Biosystematic Studies on *Clintonia* (Liliaceae-Polygonatae)
II. Somatic chromosome number and chromosomal
morphology of *Clintonia udensis* Trautvetter
et Mey

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Clintonia, a small herbaceous genus belonging to the Liliaceae-Polygonatae, consists of five species: *C. borealis* (Ait.) Raf. and *C. umbellulata* (Michx.) Morong in eastern North America; *C. uniflora* (Schult.) Kunth and *C. andrewsiana* Torr. in western North America; and *C. udensis* Trautv. et Mey. in Japan, China and the Himalayan mountains. Earlier investigators of North American *Clintonia* karyology include Smith (1911), Smith (1943), Walker (1944), Kawano (1965), Löve and Löve (1966), Utech (1972) and Utech and Thien (1973). The investigators of the Asian *C. udensis* are summarized in Table 1. Although various chromosome determinations were reported by these authors for *Clintonia* species, detailed karyotypic analyses were not made, except for brief size and centromeric description. They have, however, established $x=14$ as the generic base number, which represents a dibasic origin within the Polygonatae.

The distribution maps for *C. udensis* and var. *alpina* have been published by Kanai (1963, 1966). The specimen examination from North American herbaria (CAN, CU, DAO, F, GH, JEPS, MIN, MO, NY, UC, US, WIS, WTA, and WVA) and Europe (K and BM) has not significantly extended or altered the distributional range. The trans-Himalayan populations of *C. udensis* will be treated here, following Hara and Kurosawa (1963), as var. *alpina* (Kunth ex Baker) Hara, though they have been classified as subsp. *alpina* (Kunth ex Baker) Kitamura (Kitamura and Murata 1966).

The present study, in a series of biosystematic *Clintonia* investigations, attempts a more detailed determination of the karyotype of *C. udensis* and furthermore, provides additional information for evolutionary re-evaluation.

Materials and methods

Sixteen individuals of *Clintonia udensis* were randomly collected from the population growing in a mixed *Sasa*, *Fagus-Acer* forest (alt. 780 m) near Amihari Spa, Iwate Pref. (Prov. Rikuchû), Japan and transferred to greenhouse conditions at the University of Iwate. Excised root tips for analysis were divided between two pretreatments, colchicine and 8-hydroxyquinoline. After 4 hours in 0.01 % colchi-

cine at 24–26°C or 5 hours in 0.005 mol/l 8-hydroxyquinoline at 18–19°C, these pretreated root tips were fixed for 30 minutes in Farmer's solution (3: 1), macerated in n-HCl for 20 minutes, stained in aceto-orcein for 24 hours and the resulting squash made permanent in Canada balsam. Observations, measurements and photographs were made with a Zeiss Universal microscope at Washington University. Voucher specimens (*Suda AM 52272S-1-AM 52272S-4*) have been deposited at the University of Iwate, Morioka, Japan (IUM); slides and photographs are at the Missouri Botanical Garden, St. Louis, U. S. A. (MO).

Table 1. Chromosome numbers for *Clintonia udensis*

Taxon	Previous report				
	(2n)	(n)	Tissue*	Location	Investigator
<i>C. udensis</i> Trautv. et Mey.	—	14	PG	Japan: Mt. Sapporo- dake, Prov. Ishikari, Hokkaido	Matsuura and Sutô 1935
	28	—	RT	Japan: unknown	Satô 1942
	ca. 30	—	—	U.S.S.R.: Sakhalin Is.	Sokolovskaya 1960
	14	—	—	U.S.S.R.: Primorskiy krai	Sokolovskaya 1966
var. <i>alpina</i> (Kun- th ex Baker) Hara	—	14	PG	India: Phalut, Singalila Range	Hara and Kurosawa 1963, 1964; Kurosawa 1966
	28	—	RT	India: Sandakphoo, Sikkim, Eastern Hima- layas and Valley of Fl- owers, Kumaon, Centr- al Himalayas	Pahuja and Kumar 1971

* PG=Pollen grain; RT=Root tip.

