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Effect of X-ray Irradiation on the Male Moths of Two Tropical Races of the Silkworm *Bombyx mori* and Inheritance of Induced Sterility in the Progenies

G. Subramanya

Department of Studies in Sericulture Science, University of Mysore, Manasagangothri, Mysore—570 006, Karnataka, India.
Email: subramanyag2000@yahoo.co.in

Abstract: Newly emerged male moths of silkworm *Bombyx mori* were treated with in 24 h of eclosion irradiated with two independent doses of 50 Gy and 100 Gy X-rays. The effects of X-rays in the parental generation have indicated significant increase of unfertilized and unhatched eggs followed by significant reduction in the hatchability in the treated batches compared to control. The inheritance of induced sterility was examined in the succeeding generations by rearing the F₁, F₂ and F₃ silkworm progenies and by crossing the progenies of treated males with untreated female moths. It is evident from the results that the number of hatched eggs gradually increased from F₁–F₃ generations. Thus, the results showed that the egg hatchability do not remain constant at every generation and hatching tendency of eggs increase in the progenies of treated batches. The mechanism of inherited sterility was discussed.

Keywords: *Bombyx mori*, X-ray irradiation, rearing, unfertilized, unhatched eggs

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Lepidopteron insect *Bombyx mori* an eukaryotic bisexual organism have since long been used as a material of choice for radiobiological studies.¹ They exhibit sequential events of spermatogenesis in the homogametic (XX) males and oogenesis in the heterogametic (XY) females and mature sperms are invariably seen in the adult moths.² It is well established that, *Bombyx mori* can be conveniently be used as one of the genetical model systems and detection of wide range of genetic damage after irradiation and or chemicals could be possible either at gene or chromosomal level.³ Further, silkworm eggs are used as a valid models for estimating the biological effects of cosmic radiation and radiobiological studies.⁴ Though inherited sterility in the progenies are well documented⁵ the lepidopteron moths are also unique that they are found to be more radio resistant than any other insects due to its holokinetic nature of chromosomes.⁶ Our previous studies utilizing sublethal doses of γ -rays⁷ demonstrated how different life history stages of holometabolus insect *Bombyx mori* could be effectively used to understand the manifestation of dominant lethal mutations. With an objective to study inherited sterility using X-rays and considering such studies as an important and promising component of area wide approach for lepidopteron pest control management the present study was undertaken to determine whether inherited sterility phenomenon continues to prevail in the progenies of irradiated male moths of *Bombyx mori*.

Materials and Methods

The two silkworm varieties namely, Pure Mysore race belonging to multivoltinism and Kalimpong-A belonging to bivoltinism were drawn from the germplasm bank of the Sericulture unit. The procedures of rearing of silkworms are described below.

Hatched eggs

Freshly laid eggs of the two silkworm races were selected. The bivoltine eggs were treated with hydrochloric acid in order to break the embryonic diapause and to get hatched larvae. The standard condition for acid treatment is specific gravity of 1.10 at 15 °C, the temperature of the acid bath at 48 °C and treatment of eggs for 6 minutes.⁸ The multivoltine eggs hatch normally since they do not undergo diapause.

General rearing procedure

The larvae hatched from the eggs of both the races were reared in the silkworm rearing house following the standard procedure.⁹ providing rearing environment of 25 ± 1 °C, humidity of 80%–90% and photoperiod (12:12 L:D). The larvae were fed with succulent mulberry leaves. When the larva successfully spun the cocoons, the moth emergence takes place on the tenth day.

Parental generation treatment

Freshly emerged twenty male moths of each race were irradiated with two independent doses of 50 Gy and 100 Gy X-rays with 8 mA, 2.5 mm Aluminium filter (at a dose rate of 20 Gy/minutes) in the X-ray Unit of Department of Studies in Sericulture. The irradiated moths were crossed with untreated female moths. The non irradiated moths were used as control. The eggs laid by the female moths were carefully observed for three types of eggs namely unfertilised eggs, unhatched eggs (embryonic death), and hatched eggs. To estimate the percentage of expected eggs hatch the following the standard formula was used.²

$$\text{Exp } H_p = \frac{E_h}{E_t} \times 100$$

Table 1. Effect of two doses of X-rays on the male moths of silkworm *Bombyx mori*.

