PHYTOLOGIA BALCANICA 20(2–3): 145–153, Sofia, 2014

Variability of selected leaf traits in European beech (*Fagus sylvatica*) in relation to climatic factors – some implications for palaeoenvironmental studies

Dieter Uhl[1, 2]

[1] Senckenberg Forschungsinstitut und Naturmuseum Frankfurt, Senckenberganlage 25, 60325 Frankfurt am Main, Germany; e-mail: dieter.uhl@senckenberg.de

[2] Senckenberg Centre for Human Evolution and Palaeoenvironment, Eberhard-Karls-Universität Tübingen, Institut für Geowissenschaften, Sigwartstraße 10, 72076 Tübingen, Germany

Received: May 12, 2014 ▹ Accepted: July 21, 2014

Abstract. In the present study the intraspecific variability of selected leaf morphological traits [i.e. venation density and fluctuating asymmetry (FA)] in *Fagus sylvatica* L. across a climate gradient in SW-Germany has been studied to get some information whether these traits may have a potential for use in palaeoenvironmental studies or not.

The leaf traits have been correlated with certain climate parameters, frequently used in palaeoclimatic reconstructions (i.e. mean annual temperature, mean temperature of the coldest month, mean temperature of the warmest month, mean annual precipitation, mean precipitation of the coldest month, and mean precipitation of the warmest month) taken from nearby weather stations. Venation density in shade-leaves showed the best correlations with temperature parameters, i.e. MAT ($p < 0.01$) and WMMT ($p < 0.01$) (all other climate parameters: $p < 0.05$), whereas sun-leaves exhibited the best correlations with certain precipitation parameters, i.e. MAP ($p < 0.05$) and CMMP ($p < 0.05$) (all other climate parameters: N.S.). FA showed no clear correlation with any climate parameter, but differs statistically significant between sun- and shade-leaves from SW-Germany. In almost all samples shade-leaves displayed a higher FA than sun-leaves.

Based on our data we can emphasize previous observations, that certain leaf morphological traits, like venation density and fluctuating asymmetry, have a big potential to be used as proxies to supplement established techniques for the reconstruction of palaeoenvironmental conditions. Nevertheless it is also clear that more data from a wide variety of modern taxa, originating from different families and habitats, are needed to interpret differences or changes of individual morphological traits in fossil leaves reliably.

Key words: *Fagus sylvatica* L., leaf venation density, fluctuating asymmetry, climatic gradient, palaeoenvironmental reconstruction

---

This work is dedicated to the memory of Prof. Harald Walther (* 17.05.1929 – † 02.07.2013) one of the foremost experts in Tertiary palaeobotany of Europe.

Introduction

Morphology of angiosperm leaves is an important tool for palaeoenvironmental studies and during the last decades a number of different leaf morphological traits has been utilised for such studies. The most widely known and used traits are the form of the leaf margin, i.e. toothed versus entire (known as leaf margin analysis) to estimate mean annual temperatures (MAT) (e.g. Wolfe 1979; Wilf 1997) and leaf size to reconstruct mean annual precipitation (e.g. Wilf & al. 1998). Moreover, different multivariate methods have been developed that include, amongst leaf margin and size, various additional leaf morphological characters like outline of the leaf, form of the leaf-apex and the leaf-base etc. The most prominent and widely used of these approaches is CLAMP (Climate Leaf Analysis Multivariate Programme) a technique first introduced by Wolfe (1993) and subsequently refined by various authors (e.g. Stranks & England 1997; Wolfe & Spicer 1999; Spicer & al. 2004; Spicer 2000, 2007; Teodoridis & al. 2011). All of these approaches are based on "integrated physiognomies" of whole palaeofloras, statistically summarizing morphological/physiognomic characters from all (i.e. woody angiosperm) taxa within a fossil flora and relating these "integrated physiognomies" to environmental parameters.

So far only a few studies have dealt systematically with the intraspecific variability of selected leaf traits in modern taxa and the potential of such variability for palaeoenvironmental studies. Examples with an explicit palaeoenvironmental focus are, amongst others, studies on the intraspecific variability of leaf venation density (e.g. Manze 1968; Uhl & Mosbrugger 1999a, 1999b; Uhl & Walther 2000, 2003; Uhl & al. 2002), leaf size and shape (e.g. Uhl & Walther 2000, 2003; Royer & al. 2008) in relation to environmental, especially climatic, parameters, and studies on stomatal density/stomatal indices in relation to environmental conditions and atmospheric CO_2-concentrations (e.g. Salisbury 1927; Woodward 1987; Royer 2001). There are also a very large number of studies dealing with physiological and morphological adaptations of plants in relation to environmental factors that have an explicit neo-botanical/ecological focus.

