Cytogenetic Characterization and Nuclear DNA Content of Diploid and Tetraploid Forms of Stokes Aster

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Abstract. *Stokesia laevis* (J. Hill) Greene is a herbaceous perennial native to the southeastern United States. Most cultivars of *Stokesia* are diploid ($2n = 2x = 14$) except for ‘Omega Skyrocket’, a tetraploid ($2n = 4x = 28$) form selected from a natural population. A comparative study of the karyotypes and meiotic behavior of diploid cultivars, seed-derived accessions of ‘Omega Skyrocket’, synthetically derived autotetraploids, and triploid progeny from these taxa strongly suggest that ‘Omega Skyrocket’ is an autotetraploid form of *Stokesia*. Total karyotype length, 161 μm and 293 μm , and average chromosome length, 11.5 μm and 10.5 μm , of the diploid cultivars and tetraploid accessions of ‘Omega Skyrocket’, respectively, were determined. The karyotype of the diploid cultivars consisted of eight metacentric (m) and six submetacentric (sm) chromosomes with average arm ratio values ranging from 1.12 to 2.06. The karyotype of ‘Omega Skyrocket’ consisted of 23 m chromosomes and 5 sm chromosomes with average arm ratio values ranging from 1.22 to 2.02. Meiotic pairing in the diploids was normal. No meiotic irregularities such as laggards or bridges were observed and disjunction was balanced (7:7). Accessions of ‘Omega Skyrocket’ demonstrated a high frequency (60%) of quadrivalent formation; however, later stages of meiosis were regular with balanced disjunction (14:14) occurring in 95% of the cells. Meiotic configurations in synthetically derived autotetraploids and triploid hybrids from crosses of diploid cultivars $\times$ ‘Omega Skyrocket’ consisted of univalents, bivalents, trivalents, quadrivalents, and pentavalents. Abnormalities, including laggards, unequal and/or premature disjunction, chromosome bridges, and chromosome stickiness were observed. Average nuclear 2C DNA content was 20.3 pg for the diploid cultivars and 39.9 pg for the newly synthesized autotetraploids. Average nuclear 2C DNA content for ‘Omega Skyrocket’ was 37.3 pg, which was 8.2% less than twice the average 2C DNA content of the diploid accessions and 6.4% less than the newly synthesized autotetraploids, suggesting that genomic downsizing in ‘Omega Skyrocket’ has occurred. Similarity of the karyotypes of the diploids and ‘Omega Skyrocket’ and the slight reduction in nuclear DNA content suggest that ‘Omega Skyrocket’ has diverged little from its original diploid progenitor.

Stokesia (J. Hill) Greene is a monotypic genus comprised of a single species (*S. laevis*) that is native to the coastal plain of the southeastern United States (i.e., Mississippi,

Louisiana, Alabama, Georgia, northern Florida, and South Carolina) (Bailey, 1949; Gunn and White, 1974). *Stokesia* is a member of the Vernonieae tribe (Asteraceae) that includes 121 genera and ≈ 1500 species with global distribution (Robinson, 1999a, 1999b, 2007). There are 21 species of Vernonieae representing four genera (i.e., *Vernonia* Schreb., *Stokesia*, *Elephantopus* L., *Pseudelephantopus* Rohr) found in the southeastern United States with 19 of these being indigenous (Gunn and White, 1974; Jones, 1982). *Stokesia* is the only genus, however, that is restricted to the United States (Gettys and Werner, 2002; Jones, 1982).

Members of the Vernonieae tribe are extremely diverse in form, habitat, and ecol-

ogy, indicating that this tribe has evolved and radiated over time. Examples of this diversity include the woody tree, *Vernonia arborea* Ham., which is the tallest species (greater than 30 m) in the Asteraceae family and the small aquatic plant, *Pacourina edulis* Aubl., which has edible leaves. Additionally, there are a number of small acaulescent perennial taxa (e.g., *Vernonia guineensis* Benth., *V. acrocephala* Klatt, *V. chthonocephala* O. Hoffm.) that thrive in fire-maintained savannas in Africa (Jones, 1977, 1982). In general, members of the Vernonieae tribe are perennial, herbaceous plants, shrubs, small trees, or vines with alternate leaves showing pinnate venation. Flowers characteristically form large homogamous discoid heads with one to many perfect flowers that are deep purplish red to blue or sometimes white (Jones, 1982). Base chromosome number (x) is highly variable in the Vernonieae tribe. New World taxa have a basic chromosome number of $x = 8, 10$ –19 and often include many polyploid forms (e.g., $n = 20, 26$ –30, 33–39, 51, 68) (Jones, 1977; Ruas et al., 1991). In contrast, most Old World taxa have a lower base number of 9 or 10 (Jones, 1977). Despite their overall diversity, all members of this tribe have uniquely shaped “Vernonioid” styles that are semicylindrical with acutely branched tips that present the hirsute surface of the stigma along the interior (Jones, 1982).

