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Macroevolution of panicoid inflorescences: a history of contingency and order of trait acquisition

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• *Background and Aims* Inflorescence forms of panicoid grasses (Panicoideae *s.s.*) are remarkably diverse and they look very labile to human eyes; however, when performing a close inspection one can identify just a small subset of inflorescence types among a huge morphospace of possibilities. Consequently, some evolutionary constraints have restricted, to some extent, the diversification of their inflorescence. Developmental and genetic mechanisms, the photosynthetic type and plant longevity have been postulated as candidate constraints for angiosperms and panicoids in particular; however, it is not clear how these factors operate and which of these have played a key role during the grass inflorescence evolution. To gain insight into this matter the macroevolutionary aspects of panicoid inflorescences are investigated.

• *Methods* The inflorescence aspect (lax versus condensed), homogenization, truncation of the terminal spikelet, plant longevity and photosynthetic type were the traits selected for this study. Maximum likelihood and Bayesian Markov chain Monte Carlo methods were used to test different models of evolution and to evaluate the existence of evolutionary correlation among the traits. Both, models and evolutionary correlation were tested and analysed in a phylogenetic context by plotting the characters on a series of trees. For those cases in which the correlation was confirmed, test of contingency and order of trait acquisition were preformed to explore further the patterns of such co-evolution.

• *Key Results* The data reject the independent model of inflorescence trait evolution and confirmed the existence of evolutionary contingency. The results support the general trend of homogenization being a prerequisite for the loss of the terminal spikelet of the main axis. There was no evidence for temporal order in the gain of homogenization and condensation; consequently, the homogenization and condensation could occur simultaneously. The correlation between inflorescence traits with plant longevity and photosynthetic type is not confirmed.

• *Conclusions* The findings indicate that the lability of the panicoid inflorescence is apparent, not real. The results indicate that the history of the panicoids inflorescence is a combination of inflorescence trait contingency and order of character acquisition. These indicate that developmental and genetic mechanisms may be important constraints that have limited the diversification of the inflorescence form in panicoid grasses.

Key words: Inflorescence, morphology, evolution, panicoids, Panicoideae, Poaceae.

INTRODUCTION

Inflorescence architecture in the grasses varies enormously even among close related species (Kellogg, 2000; Doust and Kellogg, 2002; Doust *et al.*, 2005; Liu *et al.*, 2005, 2007; Reinheimer and Vegetti 2008; Reinheimer *et al.*, 2009). In fact, the evolution of the grass inflorescence seems to be very complex and the identification of clear trends appears almost impossible. Recently, we have partially investigated this issue using the panicoid grasses as a model group (also known as Panicoideae *s.s.*; Sánchez-Ken and Clark, 2010) (Reinheimer *et al.*, 2012). We have found that within the theoretical morphospace comprising all possible combinations of inflorescence traits, only a few were actually observed in the group, and among them some inflorescence architectures predominate over others. In addition, the inflorescence of each of the major clades (Arundinelleae *s.s.*, Andropogoneae, Paspaleae, and Paniceae *s.s.* tribes; Morrone *et al.*, 2011) is characterized by distinct patterns of inflorescence character state transitions. These results indicated that the evolution of the inflorescence of panicoids may follow certain patterns and cannot evolve freely.

It has been postulated that limitation in biological diversity may be due to developmental and genetic constraints as well as selection at other levels (Prusinkiewicz *et al.*, 2007). In fact, it has been empirically corroborated that the extensive variation of inflorescence architecture among angiosperms is somewhat correlated with season length, plant longevity, pollination system and seed dispersal mode (Friedman and Harder, 2004, 2005; Prusinkiewicz *et al.*, 2007). Whether, why and how this presumed co-evolution has happened in the grasses is an intriguing question that remains open for further investigations. Interestingly, Morrone *et al.* (2011) recently reported that qualitative inflorescence character changes (such as

abortion of the fertile apex of main axes and rachises, lack of branching beyond first order, and modification of the disarticulation zone of the diaspores) in Paniceae *s.l.* (Paspaleae + Paniceae *s.s.* tribes) predominates at the C_4 clades, whereas the inflorescence of the C_3 clades has not changed much during evolution. Why this happens is still unclear, although the ancestral character evolution analyses suggested that changes in qualitative traits of the Paniceae *s.l.* inflorescence may represent a delayed response to the change in photosynthetic pathway (Morrone *et al.*, 2011).

To get new insights on the underlying bases of the evolution of the grass inflorescence, we have used the molecular phylogeny of panicoid grasses and maximum likelihood (ML) and Bayesian Markov chain Monte Carlo (MCMC) methods to (*a*) test models of evolution, (*b*) identify directional trends, (*c*) test the existence of correlated evolution among inflorescence traits, and (*d*) evaluate the association between inflorescence traits with plant longevity and photosynthetic type.

MATERIALS AND METHODS

Inflorescence morphology dataset

The morphological dataset was based on the following characters: (*a*) inflorescence aspect (0, condensed; 1, lax; 2, lax to condensed); (*b*) degree of homogenization (0, non-homogenized; 1, partially homogenized; 2, fully homogenized); and (*c*) presence/absence of terminal spikelet (0, non-truncated; 1, truncated). We followed the character definitions described in Rua and Weberling (1998) and Reinheimer *et al.* (2012). The reconstruction of the homogenization character resulted in ambiguity at deep nodes when the trait was codified as a multistate character. For this reason, we have explored the reconstruction of the homogenization of the inflorescence as a binary character (0, absence; 1, presence). Character-state data were compiled from published reports (reviewed in Reinheimer *et al.*, 2012). The dataset used in this study is presented in Supplementary Data Table S1.

To calculate the incidence of the different inflorescence types within panicoids we have calculated the proportion of taxa with a given inflorescence type.

Phylogeny

Phylogenetic reconstruction and analysis of character evolution were based on *ndhF* nucleotide sequences and alignment previously published by Aliscioni *et al.* (2003), Sánchez-Ken and Clark (2010), Zuloaga *et al.* (2010) and Morrone *et al.* (2011). 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 in the literature as the Paniceae $x = 10$ clade; Giussani *et al.*, 2001). The phylogenetic position of *Steyermarkochloa angustifolia* (Spreng.) Judz. and Tristachyideae is not well resolved (Sánckez-Ken and Clark, 2010; Morrone *et al.*, 2011). Consequently, we will focus our studies on the three major, well-supported panicoid lineages: Andropogoneae + Arundinelleae *s.s.*, Paspaleae and Paniceae *s.s.* tribes which include most of the species diversity in panicoids.

We estimated phylogenetic relationships using Bayesian Markov chain Monte Carlo (MCMC) analysis as implemented in MrBayes version v3·1.2 (Huelsenbeck and Ronquist, 2001). The best-fit model (GTR + G + I) was inferred with MrModeltest v.2·3 (Nylander, 2004) based on the Akaike criterion. Two Metropolis-coupled Markov chains with an incremental heating temperature of 0·2 were run for 10 million generations and sampled every 1000th generation. The analysis was repeated twice, starting from random trees. The convergence and the effective sample size (>100) of each replicate were checked using Tracer v. 1·5 (Rambaut and Drummond, 2007). A majority-rule consensus tree was then reconstructed after applying a burn-in of 2·5 million generations (thus considering a total of 15 002 trees).

Ancestral character state reconstruction

Character states were estimated for nodes with a posterior probability equal to/higher than 0·95. We have inferred ancestral character states using ML and MCMC methods.

For the MCMC analyses, we used the 'multistate' module in BayesTraits (Pagel *et al.*, 2004; program available at www.evolution.rdg.ac.uk), and the 15 002 trees previously calculated. An exponential prior seed from a uniform prior model was chosen. To set the range of the hyperprior, preliminary chains were run under ML (Pagel *et al.*, 2004). Given these results, the range of the hyperprior was set to 0–30 for all cases. In addition, several exploratory chains were also run adjusting the value of the rate coefficient proposals (the ratedev parameter) until an approximate acceptance rate of 20–40 % was achieved (Pagel *et al.*, 2004). Final values of ratedev were 0·1 for inflorescence appearance and homogenization degree, from 0·01 to 0·05 for presence/absence of homogenization, and 2 for presence/absence of terminal spikelet. Ancestral states were estimated using the most recent common ancestor ('MRCA') of selected taxa command. Once these parameters were set, two independent analyses were run for 10 million generations and sampled every 1000th generation to ensure independence. The first 1 million generations were discarded as burn-in (convergence and ESS were checked with Tracer v1·5; Rambaut and Drummond, 2007) and the rest of the samples from the two replicates were combined (18 002 samples). Hypothesized character states at internal nodes were tested by estimating Bayes factor (BF), comparing MCMC runs in which the node in question was constrained to one state versus the other using the 'fossil' command (Pagel *et al.*, 2004). The BF approach was based on smoothed estimates of marginal likelihood analysed with Tracer v1·5 (Rambaut and Drummond, 2007), which applies the method used by Newton and Raftery (1994) with modifications by Suchard *et al.* (2001). A value between 2 and 5 indicates 'positive' support, between 5 and 10 'strong' support, and any value >10 means 'very strong' support. Similar tendencies were recovered when additional analyses of ancestral state reconstruction were run using a uniform prior (0–100).

