

ECOLOGY AND EVOLUTION OF THE DIASPORE "BURIAL SYNDROME"

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Hygroscopically active awns or "bristles" have long intrigued scientists. Experimental evidence shows that they are important for diaspore burial in the correct orientation, thereby increasing successful seed germination and seedling survival. Despite these ecological advantages, 38 of the 280 species of grasses in Danthoioideae lack awns. We provide the first study of awns in a phylogenetic context and show that although the awnless state has arisen ca. 25 times independently, the ecological disadvantage of not having an awn also applies in an evolutionary context. Only in *Tribolium* and *Schismus* have awnless ancestors diversified to form a clade of primarily awnless descendents. Several of the awnless species in these genera are annual and we find a significant correlation between the evolution of awns and the evolution of life history. A suite of other diaspore traits accompany the awned or awnless states. We interpret the awn as being the visible constituent of a compound "burial syndrome," the two ecological extremes of which may explain the correlation between awns and life history and provide an explanation why awnless species in *Tribolium* and *Schismus* persist.

KEY WORDS: Ancestral state reconstruction, character loss, correlated evolution, Danthoioideae, Poaceae.

A major goal of macroevolutionary studies is explaining imbalances in the distribution of biodiversity among clades in the tree of life. One established route through this endeavor is to test the possible role of intrinsic factors (traits, "key innovations" [Miller 1949]) promoting diversification in the group that possesses such an innovation, relative to the groups that do not (Mitter et al. 1988; Sanderson and Donoghue 1994; Barraclough et al. 1995; Hodges and Arnold 1995; Klak et al. 2004; Moore and Donoghue 2007, 2009) beyond differences in rates expected by chance (Slowinski and Guyer 1989a,b). Classic examples of biological traits that are associated with increased species richness include nectar spur length in *Aquilegia* (Hodges and Arnold 1995; Ree 2005), sexual selection by female choice in birds (Barraclough et al. 1995; Mitra et al. 1996), and phytophagy in insects (Mitter et al. 1988). The idea behind each of these cases is that if the presumed key innovation evolves in a suitable ecological setting (Miller 1949;

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de Queiroz 2002) it will allow entrance to a novel adaptive space, promote diversification and thereby generate imbalances in diversity among clades.

What, then, might the consequences of losing such innovations be? Losses of key innovations have occurred repeatedly, without necessarily conferring a detrimental effect on diversification, as might be expected based on the increased opportunities for diversification offered by the acquisition of such traits (e.g., loss of photosynthetic activity in holoparasitic plants [Kujit 1969; Barkman et al. 2007]; loss of the ability to fly in birds [Roff 1994; Harshman et al. 2008], and insects [Wagner and Liebherr 1992; Whiting et al. 2003]). "Successful" losses of innovations can only have occurred after, or simultaneously with (1) the occurrence of an ecological shift that has turned an otherwise deleterious shift to an advantage and (2) a suite of morphological and/or physiological changes that have reinforced the selective advantage of the new state. We investigate these conditions for the case of grasses (Poaceae) that have lost their awn (bristle), a structure known to be important during seed dispersal, in particular for promoting successful burial and establishment.

Many plant species have structures that increase efficiency of seed dispersal, for example, hooks and spines for catching on to fur in chestnut (*Castanea*), sail-like structures for capturing the wind in sycamore (*Acer*) or structures that coil and uncoil in response to changes in air humidity and thereby propel seeds across a soil surface or drill seeds firmly into the ground in geraniums (*Erodium*). In grasses, this hygroscopic activity is exhibited by awns attached to the diaspore. We use “diaspore” to describe the dispersal units in grasses consisting of a one-seeded nut (caryopsis or achene) in which the ovary wall is usually fused with the seed coat, together with the “chaff,” the lemma, and the palea (Fig. 1). Early accounts of hygroscopic activity of grass awns focused on describing the mechanics of the torsion activity (e.g., Hildebrand 1873; Zimmerman 1879; Murbach 1900). Later studies demonstrated that this activity caused movement of diaspores in Petri dishes upon wetting and drying (Simpson 1952), suggesting that the role of hygroscopically active awns in seed (or diaspore) dispersal was primarily in burial (Stebbins 1971, 1974). Peart (1979,

1981, 1984) showed that the depth and orientation of burial significantly influences percentage germination, that the presence of a hygroscopically active awn dramatically increases the proportion of seed lodged in suitable microsites in the soil, that removal of increasing portions of the awn decreases the percentage of seed that germinates, and that the presence of the awn affects soil type preferences, both under experimental and field conditions. Together with more recent studies on both ecological and mechanical aspects of hygroscopically active awns (Garnier and Dajoz 2001; Elbaum et al. 2007; Kulic et al. 2009) these studies have firmly established that having a well-developed awn provides an ecological advantage, enhancing seed dispersal, burial, germination, and establishment. Accordingly, we expect species that have lost their awns to be at a selective disadvantage compared to their awned competitors.

Typically, the species of the Danthonioideae (Barker et al. 2001), one of the smaller grass subfamilies, have a conspicuous, hygroscopically active awn, borne in a sinus on the lemma

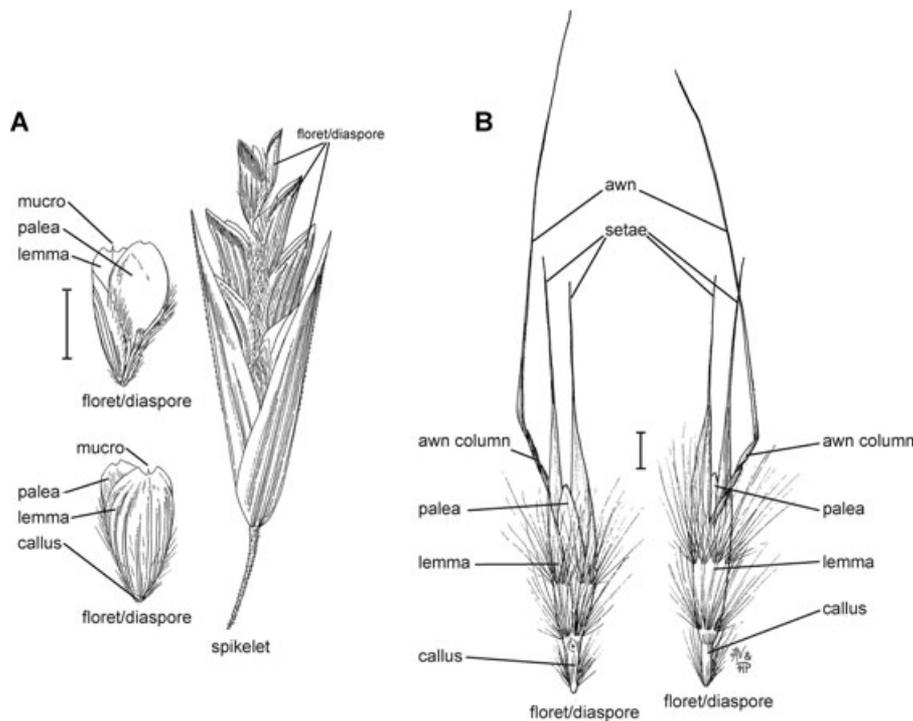


Figure 1. Diaspores that constitute two extremes of the burial syndrome. The floret encompasses the lemma and the palea that together enclose the male and female parts. After fertilization, seed maturation takes place between the lemma and palea, in the caryopsis (or achene; not visible in this figure), and together the lemma, palea, and caryopsis constitute what we refer to as the diaspore. Modified from Barkworth et al. (2007) with permission. Copyright is owned by Utah State University Press and the original illustrations were drawn by Linda A. Vorobik and Hana Pazdírková. Scale bar = 1 mm. (A) *Schismus barbatus*. Top floret/diaspore, dorsal view. Bottom floret/diaspore, ventral view. Note that the lemma lacks an awn but instead terminates in a mucro, has a short, glabrous callus and lemma indumentum (“hairs”) only at the lemma margins. We postulate that this character combination leads to a diaspore that is optimized for passive burial. (B) *Rytidosperma caespitosum*. Left floret/diaspore, dorsal view. Right floret/diaspore, ventral view. Note the deeply lobed lemma, terminating in two lateral setae, with a well-developed awn inserted at the lemma sinus. The twisted awn column is the result of hygroscopic activity. Note also the densely indumentous (hairy) lemma back and the long, hairy callus. We postulate that this character combination leads to a diaspore that is optimized for active burial in the correct orientation.

