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Cytotypes of *Andropogon gerardii* Vitman (Poaceae): fertility and reproduction of aneuploids

GUILLERMO A. NORRMANN[1]* and KATHLEEN H. KEELER[2]

[1]*Instituto de Botánica del Nordeste, Facultad de Ciencias Agrarias, CC 209, 3400 Corrientes, Argentina*
[2]*School of Biological Sciences, University of Nebraska, Lincoln, NB 68588–0118, USA*

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In many North American prairies, populations of *Andropogon gerardii* Vitman (Poaceae) are composed of hexaploid and enneaploid cytotypes ($2n = 60$, 90), with intermediates occurring occasionally. Under controlled pollination, the two common cytotypes can be crossed, producing progeny with a range of chromosome numbers. In an investigation of fertility and compatibilities of intermediate cytotypes, individuals with chromosome numbers between 60 and 90 were crossed with each other, with the $2n = 60$ and 90 cytotypes, and with South American *Andropogon* species having 60 chromosomes. Regardless of cytotype, all *A. gerardii* plants had some fertility and virtually all crosses produced seeds. Cytotype is only partially predictive of fertility. Inter-specific hybrids between *A. gerardii* and South American hexaploid species were vigorous but sterile. Gene flow in natural *A. gerardii* populations of mixed cytotype probably involves plants of all cytotypes. © 2003 The Linnean Society of London, *Botanical Journal of the Linnean Society*, 2003, **141**, 95–103.

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INTRODUCTION

Andropogon gerardii Vitman, Big Bluestem, is the dominant grass of the tallgrass prairie biome of North America. It occurs from New England to Florida, and westwards to the eastern edge of the Rocky Mountains. Where it occurs, it forms up to 80% of the total plant biomass (Weaver & Fitzpatrick, 1934; Risser *et al*., 1980). It is a useful, nutritious native forage grass (Hitchcock, 1951).

Some wild populations of *A. gerardii* consist entirely of a hexaploid cytotype with $2n = 60$ chromosomes (Gould, 1967, 1968a; Keeler, 1990). However, populations comprising hexaploid and enneaploid ($2n = 90$) cytotypes are common, especially in the western part of the range of the species (Gould, 1956, 1968b; Keeler *et al*., 1987; Keeler, 1990, 1992). Certified seed releases are hexaploid (Riley & Vogel, 1982; Keeler, 1992), but wild-collected seeds are likely to contain a mixture of cytotypes with a range of chromosome numbers between $2n = 60$ and 90. In this study we used plants produced by controlled crosses to analyse the fitness and fertility of this range.

Functionally, this system resembles a diploid: triploid complex, with the enneaploids apparently produced from the hexaploids through the fusion of a normal gamete with an unreduced one (de Wet, 1980; Bretagnolle & Thompson, 1995). Intermediate chromosome numbers are produced when enneaploids, like triploids, produce unbalanced gametes. However, the intermediate cytotypes in *A. gerardii* are often quite vigorous, possibly because of the redundancy of the genomes of higher polyploids. This paper reports on an investigation of the reproductive biology of *A. gerardii* cytotypes with 68–80 chromosomes.

MATERIAL AND METHODS

The plants of *A. gerardii* studied had chromosome numbers from 68 to 80. They were the progeny of crosses between hexaploid ($2n = 60$) and enneaploid ($2n = 90$) plants collected from natural populations (Table 1, crosses detailed in Norrmann, Quarín & Keeler, 1997). We selected more 70- and 80-chromosome individuals for study because they

*Corresponding author. E-mail: gnorrmann@hotmail.com

Table 1. Origin of plants studied in this paper (hexaploids and enneaploids studied in Norrmann *et al*., 1997)

