Вавиловский журнал генетики и селекции. 2019;23(4):496-502
DOI 10.18699/VJ19.519

Conjugacy of two types of phenotypic variability of small-leaved linden

S.G. Baranov[1] ✉, I.E. Zykov[2] ✉, D.D. Kuznetsova[2]

[1] Vladimir State University named after A.G. and N.G. Stoletovs (VlSU), Vladimir, Russia
[2] State Humanitarian Technology University, Orekhovo-Zuyevo, Moscow region, Russia
✉ e-mail: bar.serg58@gmail.com, zykov-oz@yandex.ru

The properties of five bilaterally symmetrical features of the leaf blades of the small-leaved linden (*Tilia cordata* Mill.) in four populations of the Moscow Region in 2014–2017 were studied. The angle trait was excluded, because it possessed the property of directional asymmetry. Instead, a new linear trait was used: the distance between the base of the second vein of the first order and the base of the first vein of the second order on the first vein of the first order. The population difference in fluctuating asymmetry (FA) was found only in the first two traits (leaf width and distance between the bases of the first vein of the first order and the second vein of the second order). The largest value of FA was in the urban environment, the smallest was in the rural areas. A weak negative correlation was obtained between the magnitude of linear characteristics and the value of FA, as well as a weak positive correlation relationship between the values of FA in five traits. The first trait had the highest fluctuation variability, and the second one had the highest plastic variability. The regression dependence of the fluctuation variability on the plastic variability (b_1 = 0.25, p <0.05) and the dependence of these two types of variability on the interaction of the factors "year" and "site of sampling" were revealed. Thus, the conclusion was made about the conjugacy of two types of variability: fluctuation and plastic. According to the authors, asynchronous growth, competition for light in conditions of high solar activity in 2014–2016 compared to the abnormal wet summer of 2017 led to an increase in FA due to destabilization of mechanisms of growth and regulation of gene expression, which contributed to a decrease in the stability of development. The increase in FA and the decrease in the developmental stability in urban ambient in 2016 could be due to: a) an intensive flow of vehicles in spring and summer, b) a high level of groundwater in this part of the city and c) increased hydrolytic acidity of the soil.
Key words: small-leaved linden; fluctuating asymmetry; phenotypic plasticity; stability of development; fluctuation variability.

For citation: Baranov S.G., Zykov I.E., Kuznetsova D.D. Conjugacy of two types of phenotypic variability of small-leaved linden. Vavilovskii Zhurnal Genetiki i Selektsii = Vavilov Journal of Genetics and Breeding. 2019;23(4):496-502. DOI 10.18699/VJ19.519

Сопряженность двух видов фенотипической изменчивости липы мелколистной

С.Г. Баранов[1] ✉, И.Е. Зыков[2] ✉, Д.Д. Кузнецова[2]

[1] Владимирский государственный университет им. А.Г. и Н.Г. Столетовых (ВлГУ), Владимир, Россия
[2] Государственный гуманитарно-технологический университет, Орехово-Зуево, Московская область, Россия
✉ e-mail: bar.serg58@gmail.com, zykov-oz@yandex.ru

Изучены свойства пяти билатерально симметричных признаков листовой пластины липы мелколистной (*Tilia cordata* Mill.) в четырех популяциях Московской области в 2014–2017 гг. Угловой признак был исключен, так как он обладал свойством направленной асимметрии. Вместо него использован новый линейный признак: расстояние между основанием второй жилки 1-го порядка и основанием первой жилки 2-го порядка на первой жилке 1-го порядка. Популяционное различие во флуктуирующей асимметрии (ФА) было найдено только по первым двум признакам (ширина листа и расстояние между основаниями первой жилки 1-го порядка и второй жилки 2-го порядка). Наибольшая величина ФА листовой пластины была в городской среде, наименьшая – в сельской местности. Получены слабая отрицательная корреляционная связь между величиной пяти линейных признаков листовой пластины и значением ФА, а также слабая положительная корреляционная связь между величиной ФА этих параметров. Наибольшей флуктуационной изменчивостью обладал первый признак, а наибольшей пластической изменчивостью – второй признак. Установлены регрессионная зависимость флуктуационной изменчивости от пластической изменчивости (b_1 = 0.25; p < 0.05) и зависимость этих двух видов изменчивости от взаимодействия факторов времени и места сбора листовых пластин. Сделан вывод о сопряженности двух видов изменчивости – флуктуационной и пластической. Асинхронный рост, конкуренция за свет в ус-