For conflicting nodes, we have also inferred the ancestral state reconstruction using the ML method. Maximum likelihood reconstructions were conducted using the 'multistate'

module included in the computer package BayesTraits (Pagel *et al.* 2004), and the 15 002 trees previously calculated. Ancestral states were estimated using the most recent common ancestor ('MRCA') of selected taxa command, and hypothesized character states at internal nodes were then tested by estimating the differences of the log likelihood in which the node in question was constrained to one state versus the other using the 'fossil' command (Pagel *et al.*, 2004). We applied the general rule that two log likelihood units (using the mean and median of 15 002 tree likelihoods) constitute a significant difference (Pagel *et al.*, 2004). There was no appreciable difference between the mean and the median of the 15 002 tree likelihoods. Consequently, we only calculated the mean.

For simplicity, we report ancestral state reconstructions on the majority rule consensus topology of 15 002 trees.

Models of inflorescence evolution

To test hypotheses on inflorescence evolution within panicoids, two models of character evolution were assessed. The first model assumes all character state transitions are unordered (i.e. direct transitions are possible among all character states), whereas the second model assumes ordered character state transitions as follows: transitions between lax inflorescence to condensed inflorescence are constrained to pass through a lax to condensed inflorescence, transitions between non-homogenized inflorescence and fully homogenized inflorescence are constrained to pass through a partially homogenized inflorescence, and the transitions from non-truncated to truncated inflorescences is irreversible. Models were tested on the 15 002 set of trees by ML and MCMC approaches using the 'restrict' command at the 'multistate' module of the computer package BayesTraits (Pagel *et al.*, 2004). For the ML model test we applied the general rule that two log likelihood units constitute a significant difference as explained above (Pagel *et al.* 2004). In the MCMC analysis, we used the MCMC settings and the approach to assess the convergence of the analysis as described above. The models were compared using approximate BF as explained before.

In addition, we calculated the global rate of the inflorescence aspect processes, and the homogenization processes as: $q_{\text{condensation}} = q1{,}2 + q1{,}0 + q2{,}0$ versus $q_{\text{de-condensation}} = q2{,}1 + q0{,}1 + q0{,}2$ for the inverse process, and $q_{\text{homogenization}} = q0{,}1 + q1{,}2 + q0{,}2$ versus $q_{\text{de-homogenization}} = q1{,}0 + q2{,}1 + q2{,}0$ for the inverse process, where the parameter qi, j is the transition rate from state 'i' to state 'j'. The statistical differences among pairs of rates were studied using the non-parametric Mann–Whitney *U*-test in the program InfoStat version 2009 (Grupo InfoStat, FCA, Universidad Nacional de Córdoba, Argentina).

Analysis of correlated evolution

We explored correlations between inflorescence characters across the evolutionary tree by using ML and the MCMC algorithms in the 'discrete' module implemented by BayesTraits (Pagel *et al.*, 2004). For this purpose, each trait was coded as a discrete character: condensed and lax to condensed inflorescence (state 0), lax inflorescence (state 1), non-homogenized inflorescence (state 0), partially and fully homogenized inflorescence (state 1), non-truncated inflorescence (state 0) and truncated inflorescence (state 1). For the ML test, each pair of characters was analysed under two different models (Pagel and Meade, 2006). The first model (the independent model I) assumed that the characters vary independently of each other. The second model (the dependent model D) allowed one character to vary based on the other. We determined the likelihood ratio (LR) using the equation $LR = -2[L(D) - L(I)]$ where L(D) and L(I) are the likelihood estimates of the dependent and independent models, respectively. We tested the likelihood ratio against χ^2 distribution, with four degrees of freedom (Pagel and Meade, 2006). $P < 0{\cdot}05$ is taken as positive evidence that the dependent model is favoured, $P < 0{\cdot}01$ means strong evidence, and $P < 0{\cdot}001$ is very strong evidence. When the MCMC algorithm was implemented, we used a uniform prior for the independent model and an exponential hyperprior for the model of dependent evolution (Pagel and Meade, 2006). Repeating the analyses applying a uniform hyperprior for the dependent model does not change the results. Each model, was run using the MCMC settings and the approach to assess the convergence of the analysis as described above. When necessary, the number of generations was doubled until the effective size of parameters exceeds 200 (checked with Tracer v1·5; Rambaut and Drummond, 2007). In all cases, the range of hyperprior was set to 0–30, and ratedev was set to 0·1. Then, we compared the model that assumed the independent evolution of the two binary characters being compared with that of a model that allowed for correlated evolution between these characters (dependent model) using BF as described above. Results >2 are taken as positive evidence that the dependent model is favoured, >5 is strong evidence, and >10 represents very strong evidence (Pagel and Meade, 2006).

Several factors (i.e. season length, plant longevity and photosynthesis type) are thought to be correlated with inflorescence architecture (Friedman and Harder, 2004, 2005; Prusinkiewicz, *et al.*, 2007; Morrone *et al.*, 2011). To explore better the strength of such correlations in the panicoids we statistically assessed parallelism between plant longevity (0, annual; 1, perennial) and photosynthetic pathway (0, C_3 or C_3–C_4 intermediates; 1, full-C_4) with the inflorescence traits studied here. We compiled the plant longevity and photosynthetic type datasets from published reports and on-line databases (GrassBase, http://www.kew.org/data/grasses-db.html; Watson and Dallwitz, 1992; Zuloaga *et al.*, 2000; Giussani *et al.*, 2001; Morrone *et al.*, 2011). The dataset is presented in Supplementary Data Table S1. For this analysis, we used the ML and the MCMC algorithms in the 'discrete' module implemented by BayesTraits (Pagel *et al.*, 2004), following the same methodology described above.

When we confirmed that two traits were correlated, we tested hypotheses about conditional evolution and the temporal order of trait acquisition by ML and MCMC analyses conducted in the 'discrete' module of BayesTraits (Pagel *et al.* 2004). For the ML method, we compared the likelihood estimates of two correlated evolution models: one in which all the transition rates were allowed to vary, to one that constrained the two transition rates to be equal. The likelihood ratio of the two models is tested against a χ^2 distribution with one degree of freedom (Pagel and Meade, 2006). $P < 0{\cdot}05$ is taken as positive evidence that the dependent model

is favoured, $P < 0{\cdot}01$ means strong evidence, and $P < 0{\cdot}001$ is very strong evidence. When MCMC was implemented, we analysed the posterior probability distributions of the values of the transition parameters estimated under the dependent model. For this analysis we calculated the *Z*-scores and average of the transition parameters (González-Voyer *et al.*, 2008). MCMC results are summarized in flow diagrams.

RESULTS

Phylogenetic and ancestral state reconstruction analyses

The topology of the Bayesian majority rule consensus (from 15 002 trees) is similar to that recovered using parsimony methods by Morrone *et al.* (2011). The Panicoids are divided in three major and well-supported lineages: Arundinelleae *s.s.* + Andropogoneae, Paspaleae and Paniceae *s.s.* tribes. Andropogoneae and Arundinelleae *s.s.* tribes form a well-supported clade, which is sister to the Paspaleae tribe. In addition, Paniceae *s.s.* is sister to the (Andropogoneae + Arundinelleae *s.s.*) + Paspaleae clade.

All reconstruction methods found that the ancestor of panicoid grasses had a lax, partially or fully homogenized, and non-truncated inflorescence (Fig. 1). Lax to condensed inflorescences or condensed inflorescences, as well as truncated inflorescences evolved later at the base of the Arundinelleae *s.s* and Andropogoneae tribes, and after the divergence of Paspaleae and Paniceae *s.s.* Reversal to the ancestral inflorescence aspect is observed at the Arthropogoninae and Melinidinae subtribes; however, reversal to the ancestral non-truncated inflorescence was not documented. In terms of homogenization, ML and MCMC methods suggest that the ancestor of the panicoids had a partially or fully homogenized inflorescence (Fig. 1), with 66–93 % probability and lnBF between 1 and 3·7, depending on the strategy applied. Non-homogenized inflorescences evolved only once in the Andropogoneae, and several independent times during the diversification of Paspaleae and Paniceae *s.s.* Reversals to the ancestral homogenized inflorescence occurred in the Arthropogoninae, Otachyriinae and Melinidinae subtribes, and possibly in the Cenchrinae and Boivinellinae.

Eight basic inflorescence types are identified when the three characters and their states are combined (presence/absence of branch condensation + presence/absence of homogenization + presence/absence of the terminal spikelet of the main axis; Supplementary Data Fig. S1). Lax, non-homogenized, non-truncated inflorescences are the most common inflorescence type observed in the panicoids. In contrast, lax, non-homogenized, truncated inflorescences are rare, and lax, homogenized, truncated inflorescences were not observed in the group.

