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Reconstructing shifts in diversification rates on phylogenetic trees

Michael J. Sanderson and Michael J. Donoghue

Few issues in evolutionary biology have received as much attention over the years or have generated as much controversy as those involving evolutionary rates. One unresolved issue is whether or not shifts in speciation and/or extinction rates are closely tied to the origin of 'key' innovations in evolution. This discussion has long been dominated by 'time-based' methods using data from the fossil record. Recently, however, attention has shifted to 'tree-based' methods, in which time, if it plays any role at all, is incorporated secondarily, usually based on molecular data. Tests of hypotheses about key innovations do require information about phylogenetic relationships, and some of these tests can be implemented without any information about time. However, every effort should be made to obtain information about time, which greatly increases the power of such tests.

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Many questions in evolutionary biology revolve around the observation that some groups of organisms have more species than others. We wonder, for example, why beetles are so species-rich. Is it the result of some feature that evolved in the ancestor of beetles (an intrinsic cause, such as the evolution of elytra), or is this species richness related to an environmental shift that favored the diversification of organisms that had already evolved a particular set of attributes (an extrinsic cause, such as a climatic change or changes occurring in coevolving groups of organisms) or some more complicated combination of such causes? Still other questions pertain to replicated episodes of diversification. For example, did the evolution of latex in several clades of vascular plants promote their diversification by warding off insect pests[1]? Answers to such questions hinge on an accurate reconstruction of where and when shifts in diversification rate occured in relation to the appearance of possible intrinsic or extrinsic causes.

Techniques for reconstructing the evolution of organismal features in phylogenies have been well studied[2], but reconstructing the where and when of shifts in diversification rate in phylogenies has only recently received critical attention. Our aim is to compare and contrast these methods. A key issue is how phylogenetic information can provide new insights into species diversification. But we also hope to clarify the role that other information can play, especially the absolute timing of speciation and extinction events in evolutionary history.

Trees, times and durations

Ideally, diversification would be studied in clades in which phylogenetic relationships are clear and a rich fossil record has placed tight constraints on the timing of speciation and extinction events (Fig. 1a). Although a few such cases are known (e.g. North American Neogene horses[3]), they are not common. A complete set of information about diversification includes two items: the phylogeny, and times of events (including speciation, extinction, and perhaps the origin of novelties). Durations ('lengths') of branches, which are often used in studies of diversification, can be derived from information about the timing of events. Various methods for studying diversification use these items singly or in combination (Table 1).

Much of the ambiguity in the study of diversification rates stems from the use of a subset of the necessary information. For example, inferences based on durations alone are confounded by uncertainty about what marks the beginning and end of a lineage. Is it a branching event, a true extinction event, or a pseudo-extinction or pseudo-speciation event caused by phenotypic transformation into a 'new' species[4]? Were a phylogeny available, the length of a lineage could be clearly defined. In the absence of complete information, however, compromise is inevitable. Below we consider what can and cannot be inferred when almost everything is known about a clade (as in Fig. 1a), and what can be inferred when less is known (as illustrated by Figs 1b–d). One may know relationships but know nothing about time; know time but know nothing about relationships; and so on.

A variety of models have been used to study species diversification (Box 1). Because these inevitably entail some probability of error, inferences based upon them are statistical. Such errors may involve systematic biases in estimates of rates, large average errors (deviations from the true parameter regardless of direction) in those estimates,

