

RESEARCH PAPER

Chromosome number in South American species of *Bothriochloa* (Poaceae: Andropogoneae) and evolutionary history of the genus

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ABSTRACT

Mitotic chromosome number of 14 taxa of *Bothriochloa* native to Argentina, Brazil and Uruguay were surveyed. Chromosome numbers of *B. eurylemma*, *B. meridionalis* and *B. velutina* are reported for the first time, with $2n = 6x = 60$, and this ploidy level is the most common among the studied taxa. In addition, new cytotypes were found for *B. alta* ($2n = 60$), *B. barbinodis* ($2n = 60$), *B. exaristata* ($2n = 80$), *B. laguroides* var. *torreyana* ($2n = 80$), *B. longipaniculata* ($2n = 60$ and 80), *B. perforata* ($2n = 60$) and *B. springfieldii* ($2n = 60$). These numbers differ from those reported in the literature.

INTRODUCTION

Polyploidy is an important evolutionary process that has driven and shaped evolution within the grasses, primarily by increasing the capability of individuals with different chromosome numbers to adapt to a variety of new habitats (Levy & Feldman 2002). In addition, the polyploidy background enables genomic changes that cannot be achieved at the diploid level, facilitating acceleration of genome evolution. Some authors have proposed that duplicated genes may be manifested in greater biochemical and physiological flexibility, enhanced environmental adaptability or development of novel physiologies or morphologies (Wendel 2000). In general, polyploids differ more in their geographic distribution than their diploid relatives. Polyploids usually exhibit greater ecological plasticity, easily colonise new regions and are more tolerant to extreme conditions (Wendel 2000; Leitch & Leitch 2008). About 70% of all grasses are polyploids (Chapman 1996), where all major types of polyploid, autopolyploid, segmental allopolyploid and allopolyploid can be found (Levy & Feldman 2002).

Bothriochloa Kuntze contains ca. 40 species that grow in open grassy places throughout the tropics. Members of the genus have been studied from several points of view in relation to reproduction and cytology (Gould 1956; Celarier & Harlan 1957; De Wet *et al.* 1963; Faruqi 1964; De Wet 1968; Allred & Gould 1983). In addition, several contributions have

focused on the production of essential oils and the taxonomic implications (De Wet & Scott 1965; Scrivanti 2007 and references therein), whereas others have reported different mechanisms related to the mode of reproduction (Heslop-Harrison 1961; Scrivanti *et al.* 2009). In the Americas, all native species of *Bothriochloa* reproduce sexually (De Wet *et al.* 1963; Allred & Gould 1983; Scrivanti *et al.* 2009).

Species of *Bothriochloa* display many levels of ploidy: sexual diploids endemic to India and Australia (De Wet *et al.* 1963; Yu *et al.* 2003), tetraploids widely distributed in the Old World that are facultative or obligate apomicts (Celarier & Harlan 1957; Harlan & Celarier 1961; De Wet *et al.* 1963; Harlan & De Wet 1963; Faruqi 1964) and hexaploids with different reproductive strategies. Several hexaploid species from Africa, India and Australia are obligate apomicts (De Wet *et al.* 1963; Harlan *et al.* 1964), while in North America all native species of *Bothriochloa* have been found to be sexual polyploids ($2n = 60, 120, 180, 220$) (Gould 1956; De Wet *et al.* 1963; De Wet 1968; Allred & Gould 1983). Twelve American taxa (*B. alta*, *B. barbinodis*, *B. campii*, *B. edwardsiana*, *B. exaristata*, *B. hybrida*, *B. laguroides* var. *laguroides*, *B. laguroides* var. *torreyana*, *B. longipaniculata*, *B. perforata*, *B. saccharoides* var. *saccharoides* and *B. springfieldii*) have a disjunct distribution between North and South America. The chromosome numbers of North American disjunct taxa have already been established (De Wet 1968; Allred & Gould 1983) but little information is available on chromosome

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counts and cytological data for the South American disjunct and endemic taxa. Therefore, the objectives of this work are to report the chromosome numbers in 14 taxa of *Bothriochloa* from South America and discuss the origin and evolutionary history of the genus in the Americas.

