

## Radiations and key innovations in an early branching angiosperm lineage (Annonaceae; Magnoliales)

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Biologists are fascinated by species-rich groups and have attempted to discover the causes for their abundant diversification. Comprehension of the causes and mechanisms underpinning radiations and detection of their frequency will contribute greatly to the understanding of the evolutionary origin of biodiversity and its ecological structure. A dated and well-resolved phylogenetic tree of Annonaceae was used to study diversification patterns in the family in order to identify factors that drive speciation and the evolution of morphological (key) characters. It was found that, except for *Goniothalamus*, the largest genera in the family are not the result of radiations. Furthermore, the difference in species numbers between subfamilies Annonoideae (former long branch clade) and Malmeoideae (former short branch clade) cannot be attributed to significant differences in the diversification rate. Most of the speciation in Annonaceae is not distinguishable from a random branching process (i.e. chance), and no special explanations are therefore necessary for the distribution of species richness across the major part of the phylogenetic tree for Annonaceae. Only for a small number of clades can key innovations be invoked to explain the elevated rate of diversification. © 2012 The Linnean Society of London, *Botanical Journal of the Linnean Society*, 2012, **169**, 117–134.

**ADDITIONAL KEYWORDS:** adaptive radiation – rate shift – sister-group comparison – stochastic branching pattern – temporal method – topological method.

### INTRODUCTION

Biologists are fascinated by species-rich clades because their size is associated with evolutionary success (Raikow, 1988). These clades are often thought to be the result of a radiation. Indeed, studies that invoke radiation as an explanation for the existence of species-rich plant genera are numerous in the literature (e.g. Richardson *et al.*, 2001; Cronn *et al.*, 2002; Rüber, Van Tassel & Zardoya, 2003; Beheregaray *et al.*, 2004; Cieslak *et al.*, 2005; Whittall *et al.*, 2006; Schuettelpelz & Pryer, 2009; Valente, Savolainen & Vargas, 2010; Duputié, Salick & McKey, 2011).

Radiations have also been put forward as an important factor explaining species-rich clades at higher taxonomic levels, such as families (e.g. Davies *et al.*, 2005; Couvreur *et al.*, 2010), orders (e.g. Davis *et al.*, 2005) or above (e.g. Magallon & Sanderson, 2001; Wang *et al.*, 2009). However, the term (adaptive) radiation is often uncritically used for any historic increase in species numbers (Skelton, 1993). Linder (2008) defined the term radiation simply as the increase in diversity in a lineage, and therefore all biological diversity results from radiations. However, this practice erodes the explanatory potential of the term and partly results from the fact that the term radiation is still ill-defined (Sanderson, 1998). Moreover, the causes of such a supposed radiation are

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often unclear. It is generally stated that a taxon has the built-in tendency to radiate and proliferate (perhaps because of some presumed key character or historical event) when, merely, it is observed in retrospect that it has done so (Raikow, 1988). No causal relationship between the observed characters and the diversification of the group is established, making the explanation rather speculative. This is unfortunate because an understanding of the frequency, causes and mechanisms underpinning radiations will contribute to the comprehension of the causes of biodiversity (Seehausen, 2007).

### THE MEANING OF 'RADIATION'

One of the definitions of radiation is 'the evolution of a relatively large, monophyletic group of species or higher taxa within a relatively short period of time' (adopted from Gittenberger, 2004). If, in addition, character and/or ecological divergence occurs, the radiation can be called adaptive, otherwise it is nonadaptive (Gittenberger, 1991; Sanderson, 1998; Rundell & Price, 2009). The result of a radiation is a (sometimes large) difference in species numbers between the radiating clade and its nonradiating sister clade.

Differences in species numbers between lineages can arise for many reasons, such as chance, availability of (new) resources, absence of competitors or possession of key innovations (for an elaborate discussion of these factors, see Brooks & McLennan, 2002; Sudhaus, 2004; Seehausen, 2007; Losos, 2010; Yoder *et al.*, 2010). Thus, biologically, the ancestors of a radiation can be regarded as relatively unspecialized in the first three scenarios, whereas, in the fourth case, they are specialized (Sudhaus, 2004) and would constitute an adaptive radiation. 'Adaptive' is thus about character evolution and 'radiation' is about speciation (Brooks & McLennan, 2002). Lineage diversification (speciation and extinction) and character diversification (morphological and ecological) should therefore be decoupled (although, inference mistakes can arise if the decoupled characters shaped the phylogeny under study; Sanderson, 1998; Maddison, 2006). In a radiation of species, many new taxa arise (e.g. flowering plants; Willis & McElwain, 2002), and a radiation of adaptations leads to the origin of many characters (for examples of adaptive radiations in flowering plants, see Classen-Bockhoff *et al.*, 2004). The net number of lineages arising from a radiation of adaptations is not important and adaptive radiations can therefore contain a relatively small number of species (e.g. the adaptive radiation of 28 species of Hawaiian silverswords; Schluter, 2000).

### PROBLEMS WITH THE DEFINITION OF 'RADIATION'

Questions about the diversity of evolutionary groups are questions about variation in speciation and extinction (i.e. diversification rate), which leave their signatures in the shapes of phylogenetic trees (Mooers & Heard, 1997). Dated phylogenetic trees provide important insights into the tempo and mode of evolution of clades (Glor, 2010). If, in such a tree, many lineages arise in a short period of time, it might be concluded that a radiation has taken place. However, this conclusion is highly dependent on the meaning of the vague concepts 'many lineages' and the definition of 'a short period of time' (Gittenberger, 2004). At lower taxonomic levels, the concept 'many lineages' is often equated with 'species rich'. This term covers a wide range between, for example, 62 species of *Camissonia* Link in Onagraceae (Levin *et al.*, 2003), 1000 species of *Impatiens* L. (Janssens *et al.*, 2009) and 2000 species of *Euphorbia* L. Next to this, the scope of the 'short time' for a radiation to take place is equally broad. At a macroevolutionary scale, a radiation can take tens of millions of years (Erwin, 2006). At a microevolutionary scale, the term radiation has, for instance, been applied to time frames of ten million years in the case of *Inga* Mill. (Richardson *et al.*, 2001), three million years for finches colonizing the Galápagos islands (Grant & Grant, 1996) or 100 000 years in cichlid fishes (Verheyen *et al.*, 2003).

The comparison of sister groups redefines evolutionary success (i.e. species richness) in terms of relative size rather than absolute size (Raikow, 1988; Slowinsky & Guyer, 1993), and rates of speciation and extinction are examined in the context of their relative and not absolute ages (Raikow, 1988). The use of sister groups therefore resolves the two above-mentioned problems. Sister-group comparison is a useful tool for this because sister clades are monophyletic, comparable in most basic aspects of their biology (although they can be different) and of the same age. This approach also eliminates rank-based and other taxonomic artefacts (Sanderson, 1998), eliminating any investigator bias (Brooks & McLennan, 2002). If differences between sister groups are found, there is evidence for differential rates of diversification in one group over another (Brooks & McLennan, 2002; Losos & Miles, 2002). Because the use of sister-group comparison is essential to radiation research, the definition of radiation will be extended and defined as 'the evolution of a relatively large, monophyletic group of species or higher taxa within a relatively short period of time, compared with closely related monophyletic (sister) groups'.

## DIVERSIFICATIONS IN ANNONACEAE

Few studies have focused on the superorder Magnoliidae (*sensu* Chase & Reveal, 2009; 10 000 species or 4% of all flowering plants) for the investigation of species-rich clades. Excluding the early diverging *Amborella* Baill., Nymphaeales and Austrobaileyales, Magnoliidae, together with Chloranthaceae, is sister to the rest of the angiosperms (APG III, 2009) from which it diverged some 120–130 Mya (Wikström, Savolainen & Chase, 2001; Bell, Soltis & Soltis, 2010). Thus, the analysis of patterns of diversification in Magnoliidae provides a contrast with studies that have focused on more derived clades of flowering plants, such as asterids (Bell & Donoghue, 2005; Moore & Donoghue, 2007), rosids (Wang *et al.*, 2009; Couvreur *et al.*, 2010) or monocots (Merckx *et al.*, 2008; Couvreur, Forest & Baker, 2011a).

Within Magnoliidae, the pantropically distributed Annonaceae is by far the most genus-rich family, with 110 genera and *c.* 2400 species (Couvreur *et al.*, 2011b; Chatrou *et al.*, 2012), and the third richest in terms of species [behind Piperaceae (five genera, *c.* 3100 species) and Lauraceae (50 genera, *c.* 2500 species)]. In Annonaceae, 10 genera are considered as species rich, i.e. with > 100 species (see table 2 in Chatrou *et al.*, 2012). Numerous phylogenetic studies during the past decade have led to an overall good understanding of relationships in this family (e.g. Mols *et al.*, 2004; Richardson *et al.*, 2004; Pirie *et al.*, 2006; Couvreur *et al.*, 2008b, 2011b; Erkens, Maas & Couvreur, 2009; Zhou *et al.*, 2010; Surveswaran *et al.*, 2011; Chatrou *et al.*, 2012). This provides a solid framework to study diversification patterns in order to identify factors that drive speciation and the evolution of morphological key characters in the family. Coupled to this, several family-wide molecular dating analyses have been undertaken, leading to a good understanding of the temporal dimensions of its evolution (Richardson *et al.*, 2004; Couvreur *et al.*, 2011b; Pirie & Doyle, 2012). Finally, the taxonomy of the family is well advanced as much revisionary work has been published in recent years (e.g. Saunders, 2002, 2003; Maas, Westra & Chatrou, 2003; Maas, Westra & Vermeer, 2007; Saunders & Munzinger, 2007; Erkens & Maas, 2008; Mols *et al.*, 2008; Couvreur, 2009; Schatz & Maas, 2010; Botermans *et al.*, 2011; Maas & Westra, 2011; Maas *et al.*, 2011), which provides a good knowledge of generic limitations and species, although several problems remain.

Couvreur *et al.* (2011b) recently undertook the first investigation of diversification rates in the family (and Magnoliales in general) using lineage-through-time plots and a maximum likelihood approach to test the null hypothesis of constant diversification rate versus a variable rate change in diversification.

