Changes in atmospheric CO_2 concentrations and climate from the Late Eocene to Early Miocene: palaeobotanical reconstruction based on fossil floras from Saxony, Germany

A. Roth-Nebelsick [a],*, T. Utescher [b,1], V. Mosbrugger [c,2],
L. Diester-Haass [d,3], H. Walther [e,4]

[a] Institute for Geosciences, University of Tübingen, Germany
[b] Institute for Geology, University of Bonn, Germany
[c] Institute for Geosciences, University of Tübingen, Germany
[d] Zentrum für Umweltforschung, Saarland University, Germany
[e] Staatliches Museum für Mineralogie und Geologie, Landesmuseum des Freistaates Sachsen, Germany

Abstract

In the present study, proxy data concerning changes in atmospheric CO_2 and climatic conditions from the Late Eocene to the Early Miocene were acquired by applying palaeobotanical methods. Fossil floras from 10 well-documented locations in Saxony, Germany, were investigated with respect to (1) stomatal density/index of fossil leaves from three different taxa (*Eotrigonobalanus furcinervis*, *Laurophyllum pseudoprinceps* and *Laurophyllum acutimontanum*), (2) the coexistence approach (CA) based on nearest living relatives (NLR) and (3) leaf margin analysis (LMA). Whereas the results of approach (1) indicate changes in atmospheric CO_2 concentration, approaches (2) and (3) provide climate data. The results of the analysis of stomatal parameters indicate that the atmospheric CO_2 concentration was higher during the Late Eocene than during the Early Oligocene and increased towards the Late Oligocene. A lower atmospheric pCO_2 level after the Late Eocene is also suggested by an increase in marine palaeoproductivity at this time. From the Late Oligocene onwards, no changes in atmospheric CO_2 concentration can be detected with the present data. For the considered sites, the results of the coexistence approach and of the leaf margin analysis document a significant cooling event from the Late Eocene to the Early Oligocene. The pCO_2 decrease from the Late Eocene to the Early Oligocene indicated by the stomatal data raised in this study was thus coupled to a temperature decrease which is reflected by the present datasets. From the Early Oligocene onwards, however, no further fundamental climate change can be inferred for the considered locations. The pCO_2 increase from the Early Oligocene to the Late Oligocene, which is indicated by the present data, is thus not accompanied by a climate change at the considered sites. A warming event during the Late Oligocene is, however, recorded by marine climate archives. According to the present data, no

* Corresponding author. Institut und Museum fur Geologie und Palaontologie, Universitat Tubingen, Sigwartsstrasse 10, Tubingen D-72076, Germany. Tel.: +49-7071-2973561; fax: +49-7071-295217.

E-mail addresses: anita.roth@uni-tuebingen.de (A. Roth-Nebelsick), utescher@uni-bonn.de (T. Utescher),
volker.mosbrugger@uni-tuebingen.de (V. Mosbrugger), a.l.haass@t-online.de (L. Diester-Haass), H.Walther.Dresden@t-online.de (H. Walther).
[1] Tel.: +49-228-739773; fax: +49-228-739037.
[2] Tel.: +49-7071-2972489; fax: +49-7071-295727.
[3] Tel.: +49-681-30264145; fax: +49-6841-171774.
[4] Tel.: +49-351-8926403.

change in pCO$_2$ occurred during the cooling event at the Oligocene/Miocene boundary, which is also indicated by marine data. The quality and validity of stomatal parameters as sensors for atmospheric CO$_2$ concentration are discussed.

1. Introduction

It has long been stated that CO$_2$ plays a key role in the Earth's climate changes because it represents a greenhouse gas leading to back radiation of infrared radiation (Arrhenius, 1896; Chamberlin, 1898). Intervals of high temperature are thus expected to represent intervals of high atmospheric CO$_2$ concentration or CO$_2$ partial pressure (pCO$_2$) (Crowley and Berner, 2001). The relationship between pCO$_2$ and global temperature is of high general interest particularly due to the recent anthropogenically induced rise of atmospheric CO$_2$ as monitored by direct atmospheric measurements such as at Mauna Loa since 1958 or ice core data (Neftel et al., 1985; Keeling, 1993). These data sources provide a direct access to atmospheric CO$_2$ content during time. The recent limit for direct determination of past atmospheric CO$_2$ concentration is approximately 420,000 years with data supplied by the Vostoc ice core (Petit et al., 1999). There is thus no direct means to determine atmospheric CO$_2$ concentration for time periods older than approximately 400,000 years.

There is general agreement about a global greenhouse condition across the Palaeocene/Eocene boundary with a climatic optimum during the Early Eocene (for example, Miller et al., 1987; Zachos et al., 1994). The global temperature then decreased continuously during the Eocene towards a glaciation event at the early Early Oligocene (Prothero and Berggren, 1992). Periods of warming and cooling then followed until a local climatic optimum was reached in the Middle Miocene (Zachos et al., 2001). Because these geological time periods date further back than 400,000 years before present (BP), pCO$_2$ during these time intervals has to be reconstructed by indirect evidence. This can be achieved by (1) modelling of the global CO$_2$ budget (Berner and Kothavala, 2001; Wallmann, 2001) or (2) by using palaeoatmospheric pCO$_2$ proxies. Biogeochemical proxy data comprise the deter-

mination of isotope ratios of marine carbonates (Popp et al., 1989; Freeman and Hayes, 1992) or soil carbonates (Cerling, 1991, 1999; Mora et al., 1996) and boron isotope ratios of shells (Pearson and Palmer, 1999, 2000). These different methods are summarized and discussed in, for example, Royer et al. (2001) and Boucot and Gray (2001). A palaeobotanical approach for determining past CO$_2$ concentration, which is utilized in this study, is based on the fact that, in many plant species, the density of the stomata (pores on the plant surface, usually on the leaves if present) per surface area decreases with increasing pCO$_2$ (Woodward, 1987; Beerling et al., 1993; van der Burgh et al., 1993; Kürschner, 1996). The general trend of increasing stomatal density with decreasing pCO$_2$ appears to be a stable phenomenon, especially on the long-term scale (however, not all species are sensitive to pCO$_2$ with respect to stomatal parameters; Royer, 2001). An increase or decrease of stomatal parameters is therefore expected to indicate a decrease or increase in pCO$_2$.

Based on these proxy data, several authors have suggested that the pCO$_2$ during the greenhouse period in the Eocene was higher than today (e.g., Berner, 1991; Arthur et al., 1991; Freeman and Hayes, 1992; McElwain, 1998; Kürschner et al., 2001). Recent results based on different methods mentioned above indicate, however, that, at least during parts of the Early, Middle and Late Eocene, pCO$_2$ was similar to recent values (boron isotope ratios of ancient planktonic foraminifera—Pearson and Palmer, 1999, 2000; stomatal parameters—Royer et al., 2001). By using boron isotope ratios of ancient planktonic foraminifera, Pearson and Palmer (2000) recorded a pCO$_2$ peak during the early Mid-Eocene cooling event. Additionally, their data do not reflect a long-term drop of pCO$_2$ during the Middle Miocene when global temperature decreased (Pearson and Palmer, 2000). Pagani et al. (1999) used carbon isotope analysis for CO$_2$ reconstruction during the Late Oligocene and the Miocene.

They found no evidence for a high atmospheric CO_2 concentration during the climatic optimum of the late Early Miocene. The results of Pagani et al. (1999, 2000) and of Royer et al. (2001) concerning the Miocene confirm each other by using different proxy data. Furthermore, the results of Pagani et al. (1999) suggest an increase in pCO_2 following the strong expansion of the East Antarctic ice shield. Results of Ekart et al. (1999) based on isotope data from palaeosols indicate that $pCO2$ was not constant throughout the Palaeogene/Neogene with higher levels during the Eocene and Oligocene. The biogeochemical model GEOCARB (Berner and Kothavala, 2001) indicates a general decline in CO_2 and a pCO_2 level slightly higher than today during the Palaeogene and Neogene. It shows, however, both a considerable error range as well as a low temporal resolution.

The recent studies concerning climate change and pCO_2 during the Palaeogene/Neogene based on proxy data summarized so far thus cast doubt on an inevitable "cause-and-effect" coupling between global temperature and pCO_2 and suggest an at least partial decoupling between the two. The discrepancies between the results based on isotopic studies and those based on stomatal proxy data, as well as discrepancies between different stomatal data themselves, may also be due to the following methodological reasons: (1) low stratigraphic resolution (essential for events of short duration such as the Initial Eocene Thermal Maximum); (2) possible uncertainties and broad error ranges of isotopic data; (3) sampling size, variance and therefore statistical background noise for stomatal data (Beerling and Royer, 2002a). A special problem of stomatal parameters exists with high pCO_2. The response of stomatal density and index becomes nonlinear with pCO_2 levels higher than present, and many species react insensitively in this case (Woodward and Bazzaz, 1988; Royer et al., 2001; Beerling and Royer, 2002b). It has been argued that genetic adaptation is probably the reason for the so-called "CO_2 ceiling", which is observed in short-time experiments (Kürschner et al., 1997). Low pCO_2 levels prevailed during the Neogene, and it can therefore be assumed that the extant flora is genetically adapted to this condition (Kürschner et al., 1998; Beerling and Royer, 2002b).

Many sets of different proxy data dealing with climate and pCO_2 during the Palaeogene/Neogene show temporal gaps and/or provide only data for temperature or pCO_2 (e.g., Pearson and Palmer,

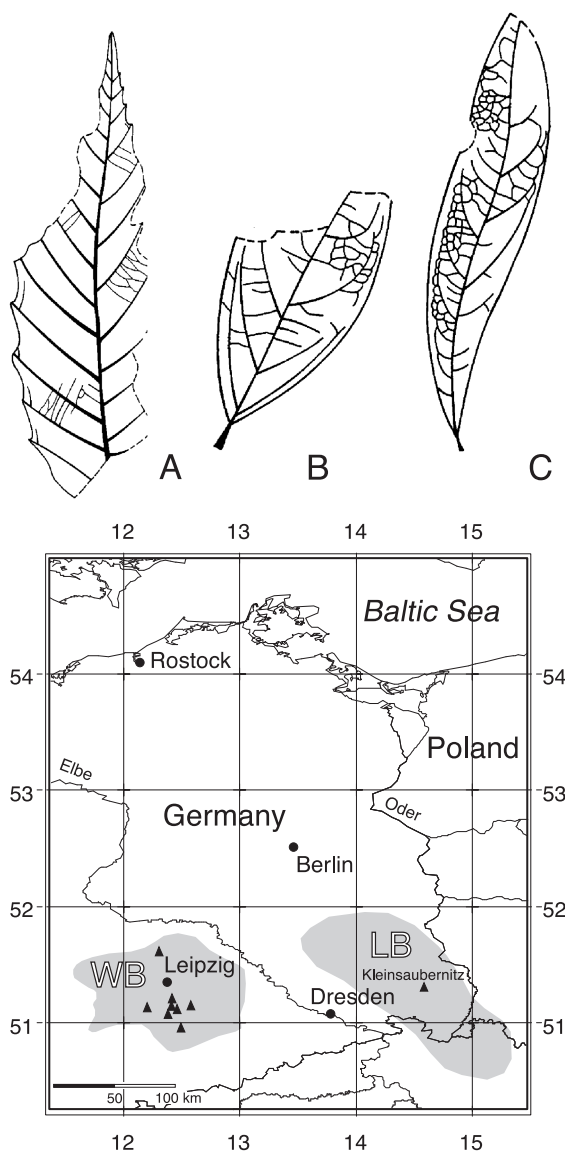Fig. 1. Lower picture: a map showing the position of the different sites (marked by ▲). Shaded area marked by WB: Weisselster basin. Shaded area marked by LB: Lausitz basin. Upper picture: typical remains of *E. furcinervis* (A), location of Knau, *L. pseudoprinceps* (B), location of Witznitz, and *L. acutimontanum* (C), location of Witznitz (after Kriegel, 2001).

