# Practical Manual

## Animal Genetics & Breeding

**(Principles of Animal and Population Genetics)**

**(UNIT-2)**


**Compiled By**

**Prof. J.S. Poonia**

**Dr. Vijay B. Sharma**

(Associate Professor)

**Dr. Yamini Choudhary**

(Assistant Professor)


Name:_____

Roll No._____

Batch: / Session:


**DEPARTMENT OF ANIMAL GENETICS & BREEDING**

**Mahatma Jyotiba Fule College of Veterinary & Animal Sciences**

**Sikar Road, Chomu, Jaipur (Rajasthan)**

# FOREWARD

I am very happy to go through the practical manual entitled, **Animal Genetics & Breeding**, prepared by **Prof. J.S. Poonia**, **Dr. Vijay B. Sharma** and **Dr. Yamini Choudhary**, Mahatma Jyotiba Fule College of Veterinary & Animal Sciences. The manual covers the practical syllabus of undergraduate courses of AGB **(Principles of Animal and Population Genetics)** prescribed by Veterinary Council of India for B.V.Sc. & A.H. programme.

The manual written by the authors are a good attempt which is based on their long experience of teaching above undergraduate courses. The language used in the manual is simple and lucid. The outline and description of practical exercises covering objectives, materials required, procedures and observations to be taken have been nicely presented which would be helpful in conducting practicals more effectively.

I congratulate the authors for the efforts put in bringing out this practical manual.

DEAN

Mahatma Jyotiba Fule College of
Veterinary & Animal Science, Chomu, Jaipur

# PREFACE

Ever since the introduction of new courses for professional B.V.Sc. & A.H. degree programme under Veterinary Council of India pattern in Veterinary College/Universities in the country, there was a dire need to have a practical manual on Animal Genetics & Breeding subject who covers the practical syllabus of undergraduate courses of AGB (Principles of Animal and Population Genetics). These new courses were not independently developed in most of the Veterinary College/Universities before the introduction of Veterinary Council of India programme. The present manual "Animal Genetics & Breeding " covers the practical's with objectives, materials required, procedure, steps to follow, precautions to be taken, observations to be recorded and exercises to be done by the students. The main objective of this manual is to meet the need of students and teachers teaching these courses. It is hoped that users will find the manual immensely useful.

Suggestions for improvement are welcome from scientists, teachers and students.

**Prof. J.S. Poonia**

**Dr. Vijay B. Sharma**

**Dr. Yamini Choudhary**

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# Exercise No. 1

## Introduction

**Genetics: -** Genetics is the branch of biological science, which deals with heredity and variation and the study of the law's governing, similarities and differences between individuals related by descent is called genetics.

**Heredity: -** It involves the transfer of biological information of parental generation to the new organism via the egg and sperm is called heredity. This biological information is present on the chromosome in the form of genes.

**Variation: -** The characters which provide individuality to a species are said to be variation. There are two types of variation, **Heredity variation** and **environmental variation**.

**Historical backgrounds of genetics are following**: -

1. Vapour and fluid theories.
2. Magnetic power theory.
3. Perforation theories
4. Epigenetic theory
5. Particulate theory

**Some important branches of genetics: -**

1. **Microbial Genetics** – The genetics of micro organism.
2. **Animal Genetics –** The genetics of animals.
3. **Human Genetics** – The genetics of man.
4. **Population Genetics** – The genetics of the different population of animal and plant species.
5. **Cytogenetics** - The genetics of cell.
6. **Molecular Genetics –** It is the most modern branch of genetics and it interprets most genetical phenomena in the term of chemical molecules.
7. **Clinical Genetics –** It applies genetical analysis in diagnosing various hereditary diseases in man and suggests the possible cures for them.
8. **Quantitative or biometric genetics –** It deals with the inheritance of quantitative traits such as body weight, mature plant height, egg or milk production records, yield of grain per acre etc.

**Criteria of genetic material by –**

1. Variation
2. Recombination
3. Controlled matting's
4. Short life cycle
5. Large number of offspring's
6. Convenience of handling

**Application of genetic**

1. Genetics and eugenics.
2. Genetics and agriculture.
3. Genetics and medical science.
4. Genetic and legality (legal problem).

Q. 1 **Define the following;**

**Genotype: -**

**Phenotype: -**

**Allele: -**

**Homozygous: -**

**Heterozygous: -**

**Epistasis:-**

**Dominance: -**

**Codominance: -**

**Incomplete dominance: -**

**Monohybrid: -**

**Dihybrid: -**

**Multiple Alleles: -**

# Exercise No. 2

## To study chromosome number in different species of livestock

Chromosomes are thread like structures found in the nucleus of cells. The chromosomes are found in pairs. They are actually the carriers of inheritance. The chromosomes are mainly two types, **Sex chromosome and Autosomes**. The chromosome numbers are different in different species of livestock.

**Q. 1. Give the chromosome number of the following species?**

| S.N. | Species | Diploid (2n) number of Chromosome |
|---|---|---|
| 1 | Cattle | |
| 2 | Goat | |
| 3 | Sheep | |
| 4 | Buffalos | |
| 5 | Horse | |
| 6 | Donkey | |
| 7 | Dog | |
| 8 | Fowl | |
| 9 | Human | |
| 10 | Drosophila | |
| 11 | Pigs | |

**Q. 2. The diploid number of chromosome in Cattle is 60. How many?**

| | | |
|---|---|---|
| A | No. of chromosome received from mother | |
| B | No. of chromosome present in gamete | |
| C | Sex chromosome present in the ovum | |
| D | Sex chromosome present in the sperm | |
| E | Autosomes found in somatic cells | |

**Q. 3. There are 54 Chromosome in somatic cells of sheep. How many chromosomes would be found in each of the following?**

| S.N. | Stages | Chromosome Number |
|---|---|---|
| A | Primary Spermatocyte | |
| B | Spermatid | |
| C | Oogonoium | |
| D | Polar body | |
| E | Secondary spermatocyte | |
| F | Spermatogonium | |
| G | Sperm | |
| H | Ovum | |
| I | Zygote | |

**Q. 4. Distinguish between autosomes and sex chromosome.**

# Exercise No. 3

## Karyotype of chromosome

Grouping of chromosome on the basis of morphology is called **Karyotyping** of chromosomes. Characterization of chromosome is generally done on the basis of length of chromosomes and the position of centromere. The pictorial representation of a Karyotype according to size is called **Ideogram** on the basis of position of centromere the chromosomes are classified as follows–

1. Metacentric – Median position of centromere.
2. Submetacentric – Submedian position of centromere.
3. Acrocentric – Subterminal position of centromere.
4. Telocentric – Terminal position of centromere.

