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ORIGINAL RESEARCH

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

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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 F_1, F_2 and F_3 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 F_1–F_3 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, silkmoth 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 documented[5] 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-rays[7] 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]

$$\text{Exp } H_P = \frac{E_h}{E_l} \times 100$$

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 Pure Mysore	50	$92.33 \pm 2.52^{c\,(37)}$	$70.00 \pm 2.00^{c\,(28)}$	$87.33 \pm 2.52^{c\,(35)}$
	100	$81.33 \pm 3.51^{d\,(37)}$	$65.67 \pm 3.06^{c\,(30)}$	$72.67 \pm 3.06^{d\,(33)}$
Bivoltine Kalimpong-A	50	$126.00 \pm 3.00^{a\,(42)}$	$90.00 \pm 2.00^{b\,(30)}$	$84.00 \pm 2.00^{c\,(28)}$
	100	$118.00 \pm 5.20^{b\,(38)}$	$112.00 \pm 4.36^{a\,(36)}$	$81.00 \pm 3.46^{cd\,(26)}$
Pure Mysore	Control	$4.00 \pm 0.00^{e\,(1)}$	$7.00 \pm 0.00^{d\,(2)}$	$324.00 \pm 10.15^{b\,(90)}$
Kalimpong-A	Control	$8.00 \pm 0.00^{e\,(2)}$	$8.00 \pm 0.00^{d\,(2)}$	$374.33 \pm 7.51^{a\,(92)}$

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 % ± SD	Unfertilized % ± SD	Hatched % ± SD
Treated groups				
Pure Mysore 50 Gy	F_1	60.33 ± 4.933^{a}	29.67 ± 6.110^{a}	10.00 ± 2.646^{d}
	F_2	66.67 ± 5.033^{a}	18.00 ± 2.646^{c,d}	14.67 ± 2.309^{d}
	F_3	66.67 ± 3.215^{a}	5.00 ± 1.00^{e}	27.67 ± 2.309^{b}
Kalimpong-A 50 Gy	F_1	63.67 ± 8.505^{a}	27.67 ± 3.512^{a,b,c}	8.33 ± 2.082^{d}
	F_2	64.67 ± 7.506^{a}	22.33 ± 2.082^{a,b,c}	13.33 ± 1.155^{d}
	F_3	64.67 ± 3.512^{a}	9.33 ± 3.055^{d,e}	25.67 ± 2.309^{b}
Pure Mysore 100 Gy	F_1	64.67 ± 7.024^{a}	28.33 ± 5.686^{a,b}	6.67 ± 1.528^{d}
	F_2	67.33 ± 7.024^{a}	19.33 ± 3.055^{b,c,d}	14.00 ± 1.732^{d}
	F_3	68.00 ± 7.810^{a}	7.33 ± 1.528^{e}	25.33 ± 2.887^{b}
Kalimpong-A 100 Gy	F_1	67.33 ± 6.658^{a}	25.67 ± 2.082^{a,b,c}	7.33 ± 2.517^{d}
	F_2	66.67 ± 4.163^{a}	21.00 ± 3.606^{a,b,c}	11.67 ± 1.528^{d}
	F_3	65.33 ± 4.041^{a}	10.00 ± 2.646^{d,e}	24.67 ± 1.528^{b,c}
Control groups				
Pure Mysore	F_1	2.33 ± 0.577^{b}	2.33 ± 0.577^{e}	92.33 ± 5.686^{a}
Kalimpong-A	F_1	3.00 ± 1.00^{b}	3.00 ± 1.000^{e}	90.33 ± 2.082^{a}
Pure Mysore	F_2	3.00 ± 1.000^{b}	4.00 ± 1.732^{e}	93.33 ± 3.786^{a}
Kalimpong-A	F_2	5.33 ± 0.577^{b}	5.00 ± 1.732^{e}	91.00 ± 4.359^{a}
Pure Mysore	F_3	4.00 ± 1.732^{b}	2.667 ± 0.577^{e}	92.33 ± 3.786^{a}
Kalimpong-A	F_3	5.67 ± 2.309^{b}	4.00 ± 1.00^{e}	90.67 ± 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 F_1 to F_3 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 F_1, F_2 and F_3 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 ± 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. Sado[11] 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 P_1 generation

