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Teaching and Learning Genetics with *Drosophila* 3. Pattern of Inheritance of Autosomal and Sex Chromosome Linked Genes/Characters.

Phenotypic characters or traits are determined by genes, and genes are physically located on different chromosomes. This is the basic tenet of the chromosomal theory of inheritance. *Drosophila melanogaster* has four pairs of chromosomes ($2n=8$) whereas humans have twenty three pairs of chromosomes ($2n=46$). We all know that differences between males and females (sexual dimorphism) are seen at different levels. One such difference lies in their chromosome complements (*Box 1*). In this part of the article, let us see how we can examine the pattern of inheritance of characters determined by genes present in autosomes and sex chromosomes of *D. melanogaster*. See [2] and [3].

Strategy of the Experiment

In this and subsequent parts, we will examine the pattern of inheritance of a few characters in *D. melanogaster* and try to learn some of the principles of formal genetics through simple experiments. For each of these experiments, genetically defined stocks are required. One of them is a standard stock, which is homozygous at all loci for normal (wild type) alleles. For instance, for *D. melanogaster* the world over, Oregon R or Canton S strains are employed as standard wild type strains. The other stock required is a true breeding mutant stock for a particular type of mutant phenotype whose pattern of inheritance is to be worked out. For instance, vestigial winged stock, white eye stock, etc. Such genetically defined and true breeding stocks are available at Drosophila Stock Centres. First, however, let us develop a general protocol which will be applicable not only for experiments described in this part, but also for the experiments of future parts (*Boxes 2, 3 and 4*).

Experiment 1. The aim here is to understand the pattern of inheritance of 'ebony body colour' (dark colour) in *D.*

Box 1.

The diploid chromosome complement of *D. melanogaster* includes eight chromosomes. Of these, three pairs are called autosomes. In each pair of autosomes, one chromosome is homologous (i.e. bears the same genes) to the other. Such three pairs of homologous chromosomes are seen in both males and females. The other pair of the complement are the sex-chromosomes. Females have a pair of homologous sex chromosomes called X-chromosomes (Figure A). On the other hand, males have one X and one Y-chromosome and these are not homologous to one another (Figure B). The Y-chromosome, which is restricted to the males only, is mostly heterochromatic and has very

few genes. The Y-chromosome is structurally and functionally different from the X-chromosome. Hence the sex chromosomes in males are called heteromorphic chromosomes. Consequently, in males, there is only one set of X-linked genes on the single X-chromosome (hemizygous condition), while in females there

are two sets of X-linked genes (in heterozygous or homozygous condition). Similarly in humans with $2n=46$ chromosomes, males have 22 pairs of autosomes plus one X and one Y-chromosome, and females have 22 pairs of autosomes and a pair of X-chromosomes.

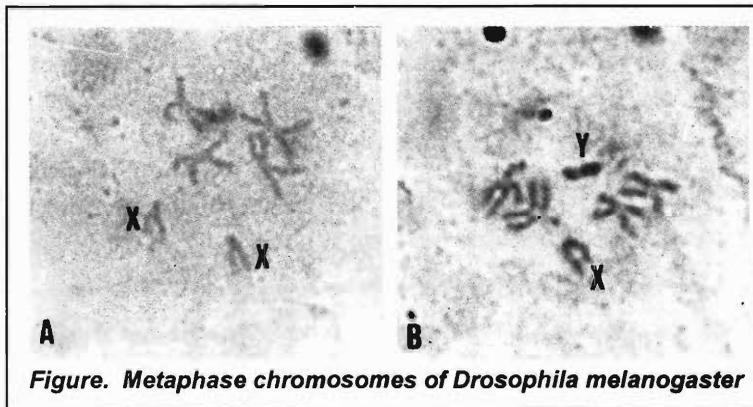


Figure. Metaphase chromosomes of *Drosophila melanogaster*

melanogaster. The normal body colour of *D. melanogaster* is grey. As per the instructions given in the Boxes 2–4, this experiment was conducted and the results are as shown on page 81:

Analysis of Results:

- a) By looking at the F_1 , one can say that the gene (s) for the normal grey body colour is dominant over the allele for the ebony colour (Law of Dominance of Mendel).
- b) The F_1 data do not tell us whether this body colour phenotype is due to one or more genes. Therefore one has to look at the F_2 progeny.
- c) Among the F_2 progeny of both the reciprocal crosses, flies with normal grey colour and flies with ebony body colour have

Box 2. Experimental Protocol

P₁ Males x P₂ virgin Females
Standard stock x Mutant stock

P₁ virgin Females x P₂ Males
Standard stock x Mutant stock

(Parental flies have to be removed from the culture before the F₁ progeny eclose from the pupae)

F₁ Generation

Analyse the phenotypes of males and females and their ratios.

