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Studies on the Control of Diploid-like Meiosis in Polyploid Taxa of *Chrysanthemum* I. Hexaploid *Ch. japonense* Nakai

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The establishment of sexually self-maintaining polyploid plants must require stable essentially diploid-like meiotic processes. The following strategies have been proposed whereby meiosis may be stabilized; 1) a strict allopolyploid amphidiploidization (Karpechenko 1927, Müntzing 1932), 2) the diploidization by selective accumulation of the many small changes of chromosome structure in an autopolyploid (Jorgensen 1928, Darlington 1932, Müntzing 1936), 3) the preferential bivalent association based on slight structural differences between homoeologous (Digby 1912, Upocott 1939), 4) the suppression of homoeologous pairing by a single major gene (Okamoto 1957, Sears and Okamoto 1958, Riley and Chapman 1958, Ladizinsky 1973), 5) the canalization of chiasmata at one chiasma per chromosome pair (Kostoff 1940, Gupta and Koak 1976), 6) the differential condensation of chromosomes during meiotic prophase (Brown 1954, Endrizzi 1962, Watanabe *et al.* 1976), 7) the suppression of homoeologous pairing by B-chromosome (Mochizuki 1964, 1965, Dover and Riley 1972, Evans and Macefield 1972, Bowman and Thomas 1973) and 8) the suppression of homoeologous pairing by the large terminal heterochromatic blocks responsible for interference with pairing initiation during premeiotic interphase or early prophase of meiosis (Thomas and Kaltisikes 1974).

In native species of *Chrysanthemum* there exists a well-known polyploid series based upon 9, i. e. 18, 36, 54, 72 and 90. Shimotomai and his collegues have reported that these polyploids showed stable meiosis, high frequency of seed setting and a distinct geographical distribution (Shimotomai 1933, Takemoto 1939, Shimotomai and Tanaka 1952, Tanaka 1952a, b, 1959a, b, 1960, Nagami 1954, Shimotomai *et al.* 1956, Kaneko 1961, Watanabe 1977a). They have observed the homoeologous chromosome pairing in the species hybrids between polyploids and argued about the nature of polyploidy, but a critical assessment of the genomic constitution of polyploids has not been made because of the lack of technique for producing F_1-hybrids between diploids and high polyploids. This barrier has been overcome by ovary culture on an artificial medium (Watanabe 1977b), and meiotic studies of diploids $\times$ high polyploids hybrids have been made. In F_1-hybrids of $2x \times 6x$ and $2x \times 10x$, the chromosomes derived from the polyploid paired both homoeologously and homologously with the diploid chromosomes, *i. e.* 18II in the former and 27II in the latter, respectively (Watanabe 1977a).

The purpose of this article is to clarify the control system of diploid-like meio-

sis in hexaploid *Ch. japonense* Nakai through the analyses of chromosome morphology, chromosome behaviour and external morphology in F_1-, F_2-, F_3-, B_1-, B_2-hybrids crossed with the diploid *Ch. boreale*.

Materials and methods

Ch. japonense Nakai is a stable hexaploid and occurs in South-Western parts of Shikoku, South-Eastern parts of Kyushu, Tanegashima Isl. and around the seashore and islets of the Inland Sea (Shimotomai *et al.* 1956, Kitamura 1967). *Ch. boreale* Makino is a stable diploid and is found in Japan, Korea, Manchuria and Northern parts of China. Plants were collected from their native localities as follows and were classified according to Kitamura's system (Kitamura 1967). In my previous papers (1977 a, b) the chrysanthemums collected from Saka were identified as *Ch. japonense* f. *debilis*, which was taxonomically nomen nudum. In this paper it is treated as *Ch. japonense* Nakai (Saka strain).

Ch. japonense (Saka strain), Saka, Hiroshima Pref.

Ch. japonense (Nakamura strain), Nakamura, Kochi Pref.

Ch. boreale, Koyaguchi, Wakayama Pref.

The cytological techniques used were identical to those reported by Watanabe *et al.* (1975). Chromosome length was measured on the photographs at a magnification of ×5000. Arm ratio (A. R.) was estimated by the ratio of the long arm of chromosome to the short. Relative length was calculated by the following formula;

$$\frac{\text{the length of individual chromosome}}{\text{the sum of the length of chromosomes}} \times \frac{\text{somatic chromosome number}}{18} \times 100$$

(Tanaka 1959 b). The expected random association of chromosomes was calculated from the table of Poisson distribution and the expansion of the binomial $(p+q)^n$, where p is the mean value of multivalent association, q is 1–p and n is 9 based on basic haploid genome x=9. Chi-square tests for goodness of fit use d.f.=N–2, since p has been calculated from the data. If all groups of homologous or homoeologous chromosomes within individual microsporocytes and all microsporocyted behave alike, the theoretical Poisson and binomial expectation should be a good fit for the data. The "t" test was carried out for the examination of significant differences between the materials in their mean chromosome pairing frequencies.

The technique of crossing did not require emasculation because the plants were mostly self-incompatible (Tanaka 1952a). The head (capitulum) of the seed-parent was covered with lens paper before and after pollination and this cover was kept on the head until the achenes were collected. Ovary culture was carried out by the technique described in the previous paper (Watanabe 1977b). The young plants were potted at the three-leaf stage and cytological examination were made.

Results

1. *Ch. japonense* Nakai (Saka strain)

Ch. japonense (Saka strain)

Ch. japonense (Saka strain) with 2n=6x=54 was used in hybridization with the diploid *Ch. boreale*.

The chromosomes of *Ch. japonense* (Saka strain), at mitotic metaphase, vary in length from 8.4 μm to 4.2 μm and in arm ratio from 1.0 to 4.9 (Fig. 1-A and Table 1). The fifty-four chromosomes are arranged in order of size in Fig. 2-A. When several chromosomes are of the same size, the chromosome with low arm ratio takes priority in the arrangement. Ten chromosomes had minute satellites. Two of these were medium-sized median chromosomes (Chromosomes 13 and 15), two medium-sized subterminals (Chromosomes 27 and 31), two medium-sized submedians (Chromosomes 26 and 28) and the rest small subterminals (Chromosomes 36, 43, 50 and 52). Satellite-chromosomes 50 and 52 could be distinguished from satellite-chromosomes 36 and 43 by their smaller size. Six extreme subterminal chromosomes with arm ratios of 3.3–4.9 (Chromosomes 37, 48, 49, 51, 53 and 54) were distinguishable. They were different from each other in their chromosome size, especially Chromosomes 53 and 54 were shorter than the rest. Karyomorphologically *Ch. japonense* (Saka strain) seems not to be an auto-hexaploid.

In meiosis 27 II was the most frequent chromosome configuration. 1 IV+25II and 26II+2I were rarely observed. The frequency of bivalent formation was 99.1%. Five bivalents were attached to the nucleolus. Ring bivalents were frequent, indicating the formation of at least two chiasmata in them. In the tetrad stage, abnormalities such as multi-ad formation were rare. Chromosome association in this species is almost entirely as bivalents (Fig. 3–A).

Ch. boreale

The chromosomes of *Ch. boreale*, at mitotic metaphase, vary in length from 6.5 μm to 3.8 μm and in arm ratio from 1.1 to 3.8 (Fig. 1–B). The eighteen chromosome are arranged in order of size in Fig. 2–B. Four chromosomes had minute satellites (Chromosomes 3, 7, 10 and 13) although three pairs of chromosomes were attached to the nucleolus at meiotic prophase. Two extreme subterminal chromosomes with arm ratios of 3.6 and 3.8 (Chromosomes 17 and 18) were distinguishable. This karyotype is nearly identical with the ones reported by Tanaka (1959 a) except that Chromosomes 15 and 16 are heteromorphic and without satellites.

In diakinesis of meiosis, 53 PMCs were scored. Of these, 51 had nine bivalents and two had 8II+2I. Three bivalents were attached to the nucleolus. Most bivalents were found to have two chiasmata each. Chromosomes 15 and 16 were heteromorphic but were capable of regular bivalent formation.

Ch. japonese (Saka strain) (♀)×*Ch. boreale* (♂)

In this cross, 2713 disc florets of 30 heads were pollinated and cultivated on the artificial medium. These ovaries developed on the medium into the following three types of achenium: fat black, schrivelled black and schrivelled lightbrown; and the former two were germinated. Eighteen ovaries developed and were germinated, representing only about 0.3% seed set. Of these, four had 2n=4x=36 and one had 2n=4x+1=37, and were accepted as being F_1-hybrids. Ten were hexaploid, 2n=6x=54, and were doubtless due to selfing. Three died before they could be karyo-

Table 1. Measurements of the somatic chromosomes of *Ch. japonense* (Saka strain)

Chromosomes	Length in μm	Relative length	Arm ratio (long/short)
1	4.2+4.2=8.4	7.3	1.0
2	3.2+5.1=8.3	7.2	1.6
3	3.2+4.6=7.8	6.8	1.4
4	3.8+3.8=7.6	6.6	1.0
5	3.4+4.2=7.6	6.6	1.2
6	2.3+5.1=7.4	6.4	2.2
7	3.6+3.7=7.3	6.4	1.0
8	2.6+4.7=7.3	6.4	1.8
9	2.5+4.8=7.3	6.4	1.9
10	3.6+3.6=7.2	6.3	1.0
11	3.2+3.9=7.1	6.2	1.2
12	2.6+4.5=7.1	6.2	1.7
13*	3.5+3.5=7.0	6.1	1.0
14	3.2+3.8=7.0	6.1	1.2
15*	3.3+3.6=6.9	6.0	1.1
16	2.9+4.0=6.9	6.0	1.4
17	2.8+4.1=6.9	6.0	1.5
18	2.4+4.5=6.9	6.0	1.9
19	2.4+4.5=6.9	6.0	1.9
20	3.4+3.4=6.8	5.9	1.0
21	3.1+3.7=6.8	5.9	1.2
22	2.6+4.1=6.7	5.8	1.6
23	2.5+4.2=6.7	5.8	1.7
24	3.2+3.3=6.5	5.7	1.0
25	2.3+4.2=6.5	5.7	1.8
26*	2.6+3.8=6.4	5.6	1.5
27*	2.4+4.0=6.4	5.6	1.7
28*	2.4+4.0=6.4	5.6	1.7
29	2.4+4.0=6.4	5.6	1.7
30	2.2+4.2=6.4	5.6	1.9
31*	1.7+4.7=6.4	5.6	2.8
32	2.8+3.5=6.3	5.5	1.3
33	2.6+3.6=6.2	5.4	1.4
34	2.2+4.0=6.2	5.4	1.8
35	2.2+3.8=6.0	5.2	1.7
36*	1.6+4.4=6.0	5.2	2.8
37	1.4+4.6=6.0	5.2	3.3
38	2.8+3.1=5.9	5.1	1.1
39	2.9+2.9=5.8	5.0	1.0
40	2.8+3.0=5.8	5.0	1.1
41	2.2+3.6=5.8	5.0	1.6
42	2.4+3.3=5.7	5.0	1.4
43*	1.7+4.0=5.7	5.0	2.4
44	2.8+2.8=5.6	4.9	1.0
45	2.6+3.0=5.6	4.9	1.2
46	2.4+3.2=5.6	4.9	1.3
47	2.4+3.1=5.5	4.9	1.3
48	0.9+4.4=5.3	4.6	4.9
49	0.9+4.2=5.1	4.4	4.7

Table 1. (continued)

Chromosomes	Length in μm	Relative length	Arm ratio (long/short)
50*	1.1+3.9=5.0	4.4	3.5
51	0.9+4.0=4.9	4.3	4.4
52*	1.1+3.7=4.8	4.2	3.4
53	0.9+3.5=4.4	3.8	3.9
54	0.8+3.4=4.2	3.7	4.3

* Chromosome with satellites.

typed.

In the reciprocal cross, 13205 disc florets of 176 heads were pollinated and cultivated on the artificial medium. Thirteen ovaries developed and were germinated, representing a seed set of only 0.1%. Three proved to be tetraploid 2n=4x=36, and presumptively were F_1-hybrids. Five were diploid 2n=2x=18, and were doubtless due to selfing. Five plants died early and could not be karyotyped.

The number of hybrids obtained in this cross, taking the two reciprocal crosses together, was eight, seven with 2n=4x=36 and one with 2n=4x+1=37. There were no significant karyotypic difference among hybrids with 2n=4x=36. The chromosomes of the F_1-hybrid, at mitotic metaphase, vary in length from 6.8 μm to 3.1 μm and in arm ratio from 1.0 to 5.2 (Fig. 1–C). The thirty-six chromosomes are arranged in order of size in Fig. 2–C. Seven chromosomes had minute satellites. One was a medium-sized median chromosome (Chromosome 8), three medium-sized submedians (Chromosomes 10, 25 and 29), one a medium-sized subterminal (Chromosome 26) and the rest small subterminals (Chromosomes 31 and 33). Three extreme subterminal chromosomes with arm ratios of 3.8–5.2 (Chromosomes 30, 35 and 36) were distinguishable.