**Q. 1. Draw the diagrams of chromosome showing the position of centromere on chromosomes.**

**Q.2. Enlist the main characters of the chromosomes that are used for preparation of karyotype.**

# Exercise No. 4

## To Study the Structure of cell and cell organelles

The Smallest unit of Life is Called Cell. A typical cell consist of outer plasma membrane, cytoplasm and nucleus .The cells are mainly of two types, Prokaryotic (These cells are composed of primitively organized cytoplasm with no defend nucleus) and Eukaryotic (The eukaryotic cells have true nucleus). The eukaryotic cytoplasm contains following cell organelle, mitochondria, endoplasmic reticulum, Golgi complex, lysosomes, plastid and microtubules etc. The eukaryotic nucleus contains nuclear membrane/envelops, Nucleoplasm, nucleolus and chromosomes.

**Cell division:** - It is a division of the cell nucleus followed by a similar division of cytoplasm and Chromosome which pass through a definite series of changes and movements adapted to ensure the transmission to each of the two daughter cells of a set of chromosomes identical with those mother cells.

Mainly cell division is two types: - (i) Mitosis (ii) Meiosis

(i) **Mitosis: -** Mitosis is regularly associated with nuclear division of somatic cells, during mitosis successive stages are known as prophase, metaphase, anaphase and telophase are recognized.

(ii) **Meiosis: -** Meiosis is a form of nuclear division differs greatly from mitosis. It consists of two successive divisions, meiosis I and meiosis II. The meiosis is a specialized type of nuclear division, which divides a diploid cell into four haploid daughter cells. It takes place in Germ Cells.

## Functions of the organelles of an animal cell

| Cell organelle | Functions |
|---|---|
| **Cell membrane** | Selective permeable membrane for exchange of substrate and cell products |
| **Nucleus** | Regulate growth and reproductive function of the cell |
| **Chromosomes** | Hereditary units and regulates cellular processes |
| **Nucleolus** | Synthesize ribosome, disappear during cellular replication |
| **Nucleoplasm** | Contain materials for building DNA and messenger molecules, which act as an intermediate between nucleolus and cytoplasm |
| **Nuclear membrane** | Selective barrier between nuclear and cytoplasmic material |
| **Cytoplasm** | Contain machinery for carrying out the instructions sent from the nucleus |
| **Endoplasmic reticulum** | Provides extensive surface area for biochemical reaction |
| **Ribosome** | Site of protein synthesis |
| **Centrioles** | Forms poles during cell division |
| **Mitochondria** | Site of energy production energy production |
| **Golgi Apparatus** | Secretary body of cell (dictyosomes in plants) |
| **Lysosomes** | Production of intracellular digestive enzyme, aid in disposal of foreign bodies, also called as suicidal bags of the cell |
| **Vacuoles** | Storage depots for excess water, waste products, soluble pigments etc. |
| **Hyaloplasm** | Contain enzymes for glycolsis and structural materials such as sugars, amino acid, water, vitamins, nucleotides, etc. |

**Cell cycle**

It consists of two stages: -

(a) Interphase: Replication of genetic material.
(b) Cell division.

Cell division of two types: -

(a) Mitosis
(b) Meiosis

**Mitosis** It takes place in somatic cells. Each division produces two identical daughter cells. Mitosis can be arrested at prophase by adding colchicines. It has following stages.

**Prophase** Formation of spindles; shortening and thickening of chromosome, nuclear membrane disintegrates, nucleolus disappears. It is the longest phase. Late prophase is best time to study chromosome as at this stage these are condensed and not confined with in nuclear membrane.

**Metaphase** Formation of metaphase plate.

**Anaphase** Sister Chromatids separate and move to the opposite poles. Meta centric chromosomes appear V – shaped, sub metacentric appear J-shaped, telocentric appear rod shaped. Shortest phase.

**Telophase** chromatids reach at the opposite ends and uncoil nuclear membrane and nucleolus reappears, cytoplasm divides to form two identical daughter cells. Daughter cells are identical with respect to type and number of chromosome i.e. passes same genetic constitution but there is no assurance of equal cytoplasmic distribution.

**Meiosis** It takes place in germ cells (gametocytes), results in the formation of four haploid daughter cells. Its stages are as follows.

**Meiosis I** DNA replicates during the Interphase preceding meiosis. I and does not replicate between telophase – I and prophase – II.

| | |
|---|---|
| **Prophase I** | It is divided in 5 phases.pairing of homologous chromosomes is known as **Synapses**. Pairing of synapsed chromosomes is known as bivalent (2 chromosomes) or tetrad (4 chromatid). |
| **Leptotene** | Chromosome appear as single thread, begin to condense. It is also khown as thin thread stage. |
| **Zygotene** | Homologous chromosome pair (bivalent). It is also known as joined thread stage. |
| **Pachytene** | chromatids further condense to form tetrad. It is also known as thick thread stage. |
| **Diplotene** | It is also known as double thread stage. |
| **Diakinesis** | Condensation continues. It is also known as double movement stages. |
| **Metaphase I** | Nuclear membrane disappears, bivalents arranged at equator. |
| **Anaphase I** | Homologous chromosomes separate and move to opposite pole. |
| **Telophase I** | Two daughter cells are formed. |
| **Interkinesis** | It is the period between first and second meiotic division and is called as Interkinesis. Depending upon the species. It can be brief or extended for a period of time. Nothing of genetic importance occurs at this stage. |
| **Meiosis II** | |
| **Prophase II** | Nuclear membrane disappear, chromosomes condense, spindle forms. |
| **Metaphase II** | chromosomes move to the equator of the spindle. |
| **Anaphase II** | Centromere divide and chromosomes move to the opposite poles. |
| **Telophase II** | Nuclear membrane reappears, chromosomes diffuse and cytoplasm divides to form four daughter cells. |

# Gametogenesis

## Spermatogenesis in male

**Origin :** Germinal epithelium of semniferous tubules of male gonads (testes) from diploid primordial cells.

**Growth Phase :** These cells undergo repeated mitotic division to form spermatozoan (spermatoza, plural) and may differentiate in to diploid primary spermatocyte.

