HOKKAIDO UNIVERSITY

Title	Analysis of chromosome variation in a population of Trillium apetalon MAKINO with reference to the breeding system
Author(s)	SAHO, Takashi
Citation	Journal of the Faculty of Science, Hokkaido University. Series 5, Botany, 10(2), 122-134
Issue Date	2007-07-27T06:13:50Z
Doc URL	https://hdl.handle.net/2115/26336
Type	departmental bulletin paper
File Information	10(2)_P122-134.pdf

Analysis of chromosome variation in a population of *Trillium apetalon* MAKINO with reference to the breeding system

By

Takashi SAHO

Botanical Institute Faculty of Science
Hokkaido University
Sapporo, Japan

In the preceding paper (SAHO, '74) chromosome variation of *Trillium apetalon* MAKINO*, a tetraploid species (4x = 20), was investigated with respect to the chromosome patterns revealed by cold treatment. In many populations it appeared that chromosomes have been fixed to certain types. The fixed types, however, are variable from locality to locality. Further, some populations were found to be polymorphic with respect to the chromosome types. For example, among thirteen plants of Onnebetsu population there were four types of chromosome A, three types of C, and so on. In spite of such a polymorphism of chromosome types, heterozygous chromosome pair was of rare occurrence and it was suggested that self-fertilization is rather operative in this species. The population of Saruru at south-west coast of Hidaka Peninsula is also one of the populations which show chromosome polymorphism. In the present paper, an analysis of the chromosome composition will be carried out with the fifty plants of Saruru population in order to investigate the mechanism of reproduction of *T. apetalon*.

Material and Method

Material plants were gathered randomly from Saruru population and transplanted to the experimental garden of our University. Immediately after flowering, the plants were removed into a cold chamber kept at 0°C and remained for 72–96 hours. Then, the cells of ovular tissue were fixed with La Cour 2 BE and stained by FEULGEN's method.

---

* *Trillium smallii* MAXIM., the former name, was used in the preceding papers, HAGA and KURABAYASHI ('53), KURABAYASHI and SAHO ('57 a, b), etc.

Result

The chromosome types found in Saruru population are demonstrated in Fig. 1 and the chromosome composition of fifty plants examined are listed in Table 1. The frequency of each chromosome type is presented in Table 2.

Chromosome A and E has only two types respectively, and their composition in all the fifty plants are *3366* and *1155* without any deviation, while each of the other three chromosomes, B, C and D, has four types. The *B-3* and *B-5* are the common types in many populations and the proportion of them attains to one third of all respectively. In spite of such high proportions of *B-3* and *B-5*, the constitution of *3355* is rather infrequent (Table 4). In many populations reported in the preceding paper (SAHO, '74), the constitution of chromosome B was *3355*. The *B-1* is the type found in Shoya population situated about 20 km south of Saruru (Fig. 2) and the *B-2* is a new type. The proportion of *C-4* and *C-6*, which are usual types in many populations, is 42.0 and 45.5% in this population. The majority of plants, thirty-five out of fifty, have *4466* constitution of chromosome C and only eight plants have one or two heteromorphic chromosome pairs. The *C-5*, being less frequent in the population, is the type found in Hiroo population about 25 km north of Saruru (Fig. 2). The *C-8* has not been found in any other populations to date. The *D-1* is contained with the proportion of only 6.5%, though it is a type having wide distribution. The *D-4* and *D-5* are the common types in many populations and contained in the population with the proportions of 44.5 and 26.5%, respectively. The *D-3*, a new type, shows rather high proportion (22.5%). In regard to chromosome D, the constitution of *3344* and *4455* are comparatively frequent.

As pointed out by SAHO ('74), it seems likely that the constituent genomes of *T. apetalon* can substitute each other at least in part. A supporting evidence can also be found in the present results. For example, the *3344*, *4455* and *3355* constitutions are all found in chromosome D. In chromosomes B and C, too, we can easily find such a situation. Further, it is found that some plants have *4444* constitution of chromosome D. This means that member of either of two genomes is completely deficient realizing autopolyploid composition so far as this chromosome is concerned. It is worthy to note here that such a partial substitution of chromosomes or lack of members of either of two genomes seems not to provide any shortcoming in viability and any detectable change in external morphology.

As shown in Table 3, the population possesses considerably high proportion of heteromorphic chromosome pairs. In chromosome B, C and D, 30.0, 9.0 and 23.0% of the total chromosome pairs respectively, are heteromorphic and consequently 80% of plants have one or more heteromorphic chromosome pairs.

