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Fluctuating asymmetry of *Betula pendula* Roth leaves – an index of environment quality

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Abstract: Fluctuating asymmetry (FA) is a non-specific stress indicator and describe developmental instability in bilateral structure. In plants, FA has been used as a tool for monitoring levels of ecological stress, however, in trees, high value of leaf FA has been assumed to indicate the level of environmental stresses. In this work, I analyzed the FA response to abiotic stress in the *Betula pendula* Roth leaves in two contrasting sites (polluted vs unpolluted). I also investigated whether another biometrical leaf feature is able to determine FA as an environmental stress indicator. After the cluster analysis and mixed-model ANOVA, I selected one feature, which was leaf apical angle. Results showed significant differences (using chi square test) in apical angle FA in the unpolluted site as compared to the polluted one. Concluding, I suggested that plants living in more stressful sites are more symmetrical and leaf FA for plant species with a wide ecological distribution such as *Betula pendula* should be used as an index of environmental quality.

Key words: fluctuating asymmetry, environmental stress, *Betula pendula*, cluster analysis

1. Introduction

Fluctuating asymmetry (FA) is one of the stress indices used recently to assess a subtle effect of environmental degradation on organisms and is expected to increase under stress condition. Developmental stability can be defined as a capacity of an individual to correct random disturbances caused by developmental noise. Increased developmental stability results in higher developmental precision and decreased FA factor. Developmental instability is a series of processes that tend to disrupt precise development (Palmer & Strobeck 2003). More symmetric individuals have greater developmental stability and usually achieve greater reproductive success and better survival rates than asymmetric individuals (Freeman *et al.* 1993). Measures of developmental instability, such as FA, may provide more sensitive indicator of stress than traditional methods (Rettig *et al.* 1997). FA changes in plants can be sometimes hardly noticeable (changes of about 1% in the value of the observed feature), but some of them are quite apparent. Abiotic environmental stress can also cause the increase of FA in plants. Such an increase was observed as a result of increase in ^{137}Cs radiation levels in tree species like *Robinia pseudacacia*, *Sorbus aucuparia* and *Matricaria perforata* (Müller 1998). Kozlov *et al.* (1996) demonstrated a positive correlation between FA of birch leaves (*Betula pubescens* and *Betula pendula*) and metal and chemical pollution.

The present study was carried out on heavily polluted site (slag industrial waste dump) and relatively clean site (control site), both located in the Upper Silesia region. The neutral pH of the waste material and high concentrations of heavy metals (Zn 100-1078 mg/kg dry weight of soil; Pb 196,9-1331 mg/kg dry weight of soil, Cd 1-9 mg/kg dry weight of soil) were of great interest, especially compared with total zinc concentration in soil under cultivation 15 to 300 mg/kg dry weight of soil, total lead 80-200 mg/kg dry weight of soil and cadmium ca. 1 mg/kg dry weight of soil (Franiel & Węski 2005).

In this study, I try to distinguish which of biometrical leaves features of *Betula pendula*, besides width and surface, is the most useful to determine fluctuating asymmetry as an environmental stress indicator. Moreover, I try to determine if leaf FA features should be considered as an environmental indicator of habitat quality.

2. Material and methods

For the present research 100 birch leaves, i.e. the second leaf from the current-year short sterile shoots in the middle of the crown of each individual, were collected. Only healthy, whole and fully developed leaves were sampled and collected during one week at the end of June 2005 year (at least 40 birch trees were selected and 8.000 leaves were collected). The leaves were pressed in a herbarium press and identified individually. After drying, each leaf was scanned and its five features were measured with ANALYSIS 5.1 software (Olympus, Japan): F1 – left and right apical angle, F2 – left and right basal angle, F3 – left and right angle between second vein and midrib, F4 – left and right distance measured from the sixth and seventh veins, F5 – left and right number of teeth between the sixth and seventh veins. In order to determine which leaf features variables discriminated between both (polluted and unpolluted) birch populations, a cluster analysis was used.

Three kinds of bilateral asymmetry (antisymmetry, directional asymmetry – DA and fluctuating asymmetry – FA) were distinguished by statistical methods. Because FA is subjected to measurement error, all leaves were measured three times to minimize the influence of measurement error in the analysis (Palmer & Strobeck 1986). To check for antisymmetry – significant differences in the R-L distribution comparing to the normal curve, Shapiro-Wilk test was used ($p>0.05$). A mixed-model ANOVA was used for each feature to find out the degree of directional asymmetry and fluctuating asymmetry. Significant difference between leaf side and individual data of trees indicate presence of fluctuating asymmetry, however, significant differences between the left and right side of analyzed feature indicate directional asymmetry. Directional symmetry appears if larger values consistently occur on one side. If data sets demonstrated that fluctuating asymmetry exist, FA value was estimated using protocol recommended by Palmer & Strobeck (1986). Absolute FA ([R-L]/size, where size=[R+L]/2) was calculated for the selected leaf feature. Dividing [R-L] by size accounts for variation in trait size.

In addition, to check significant differences between habitats for previously selected FA feature, a chi square test was used. The factor considered in the statistical analyses were the study sites (the two sampling localities: polluted and unpolluted) and individual birch trees; the variable was (log x+1) transformed FA apical angle. All analyses were performed with Statistica 7.0 PL (StatSoft, Poland) software.