1999, 2000; Retallack, 2001; Royer et al., 2001; Zachos et al., 2001; Beerling and Royer, 2002a). Moreover, the single types of proxy data are prone to several sources of uncertainty (see above; Boucot and Gray, 2001). More data about climate and pCO_2 during the Palaeogene/Neogene are thus desirable. In this contribution, a combined palaeobotanical approach is applied which (1) allows for simultaneous reconstruction of terrestrial climate data and changes in pCO_2, (2) provides data from the Late Eocene, Early and Late Oligocene, the Oligocene/Miocene boundary and the Early Miocene and (3) uses plant remains of taxa which existed in geographically adjacent regions through these time slices. It comprises (1) stomatal density and stomatal index for atmospheric CO_2 concentration, (2) the coexistence approach [CA; derived from the nearest living relative (NLR) method] for various climatic data and (3) leaf margin analysis (LMA) for temperature. The intentions of this study are to (1) provide a more or less continuous record of changes in pCO_2 during an interval of the Palaeogene/Neogene and (2) reconstruct the climate parameters of the considered location during this time interval. Three fossil dicotyledonous species commonly found in fossil beds of Saxony, Germany were utilized: *Laurophyllum acutimontanum* (Laur-

aceae), *Laurophyllum pseudoprinceps* (Lauraceae) and *Eotrigonobalanus furcinervis* (Fagaceae).

2. Material and methods

2.1. The fossil material

Fossil material from 10 localities in Saxony, Germany was used. The various locations provide rich and well-documented fossil floras spanning a time interval from the Late Eocene to the Early Miocene: Haselbach, Profen, Knau, Gröbern, Bitterfeld, Kleinsaubernitz, Witznitz, Beucha, Borna, Regis III and Espenhain (see Fig. 1 and Table 1). All cuticle preparations and the material for coexistence approach and leaf margin analysis originate from systematically well-determined and excellently preserved material from these 10 fossil-rich localities. In most cases, the sites represent brown coal formations of the Weisselster basin which were exploited by open-cast mining, with the exception of Kleinsaubernitz, which is of volcanic origin. This locality represents a sediment-filled maar and preserved a parauthochtonous floral complex. The Late Palaeogene/Neogene sediments of the Weisselster basin represent marine influenced fluviatile–lacustrine sediments of a complex

Table 1
List of leaf materials available for this study

Location—age	Species					
	E. furcinervis		L. pseudoprinceps		L. acutimontanum	
	n.l.	n.c.	n.l.	n.c.	n.l.	n.c.
Haselbach—Late Eocene	4	31	–	–	–	–
Profen—Late Eocene	7	71	–	–	–	–
Knau—Late Eocene	4	52	–	–	–	–
Beucha—early Early Oligocene	–	–	–	–	6	28
Haselbach—late Early Oligocene	–	–	2	17	3	24
Regis III—Early Oligocene	–	–	1	8	–	–
Borna—Late Oligocene	–	–	–	–	8	52
Kleinsaubernitz—Late Oligocene	8	96	1	20	4	30
Espenhain—Late Oligocene	–	–	4	54	1	10
Bitterfeld—Late Oligocene	–	–	–	–	11	66
Witznitz—Oligocene/Miocene boundary	7	55	31	134	5	21
Espenhain—Early Miocene	1	16	–	–	–	–
Gröbern—Early Miocene	–	–	–	–	21	142
Σ	31	321	39	233	59	373

n.l.: number of leaves. n.c.: total number of counts.

river system composed of various amounts of swamps, riparian forests, laurel peat forests and aquatic sites. The fossil floras of the Weisselster basin clearly document the vegetation change during the Palaeogen/Neogene. The Middle and Upper Eocene flora represents a palaeotropical laurophyllous "Mastixioid Flora" (Mai and Walther, 2000). A change to Arcto-Tertiary elements occurred at the Eocene/Oligocene boundary with the formation of Mixed Mesophytic forests (Mai and Walther, 2000). The fossil floras and the locations are described in detail in a series of publications by Mai and Walther (1978, 1985, 1991, 2000) and Walther (1999).

The three species that were selected for this study follow two criteria: (1) excellent preservation allowing for cuticle preparation and determination of stomatal density/index; and (2) wide stratigraphic range. The selected species are: *L. acutimontanum* MAI (Lauraceae; 373 cuticle preparations), *L. pseudoprinceps* WEYLAND and KILPPER (Lauraceae; 233 cuticle preparations) and *E. furcinervis* (ROSS-MAESSLER) WALTHER and KVACEK (Fagaceae; 321 cuticle preparations; see Fig. 1). The latter species represents a geographically widely distributed species (Mediterranean area, central Europe to Russia), occurring from the Middle Eocene to the Oligocene/Miocene boundary (Mai and Walther, 1978, 1985, 1991, 2000; Walther, 1999). It was therefore an important element of the palaeoflora of these locations with broad ecological amplitude. The utilized material (numbers of leaves and fragment counts) is summarized in Table 1. In the case of the material from the Early Oligocene, it is possible to resolve the age into early Early and late Early Oligocene. This differentiation was, however, only useful for the climate reconstruction [coexistence approach (CA), see Section 2.3; and leaf margin analysis (LMA), see Section 2.4]. For the stomatal density and index analysis, the data were pooled into an "Early Oligocene" group. The cuticles were prepared and are stored at the Museum for Mineralogy and Geology, Dresden, Germany.

2.2. Stomatal density and stomatal index as pCO_2 sensors

Stomatal data can show considerable scattering between individual plants, between leaves and even over a single leaf (Poole et al., 1996 and citations therein). This is due to numerous environmental parameters, especially humidity and light intensity, which influence stomatal density (Stahl, 1883; Maximov, 1929). In the case of, for example, *Quercus petraea*, it has been shown by greenhouse experiments that humidity and temperature significantly influence stomatal density (Kürschner, 1996; Kürschner et al., 1998). It is thus necessary to account for these variances if stomatal data are to be measured. Furthermore, the ratio between epidermal cells and stomata, expressed as the stomatal index, is less influenced by these parameters (Salisbury, 1927) and is thus expected to supply a clearer CO_2 signal (Poole and Kürschner, 1999). Stomatal density and index were determined after Poole and Kürschner (1999). An exact replication of the procedure described by Poole and Kürschner (1999) was, however, not possible due to the nature of the fossil material. The fossil leaves were often fragmented, and stomata counting in fields of a standard grid, which divides the leaf area into squares, could not be accomplished. Furthermore, it was often not possible to reconstruct the exact location of a fragment within the original leaf. Marginal regions, which tend to show a different stomatal density than more central regions, could also not be excluded (Poole and Kürschner, 1999). The area of a single count amounted at least to 0.03 mm^2 and, in most cases, to about 0.09 mm^2. Usually, a cuticle preparation of a leaf fragment was sufficiently large to allow for at least 3–4 counts.

The determination of stomatal density and stomatal index was completed by transferring the microscopic image into a digital image. The image-processing package (OPTIMAS, BioScan) was used for supporting the identification and counting of stomata and epidermis cells (for stomatal index). Identification of stomata and epidermis cells was performed by visual inspection of the computer-enhanced picture. The stomata and epidermis cells were marked in the image after counting. Finally, the considered area was measured by the program, and the image was stored as a file in a database. The number of leaves and counts are summarized in Table 1. Stomatal density was determined for all three species, and stomatal index was determined only for *L. acutimontanum* and *L. pseudoprinceps* because it is usually not possible to recognize

epidermal cells of the lower epidermis of *E. furci-nervis* leaves. Statistical analyses were carried out by using SPSS for Windows, Release 10.0.7.

The stomatal data are used to recognize a qualitative change in pCO_2. No attempt was made to reconstruct pCO_2 quantitatively, i.e., the calculation of pCO_2 with transfer functions, due to the following considerations. The reconstruction of palaeoatmospheric CO_2 concentration requires the calibration of the stomatal density or index of a plant species against pCO_2. This is usually achieved by conducting greenhouse experiments with extant plants or by calibration of herbarium material with actual atmospheric CO_2 measurements or ice core data (e.g., Kürschner, 1996; Rundgren and Beerling, 1999; Wagner et al., 1999; McElwain et al., 2002). In every case, extant plants are used for producing calibration curves. The quantitative reaction of stomatal density to pCO_2 is extremely species-specific, and efforts are thus made to use "living fossils" or to identify fossil leaves which probably represent a still extant species (Kürschner et al., 1996; Royer et al., 2001; Beerling and Royer, 2002b). Because all three taxa in the present study are fossil taxa with no certain extant representative, a calibration is not possible. *E. furcinervis*, for example, belongs to the Fagaceae and is "in between" the Castanoideae and the Quercoideae (Kvacek and Walther, 1989). An extant species that is phylogenetically so close to *E. furcinervis* that it can be considered as conspecific can therefore not be identified.

An alternative approach is the identification of a nearest living equivalent (NLE) (McElwain and Chaloner, 1995; McElwain, 1998). The central idea is that an ecologically equivalent species of the fossil taxon is identified which is expected to react to pCO_2 in the same way as the fossil species with respect to stomatal parameters. This approach is currently under debate (Boucot and Gray, 2001). However, we attempted to perform this method for the present dataset (see Section 4).

2.3. The coexistence approach

The coexistence approach (CA) is described in detail in Mosbrugger and Utescher (1997) and Mosbrugger (1999). Only a brief explanation will thus be provided. The distribution of plant species is—besides other factors—dependent on various climatic parameters such as mean annual temperature (MAT) or precipitation. The fossil flora of a location thus provides evidence about the climatic conditions under which the different plant taxa coexisted, provided that the climatic demands of the fossil taxa are known. This is achieved using the concept of "nearest living relatives" (NLR), which presupposes that the fossil taxon and its extant counterpart have very similar environmental demands (Hickey, 1977; Kershaw and Nix, 1988; Wing and Greenwood, 1993; Greenwood and Wing, 1995; Greenwood et al., 2003b). This key assumption dates back as far as the 19th century, and has since been extensively used (Heer, 1855–1859; Schwarzbach, 1968; van der Burgh, 1973).

