DENDROBIOLOGY
2008, vol. 60, 29–33

Włodzimierz Meissner, Katarzyna Żółkoś

What to divide leaves into ten parts for? The proposition of shape indices of leaf blade

Received: 19 June 2008, Accepted: 2 December 2008

Abstract: In this paper the method of creating indices for description leaf blade roundness and asymmetry, based on division of the leaf into 10 equal parts perpendicularly to the long axis was proposed. Along each division line the distance between the midrib and the leaf edge was measured separately for the left and right site of the blade. Sum of all 18 measurements, 9 on each side, may be treated as an index of the roundness of the leaf blade. Differences between sums of all measurements of the left and right leaf site could be a base for calculations of various asymmetry indices, including indicators of fluctuating asymmetry. Indices obtained by this method are quite sensitive and may be analysed using standard statistical methods.

Additional key words: roundness index, asymmetry index, leaf blade measurements

Addresses: W. Meissner, Avian Ecophysiology Unit, Department of Vertebrate Ecology and Zoology, University of Gdańsk, Al. Legionów 9, 80-441 Gdańsk, Poland, w.meissner@univ.gda.pl (corresponding author)
K. Żółkoś, University of Gdańsk, Department of Plant Taxonomy and Nature Protection, Al. Legionów 9, 80-441 Gdańsk, Poland, e-mail: biokz@univ.gda.pl

Introduction

The shape of leaf blade is used as a diagnostic character for distinguishing between plant species. However, the leaf shape may vary within a species or even within one specimen. This diversity is caused by several factors, among which the most important are insolation and soil fertility (Jack and Long 1991; Frazer et al. 2000; Barna 2004; Żółkoś and Meissner 2008). Differences in leaf shape may be an indicator of the influence of environmental factors on plants and through the analysis of fluctuating asymmetry could be treated as a sign of soil disturbance and stress in plants (Møller 1999; Hódar 2002; Freeman et al. 2005).

Several different leaf shape indices have been used so far. Some of them are quite straightforward, however they do not give possibility to perform statistical analysis (e.g. Jentys-Szaferowa 1959), while others require complicated computation methods, like landmarks (Dickinson et al. 1987), fractal analysis (Vlcek and Cheung 1986) or Fourier transform (Kincaid and Schneider 1983).

The aim of this paper is to present a relatively easy in computation, but also effective method of creating indices, which could be helpful in describing the shape and asymmetry of leaves. These indices are based on division of leaf blade into 10 equal parts perpendicularly to the midrib.

Material and methods

Samples of 30 fully developed leaves of European beech *(Fagus sylvatica)*, European hornbeam *(Carpinus betulus)*, black alder *(Alnus glutinosa)*, black poplar *(Populus nigra)* and white willow *(Salix alba)* were collected for the study. They were sampled randomly in July 2005 from the ends of shoots in the lower parts of tree crowns.

All leaves were scanned with the resolution of 300 dpi. The total area of each leaf as well as the area of different parts of leaf blade were measured by digiShape software (Cortex Nova). Other measurements were done in CorelDraw 9 (Corel Corporation), using standard procedures implemented in this software, although other modern software supporting vector graphics are also suitable. Statistical analysis was preformed according to Zar (1996), using STATISTICA 6.0 (StatSoft 2001).

The way of leaf blade measuring

Each leaf blade was divided into 10 equal parts perpendicularly to the long axis (Fig. 1). In CorelDraw the leaf image was rotated to put long axis of the leaf on the vertical line, which was drawn earlier. Then, two lines were drawn perpendicularly to this vertical line through outermost points of the leaf blade. Nine parallel lines were put between these two lines outlined the leaf blade length and they were uniformly aligned using command: Align and Distribute from menu Arrange (Fig. 1). Then all lines were adjusted manually to be ended on the edge of the leaf blade (Fig. 1). For this operation the picture of the leaf should be enlarged in order to obtain precise adjustment. Distances between leaf blade edge and the midrib were measured separately for the right and left side of the blade using the Freehand tool of the CorelDraw. This resulted in 9 pairs of measurements (Fig. 1), among which the fifth pair is an equivalent of perpendicular axis of the leaf blade.

