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Conduction and Observations of change in Genome after performing Mutagenic Experiments on different Drosophila Generations

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Abstract
The aim of this study is to see the differences in the behavior of the flies after exposure to UV mutation for several generations. The morphological differences are seen under a stereomicroscope and the DNA extracted is run on an agarose gel under the influence of electricity. Mutations in fruit flies are used to study various disorders like Alzheimer's and cancer.
Keywords: *Drosophila melanogaster, model organism, UV mutations, DNA extraction, stereomicroscope.*

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I. INTRODUCTION

Drosophila is a genus of small common fruit flies. In appearance the wild-type are typically pale-yellow to red-brown to black and the eyes are red. The life cycle includes 4 stages namely, egg, larvae, pupa and adult. One of the species, Drosophila melanogaster is extensively used as a model organism in genetic research. It was first used in genetic experiments by Dr. Morgan from as early as 1909 [3]. A few years after the completion of the human genome project, comparisons of the human genome to that of fruit flies were done, only to reveal that there are high homologies between the two sequences [3,6]. This information along with its short life cycle, being easily anesthetized, sexual dimorphism is present making it easy to differentiate between male and female flies, the care and culture requires minimum cost, produces hundreds of genetically similar off-springs, many genes which are essential for development of the fly are also are also critical for human growth and development [1]. The Drosophila genome is approximately 180Mb [3].

Embryos found in the earlier stages store large amounts of DNA replication enzymes [3]. These embryos can be applied to study the fundamental developmental biology which examines the formation of patterns, determines the fate of the cells, organogenesis, etc. The research on larvae stage includes studying about developmental and physiological processes. Contained within the larvae are imaginal discs, made of undifferentiated epithelium which produces the future adult fly external features such as wings, antenna, compound eyes and legs [1]. These external features can be mutated without causing total death [2]. Drosophila flies have a surprising mechanism for growth in certain tissues. DNA replication proceeds without cytokinesis, resulting in polyploid nuclei in huge cells. In a lot of the tissues, the chromosome copies are retained altogether, these have thousands of DNA strands and are called polytene chromosomes. These help in high level of gene expression, acts as binding sites for RNA pol II for replication initiation. Polytene chromosomes are large enough to be visible under a standard light microscope. In Drosophila the polytene chromosome can be found in the salivary glands of the larval stage.

The mutations in the organisms are called as "minutes" [10]. These mutations can be caused by a wide range of mutagens, most commonly UV-lights and heat are used for research purposes. Another advantage of using Drosophila as a model organism is that the mutations show the altered phenotypes. White eye mutation is due to the absence of the pigments, peridines and ommochromes, associated with the red and brown eye colours respectively. This mutation causes shorter lifespans, reduced ability to climb, males were less successful in terms of mating.

Drosophila has contributed to being the model organism of various extensive research studies such as, Aging, Cancer, Alzheimer's, Autoimmune diseases, Congenital heart problems, Musculoskeletal disorders, Huntington's Disease, Parkinson's Disease, etc. as about 75% of the genes responsible for human diseases are homologous in the flies [5]. An accelerated rate of aging which is characterized by Werner Syndrome, is due to mutations in the gene WRN causing hinderance in repair of DNA damage. Mutations in the WRN gene in Drosophila also shows accelerated aging, shorter life cycle, increase in susceptibility to tumor, muscle

degeneration. It is reported that around 90% of human cancers are originated from the epithelial [17]. The flies have been significantly contributing to organotypic cancer research. The biomarker for Alzheimer's is Aβ42, which is a 42 amino acid proteolytic product, accumulation of which in the form of extracellular plaques plays a part in the process of neurodegeneration. This aggregation can be easily determined in Drosophila [8].

1.1 Capturing Drosophila

Banana peels are mashed and kept in a clean flat bottom beaker. The beaker is covered with a plastic cling wrap and few holes are made on the wrap. The beaker is kept in an environment where flies would come and try to enter the cling wrap. The best area would be near a window, so the heat could also 'spoil' the mashed banana peels further.

Figure 1: Mashed Banana kept in a clean flat bottom beaker and covered with a cling wrap.

1.2 Preparation of Corn Media

For 1 liter preparation, 80g of Corn flour, 20g of D-Glucose, 40g of Sugar, 8g of Agar, 15g of Yeast powder are weighed and 4ml of Propionic acid, 0.6ml of Orthophosphoric acid is measured and kept aside. 1 Liter of the water is taken and heated at 60°C, corn flour is added and mixed thoroughly to remove clumps. The other substances (Sugar, Glucose, Yeast powder and Agar) are added in succession and mixed together. After the mixture is mixed, the media is cooled down. Propionic acid and Orthophosphoric acid are added and mixed thoroughly.

Figure 2: Corn Media prepared for growing *Drosophila* generations

1.3 Growing various generations

Once there is sufficient number of eggs and larvae in the beaker, transfer them carefully without harm to the corn media in conical flask. Cover the flask with a cotton plug and leave for days.

1.4 DNA Extraction

Keep 25 each of eggs, larvae and flies in 3 different tubes kept on ice. 250µl of solution A (0.1M Tris HCl pH 9.0, 0.1M EDTA, 1% SDS) is added to the tubes. Homogenize the tubes while keeping the mortar on ice pack. Incubate the solution at 70C for 30 minutes. 35µl of Potassium Acetate is added and mixed my tapping gently, incubated on ice for 30 minutes. The mixture is centrifuged for 15 minutes at 13000 rpm and the supernatant is transferred to a different tube without disturbing pellet or any precipitate. Addition of 1 vol. of Phenol Chloroform followed by thorough shaking. Centrifuge for 5 minutes at 13000 rpm. The steps from transfer of supernatant to centrifugation is repeated. The new supernatant is taken into a new tube and 150µl of

Isopropanol is added and mixed thoroughly. Spin for 5 minutes at 10000 rpm and discard supernatant without disturbing the pellet. The pellet is washed with 1ml 70% Ethanol and centrifuged at 13000 rpm for 5 minutes. Pellet is air dried and resuspended in 100µl Ethanol. The solutions are run in gel in different wells.

II. RESULT AND DISCUSSION

The wild-type and mutant stages are observed under the stereomicroscope for physical traits and differences. The morphology before mutation included yellow-beige eggs, wild type flies (red eyes) and larger egg size whereas post mutation morphology included eggs having a slight brown color, mutated flies (white eyes) and a smaller egg size. Some other observations include larvae movement before UV light exposure was faster compared to those exposed to UV light, flies had a normal flight movement as compared to a normal but erratic movement after exposure, hatch time was delayed upon exposure to UV.

Figure 4: Egg, Larvae and Fly under stereomicroscope before UV light exposure respectively

Figure 4: Egg, Larvae and Fly under stereomicroscope after UV light exposure respectively

III. CONCLUSION

Drosophila is an extremely versatile and cost-effective model which can provide a plethora of information and much more undiscovered. It can be used both, as a small-scale model and large-scale model. It may be small in size but the extensive research done on it daily is impressive and remain as the top choice for a model organism model.

Even after a hundred years, *Drosophila* are still widely used as a model organism for genetic studies. As times will evolve, so will the diseases yet to come in the future and hence would the research on them using fruit flies. The organism has provided with enough information and evidence to show its potential regarding the diseases in the human body. In the recent years, scientists are also intrigued about the molecular and cellular basis of the flies' mannerisms. This could potentially give us insights into its nervous system. Annually, a global conference regarding *Drosophila* is conducted by the Genetics Society of America (GSA). Likewise, there are hundreds of institutes and organizations conducting and participating in such events to understand the path where we stand on with advanced technology and fruit flies.

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