Biosystematic Studies on *Clintonia* (Liliaceae-Polygonatae)

III. Cytogeography, chromosome number and morphology of the North American species of *Clintonia* Raf.

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Clintonia, a small herbaceous genus belonging to the Liliaceae-Polygonatae, has four species in North America and one in Asia. Earlier investigators of the Asian *C. udensis* include Matsuura and Sutô (1935), Sato (1942), Sokolovskaya (1960, 1966), Hara and Kurosawa (1963, 1964), Kurosawa (1966), Pahuja and Kumar (1971) and Utech and Suda (1975). Past investigators of the North American species are summarized in Table 1. This cytological investigation of the North American species was designed to complete the cytological review of the genus *Clintonia* (Utech 1972, Utech and Thien 1973, Utech 1973, Utech and Suda 1975) by eliciting the following evolutionary information for each species: 1) a cytogeography survey with emphasis on somatic karyotype and chromosome number; 2) meiotic chromosome number and behavior; and 3) reinvestigation of the reported aneuploid condition in *C. borealis*.

Materials and methods

Root tips and floral buds were collected from the entire range of each North American species during a four year period (1969-1973). Detailed geographical locations are provided in Utech (1973). Clones of each species were established from field collected rhizomes and grown at the Missouri Botanical Garden. Standardized aceto-orcein and aceto-carmin squash techniques (Darlington and La Cour 1962, Utech and Thien 1973, Utech and Suda 1975) were used and the preparations made permanent in euparal. Two different root tip pretreatments were used: four hours in 0.01% colchicine at 24-26°C, or five hours in 0.005 mol/l 8-OH quinoline at 18-19°C. No significant difference in chromosomal condensations was observed between the two different pretreatment (Utech and Suda 1975). Observations, measurements and photographs were made with a Zeiss Universal microscope. Voucher specimens have been deposited at the Missouri Botanical Garden, St. Louis

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Over 20,000 miles in both eastern and western North America were extensively surveyed in 1972. Material for chromosomal study was collected during three spring months, April through June in the eastern mountains, and during two summer months, July and August, in the western mountains. Knowledge of the floral phenology, distribution and elevation of the two eastern species, *C. borealis* and *C. umbellulata* (Fig. 1), enabled collection of both actively growing root tips and meiotic floral buds, since the eastern material was obtained by following the emerging plants northward. At each sampled site, root tips and floral buds were collected separately for each clone. In the west, summer collecting was limited to the root tips and rhizomes of *C. andrewsiana* and *C. uniflora* (Fig. 2).

To establish a base level for this cytogeographic survey, a minimum of five somatic (root tip) counts and/or five meiotic figures per clone was used. In many cases, it was possible to determine both somatic and meiotic counts from the same and different clones within a population. Since PMC meiosis is synchronized in *Clintonia*, it is feasible to count large numbers of meiotic figures. In a given population where a small number of cells were counted, additional material was thoroughly scanned for univalents and other chromosomal abnormalities.

Results

1. Cytogeography of North American species of *Clintonia*

The first chromosomal observations of the genus were by R. Smith (1911), who

Table 1. Previously published chromosome numbers of the North American species of *Clintonia*

Species	2n	n	Workers	Area
<i>C. andrewsiana</i> Torrey	—	14	Walker 1944	U. S. A.: Calif.
	—	14	Cave 1970	U. S. A.: Calif.
<i>C. umbellulata</i> (Michx.) Morong	—	14	Walker 1944	U. S. A.: Pa.
	28	—	Utech 1972	U. S. A.: W. Va.
<i>C. borealis</i> (Ait.) Raf.	ca. 20	ca. 12	R. Smith 1911	Canada: Ont.
	—	16	Walker 1944	U. S. A.: Wis.
	28	—	Kawano 1965	U. S. A.: Wis.
	32	—	Löve and Löve 1966	U. S. A.: N. H.
	28	—	Kapoor 1970, 1973	Canada: N. S.
	28+2B	—	Kapoor 1970, 1973	Canada: N. S.
	32	—	Utech 1972	U. S. A.: W. Va.
	28	—	Utech 1972	U. S. A.: W. Va.
	28	14	Utech and Thien 1973	Range
<i>C. uniflora</i> (Menzies ex Schultes) Kunth	—	14	F. Smith 1943	U. S. A.: Wash.
	—	14	Walker 1944	U. S. A.: Ore.
	—	14	Cave 1970	U. S. A.: Calif.
	28	—	Utech 1972	U. S. A.: Ore.

estimated approximately 20 somatic chromosomes and 12 meiotic bivalents from his sectioned paraffin material of *C. borealis*. Since this early date numerous counts have been made for the species of *Clintonia* (Table 1; also Utech and Suda 1975).

Fig. 1. Cytogeographical survey sites and range distribution of *Clintonia* in eastern North America. A, *C. borealis* (Ait.) Raf. B, *C. umbellulata* (Michx.) Morong. Glacial maxima indicated.

The review of published counts indicates a $2n=28$ and $n=14$ generic pattern. The impetus for this reinvestigation of *Clintonia* cytology was the conflicting somatic counts of $2n=28$ (Kawano 1965), $2n=28+2B$ (Kapoor 1970, 1973) and $2n=32$ (Löve and Löve 1966) and the meiotic report of $n=16$ (Walker 1944) for *C. borealis*.

In 1970 and 1971, 23 populations of *C. borealis* were examined (Utech and Thien

1973). The $2n=28$ cytotype was found commonly throughout the western portion of its range. However, a $2n=32$ cytotype was discovered in unglaciated territory on Spruce Knob, West Virginia, as were other populations which had meiotic uni-

Fig. 2. Cytogeographical survey sites and range distribution of *Clintonia* in western North America. A, *C. uniflora* (Menzies ex Schultes) Kunth. B, *C. andrewsiana* Torrey.

valents. Clones sampled from near Oostburg, Wisconsin, for example, had univalents whose meiotic behavior suggested B-chromosomes and a basis for the dif-

ferent cytotype (Utech and Thien 1973). It is noteworthy that Walker's count of $n=16$ (1944) came from material collected near Oostburg. To determine how widespread these different cytotypes were, an extensive cytogeographic survey and chromosomal analysis of *C. borealis* and other species of *Clintonia* was undertaken.