However, their focus was to test hypotheses that might explain diversification and distribution patterns in tropical biota in general. This study focuses specifically on patterns in Annonaceae by comparing clades using topological and temporal methods of diversification analyses to pinpoint radiations of species. Major questions we wanted to address were: what genera within this family can be identified as having undergone a radiation?; can all species-rich genera in Annonaceae be considered as resulting from a radiation, or are smaller genera also possibly the result of this process, as hinted at by Couvreur *et al.* (2011b)? The results were then confronted with family-level morphological data to investigate whether any single factor or simple combination of factors might be associated with major diversification rate shifts in the family.

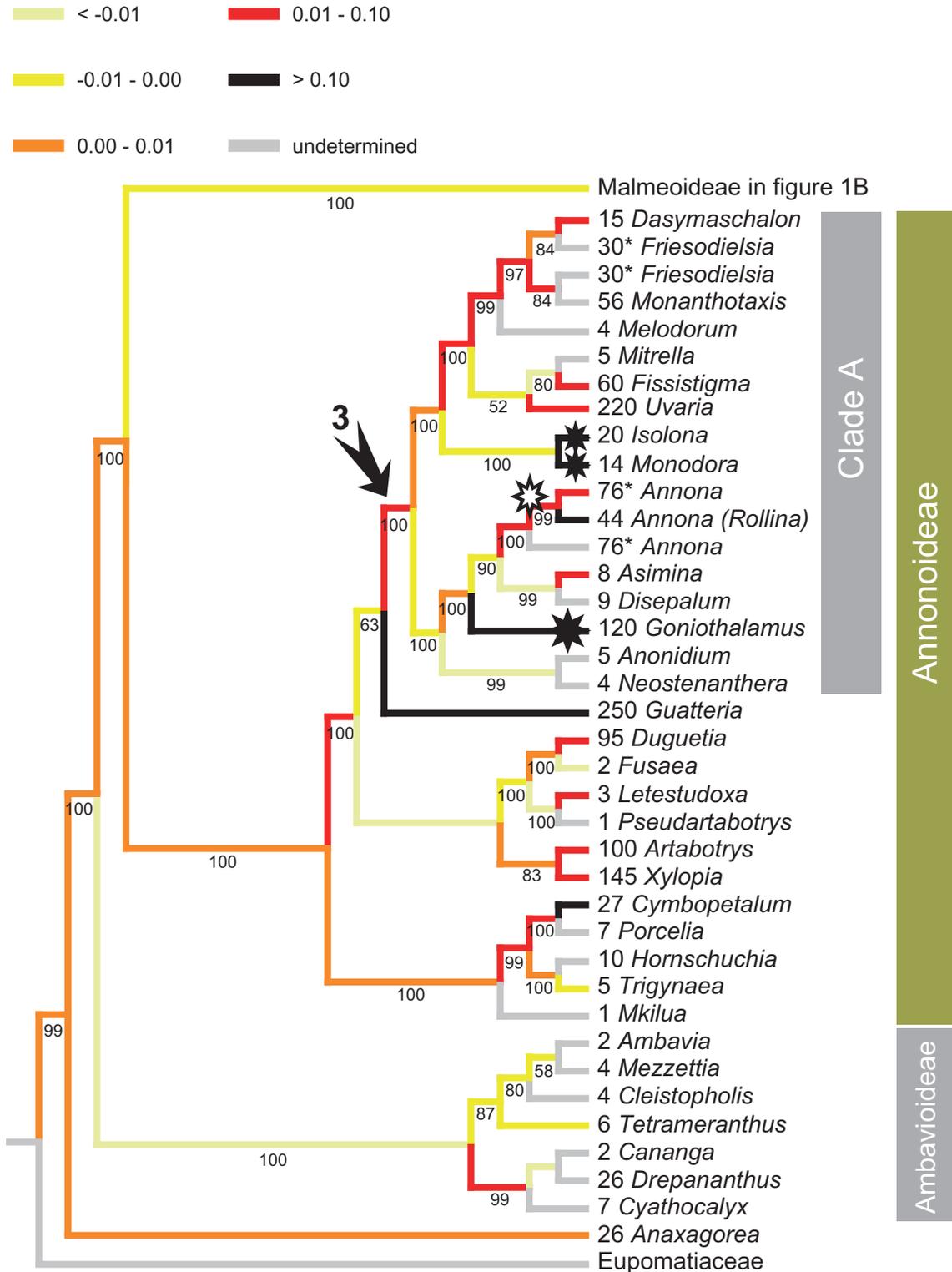
## MATERIAL AND METHODS

### PHYLOGENETIC TREE

One of the most parsimonious trees from an analysis by Chatrou *et al.*, 2012 (data not shown) was chosen as the basis for the analyses and taxa were pruned so that only one species per genus was represented (serving as a 'place holder' for the genus), except in the case of a (possible) paraphyletic constitution when two species per genus were retained (Fig. 1). This was performed to correct for bias in clade composition as a result of the different numbers of species sampled per genus (for a discussion on the different approaches to this topic, see Smith *et al.*, 2011). Because the resulting topology is derived from existing phylogenetic hypotheses, no in-depth discussion of recovered relationships is given here (for this discussion, see Couvreur *et al.*, 2011b; Chatrou *et al.*, 2012). Of 109 genera (Couvreur *et al.*, 2012; Erkens, Mennega & Westra, 2012) 75 (*c.* 70%) are represented in this study and all major clades were sampled (including the 10 largest genera). It should be noted, however, that, because of recent sampling efforts, the tree published by Chatrou *et al.* (2012) contains 99 genera and should be consulted for recent advances in the phylogenetic relationships in the family. Unfortunately, these additional taxa could not be incorporated into this study because of time constraints. The influence of this is discussed below.

### NODE AGES

The temporal method described below uses absolute node age estimates, and therefore dates for all nodes in the phylogenetic tree were taken from Pirie & Doyle (2012, see this paper for a discussion on dates). Genera for which no crown group age could be esti-



**Figure 1.** Concise cladogram for Annonaceae. Bootstrap percentages are indicated below the branches (only values > 50% are shown). Numbers of species per place holder taxon are indicated before the name. Asterisks (\*) indicate poly- or paraphyletic taxa for which the numbers of species are equally distributed over the constituent lineages because of the unavailability of a trustworthy estimate of species numbers per clade. Significant  $\Delta$  rate shifts are indicated with a numbered arrow (numbers correspond to the branches mentioned in Table 2), and significant  $\log N$  rate shifts are indicated by a filled star (terminal branches included) or open star (terminal branches excluded; details in Table 3).

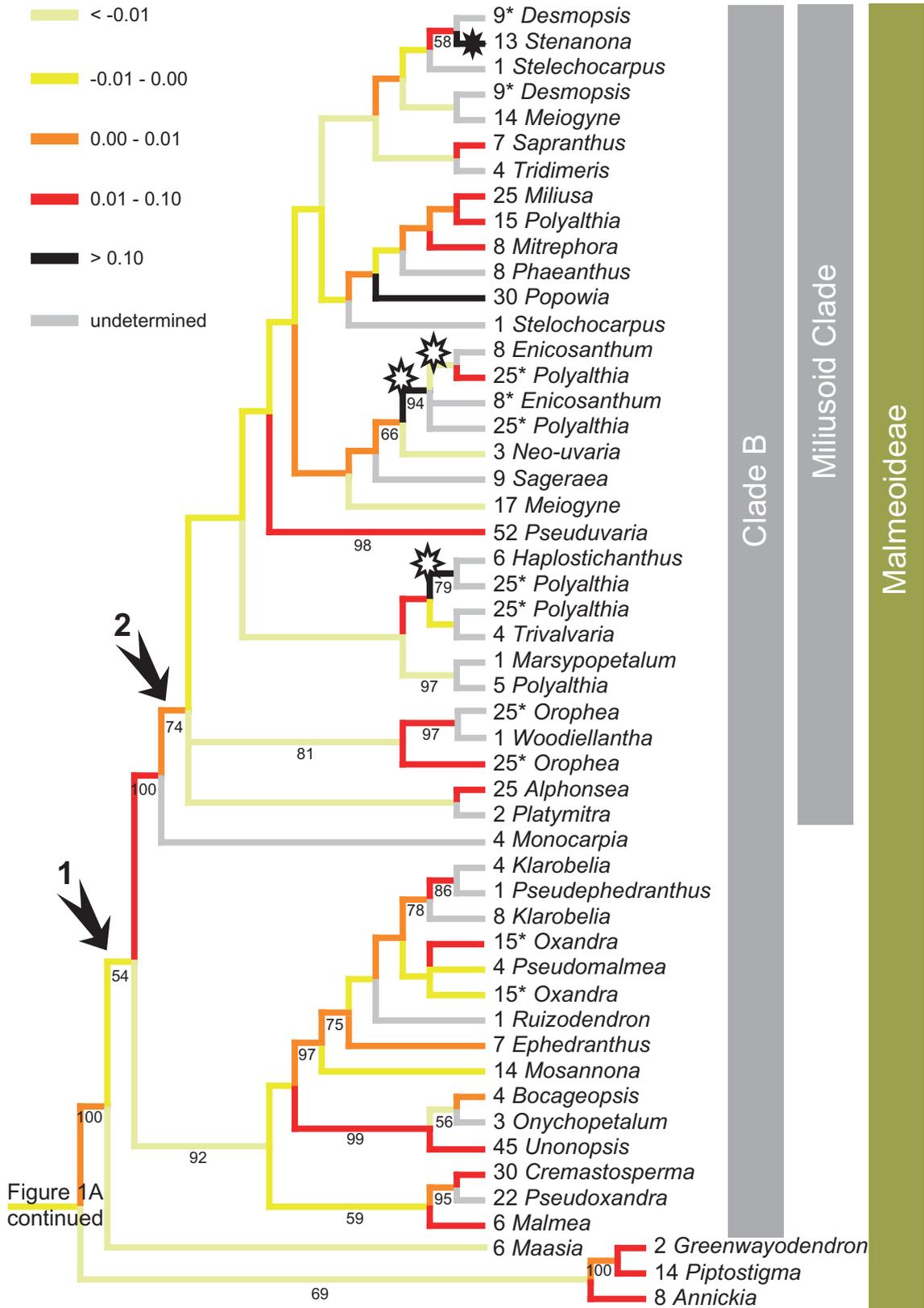


Figure 1. Continued

mated (because of the presence of only one accession in the dated tree) were not assigned an age.