Progeny	Maternal plant	Source of pollen
N230, N234	*N113* (2*n* = 90)	Mixed, from 2*n* = 60 plants
N232, N237, N238, N239, N241, N242, N243, N244	*N113* (2*n* = 90)	*N122* (2*n* = 60)
N235	*N119* (2*n* = 90)	Mixed, from 2*n* = 60 plants
N236, N248,N249, N250, N252, N253, N254, N255, N256, N257, N258	*N122* (2*n* = 60)	*N126* (2*n* = 90)
N245, N246, N247	*N122* (2*n* = 60)	*N114* (2*n* = 90)

were of particular interest as apparent 'euploids', not because they occur more frequently. Plants with 70 or 80 chromosomes would be considered 'euploids' since they have chromosome numbers that are simple multiples of the basic number in the genus ($n = x = 10$; Gould, 1967; Norrmann, 1985; Campbell & Windisch, 1986).

The following accessions belonging to South American *Andropogon* hexaploid species and hybrids were also involved in crosses with *A. gerardii*: *A. lateralis* Nees, 2*n* = 60, *Norrmann 111*, Corrientes, Argentina; *A. hypogynus* Hack. × *A. lateralis*, 2*n* = 60 + B, *Norrmann 109*, an experimental fertile hybrid (Norrmann, 1994, 1999); *A. glaucophyllus* Roseng. Arrill. et Izag., 2*n* = 60 (Norrmann & Scarel, 2000), *Valls 2411*, Porto Alegre, Rio Grande do Sul, Brazil.

The methods used for counting chromosomes and observing meiotic chromosome behaviour, pollen–pistil interactions, and embryology are standard and are described in detail in Norrmann *et al*. (1997). Chromosome numbers were established from mitotic squashes of root tips that were collected from potted plants and pretreated for 2 h with α-bromonaphthalene at room temperature. Subsequently, the material was hydrolysed with 1 mol L^{-1} HCl at 60°C for 10 min and stained with fuchsin. Squashes were made in a drop of aceto-orcein. Meiosis was studied in young inflorescences fixed in Carnoy's solution and refrigerated in 70% ethanol. Pollen mother cells (PMC) were stained with aceto-carmine. Smears were made permanent with Venetian turpentine. To determine whether there were differences in meiotic behaviour as a consequence of chromosome complement, we determined in each accession (1) frequency of formation of univalents, bivalents and multivalents, (2) presence of laggards at anaphase I, and (3) presence of micronuclei in the tetrad stage. We used fluorescence microscopy to watch pollen germination on the stigma surface and tube growth following pollination. Ovaries were fixed in FAA, placed in 1N NaOH for 15 min, transferred into 0.1% aniline blue solution for 15–30 min, mounted in a glass slide with a drop of aniline blue and gently covered with a cover glass for examination. Pollen germination was determined by count of germinated and non-germinated pollen grains on the stigmata. Penetration of the tube up to the micropylar zone was recorded

Megasporogenesis and embryo sac development were analysed in selected individuals using serial sections of ovaries, stained with safranin-fast green. We also counted the number of ovules with mature normal embryo sacs in comparison with aborted embryo sacs from a minimum of 50 mature ovules per accession using Nomarski differential interference contrast microscopy and the clearing-squash technique (Herr, 1971).

Although both the hexaploid and the enneaploid are sexual outbreeders (Bennett, 1873; Law & Anderson, 1940; Norrmann *et al*., 1997), we tested for selfing in all accessions by isolating some inflorescences. Seed set was determined by counting the number of pistilated flowers that developed fruit. Seed set in open pollination was measured in an experimental garden, with experimental plants set amidst hexaploid and enneaploid *A. gerardii* clones. We determined percentage germination for lots of 1000 seeds in the glasshouse. Ten to 15 seedlings, selected at random from each accession, were grown in an unheated glasshouse for two seasons. Survival to re-growth after winter (at 25 months) was measured, and then all plants were discarded.

