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Effects of different doses of x-rays on meiotic chromosomes of male *Physopelta schlanbuschi* (Largidae: Heteroptera)

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Abstract. Male largid bugs, *Physopelta schlanbuschi*, having $2n = 17$ chromosomes (12 autosomes + $2m + X_1X_2Y$), were irradiated with x-ray doses of 300 r, 400 r and 500 r which yielded various types of chromosome aberrations in different stages of meiosis of which the common forms were breaks, fragment of unknown origin, constriction, gap etc. Among the 3 sex chromosomes, the two conspicuously large markers, X_1 and Y, sometimes formed chiasma-like configuration in prophase I and metaphase I, while a number of anaphase I plates had a chromatid bridge, very likely formed by the X_1 and the Y. The qualitative and quantitative assessments of chromosome aberrations in spermatogonial metaphase, prophase I, metaphase I, anaphase I and metaphase II were made at 13 intervals for the doses of 300 r and 400 r and 14 intervals for 500 r between 5 min and 1 week or more. The data showed over-all dose-dependent aberration effects and the sex chromosomes appeared relatively more vulnerable than the autosomes to different doses of x-rays. The testes of untreated males taken as controls had practically no aberration.

Keywords. X-ray effects; meiotic chromosomes; *Physopelta schlanbuschi*

1. Introduction

The effects of ionizing radiations have been extensively studied among insects having chromosomes with localized centromere like grasshoppers (Ray-Chaudhuri 1961; Manna and Mazumder 1962, 1967, 1968; Mazumder and Manna 1967, 1969; Fox 1967; Westerman 1968; Kumaraswamy and Rajasekharasetty 1975) and *Drosophila* (Kvelland 1962; Lea 1962; Becker 1975; Falk and Jacoby 1975). These relate to verification of the target hypothesis, mechanism of structural rearrangements, multiple configuration at meiotic stages, intra- and interspecific radiosensitivity, radioprotection etc. On the other hand, the hemipteran species claiming to have a diffuse centromeric activity (Scharader 1953) were deployed by different workers mainly to verify the centromeric constitution of chromosomes (Hughes-Schrader and Ris 1941; Hughes-Schrader and Schrader 1961; Halkka 1965; La Chance and Degruillier 1969) while Manna and his collaborators (Manna 1984; Manna and Dey 1981, 1983; Barik and Manna 1981; Dey and Manna 1983, 1984) have conducted a series of experimental studies on the x-ray induced chromosome aberrations in some species of Heteroptera, not only to verify the centromeric problem, but also to assess the differential radiosensitivity of chromosomes, localization of breaks, induction of pseudochiasma in sex chromosomes, radioprotection by penicillin etc. In the present paper, the qualitative and quantitative effects of 3 doses of x-rays on chromosomes at different stages of meiosis in the males of *Physopelta schlanbuschi* have been dealt with in regard to the dose-dependent response, differential radiosensitivity between and within chromosomes, etc.

2. Material and method

The genus *Physopelta* has been cytologically very interesting because of the sex chromosomal polymorphisms in its different species (Manna *et al* 1985). For the present study, adult males of *P. schlanbuschi* (Largidae: Heteroptera) were irradiated with 300 r, 400 r and 500 r by the x-ray apparatus fitted with the thermax therapy tube, operated at 110 KV, 4 mA, using 1 mm thick aluminium filter. The testes of the irradiated males were fixed in acetic-alcohol (1 : 3) and each testis was squashed in 45% acetic acid on a clean slide smeared with Mayer's albumin. The squashed material on the slide was stained in iron-alum haematoxylin, dehydrated in absolute alcohol passing through up-grades, cleared in xylol and mounted in Canada balsam. The untreated males were kept in the same laboratory condition, the testes of which served as control. Assessment of the irradiation effect in different stages of meiosis could not be made from a uniform number of cells because of the rarity of some stages. The spermatogonial metaphase complement of 17 chromosomes consisted of 12 medium sized autosomes, 2 very minute *m*-chromosomes and 3 sex chromosomes (X_1X_2Y), which showed a typical heteropteran post-reductional meiosis (Manna 1951; Ray-Chaudhuri and Manna 1955). In the spermatogonial complement, the X_1 and the Y were of the same size, representing 2 largest marker chromosomes, the X_2 was of the medium size, indistinguishable from the 12 autosomes, while 2*m* constituted the minute marker pair. Disregarding the size of the very minute *m*-pair, according to the approximate size of others the proportionality of the sex elements to autosomes was 5:12, taking each X_1 and the Y as equivalent to two X_2 in size. According to the number, it was 3 (X_1X_2Y): 14 (12 autosomes plus the *m*-pair). These values could be used in calculating the expected number of aberrations between the sex chromosomes and autosomes.

