Fluctuating asymmetry of the lamina of *Betula pendula* Roth in the context of different cities and industrial load

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Abstract. The material is collected on the territory and in the vicinity of four cities of the Russian Federation (RF) that differ in climatic conditions and anthropogenic load. A total of 20 ecotopes are distinguished, and 6000 leaves are analyzed. The fluctuating asymmetry (FA) of *Betula pendula* Roth was assessed by five morphometric features of the lamina. One-way and multi-way ANOVA and principal component analysis (PCA) have been performed. The effect of three climatic and four anthropogenic factors on FA value is discussed. None of the four regions demonstrates statistically significant inter-annual differences in integrated fluctuating asymmetry (IFA). On the territory of the cities, in most ecotopes IFA is significantly higher than in natural biotopes of the same region. The analysis of the association of IFA variability and climatic and anthropogenic parameters in different ecotopes has allowed us to reveal the influence of two groups of factors, which taken together account for 89% of the changes in environmental parameters. PCA has distributed the studied ecotopes along the gradient of the anthropogenic transformation of the environment and climatic features. The greatest contribution to the ecotope variability is made by climatic factors and anthropogenic development of the region. Generally, FA of *Betula pendula* rises along the gradient of increasing industrial load, which makes it possible to conduct comparative biomonitoring studies, both on the territory of cities and in posttechnogenic territories.

1. Introduction

Fluctuating asymmetry (FA) is one of the most interesting biological phenomena; its manifestation is associated both with the factors of intraorganism nature (genetic factors, developmental noise) and with external influences on the organism [1-4]. Due to this, FA is recommended for use in environmental studies for biomonitoring and assessment of the effect of anthropogenic pollutants on environmental conditions [5-7]. Trees have a number of merits for bioindication due to their sedentary lifestyle and autotrophic nutrition; they indicate pollution of both soil cover and the atmosphere, which is why they are widely used in assessment of anthropogenic impact [8-17]. Widespread use of FA of trees is motivated mainly by the fact that there is a well-tested method of its assessment [18]. Note also that interpreting the results of assessment of environmental conditions on the territory of a city is a
complicated task, because we are dealing with a complex interaction of environmental factors of different nature. Even more difficult is assessment of the mining industry impact, because the affected area can extend for tens of kilometers, and within this area we have different soil, microclimatic, and cenotic conditions. That is why it is a matter of great interest for bioindication to develop a unified approach to assessment of the intensity of the anthropogenic load on the territories exposed to various forms of impact from industry and transport.

2. Materials and methods
The material was collected on the territory and in the vicinity of four cities with different industrial and traffic load. Yakutsk and Yoshkar-Ola are capitals of two ethnic republics in the Russian Federation, they have similar population size (over 300,000) and play the role of administrative, cultural, and scientific centers [19]. Mirny and Gubkin are centers of the mining industry, these cities are characterized by large areas affected by their presence outside of their city limits. The climate in the study areas varies from moderately continental (Yoshkar-Ola and Gubkin) to sharply continental (Yakutsk and Mirny) [19]. For the first three cities we assessed the condition of the residential area, and by the example of Gubkin we assessed the area affected by a large mining enterprise, Lebedinsky GOK, which affects the environmental conditions of a large region [20]. The cities were numbered geographically, from west to east (1, Gubkin; 2, Yoshkar-Ola; 3, Mirny; 4, Yakutsk); and the ecotopes, along the gradient of increasing anthropogenic load.

The research object is the silver birch (*Betula pendula* Roth), a species that is widespread on the study territory and is commonly used in city street plantings [21]. The material was collected by the method introduced by V.M. Zakharov et al. for *B. pendula*; in each ecotope in the middle of the growing season we collected 10 leaves from 10 plants of reproductive age [18-19]. On the territory of Yoshkar-Ola the material was collected in 2016 and 2019, in Mirny in 2011 and 2014, in Yakutsk in 2016–2019, and in the vicinity of Gubkin in 2015 and 2017–2019. The guiding principles for sampling the leaves for FA assessment and treatment of the material were described by us in detail previously [19]. During the study period a total of 6000 leaves were collected and processed and 60000 measurements of the left and right sides of the leaf were taken.

Previously, we studied the impact of 10 anthropogenic and 14 climatic factors on IFA. Comprehensive experiments using principal component analysis (PCA) made it possible to establish that only some anthropogenic and climatic parameters most objectively reflect the variability of ecotopes [19]. In the current research we used exactly these variables with some refinements. Characterizing the seasonal development of plants and assessment of their productivity take into account the sum of active temperatures, duration of the growing season, average annual precipitation, etc. [22]. It is these three climatic parameters that proved to be fitting for PCA [19].

Among the anthropogenic factors, the following were assessed in each ecotope: industrial and traffic load, city anthropogenic load, and site anthropogenic load. For characterizing the territory of the cities, the most fitting were such factors as industrial and traffic load and city anthropogenic load; the latter was assigned a score based on the population size, industrial load, air pollution index [23-24], and the nature of pollutants [19]; whereas for the territory of Lebedinsky GOK, located outside the city limits, the distance from the source of pollution was assessed. Also, we introduced a new parameter, anthropogenic development of the region, which characterizes population density and industrial load on the territory of the four discussed regions.