As such studies often focus on parameters that cannot be investigated in fossil leaves it is not always possible (or at least difficult) to extract meaningful information with regard to palaeoenvironmental studies. Nevertheless have a number of palaeoenvironmental studies successfully utilized generalized conclusions drawn from such studies on modern plants to get information about palaeoenvironmental factors or gradients (e.g. van der Burgh 2001; Tamás & Hably 2005, 2009). Such studies show, that it may also be possible to use morphological adaptations of individual taxa to get meaningful palaeoenvironmental information, which can supplement or even refine other, currently more established techniques (e.g. Tamás & Hably 2009). However, many morphological reactions to individual environmental parameters are highly species specific and two different species may react in exact opposite direction in reaction to changes in the same environmental parameters [e.g. lobing of sun- and shade-leaves in *Acer campestre* L. (deeper lobed sun-leaves; Uhl & Walther 2000) and *Sassafras albidum* (Nutt.) Nees (deeper lobed shade-leaves; DeSoyza & Kincaid 1991)]. Thus it is not always possible to generalize observations from individual or only a few taxa, to draw broad conclusions from observations in fossil leaves. To get a better data base of modern taxa it is necessary to investigate the intraspecific variability and potential morphological adaptations across climatic/environmental gradients in a larger number of taxa.

In the present study we investigate the intraspecific variability of selected leaf morphological traits in the European beech (*Fagus sylvatica* L.) across a climate gradient in SW-Germany to get some information whether these traits may have a potential for use in palaeoenvironmental studies or not.

The oldest indubitable remains of leaves and carpological remains assignable to the genus *Fagus* come from the Middle Eocene of Pacific North-western North America (Manchester & Dillhoff 2004). Although fossil representatives of this genus have been studied for more than 150 years, there is still some controversy about the taxonomy of individual species (e.g. Mai 1995; Denk 2004). However, the aim of the present study is not to resolve such taxonomic problems, but to present data about the variability of selected leaf morphological traits in modern *F. sylvatica* to explore the potential of some of these traits as palaeoenvironmental proxies. Nevertheless can these data also be used to judge the potential to use these traits for taxonomic studies of fossil representatives of the genus *Fagus*.

Material and methods

Leaf-material

In August and September 1997 mature leaves of *F. sylvatica* L. were collected at 19 different localities in the vicinity of 15 meteorological stations in SW Germany (Fig. 1), after they reached their final size and before leaf shedding started in autumn (cf. Powers 1967; Uhl & al. 2002). For a complete list of the localities and weather stations see table 1. All leaves were collected in about 1–3 m height above the ground and samples from the outer canopies were classified as sun-leaves, whereas samples from inside the canopies were classified as shade-leaves (cf. Uhl & Walther 2000; Uhl & al. 2002). Leaves were collected covering the whole range of leaf sizes available, but the majority of the leaves were sampled from the same leaf size class. As stated by Uhl & al. (2002) it should be mentioned, that the sampling frequency of the individual leaf samples is not the same as the real size frequency of the leaves in a tree, thus our samples cannot be used to establish correlations between leaf size and climate, but investigation of leaf size is necessary to avoid leaf size effects when investigating other leaf traits, therefore we included this leaf trait in our study as a control to detect potential leaf size biases.

In most cases more than 30 leaves were collected for an individual sample and for most samples 20 leaves per sample have been analysed although in a few cases 30 leaves have been measured and only one sample with less than 20 leaves (i.e. 17 sun-leaves at locality Schopfloch; Table 1) has been included in the present study (cf. Uhl & al. 2002).

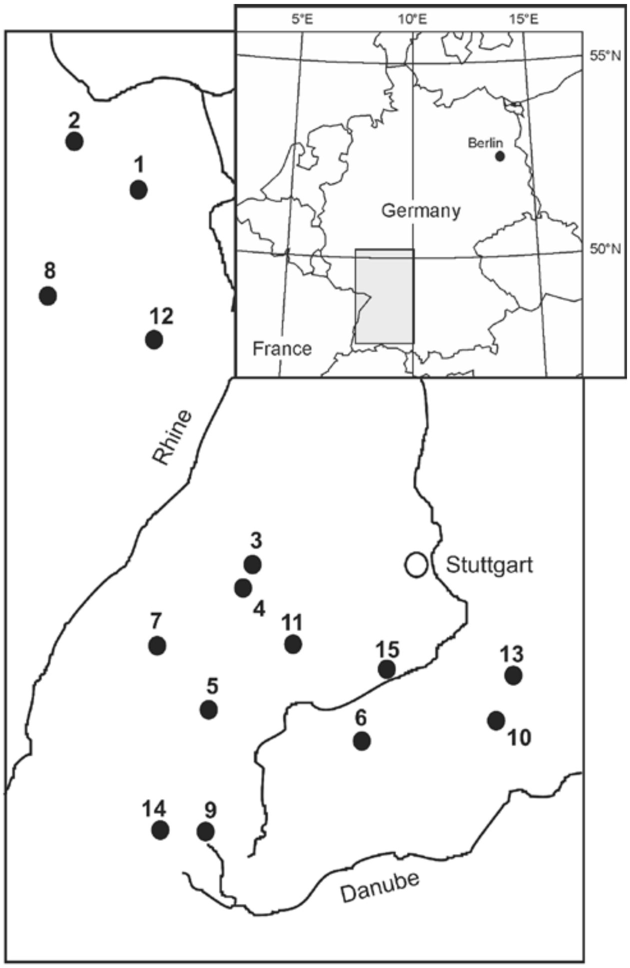

Fig. 1. Map showing the locations of the weather stations from which climate data were used for correlations with leaf traits of nearby collected leaves. 1: Alzey, 2: Bad Kreuznach, 3: Bad Wildbad-Sommerberg, 4: Bad Wildbad-Stadt, 5: Freudenstadt, 6: Hechingen, 7: Hornisgrinde, 8: Kaiserslautern,9: Königsfeld, 10: Münsingen, 11: Nagold, 12: Neustadt an der Weinstraße, 13: Schopfloch, 14: Triberg, 15: Tübingen. For further information refer to Table 1 and Uhl & al. (2002).