For intraspecific crosses we used the methods of Norrmann *et al*. (1997). From that work, we knew there was low fertility in cytotypes other than the hexaploid. To ensure that there was sufficient viable pollen, we applied a mixture of pollen from different plants of the chosen chromosome number, rather than from a single pollen donor. Interspecific crosses included hexaploid South American *A. glaucophyllus*, *A. lateralis* and a fertile hybrid between *A. hypogynus* Hack. and *A. lateralis*. Since *A. glaucophyllus* is andromonoecious and self-incompatible (Norrmann & Scarel, 2000), interspecific crosses were made similarly to the intraspecific crosses. *Andropogon lateralis* and *A. hypogynus* × *A. lateralis* are monoecious with

flowers that open at dusk (Norrmann & Quarín, 1991; Norrmann, 1994, 1999), and therefore inflorescences of the pistillate parent were bagged during the previous night.

In a comparison of the success of different crosses, 'crossability' was estimated as the number of seeds expressed as a percentage of the number of crossed spikelets. A random sample of the seeds produced was planted to check viability, and of those germinating, a random sample was tested for chromosome number.

For clarity we will term seedlings from crosses between two *A. gerardii* plants 'progeny' and reserve the term 'hybrid' for interspecific hybrids. We avoid using the terms euploid/aneuploid because of the uncertain status of the nominal 'euploid' plants with $2n = 70$ and 80 (see Results).

Statistical analysis of the relationship between reproductive measures used correlation analysis, comparison of aneuploids and 'euploids' Student's *t*-test and field studies employing the StatView 4.5 program (Abacus Concepts, 1994).

RESULTS

All cytotypes having over 60 chromosomes formed multivalent and univalent associations of chromosomes in addition to bivalents (Table 2). Laggards were observed in most cells, accounting for the loss of chromosomes and irregular segregation. The 'heptaploid' accessions ($2n = 70$) had up to ten trivalents in addition to bivalents. Meiosis in 'octoploid' cytotypes ($2n = 80$) had univalents, trivalents and quadrivalents. Aneuploids showed micronuclei in the tetrad stage, though we did not determine their meiotic chromosome associations in detail. These results point to loss of chromosomes and unequal segregation during meiosis, as is the case in enneaploid cytotypes.

Megasporogenesis was normal up to the linear tetrad stage. Then, a variable number of ovules showed deterioration and abortion of embryo sacs, mostly at the one-nucleate stage. The frequency of immature or aborted embryo sacs was so high in some genotypes as to eliminate the formation of seed almost entirely (Table 2).

All progeny were highly polymorphic in exomorphological features.

Reproductive biology

All plants of *A. gerardii* used in this study were andromonoecious and flowered early in the morning (04:00–07:00 hours). Selfed pollen germinated shortly after contacting the stigma. Pollen tubes immediately began elongating and penetrated the stigma papillae. Tubes grew a short distance down the branches, but only a few reached the central axis of the stigma, where growth stopped. None of the accessions studied (Table 1, $N = 27$) set seed when selfed. In contrast, when cross-pollinated, either from hexaploid or enneaploid plants, the pollen germinated and grew through stigma, style and ovary, reaching the micropyle in less than 2 h.

Consistent with the pollen tube growth, most of the cross-pollinated genotypes set some seeds and produced viable seedlings (Tables 2 and 3). Percentages of seedlings obtained correlated significantly with percentages of filled seed (data in Table 2, $r^2 = 0.94$, $N = 19$, $P < 0.001$), indicating that differences in the frequency of good seed carried through to germination and growth. Seedlings per 1000 seeds (Table 2) correlated significantly with percentage survival ($r^2 = 0.59$, $N = 19$, $P < 0.007$). Survival, as well as general vigour of progeny, was variable, but most accessions produced some seedlings that survived 2 years (17 of 21, Table 2).

Crosses

In all, we crossed 6896 spikelets of *A. gerardii* and obtained 29 progeny (Table 3). Using *A. gerardii* cytotypes and South American *Andropogon* species (732 spikelets), we obtained ten interspecific hybrids, from four parental combinations (Table 3). We did not assume that any of the seedlings was necessarily the result of outcrossing, but upon examining the overall results, we found that self-incompatibility was very strong and not one of the tested progeny proved to be a self.

