Studies on Chromosome Arrangement in Some Higher Plants

III. *Haplopappus gracilis* ($2n=4$) and *Crepis capillaris* ($2n=6$)¹

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Received September 8, 1979

In a previous paper (Tanaka 1981a), it has been suggested that in those species whose centromeric regions of chromosomes are constantly localized in a small portion of the nuclear envelope during the period from telophase to prophase, the relative arrangement of chromosomes tends to be retained all through the mitotic cycle. This suggestion is based chiefly on the idea that such localized distribution of the centromeric regions would minimize the disarrangement of chromosomes which might possibly occur during prometaphase. The present study was undertaken to clarify whether or not this tendency may really be present in such characterized species. As materials, two species of Compositae, of which chromosome numbers are quite low and chromosomes are readily identifiable to each other, were chosen for the present study, and the chromosomal arrangements were investigated in their root tips.

Materials and methods

Materials: Two species of Compositae; *Haplopappus gracilis* and *Crepis capillaris*, were chosen for the present study. The achenes of these two species were supplied by Prof. Dr. R. Tanaka of Botanical Institute of Hiroshima University.

Aceto-orcein staining: The observations of the relative arrangement of mitotic chromosomes and the chromosomal behavior throughout the mitotic cycle were made, using the preparations made according to the following procedure.

Achenes were germinated on moistened filter paper in petri dishes. The seedlings of which primary roots were 5~15 mm long were fixed in 45% acetic acid for 7 to 10 minutes without any pretreatment, and macerated in a 2:1 mixture of 1 N hydrochloric acid and 45% acetic acid at 60°C for about 20 seconds. Then the root tips were excised and stained with aceto-orcein for about 10 minutes. Preparations were not pressed heavily in order not to distort the nuclei or the spindle bodies. Chromosomal arrangements were observed mostly in the cells at mitotic anaphase, but when possible, the cells at metaphase or at prophase were also used for this observation.

Arm-ratios of the chromosomes (long arm/ short arm) of the two species were

¹ This work was supported, in part, by Research Grant no. 334040 from the Ministry of Education of Japan.

also calculated based on the measurements of the metaphasic chromosomes treated with 8-hydroxyquinoline (0.002 M aqueous solution) for 4 hours at 16–18°C before fixation. The number of the measured cells was 5 in case of *H. gracilis* and 3 in case of *C. capillaris*. For this measurement, the preparations which were pressed were used.

Caffeine treatment: Achenes germinated by the same way as that described above were immersed in 0.1 % aqueous solution of caffeine (Giménez-Martin *et al.* 1965) for 4 to 6 hours at 23°C in petri dishes. After washing thoroughly with running water, they were transferred onto the moistened filter paper with water in petri dishes for another 7.5 to 10 hours at 23°C. Then they were fixed, and processed according to the procedure for making the preparations as mentioned above. In this case also, preparations were not pressed. The total time from the beginning of caffeine treatment to the fixation of the seedlings was modulated to be 13.5 to 14.0 hours. This total time was determined, considering the previous reports of the total time needed for a mitotic cycle in *H. gracilis* (Yonezawa and Tanaka 1973) and in *C. capillaris* (Kuroiwa and Tanaka 1970). Arrangements of chromosomes were studied mostly in the cells at mitotic anaphase.

Observations

1. Chromosomal behavior during mitotic cycle

Observations of the chromosomal behavior throughout the mitotic cycle were made in the meristematic cells of the root tips of *Haplopappus gracilis* ($2n=4$) and *Crepis capillaris* ($2n=6$). For the observations, the preparations which were stained with aceto-orcein and not pressed were used.

In all the prophase nuclei examined, the centromeric regions of chromosomes were observed to be located very close to the inner surface of the nuclear envelope, and localized specifically in a small area (Figs. 1a, 1b, and 2a). The chromosomes were more or less curved at their centromeric regions, and their arms were also usually located close to the nuclear envelope. Some of these configurative characteristics of the prophase chromosomes are also to be recognized in the photomicrographs of the prophase chromosomes of *H. gracilis* (Tanaka 1967) and of *C. capillaris* (Kitani 1963) presented in the previously published papers.