For calculation of fluctuating asymmetry index according to Palmer (1994) the width of the left and right sides of the leaf blade were taken from the midrib to the outer leaf margin in the widest part of the leaf. This index is commonly used in botanical studies (e.g. Rettig et al. 1997; Cowart and Graham 1999; Hódar 2002).

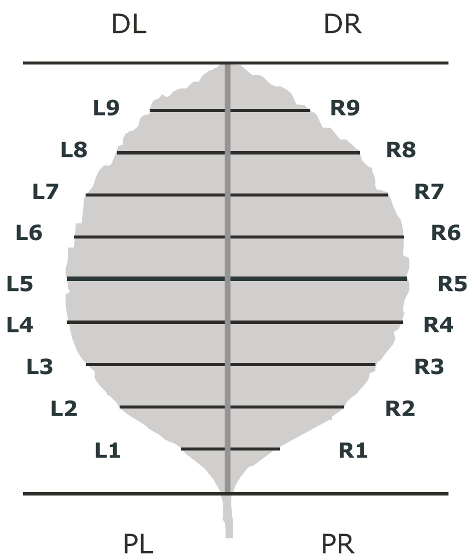

Fig. 1. Notations of all measurements and parts of the leaf blade. Grey vertical line determines midrib of the leaf. P1-P9 and L1-L9 – symbols of subsequent measurements of the left and right side of the blade. DL – left distal part, PL – left proximal part, DR – right distal part, PR – right proximal part

Roundness index of the leaf blade

The sum of all 18 measurements (9 per each side of leaf blade), can be treated as a leaf blade roundness index, because the more rounded is the leaf the greater is the distance between the leaf edge and the midrib and also the sum of all measurements.

$$W = \sum L + \sum P$$

where:

$$\sum L = L_1 + L_2 + L_3 + \ldots + L_9$$
$$\sum P = P_1 + P_2 + P_3 + \ldots + P_9$$

For symbol explanation – see figure 1.

The major disadvantage of this roundness index is its dependence on leaf blade size. The correlation coefficients between the index value and the leaf blade length were statistically significant (t-test, $p<0.05$ in all cases) and amounted to $r=0.74$ for black alder, $r=0.69$ for European beech, $r=0.74$ for European hornbeam, $r=0.87$ for black poplar and 0.64 for white willow. It doesn't allow to compare leaves of different sizes. To solve this problem the roundness index should be standardized. The best way is to divide the index calculated for particular leaf by the leaf blade length (LBL).

$$Wst = \frac{\sum L + \sum P}{LBL}$$

As the roundness index gives a numeral value to each leaf in the sample, it may be analysed using all standard statistical methods.

The mean standardized roundness indices, calculated for collected leaves of five tree species, differed significantly (ANOVA, $F_{4,145}=146.2$, $p<0.001$), except beech and hornbeam (post-hoc Tukey test, $p>0.05$) (Fig. 2).

Roundness indices may be also calculated for different parts of the leaf blade by summing measurements of the left and the right side of the blade separately for proximal and distal part of the leaf. However, in that case the fifth (central) pair of the measurements should be omitted:

$$\sum Prox = L_1 + L_2 + L_3 + L_4 + P_1 + P_2 + P_3 + P_4$$
$$\sum Dist = L_6 + L_7 + L_8 + L_9 + P_6 + P_7 + P_8 + P_9$$

In the studied material there was no significant difference between standardized roundness indices cal-

