EVOLUTION AND DEVELOPMENT OF VEGETATIVE ARCHITECTURE: BROAD SCALE PATTERNS OF BRANCHING ACROSS THE GRASS FAMILY (POACEAE) AND CHARACTERIZATION OF ARCHITECTURAL DEVELOPMENT IN *SETARIA VIRIDIS* L. P. BEAUV.

By

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Edmond, Oklahoma

2006

Submitted to the Faculty of the
Graduate College of the
Oklahoma State University
in partial fulfillment of
the requirements for
the Degree of
MASTER OF SCIENCE
July, 2012

EVOLUTION AND DEVELOPMENT OF VEGETATIVE ARCHITECTURE: BROAD SCALE PATTERNS OF BRANCHING ACROSS THE GRASS FAMILY (POACEAE) AND CHARACTERIZATION OF ARCHITECTURAL DEVELOPMENT IN WEEDY GREEN MILLET (*SETARIA VIRIDIS* L. P. BEAUV.)

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CHAPTER I

EVOLUTIONARY SURVEY OF VEGETATIVE BRANCHING ACROSS THE GRASS FAMILY (POACEAE)

INTRODUCTION

The grass family (Poaceae) is a family of monocotyledonous flowering plants that is comprised of over 10,000 species in approximately 850 genera. The family is ubiquitous, found on each continent except Antarctica, and includes annual and perennial species. Grasses may perform C3 or C4 photosynthesis and roughly 60% of all C4 species are members of Poaceae (Edwards, Osborne et al. 2010). The earliest diverging lineages of grasses have been dated via fossil phytoliths in coprolites to the late Cretaceous (~127mya) (Prasad, Stromberg et al. 2011). The crown group of grasses diverged from basal subfamilies Anomochlooideae, Pharoideae and Puelioideae approximately 83mya and accounts for over 90% of the species of Poaceae (Prasad, Stromberg et al. 2005). The crown group is comprised of 2 distinct evolutionary lineages which diverged from one another ~55mya; the BEP clade (subfamilies Bambusoideae, Pooideae and Ehrhartoideae) and the PACMAD clade (subfamilies Panicoideae, Aristidoideae, Chloridoideae, Micrairoideae, Arundinoideae and Danthonioideae) (Prasad, Stromberg et al. 2005).

Poaceae is an economically important family with several species cultivated for food, fodder for livestock, building materials and biomass for biofuels. The BEP clade contains cereal crops such as rice (Ehrhartoideae), barley, wheat and oats (Pooideae) and the timber bamboos such as *Phyllostachys* (Bambusoideae). The PACMAD clade

includes cereal crops such as maize, sorghum, the millets (Panicoideae) and tef (Chloridoideae) and potential bioenergy species switchgrass, napiergrass and pearl millet (Panicoideae).

Plant Architecture

The overall form of the grass plant, its architecture, is determined by the three-dimensional arrangement of branches throughout the plant. As in other plants, branching patterns in grasses are important for their survival and reproduction, by influencing their ability to capture light, compete with neighboring plants for nutrients, survive mechanical damage such as herbivory, and produce flowers and seeds.

Inflorescence branching has been studied extensively in the Poaceae, primarily because branching traits that affect the number of grains per inflorescence were under strong selective pressure during domestication and further selection under cultivation (Dewet and Harlan 1971; Gepts 2004). Previous research on grass architecture has concentrated on identifying the genetic pathways that control branching in the inflorescence (McSteen and Leyser 2005; Malcomber, Preston et al. 2006; McSteen 2009; Gallavotti, Long et al. 2010) and on morphological diversification in the context of phylogenetic relationships (Doust and Kellogg 2002; Malcomber, Preston et al. 2006; Liu, Peterson et al. 2007; McSteen, Malcomber et al. 2007; Reinheimer, Zuloaga et al. 2009). Inflorescence branching patterns are considered stable enough for taxonomic use, and branching traits in the inflorescence, such as number of branches, length of axes and ranks of axes are used as diagnostic characters in the Flora of North America key to Poaceae (Flora of North America editorial committee 1993).

In contrast with inflorescence branching, less is known about the patterns of vegetative architecture in the grasses. Unlike inflorescence architecture, vegetative branching exhibits more phenotypic plasticity, and thus architectural patterns can show a wide range of variation, even within species. However, differences in the architecture of domesticated grasses from long diverged groups, such as rice and wheat versus maize, sorghum and the millets suggest that stable differences in architecture across the grasses may be found. For example, domesticated cereals such as rice (subfamily Ehrhartoideae), wheat and barley (subfamily Pooideae) produce many tillers (basal branches) whereas maize, sorghum and the millets (subfamily Panicoideae) produce one or only a few tillers (Doust 2007). Previous research has shown that, like inflorescence branching, vegetative branching is under strong genetic control (McSteen and Leyser 2005). However, there has been no broad-scale study in the grass family to identify general patterns of vegetative architecture or to investigate evolutionary changes in branching patterns. In this chapter I address this gap by investigating the patterns of vegetative architecture across Poaceae in the context of a multi-gene grass phylogeny. To do so requires an understanding of vegetative architecture and its development in plants in general and grasses in particular, which I introduce below.

Vascular Plant Morphology

Vascular plants grow through the action of meristems, the pleuripotent tissues that differentiate into the mature structures that comprise the organ systems of plants, through a two-stage process of organogenesis and organ extension or elongation (Champagnat and Come 1986; Barthelemy and Caraglio 2007). Plants can be thought of as a population of meristems, each with a potentially different developmental trajectory. Branches

develop from lateral meristems that develop in the axils of leaf primordia, both of which are initiated by the shoot apical meristem (SAM) during the primary organogenesis of the primary stem (McSteen and Leyser 2005). Axillary meristems differentiate into axillary buds that may either remain dormant for some interval up to and including the entire life of the plant, or immediately begin to elongate into branches. Patterns of initiation and extension in the meristems determine the placement of branches. The number and length of these branches and their position in relation to one another and the main stem creates the vegetative architecture of the plant.

Plant development is a dynamic process of addition of repeated modules - subsets of the structures that comprise the plant body. In plants, the module of repetition includes a node, internode, bud and leaf and is referred to as a phyton (Gray 1850, Gray 1879, Arber 1934, Bell 2010). Architecture of the shoot system may be described in terms of phytons (modules) developing in succession over the life of the plant. For example, the shoot system of plants is composed of a primary axis (the main stem) that develops by repeated addition of phytons from the apical meristem, and higher order axes (branches) that are composed of phytons developing from meristems at nodes along the primary axis (Fig. 1). Architectural patterns also exist in the root system, however, rather than the repeated addition of phytons, root architecture is determined by continued elongation of the primary root through the action of the apical meristem and *de novo* formation of lateral meristems, which develop into lateral roots

Grass Morphology

Grasses are easily recognized by their distichously arranged, strap-like, sheathing leaves (although other plants, such as sedges (Cyperaceae) and rushes (Juncaceae) may also

have grass-like leaf morphologies). In grasses, the phyton is termed the phytomer, which typically consists of a node and internode, a leaf, its associated axillary bud, and adventitious roots when present (Fig. 1). The most commonly employed definition of the phytomer consists of a node, attached leaf and axillary bud and the acropetal internode (Fig. 1A) (Clark and Fisher 1986, (McMaster 2005). However, some authors have defined the phytomer differently, depending on the research question being addressed. For example, in terms of physiology, each leaf is more closely associated with the node beneath the node to which it attaches, because it is at this more basal node that the vascular bundle from the leaf joins the vascular tissue in the stem at the nodal plexus (Pizzolato 2000). Based on this physiological association, there has been debate over whether the leaf at the base of an internode or that at the distal end of the internode should properly be considered as part of the phytomer including that internode (Clark and Fisher 1986,Woods, Hope et al. 2011) In this study I am analyzing morphological patterns and will use the common definition of a phytomer as a node, attached leaf, axillary bud and the acropetal internode.

There is great variation in the spatial and temporal arrangement of branches, including caespitose tussock grasses, grasses with rhizomes or stolons (Fig. 2), grasses that produce branches on the distal portions of erect or prostrate culms (Fig. 3) and all combinations thereof. Within the vegetative portion of the culm a distinction can be made between the basal portion, where internodes are unexpanded or abbreviated— the so-called Short Internode Zone (SIZ), and the more distal portion of the culm with expanded internodes—the Long Internode Zone (LIZ) (Perreta, Ramos et al. 2011). In the basal nodes of the SIZ, axillary branches (tillers) develop, which may also produce adventitious

roots (Moore and Moser 1995). The main culm and the tillers are often very similar in their growth patterns. Grasses may also produce branches in the LIZ (termed here, aerial branches), however bud growth may be inhibited in all or some portion of the LIZ, as has been reported in many species, for example, *Melica* (Perreta and Vegetti 2004; Perreta and Vegetti 2006). There may also be a distinction between tillers and aerial branches in their developmental timing, as aerial branches may only develop when several tillers are already present and the main culm is about to flower (Doust, Devos et al. 2004). Species of grasses vary in the extent to which they produce tillers and/or aerial branches (Doust 2007).

Inflorescence development in the grasses follows the same basic pattern as vegetative architectural development. The apical meristem initiates axillary meristems, which elongate and may themselves produce secondary, tertiary and further orders of branching, some or all of which will differentiate into spikelets that contain the grass flowers, the florets. Inflorescence development and morphology have been used in a phylogenetic context (Doust and Kellogg 2002; Reinheimer, Zuloaga et al. 2009) (Reinheimer et al. 2005) and in quantitative genetics (Doust and Kellogg 2006) to identify the evolutionary origins of inflorescence diversity and its underlying genetic control.

The branching patterns of the vasculature within the branches also vary in the grasses, and have been shown to define major subgroups (Pizzolato 2000). In addition to differences in vegetative and reproductive branch placement, vascular anatomical research in grasses has revealed distinct differences in procambial development between clades (Pizzolato 2000). In leaves, the median leaf trace associated with a node is

initiated at the point of insertion of the primordium. Differentiation of each procambial trace proceeds acropetally in the leaf primordium and basipetally in the culm, where it eventually joins with the lateral traces (Pizzolato 2000; Pizzolato and Sundberg 2002). The point where the procambial traces join is known as the nodal plexus. The number of proximal nodes that each trace traverses before connecting at the nodal plexus varies between the subfamilies of the Poaceae (Pizzolato 2000; Pizzolato and Sundberg 2001). Pizzolato (2000) concluded that these differences are significant enough to use vascular anatomical characters in systematic treatments of the grasses, although other authors suggest that these characters are too subjective to be exclusively relied on (Clark and Fisher 1986).

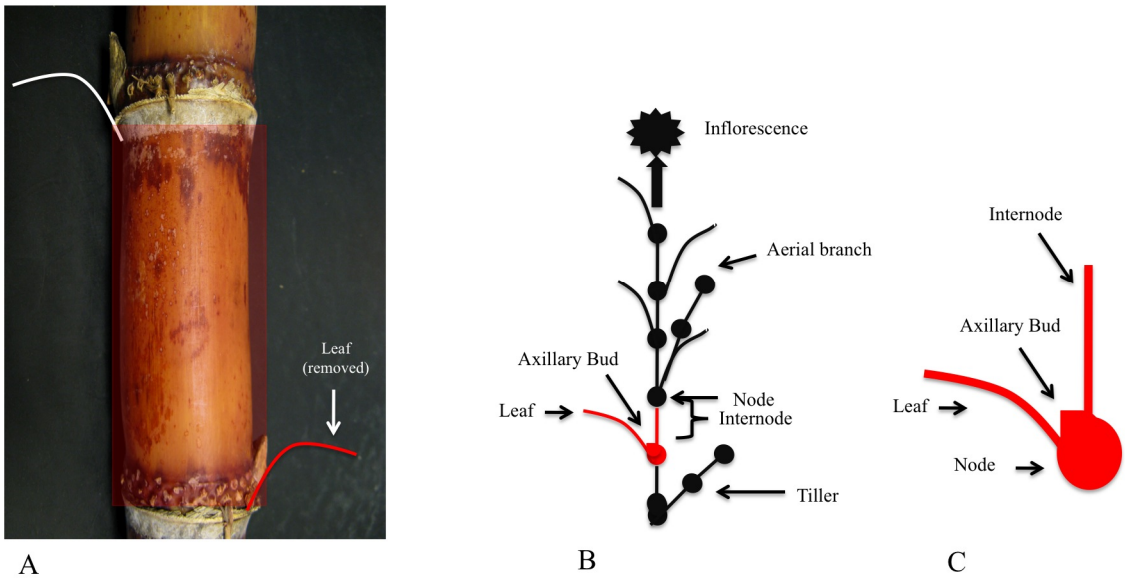


Figure 1. Grass phytomers

A) Segment of sugarcane (*Saccharum officinarum*) with leaves removed. A single phytomer is indicated in red. B) Schematic representation of a grass plant with phytomer indicated in red. C) Enlarged phytomer with constituent organs indicated.

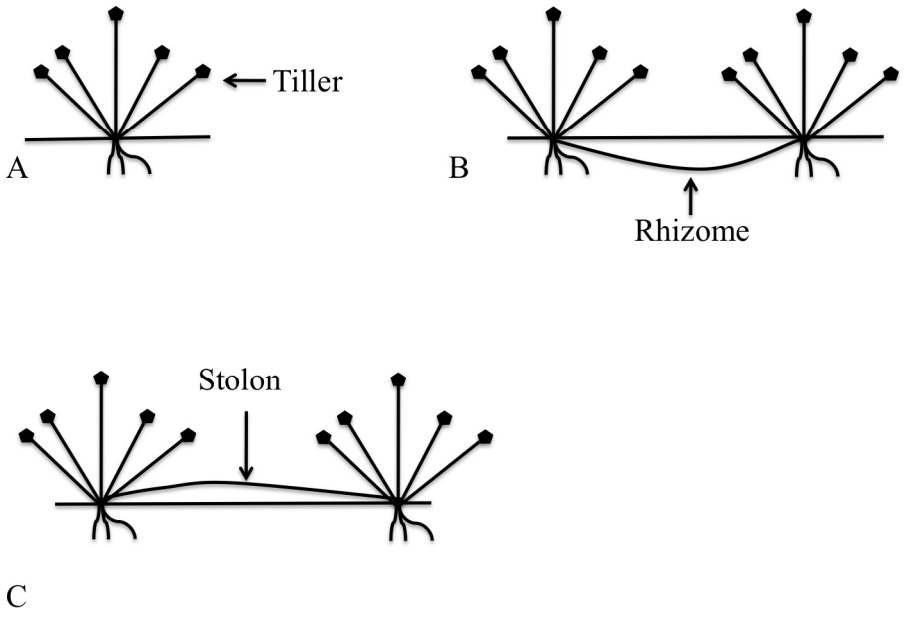


Figure 2. Branching patterns in the grasses

Plants may produce only erect branches (A: caespitose habit) or either of two types of horizontal stems, whichmay occur below the soil surface (B: rhizomes) or above the soil surface (C: stolons).

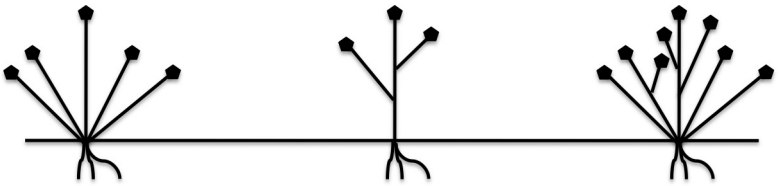


Figure 3. Variation in grass branching

Variation in the presence of the 2 main types of axillary branches found in grasses, tillers and aerial or lateral branches, determines overall architectural patterns. Plants may produce tillers only (left), aerial branches only (middle) or a combination of tillers and aerial branches (right).

Recently, it has been possible to construct large scale phylogenies from molecular data that can be used to support studies of morphological evolution across the entire grass family (Soreng and Davis 1998; Giussani, Cota-Sanchez et al. 2001; Grass_Phylogeny_Working_Group 2001; Salamin, Hodkinson et al. 2002; Barker, Galley et al. 2007; Bouchenak-Khelladi, Salamin et al. 2008; Ibrahim, Burke et al. 2009; Edwards and Smith 2010; Grass Phylogeny Working 2012). For example, in the last decade several broad scale studies have identified the origins of C4 photosynthesis in Poaceae (Giussani, Cota-Sanchez et al. 2001; Ibrahim, Burke et al. 2009; Edwards and Smith 2010). Giussani *et al.* (2001) identified multiple parallel gains of several subtypes of C4 photosynthesis within the subfamily Panicoideae. Ibrahim *et al.* (2009) took a finer-scale approach and were able to both identify specific instances of reversion from C4 to C3 photosynthesis and also potential intermediate physiological steps necessary to the transition from C3 to C4. Edwards and Smith (2010) integrated data on C4 and C3 photosynthesis, climate (temperature, atmospheric CO2 concentration), and ecosystem changes in a phylogenetic context to construct a comprehensive history of the evolution of C4 photosynthesis in the grasses.

The key to each of these studies was the availability of species level data on the mode of photosynthesis. To date, there has been no such broad study of vegetative branching in the grasses. A comprehensive dataset that includes branching data for each of the approximately 10,000 species of grasses does not currently exist, unlike the comprehensive sampling that has been conducted for photosynthetic pathways. Such a data set would require the combined effort of many researchers to sample live or preserved specimens of each species.

In this chapter I discuss the beginnings of a comprehensive investigation into the vegetative architecture of the grass family in an evolutionary context. A broad analysis of vegetative architecture has potential applications in research focused on the evolution of grasses and ecology within clades and across the family. I chose to examine both wild and domesticated species, and focused on the potential to produce aerial branches, as my preliminary observations showed that all wild species produce multiple tillers.

