ORIGINAL ARTICLE

Insights into panicoid inflorescence evolution

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Abstract Inflorescence forms can be described by different combinatorial patterns of meristem fates (indeterminate versus determinate). In theory, the model predicts that any combination is possible. Whether this is true for grasses is unknown. In this paper, the subfamily Panicoideae s.s. (panicoid grasses) was chosen as the model group to investigate this aspect of grass inflorescence evolution. We have studied the inflorescence morphology of 201 species to complement information available in the literature. We have identified the most recurrent inflorescence types and character states among panicoids. Using multivariate approaches, we have indentified correlations among different inflorescence character states. By phylogenetic reconstruction methods we have inferred the patterns of panicoid inflorescence evolution. Our results demonstrate that not all theoretical combinatorial patterns of variation are found in panicoids. The fact that each panicoid lineage has a unique pattern of inflorescence evolution adds an evolutionary component to combinatorial model.

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Introduction

Angiosperm inflorescence can be described by a simple and dynamic model of development (Kellogg 2000a). In this model, any meristem may have one of three fates: it may (1) produce indeterminate lateral branches, (2) terminate in a flower (for non-grass species) or spikelet (for grasses species), or (3) stop development without forming organs. Again, each of the indeterminate meristems could choose one of these three fates, and so on. Thus, different inflorescence architectures may be described by the combinatorial patterns of these meristem decisions and, in theory, any combination is possible.

The grass family is amazingly diverse in inflorescence forms. The high diversity of grass inflorescence morphologies might suggest that changes in inflorescence morphologies are relatively easy to achieve, and that all inflorescence types are likely to occur in this family. Whether all theoretical patterns of meristem decisions can be found in grasses or whether there have been constraints that have limited the morphological diversification of the grass inflorescences is still an open question. In order to further explore this aspect of grass inflorescence evolution, we propose here to study, as a starting point, the adult inflorescence morphology of the panicoid grasses (also knows as Panicoideae s.s., Sánchez-Ken and Clark 2010) from an evolutionary perspective.

The panicoid lineage includes some of the most important crop species, such as maize (*Zea mays* L.), sorghum [*Sorghum bicolor* (L.) Moench], sugar cane (*Saccharum officinarum* L.), common millet (*Panicum miliaceum* L.), pearl millet [*Pennisetum glaucum* (L.) R. Br.], foxtail millet

[*Setaria italica* (L.) P. Beauv.], and Shama millet [*Echinochloa colonum* (L.) Link]. Panicoids form a monophyletic group supported by plastic (*ndhF* and *rpl16* intron) and nuclear (*rbcL* and *granule-bound starch synthase I*) markers (Sánchez-Ken and Clark 2010; Morrone et al. 2011) and two unambiguous morphological synapomorphies: the presence of a spikelet with two florets, and the lack of rachilla extension (Sánchez-Ken and Clark 2010). Recent studies have suggested that panicoids should be divided into six well-supported tribes (Sánckez-Ken and Clark 2010; Zuloaga et al. 2010; Morrone et al. 2011): Steyermarkochloeae p.p., Tristachyideae, Arundinelleae s.s., Andropogoneae, Paspaleae (also known as the Paniceae *x*=9 clade; Giussani et al. 2001), and Paniceae s.s. (also referred to in the literature as the Paniceae *x*=10 clade; Giussani et al. 2001). The Andropogoneae, Paspaleae, and Paniceae s.s. tribes include most of the species diversity among panicoids. Andropogoneae and Arundinelleae s.s. tribes form a well supported lineage, which is sister to the Paspaleae tribe. In addition, Paniceae s.s. is sister to the (Andropogoneae + Arundinelleae s.s.) + Paspaleae clade. *Steyermarkochloeae angustifolia* (Spreng.) Judz. + Tristachyideae may be sister to the rest of the tribes; however, their position in the phylogeny is not well resolved (Sánckez-Ken and Clark 2010; Morrone et al. 2011).

The inflorescences of several panicoid taxa have been characterized (Cámara-Hernández and Gambino 1990; Vegetti and Pensiero 1990, 1993; Vegetti and Anton 1991; Vegetti and Tivano 1991; Cámara-Hernández and Bellón 1992; Schneider and Vegetti 1992; Vegetti 1992a, b, 1993, 1997a, b, 1998a, b, 1999; Rua 1996, 2003; Rua and Boccaloni 1996; Rua and Weberling 1998; Giraldo-Cañas 2000, 2007; Kellogg 2000b; Vegetti and Vega 2000; Pensiero and Vegetti 2001; Rua and Aliscioni 2002; Reinheimer and Vegetti 2004, 2008; Reinheimer et al. 2005; Peichoto and Vegetti 2007; among others). The inflorescence morphology of panicoids can vary in characters such as shape, symmetry, degree of branching, spikelet arrangement, presence/absence of truncation (loss of inflorescence parts such as spikelets or entire branches), and presence/absence of branch homogenization (morphological similarity among inflorescence branches) (Fig. 1). Truncation and homogenization have been proposed as common evolutionary processes in Angiosperms and, in particular, in the grasses (Troll 1964, 1969; Weberling 1965, 1985; Maresquelle 1970; Sell 1976; Kunze 1989; Vegetti and Anton 1995, 2000; Perreta et al. 2009). It has been postulated many times, that truncation and homogenization determine, at least in part, the general appearance of the inflorescence (reviewed in Perreta et al. 2009). While a considerable quantity of data are available on panicoids inflorescence morphology, this information has never been analyzed all together in the context of phylogeny.
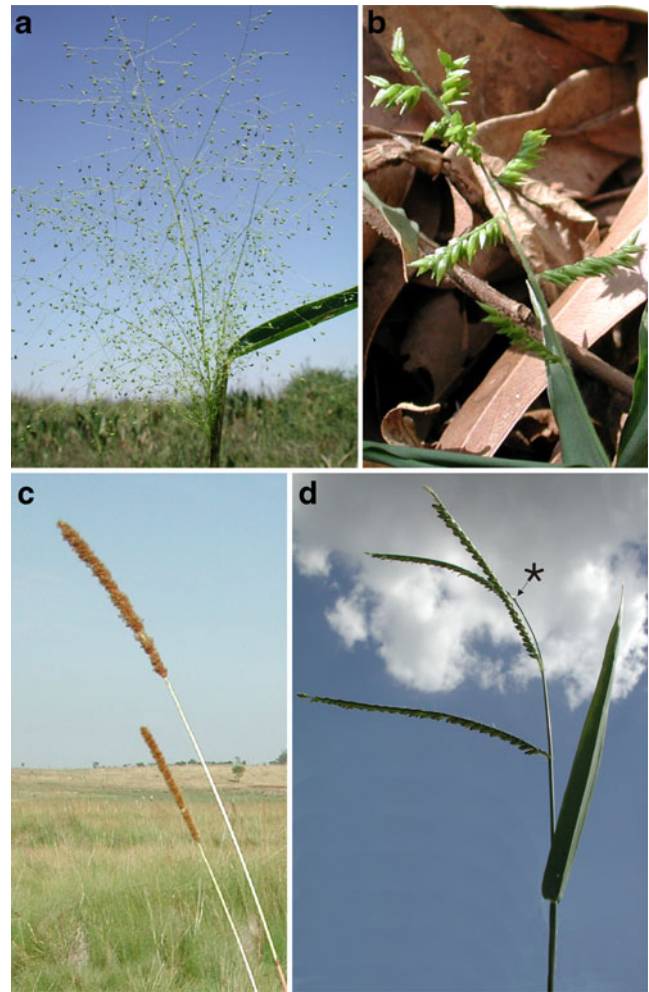
Fig. 1 a–d Contrasting inflorescence morphologies of panicoid grasses. **a** Lax, non-homogenized, and non-truncated inflorescence of *Panicum trichanthum*. **b** Lax to condensed, homogenized, and non-truncated inflorescence of *Steinchisma laxa*. **c** Condensed, non-homogenized, and non-truncated inflorescence of *Sacciolepis vilvoides*. **d** Lax-to-condensed, homogenized, and truncated inflorescence of *Urochloa ruziziensis*. *Asterisk* Truncation of the terminal spikelet of the inflorescence main axis