The results of these range-wide cytogeographic surveys are presented in Table 2 and Figures 1–2. The distributional maps of the eastern and western species pairs (*Clintonia borealis* and *C. umbellulata*, Fig. 1; *C. uniflora* and *C. andrewsiana*, Fig. 2, respectively) show the locations of sampled populations. For each population the total number of clones and counts observed for both somatic and meiotic material are indicated, as well as those clones which were cultivated (Table 2).

Table 2. Cytogeographical survey of the North American species of *Clintonia*

Species location	Voucher	2n	Root tip (clones; counts)	n	PMC (clones; counts)
<i>C. andrewsiana</i> Torrey					
Calif.: San Mateo Co., La Honda	Utech 601	28	(1;8)	—	—
Calif.: Marin Co., Mt. Tamalpais	Utech 602	28	(3;15)	—	—
* Calif.: Sonoma Co., Armstrong Red- woods	Utech 603	28	(1;5)	—	—
Calif.: Sonoma Co., Stewarts Point	Utech 604	28	(3;15)	—	—
Calif.: Mendocino Co., Mendocino	Utech 605	28	(4;20)	—	—
Calif.: Mendocino Co., Mendocino	Utech 606	28	(4;15)	—	—
Calif.: Mendocino Co., Albion	Utech 607	28	(3;17)	—	—
Calif.: Mendocino Co., Rockport	Utech 608	28	(1;8)	—	—
Calif.: Humboldt Co., Bull Creek Flats	Utech 609	28	(1;7)	—	—
* Calif.: Humboldt Co., Arcata	Utech 610	28	(4;20)	14	(5;40)
10 populations		25 clones 130 counts		5 clones 40 counts	
<i>C. umbellulata</i> (Michx.) Morong					
Tenn.: Blount Co., Rich Mt.	Utech 201	28	(1;5)	14	(1;5)
N.C.: Jackson Co., Cherokee	Utech 202	28	(3;15)	14	(5;25)
N. C.: Buncombe Co., Mt. Pisgah	Utech 203	28	(1;5)	14	(2;10)
N. C.: Buncombe Co., Mt. Pisgah	Utech 204	28	(2;9)	14	(2;10)
N. C.: Avery Co., Grandfather Mt.	Utech 205	28	(3;15)	14	(2;10)
W. Va.: Randolph Co., Elkins	Utech 206	28	(4;21)	14	(4;20)
W. Va.: Randolph Co., Alpena Gap	Utech 207	28	(2;10)	14	(2;10)
* Pa.: Fayette Co., Ohiopyle	Utech 208	28	(6;30)	14	(8;40)
* Pa.: Somerset Co., Laurel Hill	Utech 209	28	(3;17)	14	(5;25)
* Ky.: Bell Co., Pine Mt.	Utech 210	28	(4;23)	14	(3;15)
10 populations		29 clones 150 counts		34 clones 180 counts	
<i>C. borealis</i> (Ait.) Raf.					
Wis.: Lincoln Co., Irma	Thien 100	—	—	14	(4;25)
Wis.: Iron Co., Minnow Lake	Thien 101	28	(1;5)	14	(2;15)
Wis.: Forest Co., Lake Richardson	Thien 102	—	—	14	(3;25)
Wis.: Langlade Co., Neva	Thien 103	—	—	14	(4;20)
Wis.: Langlade Co., Antigo	Thien 104	—	—	14	(6;30)
Wis.: Langlade Co., Antigo	Thien 105	—	—	14	(2;10)

Table 2.—continued.

Species location	Voucher	2n	Root tip (clones; counts)	n	PMC (clones; counts)
Wis.: Oneida Co., Monico	<i>Utech 106</i>	28	(2;10)	14	(4;30)
Wis.: Menominee Co., Neopit	<i>Utech 107</i>	28	(3;15)	14	(5;30)
* Wis.: Juneau Co., Rocky Arbor	<i>Utech, Marcks + Thien 108</i>	28	(3;10)	14	(10;100) 13 _{II} +2 _I 12 _{II} +4 _I
* Wis.: Sheboygan Co., Terry Andrae	<i>Utech + van Horn 109</i>	28	(4;22)	14	(6;50) 13 _{II} +2 _I 12 _{II} +4 _I
Wis.: Manitowoc Co., Point Beach	<i>Utech + van Horn 110</i>	—		14	(5;35)
Wis.: Door Co., Cave Point	<i>Utech + van Horn 111</i>	—		14	(3;20)
Wis.: Marinette Co., Godard Park	<i>Utech + van Horn 112</i>	—		14	(3;15)
Wis.: Marinette Co., Pembine	<i>Utech + van Horn 113</i>	—		14	(6;45)
Wis.: Florence Co., Florence	<i>Utech + van Horn 114</i>	—		14	(3;15)
Wis.: Forest Co., Alvin	<i>Utech + van Horn 115</i>	28	(1;5)	14	(2;10)
Wis.: Oneida Co., Big Bearskin Creek	<i>Utech + van Horn 116</i>	—		14	(6;70)
Wis.: Oneida Co., Big Bearskin Creek	<i>Thien + Marcks 117</i>	—		14	(10;100)
Wis.: Oneida Co., Blue Lake	<i>Thien + Marcks 118</i>	—		14	(10;100)
Wis.: Vilas Co., Otto Mielke Lake	<i>Thien + Marcks 119</i>	—		14	(10;100)
Wis.: Vilas Co., Allequash Lake	<i>Thien + Marcks 120</i>	—		14	(10;100)
W. Va.: Tucker Co., Backbone Mt.	<i>Utech 1201</i>	28	(2;8)	—	
W. Va.: Randolph Co., Dolly Sods	<i>Utech 1205</i>	28	(2;12)	—	
W. Va.: Pendleton Co., Spruce Knob	<i>Utech 1208a Utech 1208b</i>	28 32	(2;10) (1;3)	—	
Mich.: Menominee Co., Spalding	<i>Utech 1302</i>	—		14	(5;25)
Minn.: St. Louis Co., Rollins	<i>Utech 1304</i>	—		14	(5;25)
Mich.: Houghton Co., Laurium	<i>Utech 1306</i>	—		14	(5;25)
* Tenn.: Sevier Co., Mt. Le Conte	<i>Utech 1</i>	28	(3;20)	14	(6;45)
* Tenn.: Sevier Co., Indian Gap	<i>Utech 2</i>	28	(2;20)	14	(2;20)
Tenn.: Sevier Co., Newfound Gap Road	<i>Utech 3</i>	28	(3;17)	14	(3;15)
Tenn.: Sevier Co., Newfound Gap Road	<i>Utech 4</i>	28	(2;10)	14	(2;15)
N. C.: Haywood Co., Wolf Ridge	<i>Utech 5</i>	—		14	(3;16)
N. C.: Buncombe Co., Craggy Mt.	<i>Utech 6</i>	28	(1;5)	14	(2;10)
N. C.: Avery Co., Grandfather Mt.	<i>Utech 7</i>	28	(1;5)	14	(1;10)
N. C.: Avery Co., Flat Rock	<i>Utech 8</i>	—		14	(1;10)
Va.: Smyth Co., Mt. Rogers	<i>Utech 9</i>	28	(2;12)	14	(3;20)