3. Results

The chromosomes in different meiotic stages of the control specimens had practically no aberration because out of 274 spermatogonial metaphases, 1300 prophase I, 2600 metaphase I, 32 anaphase I and 2600 metaphase II assessed, only a gap type aberration was encountered. However, stickiness and clumping effects were not taken into account both in control and the treated specimens. The x-ray doses of 300 r, 400 r and 500 r in different meiotic stages induced the common aberrations like breaks (figures 1–3, 5, 6, 10, 11, 13, 15, 17), fragment of unknown origin (figures 11, 16), translocation/pseudochiasma (figures 1, 4, 7–9, 14), constriction (figures 9, 12) etc, while the anaphase I plates had specially the chromatid bridges (figure 18) seemed to be formed by the X_1 and/or the Y and some laggards. Like bridges in anaphase I, the pseudochiasma was specific to the prophase I and metaphase I.

An analysis of the x-ray induced aberration frequency data assessed at 13 different intervals for the treatment of 300 r (table 1) and 400 r (table 2) and at 500 r for 14 intervals (table 3) would reveal that because of the paucity of dividing cells, the chromosome aberrations were very likely not found in most of the intervals at spermatogonial metaphases of 300 r and 400 r (tables 1, 2) and to some extent in 500 r (table 3). Even then the aberration frequency in sex chromosomes was more than that of non-marker group in all doses. In 500 r the effect was present in all intervals between

Table 1. Frequency of aberrations in sex chromosomes and autosomes (figures in parentheses) induced by 300 r in different meiotic stages of *P. schlanbuschi*.

