

NATURAL POLYPLOIDIZATION WITHIN TETRAPLOID AND HEXAPLOID POPULATIONS OF THE DESERT SHRUB *ATRIPLEX CONFERTIFOLIA*

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ABSTRACT.—Shadscale (*Atriplex confertifolia*) is a wind-pollinated dioecious shrub of western North America with an unusual development of apparently autopoloid races, showing all even ploidy levels from 2x to 12x (base x = 9). Of these, tetraploid races are the most frequently encountered, with octoploids the next most common, and hexaploids being much less common. In this study, the occurrence of neopolyploid individuals within tetraploid and hexaploid populations of shadscale was examined at natural sites in order to investigate the pathways by which octoploids may be formed and to find a possible explanation for the predominance of octoploids over hexaploids. The frequencies of apparent neopolyploid individuals among adults and the frequencies of their backcrosses were tabulated, as was the occurrence of neopolyploidy among seeds of female plants from some of the same populations. The neopolyploids encountered in these surveys were almost exclusively those expected from unions involving single unreduced gametes. 6x presumed neopolyploid plants were observed in tetraploid populations at a frequency of 0.20%, and 6x seeds were observed at a frequency of 1.91%. Within examined hexaploid populations, 9x adults were not encountered, but 9x seeds were observed at a rate of 0.52%. Evidence was obtained for the operation of some postulated autopoloid pathways for generation of octoploids, but these routes did not operate at rates comparable to those for the production of hexaploids. Although the question needs to be studied by genetic methods, the most probable reason for scarcity of hexaploids in nature appears to be that some tetraploid races are diploidized, which would result in irregularities of meiosis in their neohexaploids and a failure to form new races due to infertility and inviability.

RESUMEN.—El “shadscale” (*Atriplex confertifolia*) es un arbusto dioico del oeste de Norteamérica polinizado por el viento que exhibe el desarrollo inusual de razas aparentemente poliploides, con todos los niveles pares de ploidía desde 2x hasta 12x (x base = 9). De éstos, los más comunes son los tetraploides y luego los octoploides. Los hexaploides son mucho menos comunes. En este estudio, examinamos la presencia de individuos neopoliploides entre poblaciones tetraploides y hexaploides del shadscale en sitios naturales para investigar los medios por los cuales se pueden formar los octoploides y para encontrar una posible explicación para el predominio de los octoploides sobre los hexaploides. Tabulamos la frecuencia de individuos aparentemente neopoliploides entre adultos y su descendencia retrocruzada, así como la presencia de neopoliploides entre semillas de las plantas femeninas de algunas de las mismas poblaciones. Los neopoliploides encontrados en estos muestreos fueron casi exclusivamente los que se esperarían de la unión de un gameto no reducido y un gameto reducido. Observamos plantas 6x presuntamente neopoliploides en poblaciones tetraploides con una frecuencia del 0.20%, o del 1.91% en las semillas. Entre las poblaciones examinadas de hexaploides, no encontramos adultos 9x, aunque los observamos con una frecuencia del 0.52% en las semillas. Obtuvimos evidencia de la operación de algunos procesos autopoloides postulados para explicar la generación de octoploides, pero estos procesos no operan a frecuencias comparables a aquellas para la producción de hexaploides. Aunque es preciso investigar el tema mediante métodos genéticos, la causa más probable para la escasez de los hexaploides en entornos naturales parece ser que algunas razas tetraploides son diploidizadas, produciendo irregularidades meióticas en sus neohexaploides, los cuales no logran formar razas nuevas debido a su infertilidad o inviabilidad.

Shadscale (*Atriplex confertifolia*) is an ever-green shrub found in saline soils of western North America (Welsh et al. 2003). It is a dominant element in the vegetation over large areas and, though protected by spines, is palatable to grazing animals (Dayton 1937). It is readily killed by fire and does not reproduce vegetatively (Sapsis 1990).

Shadscale has known polyploid races of as high as 12x (Sanderson 2011). These races are fertile and meiotically regular for the most part, although multivalents are occasionally seen. Diploids are widespread, occurring from eastern

Montana and adjacent North Dakota to southern Nevada and adjacent southern California. Polyploids appear to have developed independently on the Colorado Plateau and in several different areas of the Great Basin (Stutz and Sanderson 1983, Sanderson et al. 1990, Sanderson 2011). Tetraploid races are the most commonly encountered, followed in frequency by octoploids and diploids. In contrast, hexaploids are apparently absent in most parts of the Great Basin, even though there are several regions with shadscale ploidy development to levels higher than 6x and with prominent octoploid races. A contrasting

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pattern was noted only on the Colorado Plateau, where there is a moderately common hexaploid race but no octoploids.

