

Chapter 2

Bromus

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2.1 Introduction

Bromus L. is a genus of approximately 150 C₃ grass species (Clayton and Renvoize 1986; Watson and Dallwitz 1992) that can be considered to be intermediate between the Festuceae and the Triticeae. The genus is distributed widely in Asia, Europe, Africa, and the Americas, and today, introductions are widespread in the temperate world. There is a high incidence of polyploidy, species ranging from 2x to 12x. Many of the high polyploids are of allopolyploid (hybrid) origin (Stebbins 1981; Armstrong 1991). There is a diversity of annuals and perennials with a range of bunchgrass and rhizomatous morphologies.

2.2 Evolution and Systematics

The genus is taxonomically difficult with several unresolved species complexes, especially in section *Ceratochloa*. For the purposes of this chapter, the classification of *Bromus* into seven sections (Smith 1970, 1985) will be used (Table 2.1). These sections are: *Bromus* (30–40 species), *Genea* Dumort. (seven species), *Pnigma* Dumort. (about 60 species), *Ceratochloa* (P. Beauv.) Griseb. (10–16 species), *Neobromus* (Shear.) Hitchcock (two species), and *Nevskiella*

(Krecz & Vved.) Tournay. (one species). Section *Boissiera* (Hochst. ex Steudel) P. M. Smith was included in section *Bromus* by Smith (1970) and separated as a separate section by Smith (1985). The major agricultural species come from sections *Pnigma* (*B. inermis* Leyss.) and *Ceratochloa* (*B. catharticus* Vahl, *B. sitchensis* Trin. in Bong). Polyploidy and hybridization feature strongly in the evolution of many species (Stebbins 1981). The sections are distinguished morphologically according to numbers of nerves in the glumes, spikelet shape, and lemma and awn morphology, as well as karyotypes, genome relationships (chromosome pairing), ploidal levels, and serological differences.

Because of its complexity, no worldwide taxonomic treatment exists, but many regional descriptions and identification keys have been published, including those of Alaska (Mitchell 1967), Mexico and Central America (Soderstrom and Beaman 1968), North America (Wagnon 1952; Allred 1993; Pavlick 1995), South America (Pinto-Escobar 1981, 1986; Matthei 1986; Gutierrez and Pensiero 1998; Planchuelo and Peterson 2000), Malesia (Veldkamp et al. 1991), New Zealand (Forde and Edgar 1995), South-East Asia (Chen and Kuoh 2000), and Europe and North Africa (Spalton 2002, 2004).

Stebbins (1981) hypothesized that *Bromus* probably arose in Eurasia, when the Festuceae and Triticeae were separating. The original *Bromus* species are extinct and were probably wiped out during the dramatic climatic fluctuations of the Pliocene and Pleistocene. During the Pliocene, differentiation of sections *Neobromus*, *Ceratochloa* and *Pnigma* occurred. Sections *Neobromus* and *Ceratochloa* spread to North America and later to South America. These sections became extinct in Eurasia, and even

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Table 2.1 Genus *Bromus* taxonomic delimitation by different authors [modified from Armstrong (1991)]

Tzvelev (1976)	Smith (1970, 1985)	Stebbins (1981)
7 Genera	7 Sections	7 Subgenera
<i>Anisantha</i>	<i>Genea</i>	<i>Stenobromus</i>
<i>Bromus</i>	<i>Bromus</i>	<i>Bromus</i>
<i>Bromopsis</i>	<i>Pnigma</i>	<i>Festucaria</i>
<i>Ceratochloa</i>	<i>Ceratochloa</i>	<i>Ceratochloa</i>
–	<i>Neobromus</i>	<i>Neobromus</i>
<i>Boissiera</i>	<i>Boissiera</i>	<i>Boissiera</i>
<i>Nevskiella</i>	<i>Nevskiella</i>	<i>Nevskiella</i>
<i>Littledalea</i>		

diploids and tetraploids became extinct in the New World, with the build-up of octoploids in *Ceratochloa*. In the Pleistocene, section *Pnigma* spread to Africa and North America, while polyploids increased in Eurasia. Sections *Bromus* and *Genea* have evolved more recently.

The basic chromosome number is $x = 7$, like other Poaceae. Chromosome and nuclear genome sizes have changed considerably during speciation (Armstrong 1991). Stebbins (1981) proposed that the trend was towards larger genomes. On this basis, section *Ceratochloa* would have the most primitive genome size.

Comparisons of chloroplast restriction site maps of *B. inermis* with the cereals have shown that *Bromus* is closer to the Triticeae than to the *Aveneae* and, within the Triticeae, it is closer to barley than to wheat and rye (Pillay 1993, 1995).

Attempts to intercross species from different sections indicate that the reproductive barriers between sections are strong. Although some intersection hybrids have been produced, none has shown pairing of the chromosomes from the different sections, indicating wide differentiation of the chromosomes (Armstrong 1991).

Molecular marker and DNA sequence phylogenies are revealing that some of the old sections are artificial groupings that require revision. A study of 46 species, representing a wide sample of the species diversity in *Bromus*, was conducted by Saarela et al. (2007), using nuclear and chloroplast DNA sequences. This study showed that current systematic classifications do not fully reflect phylogeny within the genus. Some differences between nuclear and chloroplast sequence

phylogenies require clarification, and much wider sampling of the species is also needed.

2.3 Agricultural Status

About ten species are used in agriculture and many more have weed status. Two sections contribute important agricultural species:

Section *Pnigma* has the winterhardy *Bromus inermis* group, *B. inermis* and *B. biebersteinii* R. & S. ($2n = 56$), *B. riparius* Rehm. ($2n = 70$), and *B. erectus* Huds. ($2n = 28$) with more than 40 cultivars in North America and Europe. Additionally, this section also contains *B. auleticus* Trin. ex Nees ($2n = 42$) used to a limited extent in southern Brazil and Uruguay.

