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Seasonal Changes in Polar Lipids in Fronds of the Ferns *Dryopteris filix-mas* and *Matteuccia struthiopteris*

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Abstract—The distribution pattern of total lipids, glyco- and phospholipids, and one betaine lipid (DGTS) in the fronds of the ferns *Dryopteris filix-mas* and *Matteuccia struthiopteris* was studied. The lipid composition of the embryo leaflets forming a bud, or "treble clef", and that of fully opened leaves changed throughout the growth season. The maximum amount of DGTS in clefs and mature leaves was detected at the beginning of the season. By midsummer, the DGTS content decreased, dropping to zero in the fully opened leaves, and then increased again. The amount of DGTS in the clefs collected in October versus those collected in May was somewhat higher in the case of *Dryopteris filix-mas* and almost twofold lower in the case of *Matteuccia struthiopteris*. The ratio between polar lipids contained in the clefs and mature leaves throughout the growth season was determined.

Key words: betaine lipid DGTS, glycolipids, total lipids, ferns, phospholipids

It is known that polar lipids include glyco- and phospholipids. In addition, this group of lipids also includes betaine lipids, which, like glyco- and phospholipids, are glycerol derivatives, but in contrast to them do not contain a phosphate or hydrocarbon fragment [1, 2]. Betaine lipids have been found in many organisms. To date, three types of betaine lipids are known: 1,2-diacylglyceryl-3-O-2′-hydroxymethyl-(N,N,N-trimethyl)-β-alanine (DGTA), 1,2-diacylglyceryl-3-O-carboxy(hydroxymethyl) choline (DGCC), and 1,2-diacylglyceryl-3-O-4′-(N,N,N-trimethyl)-homoserine (DGTS) [3]. Like phosphatidylcholine, all of these contain the positively charged trimethylammonium group. DGTS is the most frequently occurring betaine lipid. It has been found in some Protozoa species [4, 5], some fungi [3, 6-8], in algae of the orders Heterocantophyta and Cryptophyta, and in many green algae (including Charophyta, the closest relatives of the higher plants) [9-16]. In the higher, or germinal, plants (Embryophyta), DGTS was not found in the seminal or phanerogamic plants, but was detected in almost all cryptogamic plants analyzed (Bryophyta and Pteridophyta) [6, 16-19]. With regard for the fact that DGTS was found in all genera of the cryptogamic plants analyzed except for *Psilotum triquetrum* [20], it can be assumed that the presence of this lipid is a characteristic feature of this group of higher plants.

When studying the DGTS content in the fronds of different fern species, we showed that the amount of this lipid varies in the same species depending on the season, sometimes dropping to zero [21]. Because the mesomorphic state of the biological membranes required for their normal functioning is ensured by the specific composition of lipids comprising the membrane, a more thorough study of the phenomenon discovered may be useful both for understanding the role of DGTS in the membrane structure and for revealing the factors that determine the DGTS-synthesizing activity of the plant. For this reason, we studied the lipid composition of the fronds of two fern species throughout one growth season.

MATERIALS AND METHODS

Plant material. The study was performed on two members of the class Polypodiopsida: *Dryopteris filix-mas* (L.) Schott (the male fern) belonging to the family

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Abbreviations: DGDG) digalactosyldiacylglycerol; MGDG) monogalactosyldiacylglycerol; DGTS) 1,2-diacylglyceryl-3-O-4′-(N,N,N-trimethyl)-homoserine; DPG) diphosphatidylglycerol; SQDG) sulfoquinovosyldiacylglycerol; PG) phosphatidylglycerol; PA) phosphatidic acid; PC) phosphatidylcholine; PE) phosphatidylethanolamine; TLC) thin-layer chromatography.
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Aspidiaceae and *Matteuccia struthiopteris* (L.) Todaro (the ostrich fern) belonging to the family Onocleaceae. The specimens were taken in the same plot (~1 km^2) located in the flat-bottom valley of the Zhiguli Heights in the southeast of the European part of Russia in the Samara Region. The fronds at different developmental stages—from the clef-like folded primordial leaflets forming the bud (hereafter, the clefs) to the completely unfolded fronds (hereafter, the fronds)—were collected from May to September with intervals from four to six weeks. In the plot used, *M. struthiopteris* did not form the sporophylls. In view of this, the term “fronds” in the case of this species implies only the sterile fronds, or trophophylls. The fronds of *D. filix-mas* were not segregated into the trophophylls and sporophylls.

