# Laboratory Manual and Notes on Introductory Genetics

By

Adeniran M.O and Amanze C.O

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## INTRODUCTION

Biological variations arise as a result of parent organisms transferring heritable characters from their gametes to their offsprings. In plants the somatic cells through which characters are transferred are found in leaves, stems and roots. The biological individuality has patterns, limitations and characteristics that depend at least in part on the parents of an individual.

Through experimentation and study, geneticists have established certain rules that characterise the transmission of biological heredity. Early development of the science of genetics realised that genetic systems need to be understood in relation to the environments in which organisms exist.

Genetics therefore explains how biological properties are transmitted from parents to offsprings. The differences between simple and alternative genetic characters depend on particulate elements called genes. These genes are paired in an individual, each alternative pair from the paternal and maternal parents respectively. When an individual forms germ cells, new individual emerges carrying the element characters of the parents.

## MITOSIS

Mitosis not only ensures that each daughter cell has the same number of chromosomes but also that each chromosome in one cell carries exactly the same genes as a corresponding chromosome in the over-all cell. This means that there must be a duplication of each chromosome during mitosis. It can be said that this duplication takes place as a result of the attachment of nucleic acid during the prophase stage of mitosis. By Anaphase the separating chromatids or daughter chromosomes have a double series of genes. It is during the resting phase that the two series become separated to give the two chromatids which are visible in the succeeding prophase stage.

The corresponding egg formation process in female is called 'oogenesis'. There is one important difference between male and female gamete formation: In the male, four functional sperm cells are formed from each primary spermatocyte. The egg must often provide stored material for the developing embryo.

Three of the four products of meiosis in females are small abortive cells called 'polar bodies.' These contain little cytoplasmic material. They simply degenerate from the egg as meiosis proceeds. As such, in females, one primary oocyte gives rise to only a single egg cell.

The meiotic divisions in the eggs of some species occur after the sperm has entered the egg. In other species, sperm entrance occurs after the first, but before the second, meiotic divisions. In other organisms, meiosis is completed before fertilization.

GAMETE FORMATION IN PLANTS (Sporogenesis)

The germ-cell-forming tissues in plants behave in meiosis in much the same way as do those of the corresponding sexes in animals. In plants, however, the situation is somewhat complicated by the occurrence of two or more mitotic divisions of the haploid products of meiosis before fertilization occurs.

TERMINOLOGY

- Gene - a unit (hereditary factor) that determines the expression of a ...
- Allelomorph/Allele - a Greek word which means the alternate form of an attribute. It is one of a pair or series of forms of a gene which are alternatives in inheritance because they are situated at the x same locus in homologous chromosomes.
- Chromosome - a unit of inheritance that carries the genes.
- Homozygous - a pair of genes with the alleles looking alike - eg. a gene denoted by SS for smooth seed.
- Heterozygous - a pair of genes with the alleles looking different - eg. SS or Rr
- Dominant - a character that expresses itself in the appearance of an individual. It is usually denoted by capital letter - eg. LL or L - or Ll.

- Recessive - a character that fails to express itself in the appearance of an individual though present in the genetic constitution of an individual
- Filial generation - an offspring resulting from a cross from contrasting parents. It is denoted by $F_1$; $F_2$; $F_3$ etc. for first, second and third generations respectively.
- True-breeding - when an individual is capable of producing only one alternative - eg. cowpea seeds has white and brown seed coat colour but no seed has both white and brown seed coat colours together.
- Monohybrid - this is a cross involving the progeny of a single contrasting character, eg. smoothVs, wrinkled seed coat texture. Capital letters are used to denote one of the dominant characters and small letters of same for recessive character, eg. smooth Vs. wrinkled seed coat texture is denoted with; smooth - SS and wrinkled - ss.
- Dihybrid - this is a cross involving two contrasting characters eg. smooth Vs. wrinkled seed coat texture and yellow Vs. green seed coat colour - eg. smooth Vs. wrinkled - SS Vs. ss, Yellow Vs. green -YY Vs. yy

MENDELIAN GENETICS

Two laws were formulated by Gregor Mendel to support character inheritance from parent to offspring. Experiments were carried out using Garden Pea.