MATERIAL AND METHODS

Thirteen species and two subspecies of *Bothriochloa* were studied (Table 1). Seeds and pieces of plants were collected in January to May from 2003 to 2006 in Argentina, Brazil and Uruguay. All plants were collected from natural populations and grown in experimental gardens or glasshouses at the Instituto Multidisciplinario de Biología Vegetal, Córdoba, or the Instituto de Botánica del Nordeste, Corrientes. Voucher specimens of all of the plants used in this study have been deposited in the herbarium of the Museo Botánico de Córdoba (CORD). Chromosome numbers were obtained from mitotic squashes of root tips collected from potted plants according to protocols in Johansen (1940) and Norrmann (2009). Root tips were pre-treated for 2 h with α -bromonaphthalene at room temperature (20/25 °C). Material was then hydrolysed with 1 M HCl at 60 °C for 10 min and stained with fuchsin. Squashes were made in a drop of acetorcein. Preparations were made permanent with Venetian turpentine.

RESULTS

The chromosome numbers for 14 taxa of South American *Bothriochloa* are listed in Table 1. All of the counts were obtained from root tissue. Three different chromosome numbers were recorded in taxa used for this study: $2n = 60$ (Fig. 1B), $2n = 80$ (Fig. 1A, C–E) and $2n = 120$ (Fig. 1F). These numbers are multiples of $x = 10$, which is regarded as the genuine base chromosome number for the genus (Spangler *et al.* 1999). Consequently, plants with 60, 80 and 120 somatic chromosomes correspond to hexaploids, octoploids and dodecaploids, respectively. The length of the chromosomes range from 0.8 to 2.3 μm .

The chromosome numbers of three South American endemics, *B. eurylemma*, *B. meridionalis* and *B. velutina*, are reported here for the first time. In agreement with previous reports (Gould 1951, 1956, 1957, 1966; De Wet 1968; Allred & Gould 1983), we counted $2n = 60$ for *B. edwardsiana*, *B. exaristata*, *B. imperatoides*, *B. laguroides* var. *laguroides*, *B. laguroides* var. *torreyana*, *B. longipaniculata* and *B. saccharoides* var. *saccharoides*. Plants of *B. barbinodis* with $2n = 80$ were reported previously from South America (Gould 1956) and in addition we found $2n = 80$ in *B. exaristata*, *B. laguroides* var. *torreyana* and *B. longipaniculata*.

A new chromosome number of $2n = 60$ was recorded for taxa in which other chromosome numbers different from 60 were found previously: *B. alta* (we also counted $2n = 120$ in a cytotype from Chaco), *B. barbinodis*, *B. perforata* and *B. springfieldii*.

DISCUSSION

Our results indicate that in South America *Bothriochloa* are primarily hexaploids ($2n = 6x = 60$), a few octaploids

($2n = 8x = 80$), and occasionally a rare dodecaploid ($2n = 12x = 120$), whereas in North America species have higher levels of ploidy that are widely distributed ($2n = 12x = 120$, $2n = 18x = 180$, $2n = 22x = 220$).

Chromosome counts of $2n = 120$ in taxa with previous reports of $2n = 60$ are most likely the result of whole genome duplication. Considering disjunct North and South American taxa, we found South America had hexaploid taxa, although higher ploidies are usually restricted to North America (Table 1). This suggests that South America might be the origin for most American disjunct taxa. Furthermore, the finding of $2n = 60$ and 120 for the same taxon (*B. alta*) in Argentina suggests that duplication of whole genomes may occur before migration to North America. For reasons we do not understand, higher polyploid levels seem to have prospered in North America. Such a distribution pattern is often explained by adaptive differences among cytotypes to an underlying heterogeneity of environmental factors (Lewis 1980). As postulated for $2x$, $4x$ and $6x$ cytotypes of *Turnera* (Solís Neffa & Fernández 2001), the restricted geographical range and disjunct distribution of *Bothriochloa* cytotypes $6x$, $12x$, $18x$ and $22x$ suggests that these populations might be relicts, having survived periods of climatic change. Correspondingly, these climatic events might have provided favourable conditions for the establishment of the different polyploids. Hence, individuals with higher levels of polyploidy may have gained a selective advantage and been able to expand their ranges.

During the Pleistocene (18,000 years), a sequence of dry and humid climates occurred between latitudes 22°S and 39°S, as a result of extensive glaciation cycles in the Andes (Iriondo 1992; Prieto 1996). These climatic changes could explain the present distribution of cytotypes of the *Turnera sidoides* complex and *Bothriochloa* in South America. In addition, the large-scale distribution suggests that the occurrence of a cytotype is restricted by the presence of other cytotypes, as demonstrated by Van Dijk *et al.* (1992) in *Plantago media* L. A parapatric distribution may result from competitive exclusion, in which a superior cytotype replaces another geographically (Pilou 1979; Stebbins & Dawe 1987). Information about cytotype distribution in the surrounding regions and molecular data on the genetic relationships among populations are needed to elucidate this point.