F₁ Generation

Analyse the phenotypes of males and females and their ratios.

F₁ males x F₁ females

(The F₁ flies have to be removed before the eclosion of F₂ flies)

F₁ males x F₁ females

F₂ Generation

Analyse the phenotypes of males and females and their ratios.

F₂ Generation

Analyse the phenotypes of males and females and their ratios.

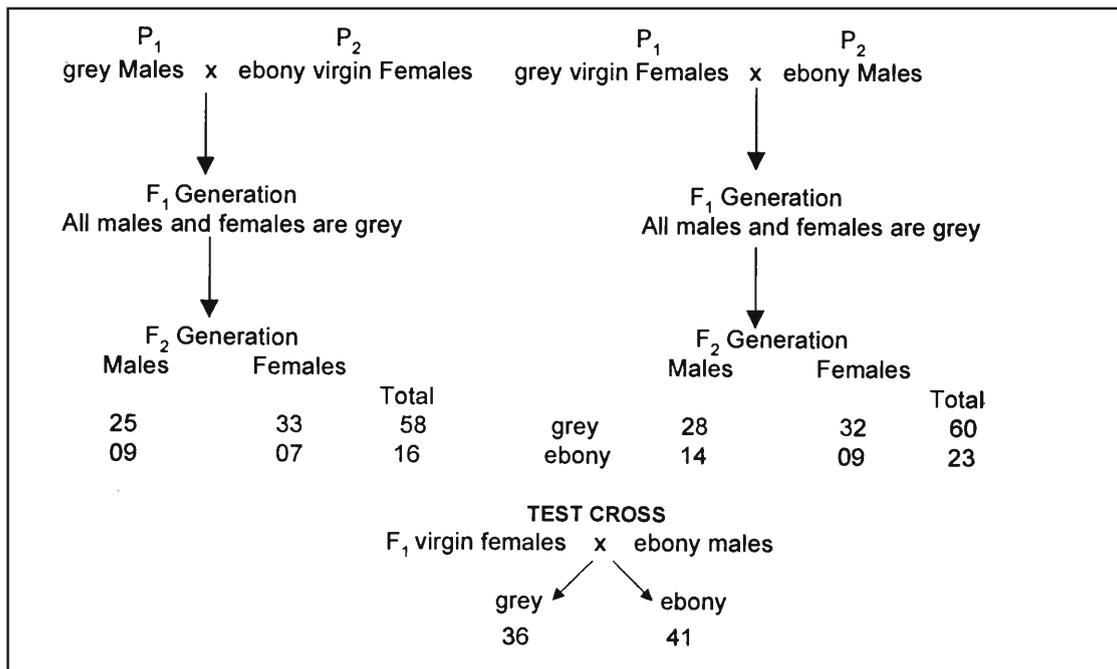
TEST CROSS

A cross between F₁ males / F₁ virgin females with the virgin female or a male of the recessive parent. Analyse the progeny of this cross.

The data has to be subjected to an appropriate statistical test and then the results interpreted (Box 4).

appeared roughly in a ratio of 3:1. This is what is expected if the character is controlled by one gene (Monohybrid ratio). This also illustrates Mendel's Law of Segregation, which means that the genes/alleles present in the F₁ will not blend/contaminate/influence one another. Rather, they segregate in the same pure form in which they have arrived from the parents.

d) The progeny of the test cross includes two types of individuals, namely grey and ebony in almost equal proportion. The F₁ heterozygotes have e^+/e alleles, while the ebony parent has e/e alleles. The alleles present in the F₁ heterozygote do not affect each other and separate pure and uncontaminated into different gametes. One of the gametes will have ' e^+ ' allele while the other



carries 'e' allele. These gametes along with a gamete of the ebony parent which brings 'e' allele, results in grey (e^+/e) or ebony (e/e) individuals in almost 1:1 frequency.

e) There is no difference between the results of the reciprocal crosses, and this suggests that the gene 'e' is located on one of the autosomes (compare this with the results of the *Experiment 3*).