Table 1. Sampling localities and corresponding weather stations (from Uhl & al., 2002).

Weather station (numbering according to Fig. 1)	Elevation [m above sea level]	Sampling locality	Leaves studied sun/shade
1: Alzey	166	Alzey	30/20
2: Bad Kreuznach	159	Bad Kreuznach-Hagenbachtal	--/20
3: Bad Wildbad-Sommerberg	740	Bad Wildbad-Sommerberg	20/20
4: Bad Wildbad-Stadt	417	Bad Wildbad-Stadt	20/20
5: Freudenstadt	797	Freudenstadt	20/20
6: Hechingen	520	Hechingen	20/20
7: Hornisgrinde	1125	Hornisgrinde	30/20
8: Kaiserslautern	248	Kaiserslautern ESA/University	20/20
		Kaiserslautern Eselsbachtal	20/20
9: Königsfeld	767	Königsfeld	20/20
10: Münsingen	721	Münsingen	20/20
11: Nagold	392	Nagold	--/20
12: Neustadt an der Weinstraße	163	Neustadt/Wstr.-Ordenswald	20/20
13: Schopfloch	758	Schopfloch	17/20
14: Triberg	683	Triberg	20/20
15: Tübingen	370	Tübingen botanical garden	20/--
		Tübingen Kirnbachtal (KBT)	20/20

Climate data

Climate data (average temperature and precipitation values 1951–1980) for the individual meteorological stations have been taken from Müller-Westermeier (1990). For the present study values for mean annual temperature (MAT), mean temperature of the coldest month (CMMT), mean temperature of the warmest month (WMMT), mean annual precipitation (MAP), mean precipitation of the coldest month (CMMP), and mean precipitation of the warmest month (WMMP) have been used for correlation with leaf morphological traits. Although it is known from previous studies that some of the leaf traits studied here show higher correlations with other climate parameters (e.g. venation density/humidity parameters; Uhl & al. 2002), we used these climate parameters because they have widely been used in previous palaeoclimatic studies based on fossil plants (e.g. Utescher & al. 2000; Mosbrugger & al. 2005; Uhl & al. 2006; Teodoridis & al. 2009).

Measurement of leaf size, length and width

Areas of individual leaves were measured with the commercial software package OPTIMAS 6.2, after digitization of individual leaves. Length and width of the leaves were measured directly with a ruler.

Measurement of venation density

Venation density was measured as distance between veins, following Uhl & Mosbrugger (1999a) and Uhl & al. (2002). This parameter was directly obtained from the leaves with the aid of a binocular and an ocular micrometre: for each leaf ten measurements of the distances between 11 veins, five that crossed a line parallel to the primary vein and five that crossed a line perpendicular to the primary vein, where done. The distance between veins is much easier to determine than the (nevertheless more precise) vein length per area and corresponds to the venation density parameter that has frequently been used in previous studies (e.g. Wylie 1951; Manze 1968; Uhl & Mosbrugger 1999a, 1999b; Uhl & Walther 2000, 2003; Uhl & al. 2002) (For a quantitative comparison of both approaches see Uhl & Mosbrugger 1999a).

Determination of fluctuating asymmetry

To establish a measure of fluctuating asymmetry (FA) the distance between the primary vein and the leaf margin have been measured at the widest point of the leaf lamina perpendicular to the primary vein for both halves of the lamina. The wider of both distances has been divided by the smaller distance, resulting in a dimensionless measure for FA. Values for this measure are by definition ≥ 1 (1 = no asymmetry at all).

Statistics

Statistics were calculated using the STATISTICA 5.1 package (Statsoft-Inc.). Correlations were treated as not significant (N.S.) if the significance levels were higher than 5 % ($p > 0.05$).

Results and discussion

Variability of leaf size

In our samples from SW Germany average leaf size does not differ significantly between sun- and shade-leaves (p = N.S.) (Fig. 2). As expected (see above) there is also no statistically significant correlation between leaf size and any of the climatic parameters considered for this study. We therefore conclude that any potential co-variation between leaf size and the other leaf traits investigated by us (leaf size effect) can be largely neglected for the present study.

Variability of leaf length : width ratio

In our samples from SW Germany the leaf length: width ratio does not differ significantly between sun- and shade-leaves (p = N.S.). There is also no statistically significant correlation between the leaf length : width ratio and any of the climatic parameters considered for this study, although there seems to be a slight trend towards higher ratios at higher temperatures (Fig. 3).

This result could be an artefact of sampling, as sampling frequency of the individual leaf samples is not the same as the real size frequency of the leaves in a tree (Uhl & al. 2002) and it is possible that length : width ratios show an allometric relationship with leaf size. However, a correlation between leaf size and length : width ratio for all leaves investigated in the present study, did not show evidence for allometric leaf growth in our *F. sylvatica* samples from SW-Germany (unpublished results). Thus it is not likely that differences in allometric growth occurred which are related to climatic conditions at the different localities. We therefore conclude that also any potential co-variation between leaf length : width ratio and the other leaf traits investigated by us can be largely neglected for the present study.