Stokesia has many of the essential features (e.g., perennial life cycle, alternate leaves, and “Vernonioid” style) that are typical of the Vernonieae tribe; however, floral and cytological characteristics of *Stokesia* are distinct. Specifically, *Stokesia* has zygomorphic flowers with ligulate ray florets that are deeply five-lobed and a reduced deciduous pappus, which are more characteristic of the Mutiseae and Lactuceae tribes (Jansen et al., 1991; Jones, 1977, 1982; Robinson, 1999c). Additionally, *Stokesia* has a low chromosome base number ($x = 7$) and large chromosomes, which are atypical of most Vernonieae (Gaus et al., 2005; Gunn and White, 1974; Jones, 1974). Despite these differences, *Stokesia* is consistently placed in the Vernonieae tribe (Jansen et al., 1991; Keeley et al., 2007; Keeley and Jansen, 1994; Kim et al., 1998; Kim and Jansen, 1995).

Stokesia is an attractive genus with large aster-like flowers that are characteristically pale blue or lavender in color with some cultivars having violet, pale yellow, pale pink, or albescent-colored flowers. Cultivars of *Stokesia* are 30 to 60 cm tall with many, moderately (three to five) branched flower scapes. Exceptions include ‘Omega Skyrocket’ [minimally (one to three) branched flower scapes greater than 1 m] and ‘Peachies Pick’ [highly (five to seven) branched flower scapes 70 to 80 cm] (Gettys and Werner, 2002).

‘Omega Skyrocket’ is an atypical example of stokes aster that was derived from a wild population discovered in Colquitt County, GA, near the town of Omega by R. Determann, S. Determann, and O. Johnson of

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the Atlanta Botanical Garden (Gettys and Werner, 2002). This natural population was uniform and consisted of plants with tall (greater than 1 m), upright flower scapes. 'Omega Skyrocket' is primarily sold by seed; however, some clonal accessions are also available.

Preliminary work demonstrated that cultivars of *Stokesia* studied thus far are diploids ($2n = 2x = 14$) except for 'Omega Skyrocket', which is a tetraploid form ($2n = 4x = 28$) (Gaus et al., 2005). The cytological characteristics of mitosis and meiosis as well as an absolute genome size estimate for this species have not been described. The objectives of this work were to: 1) prepare a karyotype; 2) characterize the meiotic behavior of diploid, triploid, and tetraploid accessions of *Stokesia laevis*, including 'Omega Skyrocket'; and 3) determine the absolute nuclear 2C DNA content of diploid and tetraploid plants using flow cytometry.

Materials and Methods

Plant material. Diploid accessions of *Stokesia* ('Alba', 'Colorwheel', 'Honeysong Purple', 'Mary Gregory', 'Peach Melba', 'Peachies Pick', 'Purple Parasols') were acquired and maintained as asexually propagated clones with the exception of 'Alba', which was grown from true-to-type seed. Accessions of the naturally occurring tetraploid, 'Omega Skyrocket', were grown from seed acquired from Jelitto Seed Co. (Schwarmstedt, Germany). Triploid plants were produced by crossing 'Alba', 'Peach Melba', 'Peachies Pick', and 'Purple Parasols' with four different accessions of 'Omega Skyrocket'. Synthetic autotetraploid forms of diploid cultivars were produced using colchicine [N-(5,6,7,9-tetrahydro-1,2,3,10-tetra-methoxy-9-oxobenzo(a)heptalen-7-yl)acetamide] or oryzalin (3,5-dinitro- N^4N^4 -dipropylsulfanilamide). Small (2.5 cm diameter) asexually reproduced plantlets were fully submerged for 24 to 48 h in a saturated solution of 0.2% oryzalin, prepared by diluting 1 mL Surflan® A.S. (United Phosphorus Inc., Trenton, NJ) (40.4% oryzalin) in 200 mL distilled water, or one to two drops ($\approx 250 \mu\text{L}$) of 2.0% colchicine were applied twice daily (0800 HR, 1600 HR) directly to the meristematic whorl of each plantlet for 3 d. Plants used for cytological examination were grown in sand under ambient light conditions in the greenhouses at North Carolina State University, Raleigh. Daytime temperatures in the greenhouse ranged from 25 to 35 °C. Plants were fertilized daily with 100 mg·L⁻¹ nitrogen of Peters 20N-8.7P-16.6K General Purpose or Peters EXCEL Cal-Mag 15N-2.2P-12.5K fertilizer (The Scotts Co., Marysville, OH).