Table 2. Pretreatment comparison of chromosomal lengths and ratio of longest and shortest chromosome

Colchicine pretreated cells (N=25)			8-hydroxyquinoline pretreated cells (N=25)		
Longest L(μ)	Shortest S(μ)	Ratio L/S	Longest L(μ)	Shortest S(μ)	Ratio L/S
$\bar{x}=16.86$	$\bar{x}=8.00$	$\bar{x}=2.11$	$\bar{x}=16.79$	$\bar{x}=8.09$	$\bar{x}=2.08$
$s=1.16$	$s=0.58$	$s=0.11$	$s=1.08$	$s=0.54$	$s=0.11$
14.5–18.5	6.5–8.9	1.95–2.36	14.7–19.0	6.7–9.0	1.92–2.39

Results

From these individuals of *Clintonia udensis*, 100 root tip cells, 50 per pretreatment, were counted and all counts were $2n=28$. No aneusomaty was observed. Furthermore, as a check on possible differential condensation, 25 cells were selected from both the colchicine and 8-hydroxyquinoline pretreatments and the longest (L) and the shortest (S) chromosome per cell at metaphase were measured (Table 2).

No difference between the two pretreatments for the long and short chromosomes was indicated by the standard 't' test. The range, average and standard deviation for the long and short chromosomal lengths are remarkably similar. For each of

Fig. 1. Somatic chromosomes of *Clintonia udensis* from Amihari Spa, Iwate, Japan. A, colchicine pretreatment ($2n=28$); B, 8-hydroxyquinoline pretreatment ($2n=28$).

Fig. 2. Somatic chromosomes of *C. udensis* with colchicine pretreatment ($2n=28$, from Amihari Spa, Iwate).

these 25 paired measures of the cell's longest and shortest chromosome, a ratio (L/S) was calculated and no difference was indicated when compared.

Five cells from each pretreatment were selected for their flatness and evenness of spread and all the chromosome of a given cell were measured at $1600\times$. Fig. 1 (A, B) represents cells from these two pretreatments, while Figs. 2 and 3 represent a karyotype of these cells respectively. Then using these 10 cells, a composite haploid idiogram was constructed (Fig. 4) with a summary of the arm lengths, centromeric index and corresponding standard deviation presented in Table 3. Each arm or total length is represented by 20 different measurements. Though the length values of the long and short chromosomes for the idiogram (Table 3) are slightly shorter

Table 3. Chromosomal lengths for composite idiogram of *Clintonia udensis* (10 cells, 20 measures, standard deviations indicated)

Pair no.	Long arm (μ)	Short arm (μ)	Total length (μ)	Centromere index
1.	8.08 (0.56)	7.65 (0.55)	15.74 (1.07)	0.95 (0.03)
2.	7.75 (0.59)	7.27 (0.67)	15.03 (1.26)	0.94 (0.03)
3.	7.67 (0.51)	4.41 (0.23)	12.08 (0.78)	0.57 (0.03)
4.	7.06 (0.65)	4.08 (0.30)	11.15 (0.96)	0.58 (0.05)
5.	6.93 (0.56)	3.57 (0.53)	10.49 (0.93)	0.47 (0.03)
6.	6.77 (0.67)	3.44 (0.30)	10.23 (0.92)	0.56 (0.05)
7.	6.03 (0.62)	3.74 (0.30)	9.77 (0.92)	0.63 (0.04)
8.	6.06 (0.64)	3.28 (0.28)	9.37 (0.91)	0.54 (0.06)
9.	4.97 (0.53)	4.07 (0.32)	8.98 (0.89)	0.82 (0.05)
10.	6.57 (0.46)	1.50+0.86 (0.19) (0.17)	8.95 (0.80)	0.35 (0.04)
11.	4.73 (0.56)	3.87 (0.27)	8.60 (0.84)	0.83 (0.05)
12.	6.49 (0.48)	2.10 (0.30)	8.58 (0.75)	0.32 (0.06)
13.	6.75 (0.48)	1.25 (0.17)	8.00 (0.71)	0.18 (0.03)
14.	5.67 (0.60)	1.95 (0.22)	7.63 (0.87)	0.34 (0.03)

than those found when checking condensation (Table 2), the selection for well-spread figures for the idiogram may have introduced a slight bias towards greater contraction.

