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TESTING FLUCTUATING ASYMMETRY IN SMALL-LEAVED LINDEN POPULATIONS

Research article

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Abstract
The metric traits of leaf blades of four populations of small-leaved linden (*Tilia cordata* Mill) in the North-West of Russia were studied. Of the four traits, only one was selected: the distance between the bases of the 1st and 2nd veins of the 1st order. The advantages of this trait were the absence of directional asymmetry and a high variation coefficient, indicating the fluctuating variability of this trait. The Kruskal-Wallis analysis showed a statistical difference (p = 0.006) in the FA index = |L-R|/(L+R) among the linden populations of four cities. The highest deviation in developmental stability (increased index of fluctuating asymmetry) was obtained in the population of Petrozavodsk, the smallest – in St. Petersburg (p = 0.005). The need for careful testing of trait properties to determine fluctuating asymmetry is noted.
Keywords: fluctuating asymmetry, developmental stability, small-leaved linden.

ПРОВЕРКА ФЛУКТУАЦИОННОЙ АСИММЕТРИИ В ПОПУЛЯЦИИ ЛИПЫ МЕЛКОЛИСТНОЙ

Научная статья

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Аннотация
Изучены метрические признаки листовых пластинок четырех популяций липы мелколистной (*Tilia cordata* Mill) на Северо-Западе России. Из четырех признаков был выбран только один – расстояние между основаниями 1-ой и 2-ой жилок 1-го порядка. Достоинствами этого признака были отсутствие направленной асимметрии и высокий коэффициент вариации, свидетельствующий о флуктуационной изменчивости этого параметра. Анализ Kruskal-Wallis показал статистическую разницу (p = 0,006) в индексе FA = |L-R|/(L+R) среди популяций липы четырех городов. Наибольшее отклонение в стабильности развития (повышенный индекс флуктуирующей асимметрии) получено в популяции Петрозаводска, наименьшее – в Санкт-Петербурге (p = 0,005). Отмечается необходимость тщательного тестирования свойств признака для определения флуктуационной асимметрии.
Ключевые слова: флуктуационная асимметрия, стабильность развития, липа мелколистная.

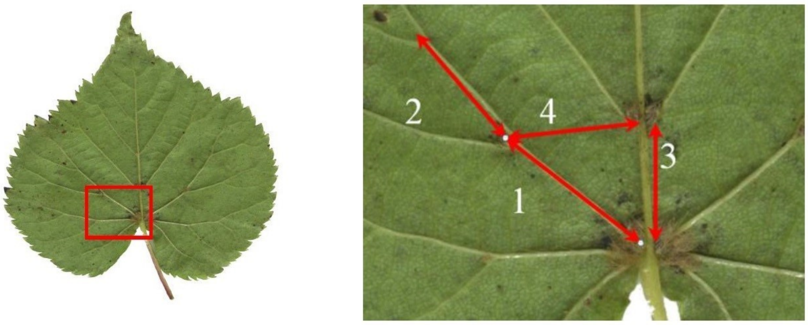
Introduction

The climate change leads to changes in the morphological and physiological properties of flora and fauna, particularly in subarctic regions. One of the population properties is a developmental stability, determined by the fluctuating asymmetry (FA) of bilaterally symmetrical organs and their parts. The leaf blades are the convenient object for studying FA, since venation in the right and left parts of the blade can be used to obtain the FA index based on several bilaterally symmetrical traits. Previous work has shown that the population variability of the FA index was higher in the northern latitudes (Kola Peninsula) compared to central Russia. A higher fluctuating asymmetry was indicated by the method of geometric morphometrics, based on a set of landmarks along the contour of the leaf blade [3]. The method of geometric morphometrics operates with high degrees of freedom, often (but not always) shows a directional asymmetry (DA) as a dominant deviation to the side, right or left. Therefore, fluctuating asymmetry is a phenomenon that is much less common, as represented using the normalizing difference L–R, where L and R are the values of the corresponding signs of the right and left. The latent directional asymmetry is a typical characteristic at low ecosystem levels of the experiment, for example, at the level of leaf blades [4]. Among the northern populations (60-67 degrees north latitude), the fourth trait was the most variable (the distance between the bases of the first and second leaf veins). We decided to study this trait in order to determine the FA measured way using the normalizing difference method, taking into account the specifics of the frequency distribution of the FA value at the level of population variability.

Among the factors influencing the magnitude of the FA are: the height of the relief above sea level, the nature of pollutants, the proximity of sources of man-made loads and climatic features [1], [2]. In the proposed work, an analysis of the FA of 4 populations with different physical-geographical relief and environmental conditions was carried out in: Murmansk (68°58′00″ N), Apatity (67°34′03″ N), Petrozavodsk (61°47′46″ N) and St. Petersburg (59°57′ N).