In this work, we studied the inflorescence morphology of panicoid grasses from an evolutionary perspective. The current study was undertaken with following goals in mind: (1) to identify the most recurrent inflorescence types among panicoids, (2) to investigate the ancestral character state, 3) to assess the most frequent character state changes on nodes over the tree, and 4) to study the number of origins and reversal of each inflorescence character. Our results demonstrate that not all theoretical combinatorial patterns of variation are found in panicoids. Despite the apparent lability of the inflorescence characters, this work supports the idea that the evolution of the grass inflorescence follows unique patterns of evolution that characterize each of the panicoid lineages.

Materials and methods

Inflorescence morphology dataset

Six morphological characters were selected for use in this study: (1) inflorescence aspect (0: condensed inflorescence with short internodes along the main axis and branches, Fig. 1c; 1: lax inflorescence with long internodes along the main axis and branches, Fig. 1a; 2: lax to condensed inflorescence with long internodes along the main axis whereas branch internodes are short, Fig. 1b, d; (2) presence/absence of homogenization (0: non-homogenized; 1: partially and fully homogenized; we followed the definition of the homogenization degree as defined in Rua and Weberling 1998); (3) degree of homogenization (0: non-homogenized; 1: partially homogenized; 2: fully homogenized; character states as defined in Rua and Weberling 1998); (4) presence/absence of terminal spikelet at the end of the main axis (0: non-truncated; 1: truncated); (5) maximum degree of ramification (0: first order; 1: second order; 2: third order; 4: fourth or more order of branching); and (6) symmetry of the main axis (0: radiate, 1: unilateral; 2: bilateral). These characters have proved the most relevant, and have been used widely by taxonomists and morphologists to describe grass inflorescence morphology at maturity.

Character state data were compiled from published reports and personal observations. We studied the inflorescence structure of 201 species using a stereoscopic microscope, to supplement information available in the literature. Morphological studies were based on herbarium specimens from MCNS, MO, SI, and SF. A complete list of specimens and vouchers studied in this work is presented in Electronic Supplementary Material 1. A detailed description of the inflorescence morphology for each of the species studied and their corresponding state assignments are presented in Electronic Supplementary Material 2 and 3, respectively.

Identification of inflorescence types and analysis of inflorescence characters

To explore and visualize similarities among taxa, and to investigate the existence of groupings, we conducted principal coordinates analysis (PCO). The PCO is a classical multidimensional scaling which allows us to analyze the interdependence between categorical variables and to find a graphical representation (in a low-dimensional space) of the *n* individuals such that it reflects the distance between them (James and McCulloch 1990; Di Rienzo et al. 2009). In addition, to search for correlations between inflorescence variables, we conducted a correspondence analysis (COA). COA is an ordination method that de-composes a two-way contingency table of counts of objects and their attributes that can be use to suggest new combined variables (James

and McCulloch 1990; Di Rienzo et al. 2009). Both multivariate analyses were implemented in Infostat version 2009 (Di Rienzo et al. 2009). For PCO, Euclidean distances were calculated on a previously standardized matrix, and results were represented in two-dimensional space (Di Rienzo et al. 2009). For COA, a chi-square deviation matrix was applied and the inertia (chi-square value divided by the total sum of the table) was used as an indicator of the contribution to reject the hypothesis of independence between the two variables (more inertia, more contribution to reject the hypothesis) (Di Rienzo et al. 2009). Cases with missing information were deleted and not analyzed.

To evaluated the incidence of different inflorescence types and character states within the panicoids, and in particular within each of its most important lineages (i.e., tribes Arundinelleae s.s. + Andropogoneae, Paspaleae, and Paniceae s.s.), we estimated the proportion of taxa with a given inflorescence type and character state.

Character optimization

To investigate the evolution of inflorescence characters over the phylogeny of panicoids, character states were optimized using one of the most parsimonious trees published by Aliscioni et al. (2003), as later adjusted by Sánchez-Ken and Clark (2010), Zuloaga et al. (2010), and Morrone et al. (2011). These studies used the plastic *ndhF* gene and produced a well supported molecular framework for comparative studies. We have inferred the history of five morphological characters (inflorescence aspect, degree of homogenization, presence/absence of terminal spikelet, degree of branching, and inflorescence symmetry) using parsimony methods and Mesquite 2.0 software (Maddison and Maddison 2011). We used the "trace character history" option and the "Markov k-state 1 parameter model" of evolution (Mk1 model), in which any particular change is equally probably. We calculated the average of character states changes across the entire tree, and inside each particular panicoid subtribe, using the "summarized state changes in selected clade" option implemented in Mesquite 2.0 (Maddison and Maddison 2011). Character states were treated as un-ordered.