#### SPECIES RICHNESS DATA

Species richness data were taken from Chatrou *et al.* (2012), who based their inventory on recent literature (Erkens *et al.*, 2012). Where genera proved to be polyphyletic and no justification could be given for a particular distribution of numbers of species per clade, the total species diversity was distributed equally over the different clades.

No complete species-level phylogenetic tree exists for Annonaceae and, with the topological method, no correction is possible for the numbers of species per place holder. For this reason, the method cannot be used to investigate species-level diversification patterns; only genus-level analyses can be conducted. Therefore, a second (temporal) method was applied that can account for the amounts of species represented by the place holder taxon.

#### TOPOLOGICAL METHOD

A measure for imbalance in species numbers per node in a tree was proposed by Slowinsky & Guyer (1993). This measure has been extended from individual nodal probabilities to whole-tree tests (Chan & Moore, 2002; Moore, Chan & Donoghue, 2004) because a single node does not represent the structure of the whole tree (Fusco & Cronk, 1995). These tests of diversification rate variation are based on cumulative equal-rates Markov (ERM) probabilities, outcomes are reported as  $M$  statistics ( $M_{\Sigma}$ ,  $M_{\Sigma}^*$ ,  $M_{\Pi}$  and  $M_{\Pi}^*$ ) and the  $P$  values obtained are called ERM nodal probabilities (for further explanation, see Methods S1 in Supporting Information). The  $M$  statistics are intended to provide differential sensitivity to asymmetry arising at different phylogenetic scales (i.e. the relative nodal depth in the tree), permitting their application to a corresponding range of associated evolutionary processes. The  $M$  statistics cannot indicate where in the tree the rate shifts have occurred. Therefore,  $\Delta_1$  and  $\Delta_2$  shift statistics were calculated. These statistics indicate the probability of a diversification rate shift along an internal branch of a local three-taxon tree comprising a local outgroup and the two earliest diverging ingroup clades. The computer program Symmetree v1.1 (Moore *et al.*, 2004) was used for all calculations. A more in-depth discussion of  $M$  and the  $\Delta_1$  and  $\Delta_2$  shift statistics is given in Chan & Moore (2002) and Moore *et al.* (2004).

For comparison, two often used tree shape indices are also reported (for an explanation of these indices, see Methods S1):  $I_C$  (Colless' index; Colless, 1982; Heard, 1992) and  $B_1$  (Shao & Sokal, 1990). The sen-

sitivity of all discussed whole-tree statistics to large-scale diversification rate variation increases from  $B_1$  via  $M_{\Sigma}$ ,  $M_{\Sigma}^*$ ,  $M_{\Pi}$ ,  $M_{\Pi}^*$  to  $I_C$ . Inconsistencies between the  $I_C$ ,  $B_1$  and  $M$  statistics do not indicate faults of the indices, but reveal the fact that they embody different definitions of imbalance (Shao & Sokal, 1990).

#### TEMPORAL METHOD

The formula  $\log(N)/t$  was applied to estimate the net diversification rates ( $R$ ) for each clade, where  $N$  is the number of species within a clade and  $t$  is the absolute time since that clade diverged from its sister (Isaac, Agapow & Harvey, 2003). Shifts in diversification rates per branch were assessed. This was performed by subtracting the  $R$  value of the stem node from that of the crown node of a branch (Isaac *et al.*, 2003; Davies *et al.*, 2004). This resulted in the so called maximum likelihood estimate of shift in diversification rate ( $\log N$ ) rate shifts (Davies *et al.*, 2004). This method shows rate changes per branch in the tree and makes it possible to identify the clades with the largest positive or negative changes in rate. Furthermore, when average shifts in  $R$  are plotted against the age of the nodes in the tree, it is possible to investigate whether significant shifts in  $R$  have occurred during any particular time window.

Whether diversification rates were conserved among close relatives was tested with the use of randomization tests. Species numbers of genera were randomized 100 times among the tips of the tree. For each randomization trial,  $R$  per node was recalculated as well as the sum of all the  $\log N$  rate shifts between the stem and crown node of a branch. If diversification rates were conserved among close relatives, the sum of the observed shifts in diversification rates across the tree should be lower than among random trials shuffling species numbers among genera (Davies *et al.*, 2004).

#### CHARACTERS CORRELATED WITH BIOTIC AND ABIOTIC RATE SHIFTS

Possible relationships between character evolution and detected shifts in diversification rates were explored by eye. Convincingly accepting or ruling out key innovations as an explanation would require more formal reconstructions of character evolution and the assessment of many more characters, which is beyond the scope of this article. The data used related to leaf architecture (Johnson, 2003), leaf anatomy (Van Setten & Koek-Noorman, 1986), flower and fruit morphology (Koek-Noorman, Setten & Zuilen, 1997), chromosome numbers (Morawetz & Waha, 1985), anther development (Tsou & Johnson, 2003), pollination characteristics (Gottsberger, 1999),

**Table 1.** Equal-rates Markov (ERM) nodal probability values (see Methods S1) corresponding to tests of ERM cladogenesis in major clades of Annonaceae

| Clade       | Tree size | Resolution | $B_1$   | $M_\Sigma$ | $M_\Sigma^*$ | $M_\Pi$ | $M_\Pi^*$ | $I_C$   |
|-------------|-----------|------------|---------|------------|--------------|---------|-----------|---------|
|             |           |            | High    | High       | High         | High    | High      | High    |
|             |           |            | Low     | Low        | Low          | Low     | Low       | Low     |
| Total       | 96        | 98         | 0.19082 | 0.00155    | 0.00006      | 0.00003 | 0.00000   | 0.00007 |
|             |           |            | 0.59265 | 0.12215    | 0.08027      | 0.04316 | 0.02823   | 0.00382 |
| Annonoideae | 31        | 100        | 0.96807 | 0.43994    | 0.30434      | 0.36617 | 0.26524   | 0.30244 |
| Malmeoideae | 55        | 94         | 0.05638 | 0.00134    | 0.00045      | 0.00019 | 0.00015   | 0.00099 |
|             |           |            | 0.19143 | 0.04268    | 0.03018      | 0.01326 | 0.00842   | 0.00495 |

Uncertainty associated with polytomies was assessed by investigating all possible alternative combinations of dichotomous resolutions, providing the upper and lower bounds of the confidence interval (the 'high' and 'low' values for high and low symmetry). These bounds correspond to the tail probabilities for the 0.025 and 0.0975 frequentiles, respectively. For Annonoideae, no confidence interval was calculated because this tree was completely resolved, and therefore singular values for this topology are indicated. The sensitivity of the whole-tree statistics to large-scale diversification rate variation increases to the right across a given row (i.e. the sensitivity of  $B_1 < M_\Sigma < M_\Sigma^* < M_\Pi < M_\Pi^* < I_C$ ). Resolution was calculated as  $k/(n - 1)$ , where  $k$  is the number of nodes in a tree of  $n$  tips; this value implicitly assumes that the underlying tree is strictly dichotomous (i.e. all polytomies are 'soft').

**Table 2.** Branches that show significant rate shifts ( $P < 0.05$ ) as indicated by two likelihood ratio-based shift statistics ( $\Delta_1$  and  $\Delta_2$ )

| Branch                             | $\Delta_1$ | $\Delta_2$ | Rate shift at branch leading to |
|------------------------------------|------------|------------|---------------------------------|
| Total tree and Malmeoideae subtree |            |            |                                 |
| 1                                  | 0.01735    | 0.02489    | Clade B                         |
| 2                                  | 0.04603    | 0.05901    | Miliusoid clade                 |
| Total tree and Annonoideae subtree |            |            |                                 |
| 3                                  | 0.02339    | 0.03509    | Clade A                         |

$\Delta_1$  and  $\Delta_2$  were calculated on the basis of the total tree and the subtrees for Malmeoideae and Annonoideae. Shifts are indicated in Figure 1 by a numbered arrow (numbers correspond to the branches in Table 3).

evolution of fragile exines (Waha, 1987), pollen characters (Doyle & Le Thomas, 1997), different trichome types, lianas and styles (Chatrou, Koek-Noorman & Maas, 2000), bilobed arils and different shapes of the floral receptacle (Johnson & Murray, 1995), oil composition (Maia *et al.*, 2005a, b) and orbicules (Huysmans *et al.*, 2010). A more elaborate discussion on morphological evolution in Annonaceae is given in Chatrou *et al.* (2012).

## RESULTS

### TOPOLOGICAL METHOD

$M$  statistics and  $I_C$  and  $B_1$  indices were calculated (Table 1). The smallest  $P$  values obtained for the tree in Figure 1 were returned by  $M_\Pi$ ,  $M_\Pi^*$  and  $I_C$ , sug-

gesting that significant diversification rate variation occurred at intermediate to larger phylogenetic scales (i.e. not near the tips or at the root of the tree). The  $\Delta_1$  and  $\Delta_2$  shift statistics (Table 2) revealed three significant diversification rate shifts at intermediate scale (indicated in Fig. 1 with an arrow). The first occurred in Annonoideae (former long branch clade of Richardson *et al.*, 2004; Chatrou *et al.*, 2012) at the origin of a strongly supported clade (arrow 3), here referred to as Clade A (containing the *Annona* L. group, *Isolona* Engl. and *Monodora* Dunal and a clade consisting of species with a climbing habit). The second rate shift occurred in Malmeoideae (former short branch clade of Richardson *et al.*, 2004; Chatrou *et al.*, 2012) at the base of a strongly supported clade that accommodates almost all genera of Malmeoideae

(arrow 1), except for the African *Annickia* Setten & Maas–*Piptostigma* Oliv.–*Greenwayodendron* Verdc. clade and the South-East Asian genus *Maasia* Mols, Kessler & Rogstad (Mols *et al.*, 2008). This subclade of Malmeoideae is hereafter referred to as Clade B. The third shift in diversification rate occurred at the base of the moderately supported miliusoid clade (arrow 2; Mols, 2004), although this shift was marginally nonsignificant for  $\Delta_2$ .

Three different analyses (whole tree, Malmeoideae only and Annonoideae only) proved to be insensitive to the specification of taxonomic scope (Table 2), because all  $\Delta_1$  and  $\Delta_2$  analyses yielded the same result (i.e. irrespective of whether the analysis was applied simultaneous to the whole tree or separately to Malmeoideae or Annonoideae). This indicates that shifts within more nested clades did not influence the estimates obtained from more inclusive clades.