Lipid extraction and assay. The samples of plants weighing 1-2 g were homogenized and extracted with the mixture of chloroform and methanol as described in [22]. Polar lipids were separated by high-performance two-dimensional thin layer chromatography (TLC) on glass plates (6 × 6 cm) with a fixed silica gel layer in the solvent systems chloroform–methanol–benzene–ammonia (130 : 60 : 20 : 12) for the first direction and chloroform–methanol–benzene–acetone–acetic acid (140 : 60 : 20 : 10 : 8) for the second direction.

The lipids were developed by spraying the chromatographic plate with 10% H_2SO_4 in methanol and subsequent charring at 180°C for 15 min. The lipids were then detected and identified using molybdenum blue [23] and malachite green [24] for phospholipids, Dragendorf’s reagent for the choline-containing lipids [25], 0.2% ninhydrin in acetone for the amino-containing lipids [25], and anthrone reagent for glycolipids [26].

The lipid content in the extracts was determined by weighing extract aliquots that were preliminarily dried under vacuum conditions to constant weight. Quantitative determination of glycolipids was performed by measuring galactose content with the anthrone reagent [25]; phospholipids, by the method of Vaskovsky [22]. The DGTS content (1-10 μg) was measured spectrophotometrically after charring in H_2SO_4. The calibration curve was plotted using known quantities of preliminarily isolated and purified DGTS.

RESULTS AND DISCUSSION

Throughout the growth season, we determined the content of total lipids and DGTS, the ratio of glyco- and phospholipids to DGTS, and the ratio between individual lipids in each class. The first samples of leaves were collected at the end of May, when the plants had already formed the fronds. The content of total lipids in the leaves of *M. struthiopteris* varied from 11.1 to 24 mg per 1 g wet weight (table). This value varied from 17.3 to 19.3 mg per 1 g wet weight in the period from May to July, increased to 24 mg in August, and dropped to 11.1 mg in September. In the clefs of this species, the content of total lipids varied from 6.5 to 14 mg per 1 g wet weight. This value did not vary significantly in the period from May to July (6.5-8.1 mg per 1 g wet weight), whereas in September it increased twofold and decreased again in October to 10.4 mg per 1 g wet weight. Similar changes in total lipids were observed in the fronds and clefs of *D. filix-mas*. Thus, the content of total lipids measured at the same intervals is always lower in the clefs compared to the fronds.

The chromatogram shown in Fig. 1 clearly demonstrates that DGTS is present in the fronds of *M. struthiopteris* at the initial developmental stages, later disappears, and then occurs again at later stages. As it is seen from the table, the DGTS content is always greater in the

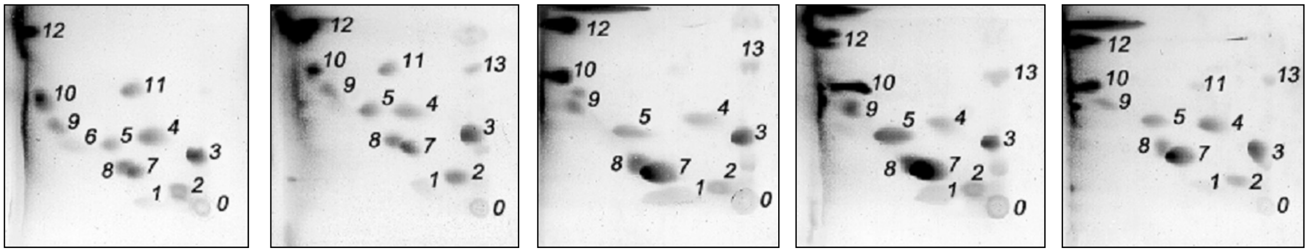
Content of total lipids (mg per 1 g wet weight) and DGTS (μg per mg lipids) in the fronds and clefs of the ferns *D. filix-mas* and *M. struthiopteris*