Properties of Organisms used for genetic studies:

1. must be easy to handle and not cumbersome.
2. must have offsprings which are distinctly enumerated.
3. the offsprings must show a continuity between the two parents.
4. the parents must have observable differences in character, and
5. the organism must be able to reproduce within a limited time possible

Mendel crossed pea plants from a smooth seeded line with pea plants from a wrinkled seed line. The $F_1$ generation had smooth seed coat. In the next generation, which was achieved by selfing the $F_1$ individuals, each of the alternatives that differentiated the original parental types reappeared.

A monohybrid cross of two contrasting parent characters gives a 3:1 $F_2$ ratio. A dihybrid cross of two contrasting parent characters gives a 9:3:3:1 $F_2$ ratio

GOODNESS OF FIT TEST

This test is used to find out the type of deviations resulting from analysed data. Deviations are mainly due to hereditary factors or due to experimental error. Chi-square ($\chi^2$) Chi-square technique is used in determining the type of deviation viz. $\chi^2 = \sum \frac{(o-e)^2}{e}$

The observed data is usually given. The expected data is the product of the observed total value and the ratio of the $F_2$ generation. The "dF" defiation "dF" depends on the number of phenotypic classes in the progeny. The calculated value and the tabulated value are compared before making conclusion on source of error.

TUTORIAL EXERCISES

1. Cell/cell division/population variation

1.1 Discuss the importance of mitosis and meiosis to variation in plant and animal population.

1.2 "It is safe to say that all traits have both heritable and environmental components". Discuss, giving distinct examples of heritable and non-heritable variations in plants and animals.

1.3 Discuss five differences between mitosis and meiosis.

2. Mendelian Genetics

2.1 State mendel's law of independent assortment and law of segregation. Explain these laws using characters from a monohybrid and dihybrid simple crosses.

2.2 In sweet peas, genes C and P are necessary for coloured flowers. In the absence of either or both (CCPP) of these genes, the flowers are white. What will be the flower colour of the offspring of the following crosses and in what proportion?

(a) CcPP x ccpp

(b) CcPP x Ccpp

(c) Ccpp x Ccpp

2.3 Flower colour in a particular plant may be purple, red or white, and these traits are known to assort independently of each other Homozygous stocks of each of these colours were raised and crossed in the following patterns - with the following results:

Cross 1 : $P_1$ Purple x Red

$F_1$ All Purple

$F_1$ x $F_1$ ¾ purple; ¼ red

Cross 2 : $P_1$ Purple x colourless

$F_1$ All purple

$F_1$ x $F_1$ = 9/16 purple; 3/16 red; 4/16 colourless

Cross 3 : $P_1$ Purple x colourless

$F_1$ All colourless

$F_1$ x $F_1$ = 12/16 colourless; 3/16 purple; 1/16 red

List the phenotypes and their ratios expected from the following crosses:

(a) $F_1$ plant from cross 1 crossed to an $F_1$ plant of cross 3

(b) $F_1$ plant from cross 1 crossed to an $F_1$ plant of cross 3

(c) $F_1$ plant from cross 2 crossed to an $F_1$ plant of cross 3

3. What is the significance of the chi-square test to Mendelian hypothesis?

GENE INTERACTION

A character may have more than one form because of environmental or hereditary differences. Mendel chose to study characters that are least affected by the environment and that are easily classified into a few categories. His first contribution to the understanding of the basis of hereditary variation was to relate a basic observed difference to a single hereditary factor. When an individual has a gene P, he has a normal pigmentation whereas when he inherits the two alleles p, p from both parents he is an albino. Thus Mendel was able to assign an observed hereditary variation to a pair of hereditary factors or genes (alleles). Therefore one can deduce that a change in a hereditary factor is usually accompanied by an observable phenotypic variation.

