The Families and Genera of Vascular Plants

Edited by K. Kubitzki

Volume XIII

Flowering Plants
Monocots

Poaceae

Elizabeth A. Kellogg

Springer

THE FAMILIES AND GENERA OF VASCULAR PLANTS

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XIII *Flowering Plants · Monocots*
Poaceae

Elizabeth A. Kellogg

With 96 Figures

Springer

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ISBN 978-3-319-15331-5 ISBN 978-3-319-15332-2 (eBook)
DOI 10.1007/978-3-319-15332-2
Springer Cham Heidelberg New York Dordrecht London

Library of Congress Control Number: 2015935331

Printed on acid-free paper

Springer is part of Springer Science+Business Media (www.springer.com)

To P.F.S.

Preface

The goal of this book is to present a state-of-the-art view of grass taxonomy and to summarize our current understanding of morphological variation in the grasses. Today, both aspects of agrostology have been engulfed in a flood of molecular sequence data and an equally large influx of developmental genetic data. I have tried throughout to incorporate these companion data as they affect our interpretation of morphological characters and our understanding of phylogeny.

The first sections of the book describe grass morphology, character by character. I also refer to many studies of developmental genetics that illuminate the genetic basis of traditional taxonomic characters. Often the data are incomplete, focused on only a handful of major cereal crops. Where possible, I include information on critical genes underlying each set of characters. As is conventional in the literature, names of genes are written in lowercase italics and proteins in uppercase Roman letters. Today, it is much less common to undertake broad surveys of particular characters across a large group of organisms than it was in the early 20th century. I hope that, by highlighting gaps in our knowledge, such survey work can be encouraged in the future.

In terms of taxonomy, this book represents an effort to update the major treatments of grass genera provided by Clayton and Renvoize (Genera Graminum, 1986) and Watson and Dallwitz (Grass Genera of the World, 1992 onward). In the decades since those publications, the major clades of grasses have been identified by the Grass Phylogeny Working Group (2001), and expanded by Sánchez-Ken et al. (2007) and the Grass Phylogeny Working Group II (2012), leading to the recognition of 12 monophyletic subfamilies. Remarkably for such a large family, all but a handful of species (fewer than ten) are confidently placed in a subfamily. Within the subfamilies, the major monophyletic tribes have been recognized, and the limits of these are largely stable. Within the tribes, broad agreement on subtribal limits is emerging, although a number of genera remain unplaced at this level.

As outlined in more detail in the section Subdivision of the Family, the major innovation of this book is its phylogenetic approach. The work of Clayton and Renvoize (1986) and Clayton et al. (2006 onward) arises from the philosophy of the evolutionary or phenetic school of taxonomy, and Watson and Dallwitz (1992 onward) also use an explicitly phenetic approach. More recent checklists are based on current phylogenies (Simon 2007; Simon et al. 2011 onward; Soreng et al. 2012 and onward), updating the classification frequently as indicated by recent molecular studies. The classification used here is similar but not identical to those in the checklists, and includes the rationale for many of the taxonomic decisions.

Remaining phylogenetic and taxonomic problems of the family are at the generic level. Many genera recognized by Clayton and Renvoize (1986) and currently accepted by Clayton et al. (2006 onward) and Watson and Dallwitz (1992 onward) are para- or polyphyletic. This book, like the online checklists, updates those generic limits based on current molecular phylogenetic studies in an effort to recognize only monophyletic genera. Nonetheless, current phylogenies leave many loose ends and not all generic

problems can be resolved by current data. Problems outstanding are discussed throughout the text.

In the formal descriptions I have used several conventions to convey phylogenetic information. I have attempted to make descriptions more or less hierarchical, so that character states are only those that apply at a particular level. This means that the descriptions of the family, subfamilies, tribes and subtribes do not encompass all possible character states, but only include those that are likely to be synapomorphic for the clade and/or applicable to the early-diverging taxa. Thus, for example, Chloridoideae are described as having bisexual flowers because dioecy is derived later in the history of the clade. However, the hierarchical aspect of the descriptions breaks down frequently because the ancestral state of a clade is often uncertain; plant habit and ligule structure are two good examples. In these cases, several states are listed.

Taxa that are clearly not monophyletic are indicated in quotes—e.g., "*Chloris*", or "*Panicum*" s.l., the latter being distinct from *Panicum* s.s., which is monophyletic. Putative synapomorphies are indicated in italics. The strength of the evidence for these varies, so that they should be considered as hypotheses to be tested.

I had initially hoped to avoid many of the arcane grass-specific floral terms, in an effort to make the entire book more accessible to non-agrostologists. This effort was not particularly successful, although I had no trouble describing bamboo inflorescences without the terms iterauctant and semelauctant. As laid out in the section on Flower Structure, recent data on the grass floret suggest that it is simply a zygomorphic monocot flower, and not as peculiar as formerly believed. Accordingly, I have used the term "flower" instead of "floret". This is certain to irritate some people, but may make things clearer to others. As described in the section on Inflorescence Structure, the terms spike, raceme, and panicle are inaccurate and so are not used. Instead, inflorescences are described according to the number of orders of branching and whether axillary branches proliferate, as in some Andropogoneae.

As noted in the section Subdivision of the Family, phylogenies show that the gross morphology of grasses is subject to substantial convergence and is not a good guide to evolutionary history. While many of the well-supported monophyletic groups are marked by strong synapomorphies, these are often characters of micromorphology or even genome structure (e.g., chromosome number) and are thus not useful in the herbarium or in the field. Identification keys are therefore cumbersome and many taxa are keyed out more than once. The presentation here thus illustrates the tension between a fully phylogenetic classification and one that is developed for identification purposes.

In summary, I hope that this book provides food for thought, encouragement for debate, and an impetus for additional research.

St. Louis, MO Elizabeth A. Kellogg

Acknowledgements

This project has required input from many people and I am grateful to everyone who helped. The project depended completely on having full access to the incomparable resources of the Missouri Botanical Garden. For this I am grateful to Peter Raven for being instrumental in bringing me to St. Louis in the first place, and being a staunch supporter of me and this work. I also depended on the help of the superb staff, particularly Jim Solomon for access to the collections and Bob Magill for the Tropicos database. The entire project required a thoroughly well-curated grass collection, which is available at the Garden because of the long-time efforts of Gerrit Davidse; I thank Gerrit for his willingness to be interrupted with questions and for sharing his taxonomic expertise. Ihsan Al-Shehbaz let me share his office for several years and gave me a place to leave books and provided great friendship. Much of this book was written while I held the E. Desmond Lee and Family Professorship in Botanical Studies, at the University of Missouri-St. Louis. That position was designed explicitly to link the university with the Botanical Garden, and this book is one result of that formal collaboration.

Many colleagues contributed expertise in their particular taxonomic groups. Neil Snow and Paul Peterson kindly shared a pre-publication manuscript on *Leptochloa* and the Eleusininae, which relieved a number of chloridoid headaches, and Paul also shared a pre-publication manuscript on placement of a substantial number of chloridoid genera. Neil Snow provided valuable updated information on *Disakisperma* and *Leptochloa*. Rob Soreng shared unpublished data on several pooid taxa and kept me up to date on changes in the very useful online Catalog of World Grasses. Jordan Teisher provided valuable comments on Arundinoideae and Micraroideae, and was able to correct several errors that had propagated in the literature. I thank Francisco Vazquez and Mary Barkworth for sharing their considerable expertise in Stipeae. Jimmy Triplett contributed helpful comments on Arundinarieae, and shared unpublished data on *Pleioblastus*. Finally, my many friends and colleagues at the Instituto de Botánica Darwinion in Buenos Aires shared their incomparable knowledge of the Panicoideae; I wish to thank particularly my long-time collaborator and director of the Institute, Fernando Zuloaga, as well as the late Osvaldo Morrone, and Liliana Giussani.

Special thanks are due to Lynn Clark who spent a huge amount of time working through the treatment of the Bambusoideae, correcting many faux-pas and clarifying points of morphology. Her input is substantial enough for me to acknowledge her specifically at the beginning of that section. Furthermore, she went through the descriptions of grass morphology at the beginning of the book, and made sure that any statements applied also to the bamboos.

Bryan Simon checked all the taxonomic treatments in detail and carefully compared the numbers of species with those in Grassworld and Grassbase. I thank him in particular for his patience with my insistence on a phylogenetic classification. I also am grateful to Maria Vorontsova and David Simpson for their enthusiasm and encouragement for this project. There were many moments during the writing phase when I

wondered how many would read this treatment—the firm support of Bryan, Maria, and David kept me pushing ahead.

The classification and description of morphology presented here incorporates much new information generated by my laboratory with steady support from the National Science Foundation. Former and current graduate students who contributed data and expertise include Emilie Bess, Paulo Camara, Ken Hiser, John Hodge, Daniel Layton, Russell Spangler, Jill Preston, Sarah Youngstrom, Cassiano Welker, and Jinshun Zhong. In addition, this project has benefited from the careful work of postdoctoral fellows Sandra Aliscioni, Janet Barber, Hugo Cota, Andrew Doust, Matt Estep, Pu Huang, Elma Kay, Simon Malcomber, Michael McKain, Roberta Mason-Gamer, Sarah Mathews, Renata Reinheimer, Jimmy Triplett, Tony Verboom, and Michael Zanis.

