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Do fossil plants signal palaeoatmospheric CO₂ concentration in the geological past?

J. C. McElwain

Department of Animal and Plant Sciences, The University of Sheffield, Western Bank, Sheffield S10 2TN, UK
(j.mcelwain@sheffield.ac.uk)

Fossil, subfossil, and herbarium leaves have been shown to provide a morphological signal of the atmospheric carbon dioxide (CO₂) environment in which they developed by means of their stomatal density and index. An inverse relationship between stomatal density/index and atmospheric CO₂ concentration has been documented for all the studies to date concerning fossil and subfossil material. Furthermore, this relationship has been demonstrated experimentally by growing plants under elevated and reduced CO₂ concentrations. To date, the mechanism that controls the stomatal density response to atmospheric CO₂ concentration remains unknown. However, stomatal parameters of fossil plants have been successfully used as a proxy indicator of palaeo-CO₂ levels. This paper presents new estimates of palaeoatmospheric CO₂ concentrations for the Middle Eocene (Lutetian), based on the stomatal ratios of fossil Lauraceae species from Bournemouth in England. Estimates of atmospheric CO₂ concentrations derived from stomatal data from plants of the Early Devonian, Late Carboniferous, Early Permian and Middle Jurassic ages are reviewed in the light of new data. Semi-quantitative palaeo-CO₂ estimates based on the stomatal ratio (a ratio of the stomatal index of a fossil plant to that of a selected nearest living equivalent) have in the past relied on the use of a Carboniferous standard. The application of a new standard based on the present-day CO₂ level is reported here for comparison. The resultant ranges of palaeo-CO₂ estimates made from standardized fossil stomatal ratio data are in good agreement with both carbon isotopic data from terrestrial and marine sources and long-term carbon cycle modelling estimates for all the time periods studied. These data indicate elevated atmospheric CO₂ concentrations during the Early Devonian, Middle Jurassic and Middle Eocene, and reduced concentrations during the Late Carboniferous and Early Permian. Such data are important in demonstrating the long-term responses of plants to changing CO₂ concentrations and in contributing to the database needed for general circulation model climatic analogues.

Keywords: stomatal density, stomatal index, Palaeozoic CO₂ concentration, Middle Jurassic CO₂, Eocene CO₂ concentration, Lauraceae

1. INTRODUCTION

The rise in atmospheric carbon dioxide (CO₂) concentration since pre-industrial times has attracted considerable attention amongst the scientific community. This is reflected in the establishment of an Intergovernmental Panel on Climate Change (IPCC) by the World Meteorological Organisation and the United Nations Environment Programme. According to global recording stations (Keeling *et al.* 1989), CO₂ concentration is rising at an annual rate of 1.8 ppm, has increased by approximately 25% since pre-industrial times (1750–1800 AD) and is predicted to double its current concentration of 360 ppm by the year 2100. Concomitant with the predicted doubling of atmospheric CO₂, global temperature is expected to rise by 1.5 to 4.5 °C according to general circulation models (GCMs) (Houghton *et al.* 1995).

GCMs are, to date, one of the most highly developed tools with which to assess the potential effects of rising levels of atmospheric CO₂ on the global climate (Schimel *et al.* 1995). However, they have their limitations (Valdes 1996). These models involve the coupling of the atmosphere component of a climate model with an ocean

model so that increasing concentrations of atmospheric CO₂ can be fed into the model and the differences in climate between ambient and elevated CO₂ conditions can be assessed. In addition to the method explained above, GCMs can be used to predict future climate change through the use of climatic analogues from the past. The precise forcing factors (such as palaeo-CO₂ concentrations, ocean circulation, and temperature) and other conditions are, however, required from the palaeo-climatic analogue in order to run these GCMs. There has therefore been a drive in recent years towards the study of past climates and the collection of proxy palaeoclimate data, in particular data on atmospheric CO₂ concentration.

Secular variations in atmospheric CO₂ concentration in the geological past have been indicated by means of computer modelling (Berner 1993, 1994; Worsley *et al.* 1994), carbon isotopic investigations of both marine organic matter (Popp *et al.* 1989; Freeman & Hayes 1992) and terrestrial soil carbonates (Cerling 1993; Mora *et al.* 1991, 1996), rare earth element analysis of marine sediments (Liu & Schmitt 1996) and, recently, by means of stomatal parameters of both subfossil (Beerling *et al.* 1993;

de Water *et al.* 1994; McElwain *et al.* 1995) and fossil ts (Van der Burgh *et al.* 1993; Kurschner *et al.* 1996; llwain & Chaloner 1995, 1996; Wagner *et al.* 1996).

This paper first reports new CO₂ estimates derived stomatal ratios of plants from the Middle Eocene etian; 44–50 Ma ago) of Bournemouth in southern and, and second reviews previous estimates of eo-CO₂ concentration from the Early Devonian (*ca.* Ma ago), Late Carboniferous (*ca.* 290 Ma ago), Early ian (*ca.* 280 Ma ago) and Middle Jurassic (*ca.* 165 Ma in the light of new data and standardization techni-

STOMATAL PARAMETERS AND ATMOSPHERIC O₂: HISTORY AND BACKGROUND

Woodward (1987) was probably the first to observe that significant inverse relationship existed between plant stomatal density (number of stomata per mm²) and atmospheric CO₂ concentration. He demonstrated that the stomatal densities and indices (a ratio of the number of stomata to the number of stomata plus epidermal cells on leaf surface expressed as a percentage) of 14 deciduous woodland species, collected approximately 200 years ago preserved as herbarium specimens, were significantly lower than those of the same plant species today, which developed in an ambient CO₂ concentration some 25% lower than that of the early nineteenth century. Many others have since confirmed Woodward's early observations in both laboratory (Woodward & Bazzaz 1988; Woodward & Kelly 1995) and field based experiments (Ghetti & Raschi 1993). In addition, the relationship has been demonstrated for subfossil material from the Pliocene (Beerling & Chaloner 1992; Paoletti & Gellini 1993; Beerling 1993; McElwain *et al.* 1995; Kurschner 1996; Wagner *et al.* 1996) and the Quaternary (Beerling *et al.* 1993; Van de Water *et al.* 1994), for which accurate estimates of the palaeo-CO₂ levels can be obtained from ice-core records (Friedli *et al.* 1986; Barnola *et al.* 1987).

Beyond the time limits for which atmospheric CO₂ data are available from ice-core records, stomatal parameters of fossil plants have been used as proxy indicators of palaeoatmospheric CO₂ concentration. Van der Burgh *et al.* (1993) have made quantitative estimates of atmospheric CO₂ concentrations for the Late Neogene period (2–10 Ma ago) from the stomatal indices of fossil *Quercus petraea* leaves, whilst Kurschner *et al.* (1996) have carried out similar investigations for *Betula subpubescens* from the same localities and time. For the pre-Tertiary, McElwain & Chaloner (1995, 1996) have made semi-quantitative estimates of palaeoatmospheric CO₂ concentration from the Cretaceous and the Mesozoic by means of the stomatal method.

Stomatal density has been found to be subject to a high degree of intraspecific variation, driven by biological and environmental parameters other than atmospheric CO₂, such as humidity (Bakker 1991) light intensity (Gay & Wood 1975) and water stress (Zalenski 1904) (see McElwain & Chaloner (1996) for further details). These environmental parameters have been found to affect the shape and spacing of the epidermal cells, therefore influencing the resultant stomatal density. The effect of these environmental parameters on stomatal density can be

removed by calculation of the stomatal index. The value of this ratio was first suggested by Salisbury (1927), who observed in a study of woodland species that there was less intraspecific variation in stomatal index than in stomatal density.

Recent studies, however, have demonstrated that the stomatal index as well as the stomatal density can be affected by light intensity (Poole *et al.* 1996; Kurschner *et al.* 1996). Poole *et al.* (1996) have demonstrated up to a 2.5-fold variation in stomatal density on the leaves of *Alnus glutinosa* sampled from a wide range of living trees from different ecological habitats in Scotland. The variation observed within the stomatal index of this species was considerably lower than that of the stomatal density, but remained significant (Poole *et al.* 1996). Kurschner *et al.* (1996) have also observed a wide range of mean stomatal densities in 'Recent' *Quercus petraea*, with up to a twofold variation in stomatal density often being observed. These authors attributed the observed variation to varying light and humidity levels, which resulted in two markedly different populations in stomatal density and index of *Q. petraea*; a 'sun' population which maintained a significantly higher stomatal density and index, and a 'shade' population which possessed significantly lower stomatal numbers. Poole *et al.* (1996) also observed these two distinct sun and shade populations in *Alnus glutinosa* and warned that they could mask or interfere with the stomatal density/index response to atmospheric CO₂ concentration.