Results

Inflorescence morphology

Inflorescence types

Taxa included in the PCO were grouped into 29 different inflorescences types (here arbitrarily named inflorescence

Table 1 Twenty-nine inflorescence types found by principal components analysis (PCA). *TS* Terminal spikelet

Type	Inflorescence aspect	Degree of homogenization	TS truncation	Branching	Symmetry	Species (example)
1	Condensed	Non-homogenized	Non-truncated	4° or more	Radiate	*Panicum condensatum*
2	Condensed	Non-homogenized	Truncated	4° or more	Radiate	*Setaria viridis*
3	Condensed	Fully homogenized	Truncated	2° order	Radiate	*Schizachyrium scoparium*
4	Condensed	Fully homogenized	Truncated	3° order	Radiate	*Ischaemum afrum*
5	Condensed	Fully homogenized	Truncated	2° order	Unilateral	*Stenotaphrum secundatum*
6	Condensed	Fully homogenized	Non-truncated	1° order	Unilateral	*Ophiochloa hydrolithica*
7	Lax	Non-homogenized	Truncated	4° or more	Radiate	*Capillipedium parviflorum*
8	Lax	Non-homogenized	Non-truncated	4° or more	Unilateral	*Melinis repens*
9	Lax	Non-homogenized	Non-truncated	3° order	Radiate	*Panicum tricholaenoides*
10	Lax	Non-homogenized	Non-truncated	4° or more	Radiate	*Urochloa maxima*
11	Lax	Partially homogenized	Non-truncated	3° order	Radiate	*Rupiochloa acuminata*
12	Lax	Partially homogenized	Non-truncated	4° or more	Radiate	*Otachyrium versicolor*
13	Lax	Fully homogenized	Non-truncated	1° order	Radiate	*Arthropogon villosus*
14	Lax	Fully homogenized	Non-truncated	2° order	Radiate	*Tatianyx arnacites*
15	Lax	Fully homogenized	Non-truncated	3° order	Radiate	*Pseudechinolaena polystachya*
16	Lax	Fully homogenized	Non-truncated	4° or more	Radiate	*Acroceras zizanioides*
17	Lax-to-condensed	Non-homogenized	Non-truncated	4° or more	Unilateral	*Echinochloa colona*
18	Lax-to-condensed	Non-homogenized	Non-truncated	4° or more	Radiate	*Hymenachne donacifolia*
19	Lax-to-condensed	Partially homogenized	Truncated	3° order	Bilateral	*Urochloa plantaginea*
20	Lax-to-condensed	Partially homogenized	Truncated	4° or more	Radiate	*Sorghum bicolor*
21	Lax-to-condensed	Partially homogenized	Non-truncated	3° order	Unilateral	*Moorochloa eruciformis*
22	Lax-to-condensed	Partially homogenized	Non-truncated	3° order	Radiate	*Phanopyrum gymnocarpon*
23	Lax-to-condensed	Partially homogenized	Non-truncated	4° or more	Radiate	*Urochloa mutica*
24	Lax-to-condensed	Partially or fully homog.	Non-truncated	3°-4° order	Unilateral	*Chaetium bromoides*
25	Lax-to-condensed	Fully homogenized	Non-truncated	2° order	Bilateral	*Oplismenus hirtellus*
26	Lax-to-condensed	Fully homogenized	Non-truncated	2° order	Radiate	*Coleataenia caricoides*
27	Lax-to-condensed	Fully homogenized	Non-truncated	3° order	Radiate	*Ocellochloa stolonifera*
28	Lax-to-condensed	Fully homogenized	Truncated	2° order	Radiate	*Paspalum vaginatum*
29	Lax-to-condensed	Fully homogenized	Truncated	3° order	Radiate	*Zea mays*

types 1–29; Table 1 and Electronic Supplementary Material 4) among at least 216 theoretical possibilities (inflorescence aspect states x degree of homogenization states x presence absence of terminal spikelet states x degree of branching states x symmetry states = total number of inflorescence morphologies [3×3×2×4×3 = 216]). The PCO showed that the two principal axes account for 62.2 % (axis 1 = 40.0 %; axis 2 = 22.2 %) of the total variation (Electronic Supplementary Material 4).

Occurrence of inflorescence types and character states among panicoids

Among the 29 different inflorescence types found by PCO analysis, type 10 (lax, non-homogenized, non-truncated, radiate, with fourth or more order of branching) is the most common inflorescence type found among panicoids (Electronic Supplementary Material 5). In general, lax, fully

homogenized, non-truncated, highly branched, and radiate inflorescences are the most frequent states found among panicoids (Table 2).

Most of the species of the Arundinelleae s.s. and Andropogoneae tribes have inflorescence morphologies type 3 (condensed, fully-homogenized, truncated, radiate, with up to second order of branching) and type 29 (lax to condensed, fully-homogenized, truncated, radiate, with up to third order of branching) (Electronic Supplementary Material 5). Type 15 (lax, fully-homogenized, non-truncated, radiate with up to third order of branching) predominates in the Paspaleae tribe and type 10 is the most frequent inflorescence type found in Paniceae s.s. (Electronic Supplementary Material 5).

In the Arundinelleae s.s. and Andropogoneae tribes condensed and lax to condensed inflorescences prevail over lax inflorescences, whereas the Paspaleae and Paniceae s.s. tribes have preferentially lax inflorescences (Table 2). The majority

Table 2 Occurrence of character states in Panicoids

Character	Panicoids	Arundinelleae s.s. +Andropogoneae	Paspaleae	Paniceae s.s.
Inflorescence aspect				
Condensed	19 %	50 %	12 %	20 %
Lax	49 %	6 %	54 %	52 %
Lax to condensed	32 %	44 %	34 %	28 %
Homogenization				
Non-homogenized	41 %	6 %	20 %	61 %
Homogenized	59 %	94 %	80 %	39 %
Degree of homogenization				
Partially-homogenized	30 %	22 %	24 %	47 %
Fully-homogenized	70 %	88 %	76 %	53 %
Truncation of the TS				
Non-truncated	76 %	0 %	85 %	80 %
Truncated	24 %	100 %	15 %	20 %
Degree of branching				
First order	5 %	0 %	12 %	0 %
Second order	11 %	33 %	11 %	8 %
Third order	36 %	50 %	43 %	28 %
Fourth or more order	48 %	17 %	34 %	64 %
Inflorescence symmetry				
Radiate	90 %	100 %	90 %	87 %
Unilateral	9 %	0 %	10 %	10 %
Bilateral	1 %	0 %	0 %	3 %

of the species of Arundinelleae s.s. + Andropogoneae and Paspaleae tribes have homogenized inflorescences, whereas most species of the Paniceae s.s. tribe have non-homogenized inflorescences (Table 2). When homogenization is present, in general, fully homogenized inflorescences are more common than partially homogenized inflorescences among the major lineages (Table 2). The species of the Arundinelleae s.s. and Andropogoneae tribes have inflorescences without a terminal spikelet at the end of the main axis (Table 2). In contrast, most species of Paspaleae, and Paniceae s.s. tribes have inflorescences with a terminal spikelet (Table 2). In terms of branch degree, most of the species of Arundinelleae s.s. and Andropogoneae tribes have inflorescences with branches from second to third order, whereas inflorescences of the Paspaleae and Paniceae s.s. tribes are, in general, highly branched (Table 2). The inflorescence symmetry is radiate in most species examined (Table 2). A low percentage of the species of the Paspaleae, and Paniceae s.s. tribes have inflorescences with unilateral or bilateral symmetry (Table 2).