METHODS

Morphological survey of the grasses

Selection of genera for analysis was initially based on the Grass Phylogeny Working Group (GPWG 2001) phylogenetic analysis, although more species per genus were measured than were in that analysis. Observations of the presence and number of aerial branches were made at the Oklahoma State University (OSU) herbarium and included 957 specimens from across the family. Observations were also made on the presence or absence of aerial branches for an additional 3,582 specimens at the Missouri Botanical Garden. In all, specimens sampled represented 10 sub-families, 65 genera (approximately 6%), and 208 species (2.08%) from across the Poaceae (Appendix 1). Live material was examined in the Climatron at the Missouri Botanical Garden for the basal grass genus *Pharus* and bambusoid genus *Streptochaeta*, and from plants collected near Stillwater, Oklahoma (Appendix 1). For all live specimens, the presence of aerial branches was examined by both visual inspection as well as stripping the leaf sheaths away from the culm to look for the presence of unexpanded buds. I utilized the Grass Genera of the World DELTA database (Watson, Dallwitz et al. 1986) to attain

information on aerial branching for genera where no herbarium or live specimens were available.

Observations were made also from herbarium specimens of the outgroup genus *Joinvillea* (Joinvilleaceae) (Grass Phylogeny Working Group 2001, Salamin et al. 2008, Grass Phylogeny Working Group II 2012).

MARKER AND ACCESSION SELECTION:

A dataset for four markers (*ndhF*, *phyB*, ITS and *rbcL*) was retrieved from NCBI, representing 132 species of the 65 grass genera that were examined for branching characteristics, as well as the outgroup genera (Appendix 2). NADH dehydrogenase subunit 5 (ndhF) and the large subunit of ribulose biphosphate carboxylase (rbcL) are both chloroplast coding genes, the internal transcribed spacer region of ribosomal RNA (ITS) is a non-coding nuclear marker, and phytochrome B (phyB) is a coding nuclear gene with both introns and exons. In several cases it was not possible to get the same species as that used for the morphological analyses, so another species was substituted, taking care to check current literature to ensure that the substitute species was still considered to be in the same genus and that the second species was morphologically similar to the originally sampled species. I considered that it was appropriate to substitute species within genera as the character optimization analysis was carried out at the generic level. This data set was compiled and concatenated in Mesquite version 2.6.

Phylogenetic analyses: The model of sequence evolution for each marker was determined in MrModelTest v2 (Nylander 2004). In all cases, a General Time Reversible substitution model (GTR) was selected using the AIC criterion, although with differing gamma values and proportions of invariable sites. Each marker was analyzed in a

preliminary maximum likelihood analysis, using PhyML version 3.0 (Guindon and Gascuel 2003), and the resulting trees visualized and compared using FigTree v1.2.2 (available for download at http://tree.bio.ed.ac.uk/software/figtree/) for the presence of differing evolutionary histories. A concatenated dataset was created with Mesquite version 2.6, and a partitioned Bayesian analysis was conducted with a GTR model with six nucleotide substitution types and a gamma distribution with 4 rate categories. The four partitions were unlinked to allow model parameters to be assessed independently and rates to vary independently. Two simultaneous runs of 4 chains each (3 cold, one heated, using default parameters) were implemented in MrBayes version 3.1 (Huelsenbeck and Ronquist 2001; Ronquist and Huelsenbeck 2003) and run for 20 million generations with trees sampled every 2,000 generations. Tracer version 1.5 (Rambaut and Drummond 2007) was used to check effective sample sizes (ESS) for all parameters in the model and to determine the length of burnin to discard from each of the runs, combined with comparison of the standard deviation of the split frequencies between runs in MrBayes. Trees were manipulated for display in FigTree.

Character optimization: Maximum parsimony optimization of the evolution of branching characters on the phylogeny was done on the majority rule consensus tree using MacClade 4.08 (Maddison and Maddison 2005).

In each genus where aerial branching was observed, each species sampled included at least one specimen in which aerial branches were present. Therefore, presence of aerial branching, as observed in herbarium and live specimens was coded as a binary character for each of the sampled genera. The presence of aerial branching in the species sampled for a given genus was considered to indicate that all members of the genus possess the potential to produce aerial branches, and the absence of branching in all species examined was required to code a genus as having branching absent. *Micraira* was coded as equivocal based on architectural and morphological characters unique to this genus.

To examine the effect of alternative phylogenetic reconstructions on the character optimization the lineage relationships within the majority rule consensus tree were rearranged to be consistent with those recovered by the GPWG II (2012). The optimization procedure was then repeated on this phylogeny.

RESULTS

Morphological survey of the grasses

All specimens exhibited tillering but there was variation in whether aerial branching was observed. Aerial branching was observed to occur in all subfamilies except the Arundinoideae and Danthonioideae. The extent of branching in each subfamily was

highly variable (Table 1 & Fig. 4). In the Pooideae only 2 of 63 total species exhibited aerial branching. The Panicoideae had the most observed branching with 57 of 70 species branching and also had the most variability in extent and location of branches.

After completing my herbarium studies I checked the presence or absence of aerial branches on a number of living specimens growing near Stillwater, Oklahoma. Specimens that did not exhibit obvious aerial branching were examined for the presence of unexpanded buds in the leaf axils, and I found that some species, such as *Bromus tectorum* and *Sorghum halepense,* did not appear to have aerial branches, yet possessed buds in their leaf axils, whereas other species, such as *Arundo donax* and *Phragmites australis* similarly appeared to be without branches, but with no buds in their axils (Table 2). However, I did also observe specimens of *Arundo donax* from a different population that possessed aerial branches.

Sub-Family	Number of species in the subfamily	Number of species examined (number of specimens in parentheses)	Number of species branching (number of specimens in parentheses)
Anomochlooideae	4	1 (106)	0 (0)
Aristidoideae	345	6 (66)	6 (61)
Arundinoideae	600	6 (73)	0 (0)
Bambusoideae	1000	6 (392)	6 (38)
Centothecoideae	75	10 (334)	10 (31)
Chloridoideae	1500	15 (291)	7 (92)
Danthonioideae	378	3 (174)	0 (0)
Ehrhartoideae	364	12 (504)	12 (90)
Panicoideae	3300	70 (1076)	57 (382)
Pharoideae	46	2 (136)	0 (0)
Pooideae	3300	63 (1250)	2 (65)
Pueliodeae	8	4 (67)	0 (0)

Table 1. Extent of aerial branching across Poaceae

Approximate number of species per subfamily, numbers of species sampled and those sampled with aerial branches arranged by sub-family for the combined datasets from the OSU herbarium and Missouri Botanical Garden. Number of specimens examined is in parentheses.

Species	Subfamily	Branches Visible	Buds present
Pharus lappulaceus	Pharoideae	no	(not dissected)
Streptochaeta sodiroana	Bambusoideae	no	(not dissected)
Lolium multiflorum	Pooideae	yes	yes
Bromus tectorum	Pooideae	no	yes
Arundo donax	Arundinoideae	no; yes	no; yes
Phragmites australis	Arundinoideae	no	no
Sorghum halepense	Panicoideae	no	yes

Table 2. Live specimens examined

P. lappulaceus and *S. sodiroana* were live accessions at the Missouri Botanical Garden and therefore not dissected. All other specimens were collected around Stillwater, Payne County, Oklahoma. I observed specimens of *Arundo donax* from 2 separate populations. In one population neither aerial branches nor buds were present. In the second population, individuals produced numerous aerial branches.

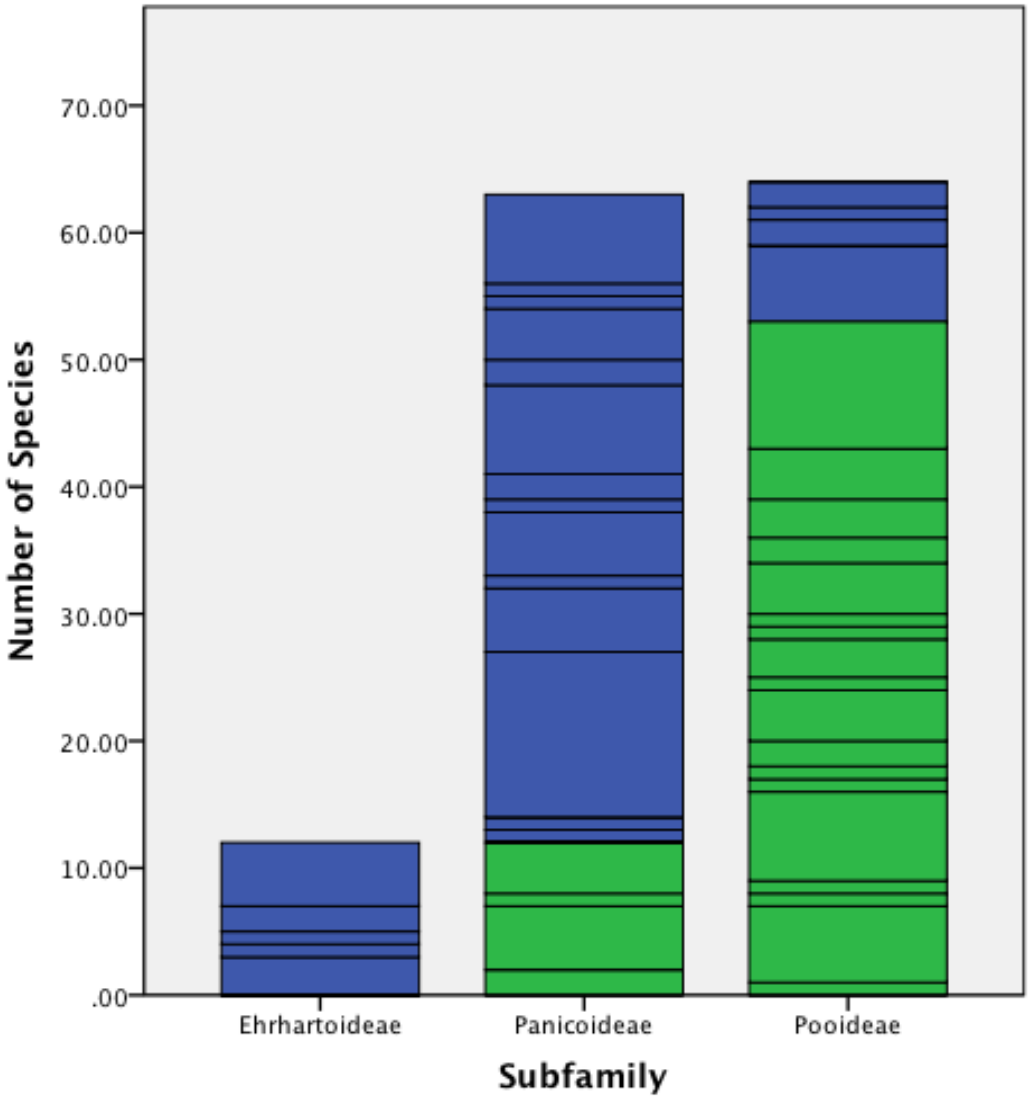


Figure 4. Proportion of species branching for the 3 most densely sampled subfamilies, using the binary coding scheme.

Each segment of a bar for a subfamily represents a genus, where the size indicates the number of species sampled. Blue bars represent genera in which aerial branches were present, green bars represent genera in which no specimen of any species possessed aerial branches. If branches were present in any specimen, the species was counted as having branches.

Phylogenetic analyses and character optimization

Bayesian phylogeny: Individual gene trees constructed using PhyML showed differences in the placement of some taxa but without strong support (trees not shown). Datasets for each marker were therefore concatenated and a partitioned Bayesian analysis was conducted. After 20 million generations the effective sample sizes for the parameters were all greater than 100, which is the minimum recommended value (Weinstock et al. 2005). Only one value, the fourth rate category of the gamma distribution was near to the 100 threshold, with a value of 138, while the others were all above 200. The length of the burnin was determined by comparing the standard deviation of the split frequencies between runs, and examining the trajectory model likelihoods runs visually in Tracer version 1.5 (Rambaut and Drummond 2007) to see whether the runs had attained stationarity and convergence. Based on these observations I discarded the first 2.5 million generations of each run as burnin and combined the remaining trees from the two runs into a majority rule consensus tree. The tree was manipulated for display in FigTree.

The majority rule consensus tree yielded through analysis of the Bayesian trees had the majority of nodes well supported (posterior probabilities [PP] $\geq$ 0.95) (Fig. 5). Subfamilies Ehrhartoideae, Bambusoideae, Pooideae and Panicoideae were all recovered as monophyletic with high support (PP = 1, 1, 1 and 0.98 respectively). In addition, the divergence of the crown group (Bambusoid-Ehrhartoid-Pooid (BEP) and Panicoid-Aristidoid-Chloridoid-Micrairoid-Arundinoid-Danthonioid (PACMAD) clades) was well supported (PP = 0.99) with the BEP and PACMAD clades recovered as monophyletic and well supported (PP = 1). Within BEP, Bambusoideae, Ehrhartoideae and Pooideae

were recovered as monophyletic with high support values (PP = 1). In the PACMAD clade, the Panicoideae (with newly added tribes Centotheceae and Chasmanthiae, Sanchez-Ken and Clark 2010) was highly supported (PP = 0.98). Chloridoideae was highly supported (PP = 1). Aristidoideae and Danthonioideae were recovered as monophyletic and well supported (PP = 1) and recovered as sister clades with high support (PP = 0.99). Arundinoideae was recovered as paraphyletic, with Micrairoideae nested between the clade containing *Amphipogon* and *Arundo* and the clade that is comprised of *Molinia* and *Phragmites.*

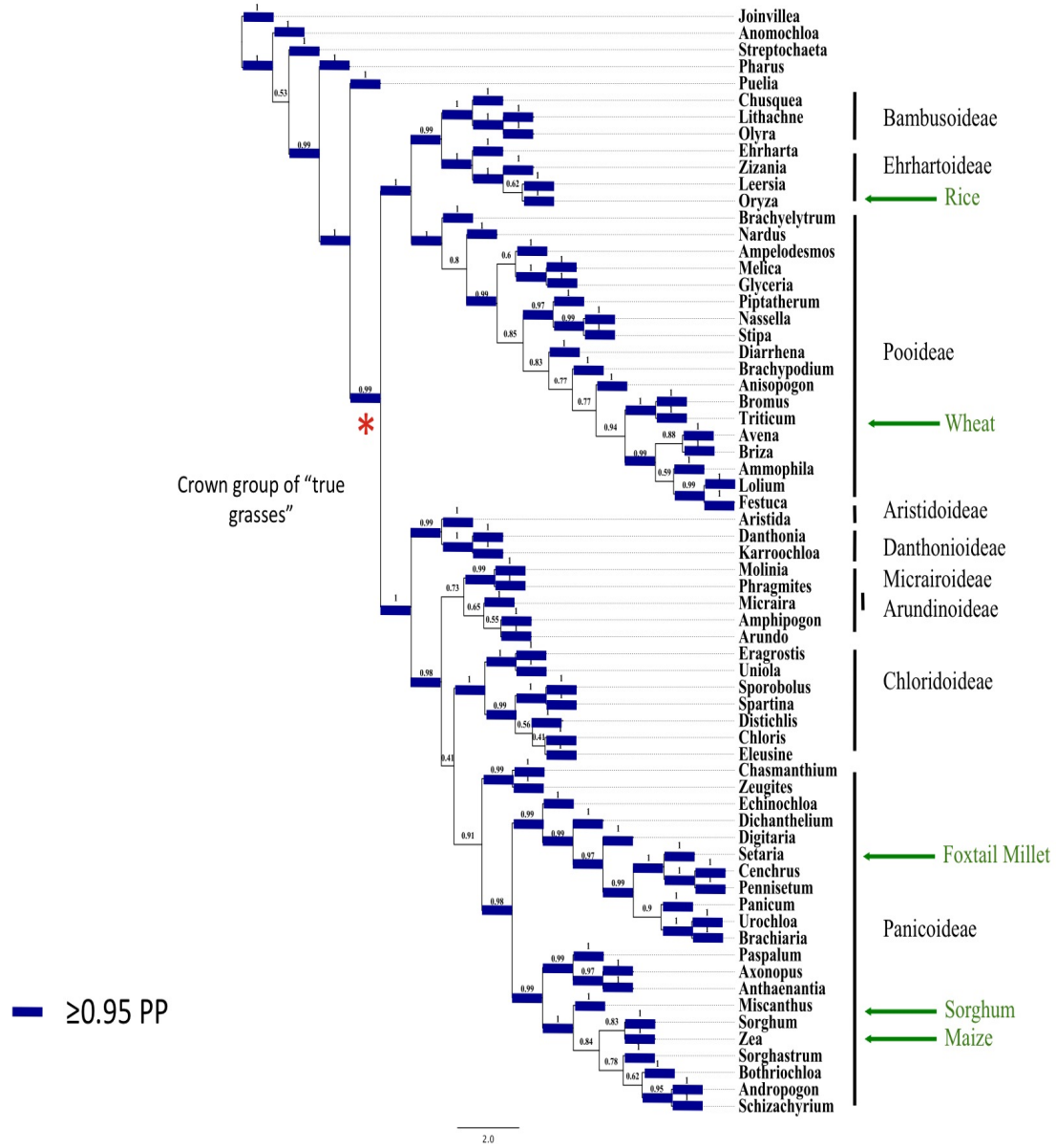


Figure 5. Phylogeny of Poaceae

Consensus tree of Bayesian analysis. Values above branches are posterior probabilities.

Character optimization

Most parsimonious optimization resulted in 2 patterns of gain and loss, but in both reconstructions, the ancestral state of the crown group (BEP and PACMAD clades) of grasses is without aerial branching. The basal grass lineages Anomochlooideae, Pharoideae and Puelioideae were unbranched, with the consensus optimization showing four gains of branching and two reversions with 2 states that vary in the reconstructions (Fig. 6). In the BEP clade I found that aerial branching is the ancestral state of Bambusoideae and Ehrhartoideae with no further state changes in this lineage. Lack of aerial branching was ancestral in the Pooid lineage with a single gain of aerial branching in *Lolium-Festuca* clade.