Section *Ceratochloa* has the hexaploid *B. catharticus* complex ($2n = 42$), including *B. stamineus* E. Desv. and *B. valdivianus* Phil. with over 30 cultivars in Argentina, France, Chile, New Zealand, Australia, and USA. The octoploid *B. carinatus* Hook. and Arn. complex ($2n = 56$), including *B. sitchensis* and *B. marginatus* Nees ex Steud., has over five cultivars in France and USA, while the South American octoploid *B. coloratus* Steud. has one cultivar in Australia. The duodecaploid *B. arizonicus* (Shear) Stebbins ($2n = 84$) has one very winter active annual cultivar, Cucamonga used in USA.

In addition, some annual species of section *Bromus* have had cultivars developed for revegetation uses. For example, *B. arvensis* Guss. ($2n = 14$) has a cultivar Dos in Russia and *B. mollis* L. ($2n = 28$) has the cultivar Blando in USA. From section *Genea*, *B. rubens* L. has the cultivar Panache (Alderson and Sharp 1995).

B. mango E.Desv. (section *Ceratochloa*) was used as a cereal grain crop in Chile. This plant was cultivated as a biennial cereal by the Araucana Indians of Chile until at least the middle of the 1800s. Thought to have become extinct (Scholz and Mos 1994), it is claimed to have been rediscovered and has been classified as a form of *B. catharticus* ssp. *catharticus* (Vahl) Herter (Massa et al. 2004).

The potential agricultural value of many of the *Bromus* species for New Zealand was assessed by Rumball (1968) and Rumball and Forde (1976).

2.4 Mediterranean and SW Asian Annual Species of Section *Genea*

Section *Genea* (subgenus *Stenobromus*; Stebbins 1981) consists of seven annual species that are self-fertilizing, with ploidies ranging from $2x$ to $8x$. The diploids are *B. sterilis* L., *B. tectorum* L., and *B. fasciculatus* C. Presl, and the tetraploids *B. madritensis* L. and *B. rubens*. In addition, there is a hexaploid *B. rigidus* Roth and an octoploid *B. diandrus* Roth.

Among the diploid species, *B. sterilis* and *B. tectorum* are distinct and widespread in Europe and Eurasia while *B. fasciculatus* is restricted to the eastern Mediterranean region. However, the widespread tetraploids have variously been classified as subspecies (Sales 1994) or separate species (Oja 2002). The hexaploid and octoploid species occur in the Mediterranean region and southern and western Europe. They have also been variously classified as separate species, subspecies, or varieties, and as a polyploid species complex. Most of the species of this section have become weeds in many countries.

An elegant molecular phylogenetic study (Fortune et al. 2008) has shown that all of the polyploid species of the section have hybrid origins and, apart from one unidentified lineage, all three other parental lineages were closely related to the three diploid species.

Designating the diploid genomes as *B. sterilis* (SS), *B. fasciculatus* (FF), and *B. tectorum* (TT), the polyploids have been proposed (female donor first) as: *B. madritensis* (FFSS), *B. rubens* (FFTT), *B. rigidus* (XXFFTT), and *B. diandrus* (XXFFTTSS), where XX is an unidentified parent (Fortune et al. 2008). The two $4x$ species were demonstrated to be distinct species, and the $8x$ *B. diandrus* appears to have originated as a hybrid between $6x$ *B. rigidus* and $2x$ *B. sterilis*. This last observation has been supported by earlier research (Fortune et al. 2008).

Species of this section have apparently donated genomes via hybridization with species of section *Bromus* to form the tetraploid *B. pectinatus* Thunb. complex (Stebbins 1981; Saarela et al. 2007) (see Sect. 2.6).

The main characteristic of this group of species is their propensity for weediness. *B. tectorum* is generally considered to be cleistogamous and has become an invasive noxious weed in dry, open areas of North America, where it is called cheatgrass or downy chess. Simple sequence repeat (SSR) marker research has

indicated that *B. tectorum* can outcross, giving populations the capacity to absorb new genetic variation and potentially to increase their invasive capacity (Ashley and Longland 2007). The high polyploids, *B. rigidus* ($6x$) (ripgut brome) and *B. diandrus* ($8x$) (great brome) are severe weeds of crops in Australia (Kon and Blacklow 1990) and New Zealand (Dastgheib et al. 2003). These weeds form acceptable early spring forage in the early growth stages, but the presence of awns on mature seeds makes them a danger to the health of grazing animals in late spring and summer.

2.5 Section *Pnigma*

Species of this large section (about 60) are native to Eurasia, Africa, and the Americas. Two distinct species groups were proposed by Armstrong (1981, 1983). The first was a group of long-lived perennials (rhizomatous or densely tufted) from Eurasia that were mostly polyploids ($4x$ – $10x$) with large anthers and small chromosomes. In this group was *B. pumpellianus* Scribn., which also occurs in North America, as well as the Eurasian species *B. inermis*, *B. erectus*, *B. variegatus* M. Bieb., and *B. riparius*. The second group included mainly diploid, relatively short-lived, loosely tufted, non-rhizomatous species from North America characterized by small anthers and large chromosomes. This group included the *B. ramosus* Huds. complex from Eurasia (Armstrong 1981, 1983, 1991; Stebbins 1981). The Eurasian and North American species were reported to be difficult to hybridize (Armstrong 1983), supporting the existence of the groups and suggesting that they may have quite different evolutionary histories.

However, further analyses of these species, while confirming the existence of groups with very different chromosome sizes, have not supported a simple evolutionary situation. Analysis has not been helped by difficult taxonomy in which similar taxa with widely different ploidy levels have been given the same species designations. Examples include *B. variegatus*, which is said to exist in a range of ploidy levels from diploid to decaploid. However, a decaploid is very unlikely to be an autopoloid form derived from the diploid. It is far more likely that hybridization has been involved and that these taxa are

morphologically similar but probably have very different genomic constitutions.

The existence of the two species groups with different chromosome sizes was established by hybridization studies and confirmed by nuclear DNA analyses. Hybrids were used to show that the chromosomes of the Eurasian *B. ramosus* complex were larger than the other Eurasian species *B. inermis*, *B. variegatus*, *B. erectus*, *B. pumpellianus*, and *B. riparius*. However, they were similar in size to *B. pacificus* Shear and *B. richardsonii* Link from North America. The North American species may all have variations of a single (L) genome. Interspecific hybrids were made between several of the diploid North American species and, despite good chromosome pairing, they were sterile. This was consistent with these species all containing the same (L) genome and in the process of differentiating by cryptic chromosomal rearrangements (Armstrong 1991).