I am indebted to Klaus Kubitzki, the editor of this Springer book series, for the invitation to undertake this project and I appreciate his patience. Its completion took far longer than he had hoped or expected—he will surely be pleasantly surprised that the end of the tunnel has been reached. I also thank Monique T. Delafontaine for her meticulous copy editing, and Andrea Schlitzberger at Springer (Heidelberg) for guiding this through to publication.

Finally, I heartily thank Peter Stevens who has encouraged this project from the start, reading the entire manuscript at least once and some parts several times, commenting on the contents, and providing a steady stream of relevant references. In addition, he has patiently cooked a very large number of nourishing meals for the two of us.

I should conclude by saying that, notwithstanding this wonderful input from colleagues, all taxonomic decisions and any remaining errors in the book are my own.

Elizabeth A. Kellogg

Contents

Poaceae – General Information

Description of the Family, Vegetative Morphology and Anatomy 3
Vegetative Morphology and Anatomy 3
Roots 3
Stems 5
Leaves 8
References 18

Inflorescence Structure 25
Transition to flowering 26
Bract formation and inflorescence phyllotaxis 28
Numbers of orders of branching 29
Numbers of branches or spikelets at each order of branching 30
Branch angle 31
Axis elongation 31
Spikelets 32
Disarticulation 34
References 35

Flower Structure 39
References 42

Embryology 45
Ovule 45
Megagametophyte 46
Embryo 46
Endosperm 48
References 51

Karyology and Genome Structure 55
Chromosome number 55
Polyploidy 56
Genome size 57
Genome mapping, sequencing and conservation of gene order 58
GC content 58
References 59

Pollen 63
Morphology and anther structure 63
Pollination 64
Pollen transfer 64
Pollen germination and pollen tube growth 65
References 66

Fruit and Seed . . . 69
Dispersal . . . 70
Unassisted or wind dispersal . . . 71
Dispersal in time: seed dormancy . . . 71
Dispersal by animals . . . 72
Role of the awn . . . 73
Forcible dispersal . . . 74
References . . . 75

Phytochemistry . . . 77
Phenolics . . . 78
Derivatives of amino acids (tryptophan, phenylalanine, tyrosine) . . . 79
Terpenoids . . . 80
Chemicals produced by symbionts or pathogens . . . 82
Phytosiderophores . . . 82
References . . . 83

Distribution, Habitats and Conservation . . . 87
Distribution and maintenance of grasslands . . . 87
Distribution of major clades . . . 88
Species diversity . . . 88
Conservation and response to climate change . . . 89
References . . . 90

Reproductive Systems . . . 93
Unisexual vs. bisexual flowers . . . 93
Self-pollination and self-incompatibility systems . . . 94
Cleistogamy . . . 95
Barriers to interspecific crossing . . . 96
Asexual reproduction . . . 97
References . . . 99

Fossil Record and Dates of Diversification . . . 103
References . . . 106

Domestication . . . 109
Origins of major cereal crops . . . 109
Morphological changes in domestication . . . 112
Genetic and genomic changes in domestication . . . 114
References . . . 116

Affinities . . . 121
References . . . 122

Classification of the Poaceae

Subdivision of the Family . . . 127
Key to the Subfamilies . . . 129
References . . . 130

I. Subfamily Anomochlooideae Pilg. ex Potztal (1957) . . . 131
Key to the Genera of Anomochlooideae . . . 131
Spikelet Clade . . . 132
References . . . 133

II. Subfamily Pharoideae L.G. Clark & Judz. (1996) . . . 135
Key to the Genera of Pharoideae . . . 135
Genera of Pharoideae . . . 135

Bistigmatic clade . . . 136
References . . . 137

III. Subfamily Puelioideae L.G. Clark et al. (2000) . . . 139
Key to the Genera . . . 139
BEP plus PACMAD clades . . . 139
BEP clade . . . 139
References . . . 142

IV. Subfamily Ehrhartoideae Link (1827) . . . 143
Key to the Genera . . . 144
Ehrhartoideae Incertae Sedis . . . 144
I. Tribe Phyllorachideae C.E. Hubbard (1939) . . . 144
II. Tribe Ehrharteae Nevski (1937) . . . 145
III. Tribe Oryzeae Dumort. (1824) . . . 146
III.1. Subtribe Oryzinae Griseb. (1853) . . . 146
III.2. Subtribe Zizaniinae Benth. (1881) . . . 147
References . . . 149

V. Subfamily Bambusoideae Luerss. (1893) . . . 151
Tribes and Subtribes of Bambusoideae . . . 154
Key to the Genera of Bambusoideae . . . 154
Group I – Herbaceous bamboos . . . 155
Group II – Branch complement of two or more subequal branches . . . 155
Group III – Branch complement with one branch only or one dominant branch and two to many smaller laterals . . . 157
Tribes and Genera of Bambusoideae . . . 159
IV. Tribe Arundinarieae Asch. & Graebn. (1902) . . . 159
V. Tribe Bambuseae Kunth ex Dumort. (1829) . . . 169
Neotropical clade . . . 169
Paleotropical clade . . . 176
VI. Tribe Olyreae Martinov (1820) . . . 189
References . . . 195

VI. Subfamily Pooideae Benth. (1861) . . . 199
Tribes and Subtribes of Pooideae . . . 200
Key to the Genera of Pooideae . . . 200
Group I – Unbranched inflorescences . . . 200
Group II – Inflorescence axis branched, spikelets without awns . . . 202
Group III – Inflorescence branched, spikelets awned, the awn straight . . . 204
Group IV – Inflorescence branched, spikelets awned, the awn curved, twisted or geniculate . . . 205
Tribes and Genera of Pooideae . . . 207
VII. Tribe Brachyelytreae Ohwi (1941) . . . 207
VIII. Tribe Nardeae W. D. J. Koch (1837) . . . 208
IX. Tribe Phaenospermateae Renvoize & Clayton (1985) . . . 209
X. Tribe Stipeae Dumort. (1824) . . . 211
XI. Tribe Meliceae Rchb. (1828) . . . 219
XII. Tribe Diarrheneae C.S. Campb. (1985) . . . 221
XIII. Tribe Brachypodieae Harz (1880) . . . 222
XIV. Tribe Bromeae Dumort. (1824) . . . 223
XV. Tribe Triticeae Dumort. (1824) . . . 223
XVI. Tribe Poeae R. Br. (1814), s.l . . . 229
References . . . 260

PACMAD Clade 267
VII. Subfamily Aristidoideae Caro (1982) 267
Key to the Genera of Aristidoideae 268
Genera of Aristidoideae 268
References 269

VIII. Subfamily Panicoideae Link (1827) 271
Tribes and Subtribes of Panicoideae 272
Key to the Genera of Panicoideae 272
Group I – Inflorescence unbranched 274
Group II – Inflorescence branched; inflorescence or branch axes disarticulating at the nodes 276
Group III – Inflorescence branches unbranched, not disarticulating 278
Group IV – Primary branches of inflorescence branched, although branches sometimes very short 279
Tribes and Genera of Panicoideae 281
XVII. Tribe Steyermarkochloeae Davidse & R.P. Ellis (1984) 281
XVIII. Tribe Tristachyideae Sánchez-Ken & L.G. Clark (2010) 282
XIX. Tribe Chasmanthieae W. V. Br. & B. N. Smith ex Sánchez-Ken & L. G. Clark (2010) 284
XX. Tribe Gynerieae Sánchez-Ken & L.G. Clark (2001) 286
XXI. Tribe Centotheceae Ridl. (1907) 286
XXII. Tribe Andropogoneae Dumort. (1824) 289
XXIII. Tribe Paspaleae J. Presl (1830) 314
XXIV. Tribe Paniceae R. Br. in Flinders (1814) 323
References 342

IX. Subfamily Danthonioideae N.P. Barker & H.P. Linder (2001) 347
Key to the Genera of Danthonioideae 347
Genera of Danthonioideae 348
References 352

X. Subfamily Chloridoideae Kunth ex Beilschm. (1833) 353
Tribes and Subtribes of Chloridoideae 354
Key to the Genera of Chloridoideae 354
Group I – Inflorescences unbranched, with spikelets sessile or pedicellate; spike-like panicles may also key here 354
Group II – Inflorescence with two or more unbranched branches, either digitate or spread along the main axis 355
Group III – Inflorescence branches themselves branched, at least some spikelets borne on secondary or higher-order branches, the spikelets mostly pedicellate 358
Tribes and Genera of Chloridoideae 359
XXV. Tribe Centropodieae P.M. Peterson, N.P. Barker & H.P. Linder (2011) 359
XXVI. Tribe Triraphideae P.M. Petersen (2010) 362
XXVII. Tribe Eragrostideae Stapf (1898) 363
XXVIII. Tribe Zoysieae Benth. (1881) 369
XXIX. Tribe Cynodonteae Dumort. (1824) 371
References 395

XI. Subfamily Micrairoideae Pilg. (1956) 399
Key to the Genera of Micrairoideae 399
Genera of Micrairoideae 400
References 402

XII. Subfamily Arundinoideae Burmeist. (1837) 403
Key to the Genera of Arundinoidae 403
Genera of Arundinoideae 404
References 408

Index 409

Poaceae – General Information

Description of the Family, Vegetative Morphology and Anatomy

Poaceae (R. Br.) Barnh. (1895).
Gramineae Juss. (1789).