ловиях высокой солнечной активности в 2014–2016 гг. (по сравнению с аномальным летом 2017 г.) приводили к повышению ФА из-за дестабилизации механизмов роста и регуляции генной экспрессии, что способствовало снижению стабильности развития. Увеличение ФА и снижение стабильности развития в городских условиях в 2016 г. могли быть обусловлены: а) интенсивным потоком автотранспорта в весенне-летний период, б) высоким уровнем залегания грунтовых вод в этой части города и в) повышенной гидролитической кислотностью почвы.
Ключевые слова: липа мелколистная; флуктуирующая асимметрия; фенотипическая пластичность; стабильность развития; флуктуационная изменчивость.

Introduction

To determine the fluctuating asymmetry (FA, a small statistically insignificant deviation from the strict symmetry of the values of the right and left parts of a homologous bilaterally symmetric trait) and the level of development stability, the dimensional or countable bilateral symmetric traits with a wide range of response to stress effect factors are used (Palmer, Strobeck, 2003).

The most common opinion is raising the FA means a reduced developmental stability, which means a decrease in the body's ability to compensate and reduce a deviation from normal ontogenetic development along a specific canalization path (Debat, David, 2001; Lens et al., 2002; Klingenberg, 2003, 2016).

The characteristics with a wide range of fluctuating asymmetry include the most genotypic variable traits with phenotypic (ecological) plasticity. They are features of many species and, for example, in small-leaved linden trees are more pronounced than in brown birch, which is associated with their species-specific properties and their affiliation to different ecological groups.

While testing developmental stability, it is important to test the magnitude of phenotypic plasticity separately from the variability associated with developmental instability caused by stress factors. According to some authors, FA is distinguished into a special type of phenotypic variability, fluctuation variability, which depends on stochastic features at the molecular-genetic level (Tikhodeyev, 2013).

For natural populations, the duration of observation of factors that influence the change in the stability of development is essential. These include climate features, biotopic characteristics, soil physicochemical status, and terrain relief. In this study, phenotypic plasticity means the variability of the size of bilateral traits.

In previous works (Baranov et al., 2015; Zykov et al., 2015), only for some traits, a high dispersion of the difference between the right and the left values (R – L) and a statistically significantly difference in FA depending on the location of the population were determined. For example, those traits were the distance between the bases of the second and the third veins of the 2nd order and the distance between the ends of the veins. The aim of this paper was to answer the question as to how stable the properties of bilateral traits during prolonged monitoring are and how the two types of variability are associated. The objectives were: to find the magnitude of the variability of traits depending on climatic conditions and the location of the population and to compare the effect of environmental factors on the level of plastic variability and fluctuation variability.

Materials and methods

Leaf blades. The collecting of leaf blades was carried out in 2014–2017 in four populations of small-leaved linden in the generative stage of development. The first population (the first site) was located in the center of the city of Orekhovo-Zuyevo, Moscow Region, 30 m away from the petrol station "British Petroleum" (BP) (55°48'13.8'' N; 38°58'23.8'' E). The second place was chosen in the western part of Orekhovo-Zuyevo, 70 m south-west of the chemical plant "Karbolit", which produces plastics based on phenol-formaldehyde resins, and 30 m away from the road parallel to the territory of this plant (55°48' 13.1'' N; 38°58'23.9'' E). The third site was located on the territory of the State Humanitarian-Technological University (SHTU) in the eastern part of Orekhovo-Zuyevo (55°47'31'' N; 38°56'14'' E). Finally, the fourth site was located within the rural settlement of Davidovo, Orekhovo-Zuyevo District, 250 m away from the "Michelin" tire plant (55°36'9'' N; 38°51'33'' E).

