

Research Article

Developmental Stability of Flowering Plants is Depending On the Amount of Rainfall

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Abstract: The purpose of this study was to quantify and to compare fluctuating asymmetry (FA) of flowers of five species of plant. The analysis was based on the Procrustes method. The first task was to test the index of fluctuating asymmetry of homologous points and identify the level of developmental stability. The second task included the comparison the index of fluctuating asymmetry of xeromorphic and succulent plants. Procrustes ANOVA showed a different picture of fluctuating asymmetry depending on adaptation species to drought season. After the year with limit of rainfall the deviation from developmental stability was essential. In succulent plants FA has been increased. In contrary the index of fluctuating asymmetry data of xeromorphic plants showed a tendency to decreasing.

Keywords: fluctuating asymmetry, developmental stability, Procrustes method.

INTRODUCTION

The influence of fluctuating asymmetry on the plants described in many guides and scientific papers. Most of the work represents a deviation from the strict bilateral asymmetry (FA) as a result of the stress of the environment. So the fluctuating asymmetry is interpreted as a measure of the developmental stability of the organisms in a population.

A large body of work on the evaluation of FA employs the use of leaves with bilateral asymmetry. The influence of drought stress on FA oak has been reported [4]. The climatic changes stimulated development instability in some species of birch and pistacia [14], [5].

Only a few field studies have focused on FA in flowers. For example some studies show a correlation between FA in flowers and FA in leaf blades, insect-pollinated flowering plants with symmetric flowers attracted bumblebees more successfully than asymmetric flowers [9], [10].

The modern approach to defining fluctuating asymmetry is based on the quantification of the properties of the form of the body or its parts. In this case, the deviation of pre-selected points from the corresponding points of some average models is taken into account. Comparing the deviations from the left and right sides permit conclusions about asymmetry including fluctuating asymmetry [8]. Most plants have bilaterally symmetrical flowers, as well as leaf blades. Flowers with radial asymmetry lie outside the scope of the study of developmental stability and require a special approach.

It is logical to assume that deviation from symmetry in the high or low value mean the changing developmental instability correspondingly in high or

low level.

The definition of FA is any deviation from bilateral symmetry, usually quantified as numerically unequal differences between the absolute (values no signs) of the right and left sides. Thus, the index of FA can be determined from the difference of coordinate (XY) values between left and right homologous points.

However, the evaluation of the FA index is a task which is continuously upgrading methodically and statistically. One of the stumbling stone is the difficulty in selecting suitable symmetry points (landmarks) and their reliability for detecting FA. Beside there are various approaches to describing FA, including comparing the area of the two halves of the leaf blade or other parts of plant.

The objective of the present work was to compare indexes of FA in five perennial plant species of different ecological groups. The first group included xeromorphic flowering herbaceous plants (structural, physiological adaptations to deficit of water), second group represented the succulents having thick, fleshy, water-storing leaves and stems.

The idea of morphometric approach to FA testing was developed in works of Rohlf in 1993 and Klingenberg, with co-authors in 2002 and performed in works on leaves tree testing [1].

In the base of the programs TPS family there is an aligning principle of all the points of interest to the user. The (XY) coordinates (in a two dimensional Cartesian coordinate system) are arranged around a zero point. The averaged model of a polygon is constructed first with known (XY) for each point.

The analysis of flower FA was based on the so-called

Procrustes method. A Procrustes superimposition analysis for the configuration of landmarks was performed using the Sage program. The first step of superimposing configurations of landmarks in two-dimensional shapes ($x_1y_1, x_2 y_2 \dots$) is a generalized least squares Procrustes superimposition that minimizes differences between landmark configurations by translation, scaling, and rotation to remove all information unrelated to shape and to obtain shape variables (Procrustes distances). After the superimposition, resulting Procrustes coordinates were averaged across all samples flowers by individual.

The task of the present study is to determine the fluctuating asymmetry of homologous points of inflorescences in five species of plants and identify the FA indexes for 3 years observation, including the year with low amount of rainfall, as a year of limit water sediments in Central part of Tanzania.

EXPERIMENTAL SECTION

Region, site and species

Dodoma region in Tanzania is among the regions severely affected by failing agriculture due to climate change, as the region is situated in semi-arid areas. This district is situated in semi-arid areas and has a dry

savannah type of climate, which is characterized by long dry season, unimodal and erratic rainfall that falls between November/December and April. The study area was about 2 km to the north-west of Dodoma town ($6^{\circ}10'23''S$ $35^{\circ}44'31''E$) in the central part of United Republic of Tanzania. The site locality was typically woodland area described as forest-steppe subtropical zone. The elevation was 1500 m and total area about 5 km².

Digital images of flowers were taken during the wet season (April-May, 2010-12) using a Panasonic DMC-FZ100 camera (mode "flowers").