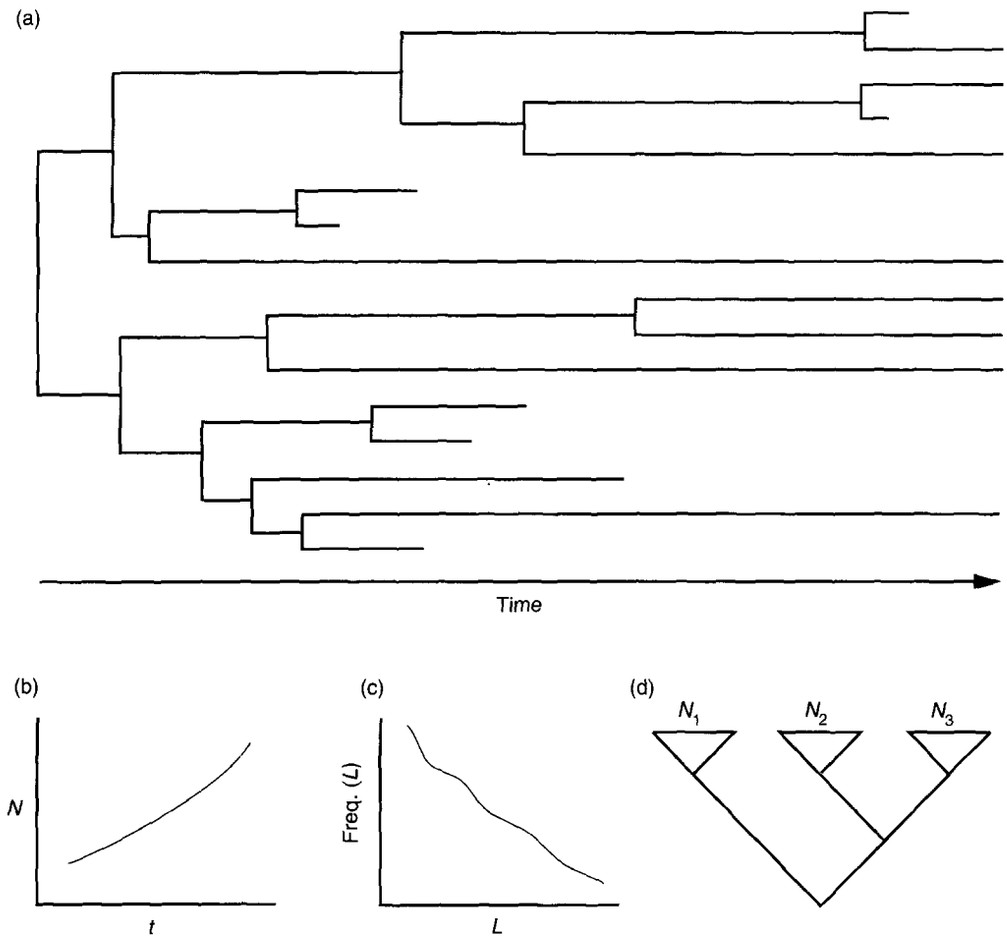

Fig. 1. Sources of information in the study of diversification. (a) Complete information consists of a phylogeny mapped onto a time scale such that speciation and extinction events are dated and lengths of branches are therefore known. This information may be obtained by a combination of phylogenetic inference, fossil evidence and reconstruction of times of splitting events by algorithmic methods using molecular data (see Box 2). (b) Incomplete information may be available in the form of just the number of lineages (N) extant at any point in time, t, or (c) a histogram of branch lengths (durations), L, of lineages, or (d) a phylogeny with no information about time at all. The number of species per clade is indicated by N_i.

or inaccurate assessments of significance levels of tested hypotheses. Even more basic are the errors stemming from lack of robustness of the evolutionary model, or measurement error in the data themselves. The latter become especially crucial if information about time is derived from an inaccurate fossil record or from erroneous assumptions about a molecular clock (Box 2).

