

# Effect of Female Age on Crossing Over Frequency in *Drosophila melanogaster* Crosses $N^{\delta} \times bcl^{\delta}$ and $N^{\delta} \times ym^{\delta}$ and Their Reciprocals

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## Abstract

Crossing over is the occurrence of disconnection and reconnection followed by a reciprocal exchange between the two chromatids in a bivalent form. The crossing event will produce parental type and recombinant type. In the event of crossing over, various factors can influence it. These factors can be due to internal and external. Recently, various factors have been reported that influence the incidence of crossing over. These factors include age, temperature, radiation, and changes in chromosome structure. This research is a type of experimental research that uses a randomized block design. Randomized block design by crossing *D. melanogaster* strains  $N^{\delta} \times bcl^{\delta}$  and  $N^{\delta} \times ym^{\delta}$  and their reciprocals. From the results of this cross (F1) then cross again  $bcl^{\delta}$  with the recessive male (from stock) then observe the phenotype of the offspring (F2) and calculate the results of the offspring. The F2 crosses were treated with age variations, namely 0, 3, 6, 9, 12, 15, and 18 days. Based on the results of these crosses, the derived strains that appeared in the F2 crosses showed the phenomenon of crossing over with the influence of the age of the female and the type of strain on crossing events. The frequency or value of crossing over (formation of recombinants) decreased with the increasing age of the female. If the age of the female affects the frequency of crossing over, then the older the female, the more likely the frequency of crossing over will decrease. However, because the data obtained were incomplete, it was not possible to know the effect of female age on the frequency of crossing over of *D. melanogaster* crosses  $bcl^{\delta} \times N^{\delta}$  and  $ym^{\delta} \times N^{\delta}$  and their reciprocals. The condition for crossing over is the formation of a synaptonemal complex. Age of *D. melanogaster* females has an effect on the frequency of crossing over in crosses  $N^{\delta} \times bcl^{\delta}$ ,  $N^{\delta} \times ym^{\delta}$ , and their reciprocals. The older *Drosophila melanogaster* is, the lower the frequency of crossing over will occur. Based on this, it was necessary to cross *D. melanogaster* with strains N, bcl, and ym. A cross consists of  $N^{\delta} \times bcl^{\delta}$  and  $N^{\delta} \times ym^{\delta}$  and their reciprocals. By crossing  $bcl^{\delta}$  with a recessive male from the stock, then observing the F2 phenotype, it is hoped that crossing over will occur. So that you can better understand by doing the practice directly. In this case, the effect of crossing over is seen from the age of the female and the type of strain.

**Keywords:** crossing over frequency; *Drosophila melanogaster*; female age; strain  $N^{\delta} \times bcl^{\delta}$ ; strain  $N^{\delta} \times ym^{\delta}$ .

**Abbreviations:** adenosine triphosphate (ATP), transverse filaments (TFs)

## INTRODUCTION

In life, all organisms try to maintain the continuity of their generations and to maintain the continuity of these generations, a reproduction process is needed. There are two types of reproduction, namely sexual and asexual reproduction. In sexual reproduction, after the meeting and fusion between the egg and sperm, a zygote is formed. In the process of development or what is called the gametogenesis process, cell division processes occur, initially one cell divides into two, then four, and so on. The process of cell division involves two events namely meiosis and mitosis.

Meiosis is a cell division event that produces gametes or sex cells and has half the number of chromosomes of each of the parents. Meanwhile, mitosis is the division of

a somatic cell into two cells containing an identical number of chromosomes. Meiosis events are divided into 2, namely meiosis 1 and meiosis 2. In meiosis 1 prophase I, paired chromosomes often show a crossed configuration. As a result, crossing over can occur and produce offspring that are different from their parents.

Crossing over is the occurrence of disconnection and reconnection followed by a reciprocal exchange between the two chromatids in a bivalent form (Corebima, 1997). Crossing over was first proposed by T. H. Morgan to explain the occurrence of recombination of factors which were concluded to be mutually linked based on genetic data (Gardner, et al. 1984 in Corebima 1997). The crossing event will produce parental type and recombinant type (Corebima, 1997). In the event of

crossing over, various factors can influence it. These factors can be due to internal and external. Recently, various factors have been reported that influence the incidence of crossing over. Rothwell (1983) in Corebima (1997) mentions these factors include age, temperature, radiation, and changes in chromosome structure. In addition, Suryo (1996) stated that one of the influential factors in crossing over was age. Where the older the age of the female, the less crossing that occurs.