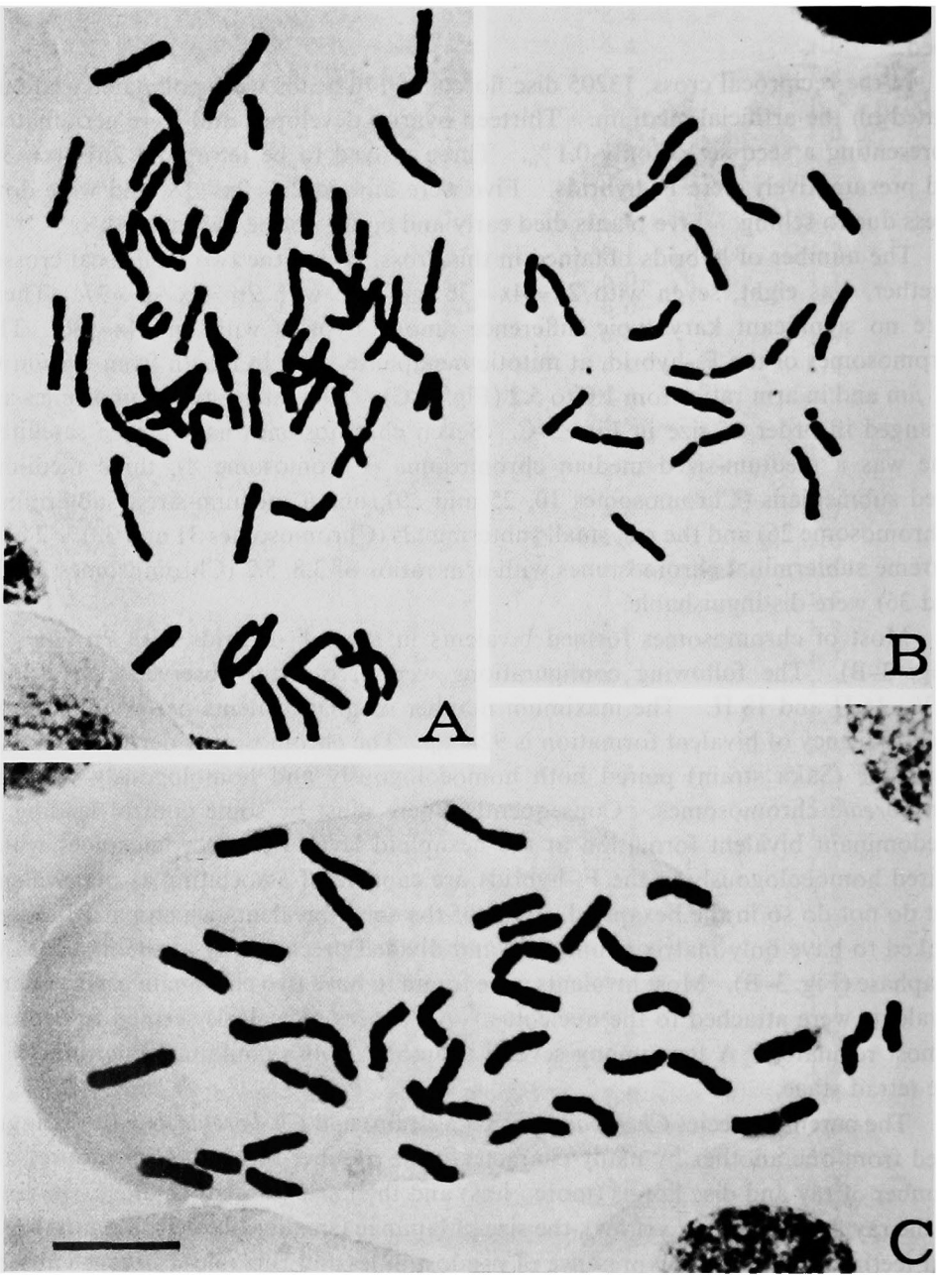
Most of chromosomes formed bivalents in these F_1-hybrids with 2n=4x=36 (Fig. 3–B). The following configurations were frequently observed; 2IV+14II, 1 IV+16 II and 18 II. The maximum number of quadrivalents per PMC is three. The frequency of bivalent formation is 92.4%. The chromosomes derived from *Ch. japonense* (Saka strain) paired both homoeologously and homologously with the *Ch. boreale* chromosomes. Consequently, there must be some control leading to predominant bivalent formation at the hexaploid level. The chromosomes which paired homoeologously in the F_1-hybrids are capable of associating as multivalents but do not do so in the hexaploid. One of the small bivalents which paired loosely looked to have only matrix connection and divided precociously at the first meiotic anaphase (Fig. 3–B). Most bivalents were found to have two chiasmata each. Three bivalents were attached to the nucleolus. All phases of meiosis seemed to procede almost regularly. A few among several thousand PMCs contained micronuclei at the tetrad stage.

The parental species *Ch. japonense* (Saka strain) and *Ch. boreale*, can be distinguished from one another by many character,—the number of heads (less / more), the number of ray and disc florets (more / less) and their size (larger / smaller), the color of the ray florets (white / yellow), the size of laminae (smaller / larger), the number of leaf teeth (less / more), the presence of psudostipules and rhizomes (present / absent)

and plant height (60–90 cm / 60–120 cm). In each comparison the form of *Ch. japonense* (Saka strain) is given first. Precise morphological analyses are summarized in Table 12.

All F_1-hybrids had rhizomes and white ray florets and they were both with and without pseudostipules. The F_1-hybrids resembled *Ch. japonense* (Saka strain) more than *Ch. boreale* in their quantitative characters (Figs. 4, 5 and Table 12).

F_1-hybrid (♀) × *Ch. boreale* (♂)



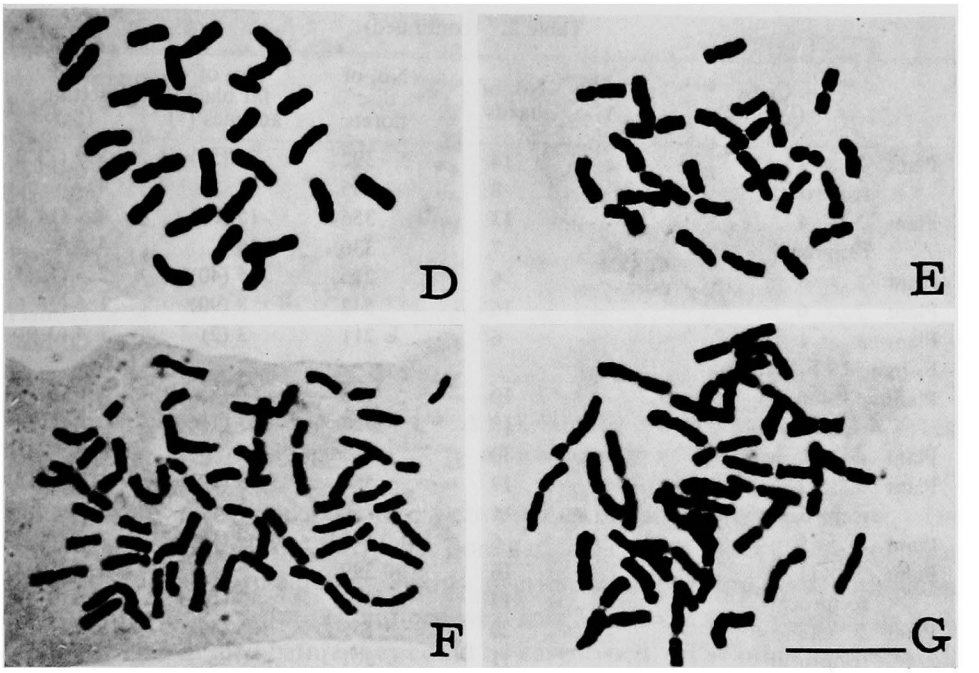


Fig. 1. A, mitotic chromosomes, *Ch. japonense* (Saka strain, 2n=6x=54). B, *Ch. boreale* (2n=2x=18). C, F_1-hybrid (2n=4x=36) between *Ch. japonense* (Saka strain)×*Ch. boreale*. D, B_1-hybrid (Plant 3, 2n=3x=27). E, B_2-hybrid (Plant 1, 2n=2x=8=26). F, F_2-hybrid (Plant 2, 2n=4x=36). G, F_3-hybrid (Plant 35, 2n=4x=36). Scale 10 μm.

Table 2. Results of hybridization

Cross (♀)×(♂)	No. of heads	No. of disc florets	No. of fat black achenes (*)	Seed setting (%)
japonense (Saka srtain) 6x ×*boreale*	30	2713	18	**
reciprocal	176	13205	13	***
F_1-hybrid×*boreale*				
Plant 2 ×*boreale*	27	1830	20	****
reciprocal	3	222	56	*****
Plant 6 ×*boreale*	7	733	0	0
reciprocal	1	66	40	*****
B_1-hybrid×*boreale*				
Plant 1 ×*boreale*	17	438	0 (11)	0 (0.5)
Plant 2 ×*boreale*	10	287	0	0
Plant 3 ×*boreale*	3	75	0	0
Plant 7 ×*boreale*	4	122	0	0
Plant 10 ×*boreale*	4	99	0	0
Plant 11 ×*boreale*	15	834	0 (10)	0 (1.2)
Plant 13 ×*boreale*	21	1052	0 (10)	0 (1.0)
Plant 14 ×*boreale*	5	140	0	0
Plant 20 ×*boreale*	12	632	1 (2)	0.2 (0.3)
F_1-hybrid×F_1-hybrid				
Plant 1 × 2	12	369	1 (1)	0.3 (0.3)
reciprocal	40	1061	40 (198)	3.8 (18.7)

Table 2. (continued)

Cross (♀)×(♂)	No. of heads	No. of disc florets	No. of fat black achenes (*)	Seed setting (%)
Plant 2 × 3	14	392	23 (52)	5.9 (13.3)
reciprocal	8	255	12	4.7
Plant 2 × 4	13	356	15 (33)	4.2 (14.9)
reciprocal	7	336	16	4.8
Plant 2 × 5	6	216	5 (40)	2.3 (18.5)
Plant 2 × 6	16	542	8 (90)	1.5 (16.6)
Plant 3 × 1	6	211	3 (2)	1.4 (0.9)
F_2-hybrid × F_2-hybrid				
Plant 4 × 6	10	130	6 (8)	4.6 (6.2)
reciprocal	18	568	263 (150)	46.3 (26.4)
Plant 4 ×11	30	568	16	2.8
Plant 5 × 7	17	564	33 (72)	5.9 (12.8)
reciprocal	11	269	0	0
Plant 5 × 9	6	148	4 (18)	2.7 (12.2)
Plant 5 ×11	16	399	5 (43)	1.3 (10.8)
reciprocal	14	321	2 (1)	0.6 (0.3)
Plant 5 ×18	20	555	105 (89)	18.9 (16.0)
reciprocal	11	301	0	0
Plant 5 ×20	8	196	8 (23)	4.1 (11.7)
Plant 6 × 7	13	424	249 (148)	58.7 (34.9)
reciprocal	19	582	0	0
Plant 6 ×10	11	295	97 (79)	32.9 (26.8)
Plant 7 ×11	21	403	0	0
reciprocal	7	188	0	0
Plant 9 ×12	4	87	0	0
reciprocal	10	193	1 (10)	0.5 (5.2)
Plant 9 ×21	3	80	1 (1)	1.3 (1.3)
Plant 10 ×12	15	224	0	0
reciprocal	9	172	2 (7)	1.2 (4.1)
Plant 12 ×13	1	12	0	0
Plant 14 × 5	5	84	0	0
Plant 14 ×19	1	13	0	0
Plant 15 ×12	2	48	0	0
Plant 16 × 2	12	176	0	0
Plant 16 × 6	16	252	1	0.4
Plant 19 ×11	7	163	6 (8)	3.7 (4.9)
Plant 20 ×14	5	50	4	8.0
Plant 20 ×19	3	35	8	22.9
Plant 22 × 5	6	81	1	1.2
Plant 22 ×18	2	19	0	0
B_1-hybrid × B_1-hybrid				
Plant 1 × 4	16	409	0	0
reciprocal	34	723	27 (49)	3.7 (6.8)
Plant 1 ×10	4	120	0	0
reciprocal	2	39	0	0
Plant 2 × 3	14	314	4	1.3
Plant 3 ×20	2	53	0	0
reciprocal	2	57	1	1.8
Plant 5 × 3	20	515	0	0

Table 2. (continued)

Plant 5 ×14	18	445	1	0.2
reciprocal	10	277	0	0
Plant 6 × 1	22	602	0	0
Plant 6 ×10	22	609	0	0
Plant 7 × 3	5	132	0	0
Plant 9 × 1	20	527	1	0.2
Plant 9 × 5	19	578	1 (5)	0.2 (0.9)
Plant 9 ×14	25	671	2	0.3
Plant 12 × 3	17	467	0	0
Plant 12 × 4	6	177	0	0
Plant 12 × 5	13	281	0	0
Plant 12 ×14	9	251	0	0

* No. of shrivelled brown achenes, ** 5 F_1-hybrids by ovary culture, *** 3 F_1-hybrids by ovary culture, **** 13 B_1-hybrid, ***** all achenes were self-fertilized *boreale*.

Two F_1-hybrids (Plants 2 and 6) were used as parents in this backcross. One thousand eight hundred and thirty disc florets of 27 heads of Plant 2 were pollinated and cultivated on the medium. Twenty ovaries developed and were germinated. Twelve plants with 2n=3x=27 and one with 2n=3x+1=28 were accepted as being F_1-hybrids. Seven died before they could be karyotyped. The other hybridization, using Plant 6, failed completely.

In the reciprocal backcross, all of the germinated progenies were diploid 2n=2x=18 and were doubtless due to selfing.

The chromosomes of the Plant 3 with 2n=3x=27, at mitotic metaphase, vary in length from 5.0 μm to 2.5 μm and in arm ratio from 1.0 to 4.0 (Fig. 1-D). The twenty-seven chromosomes are arranged in order of size in Fig. 2-D. Since the chromosomes of this karyotype are shortened to a great extent by pretreatment, the comparison of this karyotype with parental or other hybrid karyotypes should be made on the relative length and arm ratio instead of the raw value of measurement. Two small subterminal chromosomes (Chromosomes 21 and 22) had minute satellites. Chromosomes 13 was distinguishable by its length and the extreme subterminal centromere. Another four extreme subterminal chromosomes with arm ratios of 3.0–4.0 (Chromosome 23, 25, 26 and 27) were distinguishable.

Four satellite chromosomes were detected in Plant 2. Namely, two of them were medium-sized subterminal chromosomes and the rest small subterminals.

In eight of the nine B_1-hybrids with 2n=3x=27, the following meiotic configurations; 8 III+1 II+1 I, 7 III+2 II+2 I, 6 III+3 II+3 I, 5 III+4 II+4 I, 4 III+5 II+5 I, 3 III+6 II+6 I and 2 III+7 II+7 I were frequently observed. The precise meiotic analyses are summarized in Tables 3, 4 and 5. The nine B_1-hybrids are arranged in ascending order on the basis of average number of bivalent per PMC. Maximum number of trivalents per PMC varied from seven to nine among eight B_1-hybrids. The frequency of trivalent formation varied from 46.3% to 62.8% among eight B_1-hybrids (Table 4). The data of trivalent frequency in most of B_1-hybrids, except Plants 5 and 20, conform closely to the theoretical Poisson or binomial expectation based on the random hypothesis. It suggests that all the sets of three chromosomes have an equal chance of forming a trivalent. The frequency of bivalent

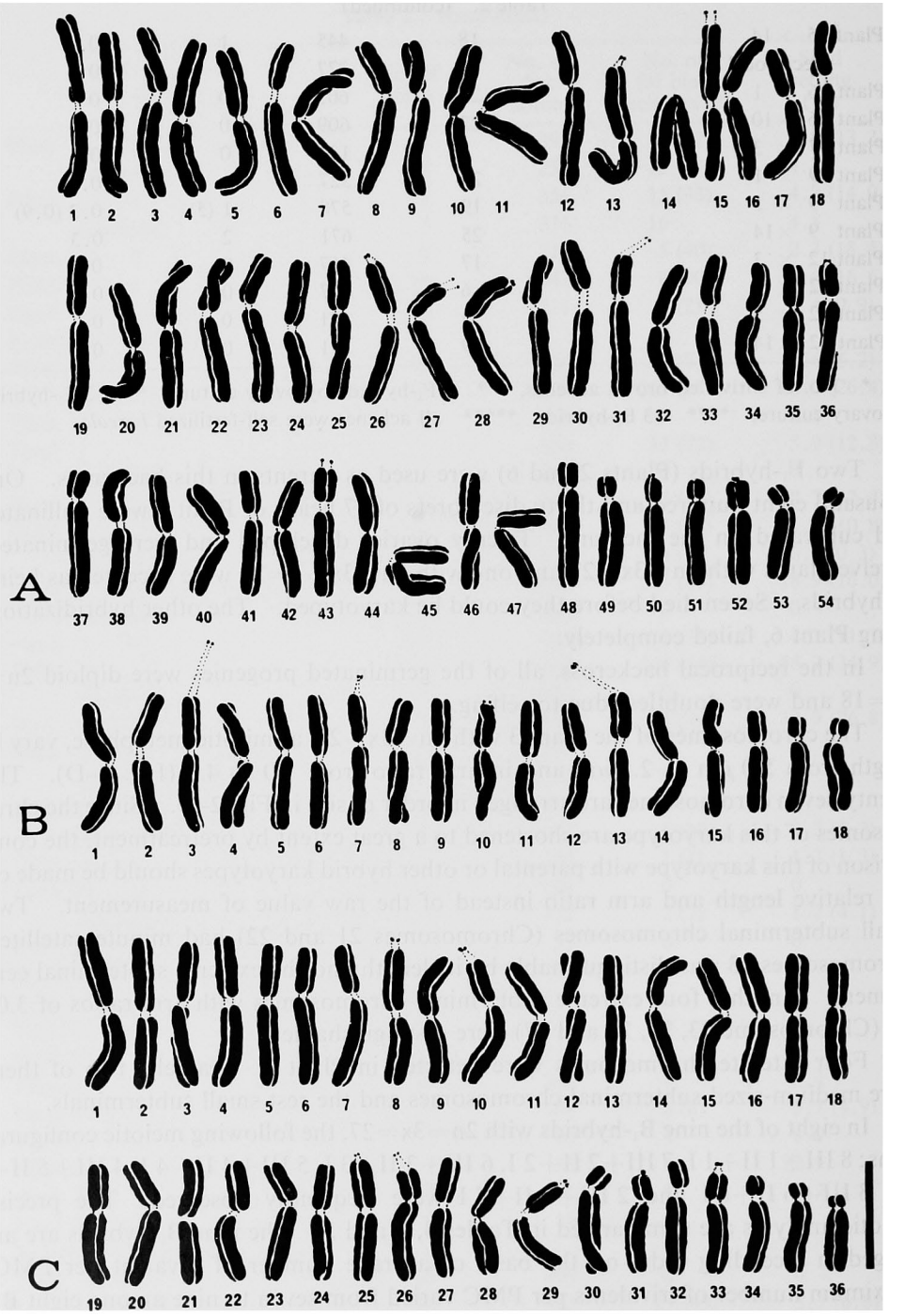


Fig. 2. Somatic karyotypes. A. *Ch. japonense* (Saka strain). B, *Ch. boreale*, C. F_1-hybrid.