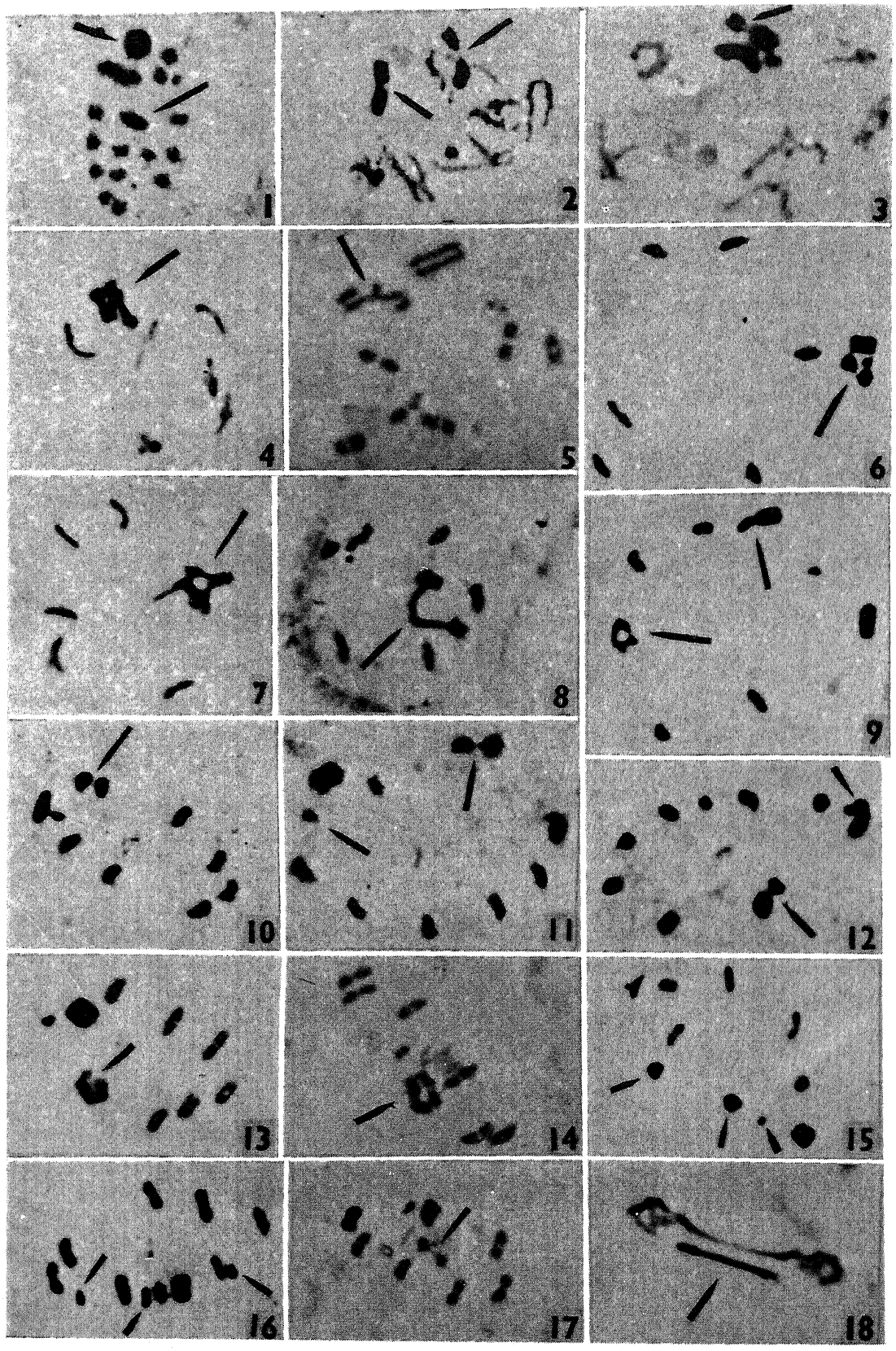
Fix. time	Sp. metaphase			Prophase I			Metaphase I			Anaphase I		Metaphase II		
	No. of cells	Break	Other types	No. of cells	Break	Other types	No. of cells	Break	Other types	No. of cells	Ana. bridge	No. of cells	Break	Other types
5 min	13	—	—	100	1	1	200	—	1	—	—	200	(1)	(1)
1 hr	5	—	—	100	1	2	200	2	1(1)	—	—	200	—	(1)
4 hr	40	1(2)	—	100	3	2	200	9(4)	5(1)	15	7	200	(1)	3(1)
8 hr	20	—	—	100	6	1(3)	200	11(4)	4(6)	5	4	200	(2)	1(4)
16 hr	62	—	3	100	8	10	200	5(2)	2(5)	3	—	200	4(2)	3(2)
24 hr	24	3(2)	—	100	3	3	200	3(2)	3(3)	—	—	200	1(3)	(3)
48 hr	27	—	—	100	1(1)	2(1)	200	2	10(1)	2	—	200	1	1
72 hr	12	—	—	100	1	5	200	5	6(2)	12	—	200	1	4
96 hr	28	—	5	100	2(1)	1	200	2	(1)	5	2	200	—	—
120 hr	15	—	—	100	8	7	200	10	1(2)	10	4	200	(2)	—
144 hr	23	—	—	100	4	—	200	4	1(2)	2	1	200	—	—
168 hr	9	1	—	100	3	3(1)	200	7	3	7	3	200	(2)	—
192 hr	29	—	—	100	1(1)	1	200	1	1(1)	5	3	200	—	1
Total	307	5(4)	8	1300	42(3)	38(5)	2600	61(12)	38(25)	66	24	2600	7(13)	13(12)

Table 2. Frequency of aberrations in sex chromosomes and autosomes (figures in parentheses) induced by 400 r in different meiotic stages of *P. schlanbuschi*.