Cytological techniques. Roots were collected between 1000 HR and 1200 HR on warm, sunny days. Roots were pretreated in a solution of 2 mM 8-hydroxyquinoline plus colchicine (0.5 g·L⁻¹) for 2 to 3 h at 4 °C (Dhesi and Stalker, 1994), fixed in freshly prepared Carnoy's solution (1 part glacial

acetic acid:3 parts chloroform:6 parts 95% ethanol) for 48 h at room temperature (RT), and stored at 4 °C in 70% ethanol. Roots were hydrolyzed in 1 N HCl at 60 °C for 8 min, washed in distilled water, and stained in Schiff's reagent for a minimum of 30 min in the dark. Root tips were removed and squashed in 1% acetocarmine. Cells were observed using a light microscope (Carl Zeiss Photomicroscope 3; Carl Zeiss MicroImaging, Inc., Thornwood, NY) under $\times 630$ and $\times 1000$ magnification. Digital photographs of well-spread metaphase plates were taken using a Sony Cybershot F717 camera (Tokyo, Japan). Total karyotype length (TKL), the total length of individual chromosomes (TCL), and the length of the short (SA) and long (LA) arm of each chromosome were measured from digital photographs using MicroMeasure version 3.3 software (Reeves, 2001). Average chromosome length (ACL) and arm ratio (AR = LA/SA) were calculated from these measurements. Ideograms were constructed based on chromosome length and arm ratios. Chromosome nomenclature followed Levan et al. (1964) (m = metacentric, sm = submetacentric, st = subtelocentric). Intrachromosome asymmetry (A_1) was calculated following the formula $A_1 = 1 - [\Sigma(s_i/l_i)/n]$, where s_i is the average length of the short arms in every chromosome, l_i is the average length of the long arm of every chromosome, and n is the number of chromosomes. This asymmetry index was adapted from the formula presented in Romero Zarco (1986), which calculated A_1 using measurements of homologous chromosome pairs, not individual chromosomes. Interchromosome asymmetry (A_2) was calculated following the formula $A_2 = SD/x$, where SD is the standard deviation and x is the mean of the chromosome lengths (Romero Zarco, 1986). The index of karyotype symmetry (TF% = $100 \Sigma S \Sigma L^{-1}$) was also calculated (Huziwara, 1962).

Meiotic chromosomes were observed in developing pollen mother cells (PMCs). Immature buds (1 to 1.5 cm in diameter) were collected on bright sunny days between 0900 HR and 1000 HR and the sepals were removed. Buds were fixed in freshly prepared Carnoy's solution for 48 h at RT and stored in 70% ethanol at 4 °C. Flower buds were dissected under a dissecting microscope and three to six anthers were removed and squashed in 1% acetocarmine. Cells were observed at $\times 630$ and $\times 1000$ magnification.

Nuclear DNA content determination. Absolute nuclear 2C DNA content was determined using a Becton-Dickinson FACSscan flow cytometer (San Jose, CA) equipped with a 488-nm argon laser. *Pisum sativum* L. 'Citrad', with a known 2C DNA content of 8.76 pg (Greilhuber et al., 2007), was used as an internal standard. Nuclei of the internal standard and sample were extracted and stained simultaneously according to protocols provided with the Partec CyStain PI Absolut P kit (Partec GmbH, Munster, Germany). Cold (0 °C) extraction buffer (500 μL) was added to $\approx 2 \text{ cm}^2$ of newly expanded

leaf tissue of the internal standard and sample and finely chopped for 30 s and incubated for 30 s at RT. The solution was filtered using Partec CellTrics™ disposable filters (Munster, Germany) with a pore size of 50 μm . Cold (0 °C) staining buffer (1 mL staining buffer, 12 μL of PI stock, 6 μL RNase stock) was added and samples were incubated for 2 h at 4 °C protected from light before they were analyzed. Replications (four to five) over 2 d were analyzed for each plant with 5,000 to 10,000 nuclei analyzed per replication. Peaks used for analysis had coefficient of variation values of 6% or less. Conversion of mass values into number of base pairs (bps) was calculated using the factor 1 pg = 978 Mbp (Doležel et al., 2003).

Statistical analysis. Data were subjected to analysis of variance using the PROC GLM procedure and means were separated by least significant difference ($P \leq 0.05$) using SAS 9.1 software (SAS Institute, Cary, NC).

Results

Karyotype. Somatic chromosomes from seven diploid ($2n = 2x = 14$) accessions ('Alba', 'Colorwheel', 'Honeysong Purple', 'Mary Gregory', 'Peach Melba', 'Peachies Pick', 'Purple Parasols') and three seed-derived accessions of 'Omega Skyrocket' (OSR) ($2n = 4x = 28$) were studied (Fig. 1). In all cases, chromosome counts were consistent with previous reports (Gaus et al., 2005; Gunn and White, 1974). There were few differences among the karyotypes of the

Fig. 1. Somatic chromosomes of *Stokesia laevis* (A) 'Peachies Pick' ($2n = 2x = 14$) and (B) 'Omega Skyrocket' ($2n = 4x = 28$). Scale bar = 10 μm .

diploid cultivars; thus, a single representative karyotype was constructed based on 33 well-spread metaphase plates (14, 'Honeysong Purple'; 14, 'Peachies Pick'; 5, 'Alba') (Fig. 2A). Similarly, there were few differences among the accessions of 'Omega Skyrocket', so one karyotype was constructed from 35 well-spread metaphase plates (14, OSR100; 15, OSR182; 6, OSR200) (Fig. 2B). Because chromosome morphology was very similar, individual chromosomes could not be definitively identified in all cases, thus preventing the direct comparison of chromosomes from the diploid accessions to the chromosomes of 'Omega Skyrocket'.

