Classroom

In this section of *Resonance*, we invite readers to pose questions likely to be raised in a classroom situation. We may suggest strategies for dealing with them, or invite responses, or both. "Classroom" is equally a forum for raising broader issues and sharing personal experiences and viewpoints on matters related to teaching and learning science.

Teaching and Learning Genetics with *Drosophila*
5. Lessons from the Experiments with Curly and Bristle Mutant Stocks*

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Even today, *Drosophila* remains as one of the potent eukaryotic systems to study different dimensions of inheritance. It provides a wide spectrum of genetic resource of mutants which are of immense help to look into the effects of different types of mutations as well the mechanisms underlying their maintenance. Experiments involving lethal mutations in *Drosophila* have helped us to realize a few more genetic lessons, and the same is discussed in this communication.

Previously, I had introduced *Drosophila* as a potential model to conduct experiments, followed by a description of a few mutant stocks (*Resonance*, Vol.4, No.2, pp.48–52, 1999; Vol.4, No.9, 95–104, 1999). In subsequent articles, based on the patterns of inheritance of a few phenotypes, I had presented experiments to demonstrate the laws of segregation and independent assortment, autosomal and sex-linked inheritance, as well as the interaction of genes and linkage (*Resonance*, Vol.4, No.10, pp.78–87, 1999; *Resonance*, Vol.5, No.7, pp.59–70, 2000.). Continuing

Keywords
Drosophila, the Queen of Genetics, is one of the most suitable model systems for laboratory exercises to teach and learn not only genetics but also many concepts in biology.

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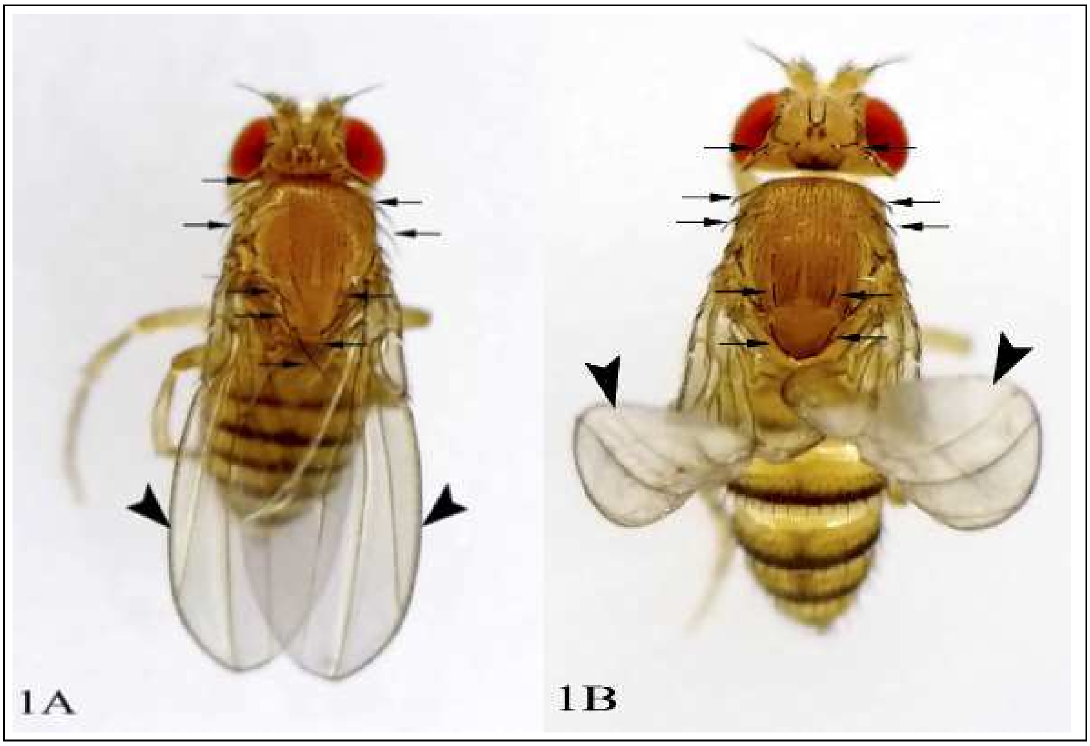

Figure 1. *D. melanogaster*: **A** with normal wings and normal bristles; **B** with curly wings and mutant bristles. Arrowhead and arrows point wings and bristles respectively. (Photo courtesy: Dr Gurudatta Baraka and Sri. Shreekrishna, Centre for Human Genetics).

along these lines, I describe an experiment here to understand how dominant lethal mutations can be maintained with the help of 'Balancer Chromosomes'.