| Race and voltinism | Dose rate (Gy) | No. of unhatched eggs ± SD | No. of unfertilized eggs ± SD | No. of hatched eggs ± SD |
|-----------------------|----------------|---------------------------------|---------------------------------|----------------------------------|
| Multivoltine | 50 | 92.33 ± 2.52 ^{c (37)} | 70.00 ± 2.00 ^{c (28)} | 87.33 ± 2.52 ^{c (35)} |
| Pure Mysore | 100 | 81.33 ± 3.51 ^{d (37)} | 65.67 ± 3.06 ^{c (30)} | 72.67 ± 3.06 ^{d (33)} |
| Bivoltine Kalimpong-A | 50 | 126.00 ± 3.00 ^{a (42)} | 90.00 ± 2.00 ^{b (30)} | 84.00 ± 2.00 ^{c (28)} |
| | 100 | 118.00 ± 5.20 ^{b (38)} | 112.00 ± 4.36 ^{a (36)} | 81.00 ± 3.46 ^{cd (26)} |
| Pure Mysore | Control | 4.00 ± 0.00 ^{e (1)} | 7.00 ± 0.00 ^{d (2)} | 324.00 ± 10.15 ^{b (90)} |
| Kalimpong-A | Control | 8.00 ± 0.00 ^{e (2)} | 8.00 ± 0.00 ^{d (2)} | 374.33 ± 7.51 ^{a (92)} |

Number in parenthesis indicates the percentage values.

**Table 2.** Progenies of irradiated male moths with 50 Gy and 100 Gy X-ray.

| Races | Generations | Unhatched % \pm SD | Unfertilized % \pm SD | Hatched % \pm SD |
|-----------------------|----------------|--------------------------------|------------------------------------|----------------------------------|
| Treated groups | | | | |
| Pure Mysore 50 Gy | F ₁ | 60.33 \pm 4.933 ^a | 29.67 \pm 6.110 ^a | 10.00 \pm 2.646 ^d |
| | F ₂ | 66.67 \pm 5.033 ^a | 18.00 \pm 2.646 ^{c,d} | 14.67 \pm 2.309 ^d |
| | F ₃ | 66.67 \pm 3.215 ^a | 5.00 \pm 1.00 ^e | 27.67 \pm 2.309 ^b |
| Kalimpong-A 50 Gy | F ₁ | 63.67 \pm 8.505 ^a | 27.67 \pm 3.512 ^{a,b,c} | 8.33 \pm 2.082 ^d |
| | F ₂ | 64.67 \pm 7.506 ^a | 22.33 \pm 2.082 ^{a,b,c} | 13.33 \pm 1.155 ^d |
| | F ₃ | 64.67 \pm 3.512 ^a | 9.33 \pm 3.055 ^{d,e} | 25.67 \pm 2.309 ^b |
| Pure Mysore 100 Gy | F ₁ | 64.67 \pm 7.024 ^a | 28.33 \pm 5.686 ^{a,b} | 6.67 \pm 1.528 ^d |
| | F ₂ | 67.33 \pm 7.024 ^a | 19.33 \pm 3.055 ^{b,c,d} | 14.00 \pm 1.732 ^d |
| | F ₃ | 68.00 \pm 7.810 ^a | 7.33 \pm 1.528 ^e | 25.33 \pm 2.887 ^b |
| Kalimpong-A 100 Gy | F ₁ | 67.33 \pm 6.658 ^a | 25.67 \pm 2.082 ^{a,b,c} | 7.33 \pm 2.517 ^d |
| | F ₂ | 66.67 \pm 4.163 ^a | 21.00 \pm 3.606 ^{a,b,c} | 11.67 \pm 1.528 ^d |
| | F ₃ | 65.33 \pm 4.041 ^a | 10.00 \pm 2.646 ^{d,e} | 24.67 \pm 1.528 ^{b,c} |
| Control groups | | | | |
| Pure Mysore | F ₁ | 2.33 \pm 0.577 ^b | 2.33 \pm 0.577 ^e | 92.33 \pm 5.686 ^a |
| Kalimpong-A | F ₁ | 3.00 \pm 1.00 ^b | 3.00 \pm 1.000 ^e | 90.33 \pm 2.082 ^a |
| Pure Mysore | F ₂ | 3.00 \pm 1.000 ^b | 4.00 \pm 1.732 ^e | 93.33 \pm 3.786 ^a |
| Kalimpong-A | F ₂ | 5.33 \pm 0.577 ^b | 5.00 \pm 1.732 ^e | 91.00 \pm 4.359 ^a |
| Pure Mysore | F ₃ | 4.00 \pm 1.732 ^b | 2.667 \pm 0.577 ^e | 92.33 \pm 3.786 ^a |
| Kalimpong-A | F ₃ | 5.67 \pm 2.309 ^b | 4.00 \pm 1.00 ^e | 90.67 \pm 3.215 ^a |