Despite inherent natural variation within species, an atmospheric CO₂ signal may be read from changing fossil stomatal numbers by applying either one of the following two strategies: (1) the method of Kurschner *et al.* (1996), who established whether the fossil *Q. petraea* leaves under investigation were sun or shade morphotypes by means of morphological and anatomical analysis; or (2) the strategy employed in this paper which involved the collection of large data sets, from a wide range of localities, from both fossil and 'Recent' nearest-living equivalent (NLE) material, so encompassing as much of the natural variation within a species as possible. Where consistent and significant differences are observed between fossil and modern stomatal index data sets, despite their respective natural variance, they may reasonably be attributed to changes in atmospheric CO₂.

3. MIDDLE EOCENE CO₂ ESTIMATES

According to carbon mass balance modelling (Berner 1993, 1994) and carbon isotopic evidence from marine phytoplankton (Freeman & Hayes 1992), the palaeoatmospheric CO₂ concentration during the Tertiary ranged between 1–2 times and 1–2.5 times the pre-industrial atmospheric level (PIL), respectively. Estimates of palaeo-CO₂ from palaeosol carbon isotopic signatures are in good agreement with these geochemical data, and suggest palaeoatmospheric CO₂ concentrations of 2.3 times the PIL for the Miocene and twice the PIL during the Eocene (Cerling 1993). According to Cerling (1993), using his reaction–diffusion model, the palaeo-CO₂ concentrations throughout the Tertiary period were generally indistinguishable from the present-day level (360 ppm). This is supported by a chronologically well-constrained study of atmospheric CO₂ estimates from

palaeosol carbon isotopes of the Paris Basin (Sinha & Stott 1994). These data for the late Palaeocene to the Early Eocene transition demonstrate palaeo-CO₂ levels of 1–2 times PIL. Fine resolution investigation of fossil stomatal parameters from the Miocene, Pliocene and Early Pleistocene have also illustrated that the palaeoatmospheric CO₂ concentrations of this time period were not significantly higher than during the last 200 years, and fluctuated several times between 280 and 370 ppm (Kurschner *et al.* 1996).

The comparison of geochemical (Berner 1994; Freeman & Hayes 1992) and biological/stomatal (Kurschner *et al.* 1996) evidence clearly illustrates that although both methods of palaeoatmospheric CO₂ reconstruction are in good agreement, the geochemical data lack the fine resolution of the stomatal density method (Kurschner *et al.* 1996). It is possible therefore that short-term fluctuations in atmospheric CO₂ concentration could be missed by mass balance modelling and carbon isotopic investigations. This paper aims to extend the biological record of palaeo-CO₂ concentration of Kurschner *et al.* (1996) into the Middle Eocene, in order to investigate whether the Eocene was indeed a period of high greenhouse gasses as geochemical evidence suggests.

4. PALAEOBOTANY OF THE BRANKSOME SAND FORMATION

Two fossil Lauraceae genera, *Lindera* and *Litsea*, from the Branksome Sand Formation of Bournemouth were investigated for this study. Correlation of the Bournemouth Freshwater Series (within the Branksome Sand Formation) with other sequences of the Late, Early and Middle Eocene strata in the Hampshire Basin by Hooker and Collinson has revealed that this unit is Lutetian in age (44–50 Ma old) (Collinson 1996).

Gardner (1882, 1886) was the first to provide a detailed description of the fossil flora contained within the Bournemouth Freshwater Series, where he attempted to correlate the beds of the Bournemouth cliffs with those at Bovey Tracey in Devon (Plint 1988). Following on from Gardner's early work on ferns and gymnosperms, Bandulska described a number of the species which occurred in these sediments in a series of papers in which she described cuticular structures and assigned a number of fossil species to modern genera (Bandulska 1923, 1924, 1926, 1928, 1931). Although Bandulska based her assignment of fossil specimens to modern genera on detailed cuticular and leaf physiognomic characteristics, her determinations have been questioned by some contemporary palaeobotanists. According to Collinson (1986), the assignment of fossil plants to modern genera assumes that little or no evolution has occurred since the Eocene among the families in question. Since Bandulska's study on the flora in the early 1900s, little systematic work has been done on her extensive cuticle collection (housed in the Natural History Museum (NHM), London), and as a result some of the species assignments are considered tenuous and further systematic investigation is believed to be required (D. Ferguson and P. Crane, personal communications). However, the original sites of Bandulska's collections between Middle and Alum Chines and Durley Chine in Bournemouth are no longer accessible.

The choice of fossil species from Bandulska's cuticle collection at the NHM was therefore restricted to those that have been published and extensively studied in terms of their systematic affinities and relationships.

5. PALAEOCLIMATE AND PALAEOECOLOGY OF THE BOURNEMOUTH FLORA

According to a wealth of palaeontological and modelling evidence, the Middle Eocene palaeoclimate was more equable than that of the present day and, according to Frakes (1979), represented a period of maximum warmth, taken together with the Early Eocene. The interpretation of Eocene terrestrial palaeoclimates of southern England has been based in the past on the classic macrofloral studies of Reid & Chandler (1926, 1933) and Chandler (1961, 1964), and the microfossil work of Pallot (1961), Sein (1961) and Daley (1972). Marine palaeotemperature reconstructions based on the oxygen isotopic record of planktonic and benthic foraminifera in the southern North Sea (Buchardt 1978) revealed a temperature maximum in the early Middle Eocene, with a typical sea-surface temperature range between 20 and 27 °C for the period of 52–50 Ma ago. The original estimate of terrestrial palaeotemperature based on the palaeofloristics of the London Clay Flora was 25–26 °C (Chandler 1964). Reid & Chandler (1933) and Chandler (1964) compared the London Clay Flora to the Indo-Malayan region, and suggested that it indicated a tropical rainforest climate during early Eocene times, with a mean annual temperature (MAT) of 21 °C, frost-free and a seasonally well-distributed rainfall. Chaney (1940) interpreted the same flora as combining subtropical and not fully tropical elements. Daley (1972) critically assessed these early palaeoclimate interpretations, outlining some important anomalies regarding the presence of a significant percentage of extra-tropical species within the London Clay Flora. These have been interpreted as representing relict species from the preceding Palaeocene, or species which perhaps inhabited contemporaneous uplands (Chandler 1964). Wolfe (1979), however, noted that the characteristically temperate taxa within modern tropical rainforests tend to occur along stream-sides and areas of secondary vegetation.

Macrofossil plant assemblages (such as those of the Branksome Sand Formation) would therefore tend to have a greater bias towards such temperate elements, resulting in an underemphasis of the tropical nature of the flora (Collinson & Hooker 1987). Daley (1972) argued that the flora of Early Eocene times must have experienced some seasonality at a palaeolatitude of approximately 40° N, and could not therefore be truly representative of a tropical forest flora. It is clear today, however, that the early interpretations of Reid (1933) and Chandler (1964) were most probably correct, as has been demonstrated for Eocene North American vegetation at palaeolatitudes of 45° N by Wolfe's CLAMP analysis (Wolfe 1993, 1995). Although less investigation has been carried out on the flora of the Branksome Sand Formation (above the London Clay Formation), there are many palaeofloristic similarities between the two that would appear to suggest that they lived in very similar 'equable' palaeoclimates (relatively free of seasonal and latitudinal extremes)

Table 1. Details of sampling strategy of fossil material

Species	Age	no. of localities	no. of specimens	observations per leaf	total <i>n</i> (counts)
<i>Litsea hirsuta</i>	Eocene	2	5	4–16	57
<i>Litsea bournensis</i>	Eocene	1	8	5–9	56
<i>Litsea edwardsii</i>	Eocene	2	6	2–15	51
<i>Litsea cinnamomifolia</i>	Eocene	1	4	3–6	18
<i>Litsea</i> sp. <i>A</i>	Eocene	1	4	3–10	25
<i>Litsea</i> sp. <i>phyllum mamillare</i> *	Mid. Jurassic	20	> 50	1–3	87
<i>Litsea</i> sp. <i>phyllum crucis</i> *	Mid. Jurassic	12	> 80	1–3	127
<i>Litsea</i> sp. <i>phyllum maculosum</i>	Mid. Jurassic	32	> 100	1–4	150
<i>Litsea</i> sp. <i>phyllum ordinatum</i>	Mid. Jurassic	13	> 70	1–4	87
<i>Litsea</i> sp. <i>phyllum kurrii</i>	Mid. Jurassic	19	> 140	1–4	170
<i>Litsea</i> sp. <i>phyllum furcata</i>	Mid. Jurassic	10	> 70	1–7	104
<i>Litsea</i> sp. <i>phyllum huttonii</i> *	Mid. Jurassic	12	> 150	3–10	210
<i>Litsea</i> sp. <i>phyllum kaneharai</i>	Mid. Jurassic	2	> 12	1–4	39
<i>Litsea</i> sp. <i>phyllum exilis</i>	Mid. Jurassic	1	> 10	1–3	23
<i>Litsea</i> sp. <i>phyllum sulcicaulis</i>	Mid. Jurassic	2	> 10	1–4	18

