

John P. Vogel *Editor*

# Genetics and Genomics of Brachypodium

# Plant Genetics and Genomics: Crops and Models

## Volume 18

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John P. Vogel  
Editor

# Genetics and Genomics of *Brachypodium*

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# Preface

Grasses dominate many natural ecosystems and produce most of the calories consumed by humans either directly in the form of grains or indirectly through forage/grain fed animals. In addition, grasses grown as biomass crops are poised to become a significant source of renewable energy. Thus, the enormous economic and environmental importance of grasses is undeniable. Despite this, research into grass biology has been hampered by the lack of a truly tractable experimental model system. While much valuable research has been conducted in crops like maize, rice, wheat, and barley, none of these grasses possess the full suite of traits necessary for a truly tractable model system (e.g., small size, rapid generation time, small diploid genome, self-fertile, simple growth requirements, and easily transformed). The power of applying a model system approach to plant biology has been amply demonstrated by the tremendous advances achieved using *Arabidopsis thaliana* as a model organism. Unfortunately, *A. thaliana* is a eudicot and about 150–200 million years of evolution separates it from the grasses. Thus, there are many biological traits unique to the grasses for which *A. thaliana* is an unsuitable model. Furthermore, even for shared traits the specific genes and regulatory networks often differ considerably between *A. thaliana* and the grasses. Thus, there is a pressing need for a grass model system. Over the past decade, *Brachypodium distachyon* has emerged to fill this void.

This book describes the current state of *B. distachyon* research tools and how they have been applied to a wide range of topics. Specific chapters describe the development of key resources and techniques including a high-quality reference genome sequence, the development of high-efficiency transformation methods, the creation of a large collection of insertional mutants, the assembly of extensive germplasm collections, genome sequencing of natural accessions, the optimization of efficient crossing techniques, and the creation of recombinant inbred lines. Chapters focused on the application of these tools cover a wide range of topics including cell wall biosynthesis, seed development including starch and storage protein biosynthesis, microRNA and small RNA biology, cytogenetics, adaptation to local environments, abiotic stress, plant–pathogen interactions, root biology, and

flowering time. In addition, recent advances using closely related *Brachypodium* species as models for perenniality and polyploidy are described.

That the current state of *Brachypodium* research can fill a book is a testament to the maturity of the system. Additional evidence in this regard includes the enormous number of seed orders distributed and the rapidly rising number of publications using *B. distachyon* as an experimental model. In comparison to other model systems, this maturity has come at an exceptional pace. In a little over a decade *B. distachyon* has gone from a curiosity to a full-blown model organism used by over 350 laboratories around the world. This explosive growth followed a new paradigm in which a genome sequencing project was initiated early on in the development of a model system. The genome project catalyzed both the rapid development of experimental tools and the adoption of the system by many researchers. While this “sequence it and they will come” strategy helps explain the rapid rise of *B. distachyon* as a model for the grasses, another crucial factor was the conscious effort by a handful of early *B. distachyon* researchers to freely share material and information long before publication. This fostered the development of a collaborative and open community ethos that is the fertile ground necessary to grow a model system. As *B. distachyon* emerges from adolescence, its future is indeed bright.

Walnut Creek, CA, USA

John P. Vogel

# Contents

<b>The Rise of <i>Brachypodium</i> as a Model System . . . . .</b>	<b>1</b>
John P. Vogel	
<b>Phylogeny and Evolution of the Genus <i>Brachypodium</i> . . . . .</b>	<b>9</b>
Pilar Catalan, Diana López-Álvarez, Antonio Díaz-Pérez, Rubén Sancho, and María Luisa López-Herránz	
<b>Molecular Cytogenetics in the Genus <i>Brachypodium</i> . . . . .</b>	<b>39</b>
Robert Hasterok, Alexander Betekhtin, Natalia Borowska-Zuchowska, Agnieszka Braszewska-Zalewska, Dominika Idziak-Helmcke, Ewa Robaszkiewicz, and Elzbieta Wolny	
<b>The <i>Brachypodium distachyon</i> Reference Genome . . . . .</b>	<b>55</b>
Robert VanBuren and Todd C. Mockler	
<b><i>Brachypodium</i> Paleogenomics: From Genome Evolution to Translational Research in Grass Crops . . . . .</b>	<b>71</b>
Jérôme Salse	
<b>Genome Size and the Role of Transposable Elements . . . . .</b>	<b>81</b>
Alan H. Schulman	
<b>Genomic Diversity and Climate Adaptation in <i>Brachypodium</i> . . . . .</b>	<b>107</b>
Pip Wilson, Jared Streich, and Justin Borevitz	
<b>The microRNAs of <i>Brachypodium</i> . . . . .</b>	<b>129</b>
Karl R. Franke and Pamela J. Green	
<b>Transformation and T-DNA Mutagenesis . . . . .</b>	<b>147</b>
Mon Mandy Hsia and John P. Vogel	



<b>Chemical and Radiation Mutagenesis: Induction and Detection by Whole Genome Sequencing</b> . . . . .	155
Fabienne Granier, Aurélie Lemaire, Yin Wang, Philippe LeBris, Sébastien Antelme, John P. Vogel, Debbie Laudencia-Chingcuanco, and Richard Sibout	
<b>Library Resources: BACs, ESTs, Full-Length cDNAs, and Y2H</b> . . . . .	171
Keiichi Mochida and Kazuo Shinozaki	
<b><i>Brachypodium distachyon</i> Genetic Resources</b> . . . . .	183
David F. Garvin	
<b><i>Brachypodium distachyon</i> as a Model Species to Understand Grass Cell Walls</b> . . . . .	197
Joshua H. Coomey and Samuel P. Hazen	
<b>Brachypodium Seed: A Potential Model for Studying Grain Development of Cereal Crops</b> . . . . .	219
Jiajie Wu, Roger Thilmony, and Yong Gu	
<b>The <i>Brachypodium distachyon</i> Root System: A Tractable Model to Investigate Grass Roots</b> . . . . .	245
Christian S. Hardtke and David Pacheco-Villalobos	
<b>Dissecting the Control of Flowering Time in Grasses Using <i>Brachypodium distachyon</i></b> . . . . .	259
Daniel P. Woods and Richard M. Amasino	
<b>Brachypodium as a Model for Grass and Cereal Diseases</b> . . . . .	275
Luis A.J. Mur, Valentina Mazzamurro, and Nicola Pecchioni	
<b><i>Brachypodium</i> and the Abiotic Environment</b> . . . . .	291
David L. Des Marais and Thomas E. Juenger	
<b>The Genus <i>Brachypodium</i> as a Model for Perenniality and Polyploidy</b> . . . . .	313
Sean P. Gordon, Lifeng Liu, and John P. Vogel	
<b>Brachypodium as an Arabidopsis for the Grasses: Are We There Yet?</b> . . . . .	327
Christopher W.P. Lyons and Karen-Beth G. Scholthof	
<b>Index</b> . . . . .	343

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# The Rise of *Brachypodium* as a Model System

John P. Vogel

**Abstract** Grasses have played a central role in the formation of human civilization. Indeed, the inception of agriculture and the production of grains that could be easily stored and traded is one of the major factors that led to the creation of cities and the specialization of labor. Today, grasses provide the bulk of calories consumed by humans either directly through the consumption of grain or indirectly through grain and grass fed animals. Furthermore, due to their high productivity, grasses are increasingly utilized as a source of renewable biomass for the sustainable production of bioenergy and liquid biofuels. Grasses also play a fundamental role in many terrestrial ecosystems that benefit humans in numerous ways. Given the importance of grasses to humanity, there is considerable value in understanding their biology in great detail. Model biological systems greatly facilitate scientific research and many of the rapid advances in molecular biology and genetics would have been difficult to achieve without them. The model plant *Arabidopsis thaliana* has been used to make tremendous gains in our understanding of plant biology. However, as a eudicot, *A. thaliana* is unsuitable to study the unique aspects of grass biology. Several crop grasses (e.g. maize and rice) have been used as model systems and while each has certain strengths, they have some disadvantages when compared to a model like *A. thaliana*. *Brachypodium distachyon* has emerged to fill the need for a truly tractable model grass that is compatible with modern high-throughput molecular-genetic experiments. An overview of the development and widespread adoption of *B. distachyon* as a model grass is presented.

