

REVIEW OF CHROMOSOME COUNTS FOR *BOTHRIOCHLOA*, *CAPILLIPEDIUM*, AND *DICHANTHIUM*
SPECIES (THE BCD CLADE)

by
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ABSTRACT

It has been perceived that multiple species of grasses are able to undergo introgression with one another forming unique hybrids of varying ploidy levels. Specifically the genera *Bothriochloa*, *Dichanthium*, and *Capillipedium*, in the generic section Bothriochloinae, tribe Andropogoneae have been studied to review the interrelated agamic complex formed from interbreeding. Extensive research into the chromosome pairing and cytogenetic affinities of each species has proven to be a useful tool in discovering the pairing of chromosomes between multiple species as well as characteristic differences of different ploidy levels and mode of reproduction. Due to 80% of its species being polyploid, it makes studying the grass family instrumental research as it is an ideal system to understand ploidy levels. Grasses are an important piece of the agricultural system as their weight in the food and fuel industries drive the economy. Thus, extensive genetic research has been conducted in grass groups to further increase knowledge on polyploidy. Through this broad research, chromosome number of species within the *Bothriochloa*, *Dichanthium*, and *Capillipedium* clade are reviewed to further be utilized for understanding polyploidy and its role in diversification.

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INTRODUCTION

There is a high level of biodiversity in angiosperms that indicate plants have undergone extensive diversification (De Storme and Mason, 2014). Some of this diversity, on the genomic level, can be explained by a high degree of variation in ploidy level and/or chromosome number (De Storme and Mason, 2014). Speciation and diversification of plants has been attributed, in part, due to polyploidy otherwise known as whole-genome duplications (WGD) (Edger and Pires, 2009; Schranz *et al.*, 2012). Changes in ploidy can result from aberrations in meiotic or mitotic processes, which includes whole genome duplications (De Storme and Mason, 2014). In the flowering plant family Poaceae (sometimes referred as Gramineae) it has been suggested that whole-genome duplications have led to diversification, but there is a long standing debate about whether this truly is driving diversification among the clade (Barker *et al.*, 2008; Barker *et al.*, 2009; Doyle *et al.*, 2008; Soltis *et al.*, 2009).

Acquiring new traits is a possible outcome of polyploidy which some researchers believe results in diversification of plants (Stebbins, 1971). The debate around whether polyploidy has led to diversification has recently received new evidence from the genomics revolution (Bekaert *et al.*, 2011; Doyle *et al.*, 2008; Flagel and Wendel, 2010; Pires and Gaeta, 2011; Pires *et al.*, 2004; Rapp *et al.*, 2009). Comparisons of sequenced genomes have revealed many WGD events within flowering plant lineages (Soltis, 2009). Polyploidization causes a group of genetic changes that include: changes in gene expression, gene loss, epigenetic release of transposons, and morphological and physical changes (Estep *et al.*, 2014). This is because when combining genetically distinct parents that belong to different species or genera, hybrid offspring contain the genetic material of both parents (Doyle *et al.*, 2008). These hybrid offspring contain twice the number of genes and other

genomic structures found in the parent taxa and the two genomes must work together. Once a WGD has occurred there is swift and extensive restructuring of the genome (De Storme and Mason, 2014). The most obvious effect being the changes in chromosome number as well as structure of the chromosomes (through translocations or deletions) (De Storme and Mason, 2014). The genome restructuring may further enhance changes in phenotype as gene expression and dosage are recalibrated through epigenetic processes (De Storme and Mason, 2014).