The CA follows the nearest living relative concept. It is based on parameter ranges that are typical for the considered taxa, which are identified as representatives of the fossil taxa. This is accomplished by listing the climatic conditions of the areas in which these extant representatives exist today. By using this database of extant taxa and their climatic requirements, a "coexistence interval" of different climatic parameters can be calculated which allowed the majority of considered plant taxa to exist at that location. Numerous taxa have to be included and overlapping of the "taxa ranges" defines the coexistence interval which represents the range of the palaeoclimate. The interval size of the different parameters decreases in general with the increasing numbers of species. The CA was tested extensively with respect to reliability and resolution, and its advantages and disadvantages are discussed thoroughly in Mosbrugger and Utescher (1997). For example, it can be demonstrated that evolutionary shifts of the climate ranges of taxa can be detected by statistical methods and by the occurrence of "outliers" which do not fit into the coexistence interval (Mosbrugger and Utescher, 1997).

The core of the coexistence approach (CA) is represented by the database CLIMBOT which contains the climatic requirements of numerous plant taxa from the Palaeogen/Neogene based on the climatic requirements of their nearest living relatives. The first step requires that the taxa of a given fossil flora represented by fruits, seeds or leaves have to be determined to the genus or species level. The database CLIMBOT is then accessed by an analysis algorithm, which calculates the intervals of coexistence for the climate parameters of the nearest living relatives (see

Fig. 2). The coexistence intervals of the single parameters are expressed as "left" (lower) and "right" (upper) boundaries. For example, the coexistence interval concerning the mean annual temperature (MAT) is expressed as lowest possible MAT (MAT_L) and highest possible MAT (MAT_R). Both values represent the lower and upper temperature limits in which the considered taxa are able to coexist. In the present study, the CA is applied to the fossil taxa of the different locations shown in Fig. 1. Taxa numbers are summarized in Section 3.2 together with the results. The taxa lists are documented in Mai and Walther (1978, 1985, 1991, 2000) and Walther (1999).
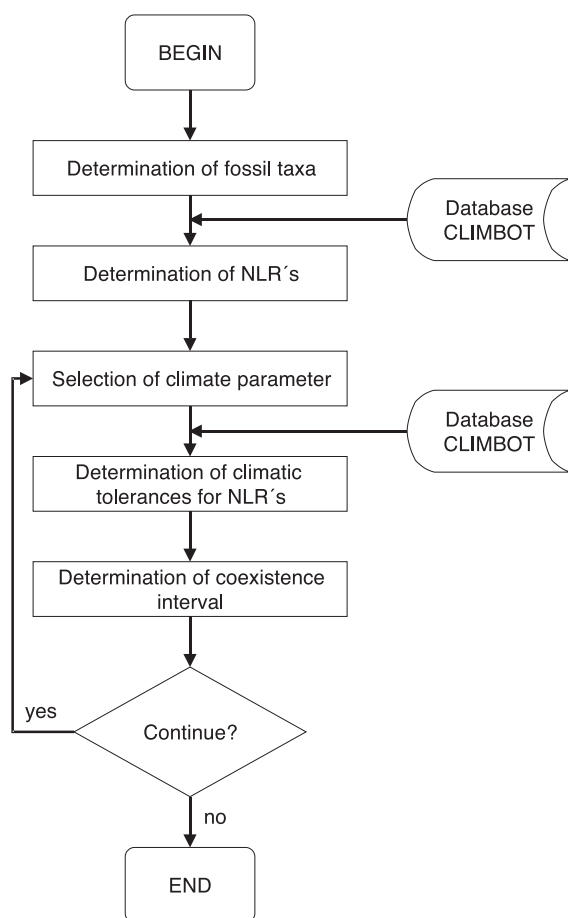
Fig. 2. Flowchart of the coexistence approach (CA). The core of the program is represented by the database CLIMBOT which contains the climatic requirements of the nearest living relatives.

2.4. Leaf margin analysis

Leaf margin analysis (LMA) represents a second proxy approach used for reconstructing palaeotemperature. It is well known that the character of the leaf margins of the different taxa composing a local flora correlates with the mean annual temperature (MAT) of that region (Bailey and Sinnott, 1915, 1916; Wolfe, 1979). This phenomenon is utilized in the LMA and represents a widespread tool in determining palaeotemperatures (Wolfe, 1979, 1993; Wilf, 1997; Wiemann et al., 1998). The leaf margin is scored as "entire" or "toothed". The percent proportion of entire-margined species is used in linear equations which return the MAT. The equations are the results of leaf margin analyses carried out on extant floras in which the varying proportions of entire-margined leaves is mathematically related to local climates. LMA therefore requires the calibration with extant vegetation. In the present paper, three formulas are used which were found for different geographic regions (summarized by Wiemann et al., 1998):

Wolfe (1979) for forests in Eastern Asia:

$$MAT = 1.14 + 0.306 \times \%_{entire} \qquad (1)$$

Greenwood (1992) for species of Australian wet forest sites (provided by Wiemann et al., 1998, who converted the original plot into the following linear equation):

$$MAT = 2.24 + 0.286 \times \%_{entire} \qquad (2)$$

Wilf (1997) for temperate and tropical America:

$$MAT = 47.4 + 6.18 \times \%_{entire} \qquad (3)$$

This approach represents thus a simple binomial score (Wilf, 1997). The LMA approach was expanded by including numerous other leaf characteristics, such as form of the leaf base or presence/absence of drip tips, in order to form a multiple character approach which is termed as CLAMP (Wolfe, 1993). In the present study, the simple LMA, considering only leaf margin type, was applied because it was demonstrated that multiple character models may not necessarily be superior to the LMA (Wilf, 1997; Wiemann et al., 1998). The LMA is carried out with the leaf floras which are documented for the various locations (see

Mai and Walther, 1978, 1985, 1991, 2000; Walther, 1999). Recent systematic revisions for single taxa were considered also in the case of the LMA.

According to Wilf (1997), (1) only vascularized leaf teeth were scored as teeth; (2) irregular serrations were not scored as teeth; and (3) only leaf extensions with an incision less than one quarter the distance from margin to midrib were considered as teeth. If no teeth were identified, then the leaf was scored as "entire". In the case of *Eotrigonobalanus*, toothed and untoothed specimens occur. In this case, a score of 0.5 was chosen according to standard practice (Wolfe, 1993; Wilf, 1997). Inspection of the data revealed, however, that this single species does not significantly influence the results. It is to be expected that the LMA result approaches the true MAT with increasing number of considered taxa (for example, see Burnham et al., 2001). Two kinds of error have to be considered for the LMA results: (1) the binomial

sampling error that results from the selection of a limited number of species from a much larger species pool and (2) the standard error. In the case of fossil floras, the binomial sampling error is generally higher than the standard error (Wilf, 1997). In the present study, only the binomial sampling error was thus considered. The binomial standard error increases with decreasing species number, and was calculated according to Wilf (1997).

3. Results

3.1. Stomatal density and index

3.1.1. E. furcinervis

Statistical tests (Kolmogoroff–Smirnoff test and Q–Q diagrams of SPSS) reveal that a normal distribution cannot be assumed for all data groups of *E.*
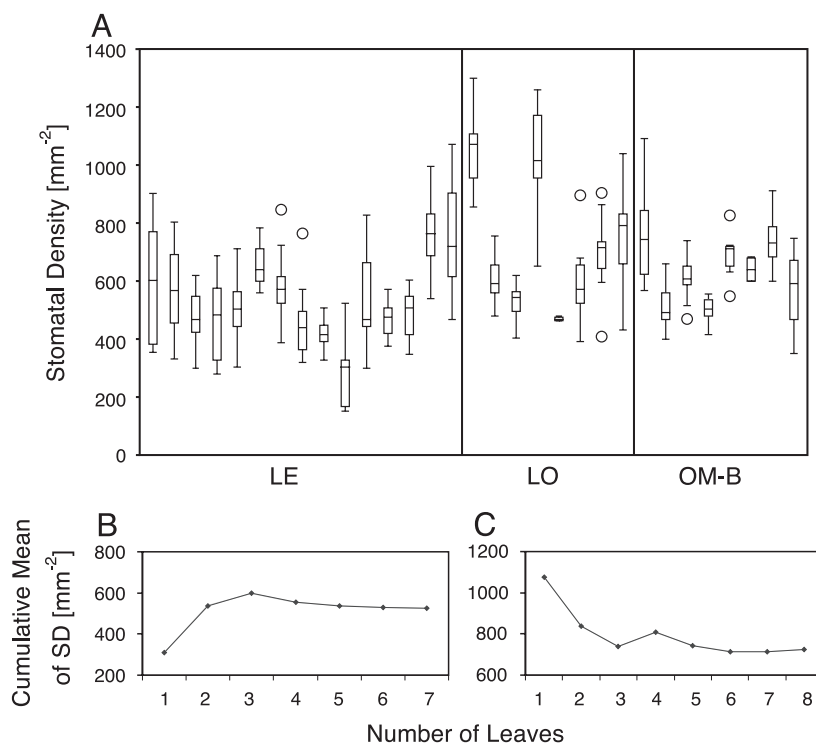

Fig. 3. (A) The results of all counts of stomatal density per leaf for *E. furcinervis* plotted against the stratigraphic age of the leaves. Within one age, the specimens are arranged randomly. The results per leaf are represented as box–whisker plots. The vertical lines mark the highest and lowest value, and the boxes span the 50% interquartile. The median is indicated by the horizontal line within the boxes. Single outliers are drawn as ○. LE: Late Eocene. LO: Late Oligocene. OM-B: Oligocene–Miocene boundary. (B) Cumulative mean of successive counts calculated for the fossil material of Profen. (C) Cumulative mean of successive counts calculated for the fossil material of Kleinsaubernitz.
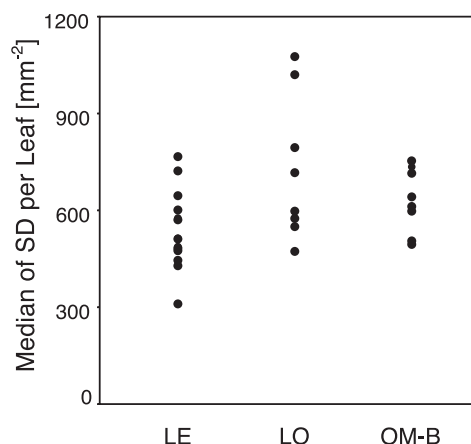
Fig. 4. Median values of stomatal density (SD) for each leaf plotted against stratigraphic age for *E. furcinervis*. LE: Late Eocene. LO: Late Oligocene. OM-B: Oligocene–Miocene boundary.

furcinervis. Therefore, median and variance are calculated for the leaf counts instead of mean values and standard deviation. This was also observed by Poole et al. (1996) studying stomatal parameters of *Alnus glutinosa*. Fig. 3A shows the counts for each leaf which are grouped according to stratigraphic age of the leaves (Late Eocene, Late Oligocene, Oligocene/Miocene boundary). The data of each single epoch show considerable scattering for both plots. High data-scatter in the case of stomatal parameters of dicotyledonous leaves is common due to the numerous factors which influence the stomatal density (Poole et al., 1996; Beerling and Royer, 2002a,b). It is especially high for fossil material. An example is the study of McElwain (1998) in which stomatal indices of fossil *Litsea* and *Lindera* genera were investigated. Another factor is the difficulty of applying standardized methods to measure stomatal parameters in fossil leaves (see Section 2). A more detailed discussion of the present data scatter is provided in Section 4.1.

