A Study on the Sampling of Drosophila Melanogaster under Various Environmental Conditions

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Abstract— Usually insects have a very low life span of about a few days. When it comes to Drosophila species, life span is around 12-14 days. Life cycle of drosophila takes place in three stages. All these three stages take place in two days of the development of the flies. On the 5th day of the development, pupa is formed. Pupa is completely developed into an adult within 7 days. Sexual dimorphism and morphological differences within the species can be seen in the adult stage. Sampling of the flies is done in the adult stage. A chemical named methyl farnesoate plays a dual role in regulating Drosophila metamorphosis. Flies were collected in the laboratories and in the canteen using rava-jaggery medium. This media was kept open in two conical flasks in the laboratory for two days. The captured flies were sampled considering the sex, eye shape, and eye colour and wing shape. After sampling, it was found that, males were more in number as compared to females at optimum room temperature without exposure of direct sunlight with no mutants. At optimum room temperature with exposure to direct sunlight, females were more in number as compared to males with no mutants and at optimum room temperature with exposure of direct sunlight along with food waste, the ratio of males and females were almost equal with one vestigial winged mutant.

Key words: Drosophila, Adult Stage, Metamorphosis, Sex Ratio

I. Introduction

Drosophila melanogaster, commonly called as fruit fly [1], is the most widely used organism in biomedical [1] and neurosciences. Although the fly has a huge difference in the anatomical structure of the brain with that of the humans, many of its functions and the development remain the same. Drosophila has been used previously in the study of neurodegenerative diseases such as Huntington's disease, Parkinson's disease and Alzheimer's disease [2]. Drosophila is used as a model organism for the study of various biological processes such as genetics and inheritance, behaviour and embryonic development. Although the anatomy of the fruit flies and humans are not similar, it has been established that the biological processes that control development and survival between them are conserved across evolution [3].

Life cycle of Drosophila includes four stages; egg, larva, pupa and fly. Imaginal discs are a group of cells by which the adult tissues are developed. Although the flies are called fruit flies, they feed on the yeast growing on the fruits [3].

Drosophila is also used as a model for regenerative biology and medicine. It has been shown that the fragments of imaginal discs injected into the abdomen of adult female Drosophila survived upto a few days. Although the imaginal discs do not differentiate, the transplanted fragments of the disc proliferate. A recent study has used Drosophila as e model for wound healing. Drosophila embryos were used as a tool for the production of regenerative medicines [3].

Genetic variations are an important aspect for the evolutionary change. Genetic variation is mainly caused by mutations. Mutations, once occurred, will either increase in frequency or it will varnish in the future generations [4]. Mutations may be caused due to insertion, deletion or substitution [5]. Mutations in gene either will have no effect on the gene product or it will alter the functioning of the gene product [6]. Lethal mutations can led to various harmful effects such as cancer. These lethal mutations can be altered by DNA repair mechanisms [7].

One study on the comparison of Drosophila species, it was reported that the mutations that lead to the change in the proteins will have adverse effects, whereas, other mutations are either weakly beneficial or neutral [6]. Another study on yeast has been reported that 7% of the mutations in noncoding DNA are deleterious, whereas, 12% of mutations in the coding DNA are deleterious and the rest of the mutations are neural or weakly beneficial [8].

Sexual dimorphism in Drosophila involves differences in size of the body, size of the wings, shape of body parts, pigmentation, number of abdominal segments, structure of genitalia, presence of combs and behaviour [9]. Drosophila genome is made up of approximately 30% of heterochromatin. Heterochromatic in Drosophila undergoes a process called Heterochromatin silencing of Position Effect Variegation (PEV). PEV effect has proven to give a pigmented red eye in Drosophila. Recent studies have shown that the heterochromatin in male and female Drosophila is different [10].

II. Materials & Methodologies

A. *Culturing of Drosophila*

There are many methods in which Drosophila can be cultured, banana mash being the oldest traditional method to culture the flies. There are many laboratory methods as well to culture the flies. One of the papers suggests cornmeal-molasses-agar culture to be one of the best ways to culture the flies [11]. But for our work, we prefer to use rava-jaggery medium [12] as it is viable in all conditions.

The ingredients for preparing the media as follows:

- Rava - 60g (approx.)
- Jaggery - 60g (approx.)
- Water – 300 ml

Approximately 60 g of rava is taken in a vessel. 150 ml of water is added to it and boiled with constant stirring for about 3-4 minutes. After about 4 minutes, approximately 60 g of jaggery and 150 ml of water is added and boiled with constant stirring for about 5 minutes till the jaggery is dissolved completely. The vessel is taken out of the flame and transferred to six conical flasks in almost equal quantities.

