Chapter 21

USING SUPERTREES TO INVESTIGATE SPECIES RICHNESS IN GRASSES AND FLOWERING PLANTS

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Abstract: Matrix representation with parsimony is the most widely used method for supertree reconstruction, due mainly to its ability to deal with incompatible source trees, and its simple and logical mathematical basis. Supertrees have the advantage over consensus methods in that the source trees do not need to contain identical terminal taxa, but only overlap. This makes supertrees a useful and attractive approach to building comprehensive phylogenetic trees, which are indispensable tools for investigating macroevolutionary patterns. Here, we highlight the use of supertrees of two plant lineages. We used the genus-level supertree of grasses (containing almost two-thirds of grass genera) and a family-level supertree of the angiosperms to investigate the influence of various putative key innovations (habit, life form, sex, mode of pollination, mode of dispersal, water resistance, salt tolerance, and habitat preference) on species richness at two different taxonomic levels within the flowering plants. The results suggest that no significant increase in speciation rates could be linked to any of these features in the angiosperms, whereas life form had a significant impact on the number of species at the family level in the grasses.

Keywords: angiosperms; grasses; key innovations; macroevolution; speciation

1. Introduction

Knowledge of the evolutionary history among groups of taxa is an essential element for classification purposes and investigations of macroevolutionary processes. Areas such as genomics (Dacks and Doolittle, 2001; Koch *et al.*, 2001; Soltis *et al.*, 2002), developmental biology (Halanych and

Bininda-Emonds, O. R. P. (ed.) Phylogenetic Supertrees: Combining Information to Reveal the Tree of Life, pp. 461–486. Computational Biology, volume 3 (Dress, A., series ed.).

Passamaneck, 2001; Jeffery *et al*., 2002; Simpson, 2002), and ecology (Christensen *et al*., 2002; Foley, 2002; Schmid-Hempel and Ebert, 2003) are also now taking advantage of the information contained in phylogenetic trees.

It has become apparent that building comprehensive phylogenetic trees is of paramount importance in evolutionary studies. Accurate inferences of macroevolutionary processes require most of the diversity of taxa within a group be sampled, either to reduce the chance of misleading the tree-reconstruction process (Graybeal, 1998; Hillis, 1998; Zwickl and Hillis, 2002) or to encapsulate most of the relevant information to make optimal use of the evolutionary history obtained (Purvis, 1996). For large groups of organisms containing tens or hundreds of thousands of species (such as the flowering plants or the grasses), the task of sampling an adequate number of taxa and gathering sufficient information to build a phylogenetic tree can easily become an immense and costly task.

The revolution in molecular techniques has eased the production of DNA sequences, and studies containing more than a hundred taxa based on molecular characters are now commonplace (e.g., Chase *et al*., 1993; Källersjö *et al*., 1998; Soltis *et al*., 1999; Qiu *et al*., 2000; Savolainen *et al*., 2000; Marvaldi *et al*., 2002). However, methods based on primary biological characters are not always applicable on a larger scale, principally because of uncoordinated data collection resulting in either a patchwork of coverage for a given taxonomic group or the extensive use of only a few types of DNA sequences (Bininda-Emonds *et al*., 2002). In such cases, a meta-analysis approach as used in supertree reconstruction might be more appropriate.

Supertree reconstruction takes advantage of existing, less comprehensive phylogenetic trees, and assembles them into a coherent and accurate representation of the relationships among the whole set of taxa at hand. Unlike consensus methods, it can deal with trees that do not have the same set of terminal taxa. It is, therefore, able to build a more comprehensive phylogenetic tree than any represented in the individual source trees on which it is based. Different methods, classified as being either “direct” or “indirect” (Wilkinson *et al*., 2001; see also Wilkinson *et al*., 2004), exist to build supertrees. Here, we concentrate on the “indirect” matrix representation with parsimony (MRP; Baum, 1992; Ragan, 1992) method and we refer the reader to the given references and Baum and Ragan (2004) for more technical information on the method. MRP is a method that is able to deal with source trees containing incompatible nodes without necessitating a loss of resolution. Its simple logical and mathematical basis, coupled with its ease of implementation makes it the most commonly used method in supertree reconstruction at the moment (Bininda-Emonds *et al*., 2002). As a consequence of their potential for complete taxonomic coverage, supertrees have been applied to a broad range of ecological and evolutionary analyses (see Bininda-Emonds *et al*., 2002; Gittleman *et al*., 2004). For example, they have been used to study rates of cladogenesis (Purvis *et al*., 1995; Paradis, 1998; Bininda-Emonds *et al*., 1999), functional relationships (e.g., Johnson, 1998; Linder, 2000; Nunn and Barton, 2000), and to associate differences in species richness with changes in phenotypic traits (Gittleman and Purvis, 1998). Their inclusive taxonomic coverage increases the power of such tests and lessens the effect of incomplete taxon sampling on comparative analyses (Bininda-Emonds *et al*., 2002).

In investigations into patterns of species richness, it has been proposed that certain traits might influence the rate of evolution and the production of new species. Such traits have been called key innovations, and it has been suggested that these traits have enabled those lineages that possess them to proliferate at an increased rate by opening up new adaptive zones (Burger, 1981; Maynard Smith and Szathmary, 1995). If this model is correct, then the expectation is that the observed differences in species richness between certain clades are correlated with the presence of particular traits. In attempting to identify correlates of species richness, the hierarchical nature of evolutionary history means that treating taxa as independent evolutionary units can result in erroneous inferences (Felsenstein, 1985). Therefore, a phylogenetic approach is essential in such studies.