The vegetation consisted of a steppe community of tall grasses and forbs. Anthropogenic factors included annual burning of dry vegetation, soil and degradation due to grazing and walking, litter and discarded building material. The plant species used were common to Central Tanzania: *Ipomea alba* (f. Convolvulaceae Vent.), *Coreopsis verticulata* (f. Asteraceae, Compositae), *Digitalis purpurea* L. (f. Scrophulariaceae), *Aspalathus capensis* (Walp.) (f. Legumiacea) and *Hibiscus engleri* K.Schum (f. Malvaceae), (Fig.1)

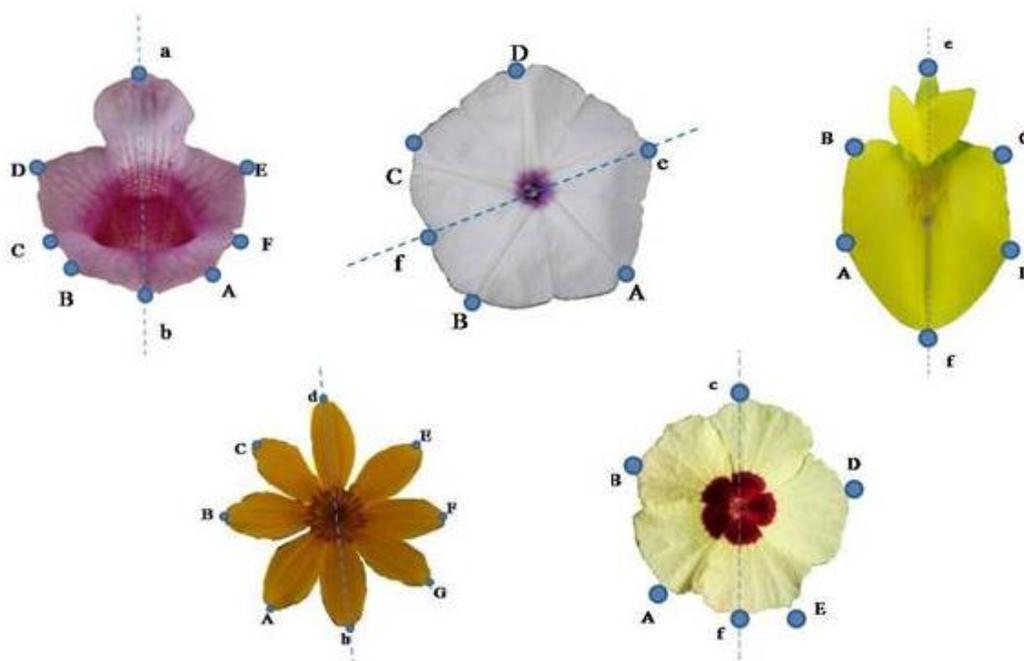


Fig-1: Bilaterally-symmetrical landmarks and axis of bilateral symmetry. Fluctuating asymmetry was analyzed using points marked capital letters, lower case letters mark axis of symmetry. Top row: *Digitalis purpurea* L, *Ipomea alba*, *Coreopsis verticulata*, bottom row: *Hibiscus engleri* K.Schum, *Aspalathus capensis* (Walp.)

Measuring and statistics

Morphometric analysis was based on unambiguous and repeatable anatomical marks on the petal marginal apex. The coordinates (x, y) of 4-6 such anatomical marks were registered for each flower image using the program TpsDig (Rohlf, 2005).

Measurement of Fluctuating Asymmetry

There are three types of deviation from perfect bilateral symmetry: fluctuating asymmetry (FA), directional asymmetry (DA), and antisymmetry (AS). FA measures the variance in left-right (L-R) differences, which are distributed around 0, whereas in

the case of DA, the L-R differences are distributed about a mean that is significantly either greater than or less than 0 (i.e., it occurs when one side of a character is consistently larger than the other). AS is the lack of symmetry in normally developing traits, and it is distinguished by a platykurtic (broad-peaked) or bimodal distribution of L-R differences about a mean of 0 [11-13]. FA corresponds to a random variation and can be used to measure developmental instability, whereas DA and AS are considered to be inappropriate as descriptors of developmental stability because both are developmentally controlled and are probably adaptive as asymmetries [12], [13].

In the software package TPS (J. Rohlf, 2010) factor "size" is supposed to reject for alignment as well as the rejection of outliers as points outside the confidence interval of the sample. The method of least squares was applied to obtain the aligned centroid. There were used 60-80 digital images for each species of plant.

Two real landmarks were pre-defined on the asymmetry/symmetry axis. Thus the assumption was that the centre of the centroid (00) coincided with the centre of the inflorescence (see Fig. 1).