#### TEMPORAL METHOD

Significant increases and decreases in diversification rates were identified. Only including internal branches, three showed a significantly increased diversification rate when compared with all other branches ( $P < 0.05$ ; Fig. 1, open star; Fig. 2A; Table 3) and one branch showed a significant decrease in rate ( $P < 0.05$ ; Fig. 1, open star; Fig. 2A; Table 3). However, this decrease occurred along a branch that was unsupported and towards a clade to which the number of species was estimated (because of taxonomic uncertainty, the number of species of *Polyalthia* Blume in this clade is unknown). When terminal branches were included, the previously mentioned rate shifts were nonsignificant, but shifts in diversification rate along several terminal branches were significant ( $P < 0.05$ ; Fig. 1, filled star; Fig. 2B; Table 3). The branches leading towards the crown groups of *Goniothalamus* Hook.f. & Thomson, *Stenanona* Standl., *Isolona* and *Monodora*, respectively, showed significant increases in diversification rate (Table 3), much larger than the significant increases when the terminal branches were excluded. The largest genus in the family, *Guatteria*, showed a marginally nonsignificant increase in diversification rate. No significant decrease in rate was observed in this second analysis.

The branch leading towards Malmeoideae as a whole showed a negative rate shift (–0.003410) and the branch subtending Annonoideae showed a positive rate shift (0.003738), but these shifts were not significantly different from the average rate shift (Fig. 2). The same was true for the branches subtending the *Anaxagorea* A.St.-Hil. clade (0.008704) and Ambavioideae (–0.012310;  $P < 0.05$ ; terminal

branches included: average, 0.022313; SD, 0.061589; terminal branches excluded: average, 0.004612; SD, 0.027794; see also Fig. 2).

The detected diversification rates were not significantly phylogenetically heritable between related lineages when the terminals were excluded, as shown by the randomization tests. The sum of rate shifts for the tree in Figure 1 (0.415113) was not significantly different from the randomized rate shifts (average, 0.364339; SD, 0.106862;  $P < 0.01$ ; Fig. 3A). This means that sister clades are *not* more likely to have similar species numbers than two clades chosen at random. Furthermore, when branches leading to the crown group of genera for which the age could be determined were included, the sum of rate shifts for the tree in Figure 1 (3.012272) was significantly higher than those from the randomized rate shifts (average, 1.825309; SD, 0.348024;  $P < 0.01$ ; Fig. 3B). Lower values are expected in comparison with randomized tests when rates are phylogenetically heritable between related lineages; thus, both randomization tests indicate that no phylogenetically heritable component is present in the rate shifts in Annonaceae.

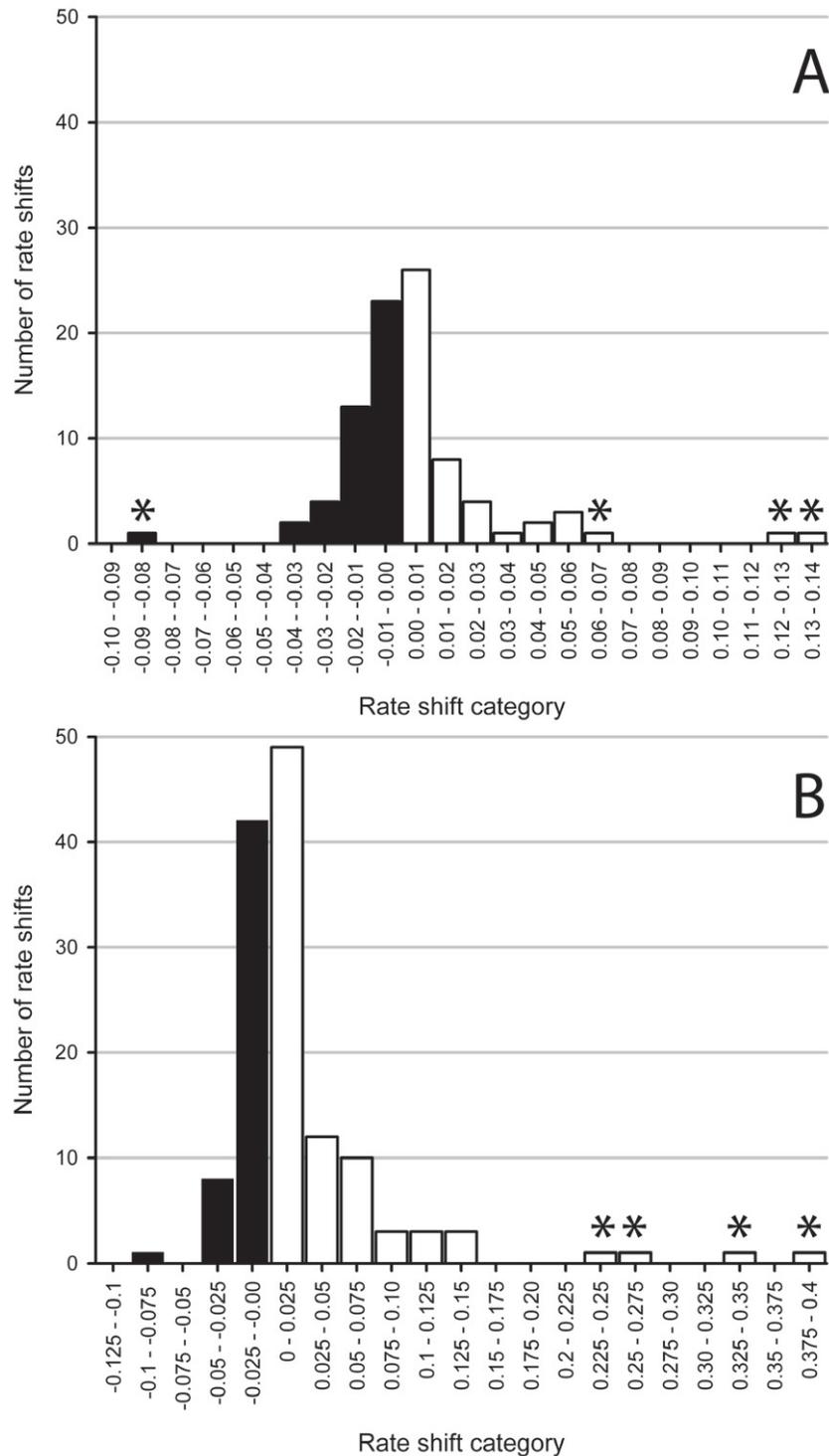
When a correlation coefficient ( $r$ ) was calculated, it was clear that the average diversification rate shifts within Annonaceae have been increasing from the past to the present ( $r = -0.79$  and  $r = -0.72$ ; Fig. 4A, B). The most recent time frame showed a significantly higher average shift in diversification rate (with and without terminal branches included) than any other time frame.

#### DISCUSSION

Imbalance and shifts in diversification rate can be used to study evolutionary processes. The radiation of a clade, for instance, results in an imbalanced tree, or the branch leading towards a radiating clade exhibits a higher diversification rate in comparison with a clade that has not radiated (Sanderson & Donoghue, 1994). After the identification of radiating clades, (a)biotic explanations can be sought for the observed patterns in (im)balance (Mooers & Heard, 1997).

#### RADIATIONS IN ANNONACEAE

Significant imbalance was revealed in net diversification rates among Annonaceae lineages by the topological method, and this imbalance was mainly located in Malmeoideae (Table 1) along the branches leading to Clade A, Clade B and the miliusoid clade (Fig. 1). No significant among-lineage diversification rate variation within Annonoideae was detected, except for the branch at the base of Clade A (Fig. 1). This single rate shift was insufficient to cause



**Figure 2.** Number of rate shifts per rate shift category plotted against the total number of observed shifts in diversification rate for the tree in Figure 1. Stars indicate significant rate shifts (details in Table 3). Filled bars indicate negative shifts, open bars positive shifts. A, Internal branches only; B, internal branches and terminals.

**Table 3.** Significant increases and decreases (95% confidence level) in diversification rates ( $\log N$ ) including and excluding terminal branches

|                                   | Rate shift<br>(+, positive; -,<br>negative)               | Diversification rate<br>shift at branch leading<br>towards  | Age (My)                              | Number of<br>species in<br>clade | Node support<br>(bootstrap)    | Geographical<br>distribution  | Putative key<br>innovation/<br>diversification<br>influencing factor |
|-----------------------------------|---|---|---------------------------------------|----------------------------------|--------------------------------|---|--|
| Terminal<br>branches<br>included* | +0.39266<br>+0.32587<br>+0.25576<br>+0.24952<br>+0.14148† | <i>Goniothalamus</i><br><i>Stenanona</i><br><i>Isolona</i><br><i>Monodora</i><br><i>Guatteria</i>   | 4.49<br>2.86<br>4.04<br>3.57<br>11.87 | 120<br>13<br>21<br>14<br>250     | 100<br>89<br>100<br>100<br>100 | South-East Asia<br>Central America<br>Africa and Madagascar<br>Africa<br>Central and South<br>America           | ?<br>?<br>x<br>x<br>Unspecialized                                    |
| Terminal<br>branches<br>excluded* | +0.13601<br>+0.12279<br>+0.06429<br>-0.08245              | <i>Enicosanthum-Polyalthia</i><br><i>s.l.</i> clade (7‡)<br><i>Haplostichanthus-Polyalthia</i><br>clade (8‡)<br><i>Annona</i> clade (14‡)<br><i>Enicosanthum-Polyalthia</i><br><i>s.s.</i> clade (1‡) | 20.75<br>7.79<br>17.33<br>10.68       | 66<br>31<br>194<br>33            | 94<br>79<br>100<br>-           | South-East Asia<br>South-East Asia<br>South-East Asia<br>Central and South<br>America, Africa<br>Sout-East Asia | ?<br>?<br>Syncarpy<br>-  |