Gametes

Progeny produced by crossing with hexaploid plants indicated that 'heptaploids' had gametes with $n = 32$, 34, 35 or 36 chromosomes, plants with $2n = 73$ and 74 had gametes with $n = 35$, 38 and 39 and 'octoploids' had gametes with $n = 37$, 38, 39 and 44 chromosomes (Table 3).

One of two combinations of 'heptaploid' × enneaploid produced only one seedling, 'octoploid' × enneaploid produced eight and aneuploid ($2n = 73$ and 74) × enneaploid yielded six. A random sample of these had $2n = 77$, 80, 81, 82, 83 and 85 (Table 3). Inter-specific crosses as well as intraspecific crosses within *A. gerardii* gave totally consistent results (Table 3). Using *A. lateralis* ($2n = 60$) as the maternal parent and 'octoploid' *A. gerardii* as the pollen parent, we obtained five hybrids: $2n = 65$, 70, 71 (two plants) and 96. 'Octoploid' *A. gerardii* × *A. glaucophyllus* ($2n = 60$) yielded four hybrids: $2n = 70$ (2), 71 and 72. *Andropogon* (*hypogynus* × *lateralis*) crossed with

Table 2. *Andropogon gerardii* intermediate cytotypes: reproductive characteristics and percentage seed set and seedling survival when outcrossed. Seedling rates calculated from the number of germinated seedlings divided by number of seeds sown. Percentage survival determined after two growing seasons in greenhouse (except for plants with $2n = 60$ and 90, in these cases 3 years in common garden)

$2n$	Meiotic behaviour Accession	% mature embryo sacs (filled seed)		% seed set random spikelets	% seedlings per 1000	% survival
60	Regular: bivalents	99	*N121* and others[1]	62.2 (59–73)		98
68	Not studied	–	*N257*	11.5	–	–
70	Irregular: univalents, bivalents, and trivalents. Laggards.	6	*N230*	1.5	1.0	73
		20.5	*N232*	3.7	–	–
		–	*N233*	7.1	–	–
		–	*N239*	7.1	3.0	67
		–	*N244*	0.5	0.3	33
71	Not studied	–	*N 247*	0.3	0	0
72	Not studied	–	*N 250*	8.8	3	47
73	Irregular: univalents, bivalents, trivalents and quadrivalents. Laggards. Micronuclei in tetrads.	–	*N243*	7.4	–	–
		12.3	*N249*	7.7	–	–
		–	*N252*	0.8	0.5	73
		–	*N258*	2.7	0.4	67
74	Irregular: univalents, bivalents, trivalents and quadrivalents. Laggards. Micronuclei in tetrads.	17	*N237*	14.8	8.0	67
		–	*N248*	2.4	2.0	86
		–	*N254*	10.2	4.1	33
		–	*N255*	0.5	0	0
75	Not studied	–	*N240*	12.8	5	92
76	Irregular: univalents, bivalents, trivalents and quadrivalents. Laggards. Micronuclei in tetrads.	12.2	*N245*	3.3	0.2	13
		–	*N253*	0.8	0.6	47
78	Not studied	–	*N238*	6.8	4	80
80	Irregular: univalents, bivalents, trivalents and quadrivalents. Laggards.	19.1	*N234*	5.2	–	–
		–	*N235*	–	–	–
		–	*N236*	1.5	0	0
		–	*N241*	9	3	73
		–	*N242*	2.3	–	–
		–	*N246*	0	0	0
		–	*N256*	8	5	80
90	Irregular: univalents, bivalents, trivalents, quadrivalents and pentavalents. Laggards.	24 (5–73)	*N118* and others[2]	9.7 (0.01–29)	2.2	42

[1]Mean and range of five different $2n = 60$ cytotypes (Norrmann *et al*., 1997).
[2]Mean and range of six different $2n = 90$ cytotypes (Norrmann *et al*., 1997).

