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Analysis of Space-Temporal Symmetry in the Early Embryogenesis of *Calla palustris* L., Araceae

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Abstract. Plants and animals have highly ordered structure both in time and in space, and one of the main questions of modern developmental biology is the transformation of genetic information into the regular structure of organism. Any multicellular plant begins its development from the universal unicellular state and acquire own species-specific structure in the course of cell divisions, cell growth and death, according to own developmental program. However the cellular mechanisms of plant development are still unknown. The aim of this work was to elaborate and verify the formalistic approach, which would allow to describe and analyze the large data of cellular architecture obtained from the real plants and to reveal the cellular mechanisms of their morphogenesis. Two multicellular embryos of *Calla palustris* L. (Araceae) was used as a model for the verification of our approach. The cellular architecture of the embryos was reconstructed from the stack of optical and serial sections in three dimensions and described as graphs of genealogy and space adjacency of cells. In result of the comparative analysis of these graphs, a set of regular cell types and highly conservative pattern of cell divisions during five cell generations were found. This mechanism of cellular development of the embryos could be considered as a developmental program, set of rules or grammars applied to the zygote. Also during the comparative analysis the finite plasticity in cell adjacency was described. The structural equivalence and the same morphogenetic potencies of some cells of the embryos were considered as the space-temporal symmetries. The symmetries were represented as a set of regular cell type permutations in the program of development of the embryo cellular architecture. Two groups of cell type permutations were revealed, each was composed of two elements and could be interpreted as the mirror and rotational space symmetries. The results obtained as well as the developed approach can be used in plant tissue modelling based on the real, large and complex structural data.

Key words: symmetry, embryogenesis, morphogenesis, Araceae, modelling
AMS subject classification: 92B05, 92C15, 92C80

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1. Introduction

Any multicellular plant or animal begins its development from the common unicellular state – the spore or zygote. Cell divisions, cell growth and cell death are the main formative processes which lead to the unique species-specific structure of the multicellular organism according to its inherited species-specific developmental program. The embryogenesis is a primary formative process where the universal developmental laws could be observed and the most basic developmental programs could be revealed.

Higher plants are the unique organisms because it is possible in most cases to trace back all divisions of their cells according to the shape of cell walls [4, 15, 24, 25]. First stages of embryogenesis – the segmentation and formation of blastomers, are critical for the elaboration of the main embryo axes of polarization and determination of most tissue initials [18, 22, 42]. Since classic investigations of Souèges [42-44] it was clearly shown that a majority of plants have their own type of embryogenesis defined by orientation of a new cell wall at the each cell generation. Diversity of the first three to five embryo divisions is finite and could be classified into "the classification of the embryogenesis types" by Souèges [44] or by Johansen [21]. However, the existing descriptions of the embryogenesis types hardly could be regarded as species-specific developmental programs, because they are shared among plant of unrelated taxons.

The cellular mechanisms of differentiation and participation of lineages of the blastomers in the embryogenesis and organogenesis remain poorly studied in modern developmental biology. A lot of cell- and species-specific genes are known to mark the cell identity in the early embryogenesis, like, for instance, *ZmWox*, *ZmOCL* in *Zea maiz* [29] or *PIN*-family and *WUS* in *Arabidopsis thaliana* [27, 30], but there is no reliable cytological criteria for the identification of cells in the multicellular embryo. The detailed cellular mechanisms of plant embryogenesis are still uncovered due to the extremely high complexity of the cellular architecture particularly in seed plants and due to the absence of suitable methods. In case of their revelation, plant development could appear as the consequence of pattern formations, as being iterative in space and time, composed of the universal elementary units – cells. However the patterns description has to be formalized due to their extreme complexity and large scales.

The aim of this work was to elaborate and verify an approach, which would allow: (1) to describe and represent unequivocally the space localization and genealogy of cells in the sample tissue without limitation in depth, (2) to compare and analyze a big amount of structural data and (3) to reveal the cellular mechanisms of morphogenesis. Here we present our developed approach of the analysis of space-temporal symmetries and results of experimental application of our approach in the reconstruction of cellular architecture of two embryos of *Calla palustris* L. (Araceae) and in the revelation of common mechanism of their development.

2. Biological background

2.1. Cellular organization of plant tissues

Plant body is composed of differentiated and non-differentiated or formative tissues. Last type of tissues is responsible for the potentially endless plant growth. Consequently, plant body is a result of the activity of formative tissues at every stage of plant ontogenesis. Actually, the formative tissues maintain plant species-specific morphology, tissue architecture and reproductive processes due to the proliferation and organizing influence of the initial or stem cells. The main biological function of any stem cell is a support of the plant body existence in the course of time owing to their cell lineages. Two main points of view exist about the term "plant stem cell" in literature. Narrow sense of this term is widely spread (for instance, [33]), when only cells of the central zones of shoot and root apices are regarded as stem cells. Another point of view implies the consideration of any initial cell contributing to the further morphogenesis as a stem cell or cell which possesses a stemness [5]. This position is closely related to the definition of Weismann's germ-cells [48]. Actually, during whole plant life-span from the zygote to the egg-cell and to the zygote again, the non-differentiated initial cells can change their quantity from only one (egg-cell or zygote) to hundreds (central zone of shoot apex) and histogenetic ability spectrum from whole plant body (zygote or initial cell of somatic embryo) to only one cell type (some epidermal initials, for example). According to this position, any plant morphogenetic process should be described as a dynamic system of initial cells with their lineages which possess the species-specific architecture and duration of activity. Consequently, the embryogenesis should be considered as the process of elaboration of tissue initials, most of which will be active throughout the postembryonic development of plant. Moreover, fist five to ten cell generations beginning from the zygote are well known to be responsible for the determination of shoot and root apices [42, 43].

2.2. Cell and cell lineage adjacency

One of the most important features of any initial cell is its space localization in the plant body. Stem cell localization is known to be a main factor of cell fate determination of its descendants [39]. For example, the apical meristems of shoot and root are composed of several cell lineages with descendants radiated by sectors [15, 25]. This domain structure of formative tissues coincides with the site-specific distribution of *PIN* family proteins of auxin symplastic transport facilitators selectively located on the cell membranes in the shoot apices [34] as well as in the root apices [6]. The established gradient of auxin in developing tissues serves as a factor of initiation of many different processes associated with cell differentiation and organogenesis like cell growth and divisions [35]. Obviously, morphogenetic processes appeared to be coordinated by cell-to-cell signaling realized via plasmodesmata between adjacent cells [36]. However, the main question is still unanswered: does the cell proliferation drive morphogenesis or some supracellular mechanisms drive the cell proliferation? [13].

Since the classical plant anatomic and morphological descriptions are based on the adjacency of elements: cells in tissue, tissues in organ, organs in shoot etc, the space localization of any

cell in plant body could be unequivocally described by its adjacency to other cells and to outer surface. In this work, the space arrangement of cells or "cellular architecture" is described in this way. The plant cells, in contrary to the animal cells, are immobile in general; their adjacency could be changed only in a predicted way after each division, that represents much information about cell origin, developmental processes and cell fate [24]. The adjacency of a cell to other cells is inherited after the division of its mother cell into two daughter cells which are always adjacent from the origin and share the adjacency of their mother cell. Thus, all the cells adjacent to each other are relatives, but in a different degree. This is a rule for most plant tissues, but the additional cell adjacency could derive from the intrusive growth of cells or in the case of postgenital tissue fusion, while the loss of adjacency could be caused by the cell separation processes [20, 37]. Finally, the adjacency of any cell without respect to the separation or fusion, is always transferred to all cell's descendants unchanged but distributed.

2.3. Description of the cellular architecture

Historically the first way of plant tissue description was a microscopic hand drawing widely spread in the era of classical anatomy and morphology. Using this approach, main laws of cellular architecture of tissues were proposed by Sachs, Hofmeister, Errera and Kelvin concerning the size of daughter cells, the orientation of cell division plate and three-dimensional shape of cells in tightly packed tissue (reviewed in [32]). Later, the precise reconstruction of three-dimensional shape of cells in the plant tissues was shown to be much complex than proposed. Consequently tt was required the formalized and exact description of each cell with indication of cell facet properties (quantity of facets, edges) represented in the tables [28, 41]. Finally, description of the cell genealogy with respect to the orientation of each division and the direction of tissue growth were found to be essential in the modeling and extrapolation of cell shape in the growing plant tissues [24]. Thus, the relation between cellular architecture and organ construction was revealed.