The aim of this study was to investigate pathways for the generation of hexaploids and octoploids in order to document their relative frequencies and find a possible explanation for the usual scarcity of hexaploids.

Generation of Polyploids Above the 4x Level in Other Species

Apparent neohexaploid individuals are observed frequently within tetraploid populations and are postulated to result from single unreduced gametes (Table 1; Rommel 1960, 1961, Bingham and Binek 1969, Hornsey 1973, Stutz and Sanderson 1979, Chinnappa and Dania-Ogbe 1984, McGinnis et al. 1988, Kumar and Minocha 1989, Xena de Enrech et al. 1991, Dawson 1997, Dobeš 1999, Urbani et al. 2002, Weiss et al. 2002, Bigazzi and Selvi 2003, Baak 2004, Ramsey 2007, Marhold et al. 2009, Trávníček et al. 2010). Under appropriate conditions, such hexaploid individuals would have the possibility of forming a hexaploid race (Thompson and Lumaret 1992, Ramsey and Schemske 2002).

Similarly, occasional nonaploids (enneaploids) are occasionally observed in hexaploid populations (Matsuda and Shinohara 1985, Holm and Ghatnekar 1996, Mahelka et al. 2005, Mandakova and Munzbergova 2006, Sonnleitner et al. 2010). Also, natural populations of the grass *Andropogon gerardii* usually consist of approximately equal numbers of hexaploid and nonaploid individuals (Keeler and Davis 1999), although efforts to find neo-nonaploids among the progeny of 6x parents were not successful (Norrman et al. 1997). Even though they are odd-ploid and therefore meiotically irregular, nonaploids represent a potential route to octoploidy because of the likely production of occasional euploid gametes by meiotic segregation. These gametes would be expected to be 4x and 5x, and male and female gametes of the former ploidy might possibly combine to form an 8x offspring.

Among the above references, Sonnleitner et al. (2010) reported finding 2 rare octoploid individuals within one hexaploid population. They also observed a few 7x and 9x individuals within hexaploid populations. These plants might serve as intermediates in the production of octoploids because of the occasional

production of 4x gametes by segregation of univalents.

Another means by which octoploids could be generated in a tetraploid race is by unreduced gametes provided simultaneously by both parents. In taxa where the endosperm balance mechanism is strongly operative, embryos with single unreduced gametes are prevented from developing so that only polyploidization by simultaneous unreduced gametes can occur (Ramsey and Schemske 1998). Although the probability of simultaneous unreduced gametes is not increased by this mechanism, it does suppress the products of single unreduced gametes, 6x in this case, which might overwhelm rare 8x innovations reproductively or vegetatively. And, since by this route octoploids are produced directly from tetraploids, without any hexaploids being involved, it might seem a potential explanation for the observed hexaploid scarcity. Nevertheless, as pointed out previously (Sanderson 2011), seeds of the order Caryophyllales, to which *Atriplex* belongs, have very little endosperm (Batygina 2006), which would probably rule out a role for endosperm balance (Ramsey and Schemske 1998).

It was suggested instead (Sanderson 2011) that the frequency of hexaploids in the areas studied might relate to the degree of diploidization of tetraploid progenitor races, which could affect hexaploid fertility and viability. Diploidization, an inevitable product of time, should probably be assumed as the reason for the observed scarcity of hexaploids in the absence of other explanations, and would accommodate the presence of hexaploids in areas where diploidization of tetraploids might not yet have occurred.

Unfortunately, it has not been possible to compare meiosis of 6x neopolyploids in different regions because of the small numbers of neopolyploid individuals encountered and a tendency for these plants to be of low vigor.

METHODS

Sampling

The occurrence of *de novo* polyploidization was examined in tetraploid populations located on the Colorado Plateau, in the Great Basin, and in the Mojave Desert of the western United States and also in hexaploid populations from the Colorado Plateau and Great Basin (Appendix). At principal sites, ploidy determinations were made on 200 adult plants. At some of these sites,

TABLE 1. Summary of autopolloid pathways for production of hexaploid and octoploid individuals by way of unreduced gametes and backcrossing, or by racial hybridization. The abbreviation "p." indicates a normal gamete from a parental population, and "unred." indicates an unreduced gamete.