**Meiosis I :** Haploid secondary spermatocytes are formed.

**Meiosis II :** Each haploid secondary spermatocyte produces two haploid spermatid.

**Maturation :** Almost entire amount of cytoplasm extrudes from each haploid, spermatid in to along whip like tail and becomes transformed into a mature gamete of sperm cells (spermatozoa).

## Oogenesis in female

**Origin :** In general epithelium of female glands (ovaries) from diploid primordial cell.

**Growth :** Storage of much cytoplasm or yolk (used as food by the early embryo) to from oogonia and transformed in a diplois primary oocytes.

**Meiosis I :** Reduce the chromosomes number by half and unequal cytokinesis (division of cytoplasm) takes place producing two cells. The larger cell is called haploid secondary oocyte and smaller cell is called primary (frist) polar body.

**Meiosis II :** Secondary oocyte again involves unequal cytokinesis producing two cells, a large yolky ova and a secondary polar body. Primary polar body undergoes division and also producing two secondary polar bodies. All polar bodies degenerate and not take part in fertilization.

**Maturation :** Ova becomes a mature female gamete called as an ovum per egg cell.

**Q. 1** Differentiate between Spermatogenesis and Oogenesis.

**Q.2** Name the stages of somatic cell division where each of the following events occurs:

| Event | Stage |
|---|---|
| Mature cell which is more or less at rest and is undergoing no growth. | |
| DNA of the chromosome is doubled | |
| Formation of spindle fibers | |
| Chromosomes oriented on the equatorial plane | |
| Nuclear membrane disappears | |

**Q.3** what is meant by the cell cycle? Name the different phases of this cycle.

**Q.4** Diagrammatically show various stages of Mitosis in an animal cell.

**Q. 5** Diagrammatically show different stages of meiosis and write the stages of Meiosis and the important events that are taking place during each stage.

# Exercise No. 5

## Solving problems based on Mendelian principle

Gregor Mendel is called Father of Genetics. He was born on 22nd July 1822 in Heizendrof, a village in Silesia and died in1884. These laws given by him laid the foundation of Genetics. He worked on garden pea (*Pisum sativum*) because the pea plant has many distinct alternative traits, short life span, flowers shows self pollination and easy to artificially cross pollinate the pea flowers. He works on seven different characteristics of Sweat Pea for which genes were present on different chromosomes.

**Q. 1. Write the following Mendel's Law's**

1. Law of segregation or purity of gametes: -

2. Law of Independent assessments: -

**Q. 2. Define: -**

**Back cross: -**

**Test Cross: -**

**Gamete: -**

**Zygote: -**

**Gene: -**

**Carrier: -**

**Hybrid: -**

**$F_1$ generation: -**

**$F_2$ generation: -**

**Segregation: -**

**Complete dominance: -**

**Co-dominance: -**

**Incomplete dominance: -**

**Lethal genes: -**

**Law of Segregation**

Q1. A pair of alleles governs coat colour in the guinea fowl. A dominant allele "B" produces black and recessive allele "b" produces white. A pure breeding black male guinea fowl is mated to pure breeding white female.

**(a)** Diagrammatically represent parental, F1 & F2 generations.

(b)What will be phenotypic and genotypic ratios in the F2 generations along with genotypes and phenotypes?

(c)Give inference with regards to the transmission of the traits from parents to the F1 and to the F2 generations.

**Examples of Test cross**

## Examples of Co-Dominance

## Examples of Lethal Genes

## Examples of Multiple Alleles

**Examples of Dihybrid cross**

**Examples of Trihybrid cross**

# PROBLEMS

# Exercise No. 6

## Sex linked inheritance and its related problems

The inheritance governed by genes present on sex chromosome is called sex linked Inheritance e.g. Colour blindness in human being, eye colour in drosophila, and Hemophilia.

The chromosomes which are identical in both the sexes are called autosomes. Each autosomal chromosome pair has its identical mate in both sexes but sex chromosome pair does not have its identical mate in males. In females the sex chromosome has its identical mate and are called X chromosomes where as in males they are called X and Y chromosome. So female has two XX and male have XY chromosome. Females are Homogametic and males are Heterogametic.

However in case of poultry females are heterogametic and males are homogametic.

1. Criss-cross Inheritance

2. Reciprocal cross

**Sex Influenced Inheritance: -** The gene governing sex – influenced traits may be on any of the autosomes in heterozygous Condition of the sex chromosome. The expression of dominant or recessiveness by the alleles of sex – influenced loci is reversed in males and females due to the difference in the internal environment provided by sex hormones. Thus examples of sex – influenced traits are most readily found in the higher animals with well – developed endocrine system e.g. the gene for baldness in man exhibits dominance in men but acts recessively in women.

**Sex- limited Inheritance:** - Some genes may only express in one of the sexes either because of differences in internal hormonal environment or because of anatomical dissimilarities when penetrance of a gene in one sex is zero, the trait will be sex limited e.g. we know that bulls have many genes for milk production that may transmit to their daughters, but all males are unable to express this trait. Other example is egg production is also limited to one sex i.e. in females. The genes for these traits are present on autosomes.

# Exercise No. 7

# Linkage and Crossing over

**Linkage:**

When two or more genes reside on the same chromosome, they are said to be linked. They may be linked together on of the autosomes or connected together on the sex chromosomes. Genes on different chromosomes are distributed into gametes independently of one another (Law of Independent Assortment). Genes on the same chromosome; however tend to stay together during the formation of gametes. Thus, the results of test crossing Dihybrid individuals will yield different results, depending upon whether the genes are linked or on different chromosomes.

**Crossing over:**

- Exchange of segments between non-sister chromatids of homologous chromosomes is termed as crossing over.
- Twice the cross over frequency is the chiasma frequency.
- The genotype AB/ab will produce the following types of gametes:

| Types of linkage | Types of gametes | Proportions | Recombination frequency |
|---|---|---|---|
| Complete | 2 (AB & ab) | Equal | 0 |
| Incomplete | 4 (AB, ab, Ab & aB) | Parental more | 0-49 |
| No linkage | 4 (AB, ab, Ab & aB) | Equal | 50 |

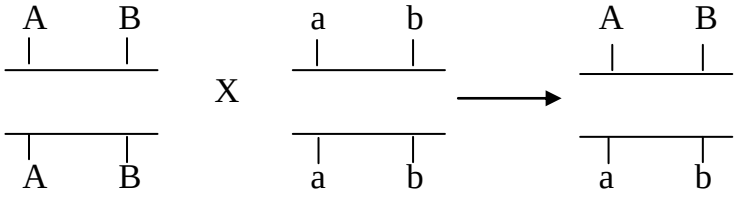
**Arrangement of Linked Genes**:

