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# Ecological Indicators

# Ecological stress memory: Evidence in two out of seven species through the examination of the relationship between leaf fluctuating asymmetry and photosynthesis

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## ARTICLE INFO

## ABSTRACT

Increased morphological fluctuating asymmetry is considered as an indication that the extent of a natural stress has surpassed the ability of the genotype to maintain the normal symmetry in the body plan. Young leaves can suffer by a variety of stresses, such as nutrient deficiencies, drought, heat and cold in combination with high light. Therefore, increased morphological fluctuating asymmetry in mature leaves may be considered as an ecological indicator of such environmental pressures. Hence, deviations from symmetry in a mature leaf may be a reminder of an adversity having occurred when the leaf was young and developing. We argued that a past stress may stigmatize future photosynthesis and we searched for correlations between leaf fluctuating asymmetry and photosynthetic parameters obtained from fast chlorophyll a fluorescence rise curves in mature leaves from seven tree species. The results were species-specific, with four species (*Arbutus unedo, Cercis siliquastrum, Platanus orientalis* and *Populus alba*) showing both maximum quantum yield of photosystem II and photosynthetic performance index (PI$_{total}$ *sensu* Strasser) to be independent of the alleged juvenile stress, while in one species (*Ceratonia siliqua*) the two parameters were negatively correlated to leaf fluctuating asymmetry. Interestingly, in two species (*Olea europaea* and *Pistacia lentiscus*), higher leaf asymmetry was linked with lower risk of chronic photoinhibitory damage and higher photosynthetic capacity. An 'ecological stress memory' may be inferred for some species improving their tolerance to future adversities and global change.

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## 1. Introduction

Although the general plant body form is asymmetric, particular organs usually display a kind of symmetry, mostly bilateral or radial. The norm for leaves, in particular, is a bilateral symmetry along the middle vein. Deviations from leaf symmetry may be genotypic, where one leaf side is constantly larger than the other (directional asymmetry) or random, where either side can be unpredictably larger (fluctuating asymmetry, FA) (Palmer and Strobeck, 1986). FA represents small, non-directional deviations from perfect symmetry in morphological characters. FA is generally accepted to increase in response to stress; therefore, FA is frequently used in ecological studies as an index of stress experienced by an organism (Kozlov and Zvereva, 2015). Furthermore, it is believed that fluctuating asymmetry appears when a stress perturbs the normal function of the developmental program (Parsons, 1990; Freeman et al., 1993). Although empirical results do not always confirm the hypothesis (Dimitriou et al., 2006; Ambo-Rappe et al., 2008; Wuytack et al., 2011), in many cases it has been shown that the extent of leaf FA is positively correlated to a variety of experimentally imposed or natural stresses (Roy and Stanton, 1999; Valkama and Kozlov, 2001; Hódar, 2002; Kozlov et al., 2002; Llorens et al., 2002; Fair and Breshears, 2005). Apparently, random morphological asymmetry is established before leaves attain their final size and further stresses during maturity cannot change their form (Møller and Dongen, 2003). Hence, it may be argued that a mature leaf's asymmetry is the memory of a stress met at juvenility. In this paper we examine whether this past stress could be somehow memorized in the leaf's future physiological attributes. Targeting this aim we investigate the relationship between FA and photosynthetic performance in several species.

Since the main function of a leaf is photosynthesis, which is negatively affected by a variety of natural stresses, i.e. nutrient deficiencies, drought, heat and cold in combination to high light (Long et al., 1994), we focus on this function and further ask whether a stress imposed in young leaves can change aspects of photosyn-

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**Table 1**
Mean values ± SD of leaf fluctuating asymmetry (FA) and number of collected leaves in the seven test species.

| Species | Mean values of FA | Number of collected leaves |
|---|---|---|
| *Pistacia lentiscus* | 0.093 ± 0.017 | 300 |
| *Olea europea* | 0.068 ± 0.012 | 324 |
| *Populus alba* | 0.039 ± 0.005 | 360 |
| *Platanus orientalis* | 0.045 ± 0.009 | 240 |
| *Cercis siliquastrum* | 0.033 ± 0.007 | 360 |
| *Arbutus unedo* | 0.030 ± 0.009 | 112 |
| *Ceratonia siliqua* | 0.033 ± 0.006 | 360 |

thetic behavior of mature leaves in the long term. We hypothesize that sensitive individuals, whose genotype cannot maintain the normally symmetric leaf form in the presence of stress, would be less photosynthetically competent. Hence, leaf fluctuating asymmetry could be a useful index of photosynthetic capability.

## 2. Materials and methods

### 2.1. Plant material and sampling

In preliminary trials, 15 species growing wild within the Patras University campus were examined for asymmetries in mature leaves. In particular, a total of one hundred leaves per species (ten leaves per plant) were harvested, scanned and finally their leaf fluctuating asymmetry was calculated electronically. Those with perfect symmetry, negligible asymmetry or directional asymmetry were not further used. The seven species (trees or woody shrubs) found to display random (fluctuating) asymmetry (FA) are shown in Table 1. Four of them are evergreens (*Arbutus unedo, Ceratonia siliqua, Olea europaea* and *Pistacia lentiscus*) and three are winter deciduous (*Cercis siliquastrum, Platanus orientalis* and *Populus alba*). All develop new leaves during early spring, and leaf duration is at least 17 months for evergreens and 9 months for deciduous species. Since the objective was to examine the photosynthetic attributes of mature leaves in relationship with FA, we decided to perform this examination in the most adverse period facing each of the investigated species. It is known that under Mediterranean field conditions photosynthesis displays a bimodal seasonal pattern with two minima, one during the cold winter and the other during the dry and hot summer (Karavatas and Manetas, 1999; Galmés et al., 2007). Therefore, sampling was performed at late summer for winter deciduous, and in two separate periods, late summer plus mid-winter, for evergreens.