(Fig. 1B). Remarkably, 38 of the 280 species of danthonioid grasses have awnless diaspores (Fig. 1A). Awnless species are distributed on several continents and among numerous genera: in Africa (*Pentameris*, *Schismus*, *Tribolium*), in South America (*Cortaderia*), and in Australasia (*Notochloe* and *Rytidosperma*). Recent molecular phylogenetic analyses (Barker et al. 2003; Verboom 2006; Galley and Linder 2007; Pirie et al. 2008; Humphreys et al. 2010) have provided a robust phylogenetic framework within which further evolutionary study can be based and which has also enabled a reconsideration of generic limits in a phylogenetic context (Linder et al. 2010). This has the practical advantage that each of the major clades corresponds to a genus and can thus be referred to by its generic name. We use this phylogenetic framework to analyze the evolution of awns and to test whether the disadvantage of not having an awn in an ecological context holds true also in an evolutionary context, in terms of lineage persistence and diversification. We also quantify variation in other diaspore traits that may be linked with the awned versus the awnless state to test if awnless species may have undergone further morphological changes following awn loss. We show that awns have been lost several times independently and that although these loss events are not associated with significantly reduced species diversity, the awnless state is only persistent in *Tribolium* and *Schismus*. We also show that the awn is the conspicuous constituent of a compound “burial syndrome” and that the evolution of awns is correlated with life-history evolution, suggesting that the awnless state is maintained if it is accompanied by an ecological shift to passive burial and into habitats where hygroscopecally active awns offer little advantage.

Materials and Methods

TAXON SAMPLING, MOLECULAR MARKERS, AND PHYLOGENY RECONSTRUCTION

We based the phylogenetic reconstruction on a modified version of the combined chloroplast DNA (cpDNA) and nuclear ribosomal DNA (nrDNA) supermatrix for the Danthonioideae generated by Pirie et al. (2008). *Rytidosperma acerosum*, *R. occidentalis*, and *R. petrosum* were added to the matrix and *R. vestitum* and *R. fortunae-hibernae* were removed, due to their markedly different positions (high patristic distance) among the set of maximum posterior probability trees found in previous analyses (Humphreys et al. 2010). Taxon sampling was thus brought to 81% overall (for taxon sampling per clade [genus] see Table 1). Noncoding markers trnT-L and trnC-ycf6-psbM-trnD, as well as the protein-coding ndhF marker, were added for most *Rytidosperma* species and coding regions rbcL and matK were added to 21 and 28 placeholder taxa, respectively, following the strategy of Pirie et al. (2008). All newly added data were generated by Humphreys et al. (2010)

and were included here to improve resolution in *Rytidosperma*. Sequences were added to the existing data matrix and aligned manually, because no regions of ambiguous alignment were encountered. Taxa for which no new data were added were scored as unknown “?” Gaps were coded as missing data “—.” The final aligned matrix comprised 299 taxa and 14,425 characters.

The best-fitting evolutionary model for the dataset was chosen using ModelTest (Posada and Crandall 1998). To overcome some of the computational difficulties in analyzing such a large dataset in a Bayesian framework (Pirie et al. 2008), we performed the phylogenetic analysis in two steps. First, a maximum likelihood (ML) analysis was run in the software GARLI 0.960 (Zwickl 2006), with seven independent runs under the GTR + Γ + I model and with stepwise sequence addition and no outgroup rooting. All runs were performed in the CIPRES cluster at the San Diego Supercomputer Center (<http://www.phylo.org/portal2>). Second, the most likely tree obtained in the GARLI analysis was used as a starting tree for phylogenetic inference in a Bayesian framework. Six independent runs of 1.2×10^6 generations each were performed in MrBayes version 3.1 (Huelsenbeck and Ronquist 2001), using four chains (one cold and three heated), sampling every 500th generation and saving branch lengths. Three different temperatures were applied for the heated chains in three sets of parallel runs: 0.1, 0.2, and 0.3. All analyses were performed at the Computational Biology Service Unit hosted by Cornell University, USA (<http://cbsuapps.tc.cornell.edu>). Performance was evaluated using Tracer version 1.4.1 (Rambaut and Drummond 2007) and AWTY (Nylander et al. 2008) and convergence was considered to have been reached when effective sample size values of the combined runs were >100 and posterior probabilities (p.p.) for nodes remained stable among generations. Node posterior probabilities were calculated on 11,406 trees, after 3000 trees were discarded as burnin.

Gene tree conflict can have an impact on phylogeny-based evolutionary inference (Pirie et al. 2009). Twenty-seven accessions for which the phylogenetic position inferred by cpDNA is significantly different from that inferred by nrDNA were therefore represented by the individual genomes separately, using the taxon duplication technique of Pirie et al. (2008, 2009). In addition, multiple accessions of taxa can influence the proportions of terminals coded for particular states (such as awns present vs. absent), which could result in a bias in character optimizations equivalent to changing the base frequencies in a nucleotide substitution model. Multiple accessions of species that are not demonstrably polyphyletic were therefore reduced to a single accession. This was achieved by pruning taxa from a random subset of 1000 post-burnin Bayesian trees in Paup* 4.0b10 (Swofford 2002) while retaining the original branch lengths. Seventeen accessions were thus removed and accessions with disparate positions were kept for two taxa: *Pentameris pallida* and *Rytidosperma caespitosum*.

Table 1. Character coding and percentage sampling per state (not counting duplicated taxa). Awns were coded as present (0) or absent (1). Absence of an awn denotes species in which the awn is altogether absent or reduced to a "micro," that is, reduced beyond hygroscopic function. Life history was coded as perennial (0) or annual (1). Four species of *Pentameris* are biannual (Linder and Ellis 1990) and these were coded as perennial to distinguish species that survive as adult plants for more than one growing season from those that do not. Callus and lemma indumentum were coded as villous (0) or glabrous (1), with "glabrous" coded only for those species that never produce hairs on these surfaces. Polymorphic species were coded as (0). Sampling per clade is not relevant for these characters because these data were not analyzed in a phylogenetic framework.

Genus (clade)	Taxon sampling	Awned (0)	Awned sampled	Awnless (1)	Awnless sampled	Perennial (0)	Perennial sampled	Annual (1)	Annual sampled
<i>Austroderia</i>	100%	5	100%	0	n/a	5	100%	0	n/a
<i>Capeochloa</i>	100%	3	100%	0	n/a	3	100%	0	n/a
<i>Chaetobromus</i>	100%	1	100%	0	n/a	1	100%	0	n/a
<i>Chimaerochloa</i>	100%	1	100%	0	n/a	1	100%	0	n/a
<i>Chionochloa</i>	92%	25	92%	0	n/a	25	92%	0	n/a
<i>Cortaderia</i>	68%	16	75%	3	33%	19	68%	0	n/a
<i>Danthonia</i>	60%	24	58%	1	100%	25	60%	0	n/a
<i>Geochloa</i>	100%	3	100%	0	n/a	3	100%	0	n/a
<i>Merxmüllera</i>	57%	7	57%	0	n/a	7	57%	0	n/a
<i>Notochloe</i>	100%	0	n/a	1	100%	1	100%	0	n/a
<i>Pentameris</i>	90%	71	89%	13	100%	76	89%	8	100%
<i>Plinthanthesis</i>	100%	1	100%	2	100%	3	100%	0	n/a
<i>Pseudopentameris</i>	100%	3	100%	0	n/a	3	100%	0	n/a
<i>Rytidosperma</i>	74%	68	72%	5	100%	73	74%	0	n/a
<i>Schismus</i>	80%	1	100%	4	75%	2	50%	3	100%
<i>Tenaxia</i>	75%	8	75%	0	n/a	8	75%	0	n/a
<i>Tribolium</i>	93%	5	80%	9	100%	9	89%	5	100%
Danthonioideae	81%	242	80%	38	92%	264	80%	16	100%

In addition, a taxon of uncertain identity was kept: *Rytidosperma* sp. (accession AMH104). Thus, a set of 1000 randomly sampled post-burnin phylograms, comprising 274 accessions, representing 228 species and including six outgroup taxa was created. This forms the phylogenetic framework in which the following analyses were carried out.

MORPHOLOGICAL DATA AND CHARACTER CODING

Information on the lemma awn, lemma indumentum, callus indumentum, callus length and life history (see below) was exported from our DELTA database (H. P. Linder, in prep.) and gaps were filled using floras and species accounts (Conert 1965; Conert and Türpe 1969, 1974; Davidse 1988; Linder and Ellis 1990; Barker and Ellis 1991; Barker 1993, 1995; Baeza 1996; Laegaard 1997; Linder and Davidse 1997; Verboom and Linder 1998; Barker 1999; Edgar and Connor 2000; Baeza 2002; Darbyshire 2003; Linder 2004; Molloy and Connor 2005; Galley and Linder 2006; Clayton et al. 2006 (onwards)). Each variable was coded as a binary character (Table 1), except callus length, for which raw measurements were used (see below). Coding/length for each species is listed in Appendix S1. These data are available in the DRYAD data repository under <http://dx.doi.org/10.5061/dryad.8013>.