During prometaphase, the centromeric regions of the chromosomes were seen to migrate toward the equatorial plane from their localized positions at one side of the prophase nucleus (Figs. 1c, 1d, and 2b–2d). Their migration toward the equator was thus one directional, almost synchronized, and not so disordered. So, it seems likely that the relative arrangement of chromosomes at prophase is largely retained during prometaphase and metaphase. Most of the arms of the chromosomes at prometaphase and early metaphase tended to be directed to the same direction, reflecting the prophase orientation of the chromosomes (Figs. 1c, 1d, and 2b–2d). The centromeric regions of the sister chromatids were already pointing to the opposite spindle poles.

At metaphase, the chromosomes were radially arranged on the equatorial plane, locating always their centromeric regions in the central part of the metaphase

plate (Figs. 1f and 2f. The side and the oblique views of the metaphase chromosomes are also shown in Figs. 1e and 2e). During metaphase, the centromeric regions were always fixed on the equatorial plane, but the other parts of the chromosomes seemed to be free from this rule. Judging from the chromosomal behavior

Fig. 1. Mitotic chromosomes of *H. gracilis*. a and b, prophase nuclei. Centromeric regions are indicated by arrows. c and d, side views of the chromosomes migrating toward the equatorial plane during prometaphase. e, side view of metaphase chromosomes. f, polar view of metaphase chromosomes. g, chromosomes at mid anaphase. h, chromosomes at late anaphase. i, chromosomes at telophase. Bar represents 10 μ .

during the period from prophase to metaphase in the two species, the radial arrangement of the metaphasic chromosomes seems to be a good reflection of the prophasic chromosomal orientation and arrangement.

At anaphase, the sister chromatids of each chromosome were observed to separate, heading for the opposite spindle poles (Figs. 1g and 2g). In all of the

anaphasic cells examined, the relative arrangement of the chromosomes was the same between the two separating groups of chromosomes. This suggests that the arrangement of chromosomes is not likely to change during anaphase in the two species.

The configurative characteristics of the anaphasic chromosomal group tended

Fig. 2. Mitotic chromosomes of *C. capillaris*. a, prophase nucleus viewed from the side where the centromeric regions (arrows) are localized. b-d, side views of the chromosomes migrating toward the equatorial plane during prometaphase. e, oblique view of metaphasic chromosomes. f, polar view of metaphasic chromosomes. g, anaphase chromosomes. h, early telophase chromosomes. i, two daughter nuclei. Bar represents 10 μ .

to be preserved during telophase (Figs. 1h, 1i, 2h, and 2i), although the chromosomes in later stages were much more structurally dispersed than those at anaphase. The chromosomes at telophase were also bent at their centromeric regions, and these centromeric regions were localized exclusively in the polar region of the nucleus.

This mode of orientation and the configurative characteristic of the telophasic chromosomes were quite similar to those of the prophasic chromosomes. So, it seems likely that the telophasic mode of chromosomal arrangement and orientation is retained during interphase and succeeding prophase, in the present materials. Similar suggestions have also been made in some other higher plants (Stack and Clarke 1974, Fussell 1975, Ghosh and Roy 1977, Roy and Ghosh 1977, Tanaka and Tanaka 1977, Tanaka 1981a).

At interphase, the greater part of the chromosomes was extremely dispersed structurally, so that the arrangement of the chromosomes was hard to analyse.

Judging from all the results obtained here, it seems highly likely that the relative arrangement of chromosomes of the two species is largely retained all through the mitotic cycle.