Correlation between inflorescence characters states

Correspondence analyses suggest several correlations among inflorescence character states; these are presented in Electronic Supplementary Material 6 and summarized in Table 3. In general, the analyses suggest five major patterns. First, the inflorescence aspect is correlated with changes in homogenization degree and truncation (lax inflorescences tend to be non-homogenized and non-truncated; lax-to-condensed inflorescences may be homogenized; condensed inflorescences may be truncated). Second, the homogenization is correlated with truncation (non- and partially homogenized inflorescences may be non-truncated; fully homogenized inflorescences tend to be truncated). Third, there is a reduction in the degree of branching from non-homogenized to fully homogenized inflorescences (non-homogenized inflorescences tend to have fourth or higher order branching; fully homogenized inflorescences may have branches up to second order). Fourth, truncation is correlated with branching (non-truncated inflorescences have first, third or higher order branching, and truncated inflorescences may have only second order branching). Finally, radiate inflorescences tend to be lax, non- or partially homogenized, non-truncated, with second or higher order branching, and unilateral inflorescences may have first order branching.

Character history

Reconstruction analyses estimate that the ancestor of the panicoids had a lax inflorescence (Fig. 2a). During panicoid

Table 3 Suggested correlations among inflorescence character states by the correspondence analyses

Correspondence analyses	Suggested correlations	Dimension 1 Inertia (%)
Inflorescence aspect and homogenization	Lax and non-homogenized inflorescences Lax to condensed and fully homogenized inflorescences	72.94
Inflorescence aspect and TS truncation	Lax and non-truncated inflorescences Condensed and truncated inflorescences	100
Inflorescence aspect and branching	Lax inflorescences and 4° or more branch order Lax to condensed inflorescences and 3° branch order	77.31
Inflorescence aspect and symmetry	Lax and radiate inflorescences	82.73
Homogenization and TS truncation	Non- and partially homogenized, and non-truncated inflorescences Fully homogenized and truncated inflorescences	100
Homogenization and branching	Non-homogenized inflorescences with 4° or more branch order Fully homogenized inflorescences with 2° or 3° branch order	98.56
Homogenization and symmetry	Non-homogenized or partially homogenized with radiate inflorescences	91.47
TS truncation and branching	Non-truncated inflorescences with 1°, 3° or 4° (or more) branch order Truncated inflorescences with 2° branch order	100
TS truncation and symmetry	Non-truncated inflorescences with radiate symmetry Truncated inflorescences with bilateral symmetry	100
Branching and symmetry	2°, 3°, and 4° or more branch order with radiate inflorescences 1° branch order with unilateral inflorescences	92.94

TS terminal spikelet of the inflorescence main axis

diversification, lax-to-condensed inflorescences may have evolved 17 different times, whereas condensed inflorescences may have appeared 6 independent times. At least seven reversals to the ancestral state are confirmed in the panicoids: one in the Andropogoneae tribe, three times in the Paspaleae tribe, and three additional times inside the Paniceae s.s. tribe.

In terms of inflorescence branch homogenization (Fig. 2b), the ancestral character state assigned at the base of the subfamily Panicoideae s.s. is a non-homogenized inflorescence. Fully homogenized inflorescences may have appeared at the base of the Arundinelleae s.s. + Andropogoneae + Paspaleae lineage and five independent times during Paniceae s.s. evolution. Partially homogenized inflorescences may have arisen 12 independent times during panicoid evolution. At least nine reversals to the ancestral character state are suggested in the panicoids: one in the Andropogoneae tribe, six different times inside the Paspaleae tribe, and two additional times within the Paniceae s.s. tribe.

Non-truncated inflorescence is the most common ancestral state assigned along the panicoids tree (Fig. 2c). Truncated inflorescences had arisen at the base of the Arundinelleae s.s. + Andropogoneae tribes, four independent times in Paspaleae tribe, and four additional times in the Paniceae s.s. tribe. Reversals to the ancestral state are not confirmed in the panicoids.

Inflorescences with fourth or higher order branching is the ancestral state assigned at the base of the panicoid grasses (Fig. 3a). Inflorescences with up to third order branching may have arisen before the diversification of the Arundinelleae s.s. + Andropogoneae + Paspaleae tribes, three later additional times inside the Andropogoneae, and once inside the Paspaleae tribe. In addition, inflorescences with up to third order branching have evolved 11 additional times in the Paniceae s.s. tribe. Inflorescences with up to second order branching evolved at least 11 different times during panicoid evolution: 3 times inside the Andropogoneae tribe, 6 times in the Paspaleae tribe, and twice in the Paniceae s.s. tribe. Inflorescence with branches up to first order happened three independent times during Paspaleae tribe diversification. At least nine reversals to the panicoid ancestral state are suggested: two independent times in the Andropogoneae tribe, six times inside the Paspaleae tribe, and once in the Paniceae s.s. tribe.

Radiate inflorescence is the most common ancestral state assigned to most of the nodes in the tree (Fig. 3b). Unilateral inflorescences arose seven times (two independent times in the Paspaleae tribe, and five times in the Paniceae s.s. tribe) and bilateral inflorescence have appeared only once inside the Paniceae s.s. tribe. No reversal to the ancestral state is observed along the evolution of the panicoids.