Rhizomatous perennials, bisexual or monoecious. Culms herbaceous to somewhat lignified, erect. Leaf blades broad, *with pseudopetioles*, ligules membranous or a fringe of hairs. Inflorescences branched or unbranched, the floral units subtended by bracts. Perianth green to brown or absent. Stamens 6; style branches and stigmas 3. Pollen monoporate, with an annulus, with channels in the exine, *lacking scrobiculi. Ovule 1*. Embryo lateral, *differentiated with clear root and shoot meristems enclosed by sheaths (coleorhiza and coleoptile), several embryonic leaves, and a lateral haustorial organ (scutellum)*. Fruit indehiscent, *with one seed, the seed coat fused to the inner wall of the pericarp*, the hilum linear. *Mesophyll with fusoid cells and cells with invaginated cell walls, midrib complex*. Epidermis with multicellular microhairs, with alternating long and short cells, the short cells developing silica bodies. Photosynthetic pathway C_3.

A full description of Poaceae including all character variation is lengthy and obscures the ancestral conditions for the family that are the basis for the description above (GPWG 2001). Other familiar characters such as reduction of the style branches and stigmas to two occurred well after the origin of the family, so are listed in clade or subfamily descriptions below. Modification of the inner perianth to form lodicules may be synapomorphic for the family and have been lost in Anomochlooideae, but it is simpler to assume that the origin of lodicules occurred before divergence of Pharoideae and the remainder of the grasses. The grasses almost certainly originated in shady moist environments; occupation of open habitats occurred several times independently well after the origin of the family.

Vegetative Morphology and Anatomy

Roots

As in most seed plants, the radicle of the grass embryo is the first structure to emerge from the caryopsis at seed germination. Additional roots also form at the scutellar node in some taxa; these have been called "transitory node roots" by Hoshikawa (1969), who notes that they are present in many (but not all) Pooideae and in *Ehrharta*, but absent in rice and in all other grasses investigated. Although the radicle and scutellar node roots (together known as seminal roots) are usually described as short lived, they have been found to survive at least 3.5 to 4 months and extend to depths of two feet (Weaver and Zink 1945). In annuals that have been investigated, including wheat, plants can survive and flower with the seminal roots alone (Weaver and Zink 1945). Roots subsequently form from the mesocotyl and the coleoptile nodes (Hoshikawa 1969), with additional roots arising from subsequent nodes of the main stem and its branches. The roots produce an extensive fibrous network (Fig. 1) (Kutschera and Lichtenegger 1982). Roots may also develop from the lowermost nodes of the plant, from decumbent stems or stolons, and from rhizomes. In the latter case, the roots bind the soil and can stabilize sand dunes. Roots emanating from rhizomes also contribute to formation of sod.

The root apical meristem has been studied in detail in maize and rice, and is presumed to be similar in other grasses. The meristem has a closed organization similar to that in the well-studied eudicot *Arabidopsis*, but unlike the eudicots root

E.A. Kellogg, *Flowering Plants. Monocots*, The Families and Genera of Vascular Plants 13,
DOI 10.1007/978-3-319-15332-2_1,

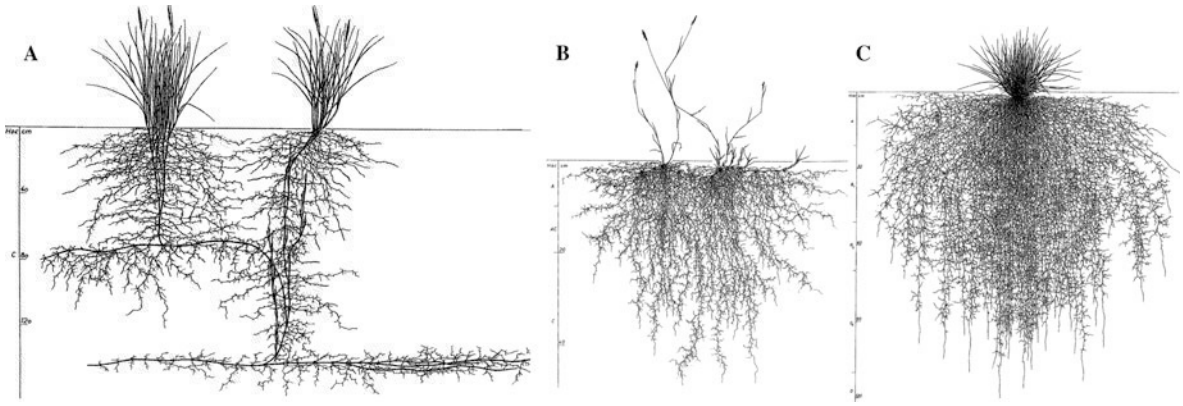

Fig. 1. Grass root systems. **A** Deep rhizomes and roots of *Ammophila arenaria*. **B** Shallow rhizomes and roots of *Poa compressa*. **C** Roots from a caespitose species, *Deschampsia caespitosa*. (From Kutschera and Lichtenegger 1982)

cap initials are wholly separate from the progenitor cells for other tissues (Coudert et al. 2010). The quiescent center, at least in maize and rice, includes hundreds of cells (Hochholdinger et al. 2004). Initiation of lateral roots begins with cell divisions in both the pericycle and endodermis, rather than just in the pericycle as in eudicots.

Cross-sectional anatomy of the mature roots of grasses is similar to that of other monocots (Clark and Fisher 1987). The root has an epidermis, an exodermis, a cortex of variable width, an endodermis, pericycle, and polyarch stele. Both the exodermis and endodermis have suberized cell walls that undergo secondary and even tertiary thickening, most notably on the inner face of the cells. However, a symplastic pathway through these walls is maintained by plasmodesmata, and some apoplastic flow appears to occur through the exodermis (Hose et al. 2001). Most species of grasses have roots with a central pith, but a few (generally annuals) have a central xylem vessel; the pericycle and pith may or may not be sclerified, and the extent of tertiary thickening varies in the endodermal cells (Goller 1977). Silica may be deposited in endodermal cells (Goller 1977; Hose et al. 2001). Goller (1977) notes that variation in root anatomy correlates with subfamilial classification, but his Table II suggests that many characters may be diagnostic for genera or tribes rather than subfamilies. The differences are generally quantitative (see summary in Clark and Fisher 1987). Enlarged storage roots are rare in the grasses (Clark and Fisher 1987).

Grasses are known to develop high root pressure. This is controlled actively by raising the concentration of ions across the suberized cell layers of the root, causing water to flow in to the xylem (Cao et al. 2012; Holloway-Phillips and Brodribb 2011). High root pressure develops at night when leaves are not actively transpiring, and effectively refills xylem vessels that have embolized during the day. Embolism is a particular problem for some grasses, which fail to close their stomata even when the leaf water potential is quite low. For example, *Lolium perenne* is able to continue gas exchange and photosynthesis even when the leaves have lost over 50 % of their hydraulic conductivity (Holloway-Phillips and Brodribb 2011). The ability of xylem to repair embolisms limits the height of plants in general; in bamboos, there is a high correlation ($r^2 = 0.81$) between root pressure and observed height (Cao et al. 2012).

Root hairs form just behind the growing point of the root, in vertical files, and hair cells alternate with non-hair cells (Clowes 2000). In many species, an epidermal cell divides asymmetrically to produce a large daughter cell (an atrichoblast) that will not become a hair and a smaller daughter cell (a trichoblast) that will become one. In the grasses, as well as in their relatives Restionaceae, Juncaceae, and Cyperaceae, the trichoblast is the daughter cell that is closest to the apical meristem, whereas it is the other way around in other monocots (Clowes 2000; Dolan and Costa 2001). In rice, however, the trichoblasts and atrichoblasts do not differ in size immediately after division, but rather undergo differential growth such that the atrichoblasts become larger (Kim and Dolan 2011). In

other grasses, the trichoblasts and atrichoblasts are not different in size at all (Rothwell 1966; Row and Reeder 1957).