Table 2.—continued.

Species location	Voucher	2n	Root tip (clones; counts)	n	PMC (clones; counts)
W. Va.: Pocahontas Co., Cranberry Glades	<i>Utech 10</i>	28	(1;5)	14	(1;5)
W. Va.: Tucker Co., Thomas	<i>Utech 11</i>	28	(1;5)	14	(1;5)
Va.: Page Co., Stoney Man Cliffs	<i>Utech 12</i>	28	(1;5)	14	(1;10)
N. J.: Sussex Co., High Point	<i>Utech 13</i>	28	(2;8)	—	—
* N. Y.: Ulster Co., Woodland	<i>Utech 14</i>	28	(3;15)	14	(4;25)
N. Y.: Greene Co., Hunter Mt.	<i>Utech 15</i>	28	(2;10)	14	(1;5)
N. Y.: Greene Co., Cave Mt.	<i>Utech 16</i>	28	(1;5)	14	(1;5)
N. Y.: Warren Co., Lake George	<i>Utech 17</i>	28	(1;5)	—	—
N. Y.: Essex Co., Big Slide Mt.	<i>Utech 18</i>	—	—	14	—
* Vt.: Lamoille Co., Mt. Mansfield	<i>Utech 19</i>	28	(3;15)	14	(4;45) 12 _{II} +4 _I 13 _{II} +2 _I 14 _{II} +2 _I
Vt.: Lamoille Co., Smugglers Notch	<i>Utech 20</i>	28	(2;10)	14	(2;10)
Vt.: Lamoille Co., Spruce	<i>Utech 21</i>	28	(1;5)	14	(1;10)
N. H.: Grafton Co., Franconia	<i>Utech 22</i>	28	(1;5)	14	(1;10)
N. H.: Grafton Co., Twin Mt.	<i>Utech 23</i>	—	—	14	(3;15)
* N. H.: Coos Co., Fabyan	<i>Utech 24</i>	28	(4;20)	14	(5;40)
N. H.: Coos Co., Crawford's Notch	<i>Utech 25</i>	28	(1;5)	14	(2;10)
N. H.: Strafford Co., Durham	<i>Utech 26</i>	28	(1;5)	—	—
Pa.: McKean Co., Kane	<i>Utech 27</i>	28	(2;10)	—	—
* Mass.: Berkshire Co., Mt. Greylock	<i>Utech 28</i>	28	(3;20)	14	(3;20)
Me.: Aroostock Co., Van Buren	<i>Utech 29</i>	—	—	14	(1;5)
Que.: Bonaventure, Port Daniel	<i>Utech 30</i>	28	(1;6)	—	—
Que.: Gaspé, Percé, Mont Ste. Anne	<i>Utech 31</i>	28	(1;5)	—	—
Que.: Gaspé, Mont Ste. Pierre	<i>Utech 32</i>	28	(1;5)	—	—
Que.: Gaspé, Mt. Albert	<i>Utech 33</i>	28	(5;25)	14	(8;400) 13 _{II} +2 _I
Que.: Rivière-du-Loup	<i>Utech 34</i>	28	(1;4)	—	—
N. H.: Coos Co., Kidderville	<i>Utech 35</i>	28	(1;5)	—	—
N. H.: Coos Co., Mt. Washington	<i>Utech 36</i>	28	(5;30)	14	(8;80)
N. Y.: Tompkin Co., Ringwood Hollow	<i>Bates s. n.</i>	28	(1;5)	14	(1;10)
Ont.: Wellington, Puslinch, Crieff Bog	<i>Pringle 1233,</i> 1233a	28	(2;2)	—	—
Ont.: Wentworth Co., Freelon	<i>Pringle 1236,</i> 1236a	28	(2;2)	—	—
Ont.: Muskoka District, Torrance	<i>Pringle 1237,</i> 1237a	28	(2;2)	—	—
Mich.: Cheboygan Co., Douglas Lake	<i>Pringle 1224</i>	28	(1;1)	—	—
67 populations		84 clones 405 counts		196 clones 1551 counts	
<i>C. uniflora</i> (Menzies ex Schultes) Kunth					
Calif.: Tuolumne Co., Calvarnes Big Trees	<i>Utech 501</i>	28	(1;5)	—	—
Ore.: Jackson Co., Aspen Point	<i>Utech 502</i>	28	(2;10)	—	—
Ore.: Klamath Co., Crater Lake National Park	<i>Utech 503</i>	28	(1;8)	—	—
Ore.: Klamath Co., Crescent	<i>Utech 504</i>	28	(1;6)	—	—
Ore.: Klamath Co., Odell Lake	<i>Utech 505</i>	28	(3;18)	—	—

Table 2.—continued.