In both reconstructions the only group with variation in aerial branching is the Chloridoideae. The two optimizations differed only in the node at which branching originated in subfamily Chloridoideae (Figure 6). In one optimization the earliest gain of aerial branching is reconstructed as occurring in the hypothetical ancestor to the Panicoideae-Chloridoideae lineage with 2 reversions in the *Sporobolus-Spartina* clade and *Uniola* for a total of 4 gains and 4 losses of aerial branching across the family. In the other optimization, the ancestral state of Chloridoideae is unbranched with parallel gain of aerial branching in the *Distichlis-Chloris-Eleusine* clade and *Eragrostis*. In both optimizations the ancestral state of Panicoideae was recovered as branched with reversions in the *Paspalum-Anthaenantia-Axonopus* clade and *Brachiaria.* Aerial branching was absent in the Arundinoideae and Danthonioideae, however, aerial branching is present in *Aristida*, which is sister to the Danthonioideae

Rearranging the lineage relationships within the tree to be congruent with GPWG II prior to character optimization resulted in 13 possible MP optimizations. The most notable difference between these reconstructions and the ones based on my analyses was that the ancestral state of aerial branching for the crown group was equivocal (Figure 7). This finding differs from my reconstructions in which the ancestral state for the crown group in both trees was unbranched. One optimization reconstructed the ancestral state of the crown group as unbranched with parallel gains of branching in subfamilies Ehrhartoideae, Bammbusoideae, Aristidoideae, Chloridoideae and Panicoideae, the *Lolium-Festuca*, *Distichlis-Eleusine-Chloris* clades and genera *Arundo* and *Eragrostis*. At the other extreme other optimizations indicate the ancestral state of the crown group as producing aerial branches with parallel reversions in subfamilies Pooideae, Arundinoideae and Danthonioideae, the *Spartina-Sporobolus* clade, *Paspalum-Anthaenantia-Axonopus* clade and genera *Uniola* and *Brachiaria.* With lineages rearranged, the subfamily Aristidoideae (branching) was now sister to the rest of the PACMAD clade. This resulted in aerial branching being the ancestral state of all of PACMAD under all optimizations. Missing data does not account for the conflicting patterns between these reconstructions, as these reflect conflicting observations in the data.

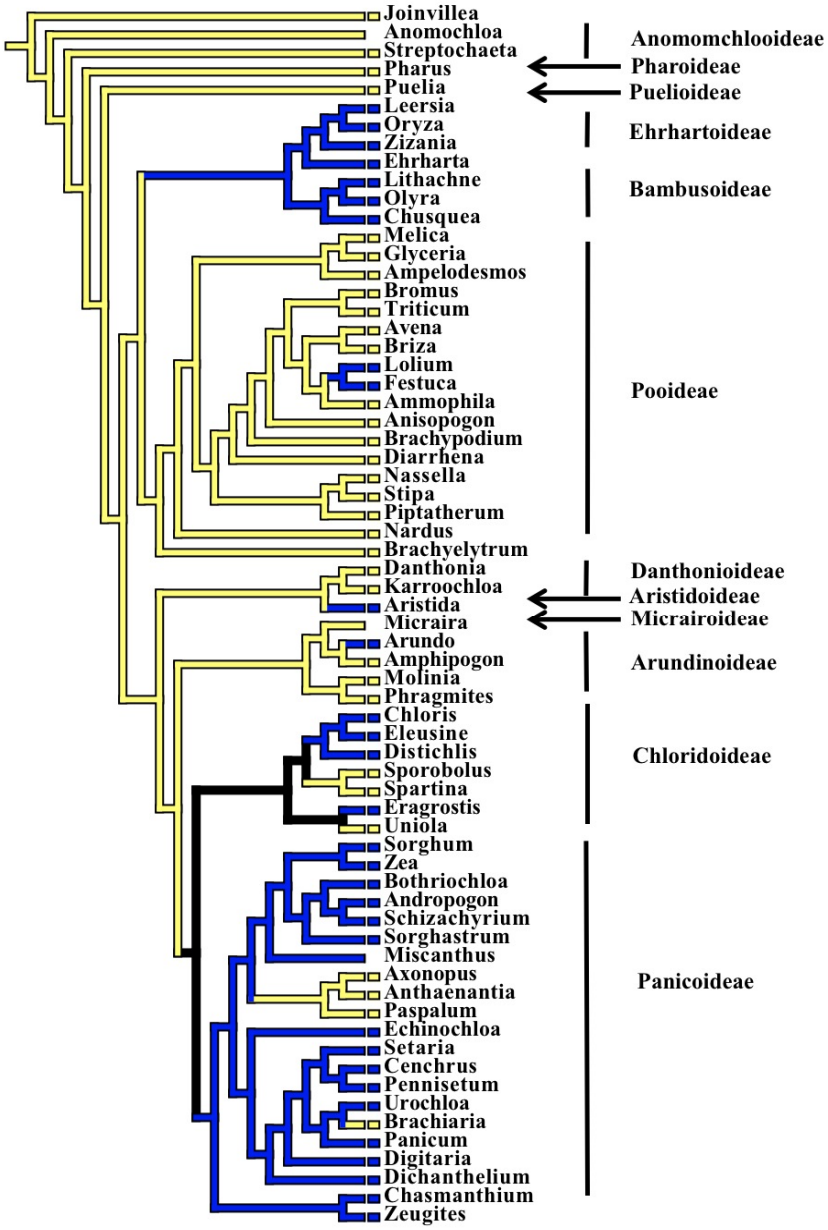


Figure 6. Branching character evolution: Binary character coding

Bayesian phylogeny with branching coded as a binary state character (either present or absent) for Most Parsimonious (MP) character optimization. Lineages reconstructed as unbranched are traced in yellow whereas lineages reconstructed as branching are traced in blue. Lineages reconstructed as equivocal are traced in black.

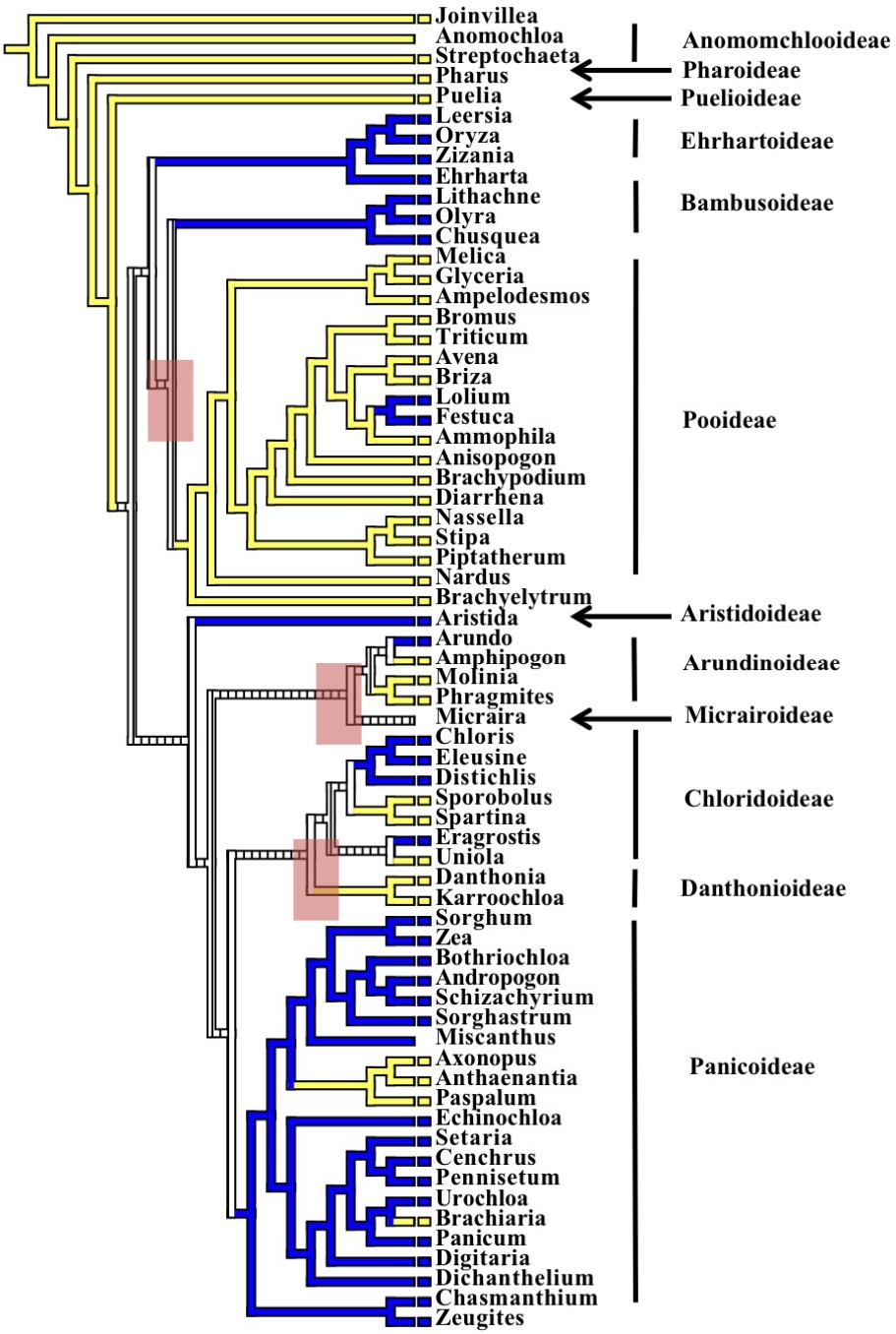


Figure 7. Branching character evolution: GPWG II

Bayesian phylogeny with lineages rearranged to be consistent with relationships recovered by the GPWG II (2012). Aerial branching is coded as a binary state character (either present or absent) for Most Parsimonious (MP) character optimization. Relationships that are changed between the two reconstructions are indicated by a red box. Lineages reconstructed as unbranched are traced in yellow whereas lineages reconstructed as branching are traced in blue. Lineages reconstructed as equivocal are traced in black.

DISCUSSION

Morphological variation

In subfamilies Pooideae and Ehrhartoideae, the majority of specimens produced many tillers but rarely produced aerial branches. This pattern is consistent with the near total suppression of aerial branching and the increase in tiller production seen in the domesticated members of those families such as wheat, barley and oats (Pooideae) and rice (Ehrhartoideae) (Fig. 5). In contrast, Panicoideae architectures ranged from a single, unbranched culm in domesticated modern maize varieties to individuals that were both profusely tillered and produced multiple orders of aerial branches.

In addition to variation across the Poaceae, there was considerable variation in vegetative branching within subfamilies. Of the 3 most densely sampled subfamilies; Pooideae, Panicoideae and Ehrhartoideae, the Ehrhartoideae was the most architecturally uniform. In the Ehrhartoideae, roughly 29% of the specimens sampled were observed to have aerial branches, and each species sampled had at least 1 branched specimen. Most specimens with branches had only 1 aerial branch on the main culm and were profusely tillered. Within the Pooideae, plants that produced aerial branches were the exception. The subfamily is largely composed of caespitose species that form dense tussocks where the tillers are similar in size and shape to the main culm. Despite the near total lack of aerial branching in this clade, there still exists variability in branching patterns. For example, wild species that were examined in tribe Triticeae typically only produced a single culm or 1 or 2 tillers. However, of the 65 specimens in Pooideae with aerial branches, all but 3 belong to a single genus within the Triticeae; *Lolium*. The remaining specimen was *Festuca arundinaceae*. My finding that *Lolium* produces aerial branches

contradicts the information for the genus contained within the Grass Genera of the World DELTA database (Watson, Dallwitz et al. 1986), emphasizing the labile nature of this character.

The Panicoideae was the most architecturally diverse clade sampled. Species varied both in the extent and placement of aerial branches and it was common to find aerial branches on the main culm, tillers and occasionally developing from nodes on other aerial branches. This is in contrast to the rest of the family in which aerial branches were only found on the main culm. The most profusely branched species were members of genera *Setaria, Pennisetum* and *Cenchrus*. In each genus, every species branched and most of the specimens per species sampled had aerial branches (average per genus: *Setaria*=77.8%, *Cenchrus*=83.9%, *Pennisetum*=100%).

Subsequent to these investigations I also dissected freshly collected specimens of a number of species from across the grasses to see whether the absence of aerial branching meant that no axillary meristems were formed or that the axillary meristems were present but remained dormant and did not elongate. Many species had axillary buds, suggesting that the absence of aerial branches was due to bud dormancy rather than the absence of buds, although some species, such as *Arundo donax* and *Phragmites australis*, from which I collected and dissected fresh material, did not have buds at many of the nodes. In the case of *Arundo donax* I observed individuals with no aerial branches and no axillary buds and individuals with numerous aerial branches. These contradictory findings suggest aerial branching can be the result of genetic variation in both meristem initiation and elongation, and that there may be variation in development dependent on environment or plant age.. Thus the observations of lack of branching on herbarium

specimens could be because of the failure of axillary meristems to make buds or of developed buds to elongate. Unfortunately, it was not possible to destructively sample the herbarium specimens to determine which of these possibilities accounted for the lack of branches in any particular case.

Phylogeny

The phylogeny generated in this thesis indicates two main lineages within the family, after the divergence of the anomochlooids, puelioids, and pharoids, these being the Bambusoid-Ehrhartoid-Pooid (BEP) clade and the Panicoid-Aristidoid-Chloridoid-Micrairoid-Arundinoid-Danthonioid (PACMAD) clade. In the BEP clade, Bambusoideae was recovered as sister to Ehrhartoideae and both were sister to Pooideae. In the PACMAD clade, my analysis indicated that Danthonioideae is sister to Aristidoideae and that the Aristidoideae-Danthonioideae clade is basal to the rest of PACMAD.

The subfamilial relationships recovered in the Bayesian phylogenetic analysis were consistent with the GPWG (2001) phylogeny of Poaceae, although several relationships conflicted with the more recent GPWG II phylogeny (GPWG II 2012). A major difference is in the sister relationship between pooids and a clade composed of bambusoid and ehrhartoid lineages, which in my analysis matches that of GPWG 2001, yet differs from recently published phylogenies which have bambusoids sister to pooids (Salamin et al 2002, Bouchenak-Khelladi et al 2008, GPWG II 2012). The source of this incongruence is likely due to discrepancy in taxon sampling, as GPWG II (2012) analyzed sequence data from 531 species in 311 genera whereas my phylogeny only comprised 132 species in 65 genera.

In the PACMAD clade, the placement of Danthonioideae as sister to Aristidoideae in my phylogeny is congruent with the relationships recovered by Salamin *et al* (2002) and Bouchenak-Khelladi *et al* (2008), yet it is inconsistent with GPWG II, in which the Aristidoideae is sister to the rest of PACMAD and Danthonioideae is sister to Chloridoideae. Placement of subfamilies Arundinoideae and Micrairoideae in my phylogeny was incongruent with each of the previously mentioned phylogenies. In my phylogeny Aristidoideae and Danthonioideae were sister to the rest of the PACMAD clade. *Micraira* nested within the Arundinoideae in a clade with *Amphipogon* and *Arundo*, sister to *Phragmites* and *Molinia*, but in Bouchenak-Khelladi *et al* (2008), Arundinoideae and Micrairoideae were sister to the Aristidoideae-Danthonioideae-Chloridoideae clade. In Salamin *et al* (2002), the Aristidoideae-Danthonioideae clade was sister to the rest of PACMAD (although Micrairoideae was not included in this analysis). In GPWG II, Arundinoideae and Micrairoideae were recovered as sister groups as in our analysis however, in GPWG II Danthonioideae was sister to Chloridoideae rather than the Arundinoideae-Micrairoideae clade as in my phylogeny.

Character optimization

The ancestral state of aerial branching for Poaceae was unbranched, however when lineage relationships were rearranged to be congruent with those in the GPWG II phylogeny, the ancestral state of the crown group is equivocal. These differing findings may well be due to differences in taxon sampling. An example of where further sampling is desirable is the loss of branching in the *Paspalum-Axonopus-Anthaenantia* tribe within mostly branched Panicoideae. This is interesting because these three taxa are the only sampled species that fall within one of the two x=10 clades of the Paniceae (as opposed

to the third major clade containing *Setaria* and *Pennisetum* where x=9) (Giussani, Cota-Sanchez et al. 2001), and these three taxa are the only species within the Panicoideae that do not have aerial branches in my phylogeny. Although other genera that fall within this clade are known to be unbranched such as *Thrasya, Ophiochloa and Leptocoryphium*, other genera are either known to produce aerial branches, such as *Icnanthus*, or information on aerial branching is not available (Watson and Dallwitz 1986). Further studies on this group are needed, both to establish patterns of branching as well as to investigate the presence or absence of developing buds in unbranched species.

It is interesting to speculate on how the evolutionary history of branching across the grasses may have affected the process and outcome of domestication (Fig. 5). The trend in domestication for pooid and ehrhartoid crops such as wheat or rice is to produce many fertile tillers, whereas the trend in panicoid crops such as maize, sorghum and millet, is the reduction or suppression of all branching. The result in panicoids is a plant comprised of essentially a single stem with occasional branches in sorghum and the millets, and consistent, but highly condensed branches in maize (the ears), though the wild progenitors of these crops exhibit much more branching. Panicoid crops also have dramatically larger inflorescences rather than an increase in number, as seen in pooids and ehrhartoids. Domesticated millets in particular have not only longer inflorescences but also increased orders of branching within the inflorescence so that the resulting inflorescence is a large, dense panicle. It is thus possible that the lack of vegetative branching in domesticated panicoid cereals is a tradeoff that favors increased resource usage for inflorescence production.

In this study, I observed variation in vegetative architecture amongst the subfamilies of Poaceae. This study represents the beginnings of a comprehensive understanding of the evolution of vegetative architecture in the family, although much more needs to be done. The results of this study will be useful in guiding more detailed analyses of architectural evolution within sub-clades of the family and in understanding how vegetative architecture varies. These findings on vegetative branching will help us integrate our knowledge of vegetative and inflorescence branching into a comprehensive understanding of branching in the grass family. However, a study of mature branching patterns may miss important details in the development of those patterns, and in the next chapter of the thesis I investigate the effects of environment on a single weedy species, *Setaria viridis*, in order to elucidate how these factors influence the development of vegetative architecture in individual plants.

CHAPTER II

DEVELOPMENT OF VEGETATIVE ARCHITECTURE IN GREEN MILLET (*SETARIA VIRIDIS*)

INTRODUCTION

In the last chapter, I discussed the idea that vegetative architecture is a dynamic process that is determined by the timing, extent and placement of axillary branches, and which develops throughout the life of the plant. I examined architectural patterns in mature specimens from across the grass family and found that in some clades architectural patterns present in wild species are still apparent in the domesticated crops that are derived from them. However, this was not the case in panicoid species. Wild panicoid species exhibited the greatest variation in branching patterns, yet panicoid crops showed the most dramatic suppression of branching, with crops, such as the millets, that produce only a single, robust culm and accompanying large inflorescence. In this chapter I examine the patterns of development in a wild/domesticate pair of panicoid grasses in an attempt to understand the branching potential of grasses in this subfamily. As branching is under both genetic and environmental control, I investigate the extent to which branching can be modified by environmental perturbation, such as changes in light quality, light intensity, and temperature conditions.

