PPRR x pprr
Gametes PR pr
F_1 PpRr

F_1 will produce purple colour of grain

Gametes	PR	Pr	pR	Pr
PR				
Pr				
pR				
Pr				

PR = alone produce red colour PP = alone produce white colour Rrpp = white colour

R combine with RRPP, RrPp, RrPP produce purple colour Phenotypic Ratio = 9 : 3 : 4

Duplicate gene action: ……..

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Example: In soybean a chlorophyll controlling system exists where at one locus the dominant allele G produce normal green seed coat colour and the recessive produces yellow. At a second locus the dominant Y3 allele results in green colour and the recessive y3 causes the leaves to turn yellow with age.

In a cross between two green parents, GGy_3y_3 x ggY_3Y_3, the F_1, Gg Y_3y_3 will be green. The F_2 will be distributed in 15 green : 1 yellow pattern as follows:

9	G__Y_3__	Green
3	gg Y_3__	Green
3	G__y_3y_3	Green
1	ggy_3y_3	Yellow

Only chlorophyll deficient plants are those that are homozygous recessive at both loci. Such interaction is called duplicate gene action.

Complementary gene action:

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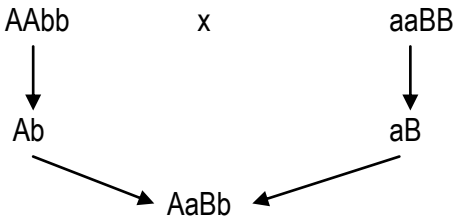
Example: AABB, AaBb, AABb and AaBb = Purple (Both dominant allele)
AAbb = White (One dominant allele A)
aaBB = White (One dominant allele B)
aabb = White (Both two allele recessive)

In garden pea interaction of two dominant gene A and B produce purple colour of the flower. Each gene separately produce white colour. Determine the phenotype of F_1 and phenotype ratio in F_2 from a cross between AABB x aaBB

A and B individually produce white colour and by interaction they produce purple colour.



F_1 will produce purple colour because both A and B genes are present

AaBb x AaBb (Selfing)

Gametes AB, Ab, aB, ab

Gametes	AB	Ab	aB	ab
AB				
Ab				
aB				
Ab				

In F_2 two types of flower colour will be produced viz. purple and white in ratio of 9:7

Additive gene action:

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Example: In wheat, the kernel colour is governed by three loci R_1, R_2 and R_3 independently assorted with two allele at each locus. Kernel colour ranged from very dark red to white, and intensity of colour depended on the number of colour adding alleles present in the genotype. The very dark red parent genotype is R_1R_1 R_2R_2 R_3R_3while the completely white parent genotype is r_1r_1 r_2r_2 r_3r_3. The $F_1$is R_1r_1 R_2r_2 R_3r_3 which is of intermediate in colour. A series of colour classes appeared in the F_2 with a nearly continuous normal distribution pattern.

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Inhibitory gene action:

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Example: In rice, green colour of plant is controlled by dominant gene I and purple colour of plant is controlled by gene P. Gene I is dominant over gene P. Phenotype of F_1 segregation ratio in F_2 from the cross between IIPP x Iipp

Green colour is controlled by I

Gene I is dominant over gene P

Cross between IIPP x iipp

Phenotype in F_1

Gamete IP x ip

F_1 IiPp (Green)

F_1 will produce green colour

F_2 Generation

Gametes	IP	Ip	iP	Ip
IP				
Ip				
iP				
ip				

Problem: In poultry, walnut combs are result of interaction of two dominant genes P and R. Determine the comb shape of parents in the following crosses, the gametes produced by them, and the genotypes and phenotypes of their progeny.

A) PPRR x PpRr B) Pp x pp rr

C) PpRr x Pprr

D) Pp Rr x ppRr

E) PpRr x PpRR

F) PpRr x PPRr

G) PPRr x pprr

H) PpRR x pprr

1. A walnut combed hen was mated to a pea combed cock; it produced only one progeny, which had single comb. Determine the genotypes of the two parents and the genotypes and phenotypes of the progeny expected from matings between such birds.

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2. In sweets peas, dominant genes C and R together produce coloured flowers. Determine the gametes, and genotypes and phenotypes of progeny obtained in the following crosses.

   a) CcRr x ccrr

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   b) CcRr x CCrr

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   c) ccRr x Ccrr

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   d) Cc Rr x cc Rr

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3. A white flowered sweet pea plant when crossed with one producing purple flowers, produced 3/8 purple and 5/8 white progeny. Determine the genotypes of the two parents and their progeny.

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4. A white flowered pea plant is crossed with another white flowered plant it yields ¾ white flowers and ¼ purple flowered progeny. Determine the genotypes of two parents and the progeny.

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5. In maize, a green plant when crossed with another green plant produced 225 green and 75 white plants, but when it was selfed it gave 153 green and 118 white off-springs. Determine the genotypes of two plants. Explain the types of gene interaction necessary to account for these findings.

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6. In maize, gene R produces red aleurone colour, while gene Pr, when present together with R, produces purple colour. Determine the phenotypes of the parents, and the genotypes and phenotypes of the progeny produced from the following crosses.

a) RRprpr x Rrprpr

b) RRprpr x RrPrPr

c) RrPrpr x Rr prpr

d) RrPrpr x rrPrPr

e) rrPrPr x Rr PrPr

f) Rr Prpr x Rr prpr

g) Rr Prpr x rrprpr

7. In mice, a dominant gene A affects the distribution with in hairs of black pigmentation produced by another dominant gene C so that CCAA genotype has grey hairs. Determine the phenotypes of the gametes produced by the parents in the following crosses, and the genotypes and phenotypes of the progeny from the following crosses.

a) CCAA x CcAa

b) Cc Aa x ccaa

c) CcAa x Cc aa

d) CcAa x ccAa

e) CcAa x CcAA

f) CcAa x CC Aa

g) CCAa x ccaa

b) CcAA x ccaa

8. In poultry, a dominant gene I inhibits plumage colour production by the dominant gene C, determine the phenotypes of and the gametes produced by the parents and the genotypes and the genotypes and phenotypes of the progeny from the following crosses.

a) Ccli x Ccii

b) Ccli x ccii

c) Ccii x ccli

d) CcII x ccli

e) Ccli x CcII

9. In summer squash , genes R1 and R2 produces round fruits when alone , but give rise to disc shaped fruits when they are together. Determine the phenotypes of and the gametes produced by the parents and the genotypes and the genotypes and phenotypes of the progeny from the following crosses.

a) R1r1R2r2 x R1r1r2r2

b) R1r1R2r2 x r1r1r2r2

c) R1r1R2r2 x r1r1R2r2

d) R1r1R2R2 x r1r1R2r2

e) R1r1R2r2 x R1r1R2R2

Practical No. 11

Objective: Study of complete dominance

Complete dominance: ……

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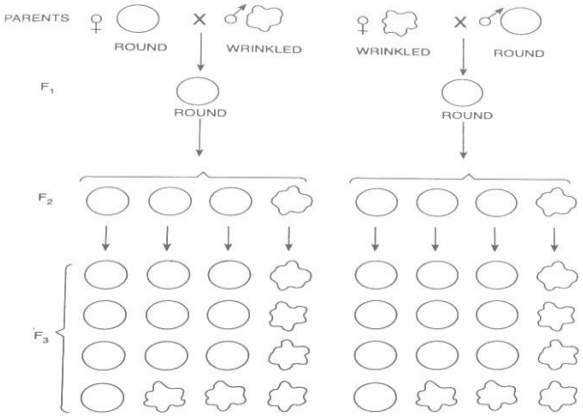
Example: Seed shape in peas. Round shape is produced by the dominant allele W; while wrinkled shape is determined by its recessive allele w. Seeds having genotype Ww are round and indistinguishable from those having the genotype WW. As a result, characters showing complete dominance yield the typical 3:1 monohybrid ratio in F_2 as shown in picture.

