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RESEARCH PAPERS

Variation of Leaf Traits and Pigment Content in Three Species of Steppe Plants Depending on the Climate Aridity

P. K. Yudina[a], *, L. A. Ivanova[a], D. A. Ronzhina[a], N. V. Zolotareva[b], and L. A. Ivanov[a]

[a] *Botanical Garden, Ural Branch, Russian Academy of Sciences, Ekaterinburg, 620144 Russia*

[b] *Institute of Plant and Animal Ecology, Ural Branch, Russian Academy of Sciences, Ekaterinburg, 620144 Russia*

**e-mail: Polina.yudina@botgard.uran.ru*

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Abstract—Mesophyll structure and content of photosynthetic pigments in the leaves of three species of steppe plants, *Centaurea scabiosa* L., *Euphorbia virgata* Waldst. et Kit., *Helichrysum arenarium* (L.) Moench, were investigated in four geographical sites of the Volga region and the Urals located in the forest-steppe and steppe zones. Variations of the studied parameters between geographical points depended both on the species and on the structural organization of the leaf. The highest level of variation was observed for leaf area and pigment content per unit leaf area, the size and the number of chloroplasts in the cell changed to a lesser extent. The leaf thickness, leaf area and mesophyll cell sizes mostly depended on the plant species. *C. scabiosa* had large leaves (40–50 cm^2) with large thickness (280–290 μm) and large mesophyll cells (up to 15000 μm^3). The leaves of *H. arenarium* and *E. virgata* were ten times smaller and characterized by 1.5 times smaller thickness and 2–3 times smaller cell size. Geographical location and climate of the region affected leaf density, proportion of partial tissue volume, and the ratio of the photosynthetic pigments. In the southern point of Volga region with the highest climate aridity, all studied species were characterized by maximum values of volumetric leaf density (LD), due to the high proportion of sclerenchyma and vascular bundles, and specificity of the mesophyll structure. With the decline in latitude, chlorophyll (Chl) and carotenoid (Car) contents in leaf area were reduced, the ratio Chl/Car was increased, and the ratio Chl *a*/*b* was declined. The reduction of the pigment content in the leaf in all species was associated with a reduction in the amount of Chl per chloroplast, and for *C. scabiosa* and *H. arenarium* it was associated also with the reduction of chloroplast amount in the leaf area. In turn, chloroplast number per leaf area and the total cell area (A_{mes}/A) depended on the ratio of the number and size of mesophyll cells inherent to this plant species. At the same time, we found a similar mechanism of spatial organization of leaf restructuring for all studied species—decrease in A_{mes}/A was accompanied by increasing in the proportion of intercellular air spaces in the leaf. It is concluded that variations in structural and functional parameters of the photosynthetic apparatus of steppe plants were associated with plant adaptation to climate features. General direction of the changes of leaf parameters of the studied species with aridity was the increase of LD and the decrease of pigment content per leaf area however the cellular mechanisms of changes in the pigment content and integral parameters of mesophyll were determined by the plant species properties.

Keywords: steppe plants, photosynthetic apparatus, intraspecific variations, mesophyll structure, partial tissue volumes, cell parameters, chlorophyll, carotenoids, climate aridization, adaptation, geographic latitude

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INTRODUCTION

Plants inhabiting semiarid lands are characterized by range of adaptations to water and nutrients deficit that affect the physiological, structural, and phenological characteristics [1]. Adaptation of plants to water deficit is accompanied by a significant variability of the structural parameters of the leaf. Previously, plant community level research identified common mechanisms of structural adaptation of leaves to climate aridity. It was shown the decrease of the proportion of the photosynthetic organs per plant mass [2], an increase of the leaf surface density and the reduction of leaf square [3–6] along the aridity gradient. At the level of photosynthetic tissues, adaptation of plants to arid climate was expressed in the reduction of mesophyll cell in increase of leaf tissue density [3, 7], and in increase of total cell surface area per leaf unit (A_{mes}/A) [8].

Abbreviations: A_{chl}/A—total chloroplast surface area per leaf area unit; A_{mes}/A—total cell surface area per leaf area unit; Car—carotenoids; Chl—chlorophyll; LD—leaf density; LMA—leaf mass area.

Fig. 1. Geographical location of the study areas. The triangles designate research areas. (1) Middle Urals, village Beklenischeva; (2) Southern Urals, Troitsk; (3) Middle Volga, village Krasnoe Pole; (4) Lower Volga, Kamishin.

However, the general patterns identified at the community level may not coincide with intraspecific changes occurring along ecological gradients. It was shown that correlations between different leaf parameters can be high at the community level, but absent at the species level [9]. This discrepancy may be due to a variety of ecological and functional properties of species in the communities. For example, it was shown that, in plants of boreal zone, mesophyll surface area (A_{mes}/A) changes depended on the ecological properties of species, while A_{mes}/A increased in xeromesophyte species *Genista tinctoria* L. in more arid conditions and it reduced in mesophytes [10]. Birch trees of different types of environmental features were characterized by multidirectional changes in the size and morphology of leaves along the zonal-climatic transect in the Urals and Western Siberia [11]. Detailed studies of intraspecific variation can improve the understanding of its role in ecological processes occurring at the community level [12].

Despite a significant amount of data on the intraspecific changes in a variety of plant parameters along natural gradients, information on intraspecific variation in leaf parameters of steppe plants in their natural habitat is scarce and fragmented. In addition, it is still not clear to what extent the parameters of leaves, directly related to the photosynthetic function of plants, are determined by the evolutionary history of species, and to what extent they are determined by the environmental conditions. The study of intraspecific changes along climatic gradients allows us to clarify this question [13]. Species that are in different conditions with respect to the environmental optimum are likely to react differently to changes in light regime and moistening and implement different mechanisms of structural adaptation of the photosynthetic apparatus to a climate. In this context, the aim of this work was to study the intraspecific variations in the structural and functional characteristics of the photosynthetic apparatus in three steppe plant species in different climatic zones.

MATERIALS AND METHODS

Investigations were conducted in two Volga regions and two regions of the Urals (Fig. 1, Table 1). The Volga climate is temperate continental, and the Urals' climate is continental. The regions on Volga were characterized by higher values of mean annual air temperature (Table 1). Aridity index (I) was determined by the De Martonne formula: $I = P/(T + 10)$, where P is the mean annual precipitation and T is the mean annual air temperature [14]. A minimal absolute value of this index corresponds to a maximum of climate aridity.

Table 1. Climatic and geographical characteristic of the sites under study

No.	Region, coordinates (latitude north, longitude east)	Vegetation zone	Type of vegetation	*H*, m	*T*, °C	*P*, mm	*I*
1	Middle Urals, village Beklenischeva, 56°26′, 61°35′	Northern forest steppe	Forb-grass meadow steppe	210	2.4	492	40
2	Southern Urals, Troitsk, 54°11′, 61°26′	Southern forest steppe	Petrophyte-forbs steppe	216	2.9	379	30
3	Middle Volga, village Krasnoe Pole, 52°51′, 46°18′	Southern forest steppe	Forb meadow steppe	237	4.4	486	34
4	Lower Volga, Kamishin, 50°18′, 45°13′	True steppe	Bunchgrass steppe	228	7.2	352	20

H—altitude; *T*—mean annual air temperature; *P*—mean annual precipitation [Global Climate Data, v. 2.01, http://climate.geog.udel.edu/~climate]; *I*—De Martonne aridity index.