Green and foxtail millet; an example of domestication in the panicoids

Foxtail millet (*Setaria italica*) and its wild relative green millet (*S. viridis*) have recently been identified as a model genetic system for studying vegetative architecture, based on their small, diploid genomes and close relationship to potential energy crops such as switchgrass (*Panicum virgatum*) (Doust, Kellogg et al. 2009; Bennetzen, Schmutz et al. 2012). In the other four domesticated cereal crops whose genome has been sequenced (maize, rice, wheat and sorghum) there exists a large body of literature on morphology and development (Dewet and Harlan 1971; Haun 1973; Zadoks, Chang et al. 1974; Belford, Klepper et al. 1987; Harlan 1992; Bos and Neuteboom 1998; Bos and Neuteboom 1998; Moulia, Loup et al. 1999; Fournier and Andrieu 2000; Asai, Satoh et al. 2002; Takeda, Suwa et al. 2003; Evers, Vos et al. 2005; Ishikawa, Maekawa et al. 2005; Jaffuel and Dauzat 2005; McMaster 2005; Borras-Gelonch, Rebetzke et al. 2012). However, there has been relatively little study of morphology and development in *Setaria*, except for inflorescence development (Doust and Kellogg 2002). Previous QTL analyses have identified a few candidate genes for control of vegetative architecture, including *barren stalk1* (*ba1*), which affects axillary meristem initiation and *teosinte branched1* (*tb1*), which suppresses the elongation of axillary buds (Doust, Devos et al. 2004; Doust and Kellogg 2006). Because of their relatively simple genomes and highly conserved genomic structure compared to rice, foxtail and green millet are a valuable system to study the evolution and genetic control of plant architecture (Doust, Kellogg et al. 2009; Li and Brutnell 2011; Bennetzen, Schmutz et al. 2012).

Green millet is characterized by the production of many tillers and aerial branches whereas foxtail millet produces only one or a few tillers. Tiller and aerial branch

production in green millet are also known to be dependent on environmental conditions (Doust and Kellogg 2006), but as yet there has been no detailed study on the effect of varying environmental factors on branch development in either species. In this chapter I detail how the characteristic branching patterns of green and foxtail millet develop and investigate to what extent the development of branching varies under the influence of differing environmental conditions. As development is an interaction between genetic control and environmental variation, I will first discuss how these two factors affect the development of vegetative architecture.

Genetic control of Plant Architecture

Architectural diversity is the outcome of the action and interaction of various developmental pathways. Vegetative branching occurs at different stages of development throughout the life of the plant and is a quantitative trait that is controlled by multiple genes, which may either promote or suppress development at each stage. What is known about the genetic control of branching in grasses is in general derived from investigating mutant phenotypes in domesticated species such as rice, wheat, and maize. Mutant analysis is a starting point for understanding the genetic control of branching, and I will discuss the genetic evidence collected so far in terms of axillary meristem formation, the maintenance of dormancy in the axillary bud, and the control of branch elongation (Leyser 2003; McSteen and Leyser 2005; McSteen 2009).

In monocots axillary meristems are initiated as part of the primary sequence of lateral organ initiation, by the shoot apical meristem (SAM) (McSteen 2009). Following initiation, axillary meristems may either enter a period of dormancy or elongate to form axillary branches. From branching mutants in crop grasses we know that branching may

be either eliminated or suppressed in the initiation or elongation stage and that there may be different effects in the vegetative and reproductive portions of the plant. In maize, the *barren inflorescence2* (*bif2*) mutant eliminates meristem initiation in the inflorescence and reduces the number of leaves and vascular bundles produced (McSteen, Malcomber et al. 2007). In rice, the *monoculm1* (*moc1*) mutant completely eliminates branching from both vegetative and reproductive parts of the plant (Li, Qian et al. 2003). On the other hand, although the *barren stalk1* (*ba1*) mutant in maize exhibits no branching in either the inflorescence or vegetative regions of the plant (Ritter, Padilla et al. 2002), the orthologous *lax panicle* mutant in rice only loses branching in the inflorescence (Kyozuka, Komatsu et al. 2002). In all three cases loss of function abolishes meristem initiation. This indicates that orthologous genes can diverge in function over evolutionary time, and suggests that there could be considerable functional divergence in gene networks across the grasses.

Once initiated, meristems differentiate into buds, which may then elongate or remain dormant. *SbDRM1* in sorghum is associated with the maintenance of bud dormancy under apical dominance (Kebrom, Burson et al. 2006), whereas *uniculm2* (*Hs-cul2*) mutants in barley produce axillary meristems that later lose their meristematic potential and through subsequent development the cells that comprise these meristems are re-incorporated into the culm (Babb and Muehlbauer 2003). In barley, *Hs-cul2* is epistatic to other branching mutants and the resulting phenotype in each plant is a single, non-tillered culm.

The elongation of buds is controlled by multiple genetic, and hormonal pathways. The balance between auxin and cytokinin plays a major role in determining the final

architecture of plants (Leyser 2003; Gallavotti, Yang et al. 2008; Ongaro and Leyser 2008; McSteen 2009; Gallavotti, Long et al. 2010; Durbak, Yao et al. 2012). Basipetal flow of auxin results in the suppression of axillary bud outgrowth during the vegetative growth phase of the apical meristem (apical dominance). Cytokinin travels acropetally from the roots into axillary buds where it promotes the elongation of these buds into branches. In addition to auxin and cytokinin, a new class of hormones was recently shown to influence vegetative architecture. Strigolactones move acropetally from the roots but suppress the elongation of axillary buds in a similar manner to auxin (McSteen 2009). Strigolactones are the product of the *MORE AXILLARY BRANCHING* (*MAX*) gene pathway in *Arabidopsis* (Bennett, Sieberer et al. 2006). The *MAX* pathway is highly conserved across monocot and eudicot lineages with orthologs to one or more *MAX* genes identified in pea (*RAMOSUS,*), *Petunia* (*DECREASED APICAL DOMINANCE*) and rice (*HIGH TILLERING DWARF1* and *DWARF3*).

Another genetic pathway controlling bud elongation that is found in grasses and *Arabidopsis* (Finlayson, BRC1 and 2) is centered on the TCP transcription factor *teosinte branched1* (*tb1*) and its orthologs (Doebley, Stec et al. 1995; Takeda, Suwa et al. 2003; Kebrom, Burson et al. 2006; Finlayson 2007). The function of *tb1* is to negatively regulate cell cycling genes, so that high expression levels of *tb1* result in suppression of cell division in the apical meristem (Hubbard, McSteen et al. 2002). Both *tb1* mutants in maize and the orthologous *OsTb1* mutants in rice produce numerous branches (Doebley, Stec et al. 1995; Takeda, Suwa et al. 2003). One of the downstream targets of *tb1* is known in maize to be *grassy tillers1* (*gt1*), whose expression is induced by a reduced red:far red (R:FR) ratio resulting from plant shading, and that promotes apical dominance

in maize (Whipple, Kebrom et al. 2011). In maize, both *tb1* and *gt1* must be expressed in order for axillary bud elongation to be suppressed (Whipple, Kebrom et al. 2011).

Vegetative branching is susceptible to environmental factors such as shading, nutrient availability and planting density. High planting densities can alter the R:FR ratio, as the proportion of far red light transmitted by neighboring plants is increased. This can result in a shade avoidance response (SAR) that includes an increase in internode length and a decrease in vegetative branching (Kebrom, Burson et al. 2006). Kebrom et al. (2006) demonstrated that TB1 and SbDRM1 expression in sorghum are responsive to light sensing via phytochrome B. The findings of Kebrom & Brutnell (2007) and Whipple et al. (2011) support the idea that decreased vegetative branching as a result of domestication in maize was due to the expression of *tb1* at high levels {Doebley, 1995 #43; Kebrom and Brutnell 2007). This pattern of expression has now become essentially constitutive, so that modern maize now exhibits obligate suppression of axillary branching and is insensitive to crowding (Moulia, Loup et al. 1999).

Phenotypic plasticity and norms of reaction

The shade avoidance response (SAR) is an example of phenotypic plasticity, where plants can modify development trajectories in response to environmental variation (Conner and Hartl 2004). In SAR, plants perceive shade from neighboring plants as a change in light quality. This perception of light quality is mediated by phytochromes, of which there are three (in grasses PhyA, PhyB, and Phy C) (Sawers, Sheehan et al. 2005). The changed ratio, as a result of shade from neighboring plants, signals competition for light resources. The shade avoidance response results in an increase in apical dominance and a decrease in axillary branching, allowing the plant to put all of its energy into

upward growth, which may allow it to grow out from under the shade of surrounding plants. Most plants exhibit some degree of morphological plasticity in response to environmental variation, including foraging for light and avoidance of shade. For example, in white clover (*Trifolium repens*) the organ responsible for perception of decreased light quality is the leaf subtending each axillary bud, and changes in the micro-environment around this leaf influences the outgrowth of axillary branches after buds have been initiated (Robin, Hay et al. 1994). In sorghum, shade avoidance is mediated via phytochrome B, which suppresses the expression of *tb1*, with the *phy-B1* mutant showing a constitutive SAR as a result of high levels of *tb1* expression (Kebrom, Burson et al. 2006; Kebrom, Brutnell et al. 2010).

One method of quantifying the response to change in the environment is by calculating reaction norms. Reaction norms represent the expected range of phenotypic variation in a trait in response to changes in a specific environmental variable. By measuring the architectural differences between plants of the same genotype in different environments, such as high light vs. low light, we can determine the effect of the particular environmental factor on phenotypic variation for that genotype (Stearns 1989). Determining the reaction norm for a given environmental influence is useful in identifying developmental constraints, range of natural variation and character independence for phenotypic characters. In this way, reaction norms are a measure of phenotypic plasticity for a given developmental program and have applications in ecological, developmental and evolutionary research. Pioneering studies on the effect of environment and genotype on morphology were performed by Clausen, Keck, and Hiesey (1940), who established common garden experiments of four subspecies of *Potentilla*

glandulosa along an east-west transect in California, to examine the extent to which genotype and environment determined phenotypic differences between the four subspecies (Clausen, Keck, and Hiesey 1940). That work has inspired generations of ecologists (Núñez-Farfán and Schlichting 2001), and for example, reaction norms have been used to investigate differences in adaptation to water stress in two species of cactus seedlings grown in stressed and normal conditions (Rosas, Zhou et al. 2012), differences in phenology of trees along altitudinal and temperature gradients (Vitasse, Bresson et al. 2010), and yield differences resulted in overall decrease in grain and seed quality in spring wheat and rape (Peltonen-Sainio, Jauhiainen et al. 2011). The use of reaction norms seems particularly appropriate in the study of branching patterns, since these are known to vary between environments and to be affected by environmental factors such as shading.

Previous work in foxtail and green millet has suggested that shading and temperature can both affect vegetative architecture, but did not explicitly test combinations of these variables (Doust and Kellogg 2006). In this chapter I examine the effects of light intensity, light quality as mediated by plant shading, and a physiologically relevant range of temperatures on branching in green millet, in order to elucidate the extent of developmental plasticity in this species.

METHODS

Growth trials-Foxtail millet and green millet

Differences between foxtail millet and green millet (FM-GM) were assessed in greenhouse and field trials in order to compare architecture and plasticity between green and foxtail millet. In each of the three trials seeds of foxtail millet (*Setaria italica* accession B100) and green millet (*S. viridis* accession A10) were planted in Metro Mix 366 potting medium and watered as needed with an aqueous solution of Jack's mix (Nitrogen, Phosphorous and Potassium (20-20-20). In all FM-GM trials seeds were germinated in a greenhouse and in the field trial plants were transplanted to the field two weeks after planting. Day length and day and night temperatures were measured for each trial. Spacing varied between 12 and 25 cm between (Table 1). Plants in all trials were arranged in a randomized block design, with six replicates per species in each of the trials. Height to the collar of the flag leaf, tiller number, and aerial branch number were measured for each replicate upon the first emergence of the culm inflorescence.

Growth trials-Green millet only (GM)

In order to probe more deeply the effect of environmental perturbation on a single genotype I conducted three more growth trials using green millet (GM) only. The same accession of green millet was used as for the FM-GM trials. I performed one growth trial to determine the pattern of vegetative development under average conditions in green millet and two further growth trials to identify effects of light quality and quantity on

vegetative architectural development. Light was manipulated both by the differing lighting intensities in growth chambers and greenhouses as well as by the use of shading from neighboring plants. The effect of shade from crowding by neighboring plants is a perceived reduction in both light quanta and change in light quality. In particular, the effect of plant shading is to decrease the ratio of red to far red light (R:FR), which is known to affect architecture in many plants.

In all three GM trials three seeds of green millet were planted per pot in 11cm x 11cm square pots containing Metro-Mix 366 growth medium, and thinned to 1 plant per pot following germination. Plants were watered ad libitum and, beginning 2 weeks after planting, an aqueous complete fertilizer mix (Jack's mix: Nitrogen, Phosphorous and Potassium (20-20-20)) was applied once a week. Germination in the first two trials was below 80%, and I decided to cold-stratify seeds in a -80°C freezer for 24 hours before planting the third trial. Potted plants were grown in a growth chamber at the Controlled Environment Research Laboratory (CERL) on the Oklahoma State University campus in the first and second trial, under low light conditions (Table 2). In the third trial, plants were grown on a single bench in a glasshouse on the Oklahoma State University campus under natural light supplemented by metal halide and high-pressure sodium growth lights in high light conditions (Table 2). There was more than a six-fold difference in light intensity between trials 1 & 2 and trial 3 (Table 2). In each trial, the photoperiod regime was 16 hours light and 8 dark, simulating summer growing conditions for this species. Temperatures varied between 22˚ and 27˚C between the trials (Table 2).

In each GM trial, the perimeter of the experimental block was composed of *S. viridis* plants that were not measured for analysis, in order to minimize edge effects on

the phenotypes of the test plants. In the first trial, 10 plants were arranged in a single randomized Intermediate Density (ID; 15.5cm between plants) block. In the second trial, 31 plants were arranged in 2 contiguous blocks with plant positions randomized following germination, with the high-density block having 16 experimental plants (HD; 11cm between plants) and the intermediate density block having 15 plants. In the third growth, plants were arranged in 4 contiguous blocks with plant positions randomized following germination. Two of these blocks were at low density (LD; 31cm between plants) and two were at high density (HD; 11 cm between plants). Each block consisted of 25 plants.

Year	Location	Day Length	Average Temperature	Plant Spacing	Growth environment
2008	Greenhouse (OSU)	13-14 hours	25.5^{o}C	15.5cm	Pots, potting mix
2010	Greenhouse (OSU)	14-15 hours	25 ^{o}C	12cm	Pots, potting mix
2010	Cimarron Valley Research Station	14-15 hours	26.7 ^{o}C	25cm	Field, soil

Table 1. Growth conditions for trials with green and foxtail millet (FM-GM)

Growing conditions for each of the FM-GM growth trials. Plant spacing is the measure of the distance between plants, not pots in which they are contained.

Table 2. Growing conditions and measurements taken for each green millet (GM) growth trial

Trial	Growing Conditions				Phenotypic Response Measurements			
	Location	Average Temperature	Light Quanta	Density	Height	Branch Number	Branch Length	Branch emergence
GM-1	Growth chamber	22 ^{o}C (23 ^{o}C day/ 21 ^{o}C night)	220μmol $cm^{-2}s^{-1}$	ID	x	x	x	x
GM-2	Growth Chamber	25 ^{o}C (25 ^{o}C day and night)	220 μmol $cm^{-2}s^{-1}$	HD, ID	x	x	x	x
GM-3	Glasshouse	27 ^{o}C (27 ^{o}C day and night	1400 μmol $cm^{-2}s^{-1}$	HD, LD	x	x	x	x

Location, growing conditions and measurements taken for each GM trial. Light intensity was measured with a LiCor LI-250A light meter. HD = high density, ID = intermediate density, LD = low density

Morphological measurements-GM trials

Morphological measurements were made at 3-day intervals in each trial. The aim of these measurements was to determine the timing and pattern of branch development and overall growth of the plant under different planting densities. To do so, measurements were made of height, tiller and aerial branch length, leaf number, number of branches and date of branch emergence (Table 2). In GM-2 and GM-3 emerging tillers were labeled with jeweler's tags to determine the relative order of tiller elongation. However, in GM-2, the tags degraded following repeated watering.

Specimens of green millet were grown for dissection in the growth chamber concurrent with the second growth trial, to determine at what stage the shoot apical meristem starts to produce an inflorescence. One plant per day was harvested each day for two weeks following planting, fixed in FAA (formalin-acetic acid-70% ethanol, 10:5:85 v/v). These plants were dissected using a Leica SP08 stereo dissecting microscope in order to ascertain at what time after germination and at what leaf stage (number of leaves visible on the plant) the apical meristem commenced transition from producing leaves to producing the primary branches of the inflorescence.

Statistical analysis

Statistical analysis was implemented in the SPSS Statistics software package (version 19; 2010) and Microsoft Excel 12.2.8 (2008), and SPSS was used to graph the results of analyses. Means, standard deviation and coefficient of variation (CV) were calculated for all trials, species with trials and treatments with trials. Correlations between height and branching variables for plants at maturity were computed for all trials to determine if there is a developmental tradeoff between these characters in response to changes in the environment.

RESULTS

Foxtail millet-green millet growth trials - FM-GM

Daylength ranged between 13 and 14 hours and average daily temperature was 25-26 °C for the three trials (Table 1). In each of the FM-GM trials, green millet produced four to seven tillers and one or two aerial branches and attained a final mean height of between 20-21.1cm (Table 3). Foxtail millet plants were significantly taller with final height ranging between 47.25-87.5cm (Tables 4 and 5). Foxtail millet individuals typically produced 1 or no tillers, and no plant produced more than 2 tillers.