Question 1: Green pods are dominant over yellow in pea. Find out the phenotype and genotypes of the F_1 and F_2 plants from a green pod (GG) and yellow pod (gg) cross?



Direct and reciprocal crosses of parents having round and wrinkled seed shape

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2. Find out the progenies of cross of F_1 obtained from Tall and dwarf parent, with the short parent?

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3. Predict the results of the following crosses.
   a. A tall variety is crossed with dwarf variety.

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   b. The resulted progeny selfed

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   c. The selfed progeny crossed with the original tall parent

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   d. The selfed progeny crossed with the original dwarf parent

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4. In the garden pea, Mendel found that yellow seed colour was dominant to green and round seed shape was dominant to shrunken.
   a) What phenotypic ratio would be expected in the F_2 from a cross of a pure yellow round x green shrunken?

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   b) What is the F_2 ratio of yellow: green and of round: shrunken?

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5. In Drosophila, ebony body colour is produced by a recessive gene e and wild type (gray) body colour by its dominant allele e+. Vestigial wings are governed by a recessive gene vg and normal wing size (wild type) by its dominant allele Vg+. If wild type dihybrid flies are crossed and produce 256 progeny, how many of these progeny flies are expected in each phenotypic class?

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6. In one strain of sesame, coloured lip of the flower is dominant over colourless (white). If a pure breeding strain of each one is crossed, what will be the fruit colour of F_1. How would you distinguish a heterozygous coloured lip flower from a homozygous coloured lip flower?

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7. Mendel crossed pea plans producing round seeds and those producing wrinkled seeds. From a total of 7324 F2 seeds, 5474 were round and 1850 were wrinkled. Using your own notation for these traits, symbolize the original parental cross, F1 progeny, F2 obtained by selfing and summarize the expected F2 results for phenotypes, genotypes, genotypic frequency and phenotypic frequency ratios.

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8 A woman has a rare abnormality of the eyelids called ptosis which makes it impossible for her to open her eyes completely. This condition has been found to depend on a single dominant gene (P). The woman's father had ptosis, but her mother had normal eyelids. Her father's mother had normal eyelids.

a) What are the probable genotypes of the woman, her father and mother?

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b) What proportion of her children would be expected to have ptosis if she marries a man with normal eyelids?

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Practical No. 12

Objective: Study of incomplete dominance

Incomplete Dominance: ……

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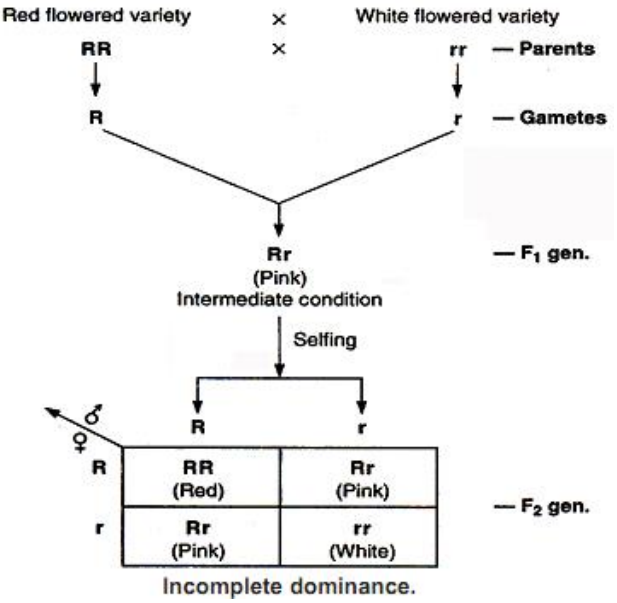
Example: Flower colour in four O'clock (*Mirabilis jalapa*). A partial dominant allele R produces red flowers in the homozygous state, while its recessive allele r produces white flowers. When a red flowered plant (RR) plant is crossed with one having white flowers (rr), the F_1 (Rr) is intermediate between those produced by the two homozygotes (RR and rr). Segregation in F_2 therefore, produces the phenotypic ratio of 1 red (RR), 2 pink (Rr) and 1 white (rr) instead of 3:1 red and white flowers. Clearly, heterozygotes for a partially dominant allele have a phenotype, which is easily distinguishable from that produced by homozygotes for the two allele.



Incomplete dominance.

1. In the four o'clock plant, white flowered variety is crossed with red flowered variety. All the F_1 were pink flowered. In the F_2 there were 142 pink, 68 white, and 73 red flowers. Find out the inheritance of flower colour**?**

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2. *Mirabilis jalapa*, a plant hybrid for red (R) and White (r) flower bears pink flowers (Rr).A plant with pink flowers is crossed with one having red flowers and other having white flowers. Give the genotypic and phenotypic ratios, expected in the progenies of the two crosses.

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3. In 4 o'clock plants, white flower is governed by recessive allele "r" and the red flower colour governed by the dominant allele "R" which has incomplete dominance over "r". Determine in following crosses genotypic and phenotypic ratio.

(A) RR x Rr

(B) Rr x rr

(C) RR x rr

(D) Rr x Rr

4. In soybean, broad leaf is incompletely dominant over narrow. The heterozygote is intermediate and purple is dominant over white.

a) What will be the phenotypic ratio of F_2 of a broad leaved plant with a homozygous purple flower crossed with a narrow leaf white flowered plant?

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c) What will be the offspring of the cross between F_1 and narrow leaf white flowered plant?

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Practical No. 13

Objective: Study of codominance

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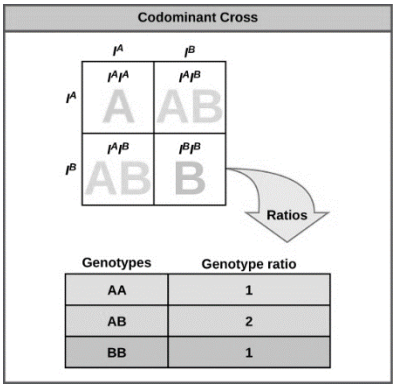
Example: Blood group antigens in man, I^A allele produces antigen A which results in blood group A.I^B allele produces antigens B which results in blood group B but heterozygote I^A I^B produces both antigen A and B resulting in AB blood group.