The appearance of octoploid cytotypes in an otherwise hexaploid geographical neighbourhood generates at least two questions regarding origin. First, are octoploids a by-product of natural secondary polyploidisation, as happens in *A. gerardii* Vitman (Keeler *et al.* 1987), or are octoploids cryptic semispecies isolated from hexaploids? Several alternatives involving duplication and loss of chromosomes are theoretically possible to explain a $2n = 60$ cytotype giving rise to a $2n = 80$ cytotype. According to Ramsey & Schemske (1998, 2002), octoploids can be produced either as (i) a secondary hybridisation process between a hexaploid and a diploid, followed by duplication, (ii) a triploid bridge can generate tetraploids and these duplicate to give octoploids, or (iii) an unreduced hexaploid gamete can give rise to an enneaploid cytotype (or from a cross between $2n = 60$ and $2n = 120$ plants) with subsequent partial loss of chromosomes. But neither diploids nor tetraploids have been found in North America, and no triploid has been recorded in the continent

Table 1. Chromosome number, ploidy level, chromosome size, voucher, distribution and published chromosome number of *Bothriochloa* taxa studied in this work.

Taxa	Chromosome number (2n)	Ploidy level (x)	Size in μm	Voucher ^a	Original distribution	Published chromosome number (2n)
<i>B. alta</i> (Hitcch.) Henrard	60	6	0.80–2.30	Argentina: Provincia de Córdoba, Capital, <i>Scrivanti</i> 117, 22-I-2004.- Colón, <i>Scrivanti</i> 39, 29-I-2003; 57 14-III-2003; 99, 07-I-2004.- Tercero Arriba, <i>Scrivanti</i> 55, 12-II-2003.- Santa María, <i>Anton</i> 340, 08-II-2003. Argentina. Provincia de Chaco, Independencia, Avia Terai, <i>JC</i> 146, V-2008.	North America (SW USA, México. Central America (Honduras, El Salvador). South America (Ecuador, Peru, Bolivia, Brazil, Argentina)	120 (Mexico, Texas, Gould 1956, 1957)
<i>B. barbinodis</i> (Lag.) Herter	120	12	s/n	Argentina: Provincia de Córdoba, Capital, <i>Scrivanti</i> 115, 20-I-2004; 204, 210, 15-II-2005.- Río Segundo, <i>Scrivanti</i> 48, 12-II-2003.- Tercero Arriba, <i>Scrivanti</i> 52, 12-II-2003.	North America (SW USA, México). Central America (El Salvador). South America (Ecuador, Peru, Bolivia, Chile, Brazil, Uruguay, Argentina)	180 (Texas, Mexico, Gould 1956, 1957; USA, De Wet 1968)
<i>B. edwardsiana</i> (Gould) Parodi	60	6	0.80–2.30	Argentina: Provincia de Córdoba, Punilla, <i>Scrivanti</i> , 113, 20-I-2004; 190, 10-V-2004; 212, 15-II-2005. Provincia de Entre Ríos, Gualeguaychú, <i>Scrivanti</i> 224, 11-III-2005.	North America (USA, Texas, México). South America (Uruguay, Argentina)	60 (Texas, Gould 1951, 1957)
<i>B. eurylemma</i> M. Marchi & Longhi-Wagner	60	6	0.80–2.30	Argentina: Provincia de Corrientes, Bella Vista, <i>Scrivanti</i> 134, 04-III-2004.- Mercedes, <i>Scrivanti</i> 130, 03-III-2004. Provincia de Entre Ríos, Federación, <i>Scrivanti</i> 243, 15-III-2005.- Paraná, <i>Scrivanti</i> 123, 02-III-2004. Argentina: Provincia de Chaco, General Belgrano, <i>Scrivanti</i> 153, 06-III-2004.	South America (Brazil, Argentina)	s/n
<i>B. exaristata</i> (Nash) Henrard	60	6	0.80–2.30	Argentina: Provincia de Chaco, General Belgrano, <i>Scrivanti</i> 155, 06-III-2004.- Colonia Benítez, <i>Scrivanti</i> 148, 06-III-2004. Provincia de Corrientes, Empedrado, <i>Scrivanti</i> 140, 04-III- 2004.- Federación, <i>Scrivanti</i> 169, 29-III-2004.	North America (USA: California, Gulf Coast). South America (Bolivia, Paraguay, Brazil, Argentina)	60 (USA, De Wet 1968; Texas, Brazil, Argentina, Gould 1956, 1957; Allred & Gould 1983)
<i>B. imperatooides</i> (Hack.) Herter	60	6	0.80–2.30	Provincia de Corrientes, Berón de Astrada, <i>Scrivanti</i> 143, 05-III-2004.- Brazil: Rio Grande do Sul, <i>CORD</i> 33. Uruguay: Maldonado, <i>Scrivanti</i> 239, 13-III-2005.	South America (Brazil, Uruguay, Argentina)	60 (Uruguay, Argentina, De Wet 1968; Allred & Gould 1983)
<i>B. lagurooides</i> var. <i>lagurooides</i> (DC.) Herter	60	6	0.80–2.30	Argentina: Provincia de Córdoba, Santa María, <i>Scrivanti</i> 56, 14-III-2003. Provincia de Corrientes, Mercedes, <i>Scrivanti</i> 131, 03-III-2003. Provincia de Entre Ríos, Gualeguaychú, <i>Scrivanti</i> 218, 11-III-2005. Uruguay, <i>Scrivanti</i> 234, 13-III-2005.	North America (S USA, México). Central America (Honduras, Panama). South America (Bolivia, Chile, Brazil, Argentina).	60 (USA, Mexico, and South America, Allred & Gould 1983)
<i>B. lagurooides</i> var. <i>torreyana</i> (Steud.) M. Marchi & Longhi-Wagner	60	6	s/n	Argentina: Provincia de Misiones, Bernardo de Irigoyen, <i>Scrivanti</i> 167, 28-III-2004. Uruguay: Maldonado, Punta del Este, <i>Scrivanti</i> 237, 13-III-2005.	North America (USA, Mexico). Central America (Honduras, Panamá). South America (Chile, Brazil, Uruguay, Argentina)	60 (Texas, Gould 1957; USA, Mexico, and South America, Allred & Gould 1983)