Based on this, it was necessary to cross *D. melanogaster* with strains N, bcl, and ym. A cross consists of ♂N × ♀bcl and ♂N × ♀ym and their reciprocals. By crossing ♀N with a recessive male from the stock, then observing the F2 phenotype, it is hoped that crossing over will occur. So that you can better understand by doing the practice directly. In this case, the effect of crossing over is seen from the age of the female and the type of strain.

## MATERIALS AND METHODS

### Study area

This research is a type of experimental research that uses a randomized block design. Randomized block design by crossing *D. melanogaster* strains ♂N × ♀bcl and ♂N × ♀ym and their reciprocals. From the results of this cross (F1) then cross again ♀N with the recessive male (from stock) then observe the phenotype of the offspring (F2) and calculate the results of the offspring. The F2 ♀N crosses were treated with age variations, namely 0, 3, 6, 9, 12, 15, and 18 days. Based on the results of these crosses, the derived strains that appeared in the F2 crosses showed the phenomenon of crossing over with the influence of the age of the female and the type of strain on crossing events.

### Procedures

#### Medium creation

1. Consider the medium ingredients, namely Rajamala banana, cassava tape, and brown sugar with a ratio of 7:2:1.
2. Cut the Rajamala banana into several parts and put it in the blender along with the cassava tape and add enough water.
3. Carry out the blending process until the tape and bananas become smooth/soft and mixed.
4. Heat the brown sugar by adding a little water until the brown sugar dissolves in the water.
5. Cook mashed tape and bananas using a saucepan for 45 minutes over medium heat by adding water so that the medium is not too thick.
6. When it starts to boil, put the brown sugar water that has been boiled into the pot and keep stirring it so it doesn't burn.
7. Evaporate the jam jar and cork over a medium saucepan, and fill the jam jar with the medium until about a quarter of the jar.

8. Close the bottle using a sponge that has been cut according to the diameter of the bottle.
9. Waiting for the bottle to cool by immersing the bottom of the bottle in cold water.
10. After the medium is cold, add approximately 4 yeast grains into the bottle containing the medium and put the pupation paper into the bottle, and close it again.

#### Stock renewal

1. Prepare a bottle of jam containing the medium that has just been heated, and after it's cold, add about 4 grains of yeast and put the pupation paper in it.
2. Transferring at least 3 pairs of flies from each strain taken from the old medium and then transferring them to the new medium that has been prepared.
3. Label according to the type of strain and date of rejuvenation.
4. Observe its development and if there are already blackened pupae, immediately collect them

#### Stock collection

1. Take carefully the black pupa in the bottle using a brush so that the pupa is not damaged
2. Insert the pupa into a short hose ( $\pm$  6-7 cm) which has been given a banana slice in the middle of the hose.
3. Closing the hose by using a small piece of sponge at each end
4. Waiting for the pupae to hatch (usually a day or two after being pulled)
5. After hatching, cross ♂N × ♀bcl and ♂N × ♀ym and their reciprocal (F1 cross)
6. For recessive males to be used in F2 crosses, the maximum age of male flies that can be crossed is 2 days.

#### F1 crosses (♂N × ♀bcl and ♂N × ♀ym, and their reciprocals)

1. Collect black pupae of N, bcl, and ym strains, and then after hatching cross ♂N × ♀bcl and ♂N × ♀ym and their reciprocals
2. After 2 days of crossing, remove the male and let the female remain in the bottle until larvae appear.
3. After the larvae appear, move the female at least 3 times (bottles A-D)
4. Gathers a black pupa from the cross for its F2 cross.
5. Look for tillers with strain ♀N from each cross (♂N × ♀bcl and ♂N × ♀ym and their reciprocals) and give them age treatments.

