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Mutagenic Effect of 5000 r Gamma Rays in *Drosophila simulans*

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ABSTRACT

Mutagenic effect of 5000 r gamma radiation was studied in *Drosophila simulans* fruit flies grown on maize meal medium. Irradiated young males (2-3 days old) were crossed to controlled virgin females. The flies of F1, F2 and F3 generations were examined to identify visible mutations. A total of six induced mutants were isolated from the irradiated strains. Out of these, three mutants were cultured successfully and their pattern of inheritance was traced, while the remaining three mutants could not be grown. All the cultured mutants were found to be autosomal recessives. No spontaneous mutant fly emerged in the controlled culture maintained for comparison.

Key Words: *Drosophila;* Gamma-rays; Mutant; Gene; Chromosome; X-rays

INTRODUCTION

Mutation is a transmissible change occurring in the linear sequence of DNA, and is the ultimate source of all genetic variations that provide raw material for the process of evolution. Genetic study of induced mutation has gained an immense importance in investigating the structure of gene and the profile of changes occurring in it. Various species of genus *Drosophila* (fruitfly) have been used for genetic analysis of spontaneous as well as induced mutations throughout the world. Considering the restrains on the use of mammals, the fruitfly still provides the most versatile eukaryotic model system available in genetics and genetic toxicology. Moreover, *Drosophila* provides an indispensable tool in the study of gene isolation, the testing of altered gene functions in higher organisms, and germ cell genetics.

The X-rays have extensively been used to study the mutagenesis in *Drosophila* (Margulies & Griffith, 1991; Ferro & Eeken, 1993). 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, deletion, and duplication or intragenic mutations are induced that involve base pair substitutions or the addition and deletion of base pairs. When X-rays act directly on the genetic material, it is postulated that the radiation disrupts covalent linkages, causing a variety of structural alterations. Acting indirectly on the genetic material, X-rays generate highly reactive free radicals from water, and these free radicals react with DNA molecule to alter its structure (Jenkins, 1975). 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).

There has been a scanty use of gamma radiation in genetic analysis of mutations in *Drosophila simulans* fruit flies, in the world. Gamma-rays are about three times less effective than X-rays in including mutations (Timofeeff-Ressovsky, 1934). Mutation frequencies vary greatly from locus to locus and show very different distributions among loci for different types of changes (Valencia & Muller, 1949). Buthionine sulfoximine mediated enhancement of gamma-radiation induced mutation frequency in *Drosophila melanogaster* has been studied (Abraham *et al.*, 1993). The results obtained suggest that the depletion of the glutathione level with buthionine sulfoximine can lead to an enhancement in the frequency of sex-linked recessive lethal mutations induced by gamma-radiation.

This paper reports the mutagenic effect of 5000 r Gamma rays in *Drosophila simulans,* a member of *melanogaster* species group.

MATERIALS AND METHODS

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

Preparation of maize meal medium. 125 g dry maize flour was soaked with 250 mL water and was stirred constantly. Powdered agar (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 5000 roentgens (r) of gamma radiation in COBALT60 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 2.28 min for 5000 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 visible mutant flies. To identify and isolate the 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, at magnification X 100. In each culture bottle, three 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 the autosomal and sex-linked dominant mutations for three successive days until there were no more flies emerging. Pairs of F1 flies were allowed to mate randomly for the production of F2 and F3 generations. The controlled culture was also grown parallel to irradiated flies for the sake of comparison. In winter, 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

Induced Mutants were identified and isolated from the culture of *Drosophila simulans* flies irradiated by 5000 r of gamma radiation and their genetic pattern was studied to the maximum extent. However, no spontaneous mutant could be recorded in the controlled strain of the fruitflies grown parallel to the irradiated culture. The induced mutants are categorized into two groups: (A) cultured mutants which were grown successfully on standard maize meal medium, and (B) uncultured mutants that could not be cultured on the maize meal medium.

I. Cultured Mutants

Radius incompletes. This mutant was traced to be autosomal recessive. Second longitudinal vein in the wing was terminally interrupted (Fig. 1). There was a reduction in the size of the wing. Usually both wings of the flies were affected by this mutation. Other veins of the wings were unaffected. Both the sexes were equally viable and fertile. Penetrance of the mutation was 70%. In the controlled flies, all veins of both the wings were complete and unbroken (Fig. 2). The radius incompletus mutation in *Drosophila simulans* was caused by the contraction of wing epithelium during the drying period following emergence from pupa case, thereby, inhibiting fusion of small spaces into a vein (Waddington, 1941).