Mechanism of origin	Gamete combination	Observed frequency
HEXAPLOIDS		
1. From 4x by single unreduced gametes.	2x (p.) + 4x (unred.) = 6x.	1.91% in seeds (data, Table 3).
2. From euploid gametes by segregation in a 5x, likely originating from crossing of 6x with 4x.	A 5x plant should produce gametes ranging from 2x to 3x; thus, 3x + 3x = 6x.	1.64% from two 5x plants from neopolyploidy (data, Table 6), or 48.8% from two 5x interracial hybrids (data, Table 7).
3. From euploid gametes by segregation in 7x plants. 7x could result from an unreduced gamete of a 4x plus a 3x gamete from a 5x, 6x, or 7x.	7x plants should produce gametes ranging from 3x to 4x; thus, 3x + 3x = 6x.	Not examined.
OCTOPLOIDS		
4. From 4x by simultaneous unreduced gametes.	4x (unred.) + 4x (unred.) = 8x.	0.15% in seeds (data, Table 3).
5. Backcrossing of a 6x neopolyploid within a 4x population, or hybridization of 4x and 6x races.	(a) 2x (p.) + 6x (unred.) = 8x. (b) 2x (p.) + 3x = 5x , then 5x (unred.) + 3x = 8x.	(a) Not observed. (b) 4.65% in seeds (latter step, data, Table 7).
6. Production of euploid gametes by segregation in a 7x. A 7x plant could be produced by a 4x unreduced gamete combining with a 3x gamete from a 6x or 5x.	4x + 4x = 8x. 7x plants should produce gametes ranging from 3x to 4x, so two 4x gametes could combine if 7x plants were intercrossed.	Not examined.
7. From 6x populations by unreduced gametes, giving 9x offspring.	(a) 3x (p.) + 6x (unred.) = 9x. (b) A 9x should occasionally produce euploid 4x and 5x gametes, so that 5x + 3x (p.) = 8x , or 4x + 4x = 8x.	(a) 0.34% in seeds (data, Table 5). (b) Not examined.

TABLE 2. Occurrence of pentaploid and hexaploid adults within tetraploid populations of *Atriplex confertifolia*. Upper and lower bounds of the percentage of unreduced gametes in the populations are for $\alpha = 0.05$ using exact equal-tail probabilities.

Location	Total	4x	5x	6x	% unreduced gametes
Colorado Plateau					
Carbon-Emery line, UT	200	199	1	0	0.0% (0%–1.83%)
Sulphur Creek, UT	204	204	0	0	0.0% (0%–1.79%)
Great Basin					
Rush Valley, UT ^a	200	199	1	0	0.0% (0.00%–1.82%)
Rachel, NV ^b	201	198	1	2	0.5% (0.01%–2.74%)
Mojave Desert					
Kramer Jct., CA	201	201	0	0	0.0% (0.00%–1.82%)
TOTALS	1006	1002	3	2	0.1% (0.00%–0.55%)

^aAt Rush Valley, a second 5x plant was later found beside the first but just outside the transect.

^bAt Rachel, there were 2 small 6x plants that were distant from each other, but one was only a few centimeters away from a 5x plant.

seeds were also collected from female plants for investigation of neopolyploidy within seed families. Additional ploidy determinations of adults or of seed progeny were also done at a number of smaller sites where neohexaploids or tetraploid-hexaploid hybrids had been accidentally discovered during geographic surveys (Sanderson 2011). Upper and lower bounds (95% confidence limits) on frequency of occurrence were calculated using formulas for exact binomial probabilities provided on a spreadsheet by the statistical unit of the USDA Forest Service, Rocky Mountain Research Station.

Flow Cytometry

For ploidy determination on leaves, labeled twigs were transported to the laboratory in an ice chest. When it was learned that ploidy determinations might also be possible from single seeds (Suda and Trávníček 2006), the study was expanded to include these. Flow cytometry was carried out on about 0.5 cm² of finely razor-chopped leaves or on individual seeds. Plant tissues were chopped in a DAPI (4,6-diamidino-2-phenylindole) solution (CyStain[®] UV Ploidy) obtained from the firm Partec GmbH, Münster, Germany, which stained the released nuclei. In the case of seeds, although these varied in size, it was most often possible to count 2000–6000 nuclei for the sample peak, except where interfering compounds were inferred to be abundant. Counts for leaf samples consisted of several times that number.