2. Relative arrangement of mitotic chromosomes

The observation of the relative arrangement of mitotic chromosomes were made in the root tip cells of *H. gracilis* and *C. capillaris*, using unpressed prepara-

Fig. 3. Two different patterns of the chromosomal arrangement at metaphase (polar view) in *H. gracilis*. a, pattern 1. b, pattern 2. Two pairs of homologous chromosomes are indicated by numbers 1 and 2, respectively. Bar represents 10 μ .

tions. These observations were made mostly in the cells at anaphase, but when possible, the cells at metaphase or at late prophase were also used. The relative arrangement of chromosomes at anaphase can relatively easily be determined by direct observations under a microscope, and we can confirm this further by checking the chromosomal arrangements of the two chromosomal groups. The arrangement of chromosomes of somewhat distorted cells which might have been caused during the process of making preparations was not included in the present data.

A. *Haplopappus gracilis* ($2n=4$)

The chromosomal complement of this species consisted of a pair of metacentric chromosomes (arm ratio, 1.4) and a pair of submetacentric ones (arm ratio, 2.3) whose short arms had nucleolar constrictions. The former was longer than the latter, and these two pairs of chromosomes were designated here as chromosomes

1 and 2, respectively (Fig. 3). As mentioned already, these four chromosomes were radially arranged on the equatorial plane at metaphase, locating always their centromeric regions in the central part of the metaphase plate. This radial arrangement of these chromosomes was also retained during anaphase. Two different patterns are distinguishable as to the relative arrangement of these four chromosomes; namely, one is the pattern in which the homologous chromosomes of each pair occupy adjacent positions as shown in Fig. 3a (this arrangement is designated here as pattern 1), and the other is the one in which the homologous chromosomes of each pair do not occupy any adjacent positions as shown in Fig. 3b (this pattern is designated here as pattern 2). The results of the observations of frequency of each pattern are shown in Table 1. That is, of 500 cells observed, 447 cells (89.4%) showed the arrangement of pattern 1, and the rest 53 cells (10.6%) showed that of pattern 2: Namely, the occurrence of the pattern 1 was much more frequent than that of the pattern 2. The results obtained here differ partly from those previously obtained by Werry *et al.* (1977) in the root tips of the same species. According to their report, the chromosome arrangement of pattern 2 (arrangement III in their report) was not found in both the cross sectioned materials and the macerated undisturbed cells. If it is assumed that the chromosomes are arranged randomly, the occurrence of the pattern 1 may be twice as frequent as that of the pattern 2 (cf. Lacadena *et al.* 1977), and thus 2/3 of the total examined cells would show the arrangement of pattern 1, and the rest 1/3 would show the pattern 2. However,

Table 1. Observed number of each of the two chromosomal arrangement patterns in *H. gracilis*

Total no. of mitotic cells observed	No. of cells with the pattern 1 (%)	No. of cells with the pattern 2 (%)
500	447 (89.4%)	53 (10.6%)

the observed frequencies of these two patterns obviously deviated significantly from the expected ones calculated based on the above assumption.

In order to determine the pattern of chromosomal arrangement in 500 cells, 54 root tips from the same number of achenes were used in all. Of these 54 root tips examined, in 24 root tips, the cells with the chromosomal arrangement of pattern 2 were not observed at all, and even in the rest 30 root tips the occurrence of the cells with pattern 2 was quite low (the highest frequency of the cells with pattern 2 per root tip was 45.5%). That is, in all of the root tips examined, the cells with pattern 1 were more frequent than those with pattern 2.

B. *Crepis capillaris* (2n=6)

The chromosomal complement of this species consisted of three well identifiable pairs of chromosomes. They were designated here as chromosomes 1, 2, and 3, respectively, in the decreasing order of size (Fig. 4). The longest pair of chromosomes (pair no. 1) was subtelocentric (arm ratio, 3.1), the second longest one of which short arms had secondary constrictions (pair no. 2) was telocentric (arm ratio, 7.3), and the shortest one (pair no. 3) was submetacentric (arm ratio, 3.0).

