CHROMOCENTER NUMBER IN *RUMEX ACETOSA* L.

YÛZÔ KUROKI AND MASAHIKE KURITA

Biological Institute, Faculty of Science, Ehime University
Matsuyama, Ehime-ken 790

Received April 27, 1971

At mid-prophase in root tip cell of *Rumex acetosa* L., it was found that there were deeply stained two Y-chromosomes (Kurita and Kuroki 1970) and the following segments of autosome arms: 1) distal segment of short arm in group-A chromosome (abb. A^{sm}) of submedian type, and 2) distal segment of one arm in group-a chromosome (a^m) of median type (Kuroki and Kurita 1969). These segments and the Y-chromosomes consist of heterochromatin, each of them usually revealing itself as a chromocenter at interphase.

The authors (Kurita and Kuroki 1971) made a study on chromocenters in *R. acetosa* nucleus, using the male and female clones in both of which an autosome complement has no such heterochromatic segment (H-segment) as mentioned above. The number of chromocenter per nucleus was reported to be larger in male than in female, being generally zero in the latter and roughly five to six in the former. This difference in number was assumed to be due to the heterochromatin of the Y-chromosomes.

In each sex, there have been made comparative studies on chromocenters between two different clones out of which one has an autosome complement with H-segments and the other has the complement without such segments. The result will be given in this paper.

MATERIALS AND METHODS

The materials used are two female clones (No. 106 collected from Kurayoshi City, Tottori Pref., and No. 16 from Matsuyama City, Ehime Pref.) and two male clones (No. 110 collected from the same city that No. 106, and No. 304 from Wake-chô, Okayama Pref.). The karyotype formula based only on autosomes is, in disregard of satellite, $10A^{st}+2a^{sm}$ for clone No. 106 (Fig. 1) and No. 110 (Figs. 3, 5), and is $3A^{sm}+7A^{st}+2a^m$ for clone No. 16 (Fig. 2) and No. 304 (Figs. 4, 6). The A^{sm} - (Fig. 7, 8) and a^m -chromosome (Fig. 7) have, as mentioned already, H-segment in short arm and in one arm respectively, while the other chromosomes, A^{st} (group-A chromosome of subterminal type) and a^{sm} (group-a chromosome of submedian type), have no such a segment.

Cytological preparations were made from root tips following the same method as in earlier study (Kurita and Kuroki 1971). In many cases, the procedures for two different clones in each sex were carried forward in parallel with each other.

Figs. 1-4. Alignment of somatic metaphase chromosomes. 1, clone No. 106 (♀). 2, No. 16 (♀). 3, No. 110 (♂). 4, No. 304 (♂).
 A, group-A chromosomes. a, group-a chromosomes: m, median type. sm, submedian type. the other autosomes, subterminal type.
 ×2600.

OBSERVATION

1. Clone No. 106 (Karyotype: $2X+10A^{st}+2a^{sm}$)

In this clone, the number of chromocenter per nucleus varies from zero to three as reported before (Kurita and Kuroki 1971). There is no chromocenter in 200 nuclei (88.4%), but 26 nuclei (11.5%) have one to three chromocenters (Table 1). The number of chromocenter per nucleus is 0.1 in average.

2. Clone No. 16 ($2X+3A^{sm}+7A^{st}+2a^m$)

The number of chromocenter per nucleus varies from one to nine in this clone (Table 1). Out of 215 nuclei, each of 63 (29.3%) nuclei has five chromocenters and each of 57 (26.5%) has four ones. These two sorts of nuclei are higher in frequency than others. In this clone, the number of chromocenter per nucleus averages 4.4.

3. Clone No. 110 ($X+2Y+10A^{st}+2a^{sm}$)

In this clone, the chromocenter had already been counted by the authors (1970). The datum in this paper (Table 1) represents one supplemented after further study. The number of chromocenter varies from zero to nine. The majority of nuclei observed

Figs. 5-8. Somatic chromosomes. 5, metaphase chromosomes of clone No. 110 (♂). 6, the same of No. 304 (♂). 7, A^{sm} - and a^m -chromosome at prophase in No. 16 (♀). 8, A^{sm} -chromosome at prophase in the same. Thin arrow, A^{sm} -chromosome. Thick arrow, a^m -chromosome. Figs. 5, 6: $\times$ ca. 2300. Figs. 7, 8: $\times$ ca. 3500.

Table 1. Calculation of chromocenters

Sex	Clone No.	No. of chromocenter per nucleus																Total No. of nuclei	Mean No. of chromocenter	
		0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15			16
♀	106 (0)	200	19	5	2	0	0	0	0	0	0	0	0	0	0	0	0	0	226	0.1
	16 (5H)	0	3	13	38	57	63	29	9	2	1	0	0	0	0	0	0	0	215	4.4
♂	110 (2Y)	5	4	18	33	37	43	45	19	12	4	0	0	0	0	0	0	0	220	4.7
	304 (2Y+5H)	0	0	0	0	0	8	18	22	36	37	33	24	22	14	5	1	0	220	9.2

Y, Y-chromosome. H, H-segment of arm.

have three to six chromocenters as follows: 33 nuclei (15.0%) with three chromocenters, 37 (16.8%) with four, 43 (19.5%) with five, and 45 (20.4%) with six. As known from Table 1, the mode is six. The chromocenter per nucleus averages 4.7 in number.

