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Analysis of Visible Mutations Induced by 2000 r Gamma Radiation in *Drosophila simulans*

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Abstract: Gamma-rays induced mutation analysis was conducted on *Drosophila simulans* grown on the standard maize meal medium. The un-etherized young males (2-3 days old) were exposed to 2000 r gamma radiation and were crossed to controlled virgin female flies, on the same day. The F_1, F_2 and F_3 generations were examined to identify visible mutations. A total of five induced mutant flies were identified and isolated from the irradiated strains. Three out of the five induced mutants were cultured successfully and their genetic pattern was studied, while the remaining two induced mutants could not be cultured due to unavoidable circumstances. The cultured mutants comprised one sex-linked recessive, and two autosomals (one autosomal dominant and one autosomal recessive). No spontaneous mutant fly could be noticed in the non-irradiated culture grown for comparison.

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

Introduction

Mutation is a fundamental genetic process essential to our understanding of genetic phenomena and is the ultimate source of all genetic variations that provide the raw material for evolution. The expression of alternative phenotypes based on gene mutations, has allowed geneticists to uncover the physical and chemical basis of heredity. Mutation and mutation process have enabled the scientists to gain insight into the structure of individual gene. Genus *Drosophila* has always remained an excellent material for the study of induced as well as spontaneous mutations. Various species of *Drosophila* have been used for mutation analysis in different parts of the world. This has added a lot to the study of organic diversities and laid the foundation of modern genetics.

Until 1927 the study of mutants was restricted to those through spontaneous mutations. Muller (1927) found that the heavy doses of X-rays produced a high rate of mutation in *Drosophila* and there is a linear relationship between doses of X-radiation and the mutation rate. Timofeeff-Ressovsky (1934) found that the dosage of gamma rays is about 3 times less effective than X-rays in inducing mutations. Tomenko (1974) compared the various mutations induced by X-rays in *Drosophila melanogaster* and the adaptive value of the mutant individuals. He found that the adaptive value of yellow gene bearing individuals was lower than normal ones. Nicoloff and Gechev (1975) explained the mechanism involved in induced mutation in the structure of chromosomes. A relationship exists between the effects of X-rays on non-disjunction and crossing over in *Drosophila melanogaster* (Savontaus, 1975). Fujikawa *et al*. (1975) noticed radiation induced fractional mutations in *Drosophila melanogaster*. They concluded that X-irradiation doses induce fractional type mutations both at the "dumpy" locus and other X-chromosome loci of *Drosophila*. Romanova *et al*. (1985) analysed the visible mutations induced by X-rays and ethylmethane sulfonate (EMS) in mature spermatozoa of *Drosophila melanogaster*. They found the recessive visible mutation spectrum of chromosome II induced by X-rays and EMS in the mature spermatozoa. Review of literature reveals that there has been a scanty use of gamma-rays to induce visible mutations in *Drosophila* species.

Radiation provides a new tool which helps to shed light on the character of the mutations produced by it and by other influences. That is, it can induce gene rearrangements and so got fragments of chromosomes containing normal or mutant genes at given loci that can then add or subtract such fragments, creating hyperploidy and can thus determine what the effects of purely quantitative changes may then be compared with the effects that are produced by the mutations themselves.

Drosophila simulans, used in the present study, is a member of *melanogaster* species group. It is abundantly available during the months from November to March, in Lahore. Culture of these flies can be easily made on maize meal medium. It is a medium-sized fly and breeds well in the laboratory conditions. Induced mutations in *Drosophila simulans* have been studied. This species has seemed to be an excellent material for genetic studies. The present work forms a part of a series of investigations made on different species of *Drosophila* genus in other parts of the world.

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 the standard maize meal medium.

Preparation of maize meal medium: To prepare the medium, 125 grams dry maize flour was soaked in 250 ml of water and was constantly stirred. Powdered agar, about 18 grams, was put in a pan with 875 ml of tap water, gently boiled and then 18 grams 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, poured into sterilized culture bottles and harden adequately. The bottles were stored in refrigerator until ready to be used. It was enough for 25 culture tubes.