Diploid and tetraploid plants of *Atriplex canescens* at the laboratory were used for standards. These standards were compared with 2x and 4x plants from Little Sahara Sand Dunes, Juab County, Utah. These plants have been measured at 1.575 pg and 3.048 pg (2c values),

respectively, by David J. Walker (personal communication). Values for the 4x standard, in multiple runs arranged to cancel machine variation, showed no significant difference with the Little Sahara plants. On the other hand, the 2x standard, which was from a seed of unknown origin obtained in Colorado, had values only 98.4% of those of diploids from Little Sahara, which would indicate a 2c value of about 1.55 pg.

In addition to machine variation, coordinated variability of peak position for sample and internal standard occurred with different plants (this was not improved by centrifugation of the nuclei and replacement of the solution) and could have affected the results to a degree; this behavior could be due to interference from plant secondary chemicals. These sources of error limited the ability to identify aneuploidy. For the purposes of this study, peaks falling within ± 9 arbitrary brightness units of expectation, where addition of a monoploid genome ($x = 9$) to nucleus size increased the peak position by 30–40 units (slightly less than 4 units per chromosome), were considered euploid. Thus, it was not possible to consistently detect aneuploidy involving only one or 2 chromosomes. To lessen interference by plant chemicals, leaves were generally abraded on both surfaces to partially remove the bladder cell layer with which *Atriplex* leaves are covered, as was done previously (Sanderson 2011).

Special treatment was necessary for ploidy determination of seeds from some locations. In order to obtain success with those from the Kramer Junction, California, study site, the filtered stain mixture was allowed to stand following leaf chopping for at least 15 minutes before introducing it into the flow cytometer.

TABLE 3. Percent occurrence of neopolyploids among seeds from tetraploid female plants, showing 95% confidence limits for unreduced gametes. The 5x seed observed at the Kramer Junction site would suggest the presence of an adult male hexaploid, although none was encountered within the transect. The 8x⁻ plant would likely be the result of union of simultaneous unreduced male and female gametes.

Location	Total	4x ⁻	4x	4x ⁺	5x	6x ⁻	6x	8x ⁻	% unreduced gametes
Kramer Jct., CA	212	1	203	0	1	0	6	1	1.9% (0.52%–4.76%)
Rush Valley, UT	241	0	234	2	0	1	4	0	1.0% (0.17%–3.28%)
Sulphur Creek, UT	227	0	225	0	0	1	1	0	0.4% (0.01%–2.43%)
TOTALS	680	1	662	2	1	2	11	1	1.1% (0.46%–2.21%)

TABLE 4. Survey of ploidy of adult plants at 3 hexaploid populations. As it happened, all were shown to lie on the boundary of populations of tetraploids, and transects at the Columbus site reached the edge of an octoploid population as well. No 9x adult individuals were found. Shown are 95% upper and lower confidence limits for percent occurrence of unreduced gametes. Because they were located near tetraploids, the 5x plants were likely 4x–6x hybrids.

Location	Total	4x	5x	6x	8x	9x	% unreduced gametes
Columbus, NV	224	79	2	141	2	0	0.0% (0%–1.63%)
Cliff Dwellers, AZ	201	1	0	200	0	0	0.0% (0%–1.82%)
Bigwater, UT	202	3	2	197	0	0	0.0% (0%–1.81%)
TOTALS	627	83	4	538	2	0	0.0% (0%–0.59%)

TABLE 5. Ploidy of seeds from 6x females. The 9x seeds from Coaldale Junction would likely represent fertilization of 6x unreduced gametes, while the 6x⁺, 7x⁻, and 10x seeds could suggest the presence of a minor 7x pollen source. Some 7x individuals were found a few blocks away at the interface between the 6x females and an 8x stand.

Location	Total	6x ⁻	6x	6x ⁺	7x ⁻	9x	10x	% unreduced gametes
Coaldale Jct., NV (3 plants)	393	3	381	4	2	2	1	0.3% (0.01%–1.41%)
Cliff Dwellers, AZ (2 plants)	200	0	200	0	0	0	0	0.0% (0.00%–1.83%)
TOTALS	593	3	581	4	2	2	1	0.17% (0.00%–0.94%)

Also, seeds from the 6x population at Lake Powell seemed to contain UV-reactive compounds that cause a rapidly increasing background fluorescence that often swamped the results. Runs had to be terminated early in order to preserve readable peaks. Leaf extracts from *Atriplex canescens* plants can sometimes show a similar, although often more vigorous, reaction.