The cellular architecture of tissues described by means of three-dimensional shape, localization in plant body and genealogy of the cells is a tissue-specific and species-specific distinct feature of any plant. Different characters of this description were shown to be determinative for the morphology of different plants. For instance, the ratio of anticlinal and periclinal divisions in the segments of fern gametophyte is responsible for the development of their heart-like shape [7]. The orientation of divisions and shape of the apical cell determine the cellular and tissue architecture of fern root [3] and *Psilotum nudum* shoot as well [4]. These generative characters of the cellular architecture were effectively used in the plant pattern modeling being used as rules or shape grammars for the self-generation in the cellular automata approach [45] or in the widely spread L-systems approach for the simulation of plant development [4, 8]. Unfortunately, these approaches describe the cellular architecture of real plants in too simplified way and usually only in two dimensions.

3. Results

3.1. Basis of description and analysis of cellular architecture development

3.1.1. Space-temporal symmetries

Plants and animals have highly ordered structure not only in space but in time owing to repetitive way of their development expressed in the life cycle. Any organism should be considered as the ordered and possibly endless sequence of generations $\{O_1, O_2, O_3, \ldots, O_n\}$. All generations are connected by corresponding transformations from one into another, all of which together construct the sequence associated with generations $\{G_1, G_2, G_3, \ldots, G_n\}$:

$$\ldots \xrightarrow{G_n} O_1 \xrightarrow{G_1} O_2 \xrightarrow{G_2} O_3 \xrightarrow{G_3} \ldots \tag{3.1}$$

Classical morphological and anatomical descriptions of plant development show the sequence of changing plant structures in the course of time. Thus, description of each generation of organism (O_n) and its reiterative transformation (G_n) in the course of life cycle could be expressed as the finite sequence of developmental stages $O_n = \{S_1, S_2, S_3, \ldots, S_k\}$ associated with the sequence of morphogenetic processes $G_n = \{g_1, g_2, g_3, \ldots, g_k\}$. Thus, transformation of organisms G_n during the life cycle is the mapping of elements of the set $O_n = \{S_1, S_2, S_3, \ldots, S_k\}$ into the set $O_{n+1} = \{S_1, S_2, S_3, \ldots, S_k\}$. Let each life cycle of plant organism O consist of 5 developmental stages generated by 5 corresponding morphogenetic processes:

$$\ldots \xrightarrow{g_5} S_1 \xrightarrow{g_1} S_2 \xrightarrow{g_2} S_3 \xrightarrow{g_3} S_4 \xrightarrow{g_4} S_5 \xrightarrow{g_5} S_1 \xrightarrow{g_1} \ldots \tag{3.2}$$

Most plants possess a cellular architecture, so the successive developmental stages are the sequence of elaboration of species-specific cell architecture during the life cycle. According to our example, each of 5 stages is composed of a set of cells:

$$S_1 = \{IC\}; S_2 = \{A, B\}; S_3 = \{M, N, B\}; S_4 = \{M, N, P, Q\}; S_5 = \{U, V, N, P, Q\}. \tag{3.3}$$

Initial developmental stage (S_1) is composed of only one initial cell (IC). Morphogenetic processes consequently transform related developmental stages (3.2) owing to a set of cell divisions, like $A \rightarrow \{M, N\}$, and cell transformations, like $V \rightarrow IC$. The set of characteristic cell types for each stages (3.3) is a set of regular cell types of the organism O. Let decide that in our case, each morphogenetic process (g_n) consists of only one cell division or a cell transformation, which consequently transform one developmental stage into another:

$$g_1 : IC \rightarrow \{A, B\}; g_2 : A \rightarrow \{M, N\}; g_3 : B \rightarrow \{P, Q\}; g_4 : M \rightarrow \{U, V\}; g_5 : V \rightarrow IC. \tag{3.4}$$

Thus, the sequence of developmental stages could be expressed as the sequence of related genealogical trees, whom vertices correspond to the cells, edges to their relations (mother to daughter

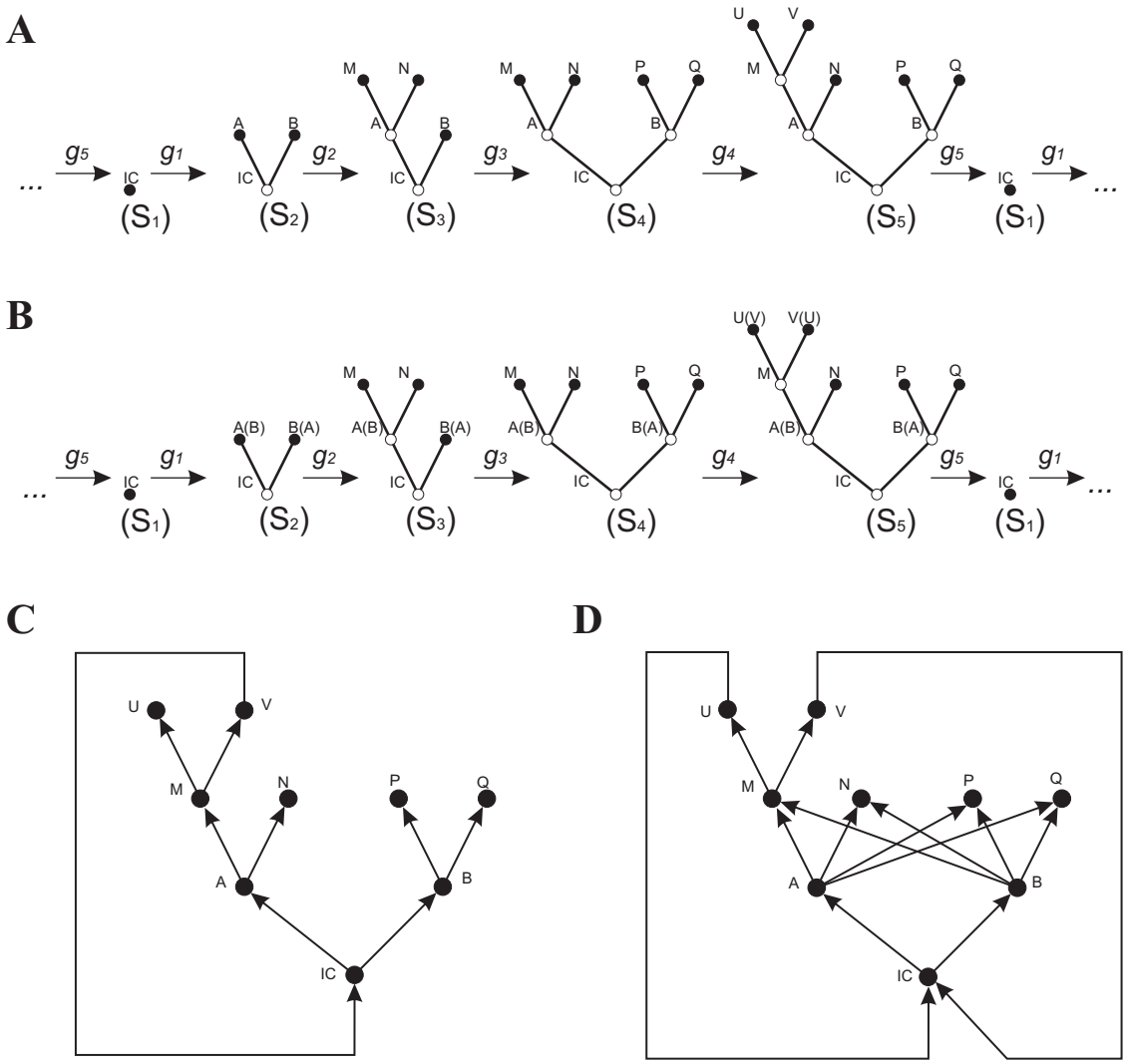


Figure 1: Space-temporal symmetries. A - representation of the developmental stages as genealogical trees of cells. B - representation of the same developmental stages complicated by equivalence of morphogenetic potencies of some cells (A and B, U and V). C, D - developmental programs of the sample organism during its life cycle in the absence (E) and in the case of presence (F) of the equivalence of some cells (A and B, U and V), shown as graphs.

cells). Pendant vertices (colored in black) correspond to a set of cells which characterize the developmental stage, while other vertices (white) represent their developmental history (Figure 1A).