Therefore, the message of this experiment is that the ebony phenotype is due to a single recessive gene (e) and it is present on one of the autosomes. The genotype of the P_1 parent is ' e^+/e^+ ' while that of P_2 is ' e/e ', and that of F_1 progeny is ' e^+/e '. *D. melanogaster* has three pairs of autosomes. To decide which one of the three autosomes has it, additional experiments have to be conducted which we will take up later on. As per the information already available for ebony, the 'e' gene is located on the chromosome 3 of *D. melanogaster*.

Experiment 2. Now let us take a mutant fly with two mutant characters and study their pattern of inheritance.

The aim here is to understand the pattern of inheritance of

Box 3. The Importance of Virgin Females.

One of the advantages of the *Drosophila* system is the possibility of making controlled mating experiments. The females of one genetically defined strain have to be mated with the males of another genetically defined strain. The entire progeny of this female have to be due to the sperms of the male parent which is involved in the experiment. In such experiments, the females have to be virgins. That means, these females should not have been mated with the males of their own strain. Therefore, care has to be taken to isolate the newly eclosed females before they are mated by the males present in the stock. In *D. melanogaster* the copulation takes place normally after five to six hours of adult eclosion from pupae. The females isolated within five hours after eclosion, are kept as such for five days, during which period they do lay unfertilised eggs. Since these eggs are not fertilised due to lack of sperms, larvae will not be seen in the vials. If larvae appear, then such females are not virgin and have to be discarded. When such tested virgin females of one genetic stock are crossed with the males of the other genetic stock, the progeny is a hybrid of two such strains. The genotype of the F_1 has to be due to the contributions of these genetically different parents.

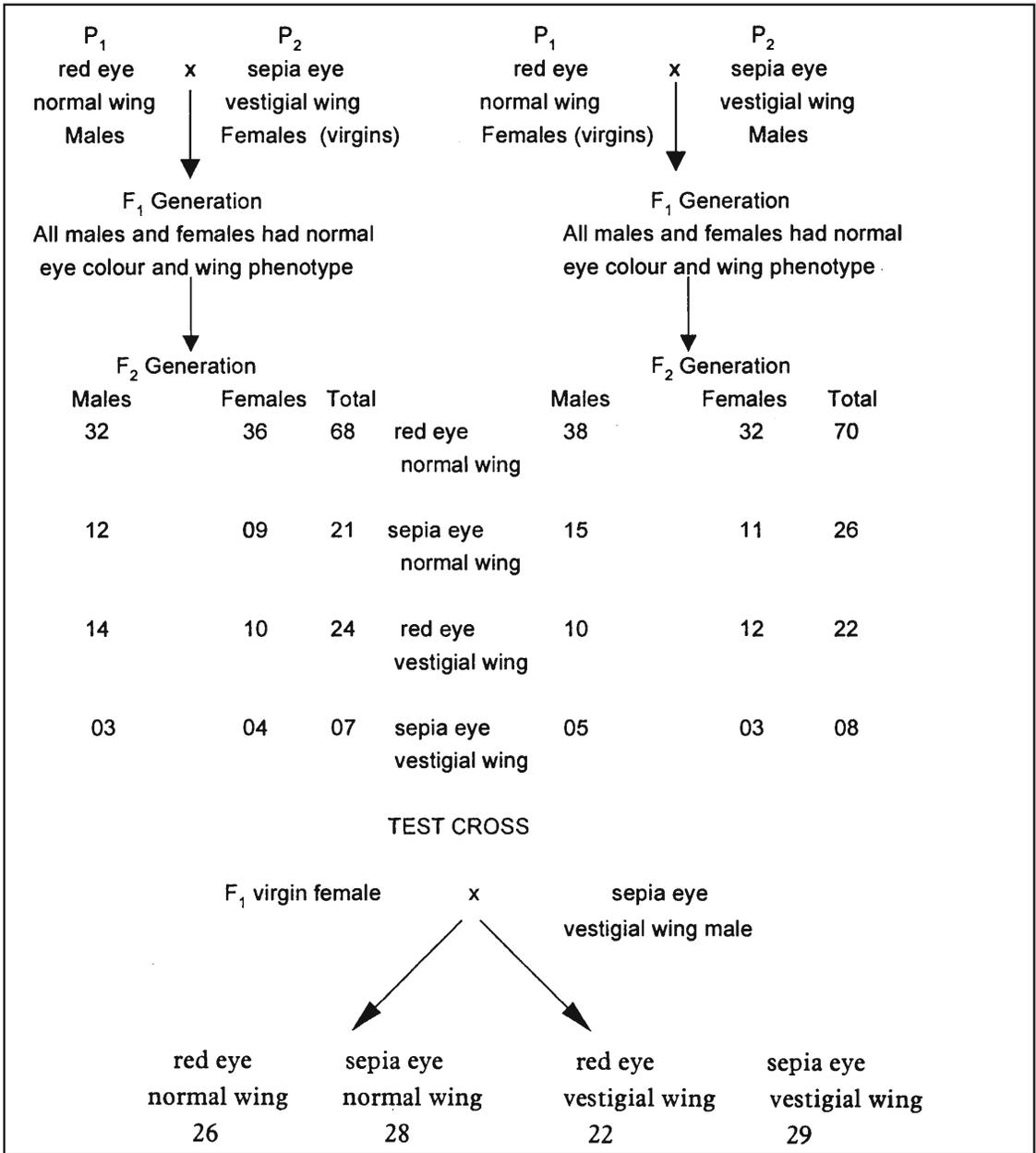
‘sepia eye colour’ (blackish) and ‘vestigial wing’ (highly reduced) in *D. melanogaster*. Normally, *D. melanogaster* has red eyes and completely developed wings. As per the instructions given in the Boxes 2–4, this experiment was conducted and the results are as on page 83.