The chromosomes at metaphase were radially arranged on the equatorial plane, locating always their centromeric regions in the central part of the metaphase plate

(Figs. 2f and 4). A total of 11 different patterns was to be distinguished as to the relative arrangement of the 6 chromosomes, as shown schematically in Fig. 5, and all of these 11 patterns could actually be observed in the root tips used (however, not all of these 11 patterns were usually to be found in a root tip, due mainly to the small sample size). Some of these patterns are shown in Fig. 4(a-d). The 11 patterns of the radial chromosomal arrangement depicted in Fig. 5 are arranged under 4 different categories, A, B, C, and D, according to the way presented by Heneen and Nichols (1972). That is, in category A, homologous chromosomes of each pair are adjacent to each other; in category B, homologous chromosomes of

Fig. 4. Some patterns of chromosomal arrangement at metaphase (polar view) in *C. capillaris*. Three pairs of homologous chromosomes are indicated by numbers, 1, 2, and 3, respectively, in the decreasing order of size. Patterns in a, b, c, and d correspond, respectively, to A-(1), B-(4), C-(6), and D-(10) in Fig. 5. Bar represents 10 μ .

only two pairs are adjacent to each other; in category C, homologous chromosomes of only one pair occupy adjacent positions; and in category D, no homologous chromosomes occupy adjacent positions. If it is assumed that the chromosomes of this species are arranged randomly, the probability of occurrence of each of the patterns 1, 5, 6, and 7 is calculated to be 2/15, while that of the other patterns 1/15. So, the probability of each of the former patterns is twice as that of each of the latter patterns, as has already been pointed out by Nur (1973), concerning the

chromosome arrangement of the Indian muntjac. In this study, pattern of chromosomal arrangement was determined in a total of 500 cells from many root tips ($N=67$). Both the observed and the expected frequencies of 11 different patterns of radial chromosome arrangement are shown in Table 2. As obvious from Table 2, both the frequencies, when analysed statistically, are not deviated so distinctly to each other. This may imply that the individual chromosomes are rather independent with each other in occupying their own positions. However, as shown in Table 3 which was prepared from Table 2 to examine further the preference for the side by side arrangement of the respective three pairs of homologues, the second pair of chromosomes (the nucleolar ones) deviated from expected frequencies and showed some tendency of taking the side by side arrangement.

Fig. 5. Diagrammatical representation of 11 different patterns of chromosomal arrangement in *C. capillaris*. Three pairs of homologous chromosomes are designated by numbers, 1, 2, and 3, respectively, in the decreasing order of size. These 11 patterns are arranged under 4 categories, as presented by Heneen and Nichols (1972).

3. Relative arrangement of mitotic chromosomes in the two nuclei of two adjacent cells

In the preparations which were macerated, stained with aceto-orcein, and not pressed, there are often masses of cells which are still associated with each other. Occasionally we can find two closely located cells that are morphologically similar to each other, and happen to be undergoing mitosis simultaneously (Fig. 6). Comparative observations of the relative chromosomal arrangements of the two nuclei of these two closely located cells or of two still adjoining cells were made. The results obtained are summarized in Table 4. That is, in case of *H. gracilis*, of 40 pairs of these two adjacent cells examined, the number of the pairs of which two

Table 2. Observed and expected frequencies of the 11 different chromosomal arrangement patterns in *C. capillaris*

Category	A			B			C			D			Total
	1	2	3	4	5	6	7	8	9	10	11		
Pattern													
Observed number (O)	79	41	30	39	55	74	69	33	27	29	24	500	
Expected value (E)	66.7	33.3	33.3	33.3	66.7	66.7	66.7	33.3	33.3	33.3	33.3	499.9	
$\frac{(O-E)^2}{E}$	2.268	1.780	0.327	0.976	2.052	0.799	0.079	0.003	1.192	0.555	2.597	12.628*	

* χ^2 , 0.20 < P < 0.30, d.f. = 10.Table 3. Observed and expected frequencies of side by side arrangement for the respective three pairs of chromosomes of *C. capillaris* (Data based on Table 2)

