PCR-analysis of direct radiation-induced mutations of *white* gene in *Drosophila melanogaster*

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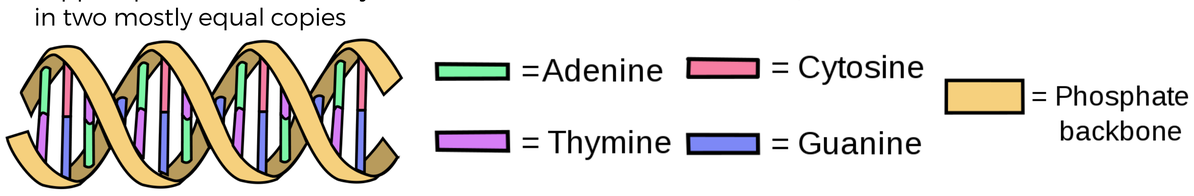
This study is showing results of molecular-biology analysis for gamma- and neutron-induced mutations in Drosophila melanogaster using range of radiation doses.

Brief introduction to genetics

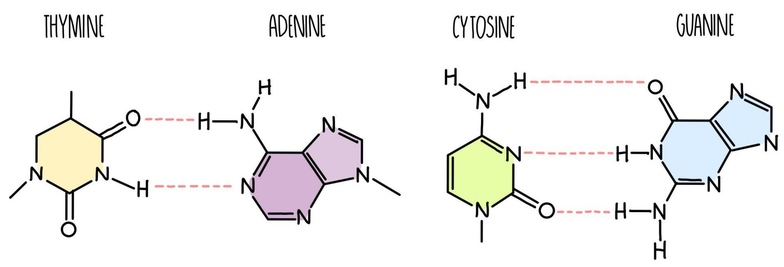
Here you can find some key terms and other info, essential to understanding genetic studies. Explanations here are simplified for better understanding.

DNA – complex organic molecule serving as a "program" for organism development. Advanced organisms like human, cats, flies, pines, sea cucumbers, mushrooms etc. have DNA present in form of chromosomal sets. In fact single chromosome consists of single DNA molecule and some support proteins. Commonly there are two sets of chromosomes, thus each DNA molecule exists in two mostly equal copies



Gene – region of DNA responsible for some organism feature (ranging from skin color, to digestive proteins production)

Nucleotides – sub-molecule "bricks" used for DNA construction. They work similar to programming language. Organism cells interpret this "source code" by specific set of rules producing organism features via making proteins.

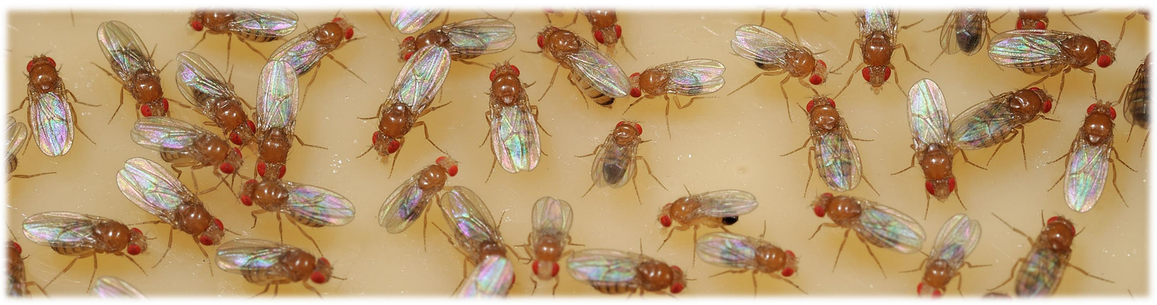


Mutations – changes to nucleotide sequence happened due to variety of reasons. Most mutations are neutral, lesser faction are harmful, and even lesser are "useful"

Note: Don't mix up terms "genetic code" and nucleotide/DNA sequence. Genetic code is set of rules for DNA interpretation, while DNA sequence is just an order of nucleotides, like an order of symbols in normal sentence.