In each population, leaf blades with half width from 3 to 4 cm were evenly collected from the lower parts of the crowns of ten even-aged trees. 100 sheets of 10 wood samples were used. Material processing was carried out according to the method of V.M. Zakharov and A.T. Chubinishvili in 2000 (Zakharov, Chubinishvili, 2001). A significant addition was the new trait, as an alternative to the angular trait (No. 5). In fact, connecting two branching points of the veins represented a segment of the secant to the angle and indirectly reflected the angle between the midrib and the first bilateral vein.

In early studies, the previously used angular trait was uneasy for measurement, due to a high degree of curvature of the first lateral vein (Baranov et al., 2015; Zykov et al., 2015). The remaining traits were used to determine FA by the formula of normalizing difference FA = |R – L|/(R + L). A threefold measurement of the first trait in a randomly selected sample of leaf blade was conducted. The standard error of the FA was equal to 0.28 % of the trait size mean value (R + L)/2. Such a standard error value (less than 1 % of the trait size) is considered acceptable for statistically significantly fluctuating asymmetry (Palmer, Strobeck, 2003).

Statistical assays. After the measurements, the data was saved in Excel spreadsheets. To test antisymmetry, one of the types of bilateral asymmetry affecting the value of FA, the values of kurtosis in samples (R – L) were tested. Directional asymmetry (DA), as the dominance of one of the bilateral sides, was tested in by the paired t-test H_0: R = L. Preliminary F-test on equality of variances was provided.

The plastic variability (PL) was calculated as: PL = 1 – (x/X), where x and X corresponded to the minimum and maximum values of the leaf blade trait (Bruschi et al., 2003).

The subsequent assays were conducted in the STATISTICA 10 (StatSoft Ink). They were:

– generalized regression analysis taking into account the components of variation (when the evaluating factors influence the variability);

– Kolmogorov–Smirnov test and the same test with the Lilliefors correction for normality;

– Kruskal–Wallis test and Spearman's non-parametric correlation analysis (for multiple comparisons of samples with a deviation from the normal distribution). In the evaluating criteria, the level of statistical significance α = 0.05 % was used in all methods.

Results

Primary data processing. The Kolmogorov–Smirnov test showed that the histogram of samples |R – L|/(R + L) grouped by location and by year of sampling deviated from the normal distribution. The Lilliefors test showed a similar result ($p < 0.01$). The magnitude of the trait and the value of FA showed a weak negative correlation (Spearman r = –0.06–0.13); (Table 1). The highlighted values of r (see Table 1 and Table 2) correspond to $p < 0.05$.

The reason for this dependence is supposedly the competition for sunlight, which results in a decrease in developmental stability and an increase in FA in a population with a small surface of leaf blades and, accordingly, with a small amount of homologous bilateral symmetrical traits (Venâncio et al., 2016). Based on this, an important part of the preliminary analysis was the homogenization of the primary data. According to the existing ideas, in the analyzed samples, the average value of the bilaterally symmetric trait in the samples should not be statistically different. Otherwise, the correlation between the FA and the size of the trait may distort the result of the comparative analysis (Palmer, Strobeck, 2003).

It was decided to screen high and low values in samples grouped into the population category. After screening, one-factor analysis of variance showed no difference in the size of each trait among the populations ($p < 0.05$).

The character of the histogram frequency in difference values (R – L) was investigated. 69 % of samples grouped by place and year of collection were characterized by kurtosis γ in the range of 0 ÷ 2. 30 % of samples showed a peak distribution with a value of γ = 2 ÷ 4. In 10 % of cases, in samples the kurtosis was less than zero, but not lower than –0.2. According to the tabular data obtained using permutation multiplication, the critical value γ, indicating antisymmetry, is equal to the value γ = –0.68 (α = 0.05; n = 100) (Palmer, Strobeck, 2003). Thus, in samples (R – L), grouped by site and year of sampling, antisymmetry was not detected.

Checking up for the presence of directional asymmetry in samples (R – L) confirmed the presence of DA in six cases in the sixth and in one case in the third trait (site Karbolit, 2017). These samples were not used in the work, since directional asymmetry, like antisymmetry, interferes with the evaluation of fluctuating asymmetry, which is the only indicator of fluctuation variability.