Three species of herbaceous perennials were chosen: *Centaurea scabiosa* L.*, Euphorbia virgata* Waldst. et Kit., and *Helichrysum arenarium* (L.) Moench. We studied two species, *C. scabiosa* and *E. virgata*, in all four regions, and we studied *H. arenarium* in three regions, since it was not found in the southern point of the Urals (point 2, Troitsk, Russia). *C. scabiosa* is related to European–Siberian southern boreal-forest-steppe-nemoral species, and *E. virgata* and *H. arenarium* are related to European–West Asian forest-steppe and steppe species [15]. Thus, *C. scabiosa* and *E. virgata* belong to a group of xeromesophytes with a wide range of environmental conditions, including steppe, forest-steppe, and anthropogenically disturbed communities. *H. arenarium* is mesoxerophyte usual for stony, sandy, and alkalinity steppes [15].

Quantitative studies of leaf mesophyll structure were performed according to the method of mesostructure [16]. For each species and for each point, we examined 10–15 individuals with fully developed leaves collected from the middle part of the plant. Plants were in the stage of bud formation–flowering. All quantitative measurements of leaves, tissues, cells, and chloroplasts were performed with the Simagis Mesoplant computer image analysis system (SIAMS, Russia) and Zeiss Axiostar light microscope (Carl Zeiss, Germany). The area of the leaf was determined by the digital photos of 10–20 freshly picked leaves. Leaf mass area (LMA) was measured in threefold replicates. For this purpose, we took cuttings from the middle part of a leaf, dried it at 80°C, and weighed it. Leaf density (dry weight per unit volume of leaf, LD) was calculated from LMA and thickness of the leaf. Anatomical studies were carried out on leaf cuttings fixed in 3.5% glutaraldehyde solution in phosphate buffer (pH 7.0). Leaf thickness, partial proportion of tissues, and chloroplast size were measured in leaves cross-sections. The number of cells was counted in a Goryaev chamber (Minimed, Russia) under light microscope with preliminary tissue maceration in 20% KOH solution in 20-fold replicates. The number of chloroplasts per cell and cell sizes were analyzed under a light microscope after leaf tissue maceration in 1 M HCl in 30-fold replicates. The volume and surface area of the mesophyll cells were determined by the method of projection [10]. Based on the measured parameters, the derivative characteristics were calculated. Number of chloroplasts per unit area ($\times 10^6/cm^2$) was calculated by multiplying the number of cells per unit leaf area and the number of chloroplasts in the cell. Total area of external cell membranes (A_{mes}/A) was calculated by multiplying the number of cells per unit leaf area and average surface area of the palisade and spongy mesophyll cells. Similarly, we calculated total surface of the outer membrane of chloroplasts (A_{Chl}/A). To determine the content of pigments (chlorophylls *a* and *b*, carotenoids), cuttings from the middle part of the leaves were frozen in liquid nitrogen in three biological replicates. Pigments extracted with 80% acetone solution and their concentration was determined spectrophotometrically (Odyssey DR/2500, HACH, United States). Content of chlorophylls and carotenoids was calculated according to Lichtenthaler and Wellburn formulas [17] with recalculation per unit leaf area.

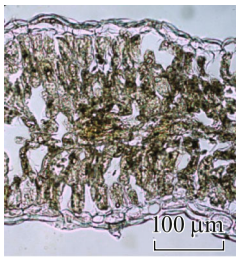
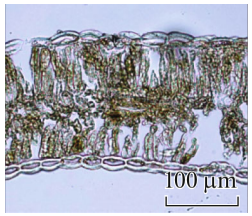
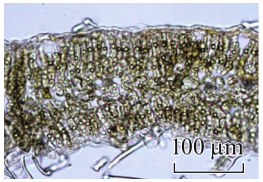
To assess the significance of differences, we used the Tukey HSD test. Analysis of the results was carried out using single-factor and two-factor analysis of variance (ANOVA) by "Species" (*C. scabiosa*, *E. virgata*, and *H. arenarium)*, "Region" (1–4 in Table 1), and "Geographic location" (Urals–Volga region) factors. To assess the level of variations between geographical areas, we calculated the index of variability: I_v—index to range between minimal (X_{min}) and maximal (X_{max}) values (in %) according to the formulae $I_v = (X_{max} - X_{min})/X_{max}$ [18].

RESULTS

Species Factor in Variation of Leaf Parameters

Table 2 shows the average values of parameters for leaves, photosynthetic tissues, cells and chloroplasts, and pigment content in steppe plants for the species. All species had similar isopalisade type of leaf mesophyll structure. Single-factor analysis of variance showed the species-specific character for some quantitative parameters of leaf structure. Thus, leaf thick-

Table 2. Mean values for leaf parameters, structure of photosynthetic tissues, and content of photosynthetic pigments

Species		*Centaurea scabiosa*		*Euphorbia virgata*		*Helichrysum arenarium*	
Cross section of leaf							
Parameter	*F*	mean value	I_v, %	mean value	I_v, %	mean value	I_v, %
Leaf area, cm^2	5.5*	44.3 ± 7.5^a	72	3.2 ± 0.2^b	48	3.2 ± 0.2^b	59
Leaf thickness, µm	33.4***	284 ± 5^a	8	189 ± 5^b	25	171 ± 5^c	26
LMA, mg/dm^2	1.0	602 ± 38^a	33	618 ± 41^a	45	498 ± 15^a	14
LD, g/cm^3	3.4	0.21 ± 0.01^a	36	0.33 ± 0.02^b	39	0.30 ± 0.02^b	34
Cell volume, $\times 10^3$ μm^3	14.0***	14.7 ± 2.4^a	47	7.4 ± 0.5^b	25	4.7 ± 0.7^b	30
Cell number, $\times 10^3/cm^2$	8.9**	832 ± 89^a	36	1310 ± 116^b	31	1556 ± 248^b	37
Number of chloroplasts per cell	12.7**	35.7 ± 3.2^a	30	35.4 ± 3.0^a	29	19.6 ± 0.5^b	7
Chloroplast volume, μm^3	14.1**	27.9 ± 2.0^a	25	19.5 ± 0.9^b	17	27.9 ± 1.6^a	14
Number of chloroplasts, $\times 10^6/cm^2$	5.2*	28.0 ± 3.1^a	34	43.3 ± 5.5^b	37	29.2 ± 4.3^{ab}	35
A_{mes}/A, cm^2/cm^2	0.6	28.1 ± 4.5^a	45	31.1 ± 2.7^a	29	25.7 ± 5.2^a	43
A_{Chl}/A, cm^2/cm^2	1.0	13.0 ± 0.7^a	19	16.2 ± 2.4^a	40	13.8 ± 2.4^a	39
Content of Chl ($a + b$), mg/dm^2	0.6	3.82 ± 0.66^a	51	3.99 ± 0.73^a	56	3.01 ± 1.00^a	60
Content of Chl ($a + b$) per chloroplast, $\times 10^{-9}$ mg	2.8	1.35 ± 0.12^a	30	1.00 ± 0.27^a	70	1.00 ± 0.19^a	38