Table 1. Continued.

Taxa	Chromosome number (2n)	Ploidy level (x)	Size in μm	Voucher ^a	Original distribution	Published chromosome number (2n)
	80	8	0.80–2.20	Argentina: Provincia de Formosa, Clorinda, Scrivanti 157, 07-III-2004.		
<i>B. longipaniculata</i> (Gould) Allred & Gould	60	6	0.90–2.20	Argentina: Provincia de Chaco, Colonia Benitez, Scrivanti 147, 06-III-2004. - Provincia de Misiones, Bernardo de Irigoyen, Scrivanti 166, 27-III-2004.	North America (Texas, Louisiana, NE Mexico). Central America (Guatemala), South America (Brazil, Paraguay, Argentina)	60 (Texas, USA, Gould 1956), 120 (Texas, USA, Mexico, Gould 1955, 1957; De Wet et al. 1963; Allred & Gould 1983)
<i>B. meridionalis</i> M. Marchi & Longhi-Wagner	60	6	0.80–2.30	Argentina: Provincia de Chaco, Resistencia, Scrivanti 146, 06-III-2004. Brazil: Rio Grande do Sul, CORD 30, 37, 40.	South America (Ecuador, Colombia, Venezuela, Brazil)	s/n
<i>B. perforata</i> (Trin ex E. Fourn.) Herter	60	6	0.80–2.30	Argentina: Provincia de Córdoba, Capital, Scrivanti 46, 06-III-2004; 118, 06-III-2004. Punilla, Scrivanti 100, 07-I-2004. Santa Maria, Scrivanti 33, 23-I-2003.	North America (USA, México). South America (Ecuador, Peru, Argentina)	120 (Texas, Gould 1953).
<i>B. saccharoides</i> var. <i>saccharoides</i> (Sw.) Rydb.	60	6	0.80–2.30	Argentina: Provincia de Córdoba, Colón, Anton 358, 09-IV-2005. - Punilla, Scrivanti 45, 29-I-2003, 104, 07-I-2004. Provincia de Chaco, Vera, Scrivanti 159, 08-III-2004. Provincia de Corrientes, Mercedes, Scrivanti 133, 03-III-2004.	North America (México). Central America (Honduras, Caribbean, El Salvador, Guatemala, Nicaragua, Panama). South America (Ecuador, Colombia, Venezuela, Peru, Bolivia, Chile, Brazil, Argentina)	60 (Texas, Mexico, Brazil, Uruguay, Argentina, Gould 1956), 120 (West Indian (inferred from pollen size), Allred & Gould 1983).
<i>B. springfieldii</i> (Gould) Parodi	60	6	0.90–2.20	Argentina: Provincia de Córdoba, Colón, Scrivanti 38, 29-I-2003. Punilla, Scrivanti 40, 29-I-2003; 199, 08-II-2005. - Rio Segundo, Scrivanti 47, 12-II-2003.	North America (SW USA, México). South America (Bolivia, Uruguay, Argentina)	120 (Texas, Gould 1957; USA, De Wet 1968)
<i>B. velutina</i> M. Marchi & Longhi-Wagner	60	6	0.80–2.20	Brazil: Santa Catarina, Scrivanti 163, 24-III-2004.	South America (S Brazil)	s/n