Species location	Voucher	2n	Root tip (clones; counts)	n	PMC (clones; counts)
Ore.: Deschutes Co., Cultus Lake	<i>Utech 506</i>	28	(1;5)	—	
Ore.: Lane Co., Limberlost	<i>Utech 507</i>	28	(4;20)	—	
Ore.: Linn Co., Santiam Junction	<i>Utech 508</i>	28	(1;9)	—	
Ore.: Linn Co., Lost Prairie	<i>Utech 509</i>	28	(3;20)	—	
* Ore.: Benton Co., Marys Peak	<i>Utech 510</i>	28	(2;15)	14	(3;25)
Ore.: Clatsop Co., Saddle Mt.	<i>Utech 511</i>	28	(1;8)	—	
* Ore.: Hood River Co., Mt. Hood	<i>Utech 512</i>	28	(5;20)	—	
Wash.: Lewis Co., La Wis Wis	<i>Utech 513</i>	28	(1;5)	—	
Wash.: Pierce Co., Silver Springs	<i>Utech 514</i>	28	(1;4)	—	
Wash.: King Co., Snoqualmie Pass	<i>Utech 515</i>	28	(1;5)	—	
Wash.: Clallam Co., Hurricane Ridge	<i>Utech 516</i>	28	(1;6)	—	
Wash.: Whatcom Co., Mt. Baker	<i>Utech 517</i>	28	(3;20)	—	
Wash.: King Co., Stevens Pass	<i>Utech 518</i>	28	(1;5)	—	
Wash.: Chelan Co., White Pine Creek	<i>Utech 519</i>	28	(1;4)	—	
Wash.: Chelan Co., Chiwaukum Creek	<i>Utech 520</i>	28	(1;3)	—	
Wash.: Spokane Co., Mt. Spokane	<i>Utech 521</i>	28	(1;5)	—	
Wash.: Pend Oreille Co., Sullivan Lake	<i>Utech 522</i>	28	(1;4)	—	
Wash.: Pend Oreille Co., Pass Creek Pass	<i>Utech 523</i>	28	(1;3)	—	
Wash.: Pend Oreille Co., Pass Creek Pass	<i>Utech 524</i>	28	(1;5)	—	
Idaho: Bonner Co., Priest River	<i>Utech 525</i>	28	(1;5)	—	
Mont.: Lincoln Co., Yaak River	<i>Utech 526</i>	28	(1;2)	—	
Mont.: Flathead Co., Lake McDonald	<i>Utech 527</i>	28	(1;4)	—	
* Mont.: Flathead Co., Yellow Bay	<i>Utech 528</i>	28	(3;25)	14	(3;30)
Wash.: Kittitas Co., Copper Lake	<i>Henderson 1</i>	—		14	(1;10)
Idaho: Latah Co., Harvard	<i>Henderson 2</i>	—		14	(1;10)
B. C.: Mt. Seymour	<i>Marchant 1</i>	28	(1;5)	—	
B. C.: Vancouver Island	<i>Marchant 2</i>	28	(1;5)	—	
B. C.: Vancouver Island	<i>Marchant 3</i>	28	(1;5)	—	
33 populations		48 clones 254 counts		6 clones 65 counts	

* Plants cultivated at the Missouri Botanical Garden, St. Louis, Missouri (MO).

These findings can be summarized as follows: in 10 populations of *C. andrewsi-ana*, $2n=28$ was observed in 130 somatic counts from 25 clones and $n=14$ in 40 meiotic counts from five clones; in *C. umbellulata*, $2n=28$ in 150 somatic counts from 29 clones and $n=14$ in 180 meiotic counts from 34 clones; in 67 populations of *C. borealis*, $2n=28$ in 402 somatic counts from 83 clones and $2n=32$ in three counts from one clone, while 1551 meiotic counts from 196 clones showed $n=14$; and in 33 populations of *C. uniflora*, $2n=28$ in 254 somatic counts from 48 clones and $n=14$ in 65 meiotic counts from six clones.

As a result of this survey, confidence can be placed in the $x=14$ chromosomal basis of the genus. It is significant that no polyploidy was found among the thousands of cells examined, nor was aneuploidy encountered in any species other than *C. borealis*. The latter species, with the most completely known cytogeography,

has a basic $2n=28$ cytotype throughout its range, while the $2n=32$ cytotype is very local. Close attention to the higher eastern mountain populations (Mt. Le Conte, Tenn., elev. 4200 ft.; Grandfather Mt., N. C., elev. 4300 ft.; Mt. Rogers, Va., elev. 4900 ft.; Spruce Knob, W. Va., elev. 4850 ft.; Mt. Mansfield, Vt., elev. 3800 ft.; Mt. Greylock, Mass., elev. 2900 ft.; Mt. Albert, Que., elev. 3000 ft.; and Mt. Washington, N. H., elev. 4900 ft.) revealed no additional $2n=32$ cytotypes. For example, five clones with 30 counts of $2n=28$ and eight clones with 80 counts of $n=14$ were determined for the Lakes-of-the-Clouds, Mt. Washington region, but no confirming $2n=32$ cytotype or univalents were encountered (Löve and Löve 1966). This survey discovered only two additional populations with univalents and meiotic abnormalities: one from Mt. Mansfield, Vermont and the other from Mt. Albert, Gaspé, Quebec.

2. Somatic karyotypes of the North American species of *Clintonia*

For each species of *Clintonia*, a pretreated root tip cell with metaphase condensed chromosomes was selected for flatness and evenness of spread and used to represent the karyotype of that species. The following karyotypic data for each species are presented: 1) a photographic plate showing the spread chromosomes (Fig. 3); 2) an idiogram plate with the same chromosomes ranked (Figs. 4 and 5); and 3) a table comparing the absolute arm and total lengths and centromeric indices (Table 3).

Fig. 3. Somatic chromosomes of the North American species of *Clintonia*. A, *C. andrewsiana* from Arcata, California (AND-610). B, *C. umbellulata* from Ohiopyle, Pennsylvania (UMB-208). D, *C. uniflora* from Flathead Lake, Montana (UNI-528). The scale is indicated with $2n=28$ for each species.