**Keywords** Grass • Bioenergy • Genome sequence • *Brachypodium* • Model system

## Importance of Grasses and the Need for a Model System

It is difficult to overstate the importance of grasses to humanity. They were a source of nutrition even before the advent of agriculture. The first known grinding of grass seed to produce flour occurred 30,000 years ago, long before the dawn of agriculture ~10,000 years ago (Aranguren et al. 2007; Revedin et al. 2010). Curiously, while no

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*Brachypodium* species are widely used as human food today, it was a species of *Brachypodium* that was ground for food ~30,000 years ago (Aranguren et al. 2007; Revedin et al. 2010). The advent of agriculture and the domestication of wheat and other grains ~10,000 years ago had certain advantages over hunting and gathering. The predictable production, high yields and storability of grains allowed larger numbers of humans to live in a single location. This ultimately led to the formation of cities and modern civilization. Grasses are still essential for modern civilization. Indeed, the majority of calories consumed by humans come directly or indirectly from grasses. In 2013, the four largest agricultural crops in terms of tonnage produced were all grasses (sugar cane, maize, rice, wheat) and these crops together had a higher production than the next 40 crops combined (<http://www.fao.org>).

Due to increasing human population and a rising standard of living in the developing world it is estimated that grain yields must increase by 70–100 % by 2050 to keep pace with demand (Editorial 2010; Godfray et al. 2011). Further complicating matters, these increases must occur despite the increasingly volatile and uncertain weather patterns associated with global climate change (Godfray et al. 2011; Wheeler and Von Braun 2013). Unfortunately, the current rate of yield increase for the major grains are not sufficient to meet projected needs (Godfray et al. 2010). As an example, in the 1990s, despite enormous investments, corn yield only increased 0.78 % annually (Kucharik and Ramankutty 2005). Thus, it will be necessary to use multiple strategies to accelerate yield increases if farmers are to meet demand (Fedoroff et al. 2010). Knowledge gained from model plants can be used to design rationale strategies for crop improvement and accelerate yield gains.

In addition to driving demand for food, increasing population and rising living standards are also driving an increase in demand for energy and transportation fuel. It is estimated that by 2024 global energy needs will increase 37 % (Development 2014). To minimize the effects of climate change, it is essential that sustainable energy sources be increasingly utilized and it is projected that biofuel production will increase 300 % by 2024 (Development 2014). The conversion of biomass derived from crop residues and dedicated energy crops into liquid transportation fuels may be a particularly important source of sustainable energy because electricity from wind or solar cannot fully substitute for gasoline and diesel with current battery technology. Due to their high productivity and ability to grow on marginal lands perennial grasses are projected to be extensively used as feedstocks for the production of biofuels (DOE 2006; Carroll and Somerville 2009). Unfortunately, the leading candidates for biomass crops such as switchgrass and *Miscanthus* are difficult to breed due to their perenniality, complex genetics, long generation time and polyploid genomes. Not surprisingly, these grasses are not fully domesticated. Indeed, current cultivars are essentially wild selections. Thus, knowledge gained from model systems may be especially useful for accelerating the domestication of these emerging crops (DOE 2006). In light of the need for fundamental knowledge to improve both food and biomass crops, it would be highly desirable to have a truly tractable model grass.

## Limitations of Other Models

Several cereal crops have served as model grasses over the years, but none possess the full suite of model organism traits: small size, rapid generation time, easily grown, self-fertile, diploid, small genome size, easily transformed (Brutnell et al. 2015). Maize and rice in particular have been widely utilized for a broad array of experiments. In some respects, maize is an ideal genetic model due to the ease with which crosses can be made, the huge amount of natural diversity and highly active endogenous transposons. However, stature and generation time present difficulties for researchers outside of large institutions with significant field programs and winter nurseries. Large size also makes growing large numbers of maize plants under controlled conditions prohibitively expensive. In addition, the complex maize genome and difficulties with transformation are impediments to many molecular genetic studies. Like maize, there is a large rice research community and significant experimental resources. Though much smaller than maize, its size and demanding growth conditions remain significant obstacles for researchers outside of large institutions in rice growing regions. In addition, the economic importance of rice has resulted in restrictive intellectual property rights and import quarantine restrictions that limit the free sharing of germplasm and other resources. Finally, as a semi-aquatic tropical grass, rice is not an ideal model for temperate grains, forage, and biomass crops.

In contrast to maize and rice, *A. thaliana* is an ideal model plant. Its small size, simple growth requirements and rapid generation time facilitate the growth of large numbers of plants under controlled conditions. Further increasing its values as a model system, it has one of the smallest genomes of any plant and is extremely easy to transform (Clough and Bent 1998; Kaul et al. 2000). Thus, it is no surprise that a large, vibrant, and open community of *A. thaliana* researchers has emerged (Meyerowitz 2001). Together, this community created a wealth of easily accessible experimental resources that have further increased the power of *A. thaliana* as a model system (<https://www.arabidopsis.org/>). Unfortunately, as a eudicot *A. thaliana* is not an ideal model for the grasses. The aspects of grass biology not shared with *A. thaliana* (e.g. cell wall composition, development, grain production, mycorrhizal associations) simply cannot be studied in *A. thaliana*. Also, given the evolutionary separation of the eudicots and grasses, even conserved areas of plant biology are expected to have significant differences at the gene level that may limit the utility of *A. thaliana* as a model for the grasses (Hayama and Coupland 2004). Thus, it would be extremely useful to have a truly tractable model grass with the attributes that have made *A. thaliana* such a powerful model.