^aHoused at CORD.

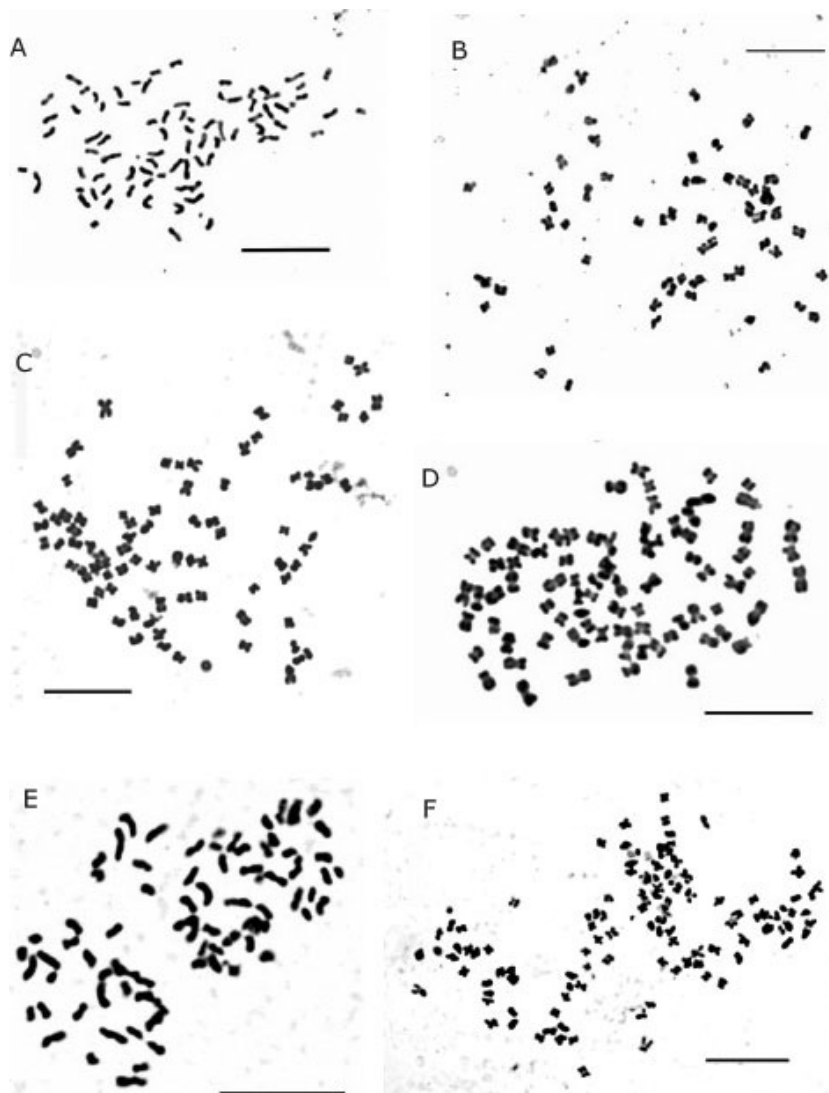


Fig. 1. XXXXXX.

up to now; so perhaps octoploidy might be very old and these cytotypes are behaving as hidden species, breeding true *via* self-pollination. At this stage of speculation, analysis of populations with mixed cytotypes is clearly needed.