Analysis and Discussion

The Saruru population is characterized by the maintainance of considerable proportion of heteromorphic chromosome pairs. It is known that various factors, such as mutation, selection and migration, participate in the diversification of population. If a certain type of chromosomes, or chromosome composition was provided with some selective advantage, in the population, it would come out predominant over the others. Since, however, the chromosome types found in this population are almostly the common types in many other populations, it seems improbable that selection pressure is so effective that brings about a drastic change of population structure. Mutation pressure may not be also excluded, but it is not likely that a certain type of chromosome is particularly in favor of mutation. Since the Saruru population is located near the tip of Hidaka Peninsula, east and west side of which are completely isolated by Hidaka Mountains except the point area of Peninsula, there is good reason to suppose that the population has been affected by the pressure of migration from both sides of Peninsula. In fact, chromosome *A–6* of this population is the type found in populations at east side of Hidaka Mountains, while *B–5** is the type found in the localities of west side of Peninsula.

The breeding system has the most immediate correlation with the change of population structure. Complete self-fertilization would result in a rapid decrease of heteromorphic chromosome pairs, and, on the contrary, panmictic fertilization would continue to produce heteromorphic pairs generation after generation maintaining them with a certain proportion in a state of eqilibrium in relation to the other factors, if population size is sufficiently large.

In polyploid species, the mode of chromosome pairing at meiosis must not be overlooked. True allopolyploids, the constituent genomes of which are sufficiently isolated not to allow pairing of chromosomes among them, may breed true without any disturbance of chromosome composition in the

---

*   Many population of east side of Hidaka Mountains include *B–5'* instead of *B–5*. However, decisive distinction between them can not be made at present due to the similarity of their morphology.

progeny, while in autopolyploids or segmental allopolyploids in the sense of STEBBINS ('50), multivalent association or pairing of chromosomes of different genomes at meiosis may inevitably result in severe disturbance of chromosome distribution to the progeny. The present data indicates that, so far as the chromosome A and E are concerned, bivalents of homologous chromosomes are regularly formed. Further, the fact that there is no deviation of chromosome number in all the five chromosomes of fifty plants, suggests that ten bivalents are usually formed at meiosis whatever the chromosome constitution may be. However, whether the two bivalents are formed randomly or not among four chromosomes, has important correlation with the chromosome composition of gametes and frequency of them, eventually with those of the next generation.

In Saruru population were found considerable proportion of heteromorphic chromosome pairs. Such a situation can not be maintained from generation to generation under complete self-fertilization. However, it seems that the proportion of heteromorphic pairs is rather small than expected from panmictic fertilization.

Then, more detailed analysis of the data was carried out with regard to the mode of chromosome pairing at meiosis and the breeding system of the species, which are the factors affecting immediately the change of population structure.

In Table 4, frequency of each chromosome composition is listed. Since the frequencies for various chromosome compositions are too small to carry out analysis and some of possible compositions are not realized in fifty plants, in order to facilitate analysis, chromosome compositions are classified into five groups as shown in Table 4. These groups are 1) *quadruple* group: four chromosomes are the same type such as *3333*, 2) *orthodox* group: four chromosomes consist of two homologous pairs such as *2255*, 3) *triple* group: three chromosomes of four are of the same type such as *1115*, 4) *three-types* group: four chromosomes consist of three types, one of which appears twice such as *1135*, and 5) *four-types* group: types of four chromosomes are different with each other such as *1235*. The frequency of each group can be seen in Table 4 and in the last column of Table 5. The great majority of plants, forty-four out of fifty with regard to chromosome B, forty-nine to chromosome C and forty-three to chromosome D, falls into the groups of the *orthodox* and *three-types*.

It is considered from the regularity of composition in chromosome A and E, two homologous chromosomes necessarily form bivalents, e. g., *3–3* and *6–6* bivalents of chromosome A and *1–1* and *5–5* bivalents of chro-

mosome E, and consequently all gametes are provided with *36* of chromosome A and *15* of chromosome E.

