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Studies on the Control of Diploid-like Meiosis in Polyploid Taxa of *Chrysanthemum* III. Decaploid *Ch. crassum* Kitamura

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The assumption that the pairing affinity can always be explained solely on the basis of homology is ruled out by works of Beadle (1933), Li *et al.* (1945) and Okamoto (1957) who found that a single mutated gene can influence dramatically on the mode of chromosome pairing. In addition to these genic controls, various kinds of control factors on the mode of chromosome pairing were put forward by several authors (Brown 1954, John and Henderson 1962, Mochizuki 1964, Thomas and Kaltisikes 1974, Gupta and Kaok 1976). In *Chrysanthemum*, a diploid-like meiotic behaviour characteristics all polyploid species although they may be composed of very closely related genomes. In the previous papers several strategies had been examined and it was suggested that the most likely control system for predominant bivalent formation in hexa- and octoploids although all of the constituent genomes of polyploids were sufficiently homologous to be able to pair with each other, was the restriction of pairing initiation at one site only per chromosome (Watanabe 1977a, 1981a, b).

The purpose of this article is to examine the penetrance of such a control system in decaploid *Ch. crassum* Kitamura through the analyses of chromosome morphology, chromosome behaviour and external morphology in the F_1- and B_1-hybrids between the diploid *Ch. boreale* and *Ch. crassum*.

Materials and methods

Chrysanthemum crassum Kitamura is a stable decaploid which is restricted to Amami Isl., Tokunoshima Isl., Kikai Isl., Yoro Isl., Uke Isl., and Kakeroma Isl. The *Ch. crassum* used in crossing was collected from Tokunoshima Isl., Kagoshima Pref. The diploid *Ch. boreale* Makino was the same strain used in the hybridization studies previously (Watanabe 1981a, b). Taxonomical treatments are followed according to Kitamura's system (1967). All methods used were identical to those reported by Watanabe (1979a, b).

Results

Ch. crassum

Ch. crassum with $2n=10x-1=89$ was used in hybridization with the diploid

Table 1. Measurements of the somatic chromosomes of *Ch. crassum* (2n=10x−1=89)

Chromosomes	Length in μm	Relative length	Arm ratio (long/short)
1	2.6+2.8=5.4	7.6	1.1
2	2.6+2.6=5.2	7.3	1.0
3	1.8+3.3=5.1	7.2	1.8
4	2.4+2.6=5.0	7.0	1.1
5	2.1+2.8=4.9	6.9	1.3
6	2.0+2.8=4.8	6.7	1.4
7	2.3+2.4=4.7	6.6	1.0
8	2.3+2.4=4.7	6.6	1.0
9	2.2+2.4=4.6	6.5	1.1
10	1.8+2.8=4.6	6.5	1.6
11	2.0+2.5=4.5	6.3	1.3
12	1.8+2.7=4.5	6.3	1.5
13	1.2+3.3=4.5	6.3	2.8
14	1.8+2.6=4.4	6.2	1.4
15	2.1+2.2=4.3	6.0	1.0
16	2.0+2.3=4.3	6.0	1.2
17	1.7+2.6=4.3	6.0	1.5
18	1.4+2.9=4.3	6.0	2.1
19	2.0+2.2=4.2	5.9	1.1
20	2.0+2.2=3.2	5.9	1.1
21*	1.9+2.3=4.2	5.9	1.2
22	1.6+2.6=4.2	5.9	1.6
23	1.6+2.6=4.2	5.9	1.6
24	1.6+2.6=4.2	5.9	1.6
25	1.6+2.6=4.2	5.9	1.6
26	1.6+2.6=4.2	5.9	1.6
27	1.4+2.8=4.2	5.9	2.0
28*	2.0+2.1=4.1	5.7	1.1
29	2.0+2.1=4.1	5.7	1.1
30	1.7+2.4=4.1	5.7	1.4
31	1.5+2.6=4.1	5.7	1.7
32	1.4+2.7=4.1	5.7	1.9
33	1.4+2.7=4.1	5.7	1.9
34	1.3+2.8=4.1	5.7	2.2
35	0.6+3.5=4.1	5.7	5.8
36	0.6+3.5=4.1	5.7	5.8
37	2.0+2.0=4.0	5.6	1.0
38	2.0+2.0=4.0	5.6	1.0
39	1.8+2.2=4.0	5.6	1.2
40	1.6+2.4=4.0	5.6	1.5
41	1.6+2.4=4.0	5.6	1.5
42	1.4+2.6=4.0	5.6	1.9
43	1.4+2.6=4.0	5.6	1.9
44	1.4+2.6=4.0	5.6	1.9
45	1.2+2.8=4.0	5.6	2.3
46*	0.9+3.1=4.0	5.6	3.4
47	1.9+2.0=3.9	5.5	1.1
48*	1.6+2.3=3.9	5.5	1.4

Table 1. (continued)

Chromosomes	Length in μm	Relative length	Arm ratio (long/short)
49	1.5+2.4=3.9	5.5	1.6
50	1.5+2.4=3.9	5.5	1.6
51	1.1+2.8=3.9	5.5	2.5
52	0.8+3.1=3.9	5.5	3.9
53	1.9+1.9=3.8	5.3	1.0
54	1.8+2.0=3.8	5.3	1.1
55	1.8+2.0=3.8	5.3	1.1
56	1.8+2.0=3.8	5.3	1.1
57	1.8+2.0=3.8	5.3	1.1
58	1.7+2.1=3.8	5.3	1.2
59	1.6+2.2=3.8	5.3	1.4
60	1.4+2.4=3.8	5.3	1.7
61	1.3+2.5=3.8	5.3	1.9
62	1.1+2.7=3.8	5.3	2.5
63	0.6+3.2=3.8	5.3	5.3
64	1.8+1.9=3.7	5.2	1.1
65	1.8+1.9=3.7	5.2	1.1
66	1.7+2.0=3.7	5.2	1.2
67	1.2+2.5=3.7	5.2	2.1
68	1.1+2.6=3.7	5.2	2.4
69	1.1+2.6=3.7	5.2	2.4
70	0.9+2.8=3.7	5.2	3.1
71	1.7+1.9=3.6	5.0	1.1
72	1.6+2.0=3.6	5.0	1.3
73	1.1+2.5=3.6	5.0	2.3
74	1.6+1.9=3.5	4.9	1.2
75	1.0+2.5=3.5	4.9	2.5
76	1.5+1.9=3.4	4.8	1.3
77	1.4+2.0=3.4	4.8	1.4
78	1.0+2.4=3.4	4.8	2.4
79	0.8+2.6=3.4	4.8	3.3
80	0.6+2.8=3.4	4.8	4.7
81	0.6+2.8=3.4	4.8	4.7
82	1.3+2.0=3.3	4.6	1.5
83	1.4+1.8=3.2	4.5	1.3
84	1.4+1.8=3.2	4.6	1.3
85	1.1+2.1=3.2	4.5	1.9
86	1.0+2.2=3.2	4.5	2.2
87	0.5+2.6=3.1	4.3	5.2
88	0.6+2.2=2.8	3.9	3.7
89*	0.8+1.7=2.5	3.5	2.1

* Chromosome with satellites.

