

Comparative Mutagenic Effects of Laboratory Dyes on *Drosophila melanogaster*

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Abstract

Mutagenic dyes are risk for the health of workers, consumers and for the environment. Mutagenicity of chemical present on textiles could be seen as a major clue towards carcinogenic activities of these chemicals. This study was conducted to determine the comparative mutagenic effects of laboratory dyes on Drosophila melanogaster. Bioaccumulation of the laboratory dyes used, malachite green, safranin, crystal violet and methylene blue were allowed to induce mutations from the parental generations up to the second filial generations of D. melanogaster with constant exposure to the laboratory dyes used at 1% and 5% concentrations. Results of the study showed that constant exposure of the fruit flies at 1% and 5% concentrations of the laboratory dyes used increased the rate of mutations on the color of the eyes, body color and wing shapes of D. melanogaster. Chi-square statistic at 0.05 level of significance showed no significant difference on the incidence of mutation on the fruit flies phenotype. Thus, this showed that constant exposure to the laboratory dyes at varying concentrations could induce mutation.

Keywords: *Drosophila melanogaster, laboratory dyes, mutagenic effect*

1. Introduction

Colorants (dyes and pigments) are important industrial chemicals. Azo dyes are the most important group of synthetic colourants that are extensively used in textile, pharmaceutical and printing industries. A wide variety of azo dyes with anthraquinone, polycyclic and triphenylmethane groups are being increasingly used in textile dyeing and printing processes. They pose toxicity (lethal effect, genotoxicity, mutagenicity and carcinogenicity) to aquatic organisms (marine life, microorganisms, etc.) as well as land animals [14]. Waste water from textile industry is a complex mixture of many polluting substances ranging from organochloride-based waste pesticides to heavy metals associated with dyes and dyeing process [3]. One of the important factors in the evaluation process of textile dyes is the knowledge of mutagenic effects. Mutagenic dyes are a risk for the health of workers and consumers and for the environment. Workers can be exposed to high concentrations when preparing the recipes for the dyeing process. Mutagenicity of chemicals present on textiles can be seen as a major clue towards carcinogenic activities of these chemicals. Different researchers have identified mutagenic effects of textile dyes [7]. There are some dye products tested that were selected for mutagenicity testing in the Ames test with *Salmonella typhimurium* OECD 471. The results confirmed previous findings that these dye products on the market which are not sufficiently tested and show mutagenic effects in *in vitro* tests. It has been reported that mentil yellow caused testicular damage in gametogenic element to arrest spermatogenesis in guinea pigs, rats and mice [5]. The effluents from dyeing industries were mutagenic and contained various types of dye [9]. There is also an increase in the incidence

of aberrant crypt in the colon of rats exposed to the dye sample as an early biomarker of carcinogenesis [8].

Due to the widespread use and potential carcinogenicity of certain dyes, there has been a growing concern in evaluating the risks associated with laboratory dyes used staining. Improper use and disposal of dyes can lead to serious consequences in terms of human health. Assessment of the mutagenicity of dyes is therefore of utmost concern. The use of biological animals has been developed to detect mutagenic substances. They have played important roles not only in screening chemicals but also provide useful information for evaluating the genetic effects of chemicals on human.

One of the assays used in somatic mutations is the use of *Drosophila melanogaster*. Fruitflies represent an exemplary investigatory tool for studying Genetics in research laboratories. They are inexpensive, easy to rear, have a short life-span (15-20 days) are easy to mate and require very little space or special equipment [1, 10]. Several factors make the assay in *D. melanogaster* advantageous. Fruit flies are eukaryotic organisms that are capable of enzymatically activating promutagens and procarcinogens. It has four pairs of chromosomes and approximately 14,000 genes, in comparison to the estimated 70,000 genes found in the human genome [4]. *Drosophila's* manageable genome size and the fact that its mutations are well-known are two more factors that make it a likely candidate for the assay [11]. Thus, comparative mutagenic of laboratory dyes in this study was tested.

2. Methodology

2.1. *Drosophila melanogaster* Culture. *Drosophila melanogaster* were collected by preparing a culture medium of mashed banana and were allowed the fruit flies to settle. This served as the starting culture of the progenies which served as the parental stock. The individuals were maintained in wide mouth bottle of the same sizes covered with stockings maintained at room temperature. The emerged adults were etherized, counted and morphologically analyzed through their body color, wing shape and eye color. Observation was aided with magnifying lens and dissecting stereomicroscope.