From the data collected in the FM-GM trials, foxtail millet appears more uniform than green millet in both tiller and axillary branch number across planting densities, yet more variable in height. Although green millet had a greater mean branch number, foxtail millet actually exhibits greater variation in branch number, when scaled for differences in the means (Table 3). However, this variation is often between having no tillers or just one tiller.

Height and branch number were not, in general significantly correlated in either green or foxtail millet, except for green millet in a single trial (Table 6).

Green millet growth trials - GM

Development of *Setaria viridis*

In the first green millet growth trial (GM1) plants began producing tillers 9 days after germination and typically produced a total of four or five primary tillers at a rate of 1 tiller every 4 days. Tiller elongation proceeded acropetally, although the second tiller appears longer than the first, and gives the false impression of being older than the first. This was also found for aerial branches, where the second aerial branch from the base appeared longer than the first or other branches. However, in all specimens aerial branch elongation is also in an acropetal order. Secondary tillers, which originate from primary tillers on the culm, began to visibly emerge from the subtending leaf sheath (3^{rd} leaf) at the 4-leaf stage, recapitulating the pattern of development on the main culm, and secondary tillers occasionally produced tertiary tillers. Aerial branches were observed on the culm, primary and secondary tillers and other aerial branches. The internodes along the culm had begun to expand three days prior to the first observations of developing aerial branches (Fig. 1).

Species	Trial	Height	Tiller Number	Aerial Branch Number
		Mean [Std.Dev.,Cv]	Mean [Std.Dev., Cv]	Mean [Std.Dev., Cv]
S. viridis	GH2008	20.5 [5.96,0.3]	6.67 [2.66,0.42]	1.03 [3.67,0.29]
S. italica	GH2008	82.5 [7.23,0.09]	1.25 [1.26,1.07]	0.5 [0.58,1.23]
S. viridis	GH2010	20.06 [2.93,0.28]	3.5 [1.4,0.57]	1.88 [1.4,1.37]
S. italica	GH2010	47.25 [14.36,0.32]	0.75 [0.96,1.37]	None
S. viridis	F2010	21.1 [7.9,0.15]	7 [3.74,0.42]	0.75 [0.96,0.75]
S. italica	F2010	62.5 [21.86,0.38]	0.33 [0.58,1.88]	None

Table 3. Architectural characteristics of *Setaria viridis* and *S. italica.*

Mean, Standard Deviation and Coefficients of Variation for foxtail and green millet.

	S. italica Mean [Std. Dev.]	*S. viridis* Mean [Std. Dev.]	Significance Value
Height	64.23[20.56]	17.51[6.53]	P<0.000
Aerial Branch number	0.15[0.38]	2.22[1.59]	P<0.000
Tiller number	0.77[0.93]	5.33[2.87]	P<0.000

Table 4. Comparison of growth characters pooled across FM-GM trials

Trial	Greenhouse 2008	Field 2010	Greenhouse 2010	Greenhouse 2010
Density	ID	HD	LD	HD
Height	P<0.0001	P<0.0001	P<0.002	P<0.011
Tiller Number	P<0.001	P<0.018	P<0.017	P<0.03
Aerial Branch Number	P<0.0001	ns	ns	ns

Table 5. ANOVA of *S. viridis* and *S. italica* growth characters within each FM-GM trial

Analysis of variance for height, tiller number, and axillary branch number within each growth trial (ns = non-significant). ID = intermediate density, HD = high density, LD = low density.

Trial	Greenhouse 2008	Field 2010	Greenhouse 2010	Greenhouse 2010
Density	ID	HD	LD	HD
S. viridis	0.19	ns	ns	ns
S. italica	ns	ns	ns	ns

Table 6. Correlations between height and tiller number for *S. viridis* and *S. italica*

Correlations within species between height and tiller number for each of the planting densities across three FM-GM growth trials (ns=non significant). ID = intermediate density, HD = high density, LD = low density.

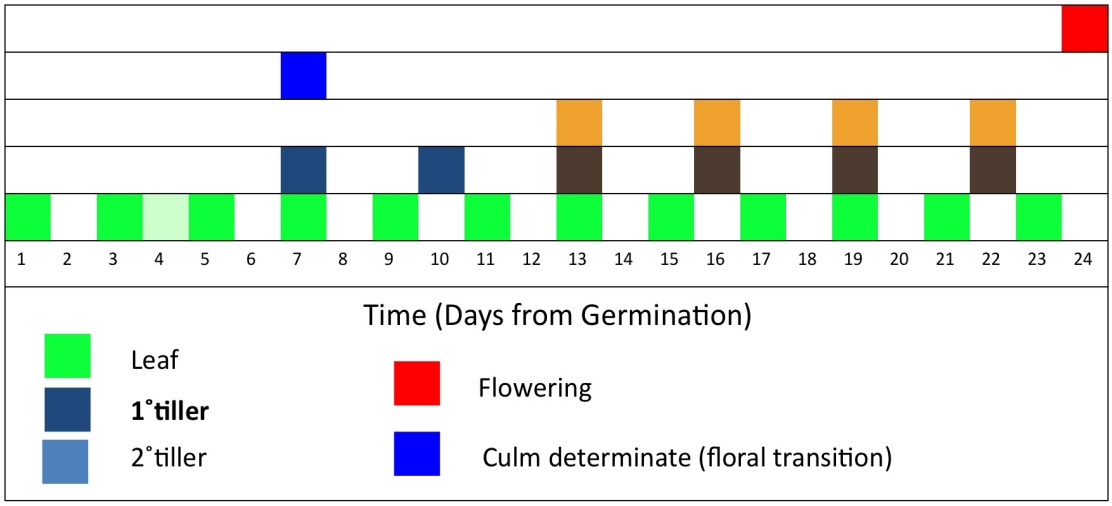


Figure 1. General phenological timeline for *Setaria viridis*

Timeline showing the orderly development of green millet based on the three GM trials. The timing of floral transition was determined from dissected specimens in GM-2.

The first expanded internode on each plant was located between node 5 and node 6. Based on this distinction, axillary branches at nodes 1-5 were tillers whereas branches at nodes 6 and above were aerial branches.

In the second growth trial (GM-2), the second tiller was also often longer than the first, even though it started elongation later. Tiller production began with the first tillers visibly emerging from the sheath of the 3rd leaf at the 4-leaf stage, and continued through development. The overall pattern of development did not change between ID treatments in GM-1 and GM-2. However, the timing of development did vary in response to both temperature and planting density. At the higher temperature in GM-2 (25^{o}C vs. 22^{o}C in growth trial 1), plants developed at an increased rate. The first tillers were observed 14 days after planting rather than 20 and emerged at a rate of 1 tiller every 3 days rather than every 4 days (Fig. 2).

Under HD in GM-2, plants produced their first aerial branch between 15 and 27 days from planting whereas in ID plants produced their first branch between 12 and 15 days from planting. In the HD treatment, plants typically produced fewer branches and were not as tall, yet the means and variance were not significantly different for either character at flowering (Table 5). In both treatments the maximum number of branches observed was 37 (Figure 1). In the HD treatment flowering was first observed 27 days from planting but 4 individuals had failed to flower by 40 days from planting. In the ID treatment, flowering was first observed 27 days from planting and all individuals had flowered by 30 days from planting.

In GM-3 the development of green millet at each of the different planting densities was less variable than in the previous two trials. The first tiller on each plant

across all 4 blocks emerged at the 4-leaf stage, between 11 and 13 days from planting (Fig. 1). Plant height at this stage ranged from 1.5cm to 4.5cm with a mean of 3.7cm. New tillers emerged simultaneously with new leaves at a rate of 1 every 2-3 days. Once branching was initiated at the base of the culm, there was no temporal delay between tiller and aerial branch emergence. Branches emerged acropetally, at regular intervals and distinction between tillers and aerial branches was only possible after the expansion of the internode between nodes 5 and 6, at the 9-leaf stage. Branch emergence ceased for all orders of branches upon the emergence of the inflorescence, which occurred between the 10-leaf and 12-leaf stage for all plants, and no plant produced more than 7 total branches (tillers and aerial branches) on the main culm. Of the 106 experimental plants across all blocks, only 4 produced fewer than 5 tillers. Timing of germination, emergence of the first tiller and subsequent tiller production was identical between treatment groups, with the general form of all plants being a somewhat taller culm and shorter tillers. Flowering time was slightly more variable however the majority of plants flowered 24 days from planting.

		ID	HD	Significance
Height	Mean [Std. Dev.]	14.73[2.21]	13.86 [5.34]	ns
Sum of Tillers and Aerial Branches	Mean [Std. Dev.]	6.5[0.53]	5.25[1.29]	0.03

Table 7. Comparison of growth characters between treatments in GM-2

Significance of comparison ** = $p < 0.01$, one-way ANOVA.

	LD Mean [Std. Dev.]	HD Mean [Std. Dev.]	Significance level
Height	23.25[2.88]	20.99[3.18]	P<0.000
Tiller number	6.14[0.66]	5.35[0.68]	P<0.000
Sum of Tillers and Aerial branches	13.48[3.75]	11.76[3.11]	P<0.008

Table 8. Comparison of growth characters between treatments in GM-3

Significance of comparison ** = p< 0.01, one-way ANOVA.

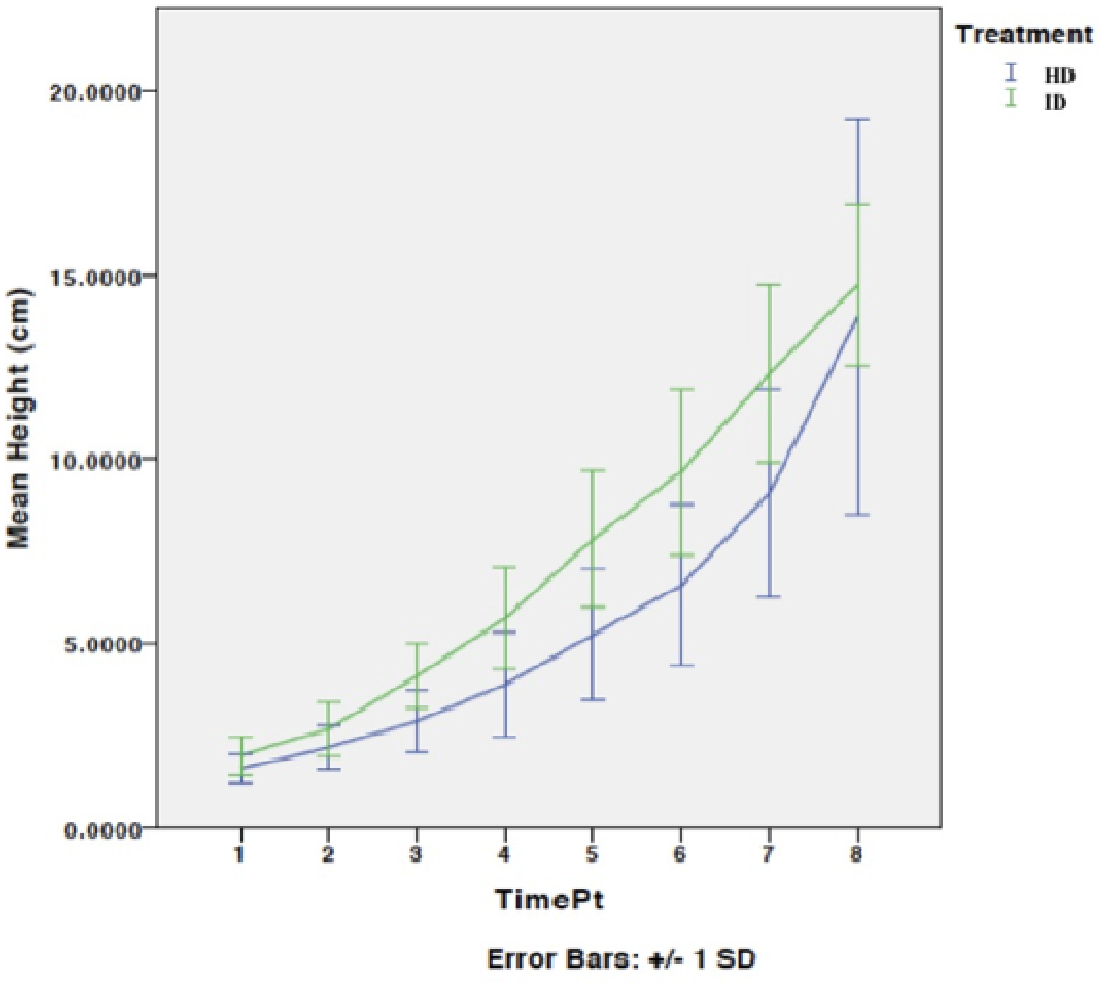
Variation between GM trials

When the temperature and light intensity under which *Setaria viridis* is grown are varied, the only modification to development is an increase in the growth rate (Fig. 4). Only under conditions meant to simulate competition (i.e., increased planting density) did I observe modifications to vegetative architecture. Plants at LD developed in a pattern similar to those in previous trials; a long main culm with many tillers that were shorter relative to the culm. The HD plants were shorter overall and had fewer tillers, with four of the plants also having markedly shorter tillers (Figs. 3 and 5). The first tiller to develop was the longest tiller for plants in each treatment group (Fig. 3). The length of each tiller is approximately equal for tiller 1 in the HD and LD trials, but each subsequent tiller is progressively shorter in the HD trials as compared to the plants in the LD trials (Fig. 3 A-E).

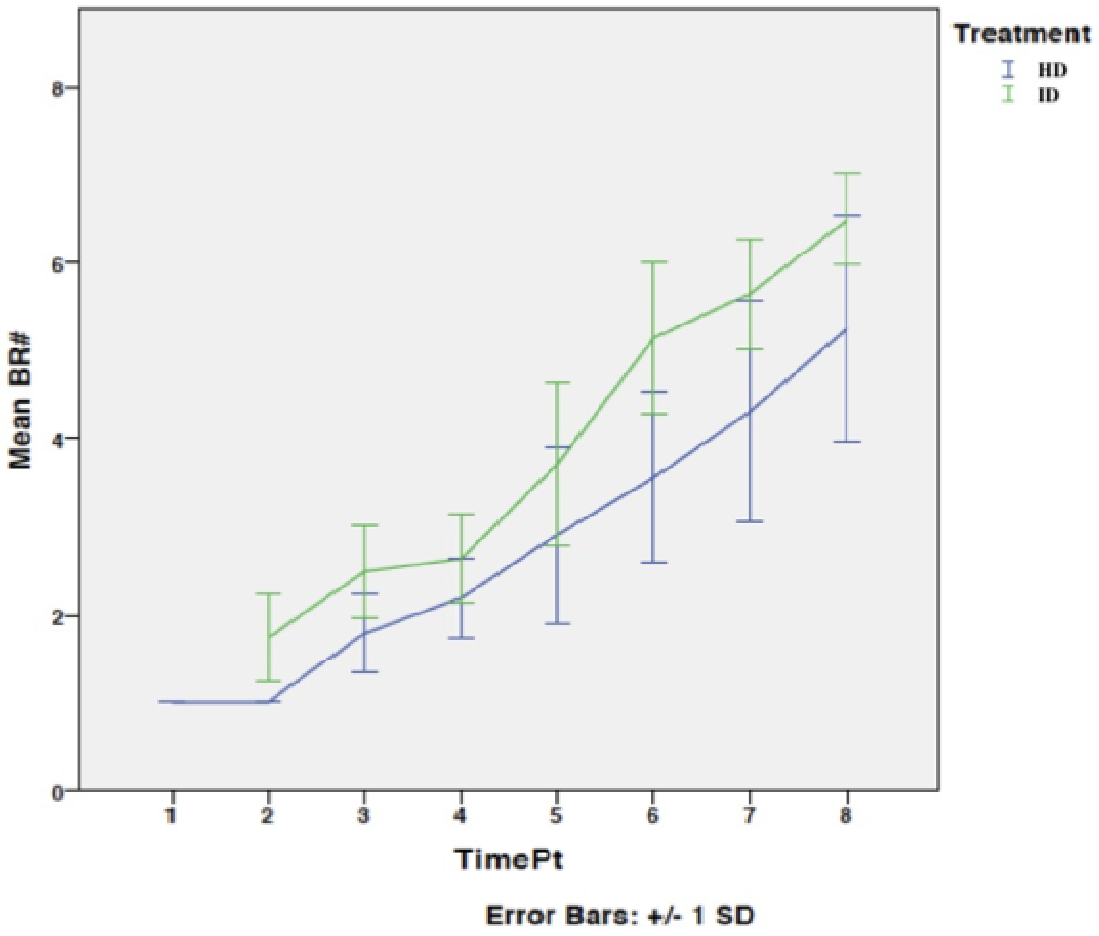
Analysis of variance between ID and HD treatments in GM-2 were non significant for height but were for tillers and aerial branches (Table 7). In GM-3 ANOVA of the differences between LD and HD were significant for all three traits (height, tiller number, and aerial branch number) (Table 8). Correlations between height and tiller number in the three GM trials ranged between significantly negative (GM-1 and GM-2 at intermediate density), to non-significant (GM-2 high density), to significantly positive (GM-3 both densities) (Table 9). Height versus total tiller length and height versus average tiller length were also significantly positive in GM-3 (and were not measured in the other trials).

Dissections

Dissections of plants collected daily in GM-2 showed that the apical meristem transitioned from producing leaves to producing the inflorescence branch primordia at approximately the 3-leaf stage. Vegetative axillary buds were also first visually evident in dissected specimens at the 3-leaf stage, however, tillers did not emerge from the subtending leaf sheath until the 4-leaf stage (Fig. 1). In these specimens, aerial branches were not yet visible.