F—value of *F*-test with one-way ANOVA. Significance level for *F*-test: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. The table shows the mean and standard error of the mean; I_v—variability index. Latin letters (*a, b, c*) denote the significant differences ($P < 0.05$) between species; the same letters indicate no significant differences; LMA—leaf mass area; LD—leaf volume density; A_{mes}/A—total cell surface area per leaf area unit; A_{Chl}/A—total chloroplast surface area per leaf area unit; Chl—chlorophylls.

ness depended on the plant species by 90% and cell parameters and chloroplasts not less than 70%. For instance, *C. scabiosa* was characterized by maximum values of leaf area and thickness, but minimum values of LD (Table 2). Also, *C. scabiosa* as compared with the other two plant species had large mesophyll cells with the smallest number per unit leaf area. *E. virgata* and *H. arenarium* compared to *C. scabiosa* possessed six times smaller leaf area and 1.6 times less its thickness. These two species were similar for cell size and cell number per unit leaf area but differed in number of chloroplasts in the cell and chloroplasts volume. Number of chloroplasts in *H. arenarium* cells was two times less, but chloroplast volume was larger. There were no inter-specific differences in the average values of integral parameters for mesophyll, such as the ratio of total surface of cells and chloroplasts in the leaf area (A_{mes}/A and A_{Chl}/A). Average values of Chl and Car contents per unit leaf area were also similar for the species. Average chlorophyll content was 3–4 mg/dm^2 and carotenoid content was 0.7–0.8 mg/dm^2 (Table 2).

The most variable characteristics on the intraspecific level were the leaf area and the content of photosynthetic pigments per unit leaf area. The difference between the minimum and maximum values of these parameters was within 50–70% (Table 2). The most stable parameters for the species were thickness of the leaf, number of chloroplasts per cell, and chloroplast volume, where the index of variability was within 10–25%. Mesophyll parameters—cell size, A_{mes}/A, and A_{Chl}/A—changed in the medium range 30–50%.

Changes in Leaf Parameters of the Studied Species, Depending on the Geographic Location and Climate

Geographic location influenced both whole leaf parameters and the internal leaf structure. In two species—*C. scabiosa* and *E. virgata*—LMA was minimal in the north point of Urals (1) and had maximum values in the most arid Volga point (4), while LMA did not change in *H. arenarium* (Fig. 2c). LD varied in all species similarly (Fig. 2e). In the south, the most arid, Volga point (4), this characteristic had the maximum values, while it had the minimum values in the Urals. Geographical location had small effect on leaf thickness, its changes were species-specific (Fig. 2a).

Analysis of the ratio of different tissue types in the leaf showed that the geographic location influenced the partial volume of sclerenchyma and vascular bundles to the greatest extent (Fig. 3). The proportion of these tissues in the leaf was the highest in the southern point of the Volga (4). Thus, the proportion of covering tissues that made up 15–20% of the leaf and did not depend on geographical location. The change in the volume fraction of intercellular spaces had a species-specific nature. For *C. scabiosa*, the minimal size of intercellular air spaces was found in the northern point of the Ural transect (1), in the area with minimal climate aridity. *E. virgata* had minimal values of the partial volume of intercellular air spaces in the northern points of Urals and Volga regions (1, 3), and it increased in more arid southern points of these regions. For *H. arenarium*, this parameter did not change significantly in different regions.

It was shown that individual cells and chloroplasts parameters have high species specificity changes depending on climatic conditions (Fig. 4). For *C. scabiosa* with large size of mesophyll cells, cell volume significantly reduced with increasing climate aridity. At the same time, small-cell leaves of species *E. virgata* and *H. arenarium* possessed sustainable cell size in different environmental conditions. Cell number changed nonlinearly for *C. scabiosa* and *E. virgata*, and reduced for *H. arenarium* under increased climate aridity (Fig. 4b). Chloroplast size showed little dependence on growing conditions, but it differed significantly between species in all studied locations. Chloroplast number per cell for *C. scabiosa* declined under increased climate aridity following the change of cell size (Figs. 4a and 4d). Chloroplast number per cell in *E. virgata* was higher in both Volga points, while it remained stable regardless of the climatic conditions in *H. arenarium*.

Integral mesophyll parameters were stable for each species in a wide range of conditions; however, 1.5–1.3-fold changes were marked in some areas, which displayed a species-specific character (Fig. 2d). For *C. scabiosa* in the northern point of the Urals (1), A_{mes}/A differed from that in other areas, while this value declined in *E. virgata* in the southern point of the Urals (2) compared to other areas. In *H. arenarium*, A_{mes}/A decline was observed in the most arid lands of the Volga (4). The total chloroplast surface (A_{Chl}/A) in all species along the aridity gradient changed similarly to changes in the number of chloroplasts per unit area (Figs. 2b and 2f). For *H. arenarium* and *C. scabiosa*, we noted a decrease in the value of these parameters with increasing aridity, while for *E. virgata* chloroplast amount per leaf area and A_{Chl}/A were significantly higher in the Volga region compared to Urals.

We found a strong dependence of the pigment complex on species habitat latitude. Chl and Car content reduced in the direction of the southern latitudes (Figs. 5a and 5b). The ratio of Chl *a*/*b* increased with the change of the latitude, and the ratio of Chl/Car, on the contrary, reduced. In addition, we did not detect interspecific differences in the ratio of pigment forms within each investigation region. In the southern point of the Volga, Chl content per chloroplast in all species had minimum values.

DISCUSSION

Characteristics of Leaf Parameters in Studied Species

We studied leave parameters (from the whole leaf to the cellular level) in three species of steppe plants—