Table 3. Somatic chromosomes of the North American species of *Clintonia*

Pair no.	<i>C. andrewsiana</i>			<i>C. umbellulata</i>			<i>C. borealis</i>			<i>C. uniflora</i>		
	Length of arms in μ	Total in μ	Index	Length of arms in μ	Total in μ	Index	Length of arms in μ	Total in μ	Index	Length of arms in μ	Total in μ	Index
1	7.35+7.35	14.7	1.00	8.0+8.0	16.0	1.00	8.25+7.50	15.75	0.91	8.0+7.3	15.3	0.91
	7.35+7.35	14.7	1.00	8.2+8.0	16.2	0.98	8.12+7.50	15.62	0.92	7.8+7.3	15.1	0.94
2	7.4+6.6	14.0	0.90	7.3+7.3	14.6	1.00	7.75+7.50	15.25	0.97	7.3+6.6	13.9	0.90
	7.4+6.6	14.0	0.90	7.3+7.3	14.6	1.00	8.00+7.25	15.25	0.91	7.3+6.6	13.9	0.90
3	8.0+4.3	12.3	0.55	8.0+4.0	12.0	0.50	7.25+4.50	11.75	0.62	8.0+4.5	12.5	0.56
	7.3+4.3	11.6	0.59	8.0+4.0	12.0	0.50	7.25+4.50	11.75	0.62	8.0+4.3	12.3	0.54
4	6.6+3.4	10.0	0.51	7.3+4.0	11.3	0.55	7.00+4.25	11.25	0.60	6.6+4.3	10.9	0.65
	6.6+3.4	10.0	0.51	7.3+4.0	11.3	0.55	7.25+4.25	11.50	0.58	6.6+4.3	10.9	0.65
5	6.3+3.0	9.3	0.47	7.3+3.3	10.6	0.45	6.75+4.25	11.00	0.63	6.65+3.35	10.0	0.50
	6.0+3.0	9.3	0.47	7.3+3.3	10.6	0.45	6.25+4.25	10.50	0.68	6.65+3.35	10.0	0.50
6	6.0+3.0	9.0	0.50	6.6+3.7	10.3	0.56	7.25+3.50	10.75	0.48	6.6+3.2	9.8	0.48
	6.0+3.0	9.0	0.50	6.6+3.8	10.4	0.57	7.50+3.50	11.00	0.47	6.6+3.2	9.8	0.48
7	5.4+3.3	8.7	0.61	6.6+3.3	9.9	0.50	5.75+3.87	9.63	0.67	5.35+3.65	9.0	0.68
	5.4+3.3	8.7	0.61	6.6+3.3	9.9	0.50	5.75+4.00	9.75	0.70	5.35+3.65	9.0	0.68
8	5.2+3.0	8.2	0.58	6.0+3.3	9.3	0.55	6.00+3.50	9.50	0.58	5.35+3.65	9.0	0.68
	5.3+3.0	8.3	0.57	6.0+3.3	9.3	0.55	6.00+3.50	9.50	0.58	5.35+3.65	9.0	0.68
9	4.3+3.7	8.0	0.86	5.0+4.0	9.0	0.80	5.25+4.25x	9.50	0.81	4.9+4.0	8.9	0.82
	4.3+3.7	8.0	0.86	5.0+4.0	9.0	0.80	5.25+4.25	9.50	0.81	4.9+4.0	8.9	0.82
10	6.0+1.3+0.8	8.2	0.35	6.6+1.35+0.85	8.8	0.33	6.75+1.75+1.0	9.50	0.41	6.7+1.3+0.9	8.9	0.33
	6.0+1.3+0.8	8.2	0.35	6.6+1.35+0.85	8.8	0.33	6.75+1.75+1.0	9.50	0.41	6.7+1.3+0.9	8.9	0.33
11	4.3+3.5	7.8	0.82	4.7+4.0	8.7	0.85	4.75+4.25	9.00	0.89	4.3+4.0	8.3	0.93
	4.1+3.3	7.4	0.85	4.7+4.0	8.7	0.85	4.75+4.25	9.00	0.89	4.3+4.0	8.3	0.93
12	5.5+2.0	7.5	0.36	6.6+2.0	8.6	0.30	6.00+3.00	9.00	0.50	6.0+2.3	8.3	0.38
	5.5+2.0	7.5	0.36	6.6+2.0	8.6	0.30	6.00+3.00	9.00	0.50	6.0+2.3	8.3	0.38
13	6.5+0.8	7.3	0.12	7.0+1.2	8.2	0.17	6.62+1.50	8.12	0.23	7.0+1.0	8.0	0.14
	6.4+0.8	7.2	0.12	7.0+1.2	8.2	0.17	6.70+1.75	8.45	0.26	7.0+1.0	8.0	0.14
14	5.0+1.6	6.6	0.32	6.0+2.0	8.0	0.33	6.0+2.25	8.25	0.37	6.0+2.0	8.0	0.33
	5.0+1.6	6.6	0.32	6.0+2.0	8.0	0.33	6.0+2.50	8.50	0.42	6.0+2.0	8.0	0.33

An additional idiogram of the $2n=32$ cytotype of *C. borealis* from Spruce Knob, West Virginia is presented in Figure 6.

CLINTONIA ANDREWSIANA

C. UMBELLULATA

Fig. 4. Idiogram for *Clintonia andrewsiana* and *C. umbellulata*. Reproduced from Fig. 3. Scale indicated.

The karyotypes of the species of *Clintonia* may roughly be symbolized as follows:
C. andrewsiana (Figs. 3A and 4; Table 3)
 $K(2n)=28=4V+10J+6v+8j$ (2 NOR+6j).

C. umbellulata (Figs. 3B and 4; Table 3)

$$K(2n)=28=4V+10J+6v+8j \text{ (2 NOR}+6j\text{)}.$$

C. borealis (Figs. 3C, 5 and 6; Table 3)

$$K(2n)=28=VV+10J+6v+8j \text{ (2 NOR}+6j\text{)}.$$

$$K(2n)=32=4V+10J+6v+8j \text{ (2 NOR}+6j\text{)}+2j+2v.$$

C. uniflora (Figs. 3D and 5; Table 3)

C. BOREALIS

C. UNIFLORA

Fig. 5. Idiogram for *Clintonia borealis* and *C. uniflora*. Reproduced from Fig. 3. Scale indicated.

$$K(2n)=28=4V+10J+6v+8j \text{ (2 NOR+6j)}.$$

C. udensis (Utech + Suda 1975)

$$K(2n)=28=4V+12J+4v+8j \text{ (2 NOR+6j)}.$$

The diploid complements for all species of *Clintonia* are very nearly identical. All have four metacentric chromosomes which are consistently larger than the rest, 10 (or 12) medium length submetacentric chromosomes, six (or four) medium to small nearly metacentric chromosomes, two medium to small subtelocentric chromosomes with short NOR-satellite regions and six small chromosomes with subtelocentric to exceedingly subtelocentric centromeres. The only difference among the karyotypes of the five species is whether one pair of chromosomes is placed in the J-chromosomal class or the v-class. The 10 or 12 J-chromosomes form a decreasing size gradient with the centromeres becoming more metacentric with decreased size (Figs. 4 and 5; Table 3). The largest v-chromosomes which have submetacentric to metacentric centromeres are approximately the same size as the smallest J-chromosomes. Their centromeric indices (Table 3) are also quite similar, making their classification both difficult and relative.