Particularly in dry environments, many grasses also develop a rhizosheath, a discrete layer of soil particles that is firmly attached to the root and fully separable from the surrounding soil (Price 1911; Thomas 1921; Wullstein et al. 1979; Wullstein and Pratt 1981). Rhizosheaths have been studied in a handful of crop plants (Duell and Peacock 1985; McCully 1995; St. Aubin et al. 1986; Young 1995) and in a small number of xerophytic grasses; there appear to be differences between crops and xerophytes, but the literature is sparse. The most comprehensive description of a rhizosheath is for *Lyginia barbata* (Restionaceae) (Shane et al. 2011), in which the sheath appears to be similar to grasses. In maize, the rhizosheath forms about 1 cm behind the root apex and extends 20–30 cm back from the apex (McCully 1995), whereas in grasses that normally grow in dry sand, the rhizosheath may be much longer, up to several meters (Buckley 1982; Price 1911). In roots with both sorts of rhizosheaths, root hairs are unusually dense, and curl around the sand grains of the sheath. In maize, the root hairs in the region of the rhizosheath are living (McCully 1995), whereas they are persistent and possibly dead in the long rhizosheaths of desert grasses, suggesting that they are no longer taking up water. The rhizosheath of maize appears only early in development, and is lost as the epidermis matures and sloughs off. In contrast, in a mature rhizosheath of a xerophyte, the hypodermis, exodermis, epidermis and root hairs plus sand grains form a layer that is largely impermeable to water. Inside the hypodermis the outer cortex breaks down, creating a long empty tube surrounding the inner cortex and stele (Buckley 1982; Wullstein and Pratt 1981).

Bacteria are found in the rhizosheath (Gochnauer et al. 1989; Wullstein and Pratt 1981) and appear to have several roles. First, they apparently secrete polysaccharides that, along with mucilage secreted from the root tip itself, glue the rhizosheath together (McCully 1995; Price 1911). Second, they fix nitrogen (Bergmann et al. 2009; Wullstein et al. 1979), although it is unclear how much of this is translocated into the plant. Third, they may have antibiotic properties and protect the root from fungi (Shane et al. 2011).

Grasses also develop associations with fungi. Most grasses can develop arbuscular endomycorrhizae, associations that can substantially improve uptake of phosphorus. Development of a functioning symbiosis is under genetic control, involving some genes that are common throughout land plants and others that appear to be grass specific (Yang et al. 2012). Ascomycetes in the family Clavicipitaceae are generally arthropod pathogens, but one clade shifted to form symbiotic associations with grasses (Spatafora et al. 2007). A member of this clade, *Metarhizium robertsii*, will invade the roots of switchgrass (*Panicum virgatum*), and stimulate root hair growth.

The genetic basis of grass root development is only beginning to be explored (Hochholdinger et al. 2004; Hochholdinger and Zimmermann 2008; Smith and De Smet 2012). For example, the gene *Rootless concerning crown and seminal roots* (*Rtcs*) has been cloned from maize, and encodes a transcription factor with a Lateral Organ Boundaries (LOB) domain; *Rtcs* controls formation of all shoot-borne roots, both seminal roots and crown roots (Majer et al. 2012). Homologues have also been cloned from rice, where they appear to have a similar function (Smith and De Smet 2012). Other loci affecting lateral root initiation and elongation have been characterized in both maize and rice (Hochholdinger et al. 2001; Hochholdinger 1998; Kitomi et al. 2011; Liu et al. 2009). Despite progress cloning genes in rice and maize, the size of the plants makes study of root systems difficult. Fortunately, the recent development of *Brachypodium distachyon* as a model system will certainly provide new tools for understanding the controls of root architecture in the grasses (Chochois et al. 2012).

Stems

The shoot apical meristem has a characteristic zonal organization like that of most seed plants; the outer layer (tunica) consists of cells that divide primarily anticlinally, whereas the inner part consists of cells with less consistent patterns of division. Brown et al. (1957) suggested that the "festucoid" grasses (an informal group that at the time included all grasses outside the Panicoideae) have two tunica layers in the meristem, whereas the panicoids have only one. In maize (a panicoid) the outer L1 layer gives rise to the

epidermis, whereas other tissues are specified by the inner cells (Jackson 2009).

As the stem matures, cells in the internodes stop dividing and differentiate basipetally. This leaves a small meristematic region, the intercalary meristem, at the base of each internode just above the next lowest node. Although the intercalary meristem is a weak spot on the stem, support is provided by the surrounding leaf sheaths. Protoxylem and protophloem are present in the meristem, so that vascular continuity is maintained (Clark and Fisher 1987 and references therein). Activity of this meristem allows lodged grass stems to right themselves.

Internodes are generally short in early development, and those near ground level often elongate little if at all. The timing of internode elongation varies between species, but is often similar within major taxa. For example, in Pooideae, internode elongation is delayed until just before flowering. In contrast, in Bambuseae and many Panicoideae, internodes elongate apparently independent of flowering. The timing of internode elongation also determines whether the plant is grazing resistant or not (Branson 1953; Holechek et al. 1998); as long as the shoot apical meristem is near ground level it cannot be easily removed by large herbivores.

Internally, internodes may be solid throughout development, or may become hollow. In some bamboos, the internodes at the base of the plant may be solid, whereas those at higher nodes are hollow. The distinction between solid and hollow internodes is not absolute, however, in that some species have aerenchyma in the internode. Variation in the internal anatomy of the internode may be taxonomically diagnostic, although it is highly homoplasious (GPWG 2001), and is probably most useful at the level of genus and species. Most Pooideae tend to have hollow internodes, whereas other subfamilies are more variable (Brown et al. 1959a). Clearly the oft-repeated jingle "sedges have edges, and rushes are round, and grasses are hollow right to the ground" is a serious over-simplification. Pooideae tend to have wider hollows than other taxa, but the ecological and evolutionary significance of hollow internodes is unknown; Brown et al. (1959a) suggest a correlation of hollow internodes with moist habitats. Genetic studies in durum wheat show that having a solid stem is dominant to hollow stem, and that the trait is controlled by a single locus (Houshmand et al. 2007). Breeding for a solid stem confers resistance to the wheat stem sawfly, suggesting that taxa with solid stems have enhanced protection against insects. The wheat mutant *tiller inhibition* (*tin*) also causes the lower three internodes of the culm to become solid, apparently by diverting sucrose away from axillary buds (Kebrom et al. 2012). This result hints that this taxonomically important character may reflect a fundamental difference among species in the way carbohydrates are partitioned.

Stems in most grasses are herbaceous, but become woody in members of Bambuseae and Arundinarieae (the woody bamboos). In these taxa, dense clusters or caps of sclerenchyma cells form both externally and internally around the vascular bundles of the stem (Liese 1998). The bundles themselves are closely spaced, resulting in an extremely hard culm. The height of bamboos is strongly correlated with root pressure, which provides the force needed to refill embolized xylem vessels (Cao et al. 2012). Other large reed-like grasses (e.g., *Phragmites*, *Thysanolaena*) and the handful of shrubby ones (e.g., *Cladoraphis spinosa*) also develop hard woody culms, but whether these are histologically and developmentally similar to bamboo stems is unknown (GPWG 2001).

Most leaves on a grass plant have a single bud in their axils. The buds are under both developmental and environmental control, and their fate also depends on where they form on the plant. Axillary buds from the short basal internodes may grow horizontally to form stolons or rhizomes, or may grow more or less vertically to form axillary branches. When the upright branches occur near ground level they are known as tillers. As the axillary branch develops, it may break through its subtending leaf sheath (an extravaginal branch) or not (an intravaginal branch). In general, rhizomes and stolons are formed from extravaginal branches, whereas tillers may be either extra- or intravaginal.

The extent and nature of basal branching controls the overall architecture of the plant. Grasses that form only tillers develop a clumped architecture (i.e., are caespitose), whereas at the other extreme those that form rhizomes or stolons are spreading and may be sod-forming. Tillers may be geniculate at the base and root from

their nodes so that they are scarcely distinct from short rhizomes. Nonetheless, the growth form of any particular species is generally reasonably constant.

The number of tillers is controlled by hormones, particularly by auxin, strigolactones, and brassinosteroids, and by carbohydrate levels. When auxin transport is inhibited, or when the apical meristem of the plant is removed, the number of tillers increases and their angle becomes wider (Li et al. 2007; Xu et al. 2005). Although this has been demonstrated experimentally only in rice, it is likely that the result is general. Proteins in the strigolactone pathway, such as HIGH TILLERING DWARF1 and DWARF10, and in the brassinosteroid pathway, such as DWARF AND LOW-TILLERING, also regulate the number of tillers in rice (Arite et al. 2007; Tong et al. 2012; Zou et al. 2006) and are likely to be involved in other grasses as well. Tiller outgrowth is affected by TEOSINTE BRANCHED1 (TB1) and its orthologues, a cell-cycle regulator that integrates input from hormonal pathways with environmental signals (McSteen 2009; Ramsay et al. 2011; Remigereau et al. 2011). Tiller production is also affected by carbohydrate partitioning; diversion of sucrose from axillary buds to the main stem in wheat, as apparently occurs in *tiller inhibition* (*tin*) mutants, leads to reduced tillering (Kebrom et al. 2012).