Here, we used supertree reconstructions for both the grass family and families of angiosperms to investigate the effect of diverse phenotypic traits on the species richness within these two plant lineages. Factors that potentially influence species richness have been the focus of intensive study. Our goal in this study was not to present new methodology or results, but rather to extend the approach to a much larger sample of taxa. Supertrees are ideally suited in our case because they both provide the capability of combining different sets of taxa into a more comprehensive analysis and put these taxa into a phylogenetic context, which is extremely important in macroevolutionary studies. We first investigate factors that could have promoted speciation in the grasses using the largest phylogeny for the grass family, a genus-level supertree containing two-third of the grass genera (Salamin *et al*., 2002). Five traits reviewed in the following section were analyzed as well as traits that are thought to have been important in grass evolution (e.g., drought resistance, salt tolerance, and adaptation to open habitat). Additionally, a supertree for the angiosperms containing the most complete set of angiosperm families (Davies *et al*., in press) was used to investigate the pattern of species richness in the flowering plants as a whole and the effect of the five putative key innovations described in the following section on the rates of diversification.

2. Phenotypic traits and species richness

Different aspects of plant biology have been suggested to influence the rate of speciation in angiosperms. Numerous studies have investigated putative key characters thought to be correlated with increased species richness in angiosperms with mixed success and with varying degrees of phylogenetic rigor (e.g., Burger, 1981; Farrell *et al*., 1991; Marzluff and Dial, 1991; Eriksson and Bremer, 1992; Manning and Linder, 1992; Ricklefs and Renner, 1994; Sanderson and Donoghue, 1994; Gaut *et al*., 1996; Dodd *et al*., 1999; Heilbuth, 2000; Silvertown *et al*., 2000). To date, there has been little consensus on the relative importance of the five main traits examined in this study in explaining differences in the rate of speciation between lineages. We now review briefly some of the principle hypotheses concerning these five traits.

2.1 Habit

It has been proposed that increased reproductive rate increases speciation and decreases the chance of extinction (Marzluff and Dial, 1991). Woody plants take longer to mature typically, and are thought to have longer generation times, which has been suggested to be correlated negatively to the rate of evolution (see Eriksson and Bremer, 1992; Gaut *et al*., 1992, 1996). Barraclough and Savolainen (2001) found increased rates of molecular evolution within angiosperm families was correlated positively with species richness, suggesting that there could be a line of causality from decreased generation time to increased species richness via the effect of the former on the rate of molecular evolution. However, Rosenheim and Tabashnik (1991) argued that the exact relationship between generation time and evolutionary rate was more complicated. Furthermore, little is actually known about the number of cell replications before reproduction or about ancestral generation times within plants, nor what effect the longevity of the seed bank could have. Moreover, it is possible that germ-line mutations can occur throughout the lifetime of a plant (Bousquet *et al*., 1992).

2.2 Life form

Bousquet *et al*. (1992) suggested that annuals might be able to fill new niches better as a result of more functionally important mutations being driven to fixation because of smaller population sizes. Evidence in support of this hypothesis comes from the faster rates of evolution, particularly in the accumulation of nonsynonymous mutations, observed in *rbc*L within annuals (Bousquet *et al*., 1992). Perennials also have longer generation times so the arguments surrounding the importance of habit will also apply. However, the finding that nonsynonymous and synonymous mutation rates in annuals varied to different degrees when compared to perennials (Bousquet *et al.*, 1992) suggests that there is a more subtle relationship between life form and rate of evolution than the simple division into annuals and perennials.

2.3 Sex

Based upon the assumption that monoecy is correlated with self-compatibility, it has been hypothesized that monoecious species might be more likely to speciate for several reasons. First, if hybridization occurs, species that can reproduce asexually are more likely to form a new species (Rieseberg, 1997). Second, according to Baker's law (Baker, 1955), self-compatible species have increased probability of establishment after long-range dispersal, and therefore increased speciation rates. Heilbuth (2000) found dioecy to be correlated with lower species richness, but no evidence in support of Baker's Law. Finally, dioecious species are more likely to have generalist pollinators (Bawa and Opler, 1975; Bawa, 1994), thereby inhibiting the reproductive isolation necessary for speciation.

2.4 Mode of pollination

Burger (1981) suggested that biotic pollination was important in the early diversification of the angiosperms by allowing outcrossing sexual reproduction in highly diverse populations of few individuals. However, it appears unlikely that this would have much impact upon established populations. Furthermore, there is evidence of insect pollination before the appearance of the angiosperms, and many diverse families within the angiosperms are predominantly wind-pollinated (e.g., Poaceae, Cyperaceae, Juncaceae, and Fagaceae; Midgley and Bond, 1991). It has also been suggested that biotic pollination is a major isolating mechanism between plant species. The occurrence of faithful pollinators could therefore increase the rate of diversification (Dodd *et al.*, 1999; Ricklefs and Renner, 1994). Pollinator-mediated reproductive isolation was demonstrated in the genus *Disperis* (Manning and Linder, 1992), where, by depositing pollen on different parts of the anatomy of a pollinator, the species became effectively reproductively isolated despite sharing a common pollinator. Gorelick (2001) suggested that biotic pollination did not in fact increase speciation rates, but influenced contemporary patterns of diversity instead by decreasing the probability of extinction, thereby resulting in the increased net speciation rates observed. He argued that biotically pollinated species were found at lower densities than abiotically pollinated species, which reduced the chances of any single event affecting all individuals in a population. Such robustness to extinction enabled populations to endure over longer evolutionary time, increasing the likelihood of speciation. Some empirical evidence supports this proposed correlation of biotic pollination (and life form) with increased species diversity (e.g., Eriksson and Bremer, 1992).

2.5 Mode of dispersal (as indicated by fruit type)

It has been argued that dispersal by animals increases long-distance dispersal, thereby promoting establishment of isolated populations (Eriksson and Bremer, 1992). Such isolated populations might be more likely to diverge through genetic drift and a founder effect. However, conversely, increased long-distance dispersal could also encourage backcrossing, breaking down reproductive isolation and decreasing rates of speciation (Ricklefs and Renner, 1994). It might be that a limited migration capacity is most likely to lead to increased speciation, enabling relatively infrequent long-range dispersal to new habitats, but inhibiting gene flow between such dispersed populations (Bousquet *et al*., 1992; Dennis *et al*., 1995). However, significant results, such as those of Smith (2001), who found a correlation between biotic dispersal and species richness, compared only plants within the same ecological conditions (the tropical understorey).