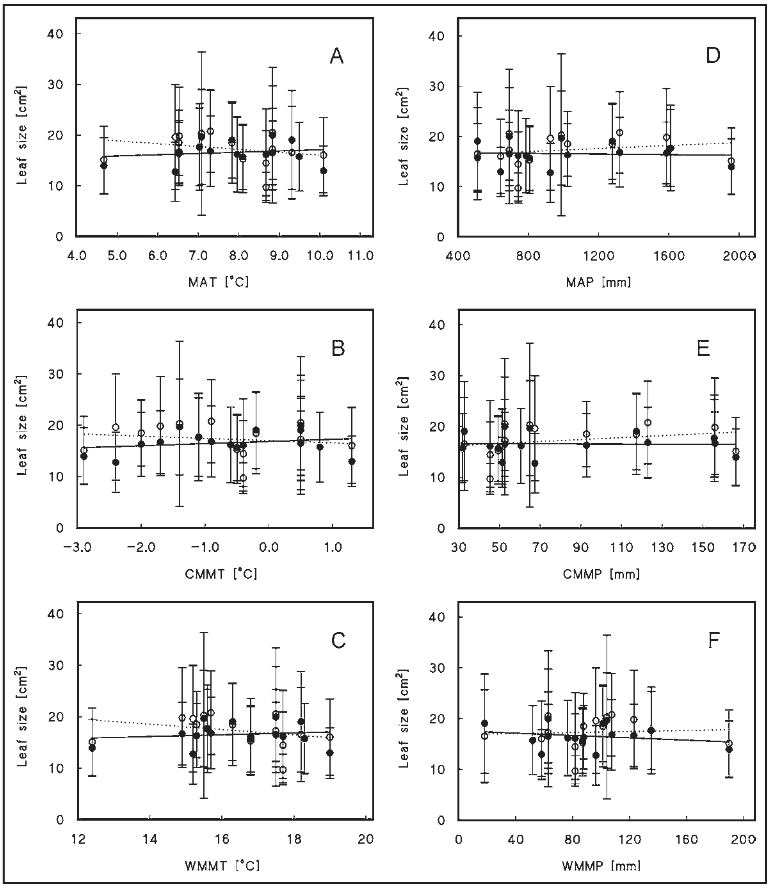

Fig. 2. Correlations between leaf size [cm^2] and the different climate parameters (see text). ● = shade leaves, solid line = regression line for shade leaves; ○ = sun leaves, dotted line = regression line for sun leaves.

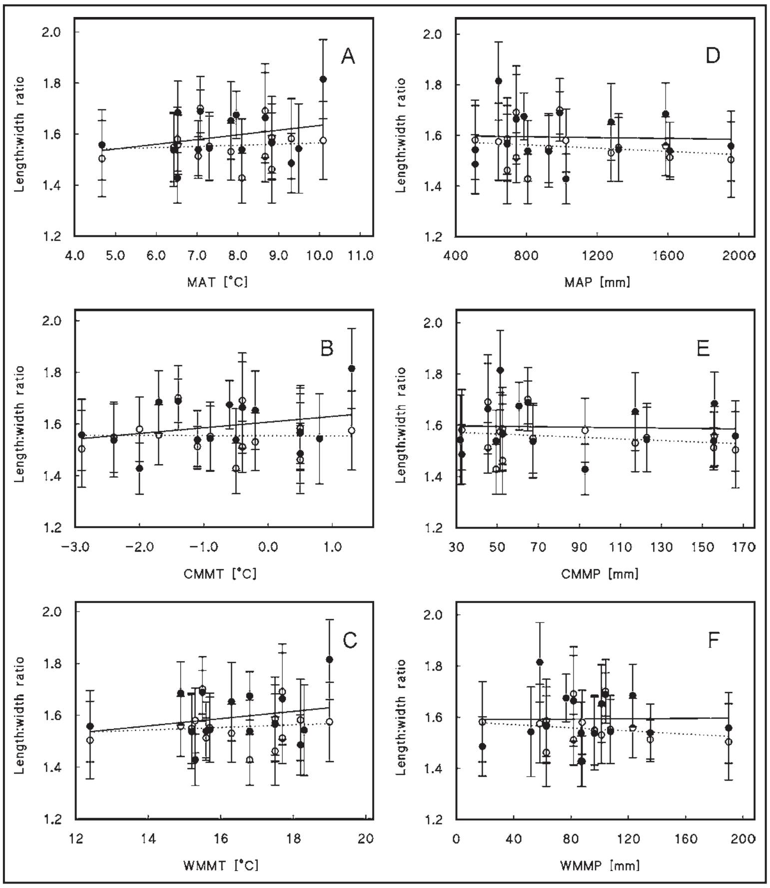

Fig. 3. Correlations between length : width ratio and the different climate parameters (see text). ● = shade leaves, solid line = regression line for shade leaves; ○ = sun leaves, dotted line = regression line for sun leaves.

Variability of venation density

Distance between veins, as a measure of venation density, differs statistically significant between sun- and shade-leaves ($p < 0.001$) from SW Germany (cf. Uhl & al. 2002). In almost all samples shade-leaves have higher distances between veins (= lower venation densities) than sun-leaves (Fig. 4). Shade-leaves show the best correlations with temperature parameters, i.e. MAT ($p < 0.01$) and WMMT ($p < 0.01$) (all other climate parameters: $p < 0.05$). In contrast, sun-leaves exhibit the best correlations with precipitation parameters, i.e. MAP ($p < 0.05$) and CMMP ($p < 0.05$) (all other climate parameters: N.S.).

