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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	http://hdl.handle.net/2115/26336
Туре	bulletin (article)
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 Fuculty 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 2BE 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 ('57a, 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 popula-The majority of plants, thirty-five out of fifty, have 4466 constitution. tion 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 The D-4 and D-5 are the common types in many populadistribution. tions 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 4455are 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.

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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, \dots, n , respectively, where $a+b+c+\dots+n=1$, $(a+b+c\dots+n)^2$ 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: orthodox1.

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

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, HIRA-IZUMI 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.

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	Chromosome Composition							
Plants No.	A	В	C	D	E			
$\begin{array}{c}1\\2\\3\\4\\5\end{array}$	$\begin{array}{c ccccccccccccccccccccccccccccccccccc$		$\begin{array}{c} 6688 \\ 4466 \\ 6688 \\ 4466 \\ 4466 \\ 4466 \end{array}$	$\begin{array}{c} 4435\\ 3344\\ 1144\\ 3344\\ 3355 \end{array}$	$ 1155 \\ 1155 \\ 1155 \\ 1155 \\ 1155 \\ 1155 \\ 1155 $			
6 7 8 9 10	3366 3366 3366 3366 3366 3366	3355 2233 2255 33 <i>31</i> 2233	$6645 \\ 4456 \\ 4466 \\ 4466 \\ 4466 \\ 4466 \\ 4466 \\ 4466 \\ $	44 <i>45</i> 4435 4435 4444 3355	1155 1155 1155 1155 1155 1155			
11 12 13 14 15	3366 3366 3366 3366 3366 3366	3325 2233 3325 5523 3315	$\begin{array}{cccc} 4466 & 44. \\ 4466 & 11. \\ 4466 & 44i \\ 4466 & 44i \\ 4466 & 44i \end{array}$		1155 1155 1155 1155 1155 1155			
16 17 18 19 20	3366 3366 3366 3366 3366 3366	$3315 \\ 5512 \\ 3355 \\ 3355 \\ 5523 \\$	$\begin{array}{c} 4466 \\ 4568 \\ 4466 \\ 4456 \\ 4466 \\ 4466 \end{array}$	4435 3345 3344 4455 4444	$1155 \\ $			
21 22 23 24 25	3366 3366 3366 3366 3366 3366	5512 2255 3333 2255 55 <i>13</i>	$\begin{array}{c} 4466 \\ 4466 \\ 8856 \\ 4466 \\ 4466 \\ 4466 \end{array}$	3344 3344 44 <i>45</i> 4444 553 <i>4</i>	1155 1155 1155 1155 1155 1155			
26 27 28 29 30	3366 3366 3366 3366 3366 3366	$2233 \\ 2233 \\ 1133 \\ 3325 \\ 1155$	$\begin{array}{c} 6688 \\ 4456 \\ 4466 \\ 5566 \\ 4466 \end{array}$	4455 55 <i>14</i> 33 <i>45</i> 4444 55 <i>14</i>	$1155 \\ $			
31 32 33 34 35	3366 3366 3366 3366 3366 3366	2233 5553 5523 1135 3325	$\begin{array}{c} 4466 \\ 4466 \\ 4466 \\ 4466 \\ 4466 \\ 4466 \end{array}$	44 <i>15</i> 3344 1155 11 <i>34</i> 55 <i>14</i>	1155 1155 1155 1155 1155 1155			
36 37 38 39 40	3366 3366 3366 3366 3366 3366	5523 3355 5513 3332 2235	$\begin{array}{r} 4466 \\ 5566 \\ 4466 \\ 4466 \\ 4466 \\ 4466 \end{array}$	33 <i>45</i> 3355 55 <i>54</i> 3344 4455	1155 1155 1155 1155 1155 1155			
41 42 43 44 45	3366 3366 3366 3366 3366 3366	55 <i>13</i> 5523 33 <i>15</i> 2255 2235	$\begin{array}{c} 4466 \\ 4466 \\ 4466 \\ 4466 \\ 4466 \\ 4466 \end{array}$	4455 44 <i>35</i> 3344 4455 3344	1155 1155 1155 1155 1155			
46 47 48 49 50	3366 3366 3366 3366 3366 3366	55 <i>15</i> 11 <i>15</i> <i>1235</i> 555 <i>2</i> 2255	$\begin{array}{c} 4488 \\ 446.8 \\ 6688 \\ 446.8 \\ 446.8 \\ 4466 \end{array}$	4455 3344 3344 55 <i>34</i> 4455	1155 1155 1155 1155 1155			

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

Туре	Frequency	%	Туре	Frequency	%
A-3	100	50.0	C-4	84	42.0
A–6	000	50.0	C-5	10	5.0
			С-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	<i>D</i> -1 13		6.5
			D-3	45	22.5
E-1	100	50.0	D-4	89	44.5
<i>E</i> -5	100	50.0	D-5	53	26.5

 TABLE 2. Frequency of each chromosome type in Saruru

 population of Trillium apetalon MAKINO

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

Chromosome	А	В	С	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	

Chromosome	В		C		D		
Group	Constitu- tion	Frequ- ency	Constitu- tion	Frequ- ency	Constitu- tion	Frequ- ency	
Quadruple	3333	1		`	4444	4	
	Total	1			Total	4	
	1133	1	4466	35	3344	10	
	1155	2	4488	1	3355	3	
Orthodox	2233	6	5566	2	4455	8	
	2255	7	8866	4	1144	1	
	3355	4			1155	1	
	Total	20	Total	42	Total	23	
	1115	1			4445	2	
Triple	33 <i>31</i>	1			5554	1	
	33 <i>32</i>	1					
	55 53	1					
	Total	4			Total	3	
	1135	1	4456	3	1134	1	
	2235	2	4468	2	1135	1	
	3315	5	664 5	1	3345	4	
Three-Types	3325	5	8856	1	4415	2	
	55 <i>12</i>	2			4435	7	
	55 <i>13</i>	3			55 <i>14</i>	3	
	5523	6			55 <i>34</i>	2	
	Total	24	Total	7	Total	20	
Four-Types	1235	1	4568	1			
	Total	1	Total	1			
TATAL		50		50		50	

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

Chromo-	0	EXPECTED						OPOPPLUD
some	Group	1	2 - p	2-s	3 - p	3 - s	3 - m	OBSERVED
	Quadruple	1.3	1.2	6.0	0.1	2.0	1.0	1
	Orthodo.x	7.9	8.3	13.2	10.2	33.5	21. 9	20
В	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
С	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

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

1. Calculated purely mathematically from the relative frequencies of chromosome types. 1

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.

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