2.2. *Drosophila melanogaster* Mutation. There were four laboratory dyes used, malachite green, crystal violet, safranin and methylene blue as the mutagens. At the starting culture, the 1%, 5% and 10% concentrations were used to determine the lethal dose for each setup. These were mixed with 100g mashed banana separately. From the initial culture of *D. melanogaster*, the second generation was used as the parental flies to ensure purity of the experimental subject. A bioaccumulation study was performed for two generations. Twelve virgin couples of females and males emerged from the stock were used to generate the first generation; for the other generations, 12 new couples were picked up among the animals emerged on the fifth day after the first adults emerged. The females were allowed to lay eggs for ten days. During the experiment, all the adults were also analyzed morphologically (body color, wing shape and eye color). Observation was aided with magnifying lens and compound stereomicroscope. The fruit flies were constantly exposed to the assigned mutagen at 1% and 5% concentrations until each group had reached the second filial generation. Morphological and phenotypic variations among the progenies were carefully recorded on the eye color, wing shapes and body color.

2.3 Data Analysis. Descriptive statistical method using the frequency tally was employed to analyze and summarize the wild-type and mutant flies in each generation. Furthermore, mutant in each eye color, wing shape and body color were separately categorized in each

generation. Inferential statistical method using Chi-square analysis was used to statistically analyzed the data in the four dyes used, concentration and phenotypes of *D. melanogaster* set at 0.05 level of significance and analyzed using SPSSv11.5.

3. Results and Discussion

Figures 1 and 2 show the frequency of fruit flies produced in the different concentrations of the laboratory dyes used in the first and second filial generations.

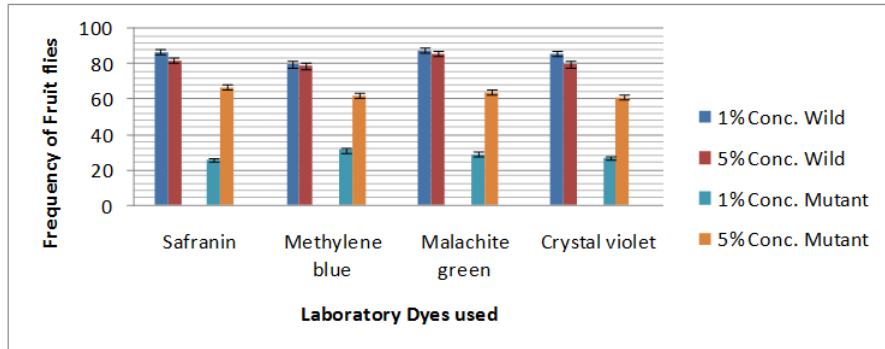


Figure 1. Frequency of First Filial Generation of Wild-type and Mutant *D. melanogaster*

In the first filial generation progenies exposed to the different laboratory dyes used, the 1% concentration of malachite green has the highest produced wild-type fruit flies while 5% concentration of methylene blue has the lowest wild-type fruit flies produced. In the production of mutant progenies, the 5% concentration of safranin has the highest number of mutants produced while the 5% concentration of crystal violet has the lowest number of mutant progenies produced.

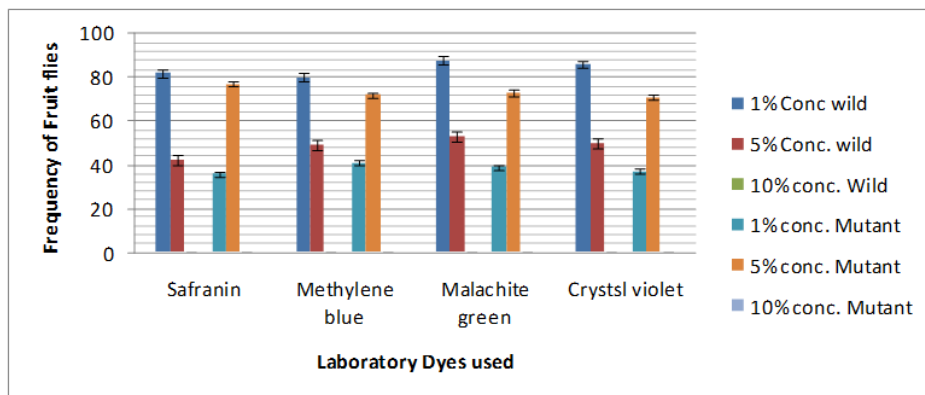


Figure 2. Frequency of Second Filial Generation of Wild-type and Mutant *D. melanogaster*

