The Origin and Evolution in *Trillium*

2. Chromosome Variation of *Trillium undulatum* in North America

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Summary Chromosome analysis by the cold-induced heterochromatin banding method was carried out for populations of the American species *Trillium undulatum*. The banded karyotypes of individuals within the species were polymorphic. Furthermore, a comparative study showed that the genome of *T. undulatum* has a close relationship with the G genome of the Asian species *T. govanianum*. It is an interesting evolutionary problem to speculate on how the American and Asian species of *Trillium* species connected in both continents across the Pacific Ocean.

Key words Heterochromatic banding chromosomes, *Trillium undulatum*, Population analysis, Comparative study, North America, Asia.

Trillium undulatum Willdenow (Fig. 1) is distributed from New Brunswick in Canada to Georgia in the United States and extends throughout the Appalachian mountain range (Fig. 2). The plants grow in a sandy and highly acidic loam under deciduous or deciduous-evergreen forests. In late spring the flowers bloom and show their most attractive white petals with a blazing red center base (Fig. 1A). This flower is commonly called the “Painted Trillium”. In late summer the fruits ripen into a bright red color.

The Somatic chromosome number for *T. undulatum* was first recorded by Wilson and Boothroyd (1941) as $2n=2x=10$. The chromosomes of this species show the heterochromatic banding patterns after the cold-treatment (Darlington and La Cour 1938, 1940).

The purpose of this article was to study the chromosome banding-karyotypes within the species of *T. undulatum*. From this study, it has been very interesting to find that the unique karyotypes of *T. undulatum* are correlated with the Himalayan species *T. govanianum* in Asia. How the characteristics of these species have developed evolutionarily has been approached in this study from both the chromosomal and morphological viewpoints.

Materials and methods

For chromosome investigation materials of *T. undulatum* were collected from 2 localities, at St. Eugene, Prescott Co., Ontario, Canada and at Holland, Erie Co., New York in U.S.A.

Chromosome experiments were carried out on meristematic cells of the root tip after a cold-treatment (0°C) for 96 h in a refrigerator (Darlington and La Cour 1938, 1940, Kurabayashi 1952). The root tips were fixed in La Cour 2BE for 15 min, hydrolyzed in 1 N HCl at 60°C for 12 min and stained in Feulgen solution for 60 min. Squash preparations were made in 45% acetic acid. Chromosome data for a comparative study was from the information that had been recorded for *T. govanianum* in Nepal (Fukuda 2001).

Specimens used for carrying out comparative studies for morphological analyses were collected at St. Eugene, Ontario, Canada for *T. undulatum*, at Chiley-La, Bhutan for *T. govanianum*, at

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Fig. 1. Photographs of *Trillium undulatum*. A. Close-up photograph of the flower. $\times 1.5$. B. Habitat photograph. $\times 0.5$.

Fig. 2. Geographical distribution of *Trillium undulatum*.

Bhuma, Kushiro, Japan for *T. camschatcense*, and at Ste. Anne de Bellevue, Quebec, Canada for *T. erectum*. Sample number was over 20 specimens in each locality.

Chromosome analyses

Cold-induced heterochromatic banding karyotypes for *T. undulatum* are clearly shown in Fig. 3. The chromosome composition in each individual is denoted by the make-up of the chromosomes and numbers are given to the different banding types. For example, sample No. 6 (Fig. 3a), showing all 10 chromosomes is denoted as, A5, A5, B3, B3, C6, C6, D2, D6, and E4, E4 (Fig. 3b).

Fig. 4 illustrates schematic drawings of the chromosome-banding karyotypes found in *T. undulatum* collections. The dark areas depict euchromatic segments, whereas the white areas illustrate heterochromatic segments. In

Fig. 3. Chromosome photographs of *Trillium undulatum*. a. Karyotypes $2n=10$ Bar represents $10\mu\text{m}$. b. Showing the heterochromatic bands. Sample No. 6 composed of A5, A5, B3, B3, C6, C6, D6, D2 and E4, E4.

Fig. 4. Diagrammatic illustration of the karyotype of *Trillium undulatum* showing the heterochromatic banding patterns for chromosome A, B, C, D and E. S shows the supernumerary chromosome.

Table 1. Chromosome compositions of natural populations in *Trillium undulatum*

Locality		St. Eugene, Ontario, Canada				1998*
Sample No.		Chromosome composition				
1	A-1·1	B-1·2	C-1·1	D-1·2	E-1·3	
2	A-3·4	B-5·6	C-2·2	D-6·8	E-1·5	
3	A-4·6	B-3·4	C-6·6	D-6·6	E-4·4	
4	A-3·4	B-4·7	C-4·4	D-1·2	E-2·3	
5	A-2·2	B-2·5	C-5·5	D-4·4	E-3·3	S-1
6	A-5·5	B-3·3	C-6·6	D-2·6	E-4·4	
7	A-2·3	B-2·6	C-5·5	D-4·4	E-3·3	S-1
8	A-3·7	B-5·8	C-3·3	D-4·5	E-9·10	
9	A-5·5	B-2·4	C-6·6	D-6·6	E-4·4	
10	A-1·3	B-2·2	C-6·6	D-3·4	E-6·6	
Total						10
Locality		Holland, New York, U.S.A.				1960
Sample No.						
1	A-1·1	B-1·1	C-7·7	D-7·7	E-7·7	
2	A-1·1	B-1·1	C-6·8	D-6·6	E-8·9	
Total						2

The chromosome composition for each sample is shown by the type of chromosome A, B, C, D and E and the banding karyotype number in Fig. 4.

