CYTOGENETICS OF *SCILLA SCILLOIDES* COMPLEX
III. HOMOEOLGY BETWEEN GENOMES
A ($x=8$) AND B ($x=9$)¹⁾

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Chromosome numbers of *Scilla scilloides* Druce ($2n=16$ to $2n=43$) make an aneuployploid series composed of various numeral combinations of two genomes, A ($x=8$) and B ($x=9$). The two genomes are differentiated distinctly not only in karyotype but also in homology. Thus $8_{II}+9_I$ and $9_{II}+8_I$ are formed in PMCs of the hybrids of the constitutions AAB and ABB respectively (Haga and Noda 1971). Trivalents are occasionally found in PMCs of AAB and ABB, indicating the genomes A and B to be homoeologous (Noda 1971). Yet the relationship between the two genomes has not been manifested in the hybrid of the constitution AB, which is the simplest and most clear-cut case for the analysis.

The present writer has succeeded in raising the hybrids AB ($2n=17$) by crossing two types of diploids, AA ($2n=16$) and BB ($2n=18$). The purpose of the present paper is to describe the homoeologous relationship between genomes A and B revealed in the hybrids, and to show how structural changes played a role in differentiating the two genomes.

MATERIALS AND METHODS

A diploid type AA was collected in Suwon, Korea, and another diploid type BB in Hakodate, Japan (Fig. 1). Both have been maintained in the *Scilla* garden of Kyushu University (cf. Noda 1967). The two types flower at different times in the garden; AA in late July and the BB employed in this study in early August.

In crossing AA and BB, flowering of AA was delayed by keeping it in a cooled room (24°C) for one week at the time the spikes emerged. Then pollen from AA was applied to stigmas of emasculated BB. The cross was successful only when AA was the pollen parent. The crossing in 1965 yielded 390 seeds of which 47 germinated in 1966, and 24 hybrids grew to maturity in 1968 in the *Scilla* garden (Fig. 1).

PMCs from 17 hybrids were fixed in Newcomer's fluid (1953) and squashed in iron-acetocarmine for meiotic study. Root-tips were pretreated with 0.002 mol 8-oxyquinoline

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Fig. 1. Flowering plants of *Scilla scilloides*. Left, AA ($2n=16$, 70 cm tall). Center, AB ($2n=17$, 44 cm tall). Right, BB ($2n=18$, 18 cm tall).

for two hours before fixation with La Cour 2BE and then treated with the Feulgen-squash method.

OBSERVATION

Chromosomes of the parents

The genome A is composed of 8 chromosomes designated by a_1 to a_8 (Fig. 2). One parent, AA, has two sets of these chromosomes in the somatic cells (Fig. 3) and shows 8_{II} at MI (Fig. 4). The genome B consists of 9 chromosomes b_1 to b_9 (Fig. 2). Two sets of these chromosomes are found in the somatic cells of the other parent, BB, which shows 9_{II} at MI (Figs. 5 and 6). Percentages of fertile pollen were very high in both parents, 98 (Table 1).

Chromosomes of the hybrids and their pairing configuration

The hybrids AB have 17 chromosomes, a_1 to a_8 plus b_1 to b_9 (Figs. 2 and 7). Pairing of chromosomes in the hybrids was variable, ranging from 17_I to $1_{IV}+3_{II}+7_I$ (Table 2

Fig. 2. Somatic metaphase chromosomes of genomes A and B. Alignment of chromosomes depicted from a cell of F_1 AB shown in Fig. 7.

and Fig. 8). The mean pairing per PMC was $0.02_{IV} + 0.10_{III} + 2.93_{II} + 10.77_I$. Anther of the hybrids scarcely dehisced and the fertile pollen percentage was as low as 1.1 (Table 1).