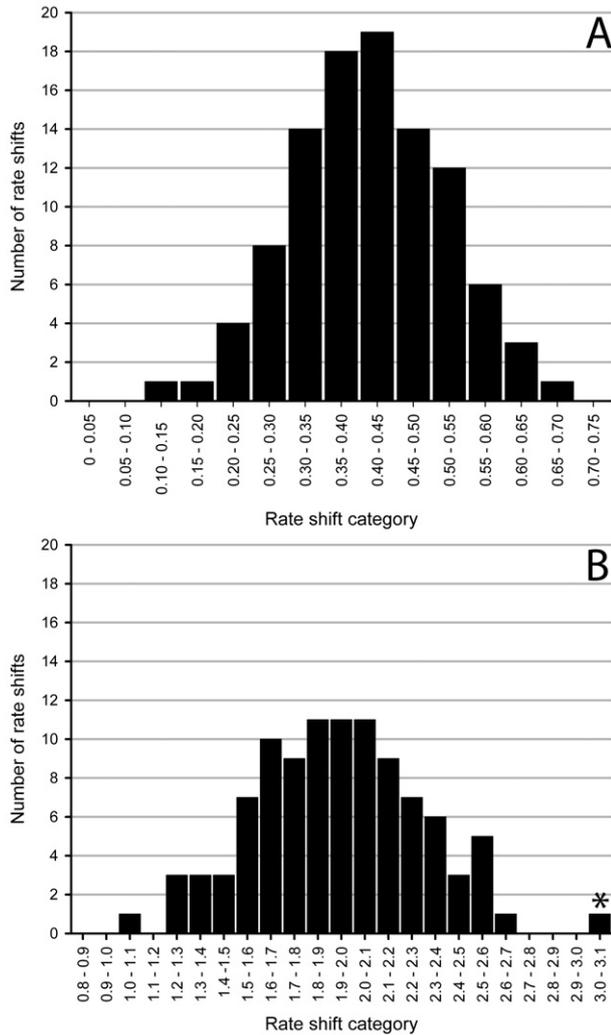
\*Terminal branches included: average, 0.022313; SD, 0.061589; terminal branches excluded: average, 0.004612; SD, 0.027794.

†*Guatteria* has the fifth largest rate shift when terminal branches are included, but this shift is marginally nonsignificant. The genus is included in this table because it is the largest genus of Annonaceae.

‡These rate shifts are not significant when the terminals are included.

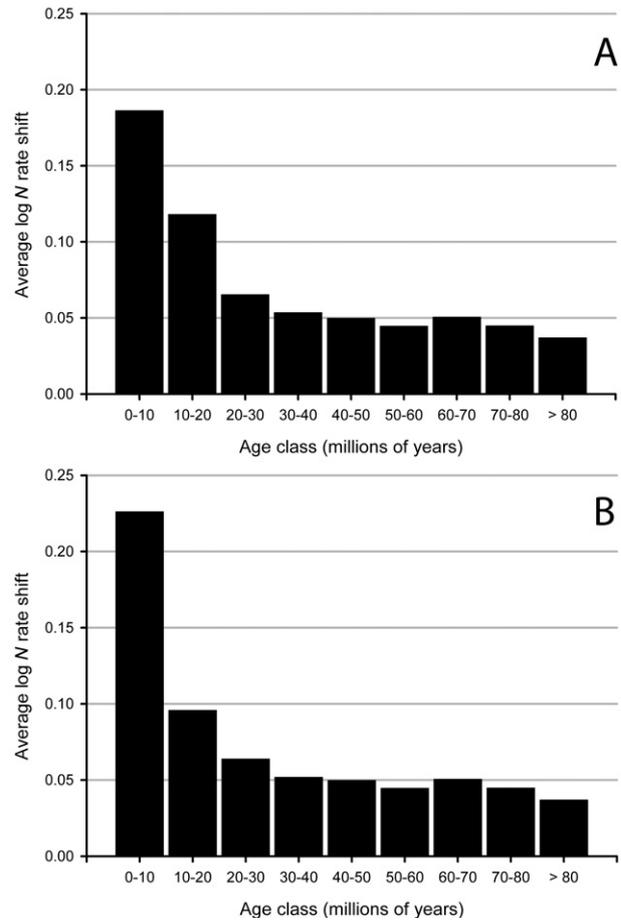
The respective nodes are indicated in Figure 1 by a star. Nodal support values were obtained from Chatrou *et al.* (2012). Values in parentheses after clade names when terminal branches are excluded indicate the position of the rate shift in the rate shift ranking when terminals are included.

For *Isolona* and *Monodora syncarpy* might be inferred as a putative key-innovation. However, the reconstructed changes in diversification rates are an artefact of undersampling as explained in the text. Therefore, no characters are mentioned in the table as putative key-innovations.



**Figure 3.** Results of randomization tests conducted to test whether log  $N$  rate shifts were phylogenetically conserved (specifications as explained in the text). The number of summed shifts in diversification rate per rate shift class is shown. The star indicates the class containing the sum of rate shifts for the tree in Figure 1. A, Internal branches only; B, internal branches and terminals.

rejection of the null hypothesis that Annonoideae as a whole diversified under a stochastic branching model. In general, this is true for most of the speciation in Annonaceae. This supports a recent study showing that the diversification of Annonaceae was not severely affected by the events of the Cretaceous–Palaeogene boundary, and that Malmeoideae and Annonoideae have a constant rate of diversification (Couvreur *et al.*, 2011b). However, that study indicated no major shifts of diversification throughout the evolutionary history of the family, whereas here three such shifts were detected (Fig. 1).



**Figure 4.** Average log  $N$  diversification rate shifts within ten million year time windows. A, Internal branches only; B, internal branches and terminals. Average changes in diversification rates increase with decreasing age ( $r = -0.79$  and  $r = -0.72$  for A and B, respectively;  $r =$  correlation coefficient). Only within the most recent time frame is the average rate shift significantly higher than the average shift over all time windows ( $P < 0.05$ ).

Annonoideae and Malmeoideae together comprise the majority of the genera and species in Annonaceae. Annonoideae and Malmeoideae both consist of approximately 50 genera, but the number of species is different (*c.* 700 vs. *c.* 1600 species for Malmeoideae and Annonoideae, respectively; Couvreur *et al.*, 2011b). In addition, Annonoideae includes the 10 largest genera of the family, comprising *c.* 1000 of its 1600 species. The cause for this Annonoideae–Malmeoideae difference is not understood. The topological method did not indicate any significant imbalance between Annonoideae and Malmeoideae. Furthermore, the temporal method indicated that the difference in species richness was not attributable to an increase in diversification rate along the stem lineages of these clades. However, a recently reported

younger age for the crown node of Malmeoideae (based on Bayesian dating) might alter this picture (Erkens *et al.*, 2009; Su & Saunders, 2009), although this should be studied further (Couvreur *et al.*, 2011b; Pirie & Doyle, 2012). The temporal method uncovered a small increase in diversification rate at the base of Annonoideae and a small decrease at the base of Malmeoideae, but neither change was significant. The distribution of significant log  $N$  rate shifts between these clades differed. Four of the five largest increases in diversification rate occurred in Annonoideae and only one in Malmeoideae (and the latter in a poorly supported part of the tree, rendering this result questionable). However, these four radiations did not produce the bulk of the species present in Annonoideae, a result similar to that of Couvreur *et al.* (2011b). Of the 10 largest genera in the family (*Annona*, *Artabotrys* R.Br., *Duguetia* A.St.-Hil., *Fisistigma* Griff., *Friesodielsia* Steenis, *Guatteria*, *Goniothalamus*, *Monanthataxis* Baill., *Uvaria* L. and *Xylophia* L.), only *Goniothalamus* constituted a radiation of species under the methods used for this study. Such a result was also supported by the study of Couvreur *et al.* (2011b). Furthermore, *Guatteria*, the largest genus in the family (Erkens *et al.*, 2007a; Erkens, Westra & Maas, 2008) did not (although only marginally nonsignificant) constitute a radiation. In relation to its sister group, *Guatteria* is smaller (200–250 *Guatteria* species vs. *c.* 700 species for Clade A) and the clade might actually be seen as species poor. This lack of species might be the result of the extinction of early lineages of *Guatteria* in the Miocene (Erkens *et al.*, 2007b, 2009), leading to a long branch in the phylogenetic tree. Taking this extinction into account, these clades might actually have been more balanced.

In Annonaceae, clade size is not a good predictor for the onset of a radiation, and this study illustrates again that the size of a group of organisms is not *a priori* evidence that the group arose from nonrandom speciation and/or extinction (Slowinsky & Guyer, 1993).

#### CHARACTERS ASSOCIATED WITH RADIATIONS

Invoking key innovations is controversial. A key innovation can be defined as an aspect of the organismal phenotype that promotes diversification (many other definitions exist, however; Hunter, 1998). The rationale is that a shift in diversification rate can be coupled to the evolution of a presumed key character along the same branch (Sanderson & Donoghue, 1994). However, these correlations should be made with great care, as traditionally this process simply entails the identification of whichever feature of a group seems to be most distinctive (Slowinsky &

Guyer, 1993). Furthermore, a lack of replication prevents statistical testing of the putative key innovation (Schluter, 2000). Even if replication is achieved, it is possible that a character that is causally involved in increasing diversification rates in one clade might not have the same effect in another clade (Brooks & McLennan, 2002). This is because key innovations by themselves are not a sufficient reason for biological expansion, as evolution always occurs in a context (Hunter, 1998). In this article, we do not provide a comprehensive study of all factors possibly functioning as key innovations, but, rather, a simple survey to pinpoint single (or a combination of simple) factors that might be associated with the detected significant rate shifts in the family. It should be further tested whether these characters are indeed responsible for the detected radiation.

The topological method indicates imbalance at the base of three clades. Clade A has one clear leaf architectural synapomorphy (Johnson, 2003). The whole clade has distichous trunk phyllotaxis (only otherwise found in *Anaxagorea* and *Cleistopholis* Pierre ex Engl., the latter belonging to the early diverging subfamily Ambavioideae), whereas the other genera have a spiral arrangement. For Clade B, as a whole, no clear synapomorphies exist, although there is a strong geographical structure, in contrast with Annonoideae. Next to a strongly supported Neotropical clade, it contains the South-East Asian miliusoid clade, at the base of which the third imbalance occurs. The miliusoid clade is found to be separated from the rest of Malmeoideae by several pollen characters. The miliusoid taxa have globose, cerebroid or echinate, disulcate pollen, whereas other Malmeoideae have monosulcate, perforate to reticulate, boat-shaped pollen (Mols *et al.*, 2004). The clade is not completely South-East Asian as it contains a small clade of Central American genera (Richardson *et al.*, 2004). Because of the geographical structure in Malmeoideae, Clade B and the miliusoid clade might be species rich as the result of a radiation after a founder event.