4. Clone No. 304 ($X+2Y+3A^{sm}+7A^{st}+2a^m$)

Figs. 9-14. Interphase nuclei. 9, clone No. 106 (♀), representing no chromocenter. 10-12, No. 16 (♀), representing three, four and seven chromocenters. 13-14, No. 304 (♂), representing eight and ten chromocenters. $\times$ ca. 1500.

In this clone, the number of chromocenter per nucleus varies from five to fifteen (Table 1). As shown in Table 1, the nuclei having nine, eight and ten chromocenters are 37 (16.8%), 36 (16.3%) and 33 (15.0%) in number respectively. In comparison with these three sorts of nuclei, the others of nuclei are fairly low in frequency. Chromocenter per nucleus shows an average of 9.3 in this clone.

In each clone, the observations are made on many prophases and metaphases in different root tips. There are never found the prophase at which the H-segments of autosomes vary in number and the metaphase at which the chromosome number and karyotype vary.

DISCUSSION

Clone No. 106 (♀) has no chromosome possessing H-segment which usually reveals itself as a chromocenter at interphase. As shown in Table 1, there is no chromocenter in the majority of nuclei observed, the chromocenter per nucleus averaging 0.1 in number. This number can be said to be due to lacking of chromosome with H-segment viz.

heterochromatin. In clone No. 16 (φ), there are three A^{sm} - and two a^m -chromosomes having H-segment. The modal number of chromocenter is five and the average number per nucleus is 4.4. In this clone, it may be admitted that the chromosomes with H-segments are identical in number with the chromocenters per nucleus.

In clone No. 110 (δ), two Y-chromosomes consist of heterochromatin and the other chromosomes have no H-segment. Then, it is guessed to find two chromocenters in each nucleus, while the chromocenter number is five and six in many nuclei and is 4.7 in average. Using this average number, the chromocenter per nucleus is larger in number than the Y-chromosomes consisting of heterochromatin by 2.7. In clone No. 304 (δ), there are five autosomes with H-segments besides two Y-chromosomes. Then, the chromocenters are supposed to be seven in number, while they are nine in mode and average 9.3. In this clone also, the chromocenters are larger in number than the chromosomes having heterochromatin.

From the above statement, it can be said that, in female, the chromocenters correspond in number to the chromosomes with H-segment and also that, in male, the chromocenters are more in number than the chromosomes having heterochromatin. Further the heterochromatin of Y-chromosomes, as mentioned before (Kurita and Kuroki 1971), is considered to have a tendency to gather euchromatin granules into a mass which must be treated as a chromocenter. The H-segments viz. heterochromatin of autosomes seem not to have connection with gathering such granules.

As mentioned already, the chromosome number, the karyotype and the H-segment of autosome are not found to vary from cell to cell in single clone. Then, it is not considered that an endomitosis, an increase in Y-chromosome and a heterochromatinization of some segment in autosome had suddenly arisen in certain cell which will show many chromocenters.

It is shown in Table 1 that, in clone No. 106 (φ) presenting no chromosome with heterochromatin, there are nuclei showing one to three chromocenters whose mean number per nucleus is 0.1 as noted already. At present, these chromocenters are thought to be really only an incidental gathering of euchromatin granules. Even if the occurrence of these false chromocenters is taken into consideration, it will be safe to admit the tendency of Y-chromosomes to gather the euchromatin granules in male clone.

SUMMARY

At interphase nuclei of *Rumex acetosa*, two Y-chromosomes and H-segments of certain autosomes usually reveal themselves as chromocenters. In each sex of the species, two different clones, one bear H-segment and the other no such segment, were studied for counting the chromocenters in relation to H-segment-bearing autosomes, to the Y-chromosomes and to both the autosomes and Y's.

In female, the number of chromocenter corresponds to that of H-segment-bearing autosomes. In male, the chromocenters are more in number than the Y-chromosomes in one clone and also more than the Y's plus the H-segment-bearing autosomes in the other clone. Supporting our earlier study, the above facts seem to show that the heterochromatin of Y-chromosomes has a tendency to gather euchromatin granules into

a mass which must be treated as a chromocenter, and that the heterochromatin of autosomes has no such a tendency.

LITERATURE CITED

- Kurita, M. and Y. Kuroki, 1970 Y-chromosome and heterochromatin in *Rumex acetosa*. Japan. J. Genetics **45**: 255-260.
- Kurita, M. and Y. Kuroki, 1971 Chromocenters in *Rumex acetosa* nucleus. Bot. Mag. Tokyo **84**: 18-23.
- Kuroki, Y. and M. Kurita, 1969 Certain autosomes in *Rumex acetosa* L. Mem. Ehime Univ., Sci., Ser. B **6**: 51-56.