No difficulties were encountered due to the presence of maternal or endosperm storage tissue in the seeds. Slight indications at expected endosperm peak positions were sometimes observed but were never strong enough to be unequivocal. Perisperm makes up approximately half of the seed. While embryo tissue gave good flow cytometry results, probably because it consists of numerous small cells and therefore contains many nuclei, a meaningful signal from perisperm was not obtainable, because perisperm consists of few large, starch-filled cells and therefore contains only a small number of nuclei.

Results from flow cytometry are corroborated by meiotic chromosome counts that had been

made previously (Stutz and Sanderson 1983, Sanderson et al. 1990, Sanderson 2011).

RESULTS

Population Surveys

Among a total of 1006 adult plants from 5 tetraploid populations (Table 2), 2 apparently neopolyploid 6x individuals were encountered. Neopolyploidy in these plants is presumably due to the action of unreduced gametes. Such action gives a mean frequency in these plants of 0.20% (95% confidence limits: 0.02% to 0.70%), equaling a frequency of 0.10% of unreduced gametes. In addition, three 5x individuals were encountered, probably formed by backcrossing of 6x individuals to parental populations, which gives a mean frequency of 0.30% (0.06% to 0.87%).

Out of 679 seeds from 8 female plants from 2 of the same populations (Table 3), there were 13 hexaploids, for a frequency of 1.91% (1.02% to 3.25%), and one octoploid for a frequency of 0.15% (0.004% to 0.82%). Neopolyploids were therefore significantly more frequent among

TABLE 6. Ploidy of seeds from 5x females in tetraploid populations, presumed backcross offspring of 6x neopolyploid plants. A 5x plant should produce gametes ranging from 2x to 3x, mostly aneuploid, which would be expected to combine with 2x gametes from the tetraploid population, producing offspring ranging from 4x to 5x, also mostly aneuploid. The numbers higher than 5x+ appear to have resulted from unreduced gametes, and the 95% confidence limits for frequency of these are shown. Unreduced gametes could have come from either the 4x population or the 5x females (4x unreduced + 2x to 3x = 6x to 7x), (5x unreduced + 2x = 7x). No 8x progeny were observed (5x unreduced + 3x = 8x), presumably due to the absence of a 6x pollen parent at the sites.

Location	Plants	Seeds	4x ⁻	4x	4x ⁺	5x ⁻	5x	5x ⁺	6x ⁻	6x	7x ⁻	7x	7x ⁺	% unreduced gametes
Point of Rocks, WY	3	74	0	11	24	27	8	1	1	0	1	1	0	2.0% (0.15%–8.39%)
Rush Valley, UT	2	170	1	39	55	51	19	1	1	2	1	1	1	1.8% (0.37%–5.07%)
TOTALS	5	244	1	50	79	78	27	2	2	2	2	2	1	1.8% (0.56%–4.43%)

TABLE 7. The occurrence of 8x progeny among seeds from two 5x females of apparent 4x × 6x racial hybrid origin, likely as the result of backcrossing to the nearby 6x populations (5x unreduced + 3x = 8x). Because of the difficulty of identifying ploidies less than 5x, in this case, due to interference from the minor tetraploid peak of the diploid flow cytometry standard, progeny attributable to backcrossing to the 4x population are not presented. The 7x+ seed could have been due to an unreduced 5x gamete or an unreduced 4x, and the 8x could represent an unreduced 5x gamete or an unreduced 6x.

Plant location	Total	5x	5x ⁺	6x ⁻	6x	7x ⁺	8x	% unreduced gametes
East of Bigwater, UT	31	3	9	12	4	1	2	9.7% (2.04%–25.75%)
Warm Cr. Wash, UT	12	3	4	4	1	0	0	0.0% (0.00%–26.46%)
TOTALS	43	6	13	16	5	1	2	7.0% (1.46%–19.06%)

TABLE 8. Ploidy of seeds from 4x and 6x females at Columbus, Nevada, and 6x from Warm Creek Wash, Utah, at the intersection of tetraploid and hexaploid populations. Because of drought, no seeds were available from Warm Creek 4x plants. The observed 5x seeds and possibly 7x would represent 4x × 6x hybrids. One of the two 4x females had all but one of the 5x seeds from that parentage, and most of the 6x, 7x, and aneuploids as well. No 8x seeds were obtained from either the 4x or 6x females.