The Eurasian species have so far had two genomes (A and B) identified by cytogenetic analyses (Armstrong 1991) and there may be a third (C) (see Sect. 2.5.1). The A and B genomes are the two genomes in tetraploid forms of *B. inermis* and *B. pumpellianus*, one of which (A) is also close to that of tetraploid *B. erectus*. The B genome has some homology (homeology) with the first but is of uncertain origin. Armstrong (1983) suggested that the species of the *B. ramosus* complex may be the progenitors, or be related to the progenitors, of the American species. Sutkowska and Mitka (2008) also hypothesized that this Eurasian complex is the source of the L genome. These authors suggested that the L genome may have originated from the B genome, in line with the suggestion (Stebbins 1981; Armstrong 1983) that the large chromosome species were derived from small chromosome species, possibly before the migration to America, and before the evolution of the polyploid species currently found in Eurasia.

In support of the existence of two groups of species, there appeared to be little or no pairing homology in hybrids between the large chromosomes of *B. ramosus* and *B. pacificus* and the small chromosomes of the Eurasian polyploids (Armstrong 1984). However, there was also little pairing homology between the chromosomes of *B. ramosus* and *B. pacificus*, possibly emphasizing the difficulties of using pairing affinities in tetraploids, where genetic control of chromosome pairing can occur (Armstrong 1984).

Sutkowska and Mitka (2008) used random amplified polymorphic DNA (RAPD) analyses to obtain evidence consistent with *B. erectus* and its close relatives being the oldest group of species in the section. This evidence was also consistent with *B. variegatus* having the B genome.

The inconsistencies in chromosome behavior are reflected by the results of molecular phylogenetic analyses. Nuclear DNA (internal transcribed spacer, ITS) sequence analysis has shown that section *Pnigma* is far from being monophyletic (Saarela et al. 2007). The Eurasian species of section *Pnigma* did not occur in one clade and the American species formed several well defined clades. The molecular data indicated that section *Pnigma* is probably an artificial group and that its current status as a section may be inappropriate. This was supported by the results of C-banding and DNA content analyses (Tuna et al. 2005). However, care is needed as reticulate evolution may have mixed the ancestral genomes such that they are apparently now distributed between continents (Sutkowska and Mitka 2008). There are many conflicting observations and there is a clear need to use further molecular analyses to resolve the species relationships in this artificial group so that more effective use of the potentially valuable wild relatives can be achieved.

Analysis of chloroplast DNA using species-specific restriction fragment length polymorphisms (RFLPs) following interspecific hybridization has indicated uniparental maternal inheritance in *Bromus* species so far investigated (Pillay and Armstrong 2001). In crosses between *B. arvensis* ($2n = 14$) as female and *B. inermis* ($2n = 28, 56$), and *B. erectus* ($2n = 42, 56, 70$), all F_1 plants had the female chloroplast restriction patterns. No paternal or biparental inheritance was detected.

In summary, there are about 35 Eurasian and 25 American species in this section. To-date, very few species have been studied, and conclusions about the evolution of the groups may be clarified as more species are given close research attention (Armstrong 1984).

2.5.1 *B. inermis* (Smooth Bromegrass, Russian Brome)

One of the most important agricultural species, *B. inermis*, belongs to this section. ITS DNA sequence

analysis (Saarela et al. 2007) placed this species in a small clade with four other Eurasian species, *B. erectus*, *B. korotkoyi* Drob., *B. pumpellianus* (also native to North America), and *B. riparius*. Somewhat surprisingly, these formed a sister clade to a larger group comprising all of the monophyletic sections *Genea*, *Neobromus*, and *Ceratochloa*, as well as *B. brachyanthera* Doll. from South America.

The form commonly grown in North America is autoallooctoploid ($2n = 8x = 56$). There are also allotetraploid ($2n = 4x = 28$) and hexaploid ($2n = 6x = 42$) forms (Tan and Dunn 1977). The >250 accessions held by USDA Plant Germplasm System are predominantly $8x$, with a few $4x$ and no $6x$ forms (Tuna et al. 2001). The tetraploid has regular meiosis (Carnahan and Hill 1960). The octoploid is irregular at meiosis, forming mostly quadrivalents and bivalents. A chlorophyll mutant has exhibited tetrasomic inheritance and an intermediate chromosome-chromatid type of segregation pattern (Ghosh and Knowles 1964). The genomic constitutions of the octoploid and tetraploid have been proposed to be AAAABBBB and AABB, respectively, and the A and B genomes appear to be closely related (Armstrong 1979). Giemsa C-banding has confirmed the allopolyploid nature of the tetraploid. However, karyotype analysis and Giemsa C-banding has indicated that the octoploid cytotype is not the same as a doubled version of the tetraploid cytotype (Tuna et al. 2004). There is evidence that the genomic constitution could be AAAABBCC, based on karyotype analyses showing that *B. inermis* has two pairs of chromosomes with large satellites and only one pair with small satellites (Ghosh and Knowles 1964; Wilton 1965; Armstrong 1973). However, this characteristic appears to be either inconsistent or polymorphic (Rychlewski 1970; Armstrong 1981). Tuna et al. (2004) confirmed the inconsistency of expression of the small satellite chromosomes and generally also confirmed the karyotypic analysis of Armstrong (1977b) showing two large satellite pairs and one small satellite pair. These authors indicated that the AAAABBBB hypothesis of Armstrong (1977b) was not supported by their chromosome analyses. To-date, the genome of *B. inermis* has remained relatively intractable to cytogenetic analyses using karyotype and C-banding. There is a need for the application of fluorescence in situ hybridization (FISH) and genomic in situ hybridization (GISH) techniques.

Interspecific hybrids indicate that the A genome may come from *B. erectus* ($2n = 4x = 28$) (Armstrong 1991; Walton 1980). However, a similar genome also occurs in a diploid form of *B. variegatus* Bieb. and a diploid that resembles *B. inermis* (Armstrong 1991). Hybrids between diploid *B. variegatus* and tetraploid and octoploid *B. inermis* had chromosome pairing patterns that suggested that the *B. variegatus* genome was not the same as either the A or B genome but was similar to both (Armstrong 1984). Karyotype and Giemsa C-banding analyses have indicated that the chromosomes of both *B. erectus* and *B. variegatus* differ from each other and from those of *B. inermis*. Therefore, if either species is a progenitor of *B. inermis*, significant chromosomal change should have occurred post-hybridization and polyploidization (Tuna et al. 2006). Natural *B. erectus* x *B. inermis* hybrids have been reported from Ukraine (Sutkowska et al. 2002).

