Oligocene–Miocene CO₂ fluctuations, climatic and palaeoecological trends inferred from fossil plant assemblages in central Europe

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Stomatal frequency data from four independently calibrated woody plant species reveal pronounced CO₂ fluctuations since the Late Oligocene. CO₂ fluctuations in the Early to Middle Miocene are of such a magnitude that they may have driven much of the temperature variation and major climatic events. Warm intervals such as in the Late Oligocene and the mid-Miocene climatic optimum are characterized by significantly elevated CO₂ levels of at least about 500 ppmv. Elevated CO₂ levels may have also increased the diversity in C3 plant communities. The major cooling events in the Early Miocene and the late Middle Miocene are characterized by CO₂ decreases down to 340 ppmv and 280 ppmv, respectively. A comparison with marine and continental climate records indicates that much of the long-term climate variation was linked to changes in atmospheric CO₂. The CO₂ fluctuations may have influenced ancient diversity of plant communities indirectly via changes in climate parameters, such as temperature or precipitation, seasonality, and/or directly by affecting plant photosynthetic performance. In the Oligocene, the type of vegetation is dominated by thermophilous paratropical–subtropical elements including palms but also with a considerable proportion of deciduous trees. The vegetation during the Oligocene–Miocene transition is slightly impoverished in species richness. There are profound differences in the physiognomy of elements, which are predominantly deciduous. During the Early to early Middle Miocene (15–17 Ma), plant assemblages are characterized by subtropical Notophyllous Evergreen forest vegetation with a maximum in species richness reflecting the middle Miocene climatic optimum (Late Mastixioid floras sensu Mai). In the late Middle Miocene (13–14 Ma), the floras are less diversified, the woody elements are mostly dicots, both evergreen and deciduous and the vegetation type belongs to the Mixed Mesophytic Forest with a higher proportion of evergreen elements but with exceptional macrofossils of palms. The youngest flora of the latest Middle Miocene is much less diversified in contrast to the previous time interval. Generally, more diversified deciduous broad-leaved trees predominate while the lauroid elements become much less dominant. The diversity trends in central Europe suggest that the highest diversification found during the Late Oligocene and Middle Miocene most likely resulted from optimal climate conditions that expanded the area available for thermophilous, paratropical–subtropical vegetation into the mid-latitudes. The Oligocene–Miocene vegetation history is hard to explain with permanent low CO₂ conditions (~200–280 ppmv) as suggested by the marine CO₂ proxy records.


Anthropogenic greenhouse gas emissions and modification of land surfaces are changing Earth’s climate profoundly. IPCC assessments indicate that global temperature will rise by about 3 °C during the next decades and will turn the present day “icehouse climate” into a “greenhouse climate” (Meehl et al. 2007). In order to assess the human impact against the natural climate variability, studies of past warm intervals are of pivotal importance. A coupling between atmospheric CO₂ and glacial–interglacial cycles over the past 600,000 years is well documented by ice core analysis (Siegenthaler et al. 2005). During this time, however, CO₂ did not exceed the present day level. Therefore, palaeoclimate studies also increasingly focus on older geological periods with presumably elevated CO₂ levels which may represent a better ancient analogue for our future greenhouse world (e.g., Sluijs et al. 2007, Zachos et al. 2008).
The Miocene is of particular interest for palaeoclimate studies as it represents a period of the Cenozoic which is characterized by extreme climate states such as the Middle Miocene climatic optimum and two major glaciations during the Early and the late Middle Miocene, when the east Antarctic ice sheet was established (e.g., Flower & Kennett 1993, Zachos et al. 2001). The middle Miocene cooling marks the founding of the modern Late Cenozoic cool mode. Associated with these key climatic events are profound vegetation changes such as the expansion of grasslands and C4 plants (Cerling et al. 1997, Jacobs et al. 1999, Stroemberg 2002). Major radiations in large mammalian herbivores have been attributed to changes in the distribution of vegetation and terrestrial primary productivity (Janis et al. 2000, MacFadden 2005). Miocene CO2 reconstructions based on marine geochemical proxies, however, indicate consistently low CO2 since the Early Miocene despite the occurrence of large-scale shifts in climate (Pagani et al. 1999, Pearson & Palmer 2000). Although both climate trends and plant physiological adaptations are thought to be influenced by long-term CO2 fluctuations (Ehleringer et al. 1991), CO2 reconstructions and modeling studies have disputed the role of CO2 as a forcing factor (Shevenell et al. 2004, Pagani et al. 2005).