Further, the fact that regular chromosome number is maintained in all the fifty plants excludes the possibility of multivalent association at meiosis, which would sometimes result in the deviation of chromosome number in the progeny. Therefore, in chromosomes B, C and D, too, it may be supposed that the homologous chromosomes preferentially accomplish bivalent association. However, as shown in the meiotic division of *T. miyabeanum*, hybrid plant between *T. apetalon* and *T. tschonoskii*, chromosome pairing is possible between the genomes S and U, which are the constituent genomes of *T. apetalon* (KURABAYASHI and SAHO, '57a, b). Moreover, chromosome types of B, C and D found in this populationcan substitute each other, and consequently some of chromosome composition represents autopolyploid condition. Therefore, it is not necessarily meaningless to test the assumption of random chromosome pairing.

The expectation of column *2-p* and *2-s* in Table 5, were calculated on the basis of random assortment of chromosomes at meiosis among four. For example, the plant with *4466* chromosome composition will produce gametes of *44*, *46* and *66* at the proportion of $1:2:1$. If the frequencies of various gametes with different chromosome composition thus calculated are a, b, c, $\cdots$, n, respectively, where $a+b+c+\cdots+n=1$, $(a+b+c\cdots+n)^2$ will give the frequencies of possible chromosome compositions in the next generation by means of panmictic fertilization (*2-p*). Self-fertilization of the plant of *4466* will give rise to the progenies of *4444*, *6666*, *4446*, *4666* and *4466* at the proportion of $1:1:4:4:6$, that is, *quadriple* 1 : *orthodox* 3 : *triple* 4. The expected values for complete self-fertilization thus calculated are listed in the column *2-s*.

The expectation of column *1* was obtained purely mathematically, expanding $(a+b+c+d)^4$, where a, b, c and d represent the relative frequencies of four types in each chromosome and $a+b+c+d=1$. The values of *1* are in good accordance with those of *2-p* indicating nearly random distribution of each type of chromosome among the groups.

The expectation of *1*, *2-p* and *2-s* does not show any agreement with the observed frequency, giving lower values for *orthodox* group, and far higher values for *triple* group than the observed values. Self-fertilization (*2-s*) improves the values of *orthodox* group but increases the difference in *triple* and *three-types* groups. Such a disagreement between the expectation and the observed frequency clearly points out that the assumption of random chromosome assortment at meiosis must be rejected.

Then, on the basis of pairing of homologous chromosomes at meiosis, expectations for both panmixis (*3-p*) and self-fertilization (*3-s*) were calculated. For example, the plant of *4466* chromosome constitution will produce only gametes of *46* and complete self-fertilization will give rise to only progenies with the same chromosome composition as the parent. The gametes of a plant of *4456* will possess *45* or *46* and their proportion will be 1 : 1. Self-pollination will produce the progenies of *4456*, *4455* and *4466* at the proportion of 2 : 1 : 1, that is, the next generation of *three-types* plant will be *three-types* 1 : *orthodox* 1.

The next generation by panmictic reproduction can be obtained from $(a+b+c+\cdots+n)^2$, where a, b, c, $\cdots$, n, represents the relative frequencies of various gametes with different chromosome composition, and $a+b+c+\cdots+n=1$.

The expectations of *3-p* and *3-s* also do not coincide with the observed frequency. However, compared with the values of *1*, *2-p* and *2-s*, pairing of homologous chromosomes considerably decreases the expectation for *quadruple* and especially *triple* groups approaching to the observed values. Further, it is pointed out that panmictic fertilization (*3-p*) expects higher proportion for *three-types* and lower proportion for *orthodox* group than that of observed, while self-fertilization (*3-s*) expects reversely lower rate of *three-types* and higher proportion in *orthodox* group. This indicates that the real nature of fertilization of the species is neither the extreme of panmixis nor of inbreeding. Then, for trial, the mean values of *3-p* and *3-s* was calculated and found to be in good accordance with the observed frequencies in all the three chromosomes.

Thus, the analysis of chromosome composition of the population suggests that the homologous chromosomes preferentially form bivalents at meiosis. As referred in the foregoing paragraph, the chromosomes of two genomes of *T. apetalon* have potentiality to pair with each other. Further, it has been reported that, in spite of such a close homological relation of the two constituent genomes, the meiotic division of *T. apetalon* is quite regular forming ten bivalents at metaphase I (KURABAYASHI, SAHO, HIRAIZUMI and SAMEJIMA, '58). However, in regard to the *three-types* chromosome constitution, the frequencies of which being considerably high in Saruru population, a direct observation of meiotic division will be needed. If any irregularity of pairing at meiosis was assumed among the four chromosomes of the *three-types* constitution, the expectation of *3-p* and *3-s* in the next generation would decrease in *orthodox* group and increase in *triple* group again getting away from the observed values.