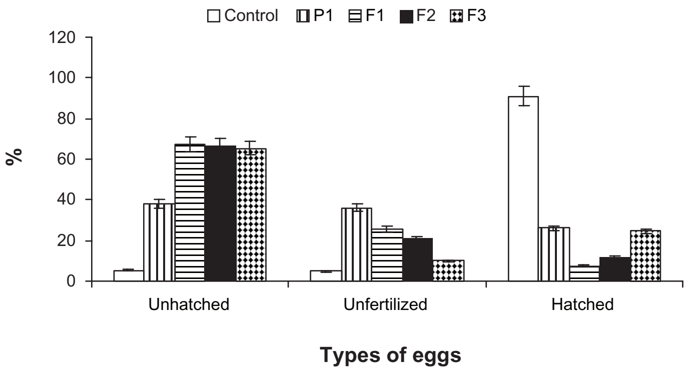

Figure 1. Pattern of different types of eggs produced (%) in Pure Mysore race after treatment with 50 Gy X-ray.

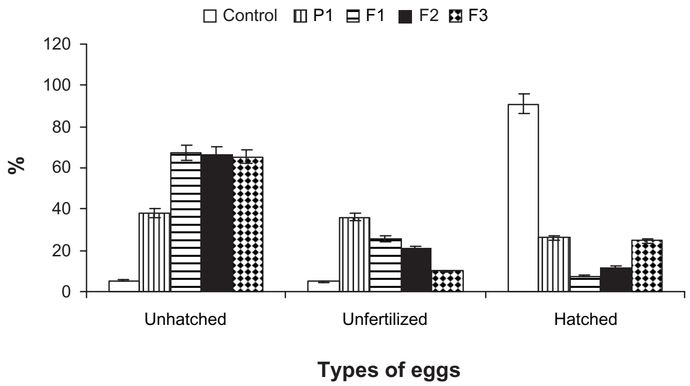

Figure 3. Pattern of different types of eggs produced (%) in Pure Mysore race after treatment with 100 Gy X-ray.

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 F_1 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 F_1, F_2 and F_3 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 F_1–F_3 generations and a significant increase of hatchability in the treated batches. Sugai and Mirumachi[5] 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 worm[16] 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 Reddy[18] 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 P_1 generation and their

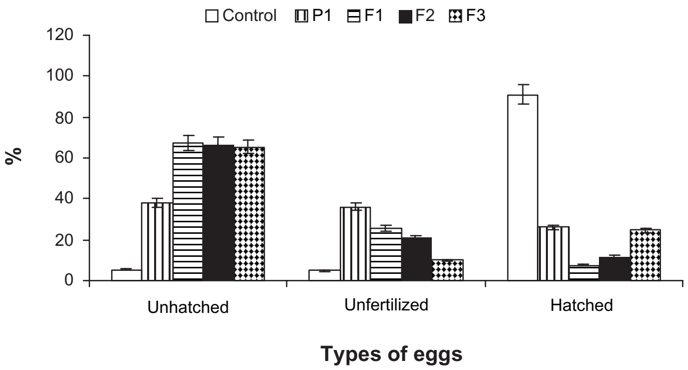

Figure 2. Pattern of different types of eggs produced (%) in Kalimpong-A race after treatment with 50 Gy X-ray.

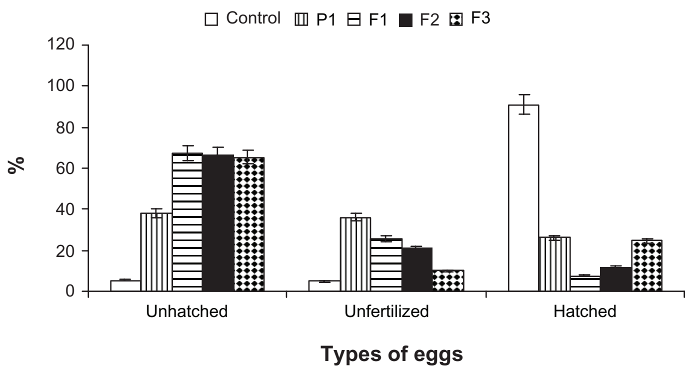

Figure 4. Pattern of different types of eggs produced (%) in Kalimpong-A race after treatment with 100 Gy X-ray.