At MI chromosomes are classifiable into three classes, V (a_1), I (a_2 to a_6 , and b_1 to b_5), and v (a_7 , a_8 , and b_6 to b_9). Within each class of I and v, precise identification of chromosome types is usually difficult, except for the smallest v, b_9 . Therefore configurations observed were categorized as follows. Bivalents: V-v (heteromorphic), V-I (heteromorphic), I-I (some are not clearly heteromorphic), I-v (heteromorphic), and v-v (heteromorphic). Trivalents: v-V-I, V-I-I, I-I-v, and I-v-v. Quadrivalents: v-V-I-I, and V-I-I-v. Frequencies of various types of bivalents, trivalents, and quadrivalents are given in Table 3.

As shown in Class 1 in Table 3, 10 I-shaped chromosomes (a_2 to a_6 , and b_1 to b_5) can pair into five I-I bivalents. Because as many as four I-I bivalents are formed in the cells with a trivalent, v-V-I, V-I-I, I-I-v, or I-v-v (Class 2 in Table 3), it is conceivable that the four definite pairs of I-shaped chromosomes do not participate in trivalent pairing. Among six v-shaped chromosomes (a_7 , a_8 , and b_6 to b_9), four pair into two v-v bivalents (Table 3).

Fortunately, in a single cell showing the highest pairing by bivalents $8_{II} + 1_I$, 1 (V-I) + 4(I-I) + 1(I-v) + 2(v-v) + v (Fig. 9), the types of the chromosomes involved in the formation of bivalents can be successfully analysed as follows: (1) Because both a_2 and b_1 are satellited (Fig. 2), and some cells at diakinesis show a bivalent attached to a nucleolus, one of the four I-I bivalents is no doubt due to the pairing of a_2 - b_1 . The remaining three I-I bivalents are assumed arbitrarily to be a_3 - b_3 , a_4 - b_4 , and a_5 - b_5 . (2) Both of the v-v bivalents are heteromorphic, but the difference between the constituents is less conspicuous in one bivalent than in the other. Based on the differences of individual chromosomes they are identified to be a_7 - b_7 and a_8 - b_8 respectively. (3) The univalent v

Figs. 3-8. Somatic metaphase in root-tip cells (odd-numbered, $\times 1200$) and MI in PMCs (even-numbered, $\times 800$). 3-4. AA ($2n=16$, 8_{II}). 5-6. BB ($2n=18$, 9_{II}). 7-8. F_1 AB ($2n=17$, 17_I). The long arrow indicates the largest chromosome a_1 , and the short arrow the smallest b_9 .

is evidently the smallest b_9 . (4) The remaining bivalents V-I and I-v should be the pairings of a_1-b_2 and a_6-b_8 respectively.

Next to be analysed are the chromosomes involved in the trivalents. As mentioned above, a trivalent, V-I-I or I-I-v, is found together with four I-I bivalents (Figs. 10 and 11). The four I-I bivalents are presumably the same ones as found in the cell with $8_{II}+1_I$, i.e., a_2-b_1 , a_3-b_3 , a_4-b_4 , and a_5-b_5 . Then, the I-I component in the trivalents should be b_2-a_6 . Hence the trivalent V-I-I comes from pairing $a_1-b_2-a_6$ and the trivalent I-I-v

Table 1. Pollen fertility and seed setting (%)

Type of plant	Plants examined	Flowers per spike	Pollen fertility	Seed setting*
AA	16	120.06±32.61	98.37±0.75	27.96±5.29
AB	26	40.54± 8.84	97.80±1.62	24.82±5.73
BB	17	59.29± 4.51	1.14±0.69	0.00

* Ripe seeds per ovule.