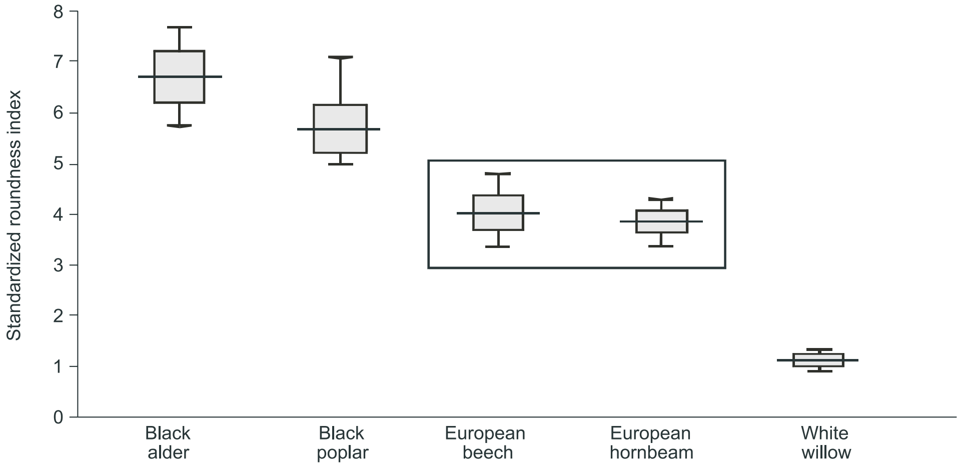

Fig. 2. Comparison of mean standardized roundness indices of leaf blades of 5 tree species. Horizontal lines – mean, rectangle – standard deviation, vertical line – range. Species which did not differ statistically were placed in the frame (post-hoc Tukey test, p>0.05)

culated for the whole leaf blades of European beech and European hornbeam. However, indices calculated separately for proximal and distal parts of the leaf blade revealed significant differences between leaves of these two tree species. The distal part of beech leaf was more rounded (convex) in comparison to hornbeam (t-test, $t=12.6$, $p<0.001$), while hornbeam had more rounded proximal part of the leaf than beech (t-test, $t=5.1$, $p<0.001$) (Fig. 3).

In all studied species there was statistically significant (t-test; $p<0.01$) and strong correlation between the sum of all measurements (unstandardized roundness index) and the total leaf area, ranging between 0.92 in European beech and 0.96 in black poplar and white willow. Similar strong correlation existed in case of the left, right, proximal and distal part of the leaf blade and corresponding sum of measurements (all above $r=0.77$). Thus, the sum of measurements is a good indicator of the leaf area, at least for leaves of regular shape.

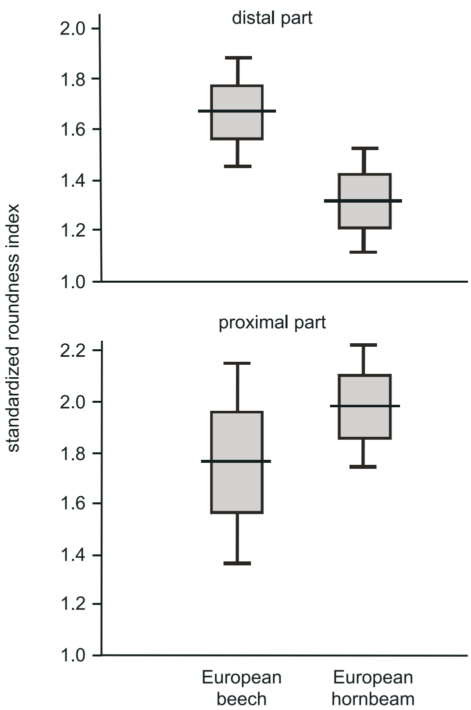

Fig. 3. Comparison of standardized roundness indices of European beech and European hornbeam leaves, calculated for distal and proximal part of the leaf blade. Symbols – see figure 2

Symmetry index of the leaf blade

The difference between the sum of measurements of the left and right side of the leaf blade may be treated as a leaf blade symmetry index. This index should be also standardized by dividing its value by the leaf blade length (LBL).

$$Sst = \frac{\sum |L_i - P_i|}{LBL}$$

Similarly to the roundness index, for each leaf only one value of symmetry index is obtained. Symmetry index calculated for leaves of four studied tree species showed that European hornbeam leaf blades revealed the highest, whereas the European beech and black alder leaf blades the lowest degrees of symmetry (Kruskal-Wallis test, $H_{3,120}=35.9$, $p<0{,}001$) (Fig. 4).

On the base of series of measurements made pairwise for both sides of the leaf blade, different symmetry indices can be calculated, for example indices concerned only distal or proximal part of the leaf.