3. Species richness in grasses

The grasses (family Poaceae) are the fifth-largest family in the angiosperms, with almost 10 000 species (Mabberley, 1993). Their importance is beyond doubt, for they provide the grass-dominated ecosystems that cover more than one-third of the Earth's land surface (Archibold, 1995), and they play an essential role in human sustenance, either as a cereal crop or as a source of forage (Raven *et al*., 1992). The success of the grasses in terms of biodiversity can be explained partly by their adaptability to changeable environments, their ability to resist grazing, and by almost endless variations based on an "all-purpose body plan" (Clayton and Renvoize, 1986; Chapman, 1996).

A great diversity in number of species can be seen between the different grass lineages within the family. For example, four of the ten most important grass subfamilies contain more than 65% of the total number of species (Kellogg, 2000). Of the 395 genera considered in this study, the ten most species-rich represent about one-third of the total grass diversity (3200+

Table 1. Character states for the eight phenotypic traits used in the angiosperms and grass analysis. “n/a” means that the data on the particular phenotypic trait was not available for the whole set of taxa considered in this study.

Trait	Character state		Supertrees	
	state 1	state 2	grass	angiosperm
Habit	trees, shrubs and lianas	herbaceous	✓	✓
Life form	annual and biennial	perennial	✓	✓
Sex	dioecious	monoecious	✓	✓
Pollination	wind	not wind	n/a	✓
Fruit	fleshy	nonfleshy	n/a	✓
Water requirement	hydrophile	xerophile	✓	n/a
Salt tolerance	halophile	glycophile	✓	n/a
Habitat	open	shade	✓	n/a

species), and half the total number of species in the family are contained in only 18 genera (Watson and Dallwitz, 1992).

Within the angiosperms, the grasses are thought to be a relatively young family. Although the earliest non-equivocal fossil evidence dates from the early Eocene (~55 million years ago; Crepet and Feldmann, 1991), the global expansion of grasses and their increasing relative abundance in terrestrial ecosystems did not occur before the early to middle Miocene (~15 million years ago; Willis and McElwain, 2002). It has been hypothesized that the co-evolution between grasses and hoofed mammals has had an important role in the expansion of the grasslands and increased speciation within the former (Janis, 1993; MacFadden, 1998). Morphological characters, such as the presence of silica bodies within the leaves, could have had an influence in the success of some species by conferring a higher resistance to herbivory (Chapman, 1996) and allowing open-habitat species to become more species rich. At the same time, compelling evidence suggests that increasing latitudinal aridity promoted the evolution and expansion of grasses (Wing and Boucher, 1998), which could indicate drought resistance as a potential important adaptation in the family. Finally, grasses are part of the angiosperms, and the different traits discussed in Section 2 could have had an influence on grass species richness. In total, we investigated the potential effect of six traits, ranging from anatomical features to life history (Table 1), on the species richness of grasses.

3.1 The grass supertree and trait / diversity relationships

The grass supertree was taken from Salamin *et al*. (2002), and is based on 61 published phylogenetic trees that contain a total of 395 grass genera (Figure

1). Because of the ability of supertrees to combine source trees into a more comprehensive tree, almost 50% of all grass genera are represented in this study, which is far greater than the taxonomic coverage offered by molecular phylogenies where typically less that 5% of the genera are represented. The MRP matrix was built using the software SuperTree0.85b (Salamin *et al*., 2002), and the Baum / Ragan coding scheme was selected with characters weighted by their bootstrap support. The characters of the MRP matrix were considered irreversible during the parsimony analysis (for details, see Salamin *et al*., 2002). The species number for each genus was recorded from the Grass Genera of the World database (Watson and Dallwitz, 1992; http://biodiversity.uno.edu/delta/grass/), and the different morphological characters considered were taken from the Delta database for the grass family (http://biodiversity.uno.edu/delta/).

The different traits of interest were mapped on the supertree using ACCTRAN and DELTRAN optimization with the software PAUP* 4.0b10 (Swofford, 2002). For equivocal character-state reconstructions, ACCTRAN favors reversals of character states over convergences, pushing the origin of the derived character state towards the root of the tree; whereas DELTRAN favors later, parallel origins of the derived character state. Based on these optimizations, all sister clades with contrasting traits were identified. When nested contrasting clades were found, we only selected the most terminal contrasting sister clades; changes from one state to another occurring in the deepest nodes were not considered. Cases where both character states were present within a given taxon could confound the effect of a trait on the species richness of that particular clade. Therefore, we also ignored polymorphic taxa, so that unequivocal contrasts only were examined.

The method of Slowinski and Guyer (1993) was used to compare the number of species belonging to each sister clade against the null hypothesis of equal speciation rates. The method obtains a test statistic from the cumulative probability of obtaining a difference in number of species between the sister clades as large as the one observed. To test the significance of the cumulative probabilities for each of the traits over the different sister clades, we used 1) Fisher's combined probability test as proposed originally by Slowinski and Guyer (1993) and 2) the method of Goudet (1999). Goudet (1999) showed that type I and II errors obtained with Fisher's test can be unduly large as a result of the non-uniform distribution of the probabilities over each sister group, and proposed a randomization procedure to avoid these biases (see also Nee *et al*., 1996). This procedure is designed to include all cases where the distribution of p-values is symmetrical about 0.5 to test the null hypothesis that the distribution of sister-group sizes follows a model of random speciation and extinction. The