Strains of *D. melanogaster* Used in the Experiment

D. melanogaster, a dipteran insect has two pairs of wings. Of these, the front pair is attached to the second thoracic segment and is completely developed. These flattened wings, when not being involved in flight, are placed on the abdomen. The hind wings, on the third thoracic segment, are reduced and transformed into balancers or halteres. Long, tapering, pointed bristles are distributed all over the body of *Drosophila* (*Figure* 1A). The number and pattern of distribution of bristles are of taxonomic importance.

In a mutant strain of *D. melanogaster*, the front pair of wings are curved upwards and forwards (*Figure* 1B), a phenotype referred to as ‘Curly Wings’ (*Cy*). Unlike the wild type with long, tapering bristles, a mutant strain has bristles that are thick and reduced in size. This phenotype (*Figure* 1B; *Box* 1) is named ‘Bristles’ (*Bl*).

The males and females of the strain used in the present experiment had ‘Curly Bristle’ phenotypes. It is a true-breeding stock, meaning that only these phenotypes appear in the progeny, generation after generation. To breed true, a first assumption would be that the stock has to be homozygous for the said mutant alleles. Let us examine this by analysing the results of the following experiment.

Experiment: 1

(Results presented here are from the experiment done by post-graduate students)

Reciprocal crosses are carried out between *D. melanogaster* Oregon K strain and Curly Bristle mutant strain. We would like to find out the genotypes of these strains with regard to these phenotypes. We find, upon crossing them, that two types of progeny are obtained in roughly equal numbers from each cross, as shown in the *Table* 1a.

Analysis of mutations has been the guiding principle of genetic analysis of any biological process during 20th century and it is more so today. What is the source for the information on mutations in *Drosophila*? FlyBase, (flybase.org) a Database of Drosophila Genes & Genomes, a boon to *Drosophila* geneticists, provides up to date information on many aspects related to *Drosophila*, particularly more so with a catalogue of detailed description of different mutations.

F_1 Progeny: Results and Interpretations

(Results presented here are from the experiment done by post-graduate students.)

a) The wing and bristle mutant phenotypes have appeared in separate individuals, in contrast to the mutant parents, in which both mutant phenotypes were in same individuals. What does it mean? A simple interpretation would be that the wing and bristle phenotypes are due to two different genes, which were together in the mutant parents, but are separated in the F_1 progeny.

b) Among the F_1 progeny, flies with normal wings and flies with curly wings have appeared, suggesting that all the individuals of the mutant parents carried an allele for the curly wing and another allele for the normal wing. They were heterozygous for these alleles. However, since all the mutant parents had curly wings, it can be inferred that the allele for the curly wings is dominant

Parents : *D. melanogaster* Oregon K strain and Curly Bristle mutant strain (*Genotypes of these not known; To be deduced after the analysis of results*) Reciprocal crosses	
Curly Bristle virgin Females X Oregon K Normal Males ↓	Curly Bristle males X Oregon K strain virgin Females ↓
2 types almost in equal numbers F_1 Progeny	2 types almost in equal numbers
a) Males and females are with Curly wings & normal bristles (Genotype?) AND b) Males and females with normal wings and Bristle phenotypes (Genotype ?)	a) Males and females are with Curly wings & normal bristles (Genotype?) AND b) Males and females with normal wings and Bristle phenotypes (Genotype ?)

Table 1a: Description of the cross and the phenotypes of the parental flies and of F_1 progeny.

over the allele for the normal wings. We can use the notation of the alleles *Cy* for the curly and Cy^+ for the normal wings. Hence, the heterozygous genotype will be $Cy//Cy^+$.