Time of sampling	*D. filix-mas*				*M. struthiopteris*			
	total lipids		DGTS		total lipids		DGTS	
	clefs	fronds	clefs	fronds	clefs	fronds	clefs	fronds
May	14.3 ± 3.6	14.7 ± 2.8	26.6 ± 0	28.9 ± 6.5	6.5 ± 0.5	19.3 ± 2.5	31.9 ± 4.0	41.0 ± 5.7
June	12.5 ± 1.0	27.0 ± 1.0	41.9 ± 0.8	20.7 ± 7.3	7.2 ± 1.0	18.4 ± 3.1	55.3 ± 12.0	40.4 ± 1.8
July	9.0 ± 0.5	14.0 ± 4.0	16.1 ± 2.3	—	7.0 ± 1.5	17.3 ± 2.4	43.0 ± 3.0	—
August	18.2 ± 3.0	20.8 ± 1.5	6.8 ± 2.0	traces	8.1 ± 2.4	24.0 ± 3.0	25.0 ± 1.0	—
September	8.0 ± 0.8	15.9 ± 4.0	26.4 ± 3.2	8.2 ± 0.3	14.0 ± 1.0	11.1 ± 2.7	11.4 ± 1.8	17.7 ± 3.2
October	12.4 ± 0.1	15.3 ± 1.6	30.0 ± 0.3	24.2 ± 5.6	10.4 ± 0.9	—	16.5 ± 3.8	—

clefs than in the unfolded fronds. Note that the amount of total lipids increases in the period from May to August by approximately 40-50%, the amount of DGTS in this period decreases, reaching minimum (zero) in the fronds and clefs in the middle and at the end of summer, respectively. We did not determine the DGTS content in the resting plants in the period from autumn to spring; however, the dynamics of DGTS content suggests that, after reaching the specific minimum in autumn, it apparently remains at the same level until spring. Because in late autumn the trophophylls of *M. struthiopteris* fade, the lipids contained in them are apparently degraded. Although the sporophylls are retained until the spring, the tissue of these fronds also fades (and, therefore, the lipids contained in the sporophylls are also degraded). However, the numerous spores contained in the sporangia remain alive. The DGTS content in the faded sporophylls is not known. Unlike the grown plants, the fronds of young *D. filix-mas* plants may winter green and remain alive until spring [27]. In view of this, it cannot be ruled out that, similar to the clefs of resting grown plants, the DGTS content in these plants reaches a specific level in autumn and remains practically unchanged until spring.

Note the reciprocal dependence between the daily mean temperature and the DGTS content: the latter drops as the former increases (Fig. 2). Because it is known that the intensity and direction of metabolic processes depend on the temperature, it is unlikely that the abovementioned phenomenon is due to a direct effect of temperature on lipid metabolism. It was also shown that, under the conditions of phosphorus deficiency in the environment, the membranes of microorganisms contain phosphorus-free lipids (DGTS, in particular) [4, 5]. Possibly, the same phenomenon occurs in the ferns that at certain times experience phosphorus starvation. Due to the competition for phosphorus between the plants, the phosphorus reserves in the soil are always limited [28]. However, in summer, as the soil warms up, the activity of heterotrophic soil organisms rises. They decompose the faded organic matter and return the phosphorus to the soil in the form accessible for the higher plants. As a result, at this period the membranes can be comprised only of phospholipids. As the reserve of accessible phosphorus in the soil decreases as a result of its utilization by the higher plants and as the activity of the heterotrophic microorganisms degrading the organic matter drops due to the decrease in the temperature of the soil, the lipid exchanges in the fern fronds changes so that they start to synthesize phosphorus-free lipids (DGTS, in particular) along with phospholipids. The assumption that the DGTS synthesis is related to the amount of phosphorus available is corroborated by the fact that we did not find DGTS in *P. triquetrum* [20]. With regard for the fact that *P. triquetrum* samples were collected at the same time and place that the samples of three *Selaginella* species, whose membranes contain DGTS [20], it seems likely that the absence of DGTS in *P. triquetrum* is related to the fact that this plant does not experience phosphorus deficiency due to symbiosis with the fungi [29]. In this study, we sought to confirm the aforementioned hypothesis by analyzing the lipid composition of the microtrophic ferns.