Another important contribution by Mendel is that hereditary factors assert independently in transmission - i e, every gene acting independently of another genes - one gene, one observable variation. It was soon found that most observable variations are not conditioned by one gene, but by several genes. Thus Mendel's observations really represent the exceptions rather than the rule.

EPISTASIS

When non-allelic genes interact to produce a phenotype, it is called epistasis; for example in poultry four basic comb types have been observed: single, walnut, rose and pea. If you cross rose and single, the F1 is rose and the F2 segregated 3:1 ratio. However, if you cross pea and rose you get walnut. From this result one would have guessed that pea and rose are co-dominant and that both alleles are dominant to single. If this hypothesis is correct, Walnut crossed to Walnut should segregate in the ratio of 1 rose: 2 Walnut: 1 pea; but in an actual cross a 9 walnut; 3 pea: 3 rose; 1 single ration was observed. From our earlier experience, this ratio suggests that two pairs of genes are segregating: viz

(a) Pea as PPrr and Single as pprr then.

PPrr x pprr
pea Single

$F_1$ = PPrr
(Pea)

$F_2$ = 1 PPrr: 2 PPrr : 1 pprr
Pea Single

or

3 P-rr : 1 pprr
pea single

This conforms to the observed 3:1 ratio If we also designate rose as ppRR then on crossing rose with pea we will expect the following phenotypic classes:

| genotypes | Phenotypes |
|---|---|
| 1 PPRR<br>2 PpRR<br>2 PPRr<br>4 PpRr | Walnut (P-R-) |
| 1 PPRR<br>2 Pprr | pea (P-rr) |
| 1 ppRR<br>2 ppRr | rose (ppR-) |

The above is an example of a trait that is conditioned by two pairs of segregating genes.

(b) In onion, red, yellow and white bulbs are commonly observed.

A colour gene in the functional form (C) must be present for onion to develop red or yellow colour and in the absence of the C gene the onion develops a white colour. When CcRr onion plant is crossed with another CcRr plant a ratio of 9 red: 3 yellow: 4 white is obtained. In light of the above explanations the $F_2$ ration have the following phenotypic distribution

C – R = red (9)

C – rr = yellow (3)

ccR- = White (3)

ccrr = White (1)

Thus, as earlier mentioned, when non-allelic genes interact to produce a phenotype it is called <u>epistasis</u>.

As a result of <u>epistasis</u>, the following phenotypic ratios have been observed when two genes are segregating. Each phenotypic class represents either one of the 4 basic phenotypes or a combination of two or more classes into one phenotypic class.

Basic Phenotypic Classes:

```
9 )          )         (       )         (
  )          )         ( = 9   ) = 9     ( = 9
  )=12       )
3 )          )         )                 (
             )  15     )                 ( = 3
             )         ) - 7  (
3 )          )         )      ( = 6      (
  ) = 4      )         )      ) = 1      ( = 4
1 )          )  1                        (
```

<u>PLEIOTROPY</u>

Another exception to Mendel's world of genetics is a situation where a gene produces more than one effect. The sickle cell trait is an example in which a gene may have more than one phenotypic effect. A sickle has a defective haemoglobin molecule thus:

$HB^A\ Hb^A$ = normal haemoglobin

$Hb^A\ Hb^S$ = sickle cell trait

$Hb^S\ Hb^S$ = Sickler

The red blood cell of $Hb^A Hb^S$ is more or lessnormal and in addition such individuals are slightly protected against the extreme symptoms of malaria; they however suffer from other physiological defects. Sicklers ($Hb^S Hb^S$) on the other hand hardly survive to reproductive age without extensive medical attention. Affected individuals suffer from anaemia, dumping of red blood corpuscles in veins and eventually death.

Another striking case of pleiotropism is albinism in which affected individuals lack skin pigmentation this condigms also affects vision.