Evolutionary history of the genus

As in the tribe, it is probable that *Bothriochloa* originated in Asia, possibly centered in India, since most diploids are endemic to this region (De Wet & Harlan 1970). Comparison of evolutionary scenarios within *Bothriochloa* from the Old World, as well as relationships with sister genera such as *Capillipedium* Stapf, *Dichanthium* Willemet and *Andropogon* L. deserve consideration. The boundaries between *Bothriochloa*, *Dichanthium* and *Capillipedium* from the Old World have been blurred due to a complex pattern of hybridisation created by introgression from *B. bladhii* (Retz.) S. T. Blake, which has been considered a compilospecies (Harlan & De Wet 1963; De Wet & Harlan 1966). Although these hybrids and introgressants were sterile,

chromosome doubling helped to lower sterility in the offspring. Celarier (1957) proposed that the origin of polyploidy in *B. ischaemum* (L.) Keng would have involved the assemblage of genomes belonging to compatible species. According to Harlan & De Wet (1963) and De Wet & Harlan (1970), the hybrid form of *B. intermedia* (R. Br.) A. Camus in the process of geographical expansion from India to Africa and Australia has subsumed its relatives, and even its own ancestral form.

Celarier (1957) and Faruqi (1964) suggested that, in the origin of hexaploids, two or three genomes could be involved; in the case of two genomes, one would be auto-duplicated. The *Bothriochloa* hexaploids from the Old World and Australia have a restricted distribution and are obligate apomictics, although a few are sexual. South American hexaploids are sexual, with regular meiotic behaviour, which suggests an allopolyploid origin even though no further cytogenetic work has been performed to address this issue (Harlan & De Wet 1975). Phylogenetic relationships among African, Australian and North American spe-

cies have been analysed by Singh & De Wet (1961), who demonstrated cytogenetically that American species of *Bothriochloa* are more closely related to Australian species than to Old World species, and that natural hybridisations are taking place in this group, suggesting a former worldwide distribution of the genus.

Bothriochloa shows a similar evolutionary pattern as *Andropogon* (Norrmann 2009). *Andropogon* has a worldwide distribution, with diploids and tetraploids in Africa, and has sexual hexaploid species in the Americas. Although apomixis has never been found in *Andropogon*, hybridisation and chromosome doubling has apparently been important in the origin of hexaploids (Norrmann *et al.* 1997; Norrmann 2009). An interesting group of South American hexaploid *Andropogon* was recently studied by Norrmann (2009): the *A. lateralis* Nees complex. Based on meiotic behaviour of natural hybrids within the complex, the origin of the *A. lateralis* complex was hypothesised as follows: one or several hexaploids of *Andropogon* were probably established multiple times in North America, in agreement with the view exposed by Hartley (1958) for the tribe. Then, from these ancestors and through small chromosome rearrangements, different species within this group developed. If *Bothriochloa* were to follow this general pattern of Hartley (1958), then one or several proto-*Bothriochloa* species might have reached North America in an 'advanced state of evolution', *i.e.*, as polyploids (hexaploids, and then followed other patterns to generate new species, such as chromosome duplication followed by absorption of whole genomes. It is possible that the hypothesis proposed by Harlan & De Wet (1963) and De Wet & Harlan (1970) for *B. intermedia* in the Old World might apply to species with high ploidy ($2n = 120, 180$) in North America. Hence, we may hypothesise that, after hexaploids were established in the south of North America, they migrated northwards assimilating germplasm from various related species during geographical expansion, and this could have involved chromosome duplication. Earlier speculation for this North American scenario was contributed by Gould (1953, 1955, 1956), who hypothesised a hybrid origin for *B. longipaniculata* ($2n = 120$) suggesting as parents *B. exaristata* ($2n = 60$) and *B. laguroides* var. *torreyana* ($2n = 60$), based on chromosome number, habitat preference, geographic distribution, pollen diameter and morphology. In addition, *B. hybrida* ($2n = 120$) is assumed to be a derivative of one or more hybrids between *B. edwardsiana* ($2n = 60$) and *B. laguroides* var. *torreyana* based on the possession of pitted sessile spikelets and pollen size (Gould 1957). In our opinion, the occurrence of octoploids ($2n = 80$) in most of these taxa does not affect Gould's hypothesis about the origin of dodecaploid species, since octoploids are likely to be by-products of occasional enneaploidy followed by loss of chromosomes.