The following chromosomes or groups of chromosomes are easily recognized in each species of *Clintonia*, including the Asian *C. udensis* (Utech and Suda 1975). The most conspicuous features are the four large metacentric chromosomes which have average lengths of 15.8μ . One pair of these four is consistently longer than the other (Figs. 4 and 5), which makes these two pairs readily discernable. The three small pairs of subtelocentric chromosomes are also easily recognized due to their size (6.0 – 9.0μ) and centromeric position. Another prominent marker is the NOR-chromosomal pair which is usually between 8.3 and 9.7μ long.

The 16 remaining chromosomes of the complement (8 pairs) have extremes which fall into two very different classes: medium length (10.5 – 12.5μ) submetacentric chromosomes, and small (7.5 – 8.7μ) metacentric chromosomes (Table 3). Usually four of the submetacentric larger pairs and two of the smaller metacentric pairs are clearly separated by size and centromeric position. There are however, usually two pairs whose lengths and centromeric position are intermediate and difficult to place into a given class. A difference of only 0.5μ for these chromosomes could change their classification. This small size is very near the source of error introduced by the pretreatment and the squash preparation.

A comparison of karyotype symmetries for the diploid *Clintonia* complements is presented in Table 4. The data for *C. udensis* (Utech and Suda 1975) indicated that there was no significant difference between the colchicine and 8-OH quinoline root tip pretreatments, since the long and short chromosome lengths, as well as their ratios, are essentially the same. For the North American species, pooled observations on 25 selected cells from both pretreatments were consequently used to calculate the long/short arm ratios and to estimate the proportion of the respective karyotypes with an arm ratio $>2:1$. The average lengths of the longest and shortest chromosomes of all five species are remarkably similar, as are their ratios and the proportion of their respective karyotypes with $>2:1$ arm ratios. Overall, the different karyotypes of the species of *Clintonia* are so remarkably similar that it is virtually impossible to infer any structural rearrangements or heterozygosity among their chromo-

somes (Figs. 3-5; Tables 3 and 4).

Each species has two nucleolar organizing chromosomes (NOR) that are subtelocentric and rank either in the tenth or ninth position according to size. The satellites (SAT) of these chromosomes were consistently found attached in all cells observed. High power observation (2000 $\times$) was rarely needed to detect the existence of connecting chromosomal material.

Table 4. Comparison of chromosomal lengths, ratio of longest to shortest chromosome and karyotype symmetry within the genus *Clintonia*

Species (Pretreatment)	Number of cells	Longest in μ	Shortest in μ	Ratio (L/S)	Karyotype proportion with > 2:1 arm ratio
<i>C. andrewsiana</i> (colchicine and 8-OH quinoline)	25	$\bar{x}=16.25$ $s=1.14$ 13.7-17.8	$\bar{x}=7.91$ $s=0.49$ 6.0-8.8	$\bar{x}=2.07$ $s=0.09$ 1.91-2.28	0.372
<i>C. umbellulata</i> (colchicine and 8-OH quinoline)	25	$\bar{x}=16.35$ $s=1.10$ 13.9-17.9	$\bar{x}=7.79$ $s=0.60$ 6.8-9.1	$\bar{x}=2.09$ $s=0.13$ 1.95-2.31	0.368
<i>C. udensis</i> ² (colchicine)	25	$\bar{x}=16.86$ $s=1.16$ 14.5-18.5	$\bar{x}=8.00$ $s=0.58$ 6.5-8.9	$\bar{x}=2.11$ $s=0.11$ 1.95-2.39	0.376
(8-OH quinoline)	25	$\bar{x}=16.79$ $s=1.08$ 14.7-19.0	$\bar{x}=8.09$ $s=0.54$ 6.7-9.0	$\bar{x}=2.08$ $s=0.11$ 1.92-2.39	0.372
<i>C. borealis</i> (colchicine and 8-OH quinoline)	25	$\bar{x}=16.45$ $s=1.20$ 14.0-18.8	$\bar{x}=8.10$ $s=0.51$ 6.4-8.8	$\bar{x}=2.08$ $s=0.15$ 1.79-2.39	0.374
<i>C. uniflora</i> (colchicine and 8-OH quinoline)	25	$\bar{x}=16.28$ $s=1.50$ 14.0-18.3	$\bar{x}=8.04$ $s=0.60$ 6.4-9.1	$\bar{x}=2.09$ $s=0.19$ 1.89-2.41	0.368

¹ Data from Utech and Suda (1975).

No aneuploidy was observed in any other species than *C. borealis*. In this species, the $2n=32$ cytotype (Fig. 6) differs from the normal $2n=28$ cytotype (Figs. 3C and 5) in having four additional chromosomes. Two are subtelocentric and two are nearly metacentric. These are pairs 15 and 16 respectively of Fig. 6.

3. Meiotic chromosomes in *Clintonia*

Considerable meiotic material was examined for each species in the cytogeographic survey (Table 2). A common feature in *Clintonia* is the continual synchrony

of the different meiotic events. The premeiotic interphase nuclei are of the reticulate or diffuse type, not the euchromocentric or prochromosomic type. The chiasma frequency is usually four to six per cell and chiefly the larger bivalents of each species are involved. They do not show any traces of terminalization. The most frequent metaphase I configuration is 14 regular bivalents (Table 2; also Utech and Thien 1973). Microphotographs of well-spread metaphase I bivalents for each species with $n=14$ are presented in Fig. 7. There is a good relative size correspondence between the meiotic karyotype and the somatic complement of each species. In such comparisons, the absolute sizes are quite different, but the relative lengths and centromeric positions are similar.

Fig. 6. Idiogram of the $2n=32$ cytotype of *Clintonia borealis* from Spruce Knob, West Virginia (BOR-1208b).