Ancestral state changes over the tree

The character optimization results presented above indicate that some transitions among character states are more recurrent over others in the panicoids. In order to better evaluate this aspect of inflorescence evolution, we calculated the average of character states changes in panicoids (Fig. 4), and also considering each particular tribe (Fig. 5).
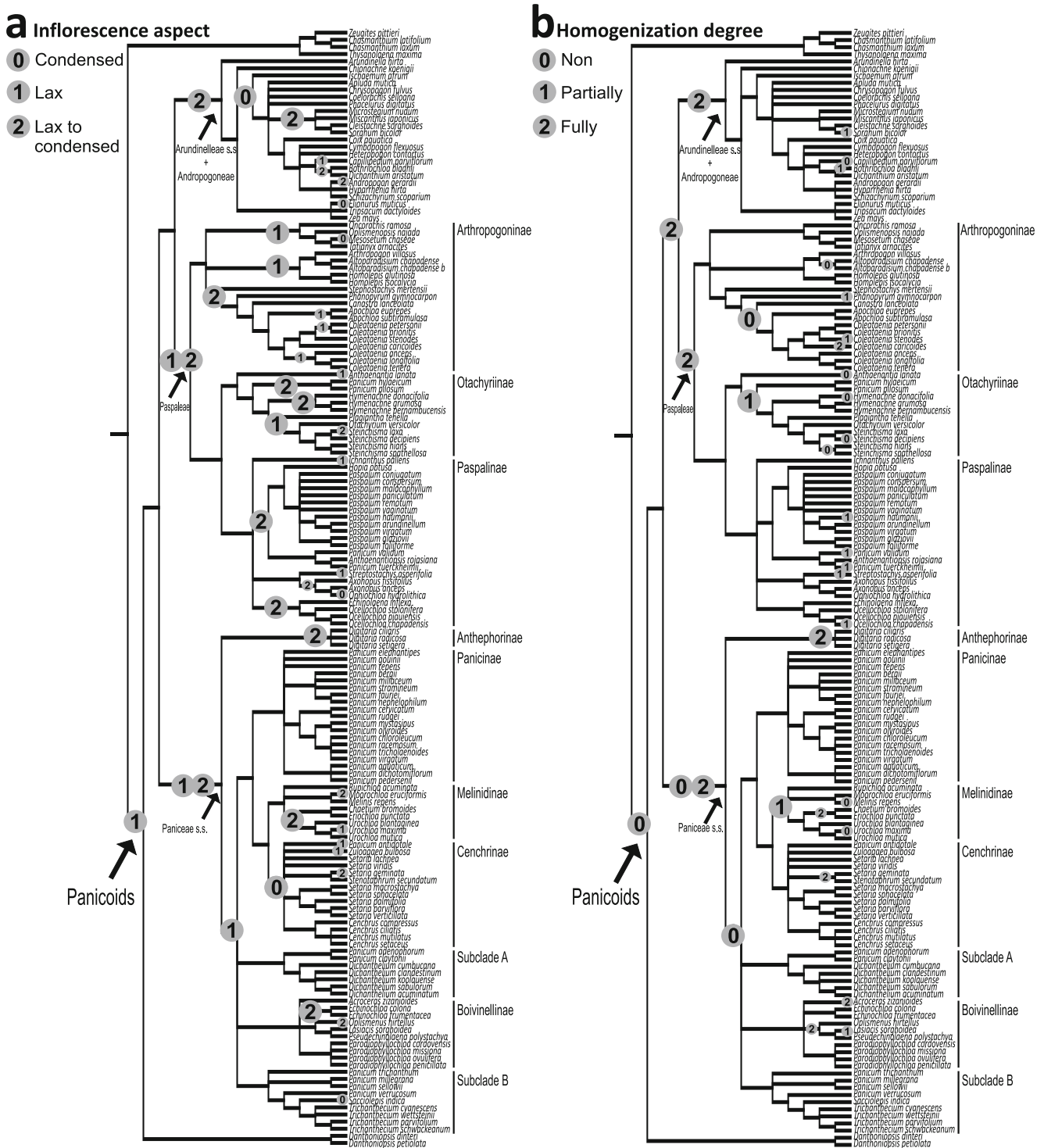
Fig. 2 **a–c** Evolution of inflorescence characters in the panicoids mapped over one of the most parsimonious trees published by Aliscioni et al. (2003), as later modified by Sánchez-Ken and Clark (2010), Zuloaga et al. (2010), and Morrone et al. (2011). **a** Optimization of the inflorescence aspect character states. **b** Optimization of the homogenization degree character states. **c** Optimization of the presence/absence of the terminal spikelet. Numbers in *grey circles* indicate the state code used to construct and analyze the dataset. Names of tribes and subtribes are according to Morrone et al. (2011)

Overall, changes from lax inflorescence to lax to condensed inflorescence, or vice versa, were the most frequent transitions that may have happened in terms of inflorescence aspect during panicoid evolution (Fig. 4a). The change from
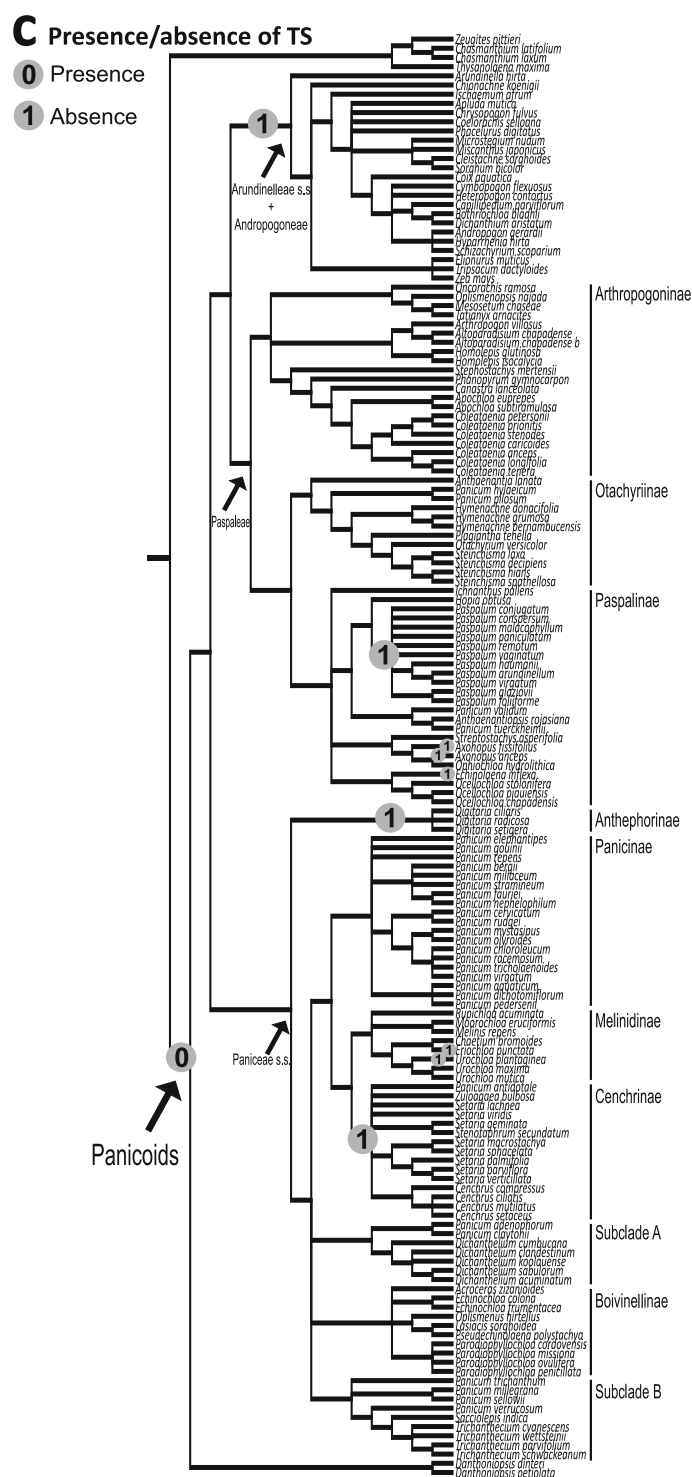
Fig. 2 (continued)

homogenized to non-homogenized inflorescences may be more common than non-homogenized to homogenized inflorescences (Fig. 4b). When the different homogenization degrees are considered, we found that changes from fully homogenized to partially homogenized inflorescences appear to be the most