Let in our example, anatomical and cytological characters of plant cells and tissues permit to distinguish, recognize and describe all the cells $\{IC, A, B, \ldots, U, V\}$ of an organism during each stage of development $\{S_1, S_2, \ldots, S_5\}$. Together with the individual cell genealogy and life time expressed in elementary morphogenetic processes $\{g_1, g_2, \ldots, g_5\}$, this set of data represents the full structural species-specific information about the organism, which could be used as a cliché in plant determination. In relation to this information the plants which belong to the same species

are to be indistinguishable. At the same time this information represents a species-specific developmental program (mechanism of development) of this organism (Figure 1C) shown as a directed graph with directed arcs that connect vertices corresponding to the regular cell types according to the possible morphogenetic processes. This program is implemented in time iteratively. Thus, at any moment of time only one of developmental stages of the organism $\{S_1, S_2, \ldots, S_5\}$ could be found after possibly endless iterations. If we find two plants of the same species but at different developmental stage (S_3, and S_5, for instance), we can find a correspondence between their cells according to their common developmental program, because this stages are directly connected by mapping $h : S_3 \to S_5, h = g_3 g_4$. This program describes the possible morphogenetic processes necessary to transform one developmental stage into any other. Thus, the correspondence between less developed stage (S_3) and more developed stage (S_5) shows the ontogenetic equivalence of their cells:

$$M = \{U, V\},\ N = N,\ B = \{P, Q\}, \tag{3.5}$$

because

$$h = g_3 g_4 : M \to \{U, V\},\ N \to N,\ B \to \{P, Q\}. \tag{3.6}$$

However, we could not find a correspondence for some cells (U, P, Q) of S_5 among the cells of S_3.

The inverse mapping $h^{-1} : S_5 \to S_3, h^{-1} = g_5 g_1 g_2$ is also possible, but the majority of cells of more developed stage S_5 does not participate in generation of the initial cell, so the single cell (V) of S_5 corresponds to all cells of S_3 derived from it:

$$V = \{M, N, B\}, \tag{3.7}$$

$$h^{-1} = g_5 g_1 g_2 : V \to IC,\ IC \to \{A, B\},\ A \to \{M, N\}. \tag{3.8}$$

Similar univocal correspondences between cells of two plants of the same species ("organism") being on any different developmental stages could be constructed, but last (3.7) is not useful in the analysis of the plant structure development, because it is connected with the structure simplification during formation of the initial cell (IC). However, exactly this mappings like (3.8) and cell correspondences like (3.7) are very important in the analysis of the reproductive biology of this plant or in case of more complex organisms than our example in other iterative formative processes like leaf formation, branching etc. Thus, if any two developmental stages of plant of the same species are found, only the single correspondence to their common developmental program could be found. In the terms of graph theory this correspondence is the isomorphism between graphs from the set $\{S_1, S_2, \ldots, S_5\}$ and graph of developmental program (Figure 1C) with strong respect to vertices labeling. Obviously, this (directed) equivalence of the cells in the course of time is an invariant or temporal symmetry of the plant with respect to its own developmental program spread and repeated in time. In other words, the temporal symmetry is the cyclic equivalence of biological structures caused by their directed "moving" in time. In the case of our sample organism the temporal symmetry could be described with a trivial group $H = \{e\}$.

In the real plants the similar developmental processes could be observed in different parts of plant body at the same time. As a result, structurally equivalent or indistinguishable parts could be found in the architecture of tissues, organs etc. This makes us to consider in plant not only the temporal but space symmetries as well.

Let consider a situation based on the previous example, where some cells of plant at the several developmental stages are indistinguishable cytologically and in their morphogenetic potencies. Let these pairs of indistinguishable cells will be A and B, U and V. If we find two plants of the same species, but on different developmental stages, we can construct not only the single (mutual) correspondence between S_3, and S_5, like (3.5) and (3.7) (Figure 1B), but a set of them due to the cell type permutations like $e, (AB), (UV), (AB)(UV)$:

$$e : M = \{U, V\},\ N = N,\ B = \{P, Q\},\ V = \{M, N, B\} \tag{3.9}$$

$$(AB) : M = \{U, V\},\ N = N,\ A = \{P, Q\},\ V = \{M, N, A\} \tag{3.10}$$

$$(UV) : M = \{U, V\},\ N = N, B = \{P, Q\},\ U = \{M, N, B\} \tag{3.11}$$

$$(AB)(UV) : M = \{U, V\},\ N = N,\ A = \{P, Q\},\ U = \{M, N, A\} \tag{3.12}$$

Plasticity in the possible development and consequently the variability of correspondence are caused by structural indistinguishability and similar morphogenetic potencies of these cells. Thus, the developmental program of our model organism (Figure 1D) is different from the previous example (Figure 1C). It is composed of the similar developmental stages and the same set of regular cell types (3.3), but possesses much more possible plasticity of morphogenetic processes. Manifold of developmental ways of the sample organism could be described as a set of isomorphisms between graphs of stages $\{S_1, S_2, \ldots, S_5\}$ and graph of developmental program from Figure 1D with strong respect to the labeling of vertices. This set of isomorphisms is determined by the structural and morphogenetic equivalence of cells (3.9)-(3.12) and should be considered as space symmetries, represented as a group H of cell types permutations, where permutations e, (AB) and (UV) are generatives. These symmetries could be observed not only in space but in time as well, when the variable correspondences between cells of two successive stages are constructed (like in 3.9 - 3.12). Thus, this allows us to consider the plant symmetries as space-temporal.

3.1.2. Representation of the source data

Cellular architecture of each *C. palustris* embryos was reconstructed in the virtual three-dimensional space (see Figure 2, and 5.2.) and represented in two graphs: cell adjacency graph and genealogical tree. The vertices in the cell adjacency graph were regarded to the cells and the edges to the cell space adjacency. Edges (directed) in the genealogical tree connect each mother cell to its daughter cells, while the vertices correspond to the cells observed (pendant vertices) and their precursors revealed after the tracing of cell lineages. The graphs of cell adjacency were represented as adjacency matrices, where columns and lines correspond to the cells and 0 or 1 correspond to

the absence or presence of adjacency between these cells. The genealogical trees were shown graphically with the vertices labeled, but without indication of the edge direction. The graphs of adjacency and genealogy of each embryo were merged by vertices according to the living cells. The space adjacency of the precursor cells to other living or to the precursor cells of other lineages was acquired according to their own living descendants, because descendants always only save or lose the precursor's adjacency. Thus, the cell genealogy tree being a part of this merged graph represents the temporal adjacency of mother cell to daughter cells. So, this graph could be termed as a space-temporal adjacency graph. In order to partly represent this graph, space adjacency matrix of each embryo was converted into the table with columns and lines supplemented with genealogy of main cell lineages (Figure 3B, D). According to such tables the space adjacency of any precursor cell can be estimated: the precursor cell had adjacency to some other cells, if at least one precursor's descendant still has adjacency to those cells.

The degree of cell proliferation in different lineages of the embryos was estimated. The vertices of genealogical trees of both embryos were weighted by subtree subdivision. The pendant vertices were considered to be with minimal weight, while vertices connected to the biggest subtrees were with the maximal weight, according to Akimov [1]. With the same result the pendant vertices could be weighted with maximal weight according to Harary [16].

There is an important difference between the space adjacency of cells and the adjacency of cell lineages. If the cell-to-cell space relation is symmetric, because two neighbor cells are always mutually adjacent, the lineage-to-lineage adjacency is not. Every cell could be the initial for own lineage, but not each the lineage consist of one cell. Each division of mother cell generates new cell lineages only for two daughter cells, but for preexisted cell lineages this division means just divisions of a cell inside of the mother cell lineage. So, two daughter cell lineages are mutually adjacent, while at least one of daughter cell lineages could be non-adjacent to aunt cell lineage, but the latter is always adjacent to whole lineage of their mother cell.