Table 2. Meiotic pairings in F₁ AB

MI configuration		Cells obs. (%)	
Class 1	17 _I	28	352 (88.4)
	1 _{II} +15 _I	54	
	2 _{II} +13 _I	75	
	3 _{II} +11 _I	67	
	4 _{II} + 9 _I	54	
	5 _{II} + 7 _I	46	
	6 _{II} + 5 _I	18	
	7 _{II} + 3 _I	9	
	8 _{II} + 1 _I	1	
Class 2	1 _{III} + +14 _I	2	39 (9.8)
	1 _{III} +1 _{II} +12 _I	5	
	1 _{III} +2 _{II} +10 _I	8	
	1 _{III} +3 _{II} + 8 _I	11	
	1 _{III} +4 _{II} + 6 _I	4	
	1 _{III} +5 _{II} + 4 _I	6	
	1 _{III} +6 _{II} + 2 _I	3	
Class 3	1 _{IV} +1 _{II} +11 _I	1	7 (1.8)
	1 _{IV} +2 _{II} + 9 _I	3	
	1 _{IV} +3 _{II} + 7 _I	3	
Total		398	(100.0)

from b_2 - a_6 - b_8 , I-v from a_6 - b_8 as mentioned in the foregoing lines. Trivalent v-V-I (Figs. 12 and 15) is explicable as the pairing b_9 - a_1 - b_2 , since the v attached to V is the smallest one and V-I is a_1 - b_2 , as mentioned in the foregoing section. The third component v of the I-v-v trivalent (Fig. 13) should be a chromosome that forms as a rule one of the two v-v bivalents and not the v that forms the V-v bivalent, because the trivalent is found with the V-v bivalent (Table 3, fourth line in Class 2). The first two components I-v of the I-v-v trivalent are a_6 - b_8 , as mentioned above, and the third component should be a_7 on the basis of chromosome size; hence the trivalent is a_6 - b_8 - a_7 .

Thus, the four trivalent configurations and the a_7 - b_7 bivalent mentioned above make it possible to relate seven chromosomes, b_9 - a_1 - b_2 - a_6 - b_8 - a_7 - b_7 , stepwise by connecting the

Table 3. Analysis of MI configurations in F₁ AB

MI pairing*	Constitution of multivalent	Frequency of various bivalents														
		V-I or V-v			I-I					I-v		v-v				
		0	V-I	V-v	0	1	2	3	4	5	0	1	0	1	2	
Class 1	—	223	—	—	36	59	62	43	20	3	191	32	146	58	19	
		—	98	—	8	30	23	25	12	—	83	15	51	34	13	
		—	—	38	3	6	13	5	4	—	24	7	18	12	1	
Class 1	Total	233	98	31	47	95	98	73	36	3	298	54	215	104	33	
		352			352					352		352				
Class 2	v-V-I	18	—	—	1	3	7	5	2	—	18	—	10	6	2	
	V-I-I	12	—	—	—	2	4	3	3	—	12	—	5	4	3	
	I-I-v	3	—	1	1	1	—	1	1	—	4	—	2	1	1	
	I-v-v	3	—	2	—	3	—	1	1	—	5	—	2	3	—	
	Class 2	Total	36	—	3	2	9	11	10	7	—	38	—	19	14	6
			39			39					39		39			
Class 3	v-V-V-I	3	—	—	—	1	2	—	—	—	3	—	1	2	—	
	V-I-I-v	4	—	—	1	3	3	—	—	—	4	—	—	3	1	
	Class 3	Total	7	—	—	1	3	3	—	—	—	7	—	1	5	1
			7			7					7		7			
Grand total		266	98	34	50	107	112	83	43	—	344	54	235	123	40	
		398			398					398		398				

* Categorized as in Table 2.

same chromosomes participating in different configurations as follows:

v—V—I

(Fig. 12)

v*—

V—I—I

(Fig. 10), (Fig. 14)*

I—I—v

(Fig. 11)

I—v—v

(Fig. 13)

v—v

(Fig. 9)

(b₉) (a₁) (b₂) (a₆) (b₈) (a₇) (b₇)

Quadrivalents of v-V-I-I (Fig. 14) and V-I-I-v are explicable from the above relationship among the seven chromosomes. Thus, all the pairing configuration recorded in Table 2 are explicable as the derivatives from potential pairing 1_{vII}+5_{II}.