Models of inflorescence evolution

Both, MCMC and ML methods showed similar results (Table 1). The free model allowing all the possible transitions among the states of inflorescence aspect fit the dataset better than the restricted model (lnBF = 3·88; log-likelihood difference = 4·21). The reconstruction of the transition rates based on the Bayesian MCMC method and the unordered model of evolution, showed that the highest rate of change was from lax inflorescences to lax to condensed inflorescences ($q1{,}2 = 2{\cdot}94 \pm 0{\cdot}78$), and from condensed inflorescences to lax to condensed inflorescences ($q0{,}2 = 2{\cdot}97 \pm 0{\cdot}83$), whereas the lowest rate of change was from lax to condensed inflorescences ($q1{,}0 = 0{\cdot}41 \pm 0{\cdot}77$; Fig. 2). Slightly different results were obtained under ML methods in which the highest rate of change was from condensed inflorescences to lax to condensed inflorescences ($q0{,}2 = 3{\cdot}7 \pm 0{\cdot}65$), and the lowest rates were to lax to condensed inflorescence as well as lax to condensed to condensed inflorescences ($q1{,}0 = 0{\cdot}66 \pm 0{\cdot}09$, and $q2{,}0 = 0{\cdot}65 \pm 0{\cdot}21$; Supplementary Data Fig. S2). The statistical differences among pairs of rates, associated with both evolutionary processes of de-condensation versus condensation, showed that the former process was favoured over the latter one independently of the method used (Bayesian MCMC inference: $q_{\text{de-condensation}} = 2{\cdot}4 \pm 1{\cdot}29$ vs. $q_{\text{condensation}} = 1{\cdot}89 \pm 1{\cdot}46$; ML inference: $q_{\text{de-condensation}} = 2{\cdot}58 \pm 1{\cdot}45$ vs. $q_{\text{condensation}} = 0{\cdot}99 \pm 0{\cdot}58$; Fig. 2 and Supplementary Data Fig. S2).

In terms of homogenization, the restricted model fits the dataset better, although there is no significant difference between the two models (lnBF = 2·00; log-likelihood difference = 0·42). The reconstruction of the transition rates based on the Bayesian MCMC method and the unordered model of evolution showed that the highest rate of change was from partially homogenized to non-homogenized inflorescences ($q1{,}0 = 8{\cdot}61 \pm 3{\cdot}45$), whereas the lowest rate of change was from fully homogenized to non-homogenized inflorescences ($q2{,}0 = 0{\cdot}13 \pm 0{\cdot}37$). The statistical differences among pairs of rates, associated to both evolutionary processes of homogenization versus de-homogenization, showed that the second process was favoured over the former ($q_{\text{de-homogenization}} = 4{\cdot}59 \pm 4{\cdot}55$ vs. $q_{\text{homogenization}} = 3{\cdot}5 \pm 4{\cdot}24$). The maximum likelihood method has produced similar tendencies (Supplementary Data Fig. S2).

Finally, we found that for truncation of the terminal spikelet the unordered model fit the dataset better than the restricted model (lnBF = 2·67; log-likelihood difference = 7·28). The reconstruction of the transition rates based on the Bayesian MCMC and ML methods and the unordered model of evolution showed that the highest rate of change was from non-truncated to truncated inflorescences (MCMC method: $q0{,}1 = 1{\cdot}06 \pm 0{\cdot}34$ vs. $1{\cdot}04 \pm 0{\cdot}36$; ML method: $q0{,}1 = 0{\cdot}97 \pm 0{\cdot}09$ vs. $q1{,}0 = 0{\cdot}96 \pm 0{\cdot}13$; Fig. 2 and Supplementary Data Fig. S2).

Evolutionary correlations

Bayesian MCMC and ML methods indicate, with positive statistical evidence, that there is correlation between homogenization and truncation (lnBF= 4·60; LR = 12·5, $P < 0{\cdot}01$; Table 2). Also, there is strong evidence for a correlation between the evolution of condensed inflorescences (condensed or lax to condensed inflorescences) the homogenization process (lnBF = 22·91; LR = 20·71, $P < 0{\cdot}001$), and truncation (lnBF = 15·16; LR = 34·92, $P < 0{\cdot}001$). In addition, MCMC and ML methods suggested a weak correlation between photosynthetic type and inflorescence aspect (lnBF = 2·02; LR = 7·9, $P = 0{\cdot}09$). None of the inflorescence characters studied show positive correlation with the plant longevity.

Having confirmed the correlation between inflorescence traits we tested hypotheses about conditional evolution and

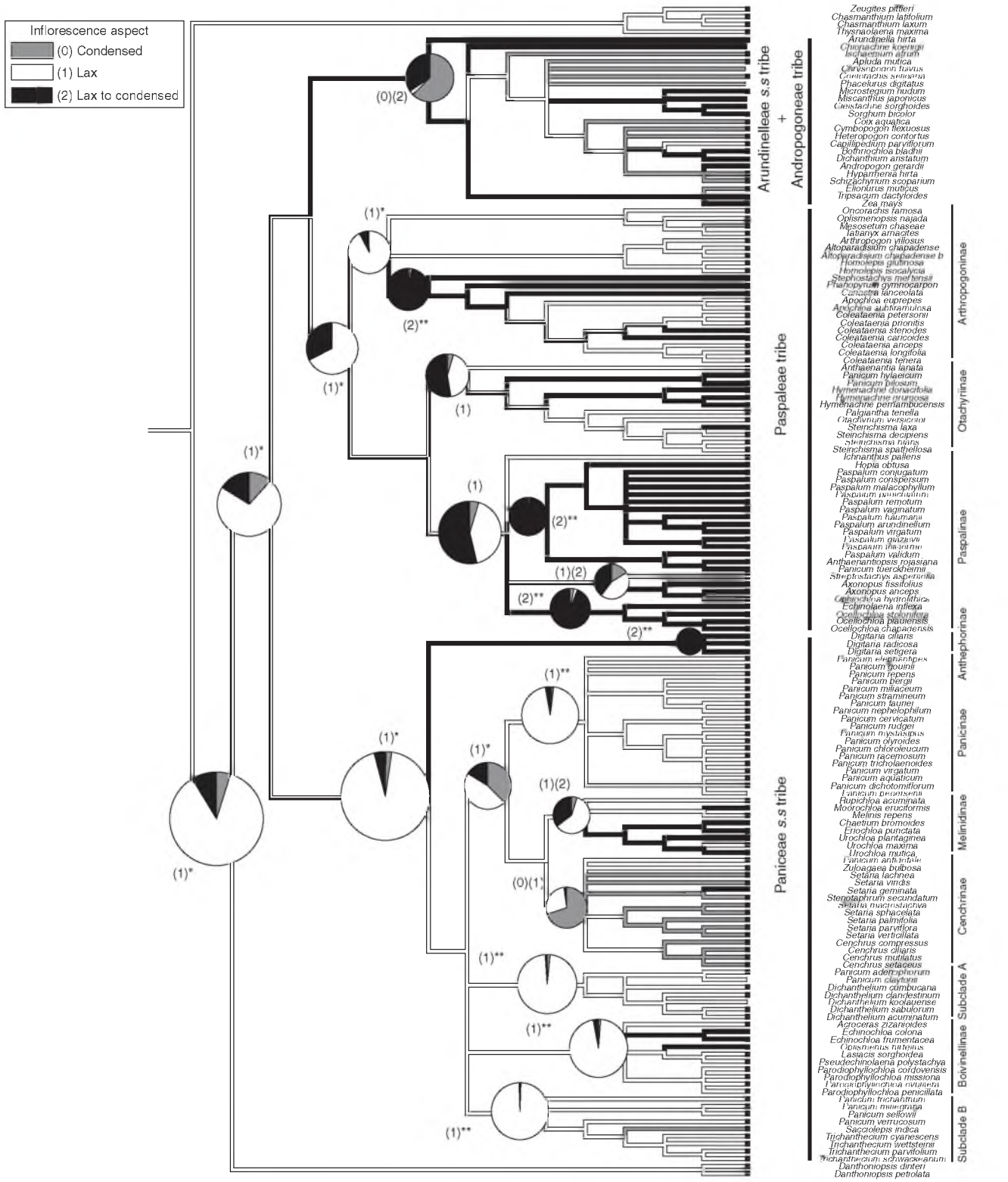


FIG. 1. Reconstructed ancestral character states on the Bayesian MCMC majority rule consensus tree. Branch shading indicates maximum parsimony reconstruction (according to Reinheimer *et al.*, 2012). Pie charts indicate Bayesian ancestral characters posterior probabilities at selected nodes. Numbers in parenthesis indicate the state with the highest likelihood based on the Bayes factor (BF) results. Two or more numbers in parenthesis indicates an ambiguous assignation of the ancestral character state. *, BF between 2 and 5 (positive support); **, BF between 5 and 10 (strong support).