Linkage between two genes in an individual heterozygous for both pairs may be either of the two types i.e. on the basis of tendency of genes to be inherited in F2 by stay close or apart, linkage is classified as:

(i) Coupling phase (ii) Repulsion Phase

(i) **Coupling phase**:

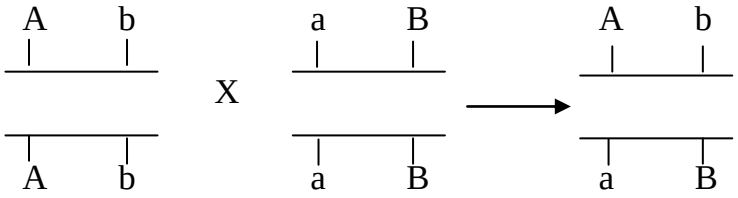
Tendency of two dominant characters inherited by $F_1$ from parents to stay together in F2 is known as **coupling phase**.




It is also termed as **Cis-arrangement**.

(ii) **Repulsion phase**:

Tendency of two dominant characters inherited one from one and other from other parent to stay apart in $F_2$ is known as **repulsion linkage**.




It is also termed as **trans arrangement**.

If "r: be the frequency of recombination, then the proportion of different gametes produced by the parent AB/ab will be as under:

**Proportion of Gametes**

| Gametes | Coupling | Repulsion |
|---|---|---|
| AB | ½ (1-r) | ½ r |
| Ab | ½ r | ½ (1-r) |
| aB | ½ r | ½ (1-r) |
| Ab | ½ (1-r) | ½ r |
| **Total** | **1** | **1** |

**Significance of linkage**

I. The phenomenon of linkage has one of the great significance for the living organisms that it reduces the possibility of variability in gametes unless crossing over occurs.

II. The frequency of crossing over is of great use in constructing genetic maps of the chromosomes.

III. It provides direct evidence for linear arrangement of linked genes in chromosomes.

IV. It increases the frequency of genetical variations which are raw materials of organic evolution.

# Exercise No. 8

# Gene Interaction

## NON EPISTATIC GENE INTERACTION

The expression of an allele will alter the expression of one of more non-allelic genes. A classical example of non epistatic gene interaction is comb shape in poultry. Gene R is rose while gene P is for pea. When genes R and P are together, they interact to produce walnut. In $F_{2,}$ classical ration of 9:3:3:1 is obtained.

Example in fowl, two pairs of alleles *(Rr and Pp)* regulate the shape of the comb. A Wyandotte cock with rose comb was mated with a Brahma hen with pea comb. The chick produced had walnut comb. The latter on interbreeding, produced 19 walnut, 6 pea, 5 rose and 2 single. How comb shape is inherited?

**Solution:**

| | | | | |
|---|---|---|---|---|
| Parents | Rose | | Pea | |
| $F_1$ | | Walnut | | |
| $F_1$ | Walnut | Rose | Pea | Single |
| Number | 19 | 6 | 5 | 2 |
| Ratio | 9 | 3 | 3 | 1 |

$F_1$ is double heterozygous as in $F_2$ 9:3:3:1 ratio is obtained. Walnut has one dominat gene at each locus, while rose and pea have one dominant gene at one or other locus and single is double homozygous recessive. Therefore: Walnut is R-P-, rose is R-pp, pea is rr-p- and single is rrpp. Genotype of cock is RRpp and that of hen is rrPP.

## EPISTASIS

When a gene at a locus masks the action of a gene present at another locus is called epistatic gene. The gene whose effect is masked called hypostatic gene. In $F_2$ the numbers of phenotypic classes are less than four. The common epistatic rations are:

1. Dominant Epitasis — Dominant allele at one locus *(A)* suppresses the expression of the other locus regardless of its allelic condition.

2. Recessive Epitasis — One recessive homozygous suppresses the expression of another locus.
3. Duplicate gene with cumulative effect — Dominant condition at either locus-produce the same phenotype.
4. Duplicate dominant gene — Each dominant locus produce the same phenotype effect, and there is no cumulative effect.
5. Duplicate recessive genes — Either recessive homozygous suppresses the expression of the other locus.
6. Dominant recessive interaction — Dominant gene at one locus and recessive homozygote at the other locus produces the same phenotype effect.

| | **A-B-** | **A-bb** | **AaB-** | **aabb** |
|---|---|---|---|---|
| Classical ration | 9 | 3 | 3 | 1 |
| Dominant epitasis | 12 | - | 3 | 1 |
| Recessive epitasis | 9 | 3 | 4 | - |
| Duplicate gene with cumulative effect | 9 | 6 | - | 1 |
| Duplicate dominant gene | 15 | - | - | 1 |
| Duplicate recessive genes | 9 | 7 | | - |
| Dominant recessive interaction | 13 | | 3 | - |

**Example:**

The feather colour in fowl depends upon the action of two loci. (i) Prevents the expression of colour gene at another independently assorting locus and produced white colour. When recessvie condition exits at the inhibitor locus (ii) the allele of the hypostatic locus expressed, iiB- producing coloured and iibb producing white. When dihybrids were crossed, determine a) the phenotypic proportion expected in the progeny, b) the chance of choosing from the white progeny a homozygous at both loci.

**Solution:**

a) Parents: White White

*IiBb* *IiBb*

$F_1$ 9/16 *I-B-* white

3/16 *I-bb* white

3/16 *iiB-* coloured

1/16 *iibb* white

Hence the ration in the progeny is 13 white: 3 coloured

b) Chance of white progeny, homozygous at both loci:

Proportion of white = 13/16

No of white homozygotes = 3

Probability of white homozygote in $F_2$ = 3/16

Probability of white homozygote among white = (1/3) x (13/16) = 3/13

Only homozygous at both loci are 1/13 *IIBB*, 1/13 *IIbb* and 1/13 *iibb* = 3/13

**PROBLEMS**

# Exercise No. 9

## Estimation of Gene and Genotype frequencies and Hardy Weinberg Law

**Population Genetic:** The study of genetics at the population level is called population genetics.

**Mendelian Population:** Group of sexually reproducing organisms a relatively close degree of genetic relationship (such as species, sub-species, population and breed etc.) residing with defined geographic boundaries where in interbreeding occurs is known as Mendelian Population.