Figs. 9-14. MI in F_1 AB. $\times 800$. 9. $8_{II}+1_I$. 10. $1_{III}+5_{II}+4_I$. 11. $1_{III}+6_{II}+2_I$. 12. $1_{III}+6_{II}+2_I$. 13. $1_{III}+6_{II}+2_I$. 14. $1_{IV}+1_{II}+11_I$.

Figs. 15-16. MI in F_1 AB. $\times 800$. 15. $1_{III}+2_{II}+10_I$. 16. $6_{II}+5_I$.

Fig. 17. Two kinds of MI configurations observed in the V-I and V-v bivalents. One of V-v bivalents, (3), is depicted from a cell shown in Fig. 16.

Fig. 18. A possible pachytene pairing of a_1 , b_2 , and b_9 (left), and the resulting MI configurations (right). MI configurations (1), (2), (3), and (4) result, respectively, from single cross-overs in the segments (1), (2), (3), and (4) indicated in the pachytene-diplotype diagram.

Evidence for inverted segments in a_1

In the hybrids, two kinds of pairing configurations were observed in V-I (a_1-b_2) and in V-v (a_1-b_9) bivalents (Figs. 9, 13, 16, and 17). Four MI configurations (1)-(4) in Fig. 17 are explicable, respectively, as the result of a single cross-over occurring in one of the four regions (1), (2), (3), and (4) indicated in a pachytene diagram of the three chromosomes a_1 , b_2 , and b_9 (Fig. 18). Frequencies of the four kinds of configurations were 68, 30, 15, and 16, respectively. Configurations (2) and (3) presented in Fig. 17 indicate that a_1 and b_2 are partially homologous and they have inverted segments, and that the same holds true for chromosomes a_1 and b_9 .

DISCUSSION

Differences in basic chromosome numbers between closely related species are of great interest with respect to evolutionary divergence. The difference may result from unequal translocation between non-homologous chromosomes followed by elimination or duplication of a centromere (Darlington 1937). Aneuploid reduction in the basic chromosome number is substantiated in *Crepis* (Tobgy 1943; cf. Babcock 1947), *Haplopappus* (Jackson 1962, 1965; Smith 1966), and *Chaenactis* (Kyhos 1965). The explanation for the relationship between two chromosomal races ($2n=4$ and $2n=6$) of *Haplopappus gracilis* (Jackson 1965) may be applicable in part to the two diploid types of the *Scilla scilloides* complex. According to Jackson (1965) a population sample of *H. gracilis* from south-central Arizona comprised $2n=4$, $2n=5$, and $2n=6$; $2n=5$ is the hybrid between the $2n=4$ and $2n=6$ types. Cytological evidence revealed that an unequal reciprocal translocation and elimination of a centromere caused the aneuploid reduction of the chromosome number from $n=3$ to $n=2$ in *H. gracilis* (Jackson 1965).

In the present case of *Scilla scilloides* the two diploid types, AA ($2n=16$) and BB ($2n=18$), are karyotypically well differentiated, but the genomes A and B are still segmentally homologous. Cytogenetic findings in the F_1 hybrids, AB, revealed a potential chain of pairing of homologous parts of seven chromosomes, $b_9-a_1-b_2-a_6-b_8-a_7-b_7$. Accordingly, the genomes A and B have differentiated by a series of translocations and subsequent loss of a centromere. In addition, the relationship between a_1 and b_2 , and between a_1 and b_9 revealed that the genomes A and B were also differentiated by inversions. A possible process through which the genome A has differentiated from the genome B is schematically shown in Fig. 19.

The above conclusion agrees in part with the view of Satô (1942) who stated "... V-shaped chromosomes belonging to the B_1 seem to have been formed by translocation or fusion of the two chromosomes belonging to the B_2 ". B_1 and B_2 in Satô's paper correspond to the genomes A and B respectively in the present paper. Later, he presented a diagram which suggests that B_1B_1 ($2n=16$) and B_2B_2 ($2n=18$) have evolved from a common prototype with $2n=16$ (Fig. 2 on p. 184, Satô 1953).