fromation varied from 53.7% to 38.6% among eight B_1-hybrids. The chromosomes derived from the F_1-hybrids associated both homoeologously and homologously with the *Ch. boreale* chromosomes. Increased homoeologous association was observed in these B_1-hybrids. Consequently, there must be some control to suppress multivalent formation at the tetraploid level of F_1-hybrids although the chromosomes which paired homoeologously in the B_1-hybrids are capable of associating as multivalents but do not do so in the tetraploids. Most of the trivalents are chain, very

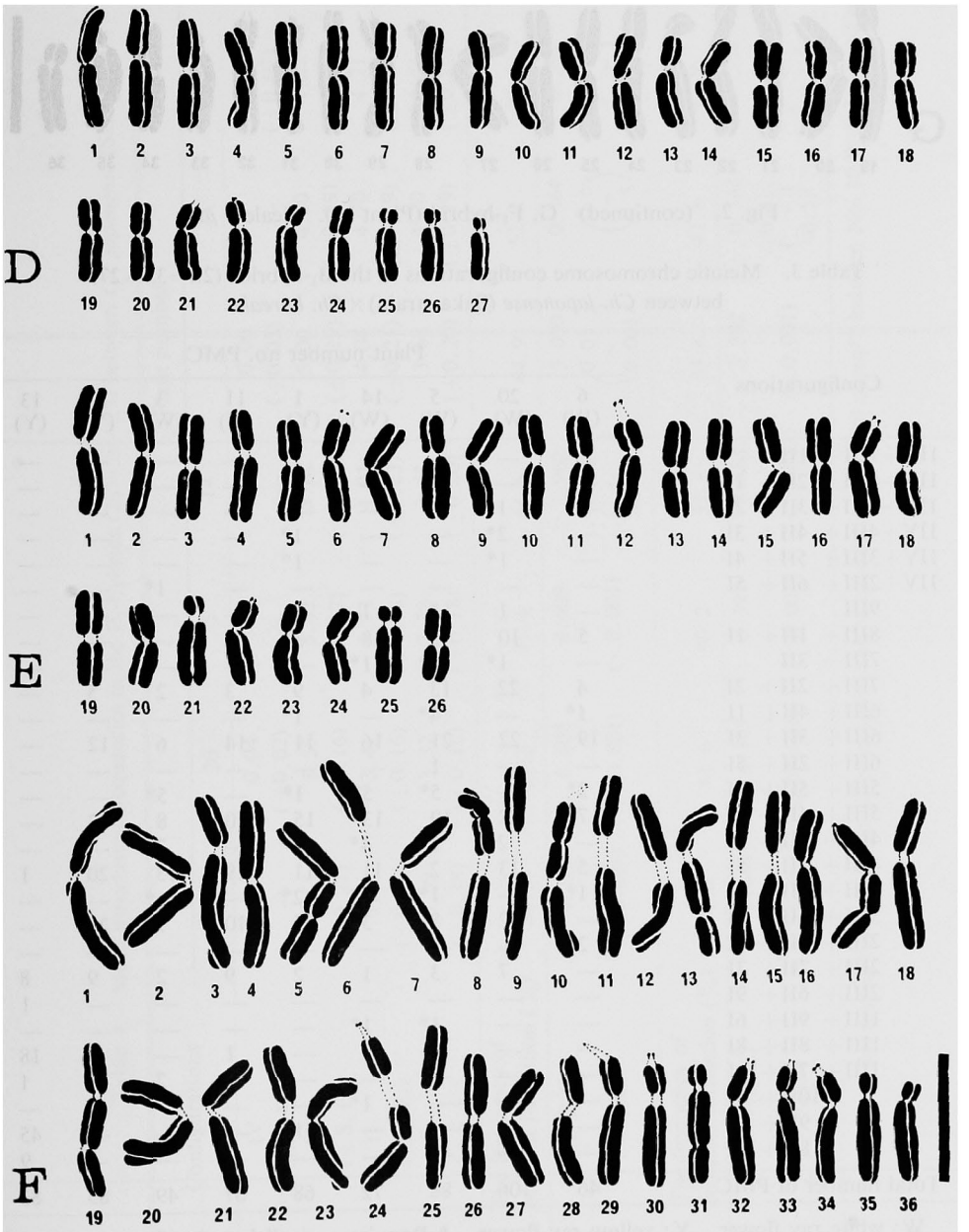


Fig. 2. (continued) D, B_1-hybrid (Plant 3). F, B_2-hybrid (Plant 1). E. F_2-hybrid (Plant 2).

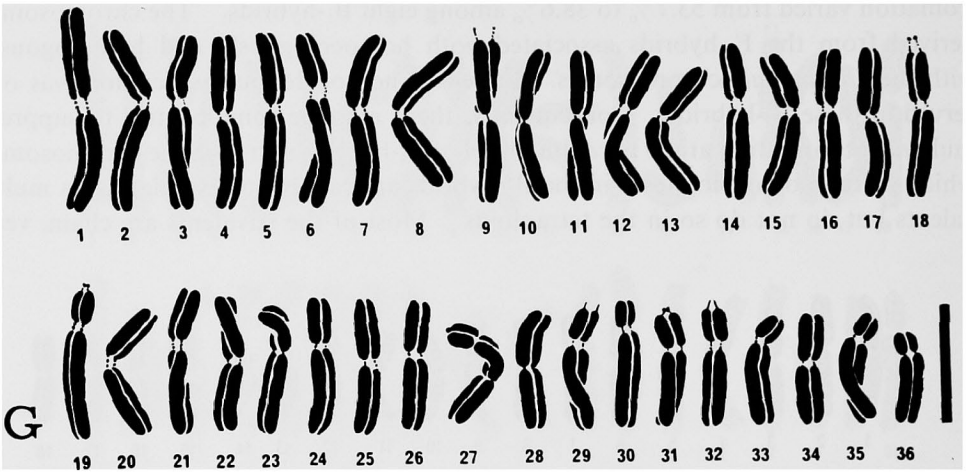


Fig. 2. (contiuned) G, F_3-hybrid (Plant 35). Scale 5 μm.

Table 3. Meiotic chromosome configurations in the B_1-hybrids (2n=3x=27) between *Ch. japonense* (Saka strain) × *Ch. boreale*

Configurations	Plant number no. PMC								
	6 (W)	20 (W)	5 (W)	14 (W)	1 (Y)	11 (Y)	3 (W)	10 (Y)	13 (Y)
1IV+7III+ 1II	—	—	—	—	1*	—	—	—	—
1IV+6III+ 2II+ 1I	—	—	—	—	1*	—	—	—	—
1IV+5III+ 3II+ 2I	—	1*	—	—	—	—	—	—	—
1IV+4III+ 4II+ 3I	—	2*	—	—	1*	—	—	—	—
1IV+3III+ 5II+ 4I	—	1*	—	—	1*	—	—	—	—
1IV+2III+ 6II+ 5I	—	—	—	—	—	—	1*	—	—
9III	—	1	1	1	—	—	—	—	—
8III+ 1II+ 1I	5	10	5	8	3	1	—	—	—
7III+ 3II	—	1*	2*	1*	—	—	—	—	—
7III+ 2II+ 2I	4	22	13	4	9	3	2	5	—
6III+ 4II+ 1I	1*	—	4*	—	1*	—	—	—	—
6III+ 3II+ 3I	19	22	21	16	11	14	6	12	—
6III+ 2II+ 5I	—	—	1	—	—	—	—	—	—
5III+ 5II+ 2I	2*	—	5*	5*	1*	—	5*	—	—
5III+ 4II+ 4I	7	22	19	13	15	10	8	17	—
4III+ 6II+ 3I	—	2*	1*	4*	3*	—	2*	—	—
4III+ 5II+ 5I	5	13	2	11	11	19	13	20	1
3III+ 7II+ 4I	1*	—	1*	2*	2*	—	1*	—	—
3III+ 6II+ 6I	—	2	8	3	5	10	7	21	—
2III+ 8II+ 5I	2*	—	—	—	—	—	—	—	—
2III+ 7II+ 7I	—	7	3	1	2	9	2	9	8
2III+ 6II+ 9I	—	—	—	—	—	—	—	—	1
1III+ 9II+ 6I	—	—	1*	1*	—	—	—	—	—
1III+ 8II+ 8I	—	—	1	1	—	1	—	1	18
1III+ 7II+10I	—	—	—	—	—	—	2	—	1
10II+ 7I	—	—	—	1*	—	—	—	—	—
9II+ 9I	—	—	—	—	1	—	—	—	45
8II+11I	—	—	—	—	—	—	—	—	9
Total number of PMC	46	106	88	72	68	67	49	85	83

W; white ray flower, Y; yellow ray flower. * Require a small but significant amount of non-homologous intra-set pairings.

Table 4. Mean association frequencies in the B_1-hybrids (2n=3x=27) between *Ch. japonense* (Saka strain) × *Ch. boreale*

Configurations	Plant number								
	6	20	5	14	1	11	3	10	13
IV	—	0.04	—	—	0.06	—	0.02	—	—
(±S. E.)	—	(±0.02)	—	—	(±0.03)	—	(±0.02)	—	—
III	5.65	5.55	5.42	5.18	5.00	4.30	4.22	4.16	0.49
(±S. E.)	(±0.21)	(±0.16)	(±0.17)	(±0.21)	(±0.19)	(±0.19)	(±0.20)	(±0.16)	(±0.09)
II	3.48	3.41	3.73	4.01	4.04	4.70	4.71	4.84	8.37
(±S. E.)	(±0.24)	(±0.16)	(±0.18)	(±0.23)	(±0.21)	(±0.19)	(±0.19)	(±0.16)	(±0.09)
I	3.09	3.32	3.28	3.43	3.68	4.70	4.41	4.84	9.98
(±S. E.)	(±0.18)	(±0.16)	(±0.18)	(±0.20)	(±0.20)	(±0.19)	(±0.26)	(±0.16)	(±0.18)
The frequency of bivalent formation (%)	38.6	38.3	41.4	44.6	44.9	52.2	52.4	53.7	93.0
t*=D/E	4.715	6.408	4.609	2.962	3.030	0.564	0.599	—	19.229
P* value	(<0.001)	(<0.001)	(<0.001)	(0.01 ~ 0.001)	(0.01 ~ 0.001)	(0.6 ~ 0.5)	(0.6 ~ 0.5)	—	(<0.001)
The frequency of trivalent formation (%)	62.8	61.6	60.2	57.6	55.6	47.8	46.9	46.3	5.5
t**=(D/E)	5.644	6.143	5.397	3.864	3.382	0.564	0.234	—	19.992
P** value	(<0.001)	(<0.001)	(<0.001)	(<0.001)	(<0.001)	(0.6 ~ 0.5)	(0.9 ~ 0.8)	—	(<0.001)

Calculated for testing the significant difference of bivalent mean* and trivalent mean** between No. 10 B_1-hybrid and other B_1-hybrids.

Table 5. Binomial and Poisson distribution of trivalent numbers in the B_1-hybrids between *Ch. japonense* (Saka strain) and *Ch. boreale*

Plant	Distribution	Cells with number of trivalents										Total cells (n)	p or λ	χ^2	P values
		0	1	2	3	4	5	6	7	8	9				
13	O	54	19	9	1	—	—	—	—	—	—	83			
	E	50.9	24.9	6.1	1.0	0.1	—	—	—	—	—	83.0	λ=0.49	3.1713	d. f.=2, (0.25 ~0.10)
10	O	—	1	9	21	20	17	12	5	—	—	85			
	E	1.4	5.5	11.5	15.9	16.6	13.8	9.5	5.7	3.0	2.1	85.0	λ=4.16	14.5434	d. f.=8, (0.10 ~0.05)
3	O	—	2	3	8	15	13	6	2	—	—	49			
	E	0.7	3.0	6.4	9.0	9.5	8.0	5.6	3.4	1.8	1.6	49.0	λ=4.22	13.0127	d. f.=7, (0.10 ~0.05)
11	O	—	1	9	10	19	10	14	3	1	—	67			
	E	0.9	3.9	8.4	12.1	12.9	11.1	8.0	4.9	2.6	2.2	67.0	λ=4.30	14.8305	d. f.=7, (0.050 ~0.025)
1	O	1	—	2	8	15	16	13	10	3	—	68			
	E	—	0.5	2.6	7.5	14.1	17.7	14.8	7.9	2.5	0.3	67.9	p=0.556	1.0487	d. f.=5, (0.975 ~0.950)
14	O	1	2	1	5	15	18	16	5	8	1	72			
	E	—	0.4	2.1	6.7	13.7	18.6	16.8	9.8	3.3	0.5	71.9	p=0.576	10.9790	d. f.=5, (0.10 ~0.05)
5	O	—	2	3	9	3	24	26	15	5	1	88			
	E	—	0.3	1.8	6.4	14.5	22.0	22.2	14.4	5.4	0.9	87.9	p=0.602	14.9143	d. f.=6, (0.025 ~0.010)
6	O	—	—	2	1	5	9	20	4	5	—	46			
	E	—	0.1	0.6	2.5	6.4	10.8	12.2	8.8	3.7	0.7	45.8	p=0.628	8.3056	d. f.=4, (0.10 ~0.05)
20	O	—	—	7	3	17	23	22	23	10	1	106			
	E	—	0.3	1.8	6.6	16.0	25.7	27.6	19.1	7.7	1.4	106.2	p=0.617	14.6137	d. f.=6, (0.025 ~0.010)

O; Observed distribution, E; Expected Poisson distribution $P_k=e^{-\lambda}(\lambda^k/k!)$, E; Expected binomial distribution $n(p+q)^k$, $q=1-p$, d. f.; degrees of freedom, N−2.