Female parent	Plants	Total seeds	4x	4x ⁺	5x ⁻	5x	5x ⁺	6x ⁻	6x	6x ⁺	7x ⁻	7x	8x	9x	9x ⁺
4x Columbus	2	163	127	3	1	27	2	0	2	0	0	1	0	0	0
6x Columbus	5	267	0	0	0	0	0	7	238	19	1	2	0	0	0
6x ⁻ Columbus	3	204	0	0	0	0	0	77	122	3	1	1	0	0	0
6x Warm Cr. Wash	3	152	0	0	0	4	0	0	135	8	0	0	0	1	2
6X TOTALS	11	623	0	0	0	4	0	84	495	30	2	3	0	1	2

seeds than among adult plants (13 of 679 vs. 2 of 1006, $\chi^2 = 11.37$, $P \leq 0.005$). The 8x plant observed might have represented an unreduced 6x gamete in pollen from an undiscovered 6x male neopolyploid (Table 1), or it could have formed from simultaneous unreduced gametes.

Frequent periods of drought occurred during this study, and the lower frequency of 6x individuals among adult plants compared to the higher frequency in seeds suggests that neopolyploids encountered more difficulty in establishment than normal plants. In addition, many of the 5x and 6x plants encountered were small and only weakly reproductive or sterile, which might suggest a lack of physiological adjustment to the increased ploidy. Because of variability in stature

and other characteristics within parental populations, as well as the small number of samples involved, no clear differences in vigor could be discerned between 5x plants from the Colorado Plateau and those from the Great Basin.

For hexaploid populations, among 627 adults from 3 locations, none of the expected 9x plants were encountered, 0.0% (0% to 0.59%) (Table 4). But, among seeds, two 9x individuals were observed among 481 seeds from five 6x females from 2 populations (Table 5), a mean percentage of seeds of 0.52% (0.06% to 1.88%). Both of these 9x seeds were produced by the same female parent. As mentioned, intercrossing between semifertile 9x plants, if they were able to reach maturity, is an avenue that might lead to 8x offspring.

TABLE 9. Ploidy of seeds produced by 6x presumed neopolyploid females within tetraploid populations, showing mean percent unreduced gametes and 95% confidence limits.

Location	Total	5x ⁻	5x	5x ⁺	6x ⁻	6x	7x	8x	% unreduced gametes
Castle Dale, UT	30	0	30	0	0	0	0	0	0.0% (0.00%–11.57%)
Hidden Springs, AZ	305	2	276	20	2	5	0	0	0.0% (0.00%–1.20%)
Totals	335	2	306	20	2	5	0	0	0.0% (0.00%–1.10%)

Progeny from 5x Females

Octoploids might also be produced from a 5x × 6x cross (Table 1; Bingham and Binek 1969), or from crossing or backcrossing 6x with 4x plants. Tables 6 and 7 show progeny that were obtained from 5x females. Table 6 shows progeny from 5x females of apparent neopolyploid origin, and Table 7 shows progeny from 5x females that most likely originated by hybridization between 4x and 6x races. For those of apparent neopolyploid origin, there was evidently no 6x pollen source present, and so only backcrossing to the 4x population occurred. Thus, no 8x seeds resulted when 5x unreduced gametes were produced (5x unreduced + 3x = 8x); only 7x seeds resulted (5x unreduced + 2x = 7x). On the other hand, in the case of the 5x plants of probable hybrid origin, numerous individuals of the 6x race were available nearby, and two 8x progeny were obtained among the 43 seeds examined. It appears likely that 8x plants could be generated from either sort of 5x female, given an appropriate pollen parent. Nevertheless, formation of octoploids by this route would be dependent upon the prior existence of 6x plants for formation of the 5x plant, as well as for the most convenient source of 3x gametes, so there is no obvious reason that this pathway should lead to a predominance of octoploids over hexaploids.

Progeny of 4x and 6x Females at Localities Where both Ploidies were Present

Although no examples were found, the production of 8x progeny should be possible from unreduced 6x gametes by 6x × 4x hybridization or by backcrossing (6x unreduced + 2x = 8x; Table 8). Table 9 shows seed production from two 6x plants of apparent neopolyploid origin that were backcrossing to tetraploids. One gave only 30 seeds over several years, and all seeds were 5x. In the other, among 323 seeds, most were 5x, although a small amount of backcrossing by a 5x male progeny was apparently also occurring. Such backcrossing would be expected to give 5x and 6x seeds and aneuploid

intermediates in between. If the 6x females had produced unreduced gametes, the products would have been 8x, or, if the 4x population produced them, 7x.