Debatable is the question of the usage of traits highly correlated in FA value. Spearman's correlation analysis showed a weak positive correlation between four pairs of traits (Table 2).

Table 1. Correlative association size – FA (Spearman's *r*)

Trait, No.	1	2	3	4	5
1	–0.05	–0.04	–0.04	–0.05	–0.02
2	–0.05	**–0.13**	0.05	0.01	–0.02
3	0.02	0.05	–0.04	–0.03	0.03
4	–0.03	**–0.07**	**–0.06**	**–0.07**	0.01
5	–0.02	**–0.06**	0.00	**–0.08**	**–0.10**

Notes: The highlighted values correspond to $p < 0.05$.

Table 2. Coefficients of pair correlation between FA value (Spearman's *r*; five traits)

Trait, No.	1	2	3	4	5
1	1.00	**0.06**	0.05	–0.01	**0.09**
2	**0.06**	1.00	0.04	0.03	**0.15**
3	0.05	0.04	1.00	0.02	0.04
4	–0.01	0.03	0.02	1.00	**0.13**
5	**0.09**	**0.15**	0.04	**0.13**	1.00

Notes: The highlighted values correspond to $p < 0.05$.

The weak Spearman's r indicated a weak positive correlation. In the case of high correlation, the traits could not be independent. The weak correlation dependence was quite natural, that is, an increase in the FA of one trait led to an increase in the FA in another trait. For example, close in location traits Nos. 2–5 showed a conjugate fluctuation with a correlation coefficient r = 0.09–0.15.

Population variability. Testing population variability by the non-parametric Kruskal–Wallis test did not show a difference in mean value of FA ($p > 0.05$). Analysis of the variability of each trait showed a statistically significant difference in the first and second traits (Fig. 1).

The first trait differed in the median test (p = 0.01), the second one differed significantly in both the median test (p = 0.039) and the Kruskal–Wallis test (p = 0.001). The pairwise comparison showed that the population in Davidovo differed from the populations in the State Humanitarian Technology University area (p = 0.002) and in the "British Petroleum" gas station area (p = 0.003). The site "State Humanitarian Technology University" had the highest value of FA and, accordingly, a reduced development stability of population.

Temporal dynamics of variability. The Kruskal–Wallis test showed that a statistically significant difference in fluctuating asymmetry depending on the year of collection was characteristic of traits Nos. 1–2 (Fig. 2).

In other traits the difference in FA was not revealed during four years of observation. In analysis of variance, the statistically significant difference revealed ca. in the same way as in the nonparametric one, i.e. the value of FA differed in 2014 and 2016 (p = 0.001). It should be noted that the first and the second traits are the largest in size, and the high variance and heterogeneity of the values of R and L contributed to the exhibit of differences in FA. Increased FA in 2014 can be explained by a high temperature in May during the formation of linden leaf blades (the air temperature was 15 to 60 % above the norm according to the report of the Hydrometeorological Centre of Russia).

The relationship between the two types of variability. It is known that the ecological plasticity of plants is determined by the buffer capacity of morphological structures, which allows them to actively adapt to environmental conditions. There are different views on the question of the relationship of developmental stability and environmental plasticity. For example, there is an opinion about the adaptation role of FA and the correlation between plasticity and developmental stability, or their partial correlation (Debat, David, 2001; Klingenberg, 2003).

A regression analysis was performed to find the relationship between fluctuation variability and plastic variability. The year of sampling was used as a fixed component of variation, the factor "site", and the interaction of "year × site" registered as random factors. The results showed that plastic variability was affected by the amount of FA (1st trait) and by the year and the mixed interaction of the factors "site" and "year of sampling" (Table 3).

Thus, the greatest impact on the plastic variability influenced the FA of the first trait (width of blade) ($p = 0.004$). The climatic conditions of the year and the interaction of the factors "year × site" were also significant ($F = 11.0$ and $F = 6.97$). A similar study was conducted on the effect of plastic variability, the year and site of sampling on the fluctuation variability (Table 4).

Plastic variability of only one, the second, trait had a statistically significant effect on FA ($p = 0.001$). The combined effect of "year × site" was significant, as was the effect of FA on plastic variability ($F = 4.19$; $p = 0.0001$). The profile graph in 3D space made it possible to estimate the impact of the site and the year of sampling on the FA value and on the value of PL (Fig. 3).

