THE BEHAVIOR OF B-CHROMOSOMES OF *TAINIA* *LAXIFLORA* IN MALE GAMETOGENESIS¹⁾

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In a previous paper (Tanaka and Matsuda 1972) we have reported that *Tainia laxiflora*, $2n=36+0-9B$, the Orchidaceae, carries B-chromosome in most of the clones (more than 95%). The mechanism of the accumulation of B-chromosomes is observed to be due to non-disjunction and preferential distribution of B-chromosomes into gametes. These observations have been reported in *Secale cereale* (Hasegawa 1934; Müntzing 1945, 1946, 1948; Müntzing and Lima-de-Faria 1953; Lima-de-Faria 1962; Håkansson 1948), *Anthoxanthum aristatum* (Östergren 1947), *Zea mays* (Roman 1947), *Festuca pratensis* (Bosemark 1950, 1954, 1956), *Lilium callosum* (Kayano, 1956, 1957, 1962), *Trillium glandiflorum* (Rutishauser 1956a, 1956b), *Plantago seraria* (Fröste 1959), *Aster ageratoides* group (Matsuda 1970), *Aegilops speltoides* (Mendelson and Zohary 1972) and so on. In these plants, however, the occurrence of B-chromosomes was not so high as found in *Tainia laxiflora*. In the present investigation, distribution of B-chromosomes during the male gametogenesis in *Tainia laxiflora* was studied to clarify the mechanism of high occurrence of B-chromosomes in the populations of this species.

MATERIALS AND METHODS

The clones of *Tainia laxiflora* Makino investigated were collected from Tainoko and Suzuko in Yaku Island, southwestern Japan. The same experimental numbers as named in our previous report (Tanaka and Matsuda 1972) were used in the clones of the present investigation.

Meiosis was observed in pollen mother cells (PMCs). The distribution of B-chromosomes in microspore division was observed in microspore tetrads and in pollinia. Anthers were fixed in 2:1:1 mixture of absolute ethyl alcohol, chloroform and glacial acetic acid for 10 min at 5°C. The anthers were stained for 3 min and then squashed in 1% aceto-orcin.

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RESULTS

Since *Tainia laxiflora* yields pollinium and its B-chromosomes were clearly heteropycnotic, the behavior of B-chromosomes could be easily observed through meiosis and successive microspore division. At each meiotic I and II interphase B-chromosomes were observed as heteropycnotic bodies of which the number was almost the same as that of B-chromosomes of the root tip.

The meiotic behavior of ordinary chromosomes was found to be normal in both the clones without B-chromosomes, $2n=36$, and those with B-chromosome, such as $2n=36+1B$, $36+2B$, $36+3B$, $36+4B$, and $36+8B$. In all of these clones the 36 ordinary chromosomes formed 18 bivalents regularly. Pairing of B-chromosomes with ordinary chromosomes was not observed.

In contrast to the ordinary chromosomes the B-chromosomes behaved somewhat different. In 10 of the 36 PMCs observed in the clone T-9-11 with two B-chromosomes, the B-chromosomes were observed to form one bivalent of end-to-end pairing at diakinesis and metaphase I (Fig. 1A). In the remaining 26 PMCs each B-chromosome formed univalents (Fig. 1B), of which 13 were located closely together (Fig. 1C). At late metaphase I the two B-chromosomes were observed to be located one at each pole in 16 PMCs, while in 25 PMCs both of the B-chromosomes to only one pole, in 38 PMCs one to one pole and the other at the equatorial plate, and in 55 PMCs both at the equatorial plate. The two B-chromosomes of the clone T-2-1 behaved similarly to those of the clone T-9-11 above mentioned. In the clone T-7 with three B-chromosomes, the B-chromosomes appeared as one trivalent (Fig. 1D), one bivalent and one univalent (Fig. 1E) or three univalents.

The distribution pattern of B-chromosomes at anaphase I was as follows. In the clone T-9-11 with two B-chromosomes, in 30 PMCs the two B-chromosomes distributed equally to each pole (Fig. 1G) and in 16 PMCs both B-chromosomes distributed to one pole (Fig. 1H). In the 51 PMCs observed in the clone T-7 with three B-chromosomes, one of the B-chromosomes distributed to one pole and the other two to the other pole (Fig. 1F).

At anaphase I, in the clone T-9-11 with two B-chromosomes lagging chromosomes were seen in 11 out of 73 PMCs and at telophase I in 6 out of 71 PMCs. The lagging chromosomes are considered to be the B-chromosomes by their irregular behavior in contrast to the regular behavior of ordinary chromosomes of the present materials. In the clone T-9-14 with two B-chromosomes, lagging chromosomes were observed in 4 out of 95 PMCs observed. In the clone T-7 with three B-chromosomes, lagging chromosomes were observed at anaphase I in 4 out of 95 PMCs and at telophase I in 2 out of 94 PMCs.