Figure 3 shows the dynamics of relative content of total phospho- and glycolipids throughout the season. The results obtained show that the relative content of total glycolipids in the fronds of both fern species is greater than that of total phospholipids. In the fronds of *M. struthiopteris*, the glycolipid-to-phospholipid ratio gradually increases from 1.2 to 2.8. The relative content of total phospholipids in the period from May to August changes from 34 to 38%, with the glycolipid-to-phospholipid ratio increasing due to an increase in the glycolipid content. In this period, the DGTS content drops to zero. In the fronds of *D. filix-mas*, the glycolipid-to-phospholipid ratio also increases from 1.6 to 3.0 in the same peri-



I *II* *III* *IV* *V*

Fig. 1. Two-dimensional TLC of *Matteuccia struthiopteris*. The solvent system contained chloroform–methanol–benzene–ammonia (130 : 60 : 20 : 12) for the first direction and chloroform–methanol–benzene–acetone–acetic acid (140 : 60 : 20 : 10 : 8) for the second direction. The lipids were developed by spraying the plates with 10% H_2SO_4 in methanol and subsequent charring at 180°C for 15 min. Denotations: *0*) origin; *1*) phosphatidic acid; *2*) phosphatidylinositol; *3*) phosphatidylcholine; *4*) phosphatidylethanolamine; *5*) phosphatidylglycerol; *6*) diphosphatidylglycerol; *7*) sulfoquinovosyldiacylglycerol; *8*) digalactosyldiacylglycerol; *9*) unidentified lipid; *10*) monogalactosyldiacylglycerol; *11*) diacylglyceryl-3-O-4′-(N,N,N-trimethyl)homoserine; *12*) neutral lipids; *13*) unidentified lipid. Collection time: I) May; II) June; III) July; IV) August; V) September.

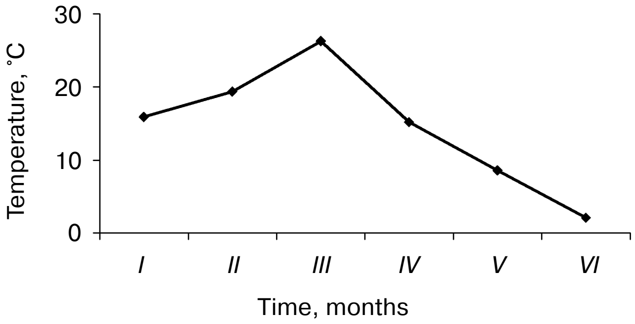


Fig. 2. Changes in the mean daily temperature according to the Bakhilova Polyana meteorological office (Zhiguli reserve). Collection time: I) May; II) June; III) July; IV) August; V) September.

od as that in the fronds of *M. struthiopteris*. As mentioned above, the amount of glycolipids in the unfolded fronds of both fern species throughout the growth period is greater than the amount of phospholipids. Conversely, the amount of glycolipids in the clefs is always lower than the amount of phospholipids. As the total amount of glycolipids changes, the proportion of individual glycolipids also changes. In all samples, we identified monogalactosyldiacylglycerol (MGDG), digalactosyldiacylglycerol (DGDG), and the sulfolipid sulfoquinovosyldiacylglycerol (SQDG), with MGDG being always predominant (although its content varies throughout the season) (Fig. 4). For example, in the fronds the MGDG content gradually decreases, whereas the DGDG content increases. The same tendency is retained in the clefs, although the glycolipid content in them is much lower. It is known that the changes in the proportion of individual glycolipids in plants are caused by numerous factors, including light and temperature [30-33]. The extent to which the proportion of individual glycolipids in these studies depends on these and other factors remains to be clarified.