Estimation of diversification rates

Estimation and hypothesis testing often raise different issues. In surveys of rate variation among higher taxa[5], estimation of rates of diversification is of interest in itself, and specific hypotheses about rates may not even be tested. In the case of complete data (Fig. 1a), maximum likelihood estimators have been derived for both the 'birth and death' (BD)[6–9] (see Box 1) and Bienaymé–Galton–Watson (BGW) processes[10,11]. Maximum likelihood estimators have many desirable properties, including convergence to the true parameter (in this case, rate) given enough data, and minimum variance, but they are often biased. Maximum likelihood (and other) estimators for rates in a BD process consistently underestimate the true rate of speciation in even the simple case of a Yule model (Box 1) with no extinction[6]. This is a consequence of Jensen's Inequality[11] (that is, the expected value of a parameter $E(\hat{\lambda}) \leq \lambda$ satisfies whenever the estimator is some concave function of the data – which is frequently the case in diversification models). Less-biased estimates can be obtained from observations taken over a brief interval of time, and unbiased estimates are known for a BGW process, but the variances of such estimates are higher, so a trade-off is inevitable[11].

In the slightly weaker case, in which branching and extinction events can be constrained to lie within an interval based on stratigraphy but perhaps not fixed precisely in time, integrated likelihood methods can be used to consider the distribution of all possible events over those intervals[8]. Currently, however, these methods are limited by the power of symbolic math programs to handle multiple integrations in large trees.

If the phylogeny is unknown but fossil evidence provides information about time, then it is still possible to estimate rates, with certain caveats that depend on the kind of temporal information available. Information about branch lengths (from point of origination of a species to its extinction or a subsequent branching event) is statistically 'sufficient' to estimate rates, and the tree is technically unnecessary, but it is difficult to imagine ways to obtain such temporal information without one (Fig. 1c). On the other hand, if, as is more common, information is solely in the form of species number over time (Fig. 1b), it is very difficult to separate speciation from extinction rates (although see Ref. 12). And, even if extinction is ignored (Yule model), the maximum likelihood estimate of rate in a clade of N taxa ($\log(N)/t$, where t is the time since origination) is still biased downward. Note that this maximum likelihood estimate is the same estimate that is obtained by equating the expected diversity over time to the observed diversity[13].

Survivorship analysis[14] and cohort analysis[15,16] have also been used when durations of lineages (lengths of branches) can be extracted from the fossil record. Survivorship analysis depends on the assumption of a stable equilibrium 'age structure' to the assemblage[17], but cohort analysis does not. The BD model makes a prediction about survivorship – the probability that lineages will persist a certain length of time, as a function of the rate of speciation and extinction. These rates can be estimated by an iterative curve-fitting method that minimizes the mean log deviation of observations from predicted values[15,16]. Properties of these estimators have not been studied, but Foote[16] used Monte Carlo simulation to construct confidence intervals, which would tend to correct for any biases in hypothesis tests.

In the absence of any information about time or durations of taxa, a phylogeny can provide reference points for

Table 1. Methods for reconstructing diversification rates

Method	Refs	Data	Model[a]	Method of estimation or test[b]	Tree necessary?	Fossil record necessary?	Statistical power	Notes
Taxonomic diversity	18,19	hollow curves[c]	BD[d]	null model	no	no	unknown	prone to taxonomic artifact
Survivorship, cohort analysis	14–16	probability of lineage survival	BD	curve fitting	no	yes	high	broad taxonomic comparisons possible
Diversity curve fitting	5,38	diversity over time	exponential	curve fitting	no	yes	high	broad taxonomic comparisons possible
BGW process estimation	10	diversity over time	BGW	ML	no, but can be used	yes	high	mathematically well understood
Sistergroup comparison	25	standing diversities	none	simple comparison	yes	no	not statistical	few assumptions
Iterated sistergroup method	26–30	standing diversities in multiple sistergroup pairs	none	test of association	yes	no	fair	power improved using null model test for each replicate
Sistergroup null model	31,32	standing diversities	Yule	null model	yes	no	low	robust
Diversity optimization	26	standing diversities	none	parsimony reconstruction	yes	no	not statistical	few assumptions
BD process estimation	7,9,12	branching times	BD	ML	yes	no, but can be used	high	optimal with complete information
BD process/ integrated likelihood	8	constraints on branching times	Yule	integrated ML	yes	no, but can be used	low	can use time constraints based on fossil record
BD process/ model-fitting	23	standing diversities	Yule	ML	yes	no, but can be used	low	localization of shifts in diversification (also fossil constraints)