Extra postvertical bristles. This was an autosomal recessive mutant. This mutation appeared in female flies. The mutant females had extra postvertical bristles (Fig. 3). The head bristles were slightly larger and thicker. Other parts of the body remained unaffected. Male flies were found to be completely normal. The mutant females were viable and fertile. Penetrance of this mutation was traced to be 60%. Each eye in the controlled fly possessed five orbital bristles (Fig. 4). It is postulated that the mutation, extra postvertical bristles were produced as the result of structural chromosome changes caused by 5000 r gamma radiation, in the present experiment.

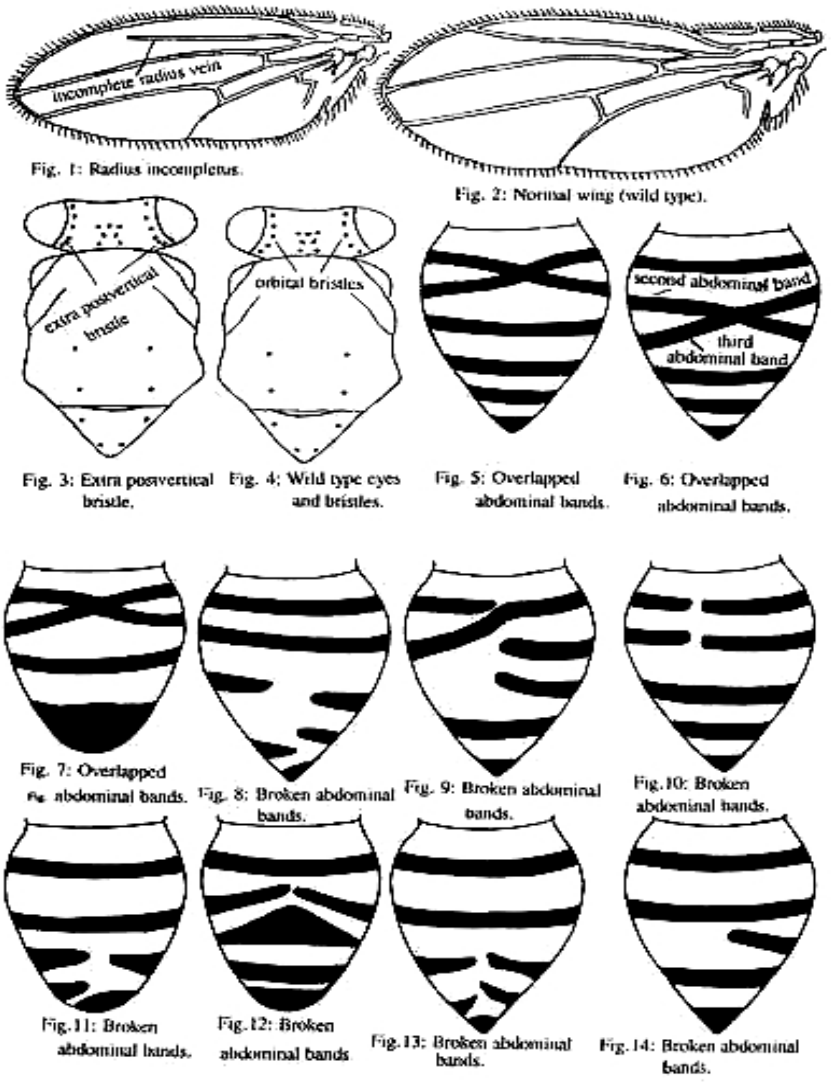

Fig. 1: Radius incompletus.
Fig. 2: Normal wing (wild type).
Fig. 3: Extra postvertical bristle.
Fig. 4: Wild type eyes and bristles.
Fig. 5: Overlapped abdominal bands.
Fig. 6: Overlapped abdominal bands.
Fig. 7: Overlapped abdominal bands.
Fig. 8: Broken abdominal bands.
Fig. 9: Broken abdominal bands.
Fig. 10: Broken abdominal bands.
Fig. 11: Broken abdominal bands.
Fig. 12: Broken abdominal bands
Fig. 13: Broken abdominal bands.
Fig. 14: Broken abdominal bands.