Analysis of Results:

a) The gene(s) for sepia eye colour and vestigial wing type are recessive to their normal allele (Law of Dominance).

Box 4. Statistical Testing

The phenotypic ratios recorded in all the crosses have to be subjected to an appropriate statistical test. The purpose is to test whether the observed ratio fits into the expected ratios calculated based on a hypothesis. For instance, if a character is controlled by one gene, based on the Mendel’s law of segregation and monohybrid ratio, the expected ratio among the F_2 generation is 3 normal phenotype: 1 mutant phenotype. Virtually, in every experiment, there will be a difference between the expected and the observed ratios. For instance the Chi-square (χ^2) test will help us to know whether the difference between the observed and the expected ratios is statistically significant or not. If the difference is not significant, it means the deviation is small enough to be ignored as being due to chance alone. Then it can be said that the experimental data fits into the hypothesis based on which the expected ratios were calculated. If the difference is significant, one of the possibilities is that it could be due to an error in the experiment, and to check this it is better to repeat the experiment. Even after repeating the experiment, if there is no change in the result, it suggests that the data do not fit the hypothesis, and the results have to be interpreted in the light of some other hypothesis.



b) The F₁ data do not tell us whether the two mutant features are controlled by the same gene or two or more different genes. For this one has to look into F₂ data.

c) Among the F₂ progeny of both the reciprocal crosses four types of individuals were seen. They are (1) flies with normal

features for eye and wing, (2) flies with sepia eye and normal wing, (3) flies with normal eye and vestigial wing and (4) flies with sepia eye and vestigial wing. These four categories have appeared roughly in a ratio of 9:3:3:1.

d) Among F_2 , if one analyses only one phenotype at a time, it could be seen that flies with normal phenotype and with the mutant phenotype have appeared roughly in a ratio of 3:1 (Monohybrid ratio and Law of Segregation).

e) Parents (P_1 and P_2) as well as F_1 flies had both the eye and the wing phenotypes either in normal form or in mutant form. But in F_2 , in addition to flies with both normal phenotypes or with both mutant phenotypes, flies with one normal and one mutant phenotype have appeared. It means that the mutant phenotypes of the eye and of the wing can be separated. This is possible only when two different genes determine these wing and eye phenotypes.

f) Therefore the genotype of the F_1 individuals with normal phenotype for both eye and wing is $se^+/se\ vg^+/vg$. Hence the genotype of the P_1 is $se^+/se^+\ vg^+/vg^+$ and of P_2 is $se/se\ vg/vg$.