	Sp. metaphase			Prophase I			Metaphase I			Anaphase I		Metaphase II		
Fix. time	No. of cells	Break	Other types	No. of cells	Break	Other types	No. of cells	Break	Other types	No. of cells	Ana. bridge	No. of cells	Break	Other types
5 min	5	—	1	100	(1)	—	200	—	2(2)	5	1	200	(1)	1(1)
1 hr	15	—	1	100	2	1	200	1(1)	2(3)	5	—	200	(2)	(1)
4 hr	9	—	—	100	2	7	200	9(3)	13(5)	4	1	200	—	(2)
8 hr	11	—	—	100	2(2)	6(7)	200	3(3)	12(5)	15	9	200	1	2(1)
16 hr	160	15(2)	5(8)	100	—	4(2)	200	8(4)	9(5)	2	1	200	—	1
24 hr	12	—	—	100	—	(2)	200	4(3)	12(5)	7	3	200	(3)	(2)
48 hr	9	—	—	100	—	—	200	5(2)	13(2)	8	7	200	(1)	—
72 hr	16	—	—	100	5	4	200	8	3(2)	6	4	200	(2)	—
96 hr	16	—	—	100	2	3(1)	200	15	2	4	—	200	—	—
120 hr	27	1	—	100	4(1)	7	200	6(2)	12(2)	4	3	200	1(1)	—
144 hr	—	—	—	100	3(2)	3	200	3	3	7	—	200	(1)	—
168 hr	10	—	—	100	2	3	200	7	6(2)	—	—	200	—	—
216 hr	6	—	—	100	8	—	200	5	1	3	1	200	(2)	—
Total	296	16(2)	7(8)	1300	30(6)	38(12)	2600	74(18)	90(33)	70	30	2600	2(13)	4(7)

Table 3. Frequency of aberrations in sex chromosomes and autosomes (figures in parentheses) induced by 500 r in different meiotic stages of *P. schlanbuschi*.

Fix. time	Sp. metaphase			Prophase I			Metaphase I			Anaphase I		Metaphase II		
	No. of cells	Break	Other types	No. of cells	Break	Other types	No. of cells	Break	Other types	No. of cells	Ana. bridge	No. of cells	Break	Other types
5 min	—	—	—	100	—	(2)	200	2	3(2)	—	—	200	—	—
1 hr	10	—	—	100	1	2(4)	200	2	2(2)	—	—	200	—	1
4 hr	28	6(2)	1(1)	100	—	11(6)	200	9(5)	13(9)	10	4	200	—	4
8 hr	93	12(1)	10(6)	100	(1)	3(3)	200	6(4)	15(9)	2	—	200	—	—
16 hr	60	13	9(6)	100	2	4(4)	200	5(3)	9(1)	—	—	200	—	—
24 hr	40	1(1)	1	100	1(1)	(1)	200	16(4)	20(6)	8	—	200	(1)	—
48 hr	13	—	1	100	(3)	1(3)	200	12(2)	19(2)	3	—	200	(2)	—
72 hr	10	—	—	100	6	—	200	7(2)	5(2)	5	2	200	(1)	(1)
96 hr	11	—	—	100	3(1)	2	200	5(4)	8(2)	—	—	200	—	—
120 hr	32	5	2(2)	100	1(2)	—	200	12(3)	—	3	1	200	—	—
168 hr	2	1	—	100	4(1)	—	200	19	2(2)	—	—	200	—	—
192 hr	—	—	—	100	—	—	200	1(1)	10(1)	3	1	200	(2)	—
216 hr	39	6	(3)	100	3(1)	1(2)	200	11(1)	3(3)	2	1	200	—	—
336 hr	22	—	—	100	—	—	200	2	—	—	—	200	(1)	—
Total	360	44(4)	24(18)	1400	21(10)	24(25)	2800	109(29)	109(41)	36	9	2800	(7)	5(1)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4 hr and 24 hr with a peak at 16 hr, while it was rare and erratic at later intervals. The absence of fragment after 24 hr in all doses did not bear testimony to the holocentric nature of the chromosomes. Further, though the number of plates in anaphase I was also limited, the chromosomes appeared more vulnerable than those of the spermatogonial metaphases as revealed by the chromosome bridges formed mainly by the marker pair of sex chromosomes and sometimes laggards or other type of aberration. First two types of aberrations were also unexpected for the holocentric chromosomes (Manna 1984). Cells in prophase I, metaphase I and metaphase II were adequate in number and most of the intervals of fixation contained some form of aberrations (tables 1–3). When the aberration frequency at different intervals induced by 300 r, 400 r and 500 r was examined, a regular mode in rise and fall of the frequency could not be seen. The peak frequency was also found not at the same fixation interval in all these stages. It was 16 hr in prophase I, 8 hr in metaphase I and 16 hr in metaphase II for 300 r (table 1); 8 hr in prophase I and 4 hr in metaphase I and 24 hr in metaphase II for 400 r (table 2) and 4 hr in prophase I, 24 hr in metaphase I and indefinite in metaphase II for 500 r (table 3). In the pooled data of aberration frequency of all intervals, there was a disproportional increase in the higher doses except for the data of prophase I and metaphase II where the frequency was decreased in higher doses. Thus, for the doses 300 r, 400 r and 500 r respectively in the spermatogonial metaphases, it was 5·5% (17 out of 307), 11·1% (33 out of 296) and 25·0% (90 out of 360); in prophase I, 6·7% (88 out of 1300), 6·6% (86 out of 1300) and 5·7% (80 out of 1400); in metaphase I, 5·2% (136 out of 2600), 8·2% (31 out of 2600) and 10·2% (288 out of 2800); in anaphase I, 36·3% (24 out of 66), 42·3% (30 out of 70) and 25·0% (9 out of 36) and in metaphase II, 1·7% (45 out of 2600), 0·9% (25 out of 2600) and 0·5% (13 out of 2800). However, when the aberration data of all stages were combined, it was 4·5% (310 out of 6873) in 300 r, 5·6% (389 out of 6866) in 400 r and 6·4% (480 out of 7396) in 500 r, indicating an average of 1% increase for the rise of 100 r from 300 r to 500 r. Some erratic results obtained sometimes with regard to the dose dependent effect were possibly due to various factors as the analysis was made from the pooled data of various types of aberrations and of different intervals, some observational difficulty due to the physiological effect and so on.