3. Results

In order to determine which leaf features (F1-F5) variables discriminated between zinc dump and control area populations a cluster analysis was used. Results of cluster analysis allowed me to make a clear distinction between apical angle (F1) and the rest of leaf features (Fig. 1). Leaf deviation from symmetry showed a statistical normal distribution as evaluated by the Shapiro-Wilk

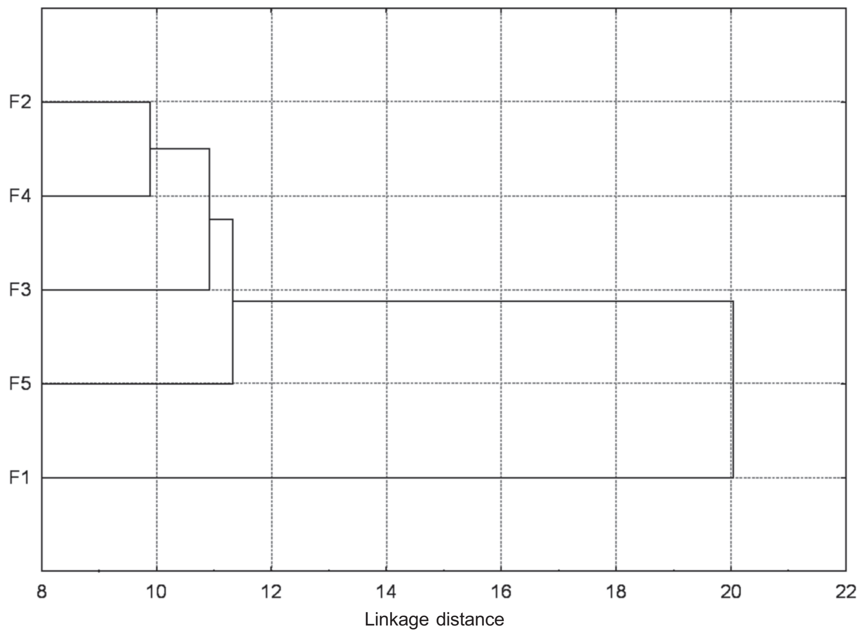

Fig. 1. Cluster analysis on measured features of leaves collected from 40 trees in the polluted and control habitats. Dendrogram based on Euclidean distance, using the Ward method

Explanations: F1 – left and right apical angle, F2 – left and right basal angle, F3 – left and right angle between second vein and midrib, F4 – left and right distance measured from the leaf sixth and seventh veins, F5 – left and right number of teeth between the sixth and seventh veins

Table 1. Results of tests for significance of FA for the leaf left and right apical angle (trait F1) in the zinc dump (polluted) and control area (unpolluted) populations

Trait	Study plot	Number of leaves collected	Mixed-model ANOVA for			(R-L)	Index of FA	Antisymmetry W
			side	individ.	side x individ.			
Apical angle	polluted	4000	0.188	0.000*	0.011*	0.461	0.119	0.992*
	unpolluted	4000	0.151	0.168	0.000*	0.481	0.124	0.984

Explanation: * – indicates significant difference at $p<0.05$

test (α = 0.05), thus suggesting the absence of antisymmetry for all features in polluted and unpolluted sites. The slight initial preference is ultimately amplified into a large consistent difference. I confirmed this result by t-test for independent samples ($p<0.01$), showing significant differences between left and right side. After the mixed-model ANOVA test, one feature, which was apical angle, was selected. Then, only for this feature Palmer's & Strobeck's (1986) protocol was used to asses environmental quality for both habitats (Table 1). Results showed a significant differences (using chi square test: chi square=91.65, p=0.000, df=1) in apical angle FA in the unpolluted site as compared to the polluted one.

4. Discussion

Genetic and environmental stress influences developmental stability, causing phenotypic traits to fluctuate above the level of natural variation. One way to measure the magnitude of environmental stress is the measurement of resident organism response to abiotic and biotic stress. Abiotic stress factors such as water limitation, as well as temperature, nutrition, pollutants and biotic stress factors, such as population density, can significantly affect FA indices. However, my results showed that trees growing on unfavorable sites are more symmetrical. Drought and concentration of heavy metals were a major factor determining angle FA in *Betula pendula*. There was significant increase in apical angle FA in the unpolluted site as compared to the polluted one. Ambo-Rappe *et al*. (2008), who study FA in plants growing in contaminated sites, suggested that there is a possibility that heavy metal exposure induced or activated some enzymes, such as phytochelatin synthase, that protectplant development from further damage. Plants from polluted sites perform better against stressful situation because they are adopted to those condition and are more symmetrical (Hódar 2002). Another possible reason for the decline of FA value in the polluted site may be an effect of natural selection leading to adaptation to life in those locations (Ambo-Rappe *et al*. 2008). A noteworthy possibility has been suggested by Hochwender & Fritz (1999), who found increased leaf FA in willow trees growing in optimal biotic conditions, suggested that it can be caused by very fast leaf growth which can disturb development processes.

A good ecological indicator must be sensitive enough to provide early warning signals. Since FA reveals small changes in tree development processes, it may be sensitive enough to show stress levels that are not high enough to affect the measures of fitness, such as growth and reproduction. Looking for a good indicator, we need to determine the level of stress in an ecosystem before it affects the critical "red line" of its resident organisms.

The investigation has showed that apical angle may be used as a good feature, beside width and surface, for evaluation of FA factor. Most contaminated sites (e.g. zinc dump) are generally not polluted by only one chemical substance but by a mixture of pollutants, which may lead to many synergistic effects. It is thus important to develop a sensitive bioassay that can monitor overall stress. If we can account for the effect of abiotic (weather conditions and pollution) stress on FA, we can use FA indices as a holistic bioassay tool for overall ecosystem level, besides the individual effects (Mal *et al*. 2002).

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