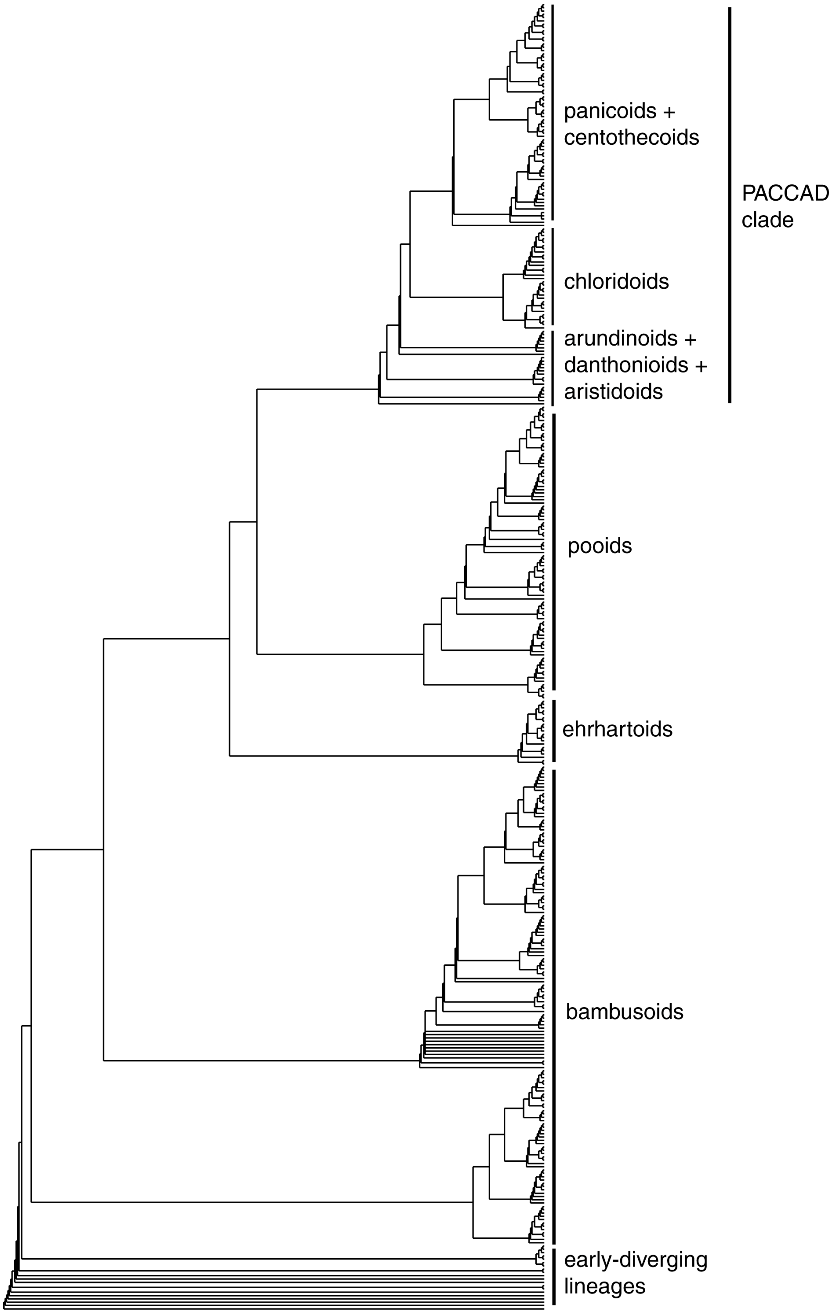

Figure 1. Grass supertree, and the position of major clades and subfamilies, based on the Baum / Ragan coding scheme with characters weighted by node support (adapted from Salamin *et al*. (2002).

Table 2. List of traits investigated in the grass family and the results of the tests based on Slowinski and Guyer (1993) and Goudet (1999) methods: a) based on ACCTRAN optimization, and b) based on DELTRAN optimization. States 1 and 2 represent the trait thought to increase species richness and the alternative trait, respectively.

a)

Trait	Character states		N	Fisher combined probability test		Randomization procedure	
	state 1	state 2		χ^2	p	G_{obs}	p
Life form	annual	perennial	22	96.839	<0.001	0.258	<0.001
Sex	bisexual / monoecy	dioecy	13	39.880	0.041	0.456	0.342
Habit	tree-like	herbaceous	6	38.764	0.015	0.316	0.036
Water requirement	hydrophile	xerophile	20	38.804	0.524	0.528	0.639
Salt tolerance	halophile	glycophile	9	20.048	0.330	0.518	0.548
Habitat	open	shaded	6	7.737	0.805	0.589	0.752

b)

Trait	Character states		N	Fisher combined probability test		Randomization procedure	
	state 1	state 2		χ^2	p	G_{obs}	p
Life form	annual	perennial	18	67.879	<0.001	0.304	<0.001
Sex	bisexual / monoecy	dioecy	12	50.475	0.001	0.445	0.192
Habit	tree-like	herbaceous	5	34.943	<0.001	0.298	0.026
Water requirement	hydrophile	xerophile	21	49.454	0.171	0.454	0.257
Salt tolerance	halophile	glycophile	9	23.287	0.179	0.365	0.304
Habitat	open	shaded	6	15.987	0.191	0.478	0.543

randomization procedure was performed using the software R–1.6.1 (http://www.r-project.org). A Bonferroni correction was used because several simultaneous non-independent tests were performed, reducing the alpha value to 0.008 from the nominal 0.05.

3.2 Results and discussion

The results showed that the ability to resist drought or salty environments, or to live in open habitat did not affect the speciation rate within the respective grass species possessing those traits significantly (Table 2). These results held regardless of whether ACCTRAN or DELTRAN optimization was used to map the morphological characters onto the supertree. Similarly, being bisexual or monoecious was not found to have a significant effect using the randomization procedure (Table 2). However, having bisexual spikelets or being a monoecious plant was found to have a significant effect on the number of species with the Fisher's combined probability test under both ACCTRAN and DELTRAN optimization (Table 2). The *p*-values obtained from Fisher's combined probability test were lower in general than those obtained from Goudet's (1999) randomization procedure, confirming the potentially elevated type I error induced by using the former test.