The temporal method indicates four significant rate shifts among genera. The largest is along the stem branch of the South-East Asian genus *Goniothalamus* (Table 3; Saunders, 2002, 2003; Saunders & Munzinger, 2007; Nakkuntod *et al.*, 2009). Although we detected a significant rate shift, the overall diversification rate calculated for the genus based on the stem age was not amongst the highest found in the family (Couvreur *et al.*, 2011b). However, this result was based on estimations from the stem node (in contrast with the crown node ages used here), which could induce some bias concerning the origin of all extant taxa in the genus. Although none of the flower or fruit characteristics is remarkable in terms of evolutionary

innovations in Annonaceae, it is known that fruit and seed structure is extremely diverse in *Goniothalamus*, presumably reflecting differing frugivores and seed dispersal mechanisms (Nakkuntod *et al.*, 2009). Thus, it might be hypothesized that divergent selection on fruit and seed dispersal mechanisms might be a driving factor for speciation in this genus.

With respect to geography, it should be noted that, although the topography of South-East Asia (and, especially, its island archipelagos) is conducive to allopatric speciation, there is no obvious reason why *Goniothalamus* should have diversified so much more rapidly than other genera in the same region. Species of Annonaceae, including *Goniothalamus* spp., often have narrow distribution patterns. There is somewhat equivocal evidence to suggest that a smaller geographical range size in birds is associated with higher rates of diversification (Isaac *et al.*, 2003). This conclusion is opposite the general view that the probability of allopatric speciation increases with range size (Rosenzweig, 1978). The factor range size should be further explored to see whether this ecological variable has any correlation with the patterns found in Annonaceae.

The second largest shift in diversification rate occurs along the stem towards the Central American genus *Stenanona* (Table 3). This genus is part of a small clade of seven Central American genera that is embedded in the large South-East Asian miliusoid clade (Mols *et al.*, 2004; Couvreur *et al.*, 2011b; Chatrou *et al.*, 2012). *Stenanona* is found from Mexico (Veracruz) to Colombia (Nariño). In the field, it is easily recognizable by the dramatically long drawn-out, aristate petal apices, a synapomorphy for the genus (Schatz & Maas, 2010). This petal morphology, in combination with the pink to blood red colour of the flower, is suggestive of a fly pollination syndrome (Schatz, 1987). If so, this would be one of the few cases of nonbeetle pollination in the family (Gottsberger, 1999; Saunders, 2012), and perhaps a cause for the radiation of the genus. *Stenanona* was also identified as having one of the highest diversification rates in Annonaceae in a previous study (Couvreur *et al.*, 2011b).

The shift in diversification rate along the stem branch of *Stenanona* indicates a radiation of species and, possibly, one of the adaptations as described above. However, the unsupported topology of this part of the tree in Figure 1 warrants caution. In addition, for many terminal taxa in Malmeoideae, the age of their most recent common ancestor is unknown (e.g. the sister group of *Stenanona*, *Desmopsis* Saff. p.p and *Stelechocarpus* Hook.f. & Thomson). An accurate estimate of the rate shifts along these terminal branches cannot therefore be given (Fig. 1). Further conclusions on the putative radiation of *Stenanona*

should therefore be postponed until age estimates for these taxa have been obtained and support values are sufficiently high.

In conclusion, the few shifts in diversification rate that occurred in the evolutionary history of Annonaceae are not easily linked to presumed key innovations. Annonaceae is not the only group for which it has proven to be difficult to correlate biotic (and abiotic) factors to shifts in diversification rate. Because of this difficulty, there has been a recent renewal of interest in the hypothesis that cladogenesis may be random, or nearly random, with respect to the intrinsic biology of the organisms (Ricklefs, 2003; Davies *et al.*, 2004).

#### SAMPLING AND DIVERSIFICATION ANALYSES

The temporal method indicates that nodes in more recent time periods tend to display greater rate shifts than older nodes (Figs 1, 4B; Table 3). Several explanations can be given for this result. For instance, this could be a consequence of frequent shifts in diversification rate, whereby differences between recent clades can be detected, but these tend to average out at deeper levels. Another explanation could be that two sister clades with balanced species numbers are joined by a relatively long stem branch. This would lead to a reconstructed high rate in both sister clades relative to the rate expected for their nesting clade, a situation not recognizable from topology alone (Davies *et al.*, 2004). Incomplete taxon sampling (or extinction) could thus be a confounding factor with respect to the balance of the tree in Figure 1, and has been shown to bias the outcome of other analyses. This is because oversampled clades will tend to have shorter branches than undersampled clades (Savolainen *et al.*, 2002). Recent data collection has shown that the long branches subtending *Goniothalamus* (Nakkuntod *et al.*, 2009; Couvreur *et al.*, 2011b) and *Guatteria* (Erkens *et al.*, 2009) are not the result of undersampling. However, for *Isolona* and *Monodora*, the effect of undersampling is clear. The branch leading to this clade is relatively long and both genera are reconstructed here as having a significant rate shift (Table 3). Further sampling of the African Long Branch Clade has broken up this branch (Couvreur *et al.*, 2008a, b, 2011b), but reveals no major topological changes. The newly added genera form a monophyletic group with *Isolona* and *Monodora*, thus not influencing internal branch lengths at deeper levels. Indeed, adding these unsampled genera to the analysis shows that there is no significant rate shift in this clade (data not shown). In addition, some estimated crown node ages are older (Couvreur *et al.*, 2008a) than others (Pirie & Doyle, 2012). These older estimates allow more time for speciation and the gradual accumulation

of species. This example again clearly demonstrates that detected rate shifts should not be taken at face value, and that undersampling (or, more worryingly, extinction) can always influence the analysis.

## CONCLUSIONS

All reconstructed shifts in diversification rate occur in strongly supported parts of the phylogenetic tree for Annonaceae, rejecting phylogenetic inaccuracy as an explanation for their origin. It is clear that (except for *Goniothalamus*) the largest genera in the family are not the result of radiations. In addition, the difference in species numbers between subfamilies Annonoideae and Malmeoideae cannot be attributed to significant differences in diversification rates along the stem branches of these clades. In general, most of the speciation in Annonaceae was not discernible from a stochastic branching model (i.e. chance). Annonaceae represents another example underlining the difficulties in understanding the variation in clade richness in terms other than stochasticity. This means that no special explanations are necessary for the distribution of species richness across a major part of the phylogenetic tree for Annonaceae. It has already been suggested that moderately low rates of dispersal (sufficiently low to interrupt gene flow, but sufficiently high to allow the occasional colonization of new habitats) can result in the highest rates of speciation (Givnish & Systma, 1997) and lead to large clade sizes. Because of the geographical structure in Malmeoideae, some clades might be species rich as the result of a radiation after a founder event. Indeed, if this is the cause for the species richness pattern observed here, it should be investigated further.

Furthermore, large clade sizes in Annonaceae have not resulted from key innovations having influenced the rate of diversification. Only for some clades (e.g. Clade A, *Stenanona* or *Goniothalamus*) might key innovations be invoked to explain the elevated rate of diversification. However, convincingly accepting or ruling out key innovations as an explanation would require more formal tests of morphological characters and phylogenetic correlation (Moore & Donoghue, 2007, 2009), the assessment of many more morphological characters and, of course, a better species-level sampling of Annonaceae. Finally, it would also be interesting to apply new maximum likelihood methods to the study of diversification shifts in order to see how these methods compare with those used here (Rabosky *et al.*, 2007; Alfaro *et al.*, 2009).

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## REFERENCES

- Alfaro ME, Santini F, Brock C, Alamillo H, Dornburg A, Rabosky DL, Carnevale G, Harmon LJ. 2009.** Nine exceptional radiations plus high turnover explain species diversity in jawed vertebrates. *Proceedings of the National Academy of Sciences of the United States of America* **106**: 13 410–13 414.
- APG III. 2009.** An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG III. *Botanical Journal of the Linnean Society* **161**: 105–121.
- Beheregaray LB, Gibbs JP, Havill N, Fritts TH, Powell JR, Caccione A. 2004.** Giant tortoises are not so slow: rapid diversification and biogeographic consensus in the Galapagos. *Proceedings of the National Academy of Sciences of the United States of America* **101**: 6514–6519.
- Bell CD, Donoghue MJ. 2005.** Dating the Dipsacales: comparing models, genes, and evolutionary implications. *American Journal of Botany* **92**: 284–296.
- Bell CD, Soltis DE, Soltis PS. 2010.** The age and diversification of the angiosperms re-visited. *American Journal of Botany* **97**: 1296–1303.
- Botermans M, Sosef MSM, Chatrou LW, Couvreur TLP. 2011.** Revision of the African genus *Hexalobus* (Annonaceae). *Systematic Botany* **36**: 33–48.
- Brooks DR, McLennan DA. 2002.** *The nature of diversity: an evolutionary voyage of discovery*. Chicago and London: University of Chicago Press.
- Chan KMA, Moore BR. 2002.** Whole-tree methods for detecting differential diversification rates. *Systematic Biology* **51**: 855–865.
- Chase MW, Reveal JL. 2009.** A phylogenetic classification of the land plants to accompany APG III. *Botanical Journal of the Linnean Society* **161**: 122–127.
- Chatrou LW, Koek-Noorman J, Maas PJM. 2000.** Studies in Annonaceae XXXVI: the *Duguetia* alliance: where the ways part. *Annals of the Missouri Botanical Garden* **87**: 234–245.
- Chatrou LW, Pirie MD, Erkens RHJ, Couvreur TLP, Neubig KM, Abbott JR, Mols JB, Maas JW, Saunders RMK, Chase MW. 2012.** A new higher-level classification of the pantropical flowering plant family Annonaceae informed by molecular phylogenetics. *Botanical Journal of the Linnean Society* **169**: 5–40.
- Cieslak T, Polepalli JS, White A, Müller K, Borsch T, Barthlott W, Steiger J, Marchant A, Legendre L. 2005.** Phylogenetic analysis of *Pinguicula* (Lentibulariaceae): chloroplast DNA sequences and morphology support several geographically distinct radiations. *American Journal of Botany* **92**: 1723–1736.
- Classen-Bockhoff R, Speck T, Tweraser E, Wester P, Thimm S, Reith M. 2004.** The staminal lever mecha-