**Gene Frequency:** Relative rarity or abundance of a gene in comparison to its alleles at that particular locus is called gene frequency. Its value ranges from 0 to 1.

**Genotypic frequency**: Relative proportion of favored genotype in a comparison to other genotype present is called genotypic frequency. It ranges from 0 to 1.

**Hardy Weinberg law:** If both gene frequencies and genotype frequencies will remain constant from generation to generation in an infinitely large interbreeding population in which mating is at random and no selection, migration or mutation occur, is called hardy Weinberg law. Hardy Weinberg law depends on following kinds of genetic equilibriums for its full attainment:

1. The population is infinitely large and mate at random.
2. No selection is operative.
3. The population is closed, i.e., no immigration or emigration occurs.
4. No mutation is operative in alleles.
5. Meiosis is normal so that chance is the only factor operative in gametogenesis.

$$\mathbf{(p+q)^2 = p^2 + 2pq + q2}$$

**Testing a Locus for Equilibrium**: Hardy Weinberg law establishes a relationship between gene and genotype frequencies. If "p" and "q" are the frequencies of the alleles A1 and A2 respectively, at a locus then the genotypic frequencies are the square of gene frequencies in a equilibrium population i.e.

$$\mathbf{(p+q)^2 = p^2 + 2pq + q^2}$$

$$\mathbf{A_1A_1 \quad A_1A_2 \quad A_2A_2}$$

Expected Number of Different Genotypes:

$$N\ [p^2\ (A_1A_1);\ 2pq(A_1A_1):\ q^2\ (A_2A_2)]$$

Methods for Testing Whether the Population is in Equilibrium or not:

1. **Chi- square method**:

   It is used to test the population for equilibrium.

   $\chi^2 = \sum (O-E)^2 / E$

   Where "O" and "E" are the observed and expected number of each genotype.

   - Degree of freedom = number of classes – number of alleles.
   - If $\chi^2_{cal} > \chi^2_{table}$ at the given degree of freedom, then the difference is significant and the population is said to be not in equilibrium.
   - If the difference between observed and expected numbers is not significant, then the population is in equilibrium.

2. In a random mating population if H,R and D are the proportions of heterozygote, recessive homozygote and dominant homozygote individual respectively then:

   $\mathbf{H^2}$ = 4DR, when population is in **equilibrium**.

   $\mathbf{H^2}$ = 4DR, when population is **not in equilibrium**.

**A. Single locus with two alleles:**

**1. No dominance/Co- dominance:**

When there is no dominance, the number of genotypes equals the number of phenotypes in the population. Lets at a locus there are two allels $A_1$ and $A_2$ with frequency "p" and "q", respectively. The three genotypes from three separate classes out of total number of individuals (N) in the population, let D, H and R denote the numbers $A_1A_1$, $A_1A_2$ and $A_2A_2$ genotypes respectively.

The gene frequency will be calculated as under:

$$P(A_1) = \frac{2D + H}{2N} \quad \text{(or)} \quad \frac{D+½\,H}{N}$$

$$q(A_2) = \frac{2R + H}{2N} \quad \text{(or)} \quad \frac{R+½\,H}{N}$$

**Problems**

**2. Complete Dominance:**

When dominance is complete, a single locus with 2 alleles would show only two phenotypic groups homozygous dominants and heteroztgotes would be indistinguishable. In such situation, the frequency of the genes can not be determined but can be estimated using H.W. principle.

**Problems**

**B. Multiple Alleles:**

For a trait controlled by multiple alleles, the concept of random mating and hardy – Weinberg principle is equally applicable.

**C. Sex Linked loci: (a) Co dominant / Incomplete Dominance:**

**(b) Complete Dominance**

**D. Testing of Hardy Weinberg Equilibrium:**

**Some Important Points**

1. If the relationship $(p+q)^2 = p^2 + 2pq + q^2$ , holds good then the population is in HW equilibrium.
2. If the population is in HW equilibrium then gene frequency and gene frequency and genotypic frequency will be same in male and female for autosomal traits.
3. Sum of $p+q+r \neq 1$ in A.B, O blood group system because AB genotype frequency is not considered in any of the formulas.
4. Calculation of gene frequency for autosomal traits is same as calculation of gene frequency in females for sex linked genes.
5. The differences in gene frequency between two sexes is reduced by half of the previous generation but of opposite sign.
6. There will be three genotypes in females and three genotypes in males for autosomal traits whereas 2 genotypes in females and genotypes in females for sex linked traits.
7. The gene and genotype frequency is same in males for sex-linked traits.

**PROBLEMS**

# Exercise No. 10

# Factors causing change in gene and genotypic frequency

The forces that change the gene frequency are mutation, migration, selection and random genetic drift.

**MUTATION**

Recurrent mutation changes gene frequency in a population. Consider at a locus there are two alleles, *A* and a, with frequencies $p_0$ and $p_0$, respectively. Let gene *A* mutates to at the rate of μ and gene a mutates back to *A* at the rate of v. the change in the gene frequency will be:

$\Delta q = u\ p_o - v\ q_o$

At equilibrium, $\Delta q = up - vq = 0$

$up = vq$

Hence $q = u/ (u + v)$

$q = v/ (u + v)$. Where, q is equilibrium frequency of a allele.

$P = 1-q$

**Example:**

The initial frequencies of alleles w and $w^+$ in Drosophila, are 0.2 and 0.8, respectively. The forward rate ($w^+$ to w) and backward rate (w to $w^+$) of mutations are $6 \times 10^{-4}$ and $1 \times 10^{-4}$ respectively. What would the gene frequencies after one generation of mutation. Calculate the equilibrium gene frequencies also.

**Solution:**

$P_0 = 0.8$, $q_0 = 0.2$,

Forward mutation rate (u) = 0.0006

Backward mutation rate (v) = 0.0001

At equilibrium, $\Delta q = u\ p_0 - v\ q_0$

$= 0.0006\ (0.8) - 0.0001\ (0.2)$

= 0.00046

Gene frequency after one generation, $q_1 = \Delta q + q_0$

= 0.00046 + 0.2 = 0.20046

$P_1 = (1-q_1) = 0.79954$

Equilibrium gene frequency q = u/(u + v)

= 0.0001/ (0.0001+0.0006)

= 0.14

P = 1-q = 0.86

**MIGRATION**

Let q and q are the frequencies of gene a in the native and migrating population and m is the proportion of the population migrated to the native population. The proportion of native population in mixed population is (1-m). the gene frequency I the mixed population is:

$q_1 = m\ q_m + (1\text{-}m)\ q_o$

$= m\ (q_m - q) + q$

The changes in gene frequency is:

$\Delta q = q_1 - q_0$

$= m\ (q_m - q_0)$

After generation of migration, the gene frequency of the mixed population become q the resulting relationship is

$(q_n - q_m) = (1\text{-}m)^n\ (q_o - q_m)$

$(1\text{-}m)^n = (q_0 - q_m)\ (q_0 - q_m)$

**Example in a** sahiwal herd of 1020 animals, frequency of gene is 0.2. A group of 180 animals with frequency 0.8 for the same allele joins the native herd. What would be the gene frequency of the mixed population? Calculate the change in gene frequency.