[a]BD = birth and death process; BGW = Bienaymé–Galton–Watson.
[b]ML = maximum likelihood.
[c]Frequency distributions of taxa of differing species diversities.
[d]Other models were also examined.

comparison of relative rates of diversification (Fig. 1d). Two (or more) extant clades with diversities of $\{N_1, N_2, \ldots\}$ that are all descended from the same node in a tree must be the same age (as long as any polytomy represents true multiple speciation rather than mere lack of resolution). Maximum likelihood estimators of their relative diversification rates under a Yule model are just $\{\log(N_1), \log(N_2), \ldots\}$. These are the simplest estimators available, but their bias and error variance is worse than that of estimators that take advantage of information about time.

Of course, the worst case is no information about time or about phylogeny. The only available information is then the apportionment of species diversity among higher taxa in a taxonomic classification. Empirically, the distribution of species in genera often follows an approximate 'hollow curve' – that is, a distribution with few large genera and very many small ones. In a pioneering paper, Yule[18] explored the relationship between such curves and the BD branching process, and Dial and Marzluff[19] considered several other models of diversification as well. Unfortunately, taxonomic artifacts in the construction of a classification by non-phylogenetic methods can bias this kind of inference – for example, the size distribution in a classification scheme developed by a taxonomic 'splitter' differing from that generated by a 'lumper'.

Testing hypotheses about shifts in rate

The first hypothesis tested in any study of diversification should be whether or not a chosen branching model actually fits the data[20,21]. It is not unusual for models to fail such a test. Nee *et al.*[22] rejected the constant rate BD process in favor of a more complex model that suggests a decrease in rate of diversification in birds. Foote[16] used cohort analysis to show that Ordovician trilobites conformed closely to a

Box 1. Mathematical models for diversification

Almost all studies of evolutionary rates use models of evolution. The simplest mathematical models are **deterministic**, with species diversity described as some function of time, t. A widely cited deterministic model is the **exponential** model, with $N(t) = e^{rt}$, where r is the difference in the rate of speciation (S) and extinction (E), $r = S - E$ (Ref. 5). More complex logistic models invoke density dependence to limit the exponential growth entailed by the simpler model[13,39].

In recognition of the uncountable number of hidden and inestimable variables influencing diversification rates, **stochastic** models are more widely used to study diversification. The '**birth and death** (BD) model' is a stochastic analog of the exponential deterministic model. Instead of a single value for the number of species observed at any time t, there is a probability distribution. That distribution has a mean of e^{rt}, in parallel with its deterministic counterpart, but it also has a variance that may be quite substantial.

A special case of the BD process is the **Yule** model, which is identical except that extinction is not allowed. A variant on the Yule model has been termed the **Markov** model[40] (although all of these stochastic models are Markov). This model allows rates to vary through time as long as they are the same across the entire clade at any instant. Because it has only been applied to cases in which no information about time is available (Fig. 1d), it is effectively equivalent to a Yule model.

A third stochastic model, the Bienaymé–Galton–Watson (BGW) **branching process**, is occasionally used because of its convenient mathematical properties[9,10]. It is essentially a continuous-time birth and death Markov process that is observed at discrete intervals – a 'skeleton' of the continuous process[10]. This, the first branching model used in biology, was used to study the extinction of family names and genes in populations, both having discrete generations[11]. Non-Markov models are used only rarely, owing to their complexity[21].

Box 2. Reconstructing times of branching in phylogenies

How feasible is it to reconstruct branching times in the absence of a fossil record? Some fossil information is necessary to reconstruct absolute branch times. However, much can be learned about diversification from relative branch times that are measured in arbitrary units from the root of the phylogeny. Some phylogenetic inference algorithms (ultrametric ones such as UPGMA or maximum-likelihood algorithms with a molecular clock) reconstruct these relative times while they build the tree. However, they are not very robust to violations of the assumption of rate constancy in the underlying characters[41].