Ch. boreale.

The chromosomes of *Ch. crassum*, at mitotic metaphase, vary in length from 5.4 μm to 2.5 μm and in arm ratio from 1.0 to 5.8 (Fig. 1–A and Table 1). The eighty-nine chromosomes are arranged in order of size in Fig. 2–A. Five chromosomes had minute satellites. Two of them were medium-sized median chromosomes (Chromo-

somes 21 and 28), one a medium-sized subterminal (Chromosome 46), one a medium-sized submedian (Chromosomes 48) and one a small subterminal (Chromosome 89). Chromosomes 35 and 36 were discriminable by their length and almost terminal centromeres. Six other extreme subterminal chromosomes with arm ratios of 3.7–5.3 (Chromosomes 52, 63, 80, 81, 87 and 88) were distinguishable. Karyomorpholo-

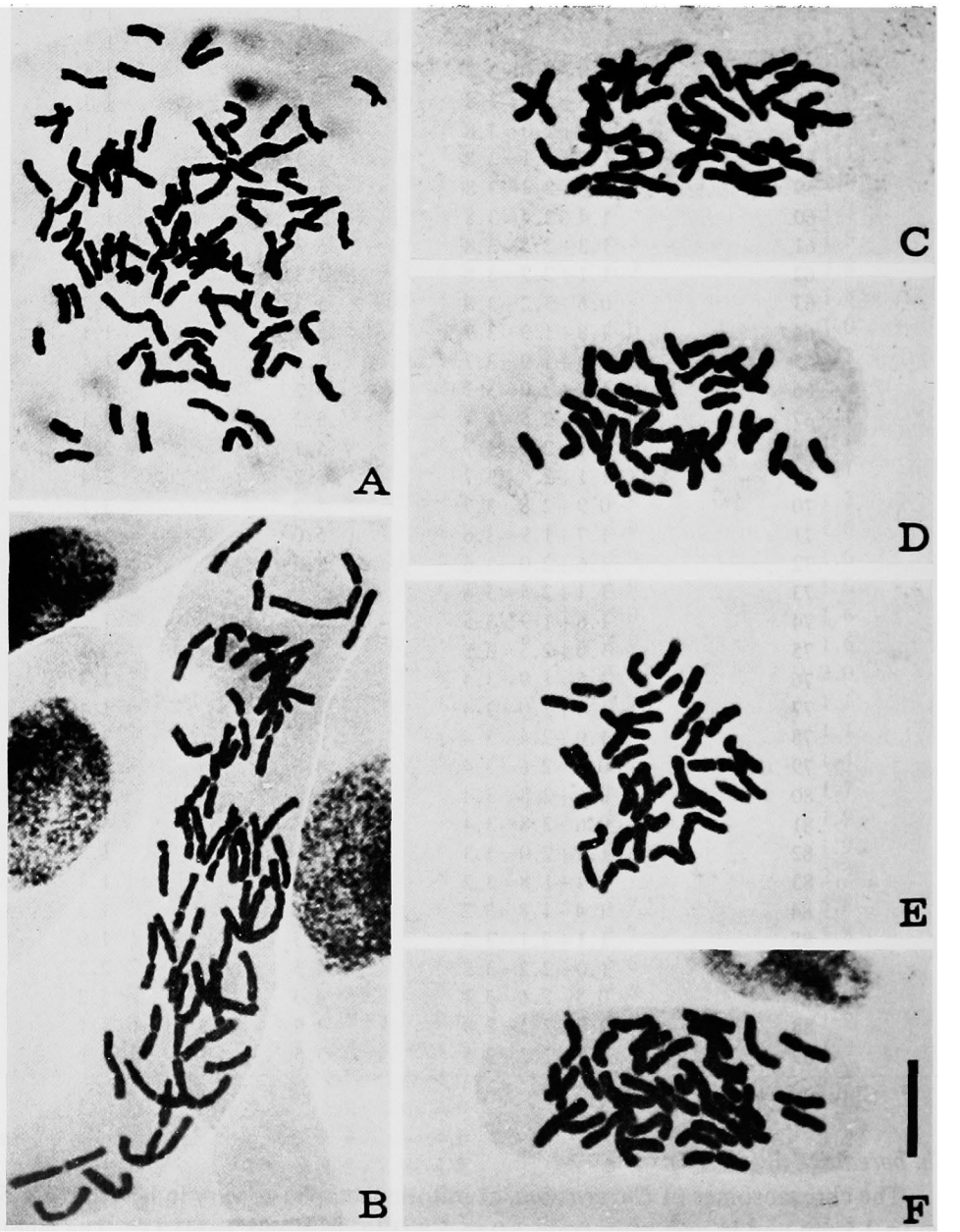

Fig. 1. Mitotic chromosomes. A, *Ch. crassum*. 2n=10x−1=89. B, F_1-hybrid between *Ch. boreale* and *Ch. crassum*. 2n=6x=54. C, D, E and F, B_1-hybrids between *Ch. boreale* × F_1-hybrid. 2n=4x=36. C, plant 23, D: plant 60, E: plant 110, F: plant 114. Scale 10 μm.

gically *Ch. crassum* seems not to be an autodecaploid.

In meiosis 44 II+1 I was the main chromosome configuration (Fig. 3-A). 43 II +3 I and 1 IV+42 II+1 I were rarely observed. The frequency of bivalent formation was 99.8%. Chromosome association in this species is primarily as bivalents.