## Early *Brachypodium* Research

Prior to 1995, most publications that mentioned *Brachypodium* species focused on ecology (e.g. Davis et al. 1985) while a few studies described the phylogenetic relationships in the genus, which was particularly interesting because of the high

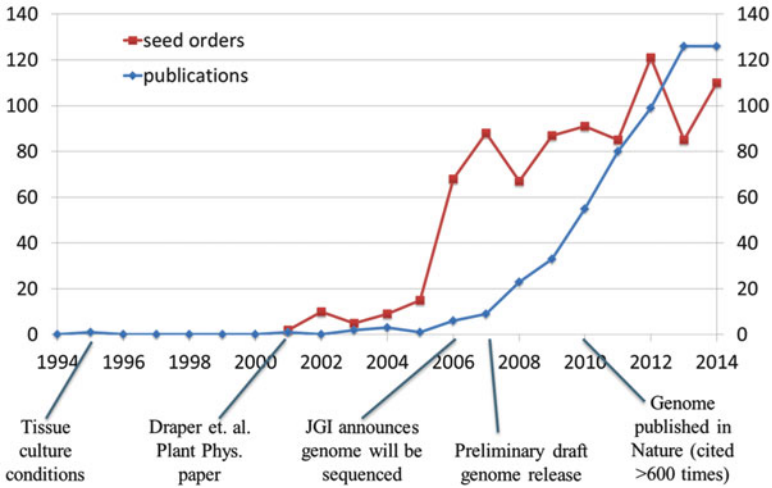


degree of variation in chromosome number and ploidy (e.g. Khan 1992; Shi et al. 1993). In 1995, Bablak et al. published a paper that explicitly proposed *B. distachyon* as model grass (Bablak et al. 1995). In this paper they described tissue culture conditions for *B. distachyon* as a first step toward developing a transformation system, a prerequisite for any modern model system. It was not until 2001 that a second paper from the same group presented a much more extensive case for using *B. distachyon* as a model system (Draper et al. 2001). This paper described biological traits that were compatible with its use as a model system including genome size, generation time, and mature size. In addition, they challenged several accessions with various pathogens and noted a range of responses indicating the potential for *B. distachyon* to serve as a model for plant-pathogen interactions. They also used biolistics to successfully transform a polyploid accession (now classified as a distinct species, *B. hybridum* (Catalán et al. 2012)). Taken together these results made a powerful case for the utility of *B. distachyon* as a model grass.

The first significant genomic resource created for the genus *Brachypodium* was a *B. sylvaticum* BAC library (Foote et al. 2004). In this case, the ultimate goal was to use the *B. sylvaticum* genome as a structural model for the much more complex wheat genome. Significantly, they noted that *B. sylvaticum* probes were more likely to work as markers for wheat than were rice probes, presumably due to greater sequence conservation between wheat and *B. sylvaticum* than between wheat and rice. The next publication developing *B. distachyon* as a model system came in 2006. This publication presented two key advances (Vogel et al. 2006). The first was a set of freely available inbred lines. These lines have since been distributed to hundreds of laboratories around the world. It is noteworthy that the lines used in the study by Draper et al. in 2001 were only available through an MTA that was too restrictive for many institutions (for an excellent review of the early days of *B. distachyon* research see Lyons and Scholthof 2015). The second advance in this paper was an *Agrobacterium*-mediated transformation protocol for the true *B. distachyon*. While the transformation efficiency was not high, it demonstrated that *B. distachyon* could be transformed using *Agrobacterium tumefaciens*, an absolute requirement for a truly tractable model system (see the chapter Phylogeny and evolution of the genus *Brachypodium*).

## A New Paradigm for Model Systems

Up to this point, the development of *B. distachyon* as a model followed a traditional trajectory of slowly increasing use and gradual development of experimental resources. However, a confluence of factors led to an accelerated developmental path in which a genome sequencing project greatly stimulated the adoption of *B. distachyon* as a model system (reviewed in Lyons and Scholthof 2015). In 2005 The U.S. Department of Energy held a workshop to develop a research plan to accelerate the development of cellulosic biofuels. During this workshop it was decided that *B. distachyon* could serve as a tractable model for the large perennial



**Fig. 1** A model takes hold. Seed distribution and publications are indicators of the size of the *B. distachyon* research community. Total seed orders distributed by the Vogel lab, the Garvin lab (prior to 2010 only) and the National Plant Germplasm System (prior to 2014 only) are plotted. The total number of seed packets shipped is 15,186. In addition, many secondary distributions are presumed to have occurred. Publications using *B. distachyon* as a molecular, genetic or physiological model system are plotted. The total number of publications is 564. Publications focused on ecology were not included. Key developments are noted below the graph

grasses being developed as biomass crops (e.g. switchgrass and *Miscanthus*). The report from this workshop outlined a plan for developing resources necessary to use *B. distachyon* as a model system (DOE 2006). Subsequent funding from the DOE led to the development of many resources for this nascent model system (Vogel and Hill 2008; IBI 2010; Bragg et al. 2012; Jeong et al. 2013; Gordon et al. 2014; Tyler et al. 2014). In addition to standard research grants, the DOE invested in *B. distachyon* research by sequencing the genome through the DOE Joint Genome Institute (IBI 2010). This represented a significant investment since it was based on Sanger sequencing alone. As soon as the genome sequencing project was announced, the demand for *B. distachyon* seed spiked upward and has remained high ever since (Fig. 1). Similarly, shortly after the first draft genome sequence was released the number of publications rose dramatically (Fig. 1).

## Widespread Adoption and Outlook

Concurrent with the genome sequencing project, other essential resources were developed (reviewed in Brkljacic et al. 2011). In 2008, two methods for high-efficiency transformation were published (Vain et al. 2008; Vogel and Hill 2008). These methods and further refinements, made *B. distachyon* one of, if not the,

easiest grass to transform (Bragg et al. 2015; Chapter Transformation and T-DNA mutagenesis). The next logical step was the creation of insertional mutant collections (Thole et al. 2010; Bragg et al. 2012; <http://jgi.doe.gov/our-science/science-programs/plant-genomics/brachypodium/brachypodium-t-dna-collection/>). These key resources further accelerated the adoption of *B. distachyon* as a model grass. The growth of the *B. distachyon* research community can be estimated by the number of seeds distributed and the number of publications. By both measures, the community is continuing to grow rapidly (Fig. 1). The resources available for *B. distachyon* are now being leveraged to use other species in the genus as models for polyploid genome evolution and regulation and for the study of perenniality (Chapter The genus *Brachypodium* as a model for perenniality and polyploidy).

The outlook for *B. distachyon* and sister species as model systems is very bright. A critical mass of researchers and resources has clearly been established. These resources are being used for an ever-expanding range of projects as described in the remainder of this book.

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## References

- Aranguren B, Becattini R, Lippi MM, Revedin A. Grinding flour in Upper Palaeolithic Europe (25 000 years bp). *Antiquity*. 2007;81(314):845–55.
- Bablak P, Draper J, Davey MR, Lynch PT. Plant regeneration and micropropagation of *Brachypodium distachyon*. *Plant Cell Tiss Org Cult*. 1995;42(1):97–107.
- Bragg JN, Anderton A, Nieu R, Vogel JP. *Brachypodium distachyon*. *Methods Mol Biol*. 2015;1223:17–33.
- Bragg JN, Wu J, Gordon SP, Guttman ME, Thilmony R, Lazo GR, et al. Generation and characterization of the Western Regional Research Center *Brachypodium* T-DNA insertional mutant collection. *PLoS One*. 2012;7(9):e41916.
- Brkljacic J, Grotewold E, Scholl S, Mockler T, Garvin D, Vain P, et al. *Brachypodium* as a model for the grasses: today and the future. *Plant Physiol*. 2011;157:3–13.
- Brtnell TP, Bennetzen JL, Vogel JP. *Brachypodium distachyon* and *Setaria viridis*: model genetic systems for the grasses. *Annu Rev Plant Biol*. 2015;66:465–85.
- Carroll A, Somerville C. Cellulosic biofuels. *Annu Rev Plant Biol*. 2009;60:165–82.
- Catalán P, Müller J, Hasterok R, Jenkins G, Mur LAJ, Langdon T, et al. Evolution and taxonomic split of the model grass *Brachypodium distachyon*. *Ann Bot*. 2012;109(2):385–405.
- Clough SJ, Bent AF. Floral dip: a simplified method for *Agrobacterium*-mediated transformation of *Arabidopsis thaliana*. *Plant J*. 1998;16(6):735–43.
- Davis BNK, Lakhani KH, Brown MC, Park DG. Early seral communities in a limestone quarry: an experimental study of treatment effects on cover and richness of vegetation. *J Appl Ecol*. 1985;22(2):473–90.
- DOE, editor. Breaking the biological barriers to cellulosic ethanol: a joint research agenda. U.S. Department of Energy, Office of Science and Office of Energy Efficiency; 2006. <http://genomicsgtl.energy.gov/biofuels/b2bworkshop.shtml>.
- Draper J, Mur LAJ, Jenkins G, Ghosh-Biswas GC, Bablak P, Hasterok R, et al. *Brachypodium distachyon*. A new model system for functional genomics in grasses. *Plant Physiol*. 2001;127(4):1539–55.