In the three clones with two B-chromosomes, S-2-2, S-3-4, and T-14-1, distribution pattern of B-chromosomes was observed at telophase II, since the B-chromosomes were much more condensed and heavily stained than ordinary chromosomes. The distribution of the daughter B-chromosomes to each nucleus of the microspore tetrad was as follows: four daughter B-chromosomes were distributed to each four nuclei (formulated as

Fig. 1. Photomicrographs of meiotic chromosome behavior in the PMCs of the clones with B-chromosomes in *Tainia laxiflora*. $18_{II}+B_{II}$ (A), $18_{II}+2B_I$ (B), and $18_{II}+2B_I$ (C) in the clone T-9-11 with two B. $18_{II}+B_{III}$ (D) and $18_{II}+B_{II}+B_I$ (E) in the clone T-7 with three B. (F) anaphase I showing the 2:1 distribution of B-chromosomes in the latter clone. (G) anaphase I showing the 1:1 distribution in the former clone. (H) anaphase I showing the 2:0 distribution in the former clone. Arrows indicate B-chromosomes. $\times 1000$.

1:1:1:1, Fig. 2A), two to only two of the four nuclei (2:2:0:0, Fig. 2B), two to one nucleus, one to each of the remaining two nuclei and none in one (2:0:1:1, Fig. 2C), and three to one nucleus, one to one nucleus and none in the remaining two (3:1:0:0, Fig. 2D). The distribution of daughter B-chromosomes at telophase II is shown in Table 1. The 1:1:1:1 distribution was most frequent (75.5% in S-2-2, 76.3% in S-3-4, 64.0% in T-14-1). The 2:2:0:0 distribution was the second highest with a mean frequency of 17.2%, the 2:0:1:1 distribution the third with 9.6%, and the 3:1:0:0 distri-

Fig. 2. Photomicrographs showing the distribution of B-chromosomes in microspore tetrad nuclei (A-D) and in the pollen grain nuclei of pollinia (E-H) in the clone S-2-2 with two B-chromosome in *Tainia laxiflora*. The B-chromosomes can be seen as heteropycnotic bodies. Of the nuclei in pollen grains, the dark one is the generative nucleus and the faint one is the vegetative nucleus. (A) and (E), the 1:1:1:1 distribution. (B) and (F), the 2:2:0:0 distribution. (C) and (G), the 2:0:1:1 distribution. (D) and (H), the 3:1:0:0 distribution. $\times 1000$.

Table 1. The distribution of B-chromosomes at telophase II nuclei of microspore tetrads in three clones with two B-chromosomes in *Tainia laxiflora*

Clones	No. of the following distribution of B (%)			
	1:1:1:1	2:2:0:0	2:0:1:1	3:1:0:0
S-2-2	71 (75.5)	14 (14.9)	8 (8.5)	1 (1.1)
S-3-4	74 (76.3)	14 (14.4)	7 (7.2)	2 (2.1)
T-14-1	64 (64.0)	22 (22.0)	13 (13.0)	1 (1.0)
Total	209 (71.8)	50 (17.2)	28 (9.6)	4 (1.4)

bution the lowest with 1.4%. The 2:0:1:1 and 3:1:0:0 distributions might have been formed by the non-disjunction of B-chromosomes at the second meiotic division.

In the clone T-10-6 with four B-chromosomes, distribution patterns were 2:2:2:2 in 71 microspore tetrads, 3:3:1:1 in 19, 3:1:2:2 in five, 4:2:1:1 in two, and 4:4:0:0 and 3:3:2:0 in one respectively. The 2:2:2:2 distribution, the highest occurrence, is considered to be of equal distribution which was similar to the 1:1:1:1 distribution occurred with the highest occurrence in the clones with two B-chromosomes. The distributions 3:1:2:2, 4:2:1:1, and 3:3:2:0 might be due to the non-disjunction.

The distribution of daughter B-chromosomes to the generative nucleus and the vegetative nucleus at the first microspore division was investigated in the three clones with two B-chromosomes, S-2-2, T-2-1, and T-2-7. At anaphase and telophase, B-chromosomes and ordinary chromosomes were equally divided and regularly distributed to both poles, and thus neither laggard nor micronucleus was observed in all of these nuclei investigated. At early interphase when the vegetative nucleus diffused, the B-chromosomes could be seen as heteropycnotic bodies in the vegetative nucleus but not in the generative nucleus as it was still condensed. In the nuclei of matured pollen grains the B-chromosomes could be observed as heteropycnotic bodies since both generative and vegetative nuclei were diffused (Fig. 2). The distribution of B-chromosomes to these two nuclei was observed to be equal in all of the pollen grains investigated. The distribution pattern of B-chromosomes in a pollinium can be formulated as follows. In these formulae the numbers of B-chromosomes in generative and vegetative nuclei are shown in parentheses: (1:1):(1:1):(1:1):(1:1) (Fig. 2E), (2:2):(2:2):(0:0):(0:0) (Fig. 2F), (2:2):(0:0):(1:1):(1:1) (Fig. 2G), and (3:3):(1:1):(0:0):(0:0) (Fig. 2H). The (1:1):(1:1):(1:1):(1:1) distribution might have resulted from the 1:1:1:1 distribution of microspore tetrad, (2:2):(2:2):(0:0):(0:0) from 2:2:0:0, (2:2):(0:0):(1:1):(1:1) from 2:0:1:1, and (3:3):(1:1):(0:0):(0:0) from 3:1:0:0. Among these the (1:1):(1:1):(1:1):(1:1) distribution occurred most often with a mean frequency of 77.0% (Table 2). The (2:2):(2:2):(0:0):(0:0) distribution was the second highest (14.2%) and the (2:2):(0:0):(1:1):(1:1) distribution the third (8.2%). The (3:3):(1:1):(0:0):(0:0) distribution was found to be a very rare case with a mean frequency of 0.6%.