The parental species *Ch. crassum* and *Ch. boreale* can be distinguished from one another by many characters, —the number of head (less / more), the number of ray and disc florets (more / less) and their size (larger / smaller), the color of ray florets (white / yellow), the size of laminae (smaller / larger), the number of leaf teeth (less / more) and the presence of pseudostipules and rhizome (present / absent). In each comparison the form of *Ch. crassum* is given first.

Ch. boreale (♀)×*Ch. crassum* (♂)

In this cross, 5024 disc florets of 67 heads were pollinated and cultivated on the artificial medium. Twenty-eight ovaries developed and were germinated, representing only about 0.5% seed set. Three plants proved to be hexaploid (2n=6x=54) and presumably F_1-hybrids. Seven plants were diploid and were doubtless due to selfing. Of the other 15, one ovary developed roots but failed to develop cotyledons, five were albinos and nine died after they were transplanted to pots. None of these could be karyotyped.

The reciprocal cross failed completely although 760 disc florets of 5 heads were pollinated and cultivated in the same manner.

The chromosomes of the F_1-hybrid with 2n=6x=54, at mitotic metaphase, vary in length from 10.5 μm to 4.8 μm and in arm ratio from 1.0 to 5.4 (Fig. 1-B). The fifty-four chromosomes are arranged in order of size in Fig. 2-B. Three satellite chromosomes were small subterminals (Chromosomes 44, 48 and 50). Chromosome 25 was distinguishable by its length and extreme subterminal centromere. Quite clearly, this chromosome is derived from *Ch. crassum*. Another five extreme subterminal chromosomes with arm ratios of 3.8–5.4 (Chromosomes 34, 42, 46, 47 and 54) were distinguishable. Karyomorphologically the F_1-hybrid seems not to be an auto-hexaploid.

The following meiotic configurations were frequently observed; 2 IV+23 II, 1 IV+25 II, 1 III+25 II+1 I, 27 II and 26 II+2 I. The maximum number of quadrivalents per PMC was two. The frequency of bivalent formation was 96.7%. This frequency is comparable to that of stable native hexaploid species (Watanabe 1977a). The chromosomes derived from *Ch. crassum* paired both homoeologously and homologously with the *Ch. boreale* chromosomes. Consequently, there must be some control leading to predominant bivalent formation at the decaploid level although the chromosomes which paired homoeologously in the F_1-hybrids are capable of associating as multivalents but do not do so in the decaploid.

In the tetrad stage, abnormalities such as multi-ad formation were rare.

All F_1-hybrids had rhizome and white ray floret and had leaves both with and without pseudostipule. They resembled *Ch. crassum* more than *Ch. boreale* in their quantitative characters (Fig. 4).

Ch. boreale (♀)×F_1-hybrid (♂)

In this backcross, 2680 disc florets of 52 heads were pollinated and cultivated on the artificial medium. Eighty ovaries developed and were germinated, representing only about 3.0% seed set. Fifteen plants proved to have the chromosome number 2n=4x=36 and two 2n=4x+1=37. Five plants were diploid and were doubtless due to selfing. Of the other 59, seven ovaries developed cotyledones but failed to develop roots, fifteen were albinos and fourteen formed callus. Ten were dwarf and twelve died after they were transplanted to pots. None of these could be karyotyped.

The reciprocal backcross failed completely although 3038 disc florets of 30 heads were pollinated and cultivated in the same manner.

The chromosomes of Plant 23 with 2n=4x=36, at mitotic metaphase, vary in length from 7.2 μm to 4.2 μm and in arm ratio from 1.0 to 5.3 (Fig. 1–C). The

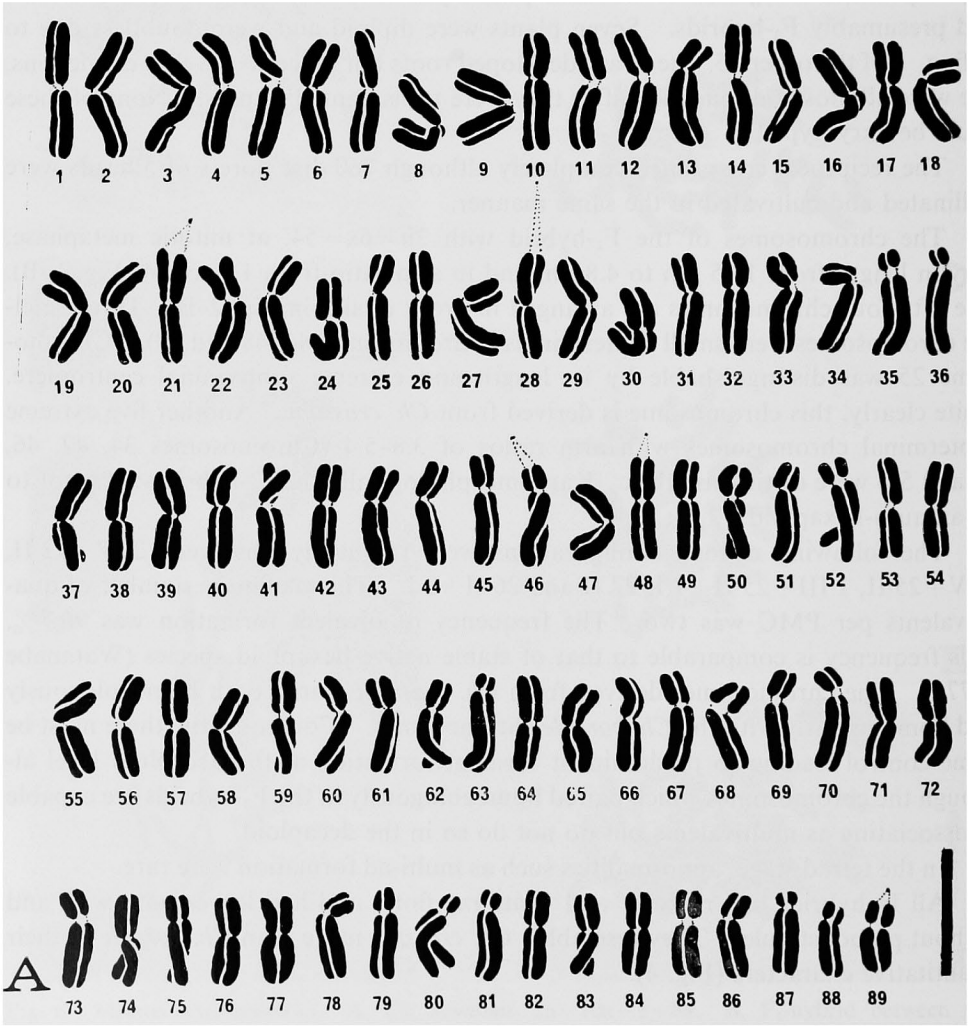

Fig. 2. Somatic karyotype. A, *Ch. crassum*. 2n=10x−1=89.