These results generally confirm the findings of earlier studies linking higher venation densities with climatic factors that increase water stress (i.e. sun- vs. shade-leaves, higher temperatures and lower precipitation) (Uhl & Mosbrugger 1999a; Uhl & al. 2002). Based on physiological and anatomical data Brodribb & al. (2007) could demonstrate in a wide range of plants that venation density, as well as leaf thickness and cell shape within mesophyll tissues, are not only strongly correlated with the maximum photosynthetic rate of foliage but also with the hydraulic conductivity of the foliage, thus linking the earlier observations with a functional/physiological explanation.

Recently Blonder & Enquist (in press) could demonstrate that community-mean venation density correlates with temperatures during the growing season as well as atmospheric CO_2-concentration in modern floras. This result, together with the results presented here and in previous studies (e.g. Manze 1968; Uhl & Mosbrugger 1999a, 1999b; Uhl & Walther 2000, 2003; Uhl & al. 2002), emphasize the potential of leaf venation density as a leaf trait that can probably be used for reconstructions of certain palaeoenvironmental parameters.

Variability of Fluctuating asymmetry

FA is considered to represent a useful indicator of (unspecific) stress in a large variety of organisms (e.g. Benitez & al. 2011; Allenbach 2013; Beasley & al. 2013, Makeleinen & al. 2013; Novak & al. 2013; Nuche & al. 2014; Wuytack & al. 2013; and citations therein). Environmental stressors that may induce FA in plants, especially leaves, include (amongst others) herbivory (e.g. Martel & al. 1999; Diaz & al. 2004),

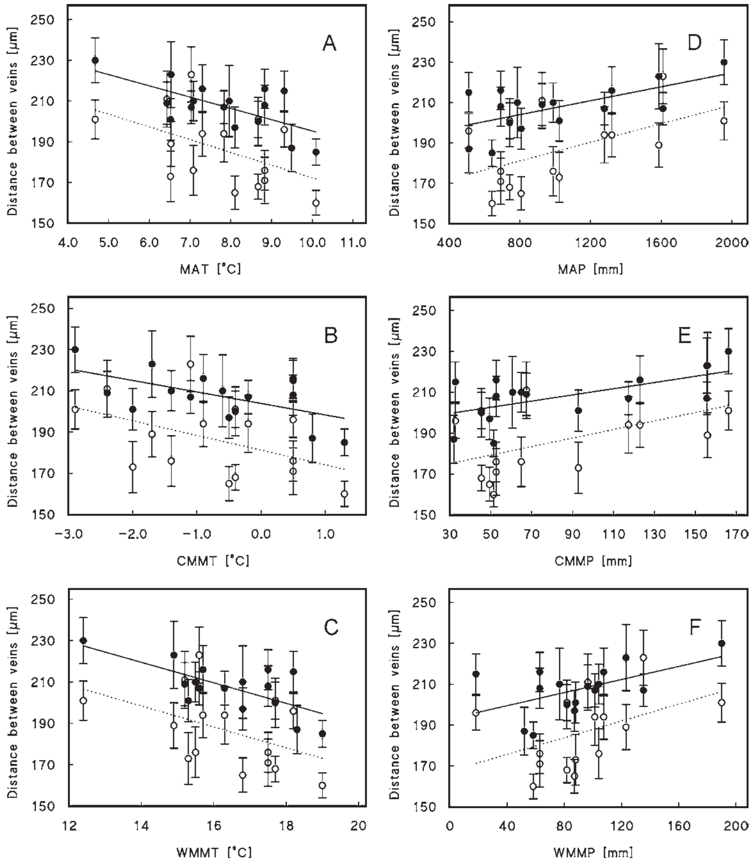

Fig. 4. Correlations between distance between veins [mm] and the different climate parameters (see text). ● = shade leaves, solid line = regression line for shade leaves; ○ = sun leaves, dotted line = regression line for sun leaves.

occurrence of wildfires (e.g. Freeman & al. 2005; Alves-Silva & Del-Claro 2013), water availability (e.g. Hódar 2002; Nuche & al. 2014), nutrient availability (e.g. Milligan & al. 2008), air- and soil-pollution (e.g. Mal & al. 2002; Kozlov & al. 2002), light intensity (e.g. Puerta-Piñero & al. 2008), (unspecified) micro-environmental factors (e.g. Wuytack & al. 2013), elevation (e.g. Hagen & al. 2008) and ontogeny (e.g. Wuytack & al. 2013).

FA differs statistically significant between sun- and shade-leaves ($p < 0.01$) from SW-Germany. In almost all samples shade-leaves display a higher FA than sun-leaves (Fig. 5). There is no statistically significant correlation between the FA and any of the climatic parameters considered for this study.