B. Collecting of Flies

Cultures are left till the imago stage of the flies is reached. Once this stage is reached, the flies are treated with ether to knock them out. These flies are then view under the microscope and sampled.

The conical flasks with the media are kept open at three conditions. One was kept at optimum room temperature without exposure to direct sunlight, one at optimum room temperature with exposure to direct sunlight and one at optimum room temperature with direct exposure to direct sunlight along with food waste. They were carefully closed to capture the flies. The following day, the flies were treated with ether to knock them off. Once the flies are knocked off, they are sampled. The flies are sampled under the simple microscope. Sampling is done considering the sex, eye shape, eye colour and wings shape.

III. Results

The results are as follows:

1) Optimum Room Temperature without Exposure to Direct Sunlight

Fig. 1: Petri Plate Showing the Flies Collected at Optimum Room Temperature without Exposure to Direct Sunlight

A. Based on Sex (Taking into Consideration the Abdominal Rings of the Size)

Sex	Frequency
Males	21
Females	09

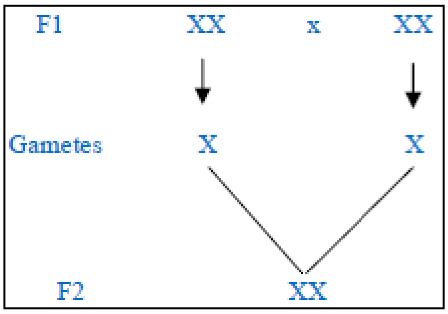
Table 1: Table Showing the Frequency of Male & Female Flies in Biotechnology Laboratory

B. Based on Eye Shape

All the flies that were sampled had round eyes. No mutants, which is bar or kidney shaped eyes were not seen.

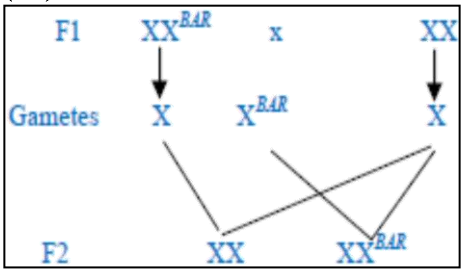
– Genetics:

1) CASE – 1.2.1: When a Homozygous Round Shaped Eyes Fly (XX) is crossed with a Homozygous Round Shaped Eyes Fly (XX)



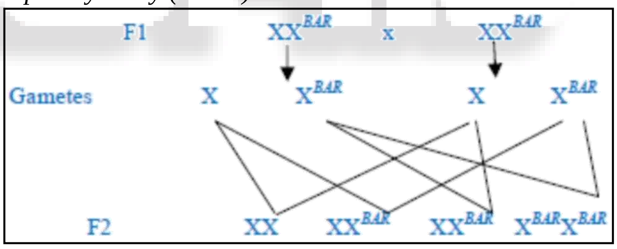
The off springs got in the above cross are 100% of homozygous round shaped eyes.

2) CASE – 1.2.2. : When a Heterozygous Round Shaped Eyes Fly (XX^{BAR}) is crossed with Homozygous Round Shaped Eyes Fly (XX)



The off springs got in the above cross are 50% of homozygous round shaped eyes and 50% of heterozygous round shaped eyes.

3) CASE – 1.2.3. : When a Heterozygous Round Shaped Eyes Fly (XX^{BAR}) is crossed with a Heterozygous Round Shaped Eyes Fly (XX^{BAR})



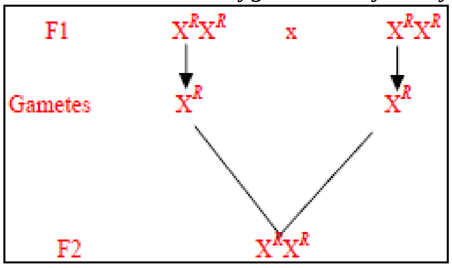
The off springs got in the above cross are 25% of homozygous round shaped eyes, 50% of heterozygous round shaped eyes and 25% of bar or kidney shaped eyes.

C. Based on Eye Colour

All the flies that were captured and sampled had red eyes. No mutants, which is white eyed was not seen.