Since 2 of the sites originally investigated as hexaploid populations turned out to be located on the interface between 4x and 6x populations, seeds from several of each ploidy near the interface were examined (Table 8). Some of the 6x females turned out to be minus aneuploid and gave frequent aneuploid progeny. Reproductive barriers between ploidies seemed considerable, and few hybrid seeds were obtained from 6x females. On the other hand, one of the 4x females at the Columbus site gave twenty-six 5x apparent hybrid seeds out of 101 seeds.

DISCUSSION

In a variety of species, polyploidization has been found to occur primarily by way of single unreduced gametes (Ramsey and Schemske 1998), although other mechanisms such as somatic doubling or polyploidization by simultaneous unreduced gametes are possible. In diploid populations, unreduced gametes usually result in production of triploids, which are largely sterile (Brown 1972). This sterility results from a lack of normal chromosome pairing in meiosis, which is not possible for an odd-ploid plant. Nevertheless, through an additional unreduced gamete or by meiotic segregation, rare tetraploid offspring can be formed (Ramsey and Schemske 1998, Bretagnolle 2001).

Polyploidization from the tetraploid level presents a somewhat different picture (Table 1), since the product of a single unreduced gamete in this case would be hexaploid, which is even-ploid and therefore potentially regular in meiosis and fertile. Because formation would be a direct, single-step process, production of neohexaploids should be relatively frequent and give a comparatively high probability of the eventual generation of new hexaploid populations and races.

In contrast, polyploidization to the octoploid level by means of single unreduced gametes

from either tetraploid or hexaploid parentage would be indirect and comparatively infrequent.

Alternative modes of polyploidization not involving single unreduced gametes could provide a means by which octoploids might arise directly from tetraploids. As discussed previously (Sanderson 2011), somatic doubling is one method but, over very extensive study, has never been found to occur in *A. confertifolia*. As mentioned above, simultaneous unreduced gametes under the influence of the endosperm balance mechanism is another possible means, but its occurrence is not to be expected in *Atriplex*. In agreement with this concept, I observed hexaploid neopolyploid products, rather than octoploid products, in tetraploid populations.

The absence of evidence for these pathways seems to leave tetraploid diploidization as the best remaining explanation for the usual scarcity of hexaploid populations in shadscale. By differentiation of initially similar chromosomes, diploidization would have the practical effect, through time, of converting a tetraploid into a functional diploid even though a tetraploid number of chromosomes might still remain. From such a diploidized parent, hexaploid neopolyploids in reality would be triploids. Triploids show sterility because they have 3 genomes, and thus 3 chromosomes of each kind. Two chromosomes of a kind may pair in meiosis, but the third will either become a stray univalent, or may partially pair with the other 2, forming a multivalent (Brown 1972, Ramsey and Schemske 2002). As a result, meiosis is strongly irregular, the progeny receive a variable number and composition of chromosomes, and most of the progeny fail to develop. Therefore, both a triploid and a formally hexaploid offspring of a tetraploid that is diploidized should suffer from sterility, and any rare offspring that might be produced would tend to likewise be sterile or inviable.

For this reason, it would be expected that few hexaploid races would establish themselves in regions in which the present tetraploid was ancient enough to have become diploidized. It was suggested previously (Sanderson 2011) that the reason that hexaploids are present on the Colorado Plateau might be that this region contains a more youthful tetraploid race that has not yet become diploidized. These questions should be investigated further by genetic methods and by examining meiosis in 6x neopolyploids if such individuals become available.

This study verifies several postulated mechanisms for the generation of higher ploidy levels in an autopolyploid system and demonstrates that the mechanisms and outcomes are somewhat different than those expected under allopolyploidy. Because of the high frequency of autopolyploid events in shadscale and other *Atriplex* species, these species might be useful candidates for further studies on autopolyploidy.

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See Appendix on page 150.

APPENDIX. Study locations, with UTM coordinates (NAD27).