- nism in *Salvia* L. (Lamiaceae): a key innovation for adaptive radiation? *Organisms, Diversity & Evolution* **4**: 189–205.
- Colless DH. 1982.** Review of phylogenetics: the theory and practice of phylogenetic systematics, by E.O. Wiley. *Systematic Zoology* **31**: 100–104.
- Couvreur TLP. 2009.** Monograph of the syncarpous African genera *Isolona* and *Monodora* (Annonaceae). *Systematic Botany Monographs* **87**: 1–150.
- Couvreur TLP, Chatrou LW, Sosef MSM, Richardson JE. 2008a.** Molecular phylogenetics reveal multiple tertiary vicariance origins of the African rain forest trees. *BMC Biology* **6**: 54.
- Couvreur TLP, Forest F, Baker WJ. 2011a.** Origin and global diversification patterns of tropical rain forests: inferences from a complete genus-level phylogeny of palms. *BMC Biology* **9**: 44.
- Couvreur TLP, Franzke A, Al-Shehbaz IA, Bakker FT, Koch MA, Mummenhoff K. 2010.** Molecular phylogenetics, temporal diversification, and principles of evolution in the mustard family (Brassicaceae). *Molecular Biology and Evolution* **27**: 55–71.
- Couvreur TLP, Maas PJM, Meinke S, Johnson DM, Keßler PJA. 2012.** Keys to the genera of Annonaceae. *Botanical Journal of the Linnean Society* **169**: 74–83.
- Couvreur TLP, Pirie MD, Chatrou LW, Saunders RMK, Su Y, Richardson JE, Erkens RHJ. 2011b.** Early evolutionary history of the flowering plant family Annonaceae: steady diversification and boreotropical geodispersal. *Journal of Biogeography* **38**: 664–680.
- Couvreur TLP, Richardson JE, Sosef MSM, Erkens RHJ, Chatrou LW. 2008b.** Evolution of syncarpy and other morphological characters in African Annonaceae: a posterior mapping approach. *Molecular Phylogenetics and Evolution* **47**: 302–318.
- Cronn RC, Small RL, Haselkorn T, Wendel JF. 2002.** Rapid diversification of the cotton genus (*Gossypium*: Malvaceae) revealed by analysis of sixteen nuclear and chloroplast genes. *American Journal of Botany* **89**: 707–725.
- Davies TJ, Barraclough TG, Chase MW, Soltis PS, Soltis DE, Savolainen V. 2004.** Darwin's abominable mystery: insights from a supertree of the angiosperms. *Proceedings of the National Academy of Sciences of the United States of America* **101**: 1904–1909.
- Davies TJ, Savolainen V, Chase MW, Goldblatt P, Barraclough TG. 2005.** Environment, area, and diversification in the species-rich flowering plant family Iridaceae. *American Naturalist* **166**: 418–425.
- Davis CC, Webb CO, Wurdack KJ, Jaramillo CA, Donoghue MJ. 2005.** Explosive radiation of Malpighiales supports a mid-Cretaceous origin of modern tropical rain forests. *American Naturalist* **165**: E36–E65.
- Doyle JA, Le Thomas A. 1997.** Significance of palynology for phylogeny of Annonaceae: experiments with removal of pollen characters. *Plant Systematics and Evolution* **206**: 133–159.
- Duputié A, Salick J, McKey D. 2011.** Evolutionary biogeography of *Manihot* (Euphorbiaceae), a rapidly radiating Neotropical genus restricted to dry environments. *Journal of Biogeography* **38**: 1033–1043.
- Erkens RHJ, Chatrou LW, Koek-Noorman J, Maas JW, Maas PJM. 2007a.** Classification of a large and widespread genus of Neotropical trees, *Guatteria* (Annonaceae) and its three satellite genera *Guatterrella*, *Guatterlopsis* and *Heteropetalum*. *Taxon* **56**: 757–774.
- Erkens RHJ, Chatrou LW, Maas JW, van der Niet T, Savolainen V. 2007b.** A rapid diversification of rainforest trees (*Guatteria*; Annonaceae) following dispersal from Central into South America. *Molecular Phylogenetics and Evolution* **44**: 399–411.
- Erkens RHJ, Maas PJM. 2008.** The *Guatteria* group disentangled: sinking *Guatterlopsis*, *Guatterrella*, and *Heteropetalum* into *Guatteria*. *Rodriguesia* **59**: 401–406.
- Erkens RHJ, Maas JW, Couvreur TLP. 2009.** From Africa via Europe to South America: migrational route of a species rich genus of Neotropical lowland rain forest trees (*Guatteria*; Annonaceae). *Journal of Biogeography* **36**: 2338–2352.
- Erkens RHJ, Mennega EA, Westra LYT. 2012.** A concise bibliographic overview of Annonaceae. *Botanical Journal of the Linnean Society* **169**: 41–73.
- Erkens RHJ, Westra LYT, Maas PJM. 2008.** Increasing diversity in the species-rich genus *Guatteria* (Annonaceae). *Blumea* **53**: 467–514.
- Erwin DH. 2006.** Dates and rates: temporal resolution in the deep time stratigraphic record. *Annual Review of Earth and Planetary Sciences* **34**: 569–590.
- Fusco G, Cronk QCB. 1995.** A new method for evaluating the shape of large phylogenies. *Journal of Theoretical Biology* **175**: 235–243.
- Gittenberger E. 1991.** What about non-adaptive radiation? *Biological Journal of the Linnean Society* **43**: 263–272.
- Gittenberger E. 2004.** Radiation and adaptation, evolutionary biology and semantics. *Organisms, Diversity & Evolution* **4**: 135–136.
- Givnish TJ, Systma KJ. 1997.** *Molecular evolution and adaptive radiation*. Cambridge: Cambridge University Press.
- Glor RE. 2010.** Phylogenetic insights on adaptive radiation. *Annual Review of Ecology, Evolution, and Systematics* **41**: 251–270.
- Gottsberger G. 1999.** Pollination and evolution in Neotropical Annonaceae. *Plant Species Biology* **14**: 143–152.
- Grant PR, Grant BR. 1996.** Speciation and hybridization in island birds. *Philosophical Transactions of the Royal Society B: Biological Sciences* **351**: 765–772.
- Heard SB. 1992.** Patterns in tree balance among cladistic, phenetic, and randomly generated phylogenetic trees. *Evolution* **46**: 1818–1826.
- Hunter JP. 1998.** Key-innovations and the ecology of macroevolution. *Trends in Ecology and Evolution* **13**: 31–36.
- Huysmans S, Verstraete B, Smets E, Chatrou LW. 2010.** Distribution of orbicules in Annonaceae mirrors evolutionary trend in angiosperms. *Plant Ecology and Evolution* **143**: 199–211.
- Isaac NJB, Agapow P, Harvey PH. 2003.** Phylogenetically

- nested comparisons for testing correlates of species richness: a simulation study of continuous variables. *Evolution* **57**: 18–26.
- Janssens SB, Knox EB, Huysmans S, Smets EF, Merckx VSFT. 2009.** Rapid radiation of *Impatiens* (Balsaminaceae) during Pliocene and Pleistocene: result of a global climate change. *Molecular Phylogenetics and Evolution* **52**: 806–824.
- Johnson DM. 2003.** Phylogenetic significance of spiral and distichous architecture in the Annonaceae. *Systematic Botany* **28**: 503–511.
- Johnson DM, Murray NA. 1995.** Synopsis of the tribe Bocageae (Annonaceae), with revisions of *Cardiopetalum*, *Froesiodendron*, *Trigynaea*, *Bocagea*, and *Hornschuchia*. *Brittonia* **47**: 248–319.
- Koek-Noorman J, Setten AKV, Zuilen CMV. 1997.** Studies in Annonaceae XXVI. Flower and fruit morphology in Annonaceae. Their contribution to patterns in cluster analysis. *Botanische Jahrbücher für Systematik, Pflanzengeschichte und Pflanzengeographie* **119**: 213–230.
- Levin RA, Wagner WL, Hoch PC, Nepokroeff M, Pires JC, Zimmer EA, Sytsma KJ. 2003.** Family-level relationships of Onagraceae based on chloroplast *rbcL* and *ndhF* data. *American Journal of Botany* **90**: 107–115.
- Linder HP. 2008.** Plant species radiations: where, when, why? *Philosophical Transactions of the Royal Society B: Biological Sciences* **363**: 3097–3105.
- Losos JB. 2010.** Adaptive radiation, ecological opportunity, and evolutionary determinism: American Society of Naturalists E. O. Wilson Award Address. *American Naturalist* **175**: 623–639.
- Losos JB, Miles DB. 2002.** Testing the hypothesis that a clade has adaptively radiated: iguanid lizard clades as a case study. *American Naturalist* **160**: 147–157.
- Maas PJM, Westra LYTH. 2011.** A taxonomic survey of *Guatteria* section *Mecocarpus* including the genera *Guatterriopsis* and *Guatteriella* p.p. (Annonaceae). *Blumea* **56**: 113–145.
- Maas PJM, Westra LYT, Chatrou LW. 2003.** *Flora neotropica monograph 88: Duguetia*. New York: New York Botanical Garden.
- Maas PJM, Westra LYT, Rainer H, Lobão AQ, Erkens RHJ. 2011.** An updated index to genera, species, and infraspecific taxa of Neotropical Annonaceae. *Nordic Journal of Botany* **29**: 257–356.
- Maas PJM, Westra LYT, Vermeer M. 2007.** Revision of the Neotropical genera *Bocageopsis*, *Onychopetalum*, and *Unonopsis* (Annonaceae). *Blumea* **52**: 413–554.
- Maddison WP. 2006.** Confounding asymmetries in evolutionary diversification and character change. *Evolution* **60**: 1743–1746.
- Magallon S, Sanderson MJ. 2001.** Absolute diversification rates in angiosperm clades. *Evolution* **55**: 1762–1780.
- Maia JGS, Andrade EHA, Carreira LMM, Oliveira J, Araújo JS. 2005a.** Essential oils of the Amazon *Guatteria* and *Guatterriopsis* species. *Flavour and Fragrance Journal* **20**: 478–480.
- Maia JGS, Andrade EHA, da Silva ACM, Oliveira J, Carreira LMM, Araújo JS. 2005b.** Leaf volatile oils from four Brazilian *Xylopi* species. *Flavour and Fragrance Journal* **20**: 474–477.
- Merckx V, Chatrou LW, Lemaire B, Sainge MN, Huysmans S, Smets EF. 2008.** Diversification of myco-heterotrophic angiosperms: evidence from Burmanniaceae. *BMC Evolutionary Biology* **8**: 16.
- Mols JB. 2004.** From Miliusa to Miliuseae to miliusoid: identifying clades in Asian Annonaceae. PhD thesis, Universiteit Leiden.
- Mols JB, Gravendeel B, Chatrou LW, Pirie MD, Bygrave PC, Chase MW, Kessler PJA. 2004.** Identifying clades in Asian Annonaceae: monophyletic genera in the polyphyletic Miliuseae. *American Journal of Botany* **91**: 590–600.
- Mols JB, Kessler PJA, Rogstad SH, Saunders RMK. 2008.** Reassignment of six *Polyalthia* species to the new genus *Maasia* (Annonaceae): molecular and morphological congruence. *Systematic Botany* **33**: 490–494.
- Moore BR, Heard SB. 1997.** Inferring evolutionary process from phylogenetic tree shape. *Quarterly Review of Biology* **72**: 31–54.
- Moore BR, Chan KMA, Donoghue MJ. 2004.** Detecting diversification rate variation in supertrees. In: Bininda-Emonds ORP, ed. *Phylogenetic supertrees: combining information to reveal the tree of life*, 3rd edn. Dordrecht: Kluwer Academic Publishers, 487–533.
- Moore BR, Donoghue MJ. 2007.** Correlates of diversification in the plant clade Dipsacales: geographic movement and evolutionary innovations. *The American Naturalist* **170**: S28–S55.
- Moore BR, Donoghue MJ. 2009.** A Bayesian approach for evaluating the impact of historical events on rates of diversification. *Proceedings of the National Academy of Sciences* **106**: 4307–4312.
- Morawetz W, Waha M. 1985.** A new pollen type, C-banded and fluorochrome counterstained chromosomes, and evolution in *Guatteria* and related genera (Annonaceae). *Plant Systematics and Evolution* **150**: 119–141.
- Nakkuntod M, Su YCF, Seelanan T, Saunders RMK. 2009.** Molecular phylogenetic and morphological evidence for the congeneric status of *Goniothalamus* and *Richella* (Annonaceae). *Taxon* **58**: 127–132.
- Pirie MD, Chatrou LW, Mols JB, Erkens RHJ, Oosterhof J. 2006.** ‘Andean-centred’ genera in the short-branch clade of Annonaceae: testing biogeographic hypotheses using phylogeny reconstruction and molecular dating. *Journal of Biogeography* **33**: 31–46.
- Pirie MD, Doyle JA. 2012.** Dating clades with fossils and molecules: the case of Annonaceae. *Botanical Journal of the Linnean Society* **169**: 84–116.
- Rabosky DL, Donnellan SC, Talaba AL, Lovette IJ. 2007.** Exceptional among-lineage variation in diversification rates during the radiation of Australia’s most diverse vertebrate clade. *Proceedings of the Royal Society B: Biological Sciences* **274**: 2915–2923.
- Raikow RJ. 1988.** The analysis of evolutionary success. *Systematic Zoology* **37**: 76–79.
- Richardson JE, Chatrou LW, Mols JB, Erkens RHJ,**