The analysis carried out in the present paper also revealed that self-fertilization may play an important role in *T. apetalon*. The plants of *T. apetalon* flowers earlier in spring than the other *Trillium* species when insects, which seems to mediate pollination of *Trillium* plants, are not yet abundant. It is often observed in field and in the experimental garden pollen grains of *T. apetalon* are released from anthers prior to the complete opening of perianths. Thus, the field observation also suggests the possibility of frequent self-fertilization.

Summary

1) The chromosome composition of fifty plants of *Trillium apetalon* MAKINO from Saruru population was investigated by means of the differential reaction of chromosomes to low temperature.

2) It was found that the population is polymorphic with respect to the chromosome types and contains considerable number of heteromorphic chromosome pairs.

3) Analysis of data indicates that homologous chromosomes preferentially accomplish bivalent association at meiosis, and that self-fertilization plays rather important role in *T. apetalon*. It is suggested, however, the real nature of fertilization of the species is neither the extreme of panmixis nor of inbreeding.

References

KURABAYASHI, M. and T. SAHO 1957 Evolution and Variation in *Trillium*. IX.
    Chromosome complement in two interspecific hybrids newly found.
    Cytologia 22 : 263-272.

KURABAYASHI, M. and T. SAHO 1957b Evolution and Variation in *Trillium*. XI.
    Mechanism of chromosomal isolation in Japanese *Trillium*.
    Cytologia 22 : 273-286.

KURABAYASHI, M., T. Saho, Y. HIRAIZUMI and K. SAMEJIMA 1958 Evolution and
    Variation in *Trillium*. X. Homological relationships among different genomes
    of Japanese *Trillium*.
    Cytologia 23 : 349-355.

SAHO, T. 1974 The chromosome variation in *Trillium apetalon* MAKINO.
    Journ. Fac. Sci. Hokkaido Univ. Ser. V (Botany), 10 (2): 113-121.

STEBBINS, G. L., Jr., 1950 *Variation and Evolution in plants.*
    Columb. Univ. Press. pp. 314-335.

TABLE 1. Chromosome composition of fifty plants of *Trillium apetalon* MAKINO from Saruru population

Plants No.	Chromosome Composition				
	A	B	C	D	E
1	3366	2255	6688	4435	1155
2	3366	1155	4466	3344	1155
3	3366	2255	6688	1144	1155
4	3366	5532	4466	3344	1155
5	3366	3351	4466	3355	1155
6	3366	3355	6645	4445	1155
7	3366	2233	4456	4435	1155
8	3366	2255	4466	4435	1155
9	3366	3331	4466	4444	1155
10	3366	2233	4466	3355	1155
11	3366	3325	4466	4435	1155
12	3366	2233	4466	1135	1155
13	3366	3325	4466	4455	1155
14	3366	5523	4466	4415	1155
15	3366	3315	4466	4435	1155
16	3366	3315	4466	4435	1155
17	3366	5512	4568	3345	1155
18	3366	3355	4466	3344	1155
19	3366	3355	4456	4455	1155
20	3366	5523	4466	4444	1155
21	3366	5512	4466	3344	1155
22	3366	2255	4466	3344	1155
23	3366	3333	8856	4445	1155
24	3366	2255	4466	4444	1155
25	3366	5513	4466	5534	1155
26	3366	2233	6688	4455	1155
27	3366	2233	4456	5514	1155
28	3366	1133	4466	3345	1155
29	3366	3325	5566	4444	1155
30	3366	1155	4466	5514	1155
31	3366	2233	4466	4415	1155
32	3366	5553	4466	3344	1155
33	3366	5523	4466	1155	1155
34	3366	1135	4466	1134	1155
35	3366	3325	4466	5514	1155
36	3366	5523	4466	3345	1155
37	3366	3355	5566	3355	1155
38	3366	5513	4466	5554	1155
39	3366	3332	4466	3344	1155
40	3366	2235	4466	4455	1155
41	3366	5513	4466	4455	1155
42	3366	5523	4466	4435	1155
43	3366	3315	4466	3344	1155
44	3366	2255	4466	4455	1155
45	3366	2235	4466	3344	1155
46	3366	5515	4488	4455	1155
47	3366	1115	4468	3344	1155
48	3366	1235	6688	3344	1155
49	3366	5552	4468	5534	1155
50	3366	2255	4466	4455	1155