Average TKL for the four diploid accessions was $161 \pm 29 \mu\text{m}$ with an ACL of $11.5 \pm 2.0 \mu\text{m}$. Chromosomes ranged in length from $8.3 \mu\text{m}$ to $16.1 \mu\text{m}$ (Table 1). The average karyotype of the diploids consisted of eight metacentric (m) chromosomes with average ARs ranging from 1.12 to 1.58 and six submetacentric (sm) chromosomes with average ARs ranging from 1.72 to 2.06 (Table 1; Fig. 2). The average TKL was $293 \pm 53 \mu\text{m}$ and ACL was $10.5 \pm 1.9 \mu\text{m}$ for the three tetraploid accessions of 'Omega Skyrocket'. Chromosomes ranged in size from $7.3 \mu\text{m}$ to $15.4 \mu\text{m}$. The average karyotype of 'Omega Skyrocket' consisted of 23 m chromosomes with average ARs ranging from 1.22 to 1.68 and five sm chromosomes with average ARs ranging from 1.71 to 2.02 (Table 1; Fig. 2). The TKL ($293 \mu\text{m}$) for 'Omega Skyrocket' was $\approx 9\%$ less than two times the average TKL ($161 \times 2 = 322 \mu\text{m}$) of the diploids. The overall symmetry of the karyotypes of 'Omega Skyrocket' and the diploid accessions were very similar. Karyotype symmetry (TF%) was 66.68% and 67.93% for the diploids and 'Omega Skyrocket', respectively. Intrachromosome asymmetry (A_1) and interchromosome asymmetry (A_2) indices were 0.341 and 0.277 for the diploids and 0.328 and 0.289 for 'Omega Skyrocket', respectively.

Meiotic behavior. Meiosis was observed in PMCs of diploids, triploids, tetraploid accessions of 'Omega Skyrocket', and synthetic autotetraploids (Tables 2 and 3; Figs. 3–8). Chromosome pairing in the diploids was normal with each bivalent, usually possessing two chiasmata (Fig. 3). No meiotic irregularities such as laggards or bridges were observed and disjunction was equal (7:7) at later stages of meiosis. Average chromosome configuration of triploid taxa was $4.9\text{I} + 5.2\text{II} + 1.9\text{III}$ (Table 2). The most frequent configurations were $2\text{III} + 5\text{II} + 5\text{I}$, $3\text{III} + 4\text{II} + 4\text{I}$, and $1\text{III} + 6\text{II} + 6\text{I}$, which accounted for 25%, 19%, and 18% of the cells, respectively (Fig. 4, data not shown). Lagging chromosomes (one to five) were present in either Anaphase I or Anaphase II in 53% of the 34 PMCs observed (Fig. 5). Disjunction at Anaphase I and II in triploid cells without lagging chromosomes produced nearly balanced cells (63%) with chromosome configurations of 10:11 and unbalanced configurations of 9:12 (31%) and 8:13 (6%), respectively (data not shown, Fig. 5). The

Fig. 2. Karyotype ideograms of *Stokesia laevis*. (A) Average karyotype of 'Honeysong Purple', 'Alba', 'Peachies Pick' ($2n = 2x = 14$). (B) 'Omega Skyrocket' ($2n = 4x = 28$). Metacentric chromosomes (AR < 1.7) in gray. Submetacentric chromosomes (AR ≥ 1.7) in black. Scale bar = $2 \mu\text{m}$.

Table 1. Karyotypic parameters of *Stokesia laevis*.