- Pirie MD. 2004.** Historical biogeography of two cosmopolitan families of flowering plants: Annonaceae and Rhamnaceae. *Philosophical Transactions of the Royal Society B: Biological Sciences* **359**: 1495–1508.
- Richardson JE, Pennington RT, Pennington TD, Hollingsworth PM. 2001.** Rapid diversification of a species-rich genus of Neotropical rainforest trees. *Science* **293**: 2242–2245.
- Ricklefs RE. 2003.** Global diversification rates of passerine birds. *Proceedings of the Royal Society B: Biological Sciences* **270**: 2285–2291.
- Rosenzweig ML. 1978.** Geographical speciation: on range size and the probability of isolate formation. In: Wolkind D, ed. *Proceedings of the Washington State University Conference on biomathematics and biostatistics*. Pullman, WA: Washington State University, 172–194.
- Rüber L, Van Tassel JL, Zardoya R. 2003.** Rapid speciation and ecological divergence in the American seven-spined gobies (Gobiidae, Gobiomatini) inferred from a molecular phylogeny. *Evolution* **57**: 1584–1598.
- Rundell RJ, Price TD. 2009.** Adaptive radiation, nonadaptive radiation, ecological speciation and nonecological speciation. *Trends in Ecology & Evolution* **24**: 394–399.
- Sanderson MJ. 1998.** Reappraising adaptive radiation. *American Journal of Botany* **85**: 1650–1655.
- Sanderson MJ, Donoghue MJ. 1994.** Shifts in diversification rate with the origin of angiosperms. *Science* **264**: 1590–1593.
- Saunders RMK. 2002.** The genus *Goniothalamus* (Annonaceae) in Sumatra. *Botanical Journal of the Linnean Society* **139**: 225–254.
- Saunders RMK. 2003.** A synopsis of *Goniothalamus* species (Annonaceae) in Peninsular Malaysia, with a description of a new species. *Botanical Journal of the Linnean Society* **142**: 321–339.
- Saunders RMK. 2012.** The diversity and evolution of pollination systems in Annonaceae. *Botanical Journal of the Linnean Society* **169**: 222–244.
- Saunders RMK, Munzinger J. 2007.** A new species of *Goniothalamus* (Annonaceae) from New Caledonia, representing a significant range extension for the genus. *Botanical Journal of the Linnean Society* **155**: 497–503.
- Savolainen V, Heard SB, Powell MP, Davies TJ, Moers AO. 2002.** Is cladogenesis heritable? *Systematic Biology* **51**: 835–843.
- Schatz GE. 1987.** Systematic and ecological studies in Central American Annonaceae. PhD thesis. University of Wisconsin, Madison, WI.
- Schatz GE, Maas PJM. 2010.** Synoptic revision of *Stenanona* (Annonaceae). *Blumea* **55**: 205–223.
- Schluter D. 2000.** *The ecology of adaptive radiation*. Oxford: Oxford University Press.
- Schuettpelz E, Pryer KM. 2009.** Evidence for a Cenozoic radiation of ferns in an angiosperm-dominated canopy. *Proceedings of the National Academy of Sciences* **106**: 11 200–11 205.
- Seehausen O. 2007.** Evolution and ecological theory: chance, historical contingency and ecological determinism jointly determine the rate of adaptive radiation. *Heredity* **99**: 361–363.
- Shao K-T, Sokal RR. 1990.** Tree balance. *Systematic Zoology* **39**: 266–276.
- Skelton PW. 1993.** Adaptive radiation: definition and diagnostic tests. In: Lees DR, Edwards D, eds. *Evolutionary patterns and processes*. London: Linnean Society of London, 45–58.
- Slowinsky JB, Guyer C. 1993.** Testing whether certain traits have caused amplified diversification: an improved method based on a model of random speciation and extinction. *American Naturalist* **142**: 1019–1024.
- Smith SA, Beaulieu JM, Stamatakis A, Donoghue MJ. 2011.** Understanding angiosperm diversification using small and large phylogenetic trees. *American Journal of Botany* **98**: 404–414.
- Su Y, Saunders R. 2009.** Evolutionary divergence times in the Annonaceae: evidence of a late Miocene origin of *Pseuduvaria* in Sundaland with subsequent diversification in New Guinea. *BMC Evolutionary Biology* **9**: 153.
- Sudhaus W. 2004.** Radiation within the framework of evolutionary ecology. *Organisms, Diversity & Evolution* **4**: 127–134.
- Surveswaran S, Wang RJ, Su YCF, Saunders RMK. 2011.** Generic delimitation and historical biogeography in the early-divergent ‘ambavioid’ lineage of Annonaceae: *Cananga*, *Cyathocalyx* and *Drepananthus*. *Taxon* **59**: 1721–1734.
- Tsou C-H, Johnson DM. 2003.** Comparative development of aseptate and septate anthers of Annonaceae. *American Journal of Botany* **90**: 832–848.
- Valente LM, Savolainen V, Vargas P. 2010.** Unparalleled rates of species diversification in Europe. *Proceedings of the Royal Society B: Biological Sciences* **277**: 1489–1496.
- Van Setten AK, Koek-Noorman J. 1986.** Studies in Annonaceae. VI. A leaf anatomical survey of genera of Annonaceae in the Neotropics. *Botanische Jahrbücher für Systematik, Pflanzengeschichte und Pflanzengeographie* **108**: 17–50.
- Verheyen S, Salzburger W, Snoeks J, Meyer A. 2003.** Origin of the superclade of cichlid fishes from Lake Victoria, East Africa. *Science* **300**: 325–329.
- Waha M. 1987.** Different origins of fragile exines within the Annonaceae. *Plant Systematics and Evolution* **158**: 23–27.
- Wang H, Moore MJ, Soltis PS, Bell CD, Brockington SF, Alexandre R, Davis CC, Latvis M, Manchester SR, Soltis DE. 2009.** Rosid radiation and the rapid rise of angiosperm-dominated forests. *Proceedings of the National Academy of Sciences of the United States of America* **106**: 3853–3858.
- Whittall JB, Medina-Marino A, Zimmer EA, Hodges SA. 2006.** Generating single-copy nuclear gene data for a recent adaptive radiation. *Molecular Phylogenetics and Evolution* **39**: 124–134.
- Wikström N, Savolainen V, Chase MW. 2001.** Evolution of

the angiosperms: calibrating the family tree. *Proceedings of the Royal Society of London: Biological Sciences* **268**: 2211–2220.

**Willis KJ, McElwain JC. 2002.** *The evolution of plants.* Oxford: Oxford University Press.

**Yoder JB, Clancey E, Des Roches S, Eastman JM, Gentry L, Godsoe W, Hagey TJ, Jochimsen D, Oswald BP, Robertson J, Sarver BAJ, Schenk JJ, Spear SF,**

**Harmon LJ. 2010.** Ecological opportunity and the origin of adaptive radiations. *Journal of Evolutionary Biology* **23**: 1581–1596.

**Zhou L, Su YCF, Chalermglin P, Saunders RMK. 2010.** Molecular phylogenetics of *Uvaria* (Annonaceae): relationships with *Balanga*, *Dasoclema* and Australian species of *Melodorum*. *Botanical Journal of the Linnean Society* **163**: 33–43.

#### SUPPORTING INFORMATION

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

**Methods S1.** Explanation of topological method.

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