another rather subtle mechanism that should protect the retina against the star light. This has to do with the irregular but rapid involuntary movements of the eye, about 30-70 times a second, over small angular excursions of about 20 arc seconds >> angular diameter of even the nearest star. The resulting motional averaging effectively does the spreading, or the smearing out of the image on the retina that now protects it from any fixated over-exposure. This is perhaps much more effective than the diffraction spreading discussed above. But, for an eye, immobilized against this movement, the diffractive smearing out is about the only protection that it has. The atmospheric refraction (twinkling) should help, but the cosmonauts obviously didn't have this protection, and they were none the worse for it.

Teaching and Learning Genetics with *Drosophila*

4. Pattern of Inheritance of Characters when there is Interaction of Genes or Linkage of Genes

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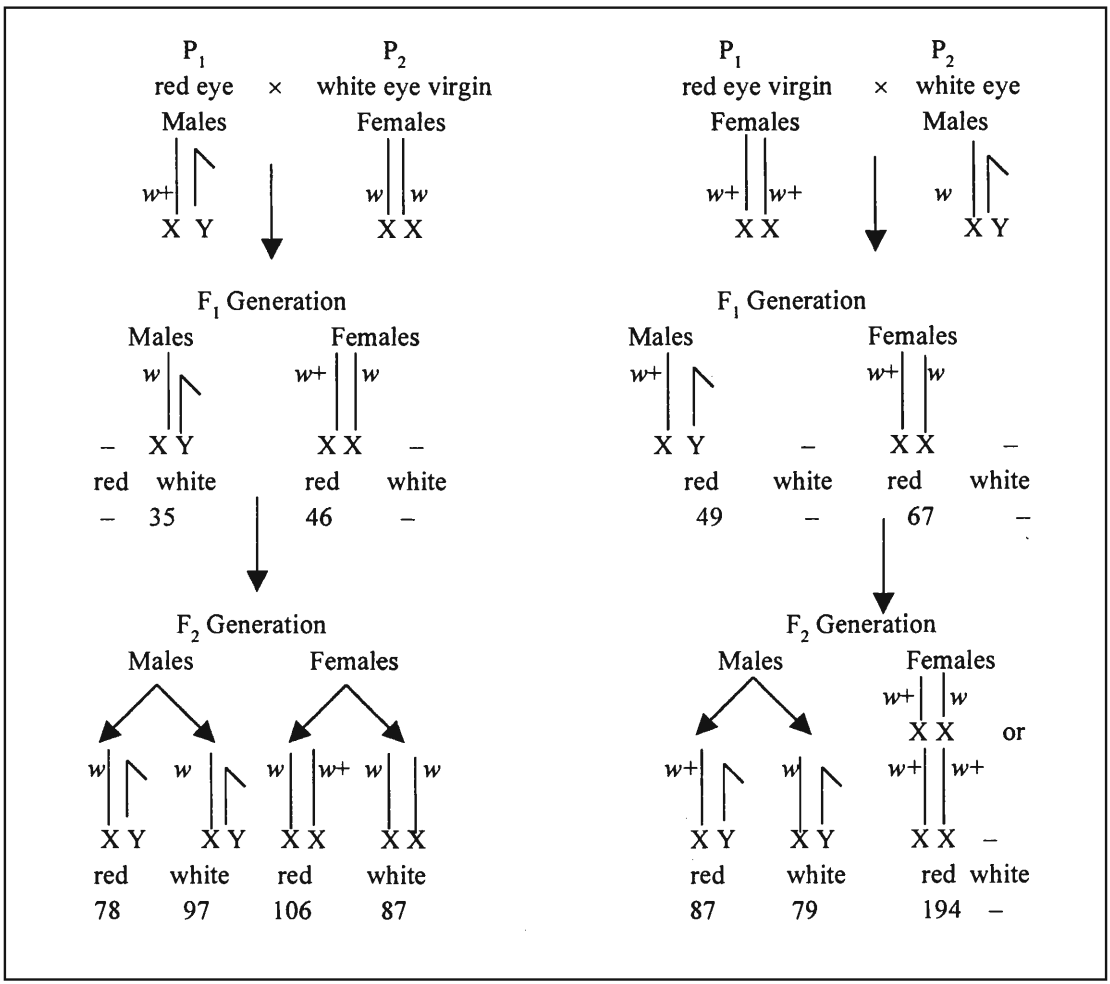
The normal (wild type) eye color in *Drosophila* is red. In Part 2 of this series, we described some mutants in which the colour is variable – such as white, brown, scarlet, etc. We also discussed that some of these eye colour phenotypes are due to genes present on different chromosomes. Now let us study the inheritance of eye colour in *D. melanogaster* in more detail. All the experiments discussed in this article are based on the general protocol that was discussed in Part 3 of the series. In that article, we described three simple experiments aimed at elucidating the pattern of inheritance of traits controlled by unlinked autosomal genes and by genes located on the X chromosome. The experiments described here are aimed at analyzing more complex situations where different genes interact to produce a particular phenotype, or when genes are linked (i.e. they are located near each other on the same chromosome, such that their transmission in gametes is not entirely independent).