Phospholipids in the ferns studied are represented by phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidyl- and diphosphatidylglycerol (PG and DPG, respectively), phosphatidylinositol (PI), and phosphatidic acid (PA). Figure 5 shows the seasonal dynamics of the relative content of the lipids listed (except for DPG). The analysis of the contribution of individual phospholipids to the total phospholipid content shows that their proportion also changes within a certain range throughout the growth period, with PC being predominant in all cases. Because the molecules of DGTS and PC contain a similar structural fragment, it was assumed that these lipids are interchangeable, i.e., a decrease in the DGTS content should cause an increase in the PC content [3, 12, 34]. Our results and the results of our previous studies do not confirm this assumption. We found that a decrease in the DGTS content in the fronds in the midsummer does not cause an increase in the PC content and

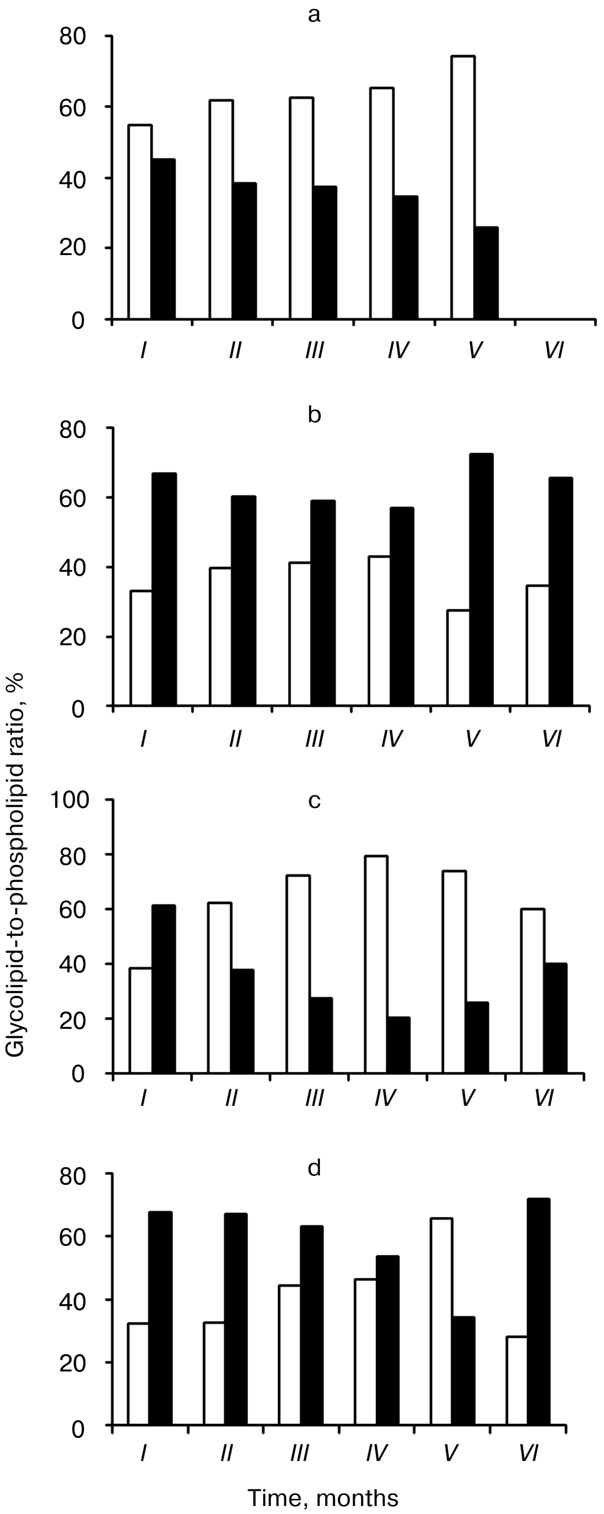


Fig. 3. Seasonal dynamics of the glycolipid-to-phospholipid ratio (shown with the open and closed squares, respectively) in percent of the sum of glycolipids and phospholipids: a, b) *M. struthiopteris* fronds and clefs; c, d) *D. filix-mas* fronds and clefs. When calculating the lipid content, we used the following molecular weights: MGDG, 774.4; DGDG, 936.5; SQDG, 837.5; PG, 768.4; PC, 748.1; PE, 744.1; PI, 856.6 daltons. Collection time: I) May; II) June; III) July; IV) August; V) September.