common transition along the panicoids phylogeny (Fig. 4c). The change from non-truncated to truncated inflorescences is more likely to happen than the conversion from truncated to non-truncated inflorescences in panicoids (Fig. 4d). When the ramification degree is considered, we found that the most
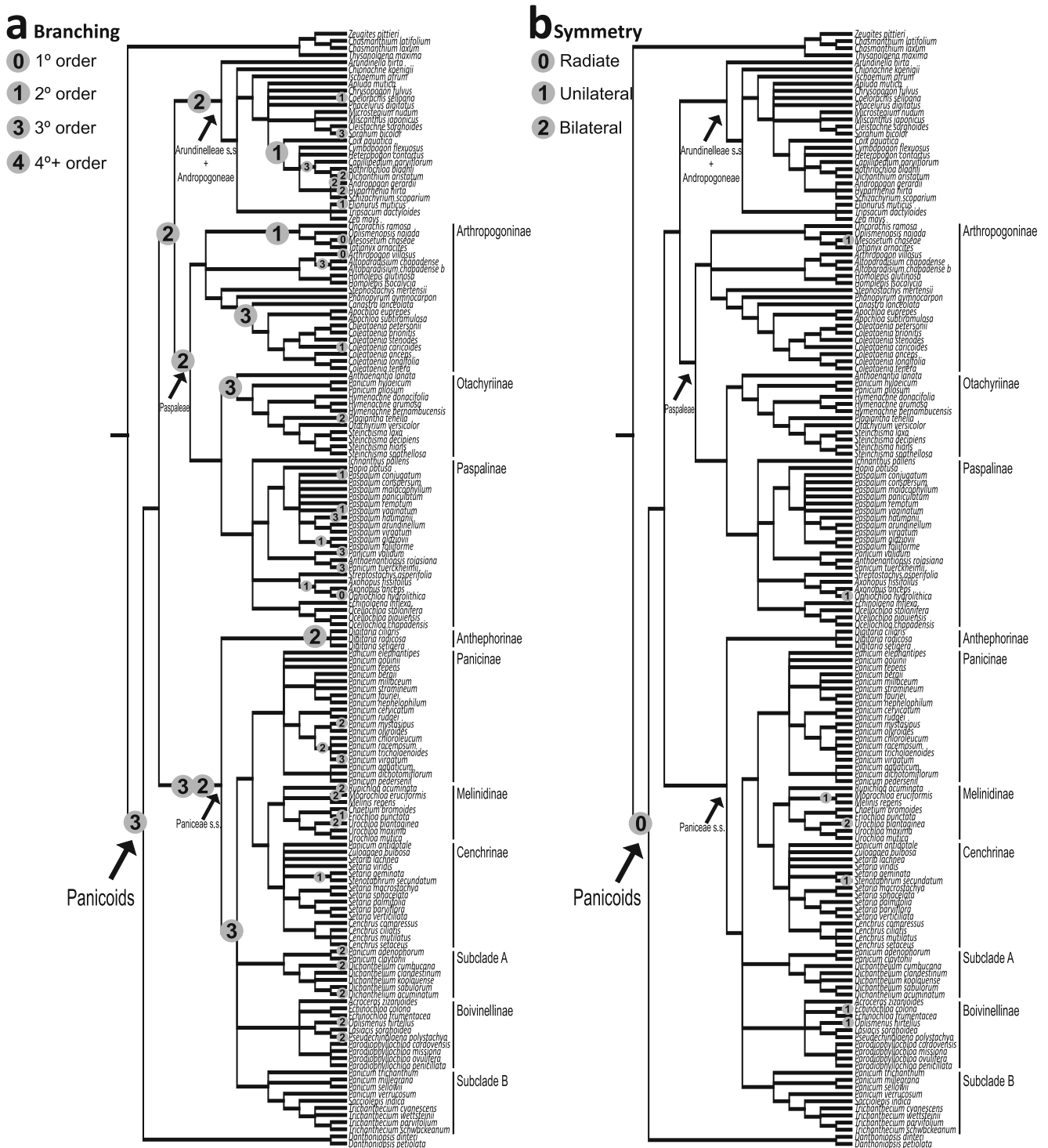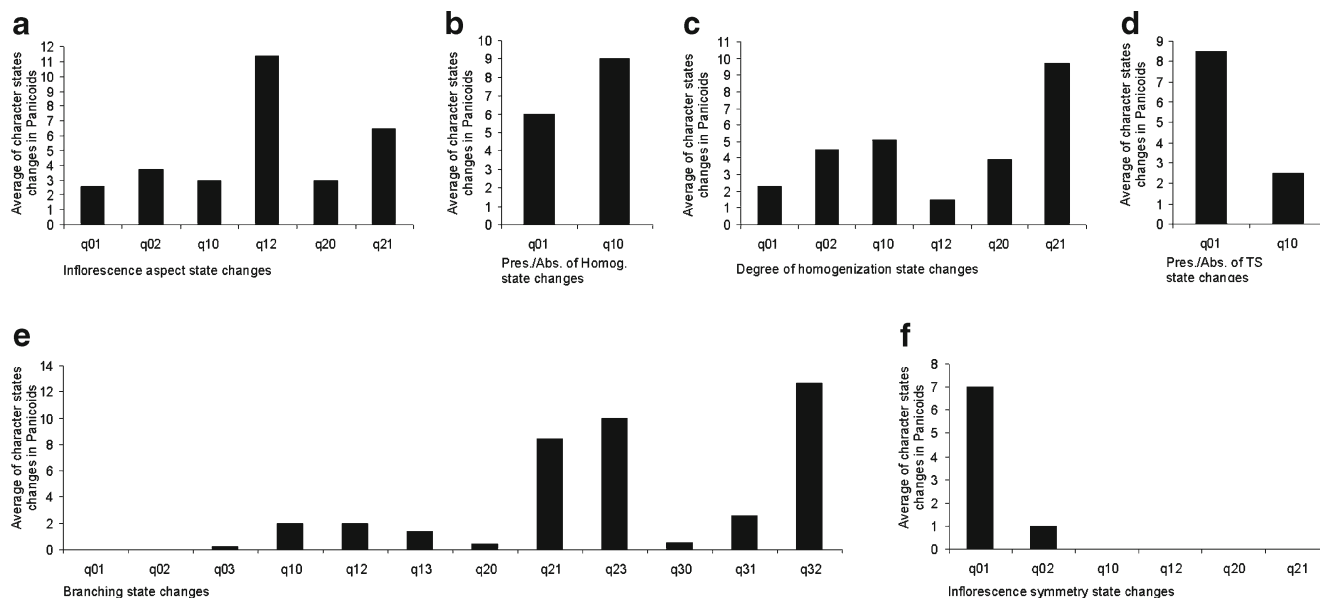
Fig. 3 a, b Evolution of inflorescence characters in the panicoids mapped over one of the most parsimonious trees published by Aliscioni et al. (2003), as later modified by Sánchez-Ken and Clark (2010), Zuloaga et al. (2010), and Morrone et al. (2011) (continuation). a Optimization of the branching degree character states. b Optimization of the inflorescence symmetry character states. Numbers in grey circles indicate the state code used to construct and analyze the dataset. Names of tribes and subtribes are according to Morrone et al. (2011)