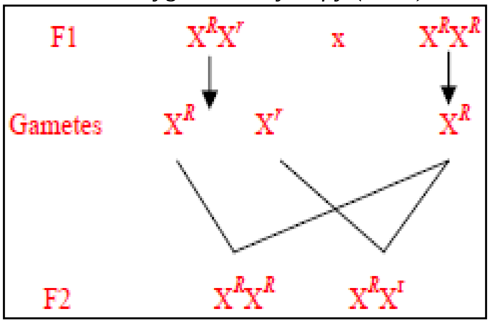
– Genetics:

1) CASE – 1.3.1. : When a Homozygous Red Eyed Fly (X^RX^R) is crossed with a Homozygous Red Eyed Fly (X^RX^R)



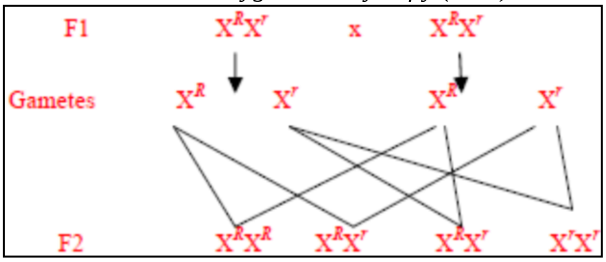
The off springs got in the above cross are 100% of homozygous red eyes.

2) CASE – 1.3.2. : When a heterozygous red eyed fly (X^RX^r) is crossed with homozygous red eyed fly (X^RX^R)



The off springs got in the above cross are 50% of homozygous red eyes and 50% of heterozygous red eyes.

3) CASE – 1.3.3. : When a heterozygous red eyed fly (X^RX^r) is crossed with a heterozygous red eyed fly (X^RX^r)



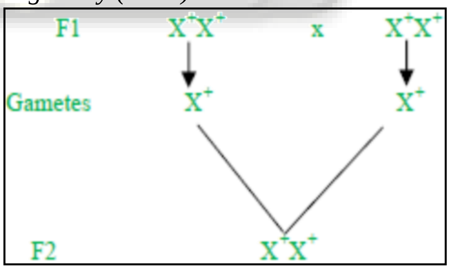
The off springs got in the above cross are 25% of homozygous red eyes, 50% of heterozygous red eyes and 25% of white eyes.

D. Based on Wing Shape

All the flies that were captured and sampled had oval wings. No mutants having curled wings or vestigial wings were seen.

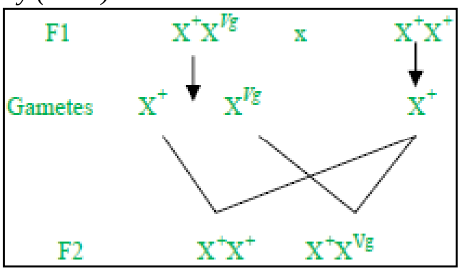
– Genetics

1) CASE – 1.4.1. : When a Homozygous Oval Shaped Winged Fly (X^+X^+) is crossed with a Homozygous Oval Shaped Winged Fly (X^+X^+)



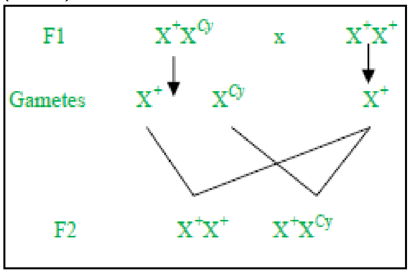
The off springs got in the above cross are 100% of homozygous oval shaped wings.

2) CASE – 1.4.2. : When a Heterozygous Oval Shaped Winged Fly (X^+X^{Vg}) crossed with a Homozygous Oval Shaped Winged Fly (X^+X^+)



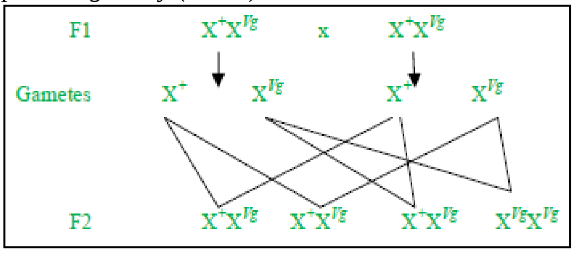
The off springs got in the above cross are 50% of homozygous oval shaped wings and 50% of heterozygous oval shaped wings.