The statistical data treatment was carried out in Statistica 10 software package using one-way ANOVA, factorial ANOVA, Scheffe test, and principal component analysis (PCA) [25].

3. Results and discussion
For the area affected by Lebedinsky GOK we analyzed the material collected over the course of four years. The studied territory is exposed to technogenic air pollution with a wide range of chemical elements, including heavy metals, due to dust emission from open pits, waste-rock dumps, and tailings ponds [20]. As opposed to the rest of the cities, the trees whose condition was assessed were not in the
residential area, but outside of the city. The IFA of the birch leaves did not change from year to year within the ecotopes 1.1–1.4 (p > 0.05). These sites are located at a distance of 10 to 30 km from the industrial complex, away from urbanized territories, and have the lowest transport and recreational load. At the same time, the IFA does not go below III points, i.e. the territory can be classified as "polluted". This is confirmed by our studies in previous years [26]. Year-to-year variability of this index was found in the trees in the pollution epicenter (ecotope 1.5), where IFA varies from 0.043 (score II) to 0.059 (score V) (p < 0.01). Perhaps such «jumps» in figures are caused by a stronger susceptibility of the plants growing there to weather factors. Two-way ANOVA showed a significant impact of the factors «year» and «ecotope» on the parameter variability (p < 0.01) only in 2018 and 2019 in all five ecotopes; in this we also assume the climate influence. Thus, the deterioration of the environmental conditions in the area affected by mining industry enterprises is manifested even at a considerable distance from the pollution source. Similar results are also demonstrated by previous studies on the territory of Lebedinsky GOK [27], Neryungrinsky coal mine [28], Nyurbinsky and Mirninsky GOKs [29, 30]. The material collected during 2 years in Yoshkar-Ola was treated with three-way ANOVA test. This revealed that the influence of such factors as «tree» and «year» on IFA of the leaves was insignificant (p > 0.05). However, a significant increase in IFA with an increase in the intensity of anthropogenic stress was found (p < 0.01). In conditions of a natural biotope in a protected territory (ecotope 2.1) IFA was 0.038–0.039, which corresponds to a relatively normal level (score I) on a five-point scale of developmental stability [18]. In one of the city's microdistricts, with a low traffic load and at a distance of 1 km from an enterprise producing construction materials (ecotope 2.2), IFA was 0.041, which indicates minor disturbances in onthogeny (score II). IFA of the birch leaves from the recreation area in the central part of the city (ecotopes 2.3 and 2.4) with the values of 0.045 and 0.047 indicates medium disturbances in the development of the trees (score III). B. pendula from the site in the vicinity of a pharmaceutical plant has IFA that varies from 0.053 (score IV, significant developmental disturbances) to 0.048 (score III). Scheffe test found significant differences between all three sites in IFA (p < 0.001). The results of the assessment of developmental stability of B. pendula by IFA value in different parts of Yoshkar-Ola are consistent with the data of physicochemical [23] and biological monitoring [16, 31, 32]. Consequently, adverse conditions in the zone of moderate pollution in Yoshkar-Ola are reflected in the morphological structure of the leaves of B. pendula as increased IFA figures.

On the territory of Mirny, birch leaves were collected during two years in five sites: in the recreational area (3.1), in the center of the city in the yards of multi-storey houses, in districts of wooden apartment houses (points 3.2–3.3), and also in the southern and northern outskirts of the city (3.4–3.5). IFA varied within 0.043–0.053. To the peculiarities of this region, as well as the city of Yakutsk, one should assign a relatively high FA value in the recreation area, which corresponded to score II on the scale by Zakharov et al. [18]. Note that the differences between the recreation area and the two sites in the center of the city are minor, environmental health there can be assigned the score of III (IFA is 0.044–0.047). For the territory of the city such figures can be described as low, while in the birches found on the outskirts of the city IFA was 0.050–0.051, score IV. This is due to the fact that the main source of pollution for the territory of the city are waste-rock dumps, tailings ponds, and industrial roads of Mirninsky GOK [14]. The studies in post-technogenic territories outside the city were carried out in three types of ecotopes: waste-rock dumps of different origin and the shore of a tailings pond. FA level of Betula pendula in these ecotopes varied within 0.052–0.056 and was significantly higher than in the recreation area and within the city territory (F = 2.67, p < 0.05). Substantial pollution of the area is in general characteristic of mining industry enterprises with open-pit mining, which we have noted previously [28-30]. MANOVA has shown that the «year» factor there, as well as in other examined sites, was insignificant; and the significance of the «ecotope» factor for the FA of the birch within the city was noticeable both when analyzing separate seasons (F = 4.52, p < 0.002) and on averaged data (F=3.72, p < 0.05).