*Data from McElwain & Chaloner (1996).

under 1964). Furthermore, recent palaeofloristic investigations of the London Clay Flora (Collinson 1983) the Bracklesham Group Flora (Early and Middle Eocene) (Collinson 1996) indicated that the coastal vegetation of southern England during the Early and Middle Eocene was a *Nyssa*-dominated mangrove, including *Nyssa* and other Rhizophoraceae. The available literature therefore suggests that palaeoecology of the Branksome Sand Formation was one of a coastal tropical/subtropical climate, similar to the conditions which exist today in South-East Asia and Indo-Malaysia. The flora of the Branksome Sand Formation, which contained elements whose present day nearest living relatives are largely or almost exclusively tropical or subtropical (Anacardiaceae, Ericaceae, Menispermaceae, Arecaceae, Vitaceae), supports the tropical palaeoclimatic interpretations for the time.

MATERIALS AND METHODS

Middle Eocene material

For Middle Eocene fossil Lauraceae genera, *Lindera* and *Litsea*, were compared with modern *Lindera* and *Litsea* species. The fossil species investigated were as follows: *Litsea hirsuta* Bandulska; *Litsea bournensis* Bandulska; *Litsea edwardsii* Bandulska; *Lindera cinnamomifolia* Bandulska; and as yet unassigned species *Lindera* sp. *A*. These fossil species were compared in terms of their stomatal parameters to the following modern species: *Litsea sebifera* Pers., *Litsea glutinosa* (Lour.) C. B. Rob., *Litsea fuscata* Thw. n., *Litsea stocksii* Hook f., *Lindera meissneri* Hook f., *Lindera rufa* Hook f., *Lindera megaphylla* (Hemsl.) and *Lindera strychnifolia* (Sieb. and Zucc.) F. Vill. 'Recent' species were derived from herbarium specimens collected between 1800 and the 1930s. For the purpose of this particular study, therefore, fossil Lauraceae material has been compared with modern material spanning a range of early post-industrial CO₂ concentrations (derived from ice core data: Friedli *et al.* 1986; Barnola *et al.* 1987). In this paper, therefore, 'Recent' refers to early post-industrial

herbarium material which developed in ambient atmospheric CO₂ concentrations between 286 and 310 ppm. (See tables 1 and 2 for the details and sampling strategies of the species used for this investigation.)

The modern genus *Litsea* includes about 400 species, and is mainly represented in the Indo-Malay archipelago (Ohwi 1965), extending north to Japan, Korea and North America and south to New Zealand and subtropical South America (Kostermans 1957). Kostermans (1957) stated that the genus *Litsea* is widely distributed though not present in Africa and Europe. The genus is predominantly comprised of trees and shrubs with alternate or rarely subopposite leaves, which are pinnately or tripinnately nerved, coriaceous and evergreen or, rarely, thinner and deciduous (Ohwi 1965).

The modern genus *Lindera*, like *Litsea*, to which this genus is closely related, is widely distributed in the Himalayas, East Africa and western Malaysia. The genus is comprised of trees and shrubs that are coriaceous and evergreen or, rarely, thinner and deciduous, similar to those of *Litsea* (Ohwi 1965).

(b) Middle Eocene NLE selection

As extant species are generally not recognized in the fossil record earlier than the Late Tertiary, a 'nearest living equivalent (NLE) species' concept was introduced by McElwain & Chaloner (McElwain & Chaloner 1995) to provide some sort of platform from which pre-Tertiary fossil stomatal data could be compared with data from the present day. NLE species have been defined as plant species from the present day that show ecological and/or structural similarity to the fossil plants with which they are being compared (in terms of their stomatal parameters). It was noted, however, that this is quite distinct from the 'nearest living relative' (NLR) of Tertiary palaeobotanists, who use tolerances of NLRs as one factor in the interpretation of palaeoenvironments (McElwain & Chaloner 1995).

The modern species of *Litsea* and *Lindera* listed in table 1 were chosen as NLE species for comparison with the fossil

Table 2. Details of sampling strategy of Recent material

species	NLE species for the following fossils	age range	no. of leaves	observations per leaf	no. of leaves per plant (plant no.)	total <i>n</i> (counts)
<i>Litsea sebifera</i>	Eocene <i>Litsea</i>	1800–1885 AD	12	6–17	12 (8)	123
<i>Litsea fuscata</i>	Eocene <i>Litsea</i>	1860–1867 AD	3	10	1 (3)	30
<i>Litsea stocksii</i>	Eocene <i>Litsea</i>	1831–1930 AD	14	5–11	12 (13)	132
<i>Lindera meisneri</i>	Eocene <i>Lindera</i>	1918 AD	4	6–10	12 (3)	31
<i>Lindera rufa</i>	Eocene <i>Lindera</i>	1911 AD	1	10	1 (1)	10
<i>Lindera megaphylla</i>	Eocene <i>Lindera</i>	1887–1994 AD	23	5–13	13 (13)	213
<i>Lindera strychnifolia</i>	Eocene <i>Lindera</i>	1887 AD	1	10	1 (1)	10
<i>Ginkgo biloba</i> *	<i>Ginkgo huttonii</i>	1994 AD	20	9–10	10 (2)	199
<i>Araucaria heterophylla</i> *	<i>Brachyphyllum</i>	1994 AD	20	1–3	20 (1)	50
<i>Athrotaxis cupressoides</i>	<i>Pagiophyllum</i>	1995 AD	50	2–8	25 (2)	192
<i>Zamia furfuracea</i>	Jurassic <i>Ctenis</i>	1995 AD	2	9–11	2 (1)	20

*Data from McElwain & Chaloner (1996)

Litsea and *Lindera* species. This was based on the suggestion made in the systematic investigations of Bandulska (1926), and the fact that both fossil and modern species in question share similar ecological habitats and clearly possess very similar cuticular and gross morphological characteristics. In the early 1900s, Bandulska studied over 74% of the genera of the Lauraceae family (which comprises about 2000–2500 species and 31 genera (Hutchinson 1964)) in an attempt to assign a number of fossil leaf specimens from the Branksome Sand Formation to present-day genera. As noted earlier, the assignment of early Tertiary fossil remains to modern genera is unacceptable to some contemporary Tertiary palaeobotanists (Collinson 1986). The outcome of this change in scientific and systematic practice over the past century has resulted in some of Bandulska's species assignments being viewed as tenuous. According to an extensive study of extant Lauraceae leaves by Hill (1986), fossil lauraceous leaves may be placed with confidence in the family, but leaf morphology within the family is so variable that, with few exceptions, more detailed affinities are impossible to determine. The extant genera *Litsea* and *Lindera* can only be separated on the basis of the number of anther locules, *Litsea* possessing four and *Lindera* possessing two. These genera therefore cannot be separated on the basis of leaf morphological characteristics in isolation from floral detail.

Despite the taxonomic difficulties associated with the separation of genera within the Lauraceae, the family may be easily recognized in the fossil record. Hill (1986) and Bandulska (1926) defined a number of cuticular characteristics that are perceived to be typically lauraceous. These include (1) possession of a stomatal complex with guard cells embedded below the overarching subsidiary cells, and a cuticular scale occurring between them; (2) hair bases, where present, are simple; (3) subsidiary cells are paracytic with a thin cuticle and pale appearance; and (4) the stomatal pore has a slit-like appearance and therefore is not extremely obvious. The presence of these cuticular characteristics support Bandulska's assignment of the fossil specimens documented in this chapter to the Lauraceae, but her identifications below the familial level must be treated with some caution.

Bandulska (1926) carried out careful investigation of both leaf physiognomy (including leaf shape, venation, texture, cuticle thickness and anatomy) and cuticular structure (including the shape and size of stomatal and epidermal cells, their arrangement on both abaxial and adaxial cuticles, presence or absence of papillae and cutinized scales, and detailed investigation into the shapes of leaf bases). On the basis of Bandulska's investigations, the most suitable morphological matches were selected for the fossil specimens of this study which meet the first requirement of selecting an NLE species.