A similar argument can be extended to the bristle phenotype. It can be inferred that the mutant parents were heterozygous, with an allele for normal bristles and another allele for mutant bristles. As in the case of curly phenotype, the mutant allele is dominant (*Bl*) over the normal allele (Bl^+). The genotype of the mutant parents will be $Bl//Bl^+$.

c) The results of the reciprocal crosses are almost identical. Therefore, the genes controlling curly wings and bristle phenotypes in the present experiment are located on one of the autosomes of *Drosophila*.

d) Two different genes are involved—one for curly wings and another for bristles. Further, mutant parents are heterozygotes for each of these characters. How are they arranged? What are the possibilities? One possibility is that they may be on different non-homologous chromosomes/different linkage groups enabling independent assortment. If so, among F_1 progeny, in addition to flies with normal wings and mutant bristles; and flies with curly wings and normal bristles, two more categories of flies, namely, flies with both the mutant features and also flies with normal phenotypes for both characters are expected. But such flies have not appeared among F_1. Therefore, the possibility of the concerned genes' presence on different linkage groups is ruled out.

e) The other possibility is, the genes concerned with these characters are on the same pair of homologous chromosomes and, therefore, belong to the same linkage group. As discussed above, each of the mutant parental individuals carries a dominant as well as a recessive allele, that is, heterozygous, $Cy//Cy^+$ and $Bl//Bl^+$. Here also, the genotype of the mutant parents can be arranged in two ways: (1) cis-arrangement: wherein both the dominant alleles are on the same chromosome, while the recessive alleles are on the other homologous chromosome, i.e. the genotype is $CyBl//Cy^+Bl^+$; (2) trans-arrangement: that is, in a pair of homologous chromosomes, each chromosome carries a dominant allele and a recessive allele for the phenotypes in question, so that the genotype is $Cy^+Bl//CyBl^+$. Whether it is cis or trans, the phenotype of the fly will be curly bristle. Therefore, whether the curly bristle phenotype comes from a cis or trans arrangement of the alleles is the question.

The composition of the F_1 progeny helps us to resolve this issue. If it is in cis-arrangement, among F_1 progeny, flies with both the mutant phenotypes (Curly and Bristle) and the flies with normal phenotypes for both (normal wings and normal bristles), that too in almost equal frequency, should have appeared. But it is not so. Therefore, cis-arrangement of these mutant markers is ruled out. On the other hand, each of the F_1 progeny has either curly or bristle phenotype. None of them had both mutant features or had normal phenotypes for both. This is a clear indication for the trans-arrangement of the mutant marker genes.

Thus, from analysing the results of Experiment 1, we can deduce the genotypes of the flies as follows. The genotype of the mutant parents for these characters is $Cy^+Bl//CyBl^+$. The genotype of the Oregon K strain flies for the characters under study is $Cy^+Bl^+//Cy^+Bl^+$. Therefore, the genotype of F_1 flies will be as follows: for curly flies with normal bristles: $CyBl^+//Cy^+Bl^+$; for flies with normal wings and mutant bristles: $Cy^+Bl^+//Cy^+Bl$.

Therefore, we can complete the chart of Experiment 1 with information on genotypes of the parents and of F_1 Progeny *Table* 1b.

<table>
<tr><td colspan="2">Parents : D. melanogaster Oregon K strain and Curly Bristle mutant strain
$Cy^+ Bl^+// Cy^+ Bl^+$ Genotypes $Cy\ Bl^+// Cy^+Bl$
Reciprocal crosses</td></tr>
<tr><td>Curly Bristle virgin Females $Cy\ Bl^+// Cy^+ Bl$
X
Oregon K Males $Cy^+ Bl^+// Cy^+ Bl^+$</td><td>Curly Bristle males $Cy\ Bl^+// Cy^+ Bl$
X
Oregon K virgin Females $Cy^+ Bl^+// Cy^+ Bl^+$</td></tr>
<tr><td colspan="2">↓ F_1 Progeny ↓</td></tr>
<tr><td>a) Males and females are with Curly wings & normal bristles $Cy^+ Bl^+// Cy\ Bl^+$
AND
b) Males and females with Bristle phenotypes and normal wings: $Cy^+ Bl // Cy^+ Bl^+$</td><td>a) Males and females are with Curly wings & normal bristles $Cy^+ Bl^+// Cy\ Bl^+$
AND
b) Males and females with Bristle phenotypes and normal wings: $Cy^+ Bl // Cy^+ Bl^+$</td></tr>
</table>

Table 1b: Description of the crosses with phenotypes and genotypes of the parental flies along with progeny of F_1.