Q1. In shorthorn cattle, hair colour is decided by a pair of codominant traits. R is the allele for red hair colour and W is the allele for white hair colour. A cow with a heterozygous genotype is roan in colour, meaning its roan contains both white and red hairs**.** Describe the expected offspring when a breeder mates cows and bulls of the following phenotypes:

1. Red X Red

2. White X White

3. Red X Roan

4. Roan X Roan

5. White X Roan

Q 2: Yellow coat colour in guinea pigs is produced by the homozygous genotype C^yC^y, cream colour by the heterozygous genotype c^yc^w. What genotypic and phenotypic ratios are produced by matings between cream coloured individuals?

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Q 3: The shape of radish may be long($S^{L}S^{L}$), round ($S^{R}S^{R}$). If long radishes are crossed to oval radishes and the F_1 allowed to cross at random among them themselves, what phenotypic ratio is expected in the F_2?

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Q 4: What are the phenotypes and genotypes of the progeny produced in a case of marriage between two heterozygotes I^{A} I^{B} (AB blood group)?

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Practical No. 14

Objective: Study of lethality

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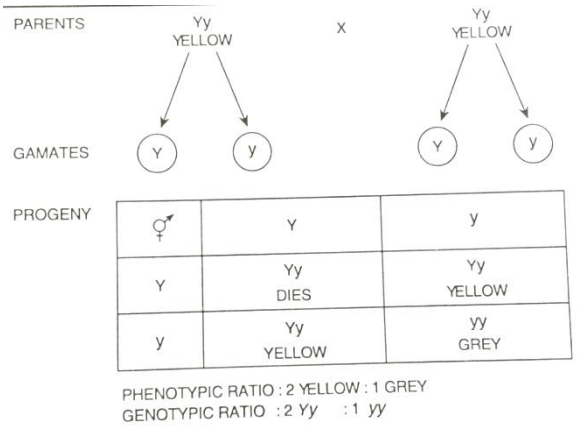
Example: A recessive lethal affecting coat colour in mice was discovered by French geneticist Cuenot in 1905. Dominant Y allele codes for yellow colour and recessive allele y codes for grey colour. Homozygous dominant yellow coat mice YY were not survived because Y was a recessive lethal.



Problems: A pair of codominant alleles is known to govern cotyledon leaf colour in soybeans. The homozygous genotype C^GC^G produces dark green, the heterozygous genotype $C^G C^Y$ produces light green, and the other homozygous genotype C^YC^Y produces yellow leaves so deficient in chloroplasts that seedlings do not grow to maturity. If dark green plants are crossed to light green plants an the F_1 crosses are made randomly to produce F_2, what phenotypic and genotypic ratios would be expected in the mature F_2 plants?

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2. Crosses of "Dexter" by "Kerry" cattle produce equal numbers of Kerry and Dexter. Crosses of Kerry by Kerry produce only Kerry. Crosses of Dexter by Dexter produce ¼ Kerry, ½ Dexter and ¼ still born calves. Give the genotypes of the parents and offspring for each of the three calves. Give the genotypes of the parents and offspring for each of the three crosses.

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3. A genetic condition on chromosome 2 in the fruit fly is lethal when homozygous (pm/pm), but when heterozygous (pm/pm+) produces a purplish eye colour called plum. The other homozygous condition (pm+/pm+) produces wild type colour. On chromosome 3, a gene called stubble produces short, thick bristles when heterozygous (sb/sb+), but is lethal when homozygous (sb/sb). The homozygous condition of its alternative allele (sb+/sb+) produces bristles of normal size (wild type).

a) What phenotypic ratio would be expected among progeny from crossed between plum and stubble parents?

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b) If the F1 progeny are allowed to mate at random to produce an F2, what is the phenotypic ratio that is expected?

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Practical No. 15

Objective: Study of multiple alleles

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Problem 1: Interpret genetic basis of ABO or Rh blood group in humans.

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Problem 2: Interpret antigen –antibody interaction.

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Problem 3: Interpret self-incompatibility in plants with suitable diagram.

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Problem 4: Interpret fur colour in rabbit with suitable diagram.

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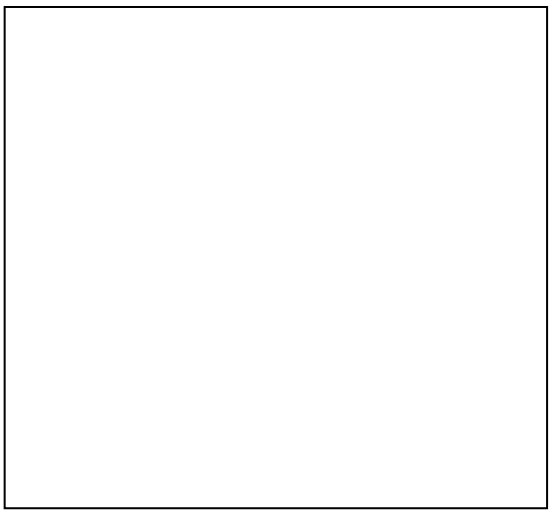

Practical No. 16

Objective: Study on Sex linked, Sex influenced and Sex limited inheritance

Sex Linked Inheritance: ……..

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Colour blindness:

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Haemophilia

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Y chromosome genes (Holandric genes)

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Sex influenced inheritance

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Sex limited characters or Secondary Sexual characters

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Write differences between the following:

S.N.	Sex linked characters	Sex limited characters
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2		
3		
4		
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Practical No. 17

Objective: Linkage and estimation of linkage from two point, three point test cross and linkage mapping.

Linkage

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Coupling:

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Repulsion:

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Problem 1: In a cross between a dihybrid parent (Aa/Bb) and a test cross parent (ab/ab) whose linkage relationships were unknown, the following progenies were obtained.

Parental combination	42% AABB
	42% aabb
Recombinant genotypes	8% AaBb
	8% AaBb

Comment on the type of inheritance and linkage relationship?

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Problem 2: Tomato plants having round peach fruits were crossed with those having elongate smooth fruits the following F_1 plant were test crossed to yield the following data.
Round smooth 14, Round peach 133, Elongate smooth 141, Elongate peach 12
Test if the gene for round and peach fruits are linked. If so in which phase? Estimate the frequency of recombination between the two genes and prepare a linkage map.

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Two point test cross

Problem: In Maize F_1 plants obtained from the cross of coloured, starchy, shrunken and colourless, waxy, full were crossed with coloured, waxy and shrunken plants. The following progenies were observed in F_2.

1.	Coloured, shrunken and starchy	2538
2.	Colourless, full and waxy	2708
3.	Coloured, full and waxy	116
4.	Colourless, shrunken and starchy	113
5.	Coloured, shrunken and waxy	601
6.	Colourless, full and starchy	624
7.	Coloured, full and starchy	4
8.	Colourless, shrunken and waxy	2

Estimate distance between genes Cs and W and determine coincidence?

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Three point test cross.

Problem: Following three pair of genes are known in maize. Determine map distance coefficient of coincidence and interference in gene mapping.