The second requirement for the selection of a suitable NLE species is that the Recent and fossil species share similar ecological habitats. It has been established from both the palaeobotanical and palaeoclimatological studies of the Branksome Sand Formation that the most suitable ecological equivalent for the fossil species would be of tropical/subtropical origin and preferably from the Indo-Malaysian, or south-east Asian regions (Reid & Chandler 1933; Chandler 1964; Bandulska 1926). All the Recent species chosen as NLE species comply with both of these ecological criteria. The systematic difficulties associated with the fossil specimens were addressed by comparing a number of fossil and NLE species within the two genera under investigation. This enabled comparison of the ranges of stomatal parameters between fossil and Recent Lauraceae, regardless of their genus or species assignments.

(c) Middle Jurassic material

McElwain & Chaloner (1996) investigated the stomatal parameters of three fossil species, *Brachyphyllum mamillare*, *B. crucis* and *Ginkgo huttonii*, from the Yorkshire Middle Jurassic. Seven additional fossil species are described here which have been added to the preliminary data set of McElwain & Chaloner (1996). They include one Ginkgoalean, *Baiera furcata* (Lindley and Hutton), three Coniferales (*Pagiophyllum kurrii* (Schimper), *P. ordinatum* Kendall, *P. maculosum* Kendall), and three Cycadales (*Ctenis sulcicaulis* (Phillips), *C. kanearai* Yokoyama and *C. exilis* sp. nov.).

(d) Middle Jurassic NLE selection

Zamia furfuracea was chosen from a preliminary investigation of six cycad species as the most suitable morphological

ecological equivalent for the three Middle Jurassic *Ctenis* species. According to Stewart (1983), the leaves of *Ctenis* and *Loxodendron* are very like those of *Zamia* and, in its natural habitat, *Z. furfuracea* is coastal, inhabits well-drained soils, and experiences a hot, tropical to subtropical climate (Stewart 1993). Harris (1964) suggests from the distribution of the remains, that all three *Ctenis* species most probably inhabited the well-drained sides of the delta and the wealthier fluvial facies (Hallam 1985; Krassilov 1981; Philippe & Levenard 1996) and geological (Frakes *et al.* 1992) evidence suggests hot, equable climates for the Middle Jurassic. On the basis of this information, *Z. furfuracea* has been chosen as the NLE species for *Ctenis kaneharai*, *C. sulcifera* and *C. exilis*.

While fossil cycads are similar to modern cycads in their fine structure, they differ significantly in their gross morphology (Harris 1961). Furthermore, based on the lack of associated evidence (i.e. stems, leaves, and cones), Harris (1961) questioned whether isolated specimens such as those of *Ctenis* were truly cycadean. The palaeobotanical evidence regarding Mesozoic cycads suggests that they experienced a wider morphological and climatic range, including deciduous and frost-tolerant species (Spicer *et al.* 1993), unlike the strictly tropical/tropical range they inhabit today. On the basis of these uncertainties regarding the Mesozoic cycads, the comparison of *Ctenis* and *Z. furfuracea* stomatal data must be treated with some caution.

Araucaria heterophylla, the Norfolk Island pine, was chosen as the NLE for the *Pagiophyllum* species. Ecologically, *A. heterophylla* is a coastal conifer in its native habitat. Harris (1979) suggested from his detailed study of the Yorkshire Jurassic flora that *Pagiophyllum ordinatum* and *P. rufum* most probably inhabited areas a few metres above floodplain, whilst the association of *Equisetum* with *P. rufum* suggests a riverbank habitat. Evidence of close microfossil associations with *P. ordinatum* following marine transgression which produced the Dogger Sea suggest that this species was subjected to coastal influences, therefore sharing a similar ecology to *A. heterophylla*. The leaves of *A. heterophylla* (mature foliage) and the genus *Pagiophyllum* share a close morphological resemblance (Stewart 1983). Both possess leaves that are densely arranged and, unlike *Brachyphyllum*, longer than they are broad, tapering to a point.

Stomatal parameters and standardizations

Stomatal densities and stomatal indices were calculated for all the species listed in tables 3 and 4 using the methodology of McElwain & Chaloner (1995, 1996). Stomatal parameters were investigated from Schulze cuticle preparations of the Eocene fossil material (prepared by Hulskamp; NHM, London) and of the Yorkshire Jurassic material (prepared by Harris; NHM, London). Investigation of the Recent material was from both silicone replicas and H₂O₂ glacial acetic acid macerations of the Recent material, where material was available, and the Jurassic species *Zamia furfuracea* and *Araucaria heterophylla* (see McElwain *et al.* (1995) for details of preparation technique). Most of the herbarium material from the Royal Botanic Gardens, Kew, and the NHM herbaria were investigated by means of silicone replicas, as this method is non-destructive. Leaves were removed from the

herbarium sheets for maceration only where numerous leaves were present on the particular specimen.

The stomatal ratio was calculated for all the fossil species listed in table 3, except *Baeira furcata* (for which no suitable NLE species could be selected), by taking a ratio of the mean stomatal index of each fossil species and the mean NLE stomatal index, or in the case of the Tertiary material, the mean generic NLE stomatal index (i.e. either *Litsea* or *Lindera*, depending on the genus of the fossil species being compared). The resultant stomatal ratios were then superimposed onto graphs of CO₂ estimates from published geochemical evidence, based on two different standards, in order to make semi-quantitative estimates of the palaeo-CO₂ concentration prevailing in Middle Eocene times.

The first standard, the 'Carboniferous standardization' was introduced by Chaloner & McElwain (1997). This standard was derived by setting the stomatal ratios of a Carboniferous conifer (*Swillingtonia denticulata*; 0.58) and a Permian conifer (*Lebachia frondosa*; 0.79), on to the Berner curve, thereby dictating the value of the stomatal ratio scale. This resulted in one stomatal ratio unit being equal to two RCO₂ units (a ratio of palaeo-CO₂ to that of the PIL of 300 ppm). To date, all stomatal data collected from the Palaeozoic (McElwain & Chaloner 1995) and Mesozoic (McElwain & Chaloner 1996) have been standardized by means of this Carboniferous standardization.

A second standard, the 'Recent standardization', is used in this paper and has been set by assuming that a stomatal ratio of 1 would have existed at the time of collection of the Recent Lauraceae material which existed in an atmospheric CO₂ concentration of approximately 300 ppm (RCO₂=1). This resulted in a scale which dictated that one stomatal ratio unit was equal to one RCO₂ unit. However, the NLE material of both Mesozoic and Palaeozoic fossils were collected in current ambient CO₂ concentrations of approximately 360 ppm. Therefore the Recent value standard dictated that one stomatal ratio unit was equal to 1.2 RCO₂ units. The Middle Eocene data has therefore been corrected by a factor of 1.2 in order to account for the fact that the Lauraceae NLE material was collected between 1800 and 1930. Data from previous investigations on Palaeozoic and Mesozoic fossil plant stomatal parameters (McElwain & Chaloner 1995, 1996; Chaloner & McElwain 1997) were subjected to this new standardization along with the additional Middle Jurassic species described here and the corrected Eocene data.

(f) Data analysis

The standard error of the mean (s.e.m.) stomatal density and index were calculated for all the fossil and Recent species listed in tables 3 and 4. Coefficients of variation (the standard deviation (s.d.) expressed as a percentage of the mean) were determined in order to assess the intraspecific variation of the stomatal density and index. Data were tested for normality using a one-sample Kolmogorov–Smirnov test and sample heterogeneity in Unistat v. 4 for Windows. All data were found to be normally distributed, and statistical analyses were carried out at the 95% confidence interval (CI). Standard ANOVAs were applied to test for any significant differences in both stomatal density and index between

Table 3. Details of fossil stomatal parameters and an assessment of their variation

(x , mean; σ , standard deviation; s.e., standard error; C_v , coefficient of variation (the s.d. expressed as a percentage of the mean). E, Middle Eocene; J, Middle Jurassic; P, Lower Permian; C, Upper Carboniferous; D, Lower Devonian.)