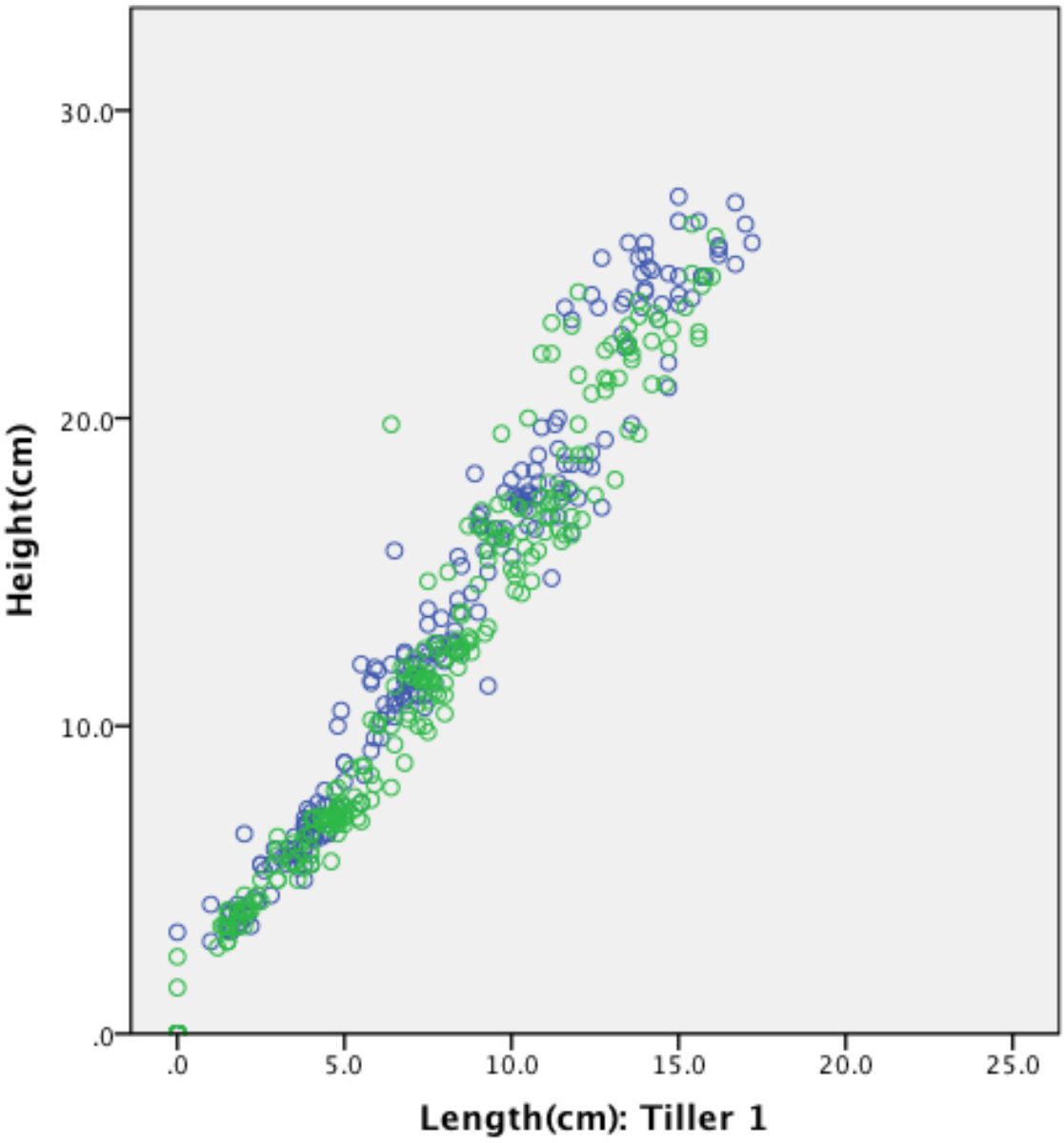
A



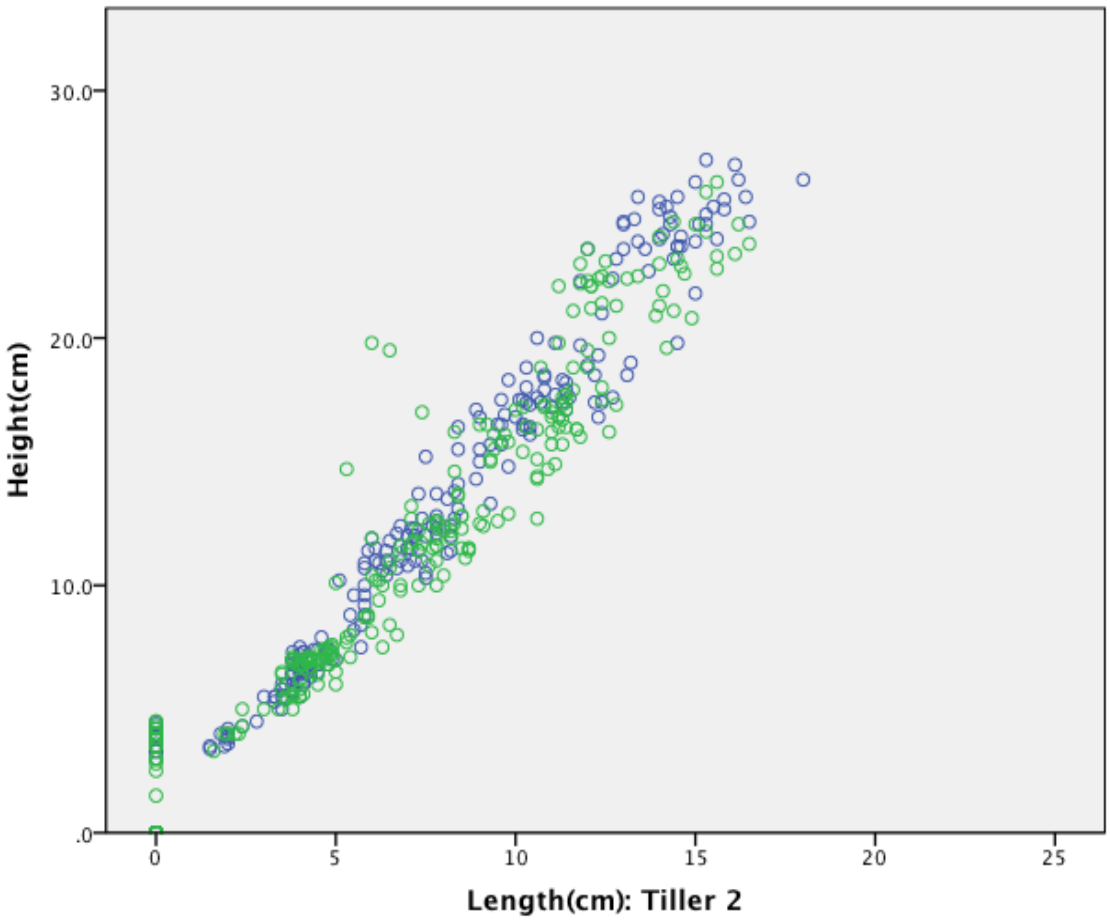
B

Figure 2. Growth curves for height and branch number in growth trial 2 (GM-2)

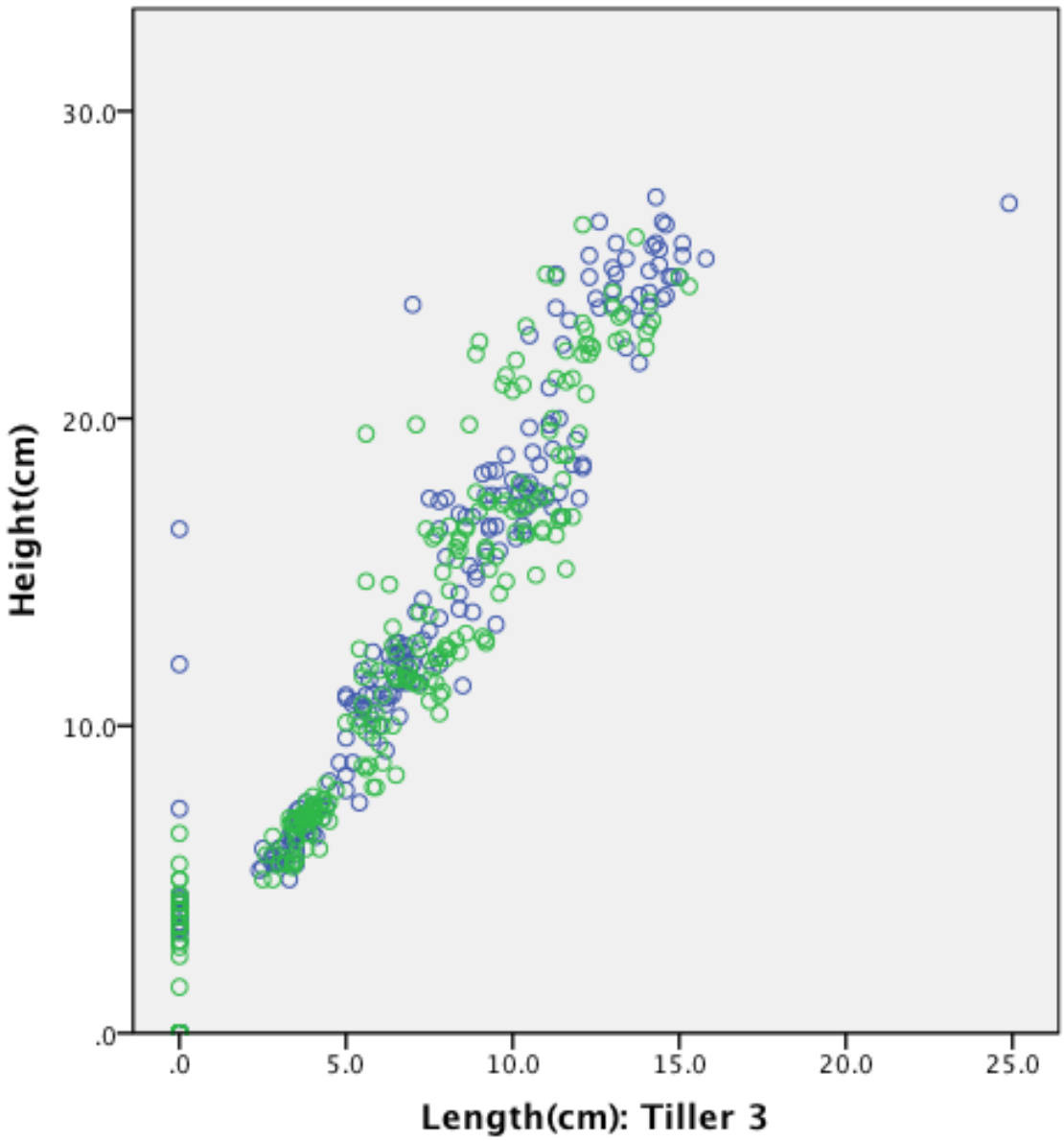
A) Relationship between mean height in each treatment group and time in growth trial 2. B) Relationship between mean secondary branch number in each treatment group and time in growth trial 2.



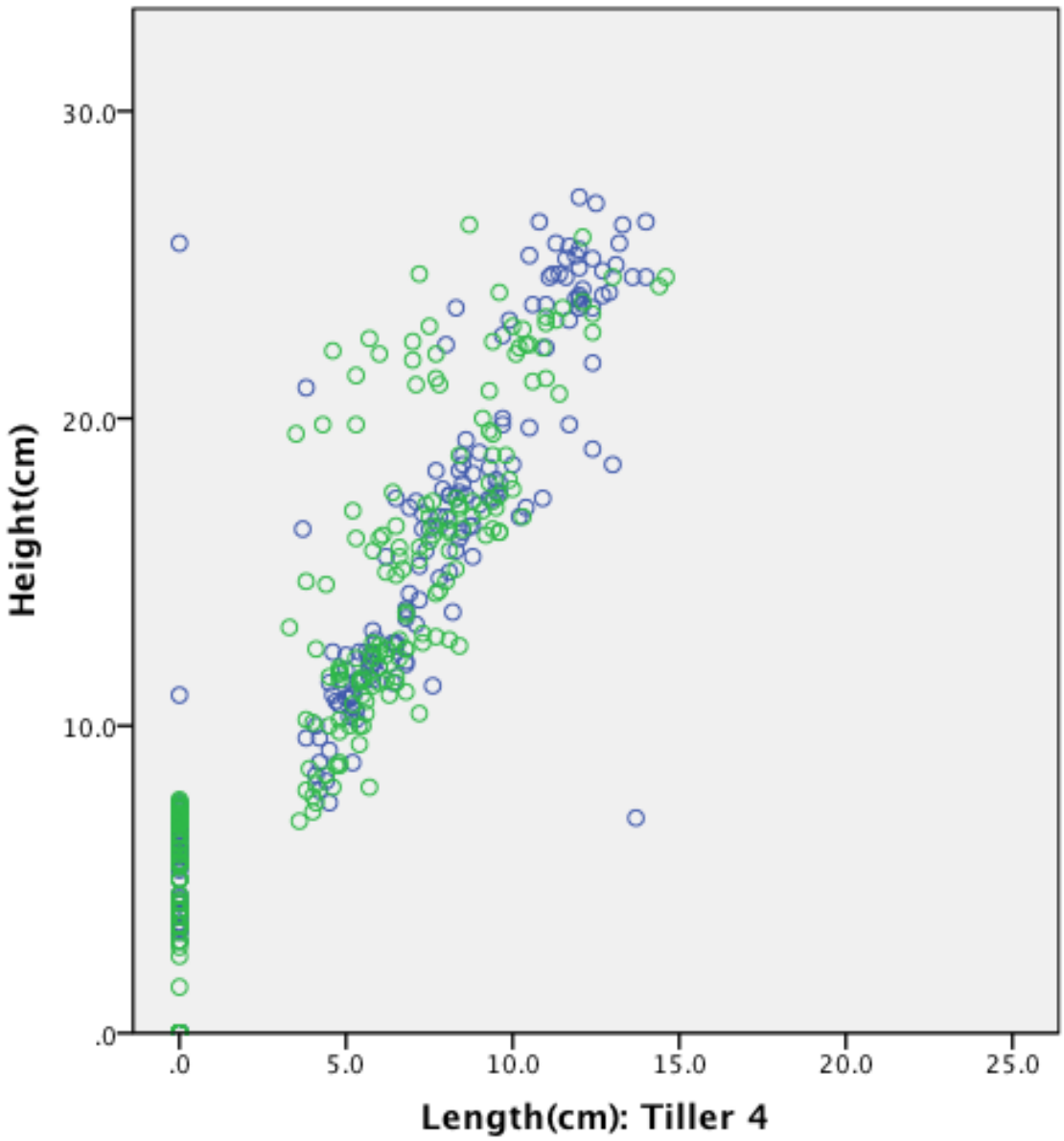
A



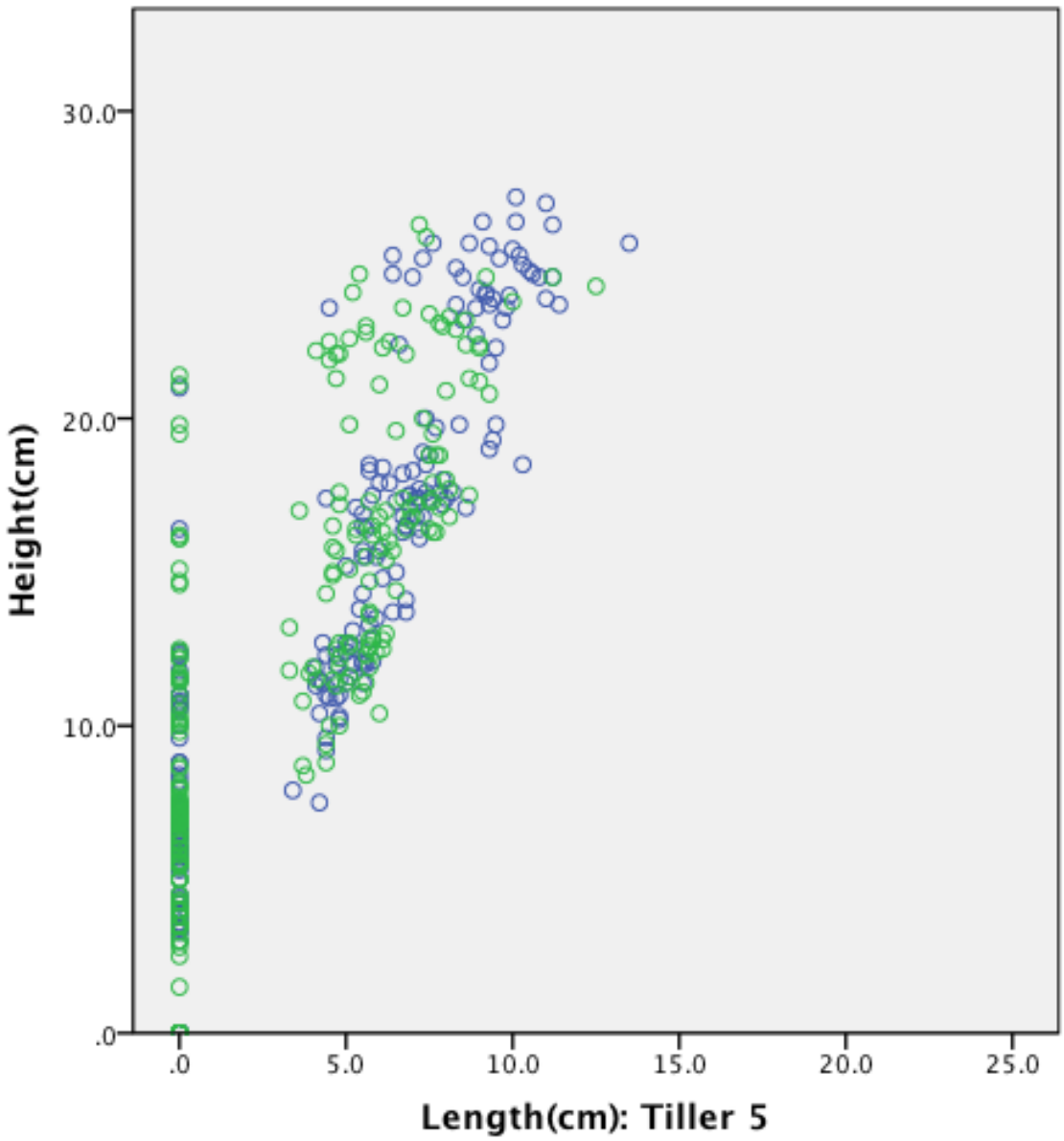
B



C



D



E

Figure 3. Culm height versus tiller length for the LD and HD treatments in growth trial 3 (GM-3)

Culm height versus tiller length for tillers 1-5 (panels A-E respectively). The HD treatment is indicated in by green circles, the LD treatment is indicated by blue circles.