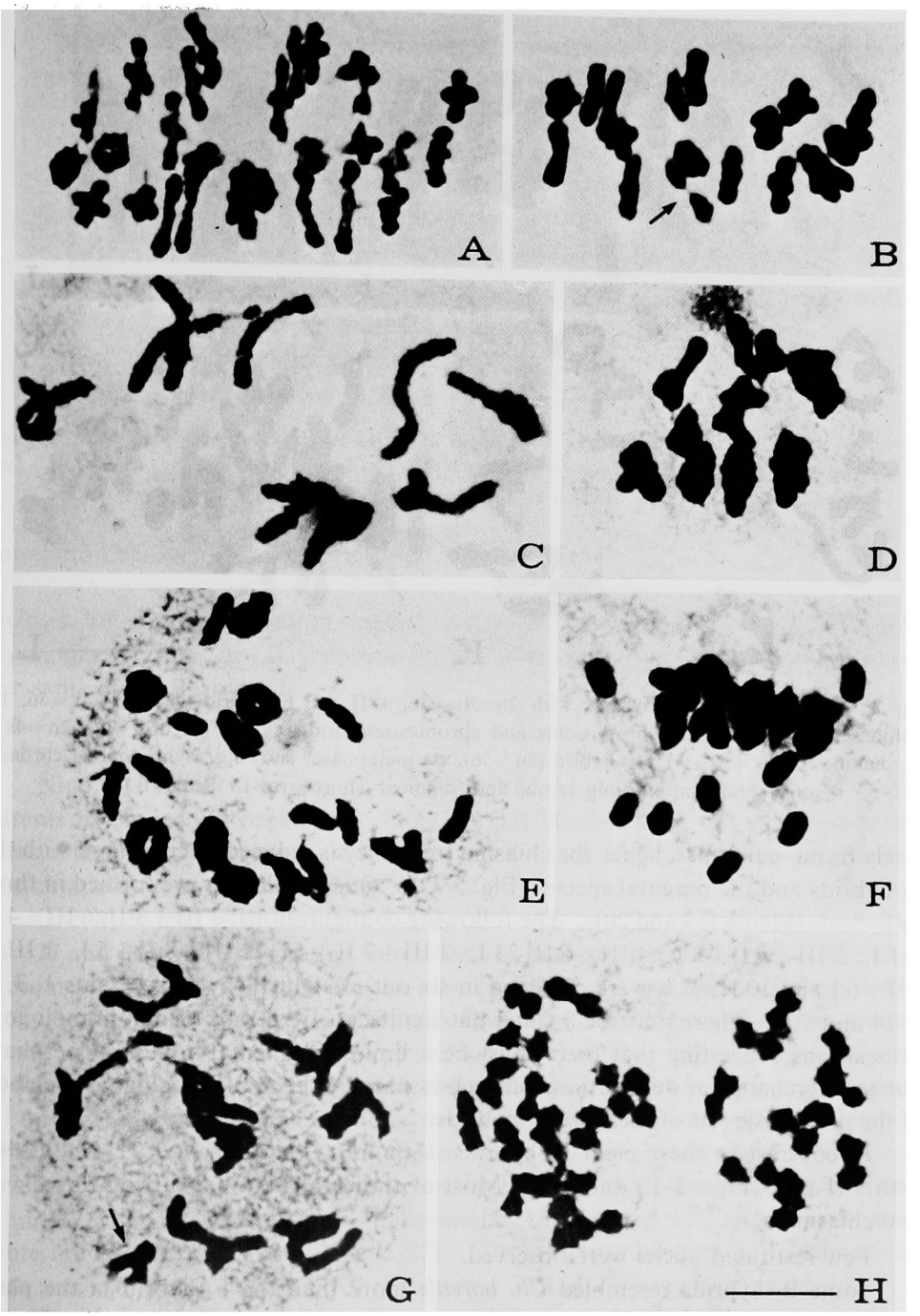


Fig. 3. Meiotic configurations. A, *Ch. japonense* (Saka strain), 27II. B, F_1-hybrid, 18II. C, B_1-hybrid with 2n=3x=27, diakinesis, 9III. D, B_1-hybrid. 1st metaphase, 9III. E, B_1-hybrid (Plant 13, 2n=27), diakinesis, 9II+9I. F, B_1-hybrid (Plant 13), 1st metaphase, 9II+9I. G, B_2-hybrid (Plant 1, 2n=2x=8=26), diakinesis, 8III+1II (bivalent, arrow). H, B_2-hybrid (Plant 1), 1st anaphase, chromosome segregation 15–11.

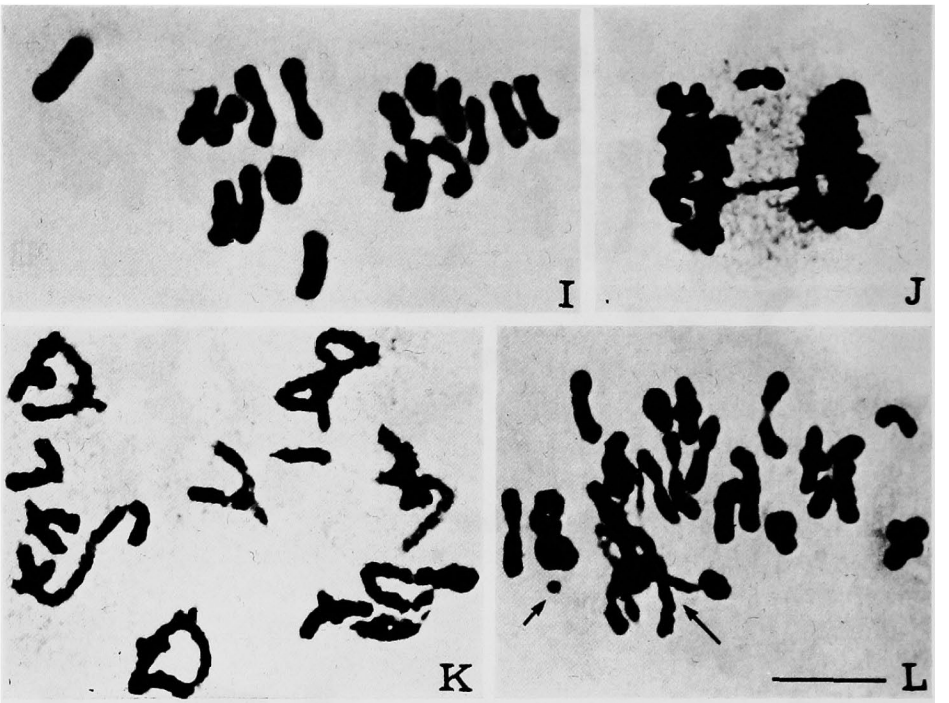


Fig. 3. (continued) I, F_2-hybrids with 2n=4x=36, 18II. J, F_3-hybrid with 2n=4x=36, 1st anaphase, showing lagging chromosome and chromosome bridge. K, F_3-hybrid with 2n=4x=36, diakinesis, 6IV+6II. L, F_2-hybrid (2n=36), 1st metaphase, showing asynchronous chromosome condensation (long arrow) and fragment (short arrow). Scale 10 μm.

rarely frying-pan types, hence the chiasma frequency is reduced as compared with the F_1-hybrids and the parental species (Fig. 3–C). Quadrivalents were formed in three B_1-hybrids (Plants 1, 3 and 20). The following configurations; 7 III+3 II, 6 III+4 II +1 I, 5 III+5 II+2 I, 4 III+6 II+3 I, 3 III+7 II+4 I, 2 III+8 II+5 I, 1 III+ 9 II+6 I and 10 II+7 I, were observed in six out of eight B_1-hybrids (Plants 1, 3, 5, 6, 14 and 20). There must be a small but significant frequency of non-homologous associations suggesting that there must be a limited segmental homology perphaps due to interchange or duplication, between essentially the non-homologous members of the three basic sets of these six B_1-hybrids.

In contrast to these eight B_1-hybrids, the ninth, Plant 13, had 93% of PMCs with 9 II+9 I (Figs. 3–E) and –F). Most of the bivalents were of ring form, with two chiasmata.

Few restituted nuclei were observed.

Some B_1-hybrids resembled *Ch. boreale* more than the F_1-hybrid in the plant height, leaf size and the number and depth of marginal teeth. One of the B_1-hybrids had cuneate basal shaped leaves instead of the cordate shape characteristic of both parents (Fig. 5–F). Flower size in ten of the thirteen B_1-hybrids is nearly the same as that in *Ch. japonense* (Saka strain). The rest have about the same flower size as *Ch. boreale*. Segregation of ray floret color was observed in this generation. Eight of the thirteen B_1-hybrids had white ray florets and the others, yellow. There was no

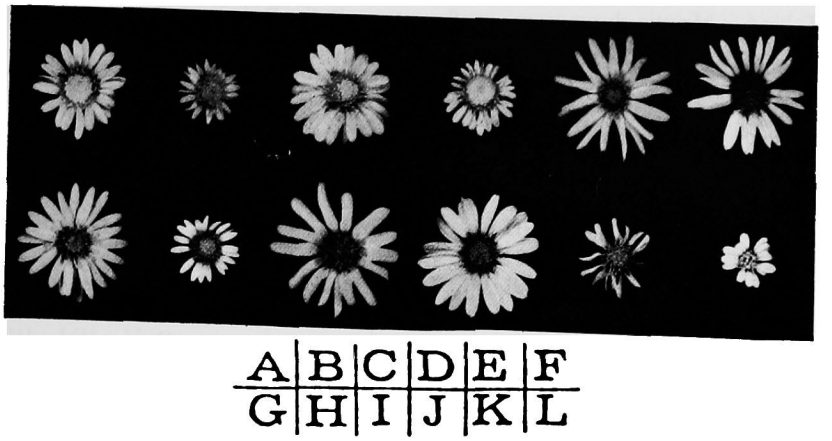


Fig. 4. Flower heads. A, *Ch. japonense* (Saka strain) (W). B, *Ch. boreale* (Y). C–D. F_1-hybrids (W). E–H, B_1-hybrids. E, plant 13 (Y). F, (W). G, (Y). H, (W). I–L, F_2-hybrids. I, K and L, (W), J, (Y). Y=yellow ray floret, w=white ray floret. ×1/2.

correlation between the ray floret color and flower size.

Plant 13 was conspicuously different from the others in pairing behaviour in meiosis, but not in the morphological characters. It had yellow ray florets, large flower size the same as *Ch. japonense* (Saka strain) (Fig. 4–E) and leaf shape nearly the same as the F_1-hybrids.

B_1-hybrid (♀)×*Ch. boreale* (♂)

Nine B_1-hybrids (Plants 1, 2, 3, 7, 10, 11, 13, 14 and 20) were used as female parents in the second backcross. Only one fat black achene was obtained in this cross, Plant 20×*Ch. boreale*. Some shrivelled black achenes obtained from four combinations were germinated.

One B_2-hybrid with 2n=2x+6=24, one with 2n=2x+8=26, two with 2n=3x=27 and one with 2n=4x=36 were obtained in the second backcross. The chromosomes of the Plant 1 with 2n=2x+8=26, at mitotic metaphase, vary in length from 4.8 μm to 2.7 μm and in arm ratio from 1.0 to 4.0 (Fig. 1–E). The twenty-six chromosomes are arranged in order of size in Fig. 2–E. Since the chromosomes of this karyotype is extremely shortened by pretreatment, the comparison with parental or other hybrid karyotypes should be made on the relative length and the arm ratio instead of the raw value of measurement. Five chromosomes had minute satellites. Two of them were medium-sized submedian chromosomes (Chromosomes 6 and 17), one a medium-sized subterminal (Chromosome 12), and the rest small subterminals (Chromosomes 22 and 23). Two extreme subterminal chromosomes with arm ratios of 3.4 and 4.0 (Chromosomes 21 and 25) were distinguishable.

There were no significant karyotypic differences between Plant 1 with 2n=2x+8=26 and Plant 2 with 2n=3x=27 except that the latter had an additional small and extreme subterminal chromosome.

In Plant 1 with 2n=2x+8=26, the following meiotic configurations were frequently observed; 7 III+2 II+1 I, 6 III+3 II+2 I, 5 III+4 II+3 I, 4 III+5 II+4 I and 3 III+6 II+5 I (Fig. 3–G). The precise meiotic analyses are summarized in

Table 6. *Meiotic chromosome configurations in the B_2-hybrid (2n=2x+8=26) between Ch. japonense (Saka strain) × Ch. boreale*

Configurations	No. PMC
8III+1II	5
7III+2II+1I	25
6III+3II+2I	31
5III+5II+1I	2*
5III+4II+3I	49
4III+6II+2I	6*
4III+5II+4I	30
3III+7II+3I	6*
3III+6II+5I	17
2III+8II+4I	9*
2III+7II+6I	2
1III+8II+7I	3
Total number of PMC	185

Mean; (4.83±0.11)III+(4.29±0.13)II+(2.92±0.10)I.
The frequency of bivalent formation is 46.7%.
The frequency of trivalent formation is 60.4%.
* Require a small but significant amount of non-homologous intra-set pairings.

Table 6. The mean number of trivalents per PMC in this B_2-hybrid was 4.83 nearly the same as that in eight of the nine B_1-hybrids. The following chromosome segregations at the first anaphase to each pole, 13–13 and 11–15 (Fig. 3–H), were observed Few restituted nuclei were observed.

B_1-hybrid × B_1-hybrid

Twenty combinations were prepared for this cross. The rate of seed setting varied from 0% to 3.7%. Fifty offsprings were obtained, but most of them died after they were transplanted to pots. One progeny with 2n=3x+3=30, four with 2n=3x+5=32, three with 2n=3x+6=33, two with 2n=3x+7=34, four with 2n=3x+8=35 and one with 2n=4x=36 were karyotyped. All these hybrids have higher chromosome numbers than the B_1-parents. The results are compatible with the observation that the first meiotic anaphase in the B_1-parents showed chromosome segregations within the range from 9 to 18 at one pole.

F_1-hybrid × F_1-hybrid

Nine combinations were prepared for this cross. The rate of seed setting varied from 0.3% to 5.9% among combinations. Japanese wild chrysanthemums are usualy self-incompatible, hence the rate of seed setting depends on the fertility of the parental gametes as shown in the results of the crosses between Plant 2 and others. Plants 2 was also used in hybridization with *Ch. boreale* and its seed setting was about 1%. Consequently, the rate of seed setting with intrabreeding was slightly higher than with interbreeding. About 200 F_2-hybrids were obtained.