**Solution:**

Proportion of population migrated (m) = 180/(180+1020) = 0.15

$q_0 = 0.2$: $q_m = 0.8$

Gene frequency in the mixed population

$q_1 = m(q_m - q_0) + q_0$

$= 0.15\ (0.8\text{-}0.2) + 0.2$

$= 0.29.$

$\Delta q = m(q_m - q_0)$

$= 0.15\ (0.8\text{-}0.2)$

$= 0.09$

Since the frequency of recessive allele is higher in migrated population of allele that increases the frequency in native population by 0.09, i.e. from 0.20 to 0.29.

**SELECTON**

It may be defined as differential survival and reproduction among genotypes. It is one of the most important factors that change the gene frequency in a population. The fitness of the genotype changes the gene frequency. Fitness is the proportionate contribution of its progeny to the next generation in comparison to a standard genotype. The intensity of selection is expressed as coefficient of selection (s), which is the proportionate reduction of gametic contribution of a genotype in comparison to standard genotype. It measures the fitness of a genotype in relation to a standard genotype.

**Selection against Recessive**

The fitness of recessive homozygote is less than other genotypes at that locus. Lets is the coefficient of selection against recessive homozygote. The fitness of recessive homozygote is 1-s. let at a locus there are two alleles (A, a) with genotype frequencies $p^2$ (AA), 2pq (Aa), $q^2$ (aa). The loss in gene frequency in allele a due to selection against recessive homozygote is sq.

| | | | |
|---|---|---|---|
| Genotype | AA | Aa | aa |
| Genotype freq | $p^2$ | 2pq | $q^2$ |
| Fitness | 1 | 1 | 1-s |
| Relative contribution to | $p^2$ | 2pq | $q^2$ (1-s) |

Next generation

Total gene frequency = $p^2 + 2pq + q^2 (1-s)$

$= 1-s\ q^2$

The gene frequency of recessive allele after one generation of selection is

$q_1 = \{pq+ q^2 (1-s)\}/(1-s\ q^2)$

$= q(1-sq)/(1-s\ q^2)$

$\Delta q = q_1-q_0$

$= -s\ q^2 (1-q)/ (1-s\ q^2)$

When complete selection is operating against recessive, then s = 1

Putting the value of s in the above formula

Gene frequency after one generation of complete selection, $q_1 = q_0/ (1+ q_0)$ and

After two generations, $q_2 = q_0/ (1+2 q_0)$

After n generations, $q_n = q_0/(1+nq_0)$

If selection against recessive is continued, then number of generations (t) required to attain desired frequency $q_t$ is

$t= (1/q_t)-(1/q_0)$ or $(q_0-q_n)/ (q_0q_n)$

When the frequency of recessive gene is low , selection is very slow to change it.

**Random Genetic Drift**

Under random genetic drift only the magnitude of change in gene in gene frequency is predictable not the direction. The effect of random drift is observed in small population i.e . when few parents are chosen to produce the next generation. In such a small sample, gene frequency may deviate widely from the frequency of the previous generation. This can be measured by the standard deviation as mentioned below:

**PROBLEMS**

# Exercise No. 11

## Calculation of Population Mean, Average effect of gene and Breeding value

Let at a single locus with two alleles ($A_1$ and $A_2$) with frequency "p" and "q" respectively. The genotypes are $A_1$ $A_1$, $A_1A_2$ and $A_2A_2$ with frequencies $p^2$, 2pq and $q^2$ and values "a", "d" and "-a" (expressed as deviation from the population mean) respectively. The population mean is calculated as:

| Genotype | Frequency | Value | Freq * value |
|---|---|---|---|
| $A_1A_1$ | $p^2$ | +a | $ap^2$ |
| $A_1A_2$ | 2pq | d | 2pqd |
| $A_2A_2$ | $q^2$ | -a | $-aq^2$ |

**Population Mean = $ap^2 + 2pqd – a\,q^2$**

**M = a (p-q) + 2pqd**

**Average Effect of A Gene:**

It is the mean deviation from the population of the individuals that received that gene from one parent and the received from the other parent having come at random from the population. It may also be defined as the difference between effects of allelic pair.

Let $\alpha'_1$ and $\alpha'_2$ are the average effect of gene $A_1$ and $A_2$ then,

$\alpha'_1 = q\,\{a+d\,(q-p)\} = q\alpha'$

$\alpha'_2 = -p\,\{a+d\,(q-p)\} = -p\alpha'$

**Average Effect of Gene Substitution:**

$\alpha' = \alpha'_1 - \alpha'_2$

$\alpha' = \{a + d\,(q-p)\}$

**Breeding Value:**

It is twice the average deviation of the progeny mean from the population mean, provided that mating is random.

| Genotype | Breeding value | Dominance deviation |
|---|---|---|
| $A_1A_1$ | $2\theta\alpha'$ | $-2q^2\,d$ |
| $A_1A_2$ | $(q-p)\,\alpha'$ | 2pqd |
| $A_2A_2$ | $-2p\,\alpha'$ | $-2p^2\,d$ |

**Q. 1** Dwarfing gene in the mouse, known as "**pygmy**" **(pg)**. it reduces body size and is recessive in its effect on size. The weights of mice of the three genotypes at 6 weeks of age were approximately as follows:

| | **Genotypes** | | |
|---|---|---|---|
| | ++ | + **pg** | **pg pg** |
| **Weight in grams** | 14 | 12 | 6 |

The genes were present at a frequency of 0.1. Find the population means, average effect of gene substitution, breeding value and dominance deviation.