In addition to the marine geochemical proxy records, the pore (stomata) frequency, preserved on the cuticle of fossil leaf remains, represents a terrestrial proxy for atmospheric CO2 that is based on the inverse relationship between atmospheric CO2 and the number of stomata (Woodward 1987). It provides a robust method for estimating CO2 levels for short (e.g., Wagner et al. 2002) and geologically long-time scales (e.g., van der Burgh et al. 1993, Royer et al. 2001, Beerling et al. 2002). Because the stomatal frequency response to CO2 is species-specific, quantitative estimates of CO2 are limited to extant species. This method requires good preservation of leaf fossils with cuticles and a good understanding of taxonomy and evolution of those elements that are followed.

The current paper is a sequel to a palaeoatmospheric CO2 study based on stomatal counts of laurel and Ginkgo leaf remains (Kürschner et al. 2008). Here we document in detail the taxonomical and biostratigraphic framework as well as the regional palaeofloristic and palaeoclimatic trends from the Late Oligocene to the Late Miocene in central Europe.

### Material and methods

We have studied three species of Lauraceae and Ginkgo, a total of 70 herbarium and 36 fossil leaf samples (Table 1) for their epidermal cell properties. The species are either ancestors distributed in the European Cenozoic and included into extant genera – *Laurus abchasica, Ocotea hradekensis, Ginkgo adiantoides* or one extinct morphospecies *Laurophyllum pseudoprinceps* with doubtful affinities. In order to convert the stomatal index (SI) values from the species *L. abchasica, O. hradekensis* to CO2 levels, CO2 inference models were established from their living equivalents *Laurus nobilis* and *Ocotea foetens* (Fig. 1). The taxonomical relationships have been established by extensive comparative studies on leaf morphology and cuticle anatomy (Ferguson 1974, Búzek et al. 1996). CO2 estimates from *G. adiantoides–biloba* lineage were based on calibration data by Royer et al. (2001). In the absence of a modern equivalent for the extinct species *L. pseudoprinceps*, the SI response of this species has been cross calibrated by using Miocene CO2 levels inferred from the three other extant species.

### Table 1. Mean raw SI values for herbarium and modern *Laurus nobilis* leaves

<table>
<thead>
<tr>
<th>sample</th>
<th>age [AD]</th>
<th>location</th>
<th>atm. CO2 [ppmv]</th>
<th>SI [%]</th>
<th>S.D.</th>
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The stomatal index calibration of CO2 concentrations results in a linear relationship with stomatal density (SD [n/mm²]). Most of the fossil cuticle SI = –0.6724 CO2 + 38.367 and a coefficient of determination (R²) of 0.64. The stomatal index calibration of L. nobilis to atmospheric CO2 concentrations results in a linear relationship with SI = –0.5448 CO2 + 30.245 and a coefficient of determination (R²) of 0.5.

To account for the non-linear response of SI to changing CO2 concentrations, both herbarium SI data and the historical CO2 concentrations were log-transformed before fitting a linear response curve through the data sets. For L. nobilis, this results in a relationship of CO2 = 10^{1.173 \times [0.5499 \times \log(SI_{fossil})]} with a coefficient of determination (R²) of 0.78 between the measured and inferred CO2 values and a root square error (RMSE) of 13.5 ppmv CO2.

For O. foetens the regression curve (Fig. 1) and statistics are: CO2 = 10^{1.9624 \times [-0.4284 \times \log(SI_{fossil})]} with a coefficient of determination (R²) of 0.5 between the measured and inferred CO2 values and a root square error (RMSE) of 19 ppmv CO2.

In the absence of a modern equivalent for the extinct species L. pseudoprinceps, the stomatal index response of this species has been cross calibrated by using Miocene CO2 levels inferred from the other three extant species. This results in a relationship of CO2 = –46.011 \times SI_{fossil} + 993.37 with a coefficient of determination (R²) of 0.68. The multiple species stomatal index record based on the three extant species allows the CO2 values to be independently inferred from the individual species-specific CO2 inference models and verified for inter-specific coherence.

**Stratigraphy of the fossil leaf samples studied**

The Oligocene site Markvartice belongs to the Děčín Fm. of the České středohoří Ms and is dated by K-Ar method to be 30.8–24.7 Ma, i.e. late Rupelian to early Chattian (Cajz 2000). The Libkovic Member of the Most Basin has been dated by palaeomagnetic study (Bucha et al. 1987). For the lower part of this member (localities Břešťany and Jezerí with cores KU 285, MR 59, lower part – chron 6) the value 20 Ma is given, for the higher levels (samples from the upper part of the core MR 59 – chron 5e) the age of about 19 Ma can be estimated. The sample from the core JZ 44 below the coal seam belongs to the Duchcov Member. Its age cannot be precisely estimated, but it is tentatively assigned to the Aquitanian. The base of the main seam (i.e. Holešice Member) is dated into the Egenburgian according to its small mammal content (zone MN 3a for the fauna from the Merkur-North Mine – Fejfar 1974).