Phenotype	Genotype	Frequency	Classes
Coloured, non-waxy, full	C W x Sh	2777	Parental
Colourless, waxy, shrunken	c w x sh	2708	
Coloured, non-waxy, shrunken	C W x Sh	643	Single cross over at 1st site
Colourless, waxy, full	c w x sh	626	
Coloured, waxy, shrunken	C w x sh	116	Single cross over at 2nd site
Colourless, non-waxy, full	c W x Sh	123	
Coloured, waxy, full	C w x Sh	4	Double cross over
Colourless, non-waxy, shrunken	c W x sh	3	

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Practical No. 18

Objective: Genetic variation in pea.

Introduction: ……..

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Material Required: ……..

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Morphological quantitative traits:

S.N.	Traits	Value	S.N.	Traits	Value
1.	Stem length (STL)		13.	Petiole length from axil to first tendril(PtLax-firstT)	
2.	No. of Pods (including first fertile node (NNod)		14.	Petiole length from axil to last tendril (PtLax-lastT)	
3.	Maximum no. of leaflets (MxNLL)		15.	peduncle length of spur (PedLsp)	
4.	Leaflet size (LLS)		16.	peduncle length from stem to first pod (PedL-1P)	
5.	Leaflet length (LLL)		17.	peduncle length between first and second pods (PedL1P-2P)	
6.	Leaflet width(LLW)		18.	pod length (PL)	
7.	Leaflet position of broadest part (LLP)		19.	Pod width (PW)	
8.	Stipule length (StL)		20.	Number of seed per pod (NSP)	
9.	Stipule width (StW)		21.	Weight of seeds per plant (WSpPL)	
10.	Stipule Size (StS)		22.	Weight of 1000 seeds (W100-S)	
11.	Stipule length from axil to tip (StLax-t)		23.	Yield per genotype (YpG)	
12.	Stipule length of lobe below axil (StLob-ax)				

Conclusion:

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GENERAL LAB RULES FOR CYTOGENETICS

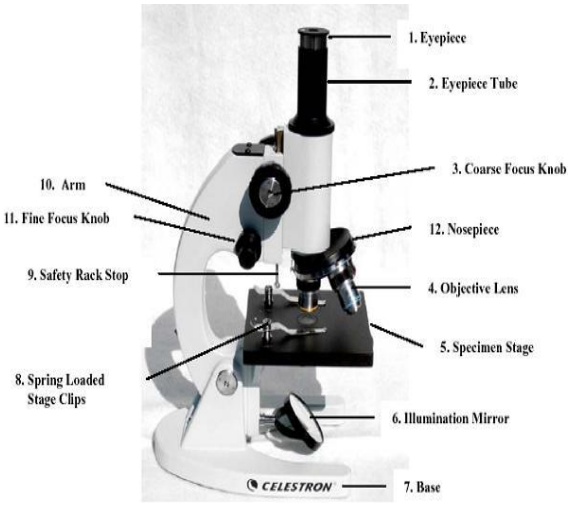
- Keep the lab tidy. Failure to do so will result in the loss of open lab privileges.
- Great care should be taken with the microscopes. Once the light source has been properly centered there should be very little adjustment needed. Immersion oil should only be used on the drawing and photo microscopes to keep the general use microscopes clean. If you find an eye piece or objective has debris on it, it can be gently cleaned with a kim wipe or cotton swab. Make sure you turn off your microscope and cover before leaving the lab.
- Never use alcohol or acetone to clean the microscopes as it can destroy the lens cement. Immersion oil can be cleaned off of objectives or slides using a cotton swab moistened with petroleum ether.
- Focus in an upward direction when using the coarse adjustment and use the fine focus when focusing downward. Preventing contact between the objective and your slide will avoid costly damage to your microscope.
- Petroleum ether should be kept at the sink and away from any open sources of flame.
- Extinguish alcohol burners immediately after use.

MICROSCOPY

APPLICATIONS OF MICROSCOPE IN CYTOLOGY:

1. Simple microscope is used for dissection and micro handling of vegetative or reproductive organs of the plants.
2. We can study and compare the structural details of given cell.
3. We can count the number of chromosomes of a given cell and identify its ploidy level.
4. Chromosomal behavior i.e., movement of chromosomes during the process of cell division can be studies and compared to processes of mitosis and meiosis in different crop plants.
5. It helps in understanding male and female gametes production.
6. Microscope with high resolving power can be made use of in studying structure and morphology of chromosomes.
7. Microscope with camera and Camera Lucida can be made use in karyotype analysis.
8. With the help of stage and ocular micrometer microscope can be used in the measurement of various cellular structure and individual chromosomes.

Compound microscope: Compound microscope has two lens the objective lens, which produces enlarge real image and an eye piece, or second lens, which produces a magnified virtual image of the first enlargement or real image.



Major parts and their uses:

Eyepiece or ocular lens: Eyepiece is the lens, present at the top and is used to see the objects under study. Eyepiece lens contains a magnification of 10X or 15X.

Tube: Tube or the body tube, connects the eyepiece to the objective lenses.

Resolving nosepiece: It is also known as the Turret. Resolving nosepiece has holders for the different objective lenses. It allows the rotation of the lenses while viewing.

Objective lenses: Generally, three or four objective lenses are found on a microscope, with ranges of 10X, 40X, 100X powers. Lenses are colour coded, the shortest lens is of the lowest power, and the longest lens is high

power lenses.

Diaphragm: Diaphragm helps in controlling the amount of light that is passing through the opening of the stage. It is helpful in the adjustment of the control of light that enters.

Coarse adjustment knob: Used for focus on scanning. Usually the low power lens is used enabling the movement of the tube.

Fine adjustment knob: Used for focus on oil. Moves the body tube for focussing the high power lens.

Arm: It supports the tube of the microscope and connects to the base of the microscope.

Stage: The platform that is flat used for placing the slides under observation.

Stage clip: Stage clips hold the slides in proper place.

Condensor: The main function of condenser lens is focussing the light on the specimen under observation. When very high powers of 400X are used, condenser lenses are very important. Presence of condenser lens gives a sharper image as compared to the microscope with no condenser lens.

Base: Provides basal support for the microscope.

FIXATIVES AND STAINS

Properties of good fixative:

1. Helps in coagulation of proteins and their precipitation, this makes the chromosomes more visible due to change in refractive index.
2. Fixative must have the property of rapid penetration so that tissue will kill instantly and arrest the chromosomes in divisional configuration (at the stage where it is). Immediate killing is essential for chromosome or cytological study.
3. It preserves the cells in their original shape.
4. It prevents the cell from autolysis or self-destruction. (As soon as the cell died protein autolysis takes place, the medium become acidic and reverse enzymatic reactions starts which changes proteins in to amino acids, due to which chromosomes structure may disorganized.)
5. Fixative prevents the bacterial decomposition of the material.

Fixative chemicals and their properties.

Acetic acid- Acetic acid is soluble in water and alcohol. It can dissolve the histone proteins present in chromosome, having highly penetration capacity and also precipitate nucleic acid. It preserves the original structure of the chromosomes without shrinkage. The problem to use the acetic acid alone is that it causes excess swelling of the chromosomes. Hence it can be used in combination with ethanol which shrink and makes hard the chromosome.