		Arm ratio ^z		Chromosome length (μm) ^y			
Diploid cultivars		Omega Skyrocket		Diploid cultivars		Omega Skyrocket	
C1 ^x	1.12 $\pm$ 0.14	C1	1.22 $\pm$ 0.17	C1	16.09 $\pm$ 3.16	C1	15.37 $\pm$ 3.42
C2	1.30 $\pm$ 0.20	C2	1.29 $\pm$ 0.25	C2	15.22 $\pm$ 2.67	C2	14.47 $\pm$ 3.05
		C3	1.36 $\pm$ 0.30			C3	14.01 $\pm$ 2.83
		C4	1.31 $\pm$ 0.22			C4	13.70 $\pm$ 2.75
C3	1.21 $\pm$ 0.18	C5	1.31 $\pm$ 0.21	C3	14.54 $\pm$ 2.56	C5	13.34 $\pm$ 2.65
C4	1.39 $\pm$ 0.16	C6	1.36 $\pm$ 0.24	C4	13.89 $\pm$ 2.54	C6	13.07 $\pm$ 2.62
		C7	1.35 $\pm$ 0.22			C7	12.76 $\pm$ 2.50
		C8	1.33 $\pm$ 0.21			C8	12.24 $\pm$ 2.43
C5	1.85 $\pm$ 0.29	C9	1.60 $\pm$ 0.30	C5	11.70 $\pm$ 1.94	C9	11.21 $\pm$ 2.35
C6	1.72 $\pm$ 0.24	C10	1.60 $\pm$ 0.30	C6	11.26 $\pm$ 1.97	C10	10.84 $\pm$ 2.13
		C11	1.80 $\pm$ 0.26			C11	10.53 $\pm$ 2.01
		C12	1.63 $\pm$ 0.24			C12	10.34 $\pm$ 1.97
C7	1.96 $\pm$ 0.24	C13	1.75 $\pm$ 0.25	C7	11.00 $\pm$ 1.98	C13	10.15 $\pm$ 1.91
C8	1.76 $\pm$ 0.29	C14	1.44 $\pm$ 0.27	C8	10.76 $\pm$ 1.93	C14	9.99 $\pm$ 1.90
		C15	1.61 $\pm$ 0.29			C15	9.85 $\pm$ 1.85
		C16	1.66 $\pm$ 0.36			C16	9.69 $\pm$ 1.78
C9	1.88 $\pm$ 0.35	C17	1.78 $\pm$ 0.40	C9	10.47 $\pm$ 1.87	C17	9.51 $\pm$ 1.77
C10	2.06 $\pm$ 0.33	C18	2.02 $\pm$ 0.37	C10	10.06 $\pm$ 1.90	C18	9.31 $\pm$ 1.68
		C19	1.61 $\pm$ 0.33			C19	9.12 $\pm$ 1.66
		C20	1.71 $\pm$ 0.32			C20	8.95 $\pm$ 1.63
C11	1.49 $\pm$ 0.31	C21	1.66 $\pm$ 0.29	C11	9.60 $\pm$ 1.75	C21	8.81 $\pm$ 1.59
C12	1.56 $\pm$ 0.33	C22	1.68 $\pm$ 0.54	C12	9.25 $\pm$ 1.80	C22	8.60 $\pm$ 1.51
		C23	1.66 $\pm$ 0.38			C23	8.45 $\pm$ 1.48
		C24	1.56 $\pm$ 0.36			C24	8.33 $\pm$ 1.48
C13	1.58 $\pm$ 0.29	C25	1.57 $\pm$ 0.58	C13	8.95 $\pm$ 1.72	C25	8.18 $\pm$ 1.47
C14	1.34 $\pm$ 0.32	C26	1.55 $\pm$ 0.31	C14	8.30 $\pm$ 1.63	C26	8.01 $\pm$ 1.47
		C27	1.50 $\pm$ 0.33			C27	7.78 $\pm$ 1.41
		C28	1.38 $\pm$ 0.29			C28	7.28 $\pm$ 1.35

^zArm ratio = length of long arm/length of short arm $\pm$ SD.

^yValue in (μm) $\pm$ SD.

^xChromosome number.

Table 2. Chromosome associations in pollen microsporocytes of different cytotypes of *Stokesia laevis*.

Sample	Ploidy	No. of plants	No. of PMCs	Frequency and type of chromosome pairing per PMC ^z					
				Univalents	Bivalents	Trivalents	Quadrivalents	5-valents	6-valents
2x	2x	6	347	0.0 b ^y	7.0 a	0.0 b	0.0 c	0.0 a	0.0 a
3x w/OSR	3x	5	415	4.9 (1–9) a	5.2 (2–9) b	1.9 (0–5) a	0.0 (0–1) c	0.0 a	0.0 a
OSR	4x	2	295	0.1 (0–2) b	5.5 (0–12) c	0.1 (0–2) b	4.2 (1–7) b	0.0 a	0.0 a
4x syn	4x	8	198	0.3 (0–4) b	2.8 (0–10) d	0.3 (0–4) b	5.3 (0–7) a	0.0 (0–1) a	0.0 (0–1) a

^zValues = average (range).

^yValues with different letters within a column are significantly different (least significant difference, $P < 0.05$). Values were transformed before analysis to accommodate statistical comparisons across different ploidy levels.

PMC = pollen mother cell; 2x = diploid cultivars; 3x w/OSR = triploids produced from crosses of diploid cultivars × ‘Omega Skyrocket’; OSR = ‘Omega Skyrocket’ accessions; 4x syn = synthetically derived tetraploids.

Table 3. Average nuclear DNA content of different *Stokesia laevis* as determined by flow cytometric measurements of PI-stained nuclei.