g) The inbreeding of F_1 flies gave four types of flies roughly in a ratio of 9:3:3:1. This is possible only when the two different sets of genes present in F_1 assort independently during meiosis. That is, F_1 with $se^+/se\ vg^+/vg$ genotype, has experienced the formation of four types of gametes in equal proportion. They are, gametes with (1) $se^+\ vg^+$ (2) $se^+\ vg$ (3) $se\ vg^+$ and (4) $se\ vg$ genotypes. Such four types of sperms and four types of eggs meet at random to produce sixteen F_2 individuals which will have a phenotypic ratio of 9:3:3:1. This is referred to as 'Dihybrid ratio' of Mendel. Therefore the present experimental data fits into the 'Law of Independent Assortment' of Mendel which states that two different sets of genes/alleles assort independently of each other during the formation of haploid gametes through meiosis. Since there is no bias or preference to any one type of gamete, four types of gametes are formed in equal proportion. Of course, under some circumstances, the genes for different characters do not assort independently, and we will

The 'Law of Independent Assortment' of Mendel states that two different sets of genes/alleles assort independently of each other during the formation of haploid gametes through meiosis.

look into this in a later experiment.

h) The purity of alleles as well as independent assortment of two sets of alleles present in F_1 heterozygote is once again seen in the progeny of the test cross, where four types of individuals are seen in almost equal proportion.

i) There is no difference between the reciprocal crosses and this suggests that gene(s) of these characters are present on the autosomes (compare this with the results of the *Experiment 3*).

The message of this experiment is that the sepia eye colour and the vestigial wing phenotypes are determined by two different genes and they assort independently. Each one of them is recessive to its normal counterpart and these genes are present on autosomes. In fact we know that the gene 'se' is present on the chromosome 3 while the gene 'vg' is on the chromosome 2 of *D. melanogaster*.

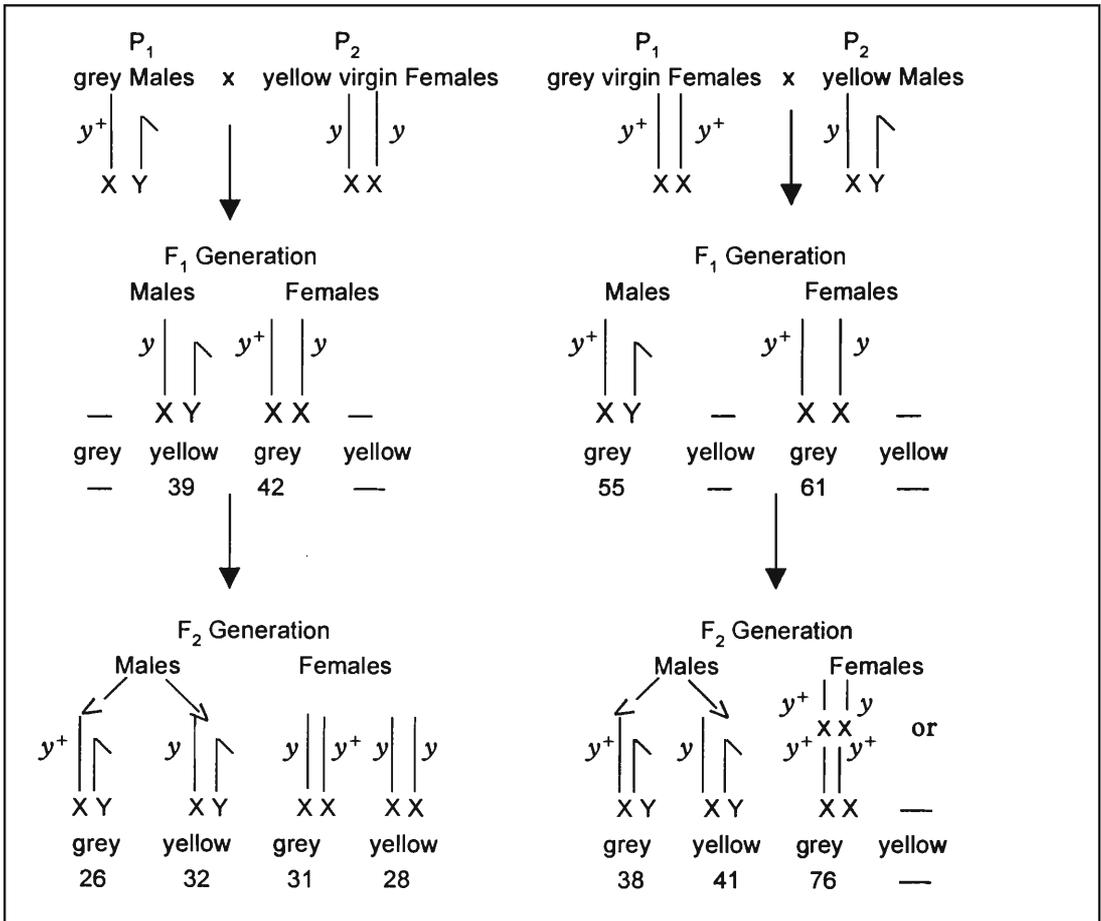
Experiment 3. Aim is to understand the pattern of inheritance of 'yellow body colour' in *D. melanogaster*. The normal body colour of *D. melanogaster* is grey. As per the instructions given in the *Boxes 2 – 4*, this experiment was conducted and the results are as follows: To facilitate an easy analysis, for each individual, the sex chromosomes are shown. For female XX and for males XY chromosomes, along with the symbol of the gene for yellow body that is $y =$ yellow body; $y^+ =$ grey body.