A. gerardii $2n = 76$ as the pollen donor resulted in one hybrid with $2n = 64$.

The hybrid with 96 chromosomes must be the result of the fertilization of a non-reduced gamete ($n = 60$) of the pistillate plant (*A. lateralis*) and a reduced gamete with 36 chromosomes of the staminate parent. It cannot be explained otherwise; if the 80 chromosomes were unreduced, the hybrid should have had 80 + 30 = 110. Thus, cytological analysis of fewer than 100 hybrids of *A. lateralis* has yielded one unreduced gamete (Table 3). In contrast, with our interest in unreduced gametes, we have seen not one seedling

Table 3. Intermediate cytotypes in controlled crosses. The pistillate (female) parent is listed first in all cases

Cross	Number of crosses	Number of seedlings (%)	Number of progeny studied: Chromosome numbers (2*n*)
Intermediate × 2*n* = 60			
2*n* = 60 × 2*n* = 70 (*N121* × *N230*)	268	52	8: 62 (3), 64 (2), 65 (2), 66
2*n* = 70 × 2*n* = 60 (*N230* × *N121*)	106	2	2: 63, 64
2*n* = 73 × 2*n* = 60 (*N249* × mix)	1000	5	2: 68, 69
2*n* = 74 × 2*n* = 60 (*N245*)	1000	1	1: 65
2*n* = 80 × 2*n* = 60 (*N234* and *N235*) × mix	280	7	5: 67, 68, 69 (2), 74
Sub-total of crosses with 2*n* = 60	2672	67 (2.5)	28
Intermediate × 2*n* = 90			
2*n* = 70 × 2*n* = 90 (*N232* × mix)	1031	30	1: 77
2*n* = 90 × 2*n* = 70 (*N118* × N232)		0	0
2*n* = 80 × 2*n* = 90 (*N234* × mix)	211	8	6: 77, 80, 81, 82 (2), 85
2*n* = 73 × 2*n* = 90	1000	6	2: 82, 83
2*n* = 74 × 2*n* = 90 (*N254* × mix)	1000	1	1: 77
2*n* = 74 × 2*n* = 90 (*N237* × mix)	1000	1	1: 80
Sub-total of crosses with 2*n* = 90	4242	46 (1.1)	11
Inter-specific crosses			
A. lateralis (2*n* = 60) × 2*n* = 80 (mixed pollen)	140	17	5: 65, 70, 71 (2), 96[1]
N236 (2*n* = 80) × *A. glaucophyllus* (2*n* = 60)	251	1	1: 70
N234 (2*n* = 80) × A. glaucophyllus (2*n* = 60)	250	3	3: 70, 71,72
A. (*hypogynus* × *lateralis*) (2*n* = 60) × *N245* (2*n* = 76)	81	3	1: 64
Subtotal of interspecific crosses	722	24 (3.3)	

[1]Derived from an unreduced gamete from *A. lateralis* (see text).

produced by an unreduced gamete of *A. gerardii*, despite examining over 1000 plants.

Crossability

The frequency of seedlings in relation to the number of spikelets crossed for matings between hexaploids ranged from 19.4% (52/268) with the 'heptaploid' as male parent to 0.02% with the 'heptaploid' as female parent. The value for 'octoploids' is similar: 2.5% (7/280) when receiving hexaploid pollen. With South American relatives *A. lateralis* and *A. glaucophyllus* as pollen parents to octoploids, crossabilities were 3% and 0.8%, respectively. The South American interspecific hybrid *A. (hypogynus* × *lateralis*) crossed with aneuploid *A. gerardii* (2*n* = 76) had a crossability of 1%. In crosses using pollen from the enneaploid, 'heptaploids' had a crossability of 2.9% (30/1031; note this is the mean of 3% and 0, Table 4), 'octoploids' had 4%, and aneuploids (2*n* = 73 and 74) 0.1%. The numbers are too low to have statistical power, but it seems clear that fertility is related to the quality of gametes. Hexaploid × hexaploid is more fertile than hexaploid by plants with more than 60 chromosomes, which is in turn more fertile than crosses where both parents have more than 60 chromosomes.