An appropriate way to account for the variance of the stomatal parameters is calculating the cumulative mean of successive counts of different leaves of one data group as suggested by Beerling and Royer (2002a). The resulting plots demonstrate the minimum number of leaves necessary for assessing the variance. This was accomplished for the leaves of each site. Two characteristic results of this method applied to *E.*

furcinervis are included in Fig. 3B and C. The plots demonstrate that a minimum sample size of about 4–5 leaves for each site is necessary for *E. furcinervis*. For *Ginkgo adiantoides*, Beerling and Royer (2002b) found a minimum sample size of about 5 whereas for *Neolitsea dealbata*, a minimum sample size of about 3 was found (Greenwood et al., 2003a). A number of 4–5 leaves appear thus to be in the range of a typical minimum sample size.

Fig. 4 shows only the median values of each leaf, again grouped according to stratigraphic age. In order to detect statistically significant differences between the stratigraphic data groups, statistical tests were applied to the median data (Fig. 4). Because the data show neither normal distribution nor homogeneous variances (according to the Levene test), possible differences between the data groups from Late Eocene, Late Oligocene and Oligocene/Miocene boundary were tested with the nonparametric Kruskal–Wallis test. The calculated $\chi^2 = 6.29$ (2 degrees of freedom) indicates that differences exist between the three datasets (with a significance of 0.043). In the next step, the nonparametric Mann–Whitney *U*-test was applied which yields the significance of differences between two groups (see Table 2). The difference between the datasets of the Late Eocene and Late Oligocene and between Late Eocene and Oligocene/Miocene boundary is significant (see Table 2). The Late Eocene group shows therefore significantly lower stomatal densities than the two other groups. However, there is no statistically significant difference between the datasets of Late Oligocene and Oligocene/Miocene boundary (see Table 2).

Table 2
Statistical results for significance of differences in stomatal density between leaves of different age for *E. furcinervis*

Dataset 1	Significance of test[a]	Dataset 2	MWU sd[b]
Late Eocene	0.03	Late Oligocene	27, $n_1 = 8$, $n_2 = 15$
Late Oligocene	ND, 0.67	Oligocene/Miocene boundary	28, $n_1 = 8$, $n_2 = 8$
Late Eocene	0.05	Oligocene/Miocene boundary	30, $n_1 = 8$, $n_2 = 15$

The significance was tested between Dataset 1 and Dataset 2, respectively, by using the nonparametric Mann–Whitney *U*-test.
[a] ND: no statistically significant difference between datasets.
[b] Mann–Whitney *U*-value for stomatal density.

3.1.2. L. pseudoprinceps

The results for stomatal density are presented in Figs. 5 and 6 in the same way as for *E. furcinervis*, because the data also did not prove to be normally distributed. Fig. 5 shows the single counts for each leaf and Fig. 6 shows the median data for the leaves, plotted against stratigraphic age. In the case of the stomatal index, Fig. 7A presents the single counts for each leaf, whereas Fig. 8 depicts the median data for the leaves. Fig. 7B and Fig. 7C show two examples of plots of the cumulative mean for successive counts of SI and indicate that a sampling size of about 3 leaves per site is sufficient in order to assess the variance within the data.

No differences appear to be visible for the different datasets of *L. pseudoprinceps*, neither for the stomatal density nor for the stomatal index data. A statistical test confirms that no significant differences can be inferred from the dataset of *L. pseudoprinceps*: the Kruskal–Wallis test yields $\chi^2 = 0.7$ (2 degrees of freedom, significance: 0.7). The data thus provide no evidence of statistically significant changes of stomatal parameters in *L. pseudoprinceps*. It is possible that this species belongs to the group of insensitive species or that a very weak signal exists in *L. pseudoprinceps*, and that more data would be necessary to detect it.
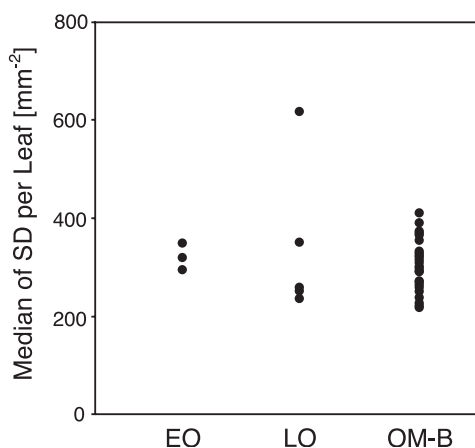

Fig. 6. Median values of stomatal density (SD) per leaf plotted against stratigraphic age for *L. pseudoprinceps*. EO: Early Oligocene. LO: Late Oligocene. OM-B: Oligocene–Miocene boundary.

Stomatal index and stomatal density are positively correlated. The single-leaf counts of stomatal density and stomatal index show a coefficient correlation of 0.73 (significance ≤ 0.01, Spearman rho; Fig. 9), and the leaf median data of stomatal density and stomatal index show a degree of correlation of 0.62 (significance ≤ 0.01, Spearman rho; Fig. 10). In the case of the single-leaf counts, the data of
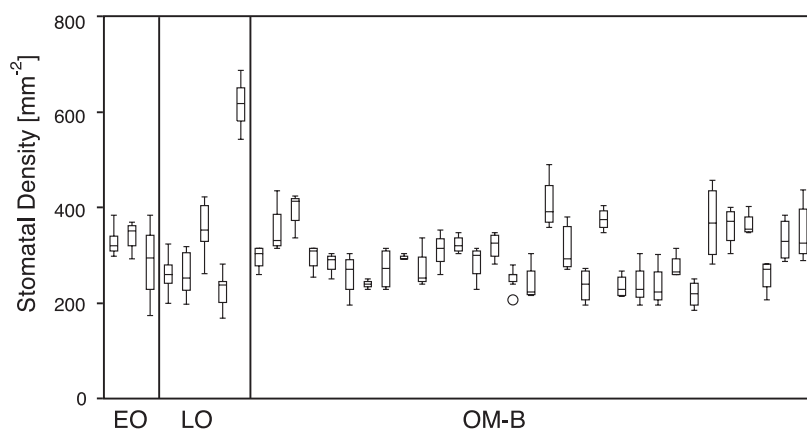

Fig. 5. The results of all counts of stomatal density for each leaf of *L. pseudoprinceps* plotted against the stratigraphic age of the leaves. Within one age, the specimens are arranged randomly. The results per leaf are represented as Box–Whisker plots. The vertical lines mark the highest and lowest value, and the boxes span the 50% interquartile. The median is indicated by the horizontal line within the boxes. Single outliers are drawn as ○. EO: Early Oligocene. LO: Late Oligocene. OM-B: Oligocene–Miocene boundary.
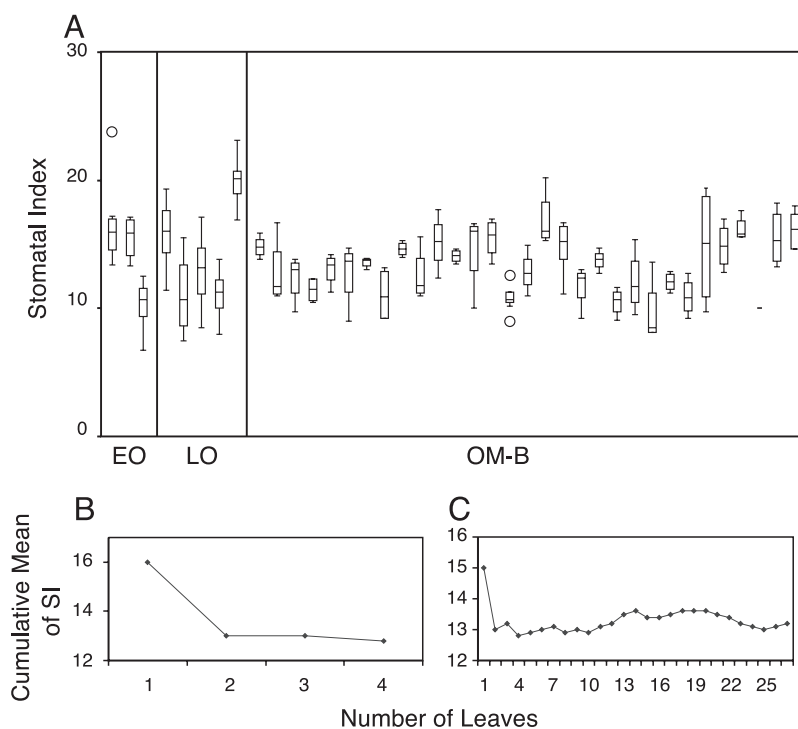
Fig. 7. (A) The results of all counts of stomatal index for each leaf of *L. pseudoprinceps* plotted against the stratigraphic age of the leaves. Within one age, the specimens are arranged randomly. The results per leaf are represented as Box–Whisker plots. The vertical lines mark the highest and lowest value, and the boxes span the 50% interquartile. The median is indicated by the horizontal line within the boxes. Single outliers are drawn as ○. EO: Early Oligocene. LO: Late Oligocene. OM-B: Oligocene–Miocene boundary. (B) Cumulative mean of successive counts calculated for the fossil material of Espenhain. (C) Cumulative mean of successive counts calculated for the fossil material of Witznitz.

individual leaves are responsible for the higher degree of correlation if compared to the leaf median data. The lower correlation in the case of the leaf
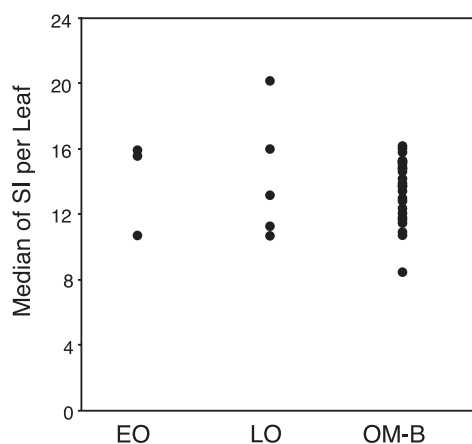

Fig. 8. Median values of stomatal index (SI) for each leaf, plotted against stratigraphic age for *L. pseudoprinceps*. EO: Early Oligocene. LO: Late Oligocene. OM-B: Oligocene–Miocene boundary.
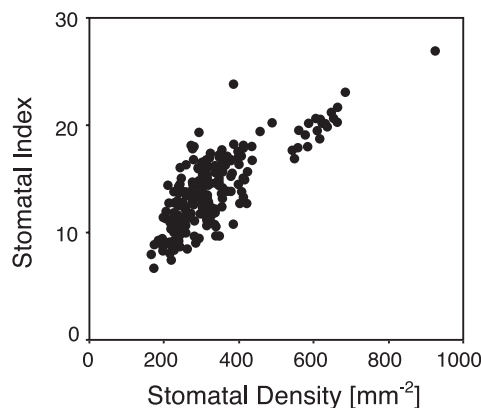