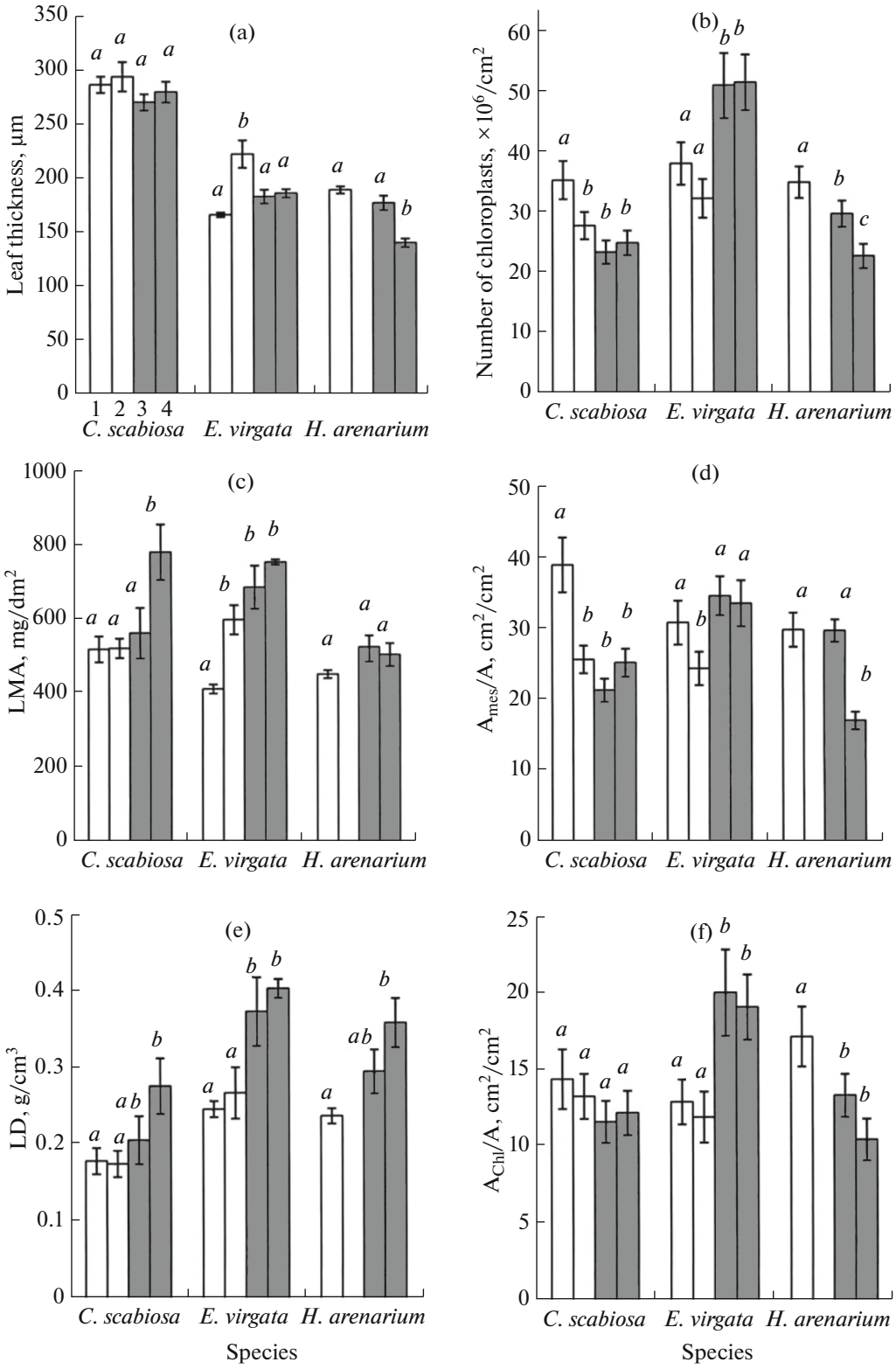


Fig. 2. Leaf parameters: (a) leaf thickness, (b) number of chloroplasts, (c) LMA, (d) A_{mes}/A, (e) LD, and (f) A_{Chl}/A of investigated species from different geographical regions. White columns correspond to the Urals; gray columns correspond to areas of the Volga region. (1) Middle Urals, village Beklenischeva; (2) Southern Urals, Troitsk; (3) Middle Volga, village Krasnoe Pole; (4) Lower Volga, Kamishin. Data for *H. arenarium* in the point 2 (Troitsk) are absent. Latin letters denote the significant differences ($P < 0.05$) between species; the same letters indicate no significant differences.

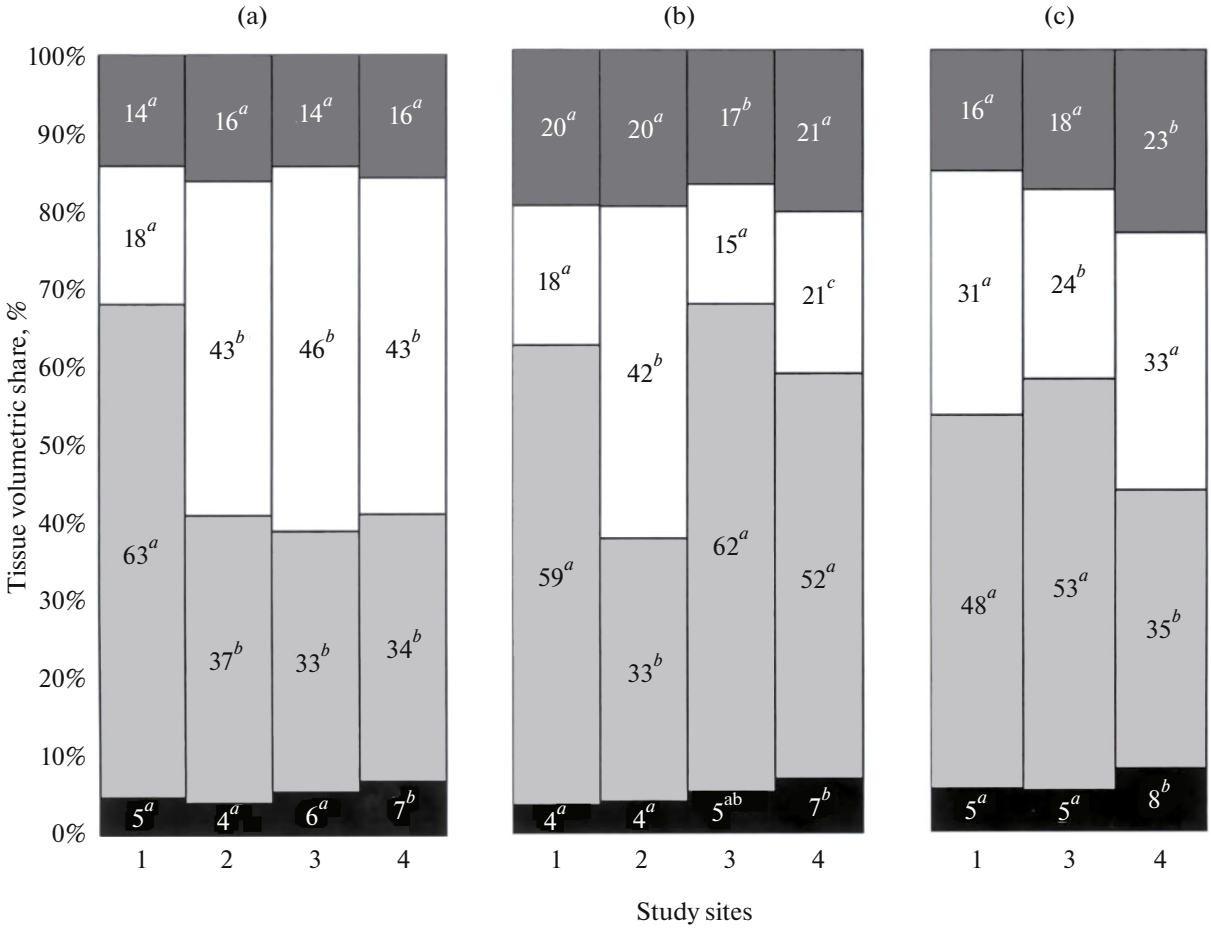


Fig. 3. Partial volumes of different tissue types for studied species: (a) *Centaurea scabiosa*, (b) *Euphorbia virgata*, (c) *Helichrysum arenarium*. (1) Middle Urals, village Beklenischeva; (2) Southern Urals, Troitsk; (3) Middle Volga, village Krasnoe Pole; (4) Lower Volga, Kamishin. Latin letters denote the significant differences ($P < 0.05$); the same letters indicate no significant differences within a species for each type of tissue. ■ covering tissues; □ intercellular spaces; ■ mesophyll; ■ sclerenchyma and conducting tissues.

