Environmental factors influencing expression of bilateral symmetrical traits

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Abstract. Fluctuating asymmetry (FA) is a kind of asymmetry used to assess the stability of development, as the body's ability to regulate its development on the phylogenetic level. Phenotypic plasticity helps plants to overcome negative effects of temperature variability, and allow to adjusting traits to adverse conditions. The aim of the study is to evaluate the level of plasticity and fluctuating asymmetry in leaf blade under influence of environmental factors. The leaf blades from urban population linden, Tilia spp. located in Moscow region, Russia were used. Fluctuating asymmetry was measured as FA = L – R / (L + R) in absolute value (L and R – left and right value of bilaterally symmetrical metric trait). Plastic variability was measured as PL = 1 – x/X (x and X – smallest and largest value of trait size). When some traits of a leaf blade had a high plasticity, other ones exposed instability of development with an increased FA value. The correlation PL - FA was changeable. In one site correlation r was – 0.69 (p<0.05; 2014; high temperature in May, up to 60% higher norm). The cold and humid vegetative season in 2017 revealed an increase in the plastic dimensions of leaf blades of the broad-leaved linden (Tilia platyphyllos Scop.) with increasing plastic variability of bilateral traits. We attribute results to the phenotypic deviation caused by low temperatures. The predominance of one type of variability was compensating by the weakness of other type variability most likely due to genetic epistasis.

1. Introduction
One of the promising areas of monitoring for the environment is a bioindication by determining the developmental stability (DS) of plants, including woody ones. Database on the developmental stability of different species of plants is to be complementing other data sets, such as chemical contamination of air, soil and water.

Fluctuating asymmetry (FA) is a kind of asymmetry used to assess the stability of development, as the body's ability to regulate its development on the phylogenetic level. The concept of developmental noise was introduced by Waddington, developed and completed in the works of foreign and Russian
scientists at the end of the 20th century [1-5]. This term originally meant the factors that lead to deviations from bilateral symmetry, such as metabolic rate, the concentration of biochemical regulator molecules, diffusion of substances, temperature gradient, growth and cell death. The value of FA is defined as a non-directional minor deviation from the strict bilateral symmetry. At the same time the value of fluctuating asymmetry means independence in phenotypic realization on the left and right lateral side. As can be seen from many sources, the large number of plastic (metric) traits and meristic (countable) traits promotes the robust FA testing. Integral index is a mean value of FA some traits. This takes into account only the trait values that are not correlated with each other [6, 7].

The fluctuating asymmetry has to be distinguished from phenodeviation having more genetic sense. Phenodeviant organisms may occur in combination of some genes in high homozygosity. Sometimes they indicate the presence of certain specific actions break or simulate normal activity substances regulators – morphogens and hormones [8]. The relation between the FA as a signal violating the DS and environmental stress of different nature, in spite of the large number of publications, is in the focus of scientific interest [9-11].

Phenotypic plasticity can help plants to overcome negative effects of some factors, first of all, temperature variability, and allow to rapidly adjust traits to adverse conditions. The genetic variation could provide potential for adaptive evolution in response to changing climate variability [12].

Expression of gene-regulators of plant development is controlled by a number of internal and external factors. The internal factors affecting their activity include hormones, sucrose and some mineral elements, and the external factors include temperature and light. The genes that contain promoters sensitive and specific to phytohormones play important role in the regulation of differentiation and development. Currently, key genes have been identified that control embryogenesis, aging, and photomorphogenesis, regulate the functioning of the apical, lateral, and floral meristem, and are responsible for the formation of the root, leaf, flower and blood vessels [13-15]. It has become possible to build so-called genetic regulatory networks (gene regulatory network, GRN), which allow to evaluate the whole range of interactions between different regulatory genes in the process of cell differentiation and the formation of plant organs. Original elements of these networks are capable of controlling several processes at different stages of development. Therefore, mutations affecting different parts of the same regulatory gene may differ in their phenotypic expression [16-18].

A co-activator is a protein that works with transcription factors to increase the rate of gene transcription, whereas a co-repressor is a protein that works with transcription factors to decrease the rate of gene transcription. Some of the transcription factors (AS1, AS2) are proteins responded for asymmetry in leaves. The epigenetic status of the organism is determined by the nature and level of DNA methylation, post-translational modifications of histones, the presence of histone isoforms and the nature of chromatin [19-20]. The most well described epigenetic mechanism of regulation is DNA methylation. Epigenetic variability is the variability of the population response rate, which leads to the realization of discrete states of morphological structures. In the era of post-industrial society, epigenetic variation becomes of particular interest. We can outline some key aspects of the field of epigenetics studies including dispersions of fluctuating asymmetry, characterizing developmental destabilization at both individual and group levels.

In many studies were stressed the importance of correlation between phenotypic expression to ambient factor effects. The correlation within phenotypic characteristics is also important. The aim of this study is to evaluate the level of plasticity and asymmetry in leaf blade under environmental factors influence and correlation between plastic and fluctuation variability.

2. Materials and methods
The leaf blades from urban population linden, *Tilia sp.* were used. The collection, storage, preparation and measure in detail reported in previous studies, as well as statistical methods of row data treat and exclusion of directional asymmetry and antisymmetry [21, 22]. Industrial pollution was estimated on a
low or on mean level, all population were located in the Moscow region, Russia (in Moscow and in Orechovo-Zuevo, western part of region).

Fluctuating asymmetry was measured as $FA = L - R / (L + R)$ in absolute value, where $L$ and $R$ are mean of left and right value of bilaterally symmetrical traits. Plastic variability was measured as $PL = 1 - x/X$, where $x$ and $X$ are mean of smallest and largest value of trait size. For each species, five traits were measured on each of 100 leaf blades from each population. Angle signs (between the veins) were not used. Only metric trait explored (length and distance between veins, width of the leaf blade). All traits were bilaterally symmetrical and probated in referenced papers.