TABLE 2.   Frequency of each chromosome type in Saruru
population of *Trillium apetalon* MAKINO

Type	Frequency	%	Type	Frequency	%
A–3	100	50.0	C–4	84	42.0
A–6	000	50.0	C–5	10	5.0
			C–6	91	45.5
B–1	23	11.5	C–8	15	7.5
B–2	45	22.5			
B–3	66	33.0			
B–5	66	33.0	D–1	13	6.5
			D–3	45	22.5
E–1	100	50.0	D–4	89	44.5
E–5	100	50.0	D–5	53	26.5

TABLE 3.   Frequency of heteromorphic pairs in each chromosome,
and of individuals bearing hetermorphic chromosome
pair in Saruru population of *Trillium apetalon* MAKINO

Chromosome	A	B	C	D	E	Total	Total of B, C, D
Freq. of Hetero-morphic Pairs. (/100)	0	30	9	23	0	62/500	62/300
%	0.0	30.0	9.0	23.0	0.0	12.4	20.7
Freq. of Plants with Hetero-morphic pair (/50)	0	29	8	23	0	40/ 50	
%	0.0	58.0	16.0	46.0	0.0	80.0	

TABLE 4. Frequency of each constitution of chromosome B, C and D in fifty plants of *Trillium apetalon* MAKINO obtained from Saruru

Chromosome Group	B		C		D	
	Constitution	Frequency	Constitution	Frequency	Constitution	Frequency
Quadruple	3333	1			4444	4
	Total	1			Total	4
Orthodox	1133	1	4466	35	3344	10
	1155	2	4488	1	3355	3
	2233	6	5566	2	4455	8
	2255	7	8866	4	1144	1
	3355	4			1155	1
	Total	20	Total	42	Total	23
Triple	111*5*	1			444*5*	2
	333*1*	1			555*4*	1
	333*2*	1				
	555*3*	1				
	Total	4			Total	3
Three-Types	113*5*	1	445*6*	3	113*4*	1
	223*5*	2	446*8*	2	113*5*	1
	331*5*	5	664*5*	1	334*5*	4
	332*5*	5	885*6*	1	441*5*	2
	551*2*	2			443*5*	7
	551*3*	3			551*4*	3
	552*3*	6			553*4*	2
	Total	24	Total	7	Total	20
Four-Types	*1235*	1	*4568*	1		
	Total	1	Total	1		
TATAL		50		50		50

TABLE 5. Analysis of data on the basis of assumptions with
respect to the chromosome pairing at meiosis and
to the breeding mechanism

Chromo-some	Group	EXPECTED						OBSERVED
		1	2-p	2-s	3-p	3-s	3-m	
B	Quadruple	1.3	1.2	6.0	0.1	2.0	1.0	1
	Orthodox	7.9	8.3	13.2	10.2	33.5	21.9	20
	Triple	11.7	11.4	18.0	3.2	2.0	2.6	4
	Three-Types	25.7	26.0	12.7	31.8	12.0	21.9	24
	Four-Types	3.4	3.1	0.2	4.7	0.5	2.6	1
	Total	50.0	50.0	50.1	50.0	50.0	50.0	50
C	Quadruple	3.7	1.9	5.7	0.0	0.0	0.0	0
	Orthodox	11.9	16.1	17.2	30.3	46.0	38.2	42
	Triple	19.1	16.8	22.8	0.0	0.0	0.0	0
	Three-Types	14.5	14.3	4.2	17.7	3.5	10.6	7
	Four-Types	0.9	0.9	0.2	2.0	0.5	1.3	1
	Total	50.1	50.0	50.1	50.0	50.0	50.1	50
D	Quadruple	2.3	2.2	8.9	0.5	4.8	2.6	4
	Orthodox	7.7	9.2	13.1	11.4	33.8	22.6	23
	Triple	16.8	14.2	18.0	7.4	1.5	4.5	3
	Three-Types	21.1	22.5	10.0	27.6	10.0	18.8	20
	Four-Types	2.1	1.8	0.0	3.1	0.0	1.6	0
	Total	50.0	49.9	50.0	50.0	50.1	50.1	50