Figures 1–18. Different stages of meiosis of X-irradiated *Physopelta schlanbuschi* showing various types of chromosome aberrations. **1.** Spermatogonial metaphase with a ring and a fragment formed by the marker sex chromosomes. **2.** Diplotene showing chromatid and isochromatid breaks in two marker sex chromosomes. **3.** Diplotene with 3 sex chromosomes associated one of which is broken in the mid-region. **4.** Diplotene showing a chiasma-like configuration formed by the X_1 and the Y, while the X_2 is attached to them. **5.** Diakinesis showing a chromatid break in the mid-region of a marker sex chromosome. **6.** Prometaphase I showing a break in the mid-region of a marker sex chromosome. 7: Metaphase I showing an interstitial chiasma-like configuration formed by the X_1 and the Y. **8.** Showing the stretched out sex chromosome pair. **9.** Metaphase I showing a translocation between two autosomes and a constriction in a marker sex chromosome. **10.** Metaphase I showing a marker sex chromosome broken in the mid-region. **11.** Metaphase I with a break in the mid-region of a marker sex chromosome, while the X_2 is near another marker and a fragment of unknown origin. **12.** Metaphase I with a constriction in marker sex chromosome, while the X_2 is associated with another marker. **13.** Metaphase with a marker sex chromosome broken in the terminal region. **14.** Metaphase I with mid-region break in two associated marker pair. **15.** Metaphase I with dispersed broken parts of a marker sex and a fragment of unknown origin. **16** and **17.** Metaphase II each with some extra elements. **18.** Anaphase I showing two stout chromatid bridges.

Table 4. Observed and expected number of aberrations induced by 300 r, 400 r and 500 r doses in *P. schlanbuschi*.

Dose		Sp. metaphase		Prophase I		Metaphase I		Metaphase II		Total	
		Sex chromosome	Autosome	Sex chromosome	Autosome	Sex chromosome	Autosome	Sex chromosome	Autosome	Sex chromosome	Autosome
300 r	Obs.	13	4	80	8	99	37	20	25	212	74
	Exp.	5	12	26	62	40	96	13	32	84	202
400 r	Obs.	23	10	68	18	164	51	6	19	261	98
	Exp.	10	23	25	61	63	152	7	18	105	244
500 r	Obs.	68	22	45	35	218	70	5	8	336	135
	Exp.	27	63	24	56	85	203	4	9	172	299
Total	Obs.	104	36	193	61	481	158	31	52	809	307
	Exp.	42	98	75	179	188	451	24	59	361	745