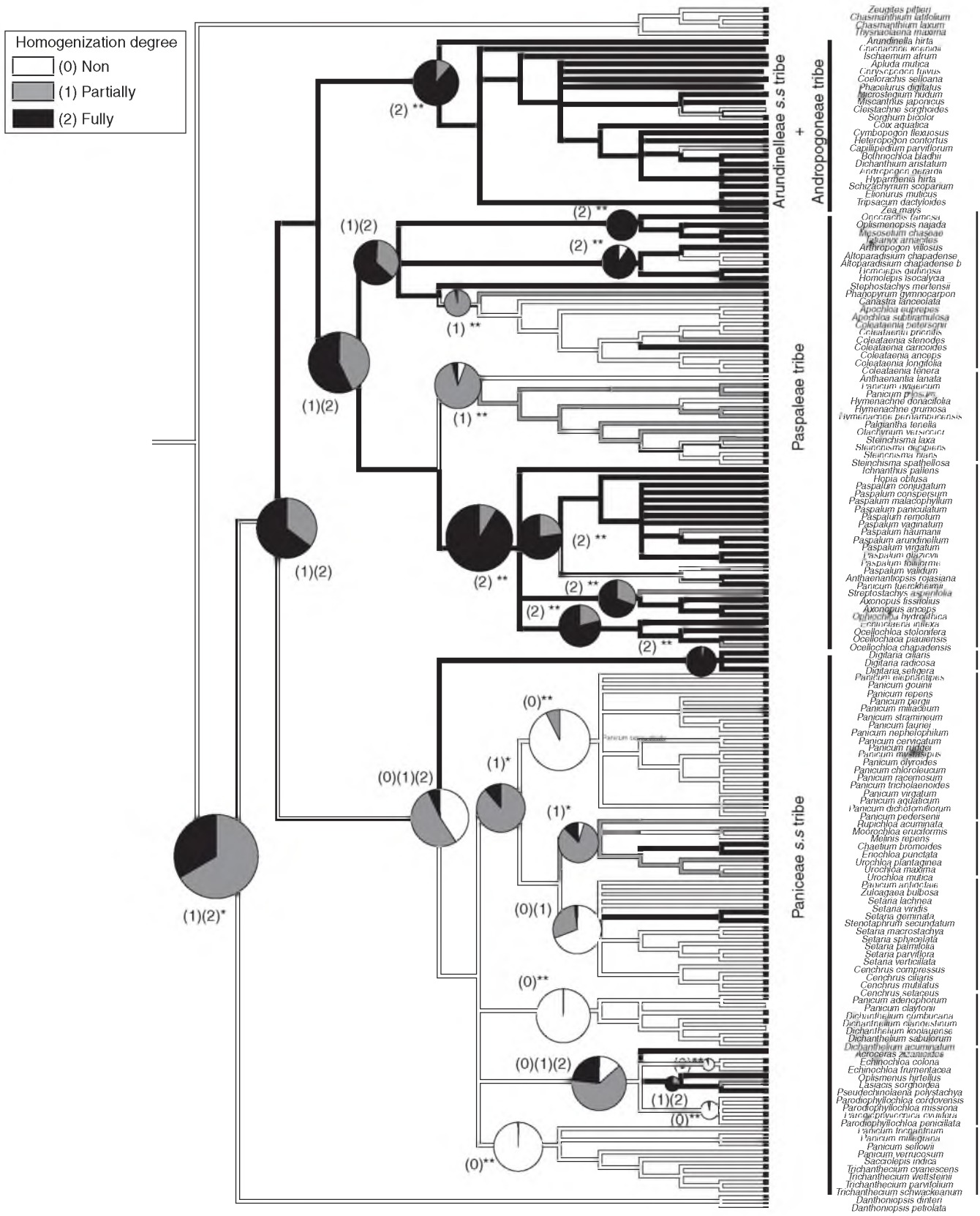


Fig. 1 *Continued*

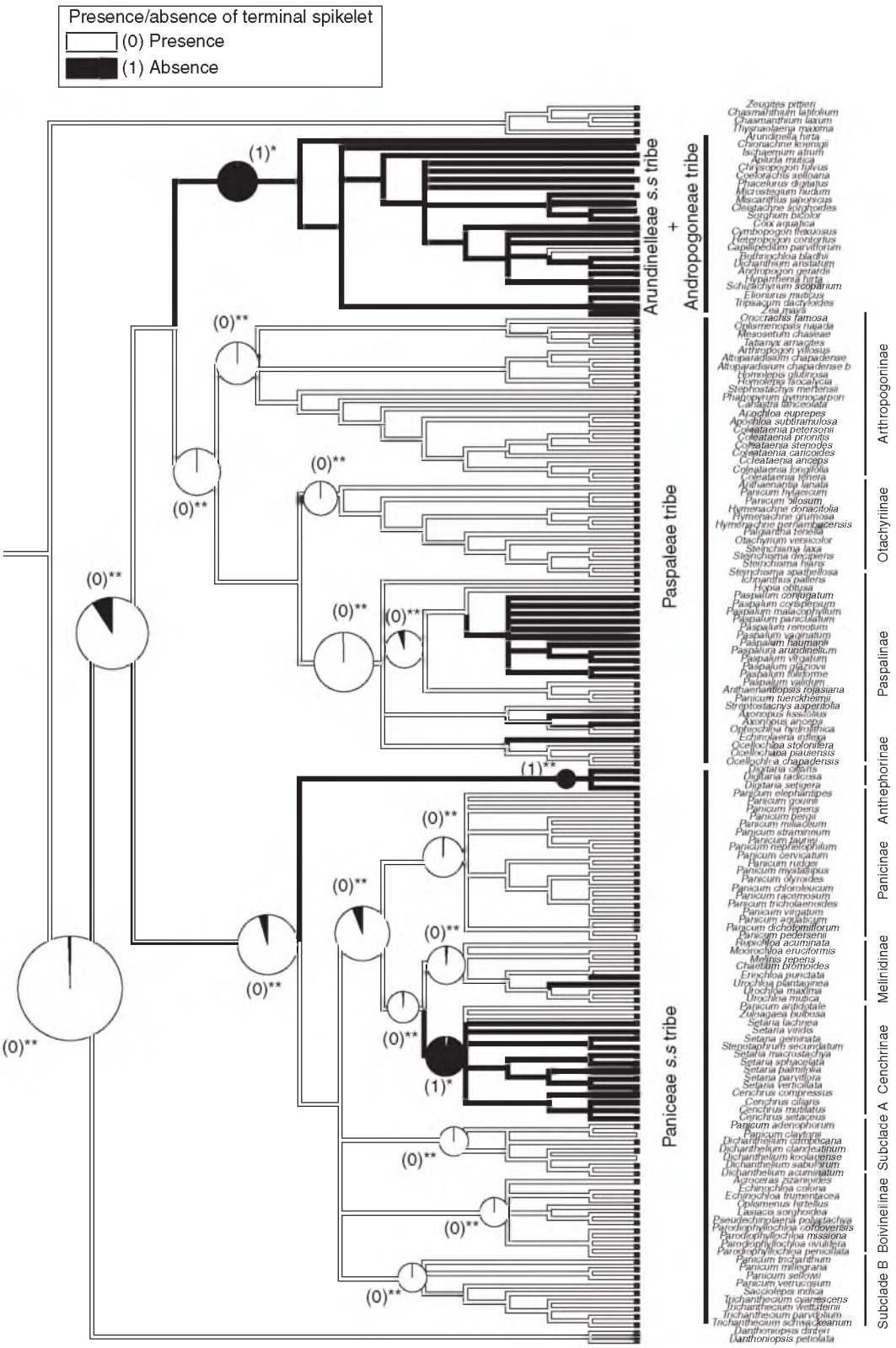
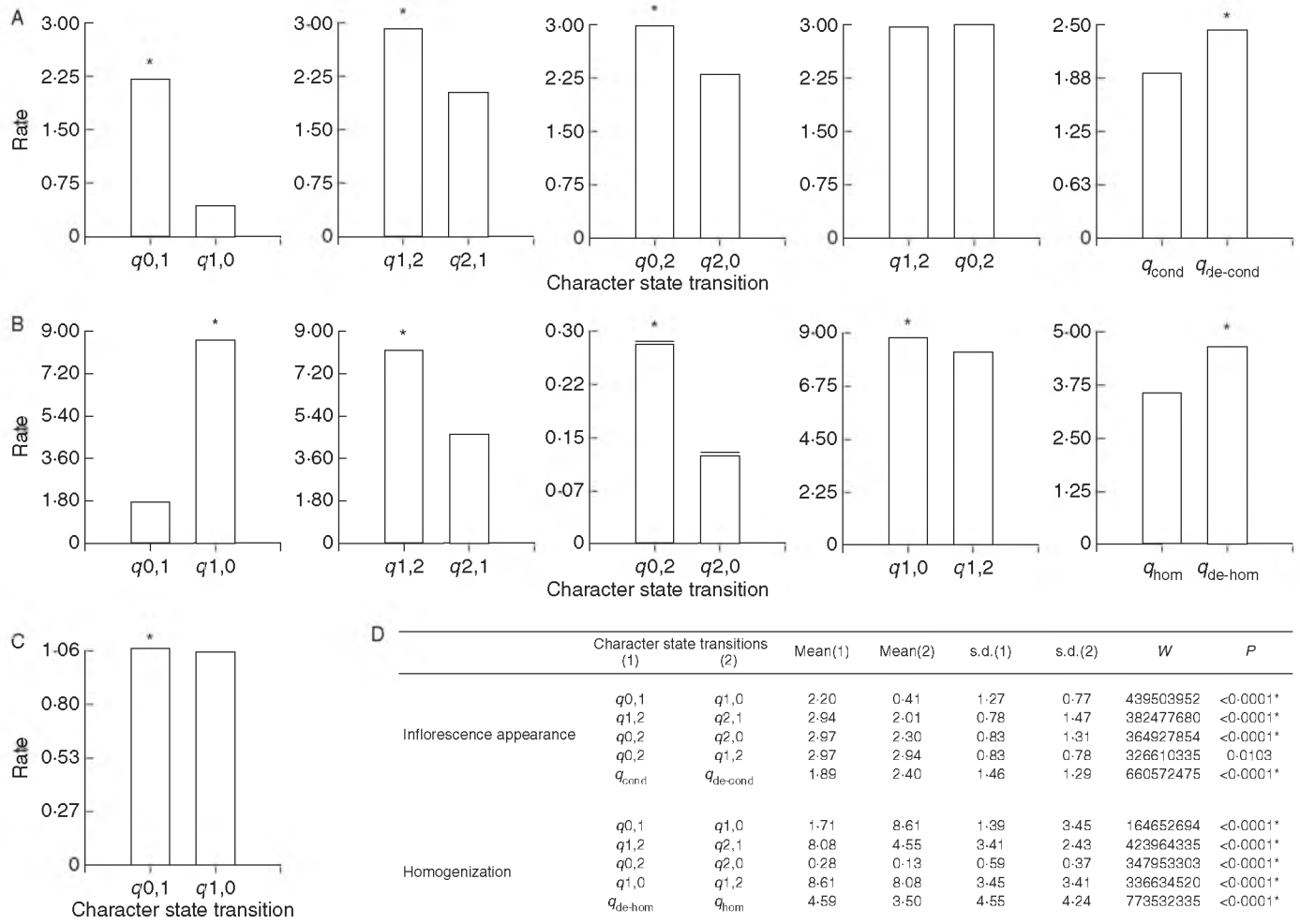