Among the F_1 progeny, the absence of flies with both normal or flies with both mutant characters indicates the absence of meiotic recombination between these maker genes. Since males of *D. melanogaster* have achiasmatic meiosis, the absence of recombination is expected. Chiasma is the point at which crossing over and exchange of genetic material occurs between the strands. Achiasmatic means the absence of chiasma and hence lack of crossing over. But recombination occurs in females. Therefore, the absence of recombinant phenotypes in the F_1 progeny where the female parents were heterozygous, needs explanation. Let us

F_1: Phenotype: Curly wing & normal bristle F_1: Genotype: $Cy\ Bl^{+}//\ Cy^{+}\ Bl^{+}$	X ↓	F_1: Phenotype: Normal wing & mutant bristle F_1: Genotype: $Cy^{+}\ Bl^{+}//\ Cy^{+}\ Bl$
Gametes	$Cy^{+}\ Bl^{+}$	$Cy^{+}\ Bl$
$Cy\ Bl^{+}$	$Cy\ Bl^{+}$ / $Cy^{+}\ Bl^{+}$ } Curly wing, normal bristle n=40	$Cy\ Bl^{+}$ / $Cy^{+}Bl$ } Curly wing, mutant Bristle n=34
$Cy^{+}Bl^{+}$	$Cy^{+}\ Bl^{+}$ / $Cy^{+}\ Bl^{+}$ } Normal wing, normal bristle n=38	$Cy^{+}\ Bl^{+}$ / $Cy^{+}\ Bl$ } Normal wing, mutant Bristle, n=36

Table 2a: Details of the cross between F_1 curly wings & normal bristle flies and normal wings & mutant bristle flies and the phenotypes and the genotypes of the F_2 progeny.

discuss it later as to why recombinant products are not seen in this experiment.

Experiment 2: Inbreeding of F_1 to get F_2 Progeny

Three types of experiments can be done. In the first, flies with curly wings and normal bristles are crossed with flies with normal wings and mutant bristles. The cross and the progeny are shown in the *Table* 2a.

As depicted in the chart, four types of F_2 flies are seen in equal proportion. They are (i) flies with normal phenotypes for wings and bristles; (ii) flies with mutant phenotypes for wings and bristle; (iii) flies with normal wings and with mutant bristles; and (iv) flies with mutant curly wings and normal bristles.

In the second experiment, flies with normal wings and mutant bristles are crossed amongst themselves. The cross and the progeny are shown in *Table* 2b.

Phenotypic ratio: Normal wing and mutant bristle: 69; Normal wing and normal bristle: 32; almost 2:1

All the F_2 flies of this cross have normal wings. Flies with mutant bristles and flies with normal bristles have appeared in a ratio of 2:1 respectively, instead of 3:1, because 25% of flies, which were

F_1: Phenotype: Normal wing and mutant bristle F_1: Genotype: $Cy^+\ Bl^+//Cy^+Bl$	X ↓	F_1: Phenotype: Normal wing and mutant bristle F_1: Genotype: $Cy^+\ Bl^+//Cy^+Bl$
Gametes	$Cy^+\ Bl^+$	Cy^+Bl
$Cy^+\ Bl^+$	$Cy^+\ Bl^+$ / $Cy^+\ Bl^+$ } Normal wing and normal bristle,	$Cy^+\ Bl$ / $Cy^+\ Bl^+$ } Normal wing and mutant bristle,
$Cy^+\ Bl$	Cy^+Bl / $Cy^+\ Bl^+$ } Normal wing, mutant bristle,	Cy^+Bl / $Cy^+\ Bl$ } Lethal, because of Bl homozygosity

Table 2b : Details of the cross between F_1 Normal wing and mutant bristle flies with F_1 Normal wing and mutant bristle flies along with the phenotypes and genotypes of F_2 progeny.