For the Cypris Formation in the Cheb Basin, age assessment has been established based on small mammal remains. The plant bearing levels are within or slightly below the fauna of Františkovo Lázěn, which corresponds to the lower part of the mammal zone MN 5, correlated with the Karpatian of the Paratethys area (Höck, personal communication 2004). The Mydlovary Formation of the South
Bohemian basins has been assigned to the Early Badenian on account of the radiometric age of the tectites 14.7 ± 0.7 Ma in the roof of the formation (Genter et al. 2003), small mammals of the MN 5 zone on the base and MN 6 within the formation (Fejfar, personal communication 2004, Ševčík et al. 2007) and the diatom flora (Reháková in Vrána 1980). The rest of the floras (Hrádek, Wackersdorf, Horní Bříza) are dated according to the regional geological and palaeofloristic correlation and the same applies to the material from the Lusatia Basin and Lower Rhinelan Embayment in Germany (see Němec et al. 2003, Czaia 2003, Ferguson et al. 1998). The fossil cuticle samples from Oberdorf, Styrian Basin in Austria (provided by Johanna Eder-Kovar) are assigned to the Ottnangian based on the mammals of the MN4 zone (Kovar-Eder & Meller 2001). The Bulgarian Sarmatian material from Karbinci that has been used from the published data (Uzunova 1995) is dated by its mollusc fauna to the Bessarabian (ca 10–12 Ma) of the Dacian Basin (Palamarev et al. 1999).

**Taxonomical remarks**

**Ginkgo adiantoides** (Unger) Heer

syn. *Ginkgo florinii* Samylina,

*Ginkgo occidentalis* Samylina

Figure 2A

This is the only representative of *Ginkgo* in the European Neogene. It has been split into smaller entities (Samylina 1967) on account of epidermal features (rare *vs* thicker cuticle, weak *vs* distinct papillae), which we consider as only ecotypic variation of a single species. It is true that the ancestors with similar leaf morphology were spread over the whole Northern Hemisphere during the Early Palaeogene. These have been rightly given different species names (for more information see Boulter & Kvaček 1989). The earliest record of *Ginkgo adiantoides* in central Europe has been enounced from the German locality Wiesa (Mai 2000), but there is some uncertainty, from which layers these fossil compressions come exactly. Most other records have been connected with the warm temperate Mixed Mesophytic forest. This is also the case of Horní Bříza in western Bohemia (Němec et al. 2003), where *Ginkgo* is accompanied by *Chamaerops* and the evergreen *Gordonia* (Theaceae) but also deciduous oaks, and other Arcto-tertiary elements. More records are available from the Late Badenian and Sarmatian of the Ukraine (Samylina 1967), Poland (Szafer 1967) and many other sites in Europe (for a more complete list of synonyms / records see Palamarev & Petkova 1987). We studied the material from the roof of the Frimmersdorf Seam in the Frimmersdorf Mine, which can be assigned to the Middle Miocene cooling period (Utescher et al. 2000). Later on *Ginkgo* was fairly well spread during the Late Miocene and Pliocene in Europe. *Ginkgo adiantoides* is not fully identi-
cal, at least with respect to epidermal anatomy (see also Samylina 1967), with the living *Ginkgo biloba* (Fig. 2B), native in East Asia, which is today probably not known in a wild state but widely cultivated in China and elsewhere in the temperate Northern Hemisphere.

**Laurus abchasica** (Kolakowskii & Shakryl) Ferguson

syn. *Laurophyllum abhasicum* Kolakowskii & Shakryl;

*Piliparicuítis triangulata* Schneider; *Litsaeophyllum miocenicum* Juchniewicz; *Laurus pliocenica* (Saporta & Marion) Kolakowskii; *Laurus canariensis pliocenica* Saporta & Marion