Fig. 1 *Continued*

TABLE 1. *Results of the unordered versus an ordered model of character evolution using MCMC and ML methods*

Character	Model	MCMC			ML	
		ln *P*(model | data)	s.e.	lnBF	−log likelihood	Difference in −log likelihood
Inflorescence aspect	Free	−102 17	0·047	3·88	−97 962 006	4·21
	Restricted	−106 052	0·078		−102 179 487	
Homogenization	Free	−93 989	0·048	2·00	−88 086 746	0·42
	Restricted	−91 985	0·046		−88 509 734	
Terminal spikelet truncation	Free	−40 959	0·026	2·67	−39 320 407	7·28
	Restricted	−43 632	0·035		−46 608 380	



D

	Character state transitions (1)	(2)	Mean(1)	Mean(2)	s.d.(1)	s.d.(2)	*W*	*P*
Inflorescence appearance	*q*0,1	*q*1,0	2·20	0·41	1·27	0·77	439503952	<0·0001*
	*q*1,2	*q*2,1	2·94	2·01	0·78	1·47	382477680	<0·0001*
	*q*0,2	*q*2,0	2·97	2·30	0·83	1·31	364927854	<0·0001*
	*q*0,2	*q*1,2	2·97	2·94	0·83	0·78	326610335	0·0103
	q_{cond}	$q_{de\text{-}cond}$	1·89	2·40	1·46	1·29	660572475	<0·0001*
Homogenization	*q*0,1	*q*1,0	1·71	8·61	1·39	3·45	164652694	<0·0001*
	*q*1,2	*q*2,1	8·08	4·55	3·41	2·43	423964335	<0·0001*
	*q*0,2	*q*2,0	0·28	0·13	0·59	0·37	347953303	<0·0001*
	*q*1,0	*q*1,2	8·61	8·08	3·45	3·41	336634520	<0·0001*
	$q_{de\text{-}hom}$	q_{hom}	4·59	3·50	4·55	4·24	773532335	<0·0001*
Truncation	*q*0,1	*q*1,0	1·06	1·04	0·34	0·36	327174653	0·0017*

FIG. 2. Rates of change between the different states of inflorescence aspect (A), homogenization (B) and truncation (C), obtained using an unordered model of character evolution and MCMC method. (D) Results of the statistical differences among pairs of rates using the non-parametric Mann–Whitney *U*-test. *qi*, *j* indicates the transition from the state *i* to the state *j*. An asterisk indicates significant differences under the non-parametric Mann–Whitney *U*-test.

the temporal order of trait acquisition with the contingency and ordered tests using ML and MCMC methods. The contingency test, using ML methods, indicated, with positive evidence, that non-homogenized inflorescences are more likely to evolve when the inflorescence is lax ($q2,1 < q4,3$), and condensed inflorescences are more likely to evolve when the inflorescence is homogenized ($q3,1 < q4,2$; Table 3 and Supplementary Data Fig. S3). However, the test could not infer with confidence, whether the homogenization happened before or after the change on the condensation aspect of the inflorescence ($q1,2 \approx q1,3$; $q4,2 \approx q4,3$). We found positive evidence for the evolution of non-truncated inflorescences when the

TABLE 2. *Bayes factor and likelihood ratio results of the BayesTraits Discrete analyses using MCMC and ML methods*

Character	Model	MCMC			ML	
		ln *P*(model | data)	s.e.	lnBF	−log likelihood	LR
Truncation with homogenization	Independent	−104 107	0·101	4·60	−101 94	12·5**
	Dependent	−99 502	0·079		−95 69	
Inflorescence condensation with homogenization	Independent	−133 763	0·105	22·91	−133 40	20·71***
	Dependent	−110 85	0·134		−123 04	
Inflorescence condensation with truncation	Independent	−106 54	0·094	15·16	−104 51	34·92***
	Dependent	−91 376	0·116		−87 05	
Inflorescence aspect with plant habit	Independent	−127 484	0·041	1·13	−101 80	1·16
	Dependent	−128 617	0·068		−101 22	
Homogenization with plant habit	Independent	−118 054	0·054	0·09	−118 92	1·7
	Dependent	−118 144	0·059		−118 07	
Truncation with plant habit	Independent	−103 229	0·053	0·15	−101 14	1·58
	Dependent	−103 588	0·05		−99 56	
Inflorescence aspect with photosynthesis	Independent	−102 714	0·087	2·02	−99 79	7·9*?
	Dependent	−100 694	0·11		−95 84	
Homogenization with photosynthesis	Independent	−92 662	0·091	0·25	−89 75	3·08
	Dependent	−92 408	0·104		−88 21	
Truncation with photosynthesis	Independent	−77 251	0·085	1·82	−73 90	7·38
	Dependent	−75 426	0·097		−70 21	

Likelihood values were tested over a χ^2 distribution. *? $P = 0{\cdot}09$, * $P < 0{\cdot}05$, ** $P < 0{\cdot}01$, *** $P < 0{\cdot}001$.

inflorescence is lax ($q2,1 < q4,3$). However, there was no evidence for temporal order in the gain of these traits (Table 3 and Supplementary Data Fig. S3). The analyses indicated very strong evidence for conditional evolution between homogenization and truncation events (Table 3 and Supplementary Data Fig. S3). The homogenization process occurred before the truncation of the terminal spikelet ($q1,2 > q1,3$), and truncation occurred if the inflorescence is homogenized ($q1,3 < q2,4$; Table 3 and Supplementary Data Fig. S3). Similar trends were inferred based on MCMC methods (Fig. 3).

Ancestral state reconstruction indicated that the ancestor of Panicoids had a lax, homogenized and non-truncated inflorescence. The most likely evolutionary pathway from the ancestral to the derived state of the traits can be inferred from the posterior probabilities of the transition rate parameters (Fig. 3). The posterior distribution shows that the most likely path from the ancestral state of lax and homogenized inflorescences to the derived state of condensed and non-homogenized inflorescences may involve (*a*) a transition toward condensed inflorescence ($q4,2 > 0$) followed by a transition toward non-homogenized inflorescence ($q2,1 > 0$), or (*b*) a transition toward non-homogenized inflorescence ($q4,3 > 0$) followed by a transition toward condensed inflorescence ($q3,1 > 0$; Fig. 4). These transitions were assigned a value of zero in <2 % of the sampled Markov chains. The inverse path from the most derived state of condensed and non-homogenized inflorescences toward the ancestral state of lax and homogenized inflorescences is not likely to occur ($q3,4 \approx 0$; $q2,4 \approx 0$; Fig. 4). Both transitions were assigned a value of zero in about 60 % of the sampled Markov chains (Fig. 3). In addition, the posterior distribution shows that the most likely path from the ancestral state of lax and non-truncated inflorescences to condensed and truncated inflorescences involved first a transition toward condensed ($q3,1 > 0$), followed by a transition toward truncated inflorescences ($q1,2 > 0$; Fig. 4). Both transitions in this evolutionary path were assigned a value of zero in $<0{\cdot}1$ % of the sampled Markov chains (Fig. 3). The alternative route which involves first a transition towards truncated inflorescences ($q3,4 \approx 0$) followed by a transition to condensed inflorescences ($q4,2 > 0$) is not supported (Fig. 4). The transition that involves first the truncation process was assigned a value of zero in over 96 % of the sampled Markov chains (Fig. 3). The inverse path from the most derived state of condensed and truncated inflorescences toward the most ancestral state of lax and non-truncated inflorescences is most likely to occur via an initial gain of lax aspect ($q2,4 > 0$) followed by the gain of a terminal spikelet ($q4,3 > 0$; Fig. 4). These transitions were assigned a value of zero in $<0{\cdot}8$ % of the sampled Markov chains (Fig. 3). The alternative inverse route may not be possible given a restriction to gain a terminal spikelet when the inflorescence is condensed ($q2,1 \approx 0$; Fig. 4). This transition was assigned a value of zero in 93 % of the sampled Markov chains (Fig. 3). Finally, the posterior distribution shows that the most likely path from the ancestral state of non-truncated and homogenized inflorescences to truncated and non-homogenized inflorescences involved first a truncation process ($q1,2 > 0$) followed by the loss of homogenization ($q2,4 > 0$; Fig. 4). None of these transitions in this evolutionary path were assigned a value of zero in the sampled Markov chains (Fig. 4). The alternative route that involves first the loss of homogenization ($q1,3 \approx 0$) followed by truncation ($q3,4 > 0$) may not be possible (Fig. 4). The transition $q1,3$ was assigned a value of zero in 80 % of the sampled Markov chains (Fig. 3). The inverse path from the derived state of non-homogenized and truncated inflorescences toward the ancestral state of homogenized and non-truncated inflorescences is most likely to occur via a loss of the terminal spikelet of the main axis first ($q4,3 > 0$) followed by the gain of homogenization ($q3,1 > 0$; Fig. 4). These transitions were assigned a value of zero in $<0{\cdot}1$ % of the sampled Markov chains (Fig. 3). The alternative inverse route may not be possible given a restriction to gain homogenization when