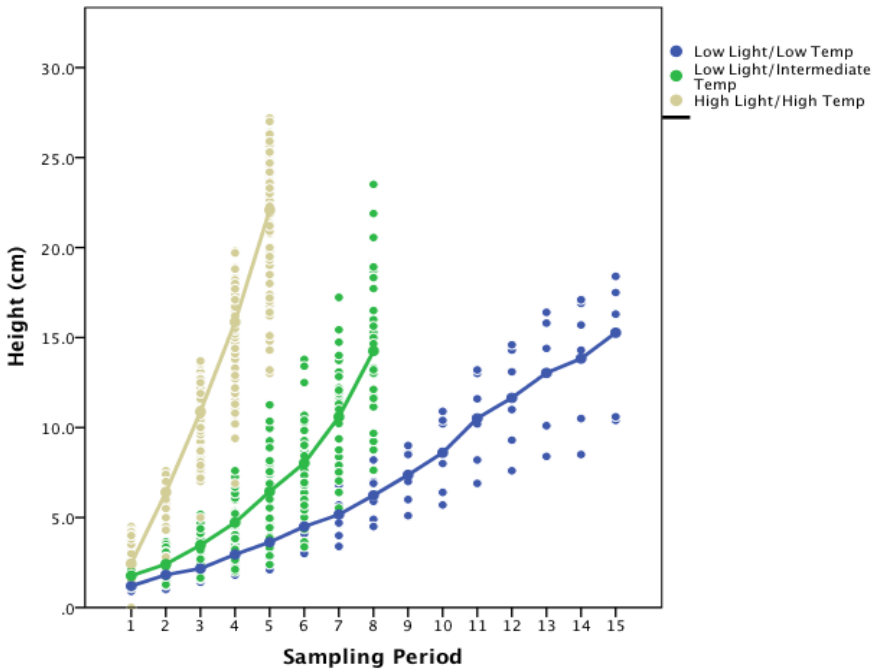


Figure 4. Temperature effects on growth rate

Differences in growth rate for *Setaria viridis* grown under 3 combinations of light intensity and temperature in the growth chamber and greenhouse. In each trial one sampling period corresponds to three calendar days. Growth trial 1=blue, Growth trial 2=green, Growth trial 3=tan (Treatment groups in trials 2 and 3 did not show significantly different means for height and have been combined in this graph.)

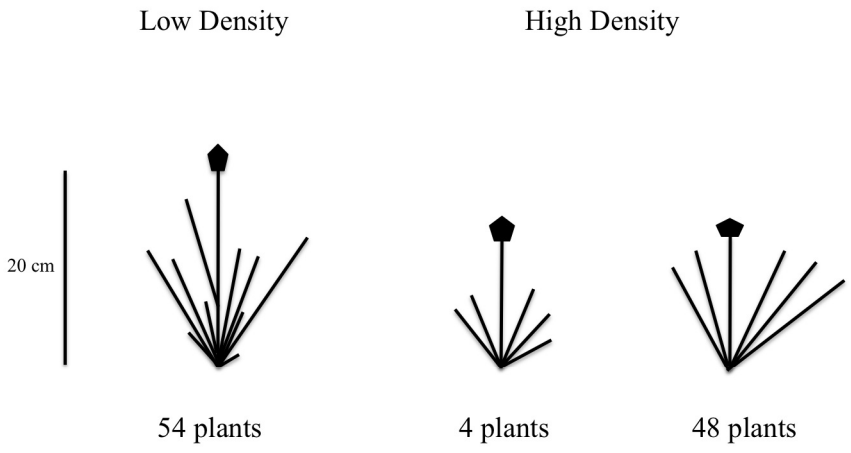


Figure 5. Architectural differences at 2 densities

Schematic representation of plants in growth trial 3 showing length of tillers relative to the main culm.

	Planting Density		Branch Number	Total Tiller Length	Average Tiller Length
GM-1	ID (15.5cm)	Height	-0.539**		
GM-2	ID (15.5cm)	Height	-0.613**		
GM-2	HD (11cm)	Height	0.142		
GM-3	LD (31cm)	Height	0.800**	0.972**	0.984**
GM-3	HD (11cm)	Height	0.847**	0.950**	0.958**

Table 9. Correlations between height and branching characters in the GM growth trials

(** indicates $p<0.01$).

DISCUSSION

Differences between foxtail and green millet-FM-GM trials

Because domesticated grasses in general, and foxtail millet specifically, appear to have experienced strong selection for suppression of branching, we would expect little variation in branching. The typically unbranched phenotype of foxtail millet has a much-reduced range of branching than does green millet, but, when corrected for differences between the means, the coefficient of variation is greater. This apparent anomaly can be explained by understanding that differences in a single tiller can increase tillering in foxtail millet by 50-100%, whereas addition of a single tiller in green millet may add much less.

Foxtail millet did produce tillers in each trial, however aerial branches were never observed. I have previously dissected specimens of the B100 and Yugu1 genotypes of foxtail millet and axillary meristems were not found on the distal portions of the culm but were present at the nodes at the base of the culm. This finding agrees with prior evidence that tiller production and aerial branching are under partially separate genetic control (Doust et al. 2004).

Phenotypic Plasticity in Green Millet trials (GM)

The density treatments in the three trials all affected morphological characteristics of green millet. The effect was stronger in the LD versus HD treatments in GM-3 than in the less extreme ID versus HD treatments in GM2, with higher densities impacting both height and branching.

The negative correlations between culm height and branch number in GM-1 and the intermediate density in GM-2 (Table 9) may indicate a tradeoff in resource allocation

between these characters. This contrasts with GM-3 in which there were strong, highly significant positive correlations between height and branch number for both the LD and HD treatments. In all three trials the watering and fertilizer regimes were consistent, while temperature and light intensity differed. The difference in light intensity was most marked between the GM-3 (1400μmol $cm^{-2}s^{-1}$) and GM-1 and GM-2 (220μmol $cm^{-2}s^{-1}$), suggesting that the low light intensity in growth trials 1 and 2 may have been a limiting resource to their growth. This is consistent with the idea that the negative correlation between height and branch number in these trials was due to a tradeoff in resource allocation in response to the light limited environment. Perhaps more surprising is the implication that the positive correlation between height and branch number in GM-3 is because of non-limiting resources, even though these plants were also grown in the same-sized pots as in GM-1 and GM-2.

The HD treatment in GM-2 did not have a significant correlation between culm height and branching, which is puzzling to explain. Height varied more in the HD as compared to the ID treatment, which may have led to reduced power to detect a statistically significant correlation.

The strong positive correlation between height and branch number in both LD and HD treatments in GM-3 indicates the high light intensity was not a factor limiting growth in this treatment. Plants in both LD and HD treatments had the same number of primary tillers but plants in the LD treatment had greater numbers of secondary tillers and aerial branches. This suggests that a shade avoidance response in the HD treatment only became apparent at later growth stages when the addition of the primary tillers markedly increased the density in the treatment. The total absence of secondary branches on the

primary tillers in the HD treatment may be the result of shade avoidance in each tiller rather than just the culm.

Although the plant is controlling the allocation and utilization of resources to each branch, it is important to remember that mature tillers are semi-autonomous and capable of surviving after being severed from the culm. It has been demonstrated in barley (*Hordeum vulgare*) (Gu and Marshall 1988) and spring wheat (*Triticum aestivum*) (Hucl and Baker 1990) that tillers within a single plant compete for nutrients and light resources. This does not necessarily negate resource allocation, however, intraplant competition may be a driving factor in the direction of resource allocation. Shade avoidance at the tiller level is consistent with the relative uniformity of tiller length and the approximately equal length of each tiller relative to the culm in the HD treatment (Fig. 5). My results suggest that there is a developmental race to form a canopy and fill space before being overtopped by neighbors. This pattern has also been observed in *Lolium multiflorum*, where plants grown at high density failed to produce new tillers once the canopy was dense enough to limit the proportion of light intercepted by each tiller, even though additional axillary buds were present (Casal, Deregibus et al. 1985).

These findings suggest that there is a consistent pattern of increasing size in green millet over time that is overlain by variation in developmental trajectories, mediated by differing environmental conditions. Green millet exhibits considerable diversity in vegetative architecture, yet branching patterns were consistent within treatments and the general pattern of development varied only by rate between temperature treatments. This suggests that the regulation of vegetative architecture is complex but not unpredictable. The complexity of architectural development in green millet together with dramatic

reduction in branching in foxtail millet, make this wild/domesticate pair a good model for phenotypic modification under domestication. Additionally, green millet presents a unique opportunity to study morphological development in a system where genetic resources are already available. Further elucidation of the architectural developmental process in this system will be valuable in investigating the developmental origins of architectural diversity across Poaceae.

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APPENDICES

Genus	***Species***	**Sub-family**
Aegilops	*cylindrica*	Pooideae
Agropyron	*hyemalis*	Pooideae
Agropyron	*pauciflorum*	Pooideae
Agropyron	*perennans*	Pooideae
Agropyron	*repens*	Pooideae
Agropyron	*scabra*	Pooideae
Agropyron	*semivesticellata*	Pooideae
Agropyron	*smithii*	Pooideae
Ampelodesmos	*mauritanica*	Pooideae
Amphipogon	*strictus*	Arundinoideae
Andropogon	*ciliaris*	Panicoideae
Andropogon	*clandestinum*	Panicoideae
Andropogon	*elliotiana*	Panicoideae
Andropogon	*fusca*	Panicoideae
Andropogon	*gerardii*	Panicoideae
Andropogon	*platyphylla*	Panicoideae
Andropogon	*saccharoides*	Panicoideae
Anomochloa	*marantoidea*	Anomochlooideae
Anthaenantia	*rufa*	Panicoideae
Anthaenantia	*villosa*	Panicoideae
Anthoxanthum	*alpinum*	Pooideae
Anthoxanthum	*aristatum*	Pooideae
Aristida	*barbinodis*	Aristidoideae
Aristida	*ciliare*	Aristidoideae

Aristida	*fendleri*	Aristidoideae
Aristida	*oligantha*	Aristidoideae
Aristida	*patens*	Aristidoideae
Arundo	*donax*	Arundinoideae
Avena	*fatua*	Pooideae
Avena	*innom - Z*	Pooideae
Avena	*sativa*	Pooideae
Axonopus	*affinis*	Panicoideae
Axonopus	*centralis*	Panicoideae
Axonopus	*compressus*	Panicoideae
Axonopus	*furcatus*	Panicoideae
Bothriochloa	*barbinodis*	Panicoideae
Brachiaria	*californica*	Panicoideae
Brachiaria	*ciliatissima*	Panicoideae
Brachiaria	*depauperatum*	Panicoideae
Brachiaria	*extensa*	Panicoideae
Brachiaria	*plantaginea*	Panicoideae
Brachiaria	*walterii*	Panicoideae
Brachyelytrum	*dolichophylla*	Pooideae
Brachyelytrum	*innom - Z*	Pooideae
Brachyelytrum	*parvigluma*	Pooideae
Brachyelytrum	*purpurea*	Pooideae
Brachypodium	*distachyon*	Pooideae
Brachypodium	*mexicanum*	Pooideae
Brachypodium	*pinnatum*	Pooideae
Brachypodium	*silvaticum*	Pooideae
Briza	*maxima*	Pooideae

Briza	*media*	Pooideae
Briza	*minor*	Pooideae
Briza	*rotundata*	Pooideae
Bromus	*anomalus*	Pooideae
Bromus	*commutatus*	Pooideae
Bromus	*diandrus*	Pooideae
Bromus	*inermis*	Pooideae
Bromus	*japonicus*	Pooideae
Bromus	*mollis*	Pooideae
Bromus	*pubescens*	Pooideae
Bromus	*secalinus*	Pooideae
Bromus	*tectorum*	Pooideae
Bromus	*unioloides*	Pooideae
Bromus	*tectorum*	Pooideae
Cenchrus	*dichotomum*	Panicoideae
Cenchrus	*gracilis*	Panicoideae
Cenchrus	*incertus*	Panicoideae
Cenchrus	*malacophyllum*	Panicoideae
Cenchrus	*viridis*	Panicoideae
Chasmanthium	*asper*	Panicoideae
Chasmanthium	*capensis*	Panicoideae
Chasmanthium	*glauca*	Panicoideae
Chasmanthium	*hexandra*	Panicoideae
Chasmanthium	*latifolium*	Panicoideae
Chasmanthium	*muricata*	Panicoideae
Chasmanthium	*parviflorus ssp. elongatus*	Panicoideae

Chasmanthium	*patens*	Panicoideae
Chasmanthium	*turgidum*	Panicoideae
Chusquea	*simpliciflora*	Bambusoideae
Dactylis	*glomerata*	Pooideae
Danthonia	*californica*	Danthonioideae
Danthonia	*innom - Z*	Danthonioideae
Danthonia	*sericea*	Danthonioideae
Diarrhena	*innom - Z*	Pooideae
Dichanthelium	*accuminatum*	Panicoideae
Dichanthelium	*macrostachya*	Panicoideae
Dichanthelium	*oligosanthes*	Panicoideae
Dichanthelium	*platyphylla*	Panicoideae
Dichanthelium	*pumila*	Panicoideae
Dichanthelium	*purpurescens*	Panicoideae
Dichanthelium	*scoparium*	Panicoideae
Digitaria	*californica*	Panicoideae
Distichlis	*spicata*	Chloridoideae
Ecdeiocolea	*monostachya*	Ecdeiocoleaceae
Echinochloa	*bicolor*	Panicoideae
Echinochloa	*crus-galli*	Panicoideae
Echinochloa	*filiformis*	Panicoideae
Echinochloa	*nitidum*	Panicoideae
Echinochloa	*oryzoides*	Panicoideae
Ehrharta	*cognata*	Ehrhartoideae
Ehrharta	*erecta*	Ehrhartoideae
Ehrharta	*longespica*	Ehrhartoideae
Eleusine	*colonum*	Chloridoideae

Eleusine	*indica*	Chloridoideae
Eleusine	*italica*	Chloridoideae
Eleusine	*longispinus*	Chloridoideae
Eleusine	*texana*	Chloridoideae
Eragrostis	*miliaceum*	Chloridoideae
Eragrostis	*oxylepis*	Chloridoideae
Eragrostis	*secundiflora*	Chloridoideae
Eragrostis	*spectabilis*	Chloridoideae
Eragrostis	*trichodes*	Chloridoideae
Festuca	*arundinacea*	Pooideae
Glyceria	*innom - Z*	Pooideae
Guaduella	*marantifolia*	Puelioideae
Joinvillea	*ascendens*	Joinvilleaceae
Joinvillea	*plicata*	Joinvilleaceae
Karroochloa	*curva*	Danthonioideae
Karroochloa	*purpurea*	Danthonioideae
Karroochloa	*schismoides*	Danthonioideae
Karroochloa	*tenella*	Danthonioideae
Leersia	*cryptandrus*	Ehrhartoideae
Leersia	*guineensis*	Ehrhartoideae
Leersia	*lenticularis*	Ehrhartoideae
Leersia	*lenticularis*	Ehrhartoideae
Leersia	*oblonga*	Ehrhartoideae
Leersia	*oryzoides*	Ehrhartoideae
Leersia	*perenne*	Ehrhartoideae
Lithachne	*pauciflora*	Bambusoideae
Lolium	*annulatus*	Pooideae

Lolium	*echinatus*	Pooideae
Lolium	*ischaemum*	Pooideae
Lolium	*latifolia*	Pooideae
Lolium	*multiflorum*	Pooideae
Lolium	*spicata*	Pooideae
Lolium	*multiflorum*	Pooideae
Melica	*alba*	Pooideae
Melica	*aristata*	Pooideae
Melica	*bulbosa*	Pooideae
Melica	*californica*	Pooideae
Melica	*ciliata*	Pooideae
Melica	*frutescens*	Pooideae
Melica	*geyeri*	Pooideae
Melica	*harfordii*	Pooideae
Melica	*imperfecta*	Pooideae
Melica	*mutica*	Pooideae
Melica	*mutica*	Pooideae
Melica	*nitens*	Pooideae
Melica	*nutans*	Pooideae
Melica	*paviflora*	Pooideae
Melica	*porteri*	Pooideae
Melica	*purpurescens*	Pooideae
Melica	*smithii*	Pooideae
Melica	*spectabilis*	Pooideae
Melica	*stricta*	Pooideae
Melica	*subulata*	Pooideae
Melica	*torreyana*	Pooideae

Melica	*uniflora*	Pooideae
Micraira	*subulifolia*	Incertae sedis
Miscanthus	*sinensis*	Panicoideae
Molinia	*caerulea*	Arundinoideae
Nardus	*stricta*	Pooideae
Nassella	*innom - Z*	Pooideae
Olyra	*adoratum*	Bambusoideae
Olyra	*glabberima*	Bambusoideae
Olyra	*latifolia*	Bambusoideae
Olyra	*longistaminata*	Bambusoideae
Oryza	*sativa*	Ehrhartoideae
Panicum	*virgatum*	Panicoideae
Paspalum	*dilatatum*	Panicoideae
Paspalum	*floridanum*	Panicoideae
Paspalum	*gigantum*	Panicoideae
Paspalum	*glaucum*	Panicoideae
Paspalum	*laeve*	Panicoideae
Pennisetum	*chilense*	Panicoideae
Pennisetum	*ciliare*	Panicoideae
Pennisetum	*gigantum*	Panicoideae
Pennisetum	*glaucum*	Panicoideae
Pennisetum	*grisebachii*	Panicoideae
Pennisetum	*leucopila*	Panicoideae
Pennisetum	*lutescens*	Panicoideae
Pennisetum	*parviflora*	Panicoideae
Pennisetum	*polystachyon*	Panicoideae
Pennisetum	*purpureum*	Panicoideae