Fig. 15: Broken abdominal bands.

Fig. 16: Abdominal bands in male (wild type).

Fig. 17: Abdominal bands in female (wild type).

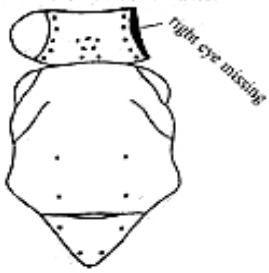

Fig. 18: Eyeless.

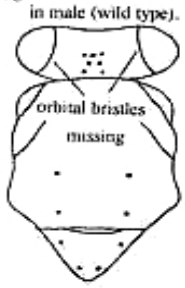

Fig. 19: Achaete.

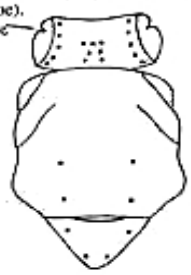

Fig. 20: Lobe eyes.

Bobbed bristles. This mutant was traced to be autosomal recessive with multiple recoveries. It had reduction in length and thickness of the bristles. Bristle character showed considerable variability and great overlapping of abdominal bands. The overlapping of abdominal bands varied greatly (Figs. 5, 6 and 7). Usually second and third anterior abdominal bands were overlapped (Fig. 6). Sometimes, posterior abdominal bands also formed overlapping. There were also cases of great variation in broken abdominal bands (Figs. 8, 9, 10, 11, 12, 13, 14 and 15). Both sexes were equally affected. Variability and fertility was normal. Penetrance of this mutation was found to be 90%. The controlled flies had long thick bristles and possessed unbroken and non-overlapping abdominal bands (Figs. 16 and 17). The bobbed mutation in *Drosophila melanogaster* was sex-linked recessive which showed females with short bristles and slight abdominal abnormalities, and it did not affect the male which was wild type (Stern, 1926). Moriwaki (1935) reported the similar situation in *Drosophila ananassae*. It was shown that bb locus contains about half as much ribosomal RNA and complementary DNA as the locus bb+. It was concluded that the locus bb was the site of ribosomal RNA synthesis. On the basis of calculations it was suggested that there was enough DNA in bb+ locus to specify approximately 130 molecules each of 28 S and 18 S ribosomal RNA. The bb locus was as highly redundant and, perhaps, was composed of a very large series of tandem duplications. The bb mutations were interpreted as partial deletions of the locus. It was further postulated that in bb flies the rate of protein synthesis was limited by the amount of ribosomal RNA, and bb phenotype resulted in part because normal bristles production represented the maximum protein synthesis on the part of trichogen cells during a particular interval in development. The bobbed bristles mutant allele was located at 67.7 map position on X-chromosome of first linkage group in *Drosophila melanogaster* (Strickberger, 1985).

II. Uncultured Mutants

Eyeless. It was an F1 female fly with right eye missing (Fig. 18). The left eye was normal. Other parts of the body were unaffected. This mutant female fly was non-viable. *Drosophila* flies normally had two eyes, both in males and females (Fig. 4). In case of *Drosophila melanogaster*, the eyeless allele was located at 2.0 map position on autosome of fourth linkage group (Strickberger, 1985).

Achaete. It was an F2 male fly in which orbital bristles were missing, on both sides of the head (Fig. 19). In wild type flies, there were five orbital bristles, on each side of the head (Fig. 4). Other parts of the mutant fly were unaffected by the gamma radiation. Viability of the fly was found to be below normal.

Lobe eyes. This mutant was an F1 female fly with small eyes (Fig. 20). The fly had nick in anterior edge of each eye. Colour of eyes was unaffected. Viability of the female was low. In the controlled flies, eyes were comparatively larger in size and had no nicks (Fig. 4). This mutant allele, lobe eyes was located at 72.0 map position on autosome of second linkage group, in *Drosophila melanogaster* (Strickberger, 1985).

These three mutant flies could not be cultured because of sterility or some other unknown factors. It seems likely that these mutants were produced due to semilethal structural chromosome mutations induced by 5000 r gamma radiation, in the present investigation.

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