## SEX DETERMINATION AND SEX LINKAGE

### Sex and Its Importance

We are used to man and our domestic animals each time we talk about sex. Each time we talk about sex what comes to mind is that there are two sexes, male and female. All organisms, however, do not have only two sexes. The lowest forms of plant and animal life may have several sexes. As example, in one variety of the ciliated protozoan *Paramecium bursaria*. There are eight (8) sexes or mating types which are all identical morphologically but each mating type is not capable of conjugating with its own type but may exchange genetic materials with any of the other types (7) within the same variety In higher plants and animals the number of sexes have been reduced to two. We know of male flower and female flowering plants. We know also of male and female animals. There are animals that have in the same body both male and female organs e.g. snails. Such animal is siad to be an hermaphrodite. In plants where staminate (male) and pistillate (female) flowers occur on the same plant we say the plant is monoecious e.g. maize, oil palm, Most of the flowering plants have both male and female parts within the same flower and so are referred to as perfect flower. Relatively, few angiosperms are having the male and female flowers in quite different individuals e.g. pawpaw, spinach, date palm, asparagus. They are siad to be dioecious.

One very important role sex plays in both plants and animals regardless of the number is that it provides for genetic variability upon which selection is based and consequently new generations are evolved.

## Sex Chromosone Mechanism

### (a) Males and Chromosome types

Normal males in man and perhaps all other mammals have XY chromosomes. Normal males are XY or referred to as heterogametic. The female on the other hand produce only one kind of gamete and is referred to as homogametic sex having XX. This mode of sex determination is referred to as XY method. This mode of sex determination produces a 1:1 sex ratio in each generation.

Diagramatically this can be explained as:

| | | | |
|---|---|---|---|
| Parents | XX (females) | | XY (Males) |
| | Gametes X | | X Y |
| F1 | 1XX female | : | 1 XY male |

In certain insects particularly those of the orders Hemiptera or true bugs and the orthoptera e.g. grasshoppers, crickets, cockroaches, males are also heterogametic but produce X bearing sperm without a sex chromosome. In mlaes of these species the X - chromosome has no homologous pairing partn[illegible] because Y chromosome is absent. Males therefore exhibit odd number in their chromosome complement.

One - X is male and XX is female.

The mode of sex determination is usually referred to as XO method wh[illegible] the O stands for absence of a chromosome analogous to the Y of the XY sys[illegible]

| | | | |
|---|---|---|---|
| Parents | XX | | XO |
| Gametes | X | | X O |
| F1 | 1XX female | : | 1 X O male |

### (b) Heterogametic Females

This method of sex determination is found in large group of animals including butterflies, silk worms, moths, birds fishes. One X determines femaleness and two X condition determines the maleness (exact opposite of what happens in man). Instead 2 X Y, the chromosomes are sometimes la[illegible] Z W in order to call the attention of female to (ZW) heterogametic and [illegible] homogametic or male.

In the domestic chicken there is no single sex chromosome as in the case of the XO mechanism previously discussed and so ZZ is used to represent the males and ZO to designate the females. A 1 : 1 sex ratio is expected also.

Diagrammatically

ZO Sex Determination method

Parents: ZZ X ZO

Male female

Gametes: Z Z and O

F1 1 ZZ male 1 ZO Female

Parents: ZZ X ZW

Male Female

Gametes: Z and Z W

F1 1ZZ (male) : 1 ZW (female)

Sex determination in bees, ants and wasps:

This group of insects of the order Hymenoptera have males and females. The male are known to develop parthenogenetically from unfertilized eggs and so are haploid, females, on the other hand originate from fertilized eggs. These are diploid. Sex chromosomes are not involved in the mechanism, sex determination. The queen bees mate only once during their life time and has a storage "bank" where sperm are kept. The queen chooses to fertilize some eggs which eventually develop into male bees. The quantity and quality of food available to the diploid larva determines whether the female will become a sterile worker or a fertile queen. Environment therefore determines sterility or fertility but does not alter the genetically determined sex. The queen determines the sex ratio.