'Euploids' and aneuploids

We find no significant differences in percentage seed set, seedlings per 1000 or percentage survival between 'euploids' and aneuploids (Student's *t*-test, Table 4). These results are unchanged if the 'euploids' are compared to all the aneuploids combined (data given in Table 2, analysis not shown). From this it appears that in this species, 2*n* = 70 or 80 could be 'euploid' cytotypes formed with seven or eight full complements of the basic 10 chromosomes, or they could be plants with unbalanced genomes (see Discussion).

Size and growth rates

Intermediate cytotypes are uncommon in the field but they do occur, with a frequency of 0–7% (Keeler, 1992; K. H. Keeler, unpubl. data). Comparison of intermediate cytotypes with hexaploids and enneaploids (Table 5) shows their great variation (as would be expected). They are, however, very similar to the enneaploids. They can apparently live a long time as well. Aneuploid clones have been measured to be more than 0.5 m in diameter (K. H. Keeler, unpubl. data). Mean clone expansion at the site is <0.05 m yr^{-1} (K. H. Keeler, unpubl. data), so clones are therefore estimated to have survived for at least a decade.

Table 4. Comparison of potential 'euploids' with aneuploids. 'Euploids' have $2n = 70, 80$; aneuploids are all other cytotypes with $2n$ between 61 and 89, thus distinguishing those with odd numbers of chromosomes as more likely to have unbalanced genomes than the even-numbered ones. The means in each column were shown by the Student's *t*-test not to be statistically different

	% Seed set		Seedlings		Seedling survival	
Group	Mean (SD)	*N*	Mean (1000 seeds SD)	*N*	Mean (SD)	*N*
Euploid	3.8 (3.3)	10	1.8 (1.9)	7	46.7 (35.3)	7
Even aneuploid	6.6 (5.1)	9	2.7 (2.7)	8	46.6 (30.5)	8
Odd aneuploid	5.3 (4.9)	6	1.5 (2.4)	4	58.1 (40.2)	4

Table 5. Field comparisons, made in Boulder, Colorado, of intermediate cytotypes of *Andropogon gerardii* to hexaploids and enneaploids

		Hexaploid		Intermediate		Enneaploid	
Character	Year	Mean (SD)	*N*	Mean (SD)	*N*	Mean	*N*
No. seeds per plant	1996	180.5 (898.0)	135	77.9 (141.9)	11	1876.8 (4977.3)	71
	1997	116.6 (830.1)	145	39.0 (86.0)	14	499.9 (1184.9)	75
	1998	148.1 (665.3)	157	196.9 (566.7)	14	695.0 (1881.7)	79
Good seeds per plant	1996	27.8 (188.1)	134	12.2 (35.8)	11	124.8 (616.9)	71
	1997	3.7 (31.9)	149	1.0 (3.9)	14	1.0 (3.9)	75
	1998	31.2 (251.1)	158	5.5 (17.2)	14	20.9 (100.1)	79
Clone area 1996	–	2365.5 (8082.5)	152	2766.6 (6678.6)	16	2798.7 (5986.4)	88
Change in clone area 1996–1998	–	765.1 (2161.5)	151	451.8 (2600.0)	16	497.3 (3224.0)	87
Foliage height	1995	28.1 (8.5)	77	22.2 (7.6)	6	39.2 (11.3)	28
	1996	24.3 (6.8)	71	20.3 (7.4)	7	28.2 (8.3)	46
Leaf width	1996	6.4 (1.8)	74	5.8 (1.2)	7	7.3 (1.5)	46

DISCUSSION

This study, combined with the previous study (Norrmann *et al*., 1997), indicates the following for the *Andropogon gerardii* polyploid complex. All plants, regardless of cytotype, are andromonoecious, undergoing anthesis early in the morning. All are also self-incompatible (Bennett, 1873; Law & Anderson, 1940; Norrmann *et al*., 1997). Failure of seed set after self-pollination is caused by the failure of the own-pollen tubes to penetrate and grow into the style, pointing to an outcrossing system functioning in all *A. gerardii* cytotypes.