3) CASE – 1.4.3. : When a Heterozygous Oval Shaped Winged Fly (X^+X^{Cy}) crossed with a Homozygous Oval Shaped Winged fly (X^+X^+)



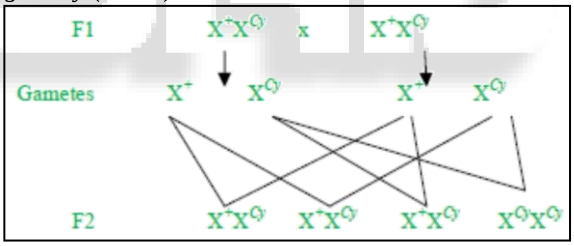
The off springs got in the above cross are 50% of homozygous oval shaped wings and 50% of heterozygous oval shaped wings.

4) CASE – 1.4.4. : When a Heterozygous Oval Shaped Winged Fly (X^+X^{Vg}) crossed with a Heterozygous Oval Shaped Winged Fly (X^+X^{Vg})



The off springs got in the above cross are 25% of homozygous oval shaped wings, 50% of heterozygous oval shaped wings and 25% of vestigial wings.

5) CASE – 1.4.5. : When a Heterozygous Oval Shaped Winged fly (X^+X^{Cy}) crossed with a Heterozygous Oval Shaped Winged Fly (X^+X^{Cy})



The off springs got in the above cross are 25% of homozygous oval shaped wings, 50% of heterozygous oval shaped wings and 25% of curled wings

2) Optimum Room Temperature with Exposure to Direct Sunlight

Fig. 2: Petri Plate Showing the Flies Obtained at Optimum Room Temperature with Exposure to Direct Sunlight

A. Based on Sex

Sex	Frequency
Males	09
Females	27

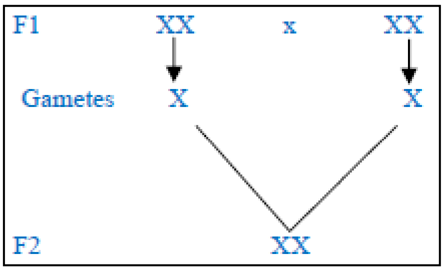
Table 2- Table Showing the Frequency of Male & Female Flies in the Zoology Laboratory

B. Based on Eye Shape

All the flies that were sampled had round eyes. No mutants, which is bar or kidney shaped eyes were no seen.

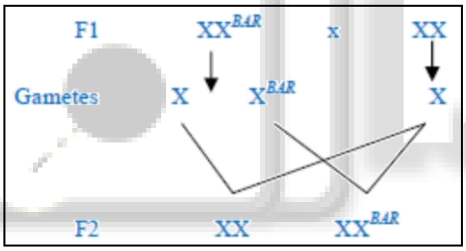
– Genetics

1) CASE – 2.2.1. : When a Homozygous Round Shaped Eyes Fly (XX) is crossed with a Homozygous Round Shaped Eyes Fly (XX)



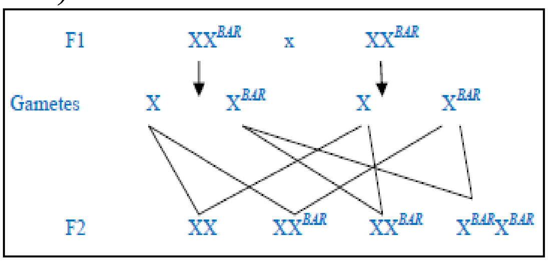
The off springs got in the above cross are 100% of homozygous round shaped eyes.

2) CASE – 2.2.2. : When a heterozygous round shaped eyes fly (XX^{BAR}) is crossed with homozygous round shaped eyes fly (XX)



The off springs got in the above cross are 50% of homozygous round shaped eyes and 50% of heterozygous round shaped eye

3) CASE – 2.2.3. : When a heterozygous round shaped eyes fly (XX^{BAR}) is crossed with a heterozygous round shaped eyes fly (XX^{BAR})



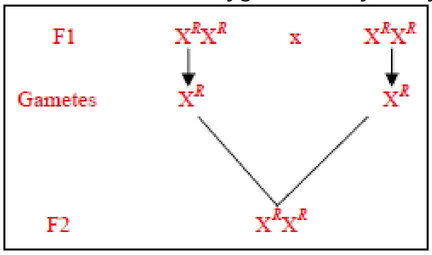
The off springs got in the above cross are 25% of homozygous round shaped eyes, 50% of heterozygous round shaped eyes and 25% of bar or kidney shaped eyes.

C. Based on Eye Colour

All the flies that were captured and sampled had red eyes. No mutants, which is white eyed was not seen.