Chloroform- It is soluble in alcohol. It is a good solvent for all fat bodies, oil and waxy substances. If the tissues will kept for long time in chloroform, they becomes brittle. Therefore, it can be used with combination of ethanol. Ethanol also prevents the decomposition of chloroform in to carbonyl chloride, a highly poisonous chemical.

Ethanol- 70 to 100% ethyl alcohol (ethanol) is suitable percentage for fixation. It penetrate in the tissues immediately. Ethanol precipitate the nucleic acid and also causes an irreversible denaturation of protein and have hardening effect on chromatin. Due to these reasons the use of ethanol alone is not suggested, but can be used in combination with acetic acid, or chloroform or formaldehyde.

Formaldehyde- Formaldehyde is a gas, which is commercially available in aqueous in 40% concentration by the name of formalin. For cytological study or fixation of chromosomes, it is used in 10-40% concentration. Chemically formaldehyde reacts with amino group of proteins and resulted precipitation. It increases the cell volume resulting in spreading of chromosomes over a large area. Formalin causes hardening and granulation of chromosomes, therefore it should be used in combination with other fixatives.

Propionic acid- It can be used in place of acetic acid. Propionic acid has less penetration power and the chromosomes less swell.

Flemming's mixture (1882)

1% aqueous chromic aced	- 15 cc
Glacial acetic acid	- 1 cc
2% aqueous osmic acid	- 4 cc

Taylor's fixing mixture (1924)

10% aqueous chromic acid	- 0.2 cc
10% aqueous acetic acid	- 2 cc
2% Osmic acid 2% chromic acid	- 1.5 cc
Distilled water	- 8.3 cc
Maltose	- 0.15g

Catchsid's fixing mixture (1934)

10% aqueous chromic acid	- 3 cc
10% aqueous acetic acid	- 2 cc
2% aqueous osmic acid in 2% chromic acid	- 1.5 cc
Distilled water	- 19 cc
Maltose	- 0.2g

LaCour's fixing mixture (1931)

2% aqueous potassium dichromate	-100 cc
2% aqueous chromic acid	- 100 cc
2% aqueous osmic acid	- 60 cc
10% aqueous acetic acid	- 20 cc
Distilled water	- 210 cc

Navashin's fixing mixture: (1912)

Solution A	
Chromic anhydride	- 1.5g
glacial acetic acid	- 10 cc
Distilled water	- 90 cc
Solution B	
40% aqueous formaldehyde solution	- 40 cc
Distilled water	- 60 cc

Carnoy's fixing mixture I

Glacial acetic acid	- 1 part
Absolute ethyl alcohol	- 3 part

Carnoy's fixing mixture II (1886)

Glacial acetic acid	- 1 part
Chloroform	- 3 part
Absolute ethyl alcohol	- 6 part

Stains for DNA, RNA and Proteins

Stain	Specific for
Feulgen	DNA
Methyl green	DNA
Pyronine	DNA
Toluidine blue	DNA and RNA
Azure A	DNA and RNA
Hematoxylin	DNA and RNA
Giemsa	DNA and RNA
Benzidine	Proteins and nucleic acid
Eosin	Proteins
Methylene blue	Proteins
Coomassie blue	Proteins

SQUASH AND SMEAR TECHNIQUES

Squash and smear techniques: In squash, the stained root tips are taken out of stain and placed over a clean slide. Remove the root cap i.e., 0.5mm or less region at the end of root. Root meristem is squashed in a drop of 45% acetic acid by applying uniform vertical pressure on the cover slip with thumb.

In smear a sample of tissue or other material taken from part of the plant, spread thinly on a microscope slide for examination.

Squashing and staining:

1. Place the prefixed root tips on a watch glass containing 9 drops of 1.5% Acetocarmine and one drop of 1N HCL.
2. Heat (without boiling) over a flame for 10-15 sec. And allow into cool.
3. Transfer a darkly stained root tip to a clean slide and add a drop of Acetocarmine stain. Place a cover slip gradually without allowing the air bubbles to enter.
4. Tap gently to scatter the cells and squash by applying uniform pressure. Tapping can be done with the blunt end (rubber end of the pencil).
5. Press the slides in the folds of a filter paper with the help of thumb to spread the cells uniformly and to remove excess stain.
6. Observe the preparation under microscope. If chromosomes are not separated satisfactorily, repeat warming, tapping and pressing after adding a drop of the stain on slide.

Feulgen staining technique:

1. Hydrolyze the root tips in 1N HCL at 60 ^{0}C for 8-10 minutes in a small vial.
2. Rinse in water and remove the adhered HCL.
3. Transfer the root tips to Feulgen stain for 10-15 minutes and keep it under dark till the root tips take magenta colour.
4. Place a drop of 1.5% acetocarmine stain in the center of the cell and transfer one root on to it.
5. Discard the elder part of the root and place a cover slip under the microscope.

Making the slide semi-permanent: Observe the slide for different stages of mitosis. If there are good stages, seal the coverslip along its edge with wax to make the slide semi-permanent let the slide be in that semi-permanent state for at least 24 hours. so that the cells are not washed away while making it permanent. Then make the slide permanent by the method mentioned in the next step.

Making the slide permanent: Pass the slide through a series of solutions as mentioned below.

a) 45% glacial acetic acid for 5-10 minutes till cover slip separates from the slide.
b) 25 ml of acetic acid + 75 ml of absolute alcohol until the coverslip slides down for (2-3 min.) (1:3 proportion).
c) Then transfer both coverslip and slide to a solution of 20 ml of acetic acid + 80 ml absolute alcohol (3 min.) (1:4 proportion).
d) 10 ml of acetic acid + 90 ml of absolute alcohol (3 min.) (1:9 proportion).
e) Absolute alcohol (3 min.)

Then dry the coverslip and slide, put a drop or two of euparol on the slide and leave the cover clip gently without allowing air bubbles in the original place.

Note: To mount the sections in Canada balsam follow the steps underlying after passing the slide in absolute alcohol.

f) Equal parts of alcohol and Xylol, for 2-3 min. and (1:1 proportion).
g) Xylol for 2-3 min.
h) Mount in Canada balsam.

CELL STRUCTURE

Nucleus: The most important organelle carries the genetic information, which inherits the physical traits from one generation to another. The passage of food and water, and the influx of nutrients in and out of the cells.

Nuclear Membrane: This membranous sheath surrounding the nucleus protects it from physical damage.

Cytoplasm: Cytoplasm of a cell is the ground substance or the matrix, which is a jelly-like material in which all the cell organelles are embedded and suspended. The main function is to keep all the cell constituents intact.

The Cell Wall: Cell wall is a rigid layer that is found outside the cell membrane and surrounds the cell. The cell wall contains not only cellulose and protein, but other polysaccharides as well. The cell wall provides structural support and protection. Pores in the cell wall allow water and nutrients to move into and out of the cell. The cell wall also prevents the plant cell from bursting when water enters the cell. Microtubules guide the formation of the plant cell wall.