* The year in which the experiments were carried.

total for *T. undulatum*, 7 karyotypes were found in chromosome A, 8 karyotypes in chromosome B, 8 karyotypes in chromosome C, 8 karyotypes in chromosome D and 11 karyotypes in chromosome E. In addition in *T. undulatum*, one telocentric supernumerary chromosome was found.

From the results in this study on the chromosome banding patterns for *T. undulatum*, the chromosome composition found for each individual sample is given in Table 1.

From the data presented, it is clearly recognized that the pattern of the chromosome banding karyotypes of *T. undulatum* show considerable variation. The breeding system in a population is an important factor on chromosome variation because autogamy and cross-fertilizing breeding system control and limit gene follow in a population and eventually bring about different population structures and evolutionary trends (Baker 1951, Grant 1981).

Now, the inbreeding coefficient can be estimated for the means of chromosome variation from the proportion of hetero-homozygotes in the population according to the following formula (Wright 1969, Fukuda 1990):

$$F = (H - H_o) / H$$

$$\text{Where } H_o = 1 - \sum_i p_i^2$$

in which H is the observed proportion of heterozygotes:

H_o is the observed proportion of homozygotes:

P_i is the frequency of i in the chromosome banding karyotype.

Generally the American *Trillium* species, *T. grandiflorum*, *T. erectum*, *T. recurvatum*, and *T. ovatum* have a comparatively homogeneous banded karyotype in contrast to the Asian *T. camschaticense* (Fukuda 1969, 1970, 1988, 1989). Up to the present time the inbreeding coefficient (F) has

been calculated for several American *Trillium* species (Fukuda 1990). Now, in this reasearch, data for *T. undulatum* has been added.

Table 2 shows the inbreeding coefficient for the American *Trillium* species including *T. undulatum*. As shown in this table, *T. undulatum* has the lowest *F* value. Among the American *Trillum* species, this highly out-breed feature is an evolutionary characteristic for *T. undulatum*.

Table 2. Inbreeding Coefficient (*F*) in North American *Trillium* species

Species	<i>F</i>
<i>Trillium erectum</i>	0.5007
<i>Trillium recurvatum</i>	0.4237
<i>Trillium grandiflorum</i>	0.3511
<i>Trillium ovatum</i>	0.2913
<i>Trillium undulatum</i>	0.2727

Fig. 5. Comparative shapes of leaves and ovary organs of *T. undulatum*, *T. govanianum*, *T. camschatcense* and *T. erectum*.

Morphological analysis

T. undulatum has 3 ovate leaves, acuminate at the apices and rounded at the bases. This leaf shape closely resembles that of the Himalayan species *T. govanianum* (Fukuda 2001).

In Fig. 5 the leaf shapes of *T. undulatum* and *T. govanianum*, and also the Asian species *T. camschatcense*, and the American species *T. erectum* are shown. As may be seen from these figures, the leaf shape of *T. undulatum* most closely resembles that of *T. govanianum*. Leaf index has been recorded from over 20 specimens in each species as *T. undulatum*=1.61, *T. govanianum*=1.70, *T. camschatcense*=1.17, and *T. erectum*=1.24. The value also shows that the relationship between *T. undulatum* and *T. govanianum* is greater than that of the other species.

Although the petal and sepal shapes of *T. undulatum* and *T. govanianum* are different, but the ovary and stigma are more closely similar than those found in *T. camschatcense* and *T. erectum* as shown in Fig. 5. The author already reported that *T. undulatum* is a diploid species and that *T. govanianum* is an allotetraploid species that originated from hybridization between species *Trillium* and *Daiswa* species (Fukuda 2001). However, it is very interesting to note the correspondence in morphological characters between *T. undulatum* and *T. govanianum*.

It is also remarkable that the fruits of *T. undulatum* and *T. govanianum* are globose with dark red center although *T. grandiflorum* and *T. camschatcense* in this respect are greenish.

Comparative chromosomal analysis in *T. undulatum* and *T. govanianum*

For a number of years, the author had been collected the cold-induced chromosome banding data for populations of *T. erectum*, *T. grandiflorum*, *T. ovatum*, *T. recurvatum* and *T. camschatcense* (Fukuda 1969, 1970, 1988, 1989, Fukuda and Kozuka 1958, Fukuda and Grant 1979) and *T. govanianum*, *T. undulatum* (Fukuda 2001, and in this paper). Comparative studies of the chromosome banding karyotypes for these seven species were carried out. As the result it was found to denote the correspondence between *T. undulatum* and *T. govanianum*, G genome has been established.

