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Genetic Study of Mutations Induced by 3000 r Gamma Rays in *Drosophila simulans*

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Abstract: Experiment was conducted on *Drosophila simulans* to isolate and characterize mutants induced by 3000 r gamma radiation. Irradiated young males (2-3 days old) were crossed to controlled virgin female flies. F₁, F₂ and F₃ generations were examined to identify visible mutations. A total of six induced mutant flies were isolated from the irradiated strains. Four out of the six mutants were cultured successfully and their genetic pattern was studied, while the remaining two mutants could not be cultured due to unavoidable circumstances. The cultured mutants were composed of one sex-linked recessive, and three autosomals (two autosomal recessive and one autosomal dominant). No spontaneous mutant fly could be found in controlled strain maintained for comparison.

Key words: *Drosophila*, gamma-rays, chromosome, mutant, X-rays

Introduction

Mutation is an important attribute of genetic material and is a universal phenomenon occurring in all organisms. It provides raw material for hereditary variations that form chief basis of organic evolution. Mutations may integrate in the population and, through sexual reproduction, are propagated from generation to generation. The expression of alternative phenotypes based on gene mutations, has enabled geneticists to unveil the physical and chemical basis of heredity. Without mutation, all genes would exist in only one form. Alleles would not exist, and thus genetic analysis would not be possible. Most important organisms would not be able to evolve and adapt to environmental changes. Knowledge of mutation and mutagenesis has played an instrumental role to gain insight into the structure of individual gene.

Various species of genus *Drosophila* (fruit fly) have been used as excellent organisms for spontaneous as well as induced mutation analysis in different parts of the world. Such mutation studies have added tremendous information to understand the phenomenon of organic diversities, and thereby, laid the foundation of modern genetics. Mutations cause some detectable phenotypic changes for their presence to be recognized. The effects of mutations on phenotype range from alterations so minor that they can be detected only by special genetic or biochemical techniques to gross modifications of morphology to lethals (Gardner *et al.*, 1991). Muller (1927) was the first to demonstrate the mutagenic effects of X-rays and reported a high rate of mutation by heavy doses of irradiation in *Drosophila*. Gamma radiation, from radium, was reported to induce a high frequency of mutation in barley (Stadler, 1928). Doses of gamma rays are about 3 times less effective than X-rays in inducing mutations (Timofeeff-Ressovsky, 1934). There is a linear relationship between radiation dose and dominant visible mutation rate in *Drosophila melanogaster* (Ives, 1959). X-rays can act directly or indirectly on the genetic material, but either way, the types of mutational events remain the same. Chromosome breaks are induced, and reconstitution produces new structures such as inversion, translocation, deletions, and duplications or intragenic mutations are induced, that involve base pair substitutions or the addition and deletion of base pairs (Jenkins, 1975; Lindsley and Zimm, 1992). Romanova *et al.* (1985) analysed visible mutations induced by X-rays and ethylmethane sulfonate in mature spermatozoa of *Drosophila melanogaster*.

Drosophila simulans, used in the present visible mutation analysis, is a member of *melanogaster* species group. These

flies can be cultured on maize meal medium under the laboratory conditions. Induced mutations in *Drosophila simulans* have been studied. This fly has been found as an excellent material for genetic studies.

Materials and Methods

Drosophila simulans flies were collected in wild by putting the banana and orange fruit baits in shady place in the orchard at University of the Punjab, New Campus, Lahore and sorted at temperature 24°C. The flies were allowed to grow on standard maize meal medium.

Preparation of maize meal medium: To prepare the medium, 125 g dry maize flour was soaked in 250 ml of water and was constantly stirred. Powdered agar, about 18 g, was put in a pan with 875 ml of tap water, gently boiled and then 18 g baker's yeast was added to it, again boiled with constant stirring. Then 125 ml molasses was added to this mixture and brought to the boiling point. The previously soaked maize flour was then poured in this boiling mixture. Just when the maize meal medium was fairly thick, 5 ml propionic acid was added. The medium was made sufficiently viscous and poured into sterilized culture bottles. The bottles were stored in refrigerator until ready to be used. It was enough for 25 culture bottles.

Irradiation treatment: For irradiation treatment 45 un-etherized young (2-3 days old) male *Drosophila simulans* flies were exposed to 3000 roentgens (r) of gamma radiation in COBALT⁸⁰ GAMMA CELL (220 Canadian make with the radiation chamber 21 x 155 mm). In the cell chamber flies within the bottles were kept approximately at the distance of 10 cm from the target. The exposure time was 27.0 seconds per 1000 r gamma radiations.