common transition may have happened from inflorescences with fourth (or more) order branching to inflorescences with up to third order branching (Fig. 4e). Changes from inflorescences with third order branching to inflorescences with fourth (or more) or second order branching may be also frequent (Fig. 4e). In addition, transitions from inflorescences with only
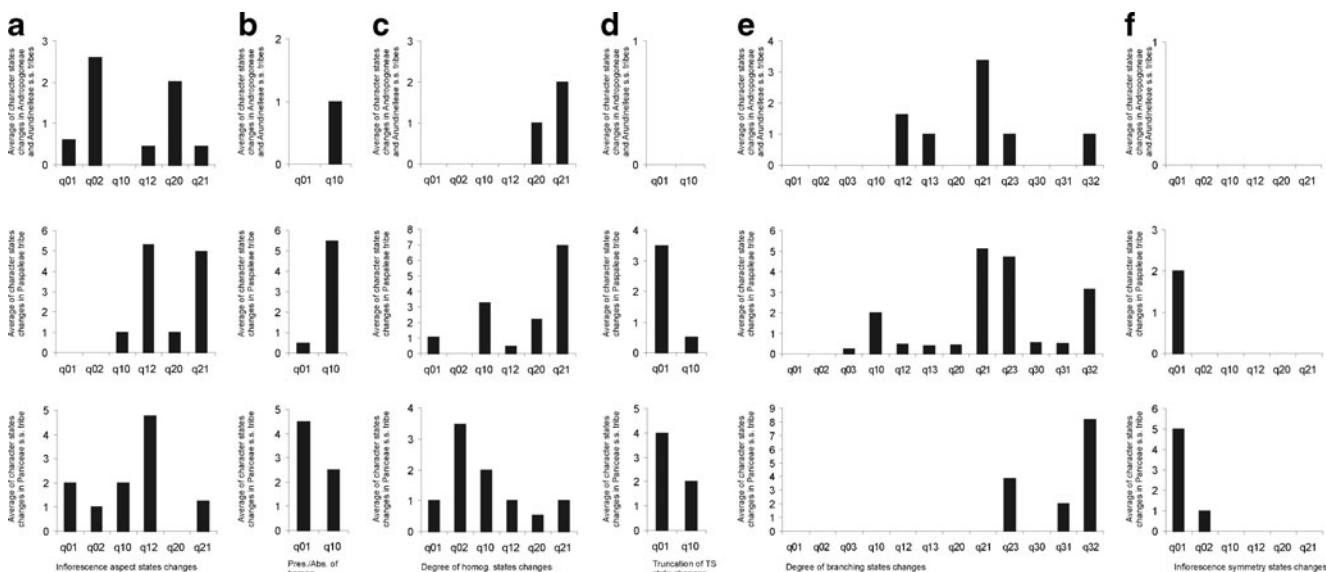
Fig. 4 a–f Average of inflorescence character states changes during the evolution of the panicoids. **a** Inflorescence aspect. **b** Presence/absence of homogenization. **c** Homogenization degree. **d** Presence/absence of terminal spikelet at the end of the main axis. **e** Branching degree. **f** Inflorescence symmetry. Numbers indicate the state code used to construct and analyze the dataset. Character transition change from one state to another is indicated by the "q" followed by the state codes. *Abs*. Absence, *Homog*. homogenization, *Pres*. presence, *TS* terminal spikelet

primary branches to inflorescences with second or third order of branching were not observed over the panicoid tree (Fig. 4e). Among six possibilities of different symmetry states changes, only two were observed among panicoids, the most recurrent change being radiate inflorescences to unilateral, rather than radiate to bilateral (Fig. 4f).

Inside the Arundinelleae s.s. + Andropogoneae tribes, the most common transition happens from condensed to lax to condensed inflorescences or viceversa, whereas the Paspaleae and Paniceae s.s. tribes are characterized by changes from lax to lax to condensed inflorescences (Fig. 5a). In addition, transition from lax to condensed to

Fig. 5 a–f Average of inflorescence character states changes during the evolution of the main panicoid lineages. **a** Inflorescence aspect. **b** Presence/absence of homogenization. **c** Homogenization degree. **d** Presence/absence of terminal spikelet at the end of the main axis. **e** Branching degree. **f** Inflorescence symmetry. Numbers indicate the state code used to construct and analyze the dataset. Character transition change from one state to another is indicated by the "q" followed by the state codes. *Abs*. Absence, *Homog*. homogenization, *Pres*. presence, *TS* terminal spikelet

lax inflorescences are also common in Paspaleae tribe (Fig. 5a). The evolutionary transition from lax to condensed inflorescences was not found in the Arundinelleae s.s. + Andropogoneae tribes. Inside the Paspaleae tribe, the transition from condensed to lax or lax to condensed inflorescences never happens (Fig. 5a). Within the Paniceae s.s. tribe the transition from lax to condensed to condensed inflorescences was not observed (Fig. 5a).

During the evolution of the Arundinelleae s.s. + Andropogoneae and the Paspaleae lineages inflorescences may change from homogenized to non-homogenized more often than non-homogenized to homogenized inflorescences (Fig. 5b). In contrast, inflorescence changes from non-homogenized inflorescence to homogenized inflorescence are well represented in the Paniceae s.s. tribe (Fig. 5b). When the degree of homogenization is considered, we found that the change from fully homogenized to partially homogenized inflorescences is a common transition among the Arundinelleae s.s. + Andropogoneae and the Paspaleae tribes (Fig. 5c). On the other hand, the transition from non-homogenized to fully homogenized inflorescences is recurrent inside the Paniceae s.s. tribe (Fig. 5c).

The inflorescences of the Arundinelleae s.s. + Andropogoneae tribes have not modified the truncation state, being always truncated inflorescences. In contrast, in the Paspaleae, and Paniceae s.s. tribes, transition from non-truncated inflorescences to truncated-inflorescences are commonly observed (Fig. 5d).

In terms of branch ramification, each major panicoid tribe is characterized by a subset of possible transitions (Fig. 5e). Within the Arundinelleae s.s. + Andropogoneae and Paspaleae tribes, inflorescences changed frequently from third order branching to second order branching (Fig. 5e). In addition, the transition from third to fourth or higher order branching is also frequent in the Paspaleae tribe. In the Paniceae s.s. tribe, the transition from inflorescences with fourth or higher order branching to inflorescences with up to third order branching prevails over other types of transition (Fig. 5e).

Changes in inflorescence symmetry were not observed in the Arundinelleae s.s. + Andropogoneae tribes, being always radiate inflorescences (Fig. 5e). In contrast, in the Paspaleae and Paniceae s.s. tribes, transition from radiate to unilateral inflorescences is at times observed (Fig. 5e).