In the second filial generation progenies, the 1% concentration of malachite green has the highest number of wild-type fruit flies produced while the 5% concentration of safranin has the lowest number of wild-type fruit flies produced. The 5% concentration of safranin has the highest frequency of mutant fruit flies produced while the 5% concentration of crystal violet has the lowest frequency of mutant fruit flies produced.

Figures 3 and 4 show the frequency of mutations in the four laboratory dyes used in the different body parts of *D. melanogaster* in the first and second filial generations respectively. The mutations were recorded on the basis of the color of the body, color of the eyes and structure of the wings of the fruit flies. In order to determine if mutation occurred in the body structures of the fruit flies, these were compared with the wild-type fruit flies. The wild-type fruit flies possess brown bodies, red eyes and full-sized wings. For the body, yellow bodies were recorded. For the eyes, vermilion eyes, sepia eyes, garnet eyes and brown eyes were observed. For the wings, cut wings, dichaeete wings, curly wings and vestigial wings were observed.

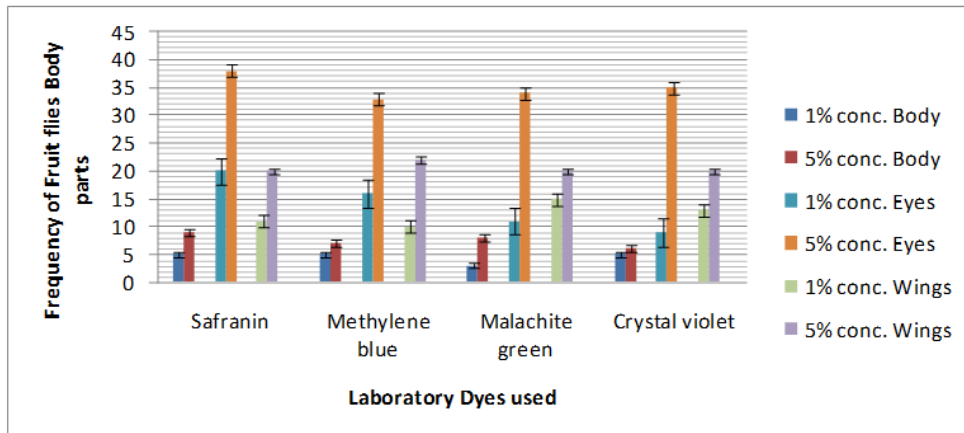


Figure 3. Frequency of First Filial Generation Mutation

In the first filial generation mutation, the 5% concentration has the highest frequency of mutation in the body, eyes and wings of *D. melanogaster*. Moreover, the 1% malachite green has the lowest frequency of mutation in the body while the 5% concentration of safranin has the highest frequency of mutation. The 5% concentration produced a yellow body in contrast to the brown body of the wild-type. The vermilion eyes, sepia eyes, garnet eyes and brown eyes were exhibited in contrast to the red eyes. The cut wings, dichaeete wings, curly wings and vestigial wings were observed in contrast to the full-sized wings. In the eyes, the 1% concentration of crystal violet has the lowest frequency of mutation while the 5% concentration of safranin is the highest. In the wings, the 1% concentration of safranin has the lowest frequency of mutation produced while the 5% concentration of methylene blue is the highest.