Chromosome pair	Observed (O) or Expected (E)	Total no. of cells with homologues		χ^2	P
		showing SSA *	not showing SSA *		
1	O	205	295	} 0.208	0.5 < P < 0.7
	E	200	300		
2	O	233	267	} 9.075	0.001 < P < 0.01
	E	200	300		
3	O	217	283	} 2.408	0.1 < P < 0.2
	E	200	300		

* abbreviation of "side by side arrangement".

nuclei showed the same relative chromosomal arrangement pattern was 39 (97.5%), and that of the pairs of which two nuclei showed the different patterns was only 1 (2.5%). In case of *C. capillaris*, of 40 pairs of two adjacent cells examined, the number of the pairs of which two nuclei showed the same relative chromosomal

Fig. 6. Two adjacent cells of which two nuclei are undergoing mitosis simultaneously (*C. capillaris*). Both the cells presented the same relative chromosomal arrangement pattern (C-6 in Fig. 5). Bar represents 10 μ .

Table 4. Results of observations on the relative arrangement of the mitotic chromosomes in the two nuclei of two adjacent cells in *H. gracilis* and *C. capillaris*

Species	No. of pairs of two adjacent cells observed	No. of pairs of two adjacent cells of which two nuclei showed	
		the same chromosomal arrangement pattern (%)	the different chromosomal arrangement patterns (%)
<i>H. gracilis</i>	40	39* (97.5%)	1 (2.5%)
<i>C. capillaris</i>	40	32** (80.0%)	8*** (20.0%)

* Of 39 pairs of cells, 33 showed the pattern 1 and the other 6 showed the pattern 2.

** Chromosomal arrangement patterns of these 32 pairs of cells are as follows (in parenthesis, the respective frequency is shown): 1(7), 3(2), 4(4), 5(3), 6(5), 7(6), 8(1), 9(2), 11(2).

*** Chromosomal arrangement patterns in the two nuclei of these 8 pairs are as follows (in parenthesis, the respective frequency is shown): 1-5(2), 3-5(1), 3-7(1), 7-10(1), 6-11(2), 8-11(1).

arrangement pattern was 32 (80%), and that of the pairs which showed the different patterns was 8 (20%). These results show that in such very closely located cells, whether or not the two cells are the sister ones produced by a preceding cell division, the occurrence of the pairs of two closely located cells of which two nuclei showed the

same relative arrangement pattern was significantly high in both of the two species. This fact seems to indicate that the relative positions of chromosomes are likely to be retained through the mitotic cycle in the two species.

Various chromosomal arrangement patterns were found in the two adjacent cells of which two nuclei showed the same relative chromosomal arrangement (Table 4). Some pairs of those cells showed the patterns in which no homologous chromosomes occupy adjacent positions. This fact may imply that the relative positions of chromosomes can be retained irrespective of the arrangement pattern.

The results obtained here seem to indicate that the relative chromosomal ar-

Fig. 7. Two binucleate cells induced by caffeine treatment. a, *H. gracilis*. Both dividing nuclei had the same relative chromosomal arrangement (pattern 2). b, *C. capillaris*. The left-hand nucleus in this cell had the chromosomal arrangement of pattern 5, while the right-hand one pattern 2. Each bar represents 10 μ .

rangment of *C. capillaris* is slightly more variable than that of *H. gracilis*. This seems to be ascribed to the differences in the chromosomal number and the karyotype of the two species.

4. Relative arrangement of mitotic chromosomes in the two sister nuclei of the binucleate cells induced by caffeine treatment

An attempt was made to observe the relative arrangement of mitotic chromosomes in the two sister nuclei of the binucleate cells which were induced by caffeine treatment.

Frequency of the binucleate cells varied to some degree among the root tips. In the root tips contained many binucleate cells, chromosome aberrations such as fragments and bridges were also found frequently. In recording the chromosomal arrangements of binucleate cells, those cells with such chromosomal aberrations were excluded. Mitoses of the two nuclei of the binucleate cells were completely synchronized (Figs. 7a and 7b). The observations of the patterns of the chromosomal arrangement were made mostly in the cells at anaphase, but when possible, the cells at metaphase were also used.