Figure 2C

This evergreen element of the Lauraceae represents the di-
rect ancestor of two extant laurels distributed today in the Mediterranean (*L. nobilis* – Fig. 2D) and Canary Islands (*L. azorica*), being more similar to the latter (Ferguson 1974). It is recognizable even in small fragments of the abaxial leaf cuticle (rhomboidal stomatal complexes, omega-shaped fine undulation of anticlinal cell walls). In central Europe it occurs sporadically in the latest Egen-
burgian and Ottnangian, mostly as solitary specimens (Most Basin, Libkovic Member – Teodoridis & Kvaček 2006; Oberdorf, Hanging wall sequence – Kovar-Eder & Meller 2001). More regularly it is recorded in Wackersdorf (Knobloch & Kvaček 1976), in the Cheb Basin, Cypris Fm. (Bůžek et al. 1996), the Czech and Polish parts of the Zittau Basin (Hrádek – Kvaček & Bůžek 1966; Turów – Juchnie-
wicz 1975), the Main (Second) Lusatia Coal Seam in Ger-
many (coal mines Greifenhain, Nohcten; *Piliparicuítis tri-
angulata* Schneider 1969, *cf. Apollonias canariensis sensu* Litke 1966), ranging into the Karpatic–Early Badenian. The last record from central Europe from the Mydlovary Formation in southern Bohemia is still of Badenian age (Le-
denice – Knobloch & Kvaček 1996). The same age can be ascribed to the site Kreuzau (Ferguson 1971, as taxon XXIII) in the Lower Rhinelan Embayment, based on the floristic correlation (Ferguson et al. 1998). In all mentioned
assemblages *Laurus* is accompanied, in addition to “Arcto-
tertiary” broad-leaved deciduous elements, by other Laurae-
ceae, evergreen Fagaceae (mainly *Trigonobalanopsis*), *En-
gelhardtia* and evergreen Hamamelidaceae (*Distylium*). La-
ter in the Miocene *Laurus abchasica* withdrew southwards
(Sarmatian of NW Bulgaria – Uzunova 1995) and survived
 till the Pliocene in Italy (Fossa – Martinetto, personal com-
 munication 2005) and Abkhasia (Kolakovskii 1958).

*Ocotea hradekensis* (Kvaček & Bůžek) Kvaček

syn. *Laurophyllum hradekense* Kvaček & Bůžek; *Piliparicus hradekensis* (Kvaček & Bůžek) Schneider; *Ocotea macrostoma sensu* Uzunova & Stojanova; *Ocotea heerii* (Gaudin) Takhtajan; *Oreodaphne heerii* Gaudin

Figure 2E

In many ways, this lauroid is similar to the previous case, but less typical in the stomatal pattern. With respect to epidermal anatomy (butterfly-like transparent stomatal complexes with broad and short ledges) and leaf architecture (glands at the base of the lowest secondary veins) and des-
pite subtle differences (Schneider 2005) *Ocotea hradekensis*
 is the best candidate for the direct ancestor of *Ocotea fo-
etens*, a relic in the Canary Island lauri-silvae (Fig. 2F). So
far, it was recorded only in the European Neogene, namely
at a few sites of the Early-Middle Miocene, first in Wacker-
sdorf (Knobloch & Kvaček 1976), the Cheb Basin, Cypris
Fm. (Bůžek et al. 1996), the Zittau and Berzdorf Basins
(Kvaček & Bůžek 1966, Schneider 2005), the Main (Se-
cond) Lusatia Coal Seam in Germany (*Laureaeceae 2 sensu*
Litke 1966), ranging into the Karpatian–Lower Badenian.
Its last record from Central Europe is again from the Myd-
lovakary Formation in southern Bohemia (Ledenice – Knob-
loch & Kvaček 1996). The dispersed cuticles from the Sar-
matian in Bulgaria (Ružinci, Karbinci – Uzunova 1995)
indicate its migration southwards. *Ocotea* (usually named
*O. heerii*) was a common evergreen element in the Late
Neogene floras of Italy (Fischer & Butzmann 2000, Marti-
netto, personal communication).

*Laurophyllum pseudoprinceps* Weyl and Kilpper

complex

syn. *Laurophyllum undulatum* Weyl and Kilpper; 
*Laurophyllum verrucosum* Weyl and Kilpper; *Ocoteophyllum verrucosum* (Weyl and Kilpper) Juchniewicz; *Nectandrophyllum czeczottiae* Juchniewicz; *Ocotea ob lanceolata* Palamarov & Petkova; *Ocotea pseudoprinceps* (Weyl and Kilpper) Uzunova & Stojanova; *Ocotea undulata* (Weyl and Kilpper) Uzunova & Stojanova; *Piliparicus pseudoprinceps* (Weyl and Kilpper) Schneider

Figure 2G, H

This apparently extinct lauroid was correlated with various
members of *Ocotea* (Juchniewicz 1976, Uzunova & Stoja-

ova 1999) without very satisfactory evidence as to the ge-

nic affinity. Indeed, *Laurophyllum pseudoprinceps* is si-
milar in the stomatal pattern to *Ocotea s.l.*, but at the same
time, also to other genera, e.g., *Aniba*, *Cryptocarya*, which
should also be taken into consideration (Kvaček 1971). Par-