Traits such as drought resistance, salt tolerance, and the ability to thrive in an open habitat are likely to be represented by a broad spectrum of adaptations and phenotypes, and could have evolved from diverse origins. Consequently, the presence of these traits was highly polymorphic within most genera, resulting in the removal of a large number of terminals from the sister-clade comparisons. The decrease in the number of species on either side of the contrasting sister clades arising from the removal of polymorphic taxa can have a large influence on the tests performed, and could have confounded any signs of enhanced diversification rates arising from these traits. The removal of polymorphic taxa could also remove the larger genera preferentially because they are more likely to be polymorphic purely by chance. Ignoring polymorphic taxa could also influence our results by removing important information. For example, the key innovation might actually have an impact on species richness, but would be ignored totally in our comparisons if it arose inside the polymorphic clade. Dioecy has been suggested to be correlated with self-incompatibility, but the interpretation of such a correlation is difficult (Weiblen *et al.*, 2000). However, the link between monoecy and bisexual spikelets and higher diversification rates is done precisely through this assumed correlation. Our results, therefore, could suggest that 1) neither monoecy nor bisexual spikelets correlate with self-compatibility in the grasses because no effect was seen on the rate of diversification, or 2) that the correlation does exist, but that Baker's law is not supported in this family. There was also no significant correlation between a herbaceous habit and an increase in net speciation rate after correction for multiple comparisons (Table 2). In contrast to the five traits presented above, an annual life cycle had a significant effect on species richness in grasses under both tests and regardless of whether ACCTRAN or DELTRAN optimization was used (Table 2). The Fisher's test again gave lower *p*-values than the randomization procedure, which can be explained by the non-uniform distribution of these *p*-values (Goudet, 1999).

Our findings for the grasses support Bousquet *et al.*'s (1992) hypothesis that annuals might be able to fit into new niches better, and, therefore, become more species rich. This hypothesis was based on the faster rates of evolution observed within annuals (Bousquet *et al.*, 1992) and the link between speciation rates and nucleotide substitution rates (e.g., Barraclough *et al.*, 1996; Savolainen and Goudet, 1998). Although the latter link has been established in other taxonomic groups (Barraclough *et al.*, 1996), evidence for higher speciation rates being correlated with higher substitution rates is inconclusive in the grasses (Gaut *et al.*, 1997). Gaut *et al.*'s (1997) analysis was restricted to a small fraction of grass diversity, and extending the sampling could change the outcome of the analysis. Our results indicate that the generation time, possibly through a change in substitution rate, could influence species richness in the grasses. However, although woody plants such as bamboos have a very long generation time, with some species only flowering every decades (Clayton and Renvoize, 1986), no link between the herbaceous / woody trait and species richness was found. Only a few sister-group comparisons were present on the grass supertree, and most were within the bamboos. Although the paucity of possible sister-group comparisons can have a large effect on the negative results we found, it is probable that factors other than those considered here have also played a role in the success of the grasses.

Another approach to investigate species richness that we did not undertake here is to first identify sister groups with significantly different species richnesses (e.g., using the methods of Moore *et al.*, 2004), and then to look for traits that also differ between these groups. This approach could be more appropriate to highlight whether the possession of a particular phenotypic character was important in the increase of the number of species within a clade. Finally, it has to be noted that no one single trait explains everything, and that the evolutionary responses leading to an increase in species richness are likely to be complex.

4. Species richness in the angiosperms

The flowering plants (angiosperms) represent one of the largest terrestrial radiations, and provide an ideal subject for statistically robust investigations into hypothesized evolutionary explanations for the contemporary pattern in species richness. Over 250 000 species are recognized currently (Wilson, 1992), although estimates vary and the final number might well be double this (Govaerts, 2001; Bramwell, 2002), with familial species richness varying over several orders of magnitude.

The angiosperm supertree (Davies *et al.*, in press; Figure 2) used here is the most complete representation of angiosperm families to date, and allowed us to investigate several alternative hypotheses that have variously been proposed as explaining the diversity of the angiosperms. It is apparent