C. scabiosa, E. virgata, and *H. arenarium*—in different climate conditions. To the best of our knowledge there was no literature on the photosynthetic structures of organs and tissues on these species. In general, there are scattered data on these parameters in steppe plant leaves. For example, the Gobi steppe plants had average thickness of leaf 500–600 μm, leaf area 1.1–1.4 cm^2, and LMA 800–1200 mg/dm^2 [8]. Compared to them, studied steppe species in the Volga region and in the Urals possessed thinner and larger leaves with a smaller surface density. These parameters of the leaves of the studied species were more similar to the herbaceous plants from steppe and southern taiga of the Urals [4, 19, 20]. At the same time, the number of cells and chloroplasts per unit leaf area of studied steppe plants were higher than the average number cells of boreal plants with $400–600 \times 10^3$ cells and $8–12 \times 10^6$ chloroplasts per 1 cm^2 of leaf area [20]. Meanwhile, the number of photosynthetic cells and chloroplasts in the leaves of studied species draw near to the Gobi steppe xerophytes [8] with cell number $1000–1800 \times 10^3/cm^2$ and number of chloroplasts $30–50 \times 10^6/cm^2$. For Tuva steppe plants with isolateral mesophyll structure, we found even greater value of the number of cells: $2500–5400 \times 10^3/cm^2$ and chloroplasts $30–95 \times 10^6/cm^2$ [7]. We found that mesophyll cell size of the steppe plants did not differ from the size of leaf cells of the boreal or steppe plants in the Gobi and Tuva. At the same time, it is often mentioned that species from dry habitats are characterized by small dimentions of mesophyll cells [13]. However, interspecies comparison of plants of different ecological groups showed no significant difference for cell size between mesophytic and xerophytic species [21]. The main differences were identified for integral parameters of mesophyll: xerophytic species had higher values of A_{mes}/A, A_{Chl}/A, and number of chloroplasts per unit leaf area compared to mesophytes. In the investigated species, high concentration of cells and chloroplasts provided high values A_{mes}/A and A_{Chl}/A, which is 2–4 times higher than the average values of these indicators for boreal mesophytes and were close

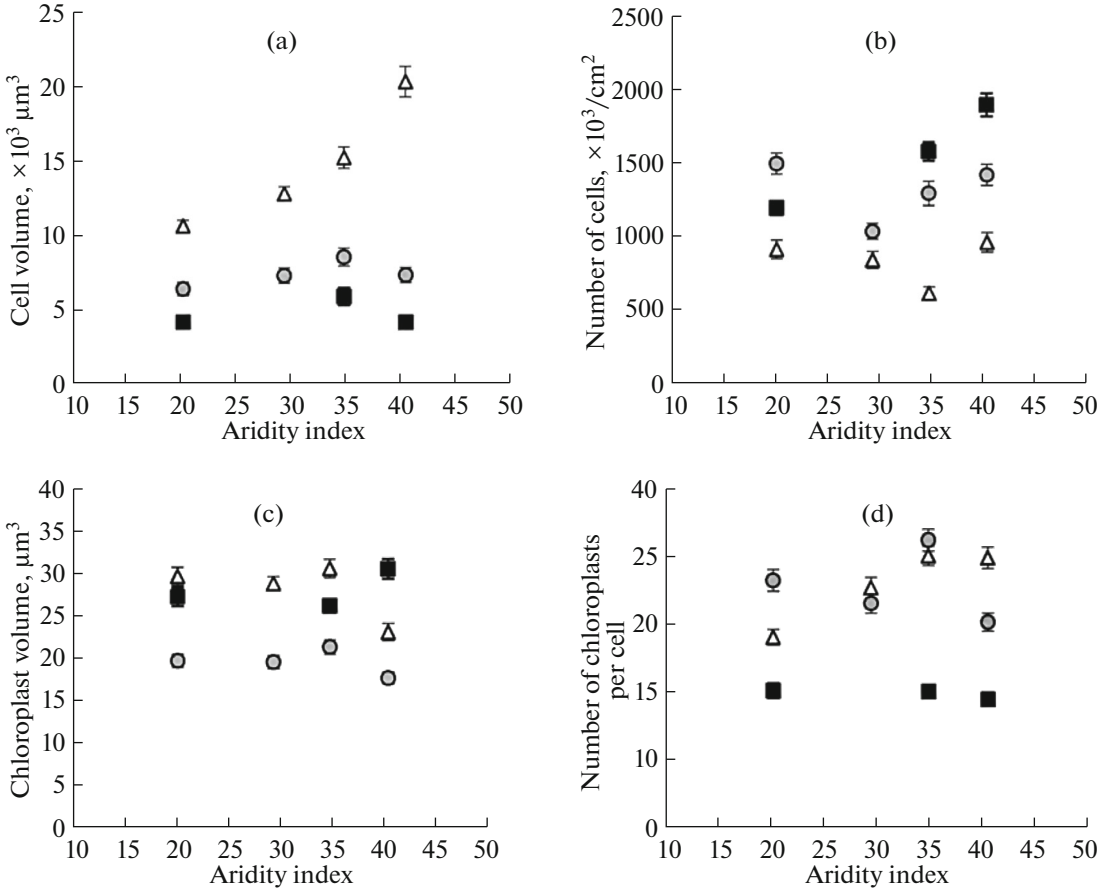


Fig. 4. Changes of parameters of (a, b) mesophyll cells and (c, d) chloroplasts in investigated species according to climate aridity. Aridity index values: Middle Urals, village Beklenischeva—40; Southern Urals, Troitsk—30; Middle Volga, village Krasnoe Pole—34; Lower Volga, Kamishin—20. The mean and standard error of the mean are shown. Triangles designate *C. scabiosa*, circles designate *E. virgata*, squares designate *H. arenarium*.

to the values of boreal mezoxerophytes [21] and xerophytes of steppes and the Gobi deserts [8]. An increase of A_{mes}/A and A_{Chl}/A, which characterizes value of exchange surface for CO_2 diffusion from intercellular spaces to the places of carboxylation in the chloroplasts, has an important physiological significance in plant adaptation to high insolation and water deficit [16, 22]. It is characteristic that the species that we studied did not differ in the values of these parameters (Table 2). Indeed, the studied species are typical representatives of forest-steppe and steppe plant communities and have similar ecological characteristics. Thus, even if the integral parameters of mesophyll changed, depending on the growing conditions, their values did not go beyond the inherent ecological group [21].