**Q. 2** A single gene such controls presence or absence of feathers in the neck region that NaNa is completely devoid of feathers around neck. The heterozygote (Na na) has relatively lesser feather growth around neck while homozygous recessive are fully feathered. In hot weather absence of feathers permits greater heat loss, which promotes greater growth? In a study of a naked allele, the body weights at 6 weeks of age in three broiler genotypes were recorded as follows:

| **Genotype** | **No. of Birds** | **Body Weight (g)** |
|---|---|---|
| Na Na | 300 | 1500 |
| Na na | 200 | 1450 |
| na na | 100 | 1300 |

(a) Calculate the arbitrary assigned genotypic values of three genotypes.
(b) Compute the population mean by referring the following table:

| **Genotype** | **No. of Birds** | **Frequency** |
|---|---|---|
| Na Na | 216 | 0.36 |
| Na na | 288 | 0.48 |
| na na | 96 | 0.16 |

# Exercise No. 12

## Estimation of Heritability and its concept

The observed phenotypic variance which is due to genes and genetic combinations and heritable to the next generation is called heritability.

$$VP = VG + VE$$

The genetic variation ($V_G$) consists of three components i.e. additive genetic variance caused by additive gene action of genes, the dominance variance ($V_D$) caused by dominant deviation from additive and epistatic variance ($V_I$) due to interaction among non allelic genes.

1. **Heritability in narrow sense**
   It is defined as the ratio of additive variance to the phenotypic variance and is denoted by $h^2$

$$h^2 = \frac{V_A}{V_P}$$

   This is transmitted in full to the next generation.

2. **Heritability is broad sense** –
   It is the ratio of total genetic variance to the phenotypic variance.

$$h^2 = \frac{V_G}{V_P}$$

The value of $h^2$ varies from 0 to 1.

- The degree of resemblance between offspring and parent is measured by **regression coefficient** and that between full or half sib is measured by **correlation**.
- Coefficients of the casual component (variance components) in the Covariance (COV) with two factor interactions of relatives.

**Variance components and their contribution**

| Relatives | $V_A$ | $V_D$ | $V_{AA}$ | $V_{AD}$ | $V_{DD}$ |
|---|---|---|---|---|---|
| **Offspring – parent : $cov_{op}$** | 1/2 | - | 1/4 | - | - |
| **Half sibs : $cov_{(HS)}$** | 1/4 | - | 1/16 | - | - |
| **Full sibs : $cov_{(FS)}$** | 1/2 | 1/4 | 1/4 | 1/8 | 1/16 |

- In case of full sib the COV includes $V_{Ec}$ (common environmental circumstances that cause differences between unrelated individual are not a cause of differences between members of the same family).
- Therefore, by observing the phenotypic covariance of different sorts of relationship the amount $V_A$ obtained is

| Relatives | Amount of $V_A$ provided by COV |
|---|---|
| Offspring and one parent | $\frac{1}{2} V_A$ |
| Offspring and mid-parent | $\frac{1}{2} V_A$ |
| Half sib | $\frac{1}{4} V_A$ |
| Full sib | $\frac{1}{2}V_A + \frac{1}{4} V_D + V_{Ec}$ |

**Heritability estimation methods**

- Regression method Regression of offspring on one parent
- Regression of offspring on mid parent
- Intra sire regression of offspring on dam

**Correlation method**

- Half sib correlation
- Full sib correlation
- Heritability can also be estimated from selection experiment data

i.e. $R = h^2S$

$h^2 = R/S$

Where R = Response to selection and S = Selection differential

The heritability estimated by this way is called Realized heritability.

1. **Correlation Method :**

   1. **Half Sib Analysis :**

**Paternal Half Sibs :** These are all the offspring by one sire with different dams. This sort of group is most often used to calculate heritability.

**Maternal Half Sibs :** These are the progeny from one dam by different sires. These are most common in **Poultry and Swine.**

**ESTIMATIN OF HERITABILITY BY VARIANCE COMPONENTS**
**(One-way classification –Half sib analysis)**

Statistical model

$$Y_{ij} = \mu + S_i + e_{ij}$$

Where,

$Y_{ij}$ is observation on jit daughter of ith sire

$\mu$ is overall mean,

$S_i$ is the random effect of $i^{th}$ sire, and

$e_{ij}$ is uncontrolled environmental and random error attributable to individuals within sire.

**Analysis of variance (ANOVA) table**

| Sources of variation | DF | SS | MS | E(MS) |
|---|---|---|---|---|
| Among sires | S – 1 | $SS_s$ | $MS_s$ | $\sigma^2_w + k\,\sigma^2_s$ |
| Among progeny/sires | N – S | $SS_w$ | $MS_w$ | $\sigma^2_w$ |

Where,

S is number of sires

N is total number of daughters

k is average number of progeny per sire.

For equal number of progeny per sire

k = number of progeny per sire

$\sigma^2_s$ is the sire component of variance and estimate ¼ $^{th}$ of additive genetic variance.

$$\sigma^2_s = MSS_B - MSS_W / K$$

$\sigma^2_w$ is within sire component of variance and estimates of the genetic variance (3/4 $\sigma^2_A$) and the environmental variance ( $\sigma^2_E$ ) and it is calculated as follows :

$$\sigma^2_w = MSS_w$$

For unequal number of progeny per sire

$k = [N - \{(\sum n^2_i) / N\}] \div$ d.f. of sires

Where,

$n_i$ is the number of progeny of $i^{th}$ sire.

**Intra-class Correlation :**

**$t = ¼\ h^2$**

**Heritability ($h^2_{H.S}$) = $4\,\sigma^2_s / (\sigma^2_s + \sigma^2_w)$**

**PROBLEMS**

## II. Full Sib Analysis

- The design is useful for species which are prolific breeder with short generation interval i.e . **Pig and poultry.**
- Full sib family data are those where we have group of progeny in each sire , coming as more than one progeny per dam from a group of dams.
- **Statistical Model :**
- $\mathbf{Y_{ijk} = \mu + S_i + D_{lj} + e_{ijk}}$

  Where : $\mathbf{Y_{ijk}}$ =

  $\boldsymbol{\mu}$ =

  $\mathbf{S_i}$ =

  $\mathbf{D_{lj}}$ =

  $\mathbf{e_{ijk}}$ =

**Analysis of variance (ANOVA) table**

| Sources of variation | DF | SS | MS | E(MS) |
|---|---|---|---|---|
| Between sire | S – 1 | $SS_s$ | $MSS_s$ | $\sigma^2_w + k\,\sigma^2_s$ |
| Between dam/ withinsires | N – S | $SS_D$ | $MSS_D$ | $\sigma^2_w + K_1\,\sigma^2_D$ |
| Between progeny / within dam/ within sire | n..-D | $SS_W$ | $MSS_W$= SSw/n..-D | $\sigma^2_w$ |

Where,

S is number of sires

N is total number of daughters

k is average number of progeny per sire.