Part 1. Drosophila as a model system, *Resonance*, Vol.4, No.2, p.48-52, 1999.
Part 2. Mutant phenotypes of *Drosophila melanogaster*, *Resonance*, Vol.4, No.9, p.95-104,1999.
Part 3. Pattern of inheritance of autosome and sex chromosome linked genes/characters, *Resonance*, Vol.4, No.10,p.78-87, 1999.

EXPERIMENT 4a: The aim here is to investigate the pattern of inheritance of white eye colour in *Drosophila melanogaster* in greater detail. The experiment is executed as per the instructions given in *Boxes* 2, 3 and 4 (Experimental protocol, The importance of virgin females and Statistical testing), of our previous article of this series.

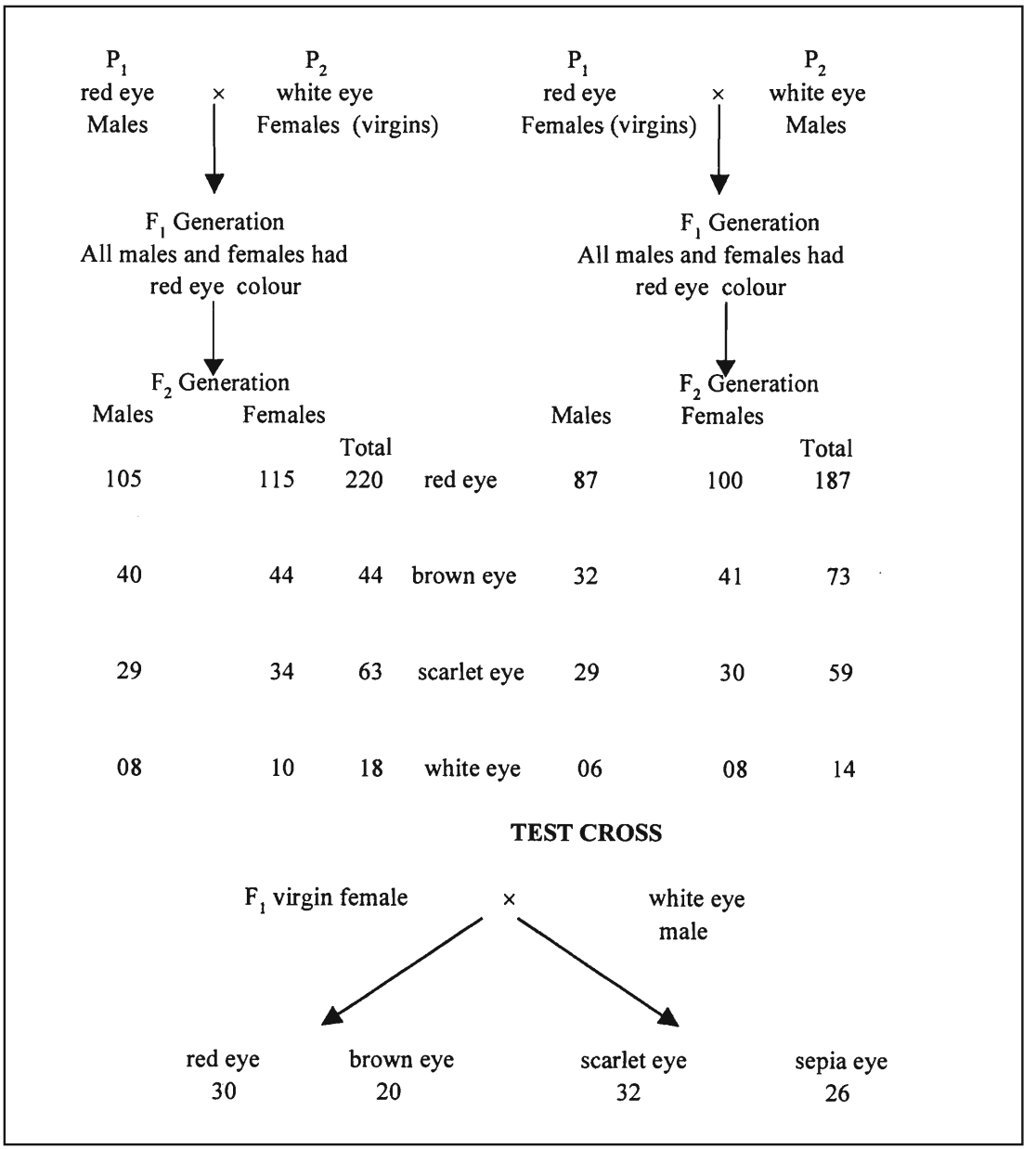
The pattern of inheritance of white eye colour in this experiment is identical to that of the inheritance of yellow body colour, which was presented in experiment 3 of our previous article. Therefore, we conclude that the white eye is due to a gene present on the X chromosome (w) and it is recessive to the normal red eye colour allele (w^+).