F_1: Phenotype: Curly wing and normal bristle F_1: Genotype: $Cy\ Bl^+//Cy^+Bl^+$	X ↓	F_1: Phenotype: Curly wing and normal bristle F_1: Genotype: $Cy\ Bl^+//Cy^+Bl^+$
Gametes	$Cy\ Bl^+$	Cy^+Bl^+
$Cy\ Bl^+$	$Cy\ Bl^+$ / $Cy\ Bl^+$ } Lethal, because of Cy homozygosity	$Cy^+\ Bl^+$ / $Cy\ Bl^+$ } Curly wing and normal bristle
Cy^+Bl^+	Cy^+Bl^+ / $Cy\ Bl^+$ } Curly wing and normal bristle	$Cy^+\ Bl^+$ / $Cy^+\ Bl^+$ } Normal wing and normal bristle

Table 2c: Details of the cross between F_1 Curly wing and normal bristle flies with F_1 Curly wing and normal bristle flies along with the phenotypes and genotypes of F_2 progeny.

homozygous for Bl/Bl did not survive. Thus Bl/Bl is lethal and Bl has to be maintained in the heterozygous state only.

In the third experiment, flies with curly wings and normal bristles are crossed amongst themselves. The cross and the progeny are shown in *Table* 2c.

Phenotypic ratio: Curly wing and normal bristle: 78; Normal wing and normal bristle: 44; almost 2:1.

As expected, all the F_2 flies have normal bristles. With regard to wing phenotype, two types of individuals are seen. They are flies with curly wings and flies with normal wings, in a ratio of about 2:1 respectively, instead of 3:1. The reason is that 25% of flies, which were homozygous for Cy/Cy did not survive. The message is that Cy/Cy is lethal and *Cy* has to be maintained in the heterozygous state only. It should be noted here that as far as survival is concerned, *Cy* is recessive as all the individuals in homozygous condition die.

Question: Since these lethal genes have to be maintained in heterozygous state only, how do we establish a pure breeding stock for such genes?

It turns out that the mutant strain used in the present set of experiments, namely 'Curly Bristle', is actually a true breeding strain. How?

Let us examine the expected outcome of a cross between males and females of the 'Curly Bristle' strain *Table 3*.

a) An important point to be reiterated is in *D. melanogaster*, meiotic recombination occurs only in females while it is absent in males.

b) Genotypes of the eight types of progeny that can arise from the cross are shown. Of these, four are without meiotic recombination (1 to 4) and four the outcome of recombination (5 to 8). Among 1 to 4, one of them is homozygous for Cy/Cy and the other one is homozygous for Bl/Bl. Since these are lethal, they do not survive The ones that survive have Curly Bristle phenotypes and these have developed from gametes with chromosomes that have not participated in the recombination. Among 5 to 8, two have lethal genotypes and do not survive. The ones that survive have normal wings with mutant bristles or curly wings with normal bristles. These are the products of gametes from recombined chromosomes. As long as these two types of flies are among the progeny, the curly bristle stock cannot be a true-breeding strain.

Balancer chromosomes are an essential and powerful part of a fly geneticist's toolbox. They are used to maintain deleterious mutations in stable stocks as well as to prevent recombination and follow chromosomes in genetic mating schemes (Bloomington Stock Center).

Phenotype: Curly wing & mutant bristle (female) X Phenotype: Curly wing & mutant bristle(male)
Genotype: $Cy\ Bl^+//Cy^+Bl$ ↓ Genotype: $Cy\ Bl^+//Cy^+Bl$

Gametes		$Cy\ Bl^+$	$Cy^+\ Bl$
$Cy\ Bl^+$		1 $Cy\ Bl^+$ / $Cy\ Bl^+$ Lethal, because of Cy homozygosity	2 $Cy^+\ Bl$ / $Cy\ Bl^+$ Curly wing and bristle
Cy^+Bl		3 $Cy^+\ Bl$ / $Cy\ Bl^+$ Curly wing and bristle	4 Cy^+Bl / Cy^+Bl Lethal, because of Bl homozygosity
$Cy\ Bl$	*Recombinant products*	5 $Cy\ Bl$ / $Cy\ Bl^+$ Lethal, because of Cy homozygosity	6 $Cy\ Bl$ / $Cy^+\ Bl$ Lethal, because of Bl homozygosity
Cy^+Bl^+		7 $Cy^+\ Bl^+$ / $Cy\ Bl^+$ Curly wing and normal bristle	8 $Cy^+\ Bl^+$ / $Cy^+\ Bl$ Normal wing and mutant bristle

Table 3: Expected results of a cross between curly wing and mutant bristle (female) flies with curly wing and mutant bristle (male) flies.