Location and racial ploidy	UTM coordinates (NAD27)	Probable event	Samples	Comments
Bitter Springs, Coconino Co., AZ	12 04 43509 E, 40 55470 N 1687 m elevation	Racial hybridization	20 adults	Adjoining 6x and 4x pops., with three 5x apparent hybrids and also a 5x (4x & 6x plus 4x <i>A. canescens</i>) <i>A. confertifolia</i> × <i>A. canescens</i> hybrid. Little studied because of tribal prohibitions.
Cliff Dwellers, Coconino Co., AZ (6x)	12 04 29647 E, 40 63173 N 1358 m elevation	None	201 adults, seeds from 6x	6x pop. with a 4x adult from a neighboring 4x pop. No neopolyploidy or hybridization observed.
Hidden Springs, Coconino Co., AZ (4x)	12 04 64665 E, 40 06348 N 1478 m elevation	Likely neopolyploidy	5 adults, seeds from 6x	4x pop. with one 6x female plant and two 5x apparent progeny.
Kramer Jct., San Bernardino Co., CA (4x)	11 04 47874 E, 38 84626 N 841 m elevation	Neopolyploidy	201 adults, seeds from 4x	Adults tested were all 4x, but seeds contained 5x, 6x, and 8x. The 5x and probably 8x would be due to an undiscovered 6x male.
Coaldale Jct., Esmeralda Co., NV (6x & 10x)	11 04 17814 E, 42 08302 N 1372 m elevation	Neopolyploidy	9 adults, seeds from 6x	One adult of 10x race was found at the site. Seeds of 7x, 9x, and 10x were obtained from 6x mothers. The 7x & 10x may indicate a 7x pollen parent in the vicinity (6x × 10x = 8x, then 8x × 6x = 7x?).
Columbus, Esmeralda Co., NV (4x, 6x, 8x)	11 04 16843 E, 42 22501 N 1883 m elevation	Racial hybridization	224 adults, seeds from 4x and 6x	Mainly 6x and 4x, with two small 5x apparent hybrids and two 8x from neighboring 8x pop.
Rachel, Lincoln Co., NV (4x)	11 06 08452 E, 41 68675 N 1451 m elevation	Neopolyploidy	201 adults	Two 6x plants plus one 5x probable backcross progeny, all small and mostly nonreproductive. One 6x was dead in 2009
Carbon–Emery county line, US 6, UT (4x)	12 05 42794 E, 43 68803 N 1617 m elevation	Neopolyploidy	200 adults	One 5x plant, probable offspring from a 6x not found.
Castle Dale, Emery Co., UT (4x)	12 05 12118 E, 43 38986 N 1727 m elevation	Neopolyploidy	9 adults, seeds from 6x	6x female plant in 4x pop. Produced seeds in some years. All seeds from 6x were 5x.
Sulphur Creek, Book Cliffs, Grand Co., UT (4x)	12 06 51075 E, 43 35883 N 1469 m elevation	Neopolyploidy	204 adults, seeds from 4x	Adults were all 4x, but seeds included 6x.
4 mi. E of Bigwater, Kane Co., UT (4x & 6x)	12 04 46844 E, 41 02639 N 1245 m elevation	Racial hybridization	9 adults, 4x and 6x, seeds from 5x	Mixed 4x and 6x site with two 5x apparent hybrids. One died, but the other gave seeds ranging from 4x to 6x, plus the 7x and 8x. The latter may be from 5x unreduced gametes backcrossing to 4x and 6x populations respectively, or the 7x from an unreduced 4x gamete.
Warm Creek Wash, Lake Powell, Kane Co., UT (6x & 4x)	12 04 46844 E, 41 02639 N 1245 m elevation	Racial hybridization	202 adults, seeds from 5x females	6x pop. with a few of 4x race, and two 5x presumed hybrids. Seeds from one 5x plant ranged from 4x to 6x.
Rush Valley at Pony Express Monument, Tooele Co., UT (4x)	12 03 90029 E, 44 51415 N 1537 m elevation	Neopolyploidy	200 adults, seeds from 4x, seeds from 5x	4x pop. with two 5x plants, only one of which was in the transect, probably offspring from a 6x not found. Seeds from the 4x included 6x. Seeds from the 5x ranged from 4x to 6x, plus 7x, the latter probably from 5x unreduced gametes.
Point of Rocks, Sweetwater Co., WY (4x)	12 06 84682 E, 46 17361 N 2125 m elevation	Neopolyploidy	8 adults, seeds from 5x females	Three 5x in 4x pop., probable offspring of a 6x no longer present. 5x gave seeds 4x to 6x plus 7x; the latter probably by 5x unreduced gametes.