RECONSTRUCTING THE EVOLUTION OF AWNS IN DANTHONIOIDEAE

Models of evolutionary change in morphological characters may make use of branching pattern alone or may include branch length information. Branch lengths may be in units of genetic divergence or time. Evidence for a correlation between rates of molecular change and rates of morphological change is contradictory (Omland 1997; Bromham et al. 2002; Davies and Savolainen 2006; Xiang et al. 2008) and under certain situations, for example, rapid radiations or selective sweeps (Cunningham 1999), neither time nor genetic divergence (of most markers) is likely to be an accurate predictor of phenotypic change. In fact, generation time may provide a more realistic approximation of phenotypic change (Pagel 1999; Smith and Donoghue 2008; Smith and Beaulieu 2009). This is clearly an area demanding further attention, but the limited empirical evidence that is available suggests that neither genetic divergence nor time is expected to be a superior predictor of morphological change (Moore and Donoghue 2007; Smith and Beaulieu 2009). We used the set of 1000 phylograms described above to analyze the evolution of awns for pragmatic reasons: to avoid zero-length branches or having to make transformations of branch lengths. Character evolution was reconstructed with ML, reversible-jump Markov chain Monte Carlo (rj-MCMC), and parsimony.

Parsimony reconstruction of awn presence at each node was implemented using the Trace Character Over Trees command in Mesquite version 2.71 (Maddison and Maddison 2009).

States were summarized for each node by counting all trees with uniquely best states. If no state is more parsimonious than the other, the reconstruction at that node will be equivocal. We tested for phylogenetic constraint in awn evolution by permuting the terminals 1000 times using the Reshuffle Terminal Taxa command. This allowed numbers of inferred gains and losses required on the observed trees to be compared to a null distribution of gains and losses.

A problem with summarizing ancestral states at individual nodes across a large sample of trees is that phylogenetic uncertainty can be confused with uncertainty in the ancestral state reconstruction. The “most recent common ancestor” (mrca) approach of Pagel et al. (2004) provides a means for combining both sources of uncertainty. Nodes of interest are defined as the mrca of a given set of taxa (Pagel et al. 2004). Where a phylogenetic hypothesis has low support, the mrca of a set of taxa will be variable across trees. If the likely ancestral state is sensitive to this variation, support for the reconstruction will be correspondingly low, limiting what useful inferences can be made about the reconstructed ancestral state. If the likely ancestral state is not sensitive to this variation, then the reconstruction may be robust irrespective of node support. To minimize the influence of phylogenetic uncertainty on posterior support for an ancestral state reconstruction, we defined nodes of interest primarily as those with $p.p. \geq 0.80$. In three cases we defined less-conservative nodes: (1) mrca of *P. reflexa* and *P. ecklonii* (average diversity across 1,000 trees: $[\bar{n}] = 9.54$), (2) mrca of *N. microdon* and *C. jubata* [ITS] ($\bar{n} = 13.7$), and (3) mrca of *R. exiguum* and *R. oreoboloides* ($\bar{n} = 10.9$). In some trees, these nodes are identical to more robust, more inclusive nodes and thus redundant. In other trees, these nodes may provide additional information about awn loss events toward the tips of the phylogeny. We did not define nodes in clades that are invariable for the awn character, for example *Chionochoa*, where all species have an awn. In total, 93 nodes were defined.

Determination of rate parameters and the most suitable model of evolution was carried out with 1×10^6 iterations of ML analysis using the Multistate commands in BayesTraits (available from <http://www.evolution.rdg.ac.uk/BayesTraits.html>). Best-fitting models were identified using a likelihood ratio (LR) test (Edwards 1972). In this approach, states at individual nodes are reconstructed as the state that maximizes the probability of arriving at the observed states in the terminals, given the model of evolution and the sample of trees (allowing the states at all other nodes to vary; Schluter et al. 1997; Pagel 1999). Ancestral state reconstruction in a Bayesian framework with rj-MCMC (Pagel and Meade 2006) was performed using the BayesMulti-state (Pagel et al. 2004) commands in BayesTraits. This approach has the advantage that all possible models of evolution are sampled in proportion to their posterior probabilities (Green 1995; Pagel and Meade 2006) as opposed to only the rate parameters

being sampled in this way, as in conventional MCMC (Pagel et al. 2004). We used an exponentially distributed hyperprior (see Pagel et al. 2004) with its mean value seeded from a uniform distribution with an interval that contained, but did not determine, the posterior distribution. To avoid autocorrelation and allow exploration of ample parameter space, we varied the amount by which the rate parameters are allowed to change between iterations of the Markov chain (ratedev) until acceptance rates averaged 20–40%. Due to initially low acceptance rates, we used a modified version of the code that accepts either a move to a new model or a move to a different tree in each iteration, rather than both simultaneously (courtesy of A. Meade). We ran 50×10^6 generations, sampling every 1000 generations, yielding a sample of 49,000 iterations after 1×10^6 iterations were removed as burnin.

AWN LOSS AND CLADE SIZE

Sister clade comparisons were carried out to estimate differences in clade size between awned and awnless clades. Based on the ancestral state reconstructions under parsimony and MCMC, 12 sister clade comparisons were made; based on the ML reconstructions, 13 comparisons were made. Differences in diversity were assessed with Wilcoxon's signed rank test and a sign test, with average clade sizes across the same sample of 1000 trees as input. To incorporate a test with an evolutionary null model, we tested for imbalances in clade size beyond those expected from stochastic differences resulting from random speciation/extinction processes, using equation (14) of Slowinski and Guyer (1989b). We did not include unsampled species in this test because although we are confident about their placement at the genus level, we do not wish to guess their placement among the tips of the phylogeny.

TIMING OF AWN LOSS EVENTS

Node ages of the 93 nodes defined in the ancestral state reconstruction (above), plus of 12 nodes leading to awn loss along a tip branch, were extracted from results of a recent dating analysis of the Danthonioideae (Antonelli et al. 2010). Each node age (95% confidence interval [CI] of variation) was associated with a "0" or "1" based on the reconstructed ancestral state for that node using ML. Equivocal nodes were coded as 0.

To assess whether the sequence of awn loss events in time is more clustered than would be expected by chance, the classical runs test, recently brought into a phylogenetics context by Ford et al. (2009), would seem appropriate. However, we note that this test does not take the length of the runs into account, meaning that in the present dataset, the long run of "0s" that separates the root at 26.1 Ma from the first awn loss event at 6.37–3.53 Ma is weighted no differently from a run constituting a single "0" occurring toward the more recent end of the sequence. Instead, we generated a null distribution of node ages associated with awn loss events (1s) by evolving a binary character across

the observed trees under a model of evolution defined as the rate matrix given by the analyses of the observed data under ML (see above). Ten simulations were carried out on 10 different starting trees as implemented in the Geiger package (Harmon et al. 2008) of *R* (www.r-project.org). This resulted in 100 simulated matrices of binary characters. Ancestral states of the simulated matrices were then estimated using ML as implemented in BayesTraits as before (see results) to generate a null distribution of node ages associated with the absence of awns, sampled from all observed node ages of the Danthonioideae. A node was considered to represent absence of an awn in the simulated dataset if the proportional likelihood of state "1" was ≥ 0.93 , based on the cut-off value identified as appropriate in the observed dataset (see above). In a few exceptional cases, nodes with lower proportional likelihoods (0.89–0.92) were included because they were part of a continuous distribution of proportional likelihoods (i.e., no gap in the distribution of values). We tested whether the observed distribution of node ages associated with "1s" differed significantly from the expected using the Wilcoxon rank sum test, separately for minimum and maximum 95% CI ages. This tests the probability that the two samples come from the same distribution and is appropriate in this case where sample sizes differ and where the data are not normally distributed.