Means having the same letters do not differ significantly at 5% level in the treated batches compared to control.

where, $Exp H_p$ = Expected hatching percentage, E_h = number of eggs hatched, E_l = number of eggs laid.

Rearing of F₁ to F₃ generations

The progenies of the X-ray irradiated moths were crossed with untreated female moths and the larvae hatched from the eggs of irradiated progenies at F₁, F₂ and F₃ were reared in the rearing house providing standard rearing procedure already described. Unhatched, unfertilised and hatched eggs were counted following the standard formula described above.²

All statistical analysis were performed using genstat 9th edition.¹⁰ Analysis of variance was carried out for the entire data set using Tukey's post-hoc test. All test were performed at a significance level 0.05. Data are presented as mean \pm SD.

Results and Discussion

Table 1 presents the data after irradiation of the male moths at parental generation with two independent

doses in the production three different types of eggs. From the table it is evident that, the mean number of hatched eggs in the control batches was 324.00 ± 10.15 and 374.33 ± 7.51 in the respective Pure Mysore and Kalimpong-A races compared to 72.67 ± 3.06 (33%) at 100 Gy and 87.33 ± 2.52 (35%) at 50 Gy in Pure Mysore race ($P < 0.05$). Similarly, the number of hatched eggs in treated Kalimpong-A race was 84.00 ± 2.00 (28%) at 50 Gy and 81.00 ± 3.46 (26%) at 100 Gy. Further, there is significant difference between number of unfertilised and unhatched eggs in both the races. Sado¹¹ found that in silkmoths the reduced fertility of irradiated moths appear due to the lack of sperms as a result of spermatogonial depletion and partly to the formation of abnormal sperms, which were unable to perform fertilization. On the other hand, the reduction in the fecundity of the female was greater when they are crossed with males treated with high doses.¹² The significant reduction in the hatchability followed by increased unfertilised and unhatched eggs in the P₁ generation

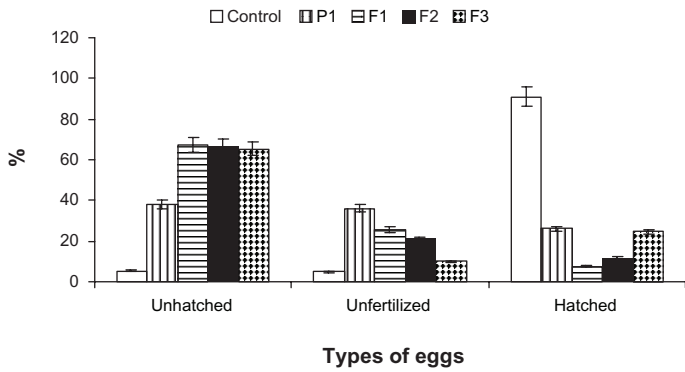


Figure 1. Pattern of different types of eggs produced (%) in Pure Mysore race after treatment with 50 Gy X-ray.

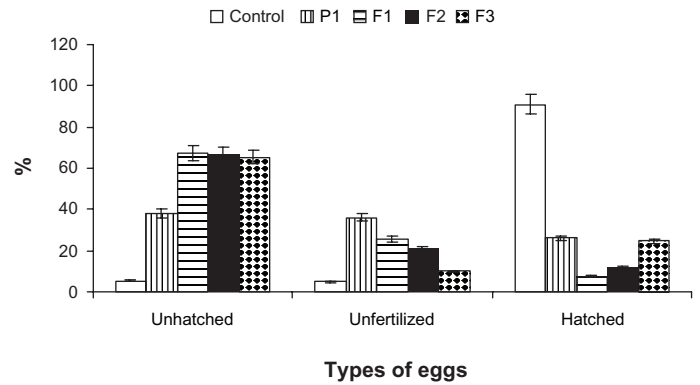


Figure 3. Pattern of different types of eggs produced (%) in Pure Mysore race after treatment with 100 Gy X-ray.

bears testimony that the two doses of X-rays at 50 Gy and 100 Gy demonstrates that, the mature spermatozoa invariably present in the adult moths are equally sensitive and resulted in higher sterility. Although mechanism causing inherited sterility in silkworm was not apparent, in a similar experiments,¹³⁻¹⁵ utilizing X-rays and γ -rays it is proposed that, inherited lethality in the F₁ progeny of treated male moths would be due to translocations which may be able to pass through meiotic divisions and a reduction in fertility owing to abnormal meiotic division can be detected by either genetic or cytogenetic method in silkworms.