The Central Vacuole: Most mature plant cells have a **central vacuole** that occupies more than 30% of the cell's volume. The central vacuole can occupy as much as 90% of the volume of certain cells. The central vacuole is surrounded by a membrane called the tonoplast. Aside from storage, the main role of the vacuole is to maintain turgor pressure against the cell wall.

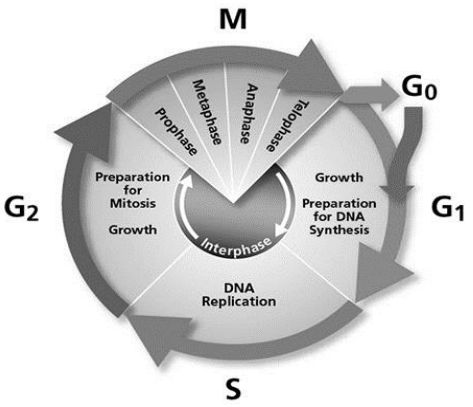
Plastids: A group of closely related membrane-bound organelles that carry out many functions. They are responsible for photosynthesis, for storage of products such as starch, and for the synthesis of many types of

molecules that are needed as cellular building blocks. Plastids have the ability to change their function between these and other forms. Plastids contain their own DNA and some ribosomes. The main types of plastids and their functions are:

- **Chloroplasts** are the organelle of photosynthesis. They capture light energy from the sun and use it with water and carbon dioxide to make food (sugar) for the plant. The arrangement of chloroplasts in a plant's cells can be seen.
- **Chromoplasts** make and store pigments that give petals and fruit their orange and yellow colours.
- **Leucoplasts** do not contain pigments and are located in roots and non-photosynthetic tissues of plants. They may become specialized for bulk storage of starch, lipid, or protein. However, in many cells, leucoplasts do not have a major storage function. Instead, they make molecules such as fatty acids and many amino acids.

MITOSIS

Cell cycle: The cell cycle is composed of 4 distinct phases:- G_1 phase, S phase, G_2 phase, M phase and C phase, G_1, S, and G_2 phase together constitute the interphase and the M stage stands for mitosis and C phase for cytokinesis. In the simplest sense, a cell duplicates its contents and then divides in two. The cycle of duplication and division is known as the cell cycle.



Interphase + Nuclear division (mitosis) + Cytokinesis = Cell cycle

Interphase:-During interphase, the cell is growing and preparing for mitosis (M phase) by accumulating nutrients and replicating DNA. Interphase is the longest phase in cell cycle. Though this phase is sometimes called resting stage, but it is in fact the most active phase of the cell cycle.

a. **G_1 phase:** G_1 stage comes between the telophase stage i.e., end of mitosis and the start of the S phase. Cell cycle ranges 8 hours to many hours.
b. **Synthesis Phase (Synthesis of DNA and histones):** This stage is present between the G_1 and G_2 phases. During S phase, new DNA is synthesized in and generates exact replication of existing DNA molecule. During this stage every double-helical DNA molecule is duplicated, making two strands of DNA that are exactly identical. Two new DNA strands are formed, which are attached together at a point called centromere.
c. **G_2 phase:** In the formation of new DNA molecule energy is utilised, to regain the energy for the cell to undergo mitosis, synthesis of RNA and protein continues, but DNA synthesis stops. The mitotic spindle fibres (proteins) are formed.

Mitosis: Mitosis is the shortest phase in cell cycle. This division itself involves the condensation and separation of the replicated chromosomes. Mitosis has been sub-divided into phases:

Prophase (condensation of chromosomes): A stage of chromosomal condensation and loss of water by them.

- At the beginning of prophase chromosomes appear as thin, filamentous uncoiled structures.
- Chromosomes become coiled, shortened and more distinct in prophase, which is of much longer duration than other stages.
- Nucleoli disappear.
- Each chromosome longitudinally splits into two sister chromatids. Double structure of each chromosome is visible at late prophase.
- The duplicated chromosome subunits (each one called chromatid) join together at the centromeres.
- Two chromatids are attached to spindle tubules (chromosomal fibres) with the help of protein plates called kinetochores a specialised structures develop on either surface of centromere of each chromosome.
- The kinetochore is the actual site of the insertion of the spindle threads and is a permanent part of the chromosome.

Metaphase

- All chromosomes line up in the middle called equatorial plate.
- Chromosome lies in the middle of the spindle fibres.
- Spindle fibres attached with centromere of the chromosomes.

Anaphase (Centromere separation and chromatid migration)

- In the beginning of Anaphase chromatids start separating from the centromeres divide and the spindle apparatus starts pulling the kinetochores towards the opposite poles.
- The chromosomes appear in the shape of V, L, J or I

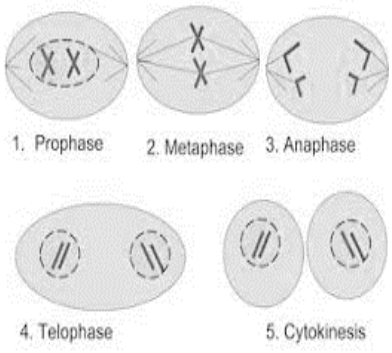
- Formation of two daughter cell begins. In anaphase the centromeres divide and two sister chromatids separates and move to the opposite ends of the cell.

Telophase: Telophase stage starts as soon as the chromatids reaches the poles of the daughter cells.

- Chromatids decadence.
- Nuclear membrane reappears around daughter nuclei
- Spindle fibres become disorganized. The spindle apparatus breaks down
- The nucleolus reappears
- Division of cytoplasm is called cytokinesis.
- The cell pinches in the middle, beginning the formation of the two cells new cell plate expanding centrifugally.
- Each daughter cell gets the same complement of chromosomes and nucleoli as of the mother cell.

Preparation of slide: The roots of onion bulb can be grown in aerated distilled water at room temperature. Two to three days old healthy roots of onion are required for study. Vigorous 1 cm length roots can be used.

1. Preparation of the vegetative material by sprouting onions (*Allium cepa*) in hydroponic solution
2. Cutting the roots at their base
3. Fixation (3 parts of ethanol, 1 part of icy cold acetic acid, time 5-10 minutes)
4. Maceration (1 part of ethanol, 1 part of concentrated hydrochloric acid, time 5-10 minutes)
5. Washing in water (10 minutes)
6. Colouring in acetocarmine (time 1 hour at least)
7. Cutting of the root tips
8. Making the microscopic preparation by compression



MEIOSIS

Meiosis: Meiosis is a type of cell division in which gametes are formed in the reproductive organs. In meiosis the mother cell produces four daughter cells called gametes with half chromosomes numbers, this division is also called reductional division.

Pre-meiotic interphase. The interphase in meiosis is called pre meiotic interphase. In this division during synthesis phase 99.4% DNA synthesis takes place, remaining 0.6% DNA synthesised during zygotene stage. In G_2 phase a special type non histone protein is synthesised which is supposed to be responsible to carry the cells under meiosis cell division. This non histone protein is absent in G_2 phase of mitosis.