Sample	2n	Avg nuclear DNA content		
		(2C/pg) ^z	Mbp ^y	N ^x
2x	14	20.3 ± 0.1 c ^w	19,900	6
OSR	28	37.3 ± 0.5 b	36,500	5
4x syn	28	39.9 ± 0.3 a	39,000	5

^zMean ± SE.

^yConversion of mass values into number of bps was calculated using the factor 1 pg = 978 Mbp (Doležel et al., 2003).

^xNumber of plants with four to five replications tested per plant.

^wValues with different letters are significantly different (least significant difference, $P \leq 0.05$).

2x = diploid cultivars; OSR = seed derived accessions of ‘Omega Skyrocket’; 4x syn = synthetically derived tetraploids; Mbp = megabase pair.

average chromosome configuration of tetraploid accessions of ‘Omega Skyrocket’ was 0.1I + 5.5II + 0.1III + 4.2IV (Table 2). The most frequent configurations were 5IV + 4II and 4IV + 6II, which accounted for 27% and 24% of the cells, respectively (Fig. 6, data not shown). Equal disjunction (14:14) was observed in 95% of the 84 anaphase cells observed (Fig. 7A). The average chromosome configuration of the eight synthetic autotetraploids was 0.3I + 2.8II + 0.3III + 5.3IV (Table 2). The most common configurations were 7IV, 6IV + 2II, 5IV + 4II, and 4IV + 6II, which accounted for 25%, 22%, 15%, and 10% of the cells, respectively (data not shown). Although laggards were observed in only one cell (Fig. 7B), only 38% of the 40 anaphase cells were equally balanced (14:14) (data not shown).

Bivalent frequency was significantly higher and quadrivalent frequency was signif-

icantly lower ($P < 0.05$) in PMCs of ‘Omega Skyrocket’ relative to that observed in PMCs of the newly synthesized tetraploids (Table 2). Triploid taxa produced more ($P < 0.05$) univalents and trivalents than the other taxa.

Nuclear DNA content estimation. 2C nuclear DNA content was determined for

six diploid accessions (‘Alba’, ‘Colorwheel’, ‘Peachies Pick’, ‘Honeysong Purple’, ‘Purple Parasols’, ‘Peach Melba’), five accessions of ‘Omega Skyrocket’, and five synthetic autotetraploids (4x-‘Alba’, 4x-‘Colorwheel’, 4x-‘Peachies Pick’, 4x-‘Purple Parasols’, 4x-‘Peach Melba’) (Table 3; Fig. 8). Chromosome number was confirmed for all of the diploids tested, three of the ‘Omega Skyrocket’ accessions, and all of the synthetic tetraploids except for 4x-‘Peach Melba’ (data not shown). There was no difference ($P < 0.001$) between measurements taken on different days, so replications were pooled for analysis. Average 2C nuclear DNA content was significantly different across all groups ($P < 0.001$) (Table 3). Average 2C DNA content of the synthetic autotetraploids (39.9 ± 0.3 pg) was approximately twice (196%) the average 2C nuclear DNA content of the diploid accessions (20.3 ± 0.1 pg). Average 2C DNA content of the natural tetraploid

Fig. 3. Meiotic chromosome associations of diploid *Stokesia laevis* ‘Peachies Pick’, 7 ring bivalents at Metaphase I ($2n = 2x = 14$). Scale bar = 10 μ m.

Fig. 4. Meiotic chromosome associations of triploid *Stokesia laevis* ($2n = 3x = 21$). Scale bar = 10 μ m. (A) ‘Purple Parasols’ × ‘Omega Skyrocket’; 1 frying pan trivalent, 6 bivalents, 6 univalents at Metaphase I. (B) ‘Peachies Pick’ × ‘Omega Skyrocket’; 3 trivalents (2 frying pans + 1 Y-shape), 4 bivalents, 4 univalents at Metaphase I.

Fig. 5. Meiotic chromosome abnormalities observed in triploid hybrids of *Stokesia laevis* ($2n = 3x = 21$). Scale bar = 10 μ m. (A) 'Peachies Pick' $\times$ 'Omega Skyrocket' with unbalanced disjunction (12:9) at Anaphase I; arrow indicates possible chromosome bridge. (B) 'Peachies Pick' $\times$ 'Omega Skyrocket' with 10 laggards (circled) at Anaphase I. (C) 'Purple Parasols' $\times$ 'Omega Skyrocket', unbalanced disjunction (10:11) at Anaphase I.

accessions of 'Omega Skyrocket' (37.3 ± 0.5 pg) was $\approx 6.4\%$ less than the synthetic autotetraploids and 8.2% less than twice the average $2C$ DNA content of the diploid accessions. The basic monoploid genome size ($1C_x$) for the diploids, 'Omega Skyrocket', and the synthetic tetraploids was 10.2 pg, 9.3 pg, and 10.0 pg, respectively. All accessions had an intermediate-sized genome (i.e., 3.5 pg $< 1C_x < 14.0$ pg) (Leitch et al., 2005).