There have also been oppositions to the hypothesis that polyploidy events contribute to diversification. Plants are a good model to study the diversification events that follow WGD as they are some of the most common genetic mutations. There have been plants (angiosperms, ferns, etc.) that have no evidence of increasing speciation due to polyploidization (Mayrose *et al.*, 2011; Wood *et al.*, 2009, Estep *et al* 2014). It has also been suggested that polyploid lineages will have lower speciation rates that contribute to higher extinction rates in comparison to diploid lineages (Estep *et al.*, 2014). When initially creating a polyploid species there is a negative association of reproductive success that occurs which leaves species in distress (De Storme and Mason, 2014). The initial phase (1st few generations) following polyploidy has been suggested to bottle-neck some species or cause decreased genetic variation and diversity so that specific genes are eliminated. This occurs due to the increased genomic stress incurred via polyploidy which can lead to lethality and/or reproductive sterility. Both outcomes negatively affect the chances of lineages passing genetic material to the next generation (De Storme and Mason, 2014). For plants that do manage to make it through the genomic upheaval following WGD, there are chances for evolution to occur, where genes that have been duplicated may be lost, retained in duplicate, or sub-functionalized (De Storme and Mason, 2014). With the incorporated evidence it is hard to come to a definitive conclusion, but increasing our understanding of the consequences of polyploidy with new techniques may yield evidence to sway the debate on the role of polyploidy in evolution.

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Studying the grass family is extremely powerful in research for multiple reasons. With 80% of its species being polyploid, grasses are an ideal system to study as it may yield knowledge for understanding ploidy levels (Stebbins, 1971). This family is similarly the only large family where having a high frequency of polyploids has been a successful strategy throughout the family (Hunziker and Stebbins, 1988). Grasses are additionally a perfect model system because they are agriculturally important. With our food source being based on grasses there is a lot of funding being poured into research of the genetics involved in the system. Grasses are extremely diverse and cover a large portion of the land on Earth making their ecological and agricultural importance much greater than many other systems. With this emphasis there is a lot that is already known about grasses. There are more genome sequences of grasses than any other plant which makes it a great area to utilize when studying genetics. With all of the exploration being done there is a plethora of genetic data that cannot be found in other plant groups. This research can also be applied to many other genera and may open the doors to new research of increasing crop yields and productivity. Opening these doors may also help in other systems distantly related to grasses if the debate of ploidy being related to diversification comes to a conclusion.

A well-studied grass clade (BCD clade) is composed of the three genera *Bothriochloa*, *Dichanthium*, and *Capillipedium*. They are in the generic section Bothriochloinae, of the grass tribe Andropogoneae within the panicoid sub-family (Harlan and De Wet, 1963a). The three genera are largely apomictic (Asexual reproduction via seeds without fertilization) and form an interrelated agamic complex or a complex that reproduces asexually. The polyploid species formed within the BCD clade have chromosome numbers that range from $2n=20$ to $2n=180$ in multiples of 10 making them simple to classify (Chheda *et al.*, 1961; Harlan *et al.*, 1961). Research in this area has a plethora of information as reviews of hybridization in controlled conditions as well as in nature between and within the genera has been investigated (Harlan *et al.*, 1958 and 1961). Multiple researches have

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shading, *Dichanthium* genomes; yellow shading, *Capillipedium* genomes; dark green and dark yellow shading, *Bothriochloa* accessions with incorporated *Dichanthium* and *Capillipedium* genomes, respectively.

It is hypothesized that diploid colonies are always a part of variable tetraploid populations as the populations are characterized to have frequent sexual reproduction that only diploid species are capable of in nature (De Wet and Harlan, 1970). Polyploids have been found to be derived from chromosome doubling in somatic cells, or more frequently come from sexual functioning of cytologically unreduced gametes (errors during meiosis) (De Wet, 1971). Diploids often produce cytologically unreduced female gametes, these unreduced gametes can fuse with normal haploid gametes (pollen) to generate triploid hybrids and, even more rarely, fuse with other unreduced gametes to produce tetraploids (having four copies of a single genome). If pollinated by facultative apomictic tetraploids, the offspring generated were frequently fertile and highly sexual tetraploid hybrids (De Wet and Harlan, 1970). This was observed in a fixed setting, but is evidently occurring in nature due to the extensive variability within tetraploid populations that are associated with diploid colonies (De Wet and Harlan, 1970). With this evidence, research on hybridization has become a necessity as understanding the pattern of inheritance can unveil how sexuality or apomictic reproduction is chosen.