A better method is to reconstruct the tree topology using a robust method such as parsimony, maximum likelihood, or neighbor joining, and then use some algorithm to estimate the times. For example, one could then apply a maximum-likelihood molecular-clock algorithm constrained by the given tree (e.g. PHYLIP's DNAMLK[42]). Maximum likelihood provides a statistical test of the underlying clock model itself[43]. Current versions of PHYLIP (version 3.5) use a so-called hidden Markov model that allows variation in rates across sites as it estimates branching times. This model sometimes greatly reduces the apparent poor fit of the clock to sequence data, because some component of the tendency to reject a clock model is due to rate heterogeneity across sites rather than across time.

Other techniques for reconstructing times on trees have been described in the literature[44], but have not been widely disseminated in software packages.

BD process but Cambrian trilobites did not. Goodness of fit can be tested with or without phylogenetic information.

However, many of the most interesting biological hypotheses entail localizing changes in rate in a phylogeny. This requires tests of hypotheses about rates that are different in different parts of a tree. The need for information about phylogeny is particularly acute for any hypothesis associating a shift in rate with the appearance of an evolutionary novelty (Fig. 2) – a so-called 'key innovation' hypothesis[23]. Almost any of the methods described above can be adapted to the study of such problems by partitioning the data among parts of the phylogeny with and without the putative novelty, estimating possibly different parameter values in those parts, and performing hypothesis tests.

If complete information is available, a likelihood framework can be used to test a hypothesis that has different rates in different parts of a phylogeny. The simplest case contrasts a two-rate model with a null model having only one rate. This method was used to reject the hypothesis that rates of branching differed between the α- and β-globin subfamilies of the globin multigene family of vertebrates[24]. It is important to remember, however, that because of biases in the maximum likelihood estimates, Monte Carlo simulation is generally necessary to develop accurate significance tests based on the likelihood ratio[7–9]. Nee *et al.*[22] used a somewhat different approach to test whether bird diversification rates are associated with body size. They tested the prediction that rapidly speciating subclades should have shorter branch lengths (shorter because speciation must be occurring more rapidly), and found that there was no correlation.

Phylogenetic information permits tests of key innovation hypotheses even in the absence of knowledge of times. The simplest approaches are deterministic and rely on numerical comparison of diversities in sistergroups descended from a common ancestor[25]. Brooks and McLennan[26] extended this approach in an analysis of diversity patterns in platyhelminth parasites. They treated species diversity as a phylogenetic character and reconstructed species-rich and species-poor portions of a phylogeny using standard character reconstruction techniques.

Although primarily descriptive, these approaches can be subjected to significance testing if instances of putative diversification are replicated. Mitter *et al.*[27] examined multiple instances of insect diversification and noted that the more species-rich sistergroup was phytophagous in 11 of 13 cases, which is significant in a nonparametric sign test. The same approach has been used to study eggshell architecture in arthropods[28], viviparity in poeciliid fishes[29], and carnivory in insects[30]. This approach can also be coupled with significance tests applied to each individual clade. Slowinski and Guyer[31] derived a simple significance test for heterogeneity in rates between two sistergroups. The cumulative probability of observing a difference in diversity equal to or more extreme than m and n in two sistergroups is just $P = 2m/(m+n-1)$, assuming diversification by a BD process with constant rates. When applied to replicated instances of a putative innovation, this has greater statistical power than nonparametric tests[32].

Comparison of sistergroup diversities alone can be of limited use. It can lead to inferences about heterogeneity in rate, but not about the direction of rate change. It cannot distinguish whether a more diverse sistergroup is a reflection of a rate increase or a rate decrease in its relative[8,26,33,34]. Moreover, sistergroup comparisons tempt the unwary to associate instances of species richness (or poorness) nested within one of the sistergroups with the entire sistergroup containing it (Fig. 2)[35].