The number of the binucleate cells of which patterns of the chromosomal arrangement of the two sister nuclei could be determined was 12 in both species (Table 5). In case of *H. gracilis*, of the 12 binucleate cells examined, the number of the binucleate cells of which two nuclei showed the same relative chromosomal arrangement pattern was 11, and that of the binucleate cells of which two nuclei showed the different patterns was only 1. In case of *C. capillaris*, the number of the binucleate cells of which two nuclei presented the same pattern was 10, and that

Table 5. Results of observations on the relative arrangement of the mitotic chromosomes in the two nuclei of the binucleate cells induced by caffeine treatment in *H. gracilis* and *C. capillaris*

Species	No. of binucleate cells observed	No. of binucleate cells of which two nuclei showed	
		the same arrangement pattern	the different arrangement patterns
<i>H. gracilis</i>	12	11*	1
<i>C. capillaris</i>	12	10**	2***

* Of 11 binucleate cells, 6 showed the pattern 1 and the other 5 showed the pattern 2.

** Chromosomal arrangement patterns of these 10 binucleate cells are as follows (in parenthesis, the respective frequency is shown): 1(6), 3(1), 5(1), 6(2).

*** Chromosomal arrangement patterns of these 2 binucleate cells are as follows (in parenthesis, the respective frequency is shown): 2-5(1), 3-5(1).

of the binucleate cells of which two nuclei showed the different patterns was only 2. That is, the frequency of the binucleate cells of which two nuclei showed the same relative chromosomal arrangement pattern was significantly high in both species. The results obtained here seem to indicate that the relative arrangement of chromosomes tends to be retained through the mitotic cycle.

About half of the patterns of the relative chromosomal arrangement of the binucleate cells of which two nuclei showed the same pattern were not the pattern 1 defined in the former chapter of this paper in both species (Table 5). Various patterns were, in fact, to be seen in such binucleate cells, particularly in case of *C. capillaris*. This probably indicates that the relative arrangement of chromosomes can be maintained irrespective of the arrangement pattern.

Meanwhile, the fact that the relative arrangement of chromosomes was different in a few cases between the two sister nuclei of binucleate cells examined may suggest that the chromosomal arrangement does change occasionally during the mitotic cycle in both species. The accumulation of these occasional changes in the

relative chromosomal arrangement may eventually give rise to the various patterns of chromosomal arrangement even in the same meristem of a root tip, especially in case of *C. capillaris*.

Discussion

From the observations of the chromosomal behavior during the mitotic cycle in the root tip cells of *H. gracilis* and *C. capillaris*, it can be deduced that the relative arrangement of chromosomes is possibly retained through the mitotic cycle. The presence of this persistent tendency of the relative chromosomal arrangement in the two species also seems to be supported by the considerably high occurrence of the same chromosomal arrangement pattern in the two nuclei of two adjacent cells or of the binucleate cells induced by caffeine treatment. The relative arrangement of chromosomes of a certain cell of the two species is, therefore, regarded as a good reflection or a modification of that of the parental cell which gave rise to the cell. In an extreme case, it would be probable that the cells with certain chromosomal arrangement pattern increase in number and come to occupy a considerable portion of the meristem, due to the continuous succession of the same chromosomal arrangement pattern through mitoses.