From studying inheritance in this clade, it is noted that the overall apomictic mode of reproduction is a dominant character over sexuality, meaning the characteristic is under genetic control (De Wet and Harlan, 1970). Harlan *et al.* (Harlan *et al.*, 1958 and 1960) has also found that there were no observable, completely sexual plants in natural collections. Again, by the overall apomictic mode of reproduction being an inherited dominant characteristic it predicts that completely sexual plants may not be present because the locus would have to be homozygous for the recessive allele to be a sexual reproducing plant (De Wet and Borgaonkar, 1963). This however,

does not mean that gametophytic apomixis and sexual reproduction are operational alternatives to each other. Within individuals there are always apomictic and sexual embryo sacs that are produced. This even occurs in the most apomictic species (De Wet and Harlan, 1970). The development of embryo sacs has been found to be dependent on genotype and environment. Thus, the offspring from a facultative apomictic tetraploid would have the possibility of being hybrids, haploids, hybrid hexaploids, or maternal tetraploids (De Wet and Harlan, 1970). These hybrids can be produced experimentally to create offspring that will reproduce via sexual or apomictic reproduction, but it is assumed that hybrids created in nature are always apomicts with the ability to be sexual. This assumption is based on the hypothesis that apomictic reproduction is dominant to sexuality. If hybrids were generated from facultative apomicts, the hybrids would then be able to reproduce either apomictically or sexually. Experimentally, if a completely sexual plant were to be crossed with an essentially obligate apomict, they would again have the ability to reproduce apomictically or sexually. There is then the question of if completely sexual offspring could be produced in nature by sexual plants crossing. This however, has not been found in nature as the only way to produce completely sexual plants would be to cross two already sexual plants which are rare in nature.

Sexually reproducing as well as obligate apomictic plants are very limited in their dispersion even though polyploid species of *Bothriochloa*, *Capillipedium* and *Dichanthium* have a wide distribution around the world (Celarier and Harlan, 1957; Harlan *et al.*, 1958). Specifically we find sexual polyploid *Bothriochloa* in North America and Australia only (Harlan *et al.* 1958). It has also been determined through genetic work done by De Wet (Harlan *et al.*, 1961) that these species from North America and Australia are closely related cytogenetically. With this information it is assumed that at one point in time this genus was world-wide (De Wet *et al.*, 1963).

With this general outline of individuals in the BCD clade having $2n$ chromosome numbers in multiples of 10 it is easy to see if there are aneuploid individuals or individuals with a $2n$ chromosome number that is not a multiple of 10 (Chheda *et al.*, 2014). Aneuploids are produced when one or more homologous pairs of chromosomes, fail to separate during meiosis producing a gamete with an unusual number of chromosomes that is not divisible by the base number. From work done by Harlan *et al.* (Harlan *et al.*, 1958 and 1960) it was seen that aneuploidy was completely absent or very rare from natural collections of plants. The absence of aneuploidy in natural collections of hybrid complexes has been widely debated. It is hypothesized that individuals not having a complete genome (aneuploids) have a selective disadvantage to euploids (individuals with a $2n$ chromosome number divisible by the base number). This hypothesis has been thoroughly investigated as aneuploids have been noted to be less vigorous than euploids. Thus natural selection may favor having complete genomes in nature resulting in rare occurrences of aneuploid plants (De Wet and Borgaonkar, 1963).