The dependence profile showed the highest value of FA in the area of the State Humanitarian Technology University (SHTU). Increased plastic variability was observed in 2014–2015 and depended on the year of sampling of leaves. Parametric estimation using univariate analysis of variance also showed a statistically significantly dependence of PL on the year of sampling (df = 3; F = 17.28; $p = 0.000$).

The described relationship of developmental stability and plastic variability characterised the 1st and 2nd traits. The main difference in the two types of variability was in their response to the ambient factors of the site and the year of sampling. The value of the plastic variability of morphological structures depended significantly on the climatic conditions of the year, as well as on the combination of these conditions with the specifics of the population station.

Developmental stability did not depend on the location of the population or on the year of sampling, but depended on the effect of the interaction of both factors. Trait No. 2 (the distance between the bases of the veins) possessed highest plastic variability; it influenced the FA mean value. The greatest fluctuation variability possessed by trait No. 1 (leaf width), which, accordingly, influenced plastic variability.

Multiple regression analysis showed the dependence of fluctuation variability on plastic variability with a regression coefficient $b = 0.25$ ($p < 0.05$).

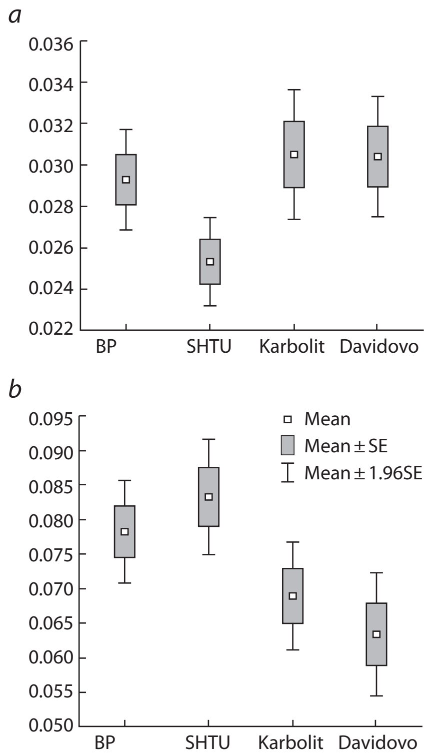

Fig. 1. Difference in FA value among populations (axis *OY*): A, *a* – first trait, *b* – second trait.
Here and in Fig. 2. Mean – mean value; SE – standart error.

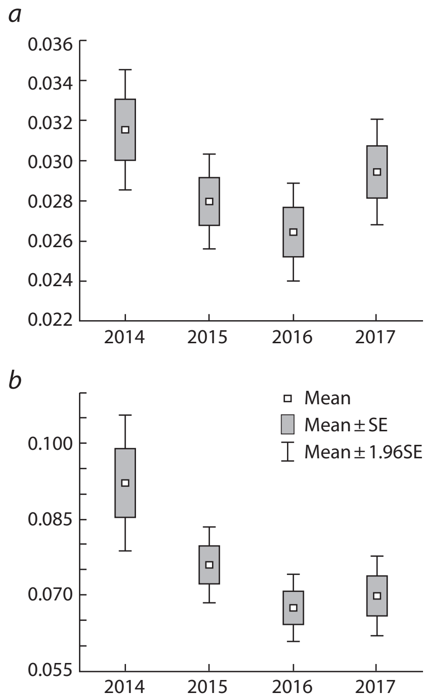

Fig. 2. Dependency FA value (axis *OY*) from year leaf blade sampling.
a – first trait, $p = 0.001$; *b* – second trait, $p = 0.012$.