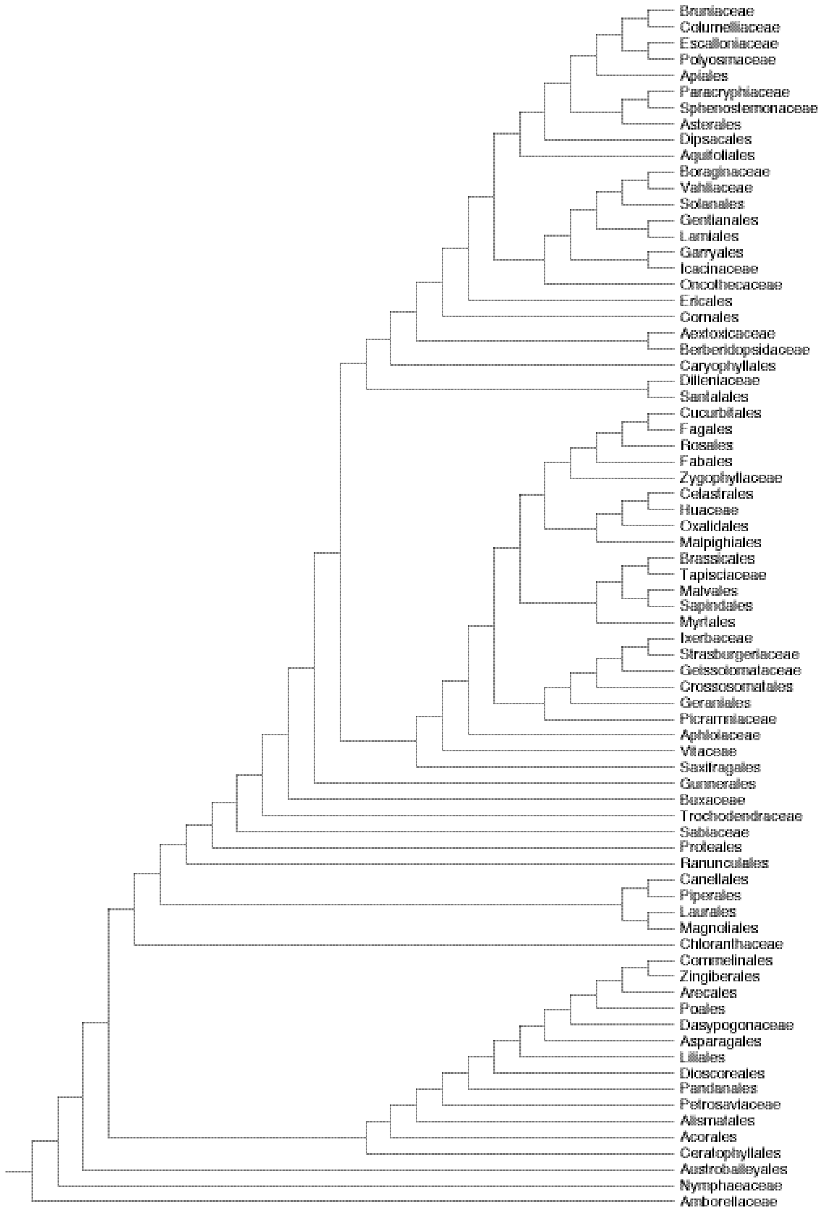

Figure 2. Angiosperm supertree based on the Baum / Ragan coding scheme with characters weighted by node support. Not all families are represented, but ordinal classification is presented when possible (adapted from Davies *et al.*, in press).

from the supertree that some lineages are much more species rich in comparison to their sister lineages and, therefore, appear to have increased rates of diversification. The net result of differential speciation rates is a highly imbalanced topology. Using Purvis *et al.*'s (2001) modification of Fusco and Cronk's (1995) imbalance measure, the weighted mean imbalance was 0.70, which was significantly different ($p < 0.001$) from the expectations of the Markovian null model (Davies *et al.*, in press). Although the Markovian null model does not predict a perfectly balanced tree (Kirkpatrick and Slatkin, 1993), the degree of skewness in the imbalance found suggests that the Markovian model is inappropriate for describing the radiation of the angiosperms. There is also evidence that such conclusion holds across a broad taxonomic spectrum (Purvis, 1996; Savolainen *et al.*, 2002).

The use of a supertree approach allowed us, for the first time, to utilize a complete family-level phylogeny of the angiosperms using currently accepted family delineations following the advice of the APG group (Bremer *et al.*, 1998 and onwards). This enabled the most thorough and robust comparisons of clade species richness with regard to putative key traits yet undertaken.

4.1 The angiosperm supertree

The angiosperm supertree combined 46 predominantly molecular phylogenetic trees encompassing the current understanding of angiosperm familial relationships. Source trees were selected based on either their comprehensive coverage or their resolution of relationships that were poorly understood previously (i.e., where support for phylogenetic affinities was weak or absent). In contrast to many situations where supertrees have been used, several large phylogenetic trees of the angiosperms already exist, such as that by Soltis *et al.* (2000) in which around 75% of families are represented. The intention behind constructing the supertree was to amass phylogenetic data to get complete familial representation rather than to produce a consensus of conflicting phylogenetic hypotheses. Consequently, sampling of source trees was not as dense as found in many other studies (e.g., primates, Purvis, 1995; carnivores, Bininda-Emonds *et al.*, 1999; grasses, Salamin *et al.*, 2002). This selection of source trees could leave the supertree construction process open to the accusations of bias, which we argue is not a valid criticism. All phylogenetic analyses suffer from some form of bias in data selection, taxon sampling, or method of analysis (to name but a few); supertree construction is no different in this way. The angiosperm supertree was intended to represent only the most recent understanding of phylogenetic relationships within the group.

Construction of the angiosperm supertree followed broadly that advocated by Salamin *et al.* (2002). As for the grasses, the software SuperTree0.85b (Salamin *et al.*, 2002) using the Baum / Ragan coding scheme was used to build the binary matrix. This matrix was analyzed using parsimony treating all characters as irreversible; further details are given in Davies *et al.* (in press).

4.2 Species richness

To investigate correlates of diversity, we used independent contrasts, which is a highly conservative approach with respect to its sensitivity to phylogenetic error (Symonds, 2002). Many previous estimates of phylogeny within the angiosperms are not in complete agreement with one other. However, despite different topologies being generated from different subsets of genes, disagreement between topologies is more likely a product of noise within the data sets rather than an indication of conflicting phylogenetic signal. Consequently, the support for relationships that differ between phylogenetic estimates is low generally. The three genes that have the broadest taxonomic sampling within the angiosperms — *rbc*L, 18S rDNA, and *atp*B — show a high degree of similarity in their phylogenetic signal and general agreement in the relationships they depict among the major angiosperm groups (Soltis *et al.*, 1998). By adopting the protocols of Salamin *et al.* (2002) for weighting relationships within the source trees in the MRP analysis by their respective bootstrap support values, well-supported nodes were able to override less well-supported nodes where there was conflict between the source trees.

Species numbers for sister taxa identified from the angiosperm supertree were obtained from Davies *et al.* (in press) and followed the family delineations of the Angiosperm Phylogeny Group (APG; Bremer *et al.*, 1998; APG II, 2003). When families appeared polyphyletic or paraphyletic in the supertree, they were merged to produce a composite taxon and species richness was calculated as the sum of the number of species in the individual families. States for the five traits mentioned above were also coded using Watson and Dallwitz's (1992) online database. Families recognized currently by the APG, but embedded within larger families in the online database were coded with a question mark. Character states were grouped to correspond to the biological traits under examination, thereby maximizing the number of contrasts. We again ignored polymorphic taxa so that only unequivocal contrasts were examined. We regard this as a conservative test of the key-innovation hypothesis.

For each trait in turn, the character states between the two sister clades were compared at each node on the phylogenetic tree. If these differed, the following species richness contrast was performed:

$$\log\left[\frac{\text{number of species in the clade possessing state 1}}{\text{number of species in the clade possessing state 2}}\right].$$

A one-sample *t*-test was then performed upon the results for all nodes to see if the mean value differed significantly from zero. A Bonferroni correction was used here as well, reducing the individual alpha value to 0.01 from 0.05.