CORRELATION ANALYSES: AWNS AND LIFE HISTORY

Most species in Danthonioideae are awned and perennial but awnless species appear to constitute a high proportion of annual species (Table 1). To test whether evolution of awns is correlated with the evolution of life history, we compared the fit of dependent and independent models of evolution to the data using the Discrete (ML) and BayesDiscrete commands (Pagel 1994; Pagel and Meade 2006) in BayesTraits. Eight rate parameters constitute the dependent model. These allow each character to evolve at different rates, both for forward and backward shifts, depending on the state of the second character. In the independent model, shifts in one character occur at the same rate regardless of the state of the second parameter (coefficients $q_{12} = q_{34}$, $q_{13} = q_{24}$, $q_{21} = q_{43}$, and $q_{31} = q_{42}$; see definitions in Table 2). Hence, a model of independent evolution has four parameters. We ran one analysis in which rate parameters were allowed to vary freely and one analysis in which sampling of models was restricted to sampling only independent models. Fit of dependent and independent models was compared with an LR test under ML (Edwards 1972) and with Bayes factors (BF) under rj-MCMC (Raftery 1996). The BF is calculated as twice the difference in log harmonic mean of the worst- and best-fitting models. To ensure that the harmonic mean remained stable within and among runs, we performed multiple, long analyses (following recommendations of A. Meade, pers. comm.). Priors were selected and nodes were defined as described above. For the dependent analyses, we ran

Table 2. Definition of rate coefficients compared in rj-MCMC correlation analyses.

Coefficient	Evolutionary transition
Forward shifts (0→1):	
q_{12}	Shift into annual in an awned background
q_{13}	Loss of awn in a perennial background
q_{24}	Loss of awn in an annual background
q_{34}	Shift into annual in unawned background
Backward shifts (1→0):	
q_{21}	Shift back to perennial in awned background
q_{31}	Secondary gain of awn in perennial background
q_{42}	Secondary gain of awn in annual background
q_{43}	Shift back to perennial in unawned background

150×10^6 iterations, sampling every 1000 iterations, yielding a sample of 110,000 iterations after burnin was removed. For the independent analyses, we ran 100×10^6 iterations, sampling every 1000 iterations, yielding a sample of 90,000 iterations after burnin was discarded. For the ML analysis, we carried out 1000 ML iterations per tree.

THE INFLUENCE OF TRIBOLIUM AND SCHISMUS

Awnless, annual species are concentrated in *Tribolium* and *Schismus*. To test the influence of these two clades on the overall results, we pruned both lineages from the set of 1000 trees as before and repeated the analyses above. The ancestral state reconstruction was carried out with 1×10^6 iterations of ML analysis or 100×10^6 iterations of rj-MCMC analysis, sampling every 1000 iterations, yielding a sample of 90,000 iterations after removal of burnin. Nodes were defined as for the entire dataset, excluding the 14 nodes in *Tribolium* and *Schismus*. Sister clade comparisons of clade size (parsimony 11, ML: 12, rj-MCMC: 10) were repeated and differences were evaluated with Wilcoxon’s signed rank test and a sign test. Analysis of the sequence in time of awn loss events was based on 78 nodes plus 12 nodes leading to terminals. Fourteen nodes were coded as “1” and simulated matrices were generated as for the entire dataset. Analysis of correlated evolution between the awn and life-history characters was performed with 1000 ML iterations per tree or 200×10^6 iterations of rj-MCMC, sampling every 1000 iterations, yielding a sample of 140,000 iterations after burnin was discarded.

QUANTIFICATION OF VARIATION IN ASSOCIATED DIASPORE TRAITS

To quantify morphological attributes of the diaspores associated with the absence of an awn, we separated awned and awnless species into two groups. Each of these groups was then subdivided again, separating species with villous lemma backs and species with glabrous lemma backs. The number of species in each group

was counted. The subdivision was repeated, instead separating species with villous calli and species with glabrous calli. The number of species in each group was recounted. Independence of the frequency of glabrous lemmas and calli in awned species compared to in unawned species was assessed with Pearson’s chi-square test with one degree of freedom. The total number of cases in each test was 280.

We also quantified the difference in the distribution of callus length variation between awned and unawned species using the nonparametric Wilcoxon rank sum test and using minimum and maximum recorded callus lengths as input. Data on callus length are not available for 75 (27%) of all Danthonioideae species and filling this gap would be beyond the scope of the present study. However, because species for which data are available constitute 13% unawned species, which is an accurate representation of the 14% unawned species in the entire dataset, we do not expect this to bias the results.

To quantify the presence of any association across all variables simultaneously, we carried out a multiple correspondence analysis (MCA) using the “ca” package in R (Nenadic and Greenacre 2007), after first having converted “callus length” into a discrete character with 10 equally spaced states (0–9; based on maximum lengths only; Appendix S4).

Results

TAXON SAMPLING AND PHYLOGENY

The standard deviation of split frequencies in the six independent MrBayes runs stabilized at 0.03 (for chain temperatures of 0.1 and 0.2) and 0.04 (temperature 0.3). The effective sample size of the combined post burn in tree samples (11,406 trees) was 305, that is, far above the recommended minimum 100 for a reliable analysis (Drummond and Rambaut 2007). All six runs reached a plateau at the same log likelihood value (Fig. S1A–C). The topology expected from previous analyses (Pirie et al. 2008), with improved resolution of *Rytidosperma* (Humphreys et al. 2010), was successfully recovered (Fig. S2). This means the phylogenetic hypothesis remains robust under addition of both taxa and data and lends confidence to its predictiveness. Therefore, it seems unlikely that addition of missing species will have a large impact on overall patterns inferred below.

NUMBER OF INFERRED AWN LOSS EVENTS AND RATE PARAMETERS

All three methods of character optimization found support for an asymmetrical model of evolution (Table 3A). Under parsimony, awns are lost on average eight times more than they are regained, but this imbalance is less severe than the one expected from the permuted data where awn loss occurs on average 18 times more

Table 3. Forward and backward shifts in the evolution of awns inferred under parsimony, ML and rj-MCMC. Observed numbers of awn loss events ($0 \rightarrow 1$) and secondary gains ($1 \rightarrow 0$) and rate parameters (q_{01} , q_{10}) estimated under different models of evolution. Permuted data were analyzed only under parsimony. (A) All taxa. (B) Excluding *Tribolium* and *Schismus*.

Parameter	parsimony: 95% confidence interval (mean)	ML: 95% confidence interval (mean)	rj-MCMC: 95% confidence interval (mean)
(A) Entire dataset			
Observed $0 \rightarrow 1$; q_{01}	24–30 (27.2)	25.00–32.88 (28.56)	14.45–39.20 (25.64)
Permuted $0 \rightarrow 1$	32–41 (37.0)	–	–
Observed $1 \rightarrow 0$; q_{10}	1–7 (3.37)	89.80–145.0 (119.1)	13.46–158.20 (68.00)
Permuted $1 \rightarrow 0$	0–6 (2.11)	–	–
(B) <i>Tribolium</i> and <i>Schismus</i> removed			
Observed $0 \rightarrow 1$; q_{01}	21–24 (22.7)	31.08–127.7 (63.73)	15.28–121.1 (59.73)
Permuted $0 \rightarrow 1$	23–28 (26.2)	–	–
Observed $1 \rightarrow 0$; q_{10}	0–3 (1.23)	204.4–1000 (475.9)	112.0–915.9 (423.1)
Permuted $1 \rightarrow 0$	0–3 (0.741)	–	–

than secondary gain occurs. These results suggest that awn loss is phylogenetically constrained. Under ML (support for asymmetrical model $P < 0.001$, under a chi-square distributed null and $df = 1$) forward rates were in the same range as the number of shifts inferred using parsimony, but backward rates were much higher. In a Bayesian framework, only asymmetrical models were sampled and forward rates were more variable, whereas backward rates were intermediate compared to those inferred with parsimony and ML (Table 3A).

ANCESTRAL STATE RECONSTRUCTION OF AWNS

Ancestral states reconstructed for each node using parsimony, ML, and rj-MCMC are presented in detail in Appendix S2 and summarized in Figure 2A. For 83 of 93 nodes, parsimony analysis provided unambiguous assignment of one or the other state across all trees. Relative likelihoods of a node adopting a particular state are summarized as mean values for each node. A plot of the mean likelihood values for each node adopting state (1) against node number reveals two gaps in the distribution of likelihood values, corresponding to the 10 highest and 10 lowest values, respectively. The 10 highest likelihood values (0.93–1.0) were interpreted as a signifying state (1) and the 10 lowest values (0.0055–0.049) as a signifying state (0). Ancestral state of the remaining 73 nodes was considered uncertain (Fig. 2). Under rj-MCMC, ancestral states were considered unambiguous if the mean (\pm standard deviation) posterior probability for a node adopting a state was ≥ 0.95 (Appendix S2). Accordingly, the presence of an awn was reconstructed with certainty at 24 nodes and the absence of an awn was reconstructed with certainty at seven nodes. Ancestral states of 62 nodes are ambiguous according to these measures. Nodes reconstructed with a $p.p. \leq 0.80$ have been indicated in Figure 2, to indicate further trends in the data.