1. Calculated purely mathematically from the relative frequencies of chromosome types.
2. Calculated from the relative frequencies of various gametes with different chromosome composition resulted from random chromosome pairing to make bivalents at meiosis.
    p: panmictic fertilization
    s: obligate self-fertilization
3. Calculated from the relative frequencies of various gametes resulted from preferential pairing of homologous chromosomes to form bivalents at meiosis.
    p: panmictic fertilization
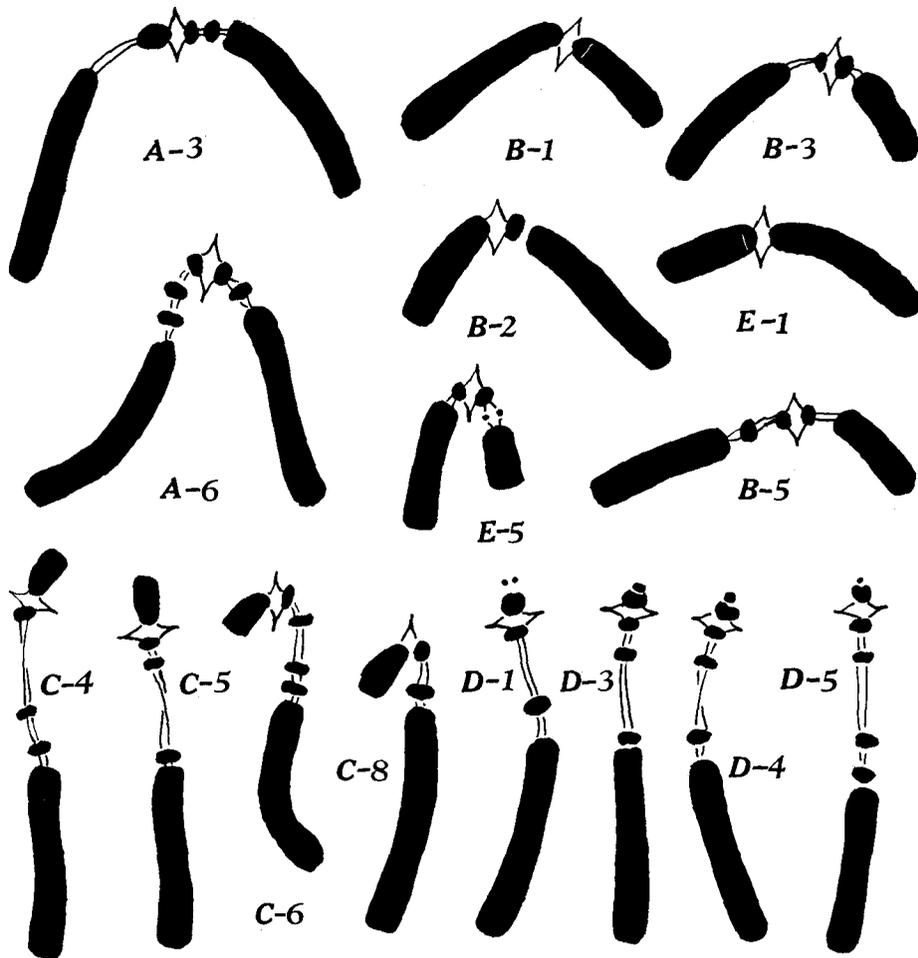   s: complete self-fertilization
    m: mean of 3-p and 3-s

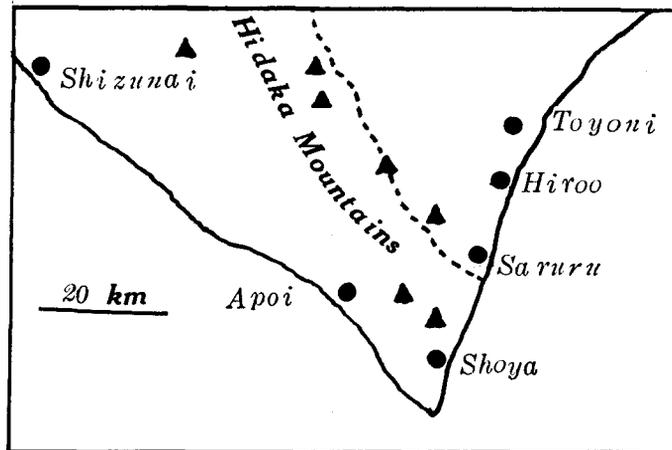**Fig. 1.** Chromosome types found in Saruru (*Srr*) population of *Trillium apetalon* MAKINO.

Fig. 2. Map of southern district of Hidaka Peninsula, Hokkaido, Japan.