FA testing

The levels of FA were obtained using the "Symmetry and Asymmetry in Geometric Data" (SAGE) program, version 1.0 (Marquez, 2006). This software analyzed the x- and y-coordinates of landmarks per individual, using a configuration protocol that divides both sides of the inflorescence.

The projection of homologous landmarks on the tangential space was used taking into account the angle to the zero point. Thus, each point received new coordinates (XY) in the tangential space; the set of points was concentrated around the point (00).

Under the each sample a set of specimens of one species per year was supposed. Antisymmetry was tested on tabulated data of kurtosis [12]. If the value of kurtosis of the difference ($XY_r - XY_l$) is higher than the tabulated values, it signalled the presence of antisymmetry, i.e. the presence of significant deviation from the normal distribution.

Procrustes superimposition analysis is performed with the original and mirrored configurations simultaneously (Fig.2). The least squares Procrustes consensus of set of landmark configurations and their relabelled mirror images is a perfectly symmetrical shape, while FA is the deviation from perfect bilateral symmetry [7].

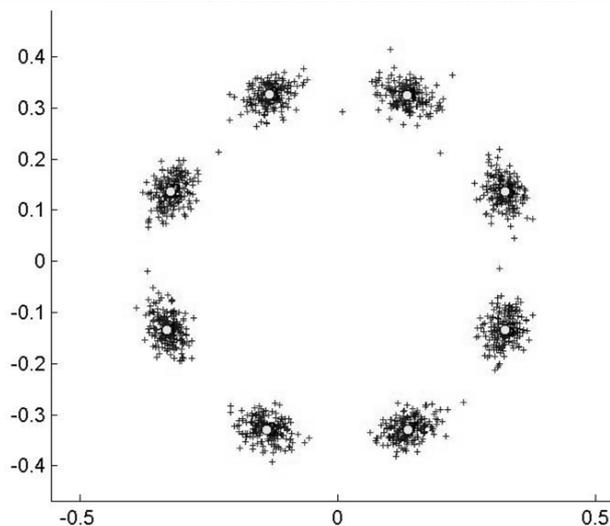


Fig-2: Procrustes fit of original and reflected data *Coreopsis verticulata*, 2011. Light marks in center of every constellation mean perfect bilaterally symmetrical points.

The squared average of Procrustes distances for all specimens is the individual contribution to the FA component of variation within a sample [15]. To detect the components of variances and deviations, a Procrustes ANOVA was used.

The aim of this analysis was:

- a) To detect individual species variation of flowers in the sample;

- b) To detect statistical significance of factor "side" (left and right) and make a conclusion about the presence of directional asymmetry;
- c) To find measurable error affecting FA value.

FA was calculated using the formula:

$$\sigma^2 = (MS_{is} - MS_m) / M, (1)$$

Where,
 MS_{is} – mean square interaction “side” and “individuals”;
 MS_m – mean square measurement error;
 M – amount of measurements (three repeats).

RESULTS AND DISCUSSION

Testing of directional asymmetry and antisymmetry

Directional asymmetry was present ($P < 0.05$; two-sample t-test with different variances) for almost all species. The result corresponded to the data of 2 way ANOVA (significant factor “side”).

The presence of antisymmetry and deviation from normality was evident for all species. The value ($XY_r - XY_l$) showed high kurtosis ($k > 7.54$) typical for leptokurtic distributions. The kurtosis value was higher than tabulated ($n = 80-90$; $\alpha = 0.05$).

The flowers with trend to radial asymmetry clearly showed directional asymmetry or undetectable FA, for example Hibiscus (Fig.2) and Ipomea in some years of observation. Even strictly bilaterally symmetrical

Aspalathus capensis (f. Legumiacea) also showed DA. Within one species, depending on landmarks, directional asymmetry on the right side or the left side was observed.

FA testing

Index of FA using the coordinates of the tangential space was determined by including the product of the coordinates of the left and right homologous points in formula (1) which provided the final result of the Procrustes ANOVA (Table 1). Fisher's exact test was used to determine statistical significance of null hypothesis of no difference between variance products (XY) left and right side.

The interaction of both factors “side” and “individuals” showed a high value of mean square and a low value of mean square measurement error. Thus the F value suggested significant FA for some samples. Only one species *Digitalis purpurea* showed presence of DA in all samples including 2012 (factor “side” is significant).

Table 1. Procrustes ANOVA results in 2012.