species	age	stomatal density (mm ⁻²)					stomatal index				
		x	σ	s.e.	n	C_v	x	σ	s.e.	n	C_v
<i>Litsea hirsuta</i>	E	315.1	37.9	5.0	57	12.0	10.7	1.4	0.3	18	13.1
<i>Litsea bournensis</i>	E	337.5	47.4	0.8	56	14.0	10.7	2.7	0.4	47	25.2
<i>Listea edwardsii</i>	E	271.3	33.6	4.7	51	12.4	10.4	3.4	1.4	6	32.7
<i>Lindera cinnamomifolia</i>	E	183.0	22.2	5.2	18	12.1	7.1	1.4	0.4	12	19.7
<i>Lindera</i> sp. A	E	322.0	35.2	7.0	25	10.9	10.9	1.9	0.4	21	17.4
<i>Brachyphyllum mamillare</i> *	J	34.2	15.4	0.2	87	45.1	5.7	2.5	0.3	62	43.8
<i>Brachyphyllum crucis</i> *	J	45.4	11.8	0.1	127	25.9	4.5	1.9	0.3	36	42.2
<i>Ginkgo huttonii</i> *	J	58.0	20.7	0.1	210	35.7	n/a	n/a	n/a	n/a	n/a
<i>Baeira furcata</i> (abaxial)	J	47.7	22.2	2.9	60	46.5	3.2	1.5	0.7	5	46.9
<i>Baeira furcata</i> (adaxial)	J	28.2	8.1	1.2	44	28.7	4.8	0.6	0.3	3	12.5
<i>Pagiophyllum maculosum</i>	J	49.8	12.3	1.0	150	24.7	5.5	1.4	0.3	15	25.4
<i>Pagiophyllum ordinatum</i>	J	49.9	8.8	0.9	87	17.6	6.3	2.0	0.4	29	31.7
<i>Pagiophyllum kurrii</i>	J	43.3	11.7	0.9	170	27.0	5.4	1.7	0.4	21	31.5
<i>Ctenis sulcicaulis</i>	J	61.9	9.4	2.2	18	15.2	n/a	n/a	n/a	n/a	n/a
<i>Ctenis kaneharai</i>	J	61.5	25.6	4.1	39	41.6	9.7	2.9	0.8	14	29.8
<i>Ctenis exilis</i>	J	84.9	29.5	6.2	23	34.7	13.4	2.4	0.2	16	17.9
<i>Lebachia frondosa</i> **	P	309.0	56.5	12.6	20	18.3	14.6	2.0	0.4	20	13.7
<i>Swillingtonia denticulata</i> **	C	787.8	158.9	37.5	18	20.2	19.7	4.3	1.2	14	21.8
<i>Aglaophyton major</i> **	D	4.5	3.9	0.8	23	86.7	2.2	1.1	0.2	19	50.0
<i>Sawdonia ornata</i> **	D	4.3	1.9	0.3	38	44.2	3.1	1.0	0.3	13	32.3

*Data from McElwain & Chaloner (1996).

**Data from McElwain & Chaloner (1995).

Table 4. Details of nearest living equivalent stomatal parameters and variance

(The species listed were the selected NLE species for fossil material from the following times: E, Middle Eocene; J, Middle Jurassic; P, Lower Permian; C, Upper Carboniferous; D, Lower Devonian. x , mean; σ , standard deviation; s.e., standard error; C_v , coefficient of variation (the s.d. expressed as a percentage of the mean).)

species	NLE for?	stomatal density (mm ⁻²)					stomatal index				
		x	σ	s.e.	n	C_v	x	σ	s.e.	n	C_v
<i>Litsea sebifera</i>	E	406.9	33.2	2.9	131	8.1	19.0	5.62	0.8	48	29.6
<i>Litsea fuscata</i>	E	454.0	41.9	7.6	30	9.2	18.3	6.01	2.1	82	32.0
<i>Litsea stocksii</i>	E	407.2	47.6	4.2	132	11.6	14.4	2.65	0.7	15	18.4
<i>Lindera meisneri</i>	E	337.7	35.6	5.6	41	10.5	18.1	4.76	1.2	16	26.3
<i>Lindera rufa</i>	E	497.3	36.8	11.7	10	7.4	n/a	n/a	n/a	n/a	n/a
<i>Lindera megaphylla</i>	E	307.0	30.6	2.1	223	9.9	15.6	3.99	0.8	22	25.6
<i>Lindera strychnifolia</i>	E	431.1	34.2	10.8	10	7.9	n/a	n/a	n/a	n/a	n/a
<i>Ginkgo biloba</i> *	J	84.8	19.5	0.1	198	22.9	n/a	n/a	n/a	n/a	n/a
<i>Araucaria heterophylla</i> *	J, C, P	140.7	26.7	3.8	50	18.9	11.5	4.2	0.9	20	36.5
<i>Athrotaxis cupressoides</i>	J	74.4	29.4	2.1	190	39.5	9.4	3.05	0.8	12	32.4
<i>Zamia furfuracea</i>	J	128	13.6	2.5	30	10.6	10.1	1.39	0.3	20	13.8
<i>Juncus effusus</i> **	D	309.1	72.8	10.3	50	23.6	12.4	2.3	0.4	50	18.5
<i>Psilotum nudum</i> **	D	40.1	16.8	1.4	145	41.9	15.5	5.6	0.6	92	36.1

*Data from McElwain & Chaloner (1995).

**Data from McElwain & Chaloner (1996).

and Recent material. An assessment of intraspecific variability (the variability in stomatal parameters within a single species) within Recent stomatal density and index was undertaken for two *Litsea* species, *L. stocksii* and *L. sebifera* and one *Lindera* species, *L. megaphylla*. This was achieved by including stomatal density and index derived from a number of (1) different positions on individual leaves, i.e. leaf apex, middle, and base; (2) different leaves from the same tree/shrub; and (3) leaves from different plants of the same species (both from the same habitat and time and from different habitats and times) within the total data set. The sampling strategy for this investigation is detailed in table 1. Interspecific variability (the variability in stomatal parameters between species) was assessed by comparing the coefficients of variation for each species detailed in table 4. Intrageneric variability in stomatal density (the variability in stomatal parameters between species within a single genus) was assessed by means of standard ANOVA in Unistat. High statistics produced by ANOVA indicated high variation between species within a genus.

RESULTS

Middle Eocene

The mean density and index values for the Recent NLE species were consistently higher than those of the Middle Eocene (tables 3 and 4). Statistical analysis (ANOVA) demonstrated that both Recent *Litsea* and *Lindera* genera possessed significantly higher stomatal densities ($F=65.016$, $p<0.0001$, and $F=78.166$, $p<0.0001$, respectively) than fossil *Litsea* and *Lindera* genera. Highly significant differences were also observed between fossil NLE *Litsea* and *Lindera* stomatal indices ($F=51.639$, $p<0.0001$, and $F=66.330$, $p<0.0001$, respectively). The variation within fossil genera of both *Litsea* and *Lindera* was significant ($F=52.917$, $p<0.0001$), as was that observed within NLE *Litsea* and *Lindera*. This within-species variability was, however, found to be insignificant in relation to the difference observed between fossil and Recent material.

Figure 1 plots the density versus index data for both fossil and NLE species within the genus *Litsea*, and it clearly demonstrates that fossil *Litsea* stomatal indices were consistently lower than those of their selected NLE species and that there were two populations of stomatal density within the Recent genus *Litsea*. Despite the fact that one of these populations possessed lower stomatal density values than the fossil *Litsea* species, the modern stomatal index values were collectively higher, and significantly so, than the fossil index values.

Figure 2 plots density versus index data for both fossil and Recent species within the genus *Lindera*. The relationship between fossil and NLE species stomatal parameters is very clear. Fossil *Lindera* species possessed consistently lower stomatal density and index values than their selected NLE species, with the exception of two outliers. These outliers demonstrate very low stomatal index values despite their high density values, which were most probably caused by environmental factors other than atmospheric CO₂, that affected the sizing and spacing of the epidermal cells but not the actual development of stomata from epidermal cells (which would alter the stomatal index).

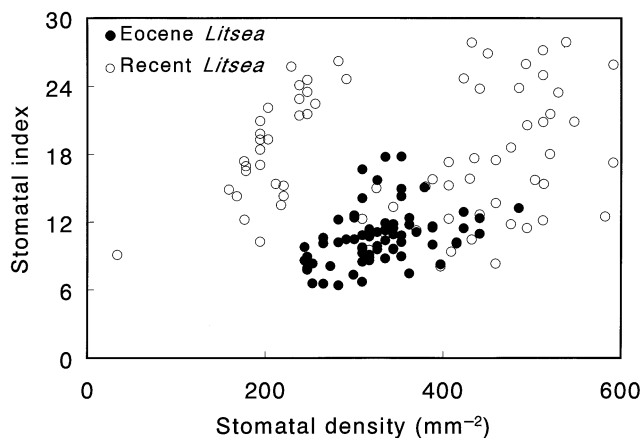


Figure 1. A comparison of Middle Eocene and Recent *Litsea* stomatal density and index. Each point represents a single stomatal density and index count.