Tetraploid and octoploid forms of *B. inermis* have 2C DNA contents of 11.74 pg and 22.15 pg (Tuna et al. 2001). These values are significantly lower than those that would have resulted from multiples of the proposed diploid progenitors. Therefore, it is likely that significant DNA loss has occurred during polyploidization. Such loss of DNA has been recorded for the Triticeae (Vogel et al. 1999).

Hybrids with *B. pumpellianus* ssp. *dicksonii* indicated that the two species have similar chromosomes, differing only by inversions and translocations (Armstrong 1982).

2.5.1.1 Genetic Diversity in *B. inermis*

Based on molecular analyses, the present distribution of *B. inermis* in Poland was attributed to post-glacial migrations from two separate refuges (Sutkowska et al. 2002). North American breeders of *B. inermis* generally classify the natural variation of the species into two ecotypes – a northern or “meadow” type adapted to valleys and moist regions of eastern Europe and temperate Asia, and a southern, or “steppe” type adapted to dry steppe regions. A third, intermediate group has developed apparently by intermixing of the first two. The ecotypes differ morphologically in root depth and leaf size (Fernandez and Coulman 2004). Amplified fragment length polymorphism (AFLP) analyses of 14 cultivars revealed that the

older (pre-1980) varieties based on southern germplasm were distinctive and less diverse than varieties based on northern germplasm and more recent southern or mixed varieties. These results suggest that intermixing of southern and northern ecotypes has occurred recently in North American breeding programs (Fernandez and Coulman 2004). A diversity analysis of cultivars based on morphological characteristics (Casler et al. 2000) indicated that similar morphological types had been developed from very diverse genetic backgrounds.

2.5.1.2 Breeding Progress

In recent years, there has been very little private sector breeding of *B. inermis* and relatively little cultivar development by public sector plant breeders. Emphasis on selection for improved quality has significantly improved digestibility and reduced fiber concentrations. Yield improvement has been slow, not only because of the low effort but also because of the complex polyploid genetics and research emphasis on genetics rather than breeding (Casler et al. 2000).

There has been almost no molecular breeding in *Bromus*. One of very few studies investigated the prospective use of marker-assisted selection for forage quality using neutral detergent fiber (NDF) concentration as a predictor of animal intake (Diaby and Casler 2005; Stendal et al. 2006). Although RAPD markers were difficult to use because of low repeatability, the association of markers with NDF was established. This has opened the way for marker-assisted selection for NDF concentration using more repeatable marker systems.

B. inermis is one of the most freezing-tolerant perennial grass species. Consequently, it is the subject of considerable research into the genetics of freezing tolerance, as well as being a potential genetic resource for isolation of important freezing tolerance genes (see Sect. 2.10).

2.5.2 *B. riparius* Rehm. (Meadow Bromegrass)

This species is often called *B. biebersteinii* and is confused with *B. erectus*, and its taxonomy is still to

be resolved (Pavlick 1995). However, its description fits that for *B. riparius* according to Smith (1980). It is a long-lived perennial with chromosome numbers of $2n = 2x = 14$, $2n = 8x = 56$, and $2n = 10x = 70$. In addition to being a useful species itself, it is a potential source of germplasm for the improvement of *B. inermis*, it has lower growing points and so recovers better from defoliation, is less aggressively rhizomatous, and has a longer growing season into the autumn than *B. inermis* (Armstrong 1991).

The common cultivars have $2n = 10x = 70$ and are believed to have the same chromosome constitution as *B. inermis* plus an additional genome of unknown origin (Armstrong 1991). Sutkowska and Mitka (2008) obtained evidence using RAPDs that this additional genome could be closely related to, or ancestral to, the L genome. Diploid *B. riparius* has a somatic cell (2C) DNA content of 6.14 ± 0.09 pg (Tuna et al. 2001) – very similar to *B. erectus* (Tuna et al. 2006). Common *B. riparius* has a 2C DNA content of 22.15 pg (Tuna et al. 2001).

The diploid form of *B. riparius* was found in Kazakhstan and is quite similar to the tetraploid *B. inermis* from the same region. This led Armstrong (1987) to suggest that diploid *B. riparius* might be a progenitor of polyploid *B. inermis*. A C-banding analysis of diploid *B. riparius* was completed by Tuna et al. (2001), and Tuna et al. (2004) analyzed the respective karyotypes and showed that *B. riparius* is an unlikely progenitor of *B. inermis*. Sterile hybrids between *B. variegatus* and diploid *B. riparius* have been produced and were reported to have normal chromosome pairing behavior (Armstrong 1991).

B. riparius is widely grown in North-central USA and in Canada. It resembles *B. inermis* in appearance but has shorter rhizomes, awned seeds, and pubescent leaves (Knowles et al. 1993). The cultivar “Regar” was released in 1966 by the Colorado Experiment Station.

Introgression of *B. riparius* traits (e.g., lower growing points and less vigorous rhizomes) into *B. inermis* would be useful. To this end, Armstrong (1990) obtained fertile hybrids between *B. inermis* ($2n = 8x = 56$), as female, and *B. riparius* ($2n = 10x = 70$) without difficulty. The F_1 plants were backcrossed to *B. inermis* as male and produced seed when open-pollinated. They had $2n = 63$ chromosomes. The F_2 population ranged from $2n = 56$ to 72 and tended to be in the 63–70 range. Chromosome

pairing results were equivocal and there was no clear evidence for or against recombination between the genomes of the two species. However, there was a suggestion that reversion to the parental types occurred, as had been observed earlier by Nielsen et al. (1965). Backcross progenies rapidly reverted to the parental chromosome numbers and appeared to lose *B. riparius* traits as they did so. Cytoplasmic effects were also apparent, and no clear strategy emerged for the use of *B. riparius* germplasm in the improvement of *B. inermis*. Nevertheless, a hybrid population was selected and interpollinated for several generations by Knowles and Baron (1990). Selections from this population were analyzed using RAPD markers and compared with the parental cultivars (Fernandez et al. 2001). A hybrid population was genetically intermediate between the parents, although closer to *B. inermis*, possibly because it had been selected for *B. inermis* traits. Thus, it is possible that recombination had occurred between *B. inermis* and *B. riparius* genomes in that population and that similar hybrids may be useful for the genetic improvement of smooth brome grass.