3.1.3. Comparative analysis and symmetry revelation

In the course of comparative analysis two main questions were verified: the degree of regularity of cellular architecture development between *C. palustris* embryos and the revelation of their common developmental program. To fulfill this analysis it was necessary to find the corresponding cells and merophytes (descendants of one cell) of less and more developed embryos. The zygote was considered as the first equivalent cell in genealogy of two embryos. All other corresponding cells and merophytes of embryos were found after the comparison of their genealogical trees and adjacency graphs by similarity of vertices. The genealogical tree of the minor embryo is required to be a subtree of the genealogical tree of major embryo with the coincidence of vertices of the zygote. Thus, cells of the minor embryo were considered as initials of cell lineages of more developed embryo and the regular cell types were revealed.

Combinations of the lineage correspondence between two embryos of *C. palustris* with maximum coincidence of adjacency were considered as a common developmental program of their cellular architecture. This program was represented as a table of cell lineage adjacency and as a directed graph with vertices corresponding to the regular cell types and arcs to the developmental

pathways. Two types of edges (arcs) were stated with respect to invariant or variable adjacency between cell lineages. Vertices of this graph were partly re-labeled where the correspondence between cell lineages of embryos was variable, to be different from those of embryos. The developmental program reconstructed by coincidence of the space adjacency and genealogy of both embryos could not exceed limits of the correspondence of embryos, i.e. the degree of development of less developed embryo.

The structural (by space adjacency and genealogy) and morphogenetic equivalence (by participation in producing of the regular cell types for next generation) of cells and corresponding vertices of the graph of common developmental program were investigated. The equivalent combinations of the correspondence of regular cell lineages between the embryos were studied to describe all possible developmental pathways in *Calla palustris* embryogenesis. Revealed combinations were considered as space-temporal symmetries and represented as groups of permutations of regular cell types. The elements of this group were represented as permutations of cells or corresponding graph vertices, which leave whole structure invariant. Each permutation was described in a cyclic form like **a** =(AB), or **a** =(A,B), that means A substitutes B, while B substitutes A.

3.2. Description and analysis of cellular architecture development in the early embryogenesis of *C. palustris*

3.2.1. Cellular architecture and genealogy of reconstructed embryos

Two embryos composed of 7 and 17 cells were reconstructed in three dimensions, their visualizations are presented in Figure 2D, E. Full cell genealogy and adjacency were revealed for both embryos and represented as genealogical trees, where living cells are labeled with numbers and their reconstructed precursors with letters (Figure 3A, C), and space adjacency matrix (Figure 3B, D). Both embryos had no internal cells and thus were fixed at the early stages of embryogenesis – the segmentation of blastomers according to Souèges [42]. Both embryos had clearly manifested apical-basal axis with the suspensor in the basal part and the sphere-shaped apical part (Figure 2D, E).

Differences in the degree of development between two embryos are mainly concerned to the cell lineages of their apical parts. The apical part of 7 celled embryo (Figure 3A) was segmented into the quadrants (cell labels O, 1, 28, 05), one of which was divided into the octants (cell labels 06 and 17), while his basal part was composed of the wedge-shaped cell and a single cell of the suspensor or basal cell (labels 4 and 6). The apical part of 17 celled embryo (Figure 3C) was segmented into the quadrants (labels P, Q, M, N) as well as into the octants (labels U, 28, V, 15, 83, 87, 75, 08). Some octants (U, V) had own descendants. The wedge-shaped cell was divided twice into 4 cells (labels 9, 39, 48, 36), and the basal cell (suspensor) was divided once forming the 2 celled suspensor (labels 17, 19). Interestingly, one of the octant's descendants (label 44nu, Figures 2E, 3C) of 17 celled embryo has no nuclear that corresponds to the final stage of the programmed cell death, according to Pennel and Lamb [31].

Degree of cell proliferation in the cell lineages of the embryos was estimated. The vertex with maximal weight was the same in both embryos – the initial cell of quadrants (weight 13

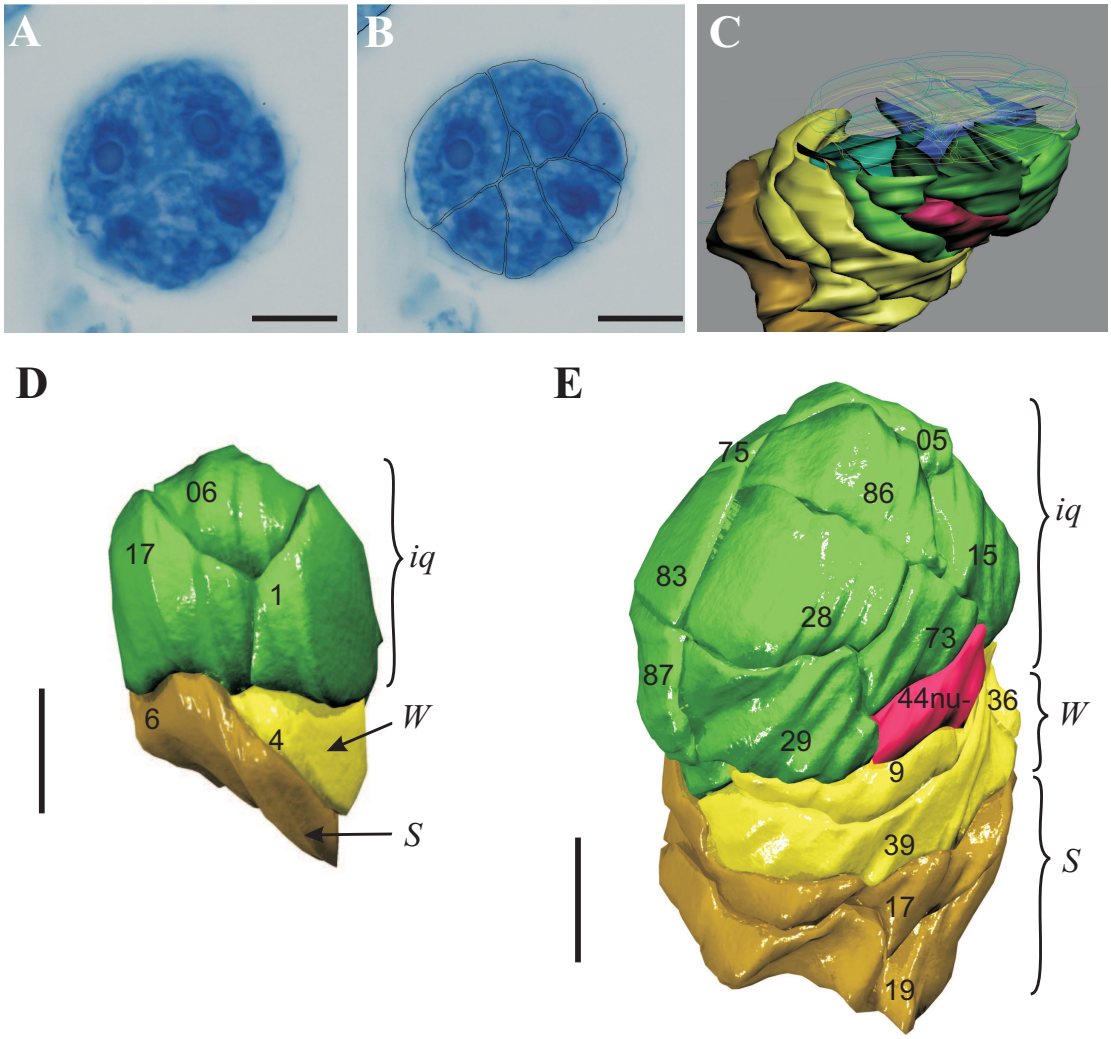
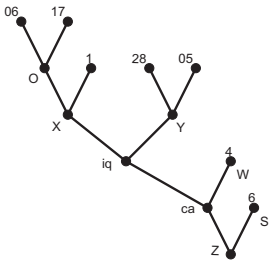


Figure 2: Three-dimensional reconstruction of two embryos of *C. palustris* upon the serial histologycal sections. A - image of one optical section from the stack of optical sections made from one histological section. B - the same image with the contoured cell boundaries. C - reconstruction of the cell surfaces according to the cell contours in 3-D graphic editor after integration of optical sections from succesive histological sections. D, E - reconstructed and visualized embryos consisted of 7 and 17 cells, respectively. *S* - suspensor, *W* - wedge-shaped cell, *iq* - initial of the quadrants. Each cell is labeled by the unique number. Numeration of the cells in different embryos is independent. Scale bar 10 mkm.

and 33 in Figure 4A and B, respectively). One lineage of the dyad (X) was much developed in the comparison to the other (Y) in both embryos (Figure 4). In both embryos the lineages of the wedge-shaped cell, being the sister cell to the initial of quadrants, and the suspensor were considerably less developed than their sister cell lineages (Figure 4).