The experimental results related to inherited sterility in F₁, F₂ and F₃ generations are summarized in Table 2 and the same is depicted in Figures 1-4. As seen from the results it is evident that, there is a significant reduction ($P < 0.05$) in the number of unfertilized and unhatched eggs from F₁-F₃ generations and a significant increase of hatchability in the treated batches. Sugai and Mirumachi⁵ in their detailed

investigations utilizing Japanese bivoltine races of silkworm *Bombyx mori* showed that X-rays irradiation results in delayed lethal effects in the progenies and proposed that reciprocal translocation may not be an obstacle and individuals with out genetic damage results in normal reproduction. In an interesting experiment it is also shown that, there was a rapid disappearance of sterility among the lepidopteron moths during the course three generations of backcrossing in pink boll worm¹⁶ and tobacco boll worm.¹⁷ Thus, an increase in the unhatched and unfertilized eggs in the parental generation and subsequent reduction in the progenies in the present study corroborates with the findings of the above authors. Subramanya and Reddy¹⁸ utilizing one of the popular tropical multivoltine Pure Mysore race has demonstrated that, pupa exposed to X-rays produce functionless spermatozoa. Thus, it is opined that, the functionless spermatozoa that is transmitted to the gametes might have been resulted in sterility and embryonic death just after the irradiation in the P₁ generation and their

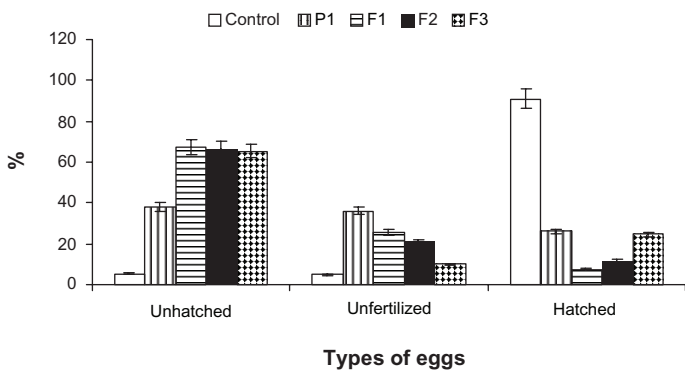


Figure 2. Pattern of different types of eggs produced (%) in Kalimpong-A race after treatment with 50 Gy X-ray.

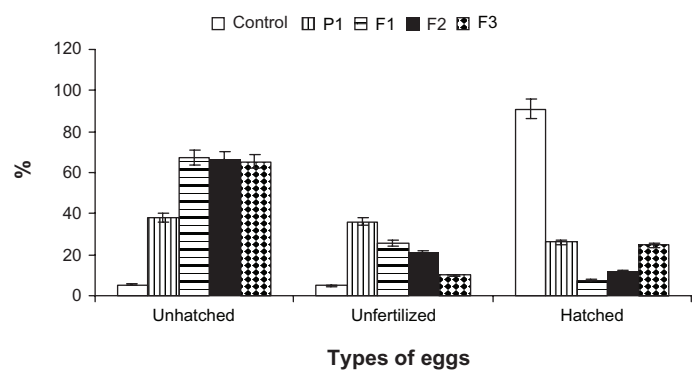


Figure 4. Pattern of different types of eggs produced (%) in Kalimpong-A race after treatment with 100 Gy X-ray.



progenies. Then the reason for increased sterility in the P₁ generation could be due to functionless spermatozoa due to irradiation but the resultant increase of hatchability in the progenies may be due to fat that F₁–F₃ progenies partially inherit the deleterious effects from the irradiated male parents supports previous work in tobacco bud worms¹⁷ and potato tuber moth *Phthorimaea operculella*.¹⁹

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Disclosure

The author reports no conflict of interests.

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