#### Preparing female parent stock

1. Transferring strain N female flies from each cross that has been ampoule into a bottle containing medium.
2. Each strain is put in a different bottle to make it easier for the practitioner to cross.
3. Label each ampoule bottle with the date and strain.
4. Waiting for the females of each strain until they are 0 days, 3 days, 6 days, 9 days, 12 days, 15 days, and 18 days.

*F2 cross*

1. Collect black pupae from each stock strain and wait for them to hatch (for recessive males)
2. Crossing ♀N from stock ♀N that has been prepared according to age treatment with recessive male P1
3. After two days, the male is released and the female is left in the bottle
4. After the larvae appear, move the female to the second bottle. Minimum of 3 female transfers (bottles A-D)
5. Observing the phenotype and counting the number of tillers emerging from each bottle for 7 days.

**Data analysis**

The data obtained were analyzed in the following way:

1. Reconstruct the body's chromosomes
2. Make an observation table F2
3. Calculating the percentage of crossing over with the formula:

Frequency of recombinant-type derivatives (%)

$$\frac{\sum \text{recombinant}}{\sum \text{parental} + \sum \text{recombinant}} \times 100\%$$

**RESULTS AND DISCUSSION****Result**

Because the observational data obtained did not allow statistical calculations to be carried out, the data analysis was carried out using descriptive analysis in the form of calculating the frequency of crossing over at each cross.

*Cross D. melanogaster strain ♀ N (from F1 bcl ♀ >< N ♂) >< bcl ♂ recessive*

1. Age ♀ 0 days, cross over frequency = 22,69%
2. Age ♀ 6 days, cross over frequency = 13,43%
3. Age ♀ 9 days, cross over frequency = 11,11%

*Cross D. melanogaster strain ♀ N (from F1 N ♂ >< bcl ♀) >< bcl ♂ recessive*

1. Age ♀ 0 days, cross over frequency = 25,51%
2. Age ♀ 3 days, cross over frequency = 24,93%
3. Age ♀ 6 days, cross over frequency = 24,93%
4. Age ♀ 9 days, cross over frequency = 15,45%

*Cross D. melanogaster strain ♀ N (from F1 N ♂ >< ym ♀) >< ym ♂ recessive*

1. Age ♀ 6 days, cross over frequency = 20,18%

**Discussion**

According to Suryo (1996), the possibility of crossing over is influenced by several factors, one of which is the age factor, where the older the female, the lower the frequency of crossing over. Whittinghill and Hinton (1950) also stated the same thing that the frequency or value of crossing over (formation of recombinants) decreased with the increasing age of the female. If the

age of the female affects the frequency of crossing over, then the older the female, the more likely the frequency of crossing over will decrease. However, because the data obtained were incomplete, it was not possible to know the effect of female age on the frequency of crossing over of *D. melanogaster* crosses ♀N >< ♂ bcl and ♀N >< ♂ ym and their reciprocals. The condition for crossing over is the formation of a synaptonemal complex.

The synaptonemal complex is a protein needed to carry out the correct pairing of homologous chromosomes (Campbell, 2000). The synaptonemal complex is a protein complex that appears to mediate synapses during the zygotene stage and then disintegrates. This complex brings together homologous chromosomes during the prophase of meiosis. According to Gardner (1991) crossing over is an enzymatic reaction like other enzymatic reactions. The older the fly, the body's metabolism will decrease. The decrease in body metabolism affects the formation of adenosine triphosphate (ATP) in the body which is a source of cellular energy that plays a role in cell activities including protein synthesis. If the process of protein synthesis decreases, the amount of protein including the synaptonemal complex and enzymes that play a role in crossing over decreases so that crossing events decrease.

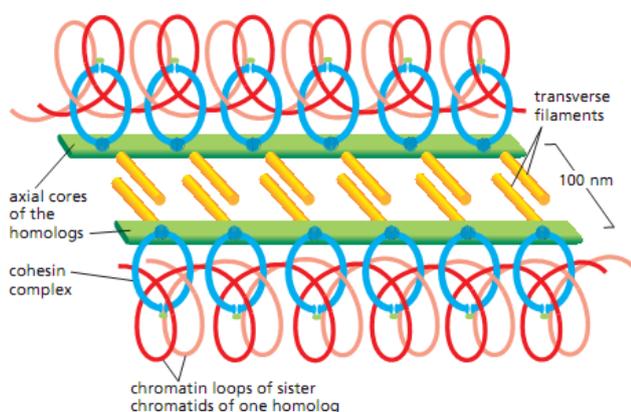
According to Ashburner (1989) under normal conditions, *D. melanogaster* can go through its entire life cycle for 30-32 days, and the time it takes to become an adult is ± 10 days from the time the eggs are released. So that the life span of imago can be known, which is around 20-22 days. The life span of the imago can be grouped into three ranges, namely young, adult, and old. The ages of *D. melanogaster*, namely 1 to 6 days are classified in the young age range, 7 to 14 days old are classified in the adult age range, and ages 15 to 20 are classified in the old age range.