Discussion

The use of non-parametric estimation methods was reasonable, since the logarithm techniques did not lead to normalization, or only part of the samples were normalized. The angular trait was replaced by a linear one, which made it easier to determine the FA index. Both types of variability, fluctuation and plastic, showed a conjugate effect: FA of the first trait influenced PL, and plastic variability of the 2nd trait influenced fluctuation variability. Such a conclusion seems to be consensus, since in the literature on this issue there is an opinion on both the one-sided effect of FA on PL (Houle, 2003; Tonsor et al., 2013; Tucić et al., 2018), and on the effect of plastic variability on FA, for example, in *Iris pumila* plants (Sultan, 2003). In other words, the traits were characterized by conjugation of 2 types of variability. The predominance of one type of variability was compensated for by the weakness of

Table 3. Effect of fluctuating asymmetry, year and site of sampling on plastic variability

Source	df effect	MS effect	df error	MS error	*F*	*p*
Fluctuation variability						
Trait 1	1	38.70	31.08	3.91	9.89	0.004
Trait 2	1	6.83	18.66	4.70	1.45	0.243
Trait 3	1	0.59	174.61	2.96	0.20	0.657
Trait 4	1	8.64	98.47	3.06	2.83	0.096
Trait 5	1	0.09	37.02	3.45	0.03	0.875
Site	3	10.61	8.51	37.57	0.28	0.837
Year	3	238.37	6.52	21.67	11.00	0.006
Site × year	7	17.03	1021.00	2.44	6.97	0.0001

Notes: Here and in Table 4: df – degree of freedom; MS effect – mean square; df error – df error degree of freedom of unexplained random error; MS error – mean square of the error; F – criterion of Fisher; *p* – statistical significance.

Table 4. Effect of factors plastic variability, year and site of sampling on fluctuation variability

Source	df effect	MS effect	df error	MS error	*F*	*p*
Plastic variability						
Trait 1	1	0.002	7.170	0.001	1.996	0.200
Trait 2	1	0.012	193.963	0.001	12.333	0.001
Trait 3	1	0.002	832.745	0.001	2.143	0.144
Trait 4	1	0.001	817.463	0.001	0.643	0.423
Trait 5	1	0.004	251.019	0.001	4.337	0.038
Site	3	0.003	7.382	0.004	0.627	0.619
Year	3	0.001	7.634	0.004	0.324	0.808
Site × year	8	0.003	1092.000	0.001	4.195	0.0001

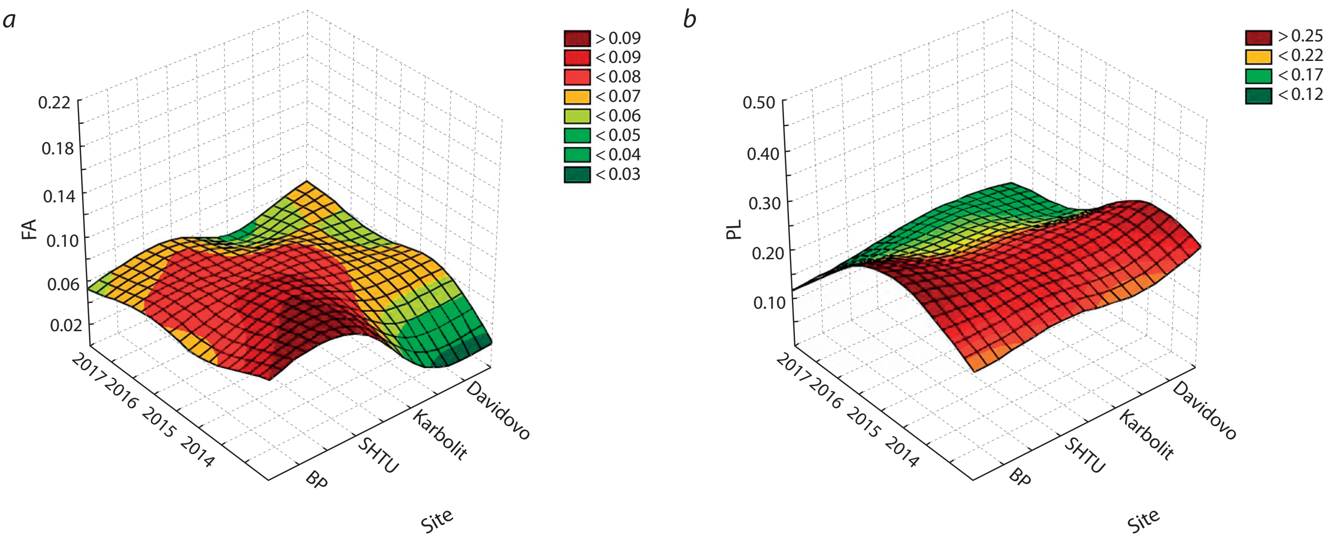

Fig. 3. Profile of the dependency of FA value (*a*) and plastic variability, PL (*b*) on the year and site of sampling.
In the tab: gradient profile FA and PL.

another type, for example, the weak fluctuation variability of trait No. 2 was compensated for by its high plastic variability.