The contents of pigments of plants from semiarid and arid lands are usually described as low [23, 24]. For instance, chlorophyll content in 11 plant species from dry steppe in the Khamar-Daban varied within 1.4–3.3 mg/dm^2 [24]. At the same time in different species of desert plants in the Karakum and Gobi, a large variety of pigment content related to species patterns was noted [23]. In these plants, Chl content ranged from 1.7 mg/dm^2 in *Artemisia frigida* Willd. to 6.2 mg/dm^2 in *Achnatherum splendens* (Trin.) Nevski. In our study, three plant species were characterized by the double-spread values of Chl content depending on the region (Fig. 5a)—from 2 to 5 mg/dm^2, that is, in the range known in the literature for plants of well-lit and dry habitats [25]. Investigated species grow in typical habitats with a high level of insolation, where only a small amount of pigment per unit leaf area was enough for efficient absorption of sunlight.

Limits of Intraspecific Variation of Leaf Parameters

Plant species may differ from each other not only on the values of leaf parameters but also on the range of their variation. Variation degree may depend on the studied feature—the structure of the low-order (chloroplasts) is usually characterized by a lower level of variation than the structure of higher orders (plant leaves), and morphological parameters will vary less than the biochemical parameters [12, 16]. Thus, in our

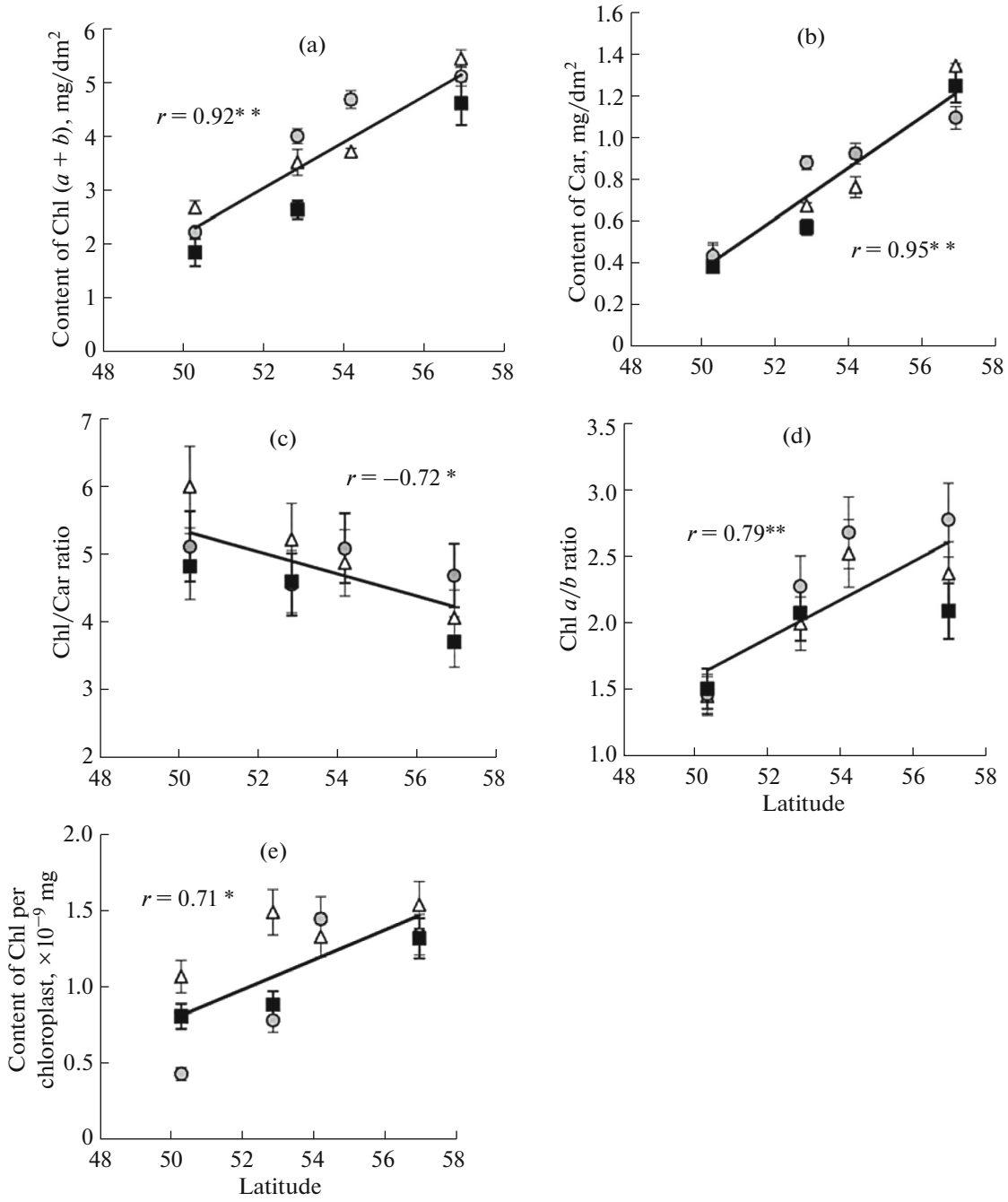


Fig. 5. Changes of chlorophyll (Chl) and carotenoid (Car) content per (a, b) unit leaf area, (c, d) ratio of pigments, and (e) Chl content per chloroplast in the studied species of steppe plants depending on latitude. The mean and standard error of the mean are shown. *r*—correlation coefficient. One asterisk denotes correlation coefficient significance for $P < 0.05$, two asterisks shows that for $P < 0.01$. The solid line is the regression line for all species. Triangles designate *C. scabiosa*, circles designate *E. virgata*, squares designate *H. arenarium*.

studies, index of variability for leaf area was 60–70%, whereas that for chloroplast volume was in the range 14–25%. Earlier, significant variation in leaf size within the species adapting to climate aridity was shown [26]. Unlike leaf area, leaf thickness and density varied to a lesser degree: 8–26 and 14–45%, respectively (Table 2). Content of photosynthetic pigments per unit leaf area has been one of the most variable indicators—index of variability was 50–70%. Other authors also showed a significant level of intraspecific variation of Chl content per unit leaf area [18, 24, 27] and in chloroplast [27]. The parameters of cells and chloroplasts in three species had an average level of variation, in the range 30–50%. Cell number in five

boreal species in contrasting conditions of humidification varied in the range 35–60%, and cell size varied in the range 6–39% [10]. The range of chloroplast number did not exceed 30% of the cell, which is consistent with the existing ideas about the stability of this index for the species [16]. Generally, intraspecific variation in the quantitative parameters of photosynthetic tissues under the influence of climatic conditions was 20–40%, and this range was much lower than interspecific differences of plants of different ecological groups within the boreal zone [21] or differences between communities in different climate zones [2, 4, 19]. When analyzing the 629 plant communities, it has been shown that intraspecific variation in plant functions was 25% of the level of variation of these parameters within the community and 32% of the total variation among communities [12]. Thus, the level of intraspecific variation of mesophyll structure and functions, due to the species adaptation to climate change, was significantly lower than the mean differences between the communities formed in different climates.