2.5.3 *B. erectus* (Erect Brome grass)

This diploid perennial species has a somatic nucleus (2C) DNA content of 6.19 ± 0.08 pg (Tuna et al. 2006). Armstrong (1973) used *B. erectus* ($2n = 4x = 28$) to make $6x$ hybrids with *B. inermis* ($2n = 8x = 56$). On the basis of chromosome pairing and karyotype analysis, he concluded that the *B. erectus* genome was present in tetrasomic condition in *B. inermis*. Armstrong (1977b) carried out further analysis of the karyotypes of these hybrids and *B. inermis* and favored the view that the *B. erectus* genome constituted the A genome in $8x$ (AAAABBBB) *B. inermis*. However, he was unable to rule out the AAAABBCC hypothesis of Ghosh and Knowles (1964).

B. erectus is naturally distributed in Europe, Britain, Ireland, and North Africa (Meusel et al. 1965) and is introduced and widespread in the North-Eastern states of USA and sporadically elsewhere (Pavlick 1995). The present distribution of *B. erectus* in Poland was studied by polymerase chain reaction (PCR) and attributed to a single post-glacial expansion (Sutkowska et al. 2002). It is a tufted perennial that occurs on roadsides

and other disturbed places, as well as in pastures. It is often confused with *B. riparius*. Because it probably shares genomes with *B. inermis*, *B. erectus* is a potential source of genetic diversity for *B. inermis* breeding. It also shares a genome with *B. pumpellianus* (Armstrong 1981).

2.5.4 *B. variegatus*

B. variegatus forms part of the meadow brome grass complex of decaploid ($2n = 70$) species, along with *B. riparius* and *B. biebersteinii*. A diploid population of this species is one of very few diploids among the Eurasian species of the section. It has a somatic cell DNA content of 6.76 ± 0.05 pg (Tuna et al. 2006). As already discussed, the role of the diploid form in the ancestry of the polyploid species remains unresolved.

2.5.5 *B. pumpellianus* (Arctic Brome grass)

This octoploid ($2n = 8x = 56$) species has populations that are native to Asia, as well as the mountains of western North America, where it provides useful high quality forage in high altitude grasslands up to 3,350 m altitude (Casler and Carlson 1995). Hybrids between $8x$ *B. inermis* and $8x$ *B. pumpellianus* are fertile, suggesting that they are subspecies (Armstrong 1991). However, there is confusion because, at the tetraploid level, they appear to be different species. Armstrong (1985) suggested that these are very heterogeneous taxa that involve allopolyploidy and have undergone introgression. A variety, "Polar" was developed from such interspecific hybrids (Hodgson et al. 1971).

2.5.6 Other North American Species in Section *Psigma*

Nodding brome (*B. anomalus* Rupr. ex Fourn.) and tetraploid ($2n = 28$) fringed brome (*B. ciliatus* L.)

provide native grazing in the western mountain regions of North America (Casler and Carlson 1995). A diploid form of *B. ciliatus* is considered to be the ancestral American species (Armstrong 1991). A detailed Giemsa C-banding chromosome analysis of *B. ciliatus* by Tuna et al. (2005) revealed that the genomes in this American species are quite different from those of any European species in the section *Pnigma*. In addition, the 2C nuclear DNA content of *B. ciliatus* was 19.13 pg, contrasting strongly with that of tetraploid *B. inermis* (11.74 pg). The chromosomes of *B. ciliatus* were almost double the length of those of Eurasian species – consistent with previous observations that the two groups of species differ in chromosome size. Additionally, the C-banding indicated quite different patterns of constitutive heterochromatin. These observations, along with the strong genetic isolation between the two groups, support the contention that they are not closely related.

B. ciliatus is of interest in North America as a potential outcrossing native grass species for restoring vegetation to ecologically important sites. Genetic diversity of Canadian populations was studied by Fu et al. (2005) using AFLPs. This identified regional Canadian forms. However, there was surprisingly little intrapopulation variation, suggesting that perhaps more self-fertilization occurred than was expected. This emphasized the need for proper breeding system studies to be carried out where genetic diversity is an important element in the end-use of a species.

Although most North American species are diploid, *B. frondosus* (Shear) Woot. and Standl., *B. richardsonii*, and *B. pacificus* all behave like allotetraploids (Armstrong 1984). *B. mucroglumis* Wagnon from Mexico and adjacent areas of the USA is similar to *B. richardsonii* (Peterson et al. 2001), but the other three tetraploid species in North America do not appear to have been studied. Hybrids between tetraploid *B. ciliatus* and *B. frondosus* indicated similar genomic structures (Barnett 1957).

2.5.7 South American Species in Section *Pnigma*

Native to southern Brazil and Uruguay, *B. auleticus* Trin. ex Nees is a perennial allogamous hexaploid ($2n = 6x = 42$) that provides outstanding forage in

native pastures (Martinello and Schifino-Wittmann 2003). It has caught the attention of agronomists and plant breeders, and some isozyme and RAPD characterizations have been done (Yanaka 2002). *B. uruguayensis* is also hexaploid, while *B. macranthus* has been reported as having 4x, 8x, 10x, and 16x chromosomes (Stebbins 1981). It is uncertain how these species are related to the remainder of section *Pnigma*.

2.6 Section *Bromus*

This section of about 40 species is considered to be the most advanced. It is native to Europe and Asia and consists of annual or biennial diploid and tetraploid species. The tetraploids are likely to have hybrid origins (Stebbins 1981). Southwest Asia and the eastern Mediterranean regions are the centers of diversity (Stebbins 1981). Several annual or biennial species have spread widely beyond their native regions and have become significant weeds, e.g., *B. briziformis* Fisch. and C.A. Mey., *B. commutatus* Schrad. (meadow brome), *B. hordeaceus* L. (*B. mollis*), *B. japonicus* Thunb. in Murr., *B. racemosus* L., *B. secalinus* L., and *B. squarrosus* L. are now widespread throughout North America (Pavlick 1995).