ticularly the bead-like thickenings on the anticlinal cell
walls in combination with the stomatal pattern (finely gra-
nular surface of the guard cells) are characteristic for this
element. The direct lineage of the fossil towards an extant
species, as is the case for *Laurus abchasica* and *Ocotea hradekensis*, is broken at the Pliocene, when *L. pseudoprinceps* became extinct in Europe. The first record comes
from the Late Eocene sandstone in North Bohemia (Knob-
loch & Kvaček 1996). Then this lauroid becomes common
in warmer parts of the Oligocene (e.g., Markvartice – Bů-
žek et al. 1976) and Early Miocene in central and Western
Europe (Kvaček 1971). The *Laurophyllum pseudoprinceps*
complex consists of two to three morphotypes sometimes
considered as independent (sub)species (Weyland & Kil-
pper 1963, *Kvaček in Knobloch et al. 1996*). We used in
our study only the typical *L. pseudoprinceps* in order to
avoid the influence of different leaf morphotypes, the for-
mer representing sun leaves while *L. undulatum* may repre-
sent a shade morphotype. A further interfering factor may
arise from polyploidy. In the case of *L. macrostoma*, a tran-
sitional form can be mistaken for *Ocotea hradekensis*
(Uzunova & Stojanova 1999). During the Late Badenian,
this species complex migrated southwards and is met again
in the Sarmatian and Pannonian of Roumania (Suraru et
al. 1978, Givulescu 1975 – partly as *Laurophyllum rhom-
hoidale*) and the Sarmatian of NW Bulgaria (Uzunova
1995, Uzunova & Stojanova 1999). The European Plio-
cene records are known so far only from Italy (Martinetto,
personal communication 2005).

Considering the role of all three lauroid species in asso-
ciated assemblages, they are typical components of the
subtropical evergreen humid forest types. The diversifica-
tion of the Lauraceae in Europe coincides with appearance
of the “Late Mastixioid Floras” in the Middle Miocene cli-

cimate optimum. Later on, central and eastern Europe was
covered by mixed mesophytic and broad-leaved deciduous
forest, in which only more hardy evergreen taxa remained
or re-appeared in southerly stations of the Paratethys and
Tethys area (Kvaček et al. 2006). This is also the time, when
*Ginkgo adiantoides* became a common component of the
forest vegetation over the whole of Europe.

Various types of the forest vegetation give only approx-
imate palaeoclimatic data and the ratio of deciduous vs.
evergreen Woody elements does not indicate very precisely
the variation in the climate / temperature regime. From the
studied Czech locations statistical calculations for two lev-
els are available. The CLAMP analysis of the diversified
classical flora of the Břešťany (Preschen) Clay (lower part
of the Libkovic Member in the Most Basin, Eggen-
burgian) indicate the following proxy data (Teodoridis & Kvaček 2006): Mean Annual Temperature (MAT) 15.9 ± 1.2 °C; mean temperature of the warmest month (WMMT) 25 ± 1.6 °C; mean temperature of the coldest month (CMMT) 7 ± 1.9 °C. According to the co-existence analysis of the Badenian assemblage of Horní Bříza (data provided by D. Uhl), similar climate data have been derived: MAT 15.7–16.5 °C, WMMT 26.4–26.7 °C, CMMT 5.0–6.2 °C.

Results and discussion

Oligocene–Miocene CO2 trends

Since the late Oligocene (Kürschner et al. 2008), the SI records of all four species (Fig. 3A) (Ginkgo adiantoides, Laurus abchasica, Ocotea hradekensis, Laurophyllum pseudoprinceps) show pronounced fluctuations. Three intervals can be recognized in the present record: During the oldest part, the Late Oligocene to Early Miocene (between 25 and 20 Ma), the SI of L. pseudoprinceps shows a significant increase from about 9% to 15% and remains high during the Early Miocene (until 20 Ma). During the middle part, the late Early to early Middle Miocene (between 20 and 16 Ma), L. pseudoprinceps shows a decrease in the SI from 15% to 12%. A simultaneous decline in SI is found for two other laurel species, L. abchasica and O. hradekensis, from 12% to 10% and from 12% to 8%, respectively. In the youngest part of the present study, the Middle Miocene (between 16 and 12 Ma), L. abchasica and G. adiantoides show pronounced changes from 10% to 20% and 8% to 15%, respectively.