4.3 Results and discussion

One of the most striking aspects of this analysis was the paucity of independent contrasts. Of the five traits examined, two (life form and mode of pollination), produced only two unequivocal sister-taxa comparisons differing in the trait. Interestingly, mode of pollination has been one of the most cited traits in previous attempts to explain the unusual success of the angiosperms. The lack of potential contrasts was as much a consequence of the large number of families that were polymorphic for the trait in question as it was of sister taxa sharing the same trait. Many of the large families such as Asteraceae and Cyperaceae contained species that were both abiotically and biotically pollinated. Those that could be classified easily as one or the other, such as abiotic pollination for the grasses, often shared this trait with their nearest species-poor relatives (Joinvilleaceae and Ecdeiocoleaceae). Of the remaining three comparisons, fruit type produced the most contrasts, but no significant correlation with species richness was apparent ($p = 0.16$; Table 3). A similar lack of significance was found for sex ($p = 0.98$; Table 3) and habit ($p = 0.62$; Table 3).

Several strategies have been adopted in the literature to increase the number of independent contrasts in an effort to test putative key innovations. These methods can be categorized broadly as clade reduction and majority rule. The former approach reduces the species number of a clade by subtracting the number of species that possess the trait deemed atypical of that clade (e.g., Heilbuth, 2000). The latter, and more common, approach characterizes a clade based upon the trait possessed by the majority of species within it (e.g., Eriksson and Bremer, 1992). Both these strategies are unsatisfactory because trait flexibility and species richness are intertwined inextricably (see below). By contrast, the current analysis is the most stringent test of the key-innovation hypothesis.

Table 3. List of traits investigated in the angiosperm supertree, number of observations, mean and standard deviation (SD) for each trait, and the one sample *t*-test statistics and associated *p*-value.

Trait	Character state		*N*	mean	SD	One sample *t*-test	
	state 1	state 2				*t*	*p*
Habit	trees, shrubs and lianas	herbaceous	14	0.49	3.57	0.51	0.62
Life Form[1]	annual and biennial	perennial	2	1.03	1.77	0.82	0.56
Sex	dioecious	monoecious	10	–0.02	2.32	–0.03	0.98
Pollination	wind	not wind	2	–4.84	3.56	–1.92	0.31
Fruit	fleshy[2]	non-fleshy[3]	19	–0.93	2.78	–1.46	0.16

[1] for herbaceous plants

[2,3] as indicators of biotic dispersal

There are many possible reasons why no key innovations were identified in the angiosperms. First, as mentioned above, the species-poor sister clade might also have the key trait associated with increased rates of cladogenesis, but subsequent adaptation in unrelated traits or niche shifts restricted its potential to diversify. Second, different traits might be advantageous at different geological times, with those taxa that happened to be pre-adapted to changes in the environment radiating rapidly. Consequently, particular traits could be correlated with increased rates of diversification only within certain geological time periods. Such a scenario has been suggested as explaining the rapid radiation of the grasses (which had been restricted previously to marginal habitats) coinciding with the late Tertiary change towards a drier climate, which enabled the exploitation of new niches and a dramatic increase in their ecological dominance (Axelrod, 1952; Chapman, 1996). Such an expansion in range size might have also influenced the probability of further speciation by increasing the likelihood of major isolating factors such as geological barriers separating populations (see Rosenzweig, 1992, 1995). The possibility that the rise to dominance of the angiosperms might be as much a consequence of environmental change as a product of evolutionary novelties gains support from the fossil record. The apparent timings of the attainment of dominance varied latitudinally (i.e., was climate specific; Crane and Lidgard, 1989), and the time lag between the origination of particular traits and the apparent increase in the proportion of taxa possessing those traits in the fossil record (Crane *et al*., 1995) suggests that some factor other than the possession of that particular novel trait was crucial for the subsequent radiations.

If rates of diversification are a product of an interaction between life-history traits and the environment, it might come as no surprise that no single trait appears to be correlated with contemporary species richness. Over evolutionary time, differing environmental conditions could have favoured the expansion of clades possessing different biological traits, such as biotic pollination in the Orchidaceae around the late Cretaceous (Crane *et al*., 1995) and abiotic pollination in the grasses in the late Tertiary (Chapman, 1996). Present day species richness is a reflection of the sum of all these historical events, and it might require a unified approach at the interface between knowledge of the fossil record and past climate together with a detailed understanding of phylogeny to tease apart the true story fully. Again, a complementary approach to identifying those traits of importance, suggested by Moore *et al.* (2004), is to identify sister groups with significantly different species richness and to look for traits that differ between them.

An alternative explanation is that traits other than the ones examined might be significant in explaining the success of the angiosperms. Gorelick (2001) lists twenty hypotheses selected from the literature that have variously been proposed to explain the apparent rapid radiation of the angiosperms in comparison to other seed plants, and which encompass co-evolution, breeding system, and numerous other life-history traits. As discussed above, there are certainly many unanswered questions surrounding the potential influence of the traits that we have examined here.

We must of course recognize one further possible explanation for the lack of significance of our findings: that the taxonomic level used in the analysis is inappropriate for identifying correlates of contemporary diversity. This would obviously be the case if the majority of present day species richness was the result of very recent rapid radiations, and if these lineages had not yet achieved sufficient taxonomic distinctiveness to be recognized as separate families. The weakly negative correlation between species richness and family age (Figure 3; see also Burger, 1981) does imply that the majority of the present day species richness could be a product of relatively recent speciation events. However, evidence from the fossil record indicates that the angiosperms attained ecological dominance around 90–130 million years ago (Crane *et al*., 1995), and that shifts in the rates of diversification within angiosperms have occurred many times over their evolutionary history and across disparate lineages (Davies *et al*., in press). It is just as probable that both these factors play a part and that contrasting generic-level species richness might give insights into the evolutionary trends favoured by the current environmental conditions, but only a limited understanding of events deeper in time.