The intraspecific variation within stomatal density was found to be low, irrespective of sample size, position on individual leaves, number of leaves sampled per plant, or number of plants sampled per species, for the three species investigated; *Litsea sebifera*, *L. stocksii* and *Lindera megaphylla*. In general, the coefficient of variation of stomatal density was greater for the fossil material than for the Recent material (tables 3 and 4). The coefficient of variation of stomatal index was greater than the variation observed in stomatal density for all species, both fossil and Recent (tables 3 and 4). Intrageneric variability in stomatal density was found to be highest in Recent *Lindera*, followed by fossil *Lindera*, fossil *Litsea* and finally Recent *Litsea*, as revealed by the *F* statistic.

(b) Middle Jurassic

Recent NLE species stomatal parameters were found to be consistently and significantly higher ($p<0.0001$ at the 95% CI) than the fossil material, which confirms McElwain & Chaloner's (1996) earlier observation for two Middle Jurassic conifers (*Brachyphyllum mamillare* and *B. crucis*) and one Ginkgoalean (*Ginkgo biloba*) compared with their nearest living equivalents.

8. DISCUSSION

(a) Middle Eocene

Highly significant differences were observed between fossil and NLE species' stomatal densities and indices (figures 1 and 2; tables 3 and 4). The fossil data comprised stomatal density and index counts from a number of different species, each collected from two-to-three different localities within the Branksome Sand Formation, and which therefore most probably originated from a varied set of ecological settings. Moreover, the modern data set included stomatal densities and indices from a wide range of altitudes, geographical areas (as indicated in table 1), and ecological settings. Despite the fact that the sampling strategy of both fossil and Recent data sets encompassed many factors which could drive or influence natural variation in stomatal density and index, a clear trend of low stomatal density and index was observed for the fossil species. Conversely, one of relatively high densities and high indices is clear in the Recent material

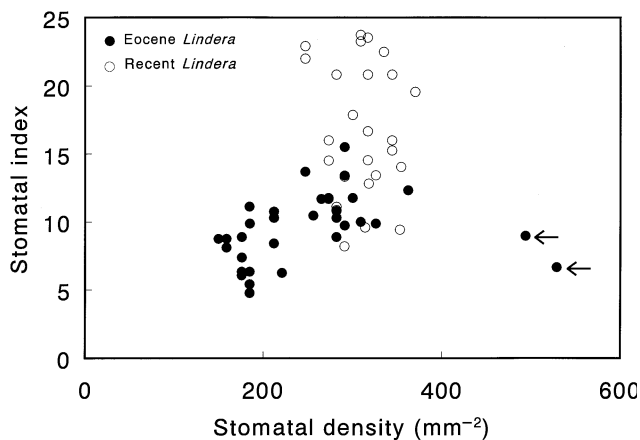


Figure 2. A comparison of fossil and Recent *Lindera* stomatal density and index. Each point represents a single stomatal density and index count. The outliers (arrows) marked on the graph belong to specimen number V396, from the Bandulka collection in the Natural History Museum, London.

(figures 1 and 2). The data consistently support the suggestion from geochemical evidence (Freeman & Hayes 1992; Berner 1993, 1994; Cerling 1993) that the atmospheric CO₂ concentration during Middle Eocene times was elevated compared to the pre-industrial ambient concentration (figure 3). The resultant ranges of CO₂ for the Middle Eocene according to the Carboniferous standardization palaeo-CO₂ concentrations were 3.2–5.8 times the PIL. These may be considered to represent the upper limits of the CO₂ concentration suggested by the stomatal ratio data, and those of the present-day standardization (figure 3), which yield CO₂ concentrations of 1.4–3 times the PIL, considered as the lower limits of the estimates. However, until the relationship between known CO₂ concentrations and stomatal density/index of *Lindera* and *Litsea* is constrained in the present day, these estimates of Eocene palaeo-CO₂ will remain semi-quantitative. These stomatal ratio derived CO₂ estimates are also consistent with marine isotopic temperature curves for ocean surfaces (Shackleton & Kennett 1975), and those from benthic molluscan shells (Buchardt 1978), as well as for terrestrial derived temperature curves from leaf physiognomy (Wolfe 1995; Dorf 1964). It is entirely possible that these semi-quantitative estimates of Middle Eocene CO₂ concentration are valid, as they are in good agreement with the modelling data of Worsley *et al.* (1994), which imply palaeo-CO₂ levels of 3.5–4.0 times the PIL.

(b) *Middle Jurassic*

McElwain & Chaloner (1996) demonstrated that the stomatal parameters of three species from the Yorkshire Middle Jurassic provided a morphological signature of elevated atmospheric CO₂ during the Middle Jurassic. This observation was found to be consistent with geochemical evidence (Cerling 1993) and modelling results (Berner 1994). The stomatal data reported here from seven additional Yorkshire Middle Jurassic species confirms the results of this preliminary investigation. The range of stomatal ratios from the preliminary study (McElwain & Chaloner 1996), and the new data described in this paper (figure 4), suggest that Middle Jurassic CO₂

concentrations ranged between 3.3 and 4.2 times the PIL, according to the Carboniferous standardization, or 2–2.5 times the PIL, according to the Recent standardization. It is interesting to note that the range of stomatal ratios for all ten species investigated from this time period was very narrow (see figure 4), therefore providing a relatively narrow band of palaeo-CO₂ estimates. A sampling strategy which incorporated both spatial and temporal variation in stomatal parameters was achieved by studying fossil specimens from many localities within a 10 Ma interval (of the Yorkshire Middle Jurassic). No statistically significant differences in stomatal parameters were observed between fossil localities or ages in relation to those observed between fossil and NLE plant species.

(c) *General discussion*

During periods of suggested elevated atmospheric CO₂ (Berner 1993, 1994; Cerling 1993; Freeman & Hayes 1992), such as the Middle Eocene, Jurassic and Early Devonian, significantly lower stomatal densities and indices were consistently observed among the 15 fossil species investigated compared with those of their NLE species (figure 4). Conversely, the Carboniferous and Permian species which, according to geochemical and modelling data, were believed to have existed in palaeo-atmospheres similar to those of today (Berner 1994; Mora *et al.* 1996), possessed somewhat higher stomatal densities and indices than their NLE species. This would suggest that Late Carboniferous and Early Permian palaeoatmospheric CO₂ levels may have been lower than previously believed. It is apparent from these data that fossil plant stomatal parameters yield palaeo-CO₂ estimates that are broadly consistent with the values determined from geochemical evidence and carbon cycle modelling results.

Figure 4 demonstrates a strong correlation between the palaeo-CO₂ estimates derived from Berner's long-term carbon cycle model and those derived from the stomatal ratios of fossil plants irrespective of the calibration used to set the stomatal ratio scale. The stomatal ratios of the Jurassic species fall between the very high stomatal ratios of the Early Devonian plants and the very low stomatal ratios of the Carboniferous and Permian species. Second, the palaeoatmospheric CO₂ estimates derived from fossil plant stomatal parameters from all four time periods investigated fall within a tight range compared with the values yielded by carbon isotopic investigations (Cerling 1993; Mora *et al.* 1996), marine (Freeman & Hayes 1992; Sinha & Stott 1994) and carbon cycle modelling (Berner 1993, 1994).

This investigation set out to establish a sampling strategy for both stomatal densities and stomatal indices of fossil and Recent leaf material that would incorporate as much of the inherent variation within these parameters as possible. Such a sampling strategy addresses current criticism regarding the interpretation of palaeo-CO₂ concentrations based on stomatal characters which, according to (Poole *et al.* 1996) should be 'treated with extreme caution' due to intrinsic environmentally controlled variability of stomatal density. These results indicate that, despite phenotypically and genotypically controlled differences in stomatal density and stomatal index for a single species, fossil stomata provide a signal of the palaeo-CO₂ concentration in which they developed.