With aneuploids generally occurring from mutations or hybridization of multiple species there has been a focus on research of the cytological associations between the three genera through intraspecific, interspecific, and intergeneric hybrids. Studies across the world have yielded evidence of *Bothriochla intermedia* being able to introgress with multiple species from all three genera (Ahsan *et al.*, 1994; Det Wet & Harlan, 1966; Faruqi *et al.*, 1979; Gill *et al.*, 1980; Harlan, 1963; Harlan & De Wet, 1963a, 1963b; Sharma *et al.*, 1978; Srivastava, 1979, 1980; Ward & Spellenberg, 1986; Yan *et al.*, 2000). In Pakistan it has been seen that *B. intermedia* introgresses with *Dichanthium annulatum* and *D. ischaemum* resulting in breakdowns of differences between the species (Faruqi *et al.*, 1979; Harlan 1963b). This is a novel observation as it is being seen that *B. intermedia* is able to steal genetic information from multiple other species and incorporate diverse genes to blur species boundaries. This again is also seen in northern Australia as *B. intermedia* is again

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the culprit of hybridization with *Capillipedium parviflorum* (De Wet and Harlan, 1966). Here introgression is so prominent that it produces a completely new introgression product termed *C. spicigerum* (De Wet and Harlan, 1966). There is a long list of geographical areas where *B. intermedia* is able to introgress with multiple species to produce hybrids, and these hybrids have been reproduced in laboratories (Harlan and De Wet, 1963a). Within each of these experiments it was necessary to use *B. intermedia* as the female (Harlan *et al.*, 1961). Using *Bothriochloa intermedia* as the female parents it has been discovered that aneuploidy occurs in some hybrids to yield $2n$ chromosome numbers not in multiples of 10 (Chheda *et al.*, 1961; Chheda and Harlan, 1962). *Bothriochloa intermedia* is a highly important species used for creating hybrids within the clade and differentiating between species. It has been seen on multiple accounts that *B. intermedia* is an introgression product between varying species. It is likewise believed that *B. intermedia* is of hybrid origin to start with as it contains multiple germplasms from at least 5 different species belonging to the BCD clade. Each accession that is collected of *B. intermedia* seems to be a cross, backcross, or introgression product (Harlan and De Wet, 1963a). There has not yet been a distinct sample of *B. intermedia* found. This is assumed to be due to its large range geographically and highly compiled heredities of its relatives (Harlan and De Wet, 1963a). The more that *B. intermedia* is able to expand geographically, the more it is able to consume heredities of its relatives (Harlan and De Wet, 1963a). Furthermore, Harlan and de Wet have shown over multiple accounts (De Wet and Harlan, 1966, 1970; Harlan, 1963a, 1963b) the genera *Bothriochloa* O. Kuntze, *Capillipedium* Stapf, and *Dichanthium* Willemet are unified by the compilospecies *B. intermedia*.

Bothriochloa intermedia is called a compilospecies with the most general definition of the word. "Compilo" comes from Latin meaning to snatch together or carry off. This accurately describes what *B. intermedia* is doing to its relative species as it is genetically aggressive towards compatible species (Harlan and De Wet, 1963a). When the compilospecies introgresses it plunders

related species of their heredities which may completely integrate a species (Harlan and De Wet, 1963a). If this occurs, the species may become extinct.

The compilospecies of *Bothriochloa-Dichanthium-Capillipedium* have chromosomes that readily pair with each other even if the pairing is not between homologous chromosomes or matching chromosomes (De Wet and Harlan, 1970). Along with homologous chromosome pairs, homeologous (homologous chromosomes that result from polyploidy events) and non-homologous chromosomes (chromosomes that do not match) are able to pair within the BCD clade. These chromosomes come from multiple species in the three genera making it impossible to trace the phylogenies through meiotic pairing of chromosomes alone (Chheda and Harlan, 1962). Chromosomes within these species are also able to pair among themselves if no homologues or homeologues are present to associate with (Chheda and Harlan, 1962). With all of the possibilities it makes it difficult to trace species within the BCD clade, but it yields a great amount of genetic data that can be utilized. This data cannot be found in many other families, resulting in grasses being much further along in genetic research.