EXPERIMENT 4b: Now we look at the data from another experiment that was performed with a different stock of *D. melanogaster*, which also had white eyes.

Analysis of Results:

a) Since the results of the reciprocal crosses are identical, it can



be inferred that the white eye colour of the stock used in this experiment is due to a gene present on one of the autosomes, and is thus different from the *w* allele in the stock used in experiment 4a.

b) White eye is recessive to the normal (Law of dominance).

c) Since the F_1 data do not tell us about the number of genes involved in determining eye colour in this stock, the F_2 progeny of both the reciprocal crosses were examined. Four types of eye colour were seen among the F_2 progeny. They are red, brown, scarlet and white. Of these four types, flies with brown and scarlet colour are new, since they are not seen in F_1 or P_1 or P_2. These four types of flies have appeared roughly in a ratio of 9:3:3:1. This suggests that two different genes, namely brown (*bw*) and scarlet (*st*) are involved and also that they have assorted independently during gametogenesis of F_1, which has resulted in the dihybrid ratio among F_2 (Law of independent assortment of Mendel. Compare this with the results of experiment 2 of our previous article).

d) Therefore, we conclude that the genotype of the F_1 (red) is *bw*/*bw*$^+$ *st*/*st*$^+$, of P_1 (red) is *bw*$^+$/*bw*$^+$ *st*$^+$/*st*$^+$ and of P_2 (white) and F_2 white flies is *bw*/*bw* *st*/*st*. Similarly, the F_2's with brown eye will have *bw*/*bw* *st*/*st*$^+$ and scarlet flies will have *bw*$^+$/*bw* *st*/*st* genotypes.

e) Additional information: In *Drosophila*, the red eye phenotype is due to coloured pigments namely drosopterin and xanthomatin. The blend of these pigments results in the characteristic red colour. The recessive gene *bw* on chromosome 2, when in homozygous state, prevents the synthesis of drosopterin. Similarly, another recessive gene *st* on chromosome 3, when in homozygous condition blocks the production of xanthomatin. If xanthomatin alone is present it gives brown eye while drosopterin alone gives scarlet eye. None of these pigments are present in white eye. The *w* gene on X-chromosome, homozygous in females and hemizygous males will not allow production of any of these pigments by the genes present on the autosomes.

	Chromosomes and Genes			
	I	II	III	
Genotype	w^+ w^+	bw^+ bw^+	st^+ st^+ = Red	Phenotype
	w^+ w	bw^+ bw	st^+ st = Red	
	w^+ w^+	bw bw	st st = White	
	w w	bw^+ bw^+	st^+ st^+ = White	

This situation, where the phenotype of a trait depends upon the genotype at several different loci is called epistasis.

Thus the results of the experiments 4a and 4b on white eye in *Drosophila* help us to demonstrate the law of segregation, the law of independent assortment, sex-linked inheritance as well as interaction of genes particularly the suppression of the expression of non-allelic genes by the *w* allele.

As an extension of these experiments related to the inheritance of white eye in *D. melanogaster*, we suggest doing the following two experiments (1) Normal red eye fly crossed to white eye fly: here white eye is due to the homozygous state of *brown* (*bw*) and *cinnabar* (*cn*) genes and they are on second chromosome; (2) Normal red eye fly is crossed to white eye fly: genotype of this white eye fly is homozygous for *white* (*w*) gene on the X-chromosome (hemizygous in males) and homozygous for *sepia* (*se*) gene on chromosome 3. The analysis of the F_1 and the F_2 data of these crosses would provide more information on genetic control of white eye in *D. melanogaster* and serve to exemplify many phenomena in classical genetics.