Methodology

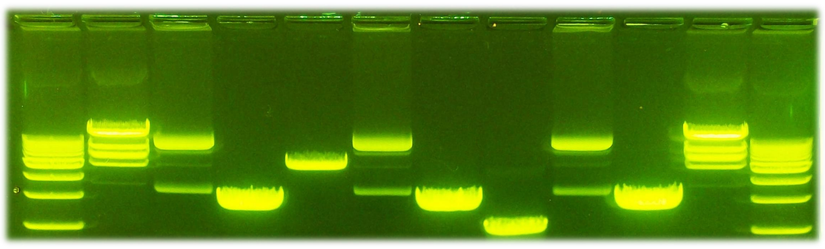
Subject for this study is *Drosophila melanogaster*. It is small fruit fly species commonly used for genetic research.



Study was performed for gene *white*, which is responsible for eye coloration of fly. When this gene is dysfunctional due to mutation, it makes drosophila eyes white colored. Compared to wild type red eye coloration this makes it easy-to-spot mutation. Thus it is good option for different studies of multiple mutations in a same gene.

Collection of mutants was made by series of drosophila males expositions to gamma-rays and neutrons ranging from 5 to 40 Gy. As result big collection of mutant lines was formed, from which 54 different variants were used for this study. Simultaneously statistics analysis was performed to estimate mutation frequency among offsprings of irradiated males.

Research itself is divided in several steps, here will be presented the first few completed.
DNA was extracted from flies with several technics all of which result in purified DNA solution.



Visualization of PCR results with electrophoresis method.

Lines represent different DNA factions.

Next we have performed PCR analysis. PCR is a method used to amplify number of copies of target DNA region for use in further studies. In this case PCR was used to detect presence or absence of fragments relying on PCR not working when major changes occur at specified locus. Whole *white* gene was divided to 7 overlapping fragments and analyzed in such way.

Mutations were classified to four types which are:
- PCR + (no fragments loss though mutant phenotype)
- Loss of single fragment
- Loss of several neighboring fragments
- Loss of several non- neighboring fragments (cluster)

Results

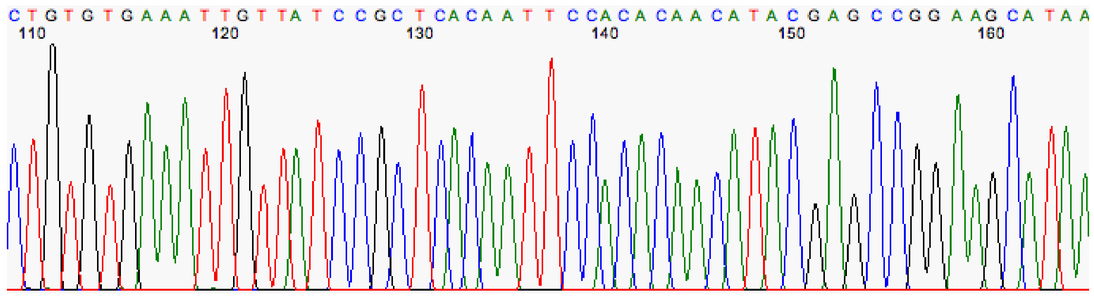
- PCR+ mutations are mostly characteristic for γ-rays
- One fragment loss is less present for γ-rays than for neutrons,
- Mutants with extended deletions arise more rarely after γ-rays than those after neutrons,
- Cluster mutations occur with similar frequency for γ-rays and neutrons.
- Linear dependency between dose and mutation frequency

Thus according to our results one-hit mutational events can be induced as a result of different introgenic changes at the DNA level at differing rates.

Mutation type	Gamma-rays (%)	Neutrons (%)
PCR +	82,9	33,3
Loss of single fragment	12,1	33,3
Loss of several neighboring fragments	2,4	26,6
Loss of several non-neighboring fragments	2,4	6,6

Further work

This study should be considered work-in-progress. There are two more things to widen and deepen data. First of them is including more drosophila mutant lines into research either from existing collection or from inducing new ones. And the second thing that currently in progress is sequencing analysis (it is another method allowing to estimate exact nucleotide sequence of nucleic acids)



There are several different techniques to perform nucleotide sequence analysis, for this project Sanger sequencing is used. This method is based on making DNA fragments differing in size by one nucleotide and marking them with fluorescent dye. Then molecules are separated in electric field by size and factions fluorescence is measured

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Image sources

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