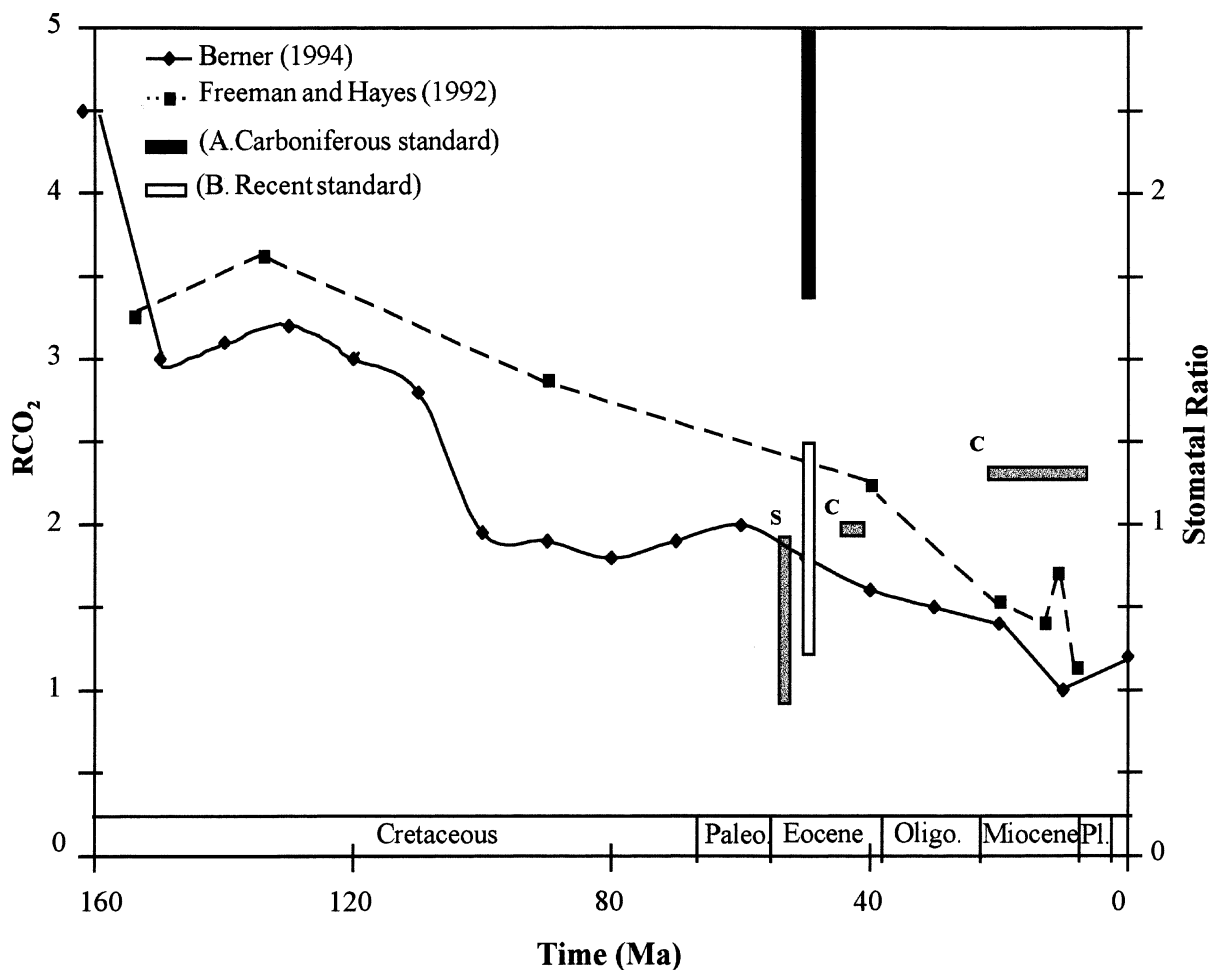


Figure 3. A comparison of Eocene Recent Lauraceae stomatal ratio (shaded bar represents the range of stomatal ratios exhibited by the Middle Eocene species listed in table 3) with the palaeosol isotopic evidence of Cerling (1992) (C) and Sinha & Stott (1994) (S) and the marine carbon isotopic evidence of Freeman & Hayes (1992) (dashed line). All data have been superimposed on Berner's long-term carbon cycle computer model (solid line). RCO_2 is a ratio of the CO_2 concentration predicted by GEOCARB II (Berner 1994) to the pre-industrial atmospheric CO_2 concentration of 300 ppm. The stomatal ratio (SR) scale for (a), the Carboniferous standard (solid black rectangle) was derived by setting the stomatal ratios of Carboniferous and Permian conifers, *Wongia denticulata* (SR=0.58) and *Lebachia frondosa* (SR=0.79), which were believed to have existed in CO_2 concentrations similar to those of today (350–400 ppmv), on the Berner curve, thereby dictating the value of the stomatal ratio scale. This standard is based on one stomatal ratio unit being equal to two RCO_2 units. The stomatal ratio scale for (b), the Recent standard (unfilled rectangle) was set by assuming that a stomatal ratio of one would have existed at the time of collection of the Recent material, which existed in atmospheric CO_2 concentrations of 280–300 ppmv (an RCO_2 =1). Therefore, one RCO_2 unit is equal to one stomatal ratio unit for (b).

in order to eliminate as much potential variation as possible caused by environmental factors other than CO_2 , it is essential to gather large data sets of fossil and Recent stomatal densities and, where possible, to calculate stomatal index.

Kurschner *et al.* (1996) noted that the fossil record may have a certain bias for the preservation of 'sun' leaves, which was originally observed in taphonomic studies in modern depositional environments by Spicer (1975, 1980, 1981). Sun morphotypes typically possess stomatal densities that are 60% higher than shade morphotypes as a direct result of high epidermal cell densities, caused by a reduction in lateral epidermal cell expansion (Kurschner *et al.* 1996). The preponderance in the fossil record of sun morphotypes, having high stomatal densities, would therefore bias the interpretation of stomatal density and, to a certain degree, stomatal index values. However, despite this

taphonomic bias in the fossil record, Early Devonian, Middle Jurassic and Middle Eocene stomatal densities and indices were found to be significantly lower than their nearest living equivalents, therefore implying elevated atmospheric CO_2 .

Stomatal data from fossil plants provide a useful insight into how plants responded to extreme fluctuations in atmospheric CO_2 concentrations during geological time. Such research provides a tool with which to help predict future responses of plants to elevated atmospheric CO_2 in terms of their stomatal parameters and water use efficiency, but also presents one of the only biological means to date of estimating palaeo- CO_2 concentrations. The fact that biological, physical/chemical and modelling data are in such good agreement provides further evidence that atmospheric CO_2 fluctuated widely over geological time.

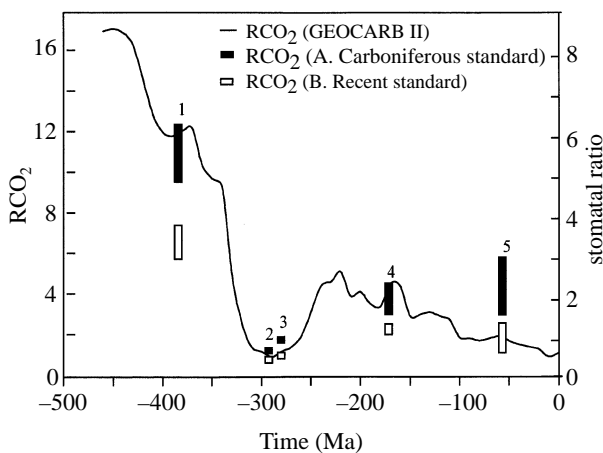


Figure 4. Estimates of palaeoatmospheric CO₂ for the Phanerozoic calibrated from the stomatal ratios (black solid bars) of the fossil plants listed in table 3 compared with model estimates of Berner (1994; GEOCARB II). (1) includes the stomatal ratios of *Aglaophyton major* (6.2) and *Sawdonia ornata* (4.8); (2) the stomatal ratio of *Swillingtonia denticulata* (0.58); (3) the stomatal ratio of *Lebachia frondosa* (0.79); (4) stomatal ratios of *Brachyphyllum mamillare* (1.65), *B. crucis* (2.08), *Pagiophyllum kurrui* (2.11), *P. ordinarum* (1.83), *P. maculosum* (2.09); and (5) stomatal ratios of *Litsea hirsuta* (1.61), *L. bournensis* (1.68), *L. edwardsii* (1.65), *Lindera cinnamomifolia* (2.54) and *L. sp. A.* (1.65). (a) The derivation of the Carboniferous standardization has been described for figure 3a. (b) The Recent standardization was set by assuming that a stomatal ratio of one would have existed at the time of collection of the Recent Lauraceae material, which existed in an atmospheric CO₂ concentration of approximately 300 ppm (RCO₂=1). This resulted in a scale which dictated that one stomatal ratio unit was equal to one RCO₂ unit. However, the NLE material of both Mesozoic and Palaeozoic fossils was collected in current ambient CO₂ concentrations of approximately 360 ppm. Therefore, the Recent standardization dictated that one stomatal ratio unit was equal to 1.2 RCO₂ units. The Middle Eocene data has therefore been corrected by a factor of 1.2 in order to account for the fact that the Lauraceae NLE material was collected between 1800 and 1930 (CO₂ concentrations between 286 and 310 ppm).