The reason of reduction in chromosome numbers is synthesis of DNA occurs only one whereas cell divides in two successive divisions in meiosis.

Meiosis I – In first division separation of chromosomes occurs.
Prophase I – First prophase is of a very long duration. It is further divided into five sub-divisions.

Leptotene – The chromatin condensed into long thin thread like structures i.e. chromosomes. The number of chromosomes remains constant. The chromosomes are visualised individually and arranged parallel.

Zygotene – The homologous chromosomes come together and form pairs called synopsis. The pairing starts one of many points in a zipper-like manner across the whole length of the homologous chromosomes.

Pachytene – The paired chromosomes are now called bivalents. They become shorter and thicker. Each of the homologous chromosomes, in meiotic prophase I consists of two closely apposed sister chromatids, thus each bivalent contains four chromatids, and is also called tetrad. The non-sister chromatids exchange the chromosomal segments with each other called crossing over. This will leads to the formation of recombinant type and original chromatids.

Diplotene –The two homologous chromosomes of the bivalent tried to pull away from each other but the separation is not completed. The homologous chromosomes remain attached with each other at some points where crossing over occurred such points of attachment are called chiasmata.

Diakinesis – Chiasmata begin to move towards the chromosome ends this is called terminalisation. The chromosomes become more condense and thick.

Metaphase I: The bivalents arranged in the equator plane forms the equatorial plate. The centromere of each chromosome is attached with spindle fibres and directed towards the opposite poles and the arms of

chromosomes remains on equatorial plate.

Anaphase I: Out of one pair of chromosomes, one set of chromosome moves towards one pole whereas other set of chromosome moves towards opposite pole. Thus each pole receives half the number of chromosomes or the haploid set of the chromosomes. Here actual reduction in number of chromosomes occurs.

Telophase I: The nuclear membranes are formed during this stage by the endoplasmic reticulum around the groups of daughter chromosomes with the appearance of one nucleolus in each nucleus. It results in the formation of two daughter cells each with haploid number of chromosomes and only half amount DNA.

Cytokinesis: It occurs by cell wall formation in plants. But in many plants cytokinesis does not take place and cell directly passes into meiosis II.

Meiosis II: First meiotic division is followed by second meiotic division without interphase.

Prophase II Chromosomes of both nuclei become shorter and thicker. The two-standard nature becomes apparent once again and the nuclear membrane disappears.

Metaphase II Spindle formation takes place. The chromosomes become oriented on the equatorial plate and have the same relationship to the spindle as in mitosis. The spindles in meiosis II are oriented at right angle to that in meiosis I.

Anaphase II The centromere divides and the two sister chromatids of each chromosome separate and move towards the poles. After separation, each chromatid behaves as a chromosome. Thus, a chromosome has one chromatid before and two chromatids after replication.

Telophase II At this stage, the four groups of chromosomes become organized into four haploid nuclei. The chromosomes return to the interphase condition. The endoplasmic reticulum forms the nuclear envelope around the chromosomes. Each nucleus at this stage contains the haploid number of chromosomes and forms four cells.

Cytokinesis occurs and the two nuclei are separated as in mitosis.

Making the slide semi-permanent:

1. Observe the slide for different stages of meiosis, if good stages found seal the edges of coverslip with wax. After minimum period of 24 hours make it permanent by following the same method as given in the squashing technique.
2. Collect the young buds in the early morning and keep in the fixative (1:3 acetone-alcohol) for about 48 hrs.
3. These buds could be preserved in 70% ethyl alcohol after removing them from fixative, and stored in a refrigerator.
3. Crush anthers in a drop of acetocarmine on a slide.
4. Remove the debris from the slide and then put a cover slip on the material.
5. Heat the slide gently put a filter paper on it and press it with thumb. Do not break the cover slip with too much press.
6. Study the slide under the microscope.

POLLEN FERTILITY AND VIABILITY

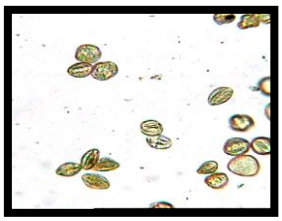
Material required: Pollen grain**,** Camel's hair brush, Whatman 1 filter paper, Beakers, Slides, Acetocarmine solution and Microscope etc.

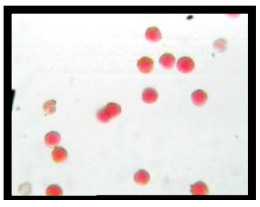
Pollen collection

a) **Solvent method:** Flowers are removed and placed in a beaker and solvent is poured over the flowers until they are submerged. The beakers are then gently agitated for 60 seconds to remove pollens from anthers. Pollens are separated from solvent by filtration through Whatman 1 filter paper and then brushed from the filter paper using sterile camel's hair brush.

b) **Direct pollen collection:** Collected at pre-anthesis stage and placed on paper sheets kept under partial sunlight. The anthers dehisced thereby liberating the pollens on the filter paper sheets which are dried under partial shade and then brushed from the filter paper using sterile camel's hair brush. All the pollen samples are collected in isolation to prevent contamination from any other source.

a) **Pollen germination and viability:** Pollen germination and viability is studied using in-vitro assay and staining method and expressed as viability percentage and germination percentage respectively.


Non-viable pollen grains


Viable pollen grains

Staining method: The pollen viability of the freshly collected as well as stored pollen is studied in 2% acetocarmine solution. Pollens are dusted on a clean haemocytometer with hair brush to which a drop of acetocarmine solution are added for 10-15 minutes to allow the pollen to absorb the stain. Several fields of the pollen mass are examined under the microscope. Deeply stained normal looking pollen grains within the five squares of the haemocytometer are counted as viable pollen, shriveled and weakly stained are recorded as nonviable and expressed in percentage.

DETERMINATION OF GAMETES AND PROBLEMS ON MONOHYBRID, DIHYBRID CROSS

Number of genes Segregating	Number of different kinds of gametes produced by F_1	Number of individuals in the perfect F_2	Number of different homozygous genotype in F_2	Number of different phenotype in F_2 (complete Dominance)
1	2	4	2	2
2	4	16	4	4
3	8	64	8	8
N	2^n	4^n	2^n	2^n

Chi SQUARE TEST AND TEST CROSS

Chi-square test is a method to determine that how close the recorded value (observed value) with the expected value. It was given by Karl Pearson to check the goodness of fit. The null hypothesis of expected result is checked against the actual result obtained

It can be determined by the following formula

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

Obtained chi square value is compared with the table chi square value which is obtained from goodness of fit chi square table against the degrees of freedom. The degree of freedom is one less than the total number of classes involved.

When the calculated chi square value more than table Chi-square value (goodness of fit), it means the observed ratio is probably not an illustration of the ratio for which it was determined and something other than chance is operating.

On the other hand if calculated chi square value is less than the table chi square value, the expected and observed value fit in to goodness of fit and null hypothesis is accepted.

Test cross: It is a cross between the F1 hybrid and its recessive parent. The purpose of test cross is to discover how many different kind of gametes are being produced by the individual whose genotype is in question.