Fluctuating asymmetry index

Usually the index of fluctuating asymmetry takes into account only one pair of measurements taken in the widest part of the leaf blade (e.g. Møller 1999, Lempa et al. 2000, Hódar 2002, Nagamitsu et al. 2004). According to Palmer (1994) it could be calculated as:

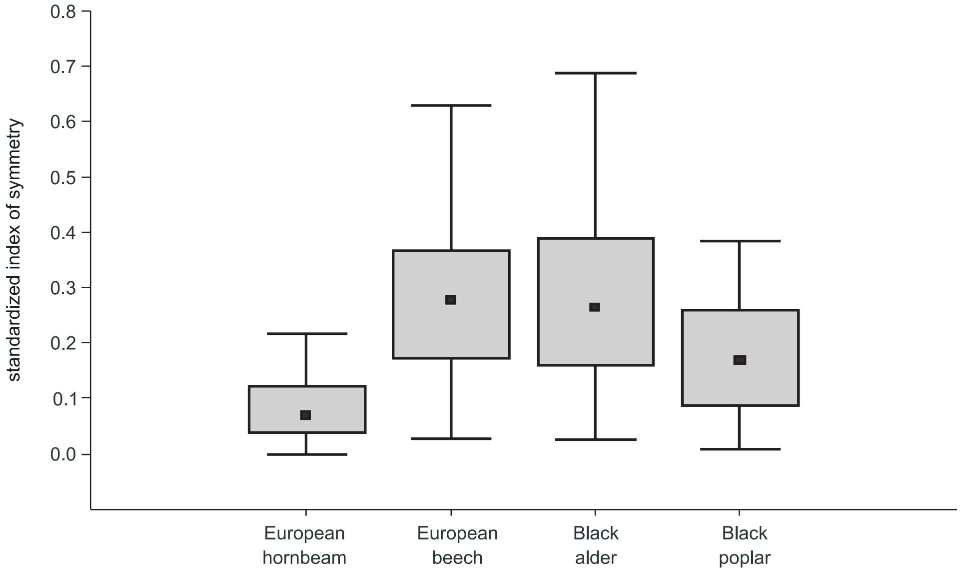

Fig. 4. Comparison of medians of leaf blade symmetry indices of 4 tree species. Point – median, rectangle – interquartile range, vertical line – range

$$FA_2 = \frac{2|WL - WP|}{WL + WP}$$

where WL and WP are the width of the left and right side of the leaf blade respectively.

For calculation of the fluctuating asymmetry index (FA_9) nine pairs of measurements were used instead of two.

$$FA_9 = \frac{2\left|\sum L - \sum P\right|}{\sum L + \sum P}$$

The Pearson's correlation coefficient between index proposed in this paper (FA_9) and the classical one (FA_2), which takes into account only one pair of measurements was different in studied species. In black alder, European beech and European hornbeam these two indices were correlated with $r=0.77$, $r=0.70$ and $r=0.60$ respectively (t-test, $p<0.001$ in all cases). However there is a lack of such correlation in black poplar ($r=0.29$, t-test, $p=0.11$).

Conclusion

Described method presents the way of creating different shape indices. According to that procedure of establishing and summing of measurements further modifications may be made. Proposed division of the leaf blade into 10 parts is a result of trade-off between the time needed to make these measurements and the sensitivity of calculated indices. Still, the higher number of measurements, the subtle differences in the shape of leaf blades can be detected. Presumptive implementation of measuring procedure into software, which provides measurement automation, enables to analyze many images in a short time.

Standardized roundness index and symmetry index can be useful in studies on variability of leave shape and area within a particular tree stands as well as between different parts of the crown. Such research subjects are common in literature (e.g. Gravano et al. 1999; Frazer et al. 2000; Barna 2004). In some cases these indices might also be an additional feature in distinguishing between similar plant species (e.g. Puntieri et al. 2003).