In the observations that have been made, crossing over is characterized by the emergence of non-parental or recombinant strains in the crosses. Based on the results of the data obtained, namely in crosses bcl♂ >< N♀ in the treatment of females aged 0 days, the percentage of crossing over was obtained, namely 22.69%, in the treatment of females aged 6 days, the percentage of crossing over was obtained by 13.44%, and in the treatment of females aged 9 days, the percentage of crossover frequency was 11.11%. The results of the cross N♂ >< bcl♀ in the treatment of females aged 0 days obtained the percentage of crossing over frequency which was 25.52%, in the treatment of females aged 3 days the percentage of crossing over was obtained in the amount of 24.93%, and in the treatment of females aged 9 days, the percentage of crossover frequency was 15.45%. The decreasing percentage of crossing over frequency of females aged 0 days, 3 days, 6 days, and 9 days indicates that there is a concordance between the practicum results and theory, that is, the older the females are, the lower the frequency of crossing over

occurs. The results of crosses  $ym^{\delta} \times N^{\delta}$  in the treatment of 6 days old females obtained the percentage of crossing over frequency which was 20.18%. However, the results obtained in this practicum were inaccurate because the data obtained were still incomplete, namely in crosses  $bcl^{\delta} \times N^{\delta}$  treated females aged 3 days, 12 days, 15 days, and 18 days, whereas  $N^{\delta} \times bcl^{\delta}$  treatments females aged 6 days, 15 days, and 18 days, and cross data  $ym^{\delta} \times N^{\delta}$  for the treatment of females aged 0 days, 3 days, 9 days, 12 days, 15 days, and 18 days.

Crossing over occurs during the synapsis of homologous chromosomes in the pachytene stage of prophase I of meiosis. Paired chromosomes at meiosis often display a crossed configuration. When the chromosomes are about to separate, the crossed chromatids attach and break off at the chiasma, then each piece is attached to the adjacent chromatid (its homolog). When the first homologous chromosomes appear as pairs during prophase I, a set of proteins called synapses joins the chromosomes together so that they are tight to one another. Apart from the synaptonemal complex, another structure responsible for crossing over is the recombination nodule. Recombination nodules are temporary structures that exist only in the middle of the pachytene stage associated with the synaptonemal complex, thus crossing over is expected to occur at that time limit (Carpenter, 1975).

Genes  $c(3)G$  and  $c(2)M$  are components of the synaptonemal complex. The  $c(3)G$  gene encodes the formation of transverse filaments (TFs) (Figure 1). According to Page and Hawley (2001), TFs are filaments that make up the synaptonemal complex in the form of coils that are in the middle of the synaptonema complex formation. The synthesis of TF will trigger the formation of a synaptonema complex between two homologous chromosomes.