Therefore, in order to avoid flies with such phenotypic combinations, the meiotic recombinant products need to be eliminated. How?

Inversion: Recombinant Suppressor

Inversion is one of the types of chromosomal rearrangements. In simple terms, the sequence of information in one of the chromosomes of a homologous pair is reversed in contrast to the normal sequence in another chromosome. For e.g., If the sequence in one chromosome is 'ABCDEFGH', the sequence in the chromosome with the inversion would be 'ABCFEDGH'. This type of inversion is called 'paracentric inversion' when the centromere does not lie within this segment, whereas, if the centromere of the chromosome lies within the inverted segment, it will be referred to as a 'pericentric inversion'. With regard to a pair of homologous chromosomes, both chromosomes may carry the same sequence, either normal or inverted. Therefore, they may be homozygous for the normal sequence or homozygous for the inverted sequence. In the heterozygous condition, in a homologous pair, one chromosome has the normal sequence while the other has the inverted sequence. During meiotic pairing followed by recombination, as long as the chromosome pair is homozygous at this sequence, the recombinant products are viable. On the other hand, since the sequence is oriented in opposite directions in heterozygotes, the pairing of the chromosomes results in the formation of a loop like configuration (*Figure* 2). Cytogenetical investigations have shown that when crossover/recombination occurs between a chromosome without an inversion and one with an inversion, the recombinant products are genetically imbalanced being either dicentric or acentric with duplications and deficiencies for certain genes. Hence do not produce viable gametes (*Figure* 2). Because of this, inversions are called 'recombinant suppressors'. Such inversions are called 'Balancers' (*Box* 1).

Drosophila geneticists have exploited this feature of inversion heterozygotes to avoid recombinant chromosomes by introducing a paracentric inversion in the chromosome with *Cy* gene, while its homologue with *Bl* gene carries the non-inverted sequence. In this heterozygous state, if recombination occurs, recombinant chromosomes are not viable. As shown in the above chart, genotypes of 5 and 6 had a lethal homozygous combination either for *Cy* or *Bl*, and they do not survive. On the other hand, due to the reasons mentioned earlier, genotypes 7 and 8, the products of recombination, are also not realized. This assures that the 'Curly Bristle' strain produce 'Curly Bristle' flies only, making it a true-breeding stock. Two lethal genes, each in the heterozygous state and in trans-arrangement with help of 'Balancer Chromosome' are being maintained as pure breeding strain and is referred to as a 'Balanced Lethal System'. Other such common examples in *Drosophila* are 'Curly Plum' and 'Lyra Dichete'.