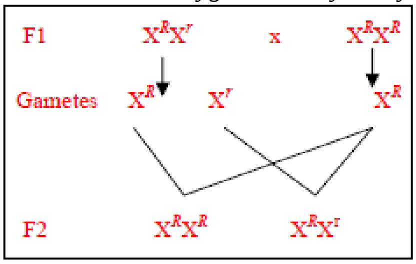
– Genetics

1) CASE – 2.3.1. : When a Homozygous Red Eyed Fly (X^RX^R) is crossed with a Homozygous Red Eyed Fly (X^RX^R)



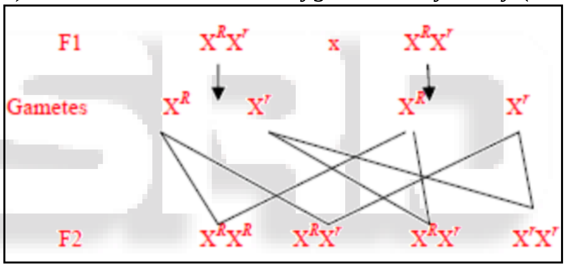
The off springs got in the above cross are 100% of homozygous red eyes.

2) CASE – 2.3.2. : When a Heterozygous Red Eyed Fly (X^RX^r) is crossed with Homozygous Red Eyed Fly (X^RX^R)



The off springs got in the above cross are 50% of homozygous red eyes and 50% of heterozygous red eyes.

3) CASE – 2.3.3. : When a Heterozygous Red Eyed Fly (X^RX^r) is crossed with a Heterozygous Red Eyed Fly (X^RX^r)



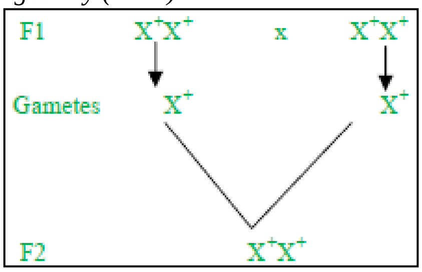
The off springs got in the above cross are 25% of homozygous red eyes, 50% of heterozygous red eyes and 25% of white eyes.

D. Based on Wing Shape

All the flies that were captured and sampled had oval wings. No mutants having curled wings or vestigial wings were seen.

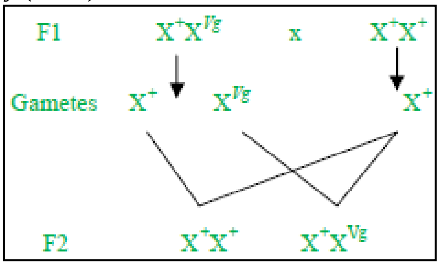
– Genetics:

1) CASE – 2.4.1. : When a Homozygous Oval Shaped Winged Fly (X^+X^+) is crossed with a Homozygous Oval Shaped Winged Fly (X^+X^+)



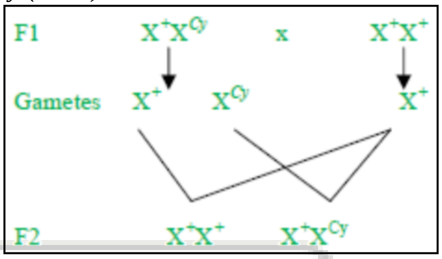
The off springs got in the above cross are 100% of homozygous oval shaped wings.

2) CASE – 2.4.2. : When a Heterozygous Oval Shaped Winged Fly (X^+X^{Vg}) crossed with a Homozygous Oval Shaped Winged Fly (X^+X^+)



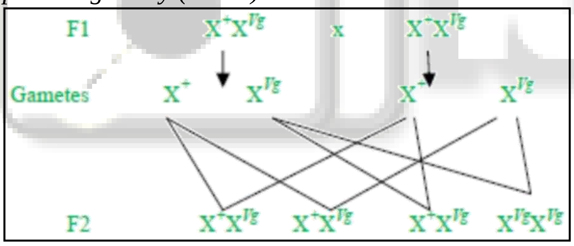
The off springs got in the above cross are 50% of homozygous oval shaped wings and 50% of heterozygous oval shaped wings.