Rate of change in leaf mesophyll parameters may also depend on the species environmental features, such as the type of its environmental strategy [10]. In plants with ruderal type of strategy, size of cells changed, whereas number of cells varied increasingly in plants with high competitiveness [10]. Other studies have shown that the volume of mesophyll cells was largely determined by a vital form [28] and was the genetically determined feature [11]. In our study, the size of cells and chloroplasts and their variation were species-specific. *C. scabiosa* differed by large mesophyll cells, the volume of which largely changed depending on growth conditions, more than the number of cells per unit leaf area (Figs. 4a, 4b). Two other species had small mesophyll cells, the size of which varies less than their number. In our opinion, the mesophyll cell sizes and the range of their varying are the result of adaptive evolution of these species, while the integrated parameters of mesophyll and pigment content were more dependent on the growing conditions. At the same time studied species differed in the structural mechanisms of formation of integral leaf parameters at adaptation to climate.

Formation Mechanisms of Leaf Parameters for Steppe Plants' Adaptation to Climate

Patterns of the whole leaf are in close relationship with its internal structure and biochemistry. For instance, LMA depends on the thickness and density of the leaf, which can be varied independently [6]. LMA depends on the leaf anatomy: sclerenchyma fraction, thickness of cuticle and epidermis, mesophyll cell packing density, and also thickness of cell walls and degree of pubescence [1]. As a rule, in the warmer and arid lands, LMA increased compared to humid areas both at the community level and at the species level [5, 6]. LMA demonstrated a positive correlation with temperature and a negative correlation with rainfall [6, 19]. However, the behavior of LMA in the steppes along the aridity gradient is not linear; it may be due to changes in zonal patterns of leaf structure characteristic of the species community [19]. One reason for LMA change is the change in the portion of nonphotosynthetic tissue leaves and the density of the leaf tissue layers [2, 4]. Another mechanism of LMA is changes of mesophyll cell structure [20].

Analysis of the data showed that there were no clear patterns in LMA change due to climate aridity in three steppe species. LMA values were stable enough for the species in the majority of areas (Fig. 2c). Of all external leaf parameters, only LD changed in a similar manner in all species (Fig. 2e). We showed an increasing LD in species of the Volga region compared to the Urals. Perhaps this is due to higher mean annual temperature in the Volga region (Table 1). We recorded LD maximum values for each species in the desert steppe of the Volga region, in a site with maximum temperature and climate aridity. In this site for each species, there was also an increase in the proportion of sclerenchyma and conductive tissues in the leaf (Fig. 3), which confirms a direct impact of the proportion of nonphotosynthetic tissue and leaf density. However, internal causes of LMA and LD changes in the studied species were different.

For *C. scabiosa*, LMA increased only in the most arid area: in the true steppe of the Volga region. This occurred due to increased LD without the change of leaf thickness. One more reason for LD increase in this species, except for increasing proportion of sclerenchyma, can be a significant reduction in cell size, found along the aridity gradient (Fig. 4a). Reducing the volume of the cell increases the cell wall fraction in the mass of the leaf [29], which leads directly to an increase in leaf density. In *E. virgata*, LMA increase in the southern point of the Urals occurred at the expense of increasing the thickness of leaf without changing LD. In turn, an increase in the leaf thickness was accompanied by an increase in the amount of leaf sclerenchyma without changing it's partial volume. In two points of the Volga region, high values of LMA in this species were maintained by the increasing of the LD. The rise in LD was also due to increased cells and chloroplasts contents per unit leaf area. In *H. arenarium*, as well as in other two species, in the most arid point of the Volga, LD increased by reducing the thickness of the leaf, while LMA remained stable in all studied regions. Thus, the mechanism of LMA and LD formation was complex comprising changes in the ratio of tissues and mesophyll cell packing, and it depended on the species. Examination of this mechanism may reveal functional differences between species and largely explain different responses to changing environmental conditions.

It is generally believed that changes in the anatomical structure of plant leaves during drought consist

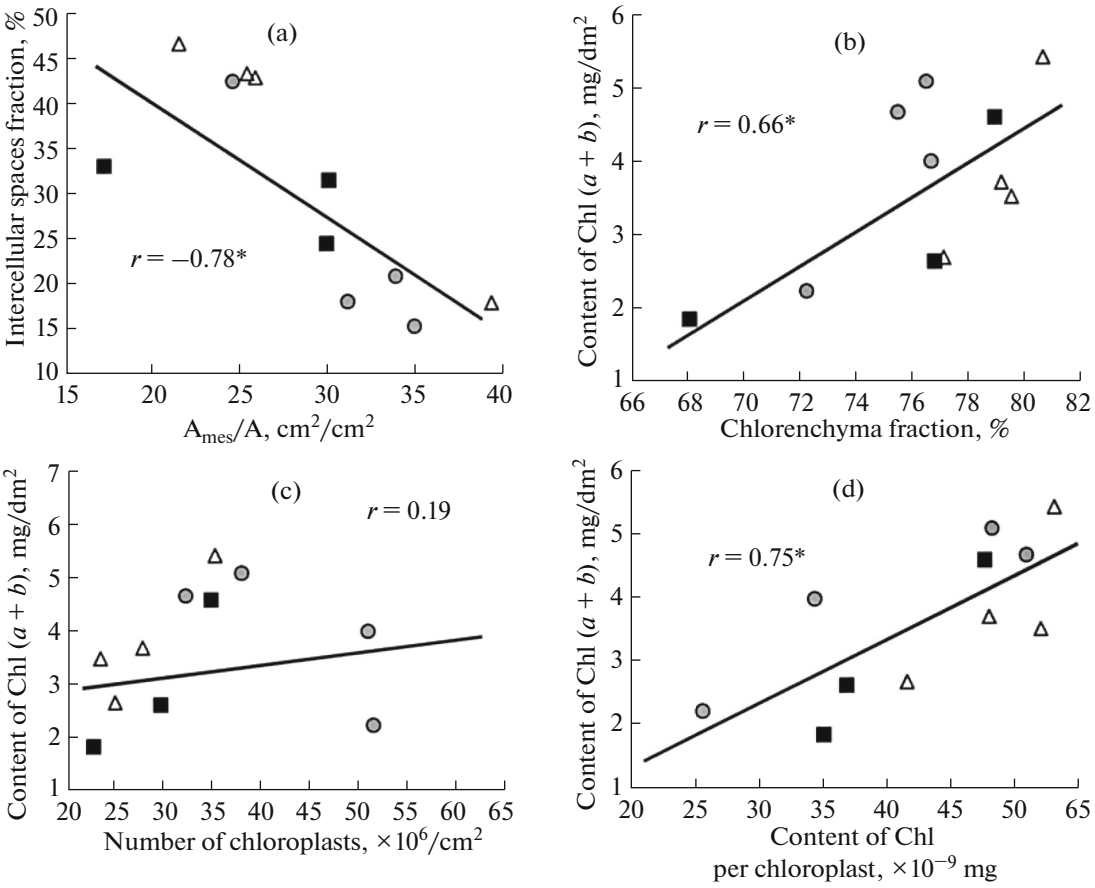


Fig. 6. Relationship between the parameters of photosynthetic tissues of three steppe species: (a) proportion of intercellular spaces and A_{mes}/A; (b) content of Chl $(a + b)$ and proportion of chlorenchyma; (c) content of Chl $(a + b)$ and number of chloroplasts per unit leaf area; (d) content of Chl $(a + b)$ per unit leaf area and Chl content per chloroplast. The solid line is regression line for all species. *r*—correlation coefficient. Asterisk denotes correlation coefficient significance for $P < 0.05$. Triangles designate *C. scabiosa*, circles designate *E. virgata*, squares designate *H. arenarium*.