- Editorial. How to feed a hungry world. *Nature*. 2010;466(7306):531–2.
- Fedoroff NV, Battisti DS, Beachy RN, Cooper PJM, Fischhoff DA, Hodges CN, et al. Radically rethinking agriculture for the 21st century. *Science*. 2010;327(5967):833–4.
- Foot T, Griffiths S, Allouis S, Moore G. Construction and analysis of a BAC library in the grass *Brachypodium sylvaticum*: its use as a tool to bridge the gap between rice and wheat in elucidating gene content. *Funct Integr Genomics*. 2004;4(1):26–33.
- Godfray HCJ, Beddington JR, Crute IR, Haddad L, Lawrence D, Muir JF, et al. Food security: the challenge of feeding 9 billion people. *Science*. 2010;327(5967):812–8.
- Godfray HCJ, Pretty J, Thomas SM, Warham EJ, Beddington JR. Linking policy on climate and food. *Science*. 2011;331(6020):1013–4.
- Gordon SP, Priest H, Des Marais DL, Schackwitz W, Figueroa M, Martin J, et al. Genome diversity in *Brachypodium distachyon*: deep sequencing of highly diverse inbred lines. *Plant J*. 2014;79(3):361–74.
- Hayama R, Coupland G. The molecular basis of diversity in the photoperiodic flowering responses of *Arabidopsis* and rice. *Plant Physiol*. 2004;135(2):677–84.
- IBI. Genome sequencing and analysis of the model grass *Brachypodium distachyon*. *Nature*. 2010;463:763–8.
- Jeong DH, Schmidt SA, Rymarquis LA, Park S, Ganssmann M, German MA, et al. Parallel analysis of RNA ends enhances global investigation of microRNAs and target RNAs of *Brachypodium distachyon*. *Genome Biol*. 2013;14(12):R145.
- Kaul S, Koo HL, Jenkins J, Rizzo M, Rooney T, Tallon LJ, et al. Analysis of the genome sequence of the flowering plant *Arabidopsis thaliana*. *Nature*. 2000;408(6814):796–815.
- Khan MA. Seed-protein electrophoretic pattern in *Brachypodium* P. Beauv. species. *Ann Bot*. 1992;70(1):61–8.
- Kucharik CJ, Ramankutty N. Trends and variability in U.S. Corn yields over the twentieth century. *Earth Interact*. 2005;9(1):1–29.
- Lyons C, Scholthof K. Watching grass grow: the emergence of *Brachypodium distachyon* as a model for the Poaceae. In: Phillips D, Kingsland S, editors. *New perspectives on the history of life sciences and agriculture*. Archimedes 40; 2015:479–501. [http://link.springer.com/chapter/10.1007%2F978-3-319-12185-7\\_23](http://link.springer.com/chapter/10.1007%2F978-3-319-12185-7_23)
- Meyerowitz EM. Prehistory and history of *Arabidopsis* research. *Plant Physiol*. 2001;125(1):15–9.
- Organisation for Economic Co-Operation and Development. *World Energy Outlook 2014*. Organisation for Economic Co-Operation and Development; 2014. ISBN: 978-92-64-20804-9.
- Revedin A, Aranguren B, Becattini R, Longo L, Marconi E, Lippi MM, et al. Thirty thousand-year-old evidence of plant food processing. *Proc Natl Acad Sci U S A*. 2010;107(44):18815–9.
- Shi Y, Draper J, Stace C. Ribosomal DNA variation and its phylogenetic implication in the genus *Brachypodium* (Poaceae). *Plant Syst Evol*. 1993;188(3–4):125–38.
- Thole V, Worland B, Wright J, Bevan MW, Vain P. Distribution and characterization of more than 1000 T-DNA tags in the genome of *Brachypodium distachyon* community standard line Bd21. *Plant Biotechnol J*. 2010;8(6):734–47.
- Tyler L, Fangel JU, Fagerström AD, Steinwand MA, Raab TK, Willats WGT, et al. Selection and phenotypic characterization of a core collection of *Brachypodium distachyon* inbred lines. *BMC Plant Biol*. 2014;14(1):25.
- Vain P, Worland B, Thole V, McKenzie N, Alves SC, Opanowicz M, et al. *Agrobacterium*-mediated transformation of the temperate grass *Brachypodium distachyon* (genotype Bd21) for T-DNA insertional mutagenesis. *Plant Biotechnol J*. 2008;6:236–45.
- Vogel J, Hill T. High-efficiency *Agrobacterium*-mediated transformation of *Brachypodium distachyon* inbred line Bd21-3. *Plant Cell Rep*. 2008;27(3):471–8.
- Vogel JP, Garvin DF, Leong OM, Hayden DM. *Agrobacterium*-mediated transformation and inbred line development in the model grass *Brachypodium distachyon*. *Plant Cell Tiss Org Cult*. 2006;85:199–211.
- Wheeler T, Von Braun J. Climate change impacts on global food security. *Science*. 2013;341(6145):508–13.

# Phylogeny and Evolution of the Genus *Brachypodium*

Pilar Catalan, Diana López-Álvarez, Antonio Díaz-Pérez, Rubén Sancho, and María Luisa López-Herránz

**Abstract** We present an updated review of the phylogenetic and evolutionary studies conducted on the model genus *Brachypodium*. The genus, which contains approximately 20 globally distributed taxa (17 species, 1 variety, and 2 undescribed cytotypes) shows an intermediate evolutionary placement within the grass temperate pooid clade, being closer to the basal than to the recent Pooideae lineages. Our comprehensive molecular phylogenetic survey of all the currently known *Brachypodium* lineages illustrates a complex reticulate scenario of recently evolved diploid and allopolyploid lineages. Haplotypic statistical parsimony networks, multilabelled (multigenic) Minimum Evolution gene tree discordances, and Bayesian dating analysis have provided a testable hypothesis for the reconstruction of the *Brachypodium* species tree and for the estimation of its nodal divergence times. Our results support the early splits of the annual and short-rhizomatose lineages (*B. stacei*, *B. mexicanum*, *B. distachyon*) in the Holarctic region during the early-Middle Miocene (and *B. hybridum* in the Pleistocene), and a profusion of rapid splits for the perennial lineages since the late Miocene to the Pleistocene in the Mediterranean and Eurasian regions, with sporadic colonizations of more remote areas. Several perennial allopolyploid species (*B. boissieri*, *B. retusum*, *B. phoenicoides*, *B. rupestre* 4x, *B. pinnatum* 4x) showed homeologous copies from both ancestral and recent genome donors. More in-depth studies of the species of the *B. distachyon* complex have demonstrated the polyphyletic origin of the allotetraploid *B. hybridum* from bidirectional crosses of its diploid *B. stacei* and

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*B. distachyon* parents. Our niche modeling analysis has also detected distinct adaptations to different ecological tolerances in the diploids and evidence of niche conservatism for *B. hybridum* and each of its parents in their native Mediterranean region. Future perspectives include ongoing comparative genomics, phylogenomic and genotype-based phylogeographic studies of *Brachypodium*.