In our opinion, asynchronous growth and competition for light in conditions of high solar activity in 2014–2016, compared with an anomalous summer of 2017, led to an increase in FA due to destabilization of the growth mechanisms and regulation of gene expression, which contributed to a decrease in developmental stability in the State Humanitarian Technology University area.

Evaluation of the components of the variance of plastic variability showed that the effect of the "year" was 26.2 % of the total dispersion, and the interaction of the factors "year" and "site" was 5.1 %.

The dispersion of fluctuating asymmetry was explained by a small fraction of the dispersion (about 2 %), which included the variance of the PL factors and "year × site".

We associated the increase in FA and the decrease in developmental stability in the State Humanitarian Technology University area in 2016: a) with a high level of intensity of the flow of vehicles, especially in spring and summer, b) with a high level of groundwater in that part of the city and c) with increased hydrolytic acidity soil. According to unpublished data, in the soil samples from Davidovo and "British Petroleum" petrol stations this indicator was 3.1–3.8 mg × eq[H^+]. In the area of SHTU, the hydrolytic acidity index was significantly higher, 5.7 mg × eq[H^+].

Environmental plasticity appears to be a highly heterogeneous type of variability, which depended on the year of sampling leaves. An abnormally wet year (the precipitation rate was over three times that in 2015) rather favorably affected the stability of the development of small-leaved linden populations in the decrease of the level fluctuating asymmetry.

It is known that the term "ecological plasticity" explains rather adaptive processes and characterizes the increased variability. The term "ecological canalization", as an attribute of development homeostasis, has the meaning of stabilizing phenotypic variability (Debat, David, 2001). Such a dialectical opposition seems to us to be a source of microadaptation of the small-leaved lime tree. In our case, first of all – to the climatic conditions. The nature of the unexplained share of the developmental stability variance remains unclear, as stated by many authors (Houle, 2003; Sultan, 2003; Lajus, Alekseev, 2004; Scheiner, 2014; Tuci et al., 2018). The results of studies conducted in 2004–2007 showed an increased fluctuating asymmetry in the area of the Karbolit on the fourth trait (distance between the bases of the first and second veins of the 1st order), which indicates a high functional variability of traits exhibiting developmental stability (Baranov, 2014).

Conclusion

The fluctuating asymmetry, associated by negative correlation with the size of the trait, manifested itself as an ontogenetic form of variability and depended on local and climatic factors.

The authors believe that long-term phenogenetic monitoring of natural populations using a set of additional environmental factors and bilaterally symmetrical features will allow a more complete analysis of the quantitative components of plastic and fluctuation variability.

References

Baranov S.G. Use of morphogeometric method for study fluctuating asymmetry in leaves *Tilia cordata* under industrial pollution. Adv. Environ. Biol. 2014;8(7):2391-2398.

Baranov S.G., Zykov I.E., Fedorova L.V. Studying *Tilia cordata* Mill. intraspecific variation on the basis of leaf bilateral asymmetry. Vestnik Tomskogo Gosudarstvennogo Universiteta. Biologiya = Tomsk State University Journal. Biology. 2015;2(30):134-145. DOI 10.17223/19988591/30/9. (in Russian)

Bruschi P., Grossoni P., Bussotti F. Within-and among-tree variation in leaf morphology of *Quercus petraea* (Matt.) Liebl. natural populations. Trees. 2003;17(2):164-172. DOI 10.4236/oje.2013.34033.

Debat V., David P. Mapping phenotypes: canalization, plasticity and developmental stability. Trends Ecol. Evol. 2001;16(10):555-561. DOI 10.1016/S0169-5347(01)02266-2.