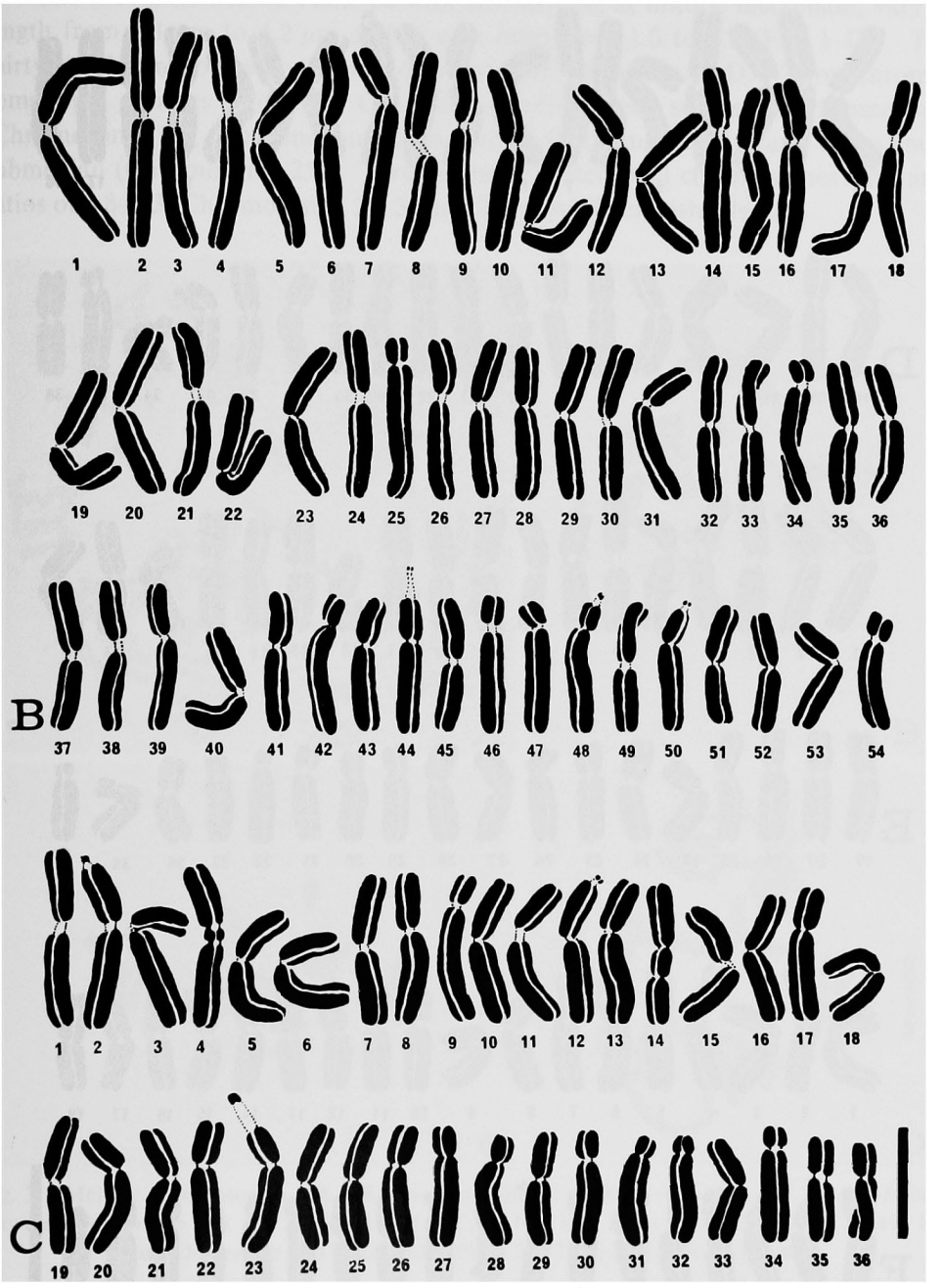

Fig. 2. (continued) B, F_1-hybrid between *Ch. boreale* and *Ch. crassum*. 2n=6x=54. C, B_1-hybrid between *Ch. boreale* and F_1-hybrid. Plant 23. 2n=4x=36.

thirty-six chromosomes are arranged in order of size in Fig. 2–C. Three chromosomes had minute satellites. One was a large submedian chromosome (Chromosome 2), one a medium-sized submedian (Chromosome 12) and one a medium-sized subterminal (Chromosome 23). Chromosome 9 was distinguishable by its