With a view to finding out if there was any differential response of the sex chromosomes and autosomes, the aberration frequency in spermatogonial metaphase, prophase I, metaphase I and metaphase II induced by the 3 doses of x-rays was analysed (table 4) which revealed that the observed number of aberrations in the sex chromosomes, occurring mainly in the X_1 and the Y was relatively quite high than that of the expected number calculated on the proportionality of size (5 : 12) and the reverse was true for autosomal aberrations. It was found that in 300 r, 400 r and 500 r and in the total of all doses respectively the observed number of aberrations in the sex chromosomes was higher than the expected number by about 2·6 times, 2·3 times, 2·5 times and 2·5 times in spermatogonial metaphases; about 4 times, 2·7 times, 1·4 times and 2·5 times in prophase I; 2·3 times, 2·4 times, 2·5 times and 2·5 times in metaphase I, and 1·5 times, 1·1 times (lower), 1·2 times and 1·3 times in metaphase II. If the aberration data of the sex chromosomes in these 4 stages were combined, the observed number as compared to the expected number was higher by about 2·5 times in 300 r and 400 r and 1·9 times in 500 r and 2·2 times in the average of all doses, indicating thereby that the sex chromosomes were highly sensitive to the effect of the 3 doses of x-rays. On the other hand, the autosomes were found quite resistant when the observed and the expected numbers were compared except some erratic situation in metaphase II. Thus, in 300 r, 400 r, 500 r and the combined data of all doses respectively the observed number was lower by about 3 times, 2·3 times, 3 times and 2·7 times in the spermatogonial metaphase; about 1·7 times, 3·4 times, 1·6 times and 2·9 times lower in prophase I; 2·6 times, 3 times, 2·9 times and 2·8 times lower in metaphase I, while in metaphase II it was lower by 1·3 times, higher by 1·0 times and 1·1 times and lower by 1·1 times in the average. However, in the combined data of all stages the observed number of aberrations in autosomes was significantly lower than the expected number by 2·7 times in 300 r, 2·5 times in 400 r, 2·2 times in 500 r and 2·4 times in the pooled data of all doses indicating clearly that the aberrations were nonrandomly distributed. Further, an analysis of the breaking points in the X_1 and the Y also showed that the mid-region was more vulnerable than the two end regions if the said chromosomes were arbitrarily divided in 3 equal regions.

4. Discussion

In the present study, the aberration frequency in the germinal chromosomes of *P. schlanbuschi* showed on the whole that the effect was dependent on different doses of x-rays used in spite of some erratic results obtained in some stages. It was mentioned before that the frequency-increase was even directly proportional when the data of all stages were pooled, showing an increase of about 1 % aberration for an increase of 100 r as it was 4·5 % in 300 r, 5·6 % in 400 r and 6·4 % in 500 r. However, it was not found when the data of different stages were separately analysed, some reasons for which were stated before.

The present study did not favour the holocentric nature of chromosomes in *Physopelta* as the number of fragments neither increased nor could they survive in cells fixed at later intervals by which time the cell could have undergone a mitotic cycle. There was also a number of chromatid bridges and some laggards at anaphase I which was not expected for dividing holocentric chromosomes (Manna 1984, 1985). Further, the breaks in marker sex chromosomes of *Physopelta* were nonrandomly distributed

between and within chromosome complement as found in chromosomes of other species with localized centromere (Kihlman 1966; Manna 1975). Lastly the occurrence of the chiasma-like configuration between X_1 and the Y, found sometimes during prophase I and its persistence in the interstitial region in metaphase I was contrary to the characteristic regular behaviour of the holocentric chromosomes. The induction of chiasma-like configuration between the marker sex chromosomes found in prophase I which continued to the metaphase I has not been recorded so far in Heteroptera. It was suggested to be due to their close association during early prophase I and the x-ray had delayed their separation otherwise the sex chromosome normally lie independently from pachytene-diplotene stage (Manna and Dey 1981). It was also suspected by these workers (Manna and Dey 1981) that the genetical exchange between sex chromosomes might normally take place during very early prophase I when they remain associated very closely. But uptill now no sex-linked genes in *Physopelta* have been discovered which could be used in verifying the proposition of genetical crossing-over of sex-linked genes during very early prophase I.

That the sex chromosomes were more susceptible to x-irradiation than the autosomes was found in spermatocyte chromosome of grasshoppers (Manna and Mazumder 1962, 1967). Bick and Brown (1975) also showed that the sex chromosomes were more radiosensitive than autosomes in cultured cells of a marsupial. Since the sex chromosomes are heterochromatic, it is tempting to suggest that they could be more sensitive to x-rays because of their heterochromatic nature.

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