Identification and isolation of mutants: The irradiated males were crossed to controlled virgin females, on the same day. The F₁, F₂ and F₃ generations were examined to identify the visible mutant flies. To identify and isolate mutant flies, the phenotypic characteristics namely, sex, body size, eyes, head, thorax, abdomen, bristles, wing shape, wing venation and genitalia were examined under Binocular microscope. In each culture bottle 3 pairs of *Drosophila simulans* flies were kept for 3-4 days and then the flies were released. The new flies of F₁ generation were counted and examined under the Binocular microscope to identify autosomal and sex-linked dominant mutations for three successive days until there were no more

Muhammad Hassen: Induced mutants of *Drosophila*.

flies emerging. Then F₂ and F₃ generations were obtained from F₁. The controlled culture was also grown parallel to irradiated flies for the sake of comparison. In winter season electric heater was used to maintain the optimum temperature (25° ± 2°C), while for the major part the flies were maintained at room temperature. The drawings of visible mutations were made through Camera Lucida on the ordinary drawing paper. Afterwards, these drawings were re-drawn on the fine paper and inked with Indian ink.

Results and Discussion

Mutants were observed and isolated from the irradiated culture. Genetic pattern of cultured mutant strains was studied minutely to the best possible extent. No spontaneous mutants could be noticed in the controlled stock grown for comparison. The mutants, for the sake of convenience, have been divided into two categories:

Cultured Mutants

Crossveinless wings: This mutant was observed and isolated from irradiated stock of *Drosophila simulans*. This mutation has been traced to be recessive and sex-linked. Posterior crossvein is reduced mostly in both wings (Fig. 1 and 1a). Anterior crossveins are unaffected. Both sexes are viable and equally fertile. Penetrance of this mutation is incomplete. It is assumed that the crossveinless mutation has been produced by the ionizing effect of gamma-rays on the specific gene required for the development of posterior crossvein in the wings. The gene for anterior crossvein has remained

unaffected by gamma radiation. Consequently, anterior crossvein has developed normally in both wings. Crossveinless allele is located at 13.7 map position on X-chromosome of first linkage group in *Drosophila melanogaster* (Gardner *et al.*, 1991).

Ski wings: This recessive autosomal mutant was identified and isolated from irradiated strains of *Drosophila simulans*. Its wings are turned upward at the tips. Male and female flies are equally affected by this mutation. Other parts of body are unaffected. Both sexes are viable and fertile. Penetrance of the mutation is incomplete. This mutation seems to be parallel to ski wings in *Drosophila ananassae* reported by Moriwaki (1934). X-rays generate highly reactive free radicals from water, and these free radicals react with DNA to alter its structure (Jenkins, 1975). Similarly gamma rays have caused the formation of wings mutation in case of *Drosophila simulans*.

Erect bristle: Erect bristle flies were also observed and isolated. Genetically, this mutation has been found to be dominant and autosomal. These mutant flies have one dorsocentral bristle erect. Other bristles are unaffected. Male and female flies are equally affected. Both sexes are viable and fertile. Penetrance is incomplete. It corresponds to erect bristle in *Drosophila melanogaster* as noticed by Neel (1942). This mutation might have been produced by the ionizing activity of gamma radiation.

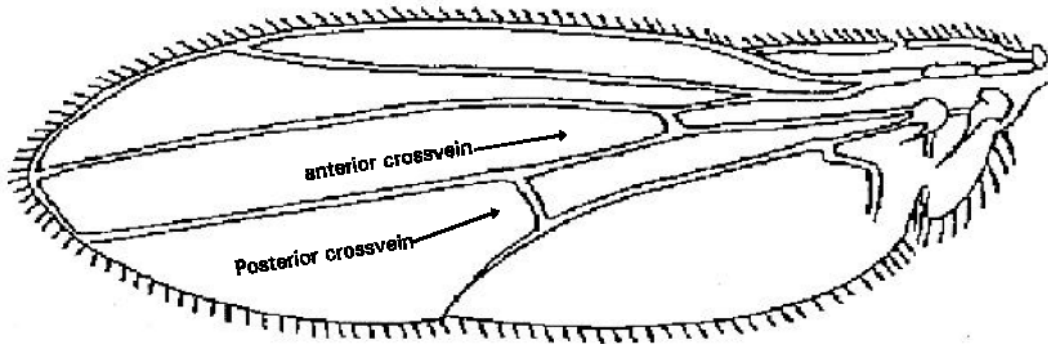


Fig. 1: Wing venation (wild type)