From $2n=28$ clones of *C. borealis*, PMC meiosis was analyzed from throughout the range. No additional populations or clones with the $2n=32$ cytotype were found, besides the initial one from Spruce Knob, West Virginia (Fig. 6), for which no meiotic material was available. However, there were 11 clones from four populations of the 207 clones and 67 populations surveyed whose univalent behavior suggested B-chromosomes (Table 5). These deviant counts represent 5.6 percent of the clones and 5.9 percent of the populations sampled. In total, 2651 meiotic figures of *C. borealis* were counted (Tables 2 and 5) and only 56 (2.1%) were deviant from the normal $n=14$. The distribution and frequency of these univalent B-chromosomes are shown in detail for these four populations (Table 5).

When considering these abnormalities it is necessary to stress that these peren-

nial clones, which have been described as atypical, actually have the normal $2n=28$ somatic karyotype. Their atypicality rests on observations of their meiotic behavior made during one spring, and it has not been established as a regular occurrence.

Table 5. Meiotic irregularities and univalent distribution in populations and clones of *Clintonia borealis*

Populations and meiotic configuration	Clone number					Totals and percents of uni- valents in:	
						Deviant clones	Whole population
Rocky Arbor, Juneau							
Co., Wis.	1-10	11	12	13	14		
14 _{II}	1000	89	97	99	94	379 (94.8%)	1379 (98.5%)
13 _{II} +2 _I	0	8	3	1	5	17 (4.2%)	17 (1.2%)
12 _{II} +4 _I	0	3	0	0	1	4 (1.0%)	4 (0.3%)
	1000	100	100	100	100	400	1400
Oostburg, Sheboygan							
Co., Wis.	1-6	7	8	9			
14 _{II}	300	87	99	95		283 (94.1%)	583 (97.2%)
13 _{II} +2 _I	0	12	1	5		16 (5.6%)	16 (2.7%)
12 _{II} +4 _I	0	1	0	0		1 (0.3%)	1 (0.1%)
	300	100	100	100		300	600
Mt. Mansfield, N. H.							
	1-4	5	6				
14 _{II}	400	95	97			192 (96.0%)	592 (98.7%)
13 _{II} +2 _I	0	4	3			7 (3.5%)	7 (1.2%)
14 _{II} +2 _I	0	1	0			1 (0.5%)	1 (0.1%)
	400	100	100			200	600
Mt. Albert, Gaspé,							
Quebec	1-8	9	10				
14 _{II}	400	99	91			190 (95.0%)	590 (98.7%)
13 _{II} +2 _I	0	1	9			10 (5.0%)	10 (1.7%)
	400	100	100			200	600

Three different types of metaphase configurations were observed: 13_{II}+2_I, 12_{II}+4_I, and 14_{II}+2_I. Miscounting is highly likely without specific attention to pairing. The occurrence of the 14_{II}+2_I configuration is the only known example of aneusomaty in *Clintonia*. The 13_{II}+2_I configuration is more common than either 12_{II}+4_I or 14_{II}+2_I. Among the four deviant populations (Table 5), the percentage of univalent cells from only atypical clones varied between 4.0 and 5.9 percent or 1.3-2.8 percent when the entire population was considered. Within these populations normal meiosis is very common. For example, at Rocky Arbor, Wisconsin, ten normal clones with a total of 1000 counts of $n=14$ were established besides the four atypicals. The percentages of deviant counts observed for all clones of this population was only 1.5 percent. These tentative values of meiotic dysfunction are probably over-estimates, since once a population was shown to have clones with univalents additional material was examined. Consequently the actual frequency of the univalents range-wide is low, probably near or below one percent.

The cytology of the B-chromosomes and other chromosomal abnormalities in *C. borealis* is presented in Fig. 8. The $12_{II}+2_I$ pattern for two different cells is shown in Fig. 8A+B. Additional cells with the $12_{II}+2_I$ and $12_{II}+4_I$ are presented in Utech and Thien (1973). All univalents in this species are small ($3.0-5.5 \mu$)

Fig. 7. Meiotic chromosomes of the North American species of *Clintonia*. A, *C. andrewsiana* from Arcata, California (AND-610). B, *C. umbellulata* from Ohiopyle, Pennsylvania (UMB-208). C, *C. borealis* from Rocky Arbor State Park, Wisconsin (BOR-108). D, *C. uniflora* from Flathead Lake, Montana (UNI-528). The scale is indicated with $n=14$ for each species.

and exhibit abnormal centromeric behavior. At both diplotene and pachytene these univalents are more tightly condensed and darkly stained than the other chromosomes. Metaphase I alignment among these abnormal chromosomes is frequently precocious. Bridges with and without fragments have also been observed at anaphase I and II (Fig. 8, C-E). Micronuclei were occasionally observed at telophase II, suggesting the non-transmission of these lagging chromosomes (Fig. 8F).

The univalents fall into two categories: 1) metacentric and small ($3.0-4.5 \mu$) and 2) submetacentric and slightly longer ($4.0-5.5 \mu$). Since the relative lengths and centromeric position of these univalents appear to correspond with chromosomal pairs 15 and 16 of the $2n=32$ cytotype (Fig. 6), it is suggested that univalents may be involved in the formation of the rare $2n=32$ cytotype.

Fig. 8. Cytology of B-chromosomes and other chromosomal abnormalities in *Clintonia borealis*. A, two precocious univalents in the 13 II + 2 I configuration from BOR-108. 900 $\times$. B, two precocious univalents in the 13 II + 2 I configuration, BOR-109. 900 $\times$. C, anaphase I bridge, BOR-108. 900 $\times$. D, fragment without centromere, first division meiosis, BOR-19. 800 $\times$. E, anaphase II bridge, BOR-109. 900 $\times$. F, micronuclei at late telophase II, BOR-33. 900 $\times$.

Discussion and summary

The genus *Clintonia* as part of the Arcto-Tertiary (Tertiario-Mesophytic) Geoflora shows the predicted disjunction between eastern Asia and eastern and western North America. Such disjunctions are well known for many plant genera (Li 1952, Graham 1972, Hara 1952, 1956, 1970, Cain 1944, Hultén 1937, Wulff 1950) and are characteristic of many genera of the liliaceous tribe Polygonatae: *Polygonatum*, *Smilacina*, *Maianthemum*, *Disporum* and *Streptopus*. Many of these genera have their major center in eastern Asia with secondary centers in western and eastern North America and even Central America, as *Smilacina* (Emons 1945, Kawano and

Iltis 1963a).