The diploid complement has 4 metacentric chromosomes which are consistently larger than the rest, 12 medium length submetacentric chromosomes, 4 medium to small nearly metacentric chromosomes, 2 medium to small subtelocentric chromosomes with short NOR—satellite regions, and 6 small chromosomes with subtelocentric to exceedingly subtelocentric centromeres. The 12 medium, submetacentric

chromosomes (Figs. 2-4; pairs 3-8) form a decreasing size gradient and when paired with their corresponding centromeric index can be readily distinguished within a given cell. As with these chromosomes, the smaller ones, including the nucleolar organizing chromosomes, can not be ranked consistently on size alone, but can be quite easily distinguished if size is combined with the respective centromeric index. The nucleolar organizing chromosomal pair is highly characteristic (Fig. 1). The satellite was usually found attached in all the cells observed. The karyotype of *Clintonia udensis* may be roughly symbolized as: $K(2n)=4V+12J+4v+8j(2\text{ NOR}+6j)$.

Fig. 3. Somatic chromosomes of *C. udensis* with 8-hydroxyquinoline pretreatment ($2n=28$, from Amihari Spa, Iwate).

Fig. 4. Composite, haploid idiogram of *C. udensis*, based on measurements of ten diploid cells from Amihari Spa, Iwate, Japan.

Discussion

The present study confirms the $2n=28$ counts and karyotypic descriptions for *Clintonia udensis* from Japan (Matsuura and Sutô 1935, Satô 1942, Table 1). The karyotype of var. *alpina* from northern India was reported by Hara and Kurosawa

(1963, 1964) and Kurosawa (1966) as agreeing with that reported from Japan. According to Pahuja and Kumar (1971), Sato's Japanese taxon *C. udensis* falls in category '2 a' of Stebbins' classification of karyotype asymmetry (Stebbins 1958). On the other hand, they found their var. *alpina* to be of the '2 b' type and inferred that the karyotype of Himalayan materials was more evolved, with marked structural heterozygosity. *C. udensis* from Amihari Spa, Japan was also found to be of the '2 b' type, for the proportion of chromosomes having arm ratio $>2:1=0.374$ (10 cells), and the ratio of the longest/shortest chromosomes $=2.09$ (Table 2).

Clintonia has long chromosomes compared to other members of the Polygonatae (Therman 1956). Chromosomal lengths during division of *C. udensis* pollen grains were reported by Matsuura and Sutô (1935) as varying between ca. 13 and 7.5 μ . Though not citing measurements, Hara and Kurosawa (1963, 1964) reported similar chromosomes in var. *alpina* pollen grain divisions from India. The authors have found Satô's (1942) figure of root tip chromosomes to give approximate values of 10–12 μ for the longest chromosomes. Table 2 compares 50 *C. udensis* cells which were analyzed, 25 pretreated with colchicine and 25 with 8-hydroxyquinoline respectively. The average of the longest was 16 μ with a range of 14.5–19.0 μ ; the average for the shortest was 8.0 μ with a range of 6.5–9.0 μ . The variation in length is much greater for the longer chromosomes. It is somewhat remarkable then to note Pahuja and Kumar's finding (1971) of 6.25 μ (longest) and 3.125 μ (shortest) for var. *alpina*, that is, only half the size consistently measured here. Such deviation warrants further investigation. Possibly this extreme condensation was due to their pretreatment, an aqueous solution of 0.029% 8-hydroxyquinoline with traces of α -bromonaphthalene for 2–3 hours at 3–4°C. The other species of *Clintonia* have long root tip chromosomes in the size range of 12–18 μ when pretreated with either colchicine or 8-hydroxyquinoline (Utech and Thien 1973, Utech, unpublished).

Summary

The detailed analysis of the *Clintonia udensis* chromosome complement demonstrated the $2n=28$ karyotype to be remarkably consistent, with no aneusomy. Root tip chromosomal lengths under both colchicine and 8-hydroxyquinoline pretreatments were virtually identical. The karyotype may be roughly symbolized as: $K(2n)=28=4V+12J+4v+8j$ (2NOR+6j).

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