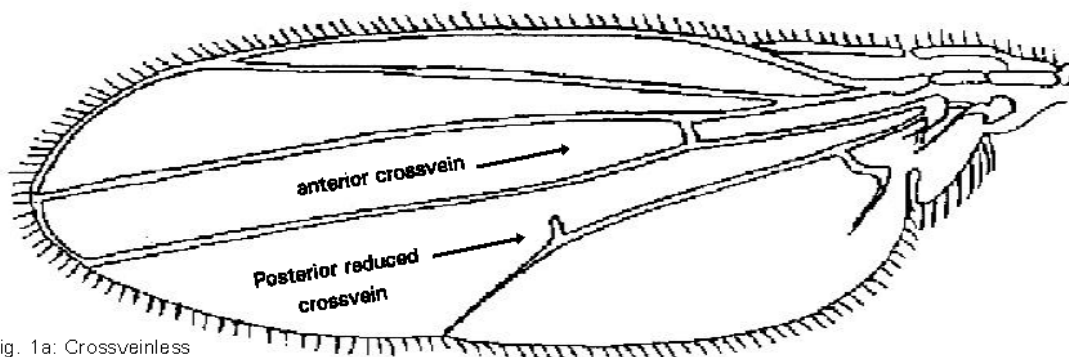


Fig. 1a: Crossveinless

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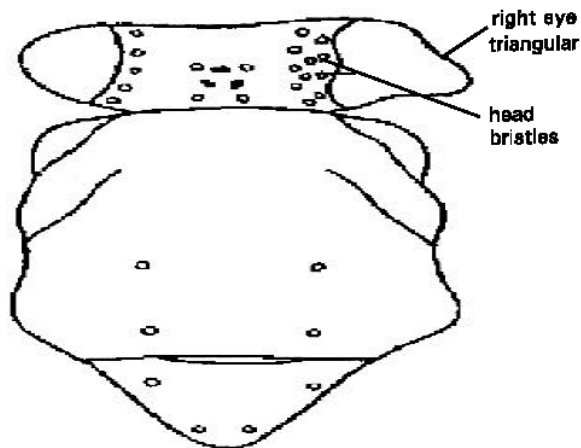


Fig. 2: Triangular eye

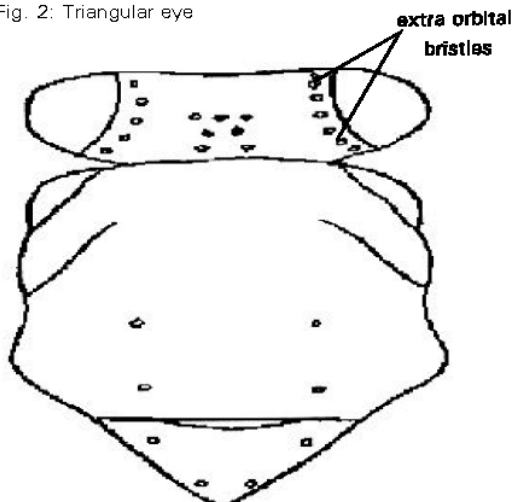


Fig. 3: Extra orbital bristles

Short bristles: This is a recessive autosomal mutant fly. It was identified and isolated from irradiated stock of *Drosophila simulans*. Bristles are slightly thin and short. Body size is slightly larger. Wings are broad. Both sexes are equally viable and fertile. Penetrance of this mutation is incomplete. In *Drosophila melanogaster*, the mutant allele short bristle is located at 54.8 map position on autosome of second linkage group (Strickberger, 1985). This mutation could also be attributed to the ionizing effect of gamma radiation.

B. Uncultured Mutants

Triangular eye: This mutant was identified and isolated from irradiated strains of *Drosophila simulans*. It is an F₃ female with right eye triangular and apex pointing forward. Left eye is wild type. Head bristles of right side are increased in number. Size of the female is larger with broad head. Bristles are larger in size. Wings are broad and blunt-tipped (Fig. 2). This mutant is viable, but sterile.

Extra orbital bristles: This mutant fly was observed and isolated from irradiated stock of *Drosophila simulans*. It is an F₂ female with extra orbital bristles (Fig. 3). Other bristles on head are slightly larger in size. Remaining parts of body are unaffected. Viability is normal.

These two mutant flies could not be cultured because of sterility or some other unknown factors. It is assumed that these mutants have been produced due to semilethal structural chromosome mutations induced by gamma rays, in present experiment.

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