Research methods and principles

The leaves (10 leaf blades per 10 trees one or similar age) were collected evenly from the lower part of the crown and scanned. Measurements were taken twice using a screen digitizer (TPSdig2, Rholf, 2017). The measurement results (cm) were first entered into Excel, then into STATISTICA 10 tables (Statsoft Ink.), where the descriptive statistics and basic statistics were performed. The work used features previously developed by V.M. Zakharov (1985) and subsequently improved by I.E. Zykov [1], [2], [3]. Thus, next four traits were measured (Fig. 1).



a *b*

Figure 1 - General view of the leaf blade of the small-leaved linden (*a*) and four dimensional features (*b*):
1 – distance between the bases of the 1st vein of the 1st order and the 2nd vein of the 2nd order; *2* – distance between the bases of the 2nd and 3rd veins of the 2nd order; *3* – distance between the bases of the 1st and 2nd veins of the 1st order; *4* – distance between the beginning of the 2nd vein of the 2nd order and the 2nd vein of the 1st order

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Note: [3]

The normality was tested using the Kolmogorov-Smirnov test (K-S test). Spearman's correlation coefficient was used to analyze the relationship between the values of each trait and the FA value (FA2) = |L – R| / (L + R) for each population. To determine the differences between populations, one-way ANOVA and its nonparametric analogue, the Kruskal-Wallis test, were used. The effect of trait variability on FA was estimated in regression analysis using the maximum likelihood (the least squares) method. All statistical analyses were performed at the significance level of α = 95%.

Main results

The leaf blades varied in size across the population. The largest average value of the chosen trait was in Murmansk (1.34 cm), the smallest (1.13 cm) – in St. Petersburg (everywhere the standard error m = ± 0.01). The largest standard deviation, characterizing the dispersion and the variation coefficient, were obtained for trait No. 3 – the distance between the bases of the first and second veins of the first order on the main median vein. This trait made the main contribution to the difference between the populations in developmental stability. Most of the FA samples showed a nonparametric frequency distribution.

To normalize the samples, we used the random number generation based on the sample mean and the standard deviation. The values of outliers with high FA2 values were removed, since linden leaves are characterized by left-sided directional asymmetry; therefore, the sample size was reduced to n =67.

After normalization, the values of kurtosis and skew were within acceptable limits [-2; ÷ 2]. The normality of the distribution was confirmed by the Kolmogorov test, since everywhere the deviation from the model sample showed no difference (p>0.2;).

The samples were predominantly characterized by deviation from the normal distribution (87%), therefore paired tests for differences, as well as correlation analysis were carried out using nonparametric criteria. Spearman's correlation coefficients were low and showed a weak negative relationship between the value of the trait and the value of FA (Spearman's r = – 0.21–0.22; p <0.01). Trait № 3, in our opinion, is more suitable for determining the FA index = |L – R| / (L + R), since it is devoid of directional asymmetry. No strong correlation was obtained between the value of the traits and the FA value for all populations. This meant the absence of an allometric relationship in pair: trait's value – FA.

According to classical concepts, when determining developmental stability level, the traits that are uncorrelated by the FA value should be used, i.e. independent in the context of developmental stability. In our case, statistically significant correlation coefficients did not exceed r = 0.31.

Traits №2 and №3 were the most suitable for comparative determination of FA. Since trait №2 had directional asymmetry, we used trait №3. The Bonferroni adjustment showed that the population of Petrozavodsk had the most significant level of statistical significance of FA (α =0.01). Further in descending order of importance: Murmansk, Apatity, St. Petersburg. The highest FA10=0.025 index (Petrozavodsk) corresponded to the smallest FA error (8.6%).

Each population had a Bonferroni significance level, reflecting the F value (side × sample) in the two-way analysis. The populations of Petrozavodsk and Apatity had the highest FA10 values (0.025 and 0.016), which were confirmed by the FA2 values obtained after normalization of the samples. We explain the highest FA values on the Kola Peninsula by the high plastic variability of the linear traits of leaf blades.

Conclusion

The population of Petrozavodsk had the highest FA value. Univariate analysis based on the assumption of normally distributed samples showed results indistinguishable from the Kruskal-Wallace test. A statistically significant difference was obtained in the pair St. Petersburg-Petrozavodsk ($p = 0.005$).

Regression analysis allowed us to estimate the contribution of each trait to the overall response to the FA model for all populations. Trait № 3 had the greatest contribution to FA variability (m = ±0.08; Wald statistics: 6.90; min. – 0.35; max. – 0.05; p =0.01).

When searching for a trait suitable for determining FA and developmental stability, it is necessary to take into account the correlation of the trait by the FA value with other traits, avoid directional asymmetry and check the allometric dependence. If the trait is correlated with others by the FA value, then the trait that is devoid of directional asymmetry should be used. Trait № 3 – the distance between the bases of the 1^{st} and 2^{nd} veins of the 1st order, we consider as the most suitable for testing FA. Note that bilateral traits vary greatly depending on the influence of the environment, even in the conditions of one small city and should be carefully tested for suitability for determining FA [4], [5], [6].

The northwestern peripheral part of the range of small-leaved linden (*Tilia cordata* Mill) was generally characterized by high variability of the leaf blade, with an admixture of directional asymmetry. The leaf sizes were smaller than in the southern populations. At the same time, the FA2 index was higher (negative correlation size – FA). The climate and atmospheric pollution of Petrozavodsk, as an industrial city, were the main factors reducing the stability of development. The method of geometric morphometrics based on the analysis of the shape showed the absence of a significant effect of the northern climate on FA. Thus, the linear traits showed the variability of the genotypic component of fluctuation variability, and the method of geometric morphometrics showed a stable exhibition of phenotypic components [3], [4]. Both properties, phenotypic and genotypic, are components of the phenomenon of developmental stability and deserve the further study.

Конфликт интересов

Не указан.

Рецензия

Все статьи проходят рецензирование. Но рецензент или автор статьи предпочли не публиковать рецензию к этой статье в открытом доступе. Рецензия может быть предоставлена компетентным органам по запросу.

Conflict of Interest

None declared.

Review

All articles are peer-reviewed. But the reviewer or the author of the article chose not to publish a review of this article in the public domain. The review can be provided to the competent authorities upon request.

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