Only a small number of artificial interspecific hybrids have been made within section *Bromus* (Armstrong 1991). *B. mollis* was crossed with *B. arenarius* (Knowles 1944), leading to an indication that *B. mollis* contains two distinct genomes and that the same two occur in *B. racemosus*. One of the genomes in *B. mollis* was partly homologous with one of the genomes in *B. arenarius* (4x).

B. commutatus, *B. macrostachys*, and *B. secalinus* are allotetraploids. Hybrids of *B. arvensis* (2x) x *B. commutatus* (4x) and *B. secalinus* (4x) showed some chromosome pairing, indicating a relationship between the genome of *B. arvensis* and those in the tetraploid species (Jahn 1959). There were also pairing homologies between some chromosomes of *B. macrostachys* and *B. mollis*.

Sections *Boissiera* (one species, *B. pumilio* (Trin.) P.M. Smith) and *Triniusia* (two species, *B. danthoniae* Trin. ex C.A. Mey., *B. pseudodanthoniae* Drobow), recognized by some authors (Smith 1985; Scholz 1998; Saarela et al. 2007), were included in Section *Bromus* by Smith (1970). They comprised annual

species from Asia and the eastern Mediterranean region. ITS and chloroplast DNA sequence analysis (Saarela et al. 2007) confirmed that the species of section *Triniusia* belong in section *Bromus*.

The *B. pectinatus* complex (five 4x species ranging from Africa to Asia) is intermediate in morphology between sections *Bromus* and *Genea*, leading Stebbins (1981) to suggest that these species arose as hybrids between species of the two sections. The DNA sequence data of Saarela et al. (2007) support this idea. If this is correct, then section *Bromus* is monophyletic.

2.6.1 *B. arvensis* (Field Brome) and Its Close Relatives

This diploid ($2n = 14$) winter annual species and its close relatives, *B. japonicus* and *B. squarrosus*, form a complex that is taxonomically difficult and has been subject to several different treatments. *B. arvensis* is allogamous (Oja et al. 2003) unlike the other two, which are almost exclusively self-fertilizing. Serological analysis (Smith 1972), isozyme analysis (Oja et al. 2003), and DNA analyses (Ainouche and Bayer 1997; Ainouche et al. 1999) indicated that *B. japonicus* and *B. squarrosus* might be sister species or a species complex distinct from *B. arvensis*. Oja et al. (2003) suggested that *B. japonicus* and *B. squarrosus* are possibly self-fertilizing derivatives of *B. arvensis*. However, a later morphological separation, using discriminant analysis to determine the most useful characters for distinguishing the taxa (Oja and Paal 2007), indicated that the three species could be fairly reliably separated using floral morphological traits, especially anther length and lemma width.

The Mediterranean self-fertilizing diploid *B. intermedius* Guss. is also very similar in morphology to *B. japonicus* var. *villosus* (Oja 2005) and apparently also belongs to this species complex. This is supported by isozyme data (Oja 2005) and ITS sequence analysis (Ainouche and Bayer 1997). It may also be a self-fertilizing derivative of *B. arvensis* (Oja 2005). Isozyme analyses (Ainouche et al. 1995) indicated that North African populations of *B. intermedius* and its close relative *B. squarrosus* were much less variable than the tetraploids *B. hordeaceus* and *B. lanceolatus*. Nevertheless, heterozygotes were present in higher

than expected frequencies, suggesting that outcrossing was a significant evolutionary factor in these predominantly self-fertilized diploids.

B. arvensis is native to the Mediterranean and into southern and central Europe and is widespread in Eurasia (Oja and Paal 2007). It has been introduced to Australia and the USA in the 1920s where it became adapted to the corn-belt region and eastward. It provides excellent winter cover, soil stabilization, and green manure as a result of its extensive root system. *B. japonicus* and *B. squarrosus* are common in the Mediterranean and Southwest Asia and have become significant weeds in several countries.

B. arvensis ($2n = 14$) has been hybridized with the perennial species *B. inermis* ($2n = 56$) and *B. erectus* ($2n = 28$) of section *Pnigma*, but the hybrids were sterile (Armstrong 1977a). This intersectional hybridization does not appear to be a fruitful source of germplasm for the widening of the *B. inermis* or *B. erectus* gene pools (Armstrong 1977a). However, Armstrong (1977b) was able to use 5x hybrids between *B. arvensis* and *B. inermis* to analyze the allopolyploid gametic (AABB) chromosome complement of *B. inermis* because the *B. arvensis* chromosomes were much larger and could be clearly distinguished.

2.6.2 *B. hordeaceus*

Also known as *B. mollis*, this is an aggressive species that has a wide climatic range that has enabled it to spread widely within Eurasia, Africa, the Americas, and Australia. It is a probable allotetraploid ($2n = 28$, Stebbins 1981). It shows wide morphological variation indicative of ecotype differentiation and, in the Mediterranean region, may hybridize with *B. lanceolatus*, a closely related tetraploid (Ainouche et al. 1995). On the basis of its morphological variation, Pavlick (1995) separated the species into four subspecies. It has been the subject of analyses using isozymes to relate population genetic diversity to habitat diversity in Australia (Brown et al. 1974). This led to the identification of fitness-related polymorphism at the alcohol dehydrogenase locus (Brown et al. 1976). Isozyme analyses have also indicated quite high outcrossing rates in some populations (Brown et al. 1974). However, Ainouche et al. (1995) found high levels of intragenomic homozygosity in North African

populations, suggesting high selfing rates in this species (and *B. lanceolatus*). High levels of intergenomic heterozygosity in these two species were consistent with their allotetraploid genomic structure.

2.7 Section *Ceratochloa*

Section or subgenus *Ceratochloa* is a small section consisting of up to 16 polyploid perennial and annual species. No diploid or tetraploid species are found in this section. The section *Ceratochloa* has the smallest genome size in the genus (Armstrong 1991). Hybridization is rife in this section, making species boundaries obscure and the taxonomy very difficult.