Fig. 9. All counts of stomatal index for *L. pseudoprinceps* plotted against corresponding stomatal density counts.
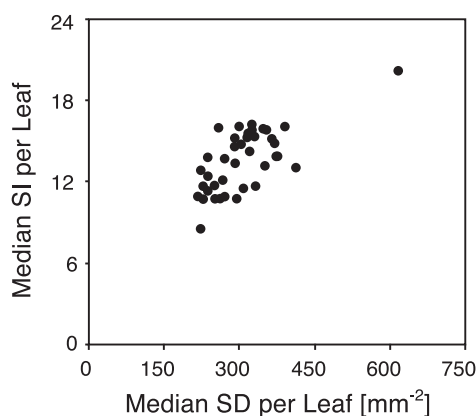
Fig. 10. Median values of stomatal index (SI) per leaf of *L. pseudoprinceps* plotted against corresponding median values of stomatal density (SD) per leaf.
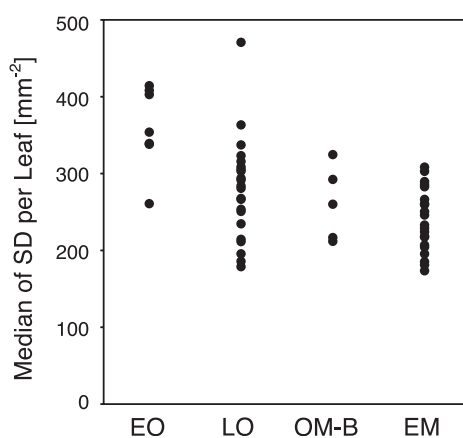

Fig. 12. Median values of stomatal density (SD) per leaf, plotted against stratigraphic age for *L. acutimontanum*. EO: Early Oligocene. LO: Late Oligocene. OM-B: Oligocene–Miocene boundary. EM: Early Miocene.

median data is due to the fact that leaves from different individuals existing at different sites and different times under different environmental conditions are pooled.

3.1.3. L. acutimontanum

The results for stomatal density are presented in Figs. 11 and 12, and results for stomatal index are presented in Figs. 13A and 14 (presentation as for *L. pseudoprinceps*). As in the two other taxa, considerable data scattering occurs for both stomatal density and index counts of the single leaves. Fig. 13B and C represents two example plots of the cumulative mean of successive counts of SI. These

indicate that a sampling size of about 3 per site is sufficient in order to account for the variance in SI for *L. acutimontanum*.

The four different leaf median groups, Early Oligocene, Late Oligocene, Oligocene/Miocene boundary and Early Miocene, were tested for normal distribution as described for *E. furcinervis*. Normal distribution can be assumed for the datasets of the four time slices. Differences between the groups were thus tested with ANOVA for both stomatal index and density. The results indicate statistically significant differences between the four
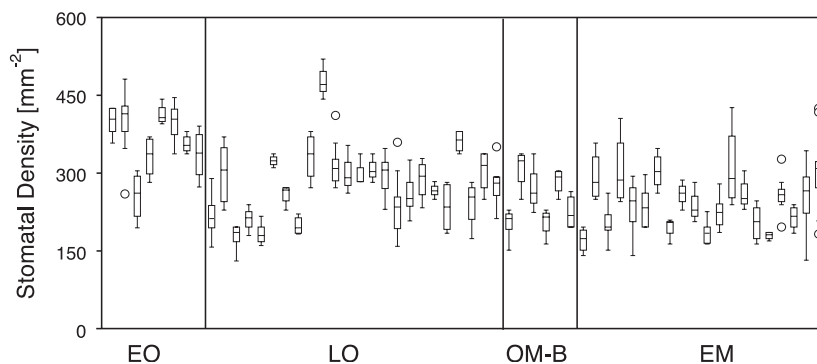

Fig. 11. The results of all counts of stomatal density per leaf of *L. acutimontanum* plotted against the stratigraphic age of the leaves. Within one age, the specimens are arranged randomly. The results per leaf are represented as box–whisker plots. The vertical lines mark the highest and lowest value, and the boxes span the 50% interquartile. The median is indicated by the horizontal line within the boxes. Single outliers are drawn as ○. EO: Early Oligocene. LO: Late Oligocene. OM-B: Oligocene–Miocene boundary. EM: Early Miocene.
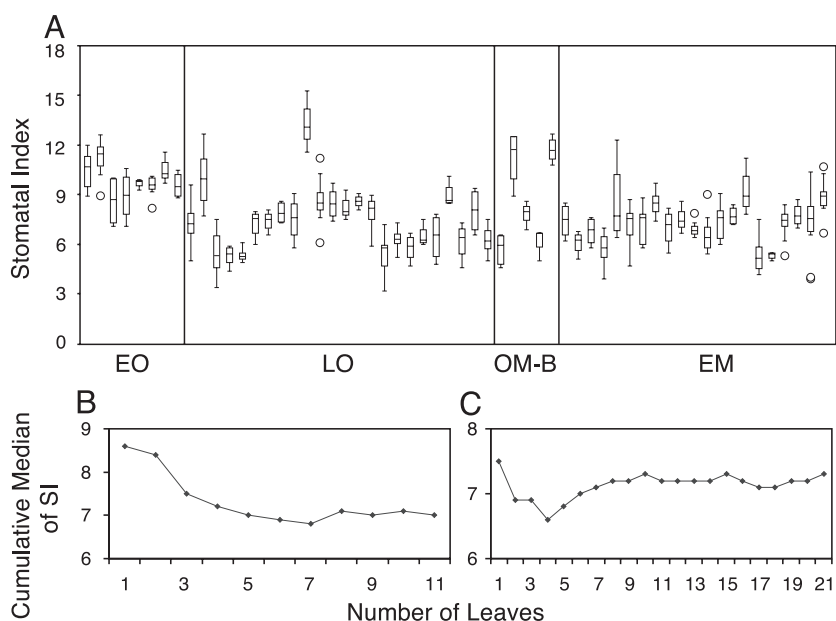
Fig. 13. The results of all counts of stomatal index for each leaf of *L. acutimontanum* plotted against the stratigraphic age of the leaves. Within one age, the specimens are arranged randomly. The results per leaf are represented as box–whisker plots. The vertical lines mark the highest and lowest value, and the boxes span the 50% interquartile. The median is indicated by the horizontal line within the boxes. Single outliers are drawn as O. EO: Early Oligocene. LO: Late Oligocene. OM-B: Oligocene–Miocene boundary. EM: Early Miocene. (B) Cumulative mean of successive counts calculated for the fossil material of Bitterfeld. (C) Cumulative mean of successive counts calculated for the fossil material of Groebern.

stratigraphic groups (see Table 3). The Student–Newman–Keuls procedure validates a significant difference between the Early Oligocene dataset

Fig. 14. Median values of stomatal index (SI) for each leaf plotted against stratigraphic age for *L. acutimontanum*. EO: Early Oligocene. LO: Late Oligocene. OM-B: Oligocene–Miocene boundary. EM: Early Miocene.

and the other datasets (Late Oligocene, Oligocene/Miocene boundary and Early Miocene) for both stomatal density and index data ($\alpha = 5\%$). The Early Oligocene dataset therefore shows significantly higher stomatal densities than the three other groups. There is, however, no statistically significant difference between the datasets of Late Oligocene, Oligocene/Miocene boundary and the Early Miocene (for $\alpha = 5\%$).

As in *L. pseudoprinceps*, no fundamental differences between the results for stomatal density and stomatal index are apparent. The relationship between stomatal density and stomatal index is shown
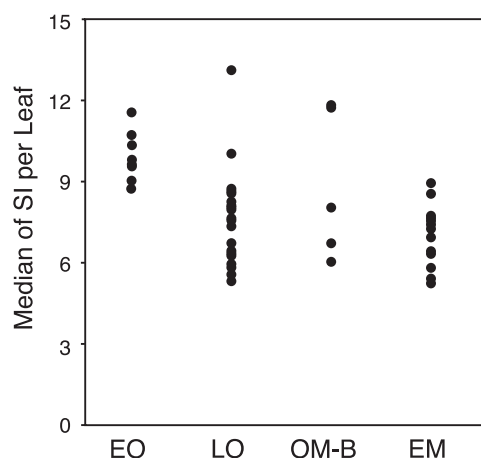
Table 3
Results of ANOVA for leaves of different stratigraphic age for *L. acutimontanum*

	F	df_b	df_w	Significance
sd	10.7	3	54	< 0.001
si	7.1	3	54	0.001

F: F-value. df_b: degrees of freedom between groups. df_w: degrees of freedom within groups. sd: stomatal density. si: stomatal index.

in Fig. 15 for the single counts and shown in Fig. 16 for the leaf median data. Stomatal density and index show a positive correlation, with a coefficient of correlation for the single-leaf counts of 0.77 (Spearman rho, significance level = 0.01) and for the median data of 0.75 (Spearman rho, significance level = 0.01).

3.1.4. Changes in pCO_2

Trends of statistically significant increasing and decreasing stomatal density and index data with stratigraphic age in the case of *E. furcinervis* and *L. acutimontanum* suggest a corresponding decrease and increase of contemporaneous pCO_2, which is depicted schematically in Fig. 17. The datasets of *E. furcinervis* indicate that the pCO_2 is lower in the Late Oligocene and at the Oligocene/Miocene boundary than during the Late Eocene. The data of *L. acutimontanum* suggest that pCO_2 was lower during the Early Oligocene than during the Late Oligocene, at the Oligocene/Miocene boundary and during the Early Miocene. Furthermore, the two datasets indicate that no significant differences between Late Oligocene, Oligocene/Miocene boundary and Early Miocene existed with respect to pCO_2.