The hexaploid cytotypes ($2n = 6x = 60$) produce some secondary associations of bivalents at meiosis I, but no laggards. They are fully fertile and produce gametes that uniformly contain 30 chromosomes (Norrmann *et al*., 1997). Minimal embryo sac abortion and good seed production follow (Table 2, see Norrmann *et al*., 1997). Enneaploids, 'heptaploids',

'octoploids' and the aneuploids with $2n = 68–78$ have similar breeding systems. Metaphase I of meiosis shows univalents and trivalents (also quadrivalents in cytotypes higher than $2n = 70$), and chromosomes lag at anaphase I. The resulting gametes frequently abort (Table 2). Pollen of these plants contains variable numbers of chromosomes, forming an apparent normal distribution with a mean of half the parental complement of chromosomes (Table 4).

Plants with $2n = 70$ and 80 are usually considered to be 'euploids' because the genus has a base number of $x = 10$ (Gould, 1967). As 'euploids', they would be expected to have meiosis that is more regular than in aneuploids. However, *A. gerardii* 'heptaploids' and 'octoploids' may not have seven and eight complete genomes, respectively, but rather unbalanced aneuploid genomes, despite their apparently 'euploid' chromosome number. Our data support this possibility. We find no significant differences between 'euploids' and aneuploids (Table 4), yet 'euploids' should be more fertile than aneuploids because of their more complete genomes. In this species, there is no indication that individuals with $2n = 70$ or 80 contain simple multiples of basic 10 chromosomes.

To put it in other words, if we consider just one of the basic set of 10 chromosomes, a hexaploid contributes $6/2 = 3$ chromosomes to its gametes during meiosis, but the enneaploid produces $9/2 = x$. Assuming that the normal type of meiotic chromosome segregation in high polyploids is roughly half – half, then $x = 4$ or 5 (occasionally 3 or 6). Now, considering all ten groups of chromosomes from the enneaploid, a gamete could have $4,4,4,4,4,4,4,4,4,4 = n = 40$ which gives a *balanced* 'heptaploid' when added to the $n = 30$ from the hexaploid. Similarly, a gamete from the enneaploid with $5,5,5,5,5,5,5,5,5,5 = n = 50$ gives a balanced 'octoploid' when added to the $n = 30$ from the hexaploid. Clearly, this type of meiotic separation would be very rare, and the 'heptaploid' progeny would more probably have come from an unbalanced gamete from the enneaploid that by chance adds up to 40, for example 5,4,4,5,3,4,4,4,3,4 or any one of many other combinations. Similarly, the 'octoploid' could have come from an enneaploid gamete with 6,5,4,5,5,5,6,5,4,5 or any one of many other combinations totalling 50. So these 'heptaploids' and 'octoploids' are genetically simply part of the unbalanced aneuploid range that has been demonstrated, and would not be expected to display any characteristic meiotic, genetic or morphological features to distinguish them from the aneuploids.

All cytotypes with $2n > 60$ form some multivalents (Norrmann *et al*., 1997; Table 2). In hexaploids, chromosomes segregate normally, producing viable gametes with $n = 30$ chromosomes. The secondary associations of bivalents seen in these hexaploids reveal their ancestral polyploidy, as suggested by the high chromosome number. In all other cytotypes studied, univalents are also present and segregation is irregular. Laggards form in these cytotypes and there is a variable but often high level of embryo sac abortion. The latter can be explained by the observed irregularities in the meiotic processes and its consequences result in low seed production (Table 2).

Pollen produced by cytotypes above the hexaploid also appears to result from poorly regulated segregation. In this study, 'heptaploids' produced gametes with $n = 32$, 33, 34, 35 and 36 chromosomes, and 'octoploids' produced gametes with $n = 35$, 36, 37, 38, 39, 40, 41, 42 and 44. Interfertility, measured as crossability, was related to the frequency of viable gametes produced by each parent. Thus crosses including a hexaploid *A. gerardii* cytotype are more fertile than if both parents are producing a high frequency of aborting gametes (Table 3). Likewise, 'heptaploids', 'octoploids', enneaploids and aneuploids are more effective as pollen parents than as seed parents, presumably because with more gametes involved there is a greater chance of including viable ones.