TABLE 3. *Likelihood ratio values for test of contingent evolution and temporal order between inflorescence characters*

Traits test	Trait contingency			Ordered change		
	$q1,2 = q3,4$	$q2,1 = q4,3$	$q1,3 = q2,4$	$q3,1 = q4,2$	$q1,2 = q1,3$	$q4,2 = q4,3$
Inflorescence aspect vs. Homogenization	0·46	3·92* Trend: loss of homogenization more likely to evolve when the inflorescence is lax	0·38	5·14* Trend: condensation process more likely to happen when the inflorescence is homogenized	0·16	0·34
Inflorescence aspect vs. Truncation	2·68	4·14* Trend: have a terminal spikelet when the inflorescence is lax	0·76	0·82	1·1	0·24
Homogenization vs. truncation	17·64*** Trend: homogenization process is likely to evolve on inflorescences that are truncated	21·00*** Trend: non-homogenized inflorescences are more likely to evolve when the inflorescence is non-truncated	22·48*** Trend: loss of terminal spikelet is more likely to happen when the inflorescence is homogenized	18·34*** Trend: the presence of a terminal spikelet is more likely to evolve when the inflorescence is non-homogenized	18·68*** Trend: gain of homogenization before truncation	17·52*** Trend: loss of homogenization before gaining a terminal spikelet

qi, j indicates the transition from the state i to the state j. Likelihood values were tested over a χ^2 distribution. * $P < 0{\cdot}05$, *** $P < 0{\cdot}001$.

the inflorescence is already truncated ($q4,2 \approx 0$; Fig. 4). This transition was assigned a value of zero in 56 % of the sampled Markov chains (Fig. 3).

DISCUSSION

Reconstructions of ML and MCMC methods show many independent origins and reversals of the panicoid inflorescence traits studied here, in agreement with previous work (Reinheimer *et al.*, 2012). This suggests that inflorescence characters can easily switch from one state to another, in any direction, without a specific pattern. However, despite the apparent lability of the panicoid inflorescence, results presented in this work support the existence of some general evolutionary trends and patterns, which are discussed below.

Both MCMC and ML ancestral state reconstructions indicate that the ancestor of the panicoid grasses had a lax, homogenized and non-truncated inflorescence. After the origin of panicoids, the inflorescence evolved following an unordered model of evolution (direct transitions are possible among all character states) in which the processes of de-condensation, de-homogenization and truncation appear favoured over the processes of condensation, homogenization and gain of a terminal spikelet. These results partially agree with previous reports (Reinheimer *et al.*, 2012). Reinheimer *et al.* (2012), using the parsimony reconstruction method, found that the panicoid ancestor may have had a non-homogenized inflorescence. This conflicting result is due to differences in the methodology used by Reinheimer *et al.* (2012) and those used here. Under parsimony reconstruction, the process of homogenization is favoured over the process of de-homogenization. In addition, Reinheimer *et al.* (2012) suggest, as do our results, that the evolution from lax to condensed inflorescences and from non-homogenized to homogenized inflorescence is not necessarily a two-step process that requires an intermediate transition from lax to condensed and partially homogenized inflorescence as was previously hypothesized by Rua (1996) and Rua and Weberling (1998).

Despite the lability of inflorescence characters in evolutionary time, ML and MCMC analyses strongly reject the independent model of evolution. The results confirm the existence of an evolutionary contingency among the inflorescence aspect, homogenization and the loss of the terminal spikelet of the main axis. Overall, the data presented here strongly support the general trend of homogenization being a prerequisite which allows the later loss of the terminal spikelet of the inflorescence main axis. Interestingly, there was no evidence for temporal order in the gain of homogenization and condensation, suggesting that homogenization and condensation could have occurred simultaneously.

Similar trends may occur in other angiosperm families, as originally suggested by Weberling (1977, 1985). In many families truncated inflorescences have also been described as homogenized (i.e. Cyperaceae, Commelinaceae, Podostemaceae, Chenopodiaceae, Amaranthaceae, Brassicaceae, Resedaceae, Fabaceae, Malvaceae, Primulaceae, Lamiaceae, Scrophulariaceae, Gesneriaceae, among others; Weberling 1965, 1985; Rua 1999; Acosta *et al.*, 2009; Panigo *et al.*, 2011; Reutemann *et al.*, 2012). In these cases, the loss of the apical organs (such as flowers or spikelets) of the angiosperm inflorescence may

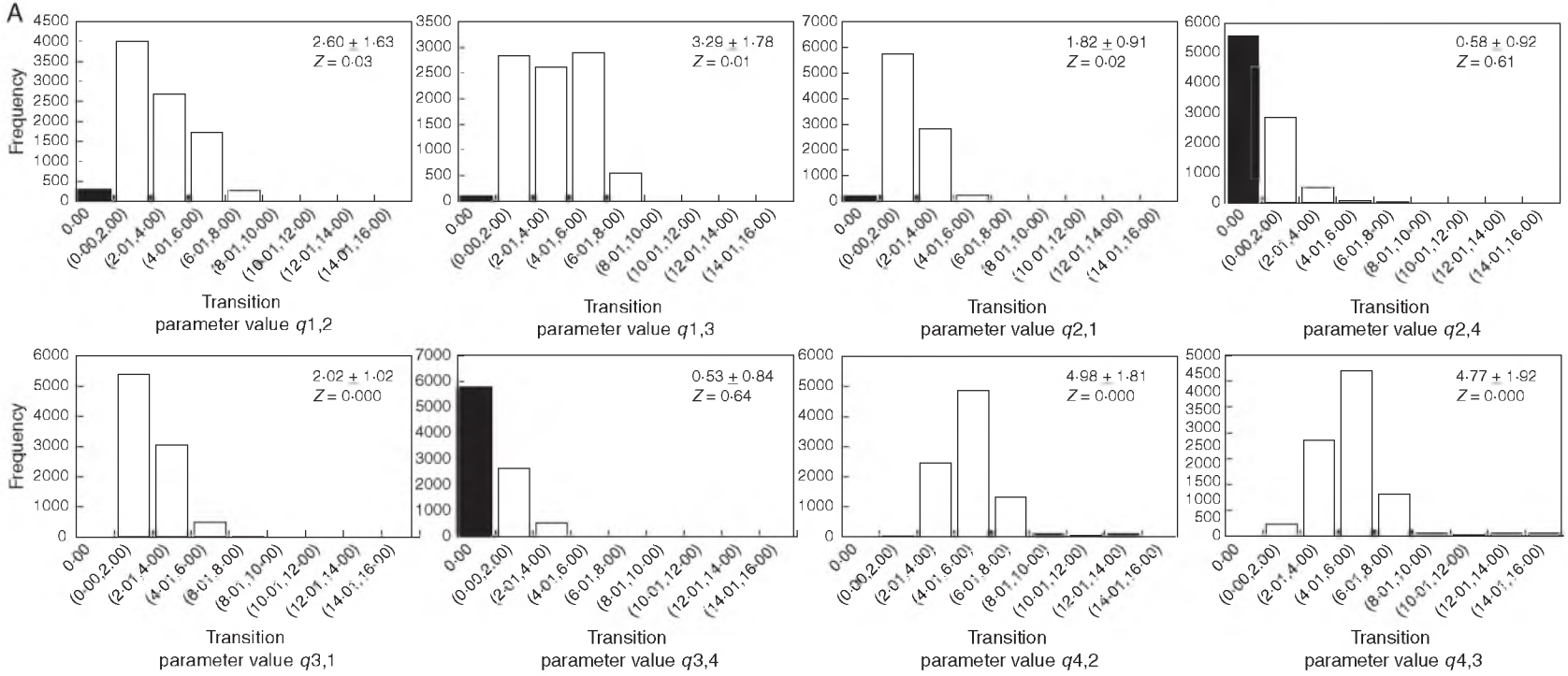


FIG. 3. Posterior probability distribution of the rate coefficients values of the correlated evolution model using the MCMC method. (A) Inflorescence aspect and homogenization, (B) inflorescence aspect and truncation, and (C) homogenization and truncation. *qi, j* indicates the transition from the state *i* to the state *j*. *Z*-values represent the proportion of the sampled runs from the Markov chain in which the parameter was assigned a value of zero. Values are the average and the standard deviation of the parameter values from the sampled runs.