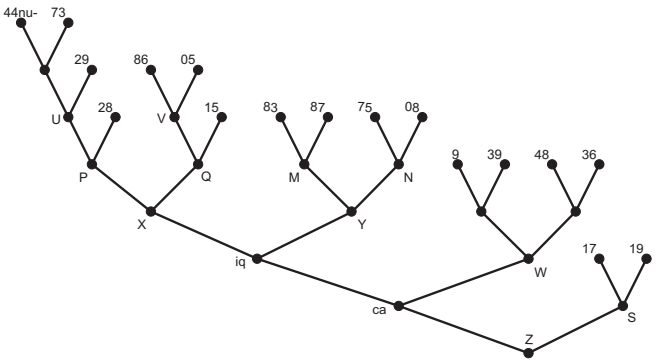
A



B

Genealogy of X, Y, W, S lineages			X			Y		W	S
			O						
		Cells	**06**	**17**	**1**	**28**	**05**	**4**	**6**
	O	**06**	0	1	1	1	1	0	0
		17	1	0	1	1	1	1	1
X		**1**	1	1	0	0	1	1	0
Y		**28**	1	1	0	0	1	1	1
		05	1	1	1	1	0	1	0
W		**4**	0	1	1	1	1	0	1
S		**6**	0	1	0	1	0	1	0

C



D

Genealogy of X, Y, W and S lineages				X							Y				W				S	
								Q			M		N							
					U			V												
			Cells	28	44nu-	73	29	86	05	15	83	87	75	08	9	39	48	36	17	19
			28	0	0	1	1	1	0	0	1	1	1	0	0	0	0	0	0	0
			44nu-	0	0	1	1	0	0	1	0	0	0	0	1	0	0	0	0	0
			73	1	1	0	1	1	0	1	0	1	0	1	1	0	1	1	0	0
	P	U	**29**	1	1	1	0	0	0	0	0	1	0	0	1	0	1	0	0	0
			86	1	0	1	0	0	1	1	1	0	1	1	0	0	0	0	0	0
		V	**05**	0	0	0	0	1	0	1	0	0	1	0	0	0	0	0	0	0
X	Q		**15**	0	1	1	0	1	1	0	0	0	0	1	0	0	0	1	0	0
			83	1	0	0	0	1	0	0	0	1	1	0	0	0	0	0	0	0
	M		**87**	1	0	1	1	0	0	0	1	0	1	1	1	1	1	0	1	0
			75		0	0	0	1	1	0	1	1	0	1	0	0	0	0	0	0
Y	N		**08**	0	0	1	0	1	0	1	0	1	1	0	0	0	1	1	0	0
			9	0	1	1	1	0	0	0	0	1	0	0	0	1	1	1	0	0
			39	0	0	0	0	0	0	0	0	1	0	0	1	0	1	1	1	0
			48	0	0	1	1	0	0	0	0	1	0	1	1	1	0	1	1	0
W			**36**	0	0	1	0	0	0	1	0	0	0	1	1	1	1	0	1	0
			17	0	0	0	0	0	0	0	0	1	0	0	0	1	1	1	0	1
S			**19**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0

Figure 3: Genealogical trees (A, C) and cell adjacency tables (B, D) of *C. palustris* embryos composed of 7 and 17 cells, respectively. Matrices of space adjacency (outlined by double lines in tables) supplemented with the genealogy of main cell lineages (X, Y, W and S). Differences in the adjacency between cells in B and D are indicated by the shading of table cells. Cells of previous generations: W - wedge-shaped cell, S - suspensor (S), U, V - octants of 17 celled embryo, P, Q, M, N - quadrants of 17 celled embryo, O - quadrant of 7 celled embryo, X, Y - cells of the dyad, iq - initial cell of quadrants, ca - apical cell, Z - zygote.

3.2.2. Mechanism of cellular architecture development of *C. palustris* embryo

The comparison of space-temporal adjacency graphs of 7 and 17 celled embryos has shown that the cellular architecture of both embryos is highly similar in the adjacency of each cell beginning from

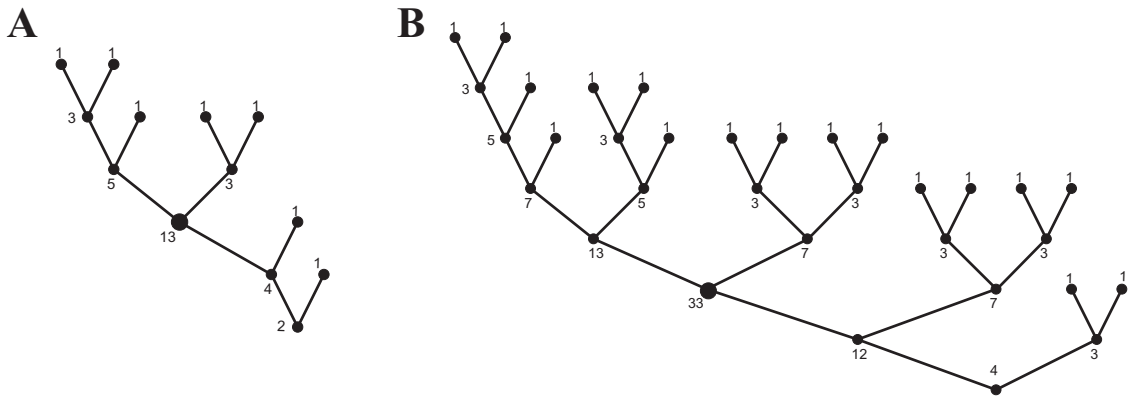


Figure 4: Genealogical trees (A, B) of *C. palustris* 7 and 17 celled embryos, respectively. All the vertices are weighted according to the order of subtree rooted at each vertex.

the zygote. The differences were revealed to be manifested in the additional adjacency between suspensor (cell label 6, Figure 3B) and two of quadrants (cell labels 17 and 28) and lack of the adjacency between quadrants originated from different dyad cells (labels 28 and 1) in 7 celled embryo, comparing to 17 celled embryo. All these differences are marked in the adjacency matrix of both 7 and 17 celled embryos (Figure 3B, D), but the variable correspondence of cell lineages of the embryos could make the difference much spread (see below).

The primary blastomers of both embryos were found to correspond unequivocally. The zygote of *C. palustris* was divided by transversal plate into the apical and basal cells (ca and cb, Figure 5A). In result of oblique division of the apical cell, two unequal cells have appeared, the smaller of them was wedge-shaped (W, Figure 5B), while the bigger cell became the initial cell of quadrants (iq, Figure 5B). The basal cell became a suspensor during further development of the embryo.

At the next stage of development the embryo of *C. palustris* is appeared to consist of 4 cells (Figure 5C). In 7 celled embryo the lineages of wedge-shaped cell and suspensor stay unicellular, so further cell divisions are basically concentrated in the lineage of the quadrant initial cell. These cells in both embryos have divided longitudinally to the apical-basal axis of embryo (Figure 5C), but the orientation of the division plate was a little different. In result, in 17 celled embryo one of the dyad cell has lost the adjacency to the suspensor (lineage X in Figure 3D). From the other hand it is difficult to find the exact correspondence between cell lineages of the dyad of both embryos (X or Y in Figure 3) and the regular cell types A and B. It is not clear what does the lineage exactly lose the adjacency. It is reflected in the tables of cell-to-cell and lineage-to-lineage adjacency (Figure 6B, C). However, the rest of space adjacency of all four cell lineages was identical in both embryos. This allow us to suppose that the orientation of division of initial cell of quadrants is variable in respect to surrounding lineages, but all the lineages are identical in their morphogenetic potential. Thus this division could be considered as a site of adjacency variability that indicated in the graph of developmental program (Figure 6A).