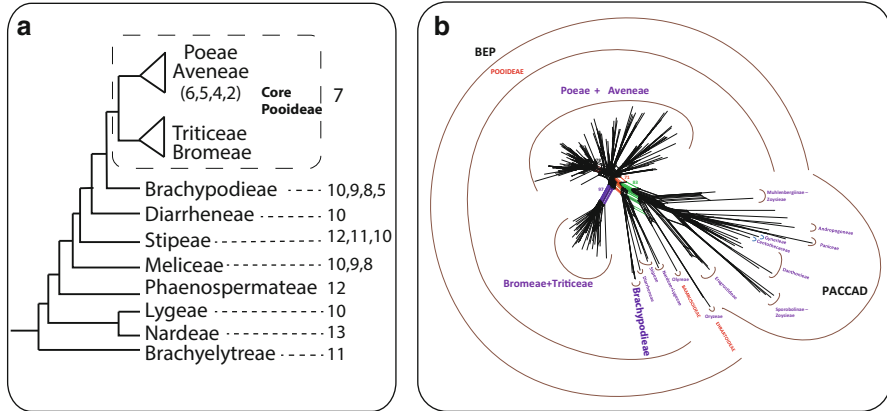
**Keywords:** Annual and perennial *Brachypodium* species • Dated phylogeny • Environmental niche modeling (*B. distachyon* group) • Haplotypic networks • Multigenic Minimum Evolution species tree • (allo)polyploid complexes

## Evolutionary Placement of *Brachypodium* within the Poaceae Tree

The genus *Brachypodium* has received considerable attention since the selection of the annual species *B. distachyon* as model functional plant for temperate cereals and biofuel grasses (IBI 2010; Catalán et al. 2014; Mur et al. 2011). Recently, the three segregated annual species of the *B. distachyon* complex (*B. distachyon*, *B. stacei*, *B. hybridum*; Catalán et al. 2012) have been proposed as a model system for grass polyploid speciation (Catalán et al. 2014) and the whole genus, containing taxa characterized by their small-size and compact genomes (Mur et al. 2011; Betekhtin et al. 2014), is also seen as an ideal candidate for comparative genomics of monocots.

Decades of systematic and phylogenetic studies were necessary, however, to frame its evolutionary position within the grasses. *Brachypodium* is considered today the single representative genus of the monotypic tribe Brachypodieae, which constitutes one of the intermediate diverging lineages of the temperate Pooideae grasses (Catalán et al. 1997; Bouchenak-Khelladi et al. 2008; Schneider et al. 2011) (Fig. 1). Its controversial position was caused by its shared or similar morphological and anatomical traits with distinct pooid groups (Catalán et al. 1995, and references therein). Consequently, it was classified in different tribes, based on the possession of embryo with mesocotyl (Poeae), hairy terminal ovary appendage and long narrow caryopsis and hilum (Bromeae), or spicate to racemose inflorescence and hairy lodicles (Triticeae), until its definitive adscription to its own tribe Brachypodieae (Jacques-Félix 1962; Schippmann 1991; Watson and Dallwitz 1992). Subsequently, its separate tribal treatment was confirmed by a number of private biological (embryo development), biochemical (exclusive seed storage proteins, seed globulins, seed storage polysaccharides and stem and leaf fructosans) (Schippmann 1991), and karyotype (large disploidy) (Robertson 1981; Khan 1984) characters.

The most recent phylogenetic works have consistently resolved Brachypodieae as the sister lineage of the recently evolved core pooid clade of temperate cereals and forages [Triticodae (Triticeae + Bromeae)/Poodae (Poeae + Aveneae)] (Fig. 1a). Its intermediate placement between the basal (Brachyelytreae, Lygeae-Nardeae, Phaenospermatæ, Meliceae, Stipeae) and the recently evolved (Triticodae/Poodae) Pooideae lineages has been recovered from both plastid and nuclear based topologies (Catalán et al. 1997; Davis and Soreng 2007; Bouchenak-



**Fig. 1** (a) Summarized plastid phylogeny of the temperate grasses showing the evolutionary placement of *Brachypodium* (Brachypodieae) between the early diverging and the recently evolved Pooideae tribes, and the intermediacy of its chromosome base numbers. (b) NeighborNet partition network tree based on nuclear  $\beta$ -amylase sequences showing the phylogenetic relationships of major tribal and subtribal grass lineages; *Brachypodium* is resolved close to the basal pooids. Pooideae (green), core pooids (red) and Triticaceae + Bromeae (purple) splits showing bootstrap support values. Subfigure (a) partially adapted and updated from Catalán et al. (1997; Fig. 4); subfigure (b) adapted from Minaya et al. (2015; Fig. 3)

Khelladi et al. 2008; Schneider et al. 2011) and from combined analysis of molecular and morphological data (GPWG 2001). An intermediate position in the Pooideae tree is also reconstructed for the isolated *Diarrhena* (Diarrheneae) lineage, which apparently split earlier than *Brachypodium* (Catalán et al. 1997; Davis and Soreng 2007; Schneider et al. 2011). Recent phylogenetic studies based on a low copy nuclear gene ( $\beta$ -amylase) showed, however, that *Brachypodium* and *Diarrhena* could be closer to the basal pooids than to the recently evolved core pooid clade (Minaya et al. 2015; Fig. 1b). The two independent and small monogeneric Brachypodieae and Diarrheneae tribes present remarkable embryo features (bambusoid-like in *Diarrhena*, first lateral stem developing from coleoptile in *Brachypodium*), with *Brachypodium* also showing intermediate chromosome base numbers when mapped into the pooid tree (Catalán et al. 1997). A karyotype evolutionary trend of increasing chromosome sizes and decreasing chromosome base numbers is observed in the Pooideae, ranging from basal tribes with small chromosomes and high chromosome base numbers (Brachyelytreae = 11; Lygeae = 10; Nardeae = 13; Phaenospermateae = 12; Meliceae = 10, 9, 8; Stipeae = 12, 11, 10; Diarrheneae = 10), through the intermediate ones of Brachypodieae (10, 9, 8, but also 5), to the large chromosomes and almost constant chromosome base number of  $x = 7$  present in the more recently evolved Triticodae + Pooideae although  $x = 6, 5, 4, 2$  occasionally occur in Aveneae (Pooideae) (Fig. 1a).