As mentioned above, *E. furcinervis* and *L. acutimontanum* are extinct. The production of a calibration curve to quantitatively reconstruct pCO_2 with the stomatal data presented here is thus not

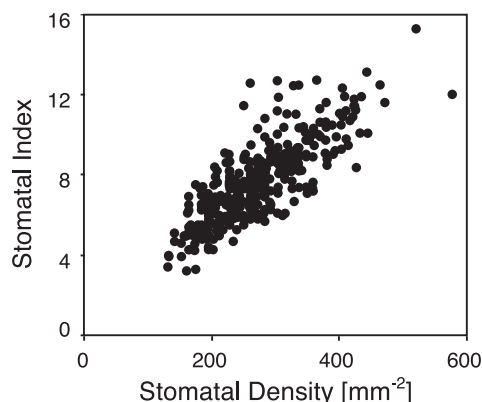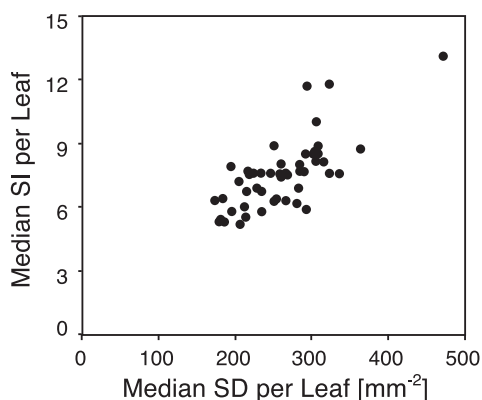Fig. 16. Median values of stomatal index (SI) per leaf of *L. acutimontanum* plotted against corresponding median values of stomatal density (SD) per leaf.

feasible. The stomatal ratio (SR) method is not based on calibration curves produced with extant taxa and is therefore claimed to be suitable for calculating pCO_2 with extinct species (McElwain and Chaloner, 1995; McElwain, 1998). Is this method suitable for the present datasets? If we consider the reaction of extant Fagaceae (to which *E. furcinervis* belongs) to changing pCO_2, then, a rather consistent qualitative reaction is apparent (stomatal density/index decreases with increasing pCO_2) (e.g., Woodward and Kelly, 1995; Kürschner, 1996). The range of stomatal densities in Fagaceae is, however, large, and the quantitative reaction of stomatal parameters on pCO_2 is strongly species specific (see review by Royer, 2001). *E. furcinervis* shows stomatal densities in the range of 500/mm^2 and higher (see Figs. 3 and 4). Comparatively high stomatal densities are expressed by species of *Quercus* (Kürschner, 1996). If a stomatal density of about 500 is taken as a representative value for a sun leaf of a *Quercus* species and used for calculating SR with *E. furcinervis* (using the mean stomatal density of *E. furcinervis* for each time period), then, for the Late Eocene, an RCO$_2$ (RCO$_2$ = fossil pCO_2/extant pCO_2) of about 1 would result. For the Late Oligocene and the Oligocene/Miocene boundary, the RCO$_2$ would amount to roughly 0.8. Apparently, these values fit into the range of pCO_2 values achieved with different proxy data for these time periods (see Fig. 17). It is, however, questionable if

Fig. 15. All counts of stomatal index for *L. acutimontanum* plotted against corresponding stomatal density counts.

a *Quercus* species is an adequate extant representative of *E. furcinervis* (see Section 2.2). If we now consider *L. acutimontanum* and the ranges of stomatal index which were provided for extant Lauraceae species, then we see that extant Lauraceae show stomatal indices of about $10-15$ (McElwain, 1998; Kürschner et al., 2001; Greenwood et al., 2003a). With the SR method applied to *L. acutimontanum*, an RCO_2 of $1-1.6$ would result for the Early Oligocene, $1.3-2$ for the Late Oligocene and $1.25-1.9$ for the Oligocene/Miocene boundary and for the Early Miocene. For the Late Oligocene, the Oligocene/Miocene boundary and the Early Miocene, the lower boundaries of these RCO_2 intervals fit into the range of RCO_2 values suggested by other data, whereas the upper boundaries appear to be very high (see Fig. 17). The SR results obtained with the present dataset do thus span almost the entire range of RCO_2 values achieved with other proxy data. Due to the species-specific quantitative reaction of stomatal parameters on pCO_2, these SR results should be regarded as a semiquantitative estimation.

3.2. Coexistence approach (CA)

Table 4 shows a summary of the results for the coexistence approach for each location, expressed as left (lower) and right (upper) boundaries of the parameter intervals in which the different taxa of the various locations could coexist. The number of taxa which were available for CA analysis are different for the various locations (see Table 4). The datasets as a whole indicate a distinct decrease in MAT occurring over the Eocene/Oligocene boundary in the Weisselster basin. In Fig. 17, the CA intervals are plotted against stratigraphic age. The temperature increase in the Early Miocene visible in Fig. 17 is caused by the single data point (Gröbern), which was available for this time slice. The precipitation values differ between about 1300 and 900 mm/year and tend be lower after the Eocene/Oligocene boundary (see Table 4).

3.3. Leaf margin analysis (LMA)

Results of the LMA are shown in Fig. 18, together with the MAT values of the CA in order to allow for comparing the results of all approaches. The results of all three LMA equations (Eqs. (1), (2) and (3)) are presented (see Section 2.4). The number of taxa which are available for LMA are different for the different sites. In many cases, the taxa number is close to $n = 20$ (see legend of Fig. 18 with a summary of the taxa numbers). The minimum sampling size which is considered to represent the critical limit ranges from 20 to 30 taxa (Wing and Greenwood, 1993; Wolfe, 1993; Burnham et al., 2001). There are, however, sites with lower taxa numbers. For the location of Profen with $n = 7$ taxa, the LMA result should be viewed with caution due to the small sample size. The largest dataset is found at the Kleinsaubernitz locality ($n = 48$). The quality of the results is also influenced by various other factors which are considered in more detail in Section 4.2.

For the Upper Eocene floras, the LMA tends to provide significantly higher MAT results than the CA. The difference between the results of the CA and the three LMA approaches may be due to (1) the low number of leaf taxa which are available for the Late Eocene and (2) taxonomic distance between the nearest living relatives and the fossil taxa. This taxonomic distance increases with increasing stratigraphic age of a fossil taxon. For all three Upper Eocene locations, the upper limits of the CA range, however, at least within the binomial sampling error of Eq. (2) which yields lower MAT values than Eqs. (1) and (3). For the Early and Late Oligocene and the Early Miocene, there is no systematic difference visible between LMA and CA. In most cases, the intervals of the LMA equations and of the CA overlap. No interval overlap occurs for Borna (Late Oligocene) and Witznitz (Oligocene/Miocene boundary). In the case of Witznitz, the lower limit of the CA is, however, close to the upper limits of the binomial sampling errors of the three LMA approaches.

3.4. Climate parameters and pCO_2

The datasets of *E. furcinervis* and *L. acutimontanum* suggest that stomatal density and/or stomatal index signal an increase or decrease in pCO_2. In Fig. 17, the general development of temperature from Late Eocene to Early Miocene is plotted together with the trends in pCO_2 change. Fig. 17
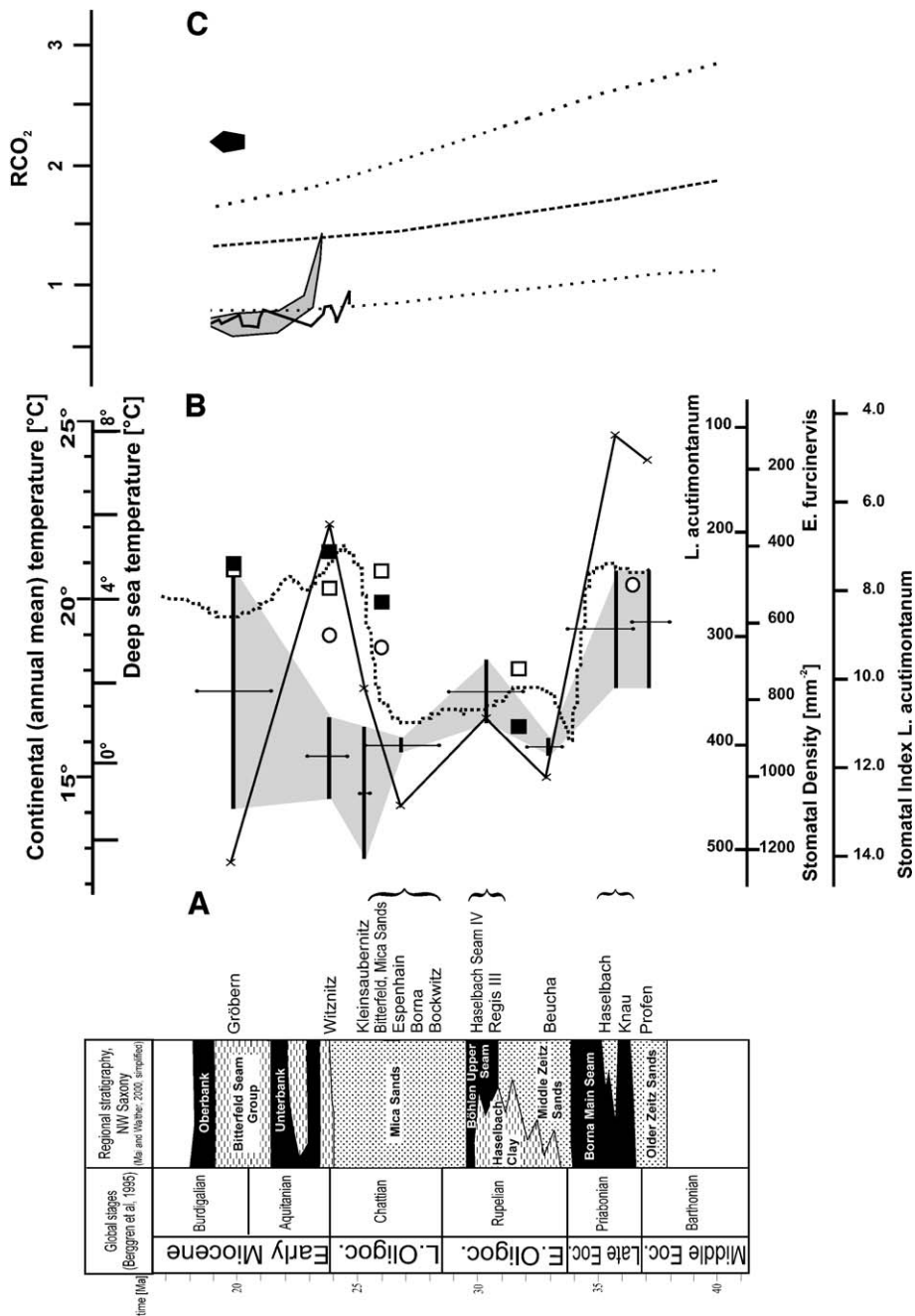
Table 4
Results of the coexistence approach (CA) for the different considered sites

Site	Age	N	MAT_L [°C]	MAT_R [°C]	CMM_L [°C]	CMM_R [°C]	WMM_L [°C]	WMM_R [°C]	MAP_L [mm]	MAP_R [mm]
Haselbach	L. Eocene	35	17.5	20.8	12.2	13.3	27.1	27.9	1122	1281
Knau	L. Eocene	29	18.0	18.6	13.3	13.3	27.1	28.1	1096	1355
Profen	L. Eocene	27	17.5	20.8	13.3	13.3	27.1	28.1	1090	1355
Beucha	E.E. Oligocene	13	15.6	16.1	5.0	5.8	24.7	25.6	897	1206
Haselbach	L.E. Oligocene	23	15.7	18.3	9.6	10.9	25.0	26.0	1231	1281
Regis III	E. Oligocene*	18	16.5	23.9	9.6	13.6	26.0	27.9	1187	1281
Bockwitz	L. Oligocene	19	13.8	16.1	1.8	6.2	25.6	25.6	1090	1213
Borna	L. Oligocene	58	15.7	16.1	3.8	7.1	25.7	26.0	1096	1355
Espenhain	L. Oligocene	13	13.3	20.8	− 0.1	13.3	25.7	28.1	897	1355
Kleinsaubernitz	L. Oligocene	36	14.0	16.1	4.3	7.8	25.6	25.6	979	1058
Glimmersande	L. Oligocene	21	13.3	19.0	− 0.1	10.9	25.7	28.5	879	1281
Witznitz	OM-B	21	14.4	16.7	3.7	7.1	27.8	28.1	1122	1355
Gröbern	E. Miocene	26	14.1	20.8	4.7	13.3	25.7	28.1	897	1281

The various time slices are: Late Eocene (L. Eocene), early Early Oligocene (E.E. Oligocene), late Early Oligocene (L.E. Oligocene). * For this site, a resolution higher than Early Oligocene (E. Oligocene) is not possible. Late Oligocene (L. Oligocene), Oligocene/Miocene boundary (OM-B), Early Miocene (E. Miocene). N: number of taxa; MAT_L: lower (left) boundary of mean annual temperature interval; MAT_R: upper (right) boundary of mean annual temperature interval; CMM_L: lower (left) boundary of mean temperature interval of coldest month; CMM_R: upper (right) boundary of mean temperature interval of coldest month; WMM_L: lower (left) boundary of mean temperature interval of warmest month; WMM_R: upper (right) boundary of mean temperature interval of warmest month; MAP_L: lower (left) boundary of mean annual precipitation interval; MAP_R: upper (right) boundary of mean annual precipitation interval.

shows the terrestrial MAT results of the present study as well as the deep-sea temperature reconstruction provided by Zachos et al. (2001). Fig. 17 shows that—for the considered terrestrial sites—a significant temperature change is only documented over the Eocene/Oligocene boundary. This decrease in temperature is also shown by the results of Zachos et al. (2001) and is accompanied by a decrease in MAT is accompanied by an increase in pCO$_2$ as is indicated by the stomatal density of *E. furcinervis*. The terrestrial MAT remains low

during the Oligocene, which is also in accordance with the results of Zachos et al. (2001) (see Fig. 17).