The stomatal index record by Kürschner et al. (2008) reveals prominent changes in CO2 since the Late Oligocene (Fig. 3B). From initially high CO2 values of about...
600 ppmv during the Late Oligocene, CO₂ declines drastically to about 340 ppmv during the early Miocene. It remains low until the mid-Early Miocene, 20 Ma and increases again to reach a maximum of about 500 ppmv in the early Middle Miocene, about 15.5 Ma. Superimposed on this increasing trend are several smaller temporary CO₂ oscillations with an amplitude of ~50 ppmv. A second major decline in CO₂ to 280 ppmv occurred during the Middle Miocene, between about 15.5 and 14 Ma which is followed by a slight increase to about 340 ppmv during the Late Miocene. It should be noted that SI data from North American Ginkgo leaf remains (Royer et al. 2001, Retallack 2002) correspond perfectly with the present study. Our data are, however, in contrast with stomatal frequency measurements on 3 dicotyledonous species (Eotrigonobalanus furcinervis, Laurophyllum pseudoprinceps, L. acutimor- tanum) from Late Eocene to Early Miocene sediments in the Weisselster Basin in Saxony, Germany (Roth-Nebelsick et al. 2004). The discrepancy may be partly explained by the insufficient stratigraphic resolution of their material. They combined SI data from different localities / stratigraphic units which may have biased original variations in SI. Another reason could be the use of leaf remains of the Laurophyllum pseudoprinceps complex which is a synthetic morphotaxon consisting of several lauroid leaf morphotypes with doubtful botanical affinity (see our taxonomical remarks above). Mixing SI data from different leaf morphotypes may yield misleading results by omitting species-specific variance of absolute SI values and SI response rates. In the present study only SI data from one leaf morphotype, L. pseudoprinceps Weyland & Kilpper sensu stricto has been used for palaeoatmospheric CO₂ estimates. During the course of our study it appeared necessary to constrain the SI analysis to only one of these lauroid leaf types as the combination of all 3 morphotypes resulted in random variance without any temporal trends in SI, which is similar to the results reported by Roth-Nebelsick et al. (2004). The variance in epidermal cell parameters of the Laurophyllum pseudoprinceps complex is subject to further investigations (Kürschner et al. in prep.).

The present leaf based CO₂ reconstruction, however, shows some similarities with the marine CO₂ proxy records as for example the CO₂ decline at the Oligocene–Miocene transition (Pagani et al. 2005, Pearson & Palmer 2001). But it deviates significantly from the alkenone based CO₂ record throughout the course of the Miocene, which shows consistently low CO₂ levels (200–280 ppmv) despite the presence of major Miocene climate events. The boron isotope based CO₂ reconstruction (Pearson & Palmer 2000) shows CO₂ variations very similar to our study although the absolute values are significantly lower, by about 200 ppmv.

Consistently low CO₂ levels during the Miocene as inferred from marine proxy records has led to the concept of a decoupled climate – carbon cycle relationship (e.g., Pagani et al. 2005). Our new results provide evidence that CO₂ fluctuations may have considerably contributed to the Miocene long-term climate evolution as recorded in marine temperature proxy records (Zachos et al. 2001).

Quantitative continental climate data, such as for example mean annual temperature (MAT), mean annual precipitation (MAP), coldest and warmest month means (CMM, WMM respectively), has been documented from European megafloras by the coexistence approach (Utescher et al. 2000, Mosbrugger et al. 2005). Both, the Late Oligocene warming and the Mid-Miocene Climate Optimum are characterized by significant increases in MAT by about 3 °C. The warming trends are even more pronounced (up to about 5 °C) in the coldest month mean (CMM). By comparison of this continental climate record with the marine CO₂ proxy records available at that time, Mosbrugger and co-workers came to the conclusion that CO₂ was not a major driving force in Cenozoic climate evolution. The present data, however, show that in fact CO₂ may well have contributed to the climate fluctuations. The radiative forcing of the CO₂ fluctuations in the global surface temperature, calculated according to equations by Kothavala et al. (1999) is shown in Fig. 3C. A 2–3 °C warming of the mean global surface temperature is indicated for the Late Oligocene and the Mid-Miocene climatic optima resulting from elevated CO₂ levels. The high CO₂ levels during the climate optima may represent conservative values as the extrapolation of the SI response function beyond the calibration interval results in an underestimation by about 200 ppmv of the actual value. In fact, CO₂ levels may have been about 650–700 ppmv and 800–900 ppmv during the Middle Miocene and Late Oligocene climatic optima, respectively (Kürschner et al. 2008).

Palaeofloristic trends

The CO₂ fluctuations may have influenced ancient diversity of plant communities indirectly via changes in climate parameters, for example temperature or precipitation, seasonality, and l or directly by affecting plant photosynthetic performance. Fossil plant assemblages do not fully reflect the species richness, being highly influenced by taphonomic processes and preservation potential of various plant organs. Therefore, only general characteristics of the sites studied or time sectors are discussed as a first order approximation (Fig. 3D). More detailed studies including both macrobotanical and palynological data will improve the present picture.