Ten to twenty-four seemingly healthy individual plants from each species, of approximately similar size (both height and volume) were tagged and visited at late afternoon of each sampling date. The number of collected leaves was determined by their availability.

At least twenty-five fully exposed, south facing leaves were harvested from each plant (i.e. a few hundred leaves per species), put in airtight plastic envelopes lined with moist filter paper and kept over-night in complete darkness at room temperature until measured (Maxwell and Johnson, 2000). Fast chlorophyll a fluorescence transients of pre-darkened leaves were recorded next morning from the upper leaf surface at two spots, one on each side of the petiole and the mean for each leaf was computed. All measurements were taken on the same day in order to calculate first, the maximum quantum yield of photosystem II ($F_v/F_m$), indicating the extent of chronic photoinhibition (Maxwell and Johnson, 2000), and second, the photosynthetic performance index ($PI_{total}$), reflecting the current photosynthetic capacity (Tsimilli-Michael and Strasser, 2008). Subsequently, each measured leaf was also scanned for the area measurement of its right and left sides along the mid vein, based

on which its FA value was calculated. In addition to the leaf level values, aggregated mean values for FA, $F_v/F_m$ and $PI_{total}$ were also calculated for each individual plant. Based on these values, we searched for possible correlations between fluctuating asymmetry and the two photosynthetic parameters which were derived from *in vivo* chlorophyll fluorescence measurements.

### 2.2. Chlorophyll fluorescence

For chlorophyll fluorescence transients, a Hansatech (Handy-PEA, Hansatech Instruments Ltd, Kings' Lynn, UK) fluorimeter was used. For excitation, a bank of three red (peak at 650 nm) light-emitting diodes provided saturating light of 3000 μmol photons $m^{-2}$ $s^{-1}$ at sample level and fluorescence was recorded from 10 μs to 1 s with data acquisition rates of $10^5$, $10^4$, $10^3$, $10^2$ and 10 readings per second in the time intervals of 10–300 μs, 0.3–3 ms, 3–30 ms, 30–300 ms and 0.3–1 s, respectively. Cardinal points in the fluorescence versus time curve used for further calculation of photosynthetic parameters were the following (see Appendix A): maximal fluorescence intensity when all PSII reaction centers are closed ($F_m$); minimal fluorescence intensity at the start of illumination (20 μs), when all PSII centers are open ($F_0$); fluorescence intensity at 300 μs ($F_{300 μs}$), needed for the calculation of the initial slope ($M_0$) of the relative variable fluorescence versus time curve; fluorescence intensity at 2 and 30 ms (i.e. at the J and I steps, respectively) ($F_J$ and $F_I$). These primary data were used to derive the maximum photochemical efficiency of PSII $\Phi_{Po} = F_v/F_m = (F_m - F_0)/F_m$ and the photosynthetic performance index $PI_{total}$ according to Strasser et al., (2004); Tsimilli-Michael and Strasser (2008). $PI_{total}$, which expresses the relative photosynthetic performance, was calculated from the formula below:

$$PI_{total} = RC/ABS X \Phi_{Po}/(1-\Phi_{Po}) X \psi_{Eo}/(1-\psi_{Eo}) X \delta_{Ro}/(1-\delta_{Ro}).$$

The four partial parameters in the formula are related to the amount of active PSII reaction centres per absorbed energy (RC/ABS), the maximum energy flux reaching the PSII reaction centres per absorbed energy ($\Phi_{Po}/(1-\Phi_{Po})$), the probability that this energy will be conserved as redox energy and drive electron transport beyond $Q_A$ ($\psi_{Eo}/(1-\psi_{Eo})$), and the probability that electrons from intermediate carriers finally reach the end acceptors of PSI ($\delta_{Ro}/(1-\delta_{Ro})$). Hence, the photosynthetic performance index takes into account the efficiency of energy and electron flow along the whole chain of events, from the absorbance of photons in PSII to the reduction of the final electron acceptors of PSI. The formulae used for the calculation of the partial parameters of $PI_{total}$ are given in Appendix A.

### 2.3. Fluctuating asymmetry

Leaf fluctuating asymmetry was calculated as the absolute value of the difference between the right and left side areas which are separate from the mid vein (|R-L|), divided by the total leaf area (R + L) to correct the fact that asymmetry may be size-dependent. Mathematically FA is expressed as: FA = (|R − L|)/(R + L), where R and L are the areas of the right and left leaf sides, respectively.

### 2.4. Statistics

A normal, bell-shaped function of probability distribution of right versus left leaf side area differences with mean equalling zero was the criterion for random asymmetry within each species. Moreover, significant area differences between the right and left leaf sides were assessed with a paired *t*-test (SPSS v.15.0 statistical package). When a preference for a specific side being consistently larger was indicated (i.e. directional asymmetry), that species was