Discussion

All members of the Vernoniae tribe classified thus far from India and North, South, and Central America, including *Stokesia*, have chromosomes that are fairly symmetrical and classified mainly as either

Fig. 6. Meiotic chromosome associations of *Stokesia laevis* 'Omega Skyrocket' ($2n = 4x = 28$). Scale bar = 10 μ m. Metaphase I configurations. (A) Four figure-8 quadrivalents and six bivalents; arrow indicates interlocking bivalents. (B) Four quadrivalents (2 figure-8s, 2 rings) and 6 bivalents. (C) Five quadrivalents (all figure-8s) and 4 bivalents (2 rings + 2 rods).

metacentric or submetacentric (Dematteis, 1998, 2002; Dematteis and Fernández, 1998; De Oliveira et al., 2007; Esteves Mansanares et al., 2007; Mathew and Mathew, 1983; Ruas et al., 1991; Smith and Jones, 1987). The TCLs of *Stokesia* (i.e., 8 to 16 μ m), however, were significantly greater than that observed for any of the other Vernoniae species (i.e., 1 to 4 μ m). *Stokesia* also differs from other species in the tribe because it has an unusually low base chromosome number of $x = 7$, which is only present in *Vernonia appendiculata* Less., a species native to Madagascar (Rabakonandrianina and Carr, 1987). *Stokesia* is a New World species but it shares a low base chromosome number (x) that is more typical of an Old World species of the Vernoniae tribe (i.e., $x = 9, 10$). This is not surprising, however, because phylogenetic studies using chloroplast DNA restriction site data and sequence data from the chloroplast, *ndhF* gene, noncoding spacer *trnL-F*, and the nuclear rRNA ITS region illustrate that New and Old World species of the Vernoniae tribe are not mutually exclusive

(Keeley and Jansen, 1994), and *Stokesia* in particular is often associated with Old World species despite its New World origin (Keeley et al., 2007).

Similarities between the karyotypes of the diploid cultivars and 'Omega Skyrocket' and the high frequency (60%) of quadrivalent formation in 'Omega Skyrocket' both indicate that 'Omega Skyrocket' is an autotetraploid form of *Stokesia laevis* (Tables 1 and 2; Figs. 2 and 6). In theory, autotetraploids exhibiting a high frequency of quadrivalents are expected to have unequal chromosomal disjunction during later stages of meiosis resulting in a corresponding reduction in fertility. Studies show that selection over several generations for increased seed set in autotetraploid populations is associated with a decrease in quadrivalent frequency and a corresponding increase in mean bivalent frequency (Bremer and Bremer-Reinders, 1954; Hilpert, 1957; Kumar et al., 1993; Swaminathan and Sulbha, 1959). However, in other studies, selection for increased seed set in autotetraploid populations is correlated with an unexpected increase in the

Fig. 7. Meiotic chromosome associations of tetraploids of *Stokesia laevis* ($2n = 4x = 28$). Scale bar = 10 μm . (A) Anaphase I of 'Omega Skyrocket'; equal disjunction (14:14); dotted line indicates division line. (B) Telophase II of 4x-'Peach Melba'; lagging chromosomes indicated by arrows.

quadrivalent frequency and a corresponding decrease in univalent and trivalent frequencies (Crowley and Rees, 1968; Hazarika and Rees, 1967; McCollum, 1958; Narasinga Rao and Pantulu, 1982). In *Stokesia*, the high frequency (60%) of quadrivalent formation in the autotetraploid accessions of 'Omega Skyrocket' did not result in a correspondingly high frequency of unequal disjunction and a reduction in fertility. In fact, later stages of meiosis in these accessions were regular and produced equally balanced gametes 95% of the time. Furthermore, the relatively high pollen viability (74%) of 'Omega Skyrocket' (unpublished data) is further evidence that quadrivalent formation does not affect fertility in this taxa.

In the synthetically derived autotetraploids, 76% of the chromosomes were involved in quadrivalent formation, a slight but significant increase relative to 'Omega Skyrocket', which is a more established autotetraploid form. This observation was not surprising because quadrivalent frequency in newly synthesized autopolyploids often decreases over subsequent generations after the initial polyploidization event (Gilles and Randolph, 1951; Santos et al., 2003). Later stages of meiosis in the synthetic autotetraploids were irregular and unequal disjunction and chromosome stickiness were observed in a high percentage of cells, unlike 'Omega Skyrocket' in which meiosis was normal. The disparity between these two groups of tetraploids may be attributed, in part, to the "types" (e.g., ring, box, zigzag chain, figure-eight) of quadrivalents formed by each group (Figs. 6 and 8). Quadrivalents of 'Omega Skyrocket' were primarily figure-eight types or zigzag chains with alternately oriented centromeres (data not shown) that presumably produced balanced 2×2 disjunction at Anaphase. In contrast, quadrivalents of the synthetic autotetraploids included zigzag chains and figure-eight types as well as rings with adjacently oriented centromeres and indifferently oriented configurations that

presumably resulted in unbalanced disjunction in some of the cells.