In result of longitudinal divisions of each cell of the dyad, the quadrants were produced, and *C. palustris* embryo has become 6 celled (Figure 5D). In each lineage of the dyad cells these di-

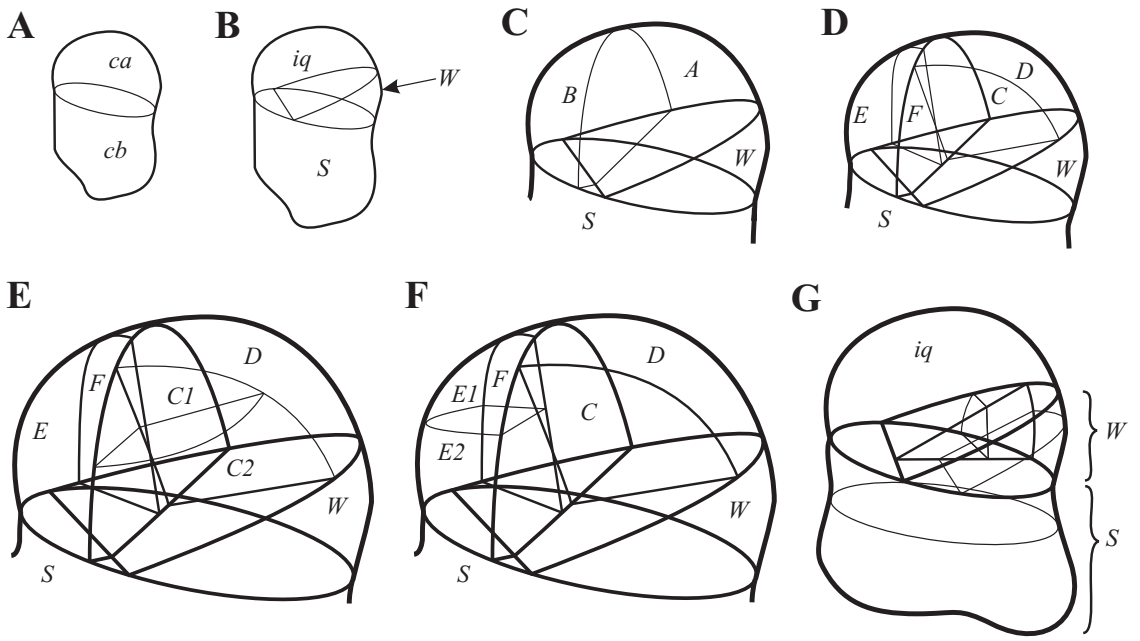


Figure 5: Schemes of regular cell divisions in the early embryogenesis of *C. palustris* revealed after the comparative analysis of cellular architecture of both embryos (A-E) and schemes of some further divisions in more developed embryo (F, G). A - transversal division of the zygote in to the apical cell (*ca*) and the basal cell (*cb*), last cell becomes a suspensor (*S*). B - oblique division of the apical cell into the wedge-shaped cell (*W*) and the initial of quadrants (*iq*). C - development of the dyad (*A*, *B*) from *iq*. D - development of quadrants (*C*, *D*, *E*. *F*). E - development of octants (*C1*, *C2*) in the lineage of the quadrant *C*. F - development of octants (*E1*, *E2*) in the lineage of the quadrant *E*. G - cell divisions in the lineages of the wedge-shaped cell and suspensor.

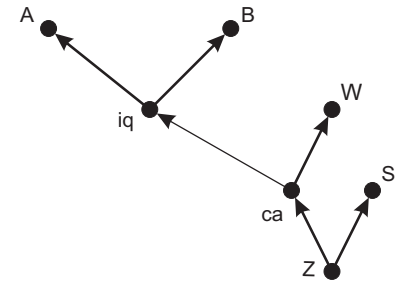
visions were similar. In the pair of relative quadrants (C and D, E and F) only one saved the adjacency of the mother cell (C and E), while the second cell have lost the adjacency to the suspensor (Figures 5D, 7B, C). As at the previous stage, the adjacency variability was found between cells and lineages of the quadrants C and E and the suspensor (Figure 7B, C). Also the variable adjacency was found between diagonally located quadrants C and F, D and E (Figure 7B). These pairs of the quadrants belong to the different (and adjacent) lineages of the dyad, so the variability in their adjacency was not influenced to the lineage adjacency (Figure 7C). Thus architecture of the apical of *C. palustris* embryo manifests the structural equivalence of the quadrants C and E, D and F. Each pair of them have originated from different cells of the dyad (A or B), that shows the equivalence of morphogenetic potency the dyad cells in addition to the structural equivalence mentioned earlier. Obviously, the equivalence of A and B cells should be considered as the space-temporal symmetry, which could be described as a group H_1 of cell type permutations:

$$H_1 = \{e, (AB)\}. \tag{3.13}$$

The equivalence of the dyad cells is represented in the graph of developmental program of *C. palustris* embryo (Figure 7A) and in the variable correspondence between regular types of cell lineages

(A and B) and lineages (X and Y) of embryos investigated (Figure 7D).

A



B

Cells	A	B	W	S
A	0	1	1	1/0
B	1	0	1	1/0
W	1	1	0	1
S	1/0	1/0	1	0

C

Cell lineages	A	B	W	S
A	0	1	1	1/0
B	1	0	1	1/0
W	1		0	1
S	1			0

Figure 6: Developmental program of main cell lineages of *C. palustris* embryo, analyzed at the (estimated) 4 celled stage. A - graph of genealogical relations between main regular cell lineages of the embryo. The less weight of the edge between vertices of apical cell (ca) and quadrants initial (iq) denotes the variable adjacency between descendants of the initial cell of quadrants (A or B) and the lineage of suspensor. B - table of space adjacency between the dyad (A, B), the wedge-shaped cell (W) and suspensor (S). C - table of space adjacency between cell lineages of 4 celled embryo. Z -label of the zygote.

At the next stage of development the embryo of *C. palustris* should consist of 7 cell in result of the division of one quadrant into two octants (Figure 5E). This stage is fully correspond to the architecture of the minor embryo investigated, so we tried to find a correspondence between these regular octants and each pairs of octants of the more developed embryo. Two octants of 7 celled embryo (06 and 17) derived from the quadrant, which was adjacent to the suspensor. Also this octants were adjacent to all other quadrants, but one of them (06) has lost the adjacency both to the suspensor and to the wedge-shaped cell (Figure 3B). Unfortunately there was no the possibility to find a correspondence for each the octant due to the limitation of development degree of 7 celled embryo. According to the cellular architecture of 17 celled embryo two ways of the correspondence to two regular octants were found. In first situation octants of regular type could derive from the quadrant C (C1, C2 in Figures 5E, 8B, C). In another possible situation they could derive from the quadrant E (E1, E2 in Figures 5F, 8E, D). Thus the equivalence of the morphogenetic potency of the regular quadrant types C and E was found. It should be considered as the space-temporal symmetry described as a group H_2 of cell type permutations:

$$H_2 = \{e, (CE)\}. \tag{3.14}$$

The equivalence of the quadrants C and E is represented in the graph of developmental program of *C. palustris* embryo (Figure 8A) and in the variable correspondence between the regular types of cell lineages (C, E) and lineages of the embryos investigated (Figure 8F). It is possible to propose that the quadrants D, F, and their octants are also equivalent in their morphogenetic potential, but to be sured at the moment the data is not enough.

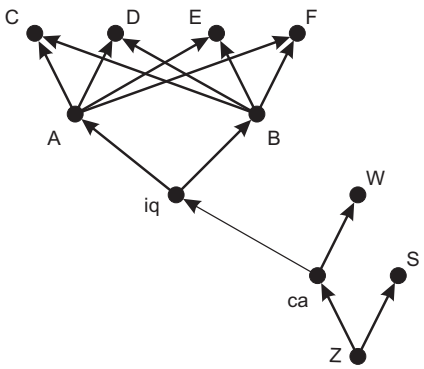
Further development of the cellular architecture was reconstructed according to more developed 17 celled embryo, and does not considered as common for both embryos. All quadrants of this embryo underwent divisions in different planes, so octants size was different and they did not manifest two tiers (Figure 5F). The wedge-shaped cell underwent longitudinal division and than two descendant cells was divided respectively longitudinally and transversally (Figure 5G). In result, four descendant cells of the wedge-shaped cell also do not manifest a common tier between each other as well as any other blastomers. The basal cell was divided transversely and developed into the 2 celled suspensor (Figure 5G).