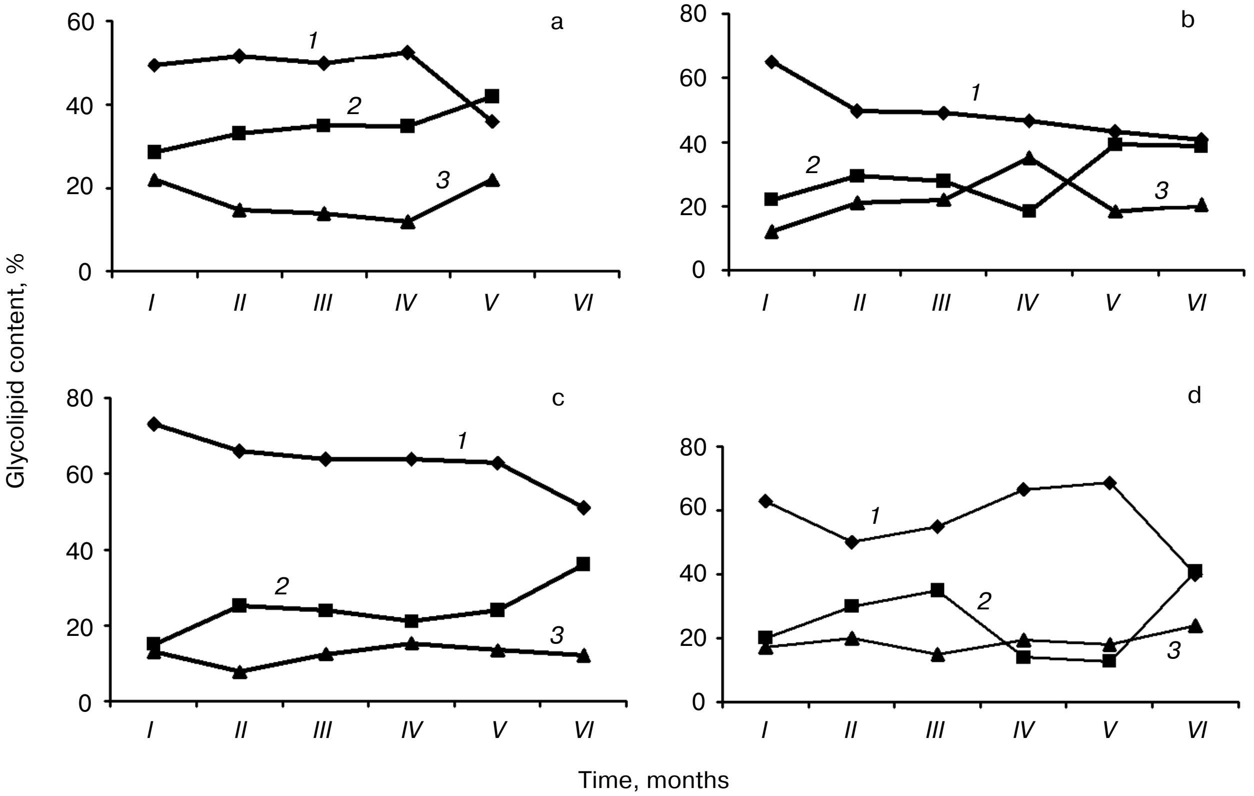


Fig. 4. Seasonal dynamics of relative glycolipid content in percent of the sum of glycolipids: *1*) MGDG; *2*) DGDG; *3*) SQDG. a) *M. struthiopteris* fronds; b) *M. struthiopteris* clefs; c) *D. filix-mas* fronds; d) *D. filix-mas* clefs. Collection time: I) May; II) June; III) July; IV) August; V) September; VI) October.