The Oligocene floras (sites Markvartice, Sušetice, Proboštov – Bůžek et al. 1976, Radoň et al. 2006) are predominantly woody, with only four conifers and ca 50 arboreal dicots and lianas. Herbaceous monocots are represented by...
a few morphotypes of foliage. The type of vegetation is rather thermophilous, containing paratropical – subtropical elements, such as Icacinaceae, Engelhardia, Sloanea, evergreen Oleaceae, well diversified Lauraceae, but also a considerable proportion of deciduous trees of Betulaceae, Acer, Carya, Ailanthus, Ulmaceae, Tiliaceae and Vitaceae. Legumes are probably also well represented but due to highly uniform leaves, their diversity is less certain with about 5 morphotypes. Palms are represented by 1–2 species.

The sites at the latest Oligocene–Miocene boundary are rare (one site in the Duchcov Mb.). The floras of this time sector belong to the Thierbach type (Teodoridis 2004). With only five conifers and about 35 dicotyledonous arboreal elements are thus slightly poorer in species richness than the previous period. Profound differences however are in the physiognomy of elements, which are predominantly deciduous. Fagus and Pseudolarix are diagnostic for long-distance correlation. Evergreen plants are represented in the shrub storey (Mahonia, Myrica) rather than in the canopy. More diversified assemblages occur in Saxony (Mai & Walther 1991) and contain remnants of subtropical evergreen plants (Mastixia, evergreen Fagaceae, Lauraceae, Theaceae), if a broader concept of this palaeo-floristic unit is accepted. Vegetation is predominantly riparian, although parts may form transitions to the Mixed Mesophytic Forest. Aquatic herbaceous plants are common (Lythraceae, Cyperaceae, Stratiotes).

The Early Miocene flora of the Břešťany clay (bottom of the Libkovic Mb. – Teodoridis & Kvaček 2006) does not differ profoundly from the previous part of the Most section. Conifers are richer, with four Cupressaceae and three species of Pinus. Woody dicots include slightly over 35 species, partly deciduous, partly evergreen. Subtropical elements slightly increased and palms again entered the riparian settings. An enigmatic index fossil Schenkiella (Wójcicki & Kvaček 2002) allows a long-distance correlation with the Brandis type of floras (Mai & Walther 1991). Vegetation of this assemblage corresponds to the mixed mesophytic forest on peripheral slopes and lowland intra-basin swamp forest. The sandy facies (Přívlaky – Teodoridis 2006) yielded purely deciduous riparian broad-leaved elements (ca 20 species) similar in composition to the flora from the Oligocene-Miocene boundary. The adjacent underlying layers contained diversified remains of aquatic plants and grasses (Kvaček et al. 2004).

Higher in the Libkovic Mb. (19 Ma) the assemblages...
come from the core samples – hence no long-term collection and not objectively reflecting species richness. The flora consists of prevailing evergreen woody taxa (diversified Lauraceae, Fagaceae, Engelhardia, Theaceae, Platanus neptuni). The horizon in the cores may laterally coincide with or slightly overlap carpological assemblages on the basin periphery, which are even richer in exotic elements (Symplocos, Theaceae, Myrica, Toddalia, Mastixia) and can form subtropical Notophyllous Evergreen green forest (Teodoridis & Kvaček 2006).

Early to early Middle Miocene floras reflect the Mid-Miocene climate optimum and belong to the Late Mastixioid floras sensu Mai (1964). Locations with the Late Mastixioid floras are frequent, including the Cypris Shale flora of the Cheb Basin, Wackersdorf at Schwandorf in Oberpfalz, Germany, the upper part of the Žitava (Zittau) Basin (Hrádek/N., Hartau, Turów) and adjacent Lusatia (Wiesa). Based on all published records including foliation and carpofloras (Mai 1964; Knobloch & Kvaček 1976; Gregor 1978, 1980; Bůžek & Mai 1991; Mai 2000; Czaja 2003) this type of floras called the Wiesa-Eichelskopf Complex (Mai 1995), is the richest in species of all Miocene plant assemblages in Europe. Conifers reached the highest diversity including 10 Pinaceae, three Cupressaceae and two Taxaceae. Woody dicotyledons are by far the prevailing form, being represented by over 80 species. Palms are represented by only a few species. The floral spectra depend greatly on the sedimentary settings so that the assemblages from fluviatile facies may contain a considerable mixture of riparian deciduous woody taxa. Herbaceous vegetation mostly consists of wetland and aquatic elements (Lythraceae, Aldrovanda, Alismataceae, Cyperaceae). Various and diversified Mastixiaceae may serve for long-distance correlation. The vegetation type is apparently subtropical Notophyllous Evergreen Forest according to the leaf size category with many exotic elements with tropical affinities. Assemblages of the same age and the same aspects are known from the Styrian Basin in Austria (Mellé et al. 1999, Kovar-Eder & Mellé 2001). The diversity of woody dicots is again high, over 60 species. Herbaceous dicot families make up less than 10%. Herbaceous monocots are mostly represented by wetland herbs and Sparganium.