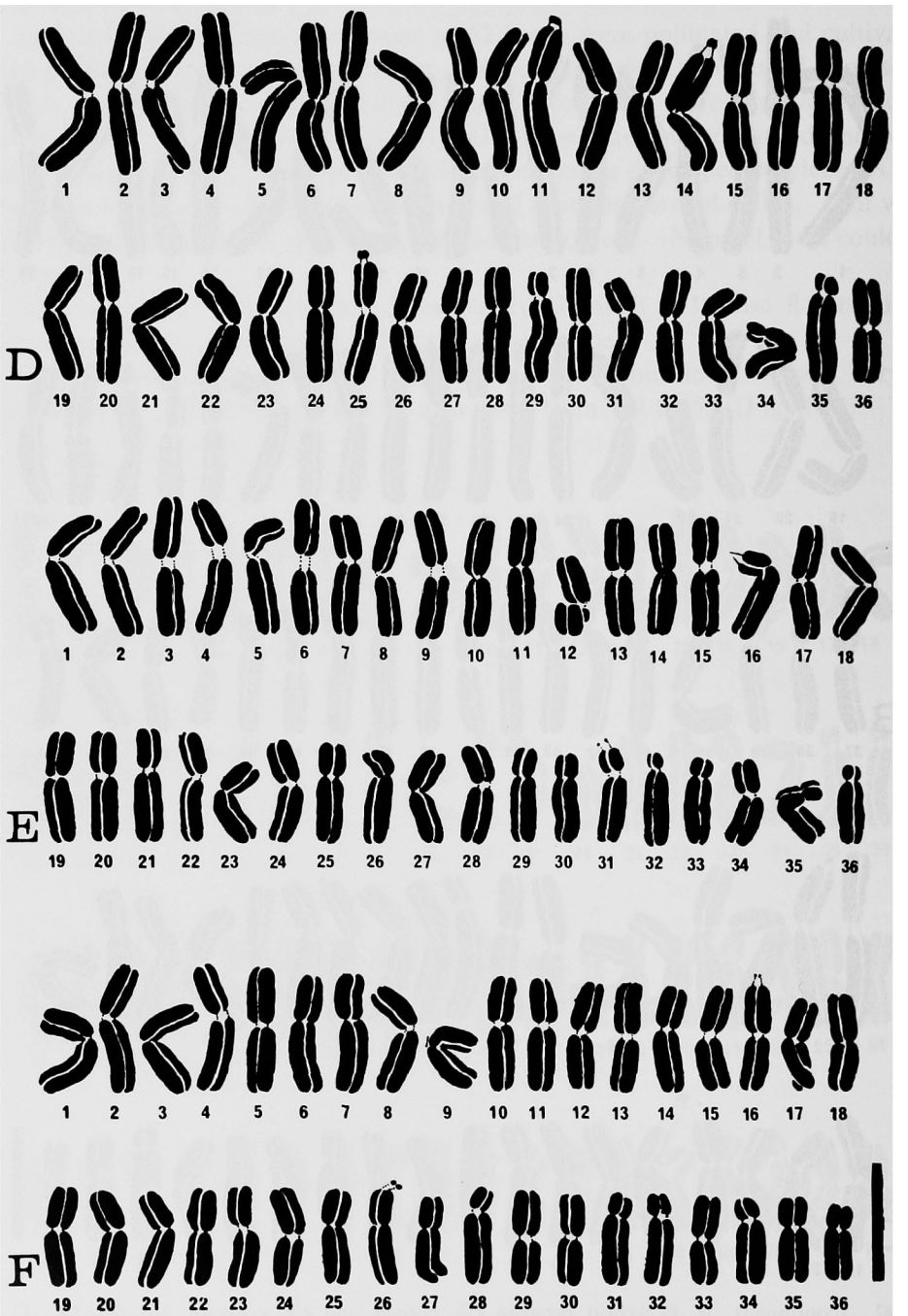

Fig. 2. (continued) D, B_1-hybrid. Plant. 60. 2n=4x=36. E, B_1-hybrid. Plant 110. 2n=4x=36. F, B_1-hybrid. Plant 114. 2n=4x=36. Scale 5 μm.

length and extreme subterminal centromere. This chromosome characterises the karyotype of grandparent, *Ch. crassum*. Three other extreme subterminal chromosomes with arm ratios of 4.4–5.3 (Chromosomes 31, 32 and 34) were distinguishable.

The chromosomes of Plant 60 with 2n=4x=36, at mitotic metaphase, vary in length from 7.1 μm to 4.2 μm and in arm ratio from 1.0 to 5.3 (Fig. 1–D). The thirty-six chromosomes are arranged in order of size in Fig. 2–D. Three chromosomes had minutes satellites. One was a medium-sized submedian chromosome (Chromosome 11), one a medium-sized median (Chromosome 14) and one a small submedian (Chromosome 25). Three extreme subterminal chromosomes with arm ratios of 3.8–5.3 (Chromosomes 29, 34 and 35) were distinguishable.

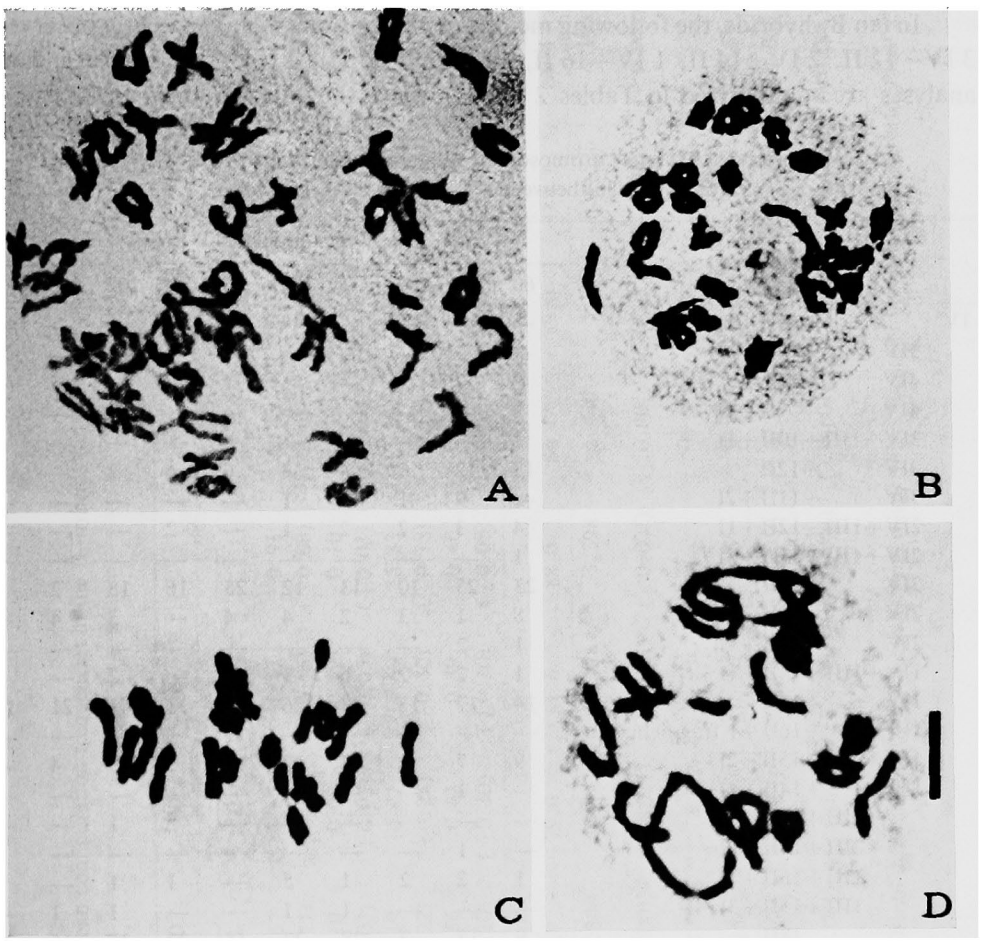

Fig. 3. Meiotic chromosomes. A, *Ch. crassum* with 2n=10x−1=89. 44II+1I. B, F_1-hybrid between *Ch. boreale* and *Ch. crassum*. 27II. C, B_1-hybrid. Plant 23. 18II. D, B_1-hybrid between *Ch. boreale* and F_1-hybrid. Plant 23. 2IV+14II. Scale 10 μm..