The isolated monophyly of *Brachypodium*, close but divergent from the core pooid clade (Fig. 1a, b), corroborates other unique genomic features reported for this genus, like the possession of small genomes with low amounts of repetitive DNA (Shi et al. 1993) and of private repetitive DNA and ribosomal DNA families and nuclear RFLP markers (Catalán et al. 1995). Recent studies have confirmed that *Brachypodium*

combines both genus-specific and core-pooids-type or basal-pooids-BEP-type genomic traits. *Brachypodium* exhibits EST (expressed sequence tag)-contig chromosomal orthology, and similar globulin gene duplication and loci controlling phenotypic traits [e.g., spiking Eps-A (m)1, earliness Mot1 and FtsH4] and pathogen resistance (e.g., stem rust resistance, Rpg1 and Rpg4) responses with the Triticeae; however, it lacks colinearity for several STS (sequence tagged sites) and other stress controlling genes with this tribe (Mur et al 2011, and references therein). Also, the *Brachypodium* genome shows greater synteny with the more ancestral *Oryza* (Ehrartoideae, early BEP lineage) genome than with the more recently evolved Triticeae genomes, probably due to accelerated genomic rearrangements in the Triticeae (Mur et al. 2011). Despite these findings, the *Brachypodium* genome is more closely related to the core pooid genomes than the rice genome, and, together with its intermediate evolutionary position within the BEP clade (Fig. 1b), is well placed to serve as model plant not only for the temperate cereals and forages but also for tropical PACCMAD grasses including species proposed as biofuel crops (e.g. *Miscanthus*, *Panicum* (switchgrass), *Paspalum*) (Mur et al. 2011; Catalán et al. 2014).

## Systematics of *Brachypodium*

*Brachypodium* is a relatively small genus that contains ca. 18 species distributed worldwide (Schippmann 1991; Catalán and Olmstead 2000; Catalán et al. 2012) (Table 1; Fig. 2). According to the most recent taxonomic updating (Catalán et al. 2012; Diaz-Pérez et al. unpub. data), 3 of them are annual species and 15 are perennial taxa. It has been recently demonstrated that the three annuals have a large distribution in their native circumMediterranean region (*B. distachyon*, *B. stacei*, *B. hybridum*) (Catalán et al. 2012; López-Alvarez et al. 2012, 2015). Among the perennials, few species show a large native Eurasian (*B. sylvaticum*, *B. pinnatum*, *B. rupestre*) or Mediterranean (*B. retusum*) distribution, whereas the rest have a restrict disjunct distributions in their respective native ranges [W Mediterranean (*B. phoenicoides*), C Mediterranean (*B. genuense*), E Mediterranean—SW Asia (*B. glaucovirens*), S Spain (*B. boissieri*), Canary isles (*B. arbuscula*), South Africa (*B. bolusii*), tropical and South Africa (*B. flexum*), Madagascar (*B. madagascariense*), Taiwan (*B. kawakamii*), SE Asia—New Guinea (*B. sylvaticum* var. *pseudodistachyon*), and America (*B. mexicanum*)] (Schippmann 1991; Diaz-Pérez et al. unpub. data; Fig. 2). Since 1812 two segregated genera were erected, *Trachynia* Link, to cover the annual species, and *Brevipodium* Lovè & Lovë, to accommodate *B. sylvaticum*; however, in almost all modern works neither of these two segregates were recognized (Catalán et al. 1995), and all the newly described species have been subsumed within *Brachypodium* (Schippmann 1991; Catalán and Olmstead 2000; Catalán et al. 2012).

The annual species are characterized by their short life-cycle, ephemeral habit and self-fertility (Catalán and Olmstead 2000; Catalán et al. 2012). Recent analysis of cryptic phenotypic, cytogenetic and molecular traits allowed us to separate the three species (Catalán et al. 2012). By contrast, most of the perennial taxa show long-rhizomes and self-incompatibility (Catalán et al. 1995; Khan and Stace 1999),



**Table 1** List of world *Brachypodium* taxa, cytotypes and ecotypes used in the phylogenetic analysis

Taxon	Code	Geographical distribution (native range)	2n	x	Ploidy	Genome size (pg/2C)
Annuals						
<i>B. distachyon</i> (L.) P. Beauv.	Bdis	circumMediterranean (Mediterranean, SW Asia)	2n = 10	5	2x	0.63
<i>B. stacei</i> Catalán, Joch. Müll., Mur & Langdon	Bsta	circumMediterranean (Mediterranean, Macaronesia, SW Asia)	2n = 20	10	2x	0.56
<i>B. hybridum</i> Catalán, Joch. Müll., Hasterok & Jenkins	Bhyb	circumMediterranean (Mediterranean, Macaronesia, SW Asia)	2n = 30	5 + 10	4x	1.26
Short-rhizomatose perennial						
<i>B. mexicanum</i> (Roem. & Schult.) Link	Bmex	America (from Mexico to N Bolivia)	2n = 40	10?	4x?	?
Long-rhizomatose perennials						
<i>B. arbuscula</i> Gay ex Knoche	Barb	Macaronesia; Canary isles (Spain)	2n = 18	9	2x	0.70
<i>B. boissieri</i> Nym.	Bboi	Spain: Betic mountain ranges (southern Spain)	2n = 42, 46	?	6x–8x?	?
<i>B. bolusii</i> Stapf	Bbol	South Africa	?	?	?	?
<i>B. flexum</i> Nees	Bflex	Tropical Africa and South Africa	?	?	?	?
<i>B. genuense</i> (D.C.) Roem. & Schult.	Bgen	Italy	2n = 18	9	2x	?
<i>B. glaucovirens</i> (Murb.) Sagorski	Bgla	East Mediterranean and SW Asia	2n = 16	8	2x	0.88
<i>B. kawakamii</i> Hayata	Bkaw	Taiwan	?	?	?	?
<i>B. madagascariense</i> Camus & Perrier	Bmad	Madagascar	?	?	?	?
<i>B. phoenicoides</i> (L.) P. Beauv. ex Roem. & Schultes	Bpho	West Mediterranean	2n = 28	5 + 9	4x	1.49
<i>B. pinnatum</i> (L.) P. Beauv. (diploid A)	Bpin2xA	Eurasia	2n = 18	9	2x	0.88
<i>B. pinnatum</i> (L.) P. Beauv. (tetraploid)	Bpin4x	Eurasia	2n = 28	5 + 9	4x	1.57
<i>B. pinnatum</i> (L.) P. Beauv. (diploid B)	Bpin2xB	SW Asia	2n = 16	8	2x	?