In summary, the hypothesis of Harlan & De Wet (1963) and De Wet & Harlan (1970) for the origin of Old World *B. intermedia* is apparently the most likely scenario for the origin of North American dodecaploid and octododecaploid cytotypes. A general picture where hexaploid species of *Bothriochloa* are common in South America and higher ploidy levels occur in North America, suggests that the origin of amphitropical disjuncts might be in South America. Never-

theless, it would be appropriate to use other tools based on molecular cytogenetics, and we are now initiating a project using genomic *in situ* hybridisation (Bennett 1995; Schwarzscher & Heslop-Harrison 2000) to determine genomic homology and phylogenetic relationships, using selected North American, South American and African species.

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REFERENCES

- Allred K.W., Gould F.W. (1983) Systematics of the *Bothriochloa saccharoides* complex (Poaceae: Andropogoneae). *Systematic Botany*, **8**, 168–184.
- Bennett M. (1995) The development and use of genomic *in situ* hybridization (GISH) as a new tool in plant biosystematics. In: Brandham P., Bennett M. (eds), *Kew Chromosome Conference IV*. Royal Botanic Gardens, Kew, pp. 167–183.
- Celarier R.P. (1957) Cytotaxonomy of the andropogoneae II. Subtribes ischaeminae, rottboellinae and the maydeae. *Cytologia*, **22**, 160–183.
- Celarier R.P., Harlan J.R. (1957) Apomixis in *Bothriochloa*, *Dichanthium* and *Campillipedium*. *Phytomorphology*, **7**, 93–102.
- Chapman G.P. (1996) *The biology of grasses*. CAB International, Wallingford, UK.
- De Wet J.M.J. (1968) Biosystematic of the *Bothriochloa barbinodis* complex (Gramineae). *American Journal of Botany*, **55**, 1246–1250.
- De Wet J.M.J., Harlan J.R. (1966) Morphology of the compilospecies *Bothriochloa intermedia*. *American Journal of Botany*, **53**, 94–98.
- De Wet J.M.J., Harlan J.R. (1970) Apomixis, polyploidy, and speciation in *Dichanthium*. *Evolution*, **24**, 270–277.
- De Wet J.M.J., Scott B.D. (1965) Essential oils as taxonomic criteria in *Bothriochloa*. *Botanical Gazette*, **126**, 209–214.
- De Wet J.M.J., Bargaonkar D.S., Richardson W.L. (1963) Chromosome number and mode of reproduction in the *Bothriochloa* inae. *Caryologia*, **16**, 47–55.
- Faruqi S.A. (1964) Cytogenetical studies of the *Bothriochloa intermedia* complex. *Cytologia*, **29**, 280–297.
- Gould F.W. (1951) A new species of *Andropogon* from Edwards Plateau of Texas. *Field & Laboratory*, **19**, 183–185.
- Gould F.W. (1953) A cytotaxonomic study in the genus *Andropogon*. *American Journal of Botany*, **40**, 297–306.
- Gould F.W. (1955) *Andropogon saccharoides* Swartz var. *longipaniculatus* var. nov. *Field & Laboratory*, **23**, 17–19.
- Gould F.W. (1956) Chromosome counts and cytotaxonomic notes on grasses of the tribe Andropogoneae. *American Journal of Botany*, **43**, 395–404.
- Gould F.W. (1957) Pollen size as related to polyploidy and speciation in the *Andropogon saccharoides* – *A. barbinodis* complex. *Brittonia*, **9**, 71–75.
- Gould F.W. (1966) Chromosome numbers of some Mexican grasses. *Canadian Journal of Botany*, **44**, 1683–1696.