For equal number of progeny per sire

k = number of progeny per sire

$\sigma^2_s$ is the sire component of variance

$\sigma^2_D$ is dam component of variance

$\sigma^2_w$ is error component of variance

**Estimation of Variance Components :**

$\sigma^2_w$ =MSSw

$\sigma^2_D = MSS_D - MSS_w / K_1$

$\sigma^2_s = MSS_B - MSS_D / K_2$

**Estimation of Heritability :**

$h^2_{F.S} = 2(\sigma^2_s + \sigma^2_D) / \sigma^2_s + \sigma^2_D + \sigma^2_w$

# Exercise No. 13

## ESTIMATION OF REPEATABILITY

Repeatability refers to measures of association among repeated records of the same trait measured at different times in the life of same individual e.g. milk production in dairy animal, wool characteristics in sheep, egg production in poultry and litter size in swine. In statistical terminology, this parameter is called as intraclass correlation between individuals. Thus, repeatability can also be defined as fraction of total phenotypic variance which is due to genetic and permanent environmental differences. The repeatability is symbolized by r, which is equal to:

$R = (V_G + V_{EP}) / (V_G + V_{EP} + V_{ET})$

= between individual variance/total phenotypic variance

Where,

$V_G$ = variance due to additive, dominance and epistasis,

$V_{EP}$ = variance due to permanent environment, and

$V_{ET}$ = variance due to temporary environment.

Repeatability also refers to the fraction of the difference from the mean in one record which is expected in another record on the same animal. Thus regression coefficient of future record on previous record or correlation between two repeated records on the same animal is also known as repeatability. Therefore repeatability can be estimated from the following two approaches.

a. Regression and correlation coefficients

b. Variance components

I. Estimation of repeatability by regression and correlation repeatability from correlation coefficient

$r_c = б p_1 p_2 / [(б^2 p_1 б^2 p_2)]^{0.5}$

Repeatability from regression coefficient

$r_б = б p_1 p_2 / б^2 p_1$

where,

$б p_1 p_2$ is covariance between first and second record.

$б^2 p_1$ is variance of first record, and

$б^2 p_2$ is variance of second record.

The estimates of repeatability obtained from regression coefficient is unbiased by selection coefficient is unbiased by selection on first record.

II. Estimation of repeatability from variance components statistical model

$y_{ij} = \mu + Ai + e_{ij}$

Where,

$y_{ij}$ is the jth measurement on ith individual,

$\mu$ is the overall mean,

Ai is the effect of ith individual, and

$e_{ij}$ is the environmental deviation of jth measurement within an individual.

**Analysis of variance Table**

| Source | df | SS | MS | E(MS) |
|---|---|---|---|---|
| Between individuals | N-1 | $SS_A$ | $MS_A$ | $\sigma^2_W + k\,\sigma^2_A$ |
| Within individuals | N(m-1) | $SS_W$ | $MS_W$ | $\sigma^2_W$ |

Where, N= The No. of animals

m= No. of records

k= Avg. No. of record

PROBLEMS

# Exercise No. 14
# ESTIMATION OF PHENOTYPIC AND GENOTYPIC CORRELATIONS

The definition of a correlation is the covariance between the two traits divided by the square root of product of variances of two traits. The correlation between two traits describes the extent to which individuals above average for one trait tend to be above, below or near average for other trait. Two traits may be correlated because of common genetic factors or common environmental factors. Thus the traits can be partitioned into genetic and environmental part with the parts having corresponding covariances and correlations. **Genetic correlation is the correlation between sets of genes which influence two or more traits on the same individual or it is the correlation between the breeding values of the traits. Phenotypic correlation is the correlation between the phenotypic values or observed values of the two traits and is caused by genetic and environmental factors.** Phenotypic correlation is also called simple correlation or total correlation or product moment correlation. Environmental correlation arises due to the similarity of environmental factors affecting the two traits. In true sense, it is not the correlation due to environmental factors alone but also due to non-additive genetic factors. The environmental correlations are less important as the environment tends to change or the data are adjusted for environmental factors.

**Correlation coefficient ranges from -1 to +1.**

**Causes of genetic correlation:**

**1. Linkage:** Genes present on the same chromosome have the tandency to remain together in the gamete and offspring, hence the character controlled by those genes are genetically correlated.

**2. Pleiotropic effect of genes:** The genes having effect on more than one character are called pleiotropic genes and the phenomenon is called pleiotropism. This is the main cause of genetic correlation between two traits.

**ESTIMATION OF GENETIC CORRELATION :**

Since the genetic correlation is a measure of the degree of association between the breeding values of the two traits, it indicates the extent to which the traits are under the control of same genes.

Estimation of genetic correlation between two traits say X and Y require the computation of the following information:

(i) Genetic Covariance $\sigma_{A(XY)}$
(ii) Phenotypic Covariance $\sigma_{P(XY)}$
(iii) Genetic variance $\sigma^2_{A(X)}$ and $\sigma^2_{P(Y)}$

Therefore, Genetic correlation ($r_G$) is calculated as:

$$r_G = \frac{Cov_{xy}}{\sqrt{\left(\sigma_x^2 \sigma_y^2\right)}}$$

$Cov_{xy}$ = Genetic covariance

$\sigma^2_x$ and $\sigma^2_y$ =Genetic variance x and Genetic variance y

The estimate of phenotypic and genetic variance are obtained by the analysis of variance method, which has been discussed under the estimation of heritability.

The computation of genetic covariance require an analysis of covariance which require multiplication of the values of the two traits recorded on the same animal.

**Analysis of covariance (ANCOVA) table**

| **Sources of variation** | **DF** | **Sum of cross product** | **Mean cross product** | **Composition of cross** |
|---|---|---|---|---|
| Between sire | S – 1 | $SCP_S$ | $MCP_S$ | $COV_W + K^*COV_S$ |
| Within sires | N – S | $SCP_W$ | $MCP_W$ | $COV_W$ |
| Total | N - 1 | $SCP_T$ | | |

Where, K* = average number of progeny per sire,

$COV_W = MCP_w$ $COV_S = MCP_S – MCP/ K$

Write formulae of genetic and phenotypic correlation.

**PROBLEMS**