In *Haplopappus*, the observed frequencies of the occurrence of the two chromosomal arrangement patterns were significantly deviated from the expected ones. This result may be taken as an indication that the individual chromosomes behave not in a completely independent manner in occupying their respective spatial positions. However, in all the materials so far studied in this series of research, including the two species of this report, no sign of a peculiar chromosomal behavior involving different chromosomes or different chromosomal segments, such as the so-called somatic homologous chromosome association, was not observed. As a possible cause for this non-random manner of chromosome disposition found in *Haplopappus*, some interaction between nucleolar chromosomes and nucleolus seems to have been involved. In fact, in both species of *Haplopappus* and *Crepis*, the interstitial portions of the short arms of the second longest pair of chromosomes were often observed associated with a nucleolus. This nucleolus-nucleolar chromosome relationship is generally known to continue during a large portion of the mitotic cell cycle. Therefore, it is conceivable that some interactive force between nucleolar chromosomes and nucleolus worked on the completely free disposition of the nucleolar chromosomes and tended to lead them to lie side by side in this species. Due to this, another pair of non-nucleolar chromosomes (No. 1) also necessarily tends to lie side by side.

On the other hand, a manner of chromosome arrangement in *Crepis* was not totally non-random one. But, in this species also, the nucleolar chromosomes showed some tendency of being located side by side (Table 3). In comparison with *Haplopappus*, weaker non-random chromosome disposition in *Crepis* is surmised to be related to some extent to the fact that this species has a higher chromosome number and a pair of smaller-sized chromosomes in its chromosome complement.

In conclusion, the individual chromosomes of both species studied seem to be-

have essentially in an independent manner in spatial disposition. But the nucleolar chromosomes may occasionally be affected in their free disposition by the interaction with the nucleolus.

Although it has been stressed in this paper that the relative arrangement of chromosomes of the two species is likely to be maintained through the mitotic cycle, this does not mean to rule out the possibility of changes of the relative arrangement of chromosomes. As a matter of fact, different arrangement patterns were to be observed not only in the same meristem, but also in the two nuclei of the binucleate cells induced by caffeine. The change in the relative chromosomal arrangement may occur as a consequence of accumulation of the minor positional changes of the centromeric regions which might possibly occur at various stages of mitotic cycle.

As discussed in a previous paper (Tanaka 1981a), in order that the relative position of chromosomes may persist to some extent through the mitotic cycle, it seems requisite that the centromeric regions of chromosomes remain localized in a relatively small area of the nuclear envelope (nuclear peripheral region) from telophase to prophase. If, on the contrary, the centromeric regions are widely distributed over the inner surface of the nuclear envelope, as in *Tofieldia coccinea* var. *kondoi* (Tanaka 1981a) and several other species listed in the Table 1 of the previous paper by Tanaka (1981b), some degree of the disarrangement in the relative positions of the centromeric regions might inevitably occur during the course of their migration toward the equatorial plane at prometaphase. The two species examined in this study are apparently referred to the former group of species.

Summary

1. Observations of the chromosomal arrangement and behavior were made in the meristematic cells of the root tips of *H. gracilis* ($2n=4$) and *C. capillaris* ($2n=6$).

2. In the prophase and telophase nuclei, the centromeric regions of chromosomes were localized in a relatively small area of the nuclear envelope. During prometaphase, the centromeric regions migrated toward the equatorial plane, keeping their arrangement at prophase. During anaphase, the relative arrangement of the chromosomes was the same between the two separating chromosomal groups. These observations suggested that the relative arrangement of chromosomes is likely to be retained through the mitotic cycle.

3. In the two nuclei of two adjacent cells, or of the binucleate cells induced by caffeine, the occurrence of the same relative chromosomal arrangement pattern was significantly high. This fact also seems to support the suggestion mentioned above.

4. In *H. gracilis*, non-random manner of chromosome disposition was revealed from a statistical approach. While in *Crepis*, a pair of nucleolar chromosomes deviated from random disposition and showed some tendency of lying side by side. Derivation of this non-random disposition was discussed briefly, and it was conjectured from all observations that the individual chromosomes are essentially indepen-

dent in occupying their spatial positions.

Acknowledgements

The author wishes to express his deep gratitude to Prof. Dr. Ryuso Tanaka of Hiroshima University and to Prof. Dr. Nobunori Tanaka of Teikyo University for their constant guidance and suggestions throughout the course of this study. He is also deeply indebted to Mr. C. M. Thompson for reading the manuscript.

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