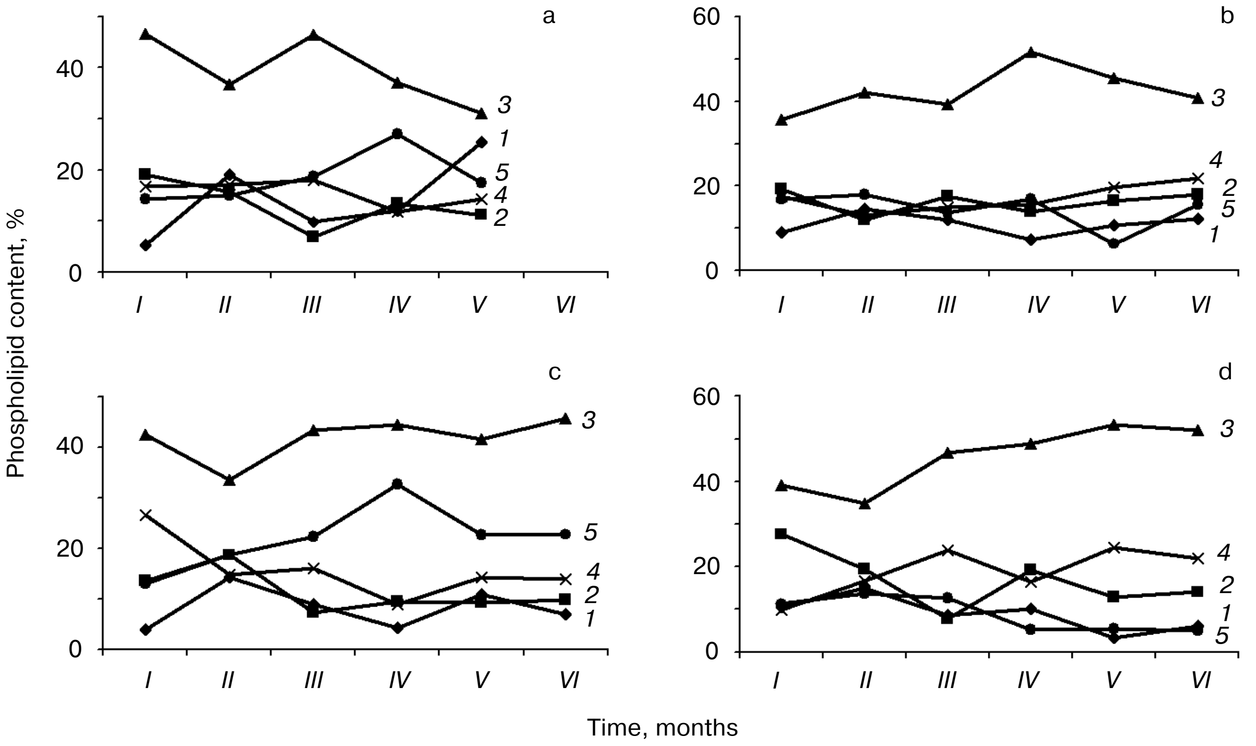


Fig. 5. Seasonal dynamics of the relative content of individual phospholipids in percent of the sum of phospholipids: *1*) phosphatidic acid; *2*) phosphatidylinositol; *3*) phosphatidylcholine; *4*) phosphatidylethanolamine; *5*) phosphatidylglycerol. a) *M. struthiopteris* fronds; b) *M. struthiopteris* clefs; c) *D. filix-mas* fronds; d) *D. filix-mas* clefs. Collection time: I) May; II) June; III) July; IV) August; V) September; VI) October.

vice versa, the maximal DGTS content in May does not correspond to a sharp decrease in the PC proportion. The PC content in the fronds of *M. struthiopteris* and *D. filix-mas* changes from 32 to 47% and from 35 to 47%, respectively; in the clefs, from 34 to 45% and from 35 to 50%, respectively. It is more likely that the decrease in the DGTS content correlates with the increase in the PG content. However, in the clefs the drop in the DGTS level is not accompanied by a sharp increase in the relative PG proportion in the midsummer. The dynamics of the PE and PI content is similar to that of PG: the relative proportion of these lipids changes in the fronds more pronouncedly than in the clefs. The analysis of the changes in each phospholipid shows that the content of phospholipids, similar to glycolipids, differentially changes throughout the growth period.

Thus, during the growth period, the clefs and unfolded fronds of the ferns studied are characterized not only by marked changes in the content of DGTS (which is predominantly located in the membranes of organelles other than plastids [12]), but also in the content of lipids contained in the chloroplasts. The question as to whether the changes in the DGTS content are the cause or the effect of the changes in the amount of lipids contained in the chloroplasts or the dynamics of the DGTS content and these lipids reflects unrelated responses of the plants to some environmental factor still remains open.

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