Clintonia is somewhat anomalous in that its present center of distribution is North America, not Asia. There are two species in both eastern and western North America, and each area has one species that is very restricted in range (paleo-endemic) and another that is widely distributed. Among the latter, both *C. borealis* and *C. uniflora* have distributions which occupy considerable areas glaciated during the Pleistocene. These two American species pairs (Figs. 1 and 2) form an interesting phyto-geographical comparison in post-Pleistocene plant migration and colonization. The Asian *C. udensis*, which is also wide-ranging and part of the Sino-Japanese floristic region (Hara 1966, Kanai 1963, 1966, Wang 1961, Utech 1973), occupies areas which have been influenced by the Pleistocene ice-age, especially in the Himalayan regions (Pahuja and Kumar 1971, Tanai 1972).

The somatic karyotypes for all species of *Clintonia* are remarkably similar. Taken together, they can be roughly represented by the formula: $K(2n)=28=4V(2V_1+2V_2)+10J+6v+8j(2\text{ NOR}+6j)$. The lack of significant structural rearrangement or chromosomal heterozygosity suggests that evolution within this genus has occurred on the gene level rather than the chromosomal level. The vast morphological and ecological differences between these species of *Clintonia* (Utech 1973) are not manifested in their karyology.

Among species of the different Polygonatae genera, a distinctive basikaryotype for a genus is not uncommon, even though there exists a wide range of morphological and ecological differentiation. In *Maianthemum* (Kawano, Ihara, Suzuki and Iltis 1967), there is virtually no karyotypic differentiation among the distinct and disjunct species. A similar situation is also observed in the genus *Convallaria* (Streveler 1966) and *Smilacina* (Kawano and Iltis 1963a, 1966). Such species, as well as others of the tribe, are rhizomatous and form stable clones of considerable longevity (Whitford 1949). This may, in part, account for their karyotypic stability and similarity in evolutionary time (Stebbins 1958, 1966, Baker 1959). On the other hand, clear examples of karyotypic differentiation are found between the sections of *Polygonatum* and *Disporum*. In the latter for example, the Asian section *Eudisporum* has large chromosomes ($10-22\ \mu$), while section *Prosartes* has small chromosomes ($4-6\ \mu$) and is restricted to North America (Therman 1956, Hasegawa 1932, Kumar 1965, Jones 1951, Ownbey 1953).

A striking similarity is evident, however, between the karyology of *Clintonia* and certain genera of the Polygonatae. Karyotypic formulas have been summarized for the following species of these genera: *Smilacina* (Kawano and Iltis 1963a, 1966, Kumar 1956, 1960): $K(2n)=36=6V+14V+14J+16v$ (or $6j+10v$); *Maianthemum* (Kawano, Ihara, Suzuki and Iltis 1967): $K(2n)=36=4V(2V_1+2V_2)+16J+16v$ (possibly $8j+8v$); *Polygonatum* 'pro parte' (Eigsti 1942, Suomalainen 1947, Therman 1949, 1950, 1956, Kawano and Iltis 1963b): $K(2n)=18=4V(2V+2\text{ NOR})+8J+6v$ and $K(2n)=20=4V(2V+2\text{ NOR})+8J+6v+2j$. The species of these different genera all have two to six large metacentric chromosomes and in each case, represent the largest chromosomes of their respective complements. These species also have two nucleolar organizing chromosomes. Two additional classes of chromosomes can also be noted in these karyotypes: a variable number of medium to small submeta-

centric to subtelocentric chromosomes, and several small subtelocentric chromosomes.

Other cytological similarities exist between these genera of the Polygonatae. They have large chromosomes, interphase nuclei of the reticulate type and a relatively low chiasma frequency with chiasmata which do not terminalize (Therman 1956, Utech 1974). There is also a second group within the tribe (*Streptopus* and *Disporum* section *Prosartes*) which has small chromosomes, euchromocentric resting nuclei and one or two chiasmata per bivalent which frequently terminalize (Therman 1956). *Clintonia* clearly has its affinities with the former group.

There is no apparent common base number for the genera of the Polygonatae. The haploid chromosome numbers for the large chromosome genera are *Polygonatum* section *Alternifolia* $n=9, 10$ (polyploids), section *Verticillata* $n=14$ and section *Oppositifolia* $n=15$; *Maianthemum* $n=18$; *Smilacina* $n=18$ (polyploids); *Clintonia* $n=14$; and *Disporum* section *Eudisporum* $n=7, 8$ and 9 , while for the small chromosome genera: *Streptopus* $n=8$ (polyploids) and *Disporum* section *Prosartes* $n=6, 8, 9$ and 11 (Utech 1973, from various sources). The high haploid number for most genera suggests that ancient allo- and/or autopolyploidy has played a role in their evolution. A comparison of these karyotypes shows that *Clintonia* is among the most symmetrical within the Polygonatae (Table 4; Pahuja and Kumar 1971). On the other hand, the karyotype symmetry of the reported polyploids of *Polygonatum*, *Smilacina* and *Streptopus* suggests a recent origin for them relative to their haploid karyotypes. Stabilized aneuploidy and B-chromosomes in euploid species are common throughout the tribe, but no linking clue for a reductional series is apparent from the reported counts. This array of karyological information indicates that the genera are long separated ends of reticulate evolution and today represent a rather heterogenous tribe or an unnatural taxonomic assemblage.

In this study of the cytology of *Clintonia*, no polyploids were observed. The $2n=28$ and $n=14$ karyotype was found consistently throughout the ranges of all species. Aneuploidy is represented by the rare $2n=32$ cytotype in *C. borealis*, and aneusomy by an even rarer occurrence of $n=14_{II}+2_I$. Four populations in this species with a normal $2n=28$ cytotype exhibited meiotic irregularities, including two to four univalents which behaved as B-chromosomes. These univalents could, if transmitted, give rise to the $2n=32$ or other cytotype. This would, however, constitute an extremely rare event. In *Clintonia*, the rhizome system is highly conservative of base chromosome numbers due to clonal longevity. Given the unvaried $2n=28$ number prevalent throughout the genus, and *C. borealis* in particular, it seems likely that the $2n=32$ cytotype constitutes a recent evolutionary event in which the number has risen, rather than representing a relictual population of an ancient $X=16$ *Clintonia* (Stebbins 1971).

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