EXPERIMENT 5: Genes on different chromosomes (non-homologous) are distributed into gametes independently of one another (Mendel's law of Independent Assortment; experiment 2 of our previous article). *D. melanogaster* has only four pairs of chromosomes but it has over 4000 genes. Since these genes are distributed on these chromosomes, clearly each chromosome has to accommodate many genes in it. Now the question is, what is the pattern of meiotic assortment of genes which lie on the same chromosome?

To address this question, let us examine the pattern of inheritance of three different mutant phenotypes, namely thread like arista, curled wing and stripes on the thorax in *D. melanogaster*. The experiment is conducted as per the instructions given in *Boxes* 2 to 4 of our previous article of this series. Particularly for this experiment, the data from test crosses will be discussed in detail.

P_1 normal arista, normal wing normal thorax Males × P_2 thread arista curled wing stripes on thorax Females (virgins)

F_1 Generation
All males and females had normal arista, wing & thorax

P_1 normal arista normal wing normal thorax Females (virgins) × P_2 thread arista curled wing stripes on thorax Males

F_1 Generation
All males and females had normal arista, wing & thorax

TEST CROSSES

F_1 Males × thread arista curled wing stripes on thorax Females (virgins)

F_1 Females (virgins) × thread arista curled wing stripes on thorax Males

Number of flies	Phenotype of flies	Number of flies
180	normal arista, normal wing, normal thorax	252
196	thread arista, curled wing, striped thorax	232
—	thread arista, normal wing, normal thorax	28
—	normal arista, curled wing, striped thorax	11
—	normal arista, normal wing, striped thorax	32
—	thread arista, curled wing, normal thorax	40
—	thread arista, normal wing, striped thorax	3
—	normal arista, curled wing, normal thorax	2
376	Total	600

Analysis of Results:

a) The F_1 flies of both the crosses showed normal phenotypes. Therefore, the gene(s) for thread arista, curled wing and striped thorax are recessive to normal allele (law of dominance).

b) Since there is no difference between the reciprocal crosses, it can be inferred that the gene(s) controlling these characters are not sex linked, that is, they are not on the X-chromosome.

c) The data of F_1 do not tell us whether these three phenotypes are controlled by one gene or two genes or three different genes. Therefore, to resolve this, one has to study the expression of these characters in the flies of the next generation. This could be F_2 or the progeny of the test cross.

d) Only two types of individuals were produced in almost equal proportion in the test cross where F_1 males were mated with virgin mutant females (P_2). The phenotype of this test cross progeny resemble either of P_1 parent with normal features or of P_2 with mutant features. Thus, in this test cross, only flies with parental combination of characters were seen. In this progeny the three different mutant phenotypes are always together and hence it gives us an impression that the same gene is controlling all these three characters. Before drawing any conclusion let us examine the progeny of the other test cross.

e) The test cross involving F_1 females and mutant males (P_2), yielded 8 types of individuals. The frequencies of these eight varieties do not fit into the expected ratios of the test cross, that is, equal proportion of different categories. Thus the result of this test cross is different from that of the other which gave only two types of individuals and also it deviates from the test cross ratios expected under the law of independent assortment. Now let us examine the phenotypes and the frequencies of these eight types of flies.

f) Of these 8 types, two types of flies, resembling either P_1 or P_2 features are much more frequent than others. The remaining 6

types of flies differ from both the parents and show new combinations for the three characters under analysis. In these six new varieties, one can see that the three mutant characters, namely thread arista, curled wing and striped thorax are separable from one another and they are not always found in the same fly. This is a very clear indication that three different genes determine these three characters of the fly. Therefore there is contradiction between the reciprocal test crosses and at a later stage we will discuss this.

f) Each of these three characters namely thread arista, curled wing and striped thorax are controlled by different genes and are recessive to their corresponding normal alleles. With this, the genes for these characters could be represented as *th*, *cu* and *sr* for mutants and for respective normal character as th^+, cu^+ and sr^+.