Houle D. A simple model of the relationship between asymmetry and developmental stability. J. Evol. Biol. 2000;13(4):720-730. DOI 10.1046/j.1420-9101.2000.00195.x.

Klingenberg C.P. A developmental perspective on developmental instability: theory, models and mechanisms. Ed. M. Polak. Developmental Instability: Causes and Consequences. New York: Oxford University Press, 2003;14-34. DOI 10.1371/journal.pone.0081824.

Klingenberg C.P. Size, shape, and form: concepts of allometry in geometric morphometrics. Dev. Genes Evol. 2016;226(3):113-137. DOI 10.1007/s00427-016-0539-2.

Lajus D.L., Alekseev V.R. Phenotypic variation and developmental instability of life-history traits: a theory and a case study on within-population variation of resting eggs formation in *Daphnia*. J. Limnol. 2004;63(Suppl. 1):37-44. DOI 10.4081/jlimnol.2004.s1.37.

Lens L.U., Van Dongen S., Kark S., Matthysen E. Fluctuating asymmetry as an indicator of fitness: can we bridge the gap between studies? Biol. Rev. 2002;77(1):27-38. DOI 10.1017/S1464793101005796.

Palmer A.R., Strobeck C.H. Fluctuating asymmetry analyses revisited. Ed. M. Polak. Developmental Instability: Causes and Consequences. New York: Oxford University Press, 2003;279-319. DOI 10.1371/journal.pone.0034689/S1464793101005796.

Scheiner S.M. The genetics of phenotypic plasticity. XIII. Interactions with developmental instability. Ecol. Evol. 2014;4(8):1347-1360. DOI 10.1002/ece3.1039.

Sultan S.E. Phenotypic plasticity in plants: a case study in ecological development. Evol. Dev. 2003;5(1):25-33. DOI 10.1046/j.1525-142X.2003.03005.x.

Tikhodeev O.N. Classification of variability forms based on phenotype determining factors: Traditional views and their revision. Ekologicheskaya Genetika = Ecological Genetics. 2013;11(3):79-92. DOI 10.17816/ecogen11379-92. (in Russian)

Tonsor S.J., Elnaccash T.W., Scheiner S.M. Developmental instability is genetically correlated with phenotypic plasticity, constraining heritability, and fitness. Evolution. 2013;67(10):2923-2935. DOI 10.1111/evo.12175.

Tucić B., Budečević S., Manitašević Jovanović S., Vuleta A., Klingenberg C.P. Phenotypic plasticity in response to environmental heterogeneity contributes to fluctuating asymmetry in plants: first empirical evidence. J. Evol. Biol. 2018;31(2):197-210. DOI 10.1111/jeb. 13207.

Venâncio H., Estevao A.-S., Jean C.S. Leaf phenotypic variation and developmental instability in relation to different light regimes. Acta Bot. Bras. 2016;30(2):296-303. DOI 10.1590/0102-33062016abb0081.

Zakharov V.M., Chubinishvili A.T. Monitoring of Environmental Health in Protected Natural Territories. Moscow: Center for the Environmental Policy of Russia, 2001. (in Russian)

Zykov I.E., Fedorova L.V., Baranov S.G. Assessment of the biological value of the level of variability of the parameters of leaf blades of small-leaved linden (*Tilia cordata* Mill.) in the city of Orekhovo-Zuyevo and the Orekhovo-Zuyevo region. Vestnik Moskovskogo Gosudarstvennogo Oblastnogo Universiteta. Seriya Estestvennye Nauki = Bulletin of the Moscow State Regional University. Ser. Natural Sciences. 2015;1:15-21. (in Russian)

ORCID ID
S.G. Baranov orcid.org/0000-0002-7778-4689
I.E. Zykov orcid.org/0000-0002-6027-3700
D.D. Kuznetsova orcid.org/0000-0001-7422-8751

Acknowledgements. The authors are grateful to everyone who assisted in the collection and preparation of herbarium material.
Conflict of interest. The authors declare no conflict of interest.
Received May 8, 2018. Revised April 9, 2019. Accepted April 9, 2019.