Pennisetum	*ramosum*	Panicoideae
Pennisetum	*ramosum*	Panicoideae
Pennisetum	*reverchonti*	Panicoideae
Pennisetum	*schimperi*	Panicoideae
Pennisetum	*setosum*	Panicoideae
Pennisetum	*setosum*	Panicoideae
Pennisetum	*trachyphylluii*	Panicoideae
Pennisetum	*villosum*	Panicoideae
Pharus	*lappulaceus*	Pharoideae
Pharus	*latifolius*	Pharoideae
Pharus	*lappulaceus*	Pharoideae
Phragmites	*australis*	Arundinoideae
Phragmites	*floccifolia*	Arundinoideae
Phragmites	*oxylepis*	Arundinoideae
Phragmites	*trichodes*	Arundinoideae
Phragmites	*australis*	Arundinoideae
Piptatherum	*innom - Z*	Pooideae
Puelia	*ciliata*	Puelioideae
Puelia	*grandiglumis*	Puelioideae
Puelia	*olyriformis*	Puelioideae
Schizachyrium	*breviligulata*	Panicoideae
Schizachyrium	*scoparium*	Panicoideae
Setaria	*glauca*	Panicoideae
Setaria	*gracilis*	Panicoideae
Setaria	*grisebachii*	Panicoideae
Setaria	*italica*	Panicoideae

Setaria	*leucopila*	Panicoideae
Setaria	*lutescens*	Panicoideae
Setaria	*macrostachya*	Panicoideae
Setaria	*parviflora*	Panicoideae
Setaria	*pumila*	Panicoideae
Setaria	*reverchonti*	Panicoideae
Setaria	*viridis*	Panicoideae
Sorghastrum	*barbinodis*	Panicoideae
Sorghastrum	*calycina*	Panicoideae
Sorghastrum	*laxiflorum*	Panicoideae
Sorghastrum	*nutans*	Panicoideae
Sorghum	*halepense*	Panicoideae
Sorghum	*sphaerocarpon*	Panicoideae
Sorghum	*halepense*	Panicoideae
Spartina	*alterniflora*	Chloridoideae
Spartina	*patens*	Chloridoideae
Spartina	*pectinata*	Chloridoideae
Sporobolus	*airoides*	Chloridoideae
Sporobolus	*asper*	Chloridoideae
Sporobolus	*clandestinus*	Chloridoideae
Sporobolus	*pittieri*	Chloridoideae
Sporobolus	*smilacinifolia*	Chloridoideae
Sporobolus	*vaginiflorus*	Chloridoideae
Stipa	*comata*	Pooideae
Stipa	*leucotricha*	Pooideae
Stipa	*leucotricha*	Pooideae
Stipa	*neomexicana*	Pooideae

Stipa	*neomexicana*	Pooideae
Streptochaeta	*sodiroana*	Bambusoideae
Triticum	*aestivum*	Pooideae
Triticum	*durum*	Pooideae
Triticum	*turgidum*	Pooideae
Uniola	*innom - Z*	Chloridoideae
Urochloa	*ciliatissima*	Panicoideae
Zea	*mays*	Panicoideae
Zeugites	*americana*	Panicoideae
Zizania	*aquatica*	Ehrhartoideae

APPENDIX 1. SPECIES EXAMINED FOR MORPHOLOGICAL ANALYSIS

Marker	Species	Subfamily	Genbank Accession
ITS	*Ampelodesmos mauritanica*	Pooideae	AF019799
	Amphipogon strictus	Pooideae	AF019848
	Andropogon gerardii	Panicoideae	AY116299
	Aristida adscensionis	Aristidoideae	DQ171974
	Arundo donax	Arundinoideae	AF019809
	Avena fatua	Pooideae	EU833742
	Axonopus polystachyus	Panicoideae	AY771920
	Bothriochloa ischaemum	Panicoideae	DQ141239
	Brachiaria deflexa	Panicoideae	AY346342
	Brachyelytrum erectum	Pooideae	EU489105
	Brachypodium mexicanum	Pooideae	AF019805
	Briza macrostachya	Pooideae	EU528599
	Bromus catharticus	Pooideae	AF521898
	Cenchrus ciliaris	Panicoideae	GQ470544
	Pennisetum purpureum	Panicoideae	FJ626357
	Chasmanthium latifolium	Centothecoideae	DQ172079
	Chloris virgata	Chloridoideae	DQ655798
	Chusquea latifolia	Bambusoideae	AF019788
	Danthonia compressa	Danthonioideae	GU359345
	Diarrhena americana	Pooideae	AF019798
	Digitaria insularis	Panicoideae	GQ478090
	Distichlis spicata	Panicoideae	GU359335
	Echinochloa crus-galli	Panicoideae	AB353365

Eleusine indica	Chloridoideae	EF153042
Eragrostis cilianensis	Chloridoideae	GU359296
Festuca arundinacea	Pooideae	HM453186
Glyceria maxima	Pooideae	FJ013226
Hordeum vulgare	Pooideae	Z11759
Joinvillea plicata	Joinvilleaceae (outgroup)	AF019784
Karroochloa purpurea	Danthonioideae	AF019874
Leersia hexandra	Ehrhartoideae	AF019793
Lithachne humilis	Bambusoideae	AF019787
Lolium perenne	Pooideae	AJ240138
Melica scabrosa	Pooideae	JF708189
Micraira subulifolia	Micrairoideae	AF019859
Miscanthus sinensis	Panicoideae	HQ822021
Molinia caerulea	Arundinoideae	AF019857
Nardus stricta	Pooideae	EU489143
Nassella hyalina	Pooideae	FN434549
Oryza sativa	Ehrhartoideae	DQ143117
Panicum bisulcatum	Panicoideae	AY129697
Paspalum notatum	Panicoideae	GQ870170
Pharus latifolius	Pharoideae	AF019786
Phragmites australis	Arundinoideae	F019810
Schizachyrium scoparium	Panicoideae	DQ005072
Setaria viridis	Panicoideae	FJ766179
Sorghastrum incompletum	Panicoideae	DQ005076
Sorghum bicolor	Panicoideae	SBU04789

	Spartina densiflora	Chloridoideae	GU359206
	Sporobolus elongatus	Chloridoideae	FJ766185
	Stipa borysthenica	Pooideae	FN434512
	Triticum aestivum	Pooideae	FJ229967
	Uniola condensata	Chloridoideae	GU359191
	Urochloa brizantha	Panicoideae	AY346349
	Zea mays	Panicoideae	DQ683016
	Zeugites americanus	Centothecoideae	AM404334
ndhF	*Ampelodesmos mauritanicus*	Pooideae	GU222746
	Amphipogon strictus	Pooideae	GU222717
	Andropogon gerardii	Panicoideae	AF117391
	Anomochloa marantoidea	Anomochlooideae	GU222697
	Anthaenantia lanata	Panicoideae	AY029640
	Aristida purpurea var. longiseta	Aristidoideae	U21966
	Arundo donax	Arundinoideae	U21998
	Avena sativa	Pooideae	DQ786814
	Axonopus anceps	Panicoideae	AY029623
	Bothriochloa ischaemum	Panicoideae	AM849131
	Brachiaria deflexa	Panicoideae	AM849200
	Brachyelytrum erectum	Pooideae	U22005
	Briza minor	Pooideae	DQ786820
	Bromus korotkiji	Pooideae	GU222751
	Pennisetum glaucum	Panicoideae	FR821361
	Cenchrus compressus	Panicoideae	AF251467

Chasmanthium latifolium	Centothecoideae	EF422909
Chloris truncata	Chloridoideae	JN681723
Chusquea circinata	Bambusoideae	U21991
Danthonia californica	Danthonioideae	GU222712
Diarrhena obovata	Pooideae	U21999
Dichanthelium clandestinum	Panicoideae	AY188461
Digitaria didactyla	Panicoideae	AM849203
Distichlis spicata	Chloridoideae	GU222709
Echinochloa crus-galli	Panicoideae	AM849149
Ehrharta calycina	Ehrhartoideae	U21996
Eleusine indica	Chloridoideae	AM849151
Eragrostis curvula	Chloridoideae	U21989
Festuca arundinacea	Pooideae	DQ786868
Glyceria grandis	Pooideae	AY622314
Hordeum vulgare	Pooideae	U22003
Joinvillea ascendens	Joinvilleaceae (outgroup)	U21973
Karroochloa purpurea	Danthonioideae	AF251458
Leersia virginica	Ehrhartoideae	U21974
Lithachne pauciflora	Bambusoideae	GU222729
Lolium perenne	Pooideae	DQ786853
Melica cupanii	Pooideae	AY622315
Micraira subulifolia	Micrairoideae	AY622316
Miscanthus japonicus	Panicoideae	AF117417
Molinia caerulea	Arundinoideae	GU222716
Nardus stricta	Pooideae	GU222733

	Nassella viridula	Pooideae	GU222742
	Olyra latifolia	Bambusoideae	GU222730
	Oryza rufipogon	Ehrhartoideae	FJ908335
	Panicum virgatum	Panicoideae	U21986
	Paspalum dilatatum	Panicoideae	AM849178
	Pharus latifolius	Pharoideae	U21993
	Phragmites australis	Arundinoideae	U21997
	Piptatherum miliaceum	Pooideae	AY622317
	Puelia olyriformis	Puelioideae	HQ604006
	Schizachyrium scoparium	Panicoideae	AF117420
	Setaria viridis	Panicoideae	U21976
	Sorghastrum nutans	Panicoideae	AF117421
	Sorghum bicolor	Panicoideae	U21981
	Spartina pectinata	Chloridoideae	GU222706
	Sporobolus indicus	Chloridoideae	U21983
	Stipa barbata	Pooideae	GU222745
	Streptochaeta angustifolia	Anomochlooideae	U21982
	Triticum aestivum	Pooideae	DQ247921
	Uniola paniculata	Chloridoideae	GU222707
	Urochloa arrecta	Panicoideae	FJ486517
	Zea mays	Panicoideae	U21985
	Zeugites pittieri	Centothecoideae	U21987
	Zizania latifolia	Ehrhartoideae	AM887888
phyB	*Andropogon gayanus*	Panicoideae	JN560778

Anomochloa marantoidea	Anomochlooideae	AF137291
Anthaenantia lanata	Panicoideae	EU272415
Aristida purpurea var. longiseta	Aristidoideae	AF137292
Axonopus fissifolius	Panicoideae	EU272418
Bothriochloa odorata	Panicoideae	AF443800
Brachypodium pinnatum	Pooideae	AF137294
Bromus inermis	Pooideae	U61193
Cenchrus americanus	Panicoideae	EU272452
Pennisetum glaucum	Panicoideae	EU272420
Chasmanthium latifolium	Centothecoideae	AF137297
Chusquea oxylepis	Bambusoideae	AF137298
Danthonia spicata	Danthonioideae	AF137299
Diarrhena obovata	Pooideae	AF137301
Dichanthelium sabulorum	Panicoideae	EU272425
Digitaria ciliaris	Panicoideae	EU272426
Echinochloa colona	Panicoideae	EU272429
Eragrostis cilianensis	Chloridoideae	U61200
Festuca pratensis	Pooideae	EU215518
Glyceria grandis	Pooideae	AF137305
Joinvillea ascendens	Joinvilleaceae (outgroup)	U61205
Lithachne pauciflora	Bambusoideae	AF137307
Lolium perenne	Pooideae	AF137308
Melica cupanii	Pooideae	AF137310
Miscanthus sinensis	Panicoideae	AF137311

	Molinia caerulea	Arundinoideae	AF137312
	Nardus stricta	Pooideae	AF137313
	Nassella viridula	Pooideae	U61217
	Olyra latifolia	Bambusoideae	AF137315
	Oryza rufipogon	Ehrhartoideae	JN594208
	Panicum capillare	Panicoideae	AF137316
	Paspalum simplex	Panicoideae	AF443814
	Pharus lappulaceus	Pharoideae	AF137321
	Phragmites australis	Arundinoideae	AF137322
	Puelia ciliata	Puelioideae	AF137324
	Schizachyrium scoparium	Panicoideae	AF443817
	Setaria viridis	Panicoideae	EU272457
	Sorghum bicolor	Panicoideae	AF182394
	Sporobolus giganteus	Chloridoideae	AF137327
	Triticum aestivum	Pooideae	AF137331
	Urochloa mutica	Panicoideae	AF443820
	Zea mays	Panicoideae	AF137332
	Zeugites pittieri	Chloridoideae	EU272465
	Zizania aquatica	Ehrhartoideae	AF137333
rbcL	*Amphipogon strictus*	Pooideae	U88403
	Andropogon gerardii	Panicoideae	AJ784818
	Anomochloa marantoidea	Anomochlooideae	EF423008
	Aristida adscensionis	Aristidoideae	EF423002
	Arundo donax		U13226

Avena fatua	Pooideae	HM849804
Axonopus compressus	Panicoideae	EF125127
Bothriochloa saccharoides	Panicoideae	AM849353
Brachiaria deflexa	Panicoideae	AM849408
Brachyelytrum aristosum	Pooideae	EF423006
Briza maxima	Pooideae	FN870384
Bromus erectus	Pooideae	AJ746286
Cenchrus americanus	Panicoideae	L14623
Chasmanthium latifolium	Centothecoideae	U31101
Chloris virgata	Chloridoideae	EF125096
Danthonia spicata	Danthonioideae	FN870387
Dichanthelium dichotomum	Panicoideae	FN870398
Digitaria ciliaris	Panicoideae	AM849336
Distichlis spicata	Chloridoideae	AY632363
Echinochloa crus-galli	Panicoideae	AM887871
Ehrharta erecta	Ehrhartoideae	AM887883
Eleusine indica	Chloridoideae	EF125108
Eragrostis obtusiflora	Chloridoideae	JN681666
Festuca rubra	Pooideae	AJ746261
Glyceria fluitans	Pooideae	HM850033
Joinvillea plicata	Joinvilleaceae (outgroup)	L01471
Karroochloa purpurea	Danthonioideae	U31437
Leersia oryzoides	Ehrhartoideae	U13228
Lithachne humilis	Bambusoideae	U13231
Lolium perenne	Pooideae	AY395547

Melica uniflora	Pooideae	AJ746263
Micraira subulifolia	Micrairoideae	AY632366
Miscanthus sinensis	Panicoideae	EF125118
Molinia caerulea	Arundinoideae	AJ746295
Nassella trichotoma	Pooideae	EF125159
Olyra latifolia	Bambusoideae	EF125090
Oryza sativa	Ehrhartoideae	D00207
Panicum virgatum	Panicoideae	EF125135
Paspalum dilatatum	Panicoideae	HM850238
Phragmites australis	Arundinoideae	EF423005
Piptatherum miliaceum	Pooideae	FN870403
Puelia olyriformis	Puelioideae	HQ604036
Setaria viridis	Panicoideae	HQ590270
Sorghastrum nutans	Panicoideae	EF125121
Sorghum bicolor	Panicoideae	AM849341
Spartina anglica	Chloridoideae	AM849382
Sporobolus festivus	Chloridoideae	AM849383
Stipa dregeana var. dregeana	Pooideae	U31442
Uniola paniculata	Chloridoideae	AY632373
Zeugites capillaris	Centhothecoideae	HM167476
Zizania texana	Ehrhartoideae	L05043

APPENDIX 2. GENBANK ACCESSIONS USED FOR BAYESIAN ANALYSIS

VITA

Michael Patrick Malahy

Candidate for the Degree of

Master of Science

Thesis: EVOLUTION AND DEVELOPMENT OF VEGETATIVE ARCHITECTURE: BROAD SCALE PATTERNS OF BRANCHING ACROSS THE GRASS FAMILY (POACEAE) AND CHARACTERIZATION OF ARCHITECTURAL DEVELOPMENT IN WEEDY GREEN MILLET (*SETARIA VIRIDIS* L. P. BEAUV.)

Major Field: Botany

Biographical:

Education:

Completed the requirements for the Master of Science in Botany at Oklahoma State University, Stillwater, Oklahoma in July, 2012.

Completed the requirements for the Bachelor of Science in Biology at University of Central Oklahoma, Edmond, Oklahoma in 2006.

Professional Memberships:
Botanical Society of America
Sigma Xi

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Name: Michael Patrick Malahy Date of Degree: July, 2012

Institution: Oklahoma State University Location: Stillwater, Oklahoma

Title of Study: EVOLUTION AND DEVELOPMENT OF VEGETATIVE ARCHITECTURE: BROAD SCALE PATTERNS OF BRANCHING ACROSS THE GRASS FAMILY (POACEAE) AND CHARACTERIZATION OF ARCHITECTURAL DEVELOPMENT IN WEEDY GREEN MILLET (*SETARIA VIRIDIS* (L.) P. BEAUV.)
Candidate for the Degree of Master of Science
Major Field: Botany

Scope and Method of Study:
The objectives of this research were to identify patterns of vegetative architecture across the grass family (Poaceae) and investigate the pattern of architectural development in foxtail (*Setaria italica* (L.) P. Beauv.) and green millet (*S. viridis* (L.) P. Beauv.). A dataset for four markers (*ndhF, phyB,* ITS and *rbcL*) was retrieved from NCBI, representing 132 species of the 65 grass genera that were examined for branching characteristics, as well as one outgroup genus. A partitioned Bayesian analysis was conducted with a GTR model with six nucleotide substitution types and a gamma distribution with 4 rate categories. Morphological character data observed in live and herbarium specimens at the Oklahoma State University and Missouri Botanical Garden herbaria were then optimized onto the phylogeny to examine the evolution aerial branching.

I examined differences in branching between green millet and its domesticated relative foxtail millet in three field and greenhouse trials to investigate the changes in branching brought about by domestication. I also conducted three controlled experiments to determine the pattern of architectural development and phenotypic plasticity for green millet in response to changes in temperature, light intensity and planting density.

Findings and Conclusions:
My work suggests that the ancestral state of branching in the Poaceae is for plants to produce tillers but not aerial branches. In addition, patterns of aerial branching are present across the grasses that are consistent with phylogenetic relationships in the family, with panicoid grasses exhibiting aerial branches and pooid grasses lacking them. Examination of live specimens suggests variation not only in the elongation of axillary buds, but also in the initiation of meristems which may be attributed to genetic or developmental variation and possibly sensitivity to environmental factors.

Foxtail millet differs from green millet in the number of tillers produced (very few to none), and a complete lack of aerial branching in foxtail millet. Green millet exhibits a stable, orderly pattern of development. Changes in temperature influence the rate of development but not the order of branch production whereas changes in light intensity influence the rate of development and the size of the plant. Under increased planting density, plants are on average shorter and produce fewer branches.

ADVISER'S APPROVAL: Dr. Andrew Doust