Specifically we look at the tribe Andropogoneae (subfamily Panicoideae) and the three genera *Bothriochloa*, *Capillipedium*, and *Dichanthium*, otherwise known as the BCD clade. This is the area of interest, as reviving cytological work from the 1960s to further investigate the compilospecies hypothesis could produce information to assist the debate of the polyploid phenomena, its relationship with diversification, and understanding compilospecies events. Knowing species ranges and chromosome numbers of the BCD clade helps to better investigate the incidence of the compilospecies existence. It is imperative to comprehend how compilospecies work due to the evidence of hybridization occurring in multiple genera. The presence of a compilospecies is unique and valued in a genetic aspect as it tells us that species are hybridizing relatives and pulling their genetic material into one species. This results in constant generating of new genetic variation,

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not through mutations, but through hybridization. By understanding this group we may be able to understand mechanisms of why polyploidy may be driving diversification. We see polyploidy happening, but we don't know why it may be driving diversification so we are hoping that this research will help unlock some of that information.

By writing this review it adds to the organization of data within the grass family to better unify and shape the continued examination of how hybridization and ploidy effect organisms. Without cytogenetic investigation, polyploidy would never be understood and tracing species hybridization would be impossible. By starting with the ideal system of grasses, it opens the door to revolutionary work in the field of genetics that can be applied to multiple other families.

METHODS

The literature and web sites were searched for research describing the number of chromosomes in a variety of BCD grasses. Multiple chromosome counts were found from tropicos (<http://www.tropicos.org/Project/IPCN>) in the Index to Plant Chromosome Numbers, and SOCGI Plant Chromosome Number Reports. Counts were found in numerous literature reviews and articles documenting how the three genera reproduce and hybridize. For each genus the species and chromosome counts observed were recorded.

Each citation of a chromosome count was quantitatively scored based on reliability. A score of 1 indicates the most reliable and 5 as the least reliable. Reliability was determined by the number of individuals counted to identify the chromosome number as well as if a photograph or drawings were included in the publication. Chromosome counts with a ranking of 1 had multiple counts, with a photograph. Counts ranked as 2 had a photograph of the chromosomes, regardless of the number of individuals. Counts ranked as 3 had multiple counts and included a detailed drawing. Those ranked with a 4 had multiple counts and counts with a score of 5 had little to no detail verifying their

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result. Most counts ranked with a 5 were numbers found in chromosome count articles with no background on how the numbers were attained.

RESULTS

The complete chromosome counts for *Bothriochloa*, *Capillipedium*, and *Dichanthium* are recorded in Table 1, 2, and 3, respectively. Along with counts recorded, the origin of the samples is included as well as the published work recording the counts.

For *Bothriochloa* there were a total of 63 species recorded with 6 species not having recorded chromosome counts. Ploidy was recorded from diploid to octodecaploid (18 copies) with the highest sporophytic count being 180 chromosomes. Only 7 species had direct evidence that yielded their chromosome count: *B. eurylemma* M. Marchi & Longhi-Wagner, *B. exaristata* (Nash) Henrard, *B. grahamii* (Haines) Bor B. *intermedia* (R. Br.) A. Camus, *B. intermedia* (R. Br.) A. Camus, *B. laguroides* var. *torreyana* (Steud.) M. Marchi & Longhi-Wagner, *B. longipaniculata* (Gould) Allred & Gould, and *B. odorata* (Lisboa) A. Camus.

Within the *Capillipedium* research there was a total of 20 species recorded, the majority of which, twelve, that did not have recorded chromosome counts. Ploidy was recorded from diploid to hexaploid (6 copies) with the highest sporophytic count being 60 chromosomes. With only 8 species having recorded chromosome counts there were 2 species that had direct evidence yielding their counts. These were *C. assimile* (Steud.) A. Camus, and *C. parviflorum* (R. Br.) Stapf.

The genus *Dichanthium* had a total of 29 individual species that had recorded chromosome counts. There were 12 species that did not have a recorded chromosome count out of the total 29 species. Ploidy was found to range from diploid to hexaploid with the highest sporophytic count being 60 chromosomes. There was one species found to be aneuploidy: *D. annulatum* (Forssk.) Stapf. Again, only 2 species had direct evidence that concluded their chromosome numbers. These were *D. annulatum* (Forssk.) Stapf, and *D. foveolatum* (Delile) Roberty.

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