The presence of an awn was inferred at the root node under parsimony and there was weak support for the presence of an awn under rj-MCMC ($p.p. = 0.71$) but not under ML (proportional likelihood $[0] = 0.54$). Restricting the likelihood model to a single rate parameter increased the likelihood of the presence of an awn at the root to 0.86, but such a model was not supported by the data (LR test, $P > 0.05$). Restricting the root node to (0) did not change the overall likelihood of the model but then neither did restricting the root node to (1) (LR test, $P > 0.05$).

DIVERSITY OF UNAUNED CLADES COMPARED TO THEIR SISTERS

No significant size differences were found between awned clades compared to their awnless sister clades ($P > 0.05$ for all three tests). However there is a trend in the data that is worth reporting. Awnless clades contained fewer species than their sisters in five of the cases compared, six cases revealed no difference in size (tip events occurring within one of the members of a single species pair) and in one (node 74 [Fig. 2A], parsimony, MCMC) or two (nodes 74 and 83 [Fig. 2A], ML) comparisons awnless clades contained more species than their awnless sisters. Low phylogenetic support in some clades limited the number of sister clade comparisons that could be made.

TIMING OF AWN LOSS EVENTS

Age of each of the defined nodes is shown in Appendix S2. The distribution of ages associated with the absence of an awn is significantly older than expected by chance when minimum ages are considered (Wilcoxon rank sum test, $n_1 = 21$, $n_2 = 23$, $W = 333$, $P = 0.032$) but no different from ages expected by chance when maximum ages are considered ($W = 303$, $P = 0.15$) (Fig. 3A). We only carried out this test based on ages of awnless

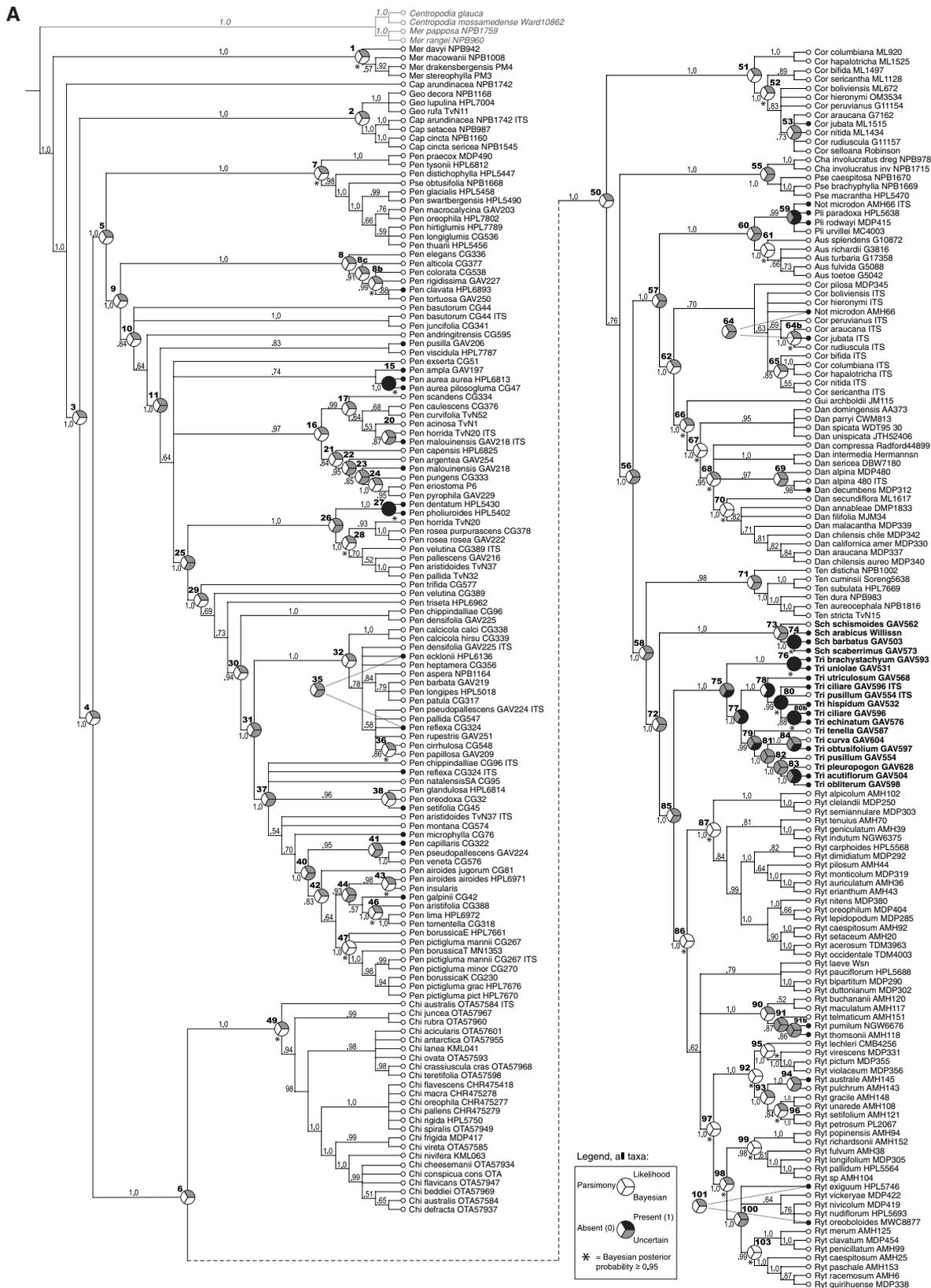


Figure 2. Ancestral state reconstructions shown on the Bayesian majority rule consensus tree. Ancestral node reconstructions at each node are shown as a pie chart split into three, each slice representing the results of the three different methods employed: parsimony, ML, and rj-MCMC. For details of the support for the results presented, refer to the text and Appendix S2. (A) All taxa. (B) Excluding *Tribolium* and *Schismus*.

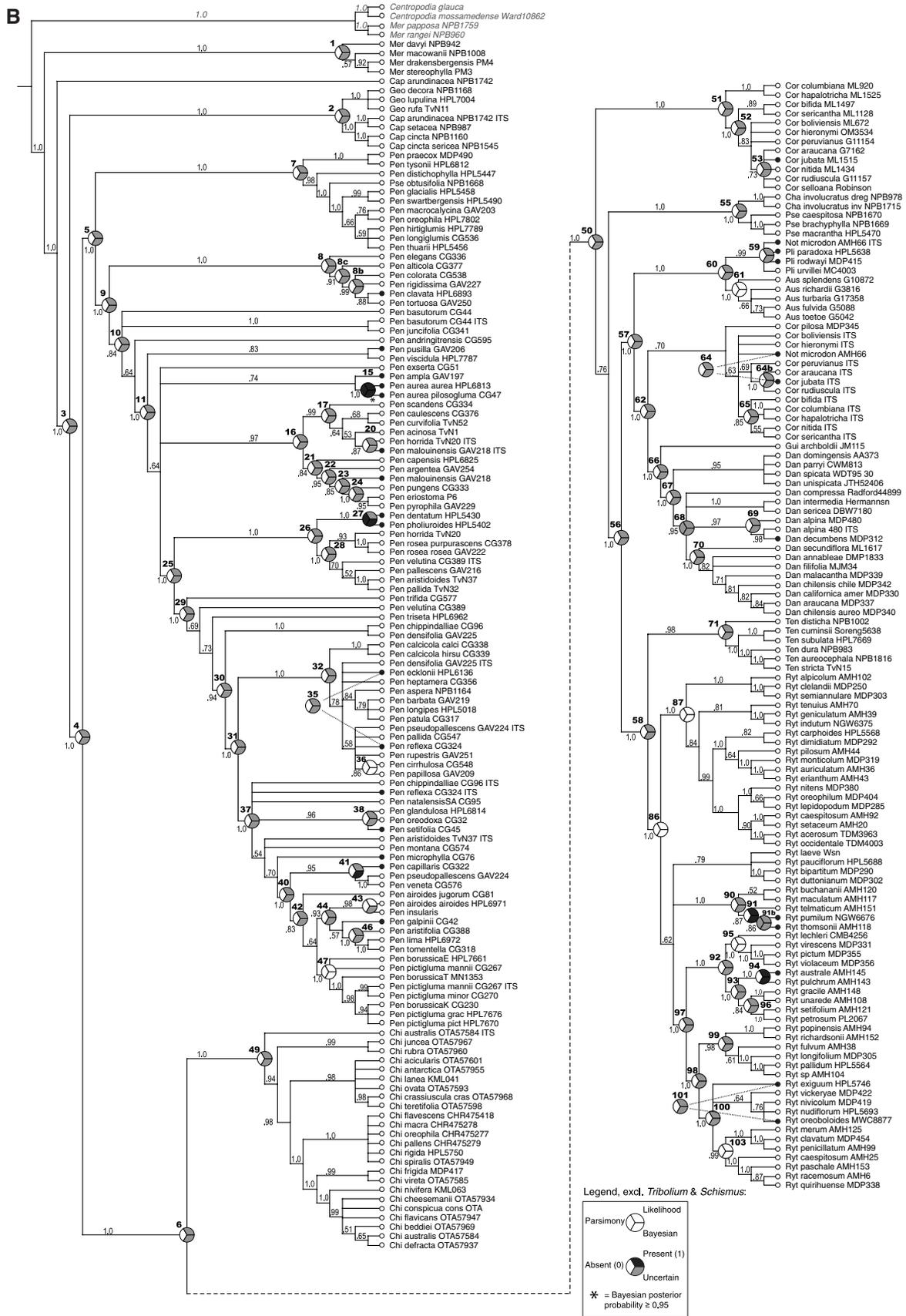


Figure 2. Continued.