4. Discussion

4.1. Formalization of plant structure and developmental processes

The genealogical tree used in our formalistic approach is appeared to be the historical and traditional form of representation of any genealogical relationships, and the additional information it possess, besides of the genealogy, was also always of interest. First of all, such a tree demonstrates the changing of structure in the course of time, if each cell generation is considered as a relative

A



B

Cells	C	D	E	F	W	S
C	0	1	1	1/0	1	1/0
D	1	0	1/0	1	1	0
E	1	1/0	0	1	1	1/0
F	1/0	1	1	0	1	0
W	1	1	1	1	0	1
S	1/0	0	1/0	0	1	0

C

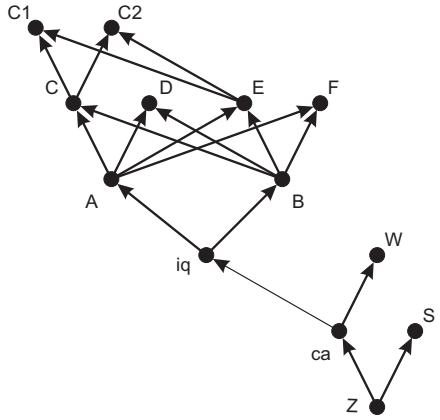
Cell lineages		A		B		W	S
		C	D	E	F		
A	C	0	1	1		1	1/0
	D	1	0	1		1	0
B	E	1		0	1	1	1/0
	F	1		1	0	1	0
W		1				0	1
S		1				1	0

D

Cell lineages	7 celled		17 celled	
	e	(AB)	e	(AB)
A	X	Y	X	Y
B	Y	X	Y	X
W	W	W	W	W
S	S	S	S	S

Figure 7: Developmental program of main cell lineages of *C. palustris* embryo, analyzed at the (estimated) 6 celled stage (stage of quadrants). A - graph of regular genealogical relations between main cell lineages of the embryo. B - table of space adjacency between the quadrants (C, D, E, F), the wedge-shaped cell (W) and the suspensor (S). C - table of space adjacency between cell lineages of 6 celled embryo. D - table of correspondence between lineages of embryos investigated and the regular cell lineage types. Permutations of group H_1 are indicated in the head of each column. iq - initial of the quadrants, ca - apical cell, Z - zygote.

A



B

Cells	C1	C2	D	E	F	W	S
C1	0	1	1	1	1/0	0	0
C2	1	0	1	1	1/0	1	1/0
D	1	1	0	0/1	1	1	0
E	1	1	0/1	0	1	1	1/0
F	1/0	1/0	1	1	0	1	0
W	0	1	1	1	1	0	1
S	0	1/0	0	1/0	0	1	0

C

Cell lineages			A			B			
			C						
			C1	**C2**	**D**	**E**	**F**	**W**	**S**
	C	**C1**	0	1	1	1		0	0
		C2	1	0	1	1		1	1/0
A		**D**	1		0	1		1	0
		E	1			0	1	1	1/0
B		**F**	1			1	0	1	0
		W	1					0	1
		S	1						0

D

Cells	C	D	E1	E2	F	W	S
C	0	1	1	1	1/0	1	1/0
D	1	0	1/0	1/0	1	1	0
E1	1	1/0	0	1	1	0	0
E2	1	1/0	1	0	1	1	1/0
F	1/0	1	1	1	0	1	0
W	1	1	0	1	1	0	1
S	1/0	0	0	1/0	0	1	0

E

Cell lineages			A		B				
					E				
			C	**D**	**E1**	**E2**	**F**	**W**	**S**
		C	0	1	1			1	1/0
A		**D**	1	0	1			1	0
	E	**E1**	1		0	1	1	0	0
		E2	1		1	0	1	1	1/0
B		**F**	1		1		0	1	0
		W	1					0	1
		S	1						0

F

Cell lineages			7 celled	17 celled	
			e	e	(CE)
	C	C1(E1)	06	28	83
		C2(E2)	17	U	87
A	D		1	Q	Q
	E(C)		28	M	P
B	F		05	N	N
W			4	W	W
S			6	S	S

Figure 8: Developmental program of main cell lineages of *C. palustris* embryo, analyzed at the (estimated) 7 celled stage. A - graph of regular genealogical relations between main cell lineages of the embryo. B - table of space adjacency between octants (C1 and C2) and other quadrants (D, E, F), the wedge-shaped cell (W) and the suspensor (S). C - table of space adjacency between regular cell lineages of 7 celled embryo. D - table of space adjacency between octants (E1 and E2) and other quadrants (C, D, F). F - table of correspondence between lineages of embryos investigated and the regular cell lineage types. Permutations of group H_2 are indicated in the head of each column. ca - apical cell, iq - initial of the quadrants, A, B - cells of the dyad, Z - zygote.

time counter. In plant and animals each cell division is a process of cell fate determination in result of variable distribution of genetic and epigenetic factors of heredity [2, 10, 24, 25] and the natural mechanism of polarity arrangement in the formative tissues [17, 33]. Secondly, the velocity of cell divisions in different lineages could be different, and, particularly in embryogenesis, has a species-specific significance according to "the law of (cell division) numbers" by Souèges [43].

However, another type of cellular genealogical trees, distinct in the construction principle from ours, was used in many works in order to estimate the temporal changes (for instance, [43]). Such

a tree is usually produced after the time-lapse successive fixation of genealogy in the tissue sample, so it represents a successive "temporal sections" marked by adding of corresponding vertices to the each branch of tree. In the moment of time fixation some cells of different lineages could divide, while some could stay undivided. Thus, in these trees some cells are represented by a set of vertices of degree 2, while all vertices in the common genealogical tree have degree 3. We think this approach is useful only for the temporal fixation of events, but not for the cellular architecture analysis, because the structure of such trees is mainly depends on the quantity of observations (time fixations).

Representation of the cellular architecture in the form of genealogical tree (Figure 3) has also some additional advantages. The genealogical tree unequivocally shows a set of transformations (divisions), which have happened with the zygote to become a multicellular embryo. Moreover, the genealogical tree of the concrete embryo demonstrates its own individual development from the zygote, which could be compared to the individual development of othe ther embryo in order to estimate the regularity of their development. This approach is completely different from known L-systems method (for instance, [4]), where the proposed transformations of cells during development are compared statistically to the morphogenetic processes found in nature.

Our study shew a higher stability of the cell lineage adjacency, comparing to the individual cell adjacency, and this was regarded as a general character of the lineage (and cell as well) space position in the plant body. Space position of the blastomers in the body of embryo was decisive in the determination of their subsequent proliferation and function according to the species-specific mechanisms, as was concluded by R. Souèges in his laws of disposition and destination [43], and indirectly supported in recent investigations of the patterning of embryonic gene expression in *Arabidopsis* and *Zea* [27, 29]. Obviously, the cellular architecture determined by space adjacency of generative cell lineages is a main factor of cell fate identity perceived as "positional information" by cells of the developing embryo or organ. Moreover, the auxin flow being a signal factor of plant organogenesis and regeneration is revealed to be regulated via cell- and tissue-specific changing of *PIN* (cellular efflux facilitator of auxin) polarity [40]. That is why it is not simple to realize the contraposition of lineage-based and position-based mechanisms of cell fate determination widely spread in the modern scientific literature (for example, [39]), where the tissue initials and auxin influence are actually implied and opposed, both being manifestation of the same mechanism on different levels of plant organization.

The patterns of relative velocity of cell divisions could be revealed after the special analysis of cell adjacency and genealogy, like we done (Figure 4). The synchronization of cell divisions in the adjacent cell lineages is one of the main marks of the symlasmically united domains of cells in the plant tissue [12]. Dynamic symplasmic domains are known to be the cytoplasmic continuity between cells in plant tissues organized via plasmodesmata, which are responsible for the cell-to-cell macromolecular trafficking, signaling, including essential morphogen auxin [12, 26, 40]. Obviously, only the adjacent cells could compose such domains, and patterns of their organization should coordinate the organogenetic activity [36] and possess the evolutionary specific features especially in the formative tissues [19].