Tiller angle is also under genetic control and has been investigated extensively in rice, in which tiller spreading affects yield and pest resistance (Wang and Li 2008a). Plants that spread too much shade their neighbors and thereby reduce grain production per unit area, whereas those that are too upright are susceptible to insect pests and pathogens because of increased contact with other plants and higher humidity within the clump. Several proteins have been identified that control tiller angle, including PROSTRATE GROWTH1 (PROG1) (Jin et al. 2008; Tan et al. 2008), LOOSE PLANT ARCHITECTURE 1 (LPA1) (Wu et al. 2013), LAZY1 (Li et al. 2007), and PIN-FORMED2 (Chen et al. 2012; Xu et al. 2005). The latter two proteins regulate auxin transport, whereas the mechanism of PROG1 influence is unknown. LPA1 affects tiller angle by controlling the growth of cells on the adaxial side of the branch; longer cells in that position lead to a more spreading tiller (Wu et al. 2013). *Tiller Angle Control1* (*TAC1*) is a quantitative trait locus that also affects tiller angle but the underlying gene has not yet been cloned (Yu et al. 2007).

Branches also form on the stem (culm) itself, although this is taxon specific. For example, branched culms are unknown in the Pooideae, whereas they are common in Panicoideae (particularly Andropogoneae) and Olyreae, and almost universal in Bambuseae and Arundinarieae. Genetic studies (Doust et al. 2004; Doust and Kellogg 2006) have shown that the genes that control the formation of culm branches differ from those that control basal branching (tillering). The genetic basis of the trait is thus consistent with the taxonomic observations.

Although the extent of branching – whether as tillers, rhizomes, or culm branches – is taxon specific, it is also controlled by the environment, particularly shade and light, and mediated by auxin, cytokinin and strigolactone, at least in the crop plants studied (Doust 2007a; McSteen 2009; Wang and Li 2008b). Many of the cellular mechanisms controlling branch formation are shared among monocots and eudicots, but others appear to be grass specific (see citations in Doust 2007b).

Not all leaves on all grasses bear axillary buds. For example, the lower culm nodes of some bamboos fail to form buds (Clark and Fisher 1987). In most such cases, whether the bud is specified but simply fails to differentiate, or whether the signal for bud formation is never transmitted or received, is unknown.

A few woody bamboos (e.g., *Chusquea*) have multiple axillary buds, which may be formed by supernumary axillary meristems in the axil of a single leaf, or may represent a highly compressed branch complex. These have never been studied developmentally.

As in most monocots, the branches produced by axillary buds bear an adaxial prophyll. This is generally two-keeled and is particularly prominent in the woody bamboos, where variation in its shape is often taxonomically useful.

The node is complex, both internally and externally. Internally it is marked by a plexus of extensively anastomosing vascular tissue that forms just above the point of insertion of the leaf (Liese 1998; Sharman 1942). Although only a handful of grass species have been investigated,

the nodal plexus is a web of transverse vessels that connect the axial vessels (Pizzolato 2000); the overwhelming majority of the latter end at the nodal plexus with only a tiny percentage extending through (Shane et al. 2000; see also André 1998). Even in taxa with hollow internodes, the node is more or less solid. In the bamboos there is a clear woody wall, the diaphragm (Liese 1998).

The vascular structure and function of the node are described in detail for rice and barley, and are presumed to be broadly similar in other grasses (Yamaji and Ma 2014). A vascular bundle that will ultimately extend into a leaf can be traced to two nodes below the leaf (i.e., leaf node minus 2) where is it relatively small in diameter (called a diffuse vascular bundle by Yamaji and Ma 2014). It connects through the next node above (leaf node minus 1; a transit vascular bundle), and at the leaf node appears enlarged, with an increased number of xylem elements and phloem sieve tubes (an enlarged vascular bundle). Thus any given node contains diffuse vascular bundles, transverse vascular bundles and expanded vascular bundles. Xylem transfer cells in the expanded vascular bundles move solutes to the diffuse vascular bundles and thus up to higher nodes in the plant. Such a pathway has been demonstrated for silicon (Si), which accumulates at nodes where it is then distributed among vessels at the nodes (Yamaji et al. 2008, 2012; Yamaji and Ma 2009). A similar pathway exists in the phloem, transporting zinc (Zn) and also the toxic metal cadmium (Cd); both Zn and Cd accumulate in the nodes and are distributed to developing tissues (Satoh-Nagasawa et al. 2012; Yamaguchi et al. 2012). Transporters for copper (Cu) and manganese (Mn) are also located at the nodes but neither mineral accumulates; rather, influx and efflux are tightly controlled to keep levels consistent throughout the plant (Deng et al. 2013; Yamaji et al. 2013).

Externally, the node is often marked by a slightly swollen area, the nodal pulvinus, which is often surrounded by a corresponding area in the sheath. This area is flexible and is involved in reorienting the stem in response to lodging, although in mature stems it may become lignified and lose the capacity to bend (Kaufman et al. 1987). Bending of the pulvinus correlates with changes in levels of auxin and gibberellin, which is consistent with the role of these hormones in cell expansion (Clore 2013; Wolbang et al. 2007), and is regulated in rice by LPA1 (Wu et al. 2013). In general, the pulvinus lacks sclerenchyma and instead is supported by collenchyma (Paiva and Machado 2003). In North American grasses, the sheath pulvinus is almost universally present, whereas the nodal pulvinus occurs primarily in Panicoideae and Chloridoideae, and is generally absent in Pooideae (Brown et al. 1959b; Clore 2013).

In a few grasses the lower nodes of the culm may be enlarged to form storage organs (Burns 1945). Whereas in a few species the thickened organs are leaves (e.g., *Poa bulbosa*), and thus the structure is a true bulb, in other species the storage organ is the stem and so is properly a corm (e.g., *Zuloagaea bulbosa*, *Melica bulbosa*, *Arrhenatherum avenaceum* var. *nodosum*, *Ehrharta capensis* and relatives). Species with corms often occur in areas with low summer rainfall (Burns 1945; Verboom et al. 2003). Most bulb- or corm-bearing species are in subfamily Pooideae, but a few are panicoid (*Zuloagaea bulbosa*; Bess et al. 2006) or ehrhartoid (Verboom et al. 2003).

In terms of life history, the ancestral condition for the grasses is herbaceous, perennial, and rhizomatous (GPWG 2001), but the annual habit has been derived repeatedly. Humans have particularly exploited the annuals (e.g., wheat, maize, rice), in which much of the photosynthate is accumulated in seeds. Multiple genes control the switch between annual and perennial. As shown in sorghum and rice, some of the same loci are involved in both species, suggesting that changes of plant habit can occur relatively easily (Hu et al. 2003).

Leaves

Morphology and development

As in all seed plants, the shoot in grasses is made up of repeating units known as phytomers or phytomeres; each unit consists of a leaf, an internode, and an axillary bud. Whether the internode and bud should be associated with the leaf above or below is a matter of debate, but however defined the phytomer is repeated over and over in the growth of the grass shoot (Clark and Fisher 1987).

Leaves form on the flanks of the shoot apical meristem. The position of a nascent leaf can be identified initially by a change in expression of the meristem identity gene *knotted1*, which is switched off in the cluster of cells that will

become the leaf primordium (Jackson et al. 1994). Auxin becomes concentrated in these cells, and their subsequent divisions lead to the formation of a leaf primordium (Reinhardt et al. 2003). The primordium develops both laterally and in the proximo-distal axis to become a broad flat structure that encircles the meristem. In maize, the leaf forms from the outer two layers of the shoot apical meristem, with the L1 layer sometimes contributing to the mesophyll as well as producing the epidermis (Poethig 1984). About 40 cells initially contribute to the circumference of the leaf primordium, and the entire primordium is made up of about 200 cells.

Leaf initiation in the grasses (as in all graminoid Poales) is strictly distichous, except in the tiny moss-like grass *Micraira*. In this species, serial sections through the shoot show a phyllotaxis of 3/8; there is no evidence of twisting of the stem or sheaths (Philipson 1935a). Spiral phyllotaxis has also been reported for the large reed-like species *Arundoclaytonia dissimilis*, but this plant has never been studied in detail. Although Judziewicz and Soderstrom (1989) cite Page (1951) to suggest that leaf initiation is spiral in *Streptochaeta*, Page was in fact referring to the inflorescence. The leaves are distichous.

Cell division and expansion initially occur throughout the young leaf primordium, but actively dividing meristematic cells become increasingly restricted to the base of the young leaf so that the leaf matures from the apex to the base (Sharman 1942). The region of active division, termed the "proliferative zone" by Sylvester and Smith (2009), is later divided into two by the developing ligule and sheath, so that two meristems are formed, one each at the base of the blade and the base of the sheath.

Mature leaves in the grasses generally consist of a distal blade and a proximal sheath. The sides of the leaf blade – on either side of the midrib – are controlled developmentally by the NARROW-SHEATH proteins, which define a lateral compartment of the leaf (Nardmann et al. 2004). The region where the blade and sheath join is known as the collar; in some taxa this corresponds to a region of more flexible tissue and less sclerenchyma (Paiva and Machado 2003). In many grasses there is a wedge-shaped region on either side of the leaf at the collar that can be identified by its color and texture. This region appears to be involved in positioning the blade (Foster and Timmermans 2009), and in rice is controlled by the gene LPA1 (Wu et al. 2013). Curiously for such an obvious morphological feature, the wedge-shaped region has no standard name. It is called a "leaf joint" in some papers on rice (e.g., Wu et al. 2013), whereas is has been called a "dewlap" by some taxonomists (e.g., Martínez-y-Pérez et al. 2008; Pohl 1980) and by agronomists working on sugarcane (Artschwager 1951), although the term is not widely used. Maize geneticists call this region an auricle. Taxonomists, however, reserve the term "auricle" for the tiny prongs or hooks that extend from the wedge-shaped region, and "auricle" is used in this sense here. In this taxonomic sense, maize lacks auricles. Bowden (1970) reports that the dewlaps of *Andropogon gayanus* var. *bisquamulatus* (Hochst.) Hack. secrete sweet nectar, but this observation appears not to have been followed up.