Irradiation treatment: For irradiation treatment 45 un-etherized young (2-3 days old) male *Drosophila simulans* flies were exposed to 2000 roentgens (r) of gamma radiation in COBALT^{60} GAMMA CELL (220 Canadian make with the radiation chamber 21 x 155 mm). In the cell chamber flies within the tubes were kept approximately at the distance of 10 cm from the target. The exposure time was 26.7 seconds per 1000 r gamma radiations.

Identification and isolation of mutants: The irradiated males were crossed to untreated virgin females, on the same day. The F_1, F_2, and F_3 generations were examined to identify 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 tube 3 pairs of *Drosophila simulans* flies were kept for

3-4 days and then the flies were released. The new flies of F_1 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 flies emerging. Then F_2 and F_3 generations were obtained from F_1. 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). This artificial temperature control was made for a shorter period, 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

In the cultured mutant strains, the genetic pattern was traced minutely to the best possible extent. The cultured mutants were composed of one sex-linked and two autosomals. No spontaneous mutant fly could be traced in the non-irradiated stock grown for comparison. For the sake of convenience, the mutants have been categorized into two major groups:

Cultured Mutants

Scute bristles: Scute bristles mutant was observed and isolated from the irradiated culture of *Drosophila simulans*. It is a sex-linked recessive mutant. Scutellar bristles are absent

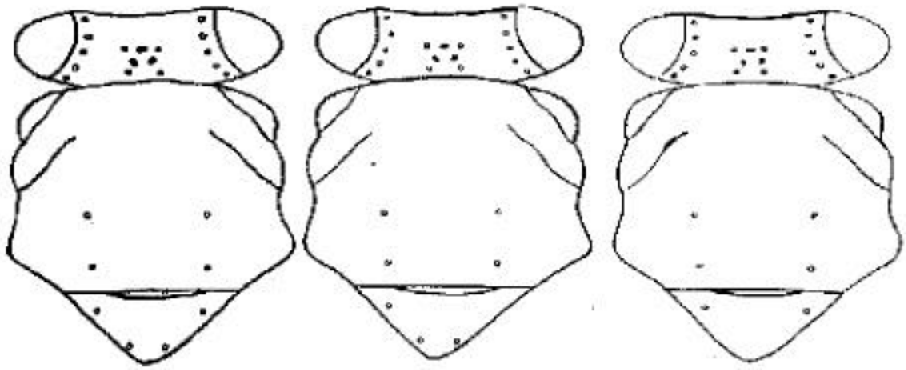

Fig. 1: Dorsocentral and scute

Fig. 1a: Scute bristles

Fig. 1b: Scute bristles

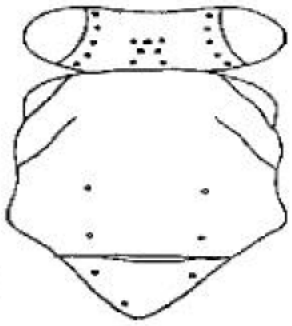

Fig. 1c: Scute bristles

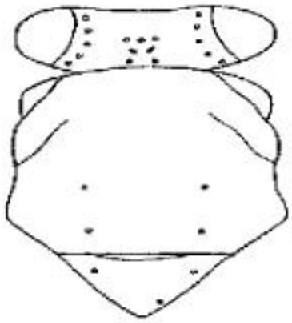

Fig. 1d: Scute bristles

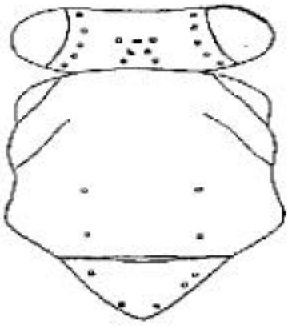

Fig. 1e: Extra scute bristles

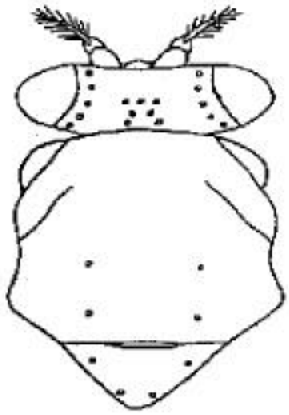

Fig. 2: Aristae (Wild type)