Table 2. The distribution of B-chromosomes at interphase in the generative nuclei of pollinia after the first microspore division in three clones with two B-chromosomes in *Tainia laxiflora*

Clones	No. of the following distribution of B (%)			
	1:1:1:1	2:2:0:0	2:0:1:1	3:1:0:0
S-2-2	88 (76.5)	17 (14.8)	9 (7.8)	1 (0.9)
T-2-1	80 (80.0)	11 (11.0)	9 (9.0)	0 (0.0)
T-2-7	86 (74.8)	19 (16.5)	9 (7.8)	1 (0.9)
Total	254 (77.0)	47 (14.2)	27 (8.2)	2 (0.6)

In the clone T-10-6 with four B-chromosomes the distribution pattern in the nuclei of 107 pollinia was as follows: the (2:2):(2:2):(2:2):(2:2) distribution in 71 pollinia (66.4%), (3:3):(3:3):(1:1):(1:1) in 25 pollinia (23.4%), (3:3):(1:1):(2:2):(2:2) in four pollinia (3.7%), (4:4):(2:2):(1:1):(1:1) and (4:4):(4:4):(0:0):(0:0) in three pollinia (2.8%), and (3:3):(3:3):(2:2):(0:0) in one pollinium (0.9%).

DISCUSSION

The B-chromosomes of *Secale cereale* (Hasegawa 1934; Müntzing 1945; etc.) are distributed to the generative nucleus by non-disjunction in microspore division and thus they can be maintained in the populations. The non-disjunction also occurred in megaspore division (Håkansson 1948). Similar non-disjunction was observed in the B-chromosomes of *Lilium callosum* (Kayano 1956, 1957, 1962) and *Trillium glandiflorum* (Rutishauser 1956a, 1956b) and some other angiosperms (Fröste 1959, 1969; Vosa 1962; Gill 1971).

In the microspore division of *Tainia laxiflora* the B-chromosomes have been observed showing the disjunction, and the disjuncted B-chromosomes were distributed regularly to both poles. Besides the 1:1:1:1 distribution and some of the 2:2:0:0 distribution, the 2:0:1:1 and 3:1:0:0 distribution were found. These latter two and some of the 2:2:0:0 distribution might be expected when the B-chromosomes are irregularly divided and distributed by non-disjunction. In the clone with four B-chromosomes the distribution of the B-chromosomes was quite similar to those of the clones with two B-chromosomes above mentioned: the 2:2:2:2 distribution, some of the 3:3:1:1 and 4:4:0:0 distributions by the regular distribution and 3:1:2:2, 4:2:1:1, 3:3:2:0, and some of the two distributions, 3:3:1:1 and 4:4:0:0, by the irregular distributions. The non-disjunction of B-chromosomes at the second meiotic division was found for the first time in plant species.

Kishikawa (1965) reported that in a Japanese cultivated variety of *Secale cereale* the occurrence of the clones with B-chromosomes was very high showing 90.8% and

the pairing of B-chromosomes was 100% at diakinesis and 92.4% at metaphase I. The pairing of B-chromosomes was attributed the cause of the high occurrence of B-chromosomes in this Japanese variety. Although the pairing of B-chromosomes in *Tainia laxiflora* was not so high as that in *Secale cereale*, the elimination of B-chromosomes in the first and the second meiotic division of PMCs was observed to be very low. In *Tainia laxiflora* the occurrence of the generative nucleus with B-chromosomes can be calculated from the distribution of two B-chromosomes in the generative nucleus in the pollinia studied (Table 2):

$$\frac{(4 \times 254) + (2 \times 47) + (3 \times 27) + (2 \times 2)}{4 \times (254 + 47 + 27 + 2)} \times 100 = 90.5\%.$$

It can be speculated that such phenomenon also occurs in the clones with three or four B-chromosomes. Furthermore, in microspore mitotic division the B-chromosomes of *Tainia laxiflora* distributed regularly to the daughter nuclei like the ordinary chromosomes. The regular behavior of B-chromosomes in meiotic and its successive mitotic divisions are considered to be a major cause of the high occurrence of B-chromosomes in the wild individuals of this species.

SUMMARY

The B-chromosomes of *Tainia laxiflora* behaved regularly like the ordinary chromosomes in both meiotic division of PMC and in the successive mitotic divisions. As a result, most of the male gametes are presumed to carry the B-chromosomes. The regular meiotic and mitotic behavior of B-chromosomes is considered to be a major cause of the high occurrence of the clones with B-chromosomes in this species.

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