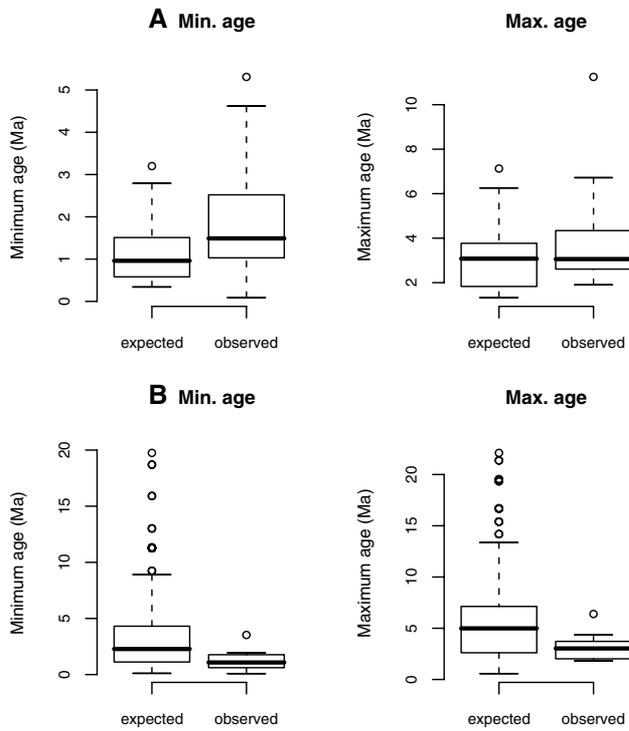


Figure 3. Distribution of observed minimum (left) and maximum (right) ages of awn loss events. (A) All taxa. Observed (max.) ages are significantly older than expected given the phylogeny and model of awn evolution ($P = 0.03$, Wilcoxon’s rank sum test). (B) Excluding of *Tribolium* and *Schismus*. Observed ages are significantly younger than expected given the phylogeny and model of awn evolution ($P < 0.01$, Wilcoxon’s rank sum test). These differences suggest that the awnless state is persistent in *Tribolium* and *Schismus* only.

nodes inferred under ML because the simulations were carried out using ML and because distribution of ages of observed nodes did not differ compared to those inferred with the other two methods (Wilcoxon rank sum test, parsimony: $n_1 = 18$, $n_2 = 21$, $W = 232.5$, $P = 0.32$ [min. ages], $W = 220.5$, $P = 0.38$ [max. ages], rj-MCMC: $n_1 = 21$, $n_2 = 25$, $W = 258$, $P = 0.93$ [min. ages], $W = 258.5$, $P = 0.94$ [max. ages]).

Table 4. Statistics of dependent (D) and independent (I) models of evolution between awn and life history. (A) All taxa. Dependent models were sampled 48,999 times and independent models were sampled only twice. (B) Excluding *Schismus* and *Tribolium*. Dependent models were sampled in all of the rj-MCMC iterations.

Data	Mean log L(I)	Mean log L(D)	LR significance level χ^2 with df = 4	Log harmonic mean (I)	Log harmonic mean (D)	n models [D/I]	Log-BF ¹
A	-166.67	-159.09	0.01	-164.18	-156.08	48,999/2	16.2
B	-125.95	-121.99	<i>n.s.</i>	-128.47	-119.89	49,001/0	17.2

¹On a logarithmic scale values of 2–5 are considered “positive” evidence that the models are different, values greater than 5 are “strong evidence” and values greater than 10 are “very strong evidence” (Raftery, 1996; Pagel and Meade, 2006).

CORRELATION ANALYSES: AWNS AND LIFE HISTORY

Both ML and rj-MCMC analyses found a better fit of a dependent model of evolution than a model of independent evolution (ML: $P < 0.01$ LR test statistic compared to a χ^2 distribution, with four degrees of freedom, considering the 95% CI of the likelihood values across trees; rj-MCMC: logBF = 16.2) (Table 4). A comparison of rate parameters of the dependent model reveals that shifts from perennial to annual do not occur in an awned background ($q_{12} = 0$) but occur frequently in an awnless background ($q_{34} > 0$). Consistently with this, awn loss is inferred to occur before a shift into an annual life history ($q_{13} > q_{12}$). None of the backward rate parameters (q_{21} , q_{31} , q_{42} , and q_{43}) can be distinguished from each other. Detailed differences are shown in Appendix S3 (A).

Individual node reconstructions under a dependent model of evolution are shown in Figure S3. The prevalent character combination (awn present; perennial) is reconstructed for one node each in *Pentameris* and *Danthonia*, three nodes in *Rytidosperma*, and ancestrally in *Austroderia* and *Rytidosperma* (p.p. ≥ 0.95 , nodes 47, 67, 87, 95, 103, 61, and 86 in Fig. S3). In addition, absence of an awn and a perennial life history is reconstructed at node 15 (p.p. ≥ 0.95). Considering also nodes reconstructed with p.p. ≥ 0.80 , reveals that awn loss probably occurred in perennial lineages (*Notochloe/Plinthanthesis* and in *Tribolium*), consistent with the findings above (awn loss before shift to annual). However, annuals have also evolved in awned lineages (in *Pentameris*).

REMOVAL OF TRIBOLIUM AND SCHISMUS

Ancestral states found when *Tribolium* and *Schismus* were excluded from the analysis are shown in Figure 2B and in detail in Appendix S2. Overall, the patterns match those found in the entire dataset but ancestral states of fewer nodes are reconstructed unambiguously. Neither forward nor backward shifts inferred under parsimony could be distinguished from those expected from the permuted data (Table 3B). Rate parameters estimated under ML and rj-MCMC were highly variable and suggest very high forward rates and extremely high backward rates compared to parsimony (Table 3B). The root was reconstructed as being awned under

parsimony but it was ambiguous under ML and rj-MCMC (ML(0) = 0.50; p.p. (0) = 0.51). Restricting the ML model to a single rate parameter increased the certainty of the state at the root (ML(0) = 0.94), but that model was not supported by the data (LR test, $P < 0.001$, $df = 1$).

As with the analysis of the entire clade, no significant difference in clade size of awned clades compared to their sister clades was found (Wilcoxon's signed rank test and sign test, $P = 0.06$ – 0.13 , with $df = 9$ – 11). We report on the trends in the data. Awnless clades contained fewer species than their sisters in five (parsimony, ML) or four (MCMC) of the cases compared and six cases revealed no difference in size (tip events occurring within one of the members of a single species pair). Only at one node did awnless clades contained more species than their awnless sisters and this reconstruction was only recovered under ML analyses (node 91 [Fig. 2B], ML).

Awn loss occurred significantly more toward the present than expected by chance (Wilcoxon rank sum test, $n_1 = 14$, $n_2 = 162$, $W = 1662$, $P = 0.0039$ [min. ages], $W = 1609$, $P = 0.0094$ [max. ages]) (Fig. 4B). Ages inferred based on the different ancestral state reconstructions were not significantly different (Wilcoxon rank sum test, parsimony: $n_1 = 14$, $n_2 = 13$, $W = 92.5$, $P = 0.96$ [min. ages], $W = 93.5$, $P = 0.92$ [max. ages], rj-MCMC: $n_1 = 14$, $n_2 = 17$, $W = 125.5$, $P = 0.81$ [min. ages], $W = 131.5$, $P = 0.63$ [max. ages]).

Correlation analyses based on ML revealed no difference in fit between a dependent and independent model of evolution ($P > 0.05$, LR test statistic compared to a χ^2 distribution with $df = 4$) whereas under rj-MCMC a dependent model fits the data much better ($\log_{10}BF = 17.2$) (Table 4B). A comparison of rate parameters again reveals that $q_{12} = 0$ and $q_{13} > 0$, suggesting that awn loss occurs before evolution of an annual life history and that $q_{34} > 0$ indicating that shifts to being annual occur in an awnless background but not an awned background (Appendix S3 (B)).