Section *Ceratochloa* appears to be monophyletic and most species display almost identical chloroplast DNA sequences (Pillay and Hilu 1990, 1995), suggesting a similar maternal genome. Similarly, the ITS sequence and other nuclear DNA sequence variation is trivial (Saarela et al. 2007), a situation found in most post glacial grass expansions. This suggests that much of *Ceratochloa* is very recent and post glacial, a conclusion drawn by Stebbins (1981). The agricultural value of many of the species in section *Ceratochloa* was reviewed by Stewart (1996).

The species of section *Ceratochloa* can be divided into four genomically distinct classes:

2.7.1 The Hexaploid *B. catharticus* Complex ($2n = 42$)

This group of species is endemic to South America (Massa et al. 2004), with members also present in Africa, *B. leptocladis* in South Africa and *B. runssoroensis* in highland Central Africa. The hexaploid species of section *Ceratochloa* are all found to be strict allopolyploids with genomic formula AABBCC (Stebbins and Tobgy 1944). These genomes are almost indistinguishable and have a nuclear DNA content of 12.7–15.1 pg (mean $1C = 2.32$ pg) (Klos et al. 2009).

Morphological variation in these hexaploids is large and it is fair to say this group is the oldest of the four groups in *Ceratochloa*. Originally, this complex was separated into a number of species but, over

time, many of these so-called species have become amalgamated into a hyper-variable species. In this sense, it may be fairer to say that the hexaploids are just over the “cusp” of speciating. It is unclear how the African and American species relate.

The commonly named taxa that constitute this hexaploid group from South America (following Planchuelo and Peterson 2000) are *B. catharticus*, *B. coloratus*, *B. lithobius*, *B. stamineus*, *B. mango*, and *B. tunicatus* Phil. Using a multivariate analysis of this group, including morphological and molecular data, Massa et al. (2001, 2004) indicated that the variation was consistent with the existence of a single hexaploid species, *B. catharticus*, with two nearly continuous subspecies, *B. catharticus* ssp. *catharticus* (Vahl) Herter and *B. catharticus* ssp. *stamineus* (E. Desv.) Massa. *B. catharticus* ssp. *catharticus* includes, among others, the following older taxa: *B. uniolooides* Kunth, *B. tunicatus*, and *B. burkartii* Munoz, as well as *B. mango*, while *B. catharticus* ssp. *stamineus* includes *B. stamineus*, *B. coloratus*, *B. lithobius*, *B. fonkii* Phil., and *B. valdivianus* Phil. These authors identified octoploid forms of *B. coloratus* and *B. lithobius* in South America, which they reclassified as *B. coloratus* Steud.

These three newly defined taxa are separable using a simple three-step morphological key. There are also differences in adaptation, the populations of ssp. *stamineus* being perennial, predominantly cleistogamous and from humid places, while those of ssp. *catharticus* are facultatively cleistogamous and are from high altitudes in open woodlands of the precordillera as well as the lower slopes of the Andes.

Species from this complex have been introduced to many countries and there are more than 30 cultivars marketed in Argentina, France, Chile, New Zealand, Australia, and USA.

2.7.2 Two Disjunct Octoploid Groups

These are the *B. carinatus* Hook. and Arn. complex ($2n = 56$), found in higher latitudes of North America, and the $8x$ *B. coloratus* referred to above, found in the higher latitudes of South America (Massa et al. 2004). They have a genomic constitution ABCL where L is a larger genome, probably from section *Pnigma*. DNA contents of *B. carinatus* (20.9–22.9 pg/cell) were more

than 70% larger than *B. catharticus* (13.0–14.9 pg/cell) (Joachimciak et al. 2001). Therefore, the octoploid species of the *B. carinatus* complex are considered to be intersectional amphidiploids between diploids of section *Pnigma* and hexaploid species of section *Ceratochloa* (Stebbins 1956). This was supported by Pillay (1996) who found rDNA sequences common between this group of species and *B. inermis*. The seven species of the *B. carinatus* complex are *B. aleutensis* Trin. ex Griseb., *B. carinatus* sens. str., *B. marginatus*, *B. maritimus* (Pip.) A.S. Hitch., *B. polyanthus* Scribn., *B. sitchensis*, and *B. subvelutinus* Shear. Based on intergrading morphologies and partial interfertilities, some taxonomists believe that all of these taxa belong to a single diverse species, *B. carinatus* sens. lato (e.g., Soderstrom and Beaman 1968; Stebbins 1981). However, Pavlick (1995) argued that the major differences in morphology and barriers of hybrid sterility justify separation into separate species. A revision of this group using molecular phylogenetic methods is required (Saarela et al. 2007).

The main agricultural species of the group are as below.

2.7.2.1 *B. carinatus* (California Brome)

This is annual, biennial, or a short-lived perennial with deep roots, strong leafy growth, and good seed production that is used for grazing animals. It is naturally distributed along the west coast of North America from the Canadian border region to Baja California. Two major varieties are often distinguished (e.g. Pavlick 1995) – var. *carinatus* generally west of the Sierra Nevada and Cascade mountain ranges and the more perennial var. *hookerianus* (Thurb.) Shear. to the east in the Columbia River Basin. The two intergrade and var. *hookerianus* intergrades with *B. marginatus* and *B. subvelutinus* at higher altitudes to the east.

2.7.2.2 *B. marginatus* (Mountain Brome)

This perennial occurs generally at high altitudes throughout the western half of North America from Canada to N. Mexico. It intergrades with *B. carinatus* and *B. subvelutinus* to the West, *B. aleutensis* to the North, and *B. polyanthus* to the Southeast. Several

cultivars have been developed and are used in the NW USA and Canada, as well as in New Zealand.

2.7.2.3 *B. sitchensis* (Sitka or Alaska Brome)

Native to mountain meadows of the Pacific coast of North America from Washington state northwards to Alaska. It is closely related to *B. aleutensis* and is used for pastures in northern Europe.