As indicated by the present stomatal data of *L. acutimontanum*, pCO$_2$ increases after the Early Oligocene. For the considered terrestrial sites, however, the results of CA and LMA do not provide an unequivocal signal with respect to a significant change of climate after the Early Oligocene. For the Witznitz site, (Oligocene/Miocene boundary) the CA provides a lower MAT than the LMA (Fig. 17). Upper boundary of the CA and lower boundaries of the LMA

Fig. 17. Compilation of climate and pCO$_2$ data of the present study and other data sources. (A) Stratigraphic chart which provides the stratigraphic data for the considered palaeosites. (B) Shaded area: coexistence intervals of the MAT obtained with the CA in the present study. Data of different palaeosites are pooled if stratigraphic age is identical. The thick lines parallel to the temperature axis give the width of the coexistence intervals. The thin lines perpendicular to the coexistence intervals represent stratigraphic range. Black line: the mean value of MAT obtained with the three LMA equations. Stippled line: deep-sea temperature according to Zachos et al. (2001). ○: Results of stomatal density for *E. furcinervis*. Pooling of data was necessary for Late Eocene and Late Oligocene. Pooled data points are placed in the middle of the corresponding stratigraphic range. ■: Results of stomatal density for *L. acutimontanum*. Pooling of data was necessary for Early Oligocene and Late Oligocene. Pooled data points are placed in the middle of the stratigraphic range. □: Results of stomatal index for *L. acutimontanum*. Pooling of data was necessary for Early Oligocene and Late Oligocene. Pooled data points are placed in the middle of the stratigraphic range. (C) Broken line: development of atmospheric CO$_2$ content expressed as RCO$_2$ (RCO$_2$ = fossil pCO$_2$/extant pCO$_2$) according to Berner and Kothavala (2001). Stippled lines: error envelope of the RCO$_2$ curve of Berner and Kothavala (2001). Shaded area: reconstruction of atmospheric CO$_2$ by marine boron data (Pearson and Palmer, 2000). Black line: reconstruction of atmospheric CO$_2$ by carbon isotope data of phytoplankton (Pagani et al., 1999). Black polygon: reconstruction of atmospheric CO$_2$ by carbon isotope data of pedogenic carbonate (Ekart et al., 1999).
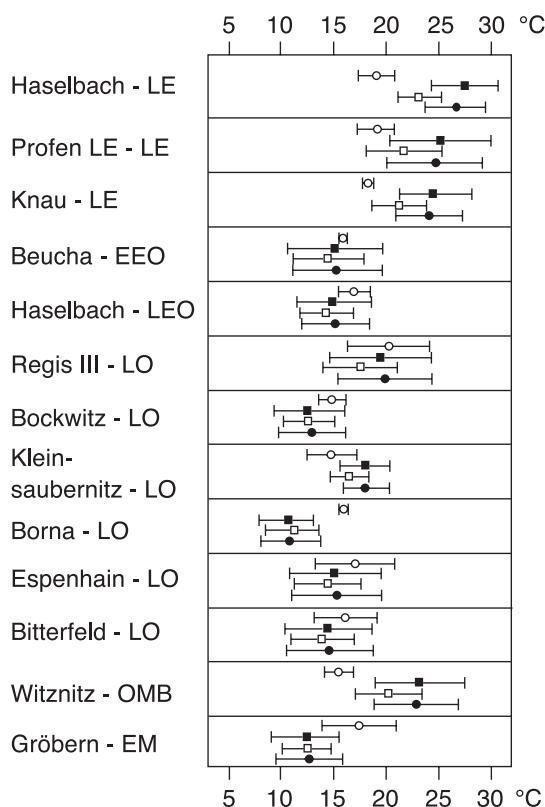
Fig. 18. MAT at the various sites in Saxony which provided the considered leaf materials. The climate parameters were reconstructed by CA and LMA (see text). ○: Results of CA. In the case of the LMA, three different formulas were used. ■: LMA after Wolfe (1979); □: LMA after Greenwood (1992); ●: LMA after Wilf (1997). The intervals indicated with the data points represent, in the case of the CA, the lowest and highest possible mean annual temperature and, for the three LMA formulas, the binomial sampling error (see text). LE: Late Eocene; EEO: early Early Oligocene; LEO: late Early Oligocene; EO: Early Oligocene; LO: Late Oligocene; OM-B: Oligocene–Miocene boundary; EM: Early Miocene. The number of taxa for the LMA which were available for the different sites are: Haselbach (UE): $n = 11$; Profen: $n = 7$; Knau: $n = 15$; Beucha: $n = 12$; Haselbach: $n = 21$; Regis III: $n = 10$; Bockwitz: $n = 20$; Kleinsaubernitz: $n = 48$; Borna: $n = 18$; Espenhain: $n = 12$; Glimmersande: $n = 15$; Witznitz: $n = 11$; Gröbern: $n = 19$.

approaches are, however, close to each other (see Fig. 18). For the site of Gröbern (Lower Miocene), the LMA equations result in lower MAT values than the CA (with, however, overlapping intervals; see Fig. 18). According to the marine data of Zachos et al. (2001), however, the temperature increased during the Late Oligocene (Fig. 17).

4. Discussion

4.1. Stomatal parameters and CO_2

In many species, the stomatal density/index is shown to react on pCO_2 with a decrease of stomatal density/index under increasing pCO_2. Stomatal parameters thus inversely respond to pCO_2 (see Section 1). This phenomenon can be observed in individuals as well as over geological periods. In the case of fossil response, more than 90% of the taxa considered so far exhibit this inverse relationship between stomatal density/index and pCO_2 (Royer, 2001). Thus, tracking the stomatal parameters of fossil taxa over time provides a reliable tool for revealing relative decreases or increases of pCO_2 (Cleal et al., 1999). It is therefore possible to use these data for studying the correlation between climate changes and fluctuations of pCO_2 (Cleal et al., 1999).

It is, however, much more difficult to reconstruct pCO_2 quantitatively. In this case, the stomatal approach requires consideration of an extant representative species because the reaction on changing pCO_2 with respect to stomatal density/index is extremely species specific. This fact is reflected by the increasing efforts to use "living fossils" for stomatal analysis (Chen et al., 2001; Retallack, 2001; Royer et al., 2001; Beerling and Royer, 2002b). The technique of producing calibration curves by experiments with extant taxa is based on the assumption that evolutionary changes in stomatal parameters as an adaptive response to pCO_2 provide a quantitative CO_2 signal which is equal or at least similar to the individual reaction which is due to genetic plasticity. The stomatal approach thus requires consideration of the same species if it is used in a *quantitative* sense. For extinct species, no calibration curve can be produced. Application of the semiquantitative SR method, which is not based on a calibration curve, to the present dataset (Section 3.1) results in a range of pCO_2 values which fit more or less into the existing range of pCO_2 proxy data.

Whereas the quantitative reconstruction of pCO_2 appears to be difficult with extinct taxa, documenting increases and decreases of pCO_2 through time qualitatively by tracking stomatal parameters is feasible. However, reconstruction of changes in atmospheric CO_2 content by stomatal parameters is hampered by

strong variance of the data. This variance is due to several factors and weakens the clarity of the qualitative CO_2 signal. One important source of variance is very probably caused by environmental parameters (see below), but strong variance can even occur over a single leaf (Poole and Kürschner, 1999; Poole et al., 2000). The differences between sun and shade leaves may also contribute to the data scatter because, at least for the Kleinsaubernitz site, the shade leaves are frequent (Walther, 1999). In general, however, the differences between sun and shade leaves are not important because sun leaves preferentially enter the fossil record (Ferguson, 1985; Gastaldo et al., 1996; Kürschner, 1996).

In the present study, the CO_2 signal of the stomatal indices is not clearer in the present study than that of the stomatal densities. Numerous observations on extant material indicate that the stomatal index is less affected by environmental parameters than the stomatal density (Beerling, 1999). For the present dataset, however, no significant difference between the stomatal density and index is discernible. That the higher reliability of the stomatal index often tends to vanish in the case of fossil material was also noted by Royer (2001). The reason for this phenomenon may be caused by fundamental differences between extant and fossil material. If fossil leaves of a certain species are considered, then strongly heterogeneous material is used compared to leaves of extant plants, especially if grown under controlled greenhouse conditions. It is to be expected that heterogeneity on various levels is present for fossil material and causes high variances of stomatal density as well as of stomatal index. Some possible sources of variance are listed in the following (there may be numerous other—even unrecognized—factors).

(1) Fossil leaves of a certain location originate from many individual plants growing in different microhabitats and environments over partially very large time intervals with often low stratigraphic resolution. It is to be expected that these environments differed in numerous conditions such as site topology, soil parameters, nutrient supply, climatic and biotic factors, etc.

(2) High-resolution studies show that pCO_2 can vary significantly over—in geological terms—short-time intervals. This is demonstrated, for example, by ice core data for the last 420,000 years (Petit et al., 1999). Short-term fluctuations of pCO_2 are also suggested by high-resolution records of stomatal density data of leaf remains in lake sediments (McElwain et al., 2002). In the case of terrestrial fossil plant material, such a stratigraphically high resolution is often not achieved. It is therefore probable that leaves of a fossil assemblage existed during a certain time interval with varying pCO_2 levels and therefore show a higher variance in stomatal parameters than if pCO_2 was constant during that time interval.