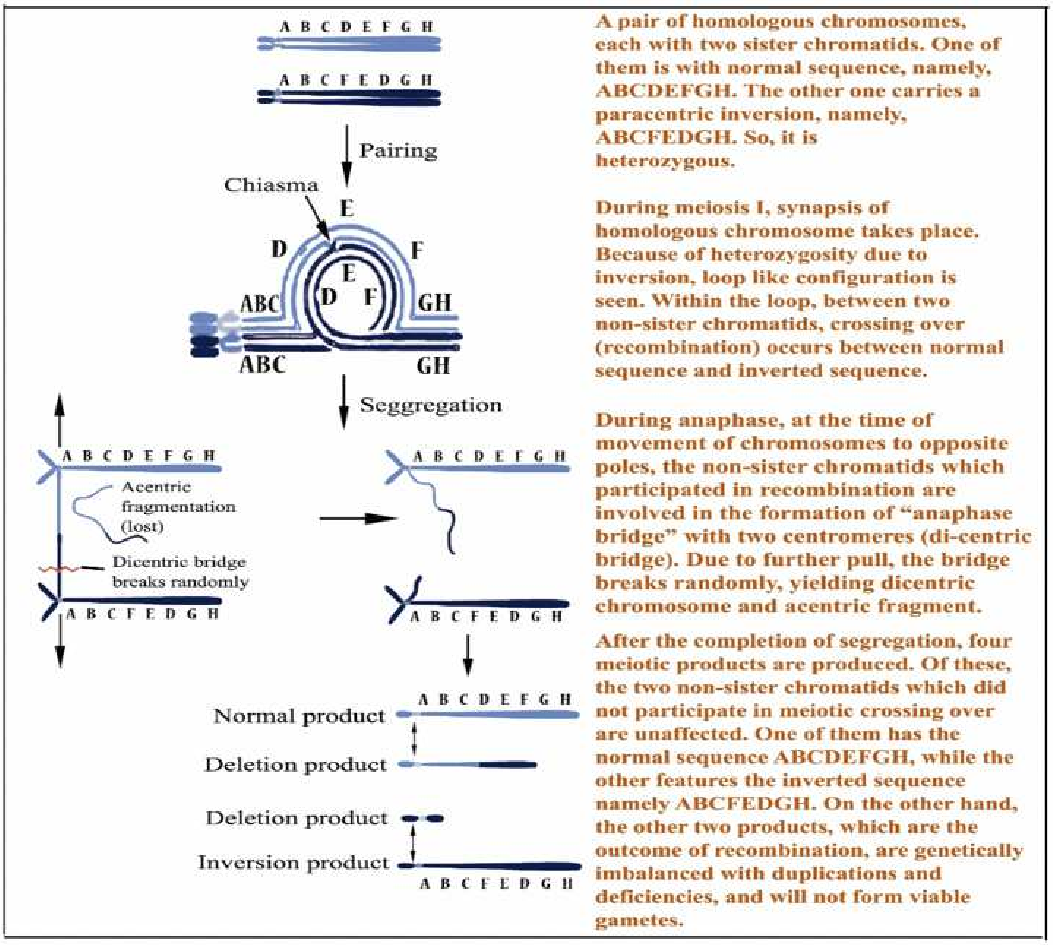

Figure 2. Consequences of meiotic recombination in a heterozygote, between the chromatid with a paracentric inversion and the chromatid with normal sequence. At the end of meiosis, two chromosomes, which are the products of recombination are genetically not balanced and do not yield viable gametes, while the other two chromosomes, not involved in recombination, give viable gametes.

Lessons

How to make a heterozygous strain a true/pure breeding stock? How to maintain lethal genes? Concept of balancer chromosomes. Balanced Lethal System.

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Box 1.

Balancers: Balancer chromosomes were first used by Hermann Muller. Balancer chromosomes are an essential and powerful part of a fly geneticist's toolbox. They are used to maintain deleterious mutations in stable stocks as well as to prevent recombination and follow chromosomes in genetic mating schemes (Bloomington Drosophila Stock Center). Balancers are available for three pairs of large chromosomes of *Drosophila*. The balancer available for the first chromosome (X chromosome) is called FM1, FM7; for the second chromosome, it is CyO, SM1, SM2, SM3, SM4, SM5 etc., for third chromosome TM2, TM3, TM6 etc. Each of these Balancers have different combination markers. (FlyBase).

Bristle: Bristle is a dominant mutation on chromosome 2 and located at 54.2 linkage map position. Bristles are one-half to two-thirds the normal length, blunt, thicker, and beaded in outline. Posterior scutellars often cross and adhere to the body. Eyes are somewhat larger and rougher. Probably affects (not clear what affects) nature of bristle secretion. Viability of heterozygote is good but erratic; homozygotes usually lethal; survivor female sterile with roughish eye character. (FlyBase)

Curly: (FlyBase) Curly is a dominant mutation on the second chromosome and is located at 7.0 of the linkage map. It is always associated with an inversion (Balancer). Hurd et al., (*Plos Genetics*, 2015 https://doi.org/10.1371/journal.pgen.1005625) have shown that *Curly* mutations arise in the gene *dual oxidase (duox)*, which encodes a reactive oxygen species (ROS) generating NADPH oxidase. They have demonstrated that Duox autonomously stabilizes the wing on the last day of pupal development. Through genetic suppression studies, they have identified a novel heme peroxidase, Curly Su (Cysu) that acts with Duox to form the wing.

Suggested Reading

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