In order to move beyond sistergroup comparisons, it is necessary to consider more of the tree's topology. Unfortunately the transition from trees with two taxa to trees with three or more taxa involves a fundamental increase in complexity, because at least one internal branching point is present with a possibly unknown time (Fig. 2). One solution is to rely on the underlying branching model to generate a probability distribution for these times and then integrate over all times between the root node and

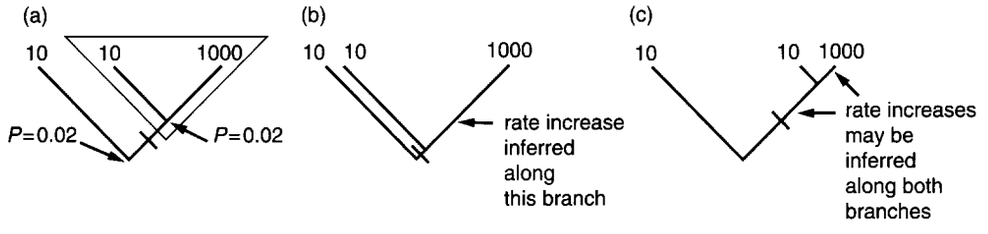

Fig. 2. Hypotheses about diversification. Numbers at tips are species diversities. Many hypotheses focus on a shift in rate associated with the evolution of some novelty in a clade (indicated by the hatchmark along the internal branch). (a) At any node, a null model[30] can be used to test for the significance of differences in species diversities of the two sistergroups descended from that node (P values shown). Such tests have been used in the literature to identify rate differences that are correlated with the evolution of a novelty. However, more complex models that take time into account reveal the limits of sistergroup comparisons. Consider models in which rates are different in the parts of the tree that do [parts inside boundary in (a)] and do not possess (parts outside boundary) the novelty. Inferences about rate now depend on when the internal branching point occurred. If early, as in (b), then a rate increase would be inferred along the terminal branch leading to the most diverse clade because the lengths of the two basal-most terminal branches are about the same. If the split were late, as in (c), then two increases in rate would probably be inferred. Only the second case (c) is consistent with the hypothesis that the novelty caused an increase in rate along the internal branch[23]. In (b), no increase in rate occurred along the branch exhibiting the novelty. These kinds of detailed inferences cannot be made on the basis of the two sistergroup comparisons shown in (a), without considering time (*contra* Ref. 45).

the present[8,23]. By considering all possible branching models on even a three-taxon tree (from a one-rate model to models with as many as four rates), and selecting the simplest model that fits the observations adequately, it may be possible to select a model that does indeed imply a direction of rate change. An application of this approach led to the conclusion that flowering plant diversity increased only after the origin of major groups within flowering plants, which argues against the popular key innovation hypotheses for flowering plant diversity[23].

Issues and recommendations

Because of the questionable assumptions invoked in reconstructing branching times by a molecular clock when the fossil record is poor (Box 2), testing hypotheses with methods that do not require temporal information is appealing. The cost of this freedom from assumptions, however, is lower statistical power. Power, a function of sample size, is the ability of a test to discriminate between alternative models. In a sistergroup null model test, the sample size is equal to the number of observations in the analysis – two. Consequently, a diversity difference of about 40:1 is required for significance at the conventional 0.05 level using this test. Similar levels of power obtain for the maximum likelihood methods that do not take advantage of information about time. Although there are many clades diverse enough to exceed this ratio (e.g. flowering plants, holometabolous insects), it is difficult to escape the perception that these are overly conservative tests. The sample size in methods that use time or branch length information is higher because there are more 'observations'. A well-resolved phylogeny with known branch times has a sample size of the order of N, where N is the number of species in the clade. The effective sample size in tree-based methods without time is now of the order of 2–10, the maximum number of clades that can be considered given available methods and computational constraints[8,23,31].