The chromosomes of Plant 110 with 2n=4x=36, at mitotic metaphase, vary in length from 6.1 μm to 3.2 μm and in arm ratio from 1.1 to 5.3 (Fig. 1–E). The thirty-six chromosomes are arranged in order of size in Fig. 2–E. Two chromosomes had minute satellites. One was a medium-sized subterminal chromosome (Chromosome 16) and the other a small subterminal (Chromosome 31). Four extreme subterminal chromosomes with arm ratios of 3.6–5.3 (Chromosomes 32, 33, 35 and 36) were distinguishable.

The chromosomes of Plant 114 with 2n=4x=36, at mitotic metaphase, vary in length from 5.6 μm to 3.2 μm and in arm ratio from 1.0 to 3.8 (Fig. 1–F). The thirty-six chromosomes are arranged in order of size in Fig. 2–F. Two chromosomes had minute satellites. One was a medium-sized median chromosomes (Chromosome 16) and the other a small subterminal (Chromosome 26). Four extreme subterminal chromosomes with arm ratios of 3.1–3.8 (Chromosomes 28, 31, 32 and 34) were distinguishable. Karyomorphologically these four B_1-hybrids seem not to be auto-tetraploids and they have different karyotypes.

In ten B_1-hybrids, the following meiotic configurations were frequently observed: 3 IV+12 II, 2 IV+14 II, 1 IV+16 II, 18 II and 17 II+2 I. The precise meiotic analyses are summarized in Tables 2 and 3, where they are arranged in ascending

Table 2. Meiotic chromosome configurations in the B_1-hybrids (2n=4x=36) between *Ch. boreale*×*Ch. crassum*

Configurations	Plant number No. PMC									
	60	30	107	5	106	23	4	102	115	130
1V +15II+1I	—	—	—	—	—	—	1	—	—	—
5IV + 8II	—	3	—	—	—	—	—	—	—	—
4IV +10II	2	3	1	—	—	1	—	—	—	—
4IV + 9II+2I	1	—	—	—	—	—	—	—	—	—
3IV+1III+10II+1I	2	—	—	—	—	—	—	—	—	—
3IV +12II	13	12	7	4	3	6	6	2	—	—
3IV +11II+2I	—	9	1	1	1	—	—	—	—	—
2IV+1III+12II+1I	4	3	2	2	1	—	2	—	—	—
2IV+1III+1III+3I	1	—	—	—	—	—	—	—	—	—
2IV +14II	23	25	10	13	12	25	16	13	2	4
2IV +13II+2I	8	1	3	2	4	4	—	3	4	—
2IV +12II+4I	1	2	—	—	—	—	—	—	—	—
1IV+1III+14II+1I	1	2	2	6	3	—	—	2	—	—
1IV +16II	14	17	13	33	26	47	31	41	21	14
1IV +16II+1 fragment	—	—	—	—	—	1	—	—	—	—
1IV +15II+2I	9	7	2	4	12	11	3	3	4	—
1IV +14II+4I	—	1	—	—	—	—	—	—	—	—
2III+15II	—	—	—	—	—	—	—	1	—	—
2III+14II+2I	—	1	—	—	—	—	—	—	—	1
1III+16II+1I	1	2	2	1	5	—	1	1	—	2
1III+15II+3I	—	—	—	1	1	—	—	1	1	—
18II	9	13	7	28	27	45	33	35	45	39
17II+2I	3	6	1	5	7	15	6	5	9	2
16II+4I	1	—	—	1	3	1	1	1	1	—
Total number of PMC	93	107	51	101	105	156	100	108	87	62

order on the basis of average number of bivalents per PMC. The maximum number of quadrivalent per PMC varied from two to five; there is a significant difference in the frequency of quadrivalent and bivalent formation. The data of quadrivalent frequencies, except plant 60 (d.f.=4, P=0.017), conform to the theoretical Poisson expectation based on the random hypothesis (d.f.=1 ~4, $0.20<P<0.90$). Probably all sets of four chromosomes have equal chances of forming quadrivalents. The

Table 3. Mean configuration frequencies in the B_1-hybrids (2n=4x=36) between *Ch. boreale*×*Ch. crassum*

Configurations	Plant number									
	60	30	107	5	106	23	4	102	115	130
V	—	—	—	—	—	—	0.01	—	—	—
(±S. E.)	—	—	—	—	—	—	(±0.01)	—	—	—
IV	1.67	1.67	1.47	0.91	0.83	0.89	0.88	0.78	0.43	0.35
(±S..E.)	(±0.11)	(±0.12)	(±0.15)	(±0.08)	(±0.08)	(±0.07)	(±0.09)	(±0.07)	(±0.07)	(±0.08)
III	0.10	0.08	0.12	0.10	0.10	—	0.03	0.06	0.01	0.06
(±S. E.)	(±0.03)	(±0.03)	(±0.05)	(±0.03)	(±0.03)	—	(±0.02)	(±0.03)	(±0.01)	(±0.04)
II	14.19	14.21	14.69	15.83	15.86	16.01	16.04	16.21	16.90	17.13
(±S. E.)	(±0.22)	(±0.24)	(±0.30)	(±0.18)	(±0.17)	(±0.14)	(±0.18)	(±0.15)	(±0.15)	(±0.16)
I	0.66	0.63	0.39	0.40	0.69	0.41	0.26	0.30	0.47	0.13
(±S. E.)	(±0.10)	(±0.10)	(±0.10)	(±0.08)	(±0.10)	(±0.07)	(±0.07)	(±0.07)	(±0.10)	(±0.06)
The frequency of bivalent formation (%)	78.9	79.0	81.6	88.0	88.1	89.0	89.1	90.1	93.9	95.2
t*=D/E	10.808	10.123	7.176	5.398	5.440	5.268	4.526	4.195	1.049	—
P* values	(<0.001)	(<0.001)	(<0.001)	(<0.001)	(<0.001)	(<0.001)	(<0.001)	(<0.001)	(0.3 ≳ 0.2)	—
The frequency of quadrivalent formation (%)	18.5	18.6	16.3	10.0	9.2	9.9	9.8	8.6	4.7	3.9
t**=D/E	9.705	9.153	6.588	4.950	4.243	5.080	4.401	4.045	0.753	—
P** values	(<0.001)	(<0.001)	(<0.001)	(<0.001)	(<0.001)	(<0.001)	(<0.001)	(<0.001)	(0.5 ≳ 0.4)	—