Finally, the polymorphic nature of many large families has also led to arguments that it is the very ability to adapt to changing conditions that has enabled some taxa to speciate so rapidly (e.g., Burger, 1981; Rickleff and

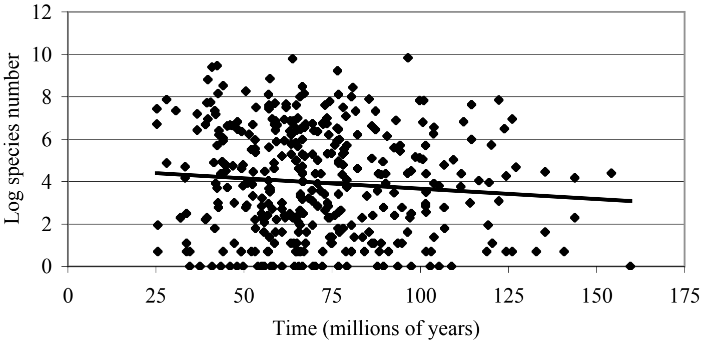

Figure 3. Plot of familial species richness against age of the node from which the family subtends. Dates were taken from Davies *et al*. (in press) and were derived from *rbc*L sequence data obtained from GenBank (http://www.ncbi.nlm.nih.gov/). Adjustments for rate heterogeneity among lineages and calibration was performed following Wikström *et al*. (2001).

Renner, 1994). Burger (1981) listed several characters that appear particularly plastic within the angiosperms, including genetic and phenotypic variability in seed production, dispersal and establishment, cell growth, gene expression, and the defining feature of the angiosperms, the flower itself. The extreme plasticity of these traits makes them unsuitable for analysis at the level of all angiosperms. Moreover, a description of these traits across the whole of the angiosperms is not available. A possible hypothesis would be that, rather than possessing a particular character state, being flexible with respect to a given phenotypic trait could lead to an advantage. Given the extraordinary diversity of the angiosperms, such a hypothesis is highly appealing. However, testing such a theory is problematic. The highly variable nature of such traits does not lend itself well to phylogenetic contrasts at higher taxonomic levels such as the family, and characterizing flexibility itself as a trait is beset by an innate circularity. It appears impossible to distinguish whether certain families are more species rich because they have the ability to be flexible in a certain trait or whether it is just more probable that a larger number of character states for a trait will evolve in larger families (see Ricklefs and Renner, 2000; Silvertown *et al*., 2000). Moreover, all measurements of flexibility are purely inferences drawn from the phylogeny rather than measurable biological characters; and Silvertown *et al*. (2000) argue that, as a consequence, they offer little explanatory power in answering questions about diversification rates. In summary, to gain a better understanding of the causes and processes of diversification, we need an even more detailed knowledge of angiosperm phylogeny. The current analysis can identify only significant traits that differ between families, and, as observed within the grasses, particular traits might be correlated with species richness within a family, which cannot be tested at this taxonomic level.

5. Conclusions

Mixed results were obtained in our investigation of species richness in the grasses and the angiosperms. In the grasses, herbaceous habit and the annual life form were found to be potential key innovations that increased diversification rates. However, for other traits, and also at the higher taxonomic level analyses for the angiosperms, no correlation with species richness was found. It is probable that no simple explanation can offer us a complete understanding of the patterns of contemporary diversity, and a knowledge of evolutionary relationships will become ever more important in providing us with answers to these questions. A goal to aid future investigations would be the creation of a complete generic-level angiosperm phylogeny or a complete species-level grass phylogeny. These are no small tasks, but one that is under consideration currently for the angiosperms. The vast taxonomic sampling required (~10 000 genera and species, respectively) and uncertainties surrounding the limitations of molecular data in resolving such complex phylogenies mean that traditional sequence-based approaches to obtaining these aims are likely to be some way into the future. As illustrated by the examples in this chapter, the use of supertree methodologies such as MRP might make the realization of this objective a much more attainable achievement in the short term.

Our analysis was constrained by several implicit assumptions. Slowinski and Guyer's (1993) method and the species-richness contrasts used here both assume a Markovian model of evolution, which might not be the correct null model of cladogenesis (Cunningham, 1995). At the same time, we can observe only the net speciation rate, and the presence of a clade that is more species rich than its sister counterpart could be a result of either an increase in diversification rates in the larger clade or an increase in extinction rates in the smaller one. Unfortunately, the tests performed here are unable to distinguish between these two cases. Furthermore, the method can be sensitive to errors in the tree used to map the characters of interest and to find contrasting sister clades. As suggested by Dodd *et al.* (1999), it could also be productive to examine interactions between traits or to calculate the variance in diversity associated with different traits that can be obtained with a non-phylogenetic approach (e.g., Ricklefs and Renner, 1994). The development of phylogenetic-based methods coupled with a multivariate approach could be an extremely useful tool in understanding the origin of differences in species richness between groups of organisms.

Finally, it is important to keep in mind the relative limitation of our approach. The influence of a trait on rates of diversification will probably be contingent upon other taxa, the possession of other traits, and the physical environment. Consequently, no single trait might be associated with species richness at all points on a phylogeny. We therefore advocate that future investigations into patterns of species richness also consider the interactions between biological traits and the environment, as neither is likely to provide definitive answers in isolation. Our goal here was not to look for global answers to the success of the angiosperms or the grasses, but rather to examine whether the possession of a particular character state at a particular point in the phylogeny would be associated with a level of imbalance in the node under consideration.

Acknowledgements

We would like to thank Olaf Bininda-Emonds for inviting us to contribute to this book; Mark Chase for his invaluable help in ensuring compliance with the APG; and Timothy Barraclough, Trevor Hodkinson, and Vincent Savolainen for their comments and support. We also would like to thank Leslie Watson for providing us with the grass morphological data set, and Elizabeth Zimmer and an anonymous reviewer for comments on the manuscript. This work has been supported by grants from the Swiss National Science Foundation (NS) and a NERC studentship (TJD).

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