Leaf length and width are variable within and between species, and are often taxonomically informative. Fiorani et al. (2000) found that differences in leaf length in species of *Poa* can be attributed to changes in the rate of growth rather than its duration – i.e., leaves grow faster for about the same length of time, a result extended by Sugiyama (2005) to several species of C_3 grasses. Arredondo and Schnyder (2003) also found a correlation between the size of the meristem at the base of the blade and the rate of leaf elongation in eight species of pooid grasses. If this result is generally true, it may point to phylogenetically correlated differences in regulation of the cell cycle.

Also at the blade-sheath boundary is an adaxial flap of epidermal tissue, the ligule; in most cases this is small, no more than a couple of millimeters long, although it may be several centimeters long in some woody bamboos. During leaf development, the blade-sheath boundary is defined by a region of increased cell division known as the pre-ligular band; this marks the position where the ligule will form (Sharman 1942; Sylvester et al. 1990). Development of the ligule has been studied extensively, especially in maize (reviewed by Foster and Timmermans 2009). Many genes are involved in the proper positioning and development of the ligule, but only a few seem to affect ligules exclusively, whereas others disrupt the entire structure of the leaf.

In a detailed description of ligule development in *Deschampsia*, *Melica* and *Phyllostachys*, Philipson (1935b) noted that the ligule appears to form from separate domains, a central epidermal one, and lateral extensions of the margins of the sheath, thus anticipating by more than 50 years the discoveries of the lateral domains in maize leaves (Scanlon and Freeling 1997). Ligule morphology is remarkably variable and is often taxonomically informative. Membranous ligules predominate among members of the BEP clade, whereas the PACMAD subfamilies often have ligules consisting of a fringe of hairs.

A ligule-like structure, which may be membranous or ciliate, may form on the abaxial side of the collar region, and is variously known as a contraligule, pseudoligule, or external ligule; the development of this structure has never been studied. It occurs in some genera such as *Puelia* (Puelioideae), *Streptogyna*, and in most, if not all, genera of Bambuseae and Arundinarieae, and can be helpful for genus and species identification.

The base of the leaf blade above the ligule may be constricted to form a pseudopetiole. Presence of a pseudopetiole is ancestral and synapomorphic among the grasses (GPWG 2001). All species of Anomochlooideae, Pharoideae, and Puelioideae have pseudopetioles, as do most species of Bambusoideae. The structure also appears in some Panicoideae. Development of pseudopetioles has never been studied and it is unknown how it affects – or is affected by – the meristem at the base of the blade, nor is there information on its contribution to the hydraulic architecture of the leaf.

In taxa such as *Streptogyna* (BEP clade, incertae sedis), most bamboos, some Panicoideae (e.g., *Thysanolaena*, tribe Centotheceae; *Gerritea*, tribe Paspaleae), Micrairoideae (*Micraira*), Arundinoideae (*Molinia*), some species of *Ehrharta*, in *Macrochloa* (Stipeae), and *Aristida* (Aristidoideae), an abscission zone forms at the collar and the leaf blades disarticulate. The anatomy of the abscission zone varies among species (Röser and Heklau 2011). In other species (e.g., in some species of *Rytidosperma*, Danthonioideae), an abscission zone forms at the base of the sheath and the entire leaf is deciduous. In the culm leaves of woody bamboos, even if the blade persists for several seasons, it will ultimately disarticulate from the sheath. Subsequent disarticulation of the sheath from the culm varies among genera or groups of genera.

Leaf angle is under the control of brassinosteroids (Tong et al. 2012), as well as other plant hormones whose effects interact with those of the brassinosteroids (Song et al. 2009). However, other controls of leaf angle operate independently of hormonal pathways, affecting cell division in and around the collar. These include the genes *Leaf inclination2* (Zhao et al. 2010), *Increased leaf angle1* (Ning et al. 2011), and *LPA1* (Wu et al. 2013).

Leaf epidermis

In most grasses, the leaf epidermis consists of a single layer of cells that form in long files parallel to the proximo-distal axis of the leaf (Fig. 2). Because the leaf matures from tip to base, these long files represent a developmental gradient that has helped in the investigation and understanding of differentiation. Many of the late cell divisions are asymmetric, with a single cell giving rise to two differently sized daughter cells (see Fig. 8A in Sylvester et al. 1990). The result of this is a characteristic alternation of long and short cells in the mature epidermis. The alternation is not perfect, and short cells may occur in pairs or files of up to five. Alternation of long and short cells occurs in other monocots as well, but only in the cell files that will produce stomata. Thus the asymmetric cell divisions that occur throughout the epidermis in the grasses represent a change in position of a developmental program (Kellogg 2000). Long-and-short cell alternation is shared with *Joinvillea*, one of the close relatives of the grasses, and provides one piece of evidence for their close relationship (see Affinities); because *Ecdeiocolea* is leafless, the ancestral condition for the grass sister group is unknown (Campbell and Kellogg 1987). The epidermis is often structured differently over the veins (costal region) than it is between them (intercostal regions), a difference that is generally consistent within a species or genus (Fig. 2). The genetic basis of the asymmetric cell divisions that will produce stomata is becoming increasingly well understood (Abrash and Bergmann 2009), but whether this machinery is also activated in other short cells is unknown.

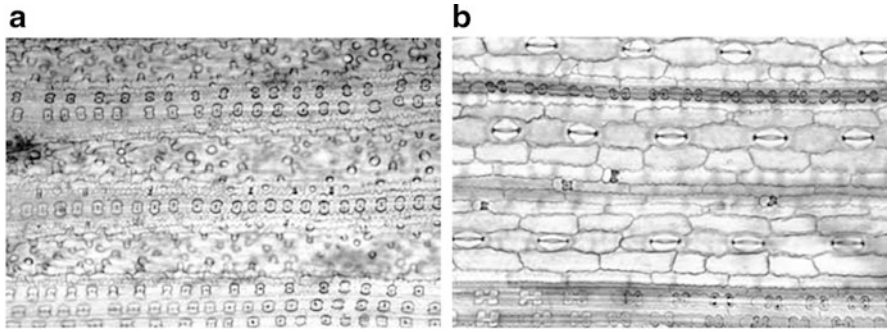

Fig. 2. Leaf epidermis showing files of cells extending along the proximo-distal axis. **A** *Oxychloris scariosa* (Chloridoideae), showing characteristic saddle-shaped silica bodies in short cells over the veins. Intercostal epidermal cells bear papillae. **B** *Monocymbium cerisiiforme* (Panicoideae), showing bilobate silica bodies in short cells over the veins. (From Watson and Dallwitz 1992 onward)

Epidermal long cells vary only slightly in shape, usually being rectangular but sometimes fusiform. Their longitudinal walls, however, may be sinuous and thus interlocking, or straight; the latter condition occurs frequently, but not universally, in Pooideae. The shape of the longitudinal wall is determined in part by localization of microtubules and actin (Frank et al. 2003). In maize, in which the longitudinal walls are normally sinuous, mutations in loci known as *Brick* (because they create brick-like cells when mutated) create long cell shapes reminiscent of those occurring in, for example, species of *Poa* or *Ehrharta*. A survey of grasses staining for tubulin and actin would help determine whether distribution of these cytoskeletal proteins is the primary determinant of taxonomic variation in cell wall morphology.

In the grasses, as in most commelinid monocots (Stevens 2012), the stomata are paracytic – there are two guard cells, with the stoma oriented parallel to the long axis of the leaf, and two subsidiary cells parallel to the guard cells. In stomatal development, a short cell divides longitudinally to give two cells and then these divide, again longitudinally, to give a set of four, more or less parallel, rectangular cells (Abrash and Bergmann 2009). The outer two differentiate into subsidiaries and the inner two into guard cells. The ancestral and most widespread condition is for subsidiaries to be somewhat dome-shaped in surface view, although in some species they are triangular. However, in the pooid clade that includes Poeae, Triticeae, Bromeae and *Brachypodium*, the subsidiaries have parallel walls, a condition that is uniquely derived. The subsidiaries overlap the guard cells in all Pooideae that originated after the divergence of Nardeae.