Calculated for testing the significant difference of bivalent means* and quadrivalent means** between the B_1-hybrid, Plant 130 and the others.

average frequency of bivalents per PMC ranged from 14.19 to 17.13 among the B_1-hybrids. The chromosomes derived from the F_1-hybrid paired both homoeologously and homologously with the *Ch. boreale* chromosomes, further homoeologous chromosome pairing was observed in this generation. There must be some control to suppress the multivalent formation still at the F_1-hybrid hexaploid level although the chromosomes which paired homoeologously in the B_1-hybrids are capable of associating as multivalents but do not do so in the hexaploid. Additionally, it is necessary to pay some attention to the significant differences in multivalent frequency among the B_1-hybrids. This might be due to a different degree of release from the mechanism for the suppression of the multivalent formation.

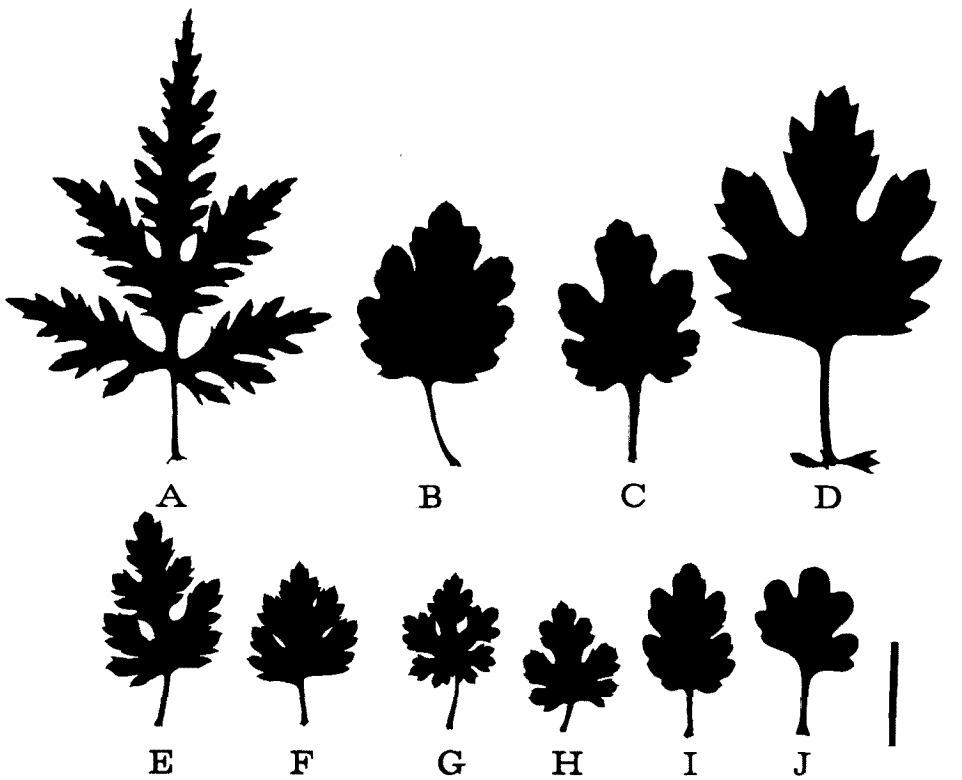

Fig. 4. Leaves. A, *Ch. boreale*. B, and C, F_1-hybrids. B, the leaf in the first year of its production. C, the leaf in the second year of its production. D, *Ch. crassum*. E, F, G, H, I and J, B_1-hybrids in the first year of their production. Scale 2.5 cm.

Segregation of various kinds of characters (ray floret color, flower, size, leaf shape) was observed in this generation (Fig. 4). All of B_1-hybrids were more weakly than *Ch. crassum* and F_1-hybrids. No triploid B_1-hybrids between *Ch. boreale* × B_1-hybrid have been obtained yet for four years although many crossings have been tried in every autumn.

Discussion

In native decaploid *Chrysanthemum crassum* Kitamura genetic stabilization of

diploid-like meiosis occurs. In F_1-hybrids between diploid *Ch. boreale* and *Ch. crassum* one of five genomes derived from decaploid paired with *Ch. boreale* genome and the remaining genomes paired homoeologously with each other. Furthermore, increased homoeologous association was observed in tetraploid B_1-hybrids. The quadrivalent frequency conforms to the Poisson distribution and all sets of four chromosomes probably had an equal chance of forming a quadrivalent even in B_1-hybrid. Chromosome and genic differentiation between the genomes is sufficient to provide a barrier of hybrid sterility, but is less than is required to prevent bivalent formation. These data indicate that there must be a restriction mechanism to form multivalent in polyploid although all of the constituent genomes of polyploid are sufficiently homologous to be able to pair with each other. The data which the quadrivalent frequency varies in each B_1-hybrid seem to be consistent with the hypothesis of multiple-gene segregation in the advanced generation. Then the quadrivalent formation must be under these suppressive genes control. Since the bivalent formation has not been disturbed, at least one of two zygomeres seems to be able to have a complete activity. Two independent and fundamentally different control systems are involved in the maintenance of efficient bivalent formation in decaploid *Ch. crassum*. These data must be explainable by the genetic control system found in hexaploid *Ch. japonense* Nakai and octoploid *Ch. ornatum* Hemsley (Watanabe 1981a, b). According to these genetic systems, meiotic chromosome behaviours in decaploid *Ch. crassum*, the hexaploid F_1- and tetraploid B_1 crossed with the diploid *Ch. boreale* are illustrated as Figure 5. When the initiation of pairing at the B site is suppressed by genic control, the pairing $(A_1\ A_2)\ (A_3\ A_4)\ (A_5\ A_6)\ (A_7\ A_8)\ (A_9\ A_{10})$ will clearly lead to the formation of five bivalents in the decaploid. In the hexaploid F_1-hybrid, A_1 is capable of pairing with A_3, A_5 with A_7, and A_9 with *Ch. boreale* A site, i. e., $(A_1\ A_3)\ (A_5\ A_7)\ (A_9\ A)$. Namely, the chromosomes derived from the decaploid paired both homoeologously and homologously with the *Ch. boreale* chromosomes. When polyploids are crossed with the diploids, without a suppressive gene on the