Proposed way of the leaf blade measurement may be also used for analysis of fluctuating asymmetry according to different equations presented by Palmer (1994). However, the fluctuating asymmetry index based on nine pair of measurements and the classical one, with only one pair of measurements, may give different results and have different interpretation. Instead of measuring asymmetry only at the widest part of the leaf, the proposed index allows to check asymmetry along the whole leaf blade. Thus, the new index seems to be more sensitive to differences in size of the left and right side of the leaf blade.

Examples presented in this paper concerned tree species of quite simple structure of the leaf blade. For calculating roundness and asymmetry indices of leaves of more complicated shape, the method should be modified. In case of compound leaves one leaflet may be chosen for analysis. In leaves with palmate venation, pairs of measurements could be done from one point at petiole base forward sinuses and lobe ends.

Acknowledgements

We would like to thank Dr Joanna Bloch-Orłowska for help in preparing the manuscript.

References

Barna M. 2004. Adaptation of european beech (*Fagus sylvatica* L.) to different ecological conditions: leaf size variation. *Polish Journal of Ecology* 52: 34–45.

Cowart N.M., Graham J.H. 1999. Within- and among-individual variation in fluctuating asymmetry of leaves in the fig (*Ficus carica* L.). *International Journal of Plant Science* 160: 116–121.

Dickinson T.A., Parker W.H., Strauss R.E. 1987. Another approach to leaf shape comparisons. *Taxon* 36: 1–20.

Frazer G.W. Trofymow J.A., Lertzman K.P. 2000. Canopy openness and leaf area in chronosequences of coastal temperate rainforest. *Canadian Journal of Forest Research* 30: 239–256.

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*. *Ecological Indicators* 5: 85–95.

Gravano E., Bussotti F., Grossoni P., Tani C. 1999. Morpho-anatomical and functional modifications in beech leaves on the top ridge of the Apennines (Central Italy). *Phyton* 39: 41–46.

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

Jack S.B., Long J.N. 1991. Response of leaf area index to density for two contrasting tree species. *Canadian Journal of Forest Research* 21: 1760–1764.

Jentys-Szaferowa J. 1959. Graficzna metoda porównywania kształtów roślinnych. *Nauka Polska* 3: 79–110.

Kincaid D.T., Schneider R.B. 1983. Quantification of leaf shape with a microcomputer and Fourier transform. *Canadian Journal of Botany* 61: 2333–2342.

Lempa K., Martel J., Koricheva J., Haukioja E., Ossipov V., Ossipova S., Pihlaja K. 2000. Covariation of fluctuating asymmetry, herbivory and chemistry during birch leaf expansion. *Oecologia* 122: 354–360.

Møller A.P. 1999. Elm, *Ulmus glabra*, leaf asymmetry and Dutch elm disease. *Oikos* 85: 109–116.

Nagamitsu T., Kawahara T., Hotta M. 2004. Phenotypic variation and leaf fluctuating asymmetry in isolated populations of an endangered dwarf birch *Betula ovalifoliaI* in Hokkaido, Japan. *Plant Species Biology* 19: 13–21.

Palmer A.R. 1994. Fluctuating asymmetry analyses: a primer. In: Developmental instability: its origins and evolutionary implications. Markow T.A. (ed.) Kluwer, Dordrecht, pp. 335–364.

Puntieri J.G., Souza M.S., Brion C., Mazzini C., Barthélémy D. 2003. Axis differentiation in two South American *Nothofagus* species (Nothofagaceae). *Annals of Botany* 92: 589–599.

Rettig J.E., Fuller R.C., Corbert A.L., Getty T. 1997. Fluctuating asymmetry indicates levels of competition in an even-aged clone. *Oikos* 80: 123–127.

StatSoft, Inc. 2001. STATISTICA (data analysis software system), version 6. www.statsoft.com.

Vlcek J., Cheung E. 1986. Fractal analysis of leaf shapes. *Canadian Journal of Forest Research* 16: 124–127.

Zar J.H. 1996. Biostatistical Analysis, 3rd editon. Prentice-Hall. London.

Żółkoś K., Meissner W. 2008. The effect of Grey Heron (*Ardea cinerea* L.) colony on the surrounding vegetation and the biometrical features of three undergrowth species. *Polish Journal of Ecology* 56: 65–74.