2.7.3 *B. arizonicus* (2n = 84) (Arizona Brome)

This duodecaploid is native to California and east to Arizona. Morphological and cytological analyses performed by Stebbins et al. (1944) indicated that *B. arizonicus* is an allopolyploid derived from *B. catharticus* and hexaploid *B. trinii* Desv. in Gay (*B. berterianus* Colla) or an unknown close relative of this species. *B. berterianus* is the representative of the section *Neobromus*, native to the Pacific coast of North and South America. The genomic constitution of *B. arizonicus* was postulated as ABCC'DE (Stebbins 1947). Klos et al. (2009) have shown that all twelve genomes in *B. arizonicus* are approximately the same average size (2.1–2.3 pg) and the same size as the genomes in the hexaploid *Ceratochloa* species. A winter active annual cultivar, Cucamonga, is used in California.

2.7.4 Duodecaploid (2n = 84) Accessions Found in Andean Regions of South America

Two 12x accessions investigated by Klos et al. (2009) differed markedly from *B. arizonicus* in nuclear DNA content and in chromosome size. In particular, these South American duodecaploids had some large chromosomes, making up about 37% of the genome. This group potentially has the genomic constitution ABCLLL (Klos et al. 2009) and is clearly different in constitution from the North American duodecaploid, *B. arizonicus*. These accessions may belong to the species *B. ayacuchensis* (Saarela et al. 2006), for which a chromosome number has not been determined.

2.8 Section *Neobromus*

This section consists of two annual hexaploids native to the western coasts of North and South America., including *B. gunkelli* Matthei and *B. berterianus* (*B. trinii*, Pavlick 1995).

2.9 Section *Nevskiella*

This section comprises one species, *B. gracillimus* Bunge from Asia and the eastern Mediterranean.

2.10 Role of *Bromus* Species in Crop Improvement Using Biotechnology

2.10.1 Tissue Culture

Callus cultures from immature inflorescences of *B. inermis* have been grown and regenerated into green plants (Wattanasiri and Walton 1993). Significant somaclonal variation was detected for several plant traits, and although none of 10 somaclones was taller than the parent plant in a replicated trial, some had more tillers and larger leaf-stem ratios. It was concluded that this approach should aid the development of superior clones of *B. inermis*.

2.10.2 Cell Culture

Non-embryogenic suspension cell cultures of *B. inermis* have been used for over 20 years and this system has been thoroughly characterized (Ishikawa et al. 1990; Robertson et al. 1994; Wilen et al. 1996). It has proved to be useful for analysis of the mechanisms of stress tolerance induced either by drought or cold treatment or by abscisic acid (ABA) in warm conditions (e.g., Lee and Chen 1993; Wu et al. 2005). Nakamura and Ishikawa (2006) have successfully transformed *B. inermis* cell cultures using *Agrobacterium tumefaciens*, opening the way for the analysis of gene functions through reverse genetics.

2.10.3 Protoplast Fusion

Fusion of protoplasts from hexaploid ($2n = 42$) wheat cv. 99P embryo-derived callus and UV-irradiated protoplasts from tetraploid ($2n = 28$) *B. inermis* resulted in asymmetric somatic hybridization (Xiang et al. 1999). Three albino hybrid explants were obtained with 42–54 chromosomes, including small chromosomes and chromosome fragments from *B. inermis*.

2.10.4 Genome Mapping

The *B. inermis* chloroplast genome was physically mapped by Pillay (1993) using barley and wheat chloroplast DNA probes to map restriction sites. The organization of the *Bromus* chloroplast genome was similar to other grasses, with a pair of inverted repeat regions flanked by single copy regions. DNA sequence colinearity was shared with wheat and other cereal grasses.

2.11 *Bromus* Species in Genetic Research

2.11.1 Herbicide Resistance

B. tectorum (downy brome) has developed resistance to herbicides based on acetolactate synthase (ALS) – inhibitors and PS II inhibitors and ACCase inhibitors. This multiple herbicide resistance was traced, in part, to a single amino acid substitution in protein product of the *psbA* gene, the target site of PS II inhibitors (Park and Mallory-Smith 2005).

2.11.2 Genetic Diversity and Weediness

Molecular markers (mainly AFLP and SSR) have been used to study the population genetics of the spread of *B. tectorum* in North America. As noted earlier, although this species is treated as an obligate self-pollinator, molecular evidence indicates some out-crossing. Nevertheless, strong associations have been

found between marker allele presence and phenotypic variation in several adaptive traits (Ramakrishnan et al. 2004, 2006), suggesting that marker diversity can be used to infer adaptive variation.

2.12 Endophytic Fungi in *Bromus*

Many grasses host choke forming *Clavicipitaceae* endophytic fungi of the genus *Epichl  * or their asexual derivatives *Neotyphodium*. The sexual stroma of *Epichl  * causes “choke disease,” which prevents seedheads emerging. Non-choke inducing asexual *Neotyphodium* endophytes occur in many grasses such as perennial ryegrass (*Lolium perenne*) and tall fescue (*Festuca arundinacea*), and they provide the host with advantages such as improved insect resistance and grazing deterrence (Schardl and Phillips 1997).

The *Bromus* species at present known to host endophytes appear to be restricted to section *Pnigma*, with reports for *B. auleticus*, *B. brachyanthera*, *B. setifolius*, *B. benekenii*, *B. erectus*, *B. ramosus*, *B. tomentellus*, and *B. anomalus* (Groppe et al. 2002; Cabral et al. 2007). These endophytes can have significant ecological effects and may well provide insect resistance and other important agricultural effects (Novas et al. 2007).

The full relevance of endophyte fungi in *Bromus* is not known. However, it will be important that endophytes are collected and studied. Germplasm collections will need to monitor and maintain endophytes where they are present.

2.13 Recommendations for Future Action

Both partially domesticated groups identified in this review consist of allopolyploid hybrid species. Therefore, there are enormous untapped opportunities for using hybridization and ploidal manipulations among members of the various species groups to achieve new grass varieties for temperate agriculture. It is recommended that grass breeders investigate this approach to using the wealth of diversity present among the wild

species. However, this work should be supported by new DNA sequence analyses to resolve species relationships, especially in the large section *Pnigma*, where hybridization and polyploidy, followed by genomic changes, have mixed the genomes and blurred species relationships.

There is an on-going need to collect *Bromus* species from the wild in both Eurasia and in the Americas in order to realize the potential offered by the genus for agricultural purposes.

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