(Heterozygous Tall) Tt x tt (Recessive Dwarf)

Uses:

1. **Test cross verifies the Mendel's factorial hypothesis:** According to Mendel, a monohybrid tall (Tt) produce two kinds of gametes in equal proportion and recessive parent produce only one kind of gamete 't'. Hence this back cross should give Tall and dwarf plants in 1:1 ratio. In actual experiment also we get tall and dwarf in '1:1' ratio. Thus Mendel's factorial hypothesis is verified.
2. **Test cross is used for identifying the genotype of an unknown parent:** A tall pea plant may be either homozygous (TT) or heterozygous (Tt). Its genotype may be determined by test cross. If the test cross progeny were tall, then the unknown tall genotype is 'homozygous'. If that test cross progeny have tall and dwarf plants in equal proportion, then the unknown genotype is heterozygous.

GLOSSARY

Acentric chromosome- Chromosome fragment without centromere.

Acrocentric- Chromosome in which centromere present near one end.

Allele- Alternative forms of a gene.

Autosomes- Chromosomes other than sex chromosomes.

Autogamy- Process of selfing.

Back cross- F_1 is crossed with any of the parents.

Centromere-Region of the chromosome where spindle fibre attached during metaphase.

Chiasma- (Chiasmata)- The points of attachments of the chromatids during diplotene stage are called chiasma.

Chromatids- Two identical strands of a chromosomes resulting from self-duplication.

Chromatin- This made up with DNA and histone proteins of a chromosome.

Chromosomal aberration- Changes in normal structure or numbers of a chromosome called chromosomal aberrations. e.g. deficiency, duplication, inversion, translocation, aneuploidy, polyploidy etc.

Codominant alleles- Alleles that produce independent effect when heterozygotes.

Codon- A set of three adjacent nucleiotides in an mRNA molecule that specify the production of an amino acid.

Crossing over- Exchange or chromosomal segments between two homologous non-sister chromatids.

Cytogenetics- Area of biology concerned with chromosomes and their implications in genetics.

Cytokinesis- Division of cytoplasm during the mitosis and meiosis.

Cytology- Study of structure and function of cells.

Cytoplasm- Protoplasm of a cell without nucleus.

Cytoplasmic inheritance- Hereditary transmission dependent on the cytoplasm, other than the nuclear genes.

***de novo*-** afresh, arising anew.

Dicentric chromosome- chromosome with two centromeres.

Diploid- An organism with two sets of chromosomes, represented by 2n.

DNA- Deoxyribonucleic acid, information carrying genetic material.

Dominance- In F_1 the character express itself is called dominant character.

Epistasis- Interactions between products of non-allelic genes. One gene masks the effect of other. Gene exhibit is called epistatic gene, suppressed gene is called hypostatic.

Eukaryotes- Members of large group of organisms.

Expressivity- Degree of expression of a trait controlled by a gene. A particular gene may produce different degrees of expression on different individuals.

Gamete- mature male and female reproductive cells.

Gametogenesis-process of formation of gametes.

Gene- Gene is the unit of inheritance (DNA) located in a fixed place on the chromosome.

Genetics- Science of inheritance and variation.

Genome- A complete set (n) of chromosomes.

Genotype- Genetic constitution or gene makeup of an organism.

Germplasm- The sum total of genetic material of a species.

Haploid- An organism with one set(n) or haploid set of chromosomes.

Hemizygous- The condition in which only one allele of a pair is present. Sex chromosome.

Heredity- Transmission of traits from parents to offspring.

Heritability- Degree to which a given trait is controlled by inheritance.

Hermaphrodite- Bisexual, or with both male and female reproductive organs.

Heterochromatin- Darkly stained regions of cell during interphase, often contains repetitive DNA, mostly genetically inactive.

Heterokaryone- A cell contains two or more different nuclei.

Heterosis- Superiority of heterozygote in one or more traits than the homozygote.

Hybrid- An offspring of homozygous parents differing in one or more genes.

Inbreeding- Mating between related individuals.

Incomplete dominance- Expression of two alleles in a heterozygote that allows the heterozygote to be distinguished from either of its homozygous parent.

Independent assortment- The random distribution of alleles to the gametes that occurs when the genes are located in different chromosomes. The distribution of one pair of alleles is independent of other located in non-homologous chromosomes.

in situ- In the natural place

Interference- Crossing over at one point which affects the chances of another crossing over nearby is called interference.

***in vitro*-** Within glass.

***in vivo*-** Within the living organism.

Karyotype- The chromosomal constitution of a cell an individual. Chromosomes are arranged in order of length and according to position of centromere.

Linkage- Tendency of two or more genes to inherited together.

Linkage map- A graphical representation of chromosome that shows the relative position of genes on chromosomes.

Locus (Pl., Loci)- Position of gene on chromosome.

Maternal effect- Trait controlled by the gene of the mother but expressed in the offspring.

Meiosis- The process of cell division in which the chromosome numbers of a reproductive cells become reduced to half and form the gametes, occur in reproductive cells.

Messenger RNA- (mRNA)- RNA that carries the information necessary for protein synthesis form the DNA to the ribosomes.

Metacentric chromosome- chromosome in which the centromere present in middle and two arms are nearly equal in size.

Mitosis- Type of cell division occur in somatic cells, in this division duplication of chromosomes and division of cytoplasm produce two genetically identical daughter cells.

Monohybrid- An offspring of two parents differs only for one pair of alleles.

Monohybrid cross- A cross between parents differing in only one trait.

Monosomic- Diploid organism lacking one chromosome (2n-1).

Multiple alleles- A condition in which a particular gene occurs in three or more allelic form.

Mutagen- Agents which causes mutation.

Mutant- Result of mutation.

Mutation- Sudden heritable changes in DNA at particular locus.

Nucleotide- Unit of DNA or RNA that contains phosphoric acid, sugar and nitrogenous base.

Nullisomic- Diploid organism lacking a pair of chromosome (2n-2).

Over-dominance- A condition in which a heterozygote are superior to either of associated homozygotes (Parents).

P- Symbol of parents

Penetrance- The percentage of individual that shows a particular phenotype among those capable off showing it.

Peptide- A compound contain amino acids.

Peptide bonds- A chemical bond holding amino acid subunits together in proteins.

Phenotype- The observable characteristics of an organism.

Pleiotropy- Single gene governs more than one traits.

Polygene-Numbers of genes are involved in expression of a quantitative trait.

Polynucleotide- A liner sequence of joined nucleotides in DNA or RNA.

Polypeptides- A liner molecule with two or more amino acids and one or more peptide groups.

Recessive- One pair of allele which could not express itself in F_1.

Reciprocal cross- Cross between different strains with sex reversed, e.g., female A x male B, male A x female B.

RNA- Ribonucleic acid

Segregation- Separation of paternal and maternal chromosomes from each other at meiosis.

Synapsis- The pairing of homologous chromosomes during meiotic prophase.

Test cross- Cross of F_1 with homozygous recessive parent.

Tetrad- The four cells arising from the second meiotic division in plants (pollen tetrad).