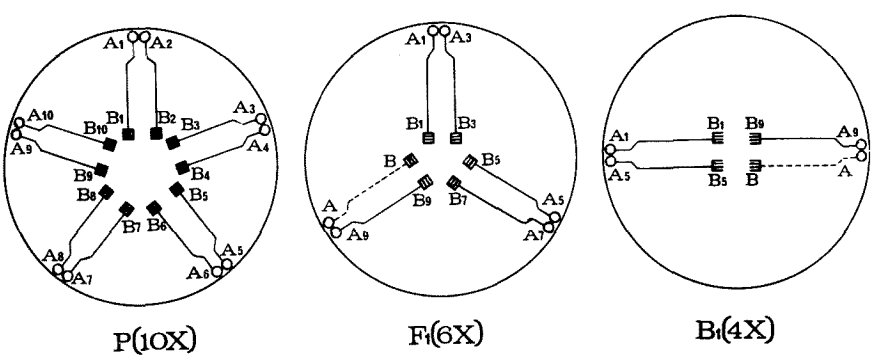

Fig. 5. The models of chromosome pairing in the decaploid *Ch. crassum*, the hexaploid F_1-hybrid and the tetraploid B_1-hybrid crossed with the diploid *Ch. boreale*. The chromosome pairing is strictly bivalent pairing owing to the suppression of initiation of pairing at the B site although all of the chromosomes are sufficiently homologous to be able to pair each other in the decaploid state. In the hexaploid F_1-hybrid the chromosomes derived from the decaploid pair both homoeologously and homologously with the *Ch. boreale* chromosome (A–B) as bivalents. In the tetraploid B_1-hybrid further homoeologous chromosome associations are evident.

initiation of pairing at the B site, the suppression will be slightly released by the reduction of suppressive gene dosage hence it will lead to the slight quadrivalent association. In the tetraploid B_1-hybrid, A_1 is capable of pairing with A_5, and A_9 with *Ch. boreale* A, i. e., (A_1 A_5) (A_9 A). Further homoeologous chromosome associations and further release from the suppression of the pairing initiation at the B site are evident in this generation. All of the constituent genomes of *Ch. crassum* are so homologous that they can pair with each other and with the *Ch. boreale* genome. The magnitude of the release from suppression seems to be equal at each B site because the frequency of quadrivalents per PMC is in correspondence with a Poisson distribution.

These data may be consistent with the hypothesis of molecular mechanisms of chromosome pairing proposed by Comings and Riggs (1971) in respect that the recognition or initial pairing site of homologous chromosomes is located only a few loci, not throughout the length of each chromosome. According to their hypothesis the existence of a class of nucleosteric proteins which bind to specific DNA sites and, on binding, undergo allosteric changes that allow them to bind to each other or to change enzymatic activity. These proteins could play a role in initiation homologous chromosome pairing. If this hypothesis is regarded as appropriate, the pairing affinity should be explained on the basis of homology of DNA base sequences of zygomere (the specific DNA segments for the recognition sites and, or, the protein binding sites) instead of the homology throughout the length of each chromosome.

The localization of zygomere at one site only per chromosome which ensures regular bivalent formation seems to have played an important role in evolution in respect to afford the extensive gene—or genome-duplication for organisms.

Summary

1) In *Ch. crassum* with 2n=10x−1=89, 44 II+1 I was the main meiotic configuration and 1 IV+42 II+1 I and 43 II+3 I were rarely observed. Eighty-nine chromosomes, at mitotic metaphase, varied in length from 5.4 μm to 2.5 μm and in arm ratio from 1.0 to 5.8. Five satellite chromosomes, two medium-sized and six small chromosomes with extreme subterminal centromeres were well distinguishable. Karyomorphologically this species is not an auto-decaploid.

2) Three F_1 hybrids with 2n=6x=54 between diploid *Ch. boreale* and *Ch. crassum* were obtained by ovary culture. The following meiotic configurations were frequently observed; 2 IV+23 II, 1 IV+25 II, 1 III+25 II+1 I, 27 II and 26 II+2 I. The chromosomes derived from *Ch. crassum* paired both homoeologously and homologously with the *Ch. boreale* chromosomes.

3) F_1-hybrids with 2n=6x=54 between *Ch. boreale* and *Ch. crassum* gave rise to tetraploid B_1-hybrids by means of ovary culture after being backcrossed with the diploid *Ch. boreale*. In B_1-hybrids with 2n=4x=36 the following meiotic configurations were frequently observed; 3 IV+12 II, 2 IV+14 II, 1 IV+16 II, 18 II and 17II +2 I. The maximum number of quadrivalents per PMC varied from five to two among B_1-hybrids. The data of quadrivalent frequencies conform to the Poisson distribution. Further homoeologous chromosome pairing was observed in this

generation.

4) The diploid-like meiosis in *Ch. crassum*, its hexaploid F_1-hybrids and tetraploid B_1-hybrids must be ensured by the following genetic system although all of the constituent genomes of these polyploids are sufficiently homologous to be able to pair each other; 1) chromosome pairing is initiated at two sites, A and B (the zygomeres localize in two loci per chromosome). 2) at either site pairing is always two-by-two, with the pairing initiated at the A site being independent of that initiated at the B site. 3) the initiation of pairing at the A site always precedes to that at the B site. 4) the initiation of pairing at the B site is usually suppressed by multiple-or polygenic control. The magnitude of release from the suppression of initiation of pairing at the B site depends on the reduction of suppressive gene dosage and seems to be equal, not differential, at each B site.

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