Analysis of Results:

a) The results of the reciprocal crosses differ (compare this with the results of the *Experiments 1 and 2*).

b) Among the F_1 progeny of the cross between grey body males and yellow females, all F_1 males are yellow and all F_1 females are grey. In the F_2 progeny of this, yellow males and females as well as grey males and females appeared in almost equal proportion. In the reciprocal cross, between grey females and yellow males, both males and females of F_1 are grey. Among the F_2 of this cross, all females are grey while fifty percent of males are grey and fifty percent of males are yellow.

The message of this experiment is that the sepia eye colour and the vestigial wing phenotypes are determined by two different genes and they assort independently.



c) This pattern of transmission of character is explained based on the sex-linked pattern of inheritance. It means that the concerned gene for the character under study, that is yellow body, is present on the X-chromosome. This is illustrated in the form of X-chromosomes with the gene for yellow body (y) along with the experimental details shown above.

d) As mentioned in *Box 1*, the Y-chromosome is devoid of alleles of the genes present on the X-chromosome. This is the reason for the differences between the reciprocal crosses.

e) The two types of alleles present in F₁ heterozygote (y^+/y) segregate in pure form to result in grey and yellow body flies among F₂ (Law of Segregation).

Box 5

One of the principles inherent in all the experiments of Mendel is “ the principle of equivalence of reciprocal hybrids”. This means the results of the reciprocal crosses of an experiment will have identical results (Results of *Experiments 1* and 2). Well-established exceptions to this are:

- (1) Traits due to genes on the sex chromosomes (sex linked inheritance, Results of *Experiment 3*),
- (2) Traits controlled by genes present in the cytoplasm (mitochondria and chloroplast genomes) and
- (3) Traits governed by genes which are susceptible to ‘genome imprinting’ (differential expression of a gene depending on whether it has come from the father or the mother).

f) Also it illustrates ‘crisscross pattern of inheritance’, that is, transmission of a gene from male parent to female child and then to male grandchild. This can be seen in the experimental details shown above.

The lesson of this experiment is that the yellow body trait is due to a recessive gene (y) and it is present on the X-chromosome.

Thus, in this part the results of the experiments have been analysed which illustrate the pattern of inheritance of characters determined by genes present on autosomes and on sex chromosomes. Also with such experiments one can demonstrate the principles of Gregor Mendel, namely the Law of Dominance, Law of Segregation and Law of Independent Assortment. These principles, elucidated in the mid-nineteenth century laid the foundations of genetics as a science. In the next part, we will examine characters, which follow other patterns of inheritance in *D. melanogaster*.

Suggested Reading

- [1] D L Lindsley and G G Zimm, *The Genome of Drosophila melanogaster*, Academic Press, San Diego, USA, 1992.
- [2] H A Ranganath, Teaching and Learning Genetics with *Drosophila*, 1. *Drosophila* as a model system, *Resonance*, Vol.4, No. 2, 48-52 1999.
- [3] H A Ranganath and M T Tanuja, *Teaching and Learning Genetics with Drosophila*, 2. Mutant phenotypes of *Drosophila melanogaster*, *Resonance*, 4, No. 9, 95-104, 1999.
- [4] M W Strickberger, *Experiments in Genetics with Drosophila*, John Wiley and Sons Inc., New York, London, Sydney, 1962.