mainly in reducing cell size [27]. In our studies, only one species *C. scabiosa* observed a significant, twofold decrease in the volume of mesophyll cells with increasing climate aridity (Fig. 4a). Two others changed cell concentration per unit leaf area. There is a certain balance between the size and the number of cells in the leaf [6, 29, 30]. Cell volume and cell number in the mesophyll linked inversely, which determines the complex mechanism of LMA and leaf thickness formation. The increase in cell volume leads to an increase in leaf thickness, which positively influences LMA rise [29]. On the other hand, increasing the cell size usually accompanied by a decrease in their number per unit leaf area, thereby reducing LMA.

A certain size ratio and the number of cells also affect the formation of integral mesophyll parameter: the total cell surface area per leaf area unit (A_{mes}/A) [10]. In our study, A_{mes}/A had dependence on the climatic growth conditions not common to all species. Moreover, for all species studied, A_{mes}/A was stable in the majority of areas and significantly changed only in one region (Fig. 2d). In this case, each species had its own cellular mechanism of A_{mes}/A changes based on the changes in the size and number of cells. For *C. scabiosa*, high values of A_{mes}/A were in an area with minimal aridity, in the northern point of the Urals (1) (Fig. 2d), defined by the maximum cell volume (Fig. 4a). For *E. virgata*, in the southern point of the Urals, A_{mes}/A reduced due to the reduction in cell number, while maintaining their size. For *H. arenarium*, in the southern point of the Volga, significant decrease of A_{mes}/A was the result of reducing cell number per unit leaf area.

Despite the different mechanisms of mesophyll restructuring depending on environment conditions, general rules of changing the ratio of the integral parameters in mesophyll were identified. The decline of A_{mes}/A was accompanied by an increase in the proportion of intercellular air spaces (Fig. 6a). This relationship between the two parameters was saved regardless of species and habitat geography, allowing us to make the assumption of the universality of the mesophyll change mechanism along ecological and geographical gradients. Increase of intercellular air

spaces leads to an increase in the free cell surface not occupied by cell–cell contact [28], which increases CO_2 transport velocity across cell surface. We assume that, for a particular species, the ratio of parameters at different structural levels of leaf organization was important. If A_{mes}/A reduced without leaf thickness changes, as in *C. scabiosa* or under its increase as for *E. virgata*, the proportion of free cell surface increased and improved conditions for CO_2 transport across the surface of mesophyll. Thus, for these species, when changing the environment conditions, A_{mes}/A decrease could be compensated by an increase in CO_2 transport velocity per unit leaf area. If A_{mes}/A decline occurred with a decrease in leaf thickness (*H. arenarium* in the southern point of the Volga), the proportion of free surface did not change, and the conditions for diffusion were worse. In this case, in order to maintain growth processes and productivity, decline in photosynthetic capacity per unit area could be compensated by the increase in leaf area. For *H. arenarium* in the southern point of the Volga, leaf area truly increased almost two times, from 1.6 to 2.6 cm^2, while leaf area reduced in the other two species in the more southern point of the Volga: from 86 to 24 cm^2 for *C. scabiosa* and from 4.3 to 3.1 cm^2 for *E. virgata*.

Another mechanism for the regulation of the photosynthetic capacity of the leaf is the restructuring of plastid apparatus associated with the change in the number of chloroplasts and chlorophyll content. In the investigated species, we showed reduced content of chlorophyll from north to south along the geographic transects of the Urals and the Volga regions and, in general, depending on the latitude (Fig. 5a). Reduction of chlorophyll content of the plants at low latitudes may be due to the protection of the photosynthetic apparatus against high insolation and overheating [23, 25]. In addition, for three species, we observed common features of the change in the balance of pigments, namely the reduction ratio Chl *a/b* and increased Chl/Car ratio in a southerly direction along the latitudinal gradient. These patterns correlate well with the data obtained for five steppe communities located along a latitudinal gradient in the Southern Urals [25]. Changing ratio of pigment forms was associated with rearrangement of the light-harvesting complex inside chloroplasts [25], while changing Chl content per unit leaf area usually occurred due to quantitative changes of cells and tissues [16]. Interspecies comparison showed that total Chl content was directly proportional to the volume of chlorenchyma fraction in a leaf [4, 23]. Positive correlation between these parameters was also found in our study (Fig. 6b). Toward southern latitudes, the decrease in Chl content of per unit leaf area has been associated with a reduction in the Chl content of a single chloroplast, but it did not depend on the number of chloroplasts in a leaf (Figs. 6c, 6d). In general, Chl content in chloroplast is an important factor that differs in different species according to their environmental features [16]. For instance, for herbaceous plants in broadleaf forests, this parameter was 4–10 mg per 10^9 chloroplasts, while the value for steppe plants varied in the range of 0.4–3.0 mg per 10^9 chloroplasts [7, 16]. According to Mokronosov [16], some desert xerophytes contained only 0.2–0.3 mg Chl per 10^9 chloroplasts. Thus, the reduction of Chl content in the chloroplast may be associated with the adaptation of steppe plants to increased insolation and water deficit.

Thus, the analysis of quantitative parameters of photosynthetic tissues of three species of steppe plants showed that intraspecific variation under the influence of climatic conditions was 20–40%. This range of variation is much lower than interspecific differences of plants of different ecological groups and the differences between the mean values of communities formed in different climates. We obtained similarly changes of LD and pigment content per unit leaf area in all species under climate change. LD increased with an increase in mean annual air temperature and climate aridity. This was due to an increase in the proportion of leaf nonphotosynthetic tissues and packing density of mesophyll. With decreasing habitat latitude and, thus, increasing climate aridity, a decrease in pigment content per unit leaf area for all species was mainly due to a decline in their content in chloroplast. In addition, we identified a total for three species pattern of changes in the ratio of integral parameters of mesophyll: A_{mes}/A decline was accompanied by an increase in the proportion of intercellular air spaces. At the same time, we found different mechanisms of structural adjustment of mesophyll. For *C. scabiosa* with relatively large cells, A_{mes}/A variation was due to changes in mesophyll cell size. For small-cell species *E. virgata* and *H. arenarium*, A_{mes}/A and A_{Chl}/A variation was due to a change in the number of cells and chloroplasts while maintaining their size. Thus, the result of leaf mesophyll restructuring was the formation of certain values of integral parameters required to optimize gas exchange of a plant in specific climatic conditions.

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