Epidermal short cells have various fates, depending on the species, position in the plant, and position in the leaf (or leaf-like structure such as the lemma). One common role of short cells is to differentiate as silica-containing cells. The accumulation of silica in the leaf is a character shared by most of the commelinid monocots, and accumulation specifically in the epidermis is a character of the Poales (Stevens 2012). Monosilicic acid, $Si(OH)_4$, is produced by weathering of soils, and is taken up by the plant both actively and passively in a highly regulated process (Yamaji et al. 2008, 2012; Yamaji and Ma 2014). Silica is deposited in an amorphous non-crystalline form as silicon dioxide ($SiO_2.nH_2O$) throughout the plant, particularly in tissues involved in support of the stems and leaves (Isa et al. 2010), but also in the walls of guard cells, subsidiary cells, and epidermal papillae (Ueno and Agaric 2005); the rate and developmental timing of accumulation is specific for different cell types (Sakai and Sanford 1984). In addition, silica commonly accumulates in specialized short epidermal cells (silica cells) in which it is deposited initially in the cell wall and then accumulates centripetally, while the cellular contents break down (reviewed by Prychid et al. 2004). Silica deposition protects the plant from bacterial and fungal pathogens, supports the stems, reduces the uptake of toxic metals, and regulates water loss (Isa et al. 2010; Ma and Yamaji 2006). In addition, silica rapidly wears down the mandibles of insect herbivores and reduces digestibility (Massey and Hartley 2009). Silica accumulation is presumed to be energetically costly, but its effect on plant biomass varies between species; this variation affects susceptibility to herbivores and thus competitive interactions (Garbuzov et al. 2011).

Silica may also be a defense against large mammalian herbivores, although the data on this are less clear. Simpson (1951) famously proposed that the evolution of hypsodont teeth in equids was driven by a shift to diets of grass, and their high concentrations of silica. However, Sanson et al. (2007) have recently questioned whether silica bodies are in fact hard enough to wear down mammalian tooth enamel, as had been suggested (Baker et al. 1959). In addition, Strömberg (2006) showed that hypsodonty appeared well after the spread of grasslands, at least in North America, weakening the hypothesized connection.

The shape of the silica deposits (silica-bodies or phytoliths) is often characteristic of particular taxonomic groups. These shapes were originally described based on their appearance in two dimensions (Metcalfe 1960; Prychid et al. 2004), and early attempts to use silica body characteristics in phylogeny reconstruction found that they were highly homoplasious (Kellogg and Campbell 1987; Kellogg and Watson 1993). The description and classification of silica bodies (phytoliths) is improving due to efforts to describe them in three dimensions rather than two (Piperno 2006; Piperno and Pearsall 1998). For example, bilobate silica bodies occur in many subfamilies of grasses (Fig. 2A, B). However, those of Stipeae are asymmetrical in cross section, while those of Panicoideae are generally symmetrical. Aristidoid bilobates tend to have a long thin shaft between the lobes, whereas in other taxa the shaft is short and in bamboos is lacking altogether. So-called "oryzoid" bilobates are elongated perpendicular to the long axis of the leaf. This form is found in Ehrhartoideae, some bambusoids (Olyreae), and arundinoids (*Eriachne*); careful analysis of these, however, shows that they can in fact be distinguished (Prasad et al. 2011). More detailed description of silica bodies will not wholly solve the problem of homoplasy in the character, however. Individual plants have a range of silica body forms (Piperno and Pearsall 1998) and thus characterization of silica bodies within a species or genus must be somewhat quantitative.

Short cells may also form trichomes. These may be unicellular (prickles, macrohairs) or bicellular (microhairs), and may accumulate silica or not (Prat 1932). Development of prickles and macrohairs begins with enlargement of the cell. As the trichome develops, the outer wall expands, so that the outline of the cell looks more or less like a muffin. The nucleus then moves up in the cell closer to the outer wall. As the outer wall continues to expand, growth becomes asymmetrical, and the cell elongates parallel to the surface of the leaf (Kellogg 1990).

In cells that will become prickles, the tip of the cell develops a sharp point and silica accumulates in the tip (Prat 1932). Silica accumulation appears to occur early before the leaf has fully opened and before silica deposition in other cells (Motomura et al. 2006). Prickles generally point toward the distal end of the structure on which they occur, but sometimes point toward the proximal end (i.e., are retrorse). Presence or absence of prickle hairs on particular structures often is a good field identification character. However, I know of no study in which this character has been evaluated in a phylogenetic context; intuitively, it seems as though it should be highly labile in evolutionary time. The function of prickle hairs is unknown, but could help deter small herbivores such as slugs and nematodes.

Bicellular microhairs are found in all non-pooid grasses (Johnston and Watson 1976), but their development has never been investigated. As their name implies, these trichomes have only two cells, one apical and one basal, but the shape of the cells is often characteristic of particular taxa. In the "panicoid type" microhair, both cells are longer than wide, and internal membranes are not readily visible (Amarasinghe and Watson 1988, 1989). In contrast, in the "chloridoid type" the apical cell is nearly as wide as long. The distinction between the two is not sharp, however, and a graph of the length-width ratio of the apical cells in all microhairs in the family shows that the variation is continuous (GPWG 2001; Kellogg, unpublished observations). Some chloridoid microhairs contain internal membranes in the basal cell and secrete salt (Liphschitz and Waisel 1974; Marcum 1999; Oi et al. 2012), but there is no evidence that the panicoid hairs are secretory (Amarasinghe and Watson 1989). Other chloridoid microhairs, the "*Enneapogon* type" have internal membranes in the apical cell, but appear to be non-secretory (Amarasinghe and Watson 1988, 1989). Lack of microhairs is synapomorphic for all Pooideae after the divergence of *Brachyelytrum*, *Nardus* and *Lygeum*, but ecological consequences of this loss are unknown. Microhairs with more than two cells are reported for *Joinvillea* (Joinvilleaceae), a

close outgroup of the grasses, and for *Streptogyna crinita* and several members of Bambusoideae (Soderstrom and Judziewicz 1987).

Macrohairs on the leaf blades are inherited independently of hairs on other plant parts (Moose et al. 2004), supporting their use as a different taxonomic character. Macrohairs are often surrounded by a multicellular and slightly raised set of epidermal cells (Prat 1932). These have been reported to be secretory (Bowden 1971), with the cells containing a variety of sugars and pectic substances. However, the number of species investigated is tiny, and macrohairs would repay closer investigation (Sylvester and Smith 2009).

In areas around some or all of the veins, adaxial epidermal cells may differentiate as bulliform cells. These cells are enlarged in the ab-adaxial direction, extending into the region normally occupied by mesophyll. They may occur as fans of cells or in irregular groups, and may or may not be associated with other colorless mesophyll cells, and thus provide a source of taxonomically informative characters. Bulliforms may occur on either side of the mid-vein only, or on the sides of lateral veins as well. Some species lack bulliform cells entirely.

Bulliform cells can rapidly take up or lose water. By expanding and contracting, bulliform cells are reported to control leaf rolling (Arber 1934; Bidlack and Jansky 2011), but there is surprisingly little evidence to support the hypothesis that changes in bulliform turgor are actually causative (Arber 1934). In the resurrection species *Sporobolus stapfianus* the outer wall of the bulliforms is thick and water is lost to the adjacent mesophyll cells (Dalla Vecchia et al. 1998). While water loss from the bulliforms may lead to leaf rolling in some species, in *S. stapfianus* the bulliforms are involved in maintaining hydration of the mesophyll. In late development of the leaf, bulliform cells may accumulate silica (Motomura et al. 2004).

A number of proteins regulate the number of bulliform cells in each group. Transcription factors that help specify identity of the ad- and abaxial sides of the leaf blade determine whether bulliforms will develop on the adaxial side (as is most common) or abaxial side, and also regulate how many bulliforms form in a cluster (Dai et al. 2007; Zhang et al. 2009; Zou et al. 2011). Various enzymes also affect bulliform cell development (Fujino et al. 2008; Hu et al. 2010; Li et al. 2010; Xiang et al. 2012), in some cases via affecting the differentiation between the ab- and adaxial sides of the leaf (Hibara et al. 2009). Comparative studies have yet to be done on most of these proteins, so it is unclear which components of the bulliform specification network may have been selected to produce the diversity of leaf anatomy observed among the grasses.

Multicellular structures known as "glands" have been observed in some grasses, particularly in Danthonioideae (Linder et al. 1990). Two-celled salt glands have also been described in *Spartina* (now part of *Sporobolus* s.l.; Peterson et al. 2014), in which an epidermal initial cell expands downward into the mesophyll, and later divides asymmetrically to form a small apical cell (Fahn 1979). Salt glands have also been characterized in *Chloris gayana*; the density of glands on leaves increases when the plant is grown in higher concentrations of salt (Oi et al. 2012). The apical cell of the gland has a complex endomembrane system and a high number of mitochondria, suggesting that salt excretion is energetically costly.

The epidermis of many grasses is coated with a layer of wax. This is characteristic of leaves on juvenile plants in maize and the presence of wax has been used as a marker of the transition from juvenile to adult morphology (Moose and Sisco 1996). In some taxa, such as *Sorghum*, the wax forms on the stem and sloughs off as large flakes. In some bambusoids, wax forms only on one portion of the leaf, indicating very precise, cell specific, genetic and developmental control.