When comparing previous studies (see citations above) it becomes rapidly evident, that FA is highly species specific and thus it is not possible to generalize our results derived from *F. sylvatica*. Nevertheless we can add an additional *caveat* whenever this parameter may be used for palaeoenvironmental studies as it became obvious that this parameter differs statistically significant between sun- and shade-leaves of this particular taxon. Maybe contrary to intuitive assumptions that the sun-leaves, which should experience more water- and heat-stress [the probable reasons for higher venation densities (e.g. Uhl & Mosbrugger 1999a; Uhl & al. 2002)], should exhibit higher FA than shade-leaves, it turned out that the opposite reaction can be observed. However, a comparable reaction, with higher FA in shade-leaves than in sun-leaves, has been described for mediterranean oak leaves (*Quercus pyrenaica* Willd.) from Spain (Puerta-Piñero & al. 2008) and here the authors concluded that low light intensity (and thus limited photosynthetic activity) may be the most important stressor for the induction of FA in this taxon. As we could not observe any obvious correlation of FA with any of the climatic parameters used in this study we can hypothesize that the same could be possible for *F. sylvatica*. However, based on our data we cannot rule out that another so far unknown environmental factor (e.g. an edaphic factor or herbivory) may also induce FA in this taxon or that the climatic gradients studied here are not long or steep enough to see any effects on FA. Thus we suggest that it is not possible at the moment to account potential differences in FA in fossil beech leaves from different localities

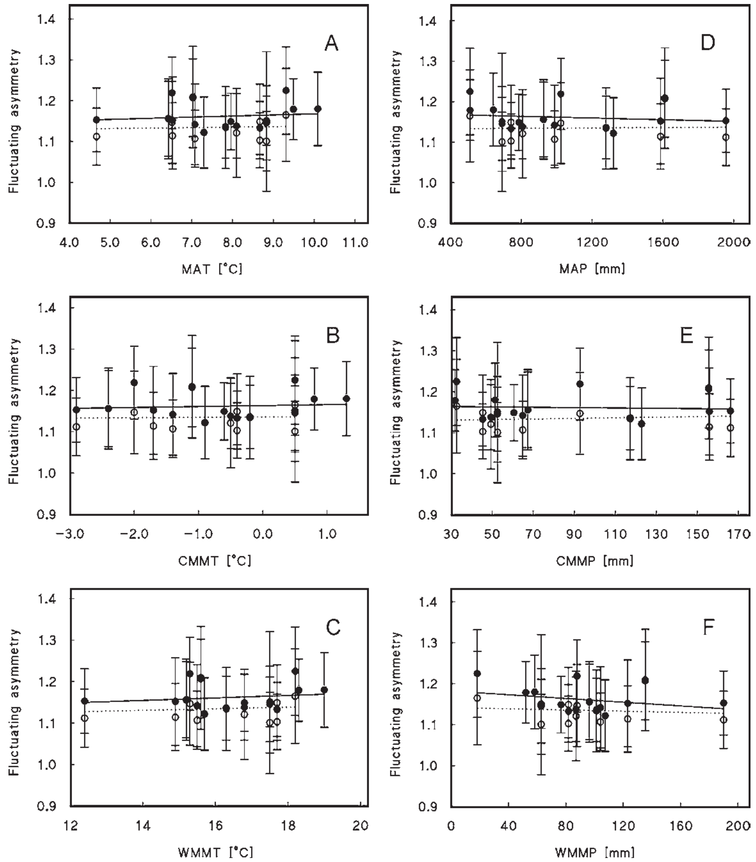

Fig. 5. Correlations between values for fluctuating asymmetry (FA) and the different climate parameters (see text). ● = shade leaves, solid line = regression line for shade leaves; ○ = sun leaves, dotted line = regression line for sun leaves.

or stratigraphic ages only to differences in the light regime experienced by such leaves. Based on the known data it is also not possible to state whether the observations in *F. sylvatica* and *Q. pyrenaica* can be generalized for other taxa of the *Fagaceae* or even other plant-families.

Studies on FA have already been used to infer palaeoenvironmental differences between different floras and there is obviously a large potential to use FA in palaeoenvironmental studies (e.g. Tamás & Hably 2005, 2009) but it is also clear, that further studies on a wider spectrum of taxa, preferably from a wide range of taxonomically unrelated groups, are needed before the use of FA in palaeoenvironmental studies will enable us to interpret differences or changes of FA in fossil leaves reliably.

Acknowledgements. The author thanks the late Prof. Dr. Harald Walther for many fruitful and encouraging discussions on the use of leaf morphology as an aid to reconstruct palaeoenvironmental conditions, as well as Dr. J. Lechterbeck (Hemmenhofen, Germany), Dr. T. Schneck (Frankfurt am Main, Germany) und S. Uhl (Neustadt an der Weinstraße, Germany) for their assistance during field work in SW Germany.

References

Allenbach, D.M. 2013. Fluctuating asymmetry and exogenous stress in fishes: a review. – Rev. Fish Biol. Fisher., **21**: 355-376.

Alves-Silva, E. & Del-Claro, K. 2013. Effect of post-fire resprouting on leaf fluctuating asymmetry, extrafloral nectar quality, and ant-plant-herbivore interactions. – Naturwiss., **100**(6): 525-532.

Beasley, D.A.E., Bonisoli-Alquati, A. & Mousseau, T.A. 2013. The use of fluctuating asymmetry as a measure of environmentally induced developmental instability: A meta-analysis. – Ecol. Indicators, **30**: 218-226 .

Benítez, H., Parra L. E., Sepulveda, E. & Sanzana, M.J. 2011. Fluctuating asymmetry: a morpho-functional tool to measure development stability. – Int. J. Morphology, **29**(4): 1459-1469.