4.2. Mechanism and uniqueness of *C. palustris* embryogenesis

In result of the comparative analysis of space-temporal adjacency graphs the corresponding cells of both embryos were recognized and common mechanism of development of embryo cellular architecture was revealed. It was represented as the graphs of developmental program and the tables of space adjacency of regular cell lineages (Figures 6-8). These tables served as a cliché for identification of corresponding cell lineages in both embryos. We have analyzed the cellular architecture of only two embryos, so the most important contribution of our article is not the structure of *C. palustris* embryo, but rather the principles of formalization and analysis of developmental processes at the cellular level.

Quite high complexity of their cell adjacency graphs demonstrate uniqueness and species-specificity of their individual cellular architecture. Each cell division in plant tissue inevitably leads to the loss of cell adjacency of daughter cells to cells of another lineages comparing to their mother cell. Loss of the adjacency is carried out in the more unique manner the more generations are taken into account. Obviously, the degree of uniqueness would increase, if the tissue samples composed of more cells are analyzed.

In the classical anatomy and embryology the general method of cell type identification was visual, according to the shape and position of cell observed in the histological sections. The degree of cell type definition was mainly limited by cell similarity in the embryonic tissues. In recent molecular genetic investigations the gene expression pattern was used for the cell type identification [27, 29]. These methods permit the cell type identification among histologically indistinguishable cells, but it was limited in application only to the model plants like *Arabidopsis* and *Zea*. The cell lineage tree of more than 40 cell generations could be reconstructed also by recent molecular genetic methods on the basis of genetic variability of somatic tissues [14]. However, this method is applicable to the species with the well known genome and each estimated cell lineage is required to consist of more than hundred cells. Our approach combines the advantages of all methods: any plant can be used and similar single cells or much celled lineages could be distinguished by their adjacency and genealogy estimated over many generations.

Adjacency of cell lineages in both embryos shew the high stability in comparison to the cell-to-cell adjacency, but only the lineage of the quadrant initial (Figures 3A, C, 6B, C) has demonstrated the variable adjacency to the suspensor. Adjacency in all the lineages of apical cell was invariant and regular for both embryos. Any difference in the cell adjacency after division could be obviously connected with different rotation of cell division axis. Thus, the changing of cell lineage adjacency is a mark of variable rotation of the cell division spindle in relation to other “immobile” lineages. So it could be concluded that Figure 8A directly describes the site of regular plasticity in the developmental history of *Calla palustris*. These our results support the investigations of cell lineage patterns in the shoot apices of maize [9] and other monocots [47], which were carried out using the clonal analysis approach. The clonal analysis allows to indirectly observe the cell lineage activity according to the distribution of variegated tissues derived from the apex inhabited with variegated initials. Analysis of the mericlinal chimeras and variegated sectors of these plants indicated, like in our study, that the high stability of shoot apex lineages was complicated by the limited reversions or transpositions in the patterns of variegation.

In this study only two embryos were investigated, that allows to consider much of our result accidental. However, among the cells of different generations only one cell lineage was indicated to have difference in relation to another lineages. The strong prevalence of invariance in the cellular architecture permits us to consider the variability in adjacency between these lineages not accidental, but regular. However for further work the homoplasy estimation is required.

According to the correspondence between cell lineages revealed in the comparative analysis of genealogy and adjacency of both embryos, two groups of regular cell type permutations were constructed and considered as the space-temporal symmetries. These symmetries are based on the equivalence of regular lineages of the dyad cells and their descendants (quadrants) and could be interpreted as the mirror and rotational symmetries in the sense of space or as a set of equivalent developmental ways in the sense of time. Equivalence of the quadrants is a necessary distinct feature of these blastomers (in contrary, they should be identified as the another cell type), which represent the native point of internal symmetry of hemispherical apical part of proembryo in the majority of plants according to the laws of origin and destination by Souèges [43]. Similar relations between groups of cells were revealed in the studies of cell lineages in nematodes and other animals, where the distinct pluripotent stem cell lineages were underwent to exchange their cell fate to substitute one another. According to the stem cell ability to exchange their fate, they were united into the groups of equivalence [46].

4.3. Modeling of plant morphogenesis

Our approach is oriented to the revelation of native grammars of morphogenesis based upon the cell space adjacency and genealogy. However it can be useful for the tissue development simulation as the source material, for model verification and as well as in the analysis of mutants with disturbances in the patterns of cell divisions peculiarly when they are obscure. Such an attempt was made by E. Coen and his colleagues [11], where four parameters of cell growth and division (growth rate, anisotropy, direction and rotation) were applied to simulate the development of species-specific leaf shape of *Antirrhinum* from the initially isotropic rectangular cells. Obviously, the final result must be in strong dependence on the isotropy/anisotropy of the initial tissues which is determined by their cell genealogy and adjacency. Indeed, our latter conclusion has been already proven in the model of plant tissue growth based not on the simplified rectangular cells, but on the naturally looked asymmetric and polarized cells [38].

Another comprehensive approach of the formative tissue development simulation was made by P. Barlow and his colleagues [4], where the maps-L systems applied to the apical cell of *Psilotum nudum* were analyzed. In this work the basic assumption meaning, that each the cell wall is subdivided into the zones labeled, was successfully verified. Thus, each new division was predicted by this labeling, which in turn determined the re-labeling of new cell wall after the next division. After some iteration of this mapping it was obvious, that the predicted labeling was determined by the adjacency to neighboring cells, like in our work. Actually, any scientific relations between biological and mathematical spheres should be fruitful, because the more carefully structure is studied the more irregularities become the hidden laws of development.

5. Materials and methods

5.1. Microscopy

Seeds of *Calla palustris* L. were collected in the field (Trubnikovo, Leningrad region, Russia), fixed in FAA (formalin 40%, alcohol 70% and acetic acid 98%, 7:100:7). After dehydration in ethanol series, acetone and chloroform, the samples were embedded in paraffin and sectioned in 5 mkm thick sections. After deparaffinization in xylene the sections were rehydrated and stained with alcyan blue and hemathoxylin by Erlich. Then sections were mounted in moviol 4-88 (Fluka, #81381) and observed in Axioplan 2ie (Carl Zeiss) microscope. The optical sections were made using 63x/1,4 PlanNeophluar objective via 600 nm along Z axis, according to the Z axis counter of the microscope. The sections were imaged using Nikon D70 (Nikon) digital camera and by means of Nikon capture 1.4.2. (Nikon) software were saved as .tiff files (Figure 2A).

5.2. Reconstruction of cellular architecture

Digital images were processed and cells were contoured using Adobe Photoshop CS (Adobe) graphic editor and Wacom PTZ-1230d (Wacom) drawing tablet (Figure 2B). Then the contours were exported as .ai files. Localization by Z axis, alignment, surface generation, material assignment and cell lineage tracing were made using 3DSMax 7.0 (Autodesk) three dimensional vector graphic editor with Mental Ray renderer (Autodesk) (Figure 2C-E). Reconstructed cells were labeled with the random unique numbers.

5.3. Cell lineage tracing

The cell wall shape of the adjacent cells of embryos in three dimensional space served as a main data source for the reconstruction of all the cell divisions in the course of embryogenesis and lineage tracing. The algorithm of cell genealogy tree construction applied in this work, implies three steps: (1) the search for any last division or any two sister cells, (2) the consecutive revealing of the cells and merophytes (descendants of one cell) sister to them from previous generations and (3) the tracing of all the indirectly related cells of sister merophytes.

Genealogical relationships between the cells were estimated after the cell shape analysis based on R. Korn approach [24]. Any two adjacent cells were regarded as sister ones with a maximum probability, if their common shape looks like one cell, i.e. convex and smooth without concavities, and their contiguous cell wall had much surface comparing to other adjacent cells. Each less relative cell or its descendants (sister merophyte) to primary determined two sister cells were considered as a result of the previous divisions according to the degree of their relationship. Or in other words, any two merophytes were considered as sister ones with a highest probability, if their common shape looks like one cell, i.e. convex and smooth without concavities, and their contiguous boundary had much surface comparing to other adjacent merophytes.

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