progenies. Then the reason for increased sterility in the P_1 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 F_1–F_3 progenies partially inherit the deleterious effects from the irradiated male parents supports previous work in tobacco bud worms[17] and potato tuber moth *Phthorimaea operculella*.[19]

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Disclosure

The author reports no conflict of interests.

References

1. Tazima Y. The genetics of silkworm. Logos press, London. 1964.
2. Murakami A. Methods for detecting mutations in silkworm. Laboratory manual for East Asian workshop on mutagenecity testing of chemicals. *Natl Inst Genet*. 1976:57–64.
3. Tazima Y. The silkworm; An important laboratory tool. Kodansha Ltd., Tokyo. 1978.
4. Kotani E, FurusawaT, Nagaoka S, et al. Somatic mutation in larvae of the silkworm, *Bombyx mori*, induced by heavy ion irradiation to diapause eggs. *J Radiat Res*. 2002;(43)Suppl:S1932–98.
5. Sugai E, Mirumachi M. Inherited sterility in progeny of irradiated male silkworm, *Bombyx mori*. L. *Japan Journal of Genetics*. 1973;48(5):329–35.
6. North DT, Holt G. Genetic and cytogenetic basis of induced sterility in the adult male cabbage looper *trichoplusia Sps*. Isotopes and radiation in entomology. International Atomic energy agency, Vienna. 1968:391–403.
7. Subramanya G, Vijayan VA, Krishnamurthy NB. Differential sensitivity of three bivoltine races of silkworm *Bombyx mori* L. to Gamma radiation. *Indian Journal of Sericulture*. 1995;20:139–41.
8. Tazima Y, Hiroshi Doira, Akai H. The domesticated silkmoth, *Bombyx mori*. Hand book of genetics. *Insects of Genetic Interest*. 1975;3:63–124.
9. Tanaka Y. Chapter-I .Silkworm rearing- in Sericolony-Central Silk Board (India). 1964:46–82.
10. Genstat 9. Lawes Agricultural Trust, Rothamsted Experimental Station, Rothamsted, UK. 2006.
11. Sado T. Histological study of radiation sensitivity of spermatogenic cells of the silkworm. *Ann Rep Natl Inst Genet*. 1958;9:101–2.
12. Saour G, Makee H. Radiation induced sterility in male potato tuber moth *Phthorimaea operculella* Zeller (Lep., Gelechiidae). *J Appl Ent*. 1997;121:411–5.
13. Tazima Y. Effect of external irradiation with Beta rays upon the germ cells of the silkworm. *Annual report of the National Institute of Genetics*. 1956;7:76–8.
14. Murakami A. Embryonic lethality in progeny of X-ray irradiated silkworm moths: Inherited lethality and sex ratio distortion. *Ann Rep Natl Inst Genet*. 1974;25:41–2.
15. Murakami A, Murota T. Annual report of the National Institute of Genetics. Japan. 1976;27:55–6.
16. Graham H, Ouye MT, Garcia RD, De La Rosa HH. Dosages of gamma irradiation for full and inherited sterility in adult pink bollworms. *J Econ Entomol*. 1972;65:645–50.
17. Proshold FI, Bartell JA. Inherited sterility in progeny irradiated male tobacco bud worms: effects on reproduction, developmental time and sex ratio. *J Econ Entomol*. 1970;63:280–5.
18. Subramanya G, Sreerama Reddy G. Isolation of a mutant line with shorter larval duration by induction of mutations in the silkworm *Bombyx mori* L. *Indian Journal of Experimental Biology*. 1982;32(1):9–13.
19. Makee H, Saour G. Non inheritted sterility in irradiated *Phthorimaea operculella* Zeller (Lep., Gelechiidae). *J Appl Ent*. 1997;121:411–5.