3) CASE – 2.4.3. : When a heterozygous Oval Shaped Winged Fly (X^+X^{Cy}) crossed with a Homozygous Oval Shaped Winged Fly (X^+X^+)



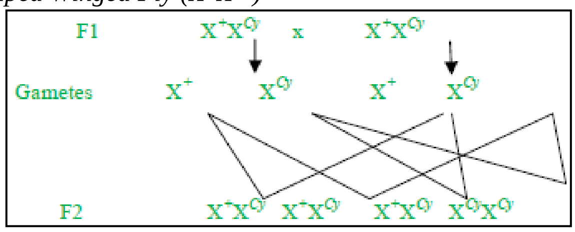
The off springs got in the above cross are 50% of homozygous oval shaped wings and 50% of heterozygous oval shaped wings.

4) CASE – 2.4.4. : When a Heterozygous Oval Shaped Winged Fly (X^+X^{Vg}) crossed with a Heterozygous Oval Shaped Winged Fly (X^+X^{Vg})



The off springs got in the above cross are 25% of homozygous oval shaped wings, 50% of heterozygous oval shaped wings and 25% of vestigial wings

5) CASE – 2.4.5. : When a Heterozygous Oval Shaped Winged Fly (X^+X^{Cy}) crossed with a Heterozygous Oval Shaped Winged Fly (X^+X^{Cy})



The off springs got in the above cross are 25% of homozygous oval shaped wings, 50% of heterozygous oval shaped wings and 25% of curled wings

3) Optimum Room Temperature With Exposure To Direct Sunlight Along With Food Waste

Fig. 3: Petri Plate Showing the Flies Collected at Optimum Room Temperature with Exposure to Direct Sunlight Along with Food Waste

A. Based on Sex

Sex	Frequency
Males	15
Females	18

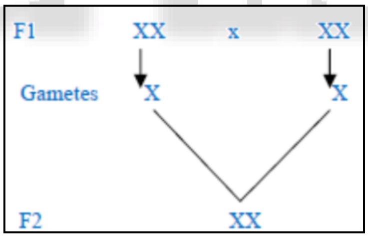
Table 3: Table Showing the Frequency of Male & Female Flies in the Hostel Canteen

B. Based on Eye Shape

All the flies that were sampled had round eyes. No mutants, which is bar or kidney shaped eyes were no seen.

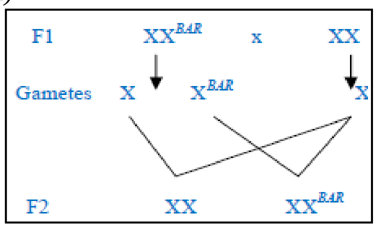
– Genetics

1) CASE – 3.2.1. : When a Homozygous Round Shaped Eyes Fly (XX) is crossed with a Homozygous Round Shaped Eyes Fly (XX)



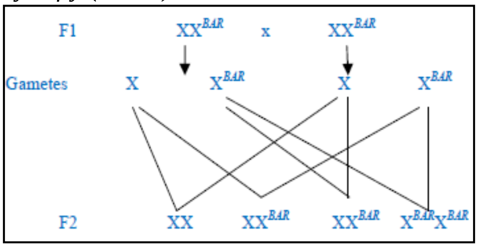
The off springs got in the above cross are 100% of homozygous round shaped eyes.

2) CASE – 3.2.2. : When a Heterozygous Round Shaped Eyes Fly (XX^{BAR}) is crossed with Homozygous Round Shaped Eyes Fly (XX)



The off springs got in the above cross are 50% of homozygous round shaped eyes and 50% of heterozygous round shaped eye

3) CASE – 3.2.3. : When a Heterozygous Round Shaped Eyes Fly (XX^{BAR}) is crossed with a Heterozygous Round Shaped Eyes fly (XX^{BAR})



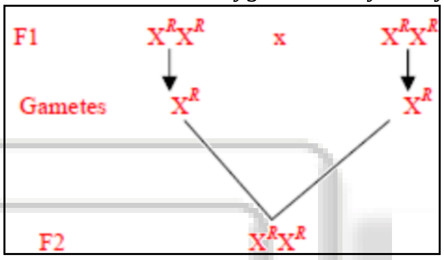
The off springs got in the above cross are 25% of homozygous round shaped eyes, 50% of heterozygous round shaped eyes and 25% of bar or kidney shaped eyes.

C. Based on Eye Colour

All the flies that were captured and sampled had red eyes. No mutants, which is white eyed was not seen.