Twenty plants proved to be tetraploid. The chromosomes of the Plant 2 with 2n=4x=36, at mitotic metaphase, vary in length from 7.3 μm to 3.9 μm and in arm from 1.0 to 4.6 (Fig. 1–F). The thirty-six chromosomes are arranged in order of

Table 7. Meiotic chromosome configuration in the F_2-hybrids (2n=4x=36) between *Ch. japonense* (Saka strain) × *Ch. boreale*

Configurations	Plant number No, PMC						
	2	1	56	3	5	6	57
4IV +10II	2	4	—	—	—	—	—
3IV +12II	12	5	—	3	2	1	—
3IV +11II+2I	2	—	—	—	—	—	—
2IV +14II	27	21	25	14	10	5	3
2IV +13II+2I	—	2	1	2	—	—	—
1IV+1III+14II+1I	1	1	1	1	1	—	1
1IV +16II	51	43	49	43	24	7	19
1IV +15II+2I	2	6	3	6	1	1	—
1III+16II+1I	2	1	—	5	—	—	2
18II	12	31	43	40	40	21	79
17II+2I	—	4	—	10	8	—	3
16II+4I	—	1	—	—	1	—	—
Total number of PMC	111	119	122	124	87	35	107

size in Fig. 2–F. Eight chromosomes had minute satellites. One was a medium-sized median chromosome (Chromosome 10), one a medium-sized submedian (Chromosome 17), one a medium-sized subterminal (Chromosome 24) and five small subterminals (Chromosomes 29, 30, 32, 33 and 34). Three extreme subterminal chromosomes with arm ratios of 3.9–4.6 (Chromosomes 31, 35 and 36) were distinguishable.

In seven F_2-hybrids, the following meiotic configurations were frequently observed; 3 IV+12 II, 2 IV+14 II, 1 IV+16 II and 18 II. The precise meiotic analyses are summarized in Table 7, where the seven F_2-hybrids are arranged in ascending order on the basis of average number of bivalents per PMC. The maximum number of quadrivalents per PMC varied from two to four among the F_2-hybrids. There is a significant difference in the frequency of quadrivalent and bivalent formation between F_1-hybrids and some of the F_2-hybrids (Plants 1, 2 and 57. Table 8). It should be noted that the frequency of bivalent formation in Plant 57 is significantly highter than that in the F_1-hybrids. The data of quadrivalent frequency in most F_2-hybrids, except Plants 2 and 56, conform to the Poisson distribution, as detailed in Table 9. The frequency of trivalent and univalent formation was extremely low.

Various kinds of abnormalities were observed throughout all stages of meiosis. Figure 3–L shows chromosome fragmentation (short arrow) and conspicuous asynchronous chromosome condensation (long arrow). One or two heteromorphic bivalents were observed in some of the F_2-hybrids.

Morphological variation in some quantitative characters transgresses the parental values. Examples are in the number of marginal teeth on the leaves, and the size of the flower head (Figs. 4–I and –J). Segregation of ray floret color was also observed in this generation. Most progeny had white ray florets but some were yellow. A few were palish yellow, intermediate between white and yellow. A gradual change of color from yellow to white, proximal to terminal, was observed in the ray florets of some F_2-hybrids. Some had no incised regions in the leaves. Various kinds

Table 8. Mean association frequencies in the F_2-hybrids (2n=4x=36) between *Ch. japonense* (Saka strain) × *Ch. boreale*

Configurations	Plant number						
	2	1	56	3	5	6	57
IV	1.42	1.07	0.86	0.73	0.60	0.60	0.24
(±S. E.)	(±0.09)	(±0.09)	(±0.07)	(±0.07)	(±0.08)	(±0.14)	(±0.05)
III	0.03	0.02	0.01	0.05	0.01	—	0.03
(±S. E.)	(±0.02)	(±0.01)	(±0.01)	(±0.02)	(±0.01)	—	(±0.02)
II	15.06	15.71	16.23	16.29	16.66	16.77	17.43
(±S. E.)	(±0.18)	(±0.18)	(±0.14)	(±0.14)	(±0.17)	(±0.30)	(±0.10)
I	0.10	0.25	0.07	0.34	0.26	0.06	0.08
(±S. E.)	(±0.04)	(±0.06)	(±0.03)	(±0.06)	(±0.08)	(±0.06)	(±0.04)
The frequency of bivalent formation (%)	83.7	87.3	90.2	90.5	92.1	93.2	96.8
t*=(D/E)	6.701	3.926	1.949	1.657	0.132	1.244	4.438
P* values	(<0.001)	(<0.001)	(0.1~0.05)	(0.1~0.05)	(0.9~0.8)	(0.3~0.2)	(<0.001)
The frequency of quadrivalent formation (%)	15.8	11.9	9.6	8.2	6.6	6.7	2.7
t**=(D/E)	6.666	3.596	2.020	0.707	0.564	0.383	4.882
P** values	(<0.001)	(<0.001)	(0.05~0.02)	(0.5~0.4)	(0.6~0.5)	(0.8~0.7)	(<0.001)

Calculated for testing the significant difference of bivalent means* and quadrivalent means** between F_1-hybrid and each F_2-hybrid.

Table 9. Poisson distribution of quadrivalent numbers in F_1-, F_2- and F_3-hybrids between *Ch. japonense* (Saka strain) and *Ch. boreale*

Plant	Distribution	Cells with number of quadrivalents										Total cells (n)	λ	χ^2	P values
		0	1	2	3	4	5	6	7	8	9				
F_1, 1	O	58	41	13	3	—	—	—	—	—	—	115			
	E	59.5	39.2	13.0	2.9	0.5	—	—	—	—	—	115.1	0.66	0.1675	d. f.=2, (0.95~0.90)
F_2, 57	O	84	20	3	—	—	—	—	—	—	—	107			
	E	84.2	20.2	2.5	0.2	—	—	—	—	—	—	107.1	0.24	0.0358	d. f.=1, (0.90~0.75)
F_2, 6	O	21	8	5	1	—	—	—	—	—	—	35			
	E	19.2	11.5	3.5	0.7	0.1	—	—	—	—	—	35.0	0.60	1.9061	d. f.=1, (0.25~0.10)
F_2, 5	O	49	26	10	2	—	—	—	—	—	—	87			
	E	47.8	28.6	8.6	1.7	0.3	—	—	—	—	—	87.0	0.60	0.4944	d. f.=2, (0.90~0.75)
F_2, 3	O	55	50	16	3	—	—	—	—	—	—	124			
	E	59.8	43.6	15.9	3.8	0.7	0.1	—	—	—	—	123.9	0.73	1.8813	d. f.=2, (0.5~0.25)
F_2, 56	O	43	53	26	—	—	—	—	—	—	—	122			
	E	51.6	44.4	19.0	5.5	1.2	0.2	—	—	—	—	121.9	0.86	12.0889	d. f.=3, (0.010~0.005)
F_2, 1	O	37	50	23	5	4	—	—	—	—	—	119			
	E	40.8	43.7	23.3	8.3	2.3	0.5	0.1	—	—	—	119.0	1.07	3.0029	d. f.=3, (0.50~0.25)
F_2, 2	O	14	54	27	14	2	—	—	—	—	—	111			
	E	26.9	38.1	27.1	12.8	4.6	1.3	0.3	—	—	—	111.1	1.42	16.0041	d. f.=4, (<0.005)
F_3, 35	O	59	17	12	—	—	—	—	—	—	—	88			
	E	55.0	25.9	6.1	1.0	0.1	—	—	—	—	—	88.1	0.47	10.1558	d. f.=2, (0.010~0.005)
F_3, 6	O	69	39	5	1	—	—	—	—	—	—	114			
	E	71.9	33.1	7.6	1.1	0.1	—	—	—	—	—	113.8	0.46	2.0913	d. f.=2, (0.50~0.25)
F_3, 25	O	21	12	13	1	—	—	—	—	—	—	47			
	E	19.7	17.1	7.5	2.2	0.5	0.1	—	—	—	—	47.1	0.87	6.7973	d. f.=2, (0.050~0.025)

Table 9. (Continued)

Plant	Distribution	Cells with number of quadrivalents										Total cells (n)	λ	χ^2	P values
		0	1	2	3	4	5	6	7	8	9				
F_3, 2	O	27	49	20	2	—	—	—	—	—	—	98			
	E	37.1	36.1	17.4	5.7	1.4	0.3	—	—	—	—	98.0	0.97	11.8496	d. f.=3, (0.010~0.005)
F_3, 4	O	25	48	24	3	—	—	—	—	—	—	100			
	E	35.0	36.7	19.3	6.8	1.8	0.4	0.1	—	—	—	100.1	1.05	12.1045	d. f.=3, (0.010~0.005)
F_3, 5	O	8	11	5	7	—	—	—	—	—	—	31			
	E	8.0	10.9	7.3	3.3	1.1	0.3	0.1	—	—	—	31.0	1.35	6.3741	d. f.=3, (0.10~0.05)
F_3, 7	O	14	42	33	20	4	—	—	—	—	—	113			
	E	22.1	36.0	29.4	15.9	6.6	2.1	0.6	0.1	—	—	112.8	1.63	9.2911	d. f.=4, (0.10~0.05)
F_3, 10	O	28	43	39	24	4	3	—	—	—	—	141			
	E	28.8	45.7	36.4	19.3	7.6	2.4	0.7	0.1	—	—	141.0	1.59	3.2298	d. f.=4, (0.75~0.50)
F_3, 62	O	10	26	14	18	4	1	—	—	—	—	73			
	E	12.4	22.0	19.5	11.5	5.1	1.8	0.5	0.1	—	—	72.9	1.77	7.4709	d. f.=4, (0.25~0.10)
F_3, 40	O	7	19	16	9	1	2	1	—	1	—	56			
	E	8.5	16.0	15.1	9.5	4.5	1.7	0.6	0.2	0.1	—	56.2	1.89	4.3832	d. f.=4, (0.50~0.25)
F_3, 34	O	9	42	38	27	10	6	3	—	—	—	135			
	E	16.1	34.2	36.5	25.8	13.8	5.7	2.0	0.7	0.1	—	134.9	2.13	6.1039	d. f.=5, (0.50~0.25)

O; Observed distribution, E; Expected Poisson distribution $P=e^{-\lambda}(\lambda^k/k!)$, d. f.; degrees freedom, N–2.

of leaf base from cordate to cuneate were observed although both parental species had cordate leaf bases (Figs. 5–K, –L, –M and –N). Some had curled leaves. Some resembled *Ch. japonense* (Saka strain) closely (Fig. 5–O).

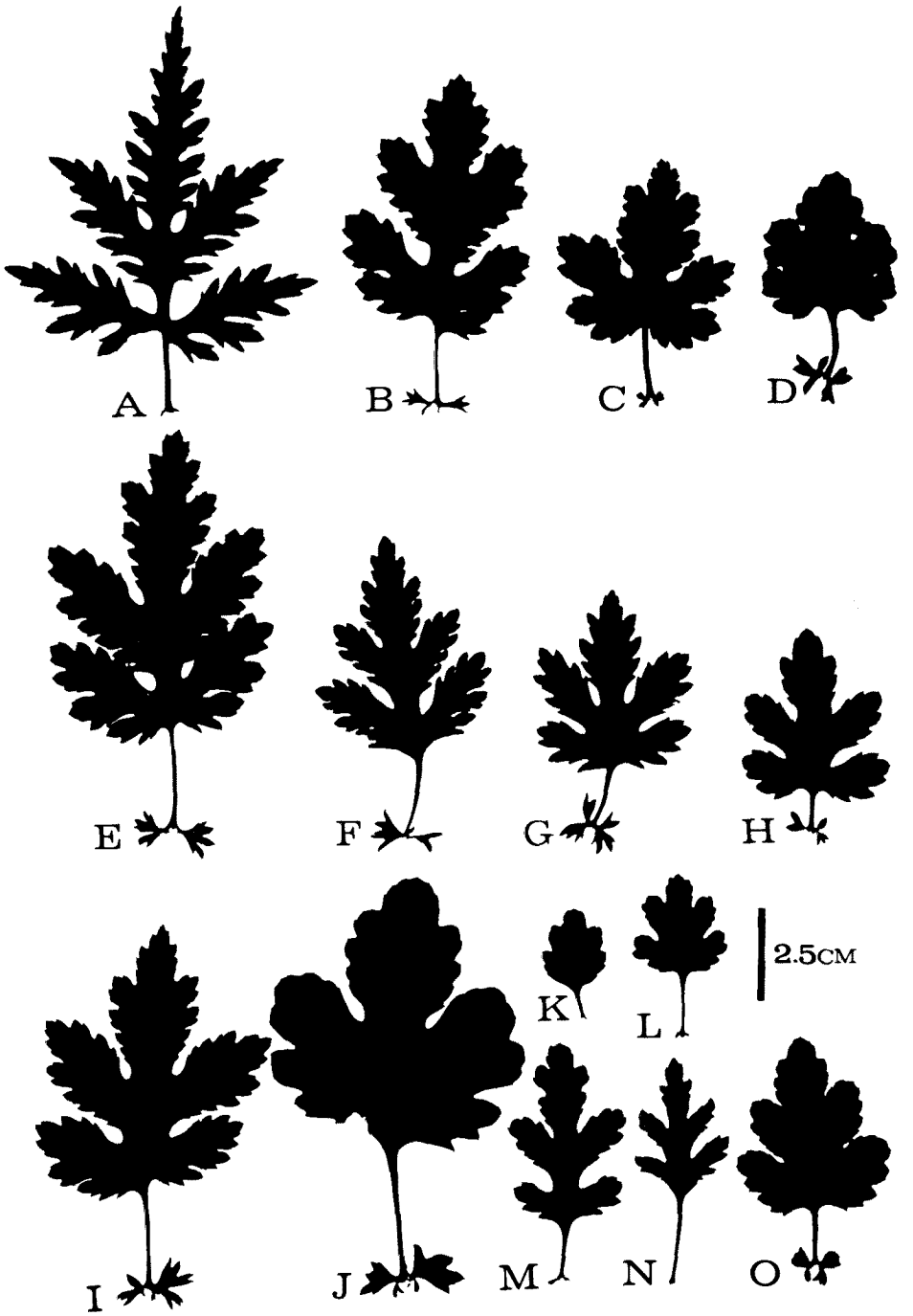


Fig. 5. Leaves. A, *Ch. boreale*. B–C, F_1-hybrids. D, *Ch. japonense* (Saka strain). E–H, B_1-hybrids, note to the cuneate leaf base of F. I–O, F_2-hybrids, note to the cuneate leaf base of K and N.

F_2-hybrid × F_2-hybrid

Thirty-two combinations were prepared for this cross. The rate of seed setting varied from 0% to 58.7% among the combinations. The extremely high rate of seed setting was observed in some of the F_2-hybrids (Plants 5, 6 and 20) when they were used as female parents (Table 2). In the combination of Plant 6×7, the set of fat black achenes was estimated at 58.7%, or, together with shrivelled black achenes, 93.8%. Fertility is almost as high as in the wild species. About 700 F_3-hybrids were obtained.

Nineteen F_3-hybrids of the twenty examined had the chromosome number 2n=4x=36 and one had 2n=4x+1=37.

The chromosomes of the Plant 35 with 2n=4x=36, at mitotic metaphase, vary in length from 8.0 μm to 3.9 μm and in arm ratio from 1.0 to 4.0 (Fig. 1–G). The thirty-six chromosomes are arranged in order of size in Fig. 2–G. Seven chromosomes had minute satellites. One was a medium-sized median chromosome (Chromosome 9), one a medium-sized submedian (Chromosome 18), one a medium-sized subterminal (Chromosome 19) and four small subterminals (Chromosomes 27, 29, 31 and 32). Four extreme subterminal chromosomes with arm ratios of 3.2–4.0 (Chromosomes 30, 33, 35 and 36) were distinguishable.

Five satellite chromosomes were detected in the karyotype of Plant 43. Four of them were medium-sized submedian chromosomes and one a small subterminal. Five extreme subterminal chromosomes were distinguishable.