3. Results

3.1. Plasticity and developmental stability in Tilia cordata Mill

The genes, responsible plasticity (genetics and environment) and stability of development are closely related. When some traits of a leaf blade (measured distances between the veins) had a high plasticity, other ones exposed instability of development with an increased FA value. The reason for the deviation was the location of the populations and climatic features of the vegetative season. In a whole the plastic variability correlated to fluctuation variability.

We call this effect of conjugacy: when the trait expressed the high index of plastic variability another trait expressed high value of fluctuation variability. In figure 1 we design the results of correlation matrix of two data plastic variability (two traits) and one data FA (one trait) in 2014-2017.

![Figure 1](image-url)

*Figure 1*. Surface plots for correlation between the magnitude of plastic variability (PL, two traits, PL1 and PL2) and fluctuation variability (FA, one trait), *Tilia cordata* Mill., 2014-2017, Moscow region, Russia (n=100). 2016 year – Person’s $r = 0.73$ (p < 0.05). Other year’s $r$ is not statistically significant.
The correlation \( PL - FA \) was changeable, and sometimes can be negative. For example in some site correlation \( r \) between \( FA \) and \( PL \) some trait (fourth) was \(-0.69\) \((p < 0.05; 2014; \text{high temperature in May, up to 60\% higher norm})\).

The level and character of expression of two types of phenotypic variability depended on some factors. For plants the temperature presumably is a dominant factor. For example the cold and humid vegetative season in 2017 (low temperature, high humidity) played a main role. Here the low correlation, statistically not significant was observed. Assuming the temperature and site location were the significant factors influencing \( FA \) and \( PL \) (2-way ANOVA; \( p <0.05\)).

3.2. The effect of climate in an example of two close species
Two close species of linden (\textit{Tilia cordata} and \textit{Tilia platyphyllos}) were studied in limit one ecosystem (small town Orechovo-Zuevo, Moscow region, 2016-2018). 2017 showed an increase in the plastic dimensions of leaf blades of the broad-leaved linden (\textit{Tilia platyphyllos}) with increasing plastic variability of those traits. We attribute this fact to the phenotypic deviation caused by low temperatures of air (figure 2).

Figure 2. Plot of means two types of variability PL (plasticity) and FA (fluctuating asymmetry) (both \( p <0.05\)) and conf. intervals (95\%) for two species of Tilia (2016-2018).

Increased fluctuating asymmetry was associated with the small size of the leaf blades. The growing plate, as is known, develops in pulsating mode, in the right/ left side and has a high asymmetry. Some signs, as we have seen, have shown a negative correlation between \( FA \) and \( PL \), so relationship size – \( PL/FA \) is also focus of interest for phenogenetics [23]. The mismatch between two types of variability in traits meant an absent of any correlation \( FA – PL \) on some dimensional bilateral traits. Proposed conclusion seems to be consensus, since in the literature on this issue there is a contradictory opinion on reciprocal effect of \( FA \) and \( PL \) [24, 25]. In other words, the traits were characterized by conjugation of two types of variability. The predominance of one type of variability is compensating by the weakness of other type variability. For example, the weak fluctuation variability of the first lateral vessel was compensated by its high plastic variability. Thus the modularity of gene regulatory network associated with external factors.

Based on the response to selection on the plasticity of a trait, we concluded that plasticity is most likely due to genetic epistasis. These models make some predictions with regard to correlations among
trait plastic variety, developmental noise and developmental instability. In evolutionary view the modularity structure may enhance the adaptability and robustness of biological systems to perturbations [26, 27].

4. Conclusion

The gene regulatory network including transcription factors responds on temperature in phase of development of meristem. We conclude that stress factor of chemical pollution plays a secondary role. The fluctuating asymmetry was highest in 2017, nevertheless, the chemical pollution was not statistically significant. We refer our study to phenogenetic one contributes to the epigenetic theory of gene networks. Bilateral asymmetry, reflecting phenotypic variation, is a convenient phenomenon for studying environmental dependencies in frame of study gene regulatory network and the direction of evolution.

It is known that the heterogeneity of the sample value of metric or counting traits in a population, i.e. dispersion in heterogeneity plays a key role in the magnitude of the revealed variability [28, 29]. Genetic architecture, associations with fitness and with compensatory growth is studied under various stressful situations.

Patterns in FA is a characteristic of both DI and canalization of development. Their common origin and role in micro and macroevolutionary processes is assumed [30, 31].

Asymmetry refers to the terms of stochastic morphogenesis, i.e. to random deviation from symmetry. The negative correlation between developmental instability and individual genetic diversity was obtained in flower petals within three Opuntia echios populations that suggest buffer effect of heterozygosity on developmental instability that concise to our study [32].

Recently, there has been an increasing interest in system information for processing of phenotypic data and their genomic sense. This system stores various relationships describing the characteristics of an individual plant, and allows to uniquely establishing the relationship between genotypic and phenotypic characteristics of plants, as well as their environmental parameters.

The database on developmental stability and other phenotypic characteristics (plasticity, fitness, productivity, sustainability to some factors) is constantly being improved. Application of the system will automate the receipt data on the relationship between genotype, phenotype and environment in plants, including economically important species. In a whole the study of environmental factors affecting the manifestation of bilaterally symmetrical traits in plants is part of the research, in the framework of the study of the subtle mechanisms of gene regulatory network that are important for the ecology and evolution of plants.

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