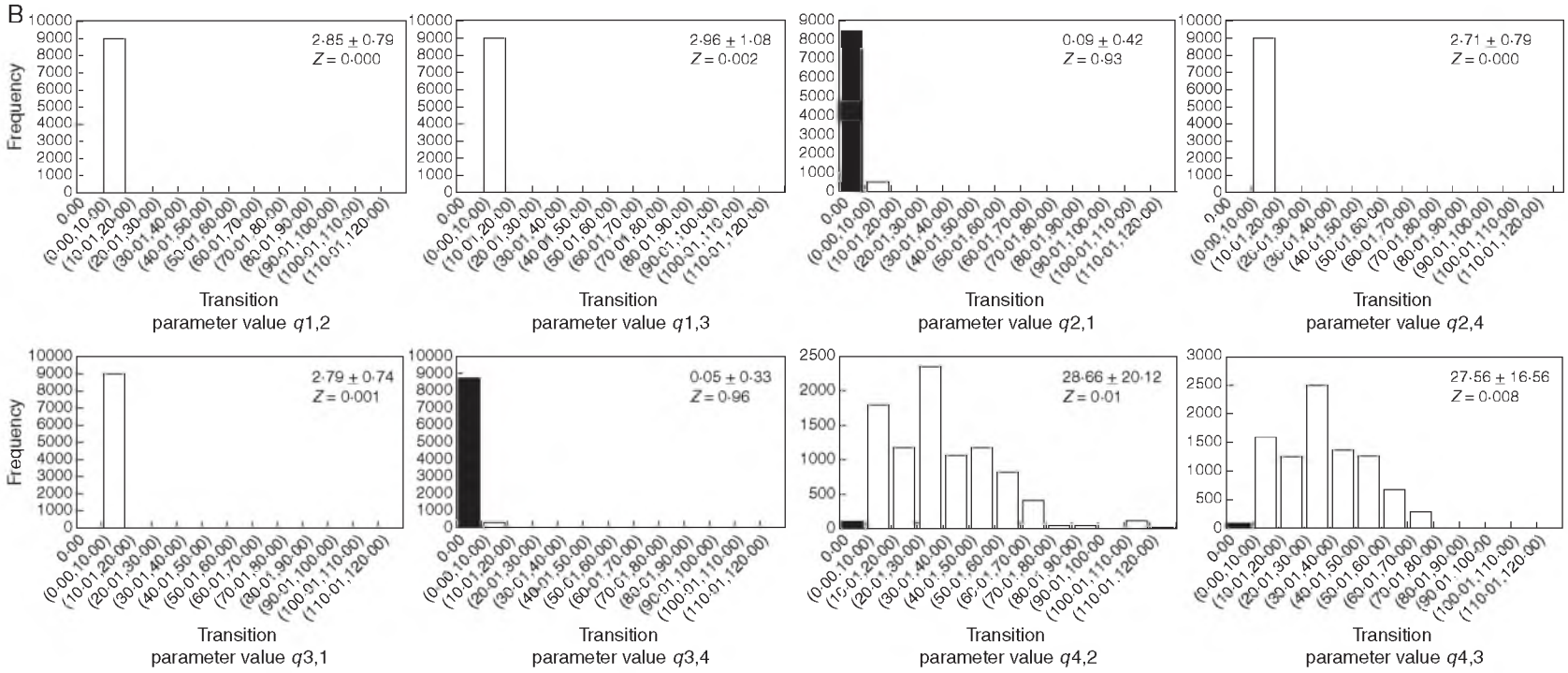


Fig. 3 *Continued*

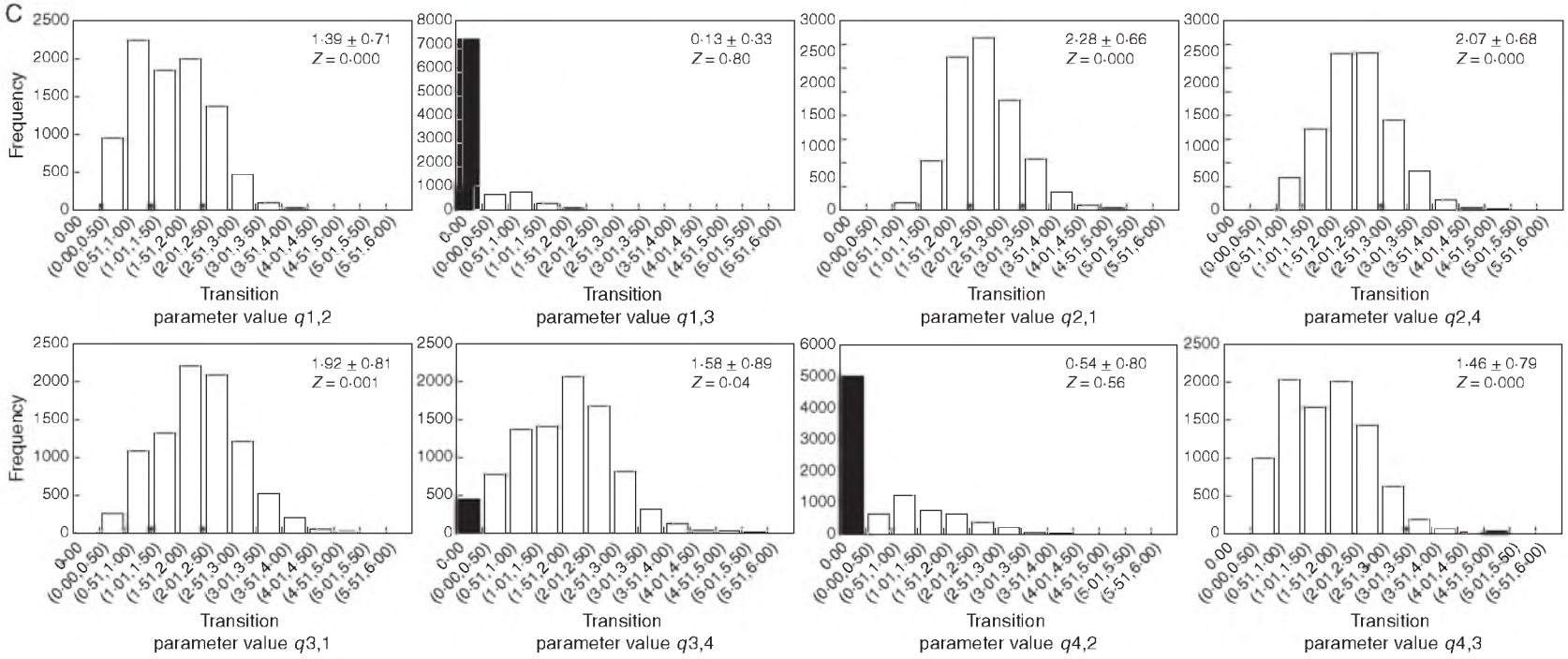


Fig. 3 *Continued*

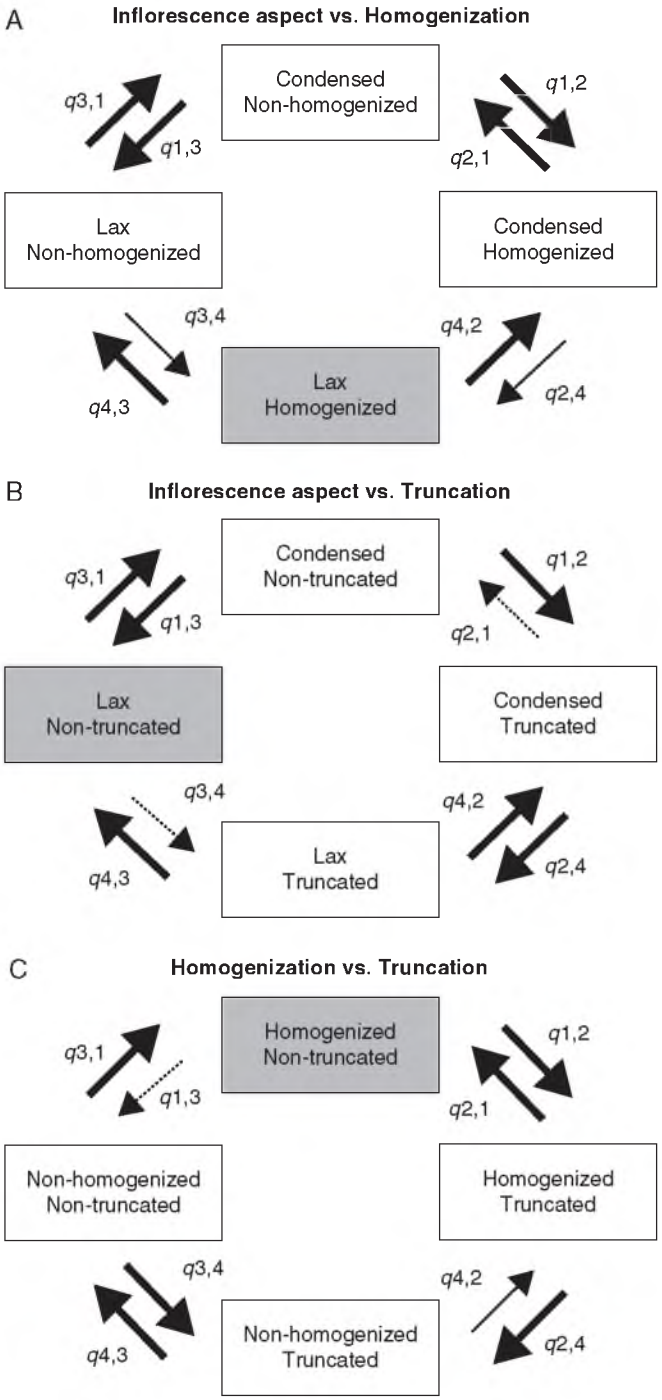


FIG. 4. Flow diagram showing the most probable evolutionary pathway from (A) the ancestral state of lax and homogenized inflorescence to the derived state of condensed and non-homogenized inflorescence, (B) the ancestral state of lax and non-truncated inflorescence to the derived state of condensed and truncated inflorescence, and (C) the ancestral state of homogenized and non-truncated inflorescence to the derived state of non-homogenized and truncated inflorescence. *qi, j* indicates the transition from the state *i* to the state *j*. Thick black arrows highlight transitions that have a low posterior probability of being zero (<5 %), thin arrows denote transitions that have higher probability of being zero (56–64 %) and dotted arrows indicate transitions that have a very high probability of being zero (>80 %). The grey shaded boxes indicate the inferred ancestral state.

require a previous organization or specialization of the axillary branches below the apex, such as simplification of the branding pattern and the presence of long and short branches (Rua and Weberling, 1998; Rua, 1999; Reinheimer *et al.*, 2012). Homogenization and truncation may represent evolutionary advantages for angiosperm taxa, and in particular for the grasses, given the reiterative pattern of acquisition of such traits for many species, or by entire angiosperm families.

The data presented here show that the evolutionary pathway from the ancestral inflorescence type of panicoids to the most-derived inflorescence type could have been accomplished either by a five- or a three-step process, indicating that the condensed, non-homogenized and truncated inflorescence may have resulted from one of two different evolutionary scenarios (Fig. 5). Our results also suggest that reversion from the derived inflorescence type to the ancestral condition is a three-step process, but one that cannot be fully completed given the constraint of a lax inflorescence to become homogenized (Fig. 5). Under this tentative scenario, the lax, non-homogenized non-truncated inflorescence type, which is the most abundant type among panicoids (Reinheimer *et al.*, 2012; this work), may represent a first step from the ancestral to the derived type, or, in several cases, an incomplete reversion to the ancestral morphology (Fig. 5). This may also explain why the lax, homogenized non-truncated inflorescence is probably one of the less abundant types of panicoid inflorescences (Reinheimer *et al.*, 2012; this work).

It has been postulated that developmental and genetic mechanisms available for selection, as well as ecological–physiological factors could impose constraints to the biological diversity of angiosperms (Kellogg, 2000; Doust and Kellogg 2002; Friedman and Harder 2004, 2005; Prusinkiewicz *et al.*, 2007; Morrone *et al.