Blonder, B. & Enquist, B. J. 2014. Inferring climate from angiosperm leaf venation networks. – New Phytol., **204**(1):116-126.

Brodribb, T.J., Feild, T.S. & Jordan, G.J. 2007. Leaf maximum photosynthetic rate and venation are linked by hydraulics. – Pl. Physiol., **144**: 1890-1898.

van der Burgh, J. 2001. Changes in the leaves of *Fagaceae* during the Late Miocene and Pliocene in the Lower Rhine embayment. – Acta Palaeobot., **41**: 283-297.

DeSoyza, A.G. & Kincaid, D.T. 1991. Patterns in leaf morphology and photosynthesis in shoots of *Sassafras albidum* (*Lauraceae*). – Amer. J. Bot., **78**(1): 89-98.

Denk, T. 2004. Revision of *Fagus* from the Cenozoic of Europe and southwestern Asia and its phylogenetic implications. – Documenta Naturae, **150**: 1-72.

Díaz, M., Pulido, F.J. & Møller, A.P. 2004. Herbivore effects on developmental instability and fecundity of holm oaks. – Oecologia, **139**: 224-234.

Freeman, D.C., Brown, M.L., Duda, J.J., Graraham, J.H., Emlen, J.M., Krzysik, A.J., Balbach, H., Kovacic, D.A. & Zak, J.C. 2005. Leaf fluctuating asymmetry, soil disturbance and plant stress: a multiple year comparison using two herbs, *Ipomoea pandurata* and *Cnidoscolus stimulosus*. – Ecol. Indicators, **5**: 85-95.

Hagen, S.B., Ims, R.A., Yoccoz, N.G. & Sørlibråten, O. 2008. Fluctuating asymmetry as an indicator of elevation stress and distribution limits in mountain birch (*Betula pubescens*). – Pl. Ecol., **195**: 157-163.

Hódar, J.A. 2002. Leaf fluctuating asymmetry of Holm oak in response to drought under contrasting climatic conditions. – J. Arid Environm., **52**: 233-243.

Kozlov, M.V., Niemelä, P. & Junttila, J. 2002. Needle fluctuating asymmetry is a sensitive indicator of pollution impact on Scots pine (*Pinus sylvestris*). – Ecol. Indicators, **1**: 271-277.

Mai, D.H. 1995. Tertiäre Vegetationsgeschichte Europas. Gustav Fischer, Jena

Makelainen, P.H., van Ginneken, A.M. & Pietiainen, H. 2013. Fluctuating asymmetry in the saddle patch shape of the Pacific Ocean killer whale (*Orcinus orca*) populations. – Ann. Zool. Fenn., **50**: 347-355.

Mal, T.K., Uveges, J.L. & Turk, K.W. 2002. Fluctuating asymmetry as an ecological indicator of heavy metal stress in *Lythrum salicaria*. – Ecol. Indicators, **1**: 189-195.

Manchester, S.R. & Dillhoff, R.M. 2004. *Fagus* (*Fagaceae*) fruits, foliage, and pollen from the Middle Eocene of Pacific Northwestern North America. – Canad. J. Bot., 82: 1509-1517.

Manze, U. 1968. Die Nervaturdichte der Blätter als Hilfsmittel der Paläoklimatologie. – Sonderveröff. Geol. Inst. Univ. Köln, **14**: 103 pp.

Martel, J., Lempa, K. & Haukioja, E. 1999. Effects of stress and rapid growth on fluctuating asymmetry and insect damage in birch leaves. – Oikos, **86**: 208-216.

Milligan, J.R., Krebs, R.A. & Mal, T.K. 2008. Separating developmental and environmental effects on fluctuating asymmetry in *Lythrum salicaria* and *Penthorum sedoides*. – Int. J. Pl. Sci., **169**(5): 625-630.

Mosbrugger, V., Utescher, T. & Dilcher, D.L. 2005. Cenozoic continental climatic evolution of Central Europe. – Proc. Natl. Acad. Sci., **102**: 14964-14969.

Müller-Westermeier, G. 1990. Klimadaten der Bundesrepublik Deutschland – Zeitraum 1951-1980. – Selbstverlag des Deutschen Wetterdienstes, 22 + 289 pp., Offenbach, Germany.

Novak, M., Abdoli, A. & Pont, D. 2013. Otolith asymmetry as a proxy of thermal stress in cold water fish: do observations on natural populations of *Cottus gobio* meet experimental results? – Cybium, **37**: 281-284.

Nuche, P., Komac, B., Camarero, J. J. & Alados, C.l. 2014. Developmental instability as an index of adaptation to drought stress in a Mediterranean oak. – Ecol. Indicators, **40**: 68-75.

Powers, H.O. 1967. A blade tissue study if forty-seven species and varieties of *Aceraceae*. – Amer. Midl. Naturalist, **78**: 301-323.

Puerta-Piñero, C, Gómez, J.M. & Hódar, J.A. 2008. Shade and herbivory induce fluctuating asymmetry in a Mediterranean oak. – Int. J. Pl. Sci., **169**(5): 631-635.

Royer, D.A. 2001. Stomatal density and stomatal index as indicators of paleoatmospheric CO_2 concentration. – Rev. Palaeobot. Palynol., **114**: 1-28.