g) Since these are different genes, the next question is about their behaviour during gametogenesis, whether they assort independently or not. Among the eight categories of test cross progeny, the flies with normal features and the flies with mutant characters, for arista, wing and thorax, virtually outnumber any other category of flies which have shown combinations of both normal and mutant characters. These two highly frequent types of flies are similar to the phenotypes of either P_1 or P_2 of this experiment. Therefore, among test cross progeny, the frequencies of individuals with parental combinations of characters are more in number than any other combination. This suggests that in the test cross progeny, these three genes tend to stay in the same combination in which they were present in P_1 or P_2 and this has resulted in high incidence of parental combinations in the test cross. Therefore one can say that these three genes have not assorted independently. This phenomenon is called *linkage of genes*. Genes present on a chromosome are physically linked to one another, and each chromosome, thus, represents a linkage group. Genes present in a linkage group do not assort independently. Therefore the law of independent assortment is not applicable to genes of a linkage group while genes of different linkage groups do assort independently. In *D. melanogaster,*

these genes namely *th, cu*, and *sr* belong to the third linkage group. *D. melanogaster* with four pairs of chromosomes has four linkage groups while man has twenty three linkage groups.

h) The next issue is to account for the appearance of six new combinations of characters in the test cross progeny. These flies differ from both the parents namely P_1 and P_2 in having different combinations of characters. The phenotypes of these flies indicate that the linkage of genes is not absolute, instead there is a possibility to break this linkage and to separate the linked genes and this results in flies having characters which are different from their parents. This is due to an event called *crossing over* and this occurs between non-sister chromatids of a pair of homologous chromosomes during meiosis (gametogenesis). With this process, now one can explain the appearance of new combinations. For this, one has to examine the possibilities of different types of gametes that can be formed in the parents of the progeny of the test cross, that is, F_1 female and the P_2 recessive male parent. The genetic constitution (genotype) of the F_1 female is $th^+ cu^+ sr^+ / th\ cu\ sr$ (heterozygous) while that of the P_2 recessive male parent is *th cu sr / th cu sr* (homozygous). The heterozygous female can yield 8 types of gametes while the homozygous male can produce one type of gamete and the fusion of these male and female gametes can result in 8 types of individuals as shown in the table on page 68.

This is how 8 types of individuals are seen in the test cross progeny. Of these, two are of parental types while six are new, and these are the products of the crossover event between the three genes. The genes, *th cu sr* are linked and therefore do not assort independently. But due to crossover, (meiotic recombination), the members of this linkage group can be separated from one another, but frequency of the process of crossing over is extremely low, which is reflected in the low frequency of each of the six new types, which are the products of recombination.

j) From this data is it possible to know the sequence in which these three genes are placed on the chromosome? Is it possible to

Female gametes:	The Male gamete has *th cu sr*. The fusion of this male gamete and the female gamete results in the following :	No. of individuals observed
I Noncrossovers:		
1. $th^+\ cu^+\ sr^+$	$th^+\ cu^+\ sr^+/\ th\ cu\ sr$ = normal arista, normal wing, normal thorax	252
2. *th cu sr*	*th cu sr / th cu sr* = thread arista, curled wing, striped thorax	232
II Crossovers between *th* and *cu* **(Single crossover)**		
3. $th^+\ cu\ sr$	$th^+\ cu\ sr/\ th\ cu\ sr$ = normal arista, curled wing, striped thorax	11
4. *th* cu^+ sr^+	*th* cu^+ sr^+ / *th cu sr* = thread arista, normal wing, normal thorax	28
III Crossovers between *cu* and *sr* **(Single crossover)**		
5. $th^+\ cu^+\ sr$	$th^+\ cu^+\ sr$ / *th cu sr* = normal arista, normal wing, striped thorax	32
6. *th cu* sr^+	*th cu* sr^+ / *th cu sr* = thread arista, curled wing, normal thorax	40
IV Crossovers between *th* and *cu* and also between *cu* and *sr* **(Double crossover)**		
7. $th^+\ cu\ sr^+$	$th^+\ cu\ sr^+/\ th\ cu\ sr$ = normal arista, curled wing, normal thorax	03
8. *th* cu^+ *sr*	*th* cu^+ *sr / th cu sr* = thread arista, normal wing, striped thorax	02

measure the distance between any two genes? The answer is yes. For this, one has to look at the incidence of single and double crossover events which is reflected in the frequencies of six new types of individuals. If the genes are linked then the distance between them can be calculated. For this, the frequency of recombination between the three gene loci must be ascertained by determining the percentage of recombination between any two genes. The frequency of crossing over giving rise to recombination of linked genes is a function of the distance between these genes on the chromosome. To find the distance between two genes, we must count all crossovers (both single and double). So, we must first decide the order of the genes on the chromosome. Since the $th^+\ cu\ sr^+$ and *th* cu^+ *sr* individuals are the least frequent, we expect them to be resulting from double crossover events (since one crossover in a given stretch of a chromosome is more likely to occur than two crossovers in the same region.) Sketch out different arrangements of genes on a paper and you can soon see that if these two groups are double

crossovers, the order of genes must be *th-cu-sr*. From the present data on *th cu sr* genes, the following calculation is possible.