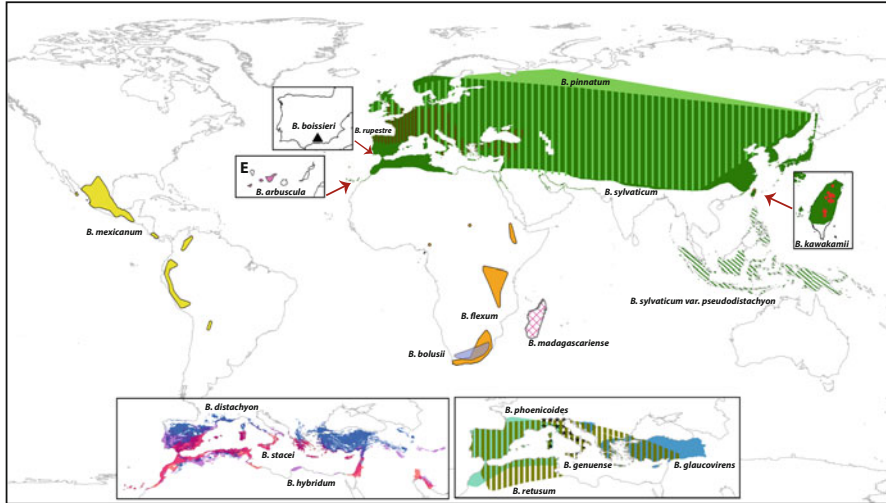
(continued)

Table 1 (continued)

Taxon	Code	Geographical distribution (native range)	2n	x	Ploidy	Genome size (pg/2C)
<i>B. retusum</i> (Pers.) P. Beauv.	Bret	Mediterranean	2n = 36	?	6x?	2.57
<i>B. rupestre</i> (Host) Roem. & Schult. (diploid)	Brup2x	West Eurasia	2n = 18	9	2x	0.84
<i>B. rupestre</i> (Host) Roem. & Schult. (tetraploid)	Brup4x	West Eurasia	2n = 28	5 + 9	4x	?
<i>B. sylvaticum</i> (Huds.) P. Beauv.	Bsyl	panEurasia (Eurasia, Macaronesia)	2n = 18	9	2x	0.87
<i>B. sylvaticum</i> (Huds.) P. Beauv. (Eastern lineage)	BsylEAs	East Asia (China)	?	?	?	?
<i>B. sylvaticum</i> var. <i>pseudodistachyon</i> J. D. Hook.	Bsyp	Malesia and New Guinea	?	?	?	?

Geographical distribution taken from Schippmann (1991), Catalán et al. (1995, 2012), Catalán and Olmstead (2000), López-Alvarez et al. (2012, 2015); 2n, x, ploidy level and genome size taken from Robertson (1981), Khan (1984), Schippmann (1991), Wolny and Hasterok (2009), Catalán et al. (2012), Betekhtin et al. (2014)

Information on geographical distribution in the native range, code, chromosome number (2n), chromosome base number (x), ploidy level and genome size is provided for each taxon. ? = unknown or unclear



**Fig. 2** Geographic distributions of the 18 world *Brachypodium* taxa (*B. arbuscula*, pink; *B. boissieri*, black triangle; *B. bolusii*, violet; *B. distachyon*, dark blue; *B. flexum*, orange; *B. genuense*, black star; *B. glaucovirens*, pale blue; *B. hybridum*, purple; *B. kawakamii*, red dot; *B. madagascariense*, open red square; *B. mexicanum*, yellow; *B. phoenicoides*, aquamarine; *B. pinnatum*, bright green; *B. retusum*, pale brown; *B. rupestre*, dark brown; *B. stacei*, red; *B. sylvaticum*, dark green; *B. sylvaticum* var. *pseudodistachyon*, diagonal dark green line)

except *B. mexicanum* and *B. sylvaticum* that are self-compatible (Khan and Stace 1999; Steinwand et al. 2013). *B. mexicanum* differs from them in its short-rhizomatous habit and self-compatibility (Khan and Stace 1999), taxonomically resembling more the annual than the perennial taxa in those traits and in seed protein contents and RFLP patterns (Khan 1992; Shi et al. 1993). The rhizomatous perennials are separated by their morphoanatomical and karyotypic traits. *B. arbuscula*, *B. retusum* and *B. boissieri* bear branched woody stems and long-lasting innovations. The Canarian *B. arbuscula* possesses top branched buds and dispersed root xylem and phloem, and grows in more humid places, whereas *B. retusum* and *B. boissieri* are adapted to xeric Mediterranean habitats and show strongly inrolled leaves. The narrow endemic *B. boissieri*, previously circumscribed within the broadly spread *B. retusum*, differs from it in its single-spikelet inflorescence, short habit and leaf blade morphology and anatomy (Schippmann 1990); the species is confined to dolomitic mountain ranges of southern Spain. The remaining taxa of the core perennial clade do not bear branched stems. The endemic alpine *B. kawakamii* and *B. bolusii* have a relatively short stature and dense, erect, and glabrous leaves; the inflorescences of *B. bolusii* present more spikelets but with less fertile florets than those of *B. kawakamii*. *B. pinnatum*, *B. rupestre* and *B. phoenicoides* show erect panicles. *B. phoenicoides*, adapted to dry places, is glabrous and presents partially inrolled leaves, semi-patent twisted spikelets and awnless lemmas, whereas the mesic *B. pinnatum* and *B. rupestre* have short awns and bright green colored leaves.

*B. rupestre*, considered until recently a subspecies of *B. pinnatum*, differs from it in its glabrous leaves and spikelets and in leaf epidermal traits (Schippmann 1991). The central Mediterranean endemic *B. genuense*, classified within *B. pinnatum* by some authors (Clayton et al. 2015), departs from it based on its particular karyotype, showing co-localized 5S and 25S rDNA loci in the same chromosome (Betekhtin pers. com.) and minor morphological differences (Valdés and Scholz 2009).

*Brachypodium sylvaticum* is the most distinct and widespread species of the genus. Its native Palearctic area ranges from Macaronesia in the west to New Guinea in the east (Fig. 2). It is characterized by its nodding panicle, densely hairy habit and long-awned lemma. Most of these features are also shared by the tropical and South African *B. flexum* and the Malagasy *B. madagascariense*, though they differ from the former in their shorter panicles, spikelets and awns, and from each other in the overall smaller habit of the mountain endemic island species. The ‘*B. sylvaticum*’ complex also includes the eastern Mediterranean—SW Asian endemic *B. glaucovirens*. This taxon, formerly synonymized to *B. sylvaticum*, or even considered a hybrid between this species and *B. pinnatum* (Schippmann 1991), has been recently recognized as a separate species (Scholz 2007). Morphologically it shows intermediate features, resembling *B. sylvaticum* in its short rhizome and long awn, and *B. pinnatum* in its bright green leaf color, broad leaf ribs and erect panicle. Furthermore, some of the six infraspecific *B. sylvaticum* taxa described in eastern Asia and Malesia—New Guinea (Schippmann 1991), like *B. sylvaticum* subsp. *pseudodistachyon*, which is characterized by its mountain dwarf habit and stiff leaves, could correspond to independent species.

Taxonomic uncertainty still persists among some poorly known extra-European taxa and within some Eurasian cryptic complex taxa (Schippmann 1991; Catalán and Olmstead 2000). Among the less known extra-European taxa, up to 5 different species have been described in America, 11 in Africa and 15 in Asia; however, most of them could probably be synonymized to currently recognized species from those regions (cf. Schippmann 1991). Regarding the Eurasian cryptic taxa, they correspond to ploidy complexes of putative diploid parents and their derived allopolyploids, involving different cytotypes of *B. pinnatum* (2x, 4x) and *B. rupestre* (2x, 4x) (Khan and Stace 1999; Wolny and Hasterok 2009; Betekhtin et al. 2014, and references therein). The intraspecific cytotypes could hardly be differentiated based on morphological traits; however, cytogenetic studies using Comparative Chromosome Painting (CCP) approaches suggest that the allopolyploids derive from interspecific crosses of distinct diploid progenitors, involving their respective diploid perennial counterparts (Wolny and Hasterok 2009; Idziak et al. 2014), or even those perennials and the annual *B. distachyon* (Wolny and Hasterok 2009; Betekhtin et al. 2014). The genus shows a remarkable disploidy, with chromosome base numbers of diploids ranging from the presumably more ancestral  $x = 10$  (*B. stacei*), through  $x = 9$  (*B. arbuscula*, *B. sylvaticum*, *B. pinnatum*, *B. rupestre*) and  $x = 8$  (*B. glaucovirens*), to  $x = 5$  (*B. distachyon*) (Robertson 1981; Betekhtin et al. 2014). Noticeably, the ‘recently evolved’ chromosome base number  $x = 7$  (Robertson 1981), which is almost fixed in most species of the large and young core pooid clade (Catalán et al. 1997), is apparently absent in *Brachypodium*, where