The early Middle Miocene is characterized by transitional floras to the previous period. The South Bohemian assemblages dated to the MN6 zone correspond in a greater part to the Late Mastixioid Floras sensu Mai (Holý 1976, Knobloch & Kvaček 1996, Ševčík et al. 2007). They are assigned to the Kleinleipisch Complex of the Berzdorf Basin in Upper Lusatia (Czaja 2003). Conifers survived as several species of Cupressaceae and Pinaceae. Woody dicots are slightly less diversified in comparison with the Wiesa type, attaining about 50 species. The vegetation becomes more open as documented by the presence of wetland herbaceous plants, both dicots and monocots, with at least 20 species each including Stellaria, Ranunculus, Teucrium etc. The vegetation type corresponds to the Mixed Mesophytic Forest with remnants of mastixioid and other thermophilous elements.

During the late Middle Miocene (13–14 Ma) the palaeofloras from the Lower Rhine Embayment (Ville Fm.) document further floristic changes. The carpological record from the Garzweiler seam in the Zukunft-West mine at Eschweiler includes several conifers (including Juniperus) (van der Burgh 1983, 1987, 1988; Günther & Gregor 1989). The woody elements are mostly dicots, partly evergreen, partly deciduous, slightly over 40 species (the number has been updated according to the revision of Symplocos by Mai & Martinetto, 2006) are all dicots, partly evergreen and partly deciduous. Herbaceous dicots (Umbelliferopsis) and monocots (Cyperaceae, Araceae) are much less common. The vegetation type belongs to the Mixed Mesophytic Forest with a higher proportion of evergreen elements. In physiognomic terms it does not differ much from the previous Kleinleipisch type and still includes some remnants of mastixioids but hardly any macrofossils of palms.

The youngest record in our study comes from Karbinci, NW Bulgaria (Uzunova 1995) and is of latest Middle Miocene – Bessarabian age. The flora is less diversified contrary to the previous time interval of the Sarmatian, called the Volhynian (Palamarev et al. 1999). Of 58 so far described species, a considerable part belongs to woody dicots, including some elements of sub-humid aspects (legumes, sclerophyllous oaks). Generally, the lauriod element declines and more diversified deciduous broad-leaved trees predominate, both in riparian and mesophytic settings. Evergreen woody plants were probably limited to the undergrowth. In addition, also swamp forest of Glyptostrobus – Byttneriophyllum – Nyssa type, and wetland herbaceous communities can be reconstructed. Herbs tend to increase in the pollen spectra. Thermophilous elements, like mastixioids and palms survived in these southern locations.

The above described palaeofloristic trends fit with quantitative estimates of woody plant diversity on a broader scale during the Miocene that have been evaluated by meta-analysis of the available Eurasian palaeobotanical records (Utescher et al. 2007, Kovar-Eder et al. 2008). Our brief, rather qualitative essay on diversity trends in central Europe (Fig. 3) suggests that the highest diversity is found during the Late Oligocene and Middle Miocene, most likely as a result of optimal climate conditions that expanded the area available for thermophilous, paratropical–subtropical vegetation into the mid-latitudes. At present we leave the question open whether the observed maxima in plant diversity are the result of climate optima alone or also caused by elevated CO2 levels. Elevated CO2 levels may have fertilized plant growth and facilitated the expansion of high-diversity forest. However, in our opinion, the Oligocene–Miocene vegetation history as summarized above
is in contradiction with permanent low CO₂ conditions (~200–280 ppmv) as suggested by the marine CO₂ proxy records (Pagani et al. 1999, 2005). Moreover, the low CO₂ scenario has been assessed physiologically with plant photosynthetic models. These modeling experiments show that photorespiration rates under a warm climate and low CO₂ would drastically reduce the Carbon assimilation rates of C₃ land plants (Cowling 1990). In fact, plant growth would have been drastically limited and plant richness would have been much lower than indicated in the fossil record. Further vegetation and climate model experiments may shed new light on the significance of CO₂ fluctuations on the evolution of Cenozoic terrestrial ecosystems.

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References


FLOWER, B.P. & KENNETT, J.P. 1993. The middle Miocene transi-


KVAČEK, Z. & BÚŽEK, Č. 1982. Tertiary assemblages of the North Bohemian brown-coal basin in connection with the lithofacial setting (Tréthorní společenstva severočešské hnědouhelné pánve ve vztažu k litofačním vývoji). MS report, Ústav geologie a geotechniky ČSAV, Praha.


P.calls, 875–878.


Woodward, F.I. 1987. Stomatal numbers are sensitive to increases in CO2, from pre-industrial levels. Nature 327, 617–618. DOI 10.1038/327617a0