Discussion

In this work, we describe some macroevolutionary aspects of mature panicoid inflorescences. Our results demonstrate that not all theoretical combinatorial patterns of variation are found in panicoids. In fact, we observed only a small subset of the theoretically possible inflorescence types, of which

lax, non-homogenized, and non-truncated inflorescences are most frequent. When the incidence of a given inflorescence type is analyzed considering each major clade separately, we found that each has preferentially adopted one type of inflorescence over others. Interestingly, inflorescence types that are present in one lineage are not well represented in the other two major clades. Similar results have been found when the occurrence of character states are analyzed separately. In addition, we observed that some character state transitions are more recurrent than others, and some never happen in panicoids; consequently, not all possible state changes have occurred throughout evolution. Moreover, the presence/absence of a given character state transition also characterized each of the major lineages. All these results reflect evolutionary constraints that restrict, to some extent, the diversification of the inflorescence architecture of panicoids. The fact that each of the major clades is characterized by a unique inflorescence pattern of frequencies and transitions may indicate differences in the evolutionary constraints that the lineages have faced during evolution.

The ancestral character states reconstruction studies suggest that most of the inflorescence changes, in aspect, homogenization, presence/absence of terminal spikelet, and branching pattern, were observed at the base of the Arundinelleae s.s. and Andropogoneae tribes, and during the diversification of the Paspaleae and Paniceae s.s. tribes. Changes in inflorescence symmetry occurred, toward the tips of the phylogeny inside the Arthropogoninae, Paspalinae, and Melinidinae subtribes. Interestingly, when it was possible to assess, we found that reversals to the ancestral states of some of the characters studied in this work are common events among panicoids. Indeed, inflorescence aspect, homogenization, and branching are characters that seem to be more likely to reverse, whereas truncation and inflorescence symmetry are characters that may not be as labile for reversal as the former. In fact, in panicoids, the loss of the terminal spikelet seems to be irreversible. Similarly, modifications of the inflorescence symmetry from radiate to unilateral or bilateral may be permanent in panicoids. It is important to note that the assessment of the correct inflorescence symmetry is sometimes blurred when dried herbarium specimens are examined in isolation. For example, it has been shown that the inflorescence of Melinis P. Beauv., which looks radiate at maturity, is indeed unilateral early in development (Reinheimer et al. 2009). Similarly, scanning electron micrographs have shown that the inflorescence of of Digitaria Haller section Trichachne (Nees) Henrard (not studied here) have proximal long branches disposed spirally, whereas distal short branches are arranged distichously (Rua 2003). Developmental studies would be of great help in further understanding the evolution of inflorescence symmetry.

Homogenization (similarities among inflorescence branches) and truncation (loss of different inflorescence

structures) have been described as processes that have played an important role in modifying the inflorescence architecture of grasses, as well as other Angiosperms, during evolution (reviewed in Perreta et al. 2009). Similarly, the multivariate analyses presented in this work suggest that the general inflorescence aspect may be correlated with changes in homogenization and truncation in panicoids. Several authors have already suggested correlations between inflorescence characters such as branch homogenization and truncation. Indeed, it has been already mentioned that most of the species that are truncated are also homogenized (Vegetti 1991; Rua and Weberling 1998; Vegetti and Anton 2000); however, Reinheimer and Vegetti (2008) have found that such correlation is asymmetric in the Melinidinae subtribe, given that all truncated inflorescences are also homogenized, but all inflorescences that are homogenized are not necessarily truncated. Our analyses on panicoids inflorescence agree with this trend, given that partially homogenized inflorescences may or may not be truncated. This work also suggests further inflorescence character correlations in panicoids such as branching pattern with homogenization degree, truncation, and inflorescence symmetry. Interestingly, when possible to assess, ancestral character state reconstructions have shown that most character state correlations can happen asynchronously. Consequently, in panicoids, some of the inflorescence character state modifications seem to be a prerequisite that may stimulate later modifications of other inflorescence characters. Better resolution of the phylogeny is need it in order to further investigate such a hypothesis. Nevertheless, hypothesis testing surrounding evolutionary models suggests that the panicoid inflorescence evolved by a combination of trait contingency and order of character acquisition (R. Reinheimer et al., unpublished).

Different degrees of homogenization are found among panicoid species: non-homogenized inflorescence, partially homogenized inflorescence, and fully homogenized inflorescence. The partially homogenized inflorescence has been described as an intermediate form between the non-homogenized and the fully-homogenized inflorescences (Rua 1996; Rua and Weberling 1998); however, this statement has never been tested in an evolutionary context. Here, ancestral character states reconstruction shows that the partially homogenized inflorescence does not necessary represent an intermediate state between non- and fully homogenized inflorescences. These findings suggest that the model of evolution of the homogenization character may not be ordered as previously postulated; indicating that the transition from non- to fully homogenized inflorescences is relatively easy and the step through a partially homogenized inflorescence is not obligatory. In the same way, we can also postulate that the lax-to-condensed inflorescence is an intermediate state between lax and condensed inflorescences; however, such directionality was not observed in panicoids so far. Given the ambiguous state assignation of several nodes,

these hypotheses should be tested further with more sensitive phylogenetic methods.

The study of inflorescence evolution in panicoids offers the exceptional opportunity to dissect the patterns of inflorescence evolution in grasses. Despite the apparent lability of inflorescence characters, this work supports the idea that the evolution of grass inflorescences follows some pattern. Such patterns of grass inflorescence evolution may reflect variations in constraints faced throughout diversification. It has been postulated previously that constraints on biological diversity may be due to three main and mutually dependent factors: (1) selection, (2) developmental and genetic mechanisms, and (3) environmental factors (Prusinkiewicz et al. 2007). Limitations found during the evolution of panicoid inflorescence may reflect constraints of developmental mechanisms available for selection, whereas selection influences the evolution of such developmental processes. In addition, it has been corroborated empirically that differences in inflorescence architecture are somewhat related with climate and life history, and may reveal diverse ways of manipulating airflow for enhance pollen export and receipt, and seed dispersal in different habitats (Friedman and Harder 2004, 2005; Prusinkiewicz, et al. 2007). Which of these factors have played a major role during the evolution of grass inflorescences is an intriguing question that remains open for further investigation.

To conclude, this work represents the first attempt to explore the lability of the inflorescence evolution in panicoids, which represents one of the most controversial aspects of grass evolution. Through the simple approach followed in this work we were able to postulate new hypothesis about trends of inflorescence evolution in the grasses. Future work should explore in more detail the inflorescence architecture in panicoids as well as in other grass lineages through a comparative approach in order to reveal new insights into grass inflorescence evolution. Such studies should also include uncertainties in the phylogenetic framework (not considered here), as well as developmental genetics data, and correlation analysis with additional environmental factors that may have influenced the plant architecture of grasses.

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