The structural changes that took place during the differentiation of AA and BB of *S. scilloides* may account for, at least in part, the reduced pairing between corresponding chromosomes of the two genomes in the hybrids (cf. Swanson 1958, p. 263). Trans-

Fig. 19. Diagram to show how the genome A differentiated from the genome B. (1) Complex translocation involving three chromosomes. (2) Reciprocal translocation. (3) Pericentric inversion. (4) Loss of a minute chromosome and increase of chromosome sizes. Break positions are indicated by cross bars.

locations, inversions, and loss of a centromere, however, do not explain the difference in total lengths of chromosomes between the two genomes A and B. As measured at metaphase in three root-tip cells of the hybrid, the total length of the chromosomes of genome A is $72.93 \pm 1.45 \mu$ on the average, and that of genome B is $57.67 \pm 0.39 \mu$ (Araki unpublished). Increase in length of individual chromosomes may be involved in the evolution of genome A.

SUMMARY

The relationship between the genomes A ($x=8$) and B ($x=9$) of *Scilla scilloides* complex has been studied in F_1 hybrids (AB, $2n=17$) between the two diploid types AA ($2n=16$) and BB ($2n=18$).

Pairing of chromosomes is variable among PMCs, the maximum number of bivalents per cell being 8. Some bivalents are clearly heteromorphic. The pairing of chromosomes in PMCs revealed that the genomes A and B are segmentally homologous, although they are karyotypically well differentiated.

A scheme is presented to interpret how the genome A has been differentiated from the genome B by translocation, inversions, and loss of a centromere.

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LITERATURE CITED

- Babcock, B., 1947 Cytogenetics and speciation in *Crepis*. *Advances in Genetics* **1**: 69-93.
- Darlington, C. D., 1937 *Recent Advances in Cytology*. 2nd ed. J. & A. Churchill, London.
- Haga, T., and S. Noda, 1971 Cytogenetics of *Scilla scilloides* complex. I. Karyotype, genome, and population. (In preparation).
- Jackson, R. C., 1962 Interspecific hybridization in *Haplopappus* and its bearing on chromosome evolution in the *Blepharodon* section. *Amer. J. Bot.* **49**: 119-132.
- Jackson, R. C., 1965 A cytogenetic study of a three-paired race of *Haplopappus gracilis*. *Amer. J. Bot.* **52**: 946-953.
- Kyhos, D. W., 1965 The independent aneuploid origin of two species of *Chaenactis* (Compositae) from a common ancestor. *Evolution* **19**: 26-43.
- Newcomer, E. H., 1953 A new cytological and histological fixing fluid. *Science* **118**: 161.
- Noda, S., 1967 Samplings in the natural populations of *Scilla scilloides*. *Bull. Osaka Gakuin Univ.* **6**: 125-132.
- Noda, S., 1971 Cytogenetics of *Scilla scilloides* complex. II. Evidence for homoeologous relationship between the genomes. (In preparation).
- Satô, D., 1942 Karyotype alteration and phylogeny in *Liliaceae* and allied families. *Japan. J. Bot.* **12**: 57-161.
- Satô, D., 1953 Karyotype analysis and law of homologous series. *Sci. Papers of Coll. Gener. Educ. Univ. Tokyo* **3**: 41-53.
- Smith, E. B., 1966 Cytogenetics and phylogeny of *Haplopappus* section *Isopappus* (Compositae). *Can. J. Genet. Cytol.* **8**: 14-36.
- Swanson, C. P., 1958 *Cytology and Cytogenetics*. Macmillan, London.
- Tobgy, H. A., 1943 A cytological study of *Crepis fuliginosa*, *C. neglecta* and their F_1 hybrid, and its bearing on the mechanism of phylogenetic reduction in chromosome number. *J. Genetics* **45**: 67-111.