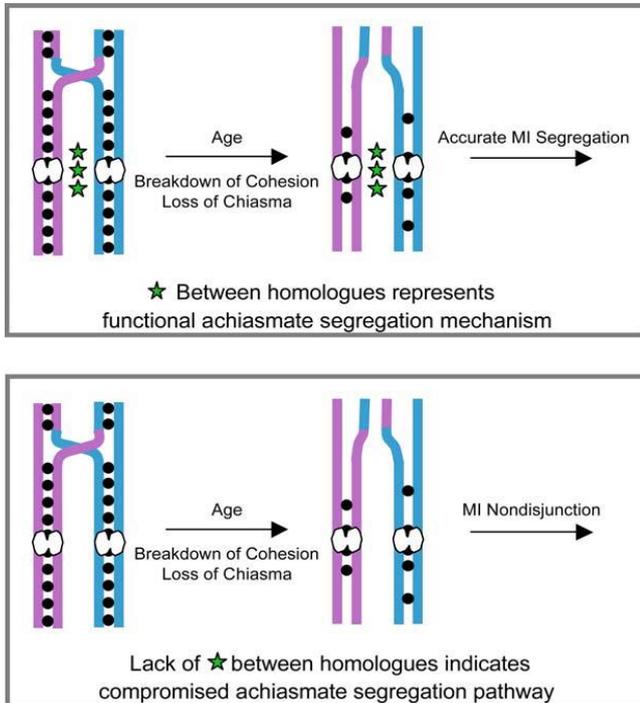
**Figure 1.** A simplified schematic of the synaptonemal complex. Before the synaptonemal complex is formed, the recombinant complex is composed of double-stranded DNA that unravels and helps catalyze crossing-over between non-sister chromatids from opposite sides of the complex. Source: Albert, et al. (2008).

It is well known that the existence of a synaptonemal complex is needed in crossing over. In addition to these genes, according to McKim and Hayashi-Hagihara (2003), the  $mei-W68$  gene is needed in the initiation of meiotic recombination. The  $mei-W68$  gene encodes the MEI-W68 protein which is a type of topoisomerase II protein. This protein is required in the event of double helix breaking during meiosis. Based on this theory, it can be concluded that there are certain enzymes that play an important role in the process of recombination. These enzymes have a role in the process of exchanging chromosome segments on homologous chromosomes that have formed pairs or are called synapses at the prophase stage which are assisted by complex proteins and synaptonemal. This complex holds homologous chromosomes together during the prophase of meiosis. So that the age of the female can be said to affect the frequency of crossing over with respect to the decrease in the working process of enzymes and body metabolism along with the increasing age of *D. melanogaster*. As it gets older, the body's metabolism in *D. melanogaster* will also decrease, so that the production of ATP as a source of energy in the body will also decrease which also results in a decrease in the work function of enzymes that play a role in the recombination process and the amount of its production, resulting in a decrease in the frequency of crossing over.

Conversely, if the age of the female does not affect the frequency of crossing over, this is possible because the increasing age of *D. melanogaster* does not affect the process of ATP formation in the body, so there is no decrease in the production of enzymes that play a role in the process of crossing over. Finally, the recombination process still occurs and there is no difference in the frequency of crossing over in each treatment of the difference in the age of the females. In addition, another possible reason for the age of females not affecting the frequency of crossing over is the presence of a DNA ligase enzyme whose job is to repair the damage that is formed so that there is no exchange of genetic material which will result in crossing over, and the number of these genes varies in each individual. Gene repair also affects the magnitude of the frequency of crossing over, namely the genes that undergo recombination have the ability to repair themselves so that the exchange of genetic material does not occur. These things resulted in no significant differences in the frequency of crossing over in various treatments of female age.

According to Subramanian and Bickel (2008), there is another mechanism, namely through the chiasmata pathway so that segregation still occurs properly (Figure 2). The chiasmata pathway in *Drosophila* oocytes ensures accurate segregation of recombinant chromosomes that have lost chiasmata as a result of the weakening of the cohesive power of the chromosomal arms during the aging process. Cohesin protein has an important role in maintaining the heterochromatin pair of achiasmata

homolog pairs in *Drosophila* oocytes and as a "glue" to hold sister chromatids together.



**Figure 2.** The Achiasmata Segregation System (shown by green stars between homologous chromosomes) allows for accurate segregation of recombinants that previously lost their chiasmata during aging (Top Figure). When the achiasmata pathway is dysfunctional (indicated by loss of green stars), recombinants that have lost chiasmata will experience inaccurate segregation (Figure Below). Source: Subramanian and Bickel (2008).

## CONCLUSIONS

Age of *D. melanogaster* females has an effect on the frequency of crossing over in crosses  $N^{\sigma} \times bcl^{\rho}$ ,  $N^{\sigma} \times ym^{\rho}$ , and their reciprocals. The older *D. melanogaster* is, the lower the frequency of crossing over will occur.

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**Competing Interests:** The authors declare that there are no competing interests.

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