Crosses with South American hexaploid species produced about as many viable plants as intraspecific crosses (Table 4), but the interspecific hybrids are sterile. The chromosome numbers of interspecific hybrids range from $n = 35$ to 44 chromosomes, consistent with the results of Norrmann *et al*. (1997). The chromosome numbers confirm the interpretations of chromosome behaviour derived from the intraspecific crosses.

The evolutionary consequences of this polyploid complex are difficult to interpret. Natural populations of *Andropogon gerardii* in the western part of its range contain high frequencies of hexaploids and enneaploids, with intermediate chromosome numbers forming less than 7% of the populations (Keeler, 1992; Norrmann *et al*., 1997; Keeler & Davis, 1999). Populations dominated by or composed of only enneaploids would be much less fertile than mixed populations (Norrmann *et al*., 1997), and indeed such populations are rare to non-existent (Keeler, 1992; K. H. Keeler, unpubl. data).

In cytotypically mixed populations, the available pollen will be a mixture of grains with $n = 30$ from hexaploids and with $n = 35–50$ from enneaploids. Hexaploids and enneaploids cross under natural conditions (K. H. Keeler, unpubl. data), so the resulting seeds could be expected to contain from $2n = 60$ to over 100. Thus, after some years of recruitment, aneuploids might dominate the population. However, aneuploids are not common in natural populations (Keeler, 1992; Keeler & Davis, 1999). The explanation of this is not known, but one possibility is that seed recruitment is of low importance in these populations

(e.g. Glenn-Lewin *et al*., 1987). Alternatively, the higher level of fitness of hexaploids could eliminate other cytotypes. These explanations are not mutually exclusive.

The origin of the enneaploids is only conjectured as no new ones have been seen. *Andropogon gerardii* and the derived species *A hallii* are the only North American hexaploids (Gould, 1967). We believe that enneaploids are produced from a hexaploid's unreduced gamete combining with a reduced gamete (i.e. $2n = 60 + 30 = 90$), although we have never actually observed it. Consequently, one of the unresolved questions in this polyploid complex is how frequently do unreduced gametes occur, an observation that would help to determine whether production of enneaploids is historic or continuing. Assuming that the unobserved non-reduction has occurred, the current populations could have been produced as follows: enneaploids were generated from hexaploids; subsequent backcrosses created aneuploids such as those observed by Gould (1968b) and studied here (Keeler, 1990, 1992). It seems most likely that strong selection at one or more stages is preventing the establishment of an aneuploid swarm in the western populations, but it is conceivable that seed recruitment is so rare that the invasion of aneuploids is only just beginning.

The most likely scenario is that under normal environmental conditions, *A. gerardii* plants do not produce unreduced gametes. However, the presence of enneaploid plants in the prairies indicates that it has occurred. Under unusual environmental conditions, such as extreme heat or cold, unreduced gametes can be produced in plants (Bretagnolle & Thompson, 1995). The same environmental conditions that produced the unreduced gametes could have reduced the plant density in the prairies sufficiently to allow the survival of enneaploids produced by the union of a normal and an unreduced gamete. The enneaploid plants are persisting in the prairies under current conditions, but produce few viable offspring and even fewer that are enneaploid. The long-term prognosis is for them to be eliminated, assuming that environmental extremes do not recur.

CONCLUSIONS

This study concludes that *Andropogon gerardii* plants with chromosome numbers between $2n = 60$ and 90 have meiotic patterns that are similar to that of the enneaploid ($2n = 90$) and reduce fertility. Like all other *A. gerardii* cytotypes, they are self-incompatible. However, they do not pose significant genetic load to replanted prairies or seed collections. They are rarely produced or established and under good management practices should be insignificant. However, when they occur, these cytotypes are very variable and established plants usually have some fertility and can be quite vigorous.

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