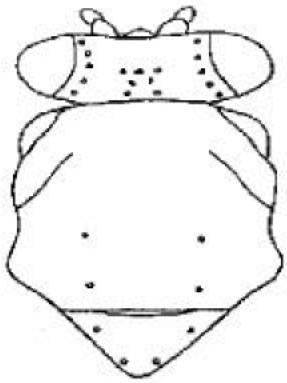

Fig. 2a: Aritaless

or reduced in number (Fig. 1, 1a, 1b, 1c and 1d). In some cases, the scute bristles are reduced in size. Cases with extra scutellar bristles are also present (Fig. 1e). Often one or two scutellar bristles are missing. Frequently posterior scutellar bristles are absent. In some flies left or right anterior bristle is missing. Other bristles are unaffected. Both sexes are equally viable and fertile. Penetrance of this mutation is incomplete. A high proportion of the scute mutation involves the breakage and reattachment of X-chromosome very close to the scute locus (Muller, 1933). Muller and Prokofyeva (1935) analyzed the position of breakage of these rearrangement of the scute region with respect to each other, and that the numerous rearrangements, although all appearing to possess one point of breakage and interchange of gene connections in one of the four definite positions near scute had the other point of breakage any where in the X or other chromosomes, so that the gene arrangements occurring in the neighbourhood of scute, after breakages and attachments had taken place, were in all cases very different from those in the normal chromosome and also from each other. Correspondingly there was very much diversity in the phenotypic expression of the "alleles", although no fixed relation was discernible between the map position of the different chromosome breaks and the kinds of scute phenotype associated with certainly the "gene" for achaete, just to left of scute, has some part in the scute complex. Series of studies on the chromosomal conformation of the other alleles shows that, just to the right of the main scute locus also, there lie one or more genes which (as compared with genes that may be substituted for them by inversion or translocation of other euchromatic regions) exert an appreciable position effect assisting in the production of the normal ("non-scute") bristle characters. Atleast one of the genes is not immediately adjoining the scute locus but lies to the right of the locus of the lethal which itself is to the right of scute, because the scute character is affected even when the chromosome is broken and rearranged to the right of this lethal locus. Similarly, X-rays have been found to induce autosomal translocations and ring-X chromosome losses in mature spermatozoa in *Drosophila melanogaster* by Sankaranarayanan and Ferro (1985). In the present study, the scute bristle mutation has likely been caused by chromosomal breakage and subsequent gene rearrangement in the X-chromosome.

Curly wings: This is an autosomal dominant mutant. It was identified and isolated from the irradiated stock of *Drosophila simulans*. In this mutant, wings are curled upward. Both male and female flies are equally viable and fertile. Penetrance is incomplete. The curvature in the wings has been caused by the unequal contraction of the upper and lower epithelia during the drying period emergence from the pupa case (Waddington, 1941). Cytological studies carried out by Muller and Painter (1929) indicate that the curly mutation in *Drosophila melanogaster* has been produced by the translocation occurred between II or III chromosome and the X-chromosome. So, it seems likely that the curly wing mutation in *Drosophila simulans* has been the outcome of some kind of structural changes in the chromosomes caused by gamma-rays, in the present study.

Aristaless: Aristaless mutant was isolated from the irradiated culture of *Drosophila simulans*. This is an autosomal recessive mutant. Aristae are absent mostly in both sexes (Fig. 2, 2a). Usually both aristae are missing. Post scutellar bristles are widely separated. Scutellum is shortened. Both sexes are equally viable and fertile. Penetrance of this mutation is incomplete. The aristaless mutation is postulated to be caused by ionizing effect of gamma-rays on the autosomal gene or genes whose normal expression is required for the development of aristae in the fruit flies.

Uncultured Mutants

Compressed body: This mutant was observed and isolated from the irradiated stock. It is an F_2 male fly with deformed body. Head, thorax, and scutellum are dorsoventrally compressed. Viability of the male is sub-normal.

Head twisted: This mutant *Drosophila simulans* fly was noticed and isolated from the irradiated culture. It is an F_2 female fly with head rotated approximately at 70° clockwise. Wings are twisted and folded. Bristles position on the head is irregular. Legs are weaker. Viability of the female fly is normal.

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

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