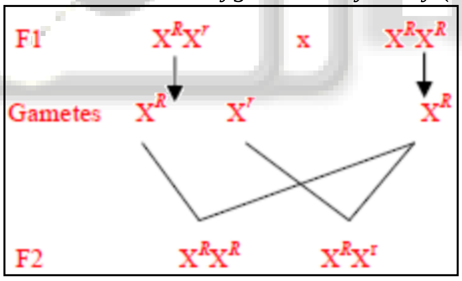
– Genetics

1) CASE – 3.3.1. : When a Homozygous Red Eyed Fly (X^RX^R) is crossed with a Homozygous Red Eyed Fly (X^RX^R)



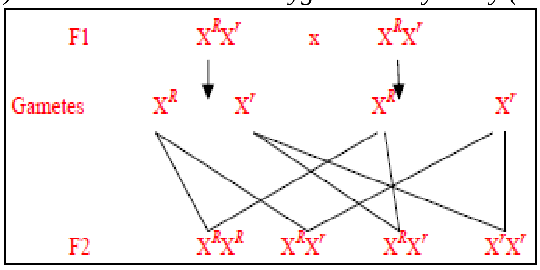
The off springs got in the above cross are 100% of homozygous red eyes.

2) CASE – 3.3.2. : When a Heterozygous Red Eyed Fly (X^RX^r) is crossed with Homozygous Red Eyed Fly (X^RX^R)



The off springs got in the above cross are 50% of homozygous red eyes and 50% of heterozygous red eyes.

3) CASE – 3.3.3. : When a Heterozygous Red Eyed Fly (X^RX^r) is crossed with a Heterozygous Red Eyed Fly (X^RX^r)



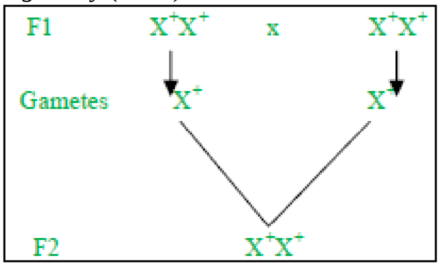
The off springs got in the above cross are 25% of homozygous red eyes, 50% of heterozygous red eyes and 25% of white eyes.

D. Based on Wing Shape

All the flies that were captured and sampled had oval wings except for one which had a vestigial wing.

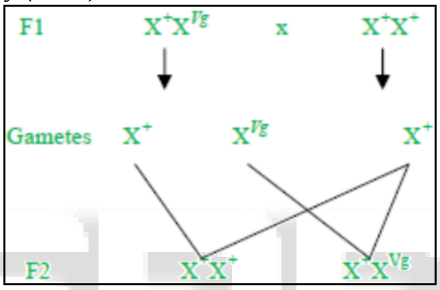
– Genetics

1) CASE – 3.4.1. : When a Homozygous Oval Shaped Winged Fly (X^+X^+) is crossed with a Homozygous Oval Shaped Winged Fly (X^+X^+)



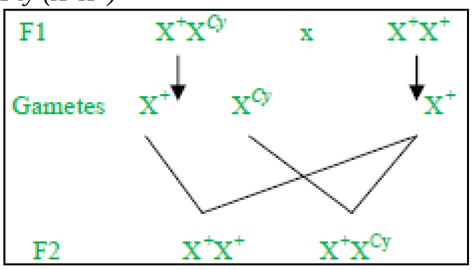
The off springs got in the above cross are 100% of homozygous oval shaped wings.

2) CASE – 3.4.2. : When a Heterozygous Oval Shaped Winged Fly (X^+X^{Vg}) crossed with a Homozygous Oval Shaped Winged Fly (X^+X^+)



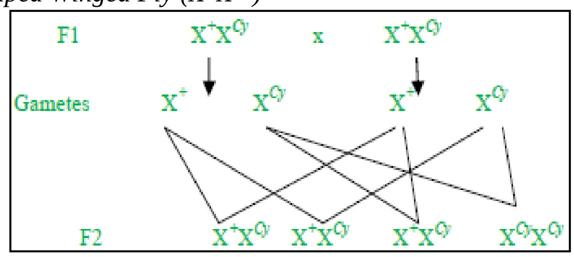
The off springs got in the above cross are 50% of homozygous oval shaped wings and 50% of heterozygous oval shaped wings.

3) CASE – 3.4.3. : When a Heterozygous Oval Shaped Winged Fly (X^+X^{Vg}) crossed with a Homozygous Oval Shaped Winged Fly (X^+X^+)



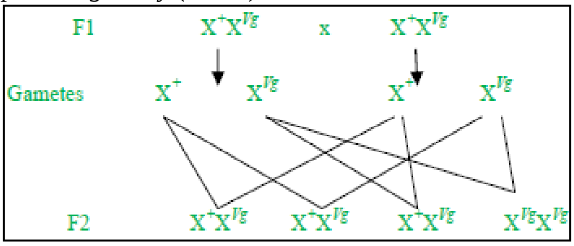
The off springs got in the above cross are 50% of homozygous oval shaped wings and 50% of heterozygous oval shaped wings.