VARIATION IN ASSOCIATED DIASPORE TRAITS

The frequency of glabrous lemmas or calli differs significantly between awned and awnless species. A higher proportion of awnless species have glabrous lemma backs (Table 5A: 11% of awnless vs. 2.9% of awned species; Pearson's χ^2 test with $df = 1$, $P = 0.024$) and glabrous calli (Table 5B: 42% of awnless vs. 0.39% of awned species; Pearson's χ^2 test with $df = 1$, $P < 0.0001$). Only two species have both a glabrous lemma and a glabrous callus and the data show no significant skew when awn presence or absence is not taken into account (Table 5C).

Overall callus length ranges from 0.01 mm (*Rytidosperra australe*) to 4 mm (*Pseudopentameris macrantha*, *P. caespitosa*). Separated into pools of awn presence or absence, callus length ranges 0.1–4 mm in awned species and 0.01–0.75 mm in awnless

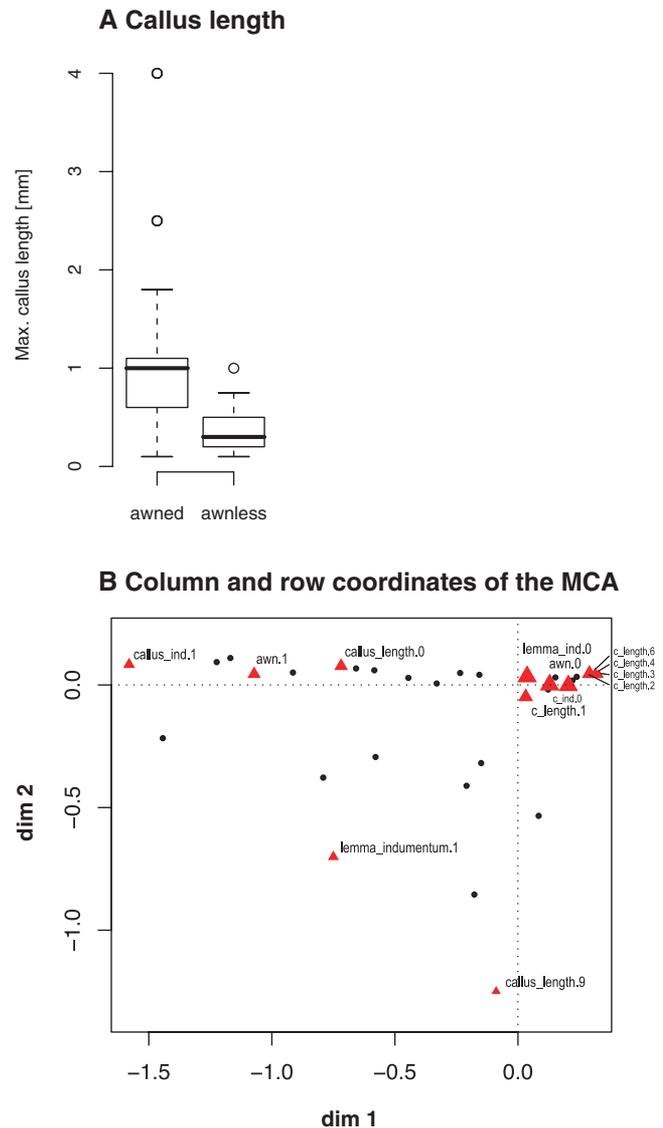


Figure 4. Diaspore traits associated with the awned and awnless state. (A) Distribution of maximum callus length compared between awned and awnless species. Differences are significant at $P < 0.01$ using Wilcoxon's rank sum test and indicate that the length of the callus is one of the traits involved in the "burial syndrome." (B) A plot of the first two dimensions resulting from a multiple correspondence analysis of four factors (awns, lemma and callus indumentum, and callus length). Triangles = column coordinates, the area of each triangle denotes relative contribution of each point (mass); filled circles = row coordinates. The first dimension (x-axis) explains 68% of the variation and separates cases that are awnless, glabrous on the lemma and callus and with a short callus from other cases (left of origo). The second dimension (y-axis) explains an additional 4.8% of the variation and separates only those cases with a glabrous lemma and a long callus from all other cases. The separation of these characters around the presence and absence of an awn suggests the presence of a burial syndrome of which the awn is the visible constituent.

Table 5. Quantification of the awn-callus-lemma “burial syndrome.” Frequency of occurrence of lemma (A) and callus (B) indumentum tested against the present or absence of an awn and tested against each other (C). Significance levels of Pearson’s χ^2 test: *significant at $P=0.05$; ***significant below $P=0.0001$; n.s.=not significant.

A	Lemma villous	Lemma glabrous*
Awn present	235	7
Awn absent	34	4
B	Callus villous	Callus glabrous***
Awn present	241	1
Awn absent	22	16
C	Lemma villous	Lemma glabrous ^{n.s}
Callus villous	254	9
Callus glabrous	15	2

species (Fig. 4A). Thus, short calli occur among both awned and awnless species but awnless species never have long calli. This difference is highly significant (Wilcoxon rank sum test, $n_1 = 26$, $n_2 = 179$, $W = 536$ [min lengths; 518 for max lengths], $P < 0.0001$) and is independent of whether minimum or maximum callus lengths are analyzed because minimum and maximum lengths are highly correlated ($r^2 = 0.77$).

The MCA analyses revealed that two axes of variation (dimensions) account for 73% of the variation among the four factors (dimension 1, 68.3%; dimension 2, 4.76%). Adding a third dimension adds very little explanation of the variation (<0.00%). Row and column coordinates along these two dimensions have been plotted in Fig. 4B and the detailed statistics are shown in Appendix S4. Dimension 1 separates awnless species with a glabrous callus and lemma and short callus (negative values) from awned species with a range of callus lengths and indumentous (“hairy”) lemmas and calli (positive values). Dimension 2 separates those species that have long calli or glabrous lemmas (negative values) from all other species and there is also a grouping of awnless species with species that have indumentous lemmas and glabrous, short calli.

Discussion

ANCESTRAL STATE RECONSTRUCTION AND THE EVOLUTION OF AWNS IN DANTHONIOIDEAE

Contrary to other recent studies (e.g., Ekman et al. 2008), we found a surprising amount of agreement among methods of ancestral state reconstruction. All three methods resulted in the inference of the absence of an awn at two nodes within *Pentameris* (nodes 15 and 27) and several nodes within *Tribolium*

and *Schismus* (Fig. 2). None of the models found support for ancestral absence of an awn in any of the other genera and all three methods support the presence of an awn ancestrally in all (parsimony, MCMC) or most (ML) of the genera or their major constituent clades. All three methods found similar rates of forward change (Table 3), with the increased variability around the mean for the ML and MCMC analyses representing the greater degree to which these methods account for uncertainty in the process of character change (Pagel 1994; Nielsen 2002; Huelsenbeck et al. 2003). Backward shifts (secondary gains) however, differed tremendously among methods (Table 3). Under parsimony one to seven reversals are inferred, depending on phylogenetic resolution, whereas ML and MCMC reconstructions require highly variable and, on average, very high reversal rates to explain the data.

Parsimony is known to provide a reasonable reconstruction of evolutionary patterns when rates of evolution are low (Harvey and Pagel 1991; Pagel 1999; Huelsenbeck et al. 2003). Our phylogenetic reconstruction of the Danthonioideae reveals that one reversal may have taken place in the clade arising from node 59 (leading to *Plinthanthesis urvillei*), two or three reversals could have occurred in *Pentameris*, one to three in *Tribolium*, and one or two in *Rytidosperma* (Fig. 2). Clearly, parsimony-inferred reversals account for these patterns very well. However, rate parameters inferred under Markov-based models are not unexpected given the distance from the root node to the nodes where the first forward shifts (0 → 1) occur. To counter any stray 0 → 1 shifts at deeper nodes in the trees, reverse rates (q_{10}) must be high (Pagel 1994, 1999). The reversal rates found in the present study are therefore probably an artifact of the Markov model, in which both rate parameters are assumed to take positive values ($q_{01}, q_{10} > 0$) along all branches. In addition, short branches along which change is inferred to occur are likely to inflate rates further in the form of multiple “unseen” changes along these branches (Pagel 1994).