There are significant karyotypic differences among the F_3-hybrids.

In eleven F_3-hybrids, the following meiotic configurations were frequently observed; 3 IV+12 II, 2 IV+14 II, 1 IV+16 II and 18 II. Hexavalent and pentavalent associations were observed in Plant 34. The precise meiotic analyses are summarized in Table 10, where the eleven F_3-hybrids are arranged in ascending order on the basis of average number of bivalents per PMC. The maximum number of quadrivalents per PMC varied from two to eight among the eleven F_3-hybrids. There is a significant difference in the frequency of quadrivalent and bivalent formation between F_1-hybrids and most of the F_3-hybrids (Table 11). The data of quadrivalent frequency in most of the F_3-hybrids, except Plants 2, 4, 25 and 35, conform closely to the theoretical Poisson expectation (Table 9). Again the frequency of trivalent and univalent associations is extremely low.

Various kinds of abnormalities were observed throughout all stages in meiosis. Figure 3–J shows a chromosome bridge and a lagging univalent chromosome. Many micro nuclei and microcytes were observed.

In F_3-hybrids, the morphological diversity is nearly the same as that in F_2-hybrids.

2. *Ch. japonense* Nakai (Nakamura strain)

Ch. japonense (Nakamura strain)

Ch. japonense (Nakamura strain) with 2n=6x=54 was used in hybridization with the diploid *Ch. boreale*.

The chromosomes of *Ch. japonense* (Nakamura strain), at mitotic metaphase, vary in length from 6.7 μm to 3.8 μm and in arm ratio from 1.0 to 4.4 (Fig. 6–A and

Table 10. Meiotic chromosome configurations in the F_3-hybrids (2n=4x=36) between *Ch. japonense* (Saka strain) × *Ch. boreale*

Configurations	Plant number No. PMC										
	34	40	62	7	10	5	4	2	25	6	35
VI+1IV +13II	1	—	—	—	—	—	—	—	—	—	—
1V+1IV +13II+1I	1	—	—	—	—	—	—	—	—	—	—
8IV + 2II	—	1	—	—	—	—	—	—	—	—	—
6IV + 6II	3	1	—	—	—	—	—	—	—	—	—
5IV+1III+ 6II+1I	1	—	—	—	—	—	—	—	—	—	—
5IV + 8II	4	2	1	—	3	—	—	—	—	—	—
5IV + 7II+2I	1	—	—	—	—	—	—	—	—	—	—
4IV+1III+ 8II+1I	—	—	—	1	—	—	—	—	—	—	—
4IV +10II	9	1	4	3	4	—	—	—	—	—	—
4IV + 9II+2I	1	—	—	—	—	—	—	—	—	—	—
3IV+1III+10II+1I	—	—	1	5	—	—	—	—	—	—	—
3IV +12II	27	8	16	15	22	7	3	2	1	1	—
3IV +11II+2I	—	1	1	—	2	—	—	—	—	—	—
2IV+1III+12II+1I	1	—	1	3	1	—	—	—	—	—	—
2IV +14II	34	15	12	30	36	5	24	19	12	4	12
2IV +13II+2I	3	1	1	—	2	—	—	1	1	1	—
1IV+2III+13II	—	—	—	1	—	—	—	—	—	—	—
1IV+1III+14II+1I	4	—	1	5	—	—	—	1	—	—	—
1IV +16II	34	19	24	32	43	11	48	45	11	36	17
1IV +15II+2I	2	—	1	4	—	—	—	2	1	3	—
1IV +14II+4I	—	—	—	—	—	—	—	1	—	—	—
1III+16II+1I	1	—	2	3	1	—	—	—	—	1	—
18II	7	7	6	10	24	8	25	26	18	55	53
17II+2I	1	—	2	1	3	—	—	1	3	12	5
16II+4I	—	—	—	—	—	—	—	—	—	1	1
Total number of PMC	135	56	73	113	141	31	100	98	47	114	88

Table 12). The fifty-four chromosomes are arranged in order of size in Fig. 7–A. Eight chromosomes had minute satellites. Two were medium-sized median chromosomes (Chromosomes 16 and 17), two medium-sized submedians (Chromosomes 21 and 26), one medium-sized subterminal (Chromosome 23) and the others small subterminals (Chromosomes 43, 46 and 48). Six extreme subterminal chromosomes with arm ratios of 3.9–4.4 (Chromosomes 49, 50, 51, 52, 53 and 54) were distinguishable. Karyomorphologically *Ch. japonense* (Nakamura strain) seems not to be an autohexaploid.

In meiosis 27 II was the most frequent configuration and configurations of 2 IV+23 II, 1 IV+25 II, 1 IV+24 II+2 I and 26 II+2 I, were rarely observed. The frequency of bivalent formation was 98.0%. Chromosome association in this species is essentially diploid (Fig. 6–B).

The parental species *Ch. japonense* (Nakamura strain) and *Ch. boreale* can be distinguished from one another by many characters, —the number of heads (less / more), the number of ray and disc floret (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), the presence of psudostipules and rhizomes

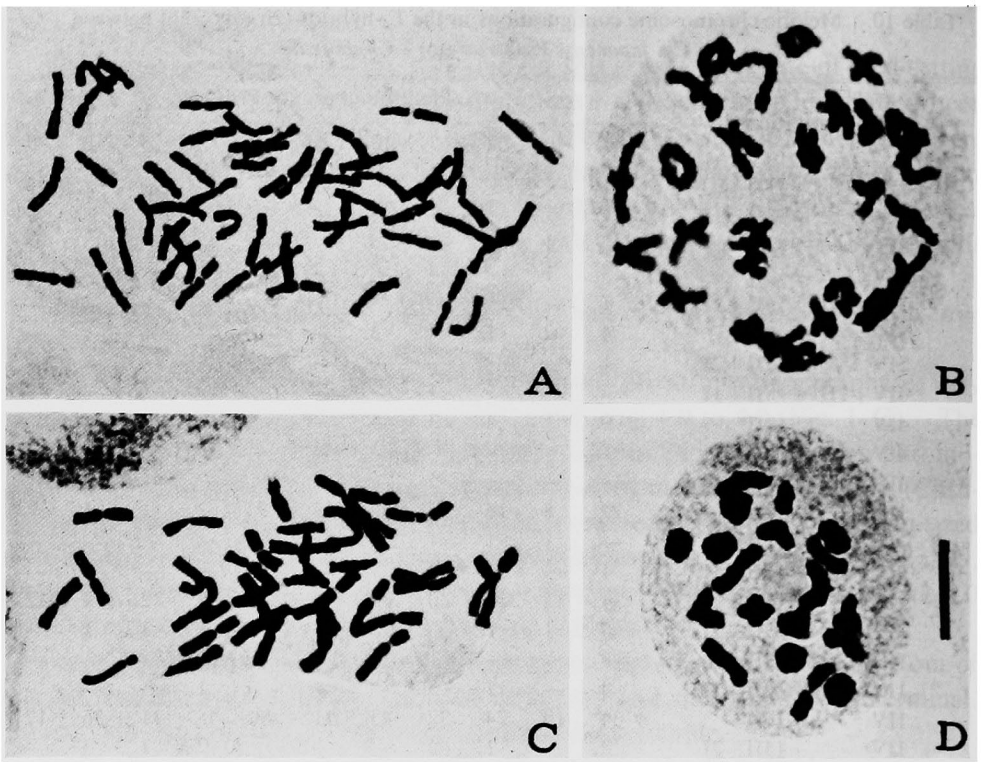


Fig. 6. Mitotic and meiotic chromosomes A and B, *Ch. japonense* (Nakamura strain). C and D, the F_1-hybrid between *Ch. boreale* and *Ch. japonense* (Nakamura strain). A, mitotic chromosomes, 2n=6x=54. B, meiotic chromosomes, 27II. C, mitotic chromosomes, 2n=4x=37. D, meiotic chromosomes, 18II+1I. Scale 10 μm.

(present / absent) and plant height (60–90 cm / 60–120 cm). In each comparison the form of *Ch. japonense* (Nakamura strain) is given first.

Ch. boreale (♀)×*Ch. japonense* (Nakamura strain) (♂)

In this cross, 3963 disc florets of 51 heads were pollinated and cultivated on the artificial medium. Nine ovaries developed and were germinated, representing only about 0.2% seed set. Of these, two plants had 2n=4x+1=37 and were accepted as being aneuploid hybrids. Two plants were diploid and were doubtless due to selfing. Five plants died after potting, and could not be karyotyped.

The chromosomes of the F_1-hybrid, at mitotic metaphase, vary in length from 7.9 μm to 4.1 μm and in arm ratio from 1.0 to 5.0 (Fig. 6–C). The thirty-seven chromosomes are arranged in order of size in Fig. 7–B. Three chromosomes had satellites. One was a medium-sized subterminal chromosome (Chromosome 11), one a small subterminal (Chromosome 24) and the third the smallest subterminal (Chromosome 37). Four extreme subterminal chromosomes with arm ratios of 3.8–5.0 (Chromosomes 25, 34, 35 and 36) were distinguishable.

In the F_1-hybrid with 2n=4x+1=37, the following meiotic configurations were frequently observed; 2 IV+14 II+1 I, 1 IV+1 III+15 II, 1 IV+16 II+1 I, 1 III+17 II and 18 II+1 I. The maximum number of quadrivalents per PMC was

Table 11. Mean association frequencies in the F_3-hybrids (2n=4x=36) between *Ch. japonense* (Saka strain) × *Ch. boreale*

Configurations	Plant number										
	34	40	62	7	10	5	4	2	25	6	35
VI	0.01	—	—	—	—	—	—	—	—	—	—
(±S. E.)	(±0.01)	—	—	—	—	—	—	—	—	—	—
V	0.01	—	—	—	—	—	—	—	—	—	—
(±S. E.)	(±0.01)	—	—	—	—	—	—	—	—	—	—
IV	2.13	1.89	1.77	1.63	1.59	1.35	1.05	0.97	0.87	0.46	0.47
(±S. E.)	(±0.12)	(±0.20)	(±0.14)	(±0.10)	(±0.10)	(±0.20)	(±0.08)	(±0.08)	(±0.13)	(±0.06)	(±0.08)
III	0.05	—	0.07	0.17	0.02	—	—	0.01	—	0.01	—
(±S. E.)	(±0.02)	—	(±0.03)	(±0.04)	(±0.01)	—	—	(±0.01)	—	(+0.01)	—
II	13.54	14.18	14.40	14.37	14.74	15.29	15.90	15.98	16.15	16.91	16.99
(±S. E.)	(±0.23)	(±0.41)	(±0.28)	(±0.21)	(±0.20)	(±0.40)	(±0.16)	(±0.16)	(+0.26)	(±0.12)	(±0.15)
I	0.18	0.07	0.21	0.24	0.11	—	—	0.13	0.21	0.32	0.16
(±S. E.)	(±0.04)	(±0.05)	(±0.06)	(±0.05)	(±0.04)	—	—	(±0.06)	(±0.10)	(±0.07)	(±0.07)
The frequency of bivalent formation (%)	75.2	78.8	79.2	79.8	81.9	84.9	88.3	88.8	89.7	94.0	94.4
t*=(D/E)	11.253	5.612	7.020	8.75	7.560	3.137	3.329	2.964	1.599	1.458	1.697
P* values	(<0.001)	(<0.001)	(<0.001)	(<0.001)	(<0.001)	(0.01 ~ 0.001)	(<0.001)	(0.01 ~ 0.001)	(0.2 ~ 0.1)	(0.05 ~ 0.02)	(0.1 ~ 0.05)
The frequency of quadrivalent formation (%)	23.6	22.4	19.6	18.1	17.7	15.1	11.7	10.8	9.7	5.1	5.2
t**=(D/E)	10.581	5.805	7.092	7.947	7.619	3.256	3.669	2.916	1.422	2.169	1.787
P** values	(<0.001)	(<0.001)	(<0.001)	(<0.001)	(<0.001)	(0.01 ~ 0.001)	(<0.001)	(0.01 ~ 0.001)	(0.2 ~ 0.1)	(0.05 ~ 0.02)	(0.1 ~ 0.05)

Calculated for testing the significant difference of bivalent means* and quadrivalent means** between F_1-hybrid and each F_3-hybrid.

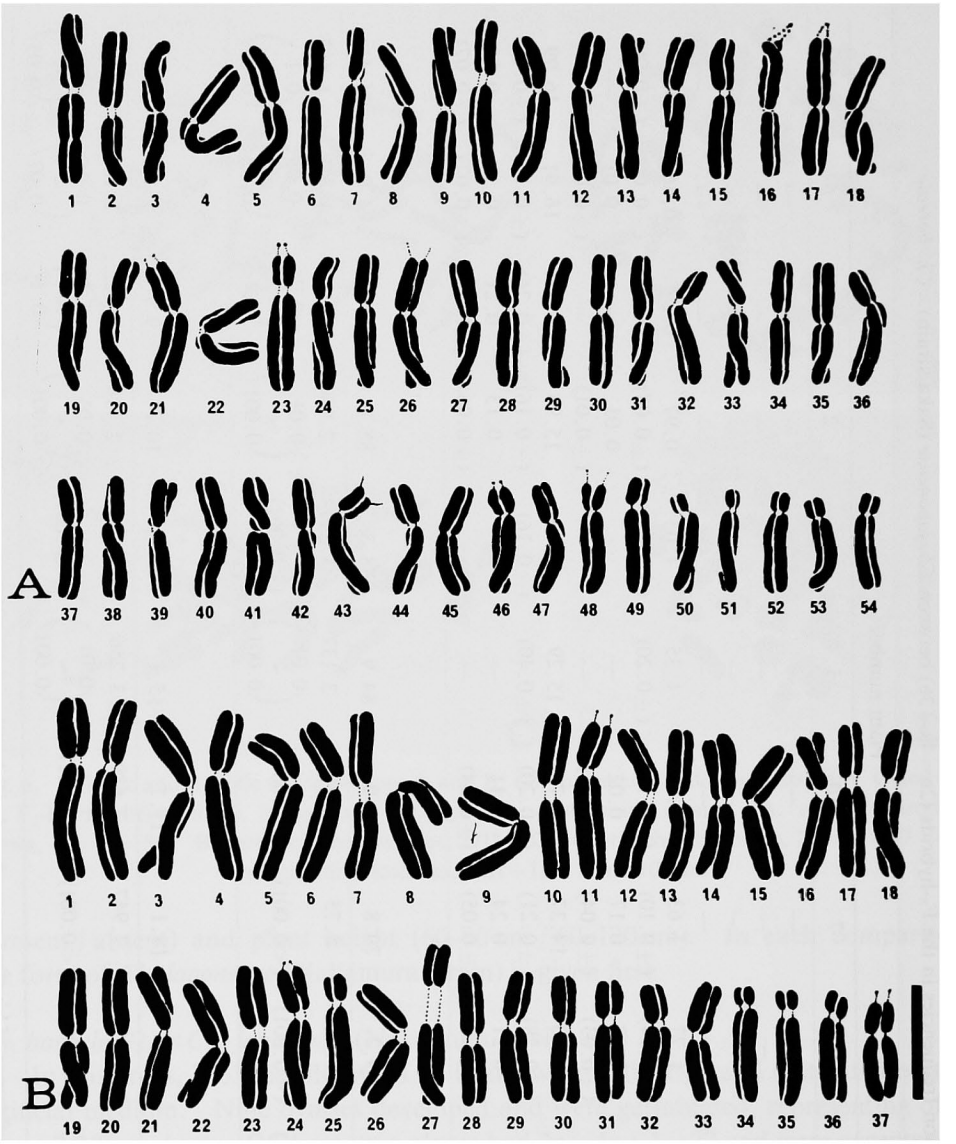


Fig. 7. Somatic karyotype. A, *Ch. japonense* (Nakamura strain). B, F_1-hybrid between *Ch. boreale* and *Ch. japonense* (Nakamura strain). Scale 5 μm.

three. The data of quadrivalent frequency conform to the Poisson distribution (d. f.=2, p=0.6). All sets of four chromosomes apparently have an equal chance of forming a quadrivalent. The frequency of bivalent formation was 89.0%. The chromosomes derived from *Ch. japonense* (Nakamura strain) paired both homoeologously and homologously with the *Ch. boreale* chromosomes. Consequently, there must be some control leading to predominant bivalent formation at the hexaploid 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 hexaploid.