4) CASE – 3.4.4. : When a Heterozygous Oval Shaped Winged Fly (X^+X^{Cy}) crossed with a Heterozygous Oval Shaped Winged Fly (X^+X^{Cy})



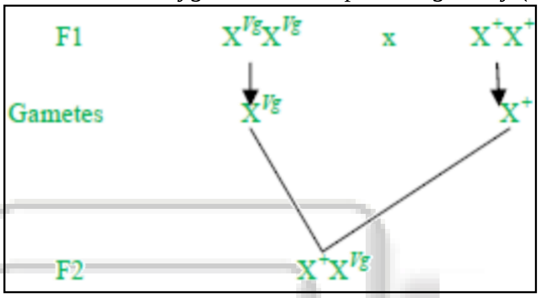
The off springs got in the above cross are 25% of homozygous oval shaped wings, 50% of heterozygous oval shaped wings and 25% of curled wings

5) CASE – 3.4.5. : When a Heterozygous Oval Shaped Winged Fly (X^+X^{Vg}) crossed with a Heterozygous Oval Shaped Winged Fly (X^+X^{Vg})



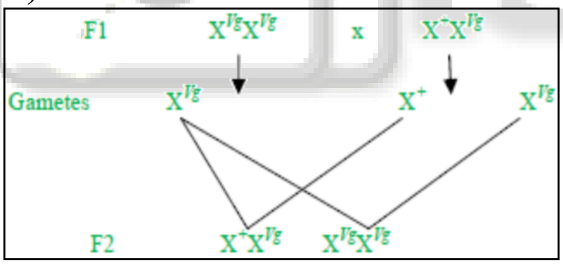
The off springs got in the above cross are 25% of homozygous oval shaped wings, 50% of heterozygous oval shaped wings and 25% of vestigial wings.

6) CASE – 3.4.6. : When a Vestigial Winged Fly ($X^{Vg}X^{Vg}$) crossed with a Homozygous Oval Shaped Winged Fly (X^+X^+)



The off springs got in the above cross are 100% of heterozygous oval shaped wings.

7) CASE – 3.4.7. : When a Vestigial Winged Fly ($X^{Vg}X^{Vg}$) crossed with a Heterozygous Oval Shaped Winged Fly (X^+X^{Vg})



The off springs got in the above cross are 50% of heterozygous oval shaped wings, 50% of homozygous vestigial wings.

IV. Discussion

Distribution of the flies, based on sex in this experiment showed that the male ratio was high at optimum room temperature without the exposure to direct sunlight wherein the movement of people in and out of the room was frequent. Female ratio on the other hand was found to be more at optimum room temperature with the exposure to direct sunlight wherein the frequency of the movement of people is not much as compared to the condition wherein there was no exposure to direct sunlight. The sex ratio at optimum room temperature with exposure to direct sunlight along with food waste did not show much difference, but, the flies collected at this condition gave one mutant. Hence, we can expect to get mutants in area wherein the frequency of movement of people is restricted and the place has food waste.

From the above results, it is evident that we can find the flies based on sex and mutants can be collected in the above mentioned conditions. The results that are obtained from the above experiment might play a significant role for positive selection in obtaining Drosophila with different traits. For large population or required sex type, sizes and even in obtaining mutant flies with different traits, this experiment might be helpful. Investigations like these offer promising opportunities to address classic questions concerning the nature and impact of selection in shaping levels and patterns of genetic variation.

The experiment may prove essential to estimate parameters of selection and demography jointly, to have plausible inferences regarding either of these processes. The expansion of population genomic surveys to include geographic sampling on the relevant demographic and ecological scales allows even broader population biological investigations.

Drosophila has many unique advantages which makes it an important model for the upcoming researches. Many sophisticated manipulations can be made in these flies which cannot be done in other organisms. The physiological functions taking place in the sleep mechanism of the fly and the human body is almost similar. Hence, this fly can also be used to study the sleeping mechanism in the vertebrates [13].

V. Conclusion

The merger of the deepening knowledge of structures and functions of genomes with full descriptions of genomic variation in natural populations represented by our analyses is an important expansion of the scope and approach of biological research.

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