Fig. 6 shows the similarity in the chromosome figures of *T. undulatum* and *T. govanianum*, G genome. In chromosome A, *T. undulatum* A3 and *T. govanianum* A1 has one heterochromatic segment similar on the left chromosome arm. The chromosomes of both species show almost the same karyotypes. In chromosome C, *T. undulatum* C3 resembles that of the *T. govanianum* C1 chromosome. In chromosome D, *T. undulatum* D7 also resembles the *T. govanianum* D1 chromosome. In

Fig. 6. Chromosome banding karyotypes in *T. undulatum* and *T. govanianum*, G genome.

chromosome B, *T. undulatum* B7 and *T. govanianum* B1 have 3 heterochromatic segments in common. When breakage occurred on the *T. undulatum* B7 chromosome, indicated by the arrow, and reunited on the opposite side, it produced the *T. govanianum* B1 chromosome. In the same manner, chromosome E of both species have 4 heterochromatic segments. When breakage occurred the *T. undulatum* E11 chromosome, as indicated by the arrow, and reunited in the opposite side, it formed the *T. govanianum* E2 chromosome.

From these data it is considered that both genomes of *T. undulatum* and *T. govanianum* G have chromosome-banding karyotypes in common. This is a very interesting phenomenon the evolutionary events in terms of chromosome participation in speciation.

Discussion

By means of chromosomal and morphological analyses the conclusion has been reached that the relationship between *T. undulatum* and *T. govanianum* is much closer than that with the other species. Information on the molecular level has recently been concerned with this problem. The *mat K* and *rbc L* chloroplast genes and amino acids sequences also showed the affinity of *T. undulatum* and *T. govanianum* separating these species phylogenetically from other *Trillium* species (Osaloo *et al.* 1999, Osaloo and Kawano 1999).

Now the most interesting problem evolutionally is how both species were geographically connected at one time and why they are separated at the present time. As mentioned earlier, *T. undulatum* is distributed in eastern North America but *T. govanianum* is located in the Himalayan mountain region in southern Asia. On the other particulars, *T. undulatum* is a diploid species but *T. govanianum* is a tetraploid species. Generally, polyploid species are of more recent origin than diploid species. Is *T. govanianum* really newer, that is a more recently arisen species, than *T. undulatum*? We must look at the paleological data in Asia and North America.

In the Cretaceous age, *Sequoia*, *Metasequoia*, *Taxodium*, *Alnus*, *Betula*, *Fagus*, *Quercus*, *Acer*, *Tilia* and *Cercidiphyllum* were distributed in Lat. 30–70°N in Asia and North America (Axelrod 1961, Tsukada 1974). It was the equable warm and wet climates in that area in the Northern Hemisphere. In the Eocene, of the Tertiary period, the flora of central and southern Asia and North America had produced the similar large floral belt in both continents across the Pacific Ocean (Axelrod 1958, Wolfe 1967). Although there are no paleological records on fossils or pollens for Trilliaceae plants, many monocotyledonous plants arose in the upper Cretaceous and developed in the Tertiary period (Stewart 1983, Takhtajan 1981, Stebbins 1974). The present *Trillium* species were associated with most of the abundant broad-leaved trees and several conifers, and grew mostly in humid habitats (Fukuda and Channell 1975). It is considered that *T. undulatum* might have developed and spread from North America to Central Asia under the floristic connection of the forests in the Tertiary period. As a first step, plants of *T. undulatum* that arrived to Central Asia were diploid. This species corresponds to *T. govanianum*, G genome. The species grew well in Central Asia in the Miocene and Pliocene periods which had a warm and cool climate but in Pleistocene, Quaternary periods, a strong cold and dry climate spread over the northern hemisphere (Flint 1957, Dunbar 1960, Roberts 1989). An immense glacier ice sheet spread down through Asia and North America. The Laurentian Ice Sheet covered in the Great Lakes region but the southern Appalachian mountain region escaped (Watts 1979). On the other hand, the great glacier did not cover the Central Asian region but instead the Asian region received severe cold and dry climatic conditions which affected living organisms (Pope 1995, Bar-yosef 1995). Especially the flora in inland Asia was greatly changed by the intense severe cold and dry conditions. As established in Part 1 of this *Trillium* series (Fukuda 2001), in which the origin of tetraploid *T. govanianum* was established a result of polyploidization from the diploid *Trillium* species by overcoming bad conditions. This resulted in the survival of *T. govanianum* in perpetuity (Fukuda 2001, Stebbins 1971, 1974). Chromosomes of *T.*

undulatum and *T. govanianum* tell us such a dramatic history on the evolutionary pathway from North America to Asia.

At last, I remember the words of Prof. Hitoshi Kihara,

“The history of the earth is recorded in the layers of its crust,
The history of all organisms is inscribed in the chromosomes”

(Kihara 1975)

This observation invokes profound thought.

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