All F_1-hybrids had rhizomes and white ray floret and had leaves both with and

Table 12. Morphological characteristics of the *Ch. japonense* (Saka strain), *Ch. boreale* and their hybrid derivatives

Characters	*Ch. boreale* (2n=2x=18)	B_1-hybrid (2n=3x=27)	F_1-hybrid (2n=4x=36)	F_2-hybrid (2n=4x=36)	*Ch. japonense* (Saka strain, 2n=6x=54)
Stem length (cm)	118	48–98	68–92	15–96	63
Leaf length (mm)	110	56–105	65–90	13–108	55
Leaf width (mm)	82	34–59	37–51	10–63	35
The depth of encision	deep	deep	deep	deep-absence	deep
No. of marginal teeth	101	30–110	44–70	5–91	36
Basal shape of leaf	cordate	cordate-cuneate	cordate	cordate-cuneate	cordate
Pseudostipule	absent	present	present	absent and present	present
Color of ray floret	yellow	yellow and white	white	yellow, palish yellow and white	white
No. of ray floret per head	13	13–21	15–24	13–24	23
Rhizome	absent	present	present	present	present

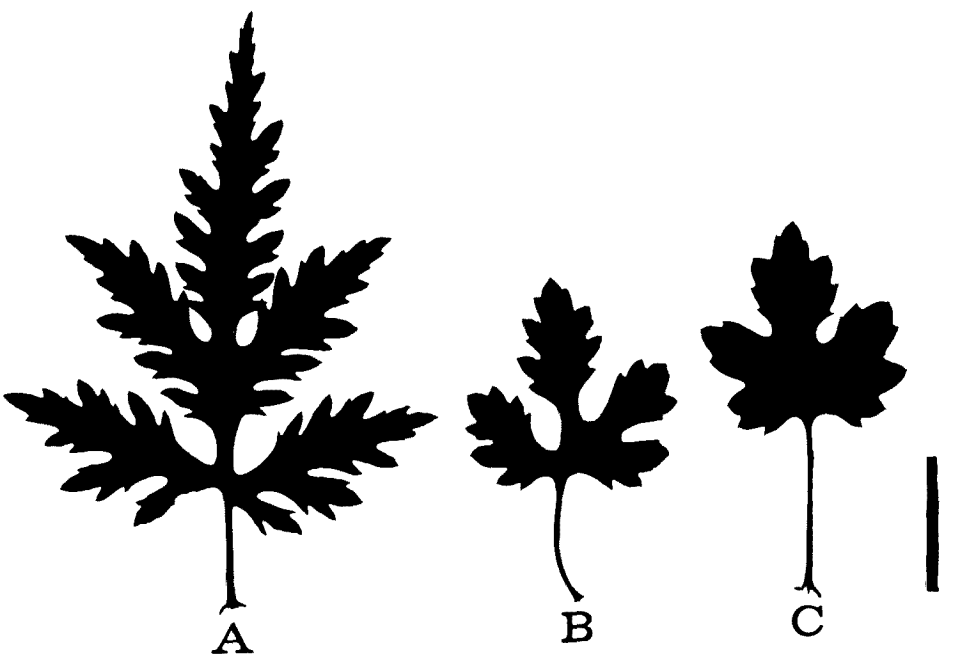


Fig. 8. Leaves. A, *Ch. boreale*. B, F_1-hybrid. D, *Ch. japonense* (Nakamura strain). Scale 2.5 cm.

without psudostipules. The F_1-hybrids resembled *Ch. japonense* (Nakamura strain) more than *Ch. boreale* in their quantitative characters (Fig. 8–B).

Similar conclusions are to be drawn from the data of different F_1-hybrids of *Ch. japonense* collected from different localities.

Table 13. Measurements of the somatic chromosomes of *Ch. japonense* (Nakamura strain)

Chromosomes	Length in μm	Relative length	Arm ratio (long/short)
1	3.1+3.6=6.7	6.8	1.2
2	3.2+3.4=6.6	6.7	1.1
3	3.2+3.2=6.4	6.5	1.0
4	3.1+3.3=6.4	6.5	1.1
5	2.4+4.0=6.4	6.5	1.7
6	2.4+4.0=6.4	6.5	1.7
7	2.2+4.1=6.3	6.4	1.9
8	3.0+3.2=6.2	6.3	1.1
9	2.9+3.3=6.2	6.3	1.1
10	2.1+4.1=6.2	6.3	2.0
11	2.3+3.9=6.2	6.3	1.7
12	2.2+4.0=6.2	6.3	1.8
13	2.3+3.9=6.2	6.3	1.7
14	2.2+3.8=6.0	6.1	1.7
15	2.2+3.6=5.8	5.9	1.6
16*	2.8+3.0=5.8	5.9	1.1
17*	2.8+3.0=5.8	5.9	1.1
18	2.7+3.0=5.7	5.8	1.1
19	1.9+3.8=5.7	5.8	2.0
20	2.2+3.5=5.7	5.8	1.6
21*	2.2+3.5=5.7	5.8	1.6
22	2.6+3.0=5.6	5.7	1.2
23*	1.2+4.4=5.6	5.7	3.7
24	1.4+3.8=5.6	5.7	2.7
25	2.1+3.5=5.6	5.7	1.7
26*	2.3+3.2=5.5	5.6	1.4
27	2.6+2.9=5.5	5.6	1.1
28	2.1+3.4=5.5	5.6	1.6
29	2.3+3.2=5.5	5.6	1.4
30	2.4+3.0=5.4	5.5	1.3
31	2.6+2.7=5.3	5.4	1.0
32	1.6+3.7=5.3	5.4	2.3
33	2.0+3.3=5.3	5.4	1.7
34	2.3+3.0=5.3	5.4	1.3
35	2.6+2.7=5.3	5.4	1.0
36	1.4+3.8=5.2	5.3	2.7
37	2.0+3.2=5.2	5.3	1.6
38	2.1+3.1=5.2	5.3	1.5
39	2.2+2.9=5.1	5.2	1.3
40	2.2+2.9=5.1	5.2	1.3
41	2.2+2.8=5.0	5.1	1.3
42	2.2+2.8=5.0	5.1	1.3
43*	1.2+3.7=4.9	5.0	3.1
44	1.8+3.1=4.9	5.0	1.7
45	2.1+2.7=4.8	5.0	1.3
46*	1.1+3.6=4.7	4.8	3.3
47	1.6+3.0=4.6	4.7	1.9
48*	1.0+3.6=4.6	4.7	3.6
49	0.9+3.7=4.6	4.7	4.1

Table 13. (continued)

Chromosomes	Length in μm	Relative length	Arm ratio (long/short)
50	0.9+3.5=4.4	4.5	3.9
51	0.8+3.5=4.3	4.4	4.4
52	0.8+3.2=4.0	4.1	4.0
53	0.8+3.2=4.0	4.1	4.0
54	0.7+3.1=3.8	3.9	4.4

* Chromosome with satellites.

Discussion

In native hexaploid *Chysanthemum japonense* Nakai genetic stabilization of diploid-like meiosis occurs. In F_1-hybrids between diploids and hexaploid homoeologous chromosome pairing from different base sets derived from the parental hexaploids was extensively observed. In a previous paper (Watanabe 1977 a), following a review of the strategies possible for the suppression of multivalent formation, it was suggested that the most likely in *Chrysanthemum* are preferentical pairing and major recessive gene control. In the latter case, a bimodal segregation with respect to multivalent formation should be found in F_2 and F_3 hybrid generations, between *Ch. japonense* (Saka strain) × *Ch. boreale*. Increased homoeologous association would not be seen in the backcrosses if the gene is recessive and ineffective in the heterozygous state. The data presented do not support this hypothesis: there is no obvious departure from a unimodal distribution in the F_2 and F_3 generations, and there is an increase in homoeologous association in backcrosses, in the hybridizations *Ch. japonense* (Saka strain) × *Ch. boreale*. The data are not consistent with an hypothesis of single gene control, but could be with ones of two or more genes, or with chromosomal rearrangements with cumulative effects on homoeologous association.

Karyomorphologically, *Ch. japonense* Nakai and their hybrid derivatives are not autopolyploids, consequently, the efficiency of preferential pairing may be ensured sufficiently by segmental allopolyploidy. The magnitude of the differentiation between the constituent genomes is large enough to discriminate them, so the preferential chromosome pairing could operate effectively to restrict association to the formation of bivalents. The predominant bivalent formation in F_1-hybrids may be due to differentiation between the constituent genomes in the polyploids. In the hybrids between the *Ch. boreale* and *Ch. japonense* one genome in the hexaploid may be much more homologous with the *Ch. boreale* genome than the rest and these residual homoeologous genomes may be able to pair with each other as bivalents.

In tetraploid hybrids bivalents were predominantly formed but nevertheless these plants were highly sterile. Chromosome and genic differentiation between the basic haploid genomes is sufficient to provide a barrier of hybrid sterility, but is less than is required to prevent bivalent formation.

As will be seen in Tables 5 and 9, however, the frequency of multivalents per PMC generally corresponds with Poisson or binomial distributions. These condi-

tions suggest that the pairing among chromosomes occurs to some extent at random (Mashima 1947, Hall 1955, McCollum 1958 and Mehra *et al.* 1971). Consequently, preferential pairing is not responsible for the restriction on multivalent formation in hexaploid and their hybrid derivatives. The distribution of multivalents in these hybrid derivatives suggests that all set of corresponding chromosomes are sufficiently homologous and have an equal chance of association.

A gradual increasing occurrence of quadrivalent formation from the F_1 to the F_3 was observed. The data seem to be consistent with the hypothesis of multiple-gene segregation in the advanced generations.

In the F_1-hybrids of *Ch. boreale* × *Ch. japonense* (Nakamura strain) and the F_1, F_2 and F_3-hybrids of *Ch. japonense* (Saka strain) × *Ch. boreale*, the average frequency of quadrivalents was extremely low in spite of the fact that all sets of corresponding chromosomes were capable of associating as quadrivalents. Increased homoeologous association was observed in the B_1-hybrids of *Ch. japonense* (Saka strain) × *Ch. boreale*. Consequently, there must be a mechanism to control or suppress multivalent formation even in F_1-hybrids, although the behaviour in the backcross shows that homoeologous chromosomes are capable of associating as multivalents.

The magnitude of homoeologous association increased dramatically with reduction in polyploidy from the hexaploid state to the triploid in the successive backrosses of *Ch. japonense* (Saka strain) × *Ch. boreale* ($6xP \rightarrow 4xF_1 \rightarrow 3xB_1$), in contrast to the gradual increasing frequency of quadrivalent formation from F_1 to F_3 seem to be governed by two independent and fundamentally different control systems.

In tetraploid-hybrids, most of chromosomes formed either bivalents or quadrivalents and the frequency of trivalent and univalent formation was extremely low. In eight of the nine triploid B_1-hybrids between *Ch. japonense* (Saka strain) and *Ch. boreale*, approximately two-thirds of the chromosomes form trivalents. In the ninth, Plant 13, two-thirds of the chromosomes form bivalents.

These data may be compatible with the slight modification of the zygomere localizing model proposed by several authors (John and Henderson 1962, Sved 1966, Sybenga 1966). According to the model of autotetraploid behaviour was put forward by John and Henderson 1962 and Sved 1966, pairing is usually initiated at two sites, i. e., A and B and homologous A sites are attached together or in close proximity on the nuclear membrane (Fig. 9–A). At either site pairing will always be two-by-two, with the pairing initiated at the A site being independent of that initiated at the B site. Then assuming, without loss of generality, that A_1 pairs with A_2 and A_3 with A_4, i. e., $(A_1\ A_2)(A_3\ A_4)$ at the A site, three different pairing may be initiated at the B site. The pairing $(B_1\ B_2)(B_3\ B_4)$ will clearly lead to the formation of two bivalents. On the other hand the two other types of pairing, *viz.* $(B_1\ B_3)(B_2\ B_4)$ or $(B_1\ B_4)(B_2\ B_3)$ will lead to quadrivalent associations. Since all three types of pairing are equally likely in an autotetraploid, two-thirds of the chromosomes will be associated as quadrivalents and one-third as bivalents.

In an autotriploid the same rule holds good and two-thirds of the chromosomes will be associated as trivalents and one-third as bivalents and univalents (Fig. 9–B).

When the initiation of pairing at the B site is suppressed by the mutated gene in these models, the chromosome association in tetraploids will be strictly bivalent for-

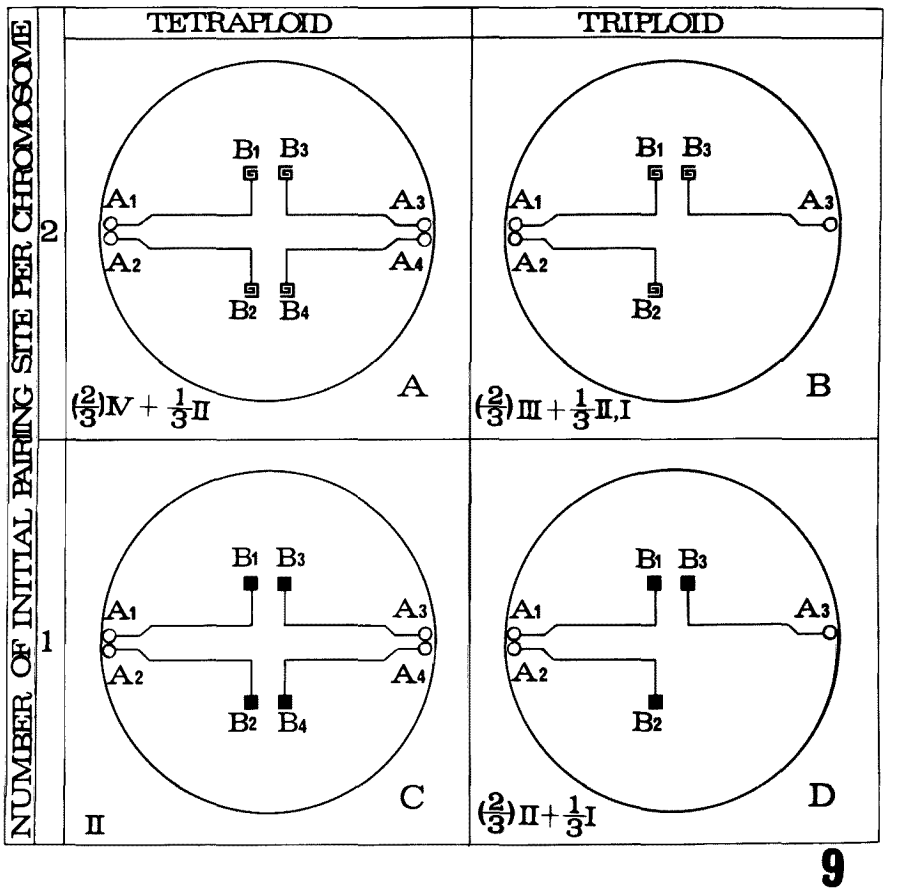
mation as shown in Fig. 9–C and two-thirds of the chromosomes will be associated as bivalents and one-third as univalents in triploids, as shown in Fig. 9–D.