Unfortunately, in many real phylogenies, time is difficult to reconstruct, either because of a poor fossil record or because the available molecular sequence data evidently are not clock-like[36]. There are three options, all of which require further investigation. First, times can be reconstructed under the assumption of a clock, even though it is clear that the clock is violated (Box 2). The robustness of this procedure is unknown but there is no reason to expect it to be high. The second possibility is to develop methods for reconstructing branching times that are less tied to the molecular clock assumption. Hasegawa and colleagues have worked towards this end in studies on branching times in Hominoids and in the human mitochondrial DNA tree, in which they examined models for variation in rate across branches[37] and across sites[38], respectively. The third possibility is to continue the development of 'robust' methods that do not require branch times, such as those that rely on integrated maximum likelihood[8,23], but with the realization that the power of such tests will probably always remain lower than methods that use time. In the meantime, however, based on what is known about currently available methods, it is possible to make recommendations about appropriate methods based on the quantity and quality of available data (Table 1).

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Palaeo-ecophysiological perspectives on plant responses to global change

D.J. Beerling and F.I. Woodward

Taxonomic classifications of plant species, based on morphological characteristics, provide a stable and robust approach for inferring taxonomic and phylogenetic relationships between extant and extinct species. This implies that, although evolution is a continuous process for a species, there is no whole-scale change in those suites of morphological characteristics that define higher order (genus and greater) relationships. Recent research suggests that a higher order characteristic – stomatal density – may reflect not only the atmospheric CO_2 concentration during initial evolution, but may also strongly constrain the responses of higher order plant groups to future CO_2-enrichment.

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Geochemical models of the long-term carbon cycle indicate that the concentration of atmospheric carbon dioxide (CO_2) has varied greatly over the course of land plant evolution[1–3]. Model results are supported by analyses of palaeosols[4,5] and the isotopic composition of fossil porphyrins[6] (Fig. 1). The implications of such large CO_2 variations for plant–atmosphere interactions are only now beginning to be explored[7–9].

Dramatic, long-term CO_2 changes have two repercussions for palaeoecophysiological research. First, the observation that leaf stomatal density (number of stomata per unit area of leaf) can be controlled by the concentration of atmospheric CO_2 (Refs 10,11) offers the possibility of investigating the relationship between stomatal density of fossil leaves and CO_2 on timescales commensurate with the entire course of land plant evolution. Investigations of fossil leaves can be used to ask a new question: do major changes in stomatal density recorded from fossil leaves correspond to predicted major changes in atmospheric CO_2 concentration? Estimates of ancient atmospheric CO_2 changes from observations on fossil leaves will always be severely limited by the lack of extant species, which are sometimes needed to calibrate the relationship between CO_2 and stomatal density, and so this approach may only be of use for testing geochemical CO_2 models and CO_2 proxies in a semi-quantitative fashion.

The second repercussion of large variations in atmospheric CO_2 is the possible effects on the ecophysiology of different taxonomic plant groups (e.g. ferns, cycads, palms, ginkgos and angiosperm and gymnosperm trees) that evolved during this time[8]. The impact on the photosynthetic 'system' appears to be small[12]; however, changes in stomatal physiology and morphology seem very likely – a response that will influence the operational range of photosynthesis.

In this review, we discuss each of these areas in turn. Our approach has been to begin with the assemblage of observations of stomatal density from fossil plants of the late Silurian through to the most recent glaciation. Building on these observations from the fossil record, we then survey the stomatal density and photosynthetic characteristics of the 'early' evolving plant groups, cycads, palms and ferns growing at the present-day to investigate the link with the environmental conditions under which the different taxonomic groups evolved.

Evidence from the palaeobotanical record

The largest CO_2 excursion in the geological past (Fig. 1) occurred from the Silurian [*c.* 435 million years ago (Mya)] through into the Carboniferous (345–280 Mya) coincident with the evolution of land plants. The stomatal densities of fossilized leaves from these two geological periods are very markedly different (Fig. 2). Plants growing in the high-CO_2