Awns are absent in 38 species of Danthonioideae and are inferred to have been lost repeatedly, on average 25.6 (rj-MCMC), 27.2 (parsimony), or 28.6 (ML) times. This rate of awn loss is significantly lower than expected by chance (Table 3). A tempting interpretation of this result is that there has been selection against awn loss such that most lineages in which awn loss occurs do not persist to leave detectable traces of these events. In support of this, we found that multiple awnless species are scattered in *Cortaderia*, *Danthonia*, *Notochloe*, *Pentameris*, *Plinthanthesis*, and *Rytidosperma* and are phylogenetically clustered only in *Tribolium* and *Schismus*. Garnier and Dajoz (2001) demonstrated that variation in awn length has a heritable component in *Hyparrhenia diplandra*, suggesting that awn characteristics indeed have a genetic, heritable basis. In the Danthonioideae, by contrast, much of the pattern surrounding the absence of an awn

on a macroevolutionary scale appears stochastic, the awnless state perhaps only being inherited in *Tribolium* and *Schismus*.

THE ECOLOGICAL DISADVANTAGE OF NOT HAVING AN AWN IN AN EVOLUTIONARY CONTEXT

No significant differences in clade size were found between awned clades and their awnless sisters, although there is a tendency for awnless clades to contain fewer species. It is well known that even seemingly dramatic imbalances between sister clades may have been generated by random speciation and extinction events alone (Raup et al. 1973; Slowinski and Guyer 1989a,b, 1993). The number of comparisons made in the present study was limited by lower phylogenetic resolution in some areas of the tree and a lack of statistical significance could reflect small sample sizes (Ree 2005). Repeatability may also constitute a measure of support for studies of this nature (de Queiroz 2002; Ree 2005) and we found that awnless clades contained fewer species than their awned sisters in five of the cases compared and only in one (parsimony, MCMC) or two (ML) comparisons did awnless clades contain more species than their awned sisters. Importantly, both these nodes are within *Tribolium* (node 83, Fig. 2A) and *Schismus* (node 74, Fig. 2A) confirming that only in these genera does the awnless state persist and do awnless lineages diversify. This finding was corroborated by the ages of “awnless” nodes. When *Tribolium* and *Schismus* are included in the analysis, the awnless state is significantly more persistent in time than expected given the phylogeny and model of awn evolution (or no different from the expected), but when these two genera are removed, the awnless state is associated with a significantly younger set of nodes than expected (Fig. 3), consistent with the lack of a phylogenetic pattern outside *Tribolium* and *Schismus* revealed by the parsimony analyses. It is plausible that the lack of persistence of the awnless state outside *Tribolium* and *Schismus* is due to high extinction rates causing a rapid turnover in these lineages (Rabosky 2009a,b; Ricklefs 2009), masking any events that may have occurred deeper in the tree. Of course, reversals may also have masked traces of awn loss events deeper in the phylogeny. Both possibilities indicate that the ecological disadvantage of not having an awn applies also in an evolutionary context.

THE “BURIAL SYNDROME”

A higher proportion of awnless danthonioid species have glabrous lemma backs or glabrous calli, with hardly any of the awned species being glabrous on these two parts of the diaspore (2.9% and 0.41%, respectively; Table 5). Awnless species on the whole also have significantly shorter calli than awned species (Fig. 4A). Despite there being only two species, both awnless, that lack hairs on both the callus and the lemma back, these variables are separa-

ble in multidimensional space via the awn: awnless diaspores have glabrous lemmas and short, glabrous calli, and awned diaspores have indumentous lemmas and calli, and calli of a range of lengths (Fig. 4B). These results are consistent with those of Peart (1981, 1984): several characters act together with the awn to increase efficiency of burial, but importantly, these are only beneficial in the presence of an awn. Together, these findings suggest that awns are the visible constituent of a compound morphological syndrome, the burial syndrome, that has been established through changes in a suite of diaspore traits over evolutionary time, promoting an ecological shift from active burial to reliance upon stochastic burial. Active burial is driven by the presence of a hygroscopically active awn and aided by a long, pointed callus that firmly anchors the caryopsis in the ground (Fig. 1B). Unidirectional movement into the soil is promoted by the presence of hairs on the lemma and callus that prevent upward movement (out of the soil) (Peart 1981). The other extreme is displayed by the awnless lemma that has a short callus and a glabrous lemma or callus, or both, culminating in a structure that is overall smaller, rounder, and smoother (Fig. 1A). Such a structure is suited to reliance on stochastic burial (Peart 1984) that, to be successful, requires reduction or complete loss of features that would render landing or burial in any particular orientation “wrong.” Because the association among these traits acts via the awn, we suggest that awn loss is the “exaptation,” that is, the prerequisite for evolution of the stochastic burial syndrome in the Gouldian sense (Gould and Vrba 1982), and that the modifications of the associated traits were subsequent and increase efficiency of stochastic burial (Miller 1949; Gould and Vrba 1982; Baum and Larson 1991).

Another example of the evolution of a compound morphological “syndrome” associated with an ecological shift in plants is the evolution of wind pollination. Although wind pollination is characterized by changes in a range of floral traits (Faegri and Van der Pijl 1979; Linder and Rudall 2005), its evolution appears to be dependent on the occurrence of dry pollen and perianth (Linder 1998). Evolution of these key morphological changes probably allowed the ecological shift to wind pollination, the efficiency of which has been fine tuned by further adaptations to the newly opened niche. In the same way, we suggest that the interplay between the diaspore traits quantified here leads to the establishment of the two extremes of the diaspore burial syndrome, each optimized to different niches.

HOW AWNLESS SPECIES PERSIST

Tribolium and *Schismus* both contain a high proportion of species without awns and a high proportion of annual species (Table 1), which are distributed in mostly semi-desert settings in Africa and the Mediterranean region (Linder and Davidse 1997; Linder et al. 2010). We found that a shift from a perennial to an annual life history is more likely in an awnless lineage than

in an awned lineage (Table 4 and Appendix S3). Annuals tend to increase in prevalence with increasing aridity (Charnov and Schaffer 1973; Axelrod 1979; Fiz et al. 2002; Verboom et al. 2003; Evans et al. 2005; Datson et al. 2008; Tank and Olmstead 2008) and are linked with climates with high seasonality (e.g., Verboom et al. 2004). The occurrence of annual species in the habitats of *Tribolium* and *Schismus* is therefore not surprising. Under these conditions adult survival is generally low and seedling survival is high (Charnov and Schaffer 1973), meaning that annual species rely entirely on reestablishment from a viable seed bank after the passing of an unfavorably dry period for their prolonged existence. In the absence of supporting experimental data, we speculate that the key to explaining the correlation between the evolution of awns and life history lies in the appreciation of the ecological shift bestowed by the two extremes of the burial syndrome: loss of the awn not only causes a shift from active to passive burial but also removes any preference for soil type (Peart 1981). Indeed, the species of *Tribolium* and *Schismus* occur on all sorts of soils (sandy soils, clay soils, well drained soils, seasonally flooded coastal sands) (Linder and Davidse 1997). Furthermore, numerous studies have shown that smaller and more spherical (compact) seeds tend to be more persistent (listed in Moles et al. 2000; Schwienbacher et al. 2010). A shift to a burial syndrome in which diaspores are smaller and therefore less costly to produce, while still allowing persistence of buried seeds, may consequently have allowed survival in habitats with temporarily unfavorable climates and on soils where hygroscopically active awns offer little benefit. According to this explanation, awnless lineages of *Tribolium* and *Schismus* persist and have diversified because they have adopted an annual reproductive strategy, produce a lot of small, compact diaspores, and can thereby rely on stochastic burial. Exploitation of this new niche has removed the disadvantage of not having an awn, a conclusion that corroborates what Verboom et al. (2006) speculated. Thus, just as other organisms that lose traits when they no longer provide an advantage, for example, animals that have adapted to life in dark caves lose their pigmentation and the ability to see (Culver 1982), birds that have escaped the constraints of predation lose the ability to fly (Roff 1994), or plants that have become holoparasitic lose their photosynthesizing appendages (Kujit 1969), awns (and the active burial syndrome) are lost when they cease to be beneficial.

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Supporting Information

The following supporting information is available for this article:

Appendix S1. Character states and callus length for each species.

Appendix S2. Ancestral state reconstructions using parsimony, ML, and rj-MCMC.

Appendix S3. Top five models in the posterior sample of dependent models, along with their probability density function (PDF), cumulative density function (CDF), and the rate classes of the constituent rate coefficients.

Appendix S4. Statistics of the MCA and coding of callus length.

Figure S1. (A–C) Likelihood values for Bayesian phylogeny reconstruction.

Figure S2. A randomly selected phylogram from optimal posterior sample of Bayesian trees.

Figure S3. Ancestral state reconstructions assuming correlated evolution between awns and life history.

Supporting Information may be found in the online version of this article.

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