Triploids from crosses of diploid cultivars $\times$ 'Omega Skyrocket' had more ($P \leq 0.05$) univalents and trivalents (Table 2), which likely contributed to the formation of a high number of unbalanced gametes. Interestingly, an average chromosome configuration of $1.9\text{III} + 5.2\text{II} + 4.9\text{I}$ observed for the triploids suggests that chromosomal homology between 'Omega Skyrocket' and the diploids was similar enough to support trivalent formation but not similar enough to prevent the formation of a relatively high number of univalents. These results indicate that some genomic rearrangement in 'Omega Skyrocket' has probably occurred since the initial doubling event; however, enough chromosomal affinity still remains between 'Omega Skyrocket' and the diploid cultivars such that chromosomal pairing still occurs in the triploid hybrids.

The $\approx 9\%$ reduction in TKL in 'Omega Skyrocket' relative to twice the karyotype of the diploids and the corresponding reduction in nuclear DNA content indicate that TKL and nuclear DNA content for this species are closely correlated, a trend frequently observed in other species (Cerbah et al., 1995; Dimitrova and Greilhuber, 2000; Garnatje et al., 2004; Moscone et al., 2003; Srivastava and Lavania, 1991; Torrell and Vallès, 2001). The reduction in nuclear DNA content and corresponding reduction in TKL indicate that differences in TKL between the diploids and 'Omega Skyrocket' is likely attributable to actual genomic downsizing and not an artifact of different chromosome condensation. Genomic downsizing in polyploid plants is a widespread phenomenon in which the monoploid genome size (i.e., $1C_x = 2C$ DNA content divided by ploidy level; Greilhuber et al., 2005) decreases with increasing ploidy level (Leitch and Bennett, 2004). In this study, the monoploid genome size of 'Omega Skyrocket' (9.3 pg) was less than the basic genome size of the diploid

Fig. 8. Flow cytometry histograms of different cytotypes of *Stokesia laevis*. *Pisum sativum* L. 'Citrad' is the internal standard. (A) 'Honeysong Purple' ($2n = 2x = 14$). (B) 'Omega Skyrocket' accession 100; naturally occurring tetraploid ($2n = 4x = 28$). (C) 4x-'Purple Parasols'; synthetic autotetraploid ($2n = 4x = 28$).

accessions (10.2 pg). Genomic downsizing of similar magnitude has been identified in other polyploids (Bennett et al., 2000; Emshwiller, 2002; Hörandl and Greilhuber, 2002; Pecinka et al., 2006; Raina et al., 1994).

According to Rees (1984), changes in DNA content (not attributable to aneuploidy or chromosome arm loss) can occur in two ways, whereby change is either proportional to chromosome size (Brandham and Doherty, 1998; Naranjo et al., 1998; Poggio et al., 2007) or change is achieved by the equal addition or subtraction of DNA to each chromosome irrespective of chromosome size (Pringle and Murray, 1993; Raina et al., 1994; Raina and Rees, 1983). Chromosomes of *Stokesia* are morphologically very similar preventing the definitive identification of individual chromosomes; thus, comparison

of homologous chromosome pairs in the diploids to the homologous chromosome pairs in 'Omega Skyrocket' was not possible. However, if the chromosomes were grouped into homologous pairs according to TCL only and compared, the reduction in DNA in 'Omega Skyrocket' appeared to be proportionally distributed relative to chromosome length such that longer chromosomes lost more DNA than shorter chromosomes (data not shown).

Mechanisms capable of reducing genome size in plants include unequal and illegitimate recombination of retroelement sequences, inefficient double-stranded DNA break repair systems, mutational bias of deletions over insertions, and the targeted elimination of specific DNA sequences (e.g., rDNA) (reviewed in Bennett and Leitch, 2005; Bennetzen, 2002; Bennetzen et al., 2005; Petrov, 2001; Tate et al., 2005; Wendel, 2000; Wendel et al., 2002). Rapid genomic rearrangement (e.g., elimination of repetitive DNA, loss of amplified fragment length polymorphism and random amplified polymorphic DNABands) in nascent allo- and autopolyploids has been identified in *Brassica* L. and *Aegilops* L. hybrids (Ozkan et al., 2001; Shaked et al., 2001; Song et al., 1995) and in autotetraploids of *Paspalum* L. and *Eragrostis* Host (Martelotto et al., 2007; Mecchia et al., 2007). These studies demonstrate that genomic rearrangement, and possibly genomic downsizing, in polyploids can occur immediately after an allo- or autopolyploidization event.

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