Sex Linked Inheritance

Any gene located on the X chromosome in mammals,drosophila and others or other species with the ZO or ZW mechanism is said to be sex linked. The first sex linked gene found in drosophila was the recessive white eye mutation. Ordinarily the drosophila has red eyes. When white eyed females are crossed with wild (red eyed) males all the females offspring have red eyes like their mother.

This type of inheritance is called criss-cross inheritance and is typical of sex linked genes. This is due to the fact that Y chromosome carries no alleles homologous to those at the white locus on the X chromosome. In most organisms with the Y- type chromosome the Y is virtually devoid of known genes.

| | | |
|---|---|---|
| $X^w X^w$ | x | $X^+ Y$ |
| white female | | wild type male |
| Gametes $X^w$ | $X^+$ | Y |
| F1 $X^w X^+$ | $X^w Y$ | |
| red females | white males | |

(Criss-cross inheritance)

Sex Influenced Traits

Genes governing sex influenced traits may reside on any of the austosomes or homologous portion of the sex chromosomes. Baldness is an example where internal environment provided by the sex hormones play a major role.

| Genotypes | Phenotypes | |
|---|---|---|
| | Men | Women |
| bb | non bald | non-bald |
| $b^1b^1$ | bald | bald |
| $bb^1$ | bald | non-bald |

Sex Limited Traits

In most higher organisms with two sexes, some genes may only be expressed in one of the two sexes because of the differences in the internal hormonal environment or because of differences in the body anatomy. For example, genes for milk production which we know are present in bulls and which can be transferred to their daughters but they or their sons cannot express the trait. Milk production is therefore limited in expression to female sex.

SEXUALITY IN PLANTS

In most flowering plants monoecy is common. That is male and female organs on the same plant. Here sex chromosomes are not known to be present. But there are genetic mechanism in some monoecious species which prevent self fertilization or the development of zygotes produced by the union of identical gametes.

This makes cross fertilization a rule in such species. There are self incompatibility system in such monoecious species and also genetic male sterility which prevent self fertilization.

However, in species having dioecism they are under the influence of genetic control. The gene Melandrium is an example of well documented case where Y-chromosome determines of sex Pistillate plants are X X and staminate plants are X Y

Exercises on Sex Determination and Sex Linkage

1. If barred feather is dominant over non barred. What will be the offspring of a barred hen mated with a non-barred roster? What is this type of cross called?
2. In drosophila, bar eye (B) behaves as a dominant sex-linked gene. Show diagramatically, the cross between a bar-eyed male and a wild type of female, and the expected genotypes, phenotypes and sex of their off spring.
3. Why can a man not pass on a sex linked gene to his son?
4. A man with normal vision marries a woman though with normal sight but whose father was colour blind, what ratios of genotypes and phenotypes would be expected among their child?
5. In man, the bleeder's disease known as haemophilia is found to be sex linked and recessive. What offspring phenotypic ratios would be expected from a marriage between
   (a) a haemophibic man and a carrier woman?
   (b) a normal man and a carrier woman?

PLANT BREEDING

Objectives of Plant Breeding

Man is almost dependent on plants for his food. The things he eats virtually without exception, are either plant materials or derived rather directly from plants as are for example, meat, eggs and dairy products.

Plants are also major source, directly or indirectly, of most clothing, fuel, drugs, and construction materials.

Plants are also used as ornamentals for beautification of surroundings.

Considering the prime importance of plants, it is not surprising that men have long been concerned with developing types of plants suited to satisfying their needs, Only recently however, and largely in conjunction with the growth of genetics have these attempts been systematized to the point where they can be called a Science. This comparative new Science is referred to as the Science of Plant Breeding.