9. GENERAL CONCLUSIONS

(1) Fifteen fossil plant species collected from numerous global localities, from the Middle Eocene, Middle Jurassic, Early Permian, Late Carboniferous and Early Devonian, were compared with 13 Recent species in terms of their stomatal density, stomatal index and stomatal ratio. Members of the Ginkgoales, Coniferales, Cycadales and Angiospermales were represented in the sample set of both fossil and Recent plants. The basis of comparison between fossil and Recent species was on ecological and/or morphological similarity for all the time periods investigated, except for the Middle Eocene where systematic relatedness was employed in addition to morphological and ecological similarity.

(2) The palaeo-CO₂ estimates provided by fossil plant stomatal parameters are also in good agreement with palaeotemperature reconstructions from the Palaeozoic (Frakes *et al.* 1992), Mesozoic (Hallam 1985) and Cenozoic

(Wolfe 1995). These data may be used by general circulation climate analogue models, such as UGAMP (Valdes & Sellwood 1992), which are used to improve the predicted estimates of future greenhouse-induced global climate change.

(3) These stomatal data provide useful information on how fossil plants responded to large-scale fluctuations in atmospheric CO₂ in the geological past in terms of their water use efficiency (as deduced from changes in their stomatal density). This knowledge is important in the light of the investigation into plant responses to the current anthropogenically driven rise in atmospheric CO₂ (Rozeema *et al.* 1991) and the global temperature rise which is predicted to ensue (Houghton *et al.* 1995). An improvement in plant water use efficiency under enriched CO₂ levels would certainly be advantageous, if the predicted 1.5–4 °C rise in global mean temperature (Houghton *et al.* 1995) does occur due to the doubling of current atmospheric CO₂ concentration.

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Discussion

F. WAGNER (*Laboratory of Palaeobotany and Palynology, Utrecht University, The Netherlands*). My question refers to the Carboniferous and Permian conifers, the stomatal frequencies of which you have studied. Some conifers have stomata arranged in bands on their leaves. Is it possible that under changing CO₂ conditions, the plants may adapt by developing additional stomata-bearing bands, e.g. on the upper surface of the needle, as observed in the modern species *Abies alba*? And, if so, would this adaptation lead to an overall increase in the stomatal density per leaf area which may not necessarily cause an increase in stomatal density per mm² on a fixed part of the needle?

J. C. McELWAIN. I agree with Dr Wagner's comment that conifers may adapt to elevated CO₂ conditions by means of a redistribution of their stomata. This response has been demonstrated for both conifer (Van de Water *et al.* 1994) and angiosperm species (Ferris & Taylor 1994). It is therefore important to monitor any changes in the number of stomatal bands or rows on both the abaxial and adaxial leaf surfaces, in order to obtain a realistic picture of a particular plant's stomatal density response. The Permian and Carboniferous conifers reviewed in this study were both hypostomatous as was their selected NLE species *Araucaria heterophylla*. The stomata of these three conifer species occurred in two broad bands on their abaxial surfaces. Both the stomatal density within the stomatal bands and the stomatal density per whole leaf were taken into account during data collection so as to remove any bias which would have resulted from an altered stomatal distribution. Furthermore, observations of all three conifers revealed that stomata were absent from the adaxial surfaces.

W. M. KÜRSCHNER (*Laboratory of Palaeobotany and Palynology, Utrecht University, The Netherlands*). I am interested in the palaeoatmospheric CO₂ (pCO₂) level, you suggest for the Middle Eocene by means of stomatal analysis of Lauraceae leaves. Your data indicate a pCO₂ concentrations 3–4 times higher than present-day level. By contrast, results from modelling exercises of the long-term global carbon budget by Berner and coworkers indicate a pCO₂ level of about twice the modern CO₂ level. Moreover, you are coming up with values similar to the modelled pCO₂ level after you have introduced a correction factor in your original data set. Could it be possible, however, that your initial interpretation is correct because the leaves originated from a dense (sub)tropical forest, where the atmospheric CO₂ concentration within

canopy is affected by plant CO₂ assimilation, respiration and degradation of organic matter? Several studies (Van der Merwe & Medina 1989; Bazzaz & Williams 1991) have demonstrated that the atmospheric CO₂ concentration can vary significantly within a dense canopy, with elevated values up to six times higher than bulk atmospheric CO₂. Consequently, the leaves you studied indeed experienced such high CO₂ values in ambient air of their immediate surroundings, and not therefore a local, but authentic, atmospheric signal other than the bulk of atmospheric CO₂ level at that time.

McELWAIN. First, the palaeoatmospheric CO₂ estimates I have derived from Eocene Lauraceae fossil leaves are merely semi-quantitative estimates and need further validation in order to quantify them fully. However, despite the fact that they require further standardization, they are in good agreement with the modelling data of Lloyd *et al.* (1994) who suggest levels of 3.5–4 times the industrial level for the Middle Eocene.

Second, to address the question of whether the palaeoatmospheric setting of the fossil Lauraceae species were uncoupled from the global CO₂ environment of the Middle Eocene, one has to consider the taphonomic environment of the 'dense tropical forest' to which Kürschner refers. Dense forest environments experience a range of both temporal (on a daily and seasonal basis) and spatial (height in the canopy) variations in CO₂ concentrations. These variations in CO₂ concentration are driven by plant assimilation, respiration and degradation of organic matter, but are also significantly influenced by geographical setting. According to Kürschner, CO₂ concentration in these forests can be up to six times the ambient concentration, however, these concentrations are low at night when the plants are not photosynthesizing at forest-floor level. In this situation, seedling development is likely to be effected, but not leaf development in early Lauraceae trees and shrubs as sampled in this study. The forest floor is most likely to be uncoupled from the global atmosphere because of CO₂ fluxes from the soils and lack of mixing. However, from 0.5–1 m above the forest floor, which should be representative of an understory vegetation, typical minimum local CO₂ concentrations being between 12% (Schmamm *et al.* 1996) for temperate forests and 30% (Grace *et al.* 1995) for an Amazonian tropical forest above the global ambient level have been recorded. Bazzaz & Williams (1991) actually measured reduced CO₂ concentrations at 1 m in the canopy during times of active photosynthesis.

It is important to note that the potential of a forest stand to be uncoupled from the global atmosphere also relies heavily on the meteorology of the region and the geographical location, as mentioned earlier. According to Grace *et al.* (1995), some regions experience significantly elevated CO₂ concentrations as a result of mesoscale meteorology. We may conclude, therefore, that the CO₂ profile of the relatively unmixed Amazonian rainforest cannot be directly compared with the coastal subtropical/tropical climate type vegetation that existed in the South of England in the Middle Eocene. Proximity to the coast and the presence of many rivers would surely have stimulated more ventilation of the canopy environment. Finally, taphonomic studies have revealed (M. E. Collinson, personal communication; Wolfe 1979) that there is a preponderance of leaves of

trees from stream sides and forest margins to be incorporated into depositional environments. This implies that fossil leaves most probably originated from sites that would not have experienced much stratification in their local CO₂ environment.

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P. F. VAN BERGEN (School of Chemistry, University of Bristol, UK). Have you considered that low stomatal densities observed in the early land plants may be more related to the fact that these plants had only 'recently' developed water transport systems, and hence only small numbers of pores, to prevent excess water loss rather than reflecting their ambient CO₂ levels?

J. C. McELWAIN. It is indeed very important to consider evolutionary changes in the role of stomata (with regard to physiological functioning) in the early vascular plants. Dr van Bergen states that the early vascular plants 'had only recently developed water transport systems, and hence only small numbers of stomatal pores, to prevent excess water loss.' First, Dr van Bergen implies from this statement that early land plant stomata were functioning in terms of their control of evapotranspirational loss of water in a similar manner as plants do today. Edwards (1993) reviewed the cells and tissues of early land plants and concluded that early land plant stomata appeared to resemble modern extant plants in both their structure and function. Assuming, therefore, that the first stomata operated in the same way as they do in plants today, one could equally argue that the control of water loss by 'few stomata' in Lower Devonian plants was only possible because of the high palaeoatmospheric CO₂ concentration in which these plants developed. An elevated CO₂ atmosphere, demonstrated by both long-term carbon cycle modelling (Berner 1994) and carbon isotopic analysis of pedogenic carbonate (Mora *et al.* 1996), would have enabled these plants to achieve the same carbon gain for photosynthesis with very few stomatal pores, thus enabling the plant to improve its water use efficiency. It is clear from the studies of early land plants that overcoming problems of water relations was the key to the establishment of a terrestrial flora in the Early Palaeozoic. Perhaps elevated Palaeozoic CO₂ concentrations were key to the development of a terrestrial flora, as they enabled such a tight control of plant water use efficiency which, along with the development of a protective waxy cuticle, and conducting tissues, were essential in order to maintain an internal hydrated environment (i.e. a homiohydric habit?)