a) Crossover between *th* and *cu* = 28 + 11 + 5 = 44

$\therefore$ the frequency of recombination is $= \frac{44 \times 100}{600} = 7.3\,\%$

(here 28+11=39 individuals result from a single crossover between *th* and *cu,* and the 5 double cross over individuals also have to be counted because they too underwent a crossover between *th* and *cu.*

b) Crossover between *cu* and *sr* = 32 + 40 + 5 = 72

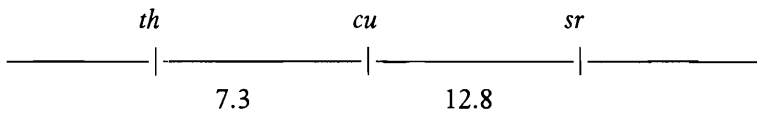
$\therefore$ the frequency of recombination is $= \frac{72 \times 100}{600} = 12.8\,\%.$

c) Crossover between *th* and *sr* = 44 + 77 = 121

$\therefore$ the frequency of recombination is $= \frac{121 \times 100}{600} = 20.3\,\%.$

These recombination values give the map distance and thus the relative distance and positions of these genes. One unit of map distance is equivalent to 1% of crossover. These map units are called 'centimorgans'. Therefore, the distance between *th* and *cu* is 7.3 units, and that between *cu* and *sr* is 12.8 units.

Based on this data, the three gene loci on the linkage map will be



k) The frequency of double crossovers in the present experiment is just 5 out of 600, that is .83%. Often the incidence of double crossover is less than expected due to a phenomenon called *interference*. Interference occurs because an event of crossing over reduces the probability of another crossover event in an adjacent region of the chromosome. The complement of interference is called coincidence and the ratio between the

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*, Vol. 4, No. 9, 95-104, 1999.

[4] H A Ranganath and M T Tanuja, Teaching and Learning Genetics with *Drosophila*, 2. Pattern of Inheritance of Autosome and Sex Chromosome Linked Genes/Characters, *Resonance*, Vol. 4, No.10, 78-87, 1999.

[5] M W Strickberger, Experiments in Genetics with *Drosophila*, John Wiley and Sons Inc., New York, London, Sydney, 1962.

observed and the expected double crossover events is referred to as *coefficient of coincidence*. When the interference is complete (1.0), no double crossovers are seen, and coefficient of coincidence is zero. If all the expected double crossovers are seen, coincidence is 1.0 and interference becomes zero.

For the present experiment, with the map distances between *th* and *cu*, and *cu* and *sr* as 7.3 and 12.8 units, respectively, the expected double crossover is $0.073 \times 0.128 = .00934$ or 0.93%, if there is no interference. The observed frequency of double crossover here is just 0.83%. Hence the coincidence 0.83/0.93 = 0.89 and interference is $1 - 0.89 = .11$. This means that 89% of the expected double crossovers have occurred and 11% of the double crossovers did not occur due to interference.

l) Let us now go back to the progeny of the test cross between F_1 male and recessive female (P_2), which gave only 2 types of individuals that showed parental phenotypes and no recombinants. This suggests the heterozygous male has produced only two types of sperms with th^+ cu^+ sr^+ or *th cu sr*. The fusion of these eggs and sperms with *th cu sr* eggs thus yield only 2 types of individuals. In other words, the F_1 male which is heterozygous (th^+ cu^+ sr^+ / *th cu sr*) like the F_1 females, instead of producing 8 types of gametes like the latter, has yielded only two types of sperms. This is because meiotic recombination does not occur in *D. melanogaster* and therefore, the meiosis in males is called achiasmatic.

The message of this experiment is that linked genes do not assort independently; with the data from the test cross one can organise a *linkage map* of the genes involved as to their relative positions and distances.

As we have seen now, every mutant is in a position to offer different types of messages and patterns of inheritance. In the next article we will continue our experiments with other mutants and learn more about inheritance.