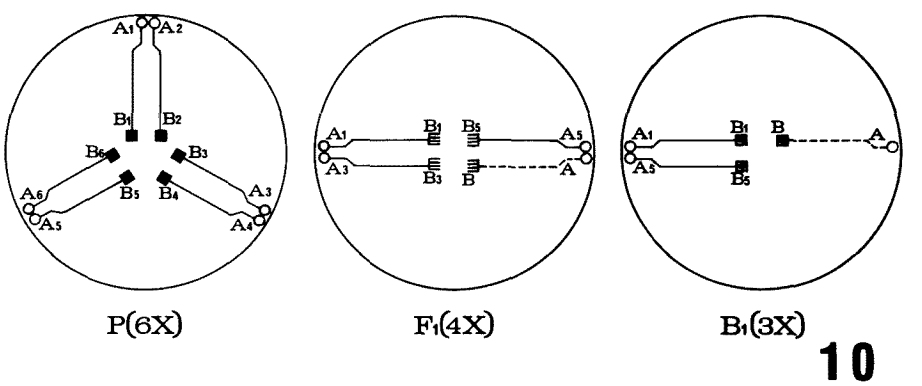
These models of Fig. 9–B, C and D may be able to explain the control system of diploid-like meiosis of polyploid species and their hybrid derivatives. Figure 10 shows the models of meiotic chromosome behaviour in the hexaploid *Ch. japonense*, the tetraploid F_1- and the triploid B_1-hybrids crossed with the diploid *Ch. boreale*. When the initiation of pairing at the B site is suppressed by gene, the pairing (A_1 A_2)(A_3 A_4)(A_5 A_6) will clearly lead to the formation of three bivalents in the hexaploid. In the tetraploid F_1-hybrid, A_1 is capable of pairing with A_3, A_5 with *Ch. boreale* A site, i. e., (A_1 A_3)(A_3 A). Namely, the chromosomes derived from the hexaploid paired both homoeologously and homologously with the *Ch. boreale* chromosomes. When polyploids are crossed with the diploids, without a suppressive gene on the initiation of pairing at the B site, the suppression will be slightly released hence it will lead to the quadrivalent association.

In eight of nine B_1-hybrids extensive trivalent formation and intragenomic pairing were observed. It seems that the release from the suppression of the initiation of pairing at the B site is complete and two-thirds of the chromosomes associate as trivalents and one thrids as bivalents and univalents in these triploid as shown in Fig. 9–B. Namely, both sites A_1 and B_1 can associate with A_5 and B_5, or *Ch. boreale* A and B, respectively (Fig. 10). Then, further homoeologous chromosome association are evident in this generation. Similar data that the frequency of trivalents is twice the frequency of bivalents and univalents in the triploid have been reported in the hybrid between *Ch. wakasaense* 4x × *Makinoi* 2x and the polyhaploid *Ch. indicum* 6x (Tanaka 1952, 1955, Watanabe 1977 a). The "lonely" chromosome (Chromosome A_3–B_3 in Fig. 9–B), without the essentially homologous partner, exhibits the enhanced pairing affinity and hunts out the essentially non-homologous ones within its own genome. This behaviour will interfere with the suppression of initiation of pairing at the B site and must lead to extensive trivalent formation and to intragenomic pairing. All of the constituent genomes of *Ch. japonense* 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 or trivalents per PMC is in correspondence with a binomial distribution. In one (Plant 13) of the nine B_1-hybrids the initiation of pairing at the B site seems to be still suppressed as shown in Fig. 9–D. The constituent genomes of this plant may include many chromosomes with suppressive genes on the initiation of pairing at the B site. According to the chromosome constitutions of the F_2-hybrids, such a chromosome segregation seems to scarcely happen in the F_1-hybrids. The possibility, however, can not be denied that chromosomes with suppressive genes might be transmitted into this B_1-hybrid from the F_1-hybrids.

The gradual increase of the frequency of quadrivalent formation from F_1 to F_3 in the crosses between *Ch. japonense* (Saka strain) and *Ch. boreale* must be explained by gradual release from the suppression of the initiation of pairing at the B site, accompanied by the replacement of chromosomes with the suppressive gene or genes to ones without, and the selective accumulation of the latter in advanced generations.

The diploid-like meiosis in hexaploid *Ch. japonense* must be ensured by the fol-





Figs. 9–10. 9–A, simplified model indicating how obligatory pairing for all zygomeres (initial pairing sites) in a system of four homologues in an autotetraploid cell will give rise to only two types of chromosome association, quadrivalents and bivalents. The former will be formed twice as commonly as the latter in the following genetic systems; 1) chromosome pairing is initiated at two sites, A, ○ and B, ▣. 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 that at the B site. B, the model of chromosome pairing in autotriploid. Two-

lowing 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 of the chromosome). they are under the independent and fundamentally different control, respectively. 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 that at the B site (the possibility can not be denied that the preferential chromosome pairing might be occurred at the A site). 4) the initiation of pairing at the B site is usually suppressed by multiple-or polygenic control (Fig. 9).

The magnitude of release from the suppression of initiation of pairing at the B site depends on either the reduction of suppressive gene dosage or the interference of the "lonely" chromosome, without the essentially homologous pairing partner in the odd-ploid. The magnitude of release from the suppression seems to be equal, not differential, at each B site.

Polyploids are traditionally classified into alloploid and autoploids (Kihara and Ono 1926). Autoploids are usually assumed to be recognizable by multivalent chromosome configurations, while the chromosomes of alloploids are assumed to synapse into bivalents during meiosis. In *Chrysanthemum*, however, homologous, homoeologous, and non-homologous chromosomes can pair in the hybrids between related species, making the isolation among the constituent genomes in the polyploids completely impossible to be due to the chromosomal or genic differentiation. For another good example to hold the same view, a single zygomere per chromosome results in exclusive bivalent formation even in true autoploid (Wilson 1932, Linnert 1961, Gupta and Koak 1976, Avivi 1976).

The recognition and initial pairing sites of homologous chromosomes seem to be located only a few loci, not throughout the length of each chromosome in *Chrysanthemum*. The number, locus of zygomere and the timing of zygomeric activation which

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thirds of the chromosomes will be associated as trivalents and the rest as bivalents and univalents. C, the model of chromosome pairing in the diploidized tetraploid owing to the suppression of the initiation of pairing at the B site, ■. The chromosome pairing will be strictly bivalent pairing. D, the model of chromosome pairing in the triploid B_1-hybrid, Plant 13. Two-thirds of the chromosomes will be associated as bivalents and the rest as univalents owing to the suppression of the initiation of pairing at the B site, ■. 10, the models of chromosome association in the hexaploid *Ch. japonense* Nakai, its tetraploid F_1-hybrid and triploid crossed with diploid *Ch. boreale*. The chromosome association can be initiated at two sites, A and B. They are under the independent and fundamentally different control, respectively. At either site pairing is always two by two. The initiation of pairing at the A site always works fine and precedes that at the B site. The initiation of pairing at the B site is usually suppressed by the poly-genic control. At the hexaploid state chromosome association is strictly bivalent pairing owing to the suppression of the initiation of pairing at the B site although all of chromosomes are so homologous to be able to pair each other. In the tetraploid F_1-hybrid, A_1 is capable of pairing A_3, A_5 with *Ch. boreale* A site. The chromosomes derived from the hexaploid paired both homoeologously and homologously with the *Ch. boreale* chromosomes. When polyploid is crossed with the diploid, without a suppressive gene on the initiation of pairing at the B site, the suppression will be slightly released in accompanying with the reduction of suppressive genes hence it will lead to the quadrivalent association. In triploid B_1-hybrids the release from the suppression of the initiation of pairing at the B site occurs completely and two-thirds of the chromosomes associated as trivalents. Then, further homoeologous chromosome association are evident in this generation.

are under genic control may come up as essential problem to analyse the meiotic configurations in the hybrids between the diploids and the high polyploids.

The exploitation of depolyploidization for conventional agricultural methods and breeding techniques has been demonstrated by several authors (Kerber 1964, Carlbom 1969 and Siddiqui 1971). The possible method of depolyploidization in hexaploid *Chrysanthemum* has been demonstrated in this study. Low ploid plants including several genomes, chromosomes or genes of polyploid species within their genetic composition could be obtained. Homoeologous chromosome pairing and multivalent formation in the hybrid derivatives could have permitted gene flow from one genome to another by intergenomic recombination.

Summary

1) In meiosis of *Ch. japonense* (Saka strain) 2n=6x=54, 27 II was the main chromosome configuration and 1 IV+25 II and 26 II+2 I were rarely observed. Fifty-four chromosomes, at mitotic metaphase, varied in length from 8.4 μm to 4.2 μm and in arm ratio from 1.0 to 4.9. Ten satellite chromosomes and six small chromosomes with extreme subterminal centromeres were well distinguishable. Karyomorphologically this species is not an autohexaploid.

2) Eight F_1-hybrids between hexaploid *Ch. japonense* (Saka strain) and diploid *Ch. boreale*, with 2n=4x=36 and 2n=4x+1=37, were obtained. 2 IV+14 II, 1 IV+16 II and 18 II were the main meiotic configurations in the F_1-hybrid with 2n=4x=36. The quadrivalent frequencies conform to the Poisson distribution. The chromosomes derived from *Ch. japonense* (Saka strain) paired both homoeologously and homologously with the *Ch. boreale* chromosomes.

3) F_1-hybrids were partially fertile (0%–1.1% in interbreeding and 0.3%–5.9% in intrabreeding) and gave rise to triploid B_1-hybrids on backcrossing to diploid *Ch. boreale*, and to tetraploid F_2-hybrids by intrabreeding.

4) In eight of the nine B_1-hybrids with 2n=3x=27 studied the average trivalent formation per PMC ranged from 4.66 to 5.55. The trivalent frequencies conform to the Poisson or binomial distribution. Further homoeologous chromosome associations were revealed in this generation. In the ninth B_1-hybrid, 9 II+9 I was the main chromosome configuration. This plant was indiscriminable morphologically from the others.

5) These B_1-hybrids with 2n=3x=27 were partially fertile (0%–0.2% in interbreeding and 0%–3.7% in intrabreeding) and gave rise to B_2-hybrids with 2n=24, 26, 27 and 36 on backcrossing to the diploid *Ch. boreale*, and progenies with 2n=30, 32, 33, 34, 35 and 36 by intrabreeding. In B_2-hybrids with 2n=26, the following meiotic configurations were frequently observed; 7 III+2 II+1 I, 6 III+3 II+2 I, 5 III+4 II+3 I, 4 III+5 II+4 I and 3 III+6 II+5 I.

6) In seven F_2-hybrids with 2n=4x=36, the following meiotic configurations were frequently observed; 3 IV+12 II, 2 IV+14 II, 1 IV+16 II and 18 II. There is a significant difference in the frequency of quadrivalent and bivalent formation among F_2-hybrids; the maximum number of quadrivalents per PMC varied from four to two. Quadrivalent frequencies conform to the Poisson distribution. The

rate of seed setting varied from 0% to 58.7% in F_2-hybrid intra-breedings. There was no correlation between the frequency of multivalent formation and the rate of seed setting.

7) Nineteen of the twenty F_3-hybrids studied had the chromosome number 2n=4x=36 and the twentieth had 2n=4x+1=37. In eleven F_3-hybrids with 2n=4x=36 the following meiotic configurations were frequently observed; 3 IV+12 II, 2 IV+14 II, 1 IV+16 II and 18 II. There was a significant difference in the frequency of quadrivalent and bivalent formation among F_3-hybrids; the maximum number of quadrivalents per PMC varied from eight to two. Quadrivalent frequencies conform to the Poisson distribution.

8) In *Ch. japonense* (Nakamura strain) with 2n=6x=54, 27 II was the main meiotic configuration and 2 IV+23 II, 1 IV+25 II, 1 IV+24 II+2 I and 26 II+2 I were rarely observed. Fifty-four chromosomes, at mitotic metaphase, varied in length from 6.7 μm to 3.8 μm and in arm ratio from 1.0 to 4.4. Eight satellite chromosomes and six small chromosomes with extreme subterminal centromeres were well distinguishable. Karyomorphologically this species is not an autohexaploid.

9) Two F_1-hybrids with 2n=4x+1=37 between diploid *Ch. boreale* and *Ch. japonense* (Nakamura strain) were obtained by ovary culture. The following meiotic configurations were frequently observed; 2 IV+14 II+1 I, 1 IV+1 III+15 II, 1 IV+16 II+1 I, 1 III+17 II and 18 II+1 I. The chromosomes derived from *Ch. japonense* (Nakamura strain) paired both homoeologously and homologously with the *Ch. boreale* chromosomes.

10) The segregation of some morphological characters was observed in F_2, F_3, B_1 and B_2-hybrids and the morphological variation conspicuously increased in these generations. Some characters, such as cuneate bases and absence of marginal teeth of the leaves, were observed in the hybrid derivatives although they did not appear in both parental species.

11) The rate of seed setting was extremely low in most of the hybrid derivatives but recovered largely in some of F_2-hybrids of *Ch. japonense* (Saka strain)$\times$*Ch. boreale*.

12) In hexaploid *Chrysanthemum japonense* Nakai genetic stabilization of diploid-like meiosis occurs. In F_1-hybrids between diploids and hexaploids homoeologous chromosome pairing from different base sets derived from the parental polyploid was extensively observed. Further homoeologous pairing was observed in the B_1-hybrids. In tetraploid-hybrids most of chromosomes formed either bivalents or quadrivalents, and the frequency of trivalents and univalents was extremely low. In most of triploid, however, approximately two-thirds of the chromosomes form trivalents. A gradual increase of quadrivalent formation from F_1 to F_3 was observed. The average frequencies of quadrivalents was extremely low in spite of the fact that all sets of corresponding chromosomes were capable of associating as quadrivalents in the tetraploid hybrids. All of these data must be explained by the following genetic system; 1) chromosome pairing is initiated at two sites A and B. they are under the independent and fundamentally different control, respectively. 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 the multiple or poly-genic control. This genetic system must ensure the diploid-like meiosis in hexaploid *Ch. japonense* although all of the constituent genomes of this species are sufficiently homologous to be able to pair with each other. The magnitude of release from the suppression of the initiation of pairing at the B site depends on either the reduction of suppressive gene dosage or the interference of the "lonely" chromosome, without the essentially homologous pairing partner in odd-ploids. The magnitude of release from the suppression is equal, not preferential at each B site.

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