<i>Effect</i>	<i>SS</i>	<i>dF</i>	<i>MS</i>	<i>F</i>
<i>Ipomea alba</i>				
Sides	0.157	3	0.005	3.397
Individuals x sides	0.463	30	0.015	5.254*
Measurement error	0.300	132	0.0029	ns
<i>Coreopsis verticulata</i>				
Sides	0.025	6	0.004	0.998
Individuals x sides	0.228	54	0.004	4.350*
Measurement	0.233	240	0.001	ns
<i>Hibiscus engleri K.Schum</i>				
Sides	0.804	3	0.014	1.310
Individualsx sides	0.282	27	0.010	9.562*
Measuremen error	0.130	120	0.001	ns
<i>Aspalathus capensis (Walp.)</i>				
Sides	0.583	2	0.293	19.687
Individuals x sides	0.297	20	0.015	8.667*
Measurement error	0.151	88	0.002	ns
<i>Digitalis purpurea L.</i>				
Sides	0.113	3	0.038	6.486*
Individuals x sides	0.489	84	0.006	2.991*
Measurement error	0.678	348	0.002	ns

Note: side = directional asymmetry; individual x sides interaction = fluctuating asymmetry; * $P < 0.001$, ns – statistically insignificant ($P > 0.05$); significance was tested with 100 permutations.

A significant index of FA was not detected for every sample. Some samples showed absence FA (in years 2010, 2011). Only one species was classified as xeromorphic (*Coreopsis verticulata*). It has

characteristics that serve as protection against excessive loss of water: thick-walled tissues, thick cuticles, long roots, stomata sunken below the surface.

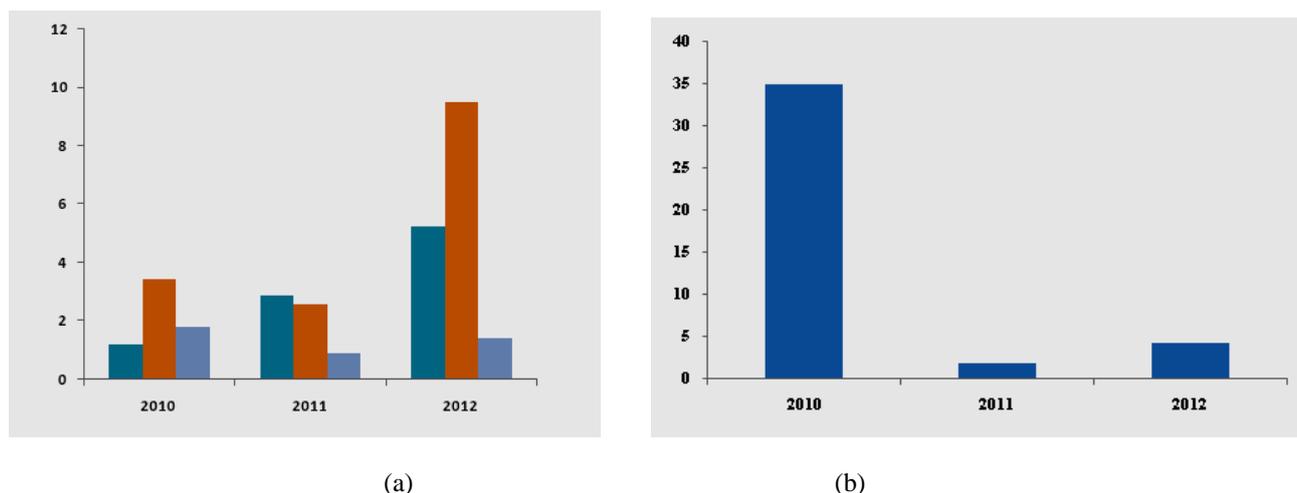


Fig-3: FA changes (F criterion) during 2010-2012. 2011 is the year of drought (twice less rainfall amount); (a) Succulents (*Ipomea alba*, *Hibiscus engleri* K.Schum, *Aspalathus capensis*, left to right) and (b) xeromorphic *Coreopsis verticulata*

The term "xeromorfizm" means a combination of morphological and physiological characteristics that appear in plants as a result of adaptation to arid environmental conditions, relating to any of the structural adaptations characteristic of xerophytes, such as water-storing stems or leaves. Decreasing of FA and increasing of developmental stability can be explained as adaptation to dry seasons. This plant (*Coreopsis verticulata*) becomes developmentally stable in year of drought. In this year (2011) coreopsis was able to transport water from deep layers of soil. In contrast, ephemeroïd like succulents with surface roots can stabilise homeostasis this year using surface water after nightly dumps sediments available for this plants root system. The next year (2012) coreopsis shows the stably low level of FA. In contrast ephemeroïd succulent plants increase the level FA after drought year (Fig.3.). It means those plants are adaptively worse to changing rainfalls volume. It is explainable as ephemeroïds are having much shorter flowering period.

CONCLUSION

As reported previously the TPS software package program was quick and easy to use and allows for FA flowers and development stability testing in context traditional ANOVA. The present study shows ability Procrustes ANOVA as a robust method confirming data of trivial 2 way ANOVA (side x individual).

Fluctuating asymmetry of flowers vary from year to year. The dry season play a role in deviation in developmental stability, particularly in xeromorphic plants.

Some succulent plants are having tendency to high developmental instability after dry year with low amount of rainfall.

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