Internal anatomy of leaves

The internal anatomy of grass leaves has been studied extensively (Brown 1977; Ellis 1976; Metcalfe 1960; Watson and Dallwitz 1992 onward). Most investigations have focused exclusively on the cross-sectional appearance of the middle portion of the adult leaf blade, so comparative data on sections in other planes, on sheaths and on early development are limited. In most species, the mesophyll cells are not tightly packed and are relatively homogeneous throughout the leaf. However, some Centotheceae have an adaxial palisade layer. In some early-diverging grasses and in virtually all bamboos the mesophyll is interrupted by fusoid cells (Fig. 3A).

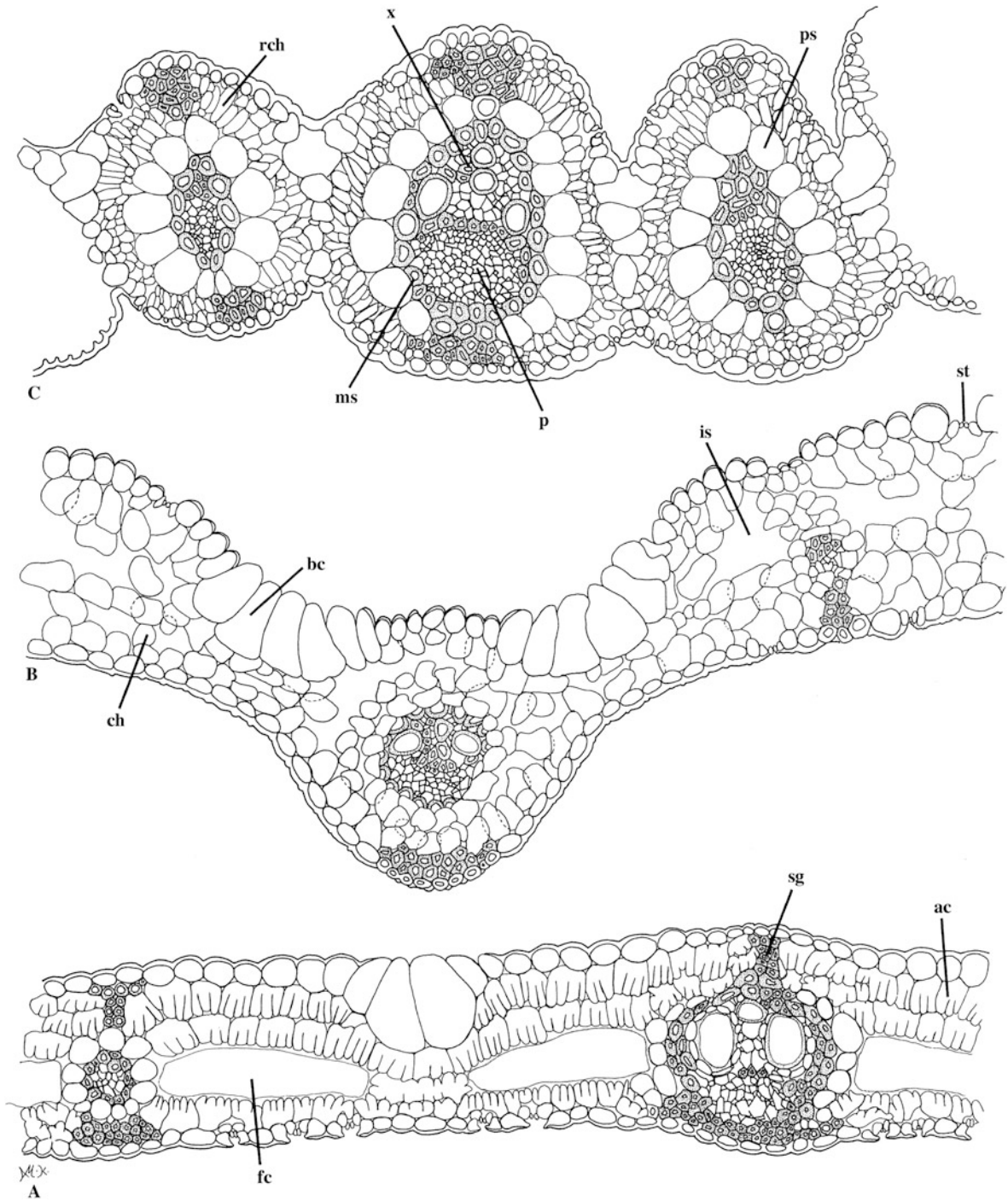

Fig. 3. Cross-sections of leaves of selected grasses. **A** *Dinochloa macclellandii* (Bambusoideae). **B** *Poa* sp. (Pooideae). **C** *Bouteloua* sp. (Chloridoideae). *ac* arm cell, *bc* bulliform cell, *ch* chlorenchyma, *fc* fusoid cell, *is* intercellular space, *ms* mestome sheath, *p* phloem, *ps* outer parenchyma sheath, *rch* radiate chlorenchyma, *sg* sclerenchyma girder, *st* stomatal apparatus, *x* xylem. (From GPWG 2001, p. 405, with permission of the Missouri Botanical Press; drawn by M. Kojima)

In all the early-diverging lineages of grasses, mesophyll cells have obvious invaginations of the cell wall when viewed in cross section; such cells are known in the literature as arm cells (Fig. 3A). Such cell wall invaginations also occur in all bamboos, and in the tribe Oryzeae of subfamily Ehrhartoideae (GPWG 2001). Invaginated cell walls also appear in *Phragmites* (subfamily Arundinoideae), where they constitute a reversal following loss at the base of the PACMAD clade. Longitudinal sections of leaves have revealed additional variation in internal morphology (Sánchez-Ken and Clark, unpubl. data).

Mesophyll cells with invaginated walls are apparently uniquely derived in the grasses. They are lacking in Joinvilleaceae. Because Ecdeiocoleaceae are leafless, there are no data for this family. However, a similar phenotype appears in other more distantly related families. For example, Restionaceae have cells with invaginated cell walls in their stems, where they are known as peg cells (Cutler 1969).

Fusoid cells are large, rectangular to cigar-shaped mesophyll cells, and appear curiously empty in leaf cross sections. They are apparently synapomorphic for the grasses; the plants in which they occur are shade loving and prefer moist habitats. They occur only in the early-diverging lineages, *Streptogyna*, the Bambusoideae, and a handful of Panicoideae in tribe Paspaleae, subtribe Arthropogoninae. Superficially similar cells have been reported in Centotheceae (Panicoideae) as well, but they appear to be laterally expanded bundle sheath cells and are more accurately described as bundle sheath extensions. The function and development of fusoid cells are unknown and it is not even clear whether they are alive or dead, although some data suggest that they may be dead at maturity.

Development of fusoid cells has been described in some detail in *Streptochaeta spicata* (Page 1947). As the primary vascular bundles begin to differentiate, most mesophyll cells are still dividing in all three planes of the leaf (ab-adaxial, lateral, and proximo-distal). However, the mesophyll cells adjacent to the vascular tissue cease to divide in the ab-adaxial and lateral planes and simply enlarge; they continue to divide along the proximo-distal axis. While cell division continues in all mesophyll, epidermis and vascular tissue, the fusoid cells develop large vacuoles. As the leaf blade emerges from the sheath of the leaf below it, the fusoid cells appear to die and their walls apparently collapse. Page (1947) then speculates that some force must be operating on the fusoid cells to preserve their regular shape during development. In long-lived leaves of some bamboos the fusoid cells accumulate silica in their lumens (Motomura et al. 2004). March and Clark (2011) find that shade grown leaves of *Chusquea*, *Phyllostachys*, and *Yushania* all developed fusoid cells, whereas sun-grown leaves did not. They speculate that perhaps fusoid cells are a way to increase light availability inside the leaf.

Grass leaves generally contain sclerenchyma associated at least with the vascular bundles, although there are often clusters of sclerenchyma at the leaf margin as well (Fig. 3A, C). The distribution of sclerenchyma within the leaf is often distinctive and can be helpful in species identification. Sclerenchyma may extend from the vascular bundle to the abaxial epidermis, the adaxial epidermis or both, or may be present as a cap over the bundle (Ellis 1976).

The functional consequences of different patterns of sclerenchyma distribution are largely unknown. Leaves with sclerenchyma girders extending from the bundle sheath to both epidermes are known as “heterobaric”, whereas those without such girders are called “homobaric”. In heterobaric leaves, such as those in *Hordeum vulgare*, the girders, called bundle sheath extensions in the physiology literature, provide a direct hydraulic connection between the vascular bundle and the epidermis (Buckley et al. 2011) allowing stomatal movements to respond rapidly and easily to water availability. Bundle sheath extensions also divide the leaf into functional compartments that affect the structural and functional aspects of the leaf, although this functional compartmentation has not been investigated in grasses. Investigations in dicotyledonous trees and shrubs suggest that heterobaric leaves have lower leaf mass per unit area, more nitrogen per unit mass, and have higher photosynthetic capacity per unit mass, than homobaric leaves (Liakoura et al. 2009). In homobaric leaves, CO_2 can diffuse laterally for a distance of several millimeters, whereas the bundle sheath extensions of a heterobaric leaf effectively prevent such