- 1 Harlan J.R., Celarier R.P. (1961) Apomixis and species formation in
2 the Bothriochloae Keng. *Recent Advances in Botany*, **1**, 706–710.
- 3 Harlan J.R., De Wet J.M.J. (1963) The compilospecies concept.
4 *Evolution*, **17**, 497–501.
- 5 Harlan J.R., De Wet J.M.J. (1975) On a wing and a prayer: the ori-
6 gins of polyploidy. *The Botanical Review*, **41**, 361–391.
- 7 Harlan J.R., Brooks M.H., Borgeonkar D.S., De Wet J.M.J. (1964)
8 Nature and inheritance of apomixis in *Bothriochloa* and *Dichan-*
9 *tium*. *Botanical Gazette*, **125**, 41–46.
- 10 Hartley W. (1958) Studies on the origin, evolution and distribu-
11 tion of the Gramineae. I. The tribe Andropogoneae. *Australian*
12 *Journal of Botany*, **6**, 116–128.
- 13 Heslop-Harrison J. (1961) The function of the glume pit and the
14 control of cleistogamy in *Bothriochloa decipiens* (Hack.) C.E.
15 Hubbard. *Phytomorphology*, **11**, 378–383.
- 16 Iriondo M.H. (1992) El Chaco. *Holoceno*, **1**, 50–63.
- 17 Johansen D.A. (1940) *Plant microtechniques*. McGraw-Hill,
18 London, UK.
- 19 Keeler K.H., Kwankin B., Barnes P.W., Galbraith D.W. (1987)
20 Polyploid polymorphism in *Andropogon gerardii*. *Genome*, **29**,
21 374–379.
- 22 Leitch A.R., Leitch I.J. (2008) Genomic plasticity and the diversity
23 of polyploid plants. *Science*, **320**, 481–483.
- 24 Levy A.A., Feldman M. (2002) The impact of polyploidy on grass
25 genome evolution. *Plant Physiology*, **130**, 1587–1593.
- 26 Lewis W.H. 1980 Polyploidy in species population. In: Lewis W.H.
27 (ed.) *Polyploidy, biological relevance*. Plenum Press, New York,
28 pp. 103–144.
- 29 Norrmann G. (2009) Natural hybridisation in the *Andropogon lat-*
30 *eralis* complex (Andropogoneae, Gramineae) and its impact on
31 taxonomic literature. *Botanical Journal of the Linnean Society*,
32 **159**, 136–154.
- 33 Norrmann G.A., Quarín C.L., Keeler K.H. (1997) Evolutionary
34 implications of meiotic chromosome behavior, reproductive
35 biology, and hybridization in 6x and 9x cytotypes of
36 *Andropogon gerardii* (Poaceae). *American Journal of Botany*, **84**,
37 201–207.
- 38 Prieto A.R. (1996) Late Quaternary vegetational and climatic
39 changes in the Pampa grassland of Argentina. *Quaternary*
40 *Research*, **45**, 73–88.
- 41 Ramsey J., Schemske D.W. (1998) Pathways, mechanisms, and
42 rates of polyploidy formation in flowering plants. *Annual Review*
43 *of Ecology and Systematics*, **29**, 467–501.
- 44 Ramsey J., Schemske D.W. (2002) Neopolyploidy in flowering
45 plants. *Annual Review of Ecology and Systematics*, **33**, 589–639.
- 46 Schwarzacher T., Heslop-Harrison J.S. 2000 *Practical in situ*
47 hybridization. Bios, Oxford, pp. xii–203.
- 48 Scrivanti L.R. 2007 *Estudios embriológicos, citológicos y fitoquímicos*
49 *en Bothriochloa (Poaceae: Andropogoneae)*. Thesis, Universidad
50 Nacional de Córdoba, Argentina.
- 51 Scrivanti L.R., Norrmann G.A., Anton A. (2009) Reproductive
52 biology of South American *Bothriochloa* (Poaceae: Andropogo-
53 *neae*). *Flora*, **204**, 644–650. 2
- 54 Singh A., De Wet J.M.J. (1961) Interspecific hybrids in *Bothrio-*
55 *chloa*. II. Relationships between some American and Australian
56 species. *Proceedings of the Oklahoma Academy of Science*, **41**, 35–
57 38.
- 58 Solís Neffa V., Fernández A. (2001) Cyto geography of the South
59 American *Turnera sidoides* L. complex (Turneraceae, Leiocar-
60 *pae*). *Botanical Journal of the Linnean Society*, **137**, 189–196.
- 61 Spangler R.E., Zaitchik B., Russo E., Kellogg E. (1999) Andropogo-
neae evolution and generic limits in *Sorghum* (Poaceae) using
ndhF sequences. *Systematic Botany*, **24**, 267–281.
- Stebbins G.L., Dawe J.C. (1987) Polyploidy and distribution in the
European flora: a reappraisal. *Botanische Jahrbücher für Systema-
tik*, **108**, 343–354.
- Van Dijk P., Hartog M., Van Delden W. (1992) Single cytotype
areas in allopolyploid *Plantago media* L. *Biological Journal of the*
Linnean Society, **46**, 315–331.
- Wendel J.F. (2000) Genome evolution in polyploids. *Plant Molecu-
lar Biology*, **42**, 225–249.
- Yu P., Prakash N., Whalley D.B. (2003) Sexual and apomictic seed
development in the vulnerable grass *Bothriochloa biloba*. *Austra-
lian Journal of Botany*, **51**, 75–84.

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