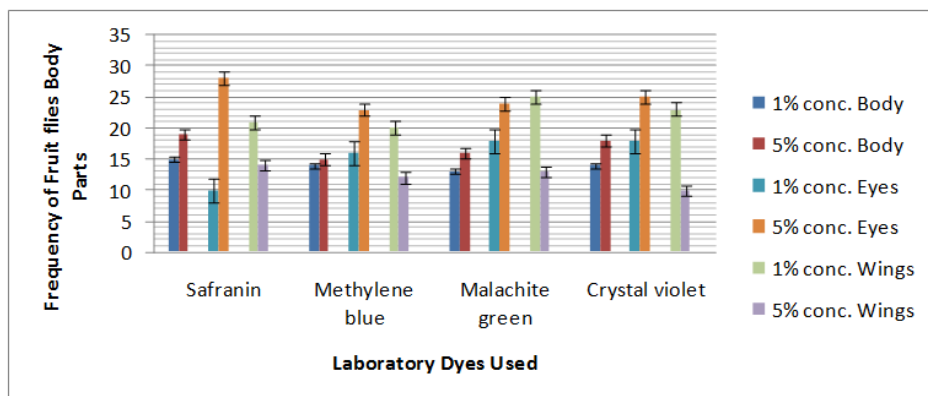


Figure 4. Frequency of Second Filial Generation Mutation

In the second filial generation mutation, the 5% concentration still has the highest frequency of mutation in the body, eyes and wings of *D. melanogaster*. In the body, the 1% concentration of malachite green produced the lowest frequency while the 5% concentration of safranin has the highest frequency produced. In the eyes, the 1% concentration of safranin produced the lowest frequency while the 5% safranin has the highest frequency. In the wings, the 1% concentration of safranin produced the lowest frequency while the 5% concentration of safranin has the highest frequency produced.

Table 1 shows the chi-square statistical test of the four laboratory dyes used. In the laboratory dyes and their respective concentrations used, there is a significant difference on the effects in inducing body or somatic mutations in *D. melanogaster*, chi-square value =8.340 (df=3), 0.39<0.05 and chi-square value=79.381(df=2),< 0.05 respectively. This supports the data in Figures 1 and 2. The 5% concentration is more potent to produce mutations in the body parts of *D. melanogaster*. However, there is no significant difference in the phenotypes produced between the wild-type and the mutant *D. melanogaster*, chi-square value=0.005 (df=1), 0.943> 0.05. This may imply that the frequency of mutant phenotypes produced is comparable to the frequency of the wild-type phenotype of *D. melanogaster* regardless of the concentration and the type of laboratory dye used to expose to the fruit flies.

Table 1. Chi-square Statistics for *D. melanogaster* Mutation

Concentration	Dye	Phenotype	
Chi-square	8.340	0.005	79.381
Df	3	1	1
Asymp. Sig.	0.039	0.943	0.00

P < 0.05 is significant.

The discharge of azo dyes on the bodies of water presents human and ecological risks, since both the original dyes and their biotransformation products can show some toxic effects, mainly causing DNA damage [2]. The azo dyes constitute an important class of environmental mutagens because they are widely used by different industries and used for coloring purposes that is discharged into the environment. This is indicated by the mutagenic and toxic effects of various dyes at different concentrations on *D. melanogaster*.

The mutagenic effects of laboratory dyes that result to mutant phenotypes are a form of induced mutations [6]. These types of mutations result from the influence of extraneous factors. It may be the result of either natural or artificial agents such as various forms of radiation, numerous natural and synthetic chemical agents. Furthermore, induced mutations arise from DNA damage caused by chemicals and radiation [6]. Mutations may or may not bring about a detectable change in phenotype [6]. The extent to which a mutation changes the characteristics of an organism depends on where the mutation occurs and the degree to which the mutation alters the function of the gene product.

One study analyzed the mutagenic, cytotoxic and genotoxic effects of the azo dye CI Disperse Blue 291, and the results clearly showed that this azo dye caused dose-dependent effects, inducing the formation of micronuclei (MN), DNA fragmentation and increasing the apoptotic index in human hepatoma cells [13].

4. Conclusions and Recommendations

The result of the experiment identified that the four common laboratory dyes used are safranin, methylene blue, crystal violet and malachite green have comparable mutagenic effects on the overall phenotype of *D. melanogaster*. The four dyes used had mutagenic effects on the body color, yellow body; eye color, vermilion eyes, sepia eyes, garnet eyes and brown eyes; and on the wings, cut wings, dichaeete wings, curly wings and vestigial wings at 1% and 5% concentrations. Mutagenic effects should also be examined at the molecular level to determine if there are any genetic mutations that are not readily observed in the phenotype. The 1% concentration may already be used for genetic studies involving mutation.

Acknowledgements

I would like to acknowledge my Doctor of Dental Medicine 2-A (2011-2012) students of Iloilo Doctors' College for the help in rearing and monitoring the fruit flies culture during the experiment in Genetics Laboratory.

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