Goals of Plant Breeding

Augmentation of Food Supplies - most of the emphasis in plant breeding has been placed on increasing agricultural productivity - in response to the pressure, on an adequate food supply caused by constant increasing population in a world of limited acres. Except during brief periods of abundance, human populations , like populations of other animals, have suffered from hunger. Each period of abundance has brought a jump in population, followed by hunger and its aftereffects, pestilence, high infant mortality and meagre sickly lives. Malthus postulated in 1798 that human populations, unless checked by ways or disaster increase until hunger takes control. He foresaw catastrophe because he believed that population was capable of increase at a geometric rate and food supply only or in arithmetic rate. On the basis he predicted that Britain would be in disaster by the midnineteenth century. This prediction did not come true befause of understimation of man's ingenuity in increasing agricultural productivity. As Malthus was writing, his countrymen about him were ushering in the age of scientific agriculture. From it came tremendous increase in food supplies as a result of better methods of pfoduction and improved variation of plants and breeds of livestock. About the same time, the machine age started which had a profound effect not only on the efficiency of production but also on the efficiency of utilization of agricultural products through better methods of preservation storage, and transportation. Many are concerned that in the broadest sense, Malthus may stil be right. Modern Malthusians maintain that catastrophe has only been delayed and that the misery associated with overpopulation is still the fate of modern civilization. If this is not to be true, constantly increasing supplies of foodstuffs will be required.

Opportunities that exist to further increase food supplies in ... development of new areas; others of more conventional and proven types also remain, but they are increasingly associated with comparatively expensive reclamation projects súch as irrigation of desserts and drainage of sewages. Most of other new sources of sustenance like algae and plantation, are un-inspiring.

Another opportunity to increase production lies in improved Agricul-tural practices, including better fertilization, more effective crop rota-tions, improved tillage methods, and more efficient weed, disease and insect control.

Finally, we can expect plant breeding to contribute substantially to greater agricultural productivity. This will be accomplished not only by the breeding of basically higher-yielding varieties, but also by the develop-ment of varieties that help to stabilize production through ressistance to disease, drought, heat, cold and wind.

End Products of Breeding Programme

1. Increased (improved) yield has been the ultimate aim of plant breeders. increased yield has been accomplished by providing varieties basically more productive, not because of specific improvements wuch as in disease resistance but as a result of generally greater physiological efficiency, e.g.

   (i) Sugar beets - increasing the sugar content

   (ii) Wheat - rust resistance
   - best agronomic characters
   - better milling qualities
   - improved grain yield

   (iii) Maize - improved yield especially through the development of HYBRID varieties.
   - Selection for desirable agronomic characters
     e.g. - Well adapted
     - non-lodging
     - uniformity in size
     - plant height.

   (iv) Potatoes, Yam or Cassava
   - big tuber
   - high yield
   - disease, insect resistance

2. Development of Better Varieties for New Agricultural Areas

- frequently accomplished by adjusting the growth circle of the variety to better suit the available growing season. e.g. modification of grain sorghums since their successful introduction to the United States about 100 years ago. This tropical grass spices was originally confined to the warmer parts of the South West and Southern-plain area.

3. Improvement of Plants in Agronomic or Horticultural Characters, e.g. development of dwarf sorghum varieties from the taller species originally introduced - to ease mechanical harvesting.

4. Development of Crop Varieties Resistant to Diseases and Insects

   - one of the most dramatic and certainly the best known contribution of plant breeding.
   - for some crops these resistant varieties have provided the only feasible control for such pests. eg. stem rust of Wheat.
   - perhaps the most important feature of resistant variety is the stabilizing effect they have on production.

5. Development of Crop Varieties

   tolerant to heat, cold or draught.

6. Advances leading to improved quality of Agricultural Products

   e.g. - breeding of stronger and longer staple cotton.
   - stringless green beams and apples with superior flavour
   - wheat with more protein
   - tomato with higher vitamin context.

Plant breeding has greater contribution to make in the future than it has made in the past. In one way the contribution to human welfare made by superior varieties is the most satisfying of all methods of increasing production. Normally such varieties and nothing to the cost of production beyond that required to handle the additional increment of yield.