tetraploid species with  $2n = 28$  chromosomes have been found to be hybrid allopolyploids, potentially derived from diploid  $2n = 18$  ( $x = 9$ ) and  $2n = 10$  ( $x = 5$ ) progenitors (Khan and Stace 1999; Wolny and Hasterok 2009; Betekhtin et al. 2014). Betekhtin et al. (2014) proposed two alternative hypotheses for karyotype evolution in *Brachypodium*, continuous descendant dispoloidy ( $x = 10$  to  $x = 9$ , 8 to  $x = 5$ ) vs. descendant + ascendant dispoloidy ( $x = 10$  to  $x = 5$  to  $x = 9$ , 8), with allotetraploid  $2n = 28$  species originating always in a later stage.

The taxonomic identity of these allotetraploid cytotypes is still unclear, though they might constitute separate species, paralleling the case of the segregated annual species of the diploid-allopolyploid *B. distachyon* complex (Catalán et al. 2012). Overall, *Brachypodium* constitutes a small isolated genus of approximately 20 species, with native ranges distributed in five continents. Two of its species, the annual *B. hybridum* and the perennial *B. sylvaticum*, are invasive plants. *B. sylvaticum* has been introduced and is spread in western N America and in Australia, and *B. hybridum* has successfully colonized C Europe, western N America (California), S America (Uruguay, Argentina), South Africa and Oceania (Australia, New Zealand) (Jenkins et al. 2003; Garvin et al. 2008; Bakker et al. 2009; Catalán et al. 2012).

## Phylogeny of *Brachypodium*

All phylogenetic studies conducted on *Brachypodium* support a rapid and relatively recent radiation of its crown ancestor, after a long time span from the earlier split of the stem ancestor and the recent split of the crown clade (Catalán and Olmstead 2000; Catalán et al. 1995, 2012; Diaz-Perez et al. unpub. data). This long isolation, followed by recent divergence, is corroborated by its exclusive nuclear genomic families (Catalán et al. 1995; Mur et al. 2011) and by its confounding assorted lineages (Catalán and Olmstead 2000; Wolny et al. 2011; Catalán et al. 2012). Successive phylogenetic works based mostly on analysis of plastid and nuclear rDNA sequences and on nuclear (RAPD) markers, including approximately half of the species of *Brachypodium*, recovered a congruent evolutionary framework for the genus (Catalán et al. 1995, 2012; Catalán and Olmstead 2000). Dated phylogenies based on combined analysis of nuclear ribosomal genes and plastid genes have estimated the origin of the common ancestor of *Brachypodium* in the mid Miocene, showing the early successive divergences of *B. boissieri*, *B. stacei*, and *B. mexicanum*, a later Pliocene split of *B. distachyon* and the recent Pliocene-Pleistocene radiation of the core perennial clade (Catalán et al. 2012). Within the latter group, a congruent trend was observed in the early divergence of *B. arbuscula*, followed by that of *B. retusum*, though uncertainty affected the rapid splits of the most recent nodes, ending in an unresolved scenario for the divergence of the *B. pinnatum*, *B. rupestre*, *B. phoenicoides*, *B. glaucovirens* and *B. sylvaticum* lineages (Catalán et al. 1995, 2012; Catalán and Olmstead 2000; Wolny et al. 2011). Phylogenetic trees reconstructed from low copy nuclear genes concurred with this hypothesis, but also showed basal homeologous copies in one allopolyploid member of the core perennial clade (*B. retusum*; Wolny et al. 2011; Catalán et al. 2012).

Deep evolutionary analysis of the perennial *Brachypodium* genomes has been hampered, however, by the intricate reticulate nature of the species in this core clade, which shows a prevalence of allopolyploid taxa, and by their explosive radiation, manifested in the mostly unresolved or weakly supported topologies (Catalán et al. 2012). Recently, a thorough taxonomic and geographic sampling of all the currently recognized species of the genus allowed us to conduct the largest and most comprehensive phylogenetic study of *Brachypodium* to date (Diaz-Pérez et al. unpub. data described below). A total of 110 samples representing the 17 recognized species plus one geographically isolated infraspecific taxon (*B. sylvaticum* var. *pseudodistachyon*) were included in the study (Table 1; Fig. 2). Six taxa (35.3 % of the total taxonomic diversity) were studied molecularly for the first time (*B. bolusii*, *B. flexum*, *B. genuense*, *B. kawakamii*, *B. madagascariense*, *B. sylvaticum* var. *pseudodistachyon*). Our study also included representatives of both diploid and allotetraploid cytotypes of the perennial *B. pinnatum* and *B. rupestre* species. Chromosomal, genome size and ploidy data information was collected for all samples except for some poorly known taxa which have not been karyotyped yet (Table 1). One thousand one hundred fifty-four DNA sequences from three nuclear (ETS, ITS, GI) and two plastid (*ndhF*, *trnLF*) loci were used to reconstruct the phylogeny of *Brachypodium*. The non-recombinant plastid *ndhF* + *trnLF* sequences were concatenated into a combined (cpDNA) data set and provided information about the maternal genomic inheritance in the hybrids, and the cloned sequences of the nuclear loci retrieved homeologous copies in the allopolyploids.

Exploratory phylogenetic and haplotypic network analyses were conducted with the respective sets of sequences (Fig. 3a–e). Phylogenetic analyses based on Maximum Likelihood (ML; RAxML) and Bayesian Inference (BI; MrBAYES) methods recovered the evolutionary relationships among the *Brachypodium* lineages, using other pooid representatives and *Oryza* (Ehrartoideae) as outgroups (Fig. 3e). Haplotypic networks were constructed to infer the genealogical relationships of the *Brachypodium* haplotypes (species and samples) obtained from each separate data set using statistical parsimony approaches (NETWORK) (Fig. 3a–d). The maternally inherited plastid haplotypic network consisted of 43 haplotypes (Fig. 3a) and was relatively well resolved for the early divergences of the monophyletic *B. boissieri*, *B. stacei*, *B. mexicanum* and *B. distachyon* clusters, each separated by a number of mutational steps in a star-like net (with highly supported divergences in the phylogenetic tree; Fig. 3e). The *B. hybridum* haplotypes were shared with its *B. stacei* and *B. distachyon* parents, though more frequently with the former. However, the cluster of the recently evolved core perennial species showed a lack of genealogical and taxonomic structure (Fig. 3a), denoted by the high number of interspecific shared haplotypes (with some haplotypes shared by up to three species; e.g., h. 28: *B. pinnatum*, *B. retusum*, *B. rupestre*), and an ambiguous resolution, manifested in the high number of internal loops and few internal mutational steps. The high number of interspecific shared maternal haplotypes reflects a history of repeated introgressions among lineages of the core perennial