*, 2011).The present work had demonstrated that in panicoids some inflorescence character-state transitions could occur frequently, some are rare, and some others are unlikely to happen. The fact that inflorescence character-state transitions occur with different rates indicates that, though such transitions are developmentally and genetically possible, their specific frequency may reflect different intensities of selection. In contrast, those character-state transitions that are unlikely to happen or that have never been documented may have such high morphogenetic costs that there is a restriction on the availability of these pathways for selection. It is also possible that, because of the high number of convergences and reversals observed, inflorescence diversification has occurred as a response to various ecological–physiological pressures. However, so far neither plant longevity nor the photosynthetic type have been shown to be strongly correlated with the evolution of the inflorescence characters analysed here.

The present study clearly reveals some comprehensive macroevolutionary trends on the evolution of the panicoid inflorescence. Overall, the differences among the frequencies of character-state transitions and the dependent evolution of inflorescence traits suggest a complex of constraints imposing restrictions to the diversification of the panicoid inflorescence. Among such constraints, morphogenetic cost (the cost to establish new developmental and genetic mechanisms versus

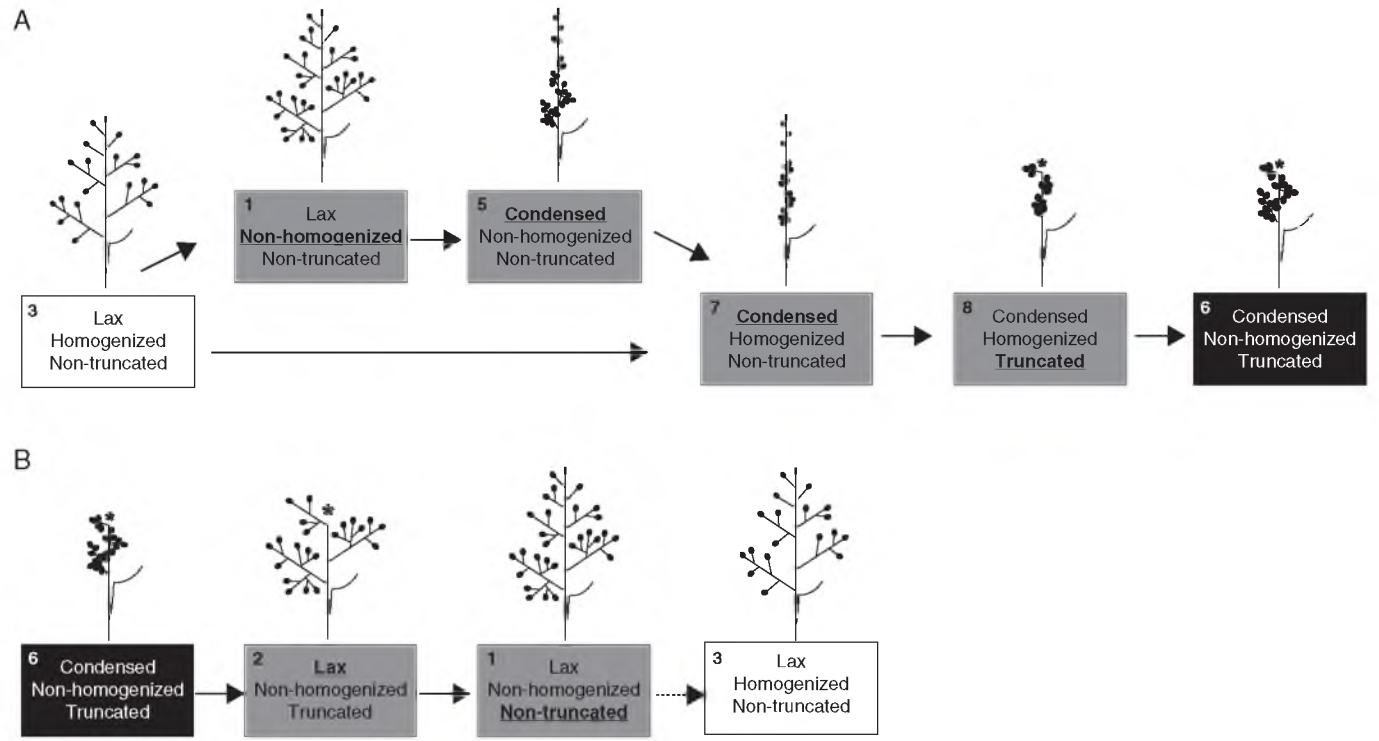


FIG. 5. Diagram summarizing our results concerning the macroevolutionary patterns of panicoid inflorescence evolution. The white box represents the ancestral inflorescence type. Grey boxes indicate intermediate inflorescence types. The black box represents the most derived inflorescence type. The dotted arrow indicates an unlikely transition according to MCMC methods. Numbers in the top left corner of the boxes indicate the inflorescence type (see Supplementary Data Fig. S1). Black ovals represent a spikelet. The asterisks represent the missing terminal spikelets of the inflorescence. (A) The evolutionary pathway from the ancestral inflorescence type to the most derived inflorescence type may have occurred via either a five- (3–1–5–7–8–6) or three-step (3–7–8–6) process. (B) The most likely evolutionary pathway from the most derived inflorescence type to the ancestral inflorescence type.

the cost of using developmental and genetic mechanisms already established) may be a key factor.

More detailed analyses of particular clades of panicoids, involving a more exhaustive taxon sampling, as well as a larger set of inflorescence traits and environmental parameters, could reveal local evolutionary pathways that do not necessarily agree with the general trends described here. It would also be interesting to investigate whether similar trends can be found in other grass or angiosperm lineages. Such investigations would reveal to what extent the models of inflorescence evolution described here can be extended to other groups.

SUPPLEMENTARY DATA

Supplementary data are available online at www.aob.oxfordjournals.org and consist of the following. Table S1: dataset used in this study. Figure S1: incidence of a given inflorescence type among panicoids main lineages. Figure S2: rates of change between the different states of inflorescence aspect, homogenization and truncation obtained using an unordered model of character evolution and the maximum likelihood method. Figure S3: posterior probability distribution of the rate coefficients values of the correlated evolution model using the maximum likelihood method.

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