Royer, D.L., McElwain, J.C., Adams, J.M. & Wilf, P. 2008. Sensitivity of leaf size and shape to climate within *Acer rubrum* and *Quercus kelloggii*. – New Phytol., **179**: 808-817.

Salisbury, E.J. 1927. On the causes and ecological significance of stomatal frequency, with special reference to the woodland flora. – Philos. Trans., Ser. B., **216**: 1-65.

Spicer, R.A. 2000. Leaf physiognomy and climate change. In: **Culver, S.J. & Rawson, P.** (Eds.), Biotic Response to Global change. The Last 145 Million Years. Cambridge Univ. Press, Cambridge.

Spicer, R.A. 2007. Recent and Future of CLAMP: building on the legacy of Jack A. Wolfe. – Courier Forschungsinst. Senckenberg, **258**: 109-118.

Spicer, R.A., Herman, A.B. & Kennedy, E.M. 2004. Foliar physiognomic record of climatic conditions during dormancy: Climate Leaf Analysis Multivariate Program (CLAMP) and the cold month mean temperature. – J. Geol., **112**: 685-702.

Stranks, L. & England, P. 1997. The use of a resemblance function in the measurement of climatic parameters from the physiognomy of woody dicotyledons. – Palaeogeogr. Palaeoclimatol. Palaeoecol., **131**: 15-28.

Tamás, J. & Hably, L. 2005. The morphometrical analysis of *Sloanea elliptica* (Andreánsky) Z. Kvacek & Hably from the Oligocene of the Buda and Bükk Mountains and its palaeogeographic implications. – Rev. Palaeobot. Palynol., **134**: 257-268.

Tamás, J. & Hably, L. 2009. Unidirectional shift in leaf morphology of coexisting species – a possible indicator of palaeoclimatic differences. – Palaeogeogr. Palaeoclimatol. Palaeoecol., **271**: 185-195.

Teodoridis, V., Kvaček, Z. & Uhl, D. 2009. Late Neogene palaeoenvironment and correlation of the Sessenheim-Auenheim floral complex. – Palaeodiversity, **2**: 1-17.

Teodoridis, V., Mazouch, P., Spicer, R.A. & Uhl, D. 2011. Refining CLAMP – investigations towards improving the Climate Leaf Analysis Multivariate Program. – Palaeogeogr. Palaeoclimatol. Palaeoecol., **239**: 39-48.

Uhl, D. & Mosbrugger, V. 1999a. Leaf venation density as a climate and/or environmental proxy – a critical review and new data. – Palaeogeogr. Palaeoclimatol. Palaeoecol., **149**: 17-30.

Uhl, D. & Mosbrugger, V. 1999b. Variabilität der Aderungsdichte in Laubblättern von Fels-Ahorn (*Acer monspessulanum* L.). – Mitt. Pollichia, **86**: 99-109.

Uhl, D. & Walther, H. 2000. Sun leaf or shade leaf? – Known facts in the light of new data with implications for palaeobotany. – Feddes Repert., **111**: 165-174.

Uhl, D. & Walther, H. 2003. Aderungsdichte und Blattgröße bei vier Arten der Gattung *Acer* L. aus SW-Deutschland. – Bot. Jahrb. Syst., **124**: 449-460.

Uhl, D., Walther, H. & Mosbrugger, V. 2002. Leaf venation density in angiosperm leaves as related to climatic and environmental conditions – problems and potential for palaeoclimatology. – Neues Jahrb. Geol. Paläontol., Abh., **224**: 49-95.

Utescher, T., Mosbrugger, V. & Ashraf, A.R. 2000. Terrestrial climate evolution in Northwest Germany over the last 25 million years. – Palaios, **15**: 430-449.

Wilf, P. 1997. When are leaves good thermometers? A new case for leaf margin analysis. – Paleobiology, **23**: 373-390.

Wilf, P., Wing, S.L., Greenwood, D.R. & Greenwood, C.L. 1998. Using fossil leaves as palaeoprecipitation indicators: an Eocene example. – Geology, **26**: 203-206.

Wolfe, J.A. 1979. Temperature parameters of humid to mesic forests of Eastern Asia and relation to forests of other regions of the Northern hemisphere and Australia. – Profess. Pap. U.S. Geol. Surv., **1106**: 1-37.

Wolfe, J.A. 1993. A method of obtaining climatic parameters from leaf assemblages. – Bull. U.S. Geol. Surv., **2040**.

Wolfe, J.A. & Spicer, R.A. 1999. Fossil leaf character states: multivariate analysis. In: **Jones, T.P. & Rowe, N.P.** (Eds.), Fossil Plants and Spores: Modern Techniques. Geological Society, London, pp. 233-239.

Woodward, F.I. 1987. Stomatal numbers are sensitive to increases in CO_2 from pre-industrial levels. – Nature, **327**: 617-618.

Wuytack, T., Samson, R., Wuyts, K. Adriaenssens, S., Kardel, F. & Verheyen, K. 2013. Do leaf characteristics of White Willow (*Salix alba* L.), Northern Red Oak (*Quercus rubra* L.), and Scots Pine (*Pinus sylvestris* L.) respond differently to ambient air pollution and other environmental stressors? – Water Air Soil Pollut., **224**(8): 1635 (14 pp.).

Wylie, R.B. 1951. Principles of foliar organization shown by sun-shade leaves from ten species of deciduous dicotyledonous trees. – Amer. J. Bot., **38**: 355-361.