38. Chromosomes of a Himalayan *Trillium* in Relation to the Japanese and American Species^{*)}

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Cytogenetical synopsis. Some thirty species are known in the genus *Trillium* ($x=5$), distributed in Eastern Asia and in North America, and not in any other parts of the world. The American species so far examined, some twenty species, are all diploid, $2n=10$, except for rare occurrences of autotriploids.^{1), 2)} According to personal communications from Prof. R. B. Channell of Vanderbilt University, there remains some confusion in the taxonomy of the American species. However, the taxonomy of the Japanese species is well established.³⁾ In striking contrast to the American species the Japanese species form a polyploid series, as summarized in the following tabulation.^{4)–13)}

Plant	Genome	Pairing	Polyploidy
<i>T. kamtschaticum</i> Pallas	K_1K_1	5_{II}	$2n=10$ (2x)
<i>T. apetalon</i> Makino	SSUU	10_{II}	$2n=20$ (4x)
<i>T. tschonoskii</i> Maximowicz	K_2K_2TT	10_{II}	$2n=20$ (4x)
<i>T. hagai</i> Miyabe et Tatewaki	$K_1K_1K_2K_2TT$	15_{II}	$2n=30$ (6x)
<i>T. smallii</i> Maximowicz	K_2K_2SSUU	15_{II}	$2n=30$ (6x)
× <i>T. hagai</i> Miyabe et Tatewaki	K_1K_2T	$5_{II}+5_I$	$2n=15$ (3x)
× <i>T. yezoense</i> Tatewaki	K_1SU	$5_{II}+5_I$	$2n=15$ (3x)
× <i>T. miyabeianum</i> Tatewaki	K_2TSU	10_{II}	$2n=20$ (4x)

Plants marked with × in the above tabulation are all of F_1 hybrid origin by natural hybridizations. Parents of the hybrids can be determined with ease from the genomic constitution of the hybrids.

Trillium govanianum Wallich ex Royle. Root-tips of this Himalayan species were fixed and preserved in Carnoy mixture on June 4, 1960, at Phalut, altitude 3500 meters, in the Singalia Range of Eastern Himalaya, by Prof. H. Hara of the Tokyo University, who headed the Japanese team of the Indo-Japanese Botanical Expedition to Sikkim and Darjeeling in 1960. This precious material was placed in the hands of the senior writer for cytological investigation.

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Dedicated to Professor Sajiro Makino, Zoological Institute, Hokkaido University, on his sixtieth birthday June 21, 1966.

On this occasion we should like to thank Prof. Hara for his generosity in giving us the opportunity of studying the species from an area little known for the genus *Trillium*.

In short, *T. govanianum* was found to be a tetraploid, $2n=20$, consisting of two pairs of chromosomes A to E. Except for chromosomes C, all are quite similar to those of the Japanese and the American species in length, and in the position of the kinetochore. Chromosome C1 is characterized by having a satellite attached to the short arm, while C2 is characterized by the presence of a slit-like constriction in the proximal region of the long arm (Fig. 1). These

Fig. 1. Chromosomes from a root-tip cell of *Trillium govanianum* ($2n=20$). $\times 780$. Feulgen or aceto-orcein squash failed to stain the chromosomes preserved in Carnoy mixture for five years. Fortunately, Ueshima's new staining technique for the material long preserved in ethyl alcohol has made it possible to give good staining.²¹⁾

two pairs are nucleolar as is obvious by the maximum number of nucleoli, 4 in the resting, telophase and/or prophase nuclei:

Number of nucleoli	1	2	3	4	Total
Nuclei observed	214	406	289	91	1000

The two pairs C1-C1 and C2-C2 are the pairs of chromosome types particular to the present species; no satellited chromosomes being present in any of the Japanese species and probably also not in the American species. These two pairs are morphologically distinct from one another. This indicates, without a doubt, an allotetraploid constitution of the present species.

In this connection it will be worthwhile to note that in all species of *Paris*, a genus related to *Trillium*, chromosome D has a satellite attached to its short arm. Further, in an octoploid species *Kinugasa japonica* Tatewaki et Sutô ($2n=40$) chromosome pairs Cj

and Dj are satellited. The somatic complement of this plant is composed of four pairs of chromosomes A to E quite similar morphologically to those present in the species of the two allied genera *Trillium* and *Paris*.¹⁴⁾ It is interesting to note from a cytogenetical point of view that the genus *Kinugasa* was described in 1935 by Tatewaki and Sutô with the single endemic species *K. japonica*; in this treatment they took into consideration in part the cytological evidence by the senior writer of the present article.^{14), 15)} Formerly, the plant at issue had been described as *Trillium japonicum* Matsumura in 1885, *Paris japonica* Franchet in 1888, and *Trillidium japonicum* Franchet et Savatier in 1879.¹⁵⁾ This historical alterations in the taxonomic treatment suggest that the morphological characters of the present species are intermediate between the two allied genera *Trillium* and *Paris*.

However, the present study has revealed the satellited C chromosomes to exist preserved in the tetraploid species *T. govanianum*, that grows now in Himalaya, very far from the present day Japanese *Paris*' and *Trilliums*. Finally, closing this section it will be worthy to note that species of the genus *Trillium* and *Paris* are reciprocally intercrossable, yielding seeds good in appearance.¹⁶⁾

Evolutional significance. Many plant taxa are known to have very wide distribution areas connecting Eastern Asia to North America.¹⁷⁾ For example a small liliaceous genus *Clintonia*, comprising only six species, is distributed in this area. Of four American species three have $n=14$ and one $n=16$; the two Asiatic species have $n=14$, of which one is a Himalayan species.¹⁸⁾ Thus, the distribution area of the genus *Clintonia* is quite parallel to that of the genus *Trillium*. History of spreading from the center of origin must be traced back carefully taking into account the geological, palaeoecological and geobotanical changes before, during and after the Pleistocene; the problem is beyond the scope of the present article.^{19), 20)} The statement above is to emphasize the time elapsed and space spanned during the long run of the evolution of the genus, in which differentiation of the genomes and hybridization between plants with differentiated genomes happened. Both fundamental processes, genetic differentiation and natural hybridization, are observable at the present time in northern Japan.⁹⁾

Here, to be emphasized first is the fact that two genomes which are missing in the present day Japanese and American *Trilliums* are found in a Himalayan allotetraploid species, *T. govanianum*. Secondly, the fact that speciation by hybridization and chromosome doubling has been recurrent in Eastern Asia but has not happened in North America is of great significance in the history of the evolution of the

genus *Trillium* as a whole. This point will be discussed elsewhere on the basis of cytogenetic evidence which is now accumulating in Japan and the United States, taking into account the evidences from the other branches of sciences.

Summary. A Himalayan species *Trillium govanianum* ($x=5$, $2n=20$) was concluded to be an allotetraploid composed of two different genomes, both of which are not to be found in the present day Japanese and American species. This finding is highly significant in confirming further the fact that speciation by hybridization and chromosome doubling had been recurrent in Eastern Asia, whereas it had not happened in North America.

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