(3) Identification of fossil material is often very complex and difficult. Even if a fossil can be identified to the species level, many difficulties and uncertainties about the detailed systematic relationships remain (e.g., Mai, 1995). For example, it is possible that subspecies, local races or hybrids of a fossil taxon existed which cannot be identified on the basis of the fossil material and therefore cannot be separated. It is also conceivable that different subtaxa may react on varying pCO_2 levels differently. Woodward et al. (2002) observed how different accessions of *Arabidopsis thaliana* show differently strong responses of stomatal density to pCO_2, and that these responses are influenced differently by water availability. It is also probable that fossil species, which existed over a large time span, experienced an evolutionary development. *E. furcinervis*, for example, one of the considered species, existed in central Europe from the Middle Eocene to the Oligocene/Miocene boundary (Mai and Walther, 1991). During this time interval, the leaves of *E. furcinervis* underwent certain evolutionary changes: the leaves tend to be less toothed and more undulated with decreasing stratigraphic age (Kriegel, 2001). Possibly, the quantitative relationships between pCO_2 and stomatal parameters are also affected if evolutionary changes occur in a taxon (see also Section 2.2).

Despite the high variance of the stomatal data, two of the three considered species, *E. furcinervis* and *L. acutimontanum*, show significant differences in stomatal density/index between certain time slices. According to the present datasets, pCO_2 was lower

during the Late Oligocene, the Oligocene/Miocene boundary and during the Early Miocene than during the Late Eocene. The datasets also indicate that pCO_2 was lower during the Early Oligocene than during the Late Oligocene, the Oligocene/Miocene boundary and during the Early Miocene. No differences in pCO_2 between Late Oligocene, the Oligocene/Miocene boundary and the Early Miocene could be detected by the stomatal data of the two reacting species. According to Pearson and Palmer (2000), who analyzed boron isotope ratios of ancient planktonic foraminifera shells, pCO_2 decreased from a high level during the Eocene and attained much lower values during the Miocene. They provide, however, no data for the Late Eocene. Stomatal data of *Ginkgo* also suggest a decrease in pCO_2 at the Eocene/Oligocene boundary (Retallack, 2002). The data of Pearson and Palmer (2000) indicate a decrease in pCO_2 at the Oligocene/Miocene boundary, as well as the data of Pagani et al. (1999). A decrease in pCO_2 at the Oligocene/Miocene boundary is not indicated by the present dataset (see Fig. 17).

4.2. CA and LMA

All upper limits of the LMA values of the Late Eocene are much higher than the MAT results reconstructed by the CA. The reasons can be attributed to several factors. The datasets of the Upper Eocene localities for LMA are with $n = 15$, 11 and 7 rather sparse. In the case of fossil leaf assemblages, the statistical problem of low number of available taxa is strongly increased by the taphonomic processes contributing to the development of a fossil assemblage, i.e., preservation potential or differential transport. Uneven species abundance can also represent a source of error (Burnham et al., 1992; Wing and DiMichele, 1995). Roth and Dilcher (1978) found that the proportion of entire-margined leaves of an assemblage deposited in a lake bottom was smaller than the proportion of entire-margined species in the surrounding flora. This effect does not appear to be the case in the present study because the MAT values reconstructed by the LMA are higher than the values of the CA. Until now, little is known about possible taphonomic distortions influencing fossil leaf assemblages (Wolfe and Spicer, 1999).

Even the leaf litter of an extant tropical or subtropical forest may not reflect forest tree composition correctly, perhaps due to the high diversity (Burnham, 1994). It is, however, possible that the LMA yields a more realistic result for the Late Eocene than the CA, even with the rather sparse material available for the LMA, because the taxonomic distance between the extant species used for the CA and the Late Eocene species is higher than is the case for younger fossil species.

For other epochs, the CA and LMA results are generally in good agreement. Exceptions (cases where no overlap exists between LMA and CA results) are Borna and Witznitz. For these two locations, comparatively few taxa are available for LMA (Borna: $n = 18$; Witznitz: $n = 11$). That a high degree of coincidence of results of LMA and CA is possible was also observed by Uhl et al. (2003), who studied the Kleinsaubernitz site and the Middle Miocene location of Schrotzburg (southern Germany). An earlier study applied both CA and leaf characteristics (CLAMP) for climate reconstruction of the Lower Rhine Embayment from the Middle Miocene to the Upper Pliocene (Mosbrugger and Utescher, 1997). The MAT results of the leaf analysis yielded significantly lower temperatures than the CA in this earlier study. A reason for this deviation may be represented by the fact that floras adjacent to riversides tend to be dominated by species with toothed leaves and can thus distort the results towards significantly lower MAT values (Burnham et al., 2001). The same is true for lakes and riparian sites (Burnham et al., 2001). This may well be the case for the data of the Lower Rhine Embayment. This effect does obviously not play a role for the present datasets because the LMA values of MAT are not systematically lower than the CA results despite the fact that the majority of the data stems from the Weisselster river system.

Besides the generally good agreement between the MAT results yielded by CA and LMA in the present study, other parameters represent additional indications of the quality of the LMA signal. Preconditions for a strong relationship between percentage of species with entire leaf margins and temperature are: (1) mean temperature of warmest month (WMM)>15 °C, (2) drought-free environment and (3) long growing season (Wing and Greenwood, 1993; Wilf, 1997; Wolfe and Spicer, 1999). The climate parameters of

the different palaeosites summarized in Table 3 fulfil these conditions and thus indicate a high reliability of the LMA results.

The combined MAT results of CA and LMA reveal a trend towards lower MAT after the Eocene (Figs. 17 and 18) and are thus in general agreement with all other evidences about the cooling event which occurred after the Eocene (Diester-Haass and Zahn, 2001; Zachos et al., 2001 and citations therein). Ice house climate was established during the Early Oligocene (Miller et al., 1991). The data obtained from oceanic sedimentary archives indicate that a warming event followed during the Late Oligocene superseded by a cooling event at the Oligocene/Miocene boundary (Zachos et al., 2001 and citations therein). The MAT results of the present study summarized in Fig. 18, however, reveal no distinct trend towards higher temperatures from the Early Oligocene onwards for the considered sites. The MAT values obtained with the CA scatter around roughly 15 °C for this time period. An unequivocal signal for a warming trend in the Late Oligocene is thus not observed for the considered locations. The LMA indicates higher temperatures at the Oligocene/Miocene boundary (site of Witznitz; Fig. 17). Walther (1999) also recognized the Witznitz flora as reflecting a more subtropical character and interpreted this as an indication for a short warming event at this site. For the Early Miocene, the LMA data indicate cooler MAT. The LMA and CA results of the Oligocene and Miocene locations thus do not provide clear evidence for further warming or cooling events during this time interval at the considered area.

4.3. Climate parameters and pCO_2

E. furcinervis shows an increase in stomatal density after the Eocene and thus signals a decrease in pCO_2. Coupled to this pCO_2 decrease, which is indicated by the stomatal density of this taxon, a decrease in temperature can be inferred by the results of both CA and LMA (see Figs. 17 and 18). When compared to the global marine temperature data provided by Zachos et al. (2001), the pCO_2 decrease suggested by the stomatal data of the present study occurs simultaneously to the cooling event at the Eocene/Oligocene boundary. This cooling event is also documented by the LMA/CA results of the present study. Marine sediments signal a strong increase in marine palaeoproductivity during the Eocene/Oligocene boundary (Diester-Haass and Zahn, 1996, 2001). It was suggested by the authors that—due to this productivity increase—CO_2 was extracted from the atmosphere and sequestered into marine sediments (Diester-Haass and Zahn, 2001). This process could have resulted in a pCO_2 decrease, which probably amplified global cooling (Diester-Haass and Zahn, 2001). The pCO_2 decrease over the Late Eocene/Early Oligocene indicated by the present dataset corroborates this hypothesis.

The present dataset further indicates that pCO_2 increases from the Early Oligocene to the Late Oligocene, signalled by lower stomatal densities/indices of the Late Oligocene, if compared to the Early Oligocene data. This pCO_2 increase after the Early Oligocene appears to be contemporaneous to the Late Oligocene warming event documented by marine data (Zachos et al., 2001). This warming event is, however, not reflected by the LMA and CA data for the considered sites (see Section 4.2). From the Late Oligocene onwards, the data indicate no further change in pCO_2. The present dataset thus provides no signal for decreasing pCO_2 during the cooling event at the Oligocene/Miocene boundary indicated by marine data (Zachos et al., 2001).

The stomata analysis of the present study thus reflects general changes in pCO_2 during the Late Eocene and Early Oligocene which appear to be simultaneous to corresponding cooling/warming events according to marine data. It should be emphasized, however, that no cause-and-effect relationship can be derived from this temporal accordance suggested by the present datasets. Due to the problem of temporal resolution, fossil data records which document a parallel increase or decrease of temperature, and pCO_2 cannot unequivocally answer the question of what comes first: pCO_2 increase or temperature increase (Boucot and Gray, 2001). Even high-resolution records of pCO_2 data by ice core technique and temperature proxy data do not always allow for a final decision on whether increase pCO_2 precedes or follows temperature increase (Fischer et al., 1999; Petit et al., 1999). Several authors discuss that various parameters other than greenhouse gases, especially tectonic events and eccentricity variations in Earth's orbit, are significant factors which cause climate

changes on Earth (e.g., Pagani et al., 1999; Zachos et al., 2001). It is, however, strongly argued that the atmospheric CO_2 content represents at least an amplifying agent of global climate change (Petit et al., 1999; Zachos et al., 2001). Further work on possible coupling/decoupling of pCO_2 and temperature at various geological epochs is therefore required in order to improve our knowledge about complexity and dynamics of the Earth's climate.

5. Conclusions

1. The stomatal data obtained in this study show a considerable degree of scattering. This variance is probably due to various classes of heterogeneity (environmental, genetic and evolutionary factors). It is, however, possible to detect qualitative pCO_2 signals in the case of *E. furcinervis* and *L. acutimontanum*.
2. According to these signals, pCO_2 was higher during the Late Eocene than during the Early Oligocene, and increased again towards the Late Oligocene. A decrease in pCO_2 from the Late Eocene to the Early Oligocene is also suggested by increasing marine palaeoproductivity during this time interval.
3. No pCO_2 change during the time interval Late Oligocene – Oligocene/Miocene boundary – Early Miocene could be detected.
4. Results obtained with LMA and CA document a significant cooling from the Late Eocene to the Early Oligocene. The data further indicate that the climate at the considered sites did not change significantly from the Early Oligocene onwards. The warming event at the Late Oligocene and the cooling event occurring at the Oligocene/Miocene boundary which are indicated by marine data are thus not reflected by the present dataset at the considered sites.
5. The pCO_2 decrease from the Late Eocene to the Early Oligocene as signalled by the stomatal data is thus coupled to a cooling event during this time which is also recorded by other data sources such as marine climate archives. This coupling is reflected in the present study by a significant correlation between stomatal density and temperature for this time interval.

6. According to the present data, the pCO_2 increase from the Early Oligocene to the Late Oligocene was not accompanied by a climate change at the considered area. Marine climate data, however, suggest a warming event during the Late Oligocene.
7. No change in pCO_2 during the cooling event at the Oligocene/Miocene boundary, which is also indicated by marine data, can be inferred from the stomatal data raised in this study.

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