The material collected over the course of four years on the territory of Yakutsk showed similar tendencies. A three-way ANOVA that we performed has revealed that IFA of the B. pendula leaf significantly increased in the city's ecotopes with an increased anthropogenic load in different years of the study (p < 0.001), and also revealed that such factors as «year» and «tree» did not have statistically
significant influence on developmental stability (p > 0.05). The average IFA value in different years varied from 0.042 to 0.058. In the recreation area of the city (ecotope 4.1) IFA was 0.042, which corresponds to minor disturbances (score II). Similarly minor developmental disturbances were observed in the ecotope 4.2, located on the outskirts of the city with predominantly private houses and low traffic load; IFA there was 0.044 (score II). The highest levels of disturbances in developmental stability of B. pendula (0.053 and 0.058) were observed in the central part of the city with multi-storey apartment houses, which has high traffic load and dust level (ecotopes 4.4 and 4.5). In other words, in these ecotopes the birch trees experience considerable (score IV) and critical (score V) disturbances in developmental stability. The largest part of the territory of the city belongs to the category «polluted areas», with IFA being 0.048 (score III). On the whole, it can be noted that a significant contribution to the pollution level of Yakutsk is made by motor transport, which is confirmed by our previous studies [11,14,19].

To understand the share and assess the significance of the studied anthropogenic and climatic variables in the differences between 20 ecotopes of 4 cities we used PCA. The analysis included 7 variables, below we present the results without factor rotation. The analysis of the association of IFA variability and climatic and anthropogenic parameters in different ecotopes of the studied territories has allowed us to reveal the influence of two groups of factors, which taken together account for 89% of the changes in parameters of the examined habitats. The greatest weight has the first principal component (PC), which reflects the climatic parameters and anthropogenic development of the region. It accounts for 61% of variability, it is negatively correlated with the anthropogenic development of the region, sum of active temperatures, duration of the growing season, and average annual precipitation. The second PC covers 28% of the variance and reflects anthropogenic characteristics. These variables are positively correlated with it: IFA of B. pendula, industrial and traffic load (table 1).

Along the first PC, the ecotopes of the four anthropogenic territories are distributed from left to right, as the anthropogenic development of the regions decreases and the climate shifts from moderately continental (Belgorod oblast, Yoshkar-Ola) to sharply continental (Mirny, Yakutsk). Along the second factor, the ecotopes are separated by IFA and industrial and traffic load. On the F2 axis, the ecotopes are distributed vertically from low to high industrial load and IFA (figure 1).

Table 1. Factor loading for ecotope variables.

| Variable                                | Principal components |
|-----------------------------------------|----------------------|
|                                         | I        | II        |
| IFA                                     | -0.42    | 0.79*     |
| Industrial and traffic load             | -0.27    | 0.83*     |
| Anthropogenic development of the region | -0.98*   | -0.14     |
| Site anthropogenic load                 | -0.64    | 0.60      |
| Sum of active temperatures              | -0.93*   | -0.29     |
| Duration of the growing season          | -0.95*   | -0.27     |
| Average annual precipitation            | -0.92*   | -0.29     |
| Dispersion                              | 0.61     | 0.28      |

*p< 0.01.
4. Conclusions
An analysis of the fluctuating asymmetry level of *Betula pendula* was performed using five morphometric features of the lamina. The material was collected on the territory of four cities of Russia differing in climatic conditions and anthropogenic load. The examined cities can be divided into administrative and industrial ones, they are located in moderately or sharply continental climatic zones. A total of 20 ecotopes with different level of industrial and traffic load were examined; for control we used natural biotopes or recreation areas. For none of the four territories statistically significant year-to-year differences in IFA were found during the study period of 4–5 years, which may indicate that weather factors have no significant effect on FA of the silver birch (within the normal range, excluding the influence of weather anomalies). In most ecotopes on the territory of the cities IFA was significantly higher than in the control, pollution-free locations.

The use of principal component analysis has allowed us to isolate from IFA and 4 anthropogenic and 3 climatic parameters only 7 variables that are grouped into 2 factors accounting for 89% of the changes in environmental parameters. The greatest contribution to variability of ecotopes is made by climatic factors and anthropogenic development of the region. IFA, industrial and traffic load affect the differences in ecotopes of the 4 territories only as second-order factors. In our previous research, on a larger set of examined cities, the opposite trend in the distribution of variables among the principal components was found; possibly these differences in the patterns are caused by the differences in the choice of the studied sites: in the first case we observed only urban territories, and in the given study we examined not only urban, but also post-technogenic territories, exposed to more intensive pollution. Note that the patterns of IFA variability along the gradient of increasing anthropogenic load are similar [19]. Fluctuating asymmetry of the *Betula pendula* leaf rises along the gradient of increasing industrial load in the cities, which allows one to perform comparative biomonitoring studies.

**Acknowledgements**
The research was carried out within the state assignment of the Ministry of Science and Higher Education of the Russian Federation (theme no. 0297-2021-0024, reg. no. AAAA-A21-121012190034-2) Study of biogeochemical cycles and adaptive reactions of plants of boreal and arctic ecosystems of the North-East Russia. We thank the director of Belogorye State Nature Reserve, Alexandr Semenovich Shapovalov for his help in material collection and a student of the North-Eastern Federal University, Natalia Alekseeva for her part in collecting and processing the material.
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